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Promoting Academic Integrity: Council on Higher Education

Academic integrity and examination cheating

Academic integrity and 'sexually transmitted marks'

Academic integrity, plagiarism and assessment

Academic integrity: Role of quality assurance agencies

Research integrity challenges require innovative approaches

Statement on Ethical Research and Scholarly Publishing Practices

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Cover caption

A white shark (*Carcharodon carcharias*) in Gansbaai, South Africa (photo: Bernard DUPONT, flickr, CC-BY-SA).
In an article on page 54, Gennari, Morse and colleagues investigate the antibiotic sensitivity of bacteria isolated from the oral cavities of white sharks.

Other articles in this issue explore a different kind of predator – an academic predator – how to identify them and to protect academic integrity from becoming their victim. These predators take various forms in this issue: exam cheating, plagiarism and sexual misconduct. This themed issue on Promoting Academic Integrity emanates from the 2019 Conference of the Council on Higher Education and its publication is sponsored by the Council on Higher Education.

Academic integrity

We highlight academic integrity in this issue, relating it to initiatives in South Africa this year. In February, the Council on Higher Education arranged a conference on the theme 'Promoting Academic Integrity in Higher Education'; select papers from this conference are published in this issue of SAJS. In July, the *Statement on Ethical Research and Scholarly Publishing Practices* was formulated and signed by five key South African agencies whose commitments to its goals are provided in their Commentaries in this issue. We announce that Cape Town has been chosen to host the 7th World Conference on Research Integrity in 2021 – the first time this event will be held in Africa. We hope readers will find our content interesting, stimulating and useful. We also take this opportunity to affirm our commitment to ethical scholarly publishing practices.

Academic integrity appears fragile in our era, and frequently the Internet is held responsible. Journals, like SAJS, as well as university staff, find themselves acting as detectives as well as teachers and editors, and it has become the norm that students' work and manuscripts are put through similarity checking (or 'plagiarism detection') programs. It was with profound dismay that we discovered that two of the manuscripts submitted to SAJS from the academic integrity conference showed a high degree of similarity (about 40–50%) with previous work. There appears to be a crisis as scholarly ethics are compromised time and again.

The principles of modern academic integrity arose with scientific professionalisation and the separation of disciplines at the end of the 19th century together with the proliferation of scholarly journals. 'Original research' was emphasised and thus ideas of 'ownership' of that research emerged. The result was that use of research, without proper acknowledgement, became akin to theft. Students are often blamed for this kind of behaviour as they jostle for jobs and citations, but the matter is more serious. Deliberate academic deceit of whatever kind – and there are many variations – has manifold consequences: it can damage the entire enterprise and place the structure of knowledge and knowledge generation at risk.

Many readers will be familiar with the fraud of 'Piltdown Man' that I use as an example. There are numerous easily accessible accounts of this scam, its unfolding, and its cover-up, but I would like to emphasise its long-term effects on South African scholarship. I begin with the bare bones of the story. In 1912, British amateur archaeologist Charles Dawson claimed to have unearthed parts of the skull of the 'missing link', a species intermediate between apes and humans, in a quarry at Piltdown, Surrey, England. He shared the news of his find with Arthur Smith Woodward, Keeper of Geology at the Natural History Museum in London, and together they found more bones at the site. In an era before reliable dating techniques were available, Woodward hypothesised the age of the bones to be around 500 000 years. There was great excitement among the upper echelons of the scientific community as *Eoanthropus dawsoni* was announced and news proliferated through learned societies. Evolutionary theory was deemed to have advanced substantially. Not all scientists were convinced; some argued it was a composition of altered parts of a modern human and an orangutan. However, the weight of opinion lay with Dawson and renowned European anatomists, physical anthropologists and naturalists, many of whom were Fellows of the Royal Society and/or associated with the Natural History Museum, including Smith Woodward, Sir Arthur Keith and Sir Grafton Elliott Smith. A painting by John Cooke (1915) shows the distinguished group examining the evidence with a portrait of Charles Darwin hanging on the wall behind them.

The Piltdown finds were very satisfying to most of the scientific community because they aligned with the scientific paradigm and ideological preconceptions of that era. It was 'accepted' that humans had evolved in the northern hemisphere and finding a protohuman in England itself was a great coup. Not surprisingly then, when young, inexperienced, upstart Australian Raymond Dart, an anatomist at Wits – then a little known, minor and new university at the southern tip of Africa – came up with fossil evidence from Taung, it was not well received by the establishment. Dart was confident – he had, after all, once been Elliott Smith's assistant. Dart named the fossil *Australopithecus africanus*, and published a short account in *Nature* on 7 February 1925. Woodward and his colleagues responded vigorously, calling the claim 'preposterous'. Not only did Piltdown contradict this fossil find, but it was 'accepted' that early hominids had large brains (like Piltdown), a juvenile specimen was unreliable, and Africa was simply out of the frame. As the old adage has it, the largest impediment to new thinking is old thinking. Only in 1953 was the Piltdown fraud exposed when Oxford physical anthropologist Joseph Weiner (interestingly, another South African) and his colleagues, who by then had fluorine dating tests at their disposal, worked out the age of Piltdown and its component parts.[1,2] Thereafter South African palaeoanthropology took its rightful place.

A veritable industry has arisen around assigning responsibility for this academic fraud. Evidence suggests that Dawson alone was the culprit (he was responsible for numerous other forgeries), but some people at the time may have known, perhaps been implicated to some extent, or simply did not publicise their knowledge or suspicions for fear of discrediting their peers and destroying reputations. Some speculation about 'who knew what' appeared in SAJS as recently as 2016.[3] But this fraud is not just a joke, not merely a 'hoax' that needs to be uncovered like a 'whodunnit'. There is a larger element to deceit, and the consequences thereof, often unintended, can cause lasting damage. Piltdown damaged South African palaeoanthropology, perhaps irreparably, retarding knowledge and research for decades.

At the time Dart announced *A. africanus*, government support for the palaeontological disciplines was strong. In 1925, Jan Smuts's Presidential Address to the South African Association for the Advancement of Science (published in full in SAJS[4]) made this clear. Robert Broom and others were given financial and other state support to continue the search and, as is well known, their efforts yielded great treasures. But after 1948 the political context changed. With the National Party in power – strongly antithetical to the philosophy of evolution – this support ended. As apartheid became entrenched, international collaboration became increasingly difficult and, not surprisingly, the centre of gravity by way of expertise and finance for palaeo-studies shifted to East Africa. Certainly, under Phillip Tobias and others at Wits, the momentum was not lost entirely, and much has been regained since 1994. But a generation of research that would have brought renown to South Africa and its scientists was lost, irrecoverably.

Dishonesty can have a profound effect, with ripples and consequences far beyond the original act of corruption.

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Discovery and significance of komatiite: 50th Anniversary

In July 1969, 50 years ago, the discovery in the Barberton area of a new type of ancient volcanic rock was announced in Pretoria at an international geological symposium.

The discovery of this new rock, which was called komatiite, stemmed from extensive research carried out as part of an international cooperative programme to study the nature of the earth's upper mantle, lying below the earth's crust. One of the six projects identified for study was that of ultrabasic rocks (rocks with ultra-high magnesium content) approaching the composition of the earth's upper mantle. In 1965, we were appointed to carry out PhD studies in the Department of Geology at the University of the Witwatersrand on a range of ultrabasic rocks which were known to occur in the lower part of the 3.5-billion-year-old Barberton Greenstone Belt. These rocks had previously been ascribed to a deep-seated intrusive igneous suite, similar to the well-known Bushveld Igneous Complex. At this time, no conclusive evidence had been found of ultrabasic magma extruded on the surface of the earth as lava flows.

However, our extensive, detailed, geological mapping over four field seasons provided, for the first time, unequivocal evidence for the widespread presence of ultrabasic lava flows in the well-preserved, lowermost formations of the Barberton Belt in the Komati River Valley. Evidence included the recognition of extensive successions of pillowed basaltic lava (indicative of sub-aqueous extrusion) with unusually high magnesium contents. Interlayered with these lava flows were ultrabasic rock sequences with features suggesting that they too were of volcanic origin.

The ultrabasic rock sequences were found to be composed of discrete thin units with fine-grained chilled contacts indicative of rapid cooling of magma at the surface. In addition, the presence of a distinctive spiky texture (later called 'spinifex texture') was found to be present in the upper part of the thin ultrabasic units. This texture was shown to be a rapid supercooling feature of the ultrabasic units which were later confirmed to be ultrabasic lava flows. The presence of cross-cutting intrusive ultrabasic, 'feeder' dykes, was also evidence for the existence of an ultrabasic magma.

Bladed 'spinifex' textured crystals of olivine passing upwards into random or 'bird track' spinifex in a komatiite lava flow. These delicate crystal structures, the result of supercooling of an ultrabasic lava flow, are now recognised as one of the iconic and diagnostic textures in geology.

The twin brothers, geologists Richard and Morris Viljoen.

An extensive literature search revealed that the geochemistry of these olivine-rich ultrabasic rocks was distinctive, and that they had no similarity to any known class of lava or ultrabasic rock previously described on our planet. Some unique features of the Barberton ultrabasic lavas included a high magnesium content (averaging 28% MgO), low aluminium and very low potassium and sodium content, together with a high calcium-to-aluminium ratio. Based on all the above evidence, a new class of high temperature, ultrabasic volcanic rock was proposed, and the name 'komatiite', after the Komati River, was introduced by us. The associated magnesium-rich basalts were also shown to be unique and to have close affinities with the ultrabasic komatiite lavas. They were termed 'basaltic komatiite' (now called komatiitic basalt).[1,2]

Numerous renowned geoscientists contributed to the recognition of komatiite. Among them was Professor Harry Hess of Princeton University, a world authority on ultrabasic rocks, who visited the outcrops of the ultrabasic lava flows prior to attending the upper mantle symposium in Pretoria in 1969. He fully endorsed the evidence presented for introducing komatiite as a major new class of ultra-high temperature volcanic rock. Komatiites are now part of the vernacular of geology, on a par with, and completing, the long-standing volcanic rock classification sequence of rhyolite, andesite, basalt, and now komatiite.

Soon after the Barberton discovery, komatiites were recognised and described from many other Archaean greenstone belts worldwide, and were described by Arndt and Nisbet in 1982 as 'one of the most important petrological advances of this century'[3]. Experimental melting and supercooling of komatiite lavas has shown that they were extruded in a primordial ocean at temperatures of more than 1600 °C. This is far hotter than the temperature of extrusion of oceanic lavas such as those from Hawaii which erupt at temperatures of about 1250 °C. It has further been shown that komatiite lavas represent an almost total melt of the earth's upper mantle. They formed the earth's earliest oceanic crust before the widespread intrusion into the primordial komatiitic crust of the major continent-forming granitic rocks which are also classically exposed in the Barberton region.

Of great significance is the fact that 3.47-billion-year-old carbonaceous sediments, in places interlayered with komatiites, contain the earliest forms of life known on our planet. These life forms occur as biomats and resemble present-day algae and cyanobacteria. The link between them and the nutrient-enriched environment supplied by the komatiite lavas is compelling. A close genetic relationship between gold mineralisation in greenstone belts and, in particular, the Barberton Belt and komatiites, has also been shown to exist and exciting new concepts are developing.

Research on komatiites continues unabated and there are now hundreds of publications on these fascinating rocks. We gave talks at the University of the Witwatersrand to mark the 50th anniversary of komatiites, and, in September 2019, we led a 4-day excursion to the Barberton area. A popular style geoheritage guidebook to the region is also being prepared and will be published by the Geological Society of South Africa.

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Suspense and discovery with Pint of Science

Nine scientists walk into a bar. Because they are there, 200 or so other people also walk into the bar. What do you have? A crowded bar full of people listening to scientists talking about their research over a drink in what has become a global science festival. This is Pint of Science!

Although a smaller event than last year's, Pint 19 was no less exciting.

South Africa is one of 24 countries that took part in the Pint of Science Festival held worldwide from 20 to 22 May 2019. 'We might not be in the same time zone, but for three days we're in this together and we are doing something,' enthused Micheline Franz, Co-Director of the South African Pint of Science Chapter. This year there was only one venue, compared with the three in 2018, but there are plans to expand again in the future. The event was appropriately held at Cause Effect Cocktail Kitchen in Cape Town – think dry ice cocktails overflowing with smoke.

This year's Pint of Science South Africa covered three themes: Our Body, Our Planet, and Atoms to Galaxies. Particularly rewarding this year was the familiar faces from the previous year's talks – both in the audience and in the speaker line-up. These are the beginnings of a new shared community that has been brought together by Pint of Science.

Last year, Dr Kerryn Warren spoke about what a human–Neanderthal hybrid might look like; she returned this year to discuss her adventures as an 'underground astronaut' in Lee Berger's team at the Cradle of Humankind in Gauteng, that involved climbing through a cave network with an 18-cm pinch point to enter the Dinaledi Chamber, where *Homo naledi* was discovered in 2015. 'I'm just here to have some fun,' she reflected during the evening as she sat with a *Homo naledi* skull from the excavation resting on the table in front of her. And she certainly seemed to have fun talking the audience through her experiences:

> *You have to actually cave in a difficult system for 30 minutes just to get into the Chamber,* [then smiling] *– but we have wifi! So we have our priorities straight.*
>
> *It's not unusual for us, while we're in the cave, to Google Hangout with Lee Berger in Command Centre so that he can see that we're safe. But he's just spying on us so he can see the cool things that we're seeing.*

Warren often talks to school groups about her work – including from inside the Dinaledi Chamber while she was there. She is currently doing her postdoc in evolution education, which complements her previous palaeontological work.

Warren is appreciative of initiatives like Pint of Science because she believes that:

> *a lot of people who opt in are really interested in science in general, and I don't think they have a lot of opportunities to just casually interact with scientists and their research, so this is a great opportunity to do this.*

Two other returning speakers, Dr John Woodland and Dr Rubina Bunjun, are also keen science communicators. Woodland spoke this year about the journey of drug development 'From Bench to Bedside', and Bunjun's talk was about the controversial possible link between increased HIV infections and injectable hormonal contraceptives.

With an audience ranging in age from 9 to 79, Pint of Science South Africa certainly has found wide appeal – and it is great to see plans being made to include interested under-18s more directly, even though the event is pub-based.

The casual setting also allows the public to appreciate the human side of scientists. After his visually rich talk about radio astronomy – including a feature on the first ever imaging of a black hole – Dr Vasaant Krishnan crouched down, pint in hand, to talk to a young lad. Young Joshua was desperate to ask Krishnan a question but had been too shy to speak up during the question session after Krishnan's talk about MeerKAT (an array telescope that forms part of the Square Kilometre Array project, where Krishnan is a Junior Commissioning Scientist). Krishnan explained:

> *The reason that MeerKAT is so good at what it does is because it is not just a single telescope, but a collection of 64 of them working together. They are all synchronised to observe the same source at the same time.*

With an array telescope you can combine data from multiple receptors to 'artificially create a telescope that is as big as the distance between them'. So, in fact, as Krishnan pointed out, 'Meerkat is essentially a telescope which is 8 km big.'

To illustrate the additive power of combining receptors, Krishnan showed an image of an orange ellipse on a black background – the centre of our galaxy imaged using two receptors. Krishnan then slowly walked the audience through the improvements in resolution from two receptors, to four, to sixteen, until the full array was complete and the blobs had resolved into separate shapes: supernova remnants, the Sagittarius A* black hole, and vertical lines that may provide clues to the direction of the galaxy's magnetic field. 'So adding those receptors has improved MeerKAT's resolutions and also its sensitivity,' concluded Krishnan as he showed the high fidelity image of the centre of the Milky Way that had been unveiled at the 2018 inauguration of MeerKAT.

Appealing to those present who were closer to the older end of the age scale, Prof. David Tabb talked about the use of biomarkers in diagnostics, and how these can reduce the need for invasive techniques, such as colonoscopies, that become more common with age. 'Turning 50: what kinds of gifts will you receive from your doctor? Number one – you will receive a colonoscopy!' Tabb went on to list other undesirable 'gifts' that are advised for screening, concluding the list with, 'Turning 50? Not advisable. Not advisable… So, how can we make things better?'

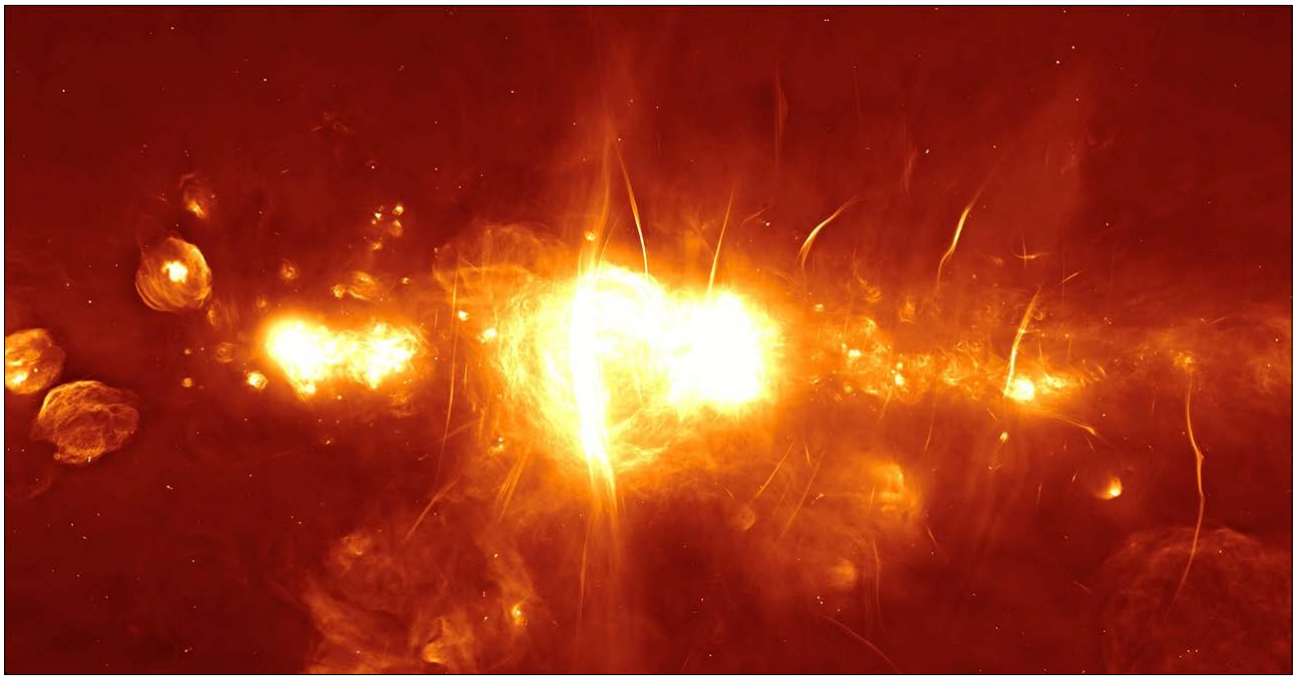


Image of the centre of our galaxy using information obtained from MeerKAT (image: South African Radio Astronomy Observatory).

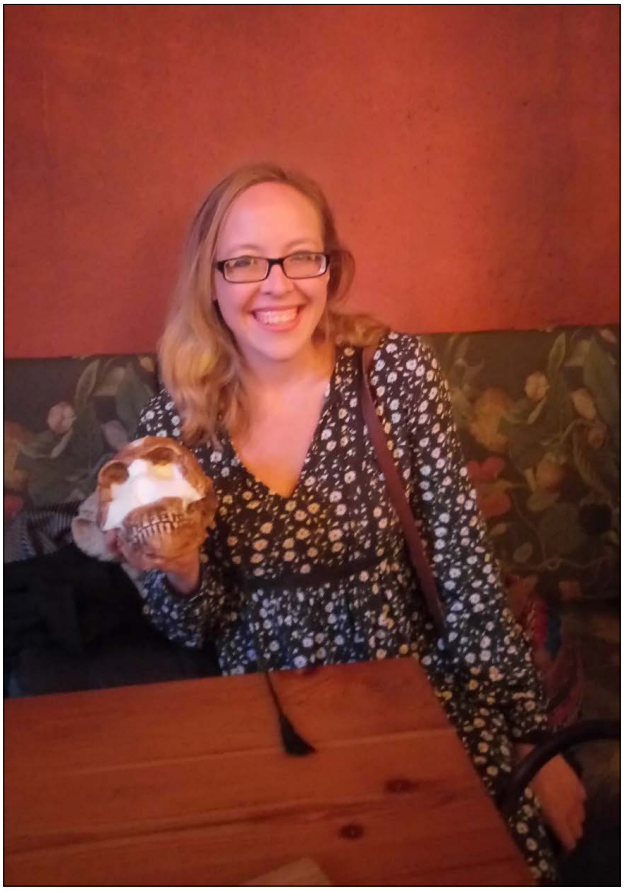
The focus of Tabb's contribution was on the research that he did while in the USA on the early detection of colon cancer, but in recent years he has become more involved in exploring urine and blood as biomarkers for tuberculosis research in South Africa. Tabb followed on from the drug discovery talk by Woodland, highlighting the striking parallels between finding a useable drug from the millions of candidates, and finding a potential biomarker out of thousands of candidates. These similarities highlight the challenges faced by scientists aiming to improve medications and diagnostics – with massive investments being made during years of disappointment, before, finally, a promising candidate drug or biomarker is identified.

'It really is a very high risk, possibly high reward game,' says Woodland. 'Fortunately, there is some cause for optimism and hope, especially locally.' He showed a picture of Dr Kelly Chibale, head of the H3D Drug Discovery Group in Cape Town, which has had some success in making new antimalarial drugs.

> *What's very exciting is we've got a clinical candidate for malaria, MMV048. It's a single dose cure that's made it all the way to Phase II clinical trials. So that's in Ethiopia at the moment. … With any luck that might be the very first drug that makes it onto the market that was developed right here, in Cape Town, in South Africa.*

It is stories like these – of suspense and discovery – that draw people to science, and keep people coming back to events like Pint of Science. 'It brings you into a space that you wouldn't usually be in,' mused Franz, 'And it takes you further in a Google search, because you can actually talk to the researchers doing the work.'

If you are interested in helping Pint of Science expand in 2020, within Cape Town or across other areas of South Africa, please email contact@pintofscience.com



Kerryn Warren with a *Homo naledi* skull (photo: Clare Garrard).

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Introducing bud bank and below-ground plant organ research to South Africa: Report on a workshop and the way forward

Bud banks are the source of vegetative reproduction of plants. They are linked to regeneration strategies of plant communities in ecosystems prone to disturbances.[1] Bud bank research is fast moving up the research agenda as an approach to better understand the dynamics and resilience of ecosystems.[2] Regeneration from seed is only one of a myriad of strategies that plants use to survive and flourish in ecosystems with seasonal rainfall, above-ground consumers such as fire and herbivores, or recurrent droughts. Because the vegetation dynamics, structure and function of southern African grassy biomes are driven by these disturbances, one would expect interesting below-ground regeneration strategies. However, studies on below-ground traits are underrepresented in the scientific literature, with only a few contributions pertaining to 'below-ground bud bank' as the main topic. Furthermore, most of these studies have been conducted in the northern hemisphere, such as in China, central Europe and the USA.[3] Bud bank related research in the southern hemisphere is represented by one study from South American grasslands[4] and one from savannas in Botswana[3]. Recent papers on 'underground trees of Africa'[5] and the underground storage organs characteristic of 'old-growth grasslands'[6] have highlighted the importance of quantifying and describing below-ground regeneration strategies to understand the evolution of our ecosystems, and appropriate ways to manage and conserve them. The savanna and grassland biomes in southern Africa host a high richness of herbaceous plants, particularly forbs, which are often more abundant below ground than in the standing vegetation.[7] Below-ground bud banks comprise an important regeneration strategy for many savanna species, and yet very little is known about below-ground strategies for plant growth-form coexistence in grassy ecosystems.

Considering the importance of understanding the 'below-ground world' for a broader comprehension of savanna ecosystem resilience in general, savanna ecologists from southern Africa and Brazil discussed the need to bring local researchers up to date with the latest approaches in regeneration strategies. In May 2019, a first hands-on workshop on how to survey bud banks and below-ground plant organs in grassy ecosystems was hosted by the Forb Ecology Research Group from the Unit for Environmental Sciences and Management, North-West University (Potchefstroom, South Africa), in collaboration with São Paulo State University (UNESP) in Brazil. This 2-day event attracted several established researchers in southern Africa and a robust community of young scientists and postgraduate students representing North-West University, University of Pretoria, University of the Witwatersrand and University of Edinburgh (Scotland). Considering the novelty of the subject and the underrepresentation of similar studies on grassy biomes in southern Africa, the workshop aimed to bring this approach to the ecological research community, broadening the understanding of the dynamics of southern African grassy biomes for improved management and conservation of these highly diverse ecosystems.

The 2-day workshop covered relevant background information on bud bank related ecological research, after which delegates had the opportunity to undertake field surveying at the Unit for Environmental Sciences and Management research facility outside Potchefstroom, followed by working group sessions to analyse and interpret results. The theoretical background of the workshop highlighted the critical role of the below-ground structures in regeneration after disturbances, and the importance of a standardised approach to investigate the morphology and terminology of below-ground bud-bearing organs. Based on the available literature on the morphology and terminology of below-ground bud organs[1,2], the workshop emphasised the importance of these less explored functional traits for future studies in grassland and savanna ecology. The event furthermore provided opportunities among scientists interested in grassy ecosystems to network and discuss projects and questions related to grassland and savanna dynamics and conservation. Young scientists had the opportunity to partake in such discussions to stimulate their thinking on the links between diversity, function, evolutionary history and disturbance in grassy biomes of South Africa.

Implications for the broader South African ecological community

Additionally to the development of skills among young scientists in southern Africa, the event stimulated discussions on the need to establish long-term monitoring sites for an improved understanding of grassland and savanna dynamics, and of potential threats to both species and functional diversity. The event also created opportunities for joint efforts to compare African and South American grassy ecosystems.

Anticipated value of this approach to ecological research in South Africa

Resilience of grasslands and savannas

South African grassland and savanna vegetation is resilient to the disturbances, including fire, herbivory and rainfall variability, within which it has evolved. In fact, these grassy ecosystems are dependent on such natural (i.e. endogenous) disturbances. However, the ecosystems are particularly sensitive to human-induced (i.e. exogenous) disturbances such as ploughing, as most herbaceous species are unable to re-establish after long-term changes in the soil.[6,8] Thus, both the evolutionary history and the history of human-induced land-use changes are contained in the below-ground plant traits of grassy ecosystems. As the majority of plants in southern African grassy systems are almost equally dependent on regeneration from below-ground bud banks as from a viable seedbank[6], exploring below-ground plant traits that articulate adaptations and/or sensitivity to endogenous and exogenous disturbances will enhance our understanding of the resilience and sensitivity of grassy ecosystems to global environmental changes[8].

The link between below-ground bud bank studies and ecosystem resilience can be applied at various spatial scales. For example, at community/landscape scale, vegetation state change (such as bush encroachment), biome shifts (particularly what drives the dominance of one life-form over the other), and coexistence of various life forms, can be better understood with knowledge of below-ground plant regeneration strategies. Below the community-level, species-specific responses to both endogenous and exogenous disturbances can inform biodiversity conservation and management practices of southern African grassy ecosystems.

Global change effects

Elevated atmospheric CO_2, climate change, nitrogen deposition, altered endogenous disturbances, anthropogenic land-use change, and alien invasions are well-known drivers of global change. Such disturbances may also impact bud banks. For instance, bush encroachment, partially driven by elevated CO_2 levels, alters natural fire frequencies which may result in a loss of the herbaceous, bud-bearing component. How reduced bud bank resources in bush-encroached grassy ecosystems are linked to changes in regeneration strategies, and hence, to the loss of resilience, remains unexplored in southern African ecosystems.

The C3 herbaceous plants in grasslands and savannas, with their extensive underground structures, contribute substantially to carbon sequestration.[9] This essential ecosystem function provided by the herbaceous communities of grassy ecosystems necessitates the maintenance and conservation of the diverse range of herbaceous functional groups. Land-use change related to losses of accumulated soil carbon, such as ploughing or tilling, is known to have significant impacts on the biodiversity and resilience of grassy ecosystems.[8] However, it is less well known how ploughing devastates the communities of plants who depend on below-ground organs for their persistence.

Effects of invasive aliens or encroachment of indigenous woody plants on plant diversity are well known. However, there is a paucity of information available on the competitive advantage of problem plants over indigenous plant communities as a result of their distinctive below-ground bud regeneration strategies. Moreover, our current understanding of the competitive advantage of problem plants over the natural communities, which relied on below-ground regeneration to persist[10], when indigenous bud banks have been destroyed after exogenous disturbances, is incomplete.

Management

Whether managing for ecosystem services, biodiversity conservation, ecosystem resilience or productivity, we argue that southern African ecologists need to consider below-ground regeneration strategies. For instance, managing for optimal rangeland productivity through bush control will require an in-depth understanding of the below-ground ecology of the particular problem plant. Restoration and rehabilitation efforts should also consider plant regeneration strategies beyond seed banks as many species with below-ground buds are disproportionately contributing to the carbon storage and resilience of grassy ecosystems. This fact also needs to be considered in agricultural land-use expansion. Lastly, southern African grassy ecosystems host a substantial abundance of indigenous food plants and medicinal plants, many of which are harvested for their underground organs, of which the below-ground bud-regeneration strategies need to be understood to ensure sustainable harvesting.

The diversity of below-ground organs in southern African flora and their contribution to ecosystem functioning, necessitate studies focused on below-ground regeneration strategies. Studying bud banks will improve our knowledge on the dynamics of grassy ecosystems, leading to more effective conservation and management of the grassy biomes of South Africa.

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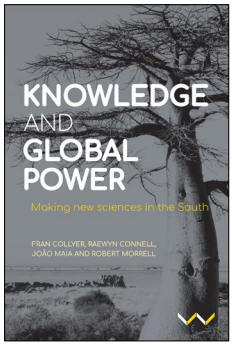
BOOK TITLE:
Knowledge and global power: Making new sciences in the South



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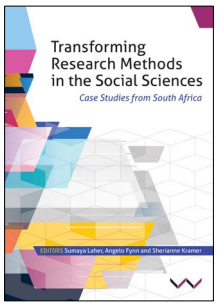
Southern crossings: Thinking inside/outside the hegemon

'Thinking from the South' has become an attractive buzz phrase for those who want to challenge the seemingly hegemonic control that the North exerts on the global production of knowledge. In a growing market of competing and competitive universities and research institutes, the proliferation of predatory journals, the conglomeration of the traditional peer-reviewed journals and the ubiquitous and relentless race for ever higher global rankings, it is becoming more complicated to define what the 'production of knowledge' actually consists of. This difficulty is in part because the term 'product' is itself controversial. On the one side are those scholars who argue that universities, especially, should not become analogous with factories and assembly lines where 'products' are produced and packaged. These scholars resent and critique the marketisation of the academy and, more often than not, they also vilify the commoditisation of publishing, promotions and other markers of academic excellence. On the other extreme are the 'beneficiaries' of the current system – the 'new' private universities, the indexing databases and the university executives whose pay is linked to performance – who will argue that the old system and the status quo that came with it, entrenched the power of universities in the North and that the current marketisation is disrupting that entrenched hierarchy. Whichever side one chooses, the 'neoliberal turn' is rapidly becoming the new norm and it is to the credit of the authors of *Knowledge and Global Power: Making New Sciences in the South* that they do not begin by assuming that this neoliberal turn is a universal occurrence. Instead, what they achieve in the book is a nuanced and articulate description of how three domains of knowledge have been shaped by the globalisation of knowledge and what the responses of knowledge producers have been. However, as is clear from the latter sentence, the vocabulary is itself quite unwieldy. Instead of writing and thinking about 'intellectuals' or the 'intelligentsia', we now have to resort to 'intellectual workers' or 'knowledge workers'. The old is indeed dying. In tracing the three domains of gender studies, HIV/Aids and climate change, the authors not only investigate the global growth of publications in these domains, but they specifically focus on the political and scholarly contexts from which these domains have grown in the South. The countries chosen – Brazil, South Africa and Australia – exemplify the complications of even applying the term 'South' to a domain of intellectual endeavour. Superficially, Australia does not seem to fit the description of a 'Southern' country, yet, as the research shows, the dominance of the Northern academic industry affects a former settler colony in much the same ways that it affects post-apartheid South Africa.

The main strength of the arguments proposed in the book is that they are all based on interviews and statistical analyses of peer-reviewed journal indices. Again, rather than merely resorting to the rhetoric about the 'neoliberal university', the authors test the applicability of this terminology by speaking to intellectuals in Brazil, Australia and South Africa about the evolution of their institutions over the last 30 years. More importantly, the authors take seriously the concept of extraversion proposed by the Beninese scholar Paulin Hountondji which describes 'the practical ways knowledge workers in the periphery are oriented to, and become dependent on, the institutions, concepts and techniques of the metropole' (p.10). This notion of extraversion functions as a neat backbone around which to hang the arguments of the book and the authors constantly return to the question of how and whether Southern scholars do shift away from or modify received ideas from the metropole. The contrast between the periphery and the metropole is further elucidated by the intellectual trajectories of the Southern scholars as many have experience of either studying in the North or being in collaborative relationships with Northern institutions. These positive attributes mean that the book is an essential and important contribution to the debates about Southern theory, postcolonialism and decolonisation.

The main limit of the book is that it is based on anonymised interviews, although the anonymity is understandable, that is, not only in the face of possible adverse consequences for the researchers and interviewees but also in terms of the repercussions that may affect the future employability of academics. The overall effect is that although the book contains ample evidence for a 'sociology of knowledge' that is based on accounting for the contributions of Southern intellectuals, these intellectuals become empty and generic ciphers who not only have anonymised names but their intellectual trajectories are also anonymised, for example in descriptions such as 'Pat…who attended a university in the North'. The latter diminishes the book's potential as a resource for other researchers working in Southern countries. Additionally, and despite its intent, the book ends up presenting Southern intellectuals as disembodied repositories of 'Southern-ness' rather than as flesh-and-bone academics who also have a vested interest in the success of their careers. The other weakness of the book is in the second phrase of the title, 'making new sciences in the South'. The authors repeatedly challenge the manner in which the three domains under consideration have been dominated by either biomedical science (in the case of HIV/Aids research especially) and the physical sciences (in the case of climate change and gender studies) and that this dominance has come at the expense of the humanities and social sciences. Yet, by titling the book using the words 'new sciences' the authors have created an expectation which they do not actually meet – which is that Southern scholars are collectively creating a novel and innovative epistemology to challenge the current American-centric worldview. In the place of radical raptures, the authors instead found the repeated invocation of local knowledge as an alternative gnosis. Although such a conception of knowledge is potentially useful in understanding the future paths of 'Southern' knowledge production, it is hardly the stuff of 'paradigm shifts' as defined by Thomas S. Kuhn. Thus, the authors seem to have anticipated their own conclusion, which is that where alternative knowledges are propounded, they are 'epistemologically loose' (p.174) and depend in large part on the intellectuals' histories as activists and/or frontline respondents to crises such as the HIV/Aids one. By ending the book with these conclusions, the authors present the big ambition of 'Southern' knowledge while factually illustrating the meagre dividends of these putatively new approaches.

BOOK TITLE:
Transforming research methods in the social sciences: Case studies from South Africa



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Engaging with the challenges of social science research methods

Many scholarly books have been written on the application and planning required for adopting appropriate research methodologies that address social complexities. Past examples include debates on the enforced notion of a 'culture of silence' by post-colonial literary theorists and cultural critics[1,2] as well as practical examples of innovative participatory research in the social sciences[3]. *Transforming Research Methods in the Social Sciences,* with its case studies from South Africa, is a welcome addition in negotiating a more inclusive exploration of research use and practice recognising diverse cultural complexities.

The ambition for the book is to address social challenges by demonstrating how past limitations and tendencies in research methodologies, applied by the social sciences, might be overcome. It is well recognised that, in contemporary social and scientific research that claims responsibility for social transformation, radical shifts in normative approaches and perceptions have introduced an element of unpredictability that impacts on the way we do research. Efforts to capture this transformation in how we generate knowledge was introduced, for example, in the theoretical framework of Mode 2, which acknowledges a Knowledge Society, a Risk Society and an Information Society that all play roles in this endeavour. This opposes Mode 1 that follows a linear (deficit) hierarchical process. It is inevitable that, within this transformed knowledge production landscape, new theories as well as new research methodologies should be adapted to fit new purposes.

In order to rise to this challenge, this book addresses how methods such as psycho-biography, critical discourse, feminism, ethnography, auto-ethnography, photo-voice and action, and community-based research methodologies might be used in ways that open up further discourse as well as provide guidance to future researchers. Most of these methods are not new and have already been applied in, for example, India and Brazil. The value of this book lies in bringing them together in a single publication focused on South Africa.

Although the editors endorse Mode 2 as well as a transdisciplinary approach, the content pays disappointingly little attention to exactly how these approaches will have impact and transgress disciplinary boundaries. Mode 2, for example, is measured through monitoring and evaluating impact of research findings, and the book falls short in demonstrating how real change takes place within communities. Although transdisciplinary approaches are mentioned in the introductory chapter, they seem to comprise no more than a buzzword in the book.

The focus remains on the dichotomies between qualitative and quantitative research, with dips into methodological varieties. One wishes for more references to seminal works on different forms of social science theories and related research methodologies but, on the other hand, the exploratory nature of the book remains useful and interesting. Moreover, the book might have been more innovative by exploring the relation between theory and practice, the current impact of socio-technical systems, the easily communicated seductive use of the creative arts and visual literacy by means of documentary films and ethno-literary approaches.

Social scientists, out of sheer necessity, use methods beyond their disciplinary training. But theories applicable to the North are seldom a 'fit to size' for scholars from the South. However, the shift into 'unmarked' territory during the process of fieldwork often provides unexpected space for creativity. Although doing so is daunting, and often with limited theoretical support or guidance, scholars in the South have provided unique, appropriate and empowering insights when they venture into research methodologies that embrace the world represented, often simultaneously, by the formally educated and by those strong in indigenous knowledge. With such research best understood as a way of both 'being in the world' and 'doing in the world', this book invites the reader to consider technology that brings about dialogical transformation.

Dialogical transformations affect many traditional structures, ranging from the political to the personal, and in a sense de-monopolise and de-compartmentalise expert knowledge by allowing *all* voices to be heard. There are challenges. The first is to manage creative approaches with research methodologies to address the specific difficulties of researchers in the South. Secondly, a more reflective conceptualisation needs to be embraced. The social sciences, in general, include invention as well as experimentation of methods to suit specific purposes, such as *bricolage*, photo-voice and visual language to generate knowledge and to continuously expand ethical reflection. This requires methods that are ahistorical, interpretive and analytical. By using the methods of the humanities, such as visual communication (film documentaries) and literature (poems and essays), the scope of traditional social science based methodologies is enlarged. This book brings to mind the challenges that current researchers face when employing social science methodologies such as interviews, ethnographies, surveys and statistical data analysis. Few academic programmes provide support or training in these areas. Social scientists also provide examples of how their research methodology might be useful to the humanities, while the latter may have methodological solutions to enrich the understanding of topics in social science fieldwork.

Despite its few shortcomings, this book is a useful guide for social science researchers in that it contextualises their methods within the South African environment. Although there is sadly still a tendency to fall back on local politics of race and culture (absurdly 'apologising' for being white), enough is provided to benefit scholars both locally and internationally. It is useful for exploring the compatibility between different purposes, based in different disciplinary relationships, that brings about different ways of conceiving knowledge, thus providing a possibility to enhance traditional research practices within challenging social structures, especially those practices that maintain an often unsuitable normative order within ever-transforming social structures. This book demonstrates sufficiently that the social sciences construct the social world through scholars who, while being the subjects of such constructions, also conceptualise social order by means of the research methodologies that they apply.

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BOOK TITLE:
The state of the South African research enterprise

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Local intellectual labour has a global effect

Johann Mouton and colleagues have compiled a magisterial volume on *The State of the South African Research Enterprise*. It originates in the DST-NRF Centre of Excellence in Scientometrics and Science, Technology and Innovation Policy based at Stellenbosch University. It builds on years of research conducted at CREST (Centre for Research on Evaluation, Science and Technology), which was established as a research centre at Stellenbosch University in 1995 and has since then been the mainstay of research on the state of research in South Africa.

The goal of this study was to conduct a 'comprehensive assessment of the state of the South African research enterprise' (p.i). This goal should be understood in the context of national and global scholarship that seeks to examine intellectual work and its outputs. In the national context, such investigations have often asked two kinds of question. The first is: to what purpose is knowledge put? Under apartheid this question often suggested ideological orientation in funding research and producing knowledge. The second relates to South Africa's national research capacity in relation to Africa and the rest of the world.

This work focuses largely on the latter question, taking a highly detailed bibliometric approach that concentrates on South Africa's global positioning and ranking. These issues dominate the first three sections of the book. The fourth section focuses on a strategic research assessment of six areas: (1) agriculture and food security, (2) climate and the environment, (3) education, (4) energy, (5) health and (6) water. Here the question is: to what extent is South African research responsive to national (and international) societal priorities and goals?

The book unfolds a clear and precise explanation of our research enterprise. It is conceptualised as consisting of three dimensions: research funding, research capacity and research performance. Funding and capacity refer to the availability of money for research and the skilled people to undertake the investigation and thinking for knowledge production. The impact of the conjunction of these two enablers is addressed in the assessment of the research performance. In this latter regard, both quantity and quality of research outputs matter.

The higher education and research landscape has been revolutionised by bibliometrics and obsession with competition and measurement. This is mirrored in the audit culture that has gripped universities. Understandably there is scepticism about the measurements. There is a reductive element to them in which the fine-grain detail is lost and qualitative analyses are ignored. Bibliometrics are also open to manipulation and outright 'cheating' in which citations, for example, can be boosted and publication rates inflated by recourse to predatory publishing.

Yet bibliometrics and measurement are here to stay; university ranking systems proliferate and universities invest a great deal into getting rated and advancing up the league tables.

What is impressive about this book is how it 'drills' down to expose trends that broad analysis often misses. This detail is an effect both of a sophisticated methodology and a very clear conceptualisation of what the book needed to do to provide a proper and helpful analysis. It is difficult to argue with the authors' own assessment that 'The report arguably constitutes the most comprehensive empirical assessment of the state of the South African research enterprise' (Preface).

What are the take-home messages of this study? South Africa does very well in an under-resourced environment. This simple statement should be evaluated against the backdrop of a current global interest in knowledge production and the continued, but not uncontested, dominance of the global North. One of the reasons for this dominance is the under-resourcing of research in the global South.

As the authors write:

> *South Africa invests too little in Research & Development (R&D). Although nominal expenditure has increased, Gross Domestic Expenditure on R&D…has remained unchanged at around 0.8% for most of the past fifteen years. South Africa's poor performance is best illustrated by the fact that, when compared to eight very similar research systems, our investment is less than half of their mean investment'.* (p.1)

Countries within the Organisation for Economic Co-operation and Development in Europe spend an average of 2.4% per annum.

Reasons for underfunding are complex but include the crisis facing the whole country and the decline in corporate/business expenditure on R&D, partly the result of the troubles of several large companies which once invested heavily (Anglo American and Eskom) and the movement of local R&D to other countries (p.2).

'The research capacity in the country is too small and needs to be expanded as a matter of urgency'(p.2). Countries similar to South Africa have, on average, twice as many full-time equivalent researchers per thousand of their workforces and three times as many per million of the countries' inhabitants. Great strides have been made to boost the PhD pipeline but the ratio of doctoral graduates to the population remains well below international benchmarks. Alarmingly, for the first time in a decade, there has been a decline in full-time equivalent researchers within universities: from 5098 in 2014/2015 to 4702 in 2015/2016.

Despite these problems, the authors conclude that, overall, South Africa's research performance is 'excellent' and describe the country as 'punching above its weight' (p.2). In terms of international benchmarking, South Africa has increased its research output and world research share and improved its world rank (28 in 2016). The output of articles (according to the Web of Science) increased from 3668 publications in 2000 to 15 550 in 2016. This annual average growth of 2.9% resulted in a doubling of South Africa's relative world output (from 0.4% in 2000 to 0.91% in 2016).

This study is a very important one that, it is hoped, will persuade funders (the state, industry and corporates, and organisations) to contribute to South African research. It will encourage our scientists because it demonstrates that local intellectual labour has a global effect. This is reassuring in the face of rising levels of competition and increases in the production of research outputs. It also offers policymakers guidance on what needs to be done to ensure that South Africa continues to contribute to the global store of what we know.

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Educating scientists in South Africa in the 21st century

In South Africa, teaching science at a tertiary institution faces a particular confluence of societal forces which can be seen as a major threat to the educational effort. These forces include strong pressure from the government to accept a mark of 30% at the school exit level as 'university entrance'; a call for a decolonised curriculum; and an increasing prevalence of conversation around the Fourth Industrial Revolution. These then translate rather crudely into a sense that we have students who are less prepared, aiming to achieve preparation for a job market no-one knows about, using a curriculum that we have to make up from scratch. Of course, to put it in these terms is to both trivialise the issue and to problematise it in such a way that we have no recourse other than to sit around wringing our hands, lamenting for the good old days when we were students.

Nonetheless, each of these issues pulls in a slightly different direction. But I contend that investigating what is actually at stake can provide a perspective which, rather than suggesting a triple threat which will sink our educational efforts, might afford a perfect opportunity to seriously interrogate our current educational efforts. This requires an acknowledgement that we may not be quite as adept at teaching as we have fancied ourselves to be. It does not take a brilliant teacher to lead a student who is well resourced and has been trained to be intellectually curious.

We are not likely to be able to substantially change the school system. Even if the current position that 30% somehow constitutes a 'pass' was reversed, it would have no impact on the reality that most learners are taught how to pass exams and maximise their marks, rather than to seriously explore any knowledge area. The long legacy of the Bantu education system will not be easily shifted at the primary and secondary levels of education. This situation, coupled with the shrinking attention span induced by social media and the information age, does not immediately incite hope. But this does not mean that the students entering higher education are not capable of intellectual curiosity. They just have not yet had sufficient exposure to the idea of the tension of inquiry which can be broken by the delicious sweetness of a flash of insight. So, the first question emerges: How do we create an environment in which we can help students to begin to tolerate the tension of inquiry?

In a different way, the call for decolonisation of curricula is often dismissed by academic scientists as being irrelevant. From the perspective of a scientist, it seems obvious that one can teach principles of literary analysis as effectively using Chinua Achebe in place of William Golding. Of course, this fails to critique the value of the current forms of literary analysis itself! But science is science is science – is it not? Is it not this that makes it science? However, if we approach the issue from a different angle, we begin to discover a world which makes many scientists slightly uneasy. What if we take seriously the notion that some of our students do genuinely experience alienation in our lecture theatres? What is the source of that alienation and is it our task to address it or attempt to manage it in any way?

Against the backdrop of this confluence of pressures, it is easy to feel slightly despondent. However, I would like to argue that this triple threat can be seen as an opportunity. An opportunity to really question what we are doing as educators on undergraduate science programmes. The sense of dis-ease in the system means that we cannot pretend that all is well with the status quo. Is there another way?

I believe the work of Bernard Lonergan[1,2] offers us a theoretical framework within which we can begin to imagine a truly educative offering in science. Those of us who choose academia, do so because somewhere along the line the satisfaction of the periodic breakthrough of insight began to outweigh the discomfort of the hours spent in the tension of inquiry. At some point the satisfaction gave way to passion – either in a memorable moment, or in the gentle shaping that takes place over hours, days and weeks of focused effort. And yet, there is a strong message which shapes our time which resists any form of discomfort. It is against this that we need to begin to talk positively about the tension of inquiry. The space of not understanding *yet* is in fact a vital part of the educative process. Precisely because new knowledge needs to be constructed in the mind, it is not simply a process of information transfer. The connections need to be made before insight can be achieved.

Lonergan offers us a four-part model of the educative process.[1] It is probably important to state from the outset that Lonergan is both a man of his time and of his tradition and as such some of his language and imagery may not be immediately palatable in South Africa today. Nonetheless, if we can forgive him his cultural formation, his model provides a coherent framework in which many aspects of educational research can find their home. His major guiding principle is that of 'self-appropriation'. Lonergan is seeking to provide an educative framework that can provide the scaffolding for the fulfillment of a person's potential. The equivalent in my mind in education-speak is 'critical citizenship'.[3] We want to facilitate the development of a person who is capable of engaging responsibly, and intelligently, in the community in which they find themselves. And that immediately summons Amartya Sen's notion of 'capabilities' to the conversation.[4]

For now, though, Lonergan's intellectual curiosity is an attempt to understand understanding. Doubtless, as a philosopher and theologian, his own route to self-appropriation was through engagement in a plethora of intellectual tasks. Whether this route is the only one to self-appropriation is perhaps a good question, but given that we are in the business of trying to educate, Lonergan's exploration into the intellectual serves us well. His route to self-appropriation is through an educative scaffolding and so has the potential to be applicable without too much adjustment to fit. It comprises experience, insight, reflection and decision-making.

Experience comprises both cognitive and physical engagement. Anything that provides stimulus will provide experience. But it is worth noting that the person who makes meaning of prior experience will create a filter through

which they will interpret current experience. Experience of a particular intellectual problem will induce the tension of inquiry. The tension of inquiry is the interior experience of knowing that there is a specific intellectual problem that I do not yet grasp.

Insight is the glorious moment of intellectual breakthrough when the tension of inquiry gives way to a flash of understanding. It is the spark in the eyes that warms the heart of any educator. But that understanding may or may not be accurate. And understanding may be partial. It must be tested.

Judgement is the outcome of the reflective process which must follow understanding. Just because I think I understand does not mean that I understand correctly. I must test my understanding in some way. Either through attempting to solve a problem that previously eluded me, or by returning to the textbook or some other primary source and reading what has been said.

Decision-making is the final step. Once I have made my judgement, I must consider the consequences. What action does my judgement require of me? If I have assessed my judgement to be accurate, do I want to move onto something new, or do I want to consolidate?

There are several important facets to this framework. Lonergan's argument is that any subject matter can provide the trajectory towards self-appropriation. Self-appropriation is the outcome of the practice of responsible decision-making based on reasonable judgement which itself requires attentiveness to experience such that shifts in understanding can be observed. To achieve this through an undergraduate science degree, we therefore need a system of education that is scientifically sound, and appropriate to the specific science which is being taught. The primary goal here is to facilitate the process whereby a graduating student would know – with some confidence – both the limits and extent of their knowledge and understanding of the field. Some graduates may indeed continue in the field, but all will know what it is to know, and therefore are well placed to be life-long learners. Note that this does not require a diminishment in any technical sense of what we are teaching. But it does mean that we have a clear criterion upon which to include or exclude things from the curriculum – depth of content knowledge is more valuable than breadth. We cannot possibly expose students to all the new and exciting emerging fields, but if they are confident in their ability to appropriate knowledge then they will be able to move into new fields as they emerge. Confidence in the ability to appropriate knowledge is contingent on having had the experience of shifting to greater and greater depths of understanding. This is only possible in a system which favours depth over breadth.

The question we must then ask is what is 'depth'? The concept of 'depth' may need to be understood and used differently in the different sciences. For example, teaching human physiology requires engagement with the whole human body whilst it is possible to teach physical chemistry without any real depth of understanding of organic chemistry. The example I offer here is from chemistry and may need to be applied and adapted within another discipline.

In chemistry, we tend to focus on the development of robust conceptual understanding. We want to make sure that the student is able to successfully use the mol concept for example. Oftentimes, that focus means that we fail to make connections between different concepts explicit. Whilst we see a web of interconnecting ideas, the student sees isolated bits of information. To use an analogy, we focus intently on each individual puzzle piece, making sure that the student can reproduce it faithfully, but we may fail to show the student the big picture or completed puzzle. In chemistry then, teaching depth requires both attention to the puzzle pieces and attention to the connections between pieces.

Lonergan's system shows that the big picture is impossible to create without attention to the individual pieces, but the real value in education comes from the capacity to assemble the bigger picture and to see where the inevitable holes are. Powerful knowledge requires that we are aware of the limits of our understanding.

Lonergan's system also makes clear that student engagement is important. Teaching in an engaging manner matters and providing memorable illustrations is important to provide a rich experience. But this alone is insufficient. We need to make sure that we are helping our students to feel and become comfortable with the tension of inquiry so that they can taste the joy of insight. And then provide them with pointers to appreciate that reflection is a necessary part of the process.

Of course, there will always be students who just want to pass the course to obtain the credits. But hopefully, if we begin to think about the greater educative possibility of our science degrees, the student will engage on this greater level in at least one of their major subjects.

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The need for improved ethics guidelines in a changing research landscape

Globally, ethics guidelines for conducting research involving human subjects have been informed by practices and procedures developed for, and with reference to, medical research.[1,2] This indication is clear from international guidelines on research ethics practices, including the Belmont Report[3] and the Declaration of Helsinki[4]. Historically, developments of guidelines in research ethics, and procedures for ethics review, have often been reactive responses to critical events (i.e. ethics breaches) in medical research practice. The context for research ethics and clinical practice changes continually owing to developments in technology and medical procedures including genetics and robotics. Thus, ethics guidelines for research involving human subjects often lag behind developments in technology and medical science. Despite such guidelines, there are limitations as to the extent to which they can be applied to research that involves human subjects but in non-medical and non-therapeutic settings (here termed human participants). In this context, the term non-medical refers to the application of social science and humanities methodologies and instruments relating to human participants outside of medical, clinical or therapeutic settings. This type of research includes data collection using qualitative and interactive methods, such as interviews, questionnaires, workshops, focus groups and ethnographic observations.

Based on the foregoing, it is appropriate to ask whether national and global guidelines on research ethics involving human participants are fit for purpose, because (1) these guidelines have been developed mainly for medical rather than non-medical research and (2) they do not speak to the specific methods of data collection and analysis, and the nature of risk and vulnerability, used in many areas of the social sciences and humanities. The important point is that new research instruments and participant groups now available to social science researchers may give rise to new types of ethical issues related to confidentiality, anonymity, privacy and consent that are not covered by existing guidelines.

I contextualise these issues here using the example of human research ethics practices in South Africa, by first discussing the regulatory framework for research ethics, and then highlighting three key characteristics of non-medical human research in the 21st century that have implications for the applicability of national and international research ethics regulations and guidelines. Finally, I explore how research ethics guidelines might be changed, at both a national and international level, to address these issues. A key argument is that, at present, national and international research ethics guidelines are not fit for purpose because they do not consider the unique challenges of non-medical research in the 21st century. Thus, alternative guidelines are needed.

Research ethics context in South Africa

The ethics of research practices involving human participants are regulated in South Africa according to the *National Health Act* (*Act 61 of 2003*). The National Health Research Ethics Council (NHREC) was established under Section 72 in 2006 as the regulatory body to: provide oversight of the conduct and practices of human research ethics committees in South Africa; set and provide guidelines on the norms and standards for research involving human subjects/participants (and animals); and act as an adjudicating and disciplinary body to handle complaints and research ethics violations. The remit and scope of the NHREC accord with Section 12(2) in the Bill of Rights in the South African Constitution. In its 2015 guidelines[5], the NHREC notes that non-medical research involving human participants should not follow the recommended ethics procedures for medical research, but there are no clear guidelines on what these procedures should be. Because there are no clear guidelines, different research institutions in South Africa have developed their own guidelines, which thus may give rise to uneven practices and procedures. Several international studies have discussed how such institutional review boards should operate, with a focus on their composition, guidelines and review workflow.[6,7] Specific issues related to the operation of institutional review boards in Africa are discussed by Kruger et al.[8] but these are framed almost entirely in a medical context.

The changing context of non-medical human research in the 21st century

The context for non-medical research has changed in recent decades in response to technological change and new political and sociocultural contexts. Previous studies reflecting on some of these changes have been viewed through narrow disciplinary lenses.[9-11] Three key overarching issues affecting all disciplines and types of data are discussed here. It is notable that none of the existing national and international ethics guidelines explicitly consider all the factors discussed here.

1. Data types and methods of data collection

Recently, more complex ideas on the definition of research 'data' in the social sciences and humanities have arisen together with the data collection methods to be used.[9] The term 'data' currently encompasses a range of evidence, or information, from primary and secondary sources, and in a range of formats, many of which are informal and transient. Previously, the main data types were written (textual) and verbal information obtained directly from individual research participants. However, multimedia and digital data types are also now used, and may be based on indirect (rather than face-to-face) interactions with individuals or larger groups, such as in online communities.[12] In addition, data may be derived from secondary sources, such as online discussion forums, vlogs or communication modes such as Twitter, where individuals respond to or report other people's thoughts or ideas either in the public domain or in semi-closed (members') forums. In addition, the instruments used to collect data of these types have also changed, to include smart phones and Internet technologies, 'big data' of different sorts (including data on individuals), telemetric systems, the Internet of Things, datafied spaces, smart cities, and streamed data and audiovisual services.[13] These data types can be considered to be transient;

they change in meaning, context and availability, often with uncertain and unclear demarcations of public and private spaces, together with varying degrees of confidentiality and anonymity of individuals.[14,15] In addition, data archiving and the ability of other researchers to validate data sources is problematic if these data are no longer available, as is often the case with digital data.

These data types and different data collection methods pose problems for the traditional model of a research project where the project is initiated, data collection takes place, and the project terminates. In the digital world, start and end points are more difficult to identify and to circumscribe within the confines of a research project. This also means that traditional concepts such as researcher–participant relationships are more complex.

2. Relationships between researcher and participant

There are few guidelines as to how researchers should engage with participants in different types of studies. This dearth may reflect a traditional viewpoint that participant groups are uniform, amorphous and characterless, and that researchers may treat individuals as powerless objects to be exploited. However, researchers no longer exist in ivory towers distant from participants. Researchers and participants now enjoy more informal, direct, lively and interactive exchanges, often in the form of interactive data collection methods including ethnographic observations and participatory methods including workshops, art activities, participatory mapping and autoethnography. Within these different research methods, participants may be active agents of data creation and collection. This approach is often collaborative, and participants are not mere passive vessels from which the researcher extracts pre-formed data. Also, participants now often have greater engagement with the researcher throughout the research process, not just in its data collection phases, and they may show greater interest in the nature, purpose and outcomes of the research. A current emphasis in social science research attempts to ensure the authentic voice of the participant. This idea is set within wider issues in social science research, and in global society more generally, of awareness of patriarchy, power relations/privilege, gender, sexuality and race, which may impact on researcher–participant relations, data quality and data interpretation.[16] In a South African context, these issues fall within the broader concept of 'transformation'.

These changed contexts of researcher–participant relationships and their changed nature of interactions require more careful consideration of consent, anonymity and confidentiality, which may be more difficult to obtain if researcher–participant interactions are informal, of short duration, or not face-to-face.

3. Managing participant confidentiality, anonymity and data protection

Issues of confidentiality and anonymity are more difficult to handle in cases in which research involves the use of digital or open-source data including social media, or in which human-subject issues are in the public domain. It may also be more difficult to guarantee confidentiality and anonymity for both data collection and results reporting. Several recent studies have concerned issues regarding social media privacy settings[17], highlighting the fact that users are commonly unaware that their personal data may be potentially shared with other commercial entities or analysed for research purposes. Issues of confidentiality and anonymity are also important where potentially sensitive data may be disclosed. The Department of Health ethics guidelines[5] list race, political opinion, religion, trade union membership, physical or mental health, sex life and criminal convictions in this category of 'sensitive data'.

Another key issue is the potential ephemeral nature of digital data (if a webpage has since been removed, does it remain a valid source of data?), the validity of data of different types, especially in an online community space ('fake news'), and the viewpoint that not all different data types are of equal value. With such data, there may be tensions with data protection rules, where restricted data access may limit the capacity of future workers to get access to, to verify or validate previous data interpretations, or to identify any incorrect, falsified or suppressed data.

In South Africa, the *Protection of Personal Information Act* (POPI) (*Act 4 of 2013*) clarifies the individual's right to privacy, with implications for how researchers manage the collection, storage and management of or access to data, and takes into account both anonymity and confidentiality issues. 'Privacy' is easier to assure with data such as old-style individual interviews, questionnaires and focus groups, and for which present guidelines can be applied. However, the use of digital and online materials as source data is potentially problematic for privacy, and with respect to the interpretation of the POPI Act when secondary data, such as those from the public domain, are reused. There may also be potential contradictions between fulfilling the requirements of data privacy under the POPI Act and fulfilling the requirements of some public funding bodies (such as South Africa's National Research Foundation) to ensure data availability/access. The ethical implications of these different data access strategies have not yet been fully explored.

Future directions in human research ethics

The nature of human research in the social sciences and humanities has changed significantly in the last decades, mainly as a consequence of changing technology which has enabled new types of interactions between researchers and participants.[18] This change has in turn led to challenges in the ways in which research may be conducted ethically, particularly with respect to participant anonymity and confidentiality. Existing national regulations and international guidelines on research ethics lag behind changing technologies and the needs of both researchers and participants, who thus may not be adequately protected under these regulations and guidelines. Moreover, these guidelines do not help researchers or institutional review boards to manage sufficiently the ethical issues related to these new modes of data collection. Action is needed at national and international levels to address this gap.

New technologies have made longitudinal and more interactive studies easier and richer, given the more complex data types, sources, data volumes and individual voices involved. However, a downside is that issues of anonymity, confidentiality and informed consent are more complex, as the research process becomes more multifaceted. These issues are particularly relevant to the developing world where consideration of risk, vulnerability and coercion are important historically[19], and where wider issues of power, privilege, gender, race and corruption also influence researcher–participant relationships. In South Africa, continued socio-economic, political and cultural change provides a dynamic landscape in which to undertake social and humanities research.[20] However, this requires research ethics practices that are responsive to the changing needs of both society (including government) and researchers, and balancing the generation of appropriate data in order to find developmental solutions for communities that are often vulnerable or marginalised, while retaining and listening to their authentic voices.

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STATEMENT ON ETHICAL RESEARCH AND SCHOLARLY PUBLISHING PRACTICES

JOINTLY ISSUED BY ASSAf, CHE, DHET, DST, NRF AND USAf

Since the global adoption of the Singapore Statement on Research Integrity in 2010 (www.singaporestatement.org), which we jointly subscribe to, adherence to its principles has not improved. In support of ensuring quality research of high integrity in South Africa and globally, we find it impelling to reiterate to the South African research community the fundamental principles of scholarly research and publishing (which we endorse) and appeal to this community to act demonstrably in advancing research integrity. The following principles should inform ethical research and scholarly publishing practices:

1. **Responsibility:** It is the responsibility of individual researchers, postgraduate students, academic societies, journal publishers and boards, universities, all university staff (including research support services) and all organisations supporting research and knowledge generation, to be aware of and adhere to regulations related to research, to actively maintain academic and research integrity and to report or act upon any unethical practices they may discover. At an institutional level, requisite policies and procedures for monitoring, investigating, censuring and reporting unethical practices, must be developed. The anonymity of those reporting such practices must be protected.
2. **Ethics and integrity:** Researchers are responsible for their own research, and for research performed under their supervision, and must take due care to ensure the publication only of authentic, accurate and reproducible findings, including findings that do not support their working hypotheses.
3. **Methodology and data:** Researchers must use appropriate research methods, assess all outcomes critically, maintain a full record of the research including all supporting data, and objectively interpret and report findings.
4. **Authorship:** All authors who made an intellectual contribution to the research publication, and only those authors, must be included as contributing authors. The sequence of authors should follow discipline-specific practices. All authors must read and approve the final draft prior to submission.
5. **Acknowledgement of contributions:** As well as acknowledging all authors, researchers must acknowledge all those who made a material contribution to the research or publication but who do not meet authorship criteria. This includes indigenous originators of the knowledge, funders, sponsors, manuscript editors and language reviewers. In addition, all knowledge (published or unpublished) used in the research must be appropriately referenced/cited and acknowledged.
6. **Peer review:** Peer-reviewers must be sufficiently qualified for the role, and the process of review must be fair, objective, and rigorous, while respecting anonymity and confidentiality where this is applicable. All research publishers and funders of research must avail their peer-review policies to authors.
7. **Social awareness:** Researchers and institutions must be sensitive to the potential impact of their research on society, marginal groups or individuals, and must consider these when weighing the benefits of the research against any harmful effects, with a view to minimising or avoiding the latter where possible.
8. **Conflicts of interest:** All possible conflicts of interest, whether financial or personal, must be declared and preferably avoided in research and in other scholarly activities such as peer review, research proposals and public comment.
9. **Editorial:** In cases where editors or members of editorial boards submit manuscripts to their own journals, editorial handling of the papers concerned must be independent of the author in process terms, up to and including the decision to publish or not, as the case may be.
10. **Research publishing environment:** Research institutions (including agencies supporting and funding research) must ensure an environment which encourages ethical research practices through education, stewardship, and clear and fair policies and practices that promote research ethics, integrity and compliance. This includes the way in which research funding or research incentives are allocated and spent. Care has to be taken to ensure that the research funding system does not incentivise perverse research and publication practices that compromise research integrity.
11. **Predatory journals and unethical editorial practices:** Researchers are responsible for avoiding falling victim to predatory publishing or unethical editorial practices. The onus is on an individual or group of researchers, and institutional processes of scrutiny, to ensure that the avenues selected for publishing their research are authentic and credible.
12. **Quality over quantity:** Researchers are reminded that publishing the outputs of their research in good quality, high-impact journals, is always preferable from a longer term career perspective, to the publication of incremental outputs in low quality journals. 'Salami slicing' of outputs to increase publication numbers should be avoided.

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Research ethics and integrity challenges require innovative approaches

Our world is growing. The number of researchers and academics is increasing. The pressure for these researchers and academics, and indeed their institutions, to publish more is ongoing. Consequently, the number of research publications in both journals and books is on an exponential upward trajectory. Coupled with this positive trend is the challenge facing all countries, both developed and developing, to uphold the ethics of research and advance research integrity. However, this task is no longer simple and requires innovative, collaborative and coordinated approaches to ensure the integrity of the research enterprise. Research integrity may be viewed as active adherence to the ethical principles and professional standards essential for the responsible practice of research.

The objective of the World Conferences on Research Integrity (WCRIs) is to foster integrity in research. The first WCRI was held in Lisbon, Portugal, in 2007. Six WCRIs later, participation has grown from 275 participants from 47 countries in 2007 in Lisbon to 701 participants from more than 50 countries at the 6th WCRI held in 2019 in Hong Kong. The majority of participants were from Asia and Europe, with only 24 participants from Africa. South Africa has been afforded the privilege of hosting the 7th WCRI in Cape Town in 2021 – the first time that a WCRI will be held on the African continent. The WCRIs have produced two global statements on research integrity, namely the Singapore Statement in 2010[1,2], of which the National Research Foundation (NRF) is a signatory, and the Montreal Statement in 2013[3,4]. In fact, the NRF translated the Singapore Statement into eight of South Africa's official languages to ensure wider dissemination of the statement.

According to the *Singapore Statement on Research Integrity*, the value and benefits of research are vitally dependent on the integrity of research. While there can be and are national and disciplinary differences in the way research is organised and conducted, there are also principles and professional responsibilities that are fundamental to the integrity of research wherever it is undertaken. The *Montreal Statement on Research Integrity in Cross-Boundary Research Collaborations* emphasises that research collaborations that cross national, institutional, disciplinary, and sector boundaries are important to the advancement of knowledge worldwide. This is particularly important to the NRF whose mandate includes research collaboration across national, regional and international borders.

The STM Report[5] estimates that there are approximately 10 000 journal publishers globally, of which around 5000 are included in the Scopus database. The main English-language trade and professional associations for journal publishers collectively comprise about 650 publishers that produce about 11 550 journals. There were about 33 100 active scholarly peer-reviewed English-language journals in mid-2018 (plus a further 9400 non-English-language journals), collectively publishing over 3 million articles a year. The increase in publications may be attributed to the growth in R&D expenditure, the inherent international competition among researchers, institutions and countries with respect to knowledge production worldwide and the increasing number of researchers, which now stands at between 7 and 8 million. China surpassed the USA in 2017 to become the pre-eminent producer of research papers globally, with a share of about 19%.[5]

The research publication output trend is no different in South Africa where a recent study commissioned by the NRF on the *State of the South African Research Enterprise*[6] showed an increase in absolute numbers of publications and a doubling of world share over the past 15 years. However, the exceptional research performance in terms of increases in scientific publication should be moderated against the background of growing concerns about increased examples of unethical and questionable publication practices, including predatory publishing, indiscriminate publication strategies and growing evidence of gaming the publishing system.[6,7]

In a recent report to the South African Department of Higher Education and Training (DHET), Mouton et al.[8] noted that predatory publishing in South Africa – at least in subsidy-earning journals – has decreased over the past 2 years. This decrease may be attributed to the high saliency of the issue, together with the interventions taken by the DHET, NRF and individual universities that have forced academics to rethink their publication strategies. However, this does not suggest that academics have stopped publishing in predatory journals. Studies from other countries in the world where academics do not benefit financially from publications suggest that predatory publishing remains a major challenge.[8]

According to the same report[8], other forms of questionable publication practices that remain common in the South African higher education system include (1) excessive publication of papers by editors in their 'own' journals; (2) excessive publication of papers by members of the editorial boards of certain journals; and (3) excessive submission of conference proceedings for subsidy by certain individual academics. All of these practices constitute unacceptable gaming of the DHET subsidy system and require firm and swift action on the part of the DHET to sanction and hence prevent such practices from continuing.

Factors that may have contributed to the current state of affairs include (1) a culture of performance management that pervades every aspect of the academic culture; (2) an incentive and reward system that has produced unintended consequences; and (3) new opportunities for fraudulent and unethical practices emerging from the digital and open access movements.[8]

In a recent editorial in *Nature*[9], it was emphasised that research integrity is about creating systems that boost the quality, relevance and reliability of all research, better record-keeping, vetting experimental designs, techniques to reduce bias, rewards for rigorous work, and incentives for sharing data, code and protocols. It is different from research misconduct that encompasses fraud, fabrication and plagiarism. According to the editorial, the conflation of integrity and misconduct is problematic because it stops researchers from talking about ways to improve their work.

The NRF is of the view that conducting research with integrity, honesty and accuracy must be acknowledged, upheld and sustained.

As South Africa's premier research funding agency, the NRF awards research grants to researchers at universities, science councils and the national research facilities based on peer review. The NRF also evaluates and rates its researchers based on their scholarship and research productivity over a period of time. As a result of its position, the NRF found it appropriate to spearhead the formulation of a joint *Statement on Ethical Research and Scholarly Publishing Practices*[10] that sets out a national position on the issue of research ethics and scholarly publishing (the Statement is also published in this issue of SAJS). The statement consists of 12 principles and is in alignment with the deliberations of the WCRIs. Adherence to these principles by researchers applying to the NRF are not negotiable. Adherence is effected through communications to institutions and the online application systems, and is monitored during the review processes.

The above constitutes a battery of NRF initiatives to ensure adherence to ethical scholarly research and publishing practices. These include, inter alia, the NRF's contribution to the Global Research Council's Principles on Peer Review; the issue of an NRF Statement on Predatory Publishing; and the issue of an NRF Statement on Open Access.

One of the objectives of the 6th WCRI in Hong Kong in 2019 was to contribute to reforming the way in which researchers are assessed.[11,12] A draft of the *Hong Kong Manifesto for Assessing Researchers: Fostering Research Integrity* was developed and discussed at the conference and the third draft posted for comment on the conference website. The closing date for comments was 13 September 2019. The manifesto suggests that the current approach to research assessment by institutions is inadequate at best and creates perverse incentives for poor research conduct at worst. The approach involves counting publications without real quality assessment beyond simply using (rather indiscriminately) the journal impact factor or H-index and adding up an individual's grant income. The manifesto suggests six principles, which might form the basis of a new more comprehensive way of assessing researchers with a special focus on strengthening and rewarding research integrity. The principles include: societal need as a goal for research; responsible indicators that broadly reflect the contribution to the research enterprise; the need to publish or report all research completely and transparently; a culture of open research; the differentiated recognition of different research types, such as exploratory research and replication; and the inclusion of other contributions to the research enterprise, such as peer review and improving the research environment. It is hoped that once the finalised version of the manifesto is endorsed by participating countries, it will be adopted for implementation.

At the 6th WCRI in Hong Kong, Australia's Chief Scientist Dr Alan Finkel, in his presentation on 'Actions to Advance Research Integrity', focused on the practical aspects in the firm conviction that the research community has a system that is fundamentally sound, but that can undoubtedly be improved through several interventions.[13,14]

Based on the aforementioned and given South Africa's own experiences, the following approaches may be considered by the NRF, in collaboration with its stakeholders and the international community, in fostering research integrity:

- Develop, in collaboration with other key role players in the research system of South Africa, a National Research Integrity Policy and Guideline, for implementation by all research entities.
- Explore the development of an online 'Research Integrity' module that is free and easily accessible to all researchers and graduate students.
- Ensure that capacities and structures for ensuring the quality assurance of research and ethical clearances exist at all NRF-funded institutions.
- Ensure that mentors are available to guide emerging researchers in respect of grant proposal writing, publications writing and optimising networking opportunities.
- Make proof of research integrity training a requirement for applying for an NRF research grant, postgraduate scholarship or NRF rating. In the case of research grants, proof of research integrity training should be applicable to all investigators listed on the application.
- Continue to request the researchers' best five publications from them when they apply for an NRF rating. This criterion should be extended to all research grant applications.
- Consider new publications only from those journals that have proven their compliance with the Publication Process Quality Assurance (PPQA), as advanced by Finkel[14].

It is hoped that the above measures will add impetus to upholding research integrity in the country. Globally, interest in research integrity and publication ethics continues to be critically important. There is growing awareness of the need to sustain ethical research practices and to avoid any form of misconduct. This is evident from the work of the Committee on Publication Ethics (COPE). The number of journal article retractions has grown substantially in the last decade. There are now more than 20 000 retracted papers in the Retraction Watch database. All these developments augur well for the future. However, as a research community, we cannot afford to become complacent.

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7th World Conference on Research Integrity 2021 – Announcement

The 7th World Conference on Research Integrity will be held in Cape Town in 2021, after the University of Cape Town (UCT) led a successful bid to host this conference for the first time in Africa. Three co-chairs have been appointed to organise and plan the 7th WCRI: Lyn Horn from UCT, Lex Bouter, Chair of the WCRI Foundation Board, and Sabine Kleinert, Editor of the *Lancet*. The co-chairs will work with the Local Organising Committee which includes members from science councils, the Department of Science and Innovation and other South African Institutions to make this conference a reality. The objectives of the 7th WCRI will be to promote and foster a culture of responsible conduct of research in Africa and globally in a rapidly changing techno-economic environment; to promote research into research integrity; to provide a platform to present the knowledge gained and explore ways this knowledge can be implemented to strengthen science, technology and innovation systems and institutions; and to explore how changes in the way science is conducted (e.g. large multi-national collaborations; open science movement) can influence the integrity of research both positively and negatively.

Conference themes will be developed in consultation with the 7th WCRI programme committee. Proposed themes include:

- Equity and social justice as components of research integrity
- Research integrity in Africa: opportunities and challenges
- The effect of the move to 'open science' on scientific integrity
- Counteracting plagiarism in multicultural and multilingual contexts
- Ethical best practice in authorship, publication and the use of scientific metrics
- Best practice in the detection, investigation and sanctioning of scientific misconduct
- Institutionalising responsible conduct of research (RCR) education and training including curriculum development and implementation in low-resource settings.

The 7th WCRI will be of great relevance to scientists and researchers in South Africa and wider afield both in Africa and globally. It is hoped that many African scientists will take the opportunity to attend this conference and actively engage with some of the themes mentioned above. The WCRI Local Organising Committee plans to offer a reduced fee for delegates from Africa as well as a significant number of scholarships with the support of both local and international sponsorship.

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NRF: Putting the 'Statement on Ethical Research and Scholarly Publishing Practices' into practice

Following a Workshop on the Ethics of Scholarly Publishing on 11 April 2018, and with the collective goal of advancing research integrity in South Africa, the Academy of Science of South Africa (ASSAf), the Council for Higher Education (CHE), the National Research Foundation (NRF), the Department of Higher Education and Training (DHET) and Universities South Africa (USAf) signed the joint **Statement on Ethical Research and Scholarly Publishing Practices** *in Pretoria on 31 July 2019.*

The signatories were invited by the South African Journal of Science *to outline to the South African research community how they individually and collectively will be 'putting the Statement into practice'.*

Given its mandate of supporting knowledge creation coupled to human capacity development, the National Research Foundation (NRF) of South Africa, as the premier Science Granting Council, considers itself to be one of the guardians of ethics in the conducting of research and publishing practices. This position has been expressed through various statements and documents over the years which include participation in the production of and commitment to advancing the principles expressed in the 2010 *Singapore Statement on Research Integrity* and the *Statement of Principles for Research Integrity* adopted by the Global Research Council in 2013, which collectively call for ethics in research and scholarly publication. Concerned with the extent to which indications of unethical behaviour emerged in scholarly publications, the NRF issued its own *Statement on Predatory Journals and Deceptive Publishers* in 2017. This Statement essentially sought to sensitise stakeholders to a growth in predatory publishing practices and to advise researchers and students of the need for more robust quality and ethical controls in the review of research proposals and in resultant scholarly publications. As a result of NRF's position on ethical research and scholarly publishing practices, the NRF found it appropriate to spearhead the formulation of a joint *Statement on Ethical Research and Scholarly Publishing Practices* that set out a national position on the issue of research ethics and scholarly publishing. This joint Statement is the first concrete outcome of the broader objective underpinning the workshop from which the Statement evolved.

The NRF remains committed to giving life and meaning to the Statement through the following actions, which are at various stages of implementation:

1. Maintaining the momentum of actions through the Working Group comprising representatives from the signatories to the joint Statement and beyond. This group will identify and drive actions that will advance the principles of the Statement and the broader objectives of the workshop. An example in this regard is developing a uniform identification system for predatory journals.
1. Distributing the Statement to stakeholders through various communication platforms. The Statement has been published on the NRF website and has been shared at the NRF's Research Administrators' Workshop – an annual event at which the NRF shares new developments and addresses NRF-related issues raised by the research community.
2. Making the document available to all research institutions as a reference/support document for the reviewing and evaluation of submissions to the NRF. The document will also be shared and engaged at training and briefing sessions for the various role players in the reviewing of NRF applications.
3. Updating, as appropriate, terms and clauses in calls, reviews and awarding documents.

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USAf: Putting the 'Statement on Ethical Research and Scholarly Publishing Practices' into practice

Following a Workshop on the Ethics of Scholarly Publishing on 11 April 2018, and with the collective goal of advancing research integrity in South Africa, the Academy of Science of South Africa (ASSAf), the Council for Higher Education (CHE), the National Research Foundation (NRF), the Department of Higher Education and Training (DHET) and Universities South Africa (USAf) signed the joint **Statement on Ethical Research and Scholarly Publishing Practices** *in Pretoria on 31 July 2019.*

The signatories were invited by the South African Journal of Science *to outline to the South African research community how they individually and collectively will be 'putting the Statement into practice'.*

While it is vital to continuously build the research integrity of South Africa's national science system, addressing the issue of research and publishing integrity is a system-wide project rather than one that can simply be addressed through interventions at the level of the individual researcher and/or individual institutions.

Following on a period of very stagnant growth, South Africa's research system has grown substantially since 2010, mainly as the result of a number of important interventions, including the introduction by the Department of Higher Education and Training (DHET) of the research output subsidy as part of the block grant subsidy system. South Africa, with its production of more than 24 000 peer-reviewed research publications in 2018, has a 1% share of the global output. More importantly, perhaps, South Africa has a 10% share of the top 10% of the world's most cited articles. What this tells us, is that even though the productiveness of the system has grown substantially over the last 10 years, the quality of the research outputs remains at a very high level. Between 80% and 90% of this output is produced in the university system, which is important because, as elsewhere in the world, the research that produces this output is tied to the generation of new cohorts of master's and doctoral graduates – the next generation of productive, ethical scholars.

With such a fast-growing system it should not be surprising that concerns are being raised about the perceived decline in the ethics of research and publishing. Much emphasis has been placed on the issue of publishing in predatory journals; while this issue is important and corrosive to the research enterprise, we have to keep in mind that these publications amount to just 3% of the total output and that 10 years ago it was at the same level. This is a complex matter because researchers chose these journals to publish in only because they were on the accredited list of journals that the DHET publishes every year. This should not be considered an excuse, but it does point towards the fact that the issue of research and publishing integrity is a system-wide project. The question of predatory journals is, of course, by no means the only ethical issue to be discussed. Plagiarism, the 'slicing and dicing' of articles, the adverse behaviour of supervisors towards their students, etc. are all of great importance.

The push to grow the high-quality research output of our system has resulted in the creation of a series of platforms and interventions that incentivise universities and individual researchers. It has been witnessed just how impactful this steering has been on the research system. It stands to reason therefore that carefully designed steering mechanisms would also contribute to improving the ethical behaviour of researchers.

One example of an intervention is to provide young scholars with the opportunity of working in mature research environments as an effective way to generate a culture of ethical research engagement. The policy and funding mechanisms at our disposal must be designed to address this kind of intervention as a matter of course. There is a strong and compelling case for the key national institutions to collaborate on such projects, as indeed they are. The key agencies – Universities South Africa (USAf), the Academy of Science of South Africa, the Council on Higher Education, the National Research Foundation, and the DHET – the signatories to the joint *Statement on Ethical Research and Scholarly Publishing Practices* – play distinct and influential roles in cultivating the science system. They have to work in concert with each other.

USAf, through its Research and Innovation Strategy Group, has the issue of research ethics high on its agenda – both in terms of advocacy and in the development of online materials for the purpose of exposing postgraduate students very early on in their careers to national and international best practice. In terms of advocacy, USAf will use its current platforms, such as the Higher Education Leadership and Management structure and the National Higher Education Conference, to focus attention on the matter and to develop the capacity of Deputy Vice Chancellors (Research), Directors of Research and Deans, to take active leadership roles in this respect. USAf will, as with all activities, undertake this goal in partnership with South Africa's universities, with DHET and with other national and international partners.

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DHET: Putting the 'Statement on Ethical Research and Scholarly Publishing Practices' into practice

Following a Workshop on the Ethics of Scholarly Publishing on 11 April 2018, and with the collective goal of advancing research integrity in South Africa, the Academy of Science of South Africa (ASSAf), the Council for Higher Education (CHE), the National Research Foundation (NRF), the Department of Higher Education and Training (DHET) and Universities South Africa (USAf) signed the joint **Statement on Ethical Research and Scholarly Publishing Practices** *in Pretoria on 31 July 2019.*

The signatories were invited by the South African Journal of Science *to outline to the South African research community how they individually and collectively will be 'putting the Statement into practice'.*

Unethical practices in the research and publishing value chain affect all of us in the higher education system. A single incident of unethical practice could tarnish the image and integrity of the whole sector and every member of the higher education system must be an agent of ethical conduct, particularly in research and publishing but also generally in academia.

The Department of Higher Education and Training (DHET) enthusiastically participates in promoting research integrity at every level because our task is to protect the integrity of higher education in South Africa and to ensure that the DHET presides over the administration of a quality system that can be counted among the most credible and trustworthy in the world. For this reason, the DHET calls upon every academic in South Africa to join hands and support the fight against the scourge of putting money ahead of ethics, quality and integrity.

In this regard, the DHET Research Outputs Policy 'aims to encourage research productivity by rewarding quality research output at public higher education institutions'. In doing so, all publications have to follow acceptable standards associated with academic publishing, such as effective peer review, norms of citation, and proper acknowledgement when using ideas from others. The Research Outputs Policy uses academic publications as a proxy for research output and emphasises the importance of research integrity by demanding that research integrity lies at the heart of submitting research output claims for subsidy.

The DHET continuously engages with administrators of the approved indices, especially when there are suspected unethical practices, so that these can be uprooted at source. The purpose of removing predatory journals from indices and journal lists is to make certain that they do not get recognised, and that articles published in them do not accrue subsidy for South African scholars. Moreover, and importantly, the DHET reserves the right to withhold research subsidy for research and publications it considers unethical.

Furthermore, the DHET participates in an international initiative to deal with unethical practices of predatory publishing. The approach of this initiative is to focus on a consensus definition of predatory journals; to create a 'one-stop shop' for educational resources regarding predatory journals; to develop an observatory for surveillance of predatory journals/publishing and to develop a Journal Authenticator which would verify the status of each journal.

Moreover, in fulfilling its obligations as a signatory to the *Statement on Ethical Research and Scholarly Publishing*, the DHET will use all avenues and platforms at its disposal to strengthen the work of purging unethical practices in research and publishing. There are several platforms to use in this regard, including the Forum of the Deputy Vice Chancellors (Research); communication of individual institutional research outputs reports and the collective sector report to all institutions; and workshops with responsible managers at all universities. The DHET continues to invest resources in ensuring that only quality work accesses state funding.

All South African universities have an obligation to ensure that their Research Ethics Committees – a requirement of the Research Outputs Policy – are effectively functional and supported in the fulfilment of their mandates.

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ASSAf: Putting the 'Statement on Ethical Research and Scholarly Publishing Practices' into practice

Following a Workshop on the Ethics of Scholarly Publishing on 11 April 2018, and with the collective goal of advancing research integrity in South Africa, the Academy of Science of South Africa (ASSAf), the Council for Higher Education (CHE), the National Research Foundation (NRF), the Department of Higher Education and Training (DHET) and Universities South Africa (USAf) signed the joint **Statement on Ethical Research and Scholarly Publishing Practices** *in Pretoria on 31 July 2019.*

The signatories were invited by the South African Journal of Science *to outline to the South African research community how they individually and collectively will be 'putting the Statement into practice'.*

When requested to endorse and co-sign the joint *Statement on Ethical Research and Scholarly Publishing Practices*, the Council of the Academy of Science of South Africa (ASSAf) was of the view that the value of such statements – in the absence of proper implementation, scrutiny, monitoring and sanctions – is largely symbolic. Although the Statement serves to frame the issues and to reinforce the commitment to ethical, high-quality and responsible research and innovation, joint and coordinated action by authorities and agencies is needed to implement the undertakings in a properly operationalised manner.

In what way does ASSAf act, monitor, support and advise the research system and the National System of Innovation more generally in the implementation of the Statement? There are three specific activities of the Scholarly Publishing Programme of ASSAf that directly promote the recommendations put forward in the Statement.

Firstly, the inception of the National Scholarly Editors' Forum (NSEF) – a forum for all scholarly journal editors in South Africa – arose from one of the key recommendations in ASSAf's *Report on a Strategic Approach to Research Publishing in South Africa*[1]. In essence, this Report focused on how to strengthen both the quality and global visibility of scholarly articles published in South Africa, through systemic adoption of the 'Code of Best Practice in Scholarly Journal Publishing, Editing and Peer Review'[2] developed through the NSEF.

Secondly, during the launch meeting of the NSEF, participants supported ASSAf in taking the lead in the implementation of a globally unique system of quality assurance and research integrity assessment of those South African scholarly journals that are accredited and subsidised by the Department of Higher Education and Training (DHET). This system took the form of peer review of journals grouped within broad disciplines and carried out by carefully selected peer review panels, drawn from the ranks of experienced scholars in and around the fields concerned in each case, as well as persons with professional publishing knowledge and experience.

Without the involvement of a well-functioning and highly participatory NSEF, these goals cannot be achieved. ASSAf is vested with the responsibility of maintaining and sustaining the Forum and acting on its behalf as mandated by the NSEF membership.

Thirdly, in 2019, ASSAf published a comprehensive report entitled *Twelve Years Later: Second ASSAf Report on Research Publishing in and from South Africa*[3], which inter alia highlights three publication practices which could be regarded as questionable, if not unethical:

- Unacceptable levels of publication intensity by the editor or a member of the editorial board of the journal.
- Unacceptable publication intensity by an individual author in the journal.
- 'Publication cartels' in which two or more individuals (sometimes also members of the editorial board) author or co-author repeatedly in the journal.

Some of the questionable practices are the direct consequence of the 'perverse incentive' aspect unfortunately intrinsic to the otherwise excellent and successful DHET Research Outputs Policy for higher education institutions. As long as (a few) authors or editors are rewarded for publishing too many poor-quality articles, ways of 'gaming' the research subsidy policy will persist, unless the recommended new control measures[3] are put in place.

The signatories to the Statement should join cooperatively and in a complementary manner in protecting the integrity both of our publication system and of the funding system, through proper quality assurance and research ethics monitoring.

It is of the utmost importance that ASSAf continues to play its specific, largely qualitative role in ensuring that scholarly publishing in South Africa is conducted ethically, without questionable behaviours on the part of authors or editors or publishers, and that this is made visible to the global audience of peers. Full adoption in the system of the NSEF's 'Code of Best Practice in Scholarly Journal Publishing, Editing and Peer Review' would to a large extent eliminate all or most of the instances of editorial misconduct that have been identified. It would also lead to a situation in which the editors and editorial boards of South African journals would be aware that their practices are being monitored and that deviations that emerge would require an explanation and could potentially have drastic consequences.

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CHE: Putting the 'Statement on Ethical Research and Scholarly Publishing Practices' into practice

Following a Workshop on the Ethics of Scholarly Publishing on 11 April 2018, and with the collective goal of advancing research integrity in South Africa, the Academy of Science of South Africa (ASSAf), the Council for Higher Education (CHE), the National Research Foundation (NRF), the Department of Higher Education and Training (DHET) and Universities South Africa (USAf) signed the joint **Statement on Ethical Research and Scholarly Publishing Practices** *in Pretoria on 31 July 2019.*

The signatories were invited by the South African Journal of Science *to outline to the South African research community how they individually and collectively will be 'putting the Statement into practice'.*

As a signatory to the *Statement on Ethical Research and Scholarly Publishing Practices*, the Council on Higher Education (CHE) affirms its total commitment to the Statement in guiding its own research and publications.

The CHE conducts or commissions research on a variety of issues in higher education, including the size and shape of the higher education system, funding, governance, student governance, transformation, curriculum development, teaching and learning, and policy. The outputs of its research include unpublished internal research reports; papers published in its journal, *Kagisano*; papers published in external journals; other CHE periodicals and monographs, including the *Higher Education Monitor*; books and/or book chapters; proceedings from its conferences, frameworks, and good practice guides.

The *Statement on Ethical Research and Scholarly Publishing Practices*, 2019, will henceforth be an integral part of the editorial policy for all internally managed and produced publications of the CHE. Key principles from the Statement, such as 'responsibility', 'ethics and integrity', 'methodology and data', 'acknowledgement of contribution', 'peer review', and 'social awareness' have also been included in the guidelines or instructions to contributors of manuscripts for publication in the CHE journal *Kagisano*. Potential contributors have to demonstrate adherence to these principles in order to have their manuscripts accepted for publication.

The CHE is in the process of formulating its policy on research, and it is envisaged that one of the chapters in the research policy will be on 'Research Ethics'. The principles in the Statement are anticipated to find expression in that chapter. A related objective is to establish a Research Ethics Committee whose membership will include external research ethicists. The policy will further stipulate that any research project to be conducted or commissioned by the CHE will have to be cleared by the Research Ethics Committee before commencement of the research.

The CHE organised a conference on the theme 'Promoting Academic Integrity in Higher Education' in February 2019, which attracted 200 participants from eight countries on the continent. Some of the papers presented at the conference are published in this issue of the *South African Journal of Science*, a themed issue focused on academic integrity and quality assurance.

In 2018, the CHE, in collaboration with the National Research Foundation, convened a workshop to highlight the breaches of academic integrity associated with publishing in predatory journals. A year earlier, in August 2017, the CHE published in its online occasional papers called *BrieflySpeaking*, an article on 'Research Publication Ethics', in which it reflected on publication in predatory journals.

The CHE has a standing practice of not paying financial rewards to researchers or authors responsible for outputs, for in the view of the CHE, this is a perverse incentive which has led to a focus on numbers and personal gain rather than quality in outputs. Our policy assists in curbing the urge to publish for the sake of earning additional personal income.

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Commentary on the 2019 Quality Promotion Conference organised by the Council on Higher Education

As part of its quality promotion initiatives, the Council on Higher Education (CHE) organised a conference themed 'Promoting Academic Integrity in Higher Education', which took place from 26 to 28 February 2019 at the International Convention Centre of the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa.

The aim of the conference was to provide a platform for sharing experiences and lessons on, and good practices for, combating acts of academic dishonesty, as well as for raising issues and stimulating dialogue on the need to maintain academic integrity throughout the higher education value chain across the higher education sector. As an outcome of the conference, it was expected that the participants would commit themselves to ensuring that the credibility and global competitiveness of higher education in South Africa would not be allowed to be compromised.

The 200 participants were mostly from South Africa, but there was also a sizeable contingent from seven other African countries: Botswana, Malawi, Seychelles, Tanzania, Uganda, Zambia and Zimbabwe. The participants were from public and private higher education institutions, quality assurance agencies or councils, statutory and non-statutory professional bodies or councils, and government departments or ministries responsible for higher education in the different countries.

The structure of the conferences included a discussion of a panel of experts on academic integrity, keynote addresses in plenary sessions, and parallel paper presentation sessions. The papers covered different conference sub-themes including discourses on academic integrity and quality; academic integrity in teaching and learning, curriculum design and review, assessment and certification, and research and publishing; threats to academic integrity and their impacts; institutional systems and services for promoting academic integrity; and leveraging technology for the promotion of academic integrity. In total, there were 36 paper presentations, including the 4 by the keynote speakers which were presented during the plenary sessions.

The formal feedback received from the participants indicated that the conference was a resounding success. Participants spoke with one voice in condemning acts of academic dishonesty in higher education institutions. They issued a clarion call for all role players in higher education to have their hands on deck in the efforts to uproot all forms of academic dishonesty and to promote academic integrity.

Of the papers from the conference subsequently submitted and accepted for publication in this themed issue of the *South African Journal of Science* (SAJS), one is on the contentious topic of cheating in examinations, which generated much interest and debate among participants at the conference. Using examples from different parts of the world, the paper documents the prevalence and risks of the cheating that takes place in examination venues.

The second paper is on the long-standing debate on whether it is possible to maintain high academic standards and integrity when there is 'massification' in higher education, understanding that 'massification' often results in abnormal lecture-to-student ratios, and the inability to ensure that all students have sufficient access to teaching and learning facilities. The paper contends that academics have to learn from their experiences in order to continuously improve their teaching and assessment methods so that their standards and integrity are not compromised by factors associated with large classes.

The conference also opened a lid on the malfeasance of illicit sexual relationships between students and lecturers. From the comments on the conference papers that focused on this topic, it was clear that this issue is a huge threat to academic integrity and one whose increasing prevalence should be a concern for all. One aspect of this issue is the so-called 'sexually transmitted marks' phenomenon, and the conference paper that explored the prevalence of this phenomenon is also published in this themed issue. The paper recommends the development, adoption and enforcement of institutional policies aimed at controlling this phenomenon from both source points – students and lecturers.

The last paper makes a case for external quality assurance agencies such as the CHE to take on the responsibility of promoting academic integrity as a foundation for quality teaching and learning, research, and engaged scholarship. It presents a 'higher education ecosystem approach' which it recommends for adoption in order to ensure that maintaining academic integrity is the business of everyone that forms part of the 'higher education ecosystem'.

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Examination cheating: Risks to the quality and integrity of higher education

We examine the exigencies and impact of examination cheating, focusing specifically on the prevalence and risk of cheating taking place in examination venues. We document the problem with global coverage and note the consistency of the scourge and highlight the different approaches of institutions to dealing with the risk. Stressing the prejudice arising from examination cheating to both universities specifically and society generally, one of the root causes of the risk, namely the moral compass and ethical norms of university students and the societies in which they function, is discussed. The innovation of students when working out cheating practices and the facilitating effects of technology are considered as a backdrop to exemplars of good practices that have been implemented to mitigate the reality and risk of examination fraud. Recognising examination cheating as a fraud on society and a critical risk to university reputation, we question whether university leadership recognises the risk and gives it adequate (and responsible) emphasis in strategic and operational organisational risk identification and management.

Significance:

- Cheating in examinations, and especially in the examination venue, is a global scourge. A comparison of global good practices is presented which provides a framework for institutional discussion to begin to address and transparently deal with the issues and impact of examination cheating.
- Acknowledging technology as one of the significant enablers of examination fraud and noting the constraints confronting universities, there is nevertheless a critical need for institutions to mitigate the risk. In not doing so, universities, which are fundamentally supported by the fiscus and public taxpayers, are committing a fraud on society.
- The attitude of some students and academic staff, as well as public perceptions to examination cheating raise the lid on a moral decay that is beginning to manifest in society globally.
- Universities are challenged to address the issue of examination cheating proactively, openly and honestly. The repercussions of failing to do so are highlighted and exemplars are provided of what can and has already been tried and tested to mitigate the risks.

Introduction

It is unarguable that the university learning experience is not just about the pass mark, academic progression, and attainment of a qualification – it is also about the *student's journey* which equally includes the acquisition of skills and expertise, development of competencies necessary for the contemporary world of work, and personal growth and development. In keeping with these broad goals, learning at a university should be a scaffolded and developmental *process*, as well as an *outcome*. However, for many students, the *process* means little or nothing – their only focus is the degree certificate (the 'outcome'); abetted by universities for which the goal is frequently the *throughput target*, at the expense of the process.

Commonly, the outcomes are measured by some form of examination or assessment activity which provides a measure of the level of learning attained in the form of knowledge, skills, and/or pre-determined competencies. These generally are accepted without much criticism or question and have a high degree of credibility. There is therefore no gainsaying the value of the examination/assessment activity within the university system specifically, but also for society, generally. Who gets a job and what offers are made to graduates is significantly dictated by the results and learning outcomes reflected on the academic record. It is startling therefore when allegations arise of widespread examination cheating. Research by Baldwin et al. reporting on the results of a study involving 2459 medical students, immediately raises a red flag – they reported that 39% of the sample tested acknowledged that they had witnessed cheating in an examination; 66.5% had heard about examination cheating; and 5% (123 doctors) admitted that they had cheated in their examinations.[1] Without derogating from the importance of the research, the caution against drawing conclusions based on a single data source is apposite. Hence the question: was this an isolated transgression from almost 20 years past, or is examination cheating a greater reality in higher education than might be readily apparent?

The idea of cheating in examinations is antithetical to the nature and purpose of higher education with its emphasis on quality, competence and individual development. Yet there is a body of reporting, especially in the public media, that when read together paints an alarming picture of the high risk that universities are facing as a result of examination fraud. A scan of the available literature adds to our sense of disquiet that the prevalent risk is considerably greater than the attention that is being given to the problem of examination cheating by institutions of higher learning. Reports discussed below point to an absence of proactive mitigation of the risks of examination cheating and rather emphasise the reactive responses by institutions after a problem is exposed. However, with the scourge seemingly becoming more rampant, universities are beginning to acknowledge the negative impact on the integrity of qualifications and the prejudice to institutional reputation that arises every time a case of examination cheating is publicly exposed. The conundrum for proactive risk management is that examination cheating is rarely identified in an institutional risk register and is often a reactive and/or ad-hoc intervention by the management.

Examination cheating is a growing global problem that has affected universities without discrimination. The following analysis illuminates the extent of the problem and impels all institutions engaged in higher education to scrutinise the integrity and credibility of institutional examination processes. Within this context, organisational awareness of the risks of examination cheating introduced by technology, as well as the factors of human greed and student creativity, must be integral to the assessment.

Discussion: A global scan

In Australia in 2014, Fairfax Media reported widespread cheating at universities across New South Wales.[2] As a consequence, the University of Sydney conducted its own investigation on academic misconduct and confirmed that 'the problem of cheating in exams is not trivial'[3]. Highlighting the risk introduced by technology, the Deputy Vice Chancellor of Education at Wollongong University acknowledged that the '[d]igital age provides more opportunities for cheating'[4]. Also acknowledging the threats created by technology, Rajiv Gandhi University of Health Sciences in India, noting the reputational damage caused by the publicity of examination cheating, sought to mitigate the opportunities enabled by technology by introducing technology jammers to prevent information sharing, and metal detectors to proactively identify students carrying devices on their person.[5] The Jawaharlal Institute of Post Graduate Medical Education and Research banned all wristwatches and even conducted tests to check students' earrings to see if they were hiding Bluetooth devices.[5] Both Bangalore University and Rajiv Gandhi University also considered installing surveillance cameras in the examination venues, but the additional staff required and the added financial commitment in the face of constrained budgets, caused the operation to be aborted.[5]

An illuminating report by Transparency International indicates that 30% of Nigerians surveyed in 2013 paid a bribe during their higher education studies.[6] Eziechina et al.[7] confirm this report noting that: 'In contemporary Nigeria corruption of examination process comes in no other way than through examination malpractice [most of which] occurs while the examination is ongoing.' Harrison[8] highlights the recurrence of the global trend with the increasing use of sophisticated technology in Nigerian universities to abet the fraudulent activities. In addition to smartwatches, he cites an example of wireless spy cameras being used to copy and transmit questions to third parties who then responded 'through linked invisible devices some of which are designed as zips and buttons'[8]. Reiterating the concerns raised earlier, Harrison points out that 'none of the said exams were cancelled post-discovery and students could continue with exams and graduate'[8]. Further analysing the influence of technology and considering social media specifically, in 2017, final-year medical students at the University of Glasgow (Scotland) were required to re-take their practical examinations after it was discovered that they had used WhatsApp, Facebook and the university's own Student Management System to share with colleagues waiting to take the practical examination the details of the cases they would encounter in the examination.[9]

In a widely publicised intervention to stop examination cheating, the Government of Algeria completely shut off the country's Internet for several hours each day for the six days of the scheduled 2018 high-school examination. Acknowledging the severe detriment caused by the proliferation of examination cheating, the Government also installed metal detectors at the entrance to examination venues, phone jammers and security cameras at more than 2000 examination centres.[10] In 2017, Ethiopia also took the extreme step of blocking all social media sites in a 'proactive' bid to mitigate the risks of cheating in the country's university entrance examinations. Describing the problem as a national risk, the Government of Algeria noted that while it recognised the impact on many people – including the economic detriment – the consequences of the risk exceeded the public inconvenience.[11]

One of the biggest education scams to grip India involved test-fixing of the admission examination for certain medical schools, as well as of the eligibility assessments for administrative positions within the state.[12] Known as the Vyapam scam (after the body conducting the admission tests), the scam operated from 2006 to 2013 and involved thousands of students paying bribes of between USD40 000 and USD70 000 to a network of 'fixers' who were responsible for running the examinations.[13] Sethi[12] describes the Vyapam scam as 'the stuff of myth and legend' and the dangerous nature of the operation was reinforced by the number of associated people found dead under mysterious circumstances. When it was eventually exposed, the investigations identified the involvement of, inter alia, high-ranking politicians, chief ministers, academics and doctors (see Supplementary note 1 for more). Niazi[13] notes that by April 2014 – a year after the investigation commenced – more than 1100 medical students admitted to various medical colleges in the state, who had taken the pre-medical test through Vyapam, had their enrolments cancelled when it became clear that they had gained admission through fraudulent means. The cancellation figure is significant: as Sethi points out, in 2013 there were only 1659 seats available for admission to the medical schools under the control of Vyapam (and 40 086 applicants).[12] Some 630 students appealed the decision to strike them from the medical fraternity, but the Indian High Court rejected the appeal on the grounds that the appellants 'had obtained admissions illegally and were therefore ineligible to hold degrees or practice medicine'[14] (Supplementary note 2).

As early as 1999, Bjrklund and Wenestam[15] highlighted the increasing problem of cheating at undergraduate level in the Nordic countries, re-iterating the reality that 'it is, however, not a new phenomenon, but a well-known problem in many European countries, as well as in the United States of America'. Analysing the facts of the different cases reported in the popular media, what is evident is institutional attitude – some universities are far more willing to acknowledge the problem and seek solutions and longer-term remediating strategies, whilst others choose to downplay the significance of the fraudulent conduct in the belief that they are protecting institutional reputation. In a reported case of examination cheating at the Royal Free and University College London Medical School, a student who was caught copying in her final examination was nevertheless allowed to graduate as the university claimed that she 'had been an exemplary student, and there was no indication that she had done this before'[16]. On the other hand, Harvard University suspended almost 2% of its undergraduate body in 2012 for collusion and collaboration in a take-home examination.[17] In the Harvard case, it is noteworthy that not all students came out in support of the university to condemn the dishonest practices of their fellow students: rather, while some students indicated satisfaction with the steps taken against cheating, a significant student reaction was anger against the university for, amongst other reasons, the time that it took to complete the investigation and the emotional 'torture' for the students charged.[17] This report provides an interesting insight into how students view and experience examination cheating and may be a reason why the real extent of the problem remains hidden. Further corroborating the proposition that examination cheating has been and is a real problem for higher education institutions, the US Education Portal recorded that, in 1940, 20% of college students in the USA admitted to cheating during their academic careers, whereas today that number ranges between 75% and 98%.[18]

As universities are pressed upon to implement contingency plans and operations to guard against the risks of examination cheating, they are also required to allocate the necessary budgets and resources to support these programmes. All these initiatives add significantly to the already high cost of education. Most universities simply do not have the necessary resources on the scale required for countermeasures that will adequately assure the integrity of their examinations in the operating budgets received through state subsidies or the national fiscus. However, institutions cannot accept the risk, share it, or transfer it – which leaves the remaining option of avoiding it or, at least, mitigating it. Under these circumstances, the decision that organisational management will need to confront is whether to choose a zero-tolerance model at exorbitant cost, or a risk-tolerance model with lower financial impact. Acknowledging the materiality of the *impact* of examination cheating, institutions will need to make their decision with a clear and informed insight on the *likelihood* of the occurrence when determining appropriate treatment plans and resourcing their risk mitigation strategies. The Makerere University degree fraud scourge in 2015 presents a clear warning of what may

result when the risk assessment activity is either not conducted or is not conducted taking proper cognisance of *all* factors.

In 2015, the University of Makerere in Uganda was confronted with a scourge of degree fraud when it was revealed that 600 of the 12 000 graduating students (i.e. 5% of their total annual throughput) had obtained their results through corrupt activity, and as the investigation unfolded, it became apparent that the 2015 incident was not isolated, and that general examination fraud was endemic at the university.[19] The Makerere University case (Supplementary note 3), like the Vyapam scam, raises a significant risk for universities – namely the involvement of unscrupulous staff colluding with students and facilitating the fraudulent activity. These integrity breaches are often aided by absent or weak systems and a poor control environment because of resource constraints (for example, in the case of Makerere University, concurrent freezing of posts). Practice shows that when jobs are at risk, it is frequently the administrative positions that are the first to be negatively impacted. The reduction in staff numbers results in jobs being consolidated, which correlates directly with poor governance and higher risk because there is now no proper segregation of roles and functions, and the appropriate checks and balances are no longer in place. In such situations, one person is given too much authority and control over critical processes, which creates a fertile environment for impropriety to take root and flourish.

Universities globally acknowledge that cheating techniques today have advanced far beyond notes on pieces of paper, with technology proving to be a significant enabler of examination cheating activities. Recognising the efficacy of smartwatches as 'wrist computers', several universities have taken steps to exclude them from the examination venue. What is interesting is that, notwithstanding the identified potential as an enabler of fraud, universities deal with the use of smartwatches rather differently. Rangongo notes that, in South Africa, the University of Cape Town explicitly excludes such devices on the person or desk of a student, whereas the University of KwaZulu-Natal bans them entirely in the examination venue. Stellenbosch University permits students to enter the examination venue with a smartwatch but they are then required to switch off the device and place it face down on their desk; a similar approach is adopted at the University of Pretoria, but it is further required that the device be switched off and placed on the floor, under a chair and out of the student's line of sight. Monash University (South Africa) also requires that all smartwatches be switched off and placed in a bag on the floor. The rule at Rhodes University is that students found wearing a 'questionable electronic device' are required to clarify its function or remove it.[20] The university approach to smartwatches and watches has reached South Africa a little later than its global counterparts: in the UK, as early as 2015, the City University of London had already introduced rules to prohibit the use of all wristwatches during examinations, Goldsmiths University had required that all watches be stored under desks, and Southampton University required all watches to be placed in a clear plastic bag on the desk. The University of London limits its rules to removal of electronic watches, and at Oxford University and Cambridge University, 'students' watches are subject to examination by invigilators'[21]. Explaining its stance on the removal of all watches, Goldsmiths University pointed out that it was (1) to avoid discrimination against students with digital watches; and (2) to compensate for the realities of under-resourcing of universities, which means that the university does not have the time to check every single watch that comes into an examination room.[21] Student reaction at Southampton University to the ban was again mixed with many being strongly critical of it but others supporting the steps taken by the University for a proactive warning to would-be cheaters.[21] In Japan, Kyoto University issued a blanket ban on all wristwatches, reiterating the justification

that it was not always easy to tell at a glance if a watch was analogue or smart.[10] At the University of New South Wales (Australia), the restriction applies to all watches which may be neither worn nor placed on desks during an examination. Le Trobe University (in Melbourne) allows for regular watches to be placed on the student's desk while the examination is being written, but expressly prescribes that no smartwatches may be brought into an examination venue.[10,22] In a conscientious effort to eliminate the risk posed by technology to the integrity of the results of the university entrance examinations, China has adopted a far more extreme approach by using drones in examination venues. The drones are geared not only to scan the examination halls but 'they also locate suspicious radio signals created by hidden earpieces used to obtain answers to examination questions'[22].

Evidence of the increasing creativity and innovation characterising examination cheating globally reinforces the concern at universities that examination fraud needs serious engagement as part of both their strategic and operational risk assessments. For example, while universities generally have an invigilation protocol for the examination venue, less attention is paid to something as mundane as toilet breaks. The literature shows that students will use the toilet for phone calls, to access materials that were hidden in the stalls, or even to use the Internet to source answers. When the University of Maastricht (the Netherlands) became aware of this exploitation during toilet breaks, it introduced specific equipment to detect the use of smartphones and other electronic devices in the toilets so that when a smartphone was turned on or data or texts transferred, the equipment gave an alert to the responsible monitor.[23,24] As part of its overall review, the University of Maastricht also reduced the number of toilet visits allowed per student; and like many of its counterparts, the University banned the wearing of smartwatches during the examination session. As a practical approach to mitigate the risk of fraud in multiple choice examinations, Maastricht University decided to present more than one examination question paper in the instance of multiple-choice examinations.[23]

It is anticipated that the list of universities and countries in which examination fraud has been identified will continue to grow as detection improves. Cheating has become a business and as the returns increase, so too do the repertoire and complexity of the methods and techniques employed. The US Education Portal correctly characterises the incentive for examination cheating by noting that 'today students are cheating not just to pass but to get ahead'[18]. With the increasingly competitive job market, economic uncertainties, and an emerging demand for 'immediate gratification', the conventional quest in university education of *time to mature* and *earning your place* appears to be overtaken by the impulsion to get ahead by any means possible. The overwhelming obsession with performance both in the university sector and in society generally is a significant spur for cheating behaviours and the pressures come from varying and often multiple sources. For instance, (1) the expectation and/or desire to be named on the university honours lists, (2) the aspiration to be awarded a scholarship or admission to postgraduate programmes, (3) the home with family expectations of success, (4) society with the intense competition for jobs, or (5) sometimes it is just a personal choice motivated by greed, dishonesty and an underdeveloped moral compass. The external factors are often enabled by an internal institutional facilitating environment which includes identified systemic loopholes including ineffective deterrents and the increasing ease to cheat, questionable academic values, and the fact that sometimes – even when cheaters are caught – the cases are handled feebly by their lecturers rather than being reported to the management structures, or are addressed weakly by the management structures with an emphasis on not exposing the problem for fear of reputational damage. The literature draws attention to the high numbers of university students who have admitted to cheating, yet who have not been caught nor disciplined by their institutions. This climate sets the tone for others to follow and is probably a key contributor to why cheating has become prevalent at institutions of higher education. The problem is exacerbated by the fact that, as the cheating culture becomes more entrenched and known in student circles, and where the sanctions for examination cheating are less stringent because of 'external factors', cheating carries less of a stigma 'because everyone is doing it' and students are more willing to take the risk. These factors – individually and collectively – pose a significant risk to the credibility and integrity of the overall academic project and universities ignore the risks at their peril.

However, while examination fraud is often linked to social drivers, a closer analysis of the problem highlights the uglier underbelly – a lack of moral rectitude. Acknowledging social and economic pressures as possible catalysts, it is, however, without question that the student who

cheats in the examination does so with a conscious decision to be *dishonest*, to take the easy route to a self-serving end and to focusing only on obtaining 'the piece of paper'. One is left to wonder whether the full impact, seriousness and risk to others of entering a job or profession without the requisite skills and competence is ever considered. From an institutional perspective, this may create a secondary risk, namely the possibility of a legal challenge from a member of society who suffers harm at the hands of an incompetent graduate who succeeded only because the university failed to properly monitor its risk universe and/or implement reasonable controls to assure the credibility and integrity of its qualifications. A scan of the available literature and case law reveals no decisions on the point but suffice it to point out that what, after all, is a risk other than 'exposure to a proposition of which one is uncertain'?[24]

Some ideas for addressing the risk

As noted by Gareth Crossman of the UK Quality Assurance Agency for Higher Education:

> *If you are realistic about it and say is it possible to create an environment where it is impossible for students to find a way to cheat, the answer is probably not.*[25]

Highlighting the worldwide trend in examination cheating, Crossman classifies the risk as 'an international issue which demands an international response'[25]. Agreeing with the need for global research on the problem, the UNESCO International Institute for Educational Planning and the International Quality Group of the US Council for Higher Education Accreditation are currently engaged in a research project to ascertain what quality assurance and accreditation bodies globally are doing to deal with academic corruption.[25] They point out that as the problem of examination cheating becomes more exposed and the concomitant risks become clearer, universities will need to become more serious about confronting cheats, as well as proactive in closing not just the systemic opportunities, but also addressing staff attitudes, and in educating students on the consequences of examination fraud because, as Carter points out, even without technology, students are incredibly innovative and can be totally ingenuous when devising activities for examination cheating.[26]

One of the first steps in addressing the problem of examination cheating, notes O'Malley[25] is to '[persuade] hard-pressed academic staff on the front line that it is not in their best interests to ignore it…'. Institutions must have a policy and the will to act against offenders and deal with cheats consistently, fairly and firmly. This aspect of punishment and deterrence was addressed succinctly by the Indian Supreme Court: 'If our country is to progress, we must maintain high educational standards, and this is only possible if malpractices in examinations are curbed with an iron hand.'[27] (Supplementary note 4)

Despite acknowledging the role of restorative justice in an environment of 'learning', allowing identified cheats to evade sanction will not send the correct message to the student body. Academic staff have a responsibility to ensure that the right thing is done in the circumstances of each case. Acknowledging this truth, several institutions – especially those emphasising punishment as a deterrent – also publish the names of the students found guilty of disciplinary infractions in student newspapers or in a register that is shared between universities. It may even be suggested that the external factors that are so often stressed in mitigation, albeit real, are no more than an excuse used by the cheaters who are caught, as human behaviour is such that one would always rather find someone or something else to blame rather than acknowledging guilt. A case in point is that of a 23-year-old law student from the UK who was desperate to become a barrister, for which a specific admission score was required. From the available facts, the student first attempted to hold up a university cleaner at gunpoint to obtain the keys to the law department, and when that attempt failed, she resorted to breaking into the offices and amending her examination marks. Her crime was discovered, and she was charged with robbery, possession of a firearm and forgery. She admitted the offences and blamed her conduct on the 'pressures to get an upper second degree to become a barrister'[28]. The case ended tragically when the student committed suicide. The father of the young student publicly criticised the university authorities for going to the police to report the alleged crimes, rather than approaching his daughter directly. 'Almost every adult in the country knows the mental stress people are under to pass their exams,' was the father's response.[28]

Examination cheating is fraud – committed both on the university and on society that accepts the performance marks as being valid, and, accordingly, awards recognition and due benefits based on the results. Understanding that the problem is often hidden and silent because innocent bystanders do not wish to become involved as accusers or witnesses, some universities have introduced Whistleblower Hotline Services with the hope that the anonymity may encourage reporting. Universities are also seeing value in re-introducing the Student Honour Code, developed with the student body, stressing the importance of moral integrity. The premise of the collectively developed Honour Code is that peer pressure and self-policing will assist in reducing the problem of dishonesty and examination cheating (Supplementary note 5). However, the steep road to success and turning the tide on examination cheating was summed up by the response of students in the USA to the inclusion of examination cheating in the Honour Code. Contrary to Management's expectations, many students were simply not prepared to follow through in reporting others for cheating, notwithstanding strong support for the notion of the Honour Code. McCabe et al., in an earlier submission for the Constitutional Rights Foundation[29], summarised the problem: 'Students sense a deterioration of general societal values, and incorporate that into their own lives.'[27] Continuing this theme, the Constitutional Rights Foundation points out that 'cheating does not have the stigma it once had in American society'[29]. A similar attitude was indicated in a study including students at four medical schools in Croatia. Notwithstanding the information that more than 99% of the Croatian respondents self-reported engaging in at least one activity of academic dishonesty and 78% admitted to frequently cheating in their assessments, only 3 students (out of the sample of 481) admitted to having reported another student for cheating.[30]

Critical to promoting trust is the requirement that examination and student disciplinary policies and procedures must firstly be applied, and secondly be applied consistently with universities ensuring that the processes are robust and that all role players understand the seriousness of the risk. Inherent in the risk mitigation strategy are robust invigilator practices and universities need to ensure that those proctoring the examinations are thoroughly trained and vigilant. Students with the intention to cheat notoriously observe the behaviours of the invigilators and often design their cheating practices according to where the invigilator stands, at what point the invigilator opens their reading material, and whether the invigilator can be distracted so that answer books may be swapped.[31] In addition, whilst cheating practices are becoming more creative, even without technology, for example, writing answers in microscopic text and sticking the paper underneath one's fingernails, using nail art to take maths formulae into the examination venue, or writing notes inside one's shoes, in all of the examples cited, the cheaters' success is materially aided by the inattentiveness of the venue invigilator.

Furthermore, and notwithstanding the financial constraint indicated by Bangalore University and referenced earlier, both Curran et al.[32] and Eziechina et al.[7] highlight the efficacy of strategically positioned CCTV cameras in the examination venues as a means of not only deterring potential cheaters but also keeping an eye on invigilator conduct. To be effective in ameliorating the risk of examination cheating and ensuring best value for what will undoubtedly be a considerable expense for the institution, Eziechina et al.[7] emphasise the importance of ensuring that (1) the parties – both students and invigilators – are aware of the existence of the cameras *and* that they are being watched, and (2) the cameras are maintained (added costs) and that they always work. The research of the Constitutional Rights Foundation shows that 'as the risk of students getting caught for cheating increases, the instances of cheating decreases'[29]. However, universities embarking on such an initiative will be advised to take note of the decision by the European Court of Human Rights which ruled that the use of camera surveillance

by the University of Montenegro in its lecture halls constituted an unjustifiable limitation of the right to privacy under Article 8 of the European Convention on Human Rights, notwithstanding the fact that there was no audio recording and thus no recording of the teaching or discussions.[32] Regarding the installation of surveillance cameras outside the Dean's office, the Court relied on the Montenegro *Personal Data Protection Act*, section 36 which permits video surveillance in official or business premises 'but only if … safety of people or property or the protection of confidential data, cannot be achieved in any other way'[33].

The longer the problem of examination cheating remains underexposed and unaddressed, the more ingrained it becomes as a facet of the normal student experience – 'everyone cheats' or 'everyone else is doing it'. If one accepts the proposition that *today's students are tomorrow's leaders* and the general principle that the university is often where character is developed, then when students succeed through dishonest means to get ahead at university, this dishonesty often shapes their behaviours in the workplace and in life more generally, thus affecting the moral fabric of the entire society. Universities turning a blind eye to the threats of examination cheating with the idea that they are avoiding reputational prejudice, are being short-sighted because if nothing is done, the danger of reputational risk remains – that is, in the workplace, the student who is now the employee is often not competent at their job and institutional standards and reputation are called into question.

Conclusion

Acknowledging the proliferation of venue-based examination cheating, the most significant query for the higher education sector is: how many institutions of higher learning have identified examination cheating as a risk on their Strategic Institutional Risk Registers? And if not, is this because it has been considered and deemed to not be a material risk? Or because it has not even been considered? Or because universities are confident in their systems? Or because of the naïve belief that examination fraud will not take place within their institutions? Or because the leadership does not want to know the extent of the problem? The integrity of examinations is a fundamental element of quality and concomitantly institutional reputation and sustainability, and it must be a strategic focus of the institutional leadership to assure and safeguard the value of qualifications offered. Examination fraud is also a significant socio-economic risk, and the importance of paying attention to the triad of factors – (1) integrity/quality of the certified examination result, (2) social expectations/acceptance/belief, and (3) economic return on investment – was persuasively summarised by Shirley Alexander, Deputy Vice Chancellor of the University of Technology in Australia:

> *Taxpayers spend a lot of money on university education. It is absolutely incumbent on us that when we put a stamp on their graduation certificate that says this person has met the requirements of the degree, that they actually have.*[2]

Authors' contributions

Both authors contributed equally to the development of the paper. The authors jointly conceptualised the research focus and format, as well as the methodology that would be followed in developing the paper. D.S. was primarily responsible for the research and information gathering and compiled the first draft of the paper, while N.B. critically reviewed the paper, validated the information and provided supporting enhancements and commentary.

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Assessment, plagiarism and its effect on academic integrity: Experiences of academics at a university in South Africa

The quality of teaching, learning and assessment is compromised by the growing problem of academic dishonesty, especially in large class sizes as a result of the 'massification' of education. In South Africa and around the world, student plagiarism and cheating has become a matter of concern, especially when it comes to teaching large classes. This concern has received much attention as it impacts negatively on the maintenance of academic standards and integrity at many universities. Academics have a major role to play in the process of maintaining academic integrity. Through an 'interpretivist' and qualitative approach, we explored the experiences of three emerging academics within the Discipline of Curriculum Studies at a university in South Africa. We used Pinar's method of currere as a lens that focuses on academics' experiences of assessment and plagiarism in teaching large classes and its effect on academic integrity. The findings suggest that although 'massification' of education in South Africa is commended for addressing past social injustices and for facilitating accessibility to education, quality teaching and learning including assessment is seriously compromised. This demands a serious rethink of assessment strategies to deter academic dishonesty, and a reconsideration of the way academics and institutions think about plagiarism detection tools in teaching large classes.

Significance:

- Understanding academics' experiences of assessment and addressing the growing problem of plagiarism can contribute significantly to efforts towards improving teaching and assessment practices in large classes, and to upholding academic honesty within higher education institutions in South Africa.
- A rethink of effective assessment strategies is needed to provide a worthwhile quality educational experience. In the context of this study, ethics within the teacher education curriculum should be prioritised.

'Massification' of higher education

A key phenomenon in education worldwide has been 'massification', characterised by high student enrolments and dominated by Neoliberal thinking, with Africa and South Arica being the latest to experience 'massification' which has been applauded in South Africa. Great strides have been made to address and to redress the problems of access to education and the low completion rates of students. Students are eligible to receive quality higher education to prepare them for employment but with the declining level of education fuelled by 'massification', quality cannot be assured.[1] The problem is that 'massification' places impossible demands on existing physical, financial and human resources, and universities cannot enrol and address the learning needs of all students desiring to study.[1] As a result, contact time with students, and quality assurance, is compromised. We further argue that, with the large numbers of students in the classroom, lecturers are overcome with the volume of assessments to be marked.

In South Africa, studies by the Council on Higher Education[2] indicated that of the students entering a 3-year undergraduate programme, less than half drop out, and 50% of students who do enrol take up to 6 years to graduate. Student enrolments rapidly increased by 67% between 2002 and 2014, and by 70% for African enrolments.[3] Redress, access, and throughput rates continue to be racially skewed with white completion rates being higher than African student rates.[3] The proportion of government funding to universities declined from 49% in 2002 to 40% by 2014.[3] Clearly, as a result of increased enrolments and the stagnation of resources and funding, university systems are under substantial pressure with the increasing enrolments, low throughputs, high staff-to-student ratios and an untenable lack of support for funding.[3]

Massification has initiated changes to the curriculum and large class pedagogies within higher education in South Africa.[2] The impact of large class teaching on academics and on academic productivity has not been considered adequately.[1] In pursuing an understanding of this impact on developing countries like South Africa, we endeavour to initiate deliberative discussions with academics within the Discipline of Curriculum Studies at a university in South Africa to provide useful insights and find solutions to problems in addressing 'massification' of education, including the matter of teaching large classes and its implications for quality, and the issue of maintenance of academic integrity.

Interplay between assessment, plagiarism, quality and academic integrity

In this section, we conceptualise and draw on the relationships between assessment, plagiarism, quality and academic integrity.

Assessment in higher education

Assessment is undoubtedly important in realising the goals of teaching and learning and in improving student performance, and it cannot be removed from the process of education.[4,5] The purpose of assessment in higher education is to: measure the level of student knowledge for quality; assess the extent to which learning outcomes have been achieved; and to judge the quality of higher education institutions and programmes in the upkeep of

standards (accreditation).[6] With the ever-changing curriculum, the aim of assessment is to measure the teaching and learning process and to guide students to monitor their own learning experiences.[5,7]

Higher education institutions worldwide are increasingly moving towards online learning management systems to offer effective and efficient assessment solutions to large class teaching and to cope with the demands of the 21st century.[8-10] However, in developing countries like South Africa, issues of affordability, access and maintenance of technological software and resources can act as barriers to using technology as a resource to promote quality education.[11] Academics play a crucial role in the adoption of and adaptation to the use of technology to enhance quality teaching, learning and assessment practices.[12]

Suitable assessment design has its roots in student plagiarism prevention.[13] Assessment activities that do not engage students in active participation, and those assessments that remain unchanged year after year are not stimulating original thinking, and this influences acts of cheating and plagiarism.[14] Research suggests that, although academics in higher education employ numerous assessment practices, the best practices are not usually shared.[6]

Quality

Universities around the world are committed to respond to the demands of globalisation and 'massification' in higher education, which raises considerable debate around the sustainability of quality education. In response to addressing historical situations, most public universities in South Africa were compelled to enrol students in excess of their capacity, resulting in the 'massification' of education with negative effects on the quality of teaching, learning and assessment.[11,15] 'Access' and 'quality' are mutually reinforcing and form the foundations for the successful transformation of higher education.[16]

Within a transformative agenda, the Chinese government had vigorously increased access and provided more opportunities for students in higher education. However, students became dissatisfied and began questioning the effectiveness of massification in higher education in achieving quality and in promoting competitiveness in the job market.[17] Clearly, 'massification' has repercussions for quality assurance, as regulating standards and guaranteeing quality becomes problematic in the context of growth and globalisation.[1,15]

At many universities worldwide, resources, staffing and physical infrastructure have not improved in proportion to increased enrolments, which has impacted negatively on the throughput ratios, graduate employment, increased staff-to-student ratios and the quality of higher education.[14,15] Undoubtedly, 'massification' compromises the quality of higher education and discounts, or severely impedes, engagement in the transmission of disciplinary knowledge with negative consequences for appropriate teaching, learning and assessment practices.[11,15,18] This scenario further adds to the fear that students are less honest and transparent where cheating and plagiarism might be entrenched.[14]

Academic integrity: Setting academic honesty and dishonesty apart

Academic integrity upholds and improves the quality of teaching, learning and assessment, while academic dishonesty compromises the quality of teaching and learning processes and undermines the credibility of the student, the academic and the institution.[19] Academic integrity in assessment within higher education institutions speaks to the ethical policy and core values of integrity in upholding the goals of the university, in respecting and protecting the knowledge of oneself and others, and in ensuring that all students are guided in the best ethical practice of learning.[19]

On the other hand, academic dishonesty is the antithesis of academic integrity and it is characterised by different ways in which students are dishonest in their academic practices, such as: plagiarism (stealing the work of others); cheating (taking information for academic credit); collusion; duplicate submission; copying; deceitfulness (lying); conspiracy; misconduct (fabrication, manipulation and misrepresentation of information); and improper use of Internet sources and the computer, including back translation. In South Africa, academic dishonesty poses a serious ethical problem facing students and academics[20], with cheating and plagiarism becoming a huge challenge in teaching and assessing large classes and maintaining academic standards of integrity.

As cheating and plagiarism is becoming more pervasive, 'back translation' is the new cyber-based form of plagiarism – a less traceable method of 'cyber-facilitated plagiarism' – to subvert academic integrity, where students intentionally run text through language translation software or through Internet translation software to camouflage the original ideas and ingeniously disguise the source.[13] To counter these acts of plagiarism, academics collect a sample of students' language and writing styles at the beginning of a module as a useful way to develop a point of reference, although they recognise that this strategy may be difficult in large classes.[13]

Student plagiarism in assessment: An ethical concern

Plagiarism is a longstanding, common, worldwide ethical issue facing universities that disrupts learning and the transmission of knowledge.[13,20-24] Plagiarism is using the intellectual work of others through means of 'kidnapping' their ideas without the appropriate sources of reference.[25] This arguably leads to questioning and the rejection of students' academic work and intelligibility.[26]

Much of the existing literature speaks to students' perspectives of plagiarism and cheating, and new plagiarism detection strategies.[14,21,23] The usual way in which students plagiarise is to 'cut and paste' and blend this into their work.[13] Students are concerned that it might occur by accident, and find it problematic when starting out in academia; they indicate being inexperienced and uncertain about referencing and reference incorrectly unknowingly.[3] It is important for academics to judge the seriousness of plagiarism and cheating with intentional devious cheating for substantial gain being considered more serious than cheating unintentionally.[14] The effect of plagiarism reduces ones' thinking, creativity and originality.[13] With the plenitude of electronic journals and literary information readily available, liberal Internet environments support plagiarism and allow students to obtain written papers at a fraction of the time, cost and effort, from writing companies[13] – a concept often referred to as ghostwriting[24].

Turnitin: Pedagogical or plagiarism detection tool?

Parallel with the uptake of Internet technology is the increase in plagiarism, as more academics complain of plagiarised work submitted for assessment by students.[22] As a result, developments made in detecting and deterring student plagiarism have complemented the uptake of Internet technology.[13] The accessibility, openness and convenience of the Internet is considered a double-edged sword for students likely to plagiarise; it can similarly be used to commit acts of plagiarism and detect acts of plagiarism.[21] Turnitin, SafeAssign and MyDropBox as plagiarism detection strategies claim to deter plagiarism. In spite of these plagiarism detection strategies, grave problems in assessment procedures prevail.[24] Arguably these strategies cannot solve plagiarism on its own, and it is still the academics' responsibility to score these assessments and to evaluate the extent of plagiarism which necessitates a systematic approach.[22,24] Similarly, Chew et al.[27] conclude that it is imperative for academics to decide if the highlighted matched text is legitimate or not within the respective disciplines and institutions.

Turnitin, as a plagiarism detection software, does not actually identify plagiarism. It merely provides a similarity report used to check a student's work for unoriginal pieces of information. Walker[24] claims that Turnitin fosters an environment of fear and mistrust amongst students with the presumption that students are guilty until 'proven innocent'. Plagiarism did not decrease as a result of awareness, but increased, and use of Turnitin has not deterred plagiarism.[24] This finding raises questions: Why do students still plagiarise despite an awareness of the risks of plagiarism and university policies on plagiarism in place? Why does use of Turnitin not deter plagiarism?

Walker[24] interrogated the reliability of Turnitin similarity reports, which undoubtedly saves hours of work for academics in establishing authenticity of work submitted. The disadvantages of Turnitin software are that it does not detect material that is password protected or texts

produced by ghostwriting companies.[24] Although Turnitin is a strong text-matching instrument, studies suggest it is easy to doctor a document to manipulate the Turnitin plagiarism check.[27] Therefore, Walker[24] suggests academics approach such reports with caution as they may not always indicate 'genuine plagiarism', accentuating that the responsibility lies with the academic to evaluate the report and to make a decision as to what extent plagiarism has occurred and whether plagiarism was intentional or accidental.

Since the change of outlook on plagiarism detection, it is essential to note the shift in thinking on Turnitin from its role as a plagiarism detection tool to a self-learning tool for students.[27] The findings of a study by Chew et al.[27] emphasise that Turnitin is not intended to be used as a 'plagiarism detection tool' (policing tool) to punish students for plagiarism but instead it should be used as an effective self-assessment learning tool used to support students. This can be done through the pedagogical use of the Turnitin originality report to improve students' academic writing practice through allowing them the option of multiple submissions. For this approach to be effective, it is suggested that: clear explanations on how to interpret the originality report are needed to thwart misunderstanding and emotional stress and anxiety amongst students; a standardised 'Turnitin policy' be put in place to provide a consistent learning experience for students across an institution; and Turnitin similarity reports should not be the solitary determinant of identifying student plagiarism.[27]

Factors influencing student plagiarism

Student plagiarism cannot be limited to a particular country, gender, age of students, uptake of technology, education levels of students, students' beliefs about plagiarism and academic honesty, or to the culture and language proficiency of international students.[21,22,24] While these factors are recognised, it is acknowledged that many acts of plagiarism go undetected, unreported and unpunished.[21]

One of the factors limiting ethical learning-oriented assessment practices is the lack of trust, and how distrust can limit assessment development and productive student learning.[28] Thus, Carless[28] advocates a shift away from 'defensive' assessment. The personal inner drive (desires, needs, ambitions and goals) can also act as a possible threat to integrity because personal desires, needs and ambitions may lure an individual to act only in the interest of oneself, dishonestly.[29]

Other factors expediting plagiarising and cheating include: students' perception that it is easy to get away with it as universities do 'not chase it up'; different methods of assessment offer different chances for plagiarising and cheating; and students consider cheating is justifiable when teaching and assessments are of poor quality. Importantly, the quality of the student experience is a priority; and lastly, teaching of large classes can result in students feeling neglected and alienated in the system.[14]

The task of completing writing assessment tasks is complex for students, but ever more perplexing for academics is designing assessment tasks to deter plagiarism and to assess these tasks.[22] Prevention of plagiarism might only be possible with the cooperation of colleagues.[22] Hence, in this study, we aimed to explore academics' experiences of student plagiarism and its effect on academic integrity within the existing context of teaching large classes intended to cope with the 'massification' of education.

Research methodology

In this qualitative research study, we explored South African academics' experiences to gain an in-depth and subjective understanding of assessment and plagiarism, and its effect on academic integrity in teaching large (undergraduate and/or postgraduate) classes in the Discipline of Curriculum Studies.[30,31]

The method of currere, which encompasses four stages, was used to reflect and examine the past and present lived educational experiences and future anticipations of academics.[32,33] These include the regressive, progressive, analytical and the 'synthetical' stages. In brief, the regressive stage is the examination of past and present experiences, insights and means of knowing of the academics, which enabled them to share and understand their experiences of teaching and assessing large classes and their experiences of academic dishonesty such as student plagiarism. The progressive stage looked to the future, consciously and deliberately thinking of and imagining the future by challenging and disrupting their own thoughts, which assists the academics on their path to envisioning acts of transformation and committed action. It also looked at the way in which they will teach and assess large classes to promote quality education and academic integrity. The third stage of analysis involved analysing these experiences for meaning-making. The fourth stage, the 'synthetical' moment, returns to the past and present experiences, and future expectations for deeper existential meaning and understanding, which is done through assimilation and interpretation of their experiences and thoughts. As represented in Figure 1, the method of 'currere' provided a methodological lens that brought to the forefront the stories the academics told of their subjective lived experiences for deeper meaning and consistency. They should reflectively recollect their past experiences and reflectively imagine the future as academics in the field of curriculum studies within the context of teaching and assessing large undergraduate classes subsequent to the conception of 'massification' of education.

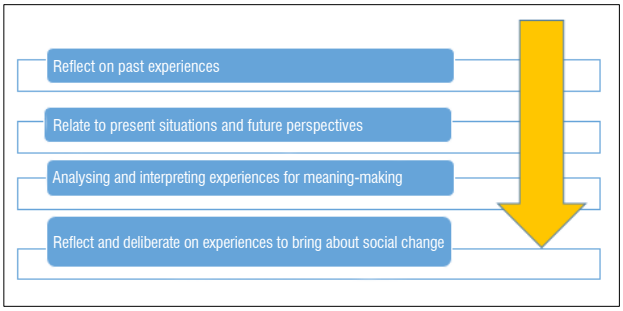

Figure 1: Using the method of currere as a lens to explore academics' experiences in the teaching and assessing of large classes (adopted from Pinar[32,33]).

Context

A 'large class' is defined differently depending on the Discipline and the pedagogical learning situation, resulting in different experiences for academics.[11] This study was located in the Discipline of Curriculum Studies in the School of Education at a university in KwaZulu-Natal in South Africa. This Discipline offers core compulsory modules to all initial teacher education students at the undergraduate level, with academics teaching classes of more than 300 students, as well as being responsible for coordinating the entire cohort of students for a particular module that often exceeds 1200 students. These student numbers become overwhelming for academics, as expressed in their stories. In light of 'massification' of education, sharing academics' experiences may benefit other academics in the way they think about effective assessment strategies and in how they deliver quality education when confronted with teaching large classes.

Participants

The sampling method utilised was purposive sampling as the participants and sites for study informed the central phenomenon of having experience in assessment, plagiarism and its effect on academic integrity and this was an attempt to ensure that the selection procedure was credible.[34-36] At the time of the study, the participants were permanent emerging African academics (lecturers) with not more than 7 years' experience in the Discipline of Curriculum Studies. All three participants (hereinafter referred to as Pearl, Ruby and Tony) were lecturers who had encountered issues with academic dishonesty in the undergraduate and/or postgraduate levels of teaching and were selected to participate based on these experiences, irrespective of race or gender.

Data collection methods

The purpose of the methods used in this study was to make sense of the data collected by showing the interplay between assessment and

plagiarism and its effects on academic integrity through the academics' lived experiences of teaching large classes.[37,38] In-depth, semi-structured questionnaires and interviews were utilised, as this approach is ideally suited to an epistemological and interpretivist research project.[38-40] An interview schedule with open-ended questions allowed the academics to express their experiences, perceptions and opinions openly. The questions were given to the participants beforehand so as to gain a more thought-out, detailed response. The participants were further contacted to clarify information.

For this study, narratives (stories) were constructed from the data collected to explore and understand participants' lived subjective experiences. The narratives were given back to the participants for confirmation.[36-38] The stories are reproduced in the supplementary material. The narratives were reflectively and reflexively analysed for emerging themes and for further interpretation and meaning.[30,41-44]

Issues of trustworthiness and ethical principles were considered in all facets of the study. Gatekeeper Permission was granted to conduct this research (HSS/0727/016). The data were collected from multiple primary sources and pseudonyms were used to guarantee anonymity of the participants so as not to infringe upon their rights in any way.[34] The purpose of this study was not to generalise the findings but rather to acquire an understanding of academics' experiences of assessment and plagiarism when teaching large classes.[34,44]

Discussion of findings

The discussion of the findings was based upon the emerging themes in response to the key research question and sub-questions.

Key research question:

1. What are academics' experiences of teaching and assessing large classes within the Discipline of Curriculum Studies?

Sub-questions:

1. Based on academics' experiences, what are some of the ways in which students displayed academic dishonesty?
2. What measures can academics put in place to diminish academic dishonesty when assessing students in large classes?

The following themes emerged from the data elicited from the participants' stories:

- Class size matters: Academics' experiences of plagiarism in teaching and assessing large classes
- Teaching large classes warrants a rethink of assessment methods
- Efficacy of Turnitin: Teaching or plagiarism policing tool?
- Going beyond the Turnitin report: Factoring in human intervention
- Avoiding plagiarism through assessment design: Hybrid assessments
- Innocent until proven guilty or guilty until proven innocent: Using Turnitin

Class size matters: Academics' experiences of plagiarism in teaching and assessing large classes

Class size matters, especially in teaching large classes. Studies indicated that students in large classes showed less commitment and lower levels of engagement, which make them more prone to cheating and plagiarism.[11] Large class sizes correlate with low student performance, goals of education, the educational experiences of teachers and students[11], and the demands placed on academics in developing effective teaching experiences[45]. However, Jawitz[45] argued that large class teaching does offer unique prospects for delivering quality learning experiences for students which require the utmost planning, support and expertise. He further opines that academics should challenge dominant taken-for-granted beliefs about large class teaching, and calls for more research on the values of large class teaching. The diversity of students in large classes is a valuable resource to the lecturer. Although literature suggests that there are initiatives in place regarding large class pedagogy, the adequacy of these initiatives needs to be re-evaluated to aid in the university's transformative goals.[16]

From the narratives, it can be deduced that the academics understand the importance of ensuring a good work ethic for both students and academics in large classes, abiding to university policies and promoting quality teaching and learning. However, the participants revealed that teaching and assessing large undergraduate classes is challenging, frustrating, 'emotionally and psychologically draining' and 'overwhelming' (Pearl). Tony recalled how difficult it is to keep 'student attention and stimulating interaction with non-responsive students' and how 'demanding it is controlling students and maintaining discipline while trying to teach at the same time'.

The emphasis of equity and redress without support for students who come poorly prepared from the school system, has destructive repercussions for the quality of education and the quality of graduates produced in universities.[3] This is especially true for the first year of study, for which many students from disadvantaged and rural backgrounds need individualised support to meet their educational needs. Large class teaching does not enable academics to provide sufficient face-to-face contact and support to students because of increased workloads and lack of time and resources to cope with teaching large classes.

Addressing these challenges is crucial to minimising the overload faced by academics, who spend hours developing material and resources for delivery of the lecture and assessments in pursuit of quality teaching and learning experiences. As the 'massification' of students increases at universities, the capacity of academics, resources and infrastructure remain a challenge.[1]

Within higher education, the aim extends beyond merely acquiring knowledge. It is about solving problems, encouraging students to engage with issues and to think critically. This level of response is fundamental to a deep quality learning experience and clearly class size does affect the quality of teaching and learning.[11] Hornsby and Osman[11] emphasise that large class sizes fail to enhance higher order cognitive skills, and students show low levels of engagement with the course material, and demonstrate low levels of commitment to and enthusiasm for their work.

Consistent with Hornsby and Osman's finding[11], the participants in our study revealed that as enrolments increased with the 'massification' of education, budgeting, staffing, resources and infrastructure did not increase proportionately. The participants in this study complained that they had not received the necessary support from the university to manage large class teaching and assessments. Managing tests for large classes is daunting. We are 'understaffed as invigilators' (Ruby), and students are more prone to cheat and plagiarise in large overcrowded spaces. This situation unquestionably affects the quality of teaching, learning and assessment.

The participants revealed that administering assessments and teaching large classes is challenging and a 'nightmare' (Pearl), hence they found themselves resorting to online assessment strategies, but this also brought about high levels of plagiarism and cheating behaviours. The academics hinted at their roles as academics changing to that of a 'security guard', 'police officer' (Tony), 'investigators' (Pearl) in monitoring and deterring plagiarism.

The participants attributed reasons for student plagiarism as: an 'easy way out' (Pearl), students are 'lazy' and 'lack commitment' and 'accountability', and students feel 'using big words is academic writing' (Tony). Participants indicated that they felt academic writing programmes offered at the university are simply not enough. Literature suggests that some acts of dishonesty occur through conscious choice as a result of laziness and demotivation to study.[14] Pearl considers that plagiarism is a result of

> *Students' poor work ethic and laziness and doing assignments at the last minute, unpreparedness, lack of understanding of assignment requirements and content, and language incompetence… students learn for assessment and not mastery of the content.*

Academics' experiences of student dishonesty included: duplicate submissions by students, copying from each other, submission of 'historic assignments' (Tony) from previous years, Turnitin submissions (including curricula vitae of students and texts in isiZulu), the submission of doctored Turnitin originality reports, and 'spin it' (Tony), which is use of an online paraphrasing tool. Tony described students who plagiarise as 'naughty' but that it is an offence committed by the 'best of students'. Pearl lamented how 'students are aggressive and cheat during tests'. The participants further identified various factors influencing student plagiarism such as: the language barrier, students' morals and beliefs, and personal inner drives. These were consistent with some of the factors influencing student plagiarism identified in the literature.[21,29]

Academics revealed that they only used Turnitin as a plagiarism detection strategy as it is university policy. They agreed that the university policy on plagiarism is 'too lenient' (Tony), and that there are 'no punitive measures in place to deal with this gross misconduct by students' and reports of incidences are not adequately followed up (Pearl). Therefore, they 'no longer bother to report it' (Pearl). The importance and transparency of university policy also places pressure on academic staff to deliver institutionally standard responses to students in certain situations rather than using their personal preference and expert judgement.[14] Generally, we found that students at this university are required to sign declarations of academic honesty when submitting assignments that confirm that all sources have been acknowledged. Student handbooks, university policy and official documents outline procedures and implications for what is not acceptable in terms of work submitted. Similarly, it is argued that university regulations place undue pressure on lecturers by demanding a universal response in terms of the university's policy to students who have plagiarised work rather than encouraging personal and professional discretion in finding a solution.[14]

Two of the participants (Pearl and Tony) expressed concern at the interference of the Student Representative Council as protector of students who commit plagiarism, leaving them reluctant to pursue any transgressions. Academics in this study indicated that they are reluctant to punish and intervene in acts of plagiarism and cheating because of the stress and uneasiness to intervene and punish the offender; the increased workload involved in detecting and punishing the students committing acts of dishonesty; the pressure on academics to sustain pass levels and enrolment figures; and the 'lack of bite' (Pearl) in universities to follow through on offences. Studies revealed that lecturers find themselves hesitant to interfere in situations that involve student plagiarism due to the large amounts of administrative workload and consequences of punishment.[13,14] Further studies should explore the reasons why academics are reluctant to take action against those committing acts of academic dishonesty.

Teaching large classes warrants a rethink of assessment methods

Considering the increasing use of the Internet amongst students, it has become the most likely source of plagiarism.[22] The participants revealed how 'some students often just copy information from the Internet sources or research papers without proper referencing' (Ruby). Despite the shift in institutional policy to the inclusion of learning management systems such as Moodle in teaching and learning, the participants reported that they find it challenging to accept and to adopt it in their practice and still resort to traditional strategies of assessment to sustain academic integrity as opposed to online assessments. As Ruby commented:

> *Online quizzes are designed on Moodle, the online teaching site, and is* [are] *open for a particular period of time for students to be able to engage with it…in the comfort of their home*[s]. *This form of assessment relieves the stress and pressure of marking large numbers of scripts because the quiz is marked and graded online. The challenge with this form of assessment is that some students may sit together and share the questions and answers; others would take screenshots of the questions and share with their friends.*

Many higher education institutions in South Africa are increasingly moving towards technology, such as online learning management systems, to offer effective and efficient assessment solutions to enhance and cope with the demands of pedagogic objectives.[8-10] As the demands for technology increase, so do the risks of plagiarism, and academics have a crucial role to play in enhancing quality teaching and assessment.[12] Assessment practices must inform and enhance teaching and learning, and it becomes vital that lecturers' reflect on the authenticity of assessment practices.[46]

The way in which assessments in higher education in South Africa are currently strategised is problematic, and emphasises a lack of assessment practices with a disengagement between teaching, learning and assessment practices.[47] Hence, Davids and Waghid[47] advocate that assessments should unfold while teaching is taking place and should be purposeful rather than standardised. Within the local context, in developing African countries like South Africa, research related to e-assessment is lacking due to the inability to integrate technology into universities.[48] Sarfo and Yidana[48] suggest that universities should use a blended learning approach (a combination of online assessment and traditional pen-and-paper assessment) as it is more effective and efficient in developing countries[49]. In the light of student plagiarism and its effect on academic integrity, academics in this study indicated that they were forced to rethink their assessment strategies in contemporary settings. The participants maintained that technology increased the risks of plagiarism, and so they reverted to traditional forms of assessment such as tests instead of online quizzes, which did not necessarily translate into effective assessment practices.

As the participants revealed, it is not impossible to counter the challenges proffered by 'massification' and teaching and assessing in large classes. It is by focusing on the structure of the curriculum, the strategies employed for instruction (teaching, learning and assessment), and the way students are assessed, that the problems associated with large class teaching environments can be addressed and quality education for all can be achieved.[11] Hence, conducive learning environments must be created by academics to maximise the quality of students' educational experiences, and the important roles teaching and assessment strategies play.[1]

Studies conducted in Lesotho highlight the contradiction between the ever-increasing enrolment at universities and the preparedness of universities to accommodate such 'massification'.[1] Academics agree, there is an overwhelming increase in enrolment at universities leading to problematic situations arising such as limited consultation time and an inability to adequately assist students who are struggling. A rethink of pedagogical practices is demanded to ensure that 'massification' of education does not compromise the quality of education.[1]

Efficacy of Turnitin: Teaching or plagiarism policing tool?

Academics lamented how Turnitin is a perplexing and cumbersome tool for deterring plagiarism in large classes, and how they resorted to alternative forms of assessment to deter student plagiarism. The Turnitin system cannot handle the increased number of submissions, so the administration becomes a 'nightmare' (Pearl). Tony stated that 'Turnitin works for honest students', while Pearl had lost 'trust in the system [Turnitin] because it brings more stress and pressure to find ways that can actually stop [a] student from cheating'. The participants further highlighted that students have 'beaten' (Tony) and 'cheat' (Pearl) the Turnitin system. Turnitin cannot detect if students pay individuals to write their assignments. When plagiarism goes hidden or undetected, the students responsible diminish the work value of honest students, it becomes tiresome at an operational level, and negatively influences the reputation of the university's qualifications.[21] Hence the importance of the accuracy of assessments cannot be overemphasised.

The participants indicated that they used Turnitin more as a policing tool and less as a teaching tool. However, Tony and Pearl noted that they do give students a second chance once they have determined the extent to which the student has plagiarised. The participants emphasised that further capacity building is needed to enhance the use of Turnitin. They noted it is 'wasteful expenditure' and 'not useful' (Tony) for undergraduate teaching and assessment. An important concern alluded to by the academics when

identifying and determining the extent of student plagiarism, was whether it was committed intentionally or unintentionally.

Good assessment strategies prevent plagiarism. To increase the reliability and efficacy of assessments, academics should plan their assessment tasks, procedures and rubrics very carefully so as to deter plagiarism.[22] A practice that concentrates on an 'educative approach' that encourages academics to manage academic dishonesty and includes instruments for deterring and detecting plagiarism when it transpires as opposed to treating it as academic misbehaviour is preferred.[19] Academics have a key role to play in the development of student moral understanding and behaviour; however, academics have been found to be unwilling to report or to take action against students who are academically dishonest.[23]

To manage classroom plagiarism, and perhaps for plagiarism to be eradicated in this ever-progressing digital age, academics need to legalise it for learning purposes by adopting diverse assessment strategies that evade plagiarism and that build a moral student culture.[13] Recognising that planning assessments with a vision to 'designing out' can possibly avoid plagiarism and cheating, can assist academics to manage plagiarism and counter back translation, because Turnitin is no longer a deterrent to students who have managed to come up with ways of 'beating' (Pearl) the system.[13]

Going beyond the Turnitin report: Factoring in human intervention

The participants have lost 'trust' (Pearl) in Turnitin and have a negative attitude toward the use of Turnitin as a plagiarism detection tool, but this has not stopped them from exploring innovative approaches to deal with the matter of plagiarism. 'Turnitin detects similarities but not all similarities are plagiarism' (Ruby). Despite the use of plagiarism detecting software, academics in this study were cognisant of the need to rely on their discretion and not solely on the Turnitin report when detecting plagiarism. They were able to use their judgement in distinguishing between intentional and unintentional plagiarism, hence bringing in the human element in detecting and determining the extent of student plagiarism.[22]

Avoiding plagiarism through assessment design: Hybrid assessments

Consistent with the findings of Agustina and Raharjo[50], academics in this study recognised that many students plagiarise because they are ill-equipped to write academically and there is a language barrier that exists among second-language English students. In providing fair assessment and evaluation, students who 'unintentionally' or 'accidentally' plagiarise because of their incapability to cite and report others' ideas in academic writing should be distinguished from those who intentionally plagiarise.[22,24] Hence, academics should focus on these two issues within the curriculum. To assist students, academics acknowledged the importance of including small assessment tasks that can help students learn how to reference and how to paraphrase within the curriculum modules they teach, that is, academic writing skills. The development and acquisition of academic writing skills is one such way to enhance the academic process rather than by solely focusing on plagiarism detection.[51]

Assessing large classes is a challenge and 'defeats the purpose of why we are assessing and the quality of these assessments' (Pearl). Assessment strategies should be carefully deliberated on, planned and executed to engage the students and to maintain their interest and commitment in promoting academic integrity. Hence, participants in this study advocated that during the teaching and assessing of large classes, it is essential that lecturers carefully consider variations of assessment strategies that are reliable, innovative, realistic, manageable, original and personalised to reduce plagiarism and cheating.

Similarly, Jones and Sheridan[13] provide some concrete and pragmatic solutions for academics to consider when thinking about assessment activities to make plagiarising less tempting. The first is 'design it out', which includes good assessment strategy planning in avoiding plagiarism. The second is to move from written assignment submissions to examinations and tests where plagiarising cannot occur. The third is to 'personalise' assessments. This relies on students' own personal experiences which cannot be copied from any external sources. The fourth, 'change', relates to the expansive alteration of assessment tasks for each student cohort so that assessments are not repeated. Lastly, 'restriction' is the limiting of sources of reference, so that the assessor will be well versed with these sources and students will realise that plagiarising is pointless.[12] To deter plagiarism, Comas-Forgas and Sureda-Negre[52] suggest that the number of assessments should be reduced, hybrid tasks that incorporate theory and practice should be introduced, and regular feedback sessions with students should be arranged to monitor the process of their academic writing. Further, acknowledging that equipping students with the appropriate academic writing skills is a viable preventative measure to plagiarism.[25]

Innocent until proven guilty or guilty until proven innocent: Using Turnitin

Academics in this study agreed that student plagiarism and cheating is the unethical practice of 'stealing people's ideas and work' (Ruby). They settled on the use of Turnitin as a 'teaching tool' instead of a 'policing tool' that will assist students to enhance their writing and to stimulate their confidence and reduce pressure, anxiety and fear when submitting work for a Turnitin plagiarism tracer test. As Ruby commented, 'Turnitin detects similarities but not all similarities result from plagiarism', that is, similarities detected included common phrases, names and concepts. Tony and Ruby elaborated on how, through using Turnitin as a teaching tool, they gave students a 'second opportunity' (Tony) to 'resubmit the assessment task' (Ruby). Carless[28] advocates that lecturers should be given greater autonomy to act responsibly in the processes of teaching and assessment that speak to the principles of sound academic integrity. Nonetheless, Elias[20] concluded that academics should stop accepting excuses of pressures and inabilities, as students are using unethical ways of simply wanting to pass and achieve the degree and not pursue knowledge.

In enhancing academic integrity, the academics in this study advocated for: a fully functional academic writing centre; a more collaborative and concerted effort with management and the Student Representative Council; and educational programmes for students that foster good ethical behaviour, accountability and acceptable academic practice. Academics should be innovative in adopting more suitable methods to enhance the quality of their teaching through 'hybrid' assessment tasks that are free of plagiarism and easier to manage. Basic academic writing skills should be taught and tested creatively in conjunction with the teaching of the module.

The findings point to the transmission of easily understandable information and vibrant awareness of cheating and plagiarism, accentuating the positives of good academic practice supported by concrete practical examples.[14] Studies suggest that compelling students to sign a pledge (a code of honour) creates awareness and commits them to academic integrity and understanding the consequences of failure to comply with this rule.[21] Changing students' mindsets on learning, assessment and plagiarism is vital to sustaining academic integrity. Academics in this study believed that plagiarism is a moral and ethical issue, and that students should be educated on ethics and academic integrity and should be made aware of the implications of fraudulent behaviour. Universities must show that they 'mean business' with a 'zero tolerance' approach; stricter measures should be put in place to monitor and control academic dishonesty.

While there are strategies in place to maintain academic integrity, trust and honesty remain key, and in the ever-changing digital context there will always be new ways to plagiarise. Therefore, it becomes imperative to revisit the dominant approaches in managing plagiarism and cheating in large classroom contexts. Plagiarism and cheating should be managed institutionally, and academics should elect to directly sanction students on the basis of learning instead of outlawing students.[13]

Martin Trow[53] suggested ways of thinking about the development of higher education in progressive societies, accentuating growth, democratisation and diversification. He enunciates the obligation for universities to monitor continuously and to evaluate higher education to guarantee quality. Arguably, the principles of the theory are largely germane to developed countries.[1] In institutions of mass education, education

becomes more integrated, thus allowing for flexible combinations of courses, and the rejection of academic forms, structures and standards extends to examinations and assessments.[53] As such, these further recommendations are put forward: (1) At the university level, operational practices, facilities, resources, and the capacity-building of academics need a rethink to guarantee quality higher education and to provide the necessary attention and support for those students who need it most. This applies especially to first-year students coming from disadvantaged and rural backgrounds. (2) To address the challenges of 'massification', traditional pedagogical approaches need a rethink to include innovative alternatives using technology to reduce overcrowding and to sustain quality in education. Jawitz[45] encourages lecturers to develop innovative pedagogies to facilitate effective large class teaching and assessment. This approach should be applauded. Universities have already commenced using online learning management systems such as Moodle and Blackboard, online courses, and 'virtual delivery' to reach large numbers of students without face-to-face contact in the class.[45] (3) The apparent disconnection between government, the university and academics should be confronted. Academics need to be given a voice. What is needed is vigorous participation and engagement with all stakeholders at all levels to deal with large class teaching, and its implications for the curriculum and for pedagogical approaches that matter. (4) The overload on academics needs to be addressed by universities to ensure quality education and to avert teachers being pressurised and experiencing burnout, stress and frustration.[1] (5) Emerging academics need: relevant educational expertise, sufficient resources and support from the university, and mentoring from skilled senior academics to improve their pedagogical practices in teaching large classes.[45]

Concluding comments

Teaching large classes is undoubtedly a daunting task with academics experiencing high levels of student academic dishonesty. Academics agreed that academic dishonesty in large classes compromised the teaching and learning process and negatively impacted on the quality of graduates produced, and on the reputation of students, academics and institutions. This situation has influenced the way academics deliberate on their methods of teaching and assessment. Although there is a university policy in place to address plagiarism, academics' felt that this policy is too lenient, and acts of plagiarism reported are not adequately monitored.

This research study prompts academics to think beyond taken-for-granted teaching and assessment strategies in large class situations to perpetuate quality education and academic integrity in warranting a relevant and meaningful educational experience.

Within the context of this study, ethics within the teacher education curriculum are fading and should be prioritised, with a focus on the professional ethics perspective and on ethics education, which is to initiate and prepare future professionals to operate in a shared community of practice that clarifies what it means to act in an ethical, principled and responsible manner, both as a student teacher and as a professional teacher.[51]

The capacity building of academics with the use of Turnitin and the issues academics face with student plagiarism needs further investigation. Further studies on assessment, plagiarism and its effect on academic integrity can probe into students' experiences of being taught in large classes and their perspectives of pedagogical approaches in the classroom. This will facilitate the analysis of plagiarism and its drivers in universities by allowing student voices to surface.

Authors' contributions

Both authors contributed to the writing of the narratives and the text, and the analysis of the data. P.M. conceptualised the study. T.P compiled the supplementary material.

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None

Exploring the prevalence of the sexually transmitted marks phenomenon in higher education institutions

Countries steadfastly pursue academia as a necessary step towards socio-economic development, which places a mandate on institutions of higher learning to stir host-country economies through university deliverables. In Zimbabwe, this entails the Ministry of Higher and Tertiary Education, Science and Technology Development's 'doctrine' spelling out the philosophy of 'Education 5.0' which emphasises teaching/learning, research, community engagement, innovation, and commercialisation of goods and services. However, academic dishonesty, such as that through 'sexually transmitted marks' (STM), threatens the realisation of such mandates. Although the norm is that such sexual transactions are initiated by academics, evidence shows students also initiate such relationships. Consequently, efforts to eliminate this threat to academic integrity should not only focus on lecturers, but also be extended to students. This paper contributes towards unmasking experiences of STM between male lecturers and female students, female lecturers and male students, and female students and male students, as determined from former university students and university alumni in Bulawayo. Exposing these practices allows for open consultation and adoption of good practices from similar institutions worldwide.

Significance:

- The majority of respondents all attested to having experienced STM directly or indirectly during their years of study.
- An explicit STM regulation policy targeting all actors across universities is needed.

Introduction

There is growing interest in issues related to academic integrity, the attribution of which is partly explained by the increasing number of academic fraud cases reported worldwide. This draws from rapid 'massification' and growth of higher education systems that have seen universities become influential organisations in society wherein integrity failures damage institutional brands and the credibility of higher education systems.[1,2] Global university branding and related influential international rankings mean positive and negative perceptions of academic integrity can have a significant impact on institutional reputations. Transparency International, a non-governmental organisation working worldwide on matters of corruption, commonly defines corruption as 'the abuse of entrusted power for private gain', adding that in higher education, corruption encompasses 'the lack of academic integrity'[1,2]. Traditionally, researchers[3,4] find that students cheat or exhibit dishonesty in five ways:

- buying a paper from an essay bank or term paper mill;
- copying a whole paper from a source without proper acknowledgement;
- submitting another student's work or a paper written by someone else and passing it off as one's own;
- copying sections of material from one or more sources and deleting the full reference; and
- paraphrasing material from one or more sources without providing appropriate documentation.

However, academic dishonesty can take other forms. This study explored the prevalence of the 'sexually transmitted marks' (STM) phenomenon among institutions of higher learning (IHL) as a facet of academic dishonesty that corrodes academic integrity. The study sought to unmask the deployment of sexual favours to influence outcomes of academic assessments as an increasingly common practice within IHLs. The study scope considered the normative belief that sexual transactions are initiated by lecturers but it is noted that there is also evidence of students initiating such transactions against lecturers. It underscores the importance of focusing not only on lecturers but also on students in efforts to eliminate this threat to academic integrity. The study deployed questionnaires designed to purposively target various university alumni, students, university administrators and lecturers with a view of contributing to the deep-seated phenomenon. Such unmasking potentially uplifts academic integrity and excellence. Furthermore, understanding the social constructions of sexual harassment is a step towards understanding how sexual harassment as a social injustice can be resolved by academics, activists and policymakers alike.[1]

Statement of the problem

Academic dishonesty is a fundamental issue for the academic integrity of IHL, and one that has lately been gaining increasing media attention.[2] Clearly, one of the key roles of IHL is to create an environment conducive to learning – one that will produce highly skilled and technically competent graduates who demonstrate high standards of honesty, ethical responsibility and commitment to serving various professions and society well.[3] The growing phenomenon of sexually transmitted marks – a form of transactional lecturer–student sexual relations amounting to academic misconduct and student cheating – directly undermines and negates efforts on this front[4-8], piling pressure on academics and institutions to manage it. Witherspoon et al.[9] summarise outcomes of lecturer–student cheating into three obvious problems for IHLs. First, it threatens the equity and efficiency of instructional measurement, thus students' relative abilities are not accurately evaluated. Second, cheating students potentially reduce their level of learning, and hence are less prepared for advanced study and application of taught concepts.

Last, at the broader, societal level, it is likely that students who disregard academic integrity while at university will treat it with equal disdain in their future professional and personal relationships.

Objectives of the study

1. To determine if the practice of 'sexually transmitted marks' occurs within Bulawayo-based state universities,
2. to highlight the manner in which the salacious relations manifest against integrity, and
3. to explore existing policy documents or regulations that outlaw sexually transmitted marks.

Theoretical framework of analysis: The socio-cultural model

American lawyer and feminist Catherine MacKinnon argues that power is at the core of feminist theories of sexual harassment, although it has rarely been measured directly in terms of workplace authority.[10,11] The sociocultural model provides a societal and political explanation that has its roots in MacKinnon's idea that the origins of sexual harassment are a patriarchal society. The model postulates that sexual harassment is a product of culturally legitimate power and status differences between men and women that stand as a manifestation of a wider system of asymmetrical power relations between men and women. For feminist theorists[4,10], sexual harassment dovetails gender socialisation processes in which men assert power and dominance over women at work and society. The sociocultural model thus argues that women experience more harassment than men.[10]

The theory suggests that the patriarchal way in which men occupy power positions in all levels of society, that is, in home and workplace decision-making processes, determines the reproduction of power inequities in the workplace.[12-17] Succinctly, the sociocultural model emphasises the role of patriarchy in establishing and maintaining male dominance in society, as a fertile ground for sprouting sexual predators who harass women in IHL. The model is, however, critiqued[5,17] for being too simplistic and for not taking into account the sociocultural context that is always shifting. Also, sexual harassment is not a normative behaviour for the majority of men and the sociocultural model does not explain why most men do not harass. The non-conforming attributes are explained[4,10] through a conceptual model of the causes and consequences of sexual harassment. Scholars such as Thomas[18], Faludi[19] and Fitzgerald et al.[20] model sexual harassment as a function of two conditions: organisational climate and job gender context[20]. In their argument, Fitzgerald et al.[20] and Lin et al.[21] conclude that sexual harassment episodes are positively correlated with the extent to which an organisation 'tolerates sexual harassment' in the workplace, as is the likelihood of working in a male-dominated job context.

Rights-based approaches

Holm[22] avows the rights-based approach is the brainchild of the United Nations' Children Education Fund (UNICEF) and ensures the meaningful and systematic inclusion and empowerment of the most vulnerable. Rights-based approaches emphasise that all calamities have perpetrators and victims and advocate for the respect, protection, and fulfilment of the rights of all, including students of IHL. The theory of rights-based development stems from the ethical assumption that all people are entitled to a certain standard in terms of material and spiritual well-being, often taking the side of people who suffer injustice.[22] By definition, a rights-based approach to development is a framework that integrates the norms, principles, standards and goals of the international human rights system into the plans and processes of task delivery among IHLs, characterised by methods and activities that link the human rights system and its inherent notion of power and struggle between women and men.[17]

Rights-based approaches recognise poverty as an injustice and view marginalisation, discrimination and sexual exploitation as human rights violations central to poverty.[22] For instance, without a degree, women earn substantially less pay, receive far fewer employee benefits, and are less likely to be financially independent.[23] Affected students avoid certain places on campus, change their schedules, and drop classes or activities to avoid sexual harassment, with telling academic effects. Concurring, Jordan et al.[24] argue that STM survivors often see their grades drop dramatically, develop post-traumatic stress disorder and anxiety, and are frequently left with no opton but to withdraw from classes or extracurriculars to avoid their perpetrators on campus. In a rights-based approach, every injustice is never simply the fault of the individual, as argued by some respondents in the manifestation of sexually transmitted marks in which female students are said to initiate STM relations. However, a rights-based approach also refuses to simply place the burden of injustice and exploitation on abstract notions such as society. For Holm[22], human rights claims always have a corresponding duty-bearer; hence a central dynamic of the rights-based approach is identifying root causes of exploitation injustices by empowering rights-holders to claim their rights and enabling duty-bearers to meet their obligations, in this case as per Sexual Harassment Policies.

Academic dishonesty has become an increasingly challenging issue in academic institutions.[6,7] Although debatable, scholars[6,25] posit that the percentage of academic dishonesty among IHL students is increasing faster in comparison to previous years.

Sexual harassment in all forms is a global issue permeating IHL and workplace fabrics wherein men and women interact.[6,7] With respect to universities and other IHLs, Morley and Lussier[26] and Taiwo et al.[27] argue sexual harassment is not limited to Africa. This harassment has often, albeit silently, taken the form of 'sexually transmitted marks' reported as sexual harassment, which has the effect of harbouring the perpetrators, victims, and the power relations involved therein, and guaranteeing the perpetuation of the salacious relations to the detriment of academic integrity. Acknowledging illicit lecturer–student sexual relations as a global challenge, scholars[27,28] propose that the 'sexually transmitted marks' phenomenon deserves mainstreaming into the academic curriculum, particularly to reduce student vulnerability and increase restorative care to victims.

The fact that universities in Ghana and Tanzania have already integrated sexual harassment into course modules on Gender, Power and Sex to address the challenge of male lecturers demanding sex from female students in exchange for higher grades[27] bears testimony to the existence of the integrity scourge. Sexual relations between lecturers and students have thus been commodified with 'sex' and 'academic marks' as the 'currency' of trade at the 'academic markets' where the most powerful currency of trade determines the form of reciprocal act by the weaker party at 'this market-place'.

Psychology students in the USA revealed a higher prevalence of sexual harassment and unethical intimacy between postgraduate students and their supervisors than between undergraduate students and their lecturers due to the frequent face-to-face interaction when postgraduates seek advice on their research studies.[6] In Africa, tertiary educational institutions in Nigeria have been no exception. For instance, Gaba[29] affirms that in Nigeria 'sexually transmitted marks' or 'sex for grades' in the tertiary institutions is a living reality where male lecturers perceive themselves as tin gods and such unprofessional behaviour can be perpetuated unchecked.[6,7,27] These views were buttressed in similar studies by Heyneman[6] and Taiwo et al.[27] who reported a high prevalence of sexual harassment in both educational institutions and the workplace.

The phenonomenon of sexually transmitted marks manifests in various forms, although most importantly it is rooted in unequal power relations that are closely associated with gender-based violence, human rights violation, as well as fraud and corruption.[27-29] Students of IHL should be given equal power to match their lecturers in case of violations and being cornered, such that students also get to determine the lecturer's future and tenure. Such a robust anti-academic-corruption policy would instil a sense of confidence in the system and limit the vulnerability of students.

Scholars concur that the harasser often is usually older, powerful and poses something of value that is beneficial to the harassed[27-29] and induces 'wilful submission' which is best described as subtle 'sexual

coercion'. Such coercion is associated with both sexual bribery and sexual intimidation, which ensnaringly lures the would-be victim of their own volition, thereby camouflaging the practice.[27-29]

The STM trends reportedly take various forms: from male lecturers to female students, female lecturers to male students, from male students to female students, female students to male students, from male lecturers to female lecturers, female lecturers to male lecturers and non-academic staff, among others. Same-sex relations were not found in a review of STM literature, although they cannot be ruled out. This study did not explore the existence of same-sex relations under STM. STM trends present disturbing scenes in an environment often believed to be a centre of excellence for moulding distinguished leadership skills, high moral qualities and intellectual capacity for human capital and future leadership.[27,29]

Scholars[23,27,29] suggest that, in most cases, female students are most at risk of being victims while male lecturers and 'high-flying' male students are more likely to be the sexual predators, although some studies have arguably presented male lecturers as victims of sexually marauding-predating female students[30]. Underpinning the 'new trend' of predator students[30], Imonikhe et al.[31] submit that 'while campus girls always accused lecturers of demanding sex for marks (STM), a survey showed a number of lecturers were actually sexually harassed by female students'.

A study[23] of undergraduate college students commissioned by the American Association of University Women Educational Foundation and conducted by Harris Interactive in 2005 found that both male and female students are more likely to be harassed by a man than by a woman. Half of the male students and almost one-third of female students admitted to sexually harassing someone in college. Equal proportions of male and female students say they harassed a student of the other gender.[23]

This study dispels the notion of female students always being the victims in such transactions and brings to light the downside inherent in transactional sexual engagements 'justified' on the basis of consenting adults. Some studies[31-33] have highlighted an increase in cases of sexual harassment, blamed, by the respondents in those studies, on what women wear. Lack of awareness as a causal factor is attributed to many students and academic, administrative and support staff not knowing various university policies and regulations against sexual harassment[33], with few having actually read it, translating to the policy evaporation lamented by Longwe[34], Risby[35] and Macdonald[36]. These scholars also lamented poor academic monitoring and mentoring systems as creating conducive environments for perpetrators to exploit and harass unabated. While students were generally concerned about the problem of 'missing marks', common in some university units, others preyed on such situations to sexually molest their mentors and lecturers in exchange for higher grades.

The roots of the STM phenomenon are traceable back to the tertiary institutions at which the lecturers were trained[7] and at which their lecturers were said to engage in illicit affairs with students. Houreld[37] and Tagoe[38] opine that sometimes corrupt lecturers entrap their victims by employing different strategies like giving low grades to their targets whom they later invite into their offices. Witch-hunting or marking down assignments of their victims and launching vendettas against those who reject them[37,38] are other strategies employed. Teodorescu and Andrei[39] highlight that, for example, 17% of students in public universities in Bucharest, Romania's capital, admitted that they had witnessed professors make sexual advances towards students. The problem under such circumstances is that, often, there are no mechanisms in place to check these lecturers' activities, and so victims are not able to talk about it to anyone. When students see these activities going on without anything being done about it, they consider it normal, thus perpetuating the cycle. Consequently, there is always the issue of conflict of interest, bias in the awarding of marks, and betraying a position of trust, hence the need for their registration and de-registration to be guided by ethical standards of professional conduct.

Methodology

As exploring matters of sexual exploitation is obviously a delicate subject, efforts were made by the author to ensure, from the onset, that every respondent would not be identifiable in subsequent published material and presentations. The study involved the collection of university policy documents and a self-administered and postal survey of Bulawayo-based universities in Zimbabwe.

Bulawayo-based universities were purposively selected for convenient access by the researcher. There are 3 universities in Bulawayo and 20 registered universities in Zimbabwe, translating to 15% of the whole university sector in Zimbabwe. This percentage is slightly above Sekaran's[40] 10% sample representation threshold. Views on STM were, however, drawn from beyond these three universities as the alumni's experiences from former institutions were also taken on board. The questionnaire included questions on whether universities consider STM and exploitation by lecturers or students to be a problem, how it manifests and what universities are doing about it to avoid compromising quality of outcomes.

Data collection and analysis

The study deployed the mail survey design to appeal to a target population stratified according to four strands: lecturers; current postgraduate students; administrators; and alumni of universities in order to get broad-based responses from all key stakeholders and interested parties. This method ensured limited time was effectively and efficiently used by the researcher whose mobility into the target population was limited, and hence would potentially affect the timelines of the research study outcomes.

A total of 30 semi-structured questionnaires eliciting responses to closed-and open-ended questions were distributed through electronic mail to potential respondents comprising alumni from universities in Bulawayo. Email addresses were obtained through cellphone and WhatsApp requests to alumni from universities in Zimbabwe known to the researcher through workplace and academic interactions. Thus, responses do not necessarily represent experiences at any particular university, but span respondents' entire tertiary university experiences.

Analysis

Pre-coded responses in which each response was allocated a value (e.g. '1 for male' and '2 for female'; '1 for yes' and '2 for no') were entered into the Statistical Package for Social Scientists (SPSS) program that was used to generate descriptive frequencies to highlight how many within each strata responded in a particular manner.

Results

The main results of the study alongside some of the key emergent themes are presented in this section. All 30 university stakeholders in Zimbabwe responded to the email questionnaires. Results of the study indicate the interactions between university education and STM as an academic fraud that threatens academic integrity. The results are presented as: respondents' demographic information; existence of STM in universities; prevalence of STM; the existence of sexual harassment policies in universities; methods lecturers employ to initiate inappropriate sexual relations with students; the extent to which lecturers initiate lecturer–student sexual relations; the transaction currency used by lecturers and students to initiate illicit sexual relations; and effects of STM on academic integrity.

Demographic information of respondents

Figure 1a depicts the gender disaggregation of respondents: 53% were women and 47% were men. All female respondents further alluded to having experienced sexual harassment of one form or another during their tenure at university. This finding concurs with others[26] that show 68% of female students have been subject to verbal or physical sexual harassment and that nearly one in four has experienced unwanted sexual contact.

Figure 1b shows the educational level of the respondents. Most (53%) respondents had a master's degree, followed by an undergraduate degree (30%), with only 13% holding a PhD and one (3%) a diploma. All female respondents stated that they had been exposed to STM at some point during their university education or employment. This finding gets expression in Gaba[29] and Imonikhe et al.[31] and in a landmark article by

Brandt[8] who suggest 50% of all women in the USA at some time or another experience some type of sexual harassment, either in the workplace or in their academic environment.

Figure 1c shows the age range of the respondents, with the highest proportion (37%) being 41–49 years of age; 30% of respondents were 26–30 years, 23% 31–40 years and 10% were 50 or more years old. STM manoeuvres are experienced with each year spent at university. Of the respondents, first degree alumni and administrators expressed exposure to STM in the first 5 years. It was found that the longer the time students and lecturers spend together, the higher the probability of lecturer–student relations sprouting. However, the time taken before female students were exposed to STM for postgraduates was much less. Reconciling the two points to calculated manoeuvres by the STM predators based on knowledge of their target's timeframe on campus. Figure 1d shows the respondent's duration at university – half were at university for less than 5 years.

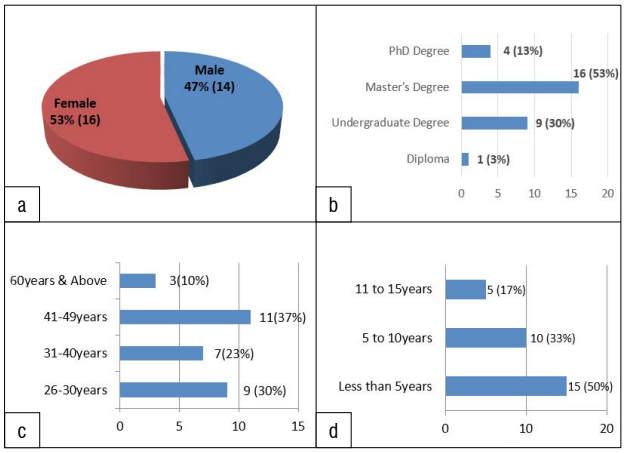

Figure 1: Respondents' (a) gender, (b) educational level, (c) age and (d) duration at university.

Existence and prevalence of sexually transmitted marks in universities

All respondents acknowledged the existence of STM in universities and agreed that STM is a concern among IHL and stands as an imminent threat to academic integrity, should it go unchecked.

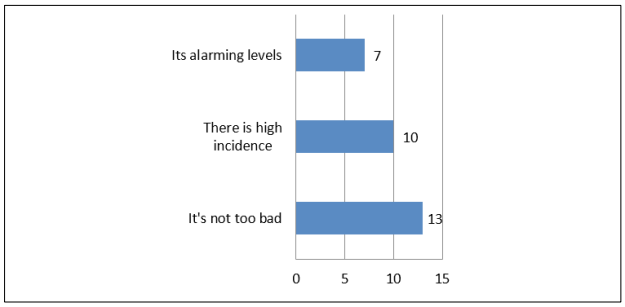

Figure 2: Respondents' perceptions on the prevalence of sexually transmitted marks.

Although 43% of respondents held the perception that the prevalence of STM was 'not too bad', 33% believed that 'there is a high incidence' and 23% lamented that STM were at 'alarming levels', albeit underreported. Reliance on volunteered data may in this case not be a reliable proxy for STM cases that go unreported for reasons of mutually accruing returns. Witherspoon et al.[9] posit that, while sexual harassment is not a new problem and has always been a reality of university life, many would like to pretend it is not happening, preferring instead to live in denial. Although they offered mixed views on the extent or prevalence of STM in Zimbabwe's IHLs, respondents were, however, unanimous that it exists in all IHLs.

Initiators of sexually transmitted marks in universities

Results shown in Figure 3 further show discordant views on the initiators of STM transactions, with lecturers and students fingered as circumstantial initiators of STM based on the strength of determining currencies of trade, which for the lecturer are the 'higher unearned marks' while for the student the currency of trade is 'sexual favours'.

On the extent to which lecturers initiate STM relations and transactions, the results show that lecturers and students have taken equal roles in initiating such transactions (Figure 4) although the literature has sought to portray the transaction as one of unequal standing on power terms.[10,11] Borrowing from the late former President Julius Nyerere of the Republic of Tanzania, no equal terms exist in the market-place and the same applies to the notion of STM.

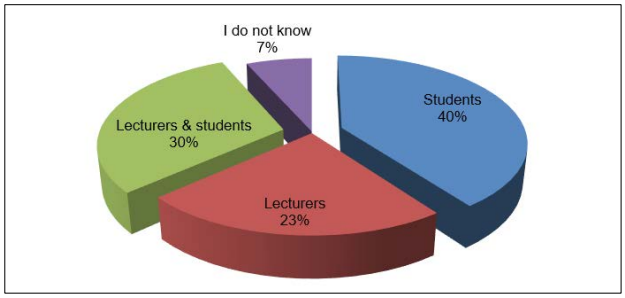

Figure 3: Respondents' perceptions on who initiates sexually transmitted marks: lecturer or student.

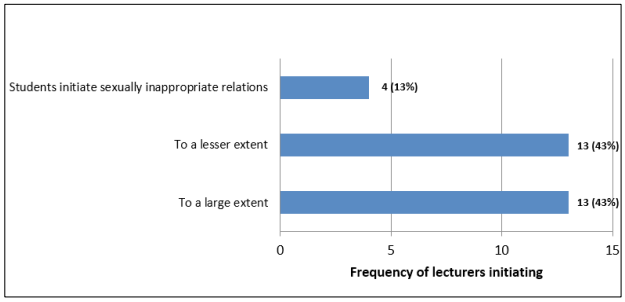

Figure 4: Extent to which lecturers initiate sexually transmitted marks according to respondents.

Existence of sexual harassment policies

The results in this study which show the existence of a belief that lecturers and students engaged in sexually inappropriate relations are in fact consenting adults in reciprocal relations have not been explored sufficiently.[10] Although policies on sexual harassment exist in all the IHLs from which respondents graduated, Figure 5 shows that the existence of these policies was largely unknown to many (60%) former students, although some may have graduated before such policies were put in place.

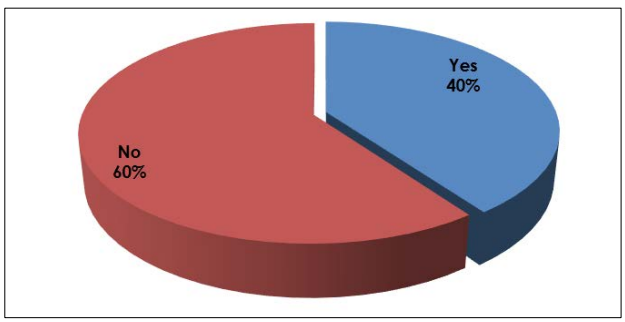

Figure 5: Percentage of respondents who indicated that their university had a sexual harassment policy and those who indicated 'no', despite all universities having such a policy.

Figure 6 shows that 87% of respondents believe that perpetrators were not punished and 13% stated that the punishment was not communicated. In the rights-based approach, in pursuit of social justice and fulfillment of human rights as well as the promotion thereof, the communication of punishment meted on perpetrators of sexual harassment (including STM) once caught (as is often the case, rather than reported), should be the means to an end to such cases, thus providing closure. On the part of justice, punishment should not only be carried out but should also be seen to be carried out to send a clear message to would-be perpetrators. Rights-holders should feel in control of their circumstances rather than feel vulnerable. Where sexual harassment policies were known to exist, respondents lamented the use of vague and abstract terms not understood by staff and students, making it difficult to enforce. Further, the policies tended to focus on staff–staff sexual relations and paid little if any attention to students as rights-holders and duty-bearers in their own right.

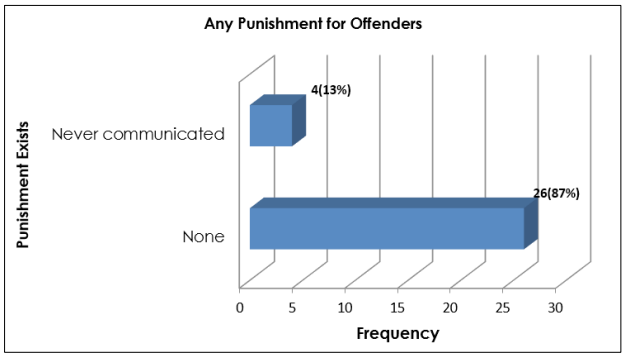

Figure 6: Respondents perceptions on whether offenders receive punishment and on whether the punishment is communicated to their victims.

Figure 6 also shows respondents' views regarding punishment meted out to STM offenders. Most (87%) respondents felt nothing was done to offenders despite exposés, hence STM perpetuates. However, others felt punishment was meted out but never communicated to the victim (13%). Neither scenarios help deter future incidents.

The key themes emergent from the text-based questions of the questionnaire were subjected to wordcloud generation. The resultant wordcloud (Figure 7) depicts two key themes: lecturers and students are equally responsible for STM transactions and thus hold similar responsibilities towards eradicating STM as both potential victims and initiators of such transactions.

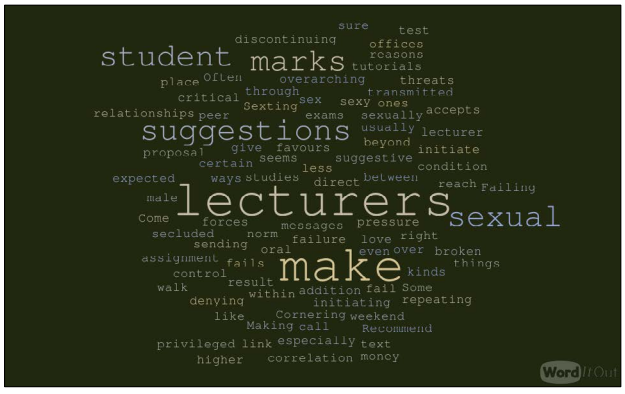

Figure 7: Word cloud generated from the responses, depicting two themes: 'Students make sexual suggestions to lecturers via 'sexting' for marks for fear of failing' or 'Lecturers make sexual suggestions to students in return for marks'.

Discussion

This study contributes to a budding discourse around STM as a growing form of dishonesty in IHLs. Results show that STM exists in all IHLs in this study in varying degrees and feeds off student vulnerability as lamented by the sociocultural model as a result of power imbalances between women and men in society. Garwe[32] cited major reasons given by students as drivers of STM as securing a place at university, awarding of undeserved marks, provision of financial and material support, as well as other favours.

Students cited that a lecturer would ask them to collect assignments from their office instead of returning them at class. Some students would even be victimised by being made to repeat courses if they failed to comply with the demands of the sexual advances of the staff member. Students denoted this practice as 'a thigh for a mark' or sex for grades.[31-33]

On rights-holders and duty-bearers regarding STM, both were found as equal actors of STM in IHLs. What determined who, between lecturers and students, would initiate was the currency value held by the one and the extent to which another one sought it. This factor underpins the notion of STM as 'transactional sex' whose currency of trade, 'sexual favours' and 'undeserved marks' fluctuates from time to time.

Staff members[32,33] argued, 'Some female students blatantly parade parts of their bodies by wearing skimpy clothes thereby exposing themselves to sexual harassment.' This view by staff on causes of STM echoes others[32,33] who lament the way female students conduct themselves in terms of behaviour and dressing as influencing their vulnerability to sexual harassment. Rights-based approaches discount sociocultural model perspectives, arguing that such views are irresponsible as every person is responsible for their actions or inactions regardless of the actions of another.

Informed by a rights-based approach core focus area, the study agitates for publicisation of policies and regulations on sexual harassment in IHL. Policies would ensure IHLs respect, protect and fulfil the human rights of lecturers, administrators and students by prohibiting all sexual relations between staff and students in the same educational institution. Strong sentiments emerged from the study: lecturers must be dismissed if found guilty of a sexual relationship with a student at the IHL at which they are employed.

The dismissal should be mandatory, regardless of whether there was consent. Such zero-tolerance policies are consistent with many laws that criminalise adults' sexual relationships based on unequal power. Scholars like Stark[10] believe that whether or not university staff are allowed to be in sexual relationships with their students is central to STM. Questions have been raised whether such liaisons between lecturers and students should be viewed as an inevitable result of mature adults meeting and working together or as examples of misuse of power by academics or students? Whatever the stance academics take, what is clear is that questions on salacious relations continue to attract interest and controversy.

Findings: Manifestations of indecent sexual relations against integrity

Qualitative responses from respondents regarding STM effects on academic integrity are shared below:

- *Lecturers offer good marks to targeted female students in return for sexual favours. The transaction is preceded by 'sexting' to elicit response as confirmation the lecturer is a willing partner in the academic crime.*
- *Lecturers give students tough assignments and timelines with personal preferences while students, for fear of failure, seek personal assistance with given tasks.*
- *Lecturers award female students more marks than deserved against written assignments.*
- *Lecturers are cornered with writing academic tasks for their partner-female students, earning them higher marks than they would earn thereby.*
- *Lecturers give tips to female students but withhold such assistance from other students.*

- *Lecturers negotiate with other lecturers for higher marks on behalf of their STM trading partner (female student), hence STM goes undetected. The lecturer returns the favour for their counterpart to avoid detection.*
- *Rights-holders deliberately invoke their right to initiate relations whereby females deliberately sit 'strategically badly' in class exposing themselves to seduce lecturers into relationships. However, the rights-based approach contends rights-holders cannot willfully allow their rights to be violated.*
- *As rights-holders, female students lure lecturers by 'sexting' them, that is, texting sexually suggestive text on WhatsApp and cellphone messages.*
- *Students visit lecturers in their offices outside normal hours of work, seduce them into sexual contact or sometimes outrightly negotiate sexually transmitted marks as consenting adults.*
- *University policies on sexual harassment are barely known, do not target lecturer–student sexual relations, which go undetected.*

Conclusion

Sexually transmitted marks exist and are pervasive among IHLs in Zimbabwe, albeit in intelligent ways such as 'wilful submission'. This is otherwise best described as subtle 'sexual coercion' and more aggressive uncamouflaged forms.

Sexual harassment policies of IHLs have tended to exclude students as duty-bearers, blindly treating them as only rights-holders. The literature supposes female students are potential STM victims, drawing from skewed patriarchal power imbalances without supposing they could potentially be perpetrators of STM in a transaction-based sexual relationship that easily disempowers male lecturers. However what determines who initiates STM is the strength of the currency of trade on the STM 'market', true to Julius Nyerere's assertions that:

'We are the only people who buy from the prices determined by the seller and sell from prices determined by the buyer.' This means 'no equal terms exist on the market place'.

Although the study found that lecturers and students are equally responsible for STM as perpetrators and victims, the rights-based approach highlights the role subtle and overt power plays in securing coercion that falsely 'presents' students as responsible for STM activities. When students' rights are respected, protected and promoted, the sociocultural dimensions of masculine power vanishes. Thus, even the socioculturally acceptable sexual relations would remain a violation under the right-based approach due to unequal power between the parties.

The spread of STM within IHLs is facilitated by current students socially learning STM behaviours from their lecturers. Alumni's exposure to STM during their learning experiences shape the manner in which they treat their students once employed in IHLs, with an inclination to practice the same STM.

Recommendations

- Duty-bearers like the Government of Zimbabwe; the Ministry of Higher and Tertiary Education, Science and Technology Development; and the Zimbabwe Council for Higher Education, must enact explicit STM targeted policies and publicise these policies to minimise STM practices in IHLs.
- IHLs must ensure student orientation programmes tackle this sensitive subject by lifting the so-called 'sensitive subject' veil and allowing open debate about it.
- IHLs must monitor staff through CCTV cameras in offices for the protection of both lecturers and students in case of allegations. Lecturers get to refute allegations while students can reinforce allegations using the same CCTV footage as the case may be. However, all legal and operational procedures for CCTV would need to be observed before such an approach is taken to respect, protect and promote the rights of actors, espousing both perpetrators and victims.
- IHLs across Africa can, and should, provide for ethical conduct and professionalisation through registering academics so as to de-register and blacklist those guilty of STM malpractices. Known offenders should never be employed again at IHL.

Relevance to other countries

A number of countries have increasingly raised concerns over compromised academic standards and quality. They highlight academic fraud including examination cheating and falsification of research data and findings to suit predetermined outcomes. This fraud includes STM practice that permeates across cultures, geography and development status. Exposure of the manifestations and effects of STM will induce IHLs to halt and reverse dishonesty concerns that do not discriminate by race, income, size or stature of university.

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Quality assurance agencies: Creating a conducive environment for academic integrity

Academic integrity is a key measure of the quality, efficiency and competitiveness of higher education systems. This article explores how a quality assurance agency can foster a conducive environment for academic quality and integrity. A self-study methodology was used, with a focus on the insights and experiences of the Zimbabwe Council for Higher Education over a 10-year period. The findings show that by assuming an innovative and transformational leadership role in instilling a culture of self-evaluation, as well as maintaining its own integrity, an external quality assurance agency can improve academic integrity. The article adds value to the existing knowledge by advancing the higher education ecosystem approach as an integrity-based panacea and conducive way to induce integrity to flow from all players as opposed to the use of heavy-handed regulatory approaches.

Significance:

- This article highlights the importance of academic integrity and situates quality assurance agencies as playing a central role in fostering academic integrity.

Introduction

Academic integrity refers to the adherence to a code of values and ideals (ethical standards) that inform the behaviour and conduct generally understood and accepted worldwide.[1,2] This code of practice demonstrates 'a commitment, even in the face of adversity, to six fundamental values: honesty, trust, fairness, respect, responsibility, and courage'[3]. It is a universal trust-bearing measure of the quality of academic and professional practices (teaching, learning, assessment, evaluation, research and community service) by individuals, groups or institutions within higher education systems.[4,5] Accordingly, the achievement of academic integrity is a critical goal that every higher education system aspires to reach, to be part of the national and global communities of integrity.

Breaches of academic integrity through engaging in behaviour and practices that are not in keeping with expectations is referred to as dishonesty, misdemeanor, fraud or corruption.[6] Denisova-Schmidt[7] highlights the global challenge of dealing with the increasing incidences of 'integrity deficiencies' that undermine the trust placed in the outcomes of higher education. In the globalised world, mobility of students and workers requires recognition of their qualifications. Lack of academic integrity at individual, institutional or national level poses a significant threat to public safety in cases in which graduates have not genuinely acquired the required competencies.[7] A case in point is that of professional courses (health and engineering) as well as programmes with economic bearing e.g. accounting and banking.

In order to uphold quality and standards, all players are collectively responsible for continuously scanning the environment to prevent, identify and rid academia of corruption.[6-8] Although several approaches to addressing academic dishonesty have been suggested,[2,9] it is generally accepted that the problem persists.

Over the last two decades, over 100 countries have established external agencies to assure quality in higher education.[10] The thesis here is that higher levels of academic quality and integrity prevent and reduce academic dishonesty. Although these agencies operate within varied contexts and apply different quality assurance mechanisms, accreditation and quality audits are the most effective and widely used methods of preventing systemic academic malpractices.

This article explores how a national quality assurance agency, the Zimbabwe Council for Higher Education (ZIMCHE), improved academic integrity in Zimbabwe, a country whose high quality education[11] contrasts with high levels of corruption in the wider society[12,13] thus posing an enigma. The case study approach is premised on using the widely recognised method of concentrating on a context/locality and generalising therefrom.

Contextualising academic integrity in Zimbabwe

Zimbabwe is a medium-sized country which gained independence from Britain on 18 April 1980. The country takes pride in its relatively well-established higher education system that spans over 60 years. The first higher education institution was the University College of Rhodesia and Nyasaland, established in 1955, in an affiliate relationship with the University of London.[14] The new government, upon gaining independence, introduced aggressive policy reforms focusing on curriculum review, inclusivity, planning and efficiency, quality and relevance. Some publications[11] position Zimbabwe as the best-educated country in Africa, with literacy levels in excess of 94%.

Zimbabwe experienced a rapid expansion of higher education characterised by increasing student enrolments and new state and public institutions between 1998 and 2005. This expansion was not supported with proportionate infrastructural, human, material and financial resources necessary to maintain the original high-quality standards. This is largely explained by the fact that the country experienced economic decline during the same period, which resulted in a brain drain of highly qualified and experienced academics and other professionals.

In its quest to safeguard quality, the country established ZIMCHE in 2006, through an Act of Parliament, to regulate and promote quality in higher education.[15] ZIMCHE developed a quality assurance framework to guide institutions to achieve ethical, legal and professional standards. ZIMCHE has recently undertaken a curriculum overhaul in line with the concept of University 5.0 introduced by the Minister of Higher and Tertiary Education, Science and Technology Development, Honourable Professor Amon Murwira. Two pillars of the university mandate (innovation and industrialisation/commercialisation) were added to teaching, research and community service. This move positioned higher education to contribute effectively to the national vision of achieving an upper-middle income status by 2030.

Corruption was reported to be the major cause of both Zimbabwe's economic downturn and the persistent failure to resolve the problem.[12,13] These authors[12,13] used the Corruption Perception Index wherein Zimbabwe featured at position 154 out of 175 most corrupt nations in the world to premise their proposition that economic improvement in the country will only commence after serious and concerted efforts to root out corruption.

Studies focusing on academic dishonesty affirmed the existence of dishonest tendencies by students, staff and management.[16-18] Media reports revealed cases in which some universities awarded unmerited degrees to public figures through coercion, or voluntarily in search of favours. A case in point is the award of a doctorate to a former first lady by a reputable university in Zimbabwe. Some unregulated non-higher education institutions also sell 'honorary' doctorates and professorships to public figures – an activity that is legally a preserve of registered and accredited higher education institutions.

Cognisant of the corruption-infested national context, its global manifestation and its consequences for higher education and the wider society, ZIMCHE has played a key role in fostering academic integrity through quality assurance.

Literature review

Academic integrity breaches

There exist different kinds of integrity breaches which negatively impact quality, effectiveness and efficiency and the sanctity of higher education.[19,20] Academic integrity breaches are complex in that all players in the higher education ecosystem are potential perpetrators.[8] In higher education institutions, students (both at undergraduate and postgraduate levels), academic and support staff, management as well as the governing council are prone to academic dishonesty. These breaches can occur during student admissions, staff recruitment, grading, promotion, teaching, supervision, assessment, research, reporting, publication and qualification award. Examples of some of the common breaches are discussed below.

Flawed student admission, staff recruitment, grading and promotion practices

Fraudulent student admissions arise due to competition for places in high-demand programmes that are perceived to be prestigious (e.g. law and medicine). Staff are either offered or demand bribes in order to circumvent the process and admit certain students ahead of others.[21] The issues of merit do not apply here because all students will be qualified but competing for limited places. Management can sometimes abuse power and appoint or promote staff members on the basis of ethnicity, gender, personal connections, family relationships, bribery or extortion.[7,22] Recruitment and promotion can also be done on the basis of misrepresented qualifications, academic achievements, as well as leadership experience.[23,24] This form of misrepresentation is usually done by padding resumes with exaggerated accomplishments and claims of fake qualifications including those obtained from unrecognised institutions.

Grade inflation or compression

Inflating or compressing grades happens when assessors award marks to increase or decrease grades inconsistent with the student's deserved grade.[25] In addition to monetary incentives, grade inflation or compression is sometimes motivated by sexual favours. In other instances, administrative staff put pressure on academics to inflate grades for the benefit of institutional reputation. This is usually motivated by the need to get higher appropriations where institutions are funded on the basis of student throughput.[25] Where students are offered merit-based scholarships, academics are inclined to give students higher grades to avoid students dropping out because they lose their scholarship, which would result in the institution losing tuition income.

Fabrication of research findings or falsification of reports

Fabrication usually occurs when research findings fail to conform to the student's or academic's preferred theory or framework. Data are then crouched or manipulated to suit the desired outcome instead of using the real data to craft new theories or create new knowledge. Academic supervisors can sometimes alter and publish the work done by students without due acknowledgement. At times academics can pay research assistants to collect data, undertake literature reviews and draft reports, which they simply spruce-up and publish as sole author.[26]

Plagiarism

Plagiarism involves academics or students copying other people's work (e.g. ideas, wording, approaches, artworks or inventions) with or without modification and without due acknowledgement.[22] Plagiarism occurs in different forms inclusive of:

- **Cyber-plagiarism, essay mills or contract cheating**, wherein known or unknown (ghostwriters) third parties are contracted to undertake assignments or research on behalf of a student, staff member or contractor either physically or online.[27]
- **Self-plagiarism** involving recycling one's own work and presenting it as new.[28]
- **Mosaic plagiarism** where synonyms are used to replace words used in the original article whilst maintaining the same ideas.[29]
- **Bureaucratic plagiarism** involving abuse of power by superiors who take ownership of work assigned and done by juniors in their day-to-day work, for example reports, grant proposals, PowerPoint presentations or speeches. The superior at times acknowledges the originators but takes the limelight with little or no contribution.[30] It is important to note that in some cultures, bureaucratic plagiarism is considered 'business as usual' as it is consistent with institutional and cultural norms.[31]

Collusion

There are still grey areas regarding the point at which collaboration becomes collusion, given that collaboration is encouraged and celebrated in academia whereas collusion is condemned.[32] The confusion results from varied understandings and practices deemed appropriate regarding assessment of students in different disciplines and contexts. Collusion captures the possibility that arises when academics or students get material and ideas from unattributed sources that are not Internet-based and hence difficult to detect using electronic anti-plagiarism software, for example interactions with other students, academics or professionals.[33] Collusion also occurs when students collaborate with peers on a piece of assessed work meant to be undertaken as an individual task. The group work is then customised to avoid detection. Another form of collusion is when a student or academic avails a completed assignment to another for money or other favours.

Academic integrity breaches in quality assurance agencies

Some quality assurance agencies accredit programmes/institutions fraudulently in return for bribes or favours.[34] There are also fake quality assurance agencies that operate as accreditation mills.[35] False audit or evaluation reports resulting from conflict of interest and bribery by peer reviewers, agency staff and board members are also common.[34] Bribing or threatening (as in the case of threats by political figures or other high-ranking officials) individuals constituting accrediting panels forces or motivates them to by-pass certain criteria and produce reports in favour of the department or give the programme or institution undue advantage.

Quality assurance agencies can also plagiarise instruments and standards designed by sister agencies from other countries. In addition, peer reviewers who are engaged by quality assurance agencies have been reported to re-use the templates that they have used before in their reports (self-plagiarism). Incidents of collusion have also been reported, wherein board members, staff and peer reviewers work in cahoots to influence decisions that would otherwise not have been made if rigour was maintained.[34]

Situating quality assurance agencies in academic integrity

Quality assurance agencies provide leadership in developing and maintaining a framework to guide institutions to achieve academic quality

and integrity in all aspects of the university mandate. Leadership is defined as the ability to inspire, support and motivate others to achieve set goals.[35] From an institutional perspective, leadership is the capacity to energise, coordinate and synergise all players towards effective goal attainment.[36] Davenport and Volpel[37] suggest that today's leadership should coordinate communities in their mandate areas, create user-friendly cultures and fend off bureaucracy.

Quality assurance frameworks embed academic integrity in the standards for programme/institutional accreditation and audit/review.[38] Institutions are required to detail the initiatives undertaken to maintain and improve academic integrity in their self-evaluation reports.[37] These claims are then validated by the accreditation and audit teams during the mandatory site visits. Placing academic integrity in the spotlight in this manner motivates higher education institutions to prioritise and actively inculcate a culture of academic integrity.[38]

Many quality assurance agencies use the philosophy of zero tolerance[39] involving use of heavy-handed approaches (e.g. legal, software and structures) to discourage, accost and discipline those who commit academic misdemeanors.[40] This approach is premised on the assumed opportunistic tendencies of human beings who largely behave according to their self-interests in order to optimise their own utility, ignoring the potential conflict of interest with their assigned duties.[41] This approach of putting emphasis on detection and sanctions to achieve academic integrity as opposed to awareness, integration and promotion of desired behaviours is fraught with many challenges.[42] To begin with, it focuses on inputs and process; some agencies spend a fortune on surveillance and oversight mechanisms rather than on productive and progressive work.[43] Furthermore, institutions incur additional costs to prove compliance to standards.[44]

Approaches that are inclusive, goal and improvement-oriented influence the choice of human behaviour.[45] An inclusive environment, in which every player is valued, inculcates a sense of belonging and a quest to contribute positively to set goals. The nature of the mentor–mentee relationship influences the awareness and acceptance of standards.[46] Students, staff and institutions acquire habits in their interactions with faculty, management and agencies through capacity building and exemplary conduct.[47] Thus the positive approach to academic integrity[48] produces better results and demands that all players play their role in encouraging good conduct through leading by example and exhibiting academic integrity at the individual level.

Higher education ecosystem

Systems theories (general, ecological, life-model, and ecosystems) embrace mutual relationships amongst elements that are part of a whole. The study adopts the ecosystem approach, a concept that has diversified from botany[49] to wider application in education and other disciplines[50,51]. Ecosystems are functional and coordinated entities characterised by dynamic bilateral and multilateral connectivity, interdependence and interaction of different players (living and non-living) for survival and growth within a specific environment. A higher education ecosystem (Figure 1) is a self-sustained, self-regulating system of players united by shared goals and mutual interdependence based on a value co-creation approach.[52]

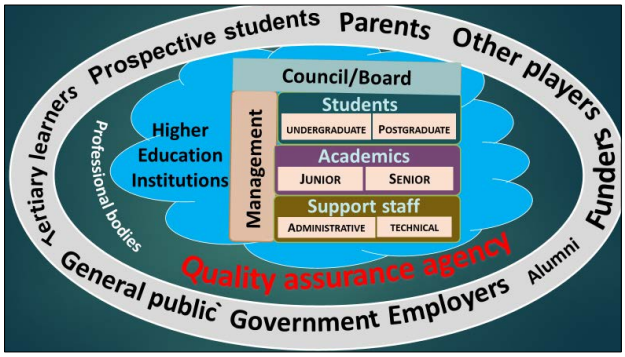


Figure 1: The higher education ecosystem.

The players in the higher education ecosystem include quality assurance agencies, professional bodies, parents, general public, alumni, prospective students, funders and higher education institutions. Higher education institutions form a sub-system within the larger ecosystem which includes the university council/board, management, academics, students and support staff.[53] The non-living components of this ecosystem that direct the ways the human players behave and interact[51] comprise physical and material resources, policies, systems and procedures, organisational cultures, leadership styles and strategies[19].

An effective ecosystem requires the cooperation of all players and the awareness of each other's presence and contributions.[46] Although quality assurance agencies coordinate and regularly monitor and evaluate results of individual and collective actions of players, it is the effective interaction of all players that is responsible for achievement of goals. Unprogressive attitudes, lack of professionalism and disagreement of players in an ecosystem disrupts the smooth flow of activities and results in pollution of the whole system.[19] For example, if issues of academic integrity are not well managed by institutions or agencies, the whole system will become polluted. In other words, the integrity of quality assurance agencies is integral to quality higher education systems; in the same vein, no agency can rise above the quality of its institutions – effective collaboration reinforces and safeguards academic integrity.

The success and reputation of institutions depend on the quality of their graduates; hence they have an intrinsic stake in upholding academic integrity. Quality assurance agencies should work together with institutions to develop strategies to maintain academic integrity. This calls for a positive approach wherein integrity is embedded in the self (both at individual, institutional and sectoral level) as opposed to viewing it from a negative perspective.[54] This approach is premised on the stewardship theory which argues that selflessness and pro-social behaviours promote collectivism as opposed to individualism. Hence the interests of agencies are aligned to those of institutions and all other players in the higher education ecosystem.[55]

Purpose of study

The study was aimed at examining the role of a quality assurance agency in providing leadership in academic quality and integrity. Specifically, the study sought to answer the following questions:

1. What challenges does ZIMCHE face in rallying Zimbabwean universities around issues of quality and academic integrity?
2. How does ZIMCHE assure quality and academic integrity in Zimbabwe?
3. What lessons and good practices can be drawn from ZIMCHE's approach to academic integrity?

Methodology

Originating from the teaching practice, the self-study methodology (intimate scholarship) has gained foothold in all disciplines as an important approach to informing and transforming practice through leveraging personal and institutional experiences.[56] This methodology is premised on the self-study theory which propounds continual reflection, critical examination, communication and comparison of personal and institutional activities, strategies and experience with the literature and development of innovative and effective interventions, in contrast to pursuing practices that are premised on tradition, habit or impulse.[57] Although often criticised on the basis of bias and an assumed lack of objectivity, the self-introspection of the distant and immediate past as well as current experiences to interrogate and identify useful insights for improvement engenders trustworthiness and transparency.[58,59] The edge of the methodology over alternatives derives from its improvement-orientation, interactivity and comparability with similar situations.[57]

ZIMCHE and the Zimbabwean higher education ecosystem were used as the institutional self and the ecological self, respectively. ZIMCHE started its operations in 2009 and hence has rich experiences spanning over 10 years. Using the five-step self-study guidelines recommended by Samaras and Roberts[57], the author worked with colleagues within and

outside ZIMCHE to brainstorm, interrogate, critique and obtain feedback regarding the three research questions identified for the study. The five steps were adapted as follows:

- **Step 1:** The study questions were designed due to their relevance to the improvement of academic quality and integrity. The questions were generated from observations, experiences and relevance to professional growth and quality improvement.
- **Step 2:** Sessions were held to brainstorm, interrogate, critique and obtain feedback from colleagues responsible for registration, accreditation, audit and compliance monitoring in ZIMCHE, quality assurance directors, registrars and academic deans, peer reviewers, ministry of higher education and professional bodies. The author held these sessions during events occurring between December 2017 to November 2018. In this way, it was possible to obtain insights and perspectives to ascertain concrete and valuable information to respond to the study questions.
- **Step 3:** Using the information collected, areas of good and bad practices on how to improve quality and academic integrity were identified.
- **Step 4:** The author packaged the study and presented the findings at a quality promotion conference on academic integrity.
- **Step 5:** After further refining the insights following dialogue and comments from colleagues at the conference, the final stage was to document the reflections, insights and recommendations for promoting academic integrity for publication and dissemination to the wider academic audience for adaptation and further improvement.

The findings are presented according to the responses to the first two research questions regarding the ZIMCHE challenges and approaches to quality and academic integrity. The discussion section deals with Question 3 on lessons and good practices derived from ZIMCHE's approach to academic quality and integrity.

Findings

Challenges

In pursuit of quality, ZIMCHE is expected to promote and protect academic quality and integrity by creating a conducive environment based on good governance, best practice and capacity development. The challenges faced by ZIMCHE in pursuit of this cause relate to: academic staff grading and promotion; autonomy of institutions; interpretation of quality assurance tools, policies, and standards; lengthy processes and procedures; existence of multiple regulatory bodies; and conflicts of interest.

Academic staff grading and promotion

In order to correct the existence of disparate criteria for academic staff grading and promotion, ZIMCHE harmonised these guidelines across the 20 registered universities in Zimbabwe. This standardisation applied pressure on academics to publish or perish. Whilst institutions reserve the right to establish promotion criteria with respect to teaching and community service, the ZIMCHE instrument harmonised issues to do with the quantum of research outputs. This puts pressure on academics to 'publish or perish' to such an extent that some may engage in academic integrity breaches inclusive of: publishing articles in low quality ('predatory') journals; manipulating research results; forming authorship cartels; making use of ghostwriters; or publishing on the basis of plagiarising work done by students or other sources.

Autonomy

Higher education institutions in Zimbabwe are autonomous institutions governed by an Act of Parliament for public higher education institutions and by a charter for private ones. As such, the perception within higher education institutions is that the state or state agencies ought not to interfere with the affairs of institutions. They argue that, for quality to prevail, academic freedom should be respected. However, for academic integrity to prevail, total autonomy is only achievable through interdependence of all players in the ecosystem. Through transparency, collaboration and engagement, trust and respect are born. Internal and external quality assurance complement each other.

Interpretation, lengthy processes and existence of multiple regulatory bodies

Many institutions report that quality assurance policies, standards, tools, and procedures are complex and difficult to interpret, which results in misunderstandings and varied interpretations and implementation. This creates a need for awareness and extensive capacity building which is resource intensive and costly. The time spent by institutions on preparing accreditation documents and self-evaluation reports is substantial, and therefore diminishes the cost:benefit ratio.

Zimbabwe has witnessed a marked increase in regulatory bodies that require compliance from different angles (academic and professional). These regulatory bodies often work in an uncoordinated fashion, thereby frustrating higher education institutions' effort. Incidents in which ZIMCHE approve degrees and professional bodies disown them and the graduates thereof were reported. An example given was that of medical students who were disowned by the relevant professional body when they had completed 4 years of study and were only left with the final year before housemanship. All but one managed to successfully complete their studies in neighbouring countries. In addition, there are additional costs associated with preparing documents and arranging visits for these regulatory bodies.

Conflicts of interest

A conflict of interest exists when one's private interests are divergent with academic or professional obligations. Experiences revealed that in cases where one has overlapping responsibilities, for example academics who serve as peer reviewers and Vice Chancellors who serve in the ZIMCHE Board, the intertwining of responsibilities poses a threat to academic integrity. There were cases where some ZIMCHE staff revealed that they faced potential compromise in their actions towards certain institutions because of the intentions of securing post-contract or post-retirement jobs at that institution. A conflict of interest may relate to anticipated material gain or loss and can also relate to non-monetary benefits relating to improvements in professional and personal status or access to facilities or classified information.

Assuring quality and academic integrity in Zimbabwe

ZIMCHE positioned itself to support the Ministry of Higher and Tertiary Education Science and Technology Development deliver an integrated higher education system that brings about convergence, transparency, comparability and consistency. The leadership was achieved through inspiring all players in the higher education system; setting standards; modelling the way; collaborating and capacitating higher education institutions as well as through self-evaluation and continuous improvement.

Inspiration

Considering the potential challenges facing ZIMCHE in its pursuit of quality and taking cognisance of this difficult and important mission, there is need for inspiration. ZIMCHE derived its inspiration and motivation from the works of Antoine de Saint-Exupéry[60], described in his book entitled *The Wisdom of the Sands*:

> *If you want to build a ship, don't drum up the men to gather wood, divide the work and give orders. Instead, teach them to yearn for the vast and endless sea.*

Thus, extrapolating from the inspiring statement in the context of providing leadership in quality assurance and academic integrity, ZIMCHE's conviction is that:

> *If you want to build academic integrity, avoid bureaucracy, straightjacketing, stifling innovation and excessive sanctions. Instead, inspire and capacitate all higher education players to yearn for communities of integrity.*

During its quality assurance missions, staff from ZIMCHE inspire individuals and institutions using the famous quote from Alan Simpson:

> *If you have integrity, nothing else matters.*
>
> *If you don't have integrity, nothing else matters.*

Setting standards

The quality assurance framework for ZIMCHE is centred around the processes of registration, accreditation, audits and compliance monitoring. In all these processes, ZIMCHE has embedded the elements of academic integrity by developing support systems, policies, standards and procedures to guide institutions.

ZIMCHE works in close collaboration with relevant academic and professional higher education players to come up with 'agreed' standards of quality assurance in areas of operation and practice. The term 'agreed' reflects the involvement and endorsement of the standards by the key players and the fact that institutions are given these standards and use them for self-evaluation during institutional (internal) quality assurance processes. The standards relate to issues of governance, leadership, academic and support staff, academic grading and promotion, infrastructure, equipment, teaching and learning facilities, minimum bodies of knowledge for each programme, ICT and bandwidth, research, student admission, student assessment, student support, and self-evaluation, among others.

Accreditation is the seal of approval by the external quality assurance agency to assure the public that the higher education institution or programme meets the 'agreed' quality standards and thus can be trusted. The accreditation process involves the use of experts and peers who benchmark with the best practices globally. This makes the process transparent as well as promotes transparency in higher education institutions. Accreditation therefore serves as an effective way of measuring and promoting academic integrity, thereby curbing academic misdemeanors in higher education institutions.

Modelling the way

In modelling the way, ZIMCHE created platforms for information sharing, recognised and rewarded best practices as well as encouraged continuous quality improvement. The voices and experiences, financial, material, intellectual and moral support of colleagues, experts, peer reviewers and partners helped the platforms to be vibrant and productive. ZIMCHE and the Ministry of Higher and Tertiary Education, Science and Technology Development created an annual platform for information sharing and recognising best practices by individuals and institutions (in all areas of the university mandate) in 2009. This platform was coined the Research and Intellectual Outputs, Science and Technology Development (RIOSET) Expo. Different themes were selected every year, to embrace the prevailing, critical and emerging national imperatives. To showcase the importance of the event, the Expo was graced by its patron, the President of the Republic of Zimbabwe, who delivered the distinguished lecture. In the spirit of sharing and benchmarking, world-renowned academics and professionals also presented and exhibited. Every stakeholder in the higher education fraternity looked forward to RIOSET.

Collaborative and collective approach to engaging all players

ZIMCHE engages all stakeholders and enhances their capacity in academic integrity and other quality assurance matters through running relevant seminars, workshops and conferences aimed at capacity building and discussing pertinent issues. ZIMCHE also guides dialogue through online and physical communication platforms. Through creating opportunities and providing multi-layered support to all stakeholders, ZIMCHE aims to engender a culture of shared responsibility and obligations to academic integrity. Teams hold focus group and targeted discussions with students, academics and management to engage on issues of welfare or any other matter that can affect the quality of the higher education experience. Efforts are made to make representations and find ways of addressing the areas of contention. In addition, ZIMCHE is open to receive complaints, grievances and suggestions on deviant behaviour and on how to address emerging challenges. ZIMCHE, through interactions will all stakeholders, has created an effective ecosystem in which all players work together seamlessly.

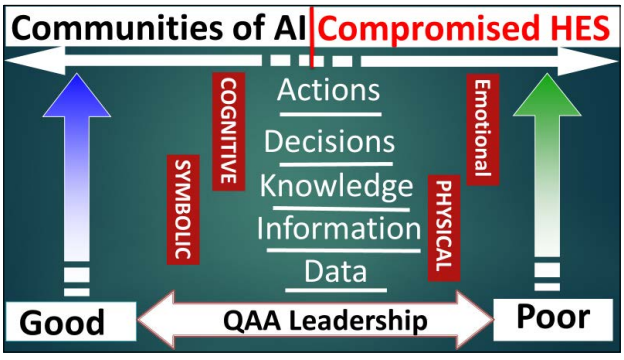
Self-evaluation and changing the approach to academic integrity leadership

By way of challenging the process, in 2018 ZIMCHE reviewed its approach to academic integrity leadership through introspection as well as gathering feedback from stakeholders over the 9 years that it had been in existence. ZIMCHE, with support from the African Union, African Quality Assurance Network and the European Union (under the auspices of the Harmonisation of African Higher Education Quality Assurance and Accreditation project), subjected itself to external assessment. The external review, undertaken by international experts who assessed the performance of ZIMCHE as a quality assurance agency, presented a good yardstick to measure performance against best practices in Africa and beyond. The process involved preparation of a self-assessment report by ZIMCHE, interviews of ZIMCHE Board and Secretariat as well as vice chancellors, chairpersons of university councils, academics, peer reviewers, students and indeed all stakeholders.

Regarding academic integrity, the findings showed that the approach that had been in use was largely effective in curtailing incidents of academic dishonesty through accreditation, audits, compliance visits and qualification assessments. All institutions had been requested to establish institutional quality assurance units manned by a Director who would act as the 'local ZIMCHE', and be responsible for ensuring institutional compliance with ZIMCHE standards. Technologies such as anti-plagiarism software became mandatory for all postgraduate and research work. However, stakeholders indicated that the approach was too intrusive, impersonal and sometimes outrightly coercive due to the compliance-driven and rule-based nature of the approach. It therefore became difficult to use it as a basis of developing a culture of academic integrity due to the perception that this approach violates academic freedom and autonomy.

ZIMCHE, being a listening agency, decided to move from the compliance-based approach towards an integrity-based approach. The new approach is premised on remediation and education and is deemed respectful and never shame-based. The approach tries to avoid homogeneity which stifles innovation as well as to avoid bureaucracy, delays or straitjacketing. This approach is hoped to create a culture of continuous self-evaluation at individual and institutional level. The results of these exciting developments are yet to be evaluated. Watch this space!

As ZIMCHE undertakes these activities, there is an overwhelming response from stakeholders that it is exhibiting good leadership which improves both quality and academic integrity as illustrated in Figure 2.



AI, academic integrity; HES, higher education system

Figure 2: Continuum and effects of quality assurance agency (QAA) leadership.

Discussion

The challenges of conflicts of interest by members of ZIMCHE Secretariat and Board that might compromise the decisions during quality assurance undertakings are consistent with the challenges reported in existing

literature.[33] ZIMCHE was, however, able to circumvent their occurrence by taking a leadership role in promoting academic quality and integrity through the ecosystem approach. By setting 'agreed' quality standards collaboratively with all stakeholders and evaluating institutions with the involvement of the internal members, peers and relevant professional bodies, the processes are transparent, and the achievement of trust was made possible. The evaluation processes of registration, accreditation and audits went a long way in promoting academic integrity in line with the assertion by Mckenzie[37]. This collaboration and engagement created an ecosystem in which all stakeholders are aware of each other's presence, needs, contributions and expectations in sync with similar research results.[46] The events and fora for capacity building, dialogue and exposition of good practices by individuals, institutions and stakeholders created vibrant platforms for information sharing integration and promotion of desired behaviours, as expounded in literature.[41,42,46]

The leadership role taken by ZIMCHE in inspiring and supporting institutions through establishment of institutional quality assurance units was developmental and geared at achieving set goals for academic quality and integrity, as suggested in other studies.[34] Engagement of students and staff in institutions, and all stakeholders in various capacities, demonstrated ZIMCHE's capacity to energise, coordinate and synergise all players towards effective goal attainment, as reported in the literature.[35,36]

The move taken by ZIMCHE to self-introspect and submit its activities for scrutiny by external assessors and stakeholders presents another example of exemplary leadership. It thus becomes possible for institutions to acquire good habits that promote transparency and academic integrity as reported in the literature.[47] The fact that ZIMCHE – in spite of already being in the right direction through employing the ecosystem approach – was ready to change its approach in line with review recommendations and literature,[48] poises the higher education system to achieve greater academic integrity levels. This is so, despite the assertion that corruption in the wider society will necessarily induce academic dishonesty.

Conclusion

This article highlights the importance of academic integrity and situates quality assurance agencies to play a central role in fostering academic integrity. The case of ZIMCHE showcases how the organisation led by example and assumed an innovative and transformational leadership role in fostering academic integrity through use of the higher education ecosystem approach. Through self-evaluation and incorporating voices of stakeholders, ZIMCHE was able to change its approach from one that relied heavily on compliance, to one that showed greater potential of cultivating a culture of academic integrity. In view of the new higher-level integrity-based approach, there is need to track and evaluate ZIMCHE's progress in this trajectory to academic integrity.

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Antibiotic sensitivity of bacteria isolated from the oral cavities of live white sharks (*Carcharodon carcharias*) in South African waters

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The white shark (*Carcharodon carcharias*) is responsible for 49% of shark-related injuries in South Africa, yet no information currently exists on the composition or antibiotic resistance of bacteria hosted by these apex predators in South African waters. This study aimed to address this gap by sampling the bacteria present in the oral cavities of 28 live *C. carcharias* along South Africa's southern coastline. The antibiotic resistance of the range of microbiota was also assessed using antibiotic disc diffusion tests. A total of 51 strains from at least 20 species of bacteria were isolated from the oral cavities of *C. carcharias*. Of these strains, the most common bacteria present were *Serratia* spp., *Proteus vulgaris* and *Vibrio alginolyticus*. The overall antibiotic resistance was relatively higher in this study than that reported for bacterial microbiota sampled from other shark species. Results indicate that the combination therapy of imipenem (carbapenem antibiotic) and vancomycin (glycopeptide antibiotic) might be the most parsimonious option to effectively treat infections resulting from white shark bites, particularly in South Africa. It is hoped that, in addition to assisting medical professionals to treat shark bite victims, these findings enhance the understanding of the microbial communities present in large coastal predators and their surrounding environments.

Significance:

- Overall antibiotic resistance of bacteria in the oral cavities of *C. carcharias* was relatively high.
- Combination therapy of imipenem (carbapenem antibiotic) and vancomycin (glycopeptide antibiotic) is recommended for the treatment of white shark bites, particularly in South Africa.
- The findings add to understanding of the microbial communities present in large coastal predators and their surrounding environments.

Introduction

The oral cavity of sharks, like many fauna, is host to a wide range of bacteria.[1-4] Therefore, victims of shark-related injuries involving shark bites require treatment for the prevention of infections caused by the transfer of pathogenic bacteria.[5,6] A review of 11 recent shark-related injuries in the USA indicated that only three of the reviewed patients received an appropriate selection of antibiotics for treatment of infection (using ciprofloxacin), and none of the reviewed patients received dual antibiotic therapy.[5] An earlier review of 83 shark-related injuries in South African waters could only confirm that 18 of the reviewed patients received any antibiotic treatment (using a variety of different antibiotics), and three of these patients continued to develop septic complications that required further surgical intervention.[6] Currently, there is no consensus on the most appropriate antibiotics to be used in treating bacterial infections resulting from shark-related injuries due to large differences in the composition of bacterial microbiota present among different shark species and their geographical locations.[4,5] Therefore, further information is needed on the antibiotic resistance of bacteria present in the oral cavities of different shark species from different regions of the world that might be responsible for shark-related injuries in humans.

There have been increasing reports of antibiotic resistance among bacteria hosted by marine predators[3,4,7], possibly because of the increased use of broad-spectrum antibiotics in humans and their subsequent entry as contaminants into coastal waters[7]. A study of bacteria found post-mortem in the oral cavities of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*) off Recife (Brazil) reported high levels of antibiotic resistance among several of the 81 isolated bacterial strains.[3] In particular, that study found a 20% resistance among *Proteus mirabilis* strains to imipenem, a broad-spectrum antibiotic commonly used to treat Gram-negative nosocomial infections.[8] Another study on bacteria isolated from cloacal swabs of *C. leucas*, blacktip sharks (*Carcharhinus limbatus*), nurse sharks (*Ginglymostoma cirratum*) and lemon sharks (*Negaprion brevirostris*) from Belize and the US East Coast, reported multidrug resistance in bacteria tested from all sampled shark species.[7] A comprehensive study undertaken by Unger et al.[4] of bacteria isolated from the oral cavities of adult, live *C. limbatus* in Florida (USA) found that the three primary bacterial types present were *Vibrio* spp., *Staphylococcus* spp. and *Pasteurella* spp. Unger et al.[4] additionally reported that 43% of isolated bacteria showed resistance to at least one antibiotic, and the overall resistance rate of all antibiotics they tested was 12%. Despite the prevalence of antibiotic resistance, these authors concluded that the best antibiotic selection for treating infections resulting from shark-related injuries involving *C. limbatus* near Florida was either a broad-spectrum flouroquinolone, or a dual antibiotic treatment using a third-generation cephalosporin and doxycycline.[4]

South Africa has the third highest incidence (after the USA and Australia) of human/shark encounters from 1580 to the present.[9] Within South Africa, white sharks (*Carcharodon carcharias*) account for 49% of reported shark-related injuries.[6] However, data on the bacterial microbiota present in *C. carcharias* oral cavities are limited to one sample that was taken port-mortem from a harpooned shark in northeast USA over two decades ago.[2] At present, very little is known about the full composition of bacterial strains present in *C. carcharias* oral cavities, or what the current resistance of these bacteria might be to available antibiotics. This study aimed to address this informational gap in order to assist medical professionals in making informed decisions when administering treatment for bacterial

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☐ Open data set
☐ All data included
☒ On request from author(s)
☐ Not available
☐ Not applicable

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infections in *C. carcharias* bite victims. Specifically, this study aimed to identify bacterial species that are present in the oral cavities of live *C. carcharias* along the southern coastline of South Africa, and to assess the sensitivity of these bacteria to 16 commonly used antibiotics. It is intended that these results might help in selecting the most appropriate antibiotic(s) for this category of injury, and also enable alternatives to broad-spectrum antibiotics that continue to raise resistance levels of pathogenic microbiota.

Methods

Animal capture and sample preparation

A total of 28 *C. carcharias* were caught using circle hooks tethered to floating buoys from four sample sites along South Africa's southern coastline (False Bay, Gansbaai, Mossel Bay and Algoa Bay) and sampled for this study (Figure 1). Sharks were led to a submerged platform, which was then lifted out of the water to minimise cross-contamination while sampling. Water supply to the gills was achieved via pumping seawater through a rigid pipe inserted into the mouth of the shark. Sterile cotton swabs were used to collect microbial samples from sharks' oral cavities. To ensure comprehensive sampling, separate swabs were taken of sharks' teeth, gums and tongue. However, these data were compiled together into a single sample category (oral cavity) due to likely cross-contamination. Additionally, attention was given to avoiding contact with the rigid pipe while sampling in order to minimise the chance of cross-contamination among individuals. All sharks were handled for a maximum of 15 min, after which they were released and monitored by a veterinarian to ensure no external signs of stress and/or capture myopathy. All interactions were approved by the South African Department of Environmental Affairs (reference RES2012/OCEARCH/JOHNSON).

Figure 1: Capture locations for white sharks (*Carcharodon carcharias*) sampled in this study.

Processing and identification of bacteria

All swabs were transported to the Ampath pathology laboratory in George (Western Cape, South Africa) within a week of sampling, where bacterial samples were each plated onto three replicates of Nutrient Agar® and streaked for single colonies. They were then incubated for 24 h at 37 °C in an aerobic atmosphere and stored in Tryptone Soya Broth®. Presumptive isolates from the Nutrient Agar® replicates were recovered from all plates, sub-cultured for purity and stored in Tryptone Soya Broth® plus 10% glycerol within microbank cryovial systems at -20 °C until further processing. Initial screening of presumptive isolates began with examining for Gram status, lactose fermentation, as well as oxidase and catalase reactions. Identifications of Gram-negative bacteria were confirmed using bioMérieux® API® 20E systems. Where possible, Gram-negative isolates were identified to species level, but in some cases could only be identified to genus level. Inconclusive Gram-negative isolates were reported as 'Gram-negative bacilli'. Gram-positive isolates were identified using Biorad Pastorex Staph Plus® and Remel® Streptex® kits.

Determining presence of beta-lactamase production

All confirmed bacterial strains were additionally examined for chromosomal beta-lactamase (Amp-C) production and extended beta-lactamase (ESBL) production at the Ampath pathology laboratory, as bacterial strains that can produce either type of these enzymes are likely to have strong resistance to all beta-lactam type antibiotics.[10] Amp-C production was assessed by observing a flattening of the cefotaxime zone on the side of the microbe adjacent to imipenem, as well as a flattening of the ceftazidime and/or cefotaxime zone on the side of the microbe adjacent to cefoxitin. Determining the presence of ESBL production among bacterial strains was performed by observing distortions of the inhibition zones around ceftazidime and/or cefepime, in the areas adjacent to the amoxicillin/clavulanate disc. Distortions indicating ESBL production took the form of (1) an increased radius of the inhibition zone for the cephalosporin(s) or (2) the presence of a lens-shaped inhibition zone between the cephalosporin and amoxicillin–clavulanic discs either when there was no inhibition zone around the cephalosporin disc or if the inhibition zone was very narrow.

For bacterial strains in which Amp-C production was present, resistance to ampicillin; amoxicillin/clavulanic acid; piperacillin/tazobactam; first-, second- and third-generation cephalosporins; and cefoxitin was reported, regardless of zone size or further testing. For any bacterial strains for which ESBL production was confirmed, resistance to ampicillin, first-, second-, third- and fourth-generation cephalosporins, piperacillin/tazobactam, amoxicillin/clavulanic acid, as well as sensitivity to cefoxitin was reported. The susceptibilities of these strains to other antibiotics, not listed above, were reported according to results of the further testing outlined below.

Assessing bacterial sensitivity to antibiotics

All bacterial strains isolated from oral cavity samples of *C. carcharias* were tested for susceptibility to a panel of 16 antibiotics (summarised in Table 1) using antibiotic disc diffusion tests according to the Kirby–Bauer methodology outlined in the guidelines of the US Clinical and Laboratory Standards Institute.[11]

Table 1: Classes and types of antibiotics used in disc diffusion tests to assess the antibiotic sensitivity of bacterial flora isolated from *Carcharodon carcharias* oral cavities

Antibiotic used in disc diffusion test	Abbreviation	Dosage	Class / type of antibiotic
Amikacin	AMK	30 mcg	Aminoglycoside
Imipenem	IPM	10 mcg	Carbapenem
Cefepime / cefpirome	FEP	30 mcg	Cephalosporin
Ceftazidime	CAZ	30 mcg	Cephalosporin
Ceftriaxone / cefotaxime	CTR	30 mcg	Cephalosporin
Cefuroxime	CFX	30 mcg	Cephalosporin
Cephalothin	CEP	30 mcg	Cephalosporin
Ciprofloxacin	CIP	5 mcg	Fluoroquinolone
Trimethoprim / sulfamethoxazole	SXT	25 mcg	Folate pathway inhibitor
Vancomycin	VAN	30 mcg	Glycopeptide
Amoxicillin	AMOX	30 mcg	Penicillin
Amoxicillin / clavulanic acid	AMC	30 mcg	Penicillin
Penicillin	PEN	10 mcg	Penicillin
Piperacillin / tazobactam	PTZ	110 mcg	Penicillin
Erythromycin / clarithromycin / azithromycin	ERY	15 mcg	Macrolide
Tetracycline	TET	30 mcg	Tetracycline

Bacteria were reported as sensitive to an antibiotic if all strains of those bacteria indicated sensitivity to the drug. Bacteria were reported as resistant to an antibiotic if all of its strains were resistant to the drug. If different strains of the same type of bacteria showed conflicting susceptibility to an antibiotic it was reported in the results as 'R*', but was considered resistant for the sake of calculating relative percentages of antibiotic effectiveness. If an antibiotic is not routinely used on an isolate, is not used for treatment of a given strain, or if there was no available information on the zone or the minimum inhibitory concentration breakpoint of the bacteria or antibiotic[11], then this combination was reported in the results as 'not applicable'.

Results

Bacterial species present

A total of 51 bacterial strains were isolated from oral cavities of 28 live *C. carcharias* (Table 2). Among these strains, there were at least 20 species of bacteria present. An additional five strains which could not be identified were grouped into the category 'Gram-negative bacilli' (Table 2). The three most common bacterial strains identified from the oral cavities (in order of frequency) were: *Serratia* spp., *Proteus vulgaris* and *Vibrio alginolyticus*. *Aeromonas hydrophyla*, *Enterococcus faecalis* and *Staphylococcus* spp. were also present in at least 10% of all sharks sampled (Table 2).

Assessment of Amp-C and ESBL production among isolated strains

Among the 51 bacterial strains isolated from samples, 6 isolates tested positive for Amp-C production. These isolates were: *E. cloacae* (n=2), *Klebsiella pneumoniae* (n=1), *Proteus mirabilis* (n=1), *P. vulgaris* (n=1) and *Serratia* spp. (n=1). All strains tested negative for ESBL production.

Bacterial sensitivity to antibiotics

All strains of bacteria isolated from the oral cavities of live *C. carcharias* in this study displayed sensitivity to a panel of at least four types of antibiotics (Table 3). Antibiotic resistance varied greatly among bacterial strains, but was observably higher in *E. cloacae, K. pneumoniae, P. mirabilis, P. vulgaris* and *Serratia* spp. which were among the strains that tested positive for Amp-C production. Of the 16 antibiotics that were tested, ciprofloxacin, amikacin and imipenem yielded the highest sensitivity among bacterial strains (Table 3). Specifically, these three antibiotics were effective in treating each of the Amp-C positive bacterial strains mentioned above. Trimethoprim-sulfamethoxazole and tetracycline followed closely in overall effectiveness; however, several of the Amp-C positive bacteria were resistant to both these antibiotics (Table 3). Isolated bacterial strains showed the highest resistance to amoxicillin, piperacillin-tazobactam and the cephalosporins (cephalothin and cefuroxime). No single antibiotic tested could effectively treat all the bacterial strains on its own (Table 3), indicating that multiple antibiotics would be necessary in treating exposure to the full panel of bacteria isolated from *C. carcharias* oral cavities in this study.

Discussion

Among the 16 antibiotics tested, both imipenem and ciprofloxacin were the only two that effectively treated each of the Gram-negative bacteria found in the oral cavities of *C. carcharias* in this study (Table 3). The Gram-positive bacteria isolated from *C. carcharias* oral cavities included *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. and these strains showed consistent sensitivity to both vancomycin and penicillin during disc diffusion tests (Table 3). Infection with these Gram-positive bacteria may also be effectively treated with imipenem; however, at the time of writing, there was no available information on the zone or minimum inhibitory concentration breakpoint of enterococci, staphylococci or streptococci with imipenem[11], and so this combination was not assessed in the disc diffusion test in the present study. Therefore, these findings indicate that treatment with imipenem, and possibly a combined regime of vancomycin would be the most effective option for preventing and/or treating infections resulting from white shark bites in South Africa. Based on the results, ciprofloxacin and penicillin, in place of imipenem and vancomycin respectively, also have potential as treatment options for white shark bite patients (Table 3). However, fluoroquinolone antibiotics, to which class ciprofloxacin belongs, often show an antagonistic effect in antibiotic combination therapies, as can the doubling up of two beta-lactam antibiotics[12] (e.g imipenem and penicillin). Nevertheless, Al-Hasan et al.[13] demonstrated that fluoroquinolones combined with beta-lactam antibiotics can contribute to a positive treatment outcome when suspecting Gram-negative bacilli. That said, combination therapy between fluoroquinolones and beta-lactam antibiotics should be considered carefully as the synergistic significance is not clear and clinical outcomes are conflicting.[14]

The overall composition of bacterial microbiota identified in the oral cavities of live *C. carcharias* in this study was similar to microbial presence reported from other shark species.[3,4] Specifically, the prevalence of *Enterobacter* spp. and *Proteus* spp. coincided with common bacteria sampled from *C. leucas* and *G. cuvier* in Recife, Brazil.[3] The occurrence of *Vibrio* spp. and *Staphylococcus* spp. strains mirrored findings from live *C. limbatus* oral cavities in Florida, USA.[4] Additionally, the presence of *Vibrio* spp. and *Shewanella putrefaciens* was consistent with bacterial microbiota previously reported from the teeth of *C. carcharias.*[2] However, findings from the present study also indicate a high incidence of many other bacterial microbiota, specifically *Enterococcus* spp. and *Serratia* spp. in

the oral cavities of *C. carcharias* in South African waters. It is additionally worth noting that the delay of up to 1 week for transporting some of the swab samples to the laboratory may have biased our findings towards the presence of faster-growing and/or persistent bacterial species. Nonetheless, at least 20 distinct species of bacteria were identified from the oral cavities of live sharks in this study, all of which could pose risk of pathogenesis to patients with shark-related injuries.

The overall antibiotic resistance observed in this study was relatively higher than that reported in other shark species.[3,4,7] Specifically, no single antibiotic tested in this study would be capable of effectively treating all pathogenic bacteria presently reported from *C. carcharias* oral cavities. This is in contrast to studies of other shark species, which have suggested that a single agent fluoroquinolone (ciprofloxacin or levofloxacin) could be used for treatment of infections in *C. leucas, C. limbatus* and *G. cuvier* bite victims.[3,4] Buck et al.[2] who assessed the antibiotic resistance of four bacterial strains isolated from the teeth of one *C. carcharias* sampled from northeast USA, reported that effective treatment of these bacteria could be achieved using aminoglycosides, tetracycline or several cephalosporin class antibiotics. These differences in bacterial composition and resistance levels reflect the dynamic nature of bacteria over space, time and host species[7,15], as well as highlight the need for species and geographic-specific microbial assessments.

Due to the limited sample size and sampling region, comparison of *C. carcharias* bacterial compositions related to geography, host age, maturity and diet were outside the scope of this study. Nonetheless, these factors are likely to have strong impacts on the presence and antibiotic resistance of pathogenic bacteria affecting shark-related injury victims.[4,7] Preliminary studies have indicated no discernible relationship between the composition of oral bacteria in *C. carcharias* and either their gut contents or sampling location in South Africa.[16] These patterns suggest that differences in feeding ecology and fine-scale geography might have minimal impact on the bacterial compositions hosted by these highly migratory[17] apex predators in South African waters. However, it is highly advocated that further microbial studies addressing the sharks' sex, age and maturity stage, as well as movement patterns over a global scale, would be very useful for making inferences between the life history of this species and the microbiota that it hosts.

Table 2: Bacterial species identified from swab samples taken from the oral cavities of 28 live white sharks (*Carcharodon carcharias*)

	False Bay			Gansbaai							Struisbaai			Mossel Bay													Algoa Bay	%
Shark	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Sex	F	F	F	M	M	F	M	F	M	M	F	F	M	F	F	F	F	F	F	F	F	F	F	F	M	F	M	F	
Total length (cm)	417	437	431	318	297	360	355	452	330	251	505	441	382	340	299	351	283	271	228	350	297	292	251	259	266	340	309	265	
Aeromonas hydrophyla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	P	-	-	10.71
Eikenella corrodens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	-	-	7.14
Enterobacter amnigenus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	3.57
Enterobacter cloacae	-	-	-	P	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	P	P	-	10.71
Enterococcus faecium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	3.57
Escherichia coli	-	-	-	-	-	-	-	-	-	-	P	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
Pantoea spp.	-	-	P	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
Photobacterium damselae	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	-	-	-	7.14
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	P	-	-	P	-	-	-	P	P	17.86
Pseudomonas oryzihabitans	-	-	-	-	-	-	-	-	-	-	P	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.14
Pseudomonas fluorescens	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	7.14
Serratia spp.	P	P	P	-	-	-	P	-	P	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.43
Shewanella putrefaciens	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	7.14
Staphylococcus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P	-	-	-	-	-	P	-	-	-	-	-	10.71
Streptococcus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	3.57
Vibrio alginolyticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	-	P	-	-	-	-	P	-	-	14.29
Vibrio parahae-molyticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	3.57
Gram-negative bacilli	P	-	-	-	P	-	-	P	-	-	-	-	P	-	-	-	-	-	-	-	-	P	-	-	-	-	-		17.86

P, present. The last column represents the raw percentage of sampled sharks that were hosts to the specified bacterial strain.

Table 3: The 20 bacterial strains and Gram-negative bacilli isolated from oral cavity samples of *Carcharodon carcharias* and their corresponding sensitivity to 16 common antibiotic types

Bacteria	Antibiotics tested															
	AMK	IPM	FEP	CAZ	CTR	CFX	CEP	CIP	SXT	VAN	AMOX	AMC	PEN	PTZ	ERY	TET
Aeromonas hydrophyla	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Eikenella corrodens	–	S	–	–	S	–	–	S	S	–	–	S	–	–	–	S
Enterobacter amnigenus	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Enterobacter cloacae	S	S	R*	R*	R*	R	R	S	R*	–	R	R*	–	R	–	S
Enterococcus faecalis	–	–	–	–	–	–	–	–	–	S	–	–	S	–	–	–
Enterococcus faecium	–	–	–	–	–	–	–	–	–	S	–	–	S	–	–	–
Escherichia coli	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Klebsiella pneumoniae	S	S	S	R	R	R	R	S	S	–	R	R	–	R	–	S
Pantoea spp.	S	S	R*	R*	R*	–	–	S	S	–	–	R	–	–	–	S
Photobacterium damselae	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Proteus mirabilis	S	S	S	R*	R*	R	R	S	S	–	R	R*	–	R	–	R
Proteus vulgaris	S	S	R*	R*	R*	R	R	S	S	–	R	R*	–	R	–	R*
Pseudomonas oryzihabitans	S	S	–	S	–	–	–	S	-	–	–	–	–	–	–	–
Pseudomonas fluorescens	S	S	S	S	–	–	–	S	S	–	–	–	–	–	–	–
Serratia spp.	S	S	S	R*	R*	R	R	S	R*	–	R	R*	–	R	–	S
Shewanella putrefaciens	S	S	–	S	S	–	–	S	S	–	–	R*	–	–	–	S
Staphylococcus spp.	S	–	–	–	–	–	S	S	S	S	S	S	S	–	S	S
Streptococcus spp.	–	–	–	–	S	–	–	–	–	S	S	-	S	–	S	S
Vibrio alginolyticus	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Vibrio parahaemolyticus	S	S	S	S	S	–	–	S	S	–	–	S	–	–	–	S
Gram-negative bacilli	S	S	S	S	S	–	–	S	S	–	–	R*	–	–	–	S
Antibiotic effectiveness (%)	**80.95**	**80.95**	**52.38**	**47.62**	**47.62**	**0**	**4.76**	**85.71**	**71.43**	**19.05**	**9.52**	**38.10**	**19.05**	**0**	**9.52**	**71.43**

S, sensitive; R, resistant; R, at least one strain of this bacteria was resistant to the corresponding antibiotic; –, test not applicable to isolate; AMK, amikacin; IPM, imipenem; FEP, cefepime/cefpirome; CAZ, ceftazidime; CTR, ceftriaxone/cefotaxime; CFX, cefuroxime; CEP, cephalothin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; VAN, vancomycin; AMOX, amoxicillin; AMC, amoxicillin/clavulanic acid; PEN, penicillin; PTZ, piperacillin/tazobactam; ERY, erythromycin/clarithromycin/azithromycin; TET, tetracycline*

Conclusion

This study presented the first assessment of antibiotic resistance among bacterial strains isolated from the oral cavities of live *C. carcharias* in South Africa. Bacteria identified in this study include pathogenic bacteria previously unreported for shark species occurring in South Africa. It is hoped that the data presented here can enable medical professionals to make more informed, and thus more effective decisions when administering antibiotic treatment to shark bite victims, and provide an increased understanding of the microbial communities present in large coastal predators and their surrounding environments. The antibiotic resistance reported here of bacterial microbiota hosted by these top predators will additionally serve as baseline information toward future studies and management processes serving human public health.

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Authors' contributions

This study was conceived and designed by E.G. Data were collected by E.G., A.A.K., M.J.S., A.T., C.F., R.J. and M.M. Data were validated by L.A.B. and curated by P.M., N.K., L.A.B. and E.G. An initial draft of this manuscript was produced by E.G., N.K. and L.A.B. Funding was acquired by C.F. and R.J. This project was overseen and managed by E.G. P.M. interpreted the data and was primary author of the final manuscript. All authors contributed to revisions of the final draft of the manuscript.

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Antimicrobial activity and toxicity profile of selected southern African medicinal plants against neglected gut pathogens

Anaerobes outnumber aerobic bacteria in the human gut. The most commonly isolated microorganisms in intra-abdominal infections include *Escherichia coli, Peptostreptococcus micros* as well as *Bacteroides* and *Clostridium* species. Several studies have been undertaken on southern African medicinal plant species and their antimicrobial efficacy against pathogens such as *E. coli* that cause stomach ailments. However, pathogens such as *Helicobacter pylori*, *Fusobacterium varium* as well as others have been neglected in medicinal plant antimicrobial research. The aim of this study was to evaluate the antimicrobial activity of selected medicinal plants documented for stomach ailments against neglected gut pathogens. A total of 102 aqueous and organic extracts were prepared from 40 different plant species. These plant samples were screened for antimicrobial efficacy against eight anaerobes and two microaerophilic strains using the micro-dilution antimicrobial assay. Plant extracts that displayed noteworthy antimicrobial activity against *Clostridium perfringens* were further evaluated for antibiofilm activity using the crystal violet staining assay. The toxicity profiles of plants that displayed noteworthy antimicrobial activity were evaluated using the brine shrimp lethality assay which revealed that most of the tested plant samples were non-toxic in nature, and the aqueous extracts proved to be safer. The organic extract of *Lippia javanica* leaf showed the best antimicrobial activity with a minimum inhibitory concentration of 0.5 μg/mL against *C. perfringens*. The organic extract of *Salvia africana-caerulea* displayed the best antibiofilm activity overall, at cell attachment (4 h) biofilm developmental stage with inhibition percentages of 82.8%.

Significance:

- *L. javanica* and *Gunnera perpensa* demonstrated the highest antimicrobial activity with minimum inhibitory concentrations of 0.5 μg/mL and 2.0 μg/mL against *C. perfringens*, respectively.
- *Salvia africana-caerulea* was the most effective plant species demonstrating biofilm attachment.
- Lowest toxic effects were observed for the organic extracts of *Aloe marlothii*, *A. tenuior*, *Bridelia cathartica*, *G. perpensa* leaf and the aqueous extracts of *G. perpensa* (leaf and rhizome).
- This study demonstrates, for the first time, both antimicrobial and antibiofilm activities for most of these plant species against neglected anaerobes.
- Noteworthy antimicrobial activities in many cases validate traditional use and safety.

Introduction

Intra-abdominal infections are infections of the stomach and are a substantial cause of mortality and morbidity.[1,2] Intra-abdominal inflictions include peritonitis, intra-abdominal abscesses, appendicitis, colorectal cancer, ulcerative colitis, food poisoning, chronic atrophic gastritis, peptic ulceration and stomach cancer.[3-5] Pathogens associated with intra-abdominal infections include *Escherichia coli*, the *Bacteroides fragilis* group, and *Clostridium* species.[6,7] *Bacteroides* species are opportunistic bacteria that form part of the normal microbiota and are often associated with polymicrobial infections such as intra-abdominal, pelvic, genital, complicated skin and soft tissue, and bloodstream infections.[6,8-10] *Clostridium* species are associated with pseudomembranous colitis which is triggered by the intake of broad-spectrum antibiotic therapy and may be the cause of infectious diarrhoea in hospital patients.[11] Other pathogens that are isolated in intra-abdominal infections include *Helicobacter pylori* as well as *Fusobacterium* species.[3,5,12] *Helicobacter pylori* infects more than 50% of the world's population; however, only a small percentage of patients develop severe disorders.[13] People that are most likely to be infected are from developing countries.[14] Another bacterial species that is associated with cancer of the gut is *Fusobacterium* spp. These species are associated with severe infections and are often related to colorectal cancer, which is the third most common cancer worldwide.[12,15]

A wide range of antibiotics and treatment regimens are used for the treatment of intra-abdominal infections. Increased antibiotic resistance is the main cause of treatment failure.[9,16] Phytomedicine has proved to be an alternative treatment for different diseases, including gastrointestinal disorders.[14,17-19] The use of the medicinal plants selected for this study have previously been reported; however, the scientific evidence for their activity against neglected pathogens of the gut has not been adequately explored.

Globally, some antimicrobial studies have focused on evaluating the activity of traditional medicinal plants against neglected gut pathogens and have shown promising antimicrobial activities against fastidious gut pathogens.[14,20,21] In southern Africa, several studies have focused on evaluating the antimicrobial efficacy of medicinal plants against commonly studied gut pathogens such as *Staphylococcus aureus*, *Shigella flexineri*, *E. coli*, *Enterococcus faecalis* and *Candida albicans*.[22] A review from a period dating almost 20 years demonstrated that very few, if any, southern African medicinal plant studies are related to gut anaerobes.[22,23] Most plant-based antimicrobial studies have focused on planktonic microorganisms, although many of the fastidious pathogens selected for this study occur not only in planktonic form but also as biofilms. Biofilms are defined as multicellular matrices of bacteria

surrounded by an extracellular polysaccharide called a glycocalyx.[24] The ability of bacteria to aggregate and form biofilms makes it difficult to treat bacterial infections as biofilms enhance the bacteria's ability to resist the host's immune system response, thus contributing to the development of antibiotic resistance.[25,26] As far as we could ascertain, no previous study has focused on the antibiofilm activity of medicinal plants against *C. perfringens* and thus, this warranted attention.

Furthermore, plants commonly used in traditional medicine are often believed to be non-toxic. However, scientific research has shown that many of them can be lethal, mutagenic and carcinogenic.[27,28] Thus the aim of this study was to evaluate the antimicrobial activity of selected medicinal plants documented for stomach ailments against neglected gut pathogens responsible for intra-abdominal infections and to further investigate biofilm activity (using *C. perfringens* as a model) and toxicity profiles of plants that demonstrated noteworthy antimicrobial activities.

Materials and methods

Ethnobotanical review, plant identification and collection

An ethnobotanical literature review was conducted to identify the southern African medicinal plants used traditionally to treat stomach ailments (Table 1). Several medicinal plant based books and scientific databases were used to search for plants that are used traditionally to treat stomach ailments.[29,30-33] Approximately 155 medicinal plant species were identified. From these, medicinal plant species which could be successfully collected from various botanical gardens (with respect to cost, season, accessibility, sustainability and time) were selected for the study. The selected plant species were collected from the Walter Sisulu National Botanical Garden (Roodepoort, Gauteng, South Africa), where the chief horticulturist, Mr Andrew Hankey, granted permission and assisted in plant identification. All documents for the transfer of materials for research purposes were completed accordingly. Medicinal plant material that was not available at Walter Sisulu National Botanical Garden was purchased from Random Harvest Indigenous Nursery (Muldersdrift, Gauteng, South Africa). Following collection, voucher specimens were prepared for each species and were housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

The collected plant samples were left to dry at room temperature. Once completely dried, samples were separated into different plant parts, i.e. roots, leaf, fruits, bark and stems. Dried plant materials were then crushed to powder using the high-speed Fritsch Pulverisette grinder (Labotec, Johannesburg, South Africa) or using a hand-held pounder (purchased at Faraday supermarkets) for harder stems and barks.

Preparation of plant extracts

Plant powder was resuspended in 1:1 dichloromethane:methanol (Sigma-Aldrich, Johannesburg, South Africa) at a ratio of plant powder:solvent of 1:2, and then placed in the platform shaker incubator (Labcon, Johannesburg, South Africa) at 37 °C for 24 h. Thereafter, the solvent was filtered and left in a fume hood to evaporate. The samples were extracted again with fresh solvent for another 24 h. Once the solvent had evaporated, the extract was transferred into suitable amber bottles for storage at ambient temperature. Aqueous extracts were prepared by immersing plant powder material in sterile distilled water. This immersion was followed by incubation in platform shaker incubator, overnight at 30 °C. Thereafter, the liquid extracts were strained and stored at -80 °C for 24 h before lyophilisation. Aqueous extracts were lyophilised using a freeze dryer (Virtis, South Africa) for approximately 7 h or overnight. Before use, aqueous extracts were placed under ultraviolet light overnight to eliminate possible microbial contaminants. All plant samples were stored in appropriate containers at room temperature. Table 1 details the plant species collected, common names, reported traditional use, plant part used and percentage yield.

Plant sample preparation

Samples were prepared by weighing out the crude extracts and calculating the volume of solvent to be added to create a sample concentration of 32 mg/mL. Acetone (Sigma-Aldrich) was used as the solvent of choice for organic samples as it has minimal antimicrobial effects. Sterile water was used to dissolve aqueous extracts.

Table 1: Southern African medicinal plants used traditionally to treat stomach ailments

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Acokanthera oppositifolia (Lam.) Codd. Apocynaceae	Bushman's poison	Leaf decoction for stomach ache, diarrhoea, anthelmintic; roots or leaves for abdominal pain; ripe fruit is for gastritis	Leaf	[b]HS245	9.5	19.5	29,32,34
			Root	[b]HS245	25.9	7.7	
Aloe arborescens Mill. Aloaceae	Krans aloe	Stomach ache	Leaf	[a]HS214	32.3	6.3	19,32,34
Aloe ferox Mill. Aloaceae	Bitter aloe	Stomach ache	Leaf	[a]HS215	10.5	3.3	34
Aloe marlothii Berger Aloaceae	Mountain aloe	Decoctions administered orally or as enemas against roundworm and for stomach ailments	Leaf	[a]HS216	12.6	7.7	19,32
Aloe tenuior Lam. Aloaceae	Slender aloe	Peptic ulcer	Leaf	[a]HS217	19.6	38.8	34
Antidesma venosum E.Mey. ex Tul. Euphorbiaceae	Tossel berry	Decoctions for abdominal cramps and dysentery	Leaf	[a]HS218	7.5	8.3	32
Artemisia afra Jacq. ex Willd. Asteraceae	African wormwood	Stomach pain	Leaf	[a]HS219	12.0	16.1	29
Boophone disticha Herb. Amaryllidaceae	Bushman's poison	Abdominal pain; gastric ulcers	bulb	[b]HS244	15.0	11.6	30
Bridelia cathartica G. Bertol. Euphorbiaceae	Blue sweet berry	Stomach ache	Leaf	[a]SVV2013.1	14.4	7.6	32
Bridelia micrantha Baill. Euphorbiaceae	Coastal golden	Stomach ache	Leaf	[a]HS220	14.8	5.8	32,35
			Stem	[a]HS220	15.1	2.6	
Catha edulis (Vahl) Forssk. ex Endl. Celastraceae	Bushman's tea	Gastrointestinal tract problems; gastritis; stomach ailments	Leaf	[a] HS221	10.2	11.7	32

Table 1: Continued.

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Dichrostachys cinerea (L.) Wight & Arn. Fabaceae	Sickle bush	Used for abdominal pain	Bulb	[a]HS223	6.5	14.1	32
Dodonaea viscosa Jacq. Sapindaceae	Sand olive	Decoction is used for stomach trouble	Leaf	[a]HS222	16.8	9.6	30
Dombeya rotundifolia Planch. Sterculiaceae	Wild plum	Leaves for internal ulcers; bark for ulcerative colitis and intestinal ulceration; roots are used for abdominal pain; stems and leaves are used for stomach cramps	Leaf	[a]HS224	8.3	7.0	29,32
			Stem	[a]HS224	6.8	4.9	
Drimiopsis maculata Lindl. & Paxton Hyacinthaceae	Little white soldiers	Stomach ailments	bulb	[a]HS225	13.4	4.9	32
Ekebergia capensis Sparrm. Meliaceae	Cape ash	Dysentery and acute gastritis	Leaf	[a]HS226	4.6	12.7	30
Elephantorrhiza elephantina (Burch.) Skeels. Fabaceae	Elephant's root	Diarrhoea, dysentery, stomach disorders, peptic ulcers	Root + rhizome	[a]UM172	15.9	10.8	30
Eucomis autumnalis (Mill.) Chitt. Hyacinthaceae	Pineapple lily	Boil bulb for abdominal problems; stomach ache	Leaf	[a]HS229	32.4	13.2	30
Gunnera perpensa L. Gunneraceae	River pumpkin	Roots are used for stomach ailments; unspecified plant parts used for stomach bleeding	Leaf	[a]UM168	26.9	11.8	30
			Rhizome	[a]UM176	27.5	14.1	
Heteromorpha arborescens Cham. & Schltdl Apiaceae	Parsley tree	Abdominal pain; dysentery	Leaf	[a]HS246	2.4	11.3	30
Ipomoea purpurea (L.) Roth. onvolvulaceae	Morning glory	Stems are used for stomach disorders	Stem	[a]HS230	3.1	3.3	32
Kigelia africana (Lam.) Benth. Bignoniaceae	Sausage tree	Fruit is used for ulcers; fruit and ground bark used for stomach ailments	Fruit	[a]HS231	7.4	1.9	30,32
			Leaf	[a]HS231	5.1	5.0	
			Stem	[a]HS231	4.8	2.0	
Lippia javanica Spreng. Verbenaceae	Fever tea	Leaf infusions for diarrhoea and stomach disorders	Leaf	[a]HS232	9.9	9.0	30,32,35
			Twigs	[a]HS232	15.2	3.3	
Mentha longifolia Huds. Lamiaceae	Mint	Leaf is used for stomach ache	Leaf	[a]UM148	14.3	15.3	32,34
Osmitopsis asteriscoides Cass. Asteraceae	Mountain daisy	Colic	Leaf	[a]HS234	14.3	9.3	30
Oxalis corniculata L. Oxalidaceae	Creeping wood	Stomach ache; peptic ulcers	Leaf	[a]HS232	16.2	11.3	34
Peltophorum africanum Sond. Leguminosae	African blackwood	Diarrhoea, dysentery, abdominal pain	Leaf	[a]HS235	15.5	7.8	29
Polygala fruticosa P.J. Bergius Polygalaceae	Petite butterfly	Intestinal sores	Leaf	[a]SVV2013.2	12.4	22.4	30
Rapanea melanophloeos Mez Myrsinaceae	Cape beech	Ground bark decoctions are used for stomach ache	Leaf	[a]HS236	7.3	6.5	32
			Stem	[a]HS236	0.3	4.9	
Rauvolfia caffra Sond. Apocynaceae	Kinaboom	Bark for abdominal pain	Leaf	[a]UM137	14.5	6.6	32
Salvia africana L. *caerulea* Lamiaceae	Purple sage	Unspecified plant part is used for stomach pain	Leaf + young twigs	SWC AV 875	17.4	12.4	30
Scadoxus puniceus (L.) Friis & Nordal Amaryllidaceae	Paintbrush lily	Bulb and leaves for abdominal pain, stomach ailments, diarrhoea, and nausea	Root + rhizome	[a]UM143	9.8	4.3	30
Solanum incanum Ruiz & Pav. Solanaceae	Bitter apple	Roots and leaves for abdominal pain	Leaf	[a]UM158	25.8	9.7	29,33
Spirostachys africana Sond. Euphorbiaceae	Jumping-bean tree	Stomach ulcers; stomach pain; dysentery; acute gastritis; diarrhoea	Leaf	[a]HS247	28.6	11.1	32,35
			Stem	[a]HS247	5.8	4.6	

Table 1: Continued.

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Syzygium cordatum Hochst Myrtaceae	Water berry	Unspecified plant parts for stomach ache and diarrhoea	Leaf	[a]HS237	10.0	8.9	29–32
Tarchonanthus camphoratus Houtt. ex DC Asteraceae	Camphor bush	Infusions for abdominal pains	Leaf	[a]SVV1100	10.2	10.8	30–32
Tetradenia riparia (Hochst.) Codd Lamiaceae	Ginger bush	Stomach ache; diarrhoea; ulcers; gastroenteritis	Leaf	[a]HS238	10.4	13.4	29,32
Warburgia salutaris (Berto.f.) Chiov. Canellacea	Fever tree	Gastric ulcers	Leaf	[a]HS239	10.6	10.0	30,32
			Stem		3.2	4.0	
Zanthoxylum capense Harv. Rutaceae	Small knob wood	Gastric and intestinal disorders	Leaf	[a]HS240	8.9	8.0	32

[a]*Walter Sisulu National Botanical Garden;* [b]*Random Harvest Indigenous Nursery*

Test microorganisms

Test pathogens were selected according to their propensity to cause stomach ailments. Most of the selected microorganisms were obtained from the American Type Culture Collection (ATCC) and were purchased from Davies Diagnostics (Johannesburg, South Africa). Eight members of the Gram-negative anaerobic bacilli were selected. Two non-fastidious pathogens, *E. coli* (ATCC 8739) and *E. faecalis* (ATCC 29212), were included as comparators of activity (Table 2). These microorganisms were cultured in the respective media and under the incubation conditions prescribed by the Clinical Laboratory Standards Institute[34], with slight modifications as described in Table 2. Two ethics waivers for the use of these microorganisms were obtained from the University of the Witwatersrand Human Research Ethics Committee (reference no. W-CBP-180509-01 for anaerobes and aerobic bacteria; and M170582 for *H. pylori* strains).

For *H. pylori*, the clinical strain was obtained from Chris Hani Baragwanath Academic Hospital (Johannesburg, South Africa). Methods as previously described[35] were used to isolate the strains from patients. This isolation was achieved by obtaining biopsies from the antrum and corpus. These specimens were then placed in sterile bijou bottles containing a mixture of cysteine (200 mg/mL) and glycerol (20%) in brain heart infusion broth and transported on ice to the laboratory within 2 h of collection. *Helicobacter pylori* isolates were then confirmed by: polymerase chain reaction using *glmM* as the target gene; colony morphology and characteristic spiral morphology on Gram staining; and positive catalase, urease and oxidase tests. Confirmed isolates were suspended in 20% glycerol and stored at -80 °C in a freezer for future use. A reference strain, namely *H. pylori* (B8), was also tested. This strain was obtained from the Ludwig Maximilian University of Munich (Germany) medical microbiology laboratory, through the University of the Witwatersrand's Department of Surgery.

Antimicrobial analysis

Antimicrobial susceptibility was evaluated using the minimum inhibitory concentration (MIC) assay with specific modifications to facilitate fastidious growth of pathogens.[34,36] Using aseptic techniques, 100 μL of broth, selected depending on the microorganism being tested, was introduced to all wells of the 96-well microtitre plates. Thereafter, 100 μL of respective plant sample to be tested was placed in the top row of the microtitre plate.

Controls (positive, negative and culture) were included in all assays. The role of the negative control was to ensure that the solvent (acetone) exerted no or minimal antimicrobial effect. Positive controls at starting concentrations of 0.01 mg/mL were used to validate the microbial susceptibility: ciprofloxacin was used for *E. coli*, *E. faecalis, C. perfringens* and *Fusobacterium* species; an equal ratio mix of clarithromycin and amoxicillin was used for *H. pylori* species; imipenem for *Bacteroides* species; and metronidazole for *C. difficile*. Ciprofloxacin was used as a broad-spectrum antibiotic. Metronidazole, imipenem, clarithromycin and amoxicillin were selected based on their antimicrobial susceptibility. A culture control was added to ensure the broth's ability to support microbial growth. Serial dilutions were then performed, and the plant extracts were diluted to concentrations of 8000, 4000, 2000, 1000, 500, 250, 130 and 60 μL/mL. A 100-μL volume of a standardised culture suspension (1 x 10^8 CFU/mL) prepared as a 0.5 McFarland's standard was added to all the wells of the microtitre plates. This resulted in two-fold dilutions descending along each row. Assays were undertaken at least in duplicate to ensure accuracy. The microtitre plates were incubated at optimal conditions (Table 2) without an adhesive seal film to allow the exposure of the cultures to required atmospheric conditions.

Antibiofilm analysis

Plant extracts that exhibited noteworthy activity (MIC ≤ 160 μg/mL) against *C. perfringens* were selected for biofilm studies. *Clostridium perfringens* was also selected because it was the most susceptible of all the pathogens studied. Plant samples were immersed in sterile water and thereafter sonicated at room temperature and low speed using ultrasonic waves (SCIENTECH). The effect of plant extracts on biofilm attachment was tested using the method described by Sandasi et al.[37] Using spectrophotometric methods, microbial cultures containing approximately 1x10^6 CFU/mL were prepared and added to the wells of a new 96-well microtitre plate, and a blank column containing sterile broth was also included. Prior to testing, the plate was incubated anaerobically for 4 h at 37 °C.

To test for the effect of plant extracts on established biofilms, the method described above was used, except stock cultures were incubated for 24 h, 48 h and 72 h at 37 °C. After incubation, 100 μL of each plant extract was transferred to a final concentration of 1 mg/mL in the wells. Plates were incubated overnight at 37 °C, after which the crystal violet assay was performed at selected time intervals and the biofilm biomass determined. The percentage inhibition was calculated using Equation 1[37]:

$$\% \text{ Inhibition} = \frac{\text{Optical density (OD) culture control} - \text{OD experimental} \times 100}{\text{OD culture control}} \quad \text{Equation 1}$$

The crystal violet assay was undertaken to evaluate the ability of the extracts to prevent and inhibit the development of biofilms. This was done by washing the incubated plates with sterile water and oven drying them at 60 °C for 45 min. Once dried, all the wells were stained with 200 μL of 1% crystal violet and left at room temperature for 15 min to allow for proper absorption of the stain. This was followed by washing the plates with sterile water three times to remove the unabsorbed stain and adding 125 μL ethanol as a de-staining solution. A volume of 100 μL of the de-staining solution was transferred to a new microtitre plate and the absorbance was determined at 590 nm using a microplate reader (Universal microplate reader ELX 800). The mean absorbance of the extracts was determined prior to calculating the percentage inhibition. All tests were repeated at least in triplicate for reproducibility.

Table 2: Growing conditions for cultures

Pathogen	Agar	Broth	Incubation conditions
Bacteroides species: *B. fragilis* (ATCC 23745) *B. ovatus* (ATCC 8483) *B. thetaiotaomicron* (ATCC 29741) *B. vulgatus* (ATCC 8482)	Tryptone Soya agar (TSA) (Oxoid) with 5% defibrinated sheep blood (NHLS)	Muller–Hinton broth with 5% yeast extract (Oxoid) and *Haemophilus* supplement (Oxoid)	37 °C for 24–48 h using anaerobic gas packs (Oxoid)
Clostridium species: *C. difficile* (ATCC 43593) *C. perfringens* (ATCC 13124)	TSA with 5% defibrinated sheep blood	Thioglycolate broth (Oxoid)	37 °C for 24 h using anaerobic gas packs
Fusobacterium species: *F. nucleatum* (ATCC 25586) *F. varium* (ATCC 27725)	Todd' Hewitt broth (Oxoid) 5% defibrinated sheep blood	Muller–Hinton broth (Oxoid) supplemented with 5% yeast (Oxoid) and *Haemophilus* supplement	35–37 °C for 48–96 h using anaerobic gas packs
Helicobacter pylori strains: (B8) (reference strain) (clinical strain)	Columbia agar base (Oxoid) supplemented with: 7% foetal bovine serum /sheep blood (Davies Diagnostics), 10 mL Vitox (Oxoid), 2 mL *H. pylori* selective supplement (Dent) (Oxoid)	Brain heart infusion broth (Oxoid) supplemented with: 7% foetal bovine serum, 10 mL Vitox, 2 mL Dent	37 °C, 4–9 days using Pack Microaero (Camphylo) generating kit (Oxoid)
E. coli (ATCC 8739) *E. faecalis* (ATCC 29212)	TSA	Tryptone Soya broth (Oxoid)	37 °C for 24 h using anaerobic gas packs

Toxicity of plant extracts

In order to hatch brine shrimp larvae, artificial seawater was prepared by dissolving 16 g of Tropic Marine® salt in 500 mL sterile water. Thereafter, 0.5 g of brine shrimp larvae (*Artemia franciscana*) (Ocean Nutrition) was added to the prepared seawater. Seawater was selected because it promotes the growth of brine shrimp larvae. A mixture containing the brine shrimp larvae and seawater was exposed to constant light from a light emitting diode (LED) bulb. Then larvae were aerated using a rotary pump (Kiho) to promote a better hatch. The mixture was then left at room temperature (25 °C) for 1–2 days. Toxicity was investigated for all extracts that displayed noteworthy antimicrobial activities (MIC≤160 μg/mL) against any of the tested pathogens (Table 3). Both the dichloromethane:methanol and aqueous plant extracts were prepared to a stock concentration of 2 mg/mL, and then a starting concentration of 1 mg/mL was achieved after dilution. Organic extracts were dissolved in 2% v/v dimethyl sulfoxide and aqueous extracts were dissolved in sterile water.

Hatched shrimp were transferred into a shallow, four-sided container, and then the LED study lamp was placed next to the container facing the opening of the container. This placement allowed for maximum light exposure, which in turn allowed the shrimp to gather in one place for easy collection. A volume of 400 μL seawater containing the brine shrimp (numbering 39–75) was transferred to each well of the 48-well microtitre plate. Viability of the brine shrimp was confirmed by observation under a light microscope (Olympus) prior to adding the samples. A volume of 400 μL of each organic and aqueous plant sample was added to 48-well microtitre plates. Each test was done in triplicate. Thereafter, 32 mg/mL seawater and 1.6 mg/mL potassium dichromate (Sigma) were added as positive and negative controls, respectively. All shrimp that were found dead after 24 h and 48 h incubation were counted under the light microscope.

Plant extracts that displayed toxic effects were further tested at six concentrations (1000, 500, 250, 125, 63 and 31 μg/mL) to generate LC_{50} values that were determined using IBM® SPSS statistics and probit analysis. The LC_{50} value is defined as the concentration of a test material that possesses a toxic effect on half (50%) the tested shrimp. A lower LC_{50} value indicates a higher toxic profile of a material. Extracts with LC_{50} values lower than 249 μg/mL were considered highly toxic, 250 to 499 μg/mL moderately toxic, 500 to 999 μg/mL of low toxicity and values ≥1000 were considered non-toxic.[38]

Results and discussion

Antimicrobial analysis

The results of the antimicrobial assay expressed as MIC values are represented in Table 3. Antimicrobial activity was considered noteworthy for plant extracts when MIC values were ≤160 μg/mL. Moderate values were between 160 μg/mL and 1000 μg/mL and weak activity was classified as MICs of >1000 μg/mL. Poor activity is expressed by MICs greater than 8000 μg/mL.[22,39,40] For the aqueous extracts, *G. perpensa* (leaf and rhizome) was the most active with a MIC of 130 μg/mL against the *Clostridium* species. As the organic extracts showed better activity, only these results are presented in Table 3.

Antimicrobial activity was compared for leaf and other plant parts; 7 of the 10 plants evaluated (70%) showed better activity for leaves than for other plant parts. Interestingly, none of the plant extracts displayed noteworthy antimicrobial activity against the common gut pathogens *E. coli* and *E. faecalis.*

Antimicrobial activity against Gram-positive bacteria

Gram-positive bacteria included two *Clostridium* species: *C. perfringens* and *C. difficile*. The Gram-positive bacteria were more vulnerable to the extracts than were the Gram-negative bacteria. *Clostridium perfringens* was the most susceptible. Approximately 10% of the extracts displayed noteworthy antimicrobial activity against *C. difficile,* whereas 39% of the extracts displayed moderate activity. Approximately 39% of the extracts displayed noteworthy activity against *C. perfringens* and another 39% displayed moderate activity.

The organic extracts of *L. javanica* leaf showed the best antimicrobial activity with an MIC of 0.5 μg/mL against *C. perfringens*. This value was comparable to the control antibiotic ciprofloxacin (MIC=0.2 μg/mL). The traditional use of *L. javanica* corroborates with the antimicrobial activity against *Clostridium* species, as the leaf infusion is traditionally used to treat diarrhoea, which is one of the symptoms of food poisoning or pseudomembranous colitis.[41] Even though *L. javanica* displayed the best antimicrobial activity, to the best of our knowledge, this plant species has not been tested previously against *Clostridium* species. Other studies have instead focused on the antimicrobial activity of this plant species against common pathogens such as *S. aureus, E. coli, E. faecalis,* and *Pseudomonas aeuruginosa.*[42]

Table 3: The antimicrobial (MIC values in μg/mL) efficacy of organic plants extracts against neglected and common pathogens of the gut

Plant extract	Plant part used	Mean MIC value (μg/mL)											
		Gram positive		Gram negative								Commonly screened pathogens	
		Clostridium species		*Bacteroides fragilis* group				*Fusobacterium* species		*Helicobacter pylori*			
		C. d	C. p	B. f	B. o	B. v	B.t	F. n	F. v	H. p c	H. p r	E. c	E. f
Acokanthera oppositifolia	Leaf	>8000	**130**	2000	>8000	8000	2000	*1000*	4000	4000	*1000*	2000	4000
	Root	2000	*250*	*500*	>8000	>8000	2000	*1000*	4000	8000	2000	4000	4000
Aloe arborescens	Leaf	>8000	**30**	4000	4000	>8000	2000	2000	6000	>8000	**130**	4000	4000
Aloe ferox	Leaf	*750*	**130**	4000	2000	2000	2000	*1000*	2000	4000	*1000*	4000	2000
Aloe marlothii	Leaf	>8000	**130**	2000	>8000	4000	2000	4000	>8000	>8000	8000	4000	4000
Aloe tenuior	Leaf	2000	**2**	*380*	8000	4000	1000	2000	6000	2000	*500*	4000	2000
Antidesma venosum	Leaf	3000	**60**	*1000*	4000	4000	2000	4000	6000	*500*	*250*	4000	2000
Artemisia afra	Leaf	>8000	**8**	8000	2000	2000	*1000*	*500*	4000	*1000*	2000	2000	2000
Boophone disticha	Bulb	*1000*	*250*	*1000*	>8000	>8000	2000	4000	4000	>8000	>8000	4000	2000
Bridelia cathartica	Leaf	2000	**130**	2000	8000	2000	2000	*1000*	2000	4000	>8000	*1000*	2000
Bridelia micrantha	Leaf	>8000	*500*	*1000*	8000	8000	2000	2000	2000	*500*	*250*	2000	2000
	Stem	*750*	**130**	1500	*1000*	*1000*	*1000*	2000	4000	8000	8000	*1000*	2000
Catha edulis	Leaf	>8000	*1000*	*380*	*1000*	4000	>8000	4000	2000	4000	2000	2000	*1000*
Clematis brachiata	Stems	>8000	2000	2000	>8000	>8000	4000	4000	*1000*	*1000*	*1000*	4000	2000
Dichrostachys cinerea	Bulb	>8000	2000	>8000	2000	4000	2000	2000	2000	*1000*	*500*	*1000*	2000
Dodonaea viscosa	Leaf	>8000	*250*	2000	>8000	8000	*1000*	*500*	>8000	*1000*	*500*	>8000	>8000
Dombeya rotundifolia	Leaf	2000	*500*	>8000	2000	2000	4000	2000	2000	*500*	*1000*	4000	2000
	Stem	2000	2000	>8000	4000	4000	4000	2000	3000	>8000	>8000	4000	2000
Drimiopsis maculata	Bulb	*750*	*250*	3000	2000	2000	*500*	*500*	1500	>8000	2000	2000	2000
Ekebergia capensis	Leaf	2000	2000	4000	>8000	4000	2000	2000	2000	8000	2000	2000	2000
Elephantorrhiza elephantina	Roots	*500*	*500*	*1000*	*500*	1500	*1000*	*1000*	2000	>8000	8000	*500*	*500*
Eucomis autumnalis	Leaf	2000	>8000	4000	>8000	8000	2000	2000	4000	>8000	2000	4000	2000
Gunnera perpensa	Leaf	**130**	**2**	*750*	*250*	*750*	*1000*	*500*	*250*	8000	*1000*	2000	*1000*
	Rhizomes	**130**	**60**	*1000*	*500*	*1000*	*1000*	*500*	2250	>8000	4000	2000	4000
Hydrangea arborescens	Leaf	*1000*	4000	8000	>8000	>8000	>8000	2000	3000	2000	2000	4000	2000
Ipomoea purpurea	Leaf	2000	2000	8000	>8000	>8000	4000	4000	2000	4000	2000	4000	4000
Kigelia africana	Leaf	2000	*1000*	4000	8000	8000	*1000*	*1000*	2000	2000	*500*	*1000*	2000
	Fruit	3000	*1000*	4000	>8000	2000	*1000*	*1000*	2000	>8000	>8000	*1000*	2000
	Stem	*1000*	*500*	4000	8000	4000	*1000*	*1000*	4000	>8000	8000	4000	4000
Lippia javanica	Leaf	*1000*	**0.5**	20	4000	20	*250*	*190*	*250*	2000	2000	4000	2000
	Twigs	2000	**2**	2000	4000	2000	*500*	*500*	*310*	>8000	4000	2000	500
Mentha longifolia	Leaf	**60**	*250*	2000	2000	2000	*1000*	*500*	*750*	*1000*	3000	2000	2000
Osmitopsis asteriscoides	Leaf	*500*	*250*	2000	8000	2000	*500*	*500*	*750*	4000	2000	*1000*	*1000*
Oxalis corniculata	Leaf	2000	2000	>8000	8000	>8000	2000	4000	2000	2000	*1000*	2000	2000
Peltophorum africanum	Leaf	1500	*1000*	4000	4000	6000	2000	2000	4000	2000	2000	4000	2000
	Stems	*1000*	*1000*	2000	*1000*	4000	2000	2000	4000	4000	4000	*1000*	2000
Polygala fruticosa	Leaf	*250*	**20**	*500*	*250*	*1000*	**130**	**130**	*250*	*500*	*1000*	*500*	*500*
Rapanea melanophloeos	Leaf	4000	*1000*	4000	>8000	8000	*1000*	2000	*500*	*500*	2000	2000	4000
	Stem	4000	*500*	4000	4000	6000	2000	4000	2000	4000	4000	2000	2000
Rauvolfia caffra	Leaf	8000	2000	2000	>8000	8000	*1000*	*1000*	*1000*	*250*	*380*	*1000*	*1000*
Salvia africana-caerulea	Leaf	**130**	**30**	*380*	*250*	*380*	*1000*	*250*	1300	*500*	*750*	*500*	*500*
Scadoxus puniceus	Rhizomes	2000	2000	*1000*	2000	*1000*	**130**	*250*	2000	>8000	4000	2000	2000
Solanum incanum	Leaf	2000	**130**	8000	>8000	8000	*1000*	*1000*	3000	*1000*	*1000*	2000	2000
Salvia africana	Leaf	**130**	**130**	1000	1000	2000	1000	1000	2000	500	2000	2000	4000
	Stems	*1000*	*500*	2000	2000	4000	*500*	*1000*	*250*	8000	4000	2000	4000
Syzygium cordatum	Leaf	**130**	**130**	1000	2000	2000	2000	2000	*500*	*500*	1500	2000	2000
Tarchonanthus camphoratus	Leaf	*1000*	*500*	1500	2000	3000	*500*	*1000*	*250*	2000	2000	2000	2000
Tetradenia riparia	Leaf	**130**	**130**	*380*	2000	*250*	*250*	*250*	1300	2000	2000	*500*	*500*
Warburgia salutaris	Leaf	1500	2000	3000	2000	4000	*500*	*1000*	1500	*1000*	*1000*	2000	2000
	Stem	2000	*1000*	2000	2000	4000	*500*	*1000*	*1000*	8000	4000	2000	2000
Zanthoxylum capense	Leaf	>8000	4000	>8000	>8000	8000	8000	>8000	>8000	*500*	2000	4000	4000
Positive control		**0.156**[a]	**0.156**[b]	**0.04**[c]	**0.08**[c]	**0.08**[c]	**0.08**[c]	**0.08**[b]	**0.08**[b]	**0.08**[d]	**0.04**[d]	**0.04**[b]	**0.63**[b]
Negative control		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000
Culture control		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000

C. d: C. difficile *(ATCC 43593)*; ***C. p***: C. perfringens *(ATCC 13124)*; ***B. f***: B. fragilis *(ATCC 23745)*; ***B. o***: B. ovatus *(ATCC 8483)*; ***B. t***: B. thetaiotaomicron *(ATCC 29741)*; ***B. v***: B. vulgatus *(ATCC 8482)*; ***F. o***: F. varium *(ATCC 27725)*; ***F. n***: F. nucleatum *(ATCC 25586)*; ***H. p c***: H. pylori *(548) clinical strain*; ***H. p r***: H. pylori *(B8) reference strain*; ***E. c***: E. coli; ***E. f***: E. faecalis; [a]*metronidazole*; [b]*ciprofloxacin*; [c]*imipenem*; [d]*amoxicillin; clarithromycin. Values in bold = noteworthy activity; values in italics = moderate activity*

The organic extracts of *G. perpensa* (leaf 2 μg/mL and rhizome 130 μg/mL), as well as the leaf extracts of *S. africana-caerulea* (130 μg/mL and 30 μg/mL), *S. africana* (130 μg/mL), *Syzygium cordatum* (130 μg/mL) and *Tetradenia riparia* (130 μg/mL) displayed noteworthy antimicrobial activity against both *Clostridium* species. Traditionally, unspecified parts of *G. perpensa* are used for the treatment of stomach bleeding and the roots are used for other stomach ailments.[30] To date, no previous studies have reported on the antimicrobial effectiveness of this plant on neglected pathogens. Nevertheless, findings from the current study were comparable to those reported in the literature, that is, Madikizela et al.[43] reported good activity for the organic extracts of *G. perpensa* (leaf) against the gut pathogens *Campylobacter jejuni*, *E. coli*, *S. aureus* and *Shigella flexineri,* with MICs between 0.39 mg/mL and 0.78 mg/mL.

Traditionally, twig and leaf infusions of *S. africana-caerulea* are mixed with Epsom salts (magnesium sulfate) and lemon to treat stomach illnesses such as colic, diarrhoea, indigestion and stomach pain.[30] To the best of our knowledge, no antimicrobial study was found with regard to *S. africana-caerulea* and the gut pathogens selected for this study. However, several other studies have reported on the antimicrobial activity of *S. africana-caerulea* against other gut microorganisms.[44]

Spirostachys africana is commonly known as the jumping-bean tree and it is traditionally used for the treatment of stomach ulcers, stomach pain, dysentery, acute gastritis and diarrhoea.[31] The antimicrobial effects of *S. africana* on other pathogens has also been reported,[45] with leaf and twig extracts showing good activity against *S. aureus* at a mean MIC value of 0.78 mg/mL.

The antimicrobial activity of *S. cordatum* validates the traditional use as the bark is boiled in water, then the mixture is taken orally three times a day until diarrhoea resolves.[30] Mathabe et al.[31] reported *S. cordatum* to be effective against a wide variation of diarrhoeal pathogens, including *S. aureus*, *E. coli, S. typhimurium*, *Vibrio cholerae* as well as *Shigella* species, with MIC values in the range of 0.16–0.31 mg/mL.

In previous studies, *T. riparia* showed good antimicrobial activity against common pathogens of the gut.[28,44] *Tetradenia riparia* is a multi-branched shrub or small tree, the leaves of which are traditionally used in infusions to treat stomach aches and diarrhoea.[30] No study was found on the antimicrobial activity of *T. riparia* against *Clostridium* species. In a previous study[44], *T. riparia* was found to be active against *S. aureus* with an MIC value of 0.78 mg/mL. Good antimicrobial activity of *T. riparia* was also noted against oral pathogens.[28] Other extracts that displayed noteworthy activity against *C. perfringens* include *Acokanthera oppositifolia* (MIC=130 μg/mL), *Aloe arborescens* (MIC=30 μg/mL), *Aloe marlothii* (MIC=130 μg/mL), *Aloe tenuior* (MIC=2 μg/mL), *Antidesma venosum* (MIC=60 μg/mL), *Artemisia afra* (MIC=8 μg/mL), *Bridelia micrantha* (MIC=130 μg/mL), *Polygala fruticosa* (MIC=20 μg/mL), *Solanum incanum* (MIC=130 μg/mL) and *S. cordatum* (MIC=130 μg/mL).

Antimicrobial activity of organic extracts against Gram-negative bacteria

Gram-negative bacteria included eight bacterial groups which were further divided into two classes': (1) *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus, F. nucleatum* and *F. varium* and (2) Gram-negative micro-aerophiles (*H. pylori* reference and the clinical strain).

Gram-negative anaerobes

Three extracts displayed noteworthy pathogen-specific activity. A total of 37 of the organic extracts displayed moderate activity against one or more Gram-negative anaerobes. The organic extracts of *L. javanica* (leaf) exhibited the best antimicrobial activity in this category, being active against *B. fragilis* and *B. vulgatus*, with MIC values of 20 μg/mL for both bacteria. Other plant extracts that were active in this category include *P. fruticosa*, which was active against *B. thetaiotaomicron* and *F. nucleatum* with an MIC value of 130 μg/mL for both bacteria. *S. puniceus* was active against *B. thetaiotaomicron* with an MIC value of 130 μg/mL. *Polygala fruticosa* roots are used traditionally for the management of intestinal sores.[30]

Gram-negative microaerophiles

Microaerophiles included the *Helicobacter* spp. which are a group of microorganisms that require a lower concentration of oxygen to survive.[46,47] The organic extracts of *A. arborescens* displayed the best antimicrobial activity with an MIC value of 130 μg/mL against the reference strain. Comparative studies regarding anti-*Helicobacter* activities of *A. arborescens* were not found in the literature; however, it is not surprising that this species displayed good antimicrobial activity against *H. pylori*, because a decoction of the fresh leaves of *Aloe* species is traditionally used for management of *H. pylori* related infections.[18]

Antibiofilm assay

Results for the antibiofilm activities are categorised into four phases corresponding to biofilm developmental stages. First, the initial attachment of biofilms is represented at 4 h; biofilm formation at 24 h; and development of a mature biofilm at 48 h and 72 h. The results are presented in Table 4 and are interpreted either as weak antibiofilm activity (0–49%) or good antibiofilm activity (50–100%).[48] Negative percentage inhibition denotes enhancement rather than inhibition of biofilms. Values in bold typeface denote good antibiofilm activity. At initial cell attachment stage (4 h), 19% of the extracts had antibiofilm inhibitory activity with at least 50% reduction in cell attachment. Approximately 57% of the extracts displayed good antibiofilm development (24 h) with percentage >50%. Most of the extracts had better activity than ciprofloxacin, whereas 38% of extracts displayed good antibiofilm activity and stopped the development of mature biofilms at 48 h and 72 h. With the exception of the organic extracts of *A. tenuior*, *Bridelia cathartica* and *B. micrantha,* all extracts displayed good antibiofilm activity for at least one stage of biofilm development.

Table 4: The antibiofilm activity of plant extracts against *C. perfringens*

Plant extracts		% Inhibition			
	Plant part used	4 h biofilm	24 h biofilm	48 h biofilm	72 h biofilm
Acokanthera oppositifolia	Leaf	30.4	**64.8**	**52.1**	**59.6**
Aloe arborescens	Leaf	43.9	**62.9**	46.5	10.1
Aloe ferox	Leaf	31.4	**65.8**	45.8	**61.4**
Aloe marlothii	Leaf	43.4	39.8	**57.1**	**61.6**
Aloe tenuior	Leaf	38.9	40.4	14.0	07.9
Antidesma venosum	Leaf	17.6	**59.9**	12.2	40.2
Artemisia afra	Leaf	37.5	**53.7**	23.5	**75.1**
Bridelia cathartica	Leaf	-95.1	-33.0	-31.3	21.7
Bridelia micrantha	Stem	18.2	46,1	8.7	45.3
Gunnera perpensa	Leaf	**57.7**	**77.6**	**50.1**	23.8
	Rhizomes	39.1	**78.8**	**60.9**	**55.8**
Lippia javanica	Leaf	45.4	**77.2**	34.4	42.7
	Twigs	**59.5**	**57.5**	**52.6**	39.2
Polygala fruticosa	Leaf	42.4	41,3	**50.4**	38.9
Salvia africana-caerulea	Leaf	**82.8**	49.1	37,1	16.2
Solanum incanum	Leaf	34.9	-5.5	**55.3**	15.6
Spirostachys africana	Leaf	30.8	**71.6**	16.8	**51.5**
Syzygium cordatum	Leaf	24.2	4.0	32.6	**62.1**
Tetradenia riparia	Leaf	**51.9**	**73.2**	**77.9**	13.3
Aqueous extracts					
Gunnera perpensa	Leaf	32.4	5.1	42.5	26.7
	Rhizomes	-35.9	-36.9	42.5	**73.8**
Ciprofloxacin		**70.4**	**58.7**	**68.5**	**68.3**

The bold percentage inhibition values denote the active samples

The organic extract of *S. africana-caerulea* leaf displayed the best antibiofilm activity overall, at 4 h at which it exhibited a percentage inhibition of 82.8%. The organic extracts of *A. oppositifolia* (leaf), *G. perpensa* (leaf), *L. javanica* (twigs) and *T. riparia* (leaf) displayed good antibiofilm activities for at least three biofilm developmental stages. *Acokanthera oppositifolia* displayed good antibiofilm activity at 24 h, 48 h and 72 h, preventing both initial biofilm formation and development of mature biofilms. *Acokanthera oppositifolia* displayed poor activity at 4 h. It can thus be concluded from these results that *A. oppositifolia* was more effective on older biofilms. At 24 h, the activity of *A. oppositifolia* was greater than that of ciprofloxacin (64.8% vs 58.7%). To the best of our knowledge, this study is the first antibiofilm study of *A. oppositifolia.*

The organic extracts of *G. perpensa* (leaf) were active at 4 h, 24 h and 48 h, preventing the attachment, formation and development of mature biofilms, whereas the organic extracts of *G. perpensa* rhizomes were active at 24 h, 48 h and 72 h. *Gunnera perpensa* extracts were mostly active against mature biofilms.

Lippia javanica twigs were active at 4 h, 24 h and 48 h with similar inhibition percentages. This finding suggests that the activity of *L. javanica* is not dependent on the incubation period and can work at any stage of biofilm development. Concerning the best activity in the MIC assay (Table 3), it is very interesting to note that the *L. javanica* extract was not only active against planktonic cells of *C. perfringens* but also displayed activity at an additional three biofilm developmental stages. These results support a previous study in which it was found that the same plant extracts that had good antibacterial activity also had good antibiofilm activity.[48]

The organic extracts of *S. africana-caerulea* leaf stood out, with the highest antibiofilm activities at 4 h. At 4 h, *S. africana-caerulea* reduced cell attachment with a better reduction percentage (82.79%) than that of ciprofloxacin (70.35%). The antimicrobial activity of *S. africana-caerulea* decreased with an increase in incubation period, thus it can be concluded that *S. africana-caerulea* was more effective on new biofilms.

The organic extracts of *T. riparia* (leaf) demonstrated notable antibiofilm activity at 4 h, 24 h and 48 h, preventing cell attachment, stopping development of biofilms and development of mature biofilms. At 72 h, *T. riparia* displayed poor antibiofilm activity, meaning that it is more effective on premature biofilms than on mature biofilms.

This study is the first to report on the antibiofilm activity of plant extracts on *C. perfringens* biofilms. Globally, most plant-based studies have focused on the antibiofilm activity of medicinal plants against biofilm formers such as *E. coli*, *S. aureus* and *P. aeruginosa.*[49,50] For southern African plant species, studies undertaken on antibiofilm activity have been neglected. Only a few relevant studies have been investigated.[22] Most of these have focused on the antibiofilm activities of southern African medicinal plants against clinically important pathogens such as *Listeria monocytogenes*, *P. aeruginosa* and *C. albicans.*[48-52] The antibiofilm activity of southern African medicinal plants has been investigated against the oral pathogen *Streptococcus mutans.*[28]

The current study showed that some plant extracts that showed good antimicrobial activity against *C. perfringens* in the MIC assay are capable of inhibiting *C. perfringens* biofilms. Prevention of cell attachment proved to be more difficult to achieve than prevention of biofilm development in a mature biofilm. It is very surprising that many extracts displayed better activity at biofilm development stage (24 h) than at cell attachment stage (4 h), as a previous study reported that inhibiting initial cell attachment is easier than inhibiting preformed biofilms.[47]

Toxicity assay

The 22 medicinal plant extracts that displayed noteworthy antimicrobial activity (MIC ≤160 μg/mL) (Table 3) against neglected gut pathogens, were screened for toxicity. The results of the brine shrimp lethality assay for both organic and aqueous extracts are shown in Table 5. None of the aqueous extracts possessed toxic effects. At 24 h, none of the extracts displayed toxic effects. At 48 h, 82% of the tested extracts were non-cytotoxic and 18% of the extracts possessed toxic effects. Organic extracts of *A. oppositifolia*, *A. venosum, L. javanica* and *T. riparia* leaves were toxic, with percentage mortalities of 73.23%, 100%, 94.70% and 59.65%, respectively.

The majority of the tested plant extracts were non-toxic. The lowest toxic effects were observed for the leaf organic extracts of *A. marlothii, A. tenuior, B. cathartica* and *G. perpensa*, and the aqueous extracts of *G. perpensa* leaf and rhizome for which the percentage mortalities of 0% were displayed at both 24 h and 48 h. Similar conclusions were reached in a study by Gehring et al.[53] They found that the dichloromethane extracts of *G. perpensa* rhizome had no toxic effects on brine shrimp at a concentration of 1 mg/mL.

When the LC_{50} values of extracts of the plants that displayed toxic effects were tested (Table 6), *A. oppositifolia* leaf demonstrated low toxicity on the brine shrimp with an LC_{50} of 984 μg/mL. *Antidesma venosum* leaf was moderately toxic with an LC_{50} of 297 μg/mL after 48 h, whereas *L. javanica* and *T. riparia* leaves were highly toxic after 48 h with LC_{50} values of 88 μg/mL and 77 μg/mL, respectively. These plant extracts were highly active against planktonic bacteria and biofilms, but the high toxicity demonstrates a very low therapeutic index.

Table 5: Average % mortality of organic and aqueous extracts in brine shrimp lethality assay

Plant species	Plant part used	Average (%) mortality	
		24 h	48 h
Organic extracts			
Acokanthera oppositifolia	Leaf	0.0	**73.2**
Aloe arborescens	Leaf	0.0	6.0
Aloe ferox	Leaf	0.0	1.4
Aloe marlothii	Leaf	0.0	0.0
Aloe tenuior	Leaf	0.0	0.0
Antidesma venosum	Leaf	28.0	**100.0**
Artemisia afra	Leaf	2.4	11.3
Bridelia cathartica	Stem	0.0	0.0
Gunnera perpensa	Leaf	0.0	0.0
	Rhizomes	0.0	0.0
Lippia javanica	Leaf	6.0	**94.7**
	Small twigs	0.0	3.4
Mentha longifolia	Leaf	0.0	10.6
Polygala fruticose	Leaf	5.2	6.6
Salvia africana-caerulea	Leaf	3.3	7.4
Scadoxus puniceus	Rhizomes	6.7	25.2
Solanum incanum	Leaf	2.8	11.2
Spirostachys africana	Leaf	3.6	17.0
Syzygium cordatum	Leaf	1.6	2.4
Tetradenia riparia	Leaf	1.7	**59.7**
Aqueous extracts			
Gunnera perpensa	Leaf	0.0	0.0
	Rhizomes	0.0	0.0
Lippia javanica	Leaf	2.0	41.0
Controls			
Tropic marine water	Negative control	0.0	0.0
Potassium dichromate	Positive control	**100.0**	**100.0**

Values marked in bold denote mortality greater than 50% and are considered toxic.

Table 6: LC_{50} (μg/mL) values of plant samples that displayed cytotoxic effects

Plant extract	Plant part used	LC_{50} (μg/mL)	
		24 h	48 h
Acokanthera oppositifolia	Leaf	984	984
Antidesma venosum	Leaf	690	297
Lippia javanica	Leaf	624	**88**
Tetradenia riparia	Leaf	492	**77**

Values marked in bold signify highly toxic medicinal plants ($LC_{50} \leq 249$ μg/mL)

Table 7 displays a complete overview of the plant extracts that were active against at least one pathogen, displayed good antibiofilm activity at one biofilm development stage and had low cytotoxic effect. These plant species warrant further investigation.

Conclusion

The results from the MIC assay favour the traditional use of some plant extracts for intra-abdominal infections. The *Gunnera perpensa* organic extract was the most interesting of all the tested extracts, in that it displayed very good antimicrobial activity against *Clostridium* species (MIC = 2–130 μg/mL). The plant species also displayed good antibiofilm activity against new and older biofilms (average inhibition = 52.3% for leaf extract and 58.7% for rhizome), with no toxic effects (mortality = 0%). A notable result was seen in the aqueous extracts of *G. perpensa* (leaf and rhizomes), where noteworthy activity was observed against *Clostridium* species with MIC values of 130 μg/mL. In some instances, there was a direct relationship between the antimicrobial activity and the traditional use. For example, *S. africana* is traditionally used for diarrhoea. In the current study the organic extract of the leaf displayed noteworthy activity against *C. difficile* and *C. perfringens*. Also interesting is that none of the plant extracts displayed noteworthy activity against the common pathogens *E. coli* and *E. faecalis*. Biofilm results indicated that most of the plants that were active against *C. perfringens* were also effective against *C. perfringens* biofilms. The brine shrimp lethality assay results revealed that most of the plant samples were non-toxic to the brine shrimps. This study demonstrates that investigations should not only focus on common pathogens, but also on neglected pathogens which may yield excellent results not previously reported. This study contributes to the knowledge of the antimicrobial properties of plants commonly found in southern Africa.

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Authors' contributions

H.S.: method development; data collection; sample analysis; data analysis; writing – the initial draft; writing – revisions. C.L.: Assisted with biofilm assay; edited final draft of manuscript. G.C.: Method development; editing drafts; student supervision; project leadership; funding acquisition. S.v.V.: Conceptualisation of project; method development; data collection; sample analysis; data analysis; editing drafts; primary student supervision; project leadership; project management; funding acquisition.

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Table 7: Overall summary of the study

Plant species	MIC (≤160 μg/mL)	Biofilms mortality (>50%)	Toxicity mortality (>50%)
Aloe marlothii (L) (O)	130 μg/mL against *C. perfringens*	Active against mature biofilms (48 and 72 h)	0.0
Gunnera perpensa (L) (O)	2–130 μg/mL against *Clostridium* species	Active against biofilm attachment, development and mature biofilm (4, 24 and 48 h)	0.0
Gunnera perpensa (R) (O)	60 and 130 μg/mL against *Clostridium* species	Active against biofilm development and mature biofilm (24, 48 and 72 h)	0.0
Salvia africana-caerulea (L and T) (O)	30 and 130 μg/mL against *Clostridium* species	Active against biofilm attachment (4 h)	5.4

L, leaf; R, rhizomes; T, twigs; O, organic extracts

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Bacteria and yeast isolation and characterisation from a South African fermented beverage

Spontaneous fermentation of *motoho*, a southern African non-alcoholic sorghum beverage, results in products with inconsistent microbiological and sensory quality. We aimed to identify the microorganisms involved in the fermentation of *motoho* by using culture-dependent techniques as well as culture-independent polymerase chain reaction (PCR) screening and matrix-assisted laser desorption/ionisation time-of-flight analysis (MALDI-TOF). *Lactobacillus*, *Candida*, *Rhodotorula* and *Geotrichum* species were identified. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to evaluate the protein profiles of the isolated *Lactobacillus* species which produced protein bands of 14 kDa to 160 kDa, similar to those of other lactic acid bacteria isolated from various foods. A sensory panel evaluated and found significant differences ($p<0.05$) between the mouth feel, aroma and flavour of the traditional and modified *motoho*, with the latter being preferred. The microorganisms identified in this study could be used as starter cultures to optimise upscaled production of *motoho*.

Significance:

- Traditionally fermented products have variable quality and the microorganisms isolated in this study could be used to decrease the variability in this fermented sorghum beverage.

Introduction

Motoho is a traditionally fermented non-alcoholic sorghum beverage produced by the Sotho people of southern Africa using spontaneous fermentation. Traditional fermentation is an unrestrained process and depends upon microorganisms present in the raw material or utensils, or added as a starter culture, and often results in variations in the microbiological safety as well as the quality of products.[1] This variability can be overcome with the use of starter cultures[2] and through understanding the fermentation process so that end products with consistent quality can be produced[3]. The lack of durability in terms of shelf life as well as variability in the quality of traditionally fermented products could inconvenience the consumer and result in a decline in the purchasing of the product.[4] It was presumed that the phenotypic and genotypic characterisation of *motoho* would indicate that yeasts and lactic acid bacteria (LAB) would be the principal microorganisms responsible for fermentation, as is the case with the majority of African fermented foods.[4-9] Previous studies have included the preparation method of motoho[10] as well as the inhibitory effects of *motoho* fermentation on pathogenic microorganisms such as *Salmonella typhi*, *Salmonella* sp., *Escherichia coli* 0126 and *Shigella boydii*.[11] However, to our knowledge, this study is the first to isolate and characterise the microorganisms involved in the fermentation of *motoho*.

The aim of this study was to assist a small to medium enterprise (SME) owner with the upscaling of *motoho* production from household to more industrialised scale and also to assist with improving the shelf life and quality of the product so that market accessibility could be promoted. To achieve this, it was necessary to identify the microorganisms involved in the fermentation, using both phenotypic and genotypic methods. The identified microorganisms could then be used as potential starter cultures to decrease the variability during the upscaling of *motoho* production. Upscaling would provide more job opportunities and thereby uplift the local community in Welkom, South Africa, from which this SME operates.

Materials and methods

Traditional and modified production of motoho

Motoho was produced in the Department of Food Science pilot plant, University of Pretoria, South Africa, using traditional and modified methods and the methods described in detail in Figure 1. The modified *motoho* fermentation was prepared in triplicate (B, C and D) using the starter culture prepared from the back-slopping process, as described for the traditional process; and this preparation was added to a mixture of 2 kg sorghum flour in 20 L tap water. The mixture was added to a pasteuriser, heated to 30 °C and left to ferment for 14 h. This was followed by the addition of sodium benzoate and heating of the slurry to 90 °C with constant stirring for 20 min. The corn starch slurry was prepared in cold water and was added to the boiling mixture, followed by boiling for a further 20 min. The boiled product was cooled to temperatures of 39–49 °C, followed by the addition of sugar and mixing. The product was sieved using a hand-held household sieve and bottled in 1-L polyethylene terephthalate (PET) bottles. The bottled *motoho* products were stored at 4 °C and a shelf-life study was undertaken during 5 weeks of storage. Samples were taken at each process point during the manufacture of *motoho* for measurement of pH (Crison, Barcelona, Spain) and microbial evaluation.

Microbial enumeration, isolation and primary phenotypic characterisation

Triplicate samples of liquid suspension were collected aseptically at each process point for microbial analysis. The collection was done at the following stages: (1) sorghum + water, (2) sorghum + water heated to 30 °C, (3) fermented slurry, (4) heated and cooled sample, (5) sample after adding sugar and (6) bottling.

A mass of 5 g of each sample was diluted with 45 mL of sterile Maximum Recovery Diluent (MRD) consisting of 8.5 g NaCl (Merck, Darmstadt, Germany) and 1 g bacteriological peptone (Oxoid, Basingstoke, United Kingdom) per litre of distilled water. This solution was then serially diluted using MRD and the dilutions were surface inoculated

onto selective agar plates as follows: Tryptone Soy Agar (Oxoid) for the total plate counts of all viable mesophilic aerobic organisms and de Man, Rogosa and Sharpe Agar (MRS; Merck) for the isolation of LAB. The plates were incubated at 30 °C for 48 h. Yeasts were quantified on Potato Dextrose Agar (Merck) which was acidified using 10% tartaric acid. The plates were incubated for 120 h at 25 °C. The most probable number method was selected to test for the presence of *E. coli* using test tubes containing Lauryl Tryptose Broth (Oxoid). Tubes were incubated at 37 °C for 24 h and thereafter checked for the presence of gas production.

A total of 24 colonies of both yeasts and LAB were randomly selected from the Potato Dextrose Agar and MRS plates, respectively, that displayed counts of between 30 and 300 colony forming units (CFUs) and sub-cultured to obtain pure cultures.

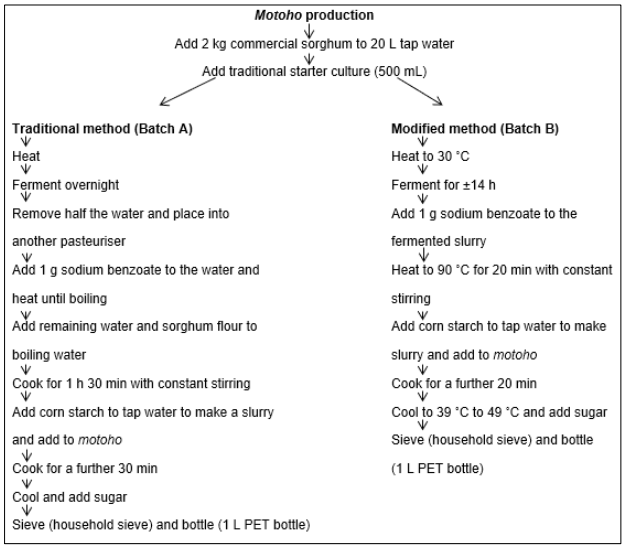


Figure 1: Process steps outlining the production of the traditional and modified *motoho*. Batch A was produced using the traditional process while Batch B was produced using the standardised, modified process.

Preparation of pure cultures for characterisation

For characterisation through polymerase chain reaction (PCR), pure cultures of LAB were inoculated into 10 mL of MRS Broth (Merck) and incubated at 30 °C for 72 h with agitation at 80 rpm in a New Brunswick 25D incubator shaker (New Brunswick, NJ, USA). The yeast colonies were inoculated into 10 mL of Yeast and Mould Broth (5 g enzymatic digest of gelatine (Sigma Aldrich, St. Louis, MO, USA), 3 g malt extract (Merck), 12 g dextrose.H_2O (BDH Chemicals, Poole, United Kingdom), 3 g yeast extract (Oxoid) in 1 L of purified H_2O) and incubated at 25 °C for 120 h with agitation at 80 rpm (New Brunswick).

PCR analyses of LAB and yeasts isolated from motoho

Extraction of LAB and yeast DNA

The LAB pure cultures inoculated in MRS Broth and incubated for 72 h were centrifuged at 17 000 x g for 30 min to obtain cell pellets. The supernatant was discarded, the cell pellets were frozen in liquid nitrogen and then ground to a fine powder. DNA extraction was then performed according to the method of Sakallah et al.[12] Yeast cell pellets were prepared from the pure yeast cultures that had been inoculated and incubated in Yeast and Mould Broth, as described, and the pellets were obtained by centrifugation at 17 000 x g for 30 min. The resulting pellets were washed once with 100 μL of a 0.1% sarkosyl (Sigma Aldrich) solution in distilled water and centrifuged again at 17 000 x g for 10 min and the sarkosyl discarded. The pellets were then frozen with liquid nitrogen and ground to a fine powder. Extraction of yeast DNA was performed according to the method of Rivas et al.[13]

PCR of isolated yeast and LAB from *motoho*

All PCR reaction mixtures (total volume 25 μL) contained 12.5 μL DreamTaq Green PCR Master Mix (2X) (Thermo Scientific, MA, USA) which comprised 0.4 mM deoxynucleotide triphosphates, Taq polymerase, buffer and 4 mM $MgCl_2$. Also added was 2.5 μL (1 μM) of the forward primer, 2.5 μL (1 μM) of the reverse primer, 2 μL of DNA (about 10 ng) and 5.5 μL nuclease free water. All PCR reactions were performed using an Applied Biosystems 2720 Thermocycler (Life Technologies, CA, USA).

The bacterial (LAB) 16S rRNA gene was amplified using the primers 27F modified (5'-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5'-TACGGYTAC-CTTGTTACGACTT-3') according to the method described by Zhang et al.[14] The universal primer N21 (5'-GGATCCGAGGGTGGCGGTTCT-3') was used to amplify yeast genomic DNA according to the method of Naumova et al.[15] The reference strains used (obtained from the culture collection of the Council for Scientific and Industrial Research, Pretoria, South Africa) were *Lactobacillus casei* ATCC 7469 (*Lb. casei)* as a positive control for LAB, while *Candida krusei* and *Rhodotorula* species were used as positive controls for the yeasts.

Electrophoresis of LAB and yeast PCR products

Electrophoresis was performed for LAB and yeast PCR products obtained from the amplification of LAB using the 27F modified primer sequence and that of yeast using the N21 primer. Amplicons (10 μL of PCR products) of the resulting LAB, yeast and reference strains were subjected to electrophoresis on a 1.2% agarose gel containing 0.5 μg/mL ethidium bromide. A GeneRuler 1-kb molecular ladder was used (Thermo Scientific). Electrophoresis was performed in 1 x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) (Biorad, CA, USA) using a current of 80 V. Gels were visualised via a UV transilluminator and images were acquired through a Syngene GBox Gel system (Syngene, Cambridge, UK) mounted with a camera (Syngene).

Matrix-assisted laser desorption/ionisation time-of-flight analysis

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) analysis was undertaken for LAB and yeasts obtained from various stages of *motoho* production using traditional and modified production methods. A total of 24 yeast and LAB isolates were appropriated from each stage of *motoho* production and sub-cultured onto acidified Potato Dextrose and MRS Agars, respectively, to obtain pure cultures. After 24 h, colonies were smeared onto the MALDI-TOF stainless steel target plate as a thin film (Bruker Daltonics, Bremen, Germany) and allowed to air dry. A volume of 1 μL of matrix solution formulated using α-cyano-4-hydrocinnamic-acid (Bruker Daltonics), 500 μL acetonitrile (50%; (Sigma Aldrich), 75 μL ultra-pure water (47.5%) and 25 μL trifluoroacetic acid (25%; (Sigma) was applied to the colony smear and allowed to air dry. Dried formulations were subjected to 40 laser pulses on six different positions of the sample for 15 s. The resulting spectra were compared to the reference spectra of microorganisms that were already present in the MALDI-TOF database. The MALDI-Biotyper (Bruker Daltonics) software was used to analyse the captured spectra. A standard bacterial sample of *E. coli* was included to validate and calibrate each run.

Protein analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis

LAB isolates confirmed via MALDI-TOF were further subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis in order to verify their identification. Only the LAB isolates which were identified to the genus level using MALDI-TOF analysis were chosen for SDS-PAGE protein profiling.

Single colonies were inoculated into 50 mL of MRS broth and incubated for 72 h at 30 °C with continuous agitation at 80 rpm using a temperature-controlled benchtop shaker (New Brunswick). The MRS broth was centrifuged at 17 000 x g for 30 min and the resultant pellets were frozen with liquid nitrogen and ground to a fine powder with a pestle and mortar. A volume of 500 μL of protein extraction buffer (750 mM Tris-HCl, pH 8.0; 15% sucrose; 0.25% protease inhibitor mix) (Sigma Aldrich) and 1%

mercaptoethanol was added and the ensuing suspension was centrifuged at 17 000 x g for 20 min. The supernatant containing total soluble protein was removed and placed into 2-mL Eppendorf tubes and stored at -20 °C.

Casting and running of SDS-PAGE

The casting and running of SDS-PAGE protein gels was performed according to the Laemmli method.[16] The resolving gel comprised 15% T, 3.3% Cbis and 40% SDS (w/v) while the stacking gel consisted of 6% T, 3.3% Cbis and 40% SDS (w/v). The electrophoresis buffer used was 1 x Tris/Glycine/SDS buffer (Biorad). About 20 μg of protein from each sample was loaded onto gels using 4 x sample buffer.[16] Samples were heated at 100 °C for 4 min, placed on ice to cool, pulsed to collect at the bottom of the tube and then loaded onto SDS-PAGE gels.

A PageRuler Prestained Protein Ladder (Thermo Scientific) was included in each gel to use to estimate the protein band size. A current of 120 V was used to conduct electrophoresis. Following three washes of 10 min each with sterile distilled water, proteins were stained for about 3 h or overnight by immersing the gels in PageBlue Protein Staining Solution (Thermo Scientific) with gentle agitation at room temperature. Gels were then washed repeatedly with sterile distilled water until all excess dye was removed and the gels scanned.

Shelf life of bottled motoho

The shelf life of bottled *motoho* stored at 4 °C was investigated over a 5-week period by determining the total plate counts of all viable mesophilic aerobic organisms determined using Tryptone Soy Agar (Oxoid) incubated at 30 °C for 48 h.

Sensory evaluation of traditional and modified motoho

A consumer panel was recruited at the University of Pretoria (Pretoria, South Africa) to evaluate the sensory differences between *motoho* produced using the traditional versus modified processes. The *motoho* was initially assessed for microbial quality and declared to be safe for consumption. The panel, which consisted of 21 men and 30 women between the age of 19 and 37 years, was selected based on their familiarity with fermented cereal products similar to *motoho*. The appearance, aroma, mouth feel, and flavour or overall impression (like or dislike) of the *motoho* samples were assessed. A nine-point hedonic scale was used to rate the products.[17]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the University of Pretoria, Division of Food Science. The ethical clearance number for this study was EC100811-045.

Statistical analysis

A one-way analysis of variance with a significance level of $p<0.05$ was used[18] for the microbiological and sensory analyses. Principal component analysis was used to classify the results from the sensory analysis according to selected descriptors using the PRINCOMP procedure of the SAS statistical software.

Results

Change in pH during manufacture of traditional and modified motoho

The initial pH of the traditional fermentation mixture was 6.6±0.2, which dropped overnight to 4.2±0.6. The initial pH of the *motoho* mixture from the modified process was 6.2±0.1, which dropped to 3±0.3 overnight. The pH of *motoho* from the modified process was therefore lower than that of the traditionally processed *motoho*.

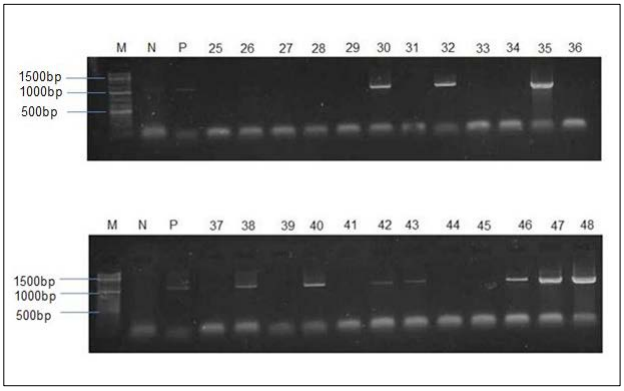
Microbiological counts of motoho *produced using two production methods*

The total plate counts, LAB and yeast counts increased at each process point – for both the traditional and modified processes – from the time the sorghum was mixed with water, after heating the mixture and after fermentation. The counts then decreased after cooking (Table 1). The microbial levels – namely total plate counts, LAB and yeast counts – at each corresponding process point were not significantly different ($p>0.05$) between the traditional and modified *motoho* (Table 1).

PCR analysis of LAB and yeasts isolated from motoho

PCR amplification of 16S rRNA for identification of LAB cultures

The positive control *Lb. casei* showed similar banding patterns to 10 of the 24 selected isolates which also produced a band of between 1000 and 1500 base pairs (bp) and were thus considered most likely to be *Lactobacillus* strains. The agarose gel electrophoresis of the PCR amplification products with primers 27F and 1492R is illustrated in Figure 2.



M, molecular size standard (1 Kb); P1, positive control Candida krusei*; P2, positive control* Rhodotorula*; N, negative control (nuclease free water)*

Figure 2: Polymerase chain reaction amplification of 16S rRNA for identification of lactic acid bacteria cultures.

Table 1: Mean microbiological counts (log CFU/g) of the traditional and modified *motoho* obtained during processing and on various selective media

	Total viable count (CFU/g)				Yeast count (CFU/g)				Lactic acid bacteria count (CFU/g)			
	A	B	C	D	A	B	C	D	A	B	C	D
Sorghum + water	2.6±0.4	2.7±0.4	3.5±0.9	3.1±0.2	1.1±1.1	<1.0	1.9±1.3	<1.0	<1.0	2.1±0.2	3.6±0.7	2.2±0.2
Sorghum + water heated	6.6±0.1	6.7±0.1	7.0±0.3	5.3±0.5	5.6±0.2	6.1±0.1	5.3±1.5	2.4±0.3	6.8±0.2	6.6±0.1	7.7±1.6	5.0±0.1
Fermented slurry	7.9±0.2	8.3±0.4	7.8±0.5	7.0±0.1	7.5±0.2	6.1±0.5	4.3±0.1	2.5±0.3	7.7±0.3	8.3±0.4	8.0±0.2	6.7±0.3
Cooled sample	2.7±2.1	<1.0	1.7±2.6	0.2±0.4	<1.0	<1.0	<1.0	<1.0	2.1±2.6	0.5±0.8	2.2±2.2	0.4±0.6
After sugar	5.4±0.1	3.3±0.6	0.2±0.4	<1.0	5.4±0.2	2.5±1.3	<1.0	<1.0	5.5±0.1	3.2±0.6	<1.0	<1.0
Bottled *motoho*	5.7±0.1	3.7±0.3	0.9±0.8	5.4±0.7	5.6±0.3	2.8±0.6	<1.0	<1.0	5.9±0.1	3.5±0.9	<1.0	5.7±0.5

Total viable count was obtained from Tryptone Soy Agar, yeast count from Potato Dextrose Agar and lactic acid bacteria count from de Man, Rogosa and Sharpe Agar.
A, traditionally produced motoho; only one batch was produced. B, C, D, modified production of motoho; three batches were produced.
Number of replicates (n)*=3 for all six process points.*

PCR amplification using the N21 primer for identification of yeast cultures

The primer N21 produced two characteristic bands of about 600 bp and 400 bp when assayed against the positive control *C. krusei*. The same primer produced four characteristic bands of 650 bp, 600 bp, 500 bp and 350 bp when *Rhodotorula* sp. was used as the positive control. Of the yeast isolates, 5 produced banding similar to *C. krusei* and 13 produced the four characteristic bands of *Rhodotorula* species. The agarose gel electrophoresis of the PCR products is illustrated in Figure 3.

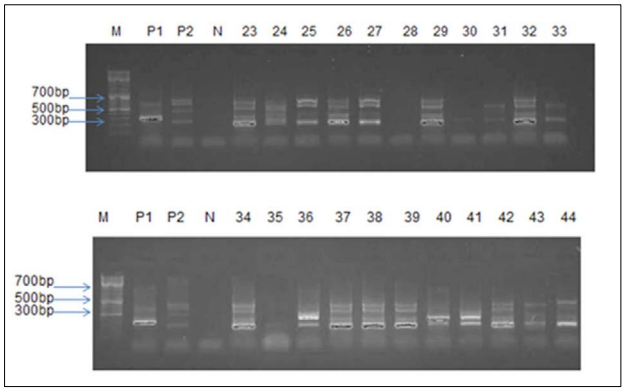


Figure 3: Polymerase chain reaction amplification using N21 universal primer for identification of pure yeast cultures.

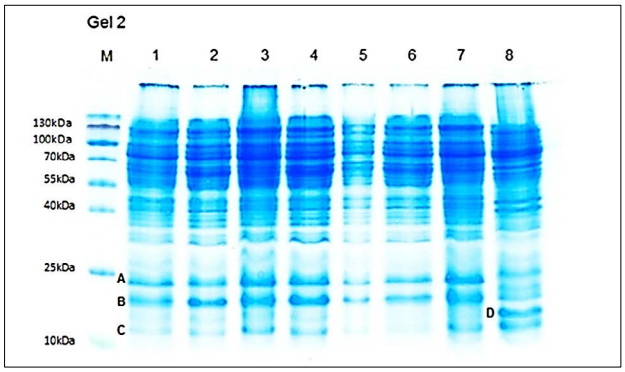
MALDI-TOF identification of LAB and yeasts isolated from motoho

According to the standards recommended by the manufacturer, a log score of between 2.300 and 3.000 is considered a highly probable identification to species level while values of between 2.000 and 2.299 signify a secure classification to the genus level and a possible identification to the species level. Levels of 1.700 to 1.999 denote a likely classification to the genus level while values below 1.699 are regarded as not reliable.

In the current study, out of a total of 24 putative LAB isolates, 2 *Lb. plantarum*, 10 *Lb. fermentum*, 2 *Lb. coryniformis* and 4 *Lb. paracasei* were identified to the probable species level. Of the yeast isolates, 12 were identified as *C. lambica,* 4 were identified as *C. kefyr*, 1 as *C. glabarata* and 1 as *C. pelliculosa*; 1 *R. mucilaginosa* and 2 *Geotrichum*, namely *G. candidum* and *G. silvicola,* were also detected.

Protein characterisation of LAB using SDS-PAGE

Isolates identified as *Lb. fermentum* (Lanes 1, 3, 4, 5; Figure 4) showed similarities in protein band patterns. In Lane 1, two distinct bands (A and B) of between 10 and 25 kDa can be seen.



M, PageRuler Prestained Protein Ladder; A–D represent prominent protein bands. The protein profile of Lb. fermentum *can be seen in Lanes 1, 3, 4, and 5, of* Lb. casei *can be seen in Lanes 6 and 8, and of* Lb. plantarum *can be seen in Lanes 2 and 7.*

Figure 4: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of total proteins from lactic acid bacteria showing similarities in protein patterns.

These bands are apparent throughout Lanes 1 to 7. A minor band of slightly more than 10 kDa is represented by C in Lane 1. This band can also be seen in Lanes 2 to 8. The protein profiles of *Lb. plantarum* are found in Lanes 2 and 7 while the profiles of the two *Lb. paracasei* isolates, seen in Lanes 6 and 8, differed slightly. A single prominent band of between 10 and 25 kDa is seen in Lane 8 (D) but not in Lane 7 nor in the other lanes.

Shelf life of bottled motoho

The shelf life of *motoho* was assessed using total plate counts over a 5-week period; the results are shown in Figure 5. *E. coli* was not detected throughout the production process of *motoho* or during the 5-week shelf-life study.

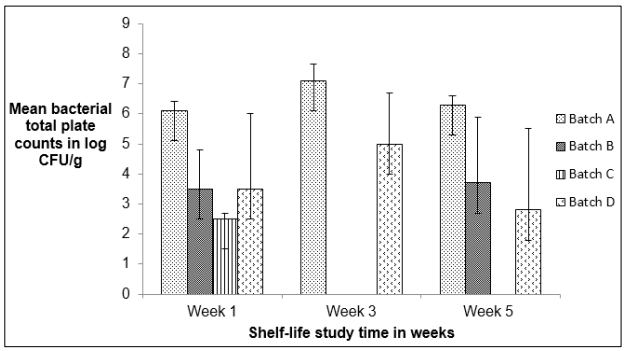


Figure 5: Total bacterial plate counts (log CFU/g) obtained for modified (Batches B, C, D) and traditional (Batch A) *motoho* over a 5-week shelf-life study.

Sensory analysis of traditional vs modified motoho

In the current study, there was no significant difference in the appearance of modified and traditional *motoho* ($p>0.05$). However, there were differences in aroma, mouth feel, and flavour or overall preference, with a preference for modified *motoho* ($p<0.05$). Figure 6 shows the principal component analysis of the sensory attributes of both the traditional and modified *motoho*.

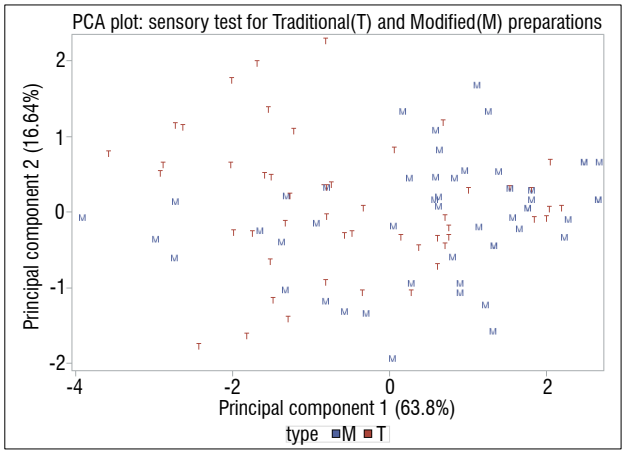


Figure 6: Principal component analysis (PCA) of the sensory evaluation of traditional (T) and modified (M) *motoho* by 51 panellists who rated the appearance, aroma, mouth feel and flavour of *motoho* using a nine-point hedonic scale.

Principal component 1 (PC1) and principal component 2 (PC2) were extracted and accounted for 63.8% and 16.64% of the variance across the samples, respectively, within a four-variable system which included aroma, flavour, mouth feel and appearance. Along PC1, there is a separation between the traditional and modified *motoho* with the modified *motoho* appearing predominantly towards the right, indicating high levels for flavour and mouth feel, while the traditional *motoho* was found towards the left of PC1, showing a high intensity in the negative side for flavour and mouth feel. Along PC2 there appears to be an even

distribution of both the modified and traditional towards the left and right sides, showing a high intensity in both the negative and positive sides in terms of appearance for both the traditional and modified *motoho*.

Discussion

This study is the first to evaluate the phenotypic, genotypic and sensory attributes of both traditional and modified *motoho*. Of the 24 LAB isolated from both the traditional and modified *motoho*, 10 were identified with 16S rRNA sequencing using the universal primers 27F and 1492R and produced bands of 1000–1500 bp, consistent with the band sizes generated by Zhang et al.[14] from extracted bacterial DNA obtained from soil iron-manganese nodules. Nucleic acid purification can be challenging because foods, like *motoho*, are not simple matrices and the levels of DNA extracted from a mixed bacterial culture can also vary.[19]

Of the 24 yeasts that were isolated from *motoho* and amplified using the N21 universal primer, 75% showed positive amplification. Using the N21 primer, Naumova et al.[15] also achieved effective amplification profiles of all 24 yeasts isolated from fermented sorghum beer.

No significant differences were found between the modified versus traditional production of *motoho* in terms of the yeast and LAB counts. However, simultaneous increases in the yeast and LAB counts were seen in the *motoho* produced by both methods. Mohamed et al.[20] and Muyanja et al.[21] obtained similar findings during the fermentation of kisra and togwa, respectively.

Yeasts and LAB are described as the principal microorganisms in fermented foods like ogi[22-23], kisra[20], hussuwa[24] and togwa[25]. This is consistent with the microbial populations of both the traditional and modified *motoho* which showed the predominance of four LAB strains, namely *Lb. fermentum*, *Lb. plantarum*, *Lb. coryniformis* and *Lb. paracasei*, and yeasts C. *lambica*, *C. glabarata*, *C. pelliculosa*, *C. kefyr*, *G. candidum*, *G. silvicola* and *R. mucilaginosa*. The strains *Lb. fermentum* and *Lb. plantarum* were isolated from a number of products including obushera, a collection of traditionally fermented cereal beverages[26], kenkey and ogi (fermented maize), fufu (fermented cassava) and kunun-zaki (fermented millet)[27]. *Lb. paracasei* forms part of the *Lb. casei* group and has been sequestered from fermented milk.[28] *C. krusei*, *G. candidum* and *R. graminis* were all isolated during the fermentation of maize for the production of ogi[29], while *R. mucilaginosa* was isolated from bili bili, a traditional beer from Chad made from sorghum[30].

Lactic acid bacteria that were isolated from *motoho* produced protein bands within the range of 14–160 kDa and showed similar protein banding patterns to LAB isolated from various food products, similarly to the study by Hébert et al.[31] on regional cheese. Slight variations in protein profiles exist within each LAB species isolated from *motoho*. This variation could be ascribed to genomic heterogeneity of the different species, as was proposed by Sánchez et al.[32] in relation to differences in the banding intensities of strains characterised as *Lb. plantarum* and *Lb. paracasei*, which were appropriated from sour dough[33]. The slight irregularities in the protein profiles could also be attributed to the differing origins, or process points of sampling the strains, as outlined by Pérez et al.[34] with reference to a study conducted by Ghazi et al.[35] Ghazi et al. aimed to identify the LAB, namely *Leu. mesenteroides* subsp. *dextranicum*, isolated from raw milk in Algeria using protein profiling, and also found differences in protein profiles.

Overall, the sensory profile (mouth feel, flavour and aroma) of the modified *motoho* was preferred over the traditional *motoho*. The higher yeast counts in the traditional *motoho* could have contributed to the lower sensory quality as yeasts are able to contribute to the organoleptic characteristics of the end products derived from fermentations.[36]

The microbiological counts of the *motoho* during the shelf-life study were unpredictable. This result could be because of the natural microflora of spontaneous food fermentations being uncontrollable, unpredictable and inefficient, or because they were destroyed by the heat treatments applied to the food.[37] The modified *motoho* had lower microbiological counts over the 5-week period, possibly because the lower pH of the modified *motoho* may have had a preservation effect.[38] Overall, the *motoho* microbial counts decreased for the modified process by Week 5, while there was a slight increase in microbial counts for the traditional *motoho*. These results are consistent with the results obtained in a shelf-life study on togwa[25] in which the CFU/g decreased with increased storage time. A limitation of this study was that not all putative LAB isolates were identified using PCR and the low percentage of amplification of LAB DNA could be accounted for by the variability in DNA extraction.

Conclusion

The LAB and yeasts were identified to the species level using phenotypic and genotypic techniques. *Lb. fermentum* seemed to be the predominant microorganism during the production of *motoho* and will be characterised as a potential starter culture for future research on the large-scale production of *motoho*. Also, the modified method of *motoho* production resulted in a product with preferable sensory attributes and this method could be used during the upscaling of *motoho*. The modified *motoho* showed lower microbial counts than the traditional *motoho* throughout the 5-week shelf-life study and could have a longer shelf life. The results of this study may assist the SME in producing a more consistent product during upscaling. Future studies could include selecting specific microorganisms from those isolated and identified during this study to be used as starter cultures for the production of *motoho*.

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Authors' contributions

S.S.M. conducted the data analysis, collected the samples and wrote the initial draft. N.R.D. was responsible for the conceptualisation, assistance with methodology and writing revisions as well as student supervision and funding acquisition. L.S. assisted with writing revisions. E.M.B. was responsible for conceptualisation, assistance with methodology, student supervision, and writing revisions.

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Identification of lactic acid bacteria and determination of selected biochemical properties in *emasi* and *emahewu*

Fermented foods are produced at household level for personal consumption in the Kingdom of Eswatini (formerly Swaziland). In this study, we determined the biochemical aspects, enumeration, isolation and identification of lactic acid bacteria (LAB) in *emasi* and *emahewu* – two Swazi traditional fermented foods. *Emasi* had an average pH of 4.68, titratable acidity of 0.9% and LAB count of 8.25 log CFU/mL. *Emahewu* had a pH of 3.62, titratable acidity of 0.4% and LAB count of 8.10 log CFU/mL. The LAB counts were consistent with observations for similar African fermented foods. The LAB from *emasi* and *emahewu* were identified through Gram stain, catalase reaction, sugar assimilation tests using API 50 CH test strips, and sequencing of 16S rDNA. It was found (from nine isolates) that *Lactococcus lactis* subsp. *lactis* and *Leuconostoc mesenteroides* were the common strains in *emasi*. *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *paracasei* and *Lactobacillus brevis* were also detected. *Lb. plantarum, L. mesenteroides* ssp. *mesenteroides, Lactobacillus fermentum, Lb. brevis, Wessella confusa, Lactobacillus acidophilus* and *Lb. lactis* were found in *emahewu* (from 16 isolates). This finding was consistent with LAB found in a South African fermented milk, in which common genera were *Leuconostoc, Lactococcus* and *Lactobacillus.* Strains found in *emahewu* – mainly *Lactobacillus* spp., *Weissella* and *Enterococcus* – are similar to those found in *ting*, a South African fermented non-alcoholic beverage.

Significance:

- This study provides the first documentation of microbial and biochemical aspects of the Swazi traditional fermented foods, *emasi* and *emahewu*.

Introduction

Fermentation of food is one of the oldest forms of food preservation.[1] Several studies have shown how this technique helps in preventing food-borne illnesses, including childhood diarrhoea.[2] Consumption of fermented foods is thought to contribute to good health because of the benefit of their microflora to the human gut.[3]

Fermented foods can be grouped into four categories: alcohol, lactic acid, acetic acid and alkali fermented foods.[1,4] Several traditional African fermented cereal grain foods, such as *mahewu* (sour sorghum or maize meal non-alcoholic beverage from South Africa, Zimbabwe and Lesotho), *togwa* (thin sour maize meal porridge from Tanzania), *kenkey* (thick sour maize meal porridge from Ghana), *amasi* (spontaneously fermented milk from southern Africa) and *motoho* (thin sour sorghum porridge or beverage from Lesotho), are largely products of lactic acid fermentation.

Lactic acid bacteria (LAB) have been found to be the predominant microorganisms in most of these products.[1] However, yeasts are also important in alcoholic fermented foods[4], and may be accidental contaminants in fermented milk[5,6]. Mathara et al.[7] found that *Lactobacillus* species (*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus paracasei* and *Lactobacillus acidophilus*) were predominant in *kule naoto*, Kenyan traditional fermented milk produced by the Maasai. Other genera isolated from *kule naoto* were *Enterococcus*, *Lactococcus* and *Leuconostoc*. Schoustra et al.[8] reported that *Lactobacillus* and *Weissella* were common genera, together with *Lactococcus*, *Streptococcus* and *Leuconostoc,* in Munkoyo and Chibwantu, traditional non-alcoholic fermented beverages popularly consumed in Zambia. *Emasi* and *emahewu* are non-alcoholic lactic acid fermented traditional foods produced by households in Eswatini.

The preparation methods of *sancta* (fermented maize meal), *incwancwa* (fermented porridge), *emasi* (fermented milk), *emahewu* (non-alcoholic cereal beverage), *umcombotsi* (alcoholic sorghum or millet beverage), *mankanjane* (malt distilled spirit), *buganu*/*marula* wine and papaya beer (fermented fruit mashes) have been previously outlined.[9] However, the microbial flora responsible for the fermentation has not been studied. The aim of this study, therefore, was to investigate the microbial diversity, to isolate potential probiotic LAB strains and to identify LAB in *emasi* and *emahewu*. Some of the biochemical properties were also investigated. This step is important in up-scaling and possible commercialisation of these products.

Materials and methods

Location of study

This study was done in the Hhohho Region of the Kingdom of Eswatini (formerly Swaziland). Hhohho Region is in the highveld of the country where the temperatures range from very cold to warm. The Region is divided into 14 local administrations called *tinkhundla*. Samples were collected from 5 *tinkhundla* that were randomly selected from the 14 *tinkhundla* using a lottery system.

Sample collection

Samples of *emasi* and *emahewu* were collected from nine locations within the five *tinkhundla*: Lobamba (coded L), Mangwaneni (M), Zone 4 (Z4), Motshane (Mot), Mbabane (Mb), Ezulwini (Ez), Mvutjini (Mv), Sitjeni (S) and

Ntfonjeni (Nt) areas in Hhohho, Swaziland. At each *inkhundla*, a list of members of the community who were known to prepare fermented foods was compiled with the assistance of community leaders, such as the *umphakatsi* or schoolteachers. Samples of fermented food were then collected from the households that were randomly selected from the list. The samples were collected in sterile screw-capped bottles and ferried in a cooler box to the laboratory at the University of Swaziland (a distance of 5–75 km) for analysis.

Preparation of emahewu and emasi

Women who prepared *emahewu* explained that the product was prepared by thoroughly mixing 1 kg maize meal with 5 L of water. The mixture was then cooked until well gelatinised into a soft porridge called *umhidvo,* then cooled (to 25–30 °C) and left to ferment at room temperature (25–30 °C; 2–5 days). Malt was not added during preparation of the product, therefore *emahewu* lacked the enzymes that come with addition of malt to trigger the start of fermentation. However, some households reported adding sugar or a peeled potato, therefore some bacterial inoculum may have originated from addition of potato and/or from bacteria that may have been present on utensils used during preparation.

Emasi was prepared by leaving raw milk to naturally ferment at room temperature (25–30 °C; 2–3 days) using plastic or metal containers or clay pots. The whey was sometimes strained to give a thick product. Back-slopping – which is inoculation using substrate from a previous fermentation – is often used during fermentation of milk to *emasi*.

Determination of pH and titratable acidity

The pH was determined using a Hanna Instruments pH meter (HI 8314, Leighton Buzzard Bedfordshire, UK) after calibrating with buffers at pH 4 and pH 7. Titratable acidity (TA) was determined using standardised 0.1 N NaOH (Rochelle Chemicals, Johannesburg, South Africa) according to the Association of Official Analytical Chemists (AOAC) method no. 947.05.[10]

Microbiological analysis

Enumeration of LAB

The fermented samples were analysed immediately upon arrival at the laboratory. LAB were enumerated on De Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK; CM0361) (selective agar) by spreading 0.1 mL of appropriate serial dilutions and incubating anaerobically at 30 °C for 48 h. Anaerobic conditions were created using an Oxoid anaerobic gas generating system (Oxoid, Basingstoke, UK, BR0038B) according to the manufacturer's instructions.

Isolation and selection of LAB strains

Colonies with a different appearance (based on colour, shape and size) were extracted from the MRS agar and purified by streaking on a fresh MRS agar plate. The purification process was repeated until single colonies with distinct appearance were obtained. The pure isolates were tested for Gram and catalase reactions. Cell morphology was observed under the microscope. The isolates that were Gram positive and catalase negative were taken as presumptive LAB. The LAB isolates were stored at -20 °C in MRS broth (Biolab, Wadeville, South Africa; HG000C87.500) containing 20% (v/v) glycerol until required for further tests.

Identification of LAB using Analytical Profile Index kits

The frozen LAB isolates were thawed and resuscitated by inoculating into fresh MRS broth and incubating at 30 °C for 24 h. A portion of the fresh culture was streaked onto MRS agar, which was then incubated anaerobically for 48 h. The pure colonies were extracted and inoculated onto Analytical Profile Index (API) 50 CH (bioMerieux, Marcy l'Etloile, France; Ref 50 300) test strips according to the manufacturer's instructions. The sugar fermentation profiles were then used to identify the isolates using API identification software (APIWEB™). A total of 16 LAB strains from *emahewu* and 9 LAB strains from *emasi* were identified using the API 50 CH kit. The carbohydrate profile was generated based on substrate metabolism using the API 50 CH kit. The API 50 CH approach is a well-established accurate method for manual microorganism identification for Gram-positive and Gram-negative bacteria and yeast to the species level based on extensive databases. The system offers a large and robust database accessible through the Internet-based APIWEB™ service. The method is economical to run and user-friendly.

Identification of LAB by sequencing 16S rDNA

Identification of LAB was performed at Inqaba Biotec Industries (Pretoria, South Africa). Briefly, DNA was extracted using ZR Fungal/Bacteria DNA™ kit (Zymo Research, Irvine, CA, USA). The 16S rDNA target region was amplified using DreamTaq™ DNA polymerase (Thermo Scientific™, Waltham, MA, USA) and the primers 16S-27F (sequence 5′-AGAGTTTGATCMTGGCTCAG-3′) and 16S-1492R (sequence 5′-CGG-TTACCTTGTTACGACTT-3′). Polymerase chain reaction (PCR) products were gel extracted (Zymo Research, Zymoclean™ Gel DNA Recovery kit), and sequenced in the forward and reverse directions on the ABI PRISM™ 3500 x l Genetic Analyser. Purified sequencing products (Zymo Research, ZR-96 DNA Sequencing Clean-up™ kit) were analysed using CLC Main Workbench 7 followed by a BLAST search on the database of the US National Center for Biotechnology Information.[11] Of the 16 LAB strains initially identified from *emahewu* using the API 50 CH kit, 9 were identified using the 16S rDNA method.

Statistical analysis

Mean (± standard deviation) was calculated for the pH, TA and microbial counts for the samples in the different categories using Microsoft Excel. The statistical significance ($p<0.05$) of the data sets was evaluated using Statistical Package for Social Science (SPSS) software.

Results and discussion

Emasi

pH and TA

The average pH of *emasi* was 4.68±0.25, and TA was 0.9±0.08% (Table 1), which corresponds well with values obtained in other studies for naturally fermented milk. For example, Kebede et al.[12] reported that *sethemi*, South African naturally fermented milk similar to *emasi,* had pH values of about 4.1–4.3. Beukes et al.[13] also reported that the pH of indigenous fermented milks from South Africa and Namibia ranged from 4.0 to 5.4, with an average of 4.6. *Amasi* produced at household level in Zimbabwe was found to have a mean pH of 3.98 and 1.0% TA.[14] Gran et al.[15] found that the pH of naturally fermented *amasi* produced by smallholder producers in Zimbabwe was about 4.2 after 48 h fermentation. *Nunu* is a Ghanaian spontaneously fermented milk with the consistency of yoghurt and a pH of about 3.4 after 48 h of fermentation.[16] However, the reported TA of 4.5% for *nunu* was uncharacteristically high compared with the values recorded for *emasi*, *amasi* and other similar products in southern Africa. In comparison, Moyane and Jideani[17] found that the pH of commercially produced *amasi* in Venda, South Africa, ranged from 4.22 to 4.34, with an average TA of 0.8%, which is close to what was recorded for spontaneously fermented *emasi*.

Table 1: The pH, titratable acidity and lactic acid bacteria (LAB) count of *emasi*, a Swazi naturally fermented milk

Sample code	pH*	Titratable acidity (% lactic acid)	LAB count (log CFU/mL)
MOT-emasi	4.31±0.01	1.0±0.03	8.34
Nt-emasi-1	4.57±0.01	1.0±0.04	8.69
Nt-emasi	4.52±0.51	0.8±0.03	8.82
L-emasi	5.03±0.07	0.8±0.07	7.30
Mb-emasi-1	4.98±0.11	0.9±0.04	7.78
Mb-emasi-2	4.87±0.03	0.9±0.01	8.24
Mb-emasi-3	4.62±0.1	0.9±0.03	8.36
Mb-emasi-4	4.55±0.03	0.9±0.01	8.45
Average	4.68±0.25	0.9±0.08	8.25±0.49

MOT, Motshane; Nt, Ntfonjeni; L, Lobamba; Mb, Mbabane (locations from where samples were collected)
**There were no significant differences in the column (p>0.05).*

The pH range for *emasi* (Table 1) was 4.31–5.03. Although there were variations in pH of the samples, the deviations were not significant ($p>0.05$). The differences in varying values of pH in Table 1 may be attributed to the variations in the amount of available substrate for LAB to ferment, the type and quantity of predominant fermenting LAB (*emasi* production often involves back-slopping), and the duration of fermentation.

Enumeration of LAB

The LAB counts in *emasi* ranged from 7.30 to 8.82 log CFU/mL (translating to an average of 8.25±0.49 log CFU/mL) (Table 1). The LAB counts were very comparable to those of similar African naturally fermented milk products. For instance, the presumptive LAB counts in indigenous spontaneously fermented *amasi* from South Africa were about 7.7 x 10^8 CFU/mL (8.89 log CFU/mL).[13] Zimbabwean *amasi* had a LAB ranging from 8.29 to 9.88 log CFU/g[14], while a Nigerian fermented milk, *nono*, was found to have LAB counts of about 9.8 x 10^6 CFU/mL (6.99 log CFU/mL). In addition, Egyptian traditional fermented milk, Laban Zeer, had LAB counts of up to 7.4 log CFU/g. The Ghanaian *nunu* was also reported to have LAB counts of up to 9 log CFU/mL after 48 h fermentation.[16] In contrast, Matsheka et al.[18] reported a much lower value of 5.3 log CFU/mL LAB in *madila*, Botswanan spontaneously fermented milk.

Other studies on non-African fermented milks showed similar trends for LAB counts. Traditional naturally fermented goat's milk collected from households in the Haixi Region of China had LAB counts of 2.5 x 10^8–3.0 x 10^9 CFU/mL (8.4–9.5 log CFU/m).[19]

There was a relationship amongst the pH, TA and LAB of *emasi*. The LAB fermented the lactose in raw milk that led to production of organic acids. The organic acids lowered the pH and increased the TA. As the acidity in *emasi* increased over the processing time, it inhibited the growth of low tolerant LAB. The amount of fermentable lactose in raw milk therefore had an influence on pH and TA.

Emahewu

pH and TA

The average pH of *emahewu* was 3.61±0.55, ranging from 2.95 to 4.51. The TA was 0.42±0.17% (Table 2). A similar product prepared in Zimbabwe, which is also called *mahewu*, had a final pH of 3.0.[20] This product had a TA of about 0.9% after 48 h fermentation, which is higher than that observed for *emahewu*. The Zimbabwean *mahewu* is made with maize meal and sorghum malt flour, which is probably the reason for production of higher amounts of organic acids. Sorghum malt is not added during preparation of *emahewu*.[9] The pH in *bushera,* a non-alcoholic sorghum-based beverage from Uganda, was found to range from 3.7 to 4.5,[21] which is close to the values obtained for *emahewu*. The TA of this product was 0.5%, which tallies with the results of the current study and the pH values obtained.

Table 2: The pH, titratable acidity and lactic acid bacteria (LAB) count of *emahewu*, a Swazi non-alcoholic fermented beverage

Sample code	pH*	Titratable acidity (% lactic acid)	LAB count (log CFU/mL)
L-emah-1	4.34±0.2	0.2±0.03	6.91
L-emah-2	4.17±0.1	0.4±0.03	7.78
L-emah	3.28±0.04	0.5±0.06	9.30
L-emah-3	3.86±0.06	0.5±0.04	8.75
Nt-emah	3.84±0.06	0.5±0.03	8.14
Z4-emah-1	4.51±0.08	0.8±0.03	6.88
L-emah-20	2.95±0.13	0.4±0.04	8.11
L-emah-21	3.09±0.1	0.4±0.03	8.67
L-emah-22	3.15±0.21	0.3±0.04	8.43
Ez-emah	3.30±0.21	0.2±0.01	7.74
Mv-emah	3.24±0.2	0.4±0.06	8.41
Mean	3.61±0.55	0.42±0.17	8.10±0.74

L, Lobamba; Nt, Ntfonjeni; Z4, Zone 4; Ez, Ezulwini; Mv, Mvutjini (locations from where samples were collected)
**There were no significant differences in the column ($p>0.05$).*

The pH range for *emahewu* (Table 2) was 3.09–4.51. Although there were variations in pH of the samples, the deviations were not significant ($p>0.05$). The differences in varying values of pH in Table 2 may be attributed to the variations in the amount of available substrate for LAB to ferment, the type and quantity of predominant fermenting LAB, and the duration of fermentation.

Enumeration of LAB

The LAB counts in *emahewu* ranged from 6.88 to 9.30 log CFU/mL (translating to an average of 8.10±0.74 log CFU/mL) (Table 2). The LAB counts were within the range expected when compared to those of other studies. Muyanja et al.[21], in their study of *bushera*, found that the LAB counts varied between 7.1 and 9.4 log CFU/mL. LAB counts in homemade *mahewu* from Zimbabwe increased from 2.0 to 8.0 log CFU/mL after 72 h of fermentation.[20] *Ting* is a non-alcoholic beverage prepared in Botswana and is made from sorghum meal and malt. The LAB counts of *ting* were found to range between 8.08 and 10.1 log CFU/g.[22]

As with *emasi*, there was a relationship amongst the pH, TA and LAB of *emhewu*. The LAB fermented the carbohydrates (starch and some sugars) in maize meal used to make *emahewu* that led to production of organic acids. The organic acids lowered the pH and increased the TA. As the acidity in *emahewu* increased over the processing time, it inhibited the growth of low tolerant LAB. The amount of fermentable carbohydrates in maize meal therefore had an influence on pH and TA.

Identification of LAB

The isolates were initially screened as presumptive LAB using the Gram stain, catalase test and microscopic examination. The Gram-positive, catalase-negative isolates were identified to species level using API 50 CH test strips and by sequencing the 16S rDNA as shown in Table 3 and Table 4 and carbohydrate profile of LAB was as shown in Table 5.

Emasi

Among the 9 *emasi* isolates identified using the API 50 CH kits, 4 were identified as *Leuconostoc mesenteroides* ssp. *mesenteroides*/*dextranicum*, 2 as *Lactococcus lactis*, 1 as *Lb. plantarum* and the other 2 as *Lactobacillus brevis* (Table 3). The four *Leuconostoc* isolates were characterised by sequencing the 16S rDNA and were identified as *Leuconostoc pseudomesenteroides* (Table 4), which was in close agreement with the API identification. The small difference in the identification of LAB between API and sequencing 16S rDNA methods is because the latter method is much more accurate than API.

In a study on South African naturally fermented milk, Beukes et al.[13] reported that the genera *Leuconostoc*, *Lactococcus* and *Lactobacillus* were the main flora. The dominant lactococci species in the South African product was *Lactococcus lactis* subsp. *lactis*, while most of the *Leuconostoc* isolates were identified as *Leuconostoc mesenteroides* subsp. *dextranicum,* similarly to the findings for Swazi *emasi*. Other species identified in that study include *Leuconostoc citreum*, *Leuconostoc lactis*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lb. plantarum*.

Mutukumira[14] observed that *Lactococcus lactis* subsp. *lactis* was the predominant strain isolated from *amasi,* spontaneously fermented milk produced in Zimbabwe. The Zimbabwean *amasi* is produced in a similar way to *emasi*, which may explain the similarity in microbial ecology. Slight differences that may be found in the microbial diversity can be attributed to different types of containers used, as well as the environment under which the fermentation is done. Clay pots, metal containers, calabashes and gourds are often used and have been found to impact the microbial diversity.[12] The current observations also agree with recent work by Osvik et al.[23] who studied the bacterial diversity of *amasi* from the EkuPindiseni community of KwaZulu-Natal in South Africa using 16S rRNA and denaturing gradient gel electrophoresis for identification. The majority of the strains found were in the genus *Lactococcus*, as well as *Lactobacillus*, *Leuconostoc* and *Enterococcus*. However, a study by Mathara et al.[7] showed that the genus *Lactobacillus* was predominant in *kule naoto*, Kenyan traditional fermented milk produced by the Maasai, in which the major *Lactobacillus* species was *Lb. plantarum*, followed by *Lb. fermentum*, *Lb. paracasei* and *Lb. acidophilus*. Other genera that were isolated in *kule naoto* were *Enterococcus*, *Lactococcus* and *Leuconostoc*.

Laban Zeer produced in Egypt seems to have similar flora to that of *emasi*. Saleh[24] identified the LAB species in Laban Zeer as *Leuconostoc mesenteroides* subsp. *cremoris*, *Lb*. *rhamnosus*, *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbruekii* subsp. *bulgaricus*, *Lb. curvatus* subsp. *curvatus* and *Lb. acidophilus*. The most frequently isolated LAB species were found to be *Leuconostoc mesenteroides* subsp. *cremoris* and *Lb. rhamnosus*.

The Swazi fermented milk's microflora is therefore similar to that in other naturally fermented products from southern Africa, in particular *amasi* from South Africa and Zimbabwe, in which the dominant genera are *Leuconostoc, Lactobacillus and Lactococcus.*

Emahewu

Of the 16 isolates from *emahewu* identified using the API 50 CH test kit, 6 were identified as *Lb. plantarum*, 3 as *Leuconostoc mesenteroides* ssp*. mesenteroides*, 2 as *Lb. fermentum*, 2 as *Lb. brevis*, and 1 as *Lb. collinoides* (Table 3).

Table 3: Identification of lactic acid bacteria isolated from Swazi traditional fermented *emasi* and *emahewu* using API 50CH kit

	Isolate code	Identity
		Emasi
1	L-emasi-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
2	L-emasi-5	*Leuconostoc mesenteroides* ssp. *mesenteroides*
3	L-emasi-7	*Leuconostoc mesenteroides* ssp. *mesenteroides/*dextranicum
4	L-emasi-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
5	Mot-emasi-7	*Lactococcus lactis* ssp. *lactis*
6	Nt-emas-2	*Lactococcus lactis* ssp*. lactis*
7	Nt-emas2-6	*Lactobacillus paracasei* ssp. *paracasei*
8	Nt-emas-5	*Lactobacillus plantarum*
9	Nt-emas-6	*Lactobacillus brevis*
		Emahewu
10	L-emah-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
11	L-emah-3	*Lactobacillus plantarum*
12	L-emah-5	*Lactobacillus brevis*
13	L-emah-6	*Lactobacillus brevis*
14	L-emah-7	*Lactobacillus collinoides / Lb. fermentum*
15	L-emah-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
16	L-emah-9	*Lactobacillus plantarum*
17	L-emah-13	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
18	L-emah-16	*Lactobacillus plantarum*
19	L-emah-18	*Lactobacillus plantarum*
20	Mot-emah-4	*Lactobacillus fermentum*
21	Mot-emah-6	*Lactobacillus collinoides*
22	Nt-emah-2	*Lactobacillus fermentum*
23	Nt-emah-6	*Lactobacillus paracasei* ssp. *paracasei*
24	S-emah	*Lactobacillus plantarum*
25	S-emah-5	*Lactobacillus plantarum*

L, Lobamba; MOT, Motshane; Nt, Ntfonjeni; S, Sitjeni (locations from where samples were collected)

The predominant isolates were therefore *Lb. plantarum* strains. Of the 9 isolates further characterised by sequencing the 16S rDNA, 4 were confirmed as *Lb. plantarum*, while the others were identified as *Leuconostoc lactis*, *Weissella confusa*, *Lactobacillus acidophilus* and *Lactococcus lactis* (Table 4).

Table 4: Identification of lactic acid bacterial isolates from Swazi traditional fermented *emasi* and *emahewu* using API 50 CH kit and by sequencing 16S rDNA

	Isolate code	Identity using API 50 CH kit	Identity using 16S rDNA†
		Emasi	
1	L-emasi-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	*Leuconostoc pseudomesenteroides*
2	L-emasi-5	*Leuconostoc mesenteroides* ssp. *mesenteroides*	*Leuconostoc pseudomesenteroides*
3	L-emasi-7	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	*Leuconostoc pseudomesenteroides*
4	L-emasi-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
5	Mot-emasi-7	*Lactococcus lactis* ssp. *lactis*	Not identified
6	Nt-emas-2	*Lactococcus lactis* ssp. *lactis*	Not identified
7	Nt-emas2-6	*Lactobacillus paracasei* ssp. *paracasei*	Not identified
8	Nt-emas-5	*Lactobacillus plantarum*	Not identified
9	Nt-emas-6	*Lactobacillus brevis*	Not identified
10	L-emasi-13		*Leuconostoc pseudomesenteroides*
		Emahewu	
11	L-emah-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
12	L-emah-3	*Lactobacillus plantarum*	*Lactobacillus plantarum*
13	L-emah-5	*Lactobacillus brevis*	Not identified
14	L-emah-6	*Lactobacillus brevis*	Not identified
15	L-emah-7	*Lactobacillus collinoides / Lb. fermentum*	*Weissella confusa*
16	L-emah-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
17	L-emah-9	*Lactobacillus plantarum*	*Lactobacillus plantarum*
18	L-emah-13	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
19	L-emah-16	*Lactobacillus plantarum*	*Lactobacillus plantarum*
20	L-emah-18	*Lactobacillus plantarum*	*Leuconostoc lactis*
22	L-emah-19		*Lactobacillus plantarum*
23	Mot-emah-4	*Lactobacillus fermentum*	*Lactococcus lactis*
24	Mot-emah-6	*Lactobacillus collinoides*	*Lactobacillus acidophilus*
25	Nt-emah-2	*Lactobacillus fermentum*	Not identified
26	Nt-emah-6	*Lactobacillus paracasei* ssp. *paracasei*	*Leuconostoc pseudomesenteroides*
27	S-emah	*Lactobacillus plantarum*	Not identified
28	S-emah-5	*Lactobacillus plantarum*	Not identified

L, Lobamba; MOT, Motshane; Nt, Ntfonjeni; S, Sitjeni (locations from where samples were collected)
†Only representative strains were further identified by molecular method by sequencing 16S rDNA.

In comparison, the main LAB in *ogi*, a Nigerian fermented cereal beverage, were found to be *Lb. plantarum*, *Lb. casei*, *Lb. brevis, Lb. fermentum*, *Lb. delbrueckii*, *Lb. acidophilus*, *Leuconostoc mesenteroides* and *Pediococcus acidilacti*.[25] In a separate study, Madoroba et al.[26] isolated and identified LAB in *ting*, a South African spontaneously fermented sorghum non-alcoholic beverage, and found that the predominant LAB were *Lb. plantarum, Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Weissella cibaria* and *Enterococcus faecalis*. Some Enterobacteriaceae were also isolated. The Swazi *emahewu* samples were prepared from maize meal. The predominant microorganisms in *koko*, a Ghanaian spontaneously fermented porridge from millet, were identified as *Weissella confusa* and *Lactobacillus fermentum*[27], while Yousif et al.[28] found that *Lactobacillus fermentum* and *Pediococcus acidilacti* were

Table 5: Carbohydrate fermentation (+ = positive reaction, - = negative reaction) by lactic acid bacteria species isolated from *emasi* and *emahewu*

Substrate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-arabinose	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+
Ribose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
D-xylose	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-	-	-	+	-	-	-	-
L-xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-methyl-xyloside	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
L-sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	+	+	+
Sorbitol	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
α-Methyl-D-mannoside	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
α-Methyl-D-glucoside	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
N-acetylglucosamide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Amygdaline	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	+	+	+
Arbutine	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+
Esculine	-	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+
Salicine	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	-	-	-	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+
Melibiose	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
Saccharose	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	+
Inuline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melizitose	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
D-raffinose	-	+	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	+
Starch	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-gentiobiose	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+
D-turanose	+	+	+	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+
D-lyxose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tagatose	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D-fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-arabitol	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+
L-arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
2-Ketogluconate	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
5-Ketogluconate	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Key: *1:* Leuconostoc mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-1); 2:* L. mesenteroides *ssp.* mesenteroides *(L-emasi-5); 3:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-7); 4:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-8); 5:* Lactococcus lactis *ssp.* lactis *(Mot-emasi-7); 6:* Lc. lactis *ssp.* lactis *(Nt-emas-2); 7:* Lactobacillus paracasei *ssp.* paracasei *(Nt-emas2-6); 8:* Lb. plantarum *(Nt-emas-5); 9:* Lb. brevis *(Nt-emas-6); 10:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-1); 11:* Lb. plantarum *(L-emah-3); 12:* Lb. brevis *(L-emah-5); 13:* Lb. brevis *(L-emah-6); 14:* Lb. collinoides/Lb. fermentum *(L-emah-7); 15:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-8); 16:* Lb. plantarum *(L-emah-9); 17:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-13); 18:* Lb. plantarum *(L-emah-16); 19:* Lb. plantarum *(L-emah-18); 20:* Lb. fermentum *(Mot-emah-4); 21:* Lb. collinoides *(Mot-emah-6); 22:* Lb. fermentum *(Nt-emah-2); 23:* Lb. paracasei *ssp.* paracasei *(Nt-emah-6); 24:* Lb. plantarum *(S-emah); 25:* Lb. plantarum *(S-emah-5)*

the predominant strains in *hussuwa*, a Sudanese fermented sorghum food. Also, in *gari*, a cassava-based fermented food from Benin, *Lb. plantarum* was the most commonly isolated species followed by *Leuconostoc fallax* and *Lactobacillus fermentum*.[29] Muyanja et al.[21] also identified the LAB isolated from the spontaneously fermented Ugandan *bushera* as *Lb. plantarum*, *L. paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb*. *brevis* and *Lb. delbrueckii* subsp. *delbrueckii*. Similarly, *Lactobacillus* and *Weissella* were the common genera isolated from Munkoyo and Chibwantu, traditional non-alcoholic fermented beverages popularly consumed in Zambia.[8] Therefore, the common LAB strains in Swazi *emahewu* belong to *Lb. plantarum, Lactobacillus* spp., *Leuconostoc* spp., *Lactococcus* spp. and *Weissella* spp. This finding is consistent with LAB strains reported in other products similar to Swazi *emahewu*. Notably, there were very few differences in identification of LAB for some isolates between the two methods (API 50 CH test and sequencing 16S rDNA). The accuracy of the API 50 CH test is limited to species available on the databases on the Internet-based APIWEB™ service, and the accuracy of 16S rDNA analyses strongly depends on the choice of primers.

Notably, the common LAB strains in Swazi *emahewu* belong to *Lactobacillus,* which suggests that *Lb. plantarum*, in particular, is a typical biota of spontaneously fermented maize and sorghum non-alcoholic beverages and plays a key role in defining the attributes of these products. Some strains of *Lb. plantarum* have been found to be amylolytic, that is, they break down starch in pearl millet slurries[30]; further studies on these *emahewu* strains is needed.

Carbohydrate profile of LAB

Almost all *Lactobacillus* spp. were able to utilise mainly ribose, galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamide, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose, melibiose, saccharose and trehalose (Table 5). *Lactococcus* ssp. metabolised carbon source ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamide, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose and trehalose. Most *Leuconostoc mesenteroides* ssp. utilised substrate ribose, galactose, D-glucose, D-fructose, D-mannose, cellobiose, maltose, lactose, melibiose, saccharose and trehalose. In general, LAB in the current study fermented other carbohydrates such as L-arabinose, rhamnose, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, melizitose, D-raffinose, starch, B gentiobiose, D-turanose, D-tagatose, D-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate (Table 5).

The metabolism of carbohydrates by LAB is a similar observation to that made by Negussie et al.[31] who observed that LAB isolated from Ethiopian naturally fermented buttermilk were able to utilise carbohydrates such as galactose, maltose, glucose, fructose, mannose and lactose. The results of the current study are supported by those of Ashmaig et al.[32] who observed that LAB isolated from traditional Sudanese fermented camel's milk were able to ferment some carbohydrates. The common substrates that were fermented include carbohydrates such as lactose, fructose, galactose, trehalose, melibiose, mannose, xylitol and sorbose.

Conclusions

Emasi and *emahewu* are fermented foods of Swaziland. *Leuconostoc mesenteroides, Lb. plantarum* and *Lb. lactis* subsp. *lactis* were typical strains in *emasi*, while the *Lactobacillus* genus, especially *Lb. plantarum,* was typical in *emahewu*. Other LAB strains commonly found in *emahewu* were *Lb. acidophilus, Leuconostoc lactis, Lactococcus lactis* and *Weissella confusa*. Nevertheless, there is still a need to broaden the LAB isolates to be identified by sequencing 16S rDNA, carefully considering the choice of primers. *Emasi* and *emahewu* enhance dietary diversity and are popular foods for both children and adults in Eswatini. Studies are therefore needed to develop starter cultures for easier production of these foods.

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Authors' contributions

P.S. performed all the methodology, including data collection, sample analysis and data analysis as part of PhD studies; worked on the original concept of the manuscript write-up and revisions of the manuscript. M.S. and T.H.G. provided supervision and contributed significantly to the final version of the manuscript.

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Poultry and cattle manure effects on sunflower performance, grain yield and selected soil properties in Limpopo Province, South Africa

The application of organic manures as alternatives to reduce the use of mineral fertilisers is considered a good agricultural practice for smallholder farmers. However, the effect of organic manure on soil properties and crop yield depends upon its application rate and its chemical composition. A field experiment was carried out during the 2013/2014 and 2014/2015 seasons at the University of Venda experimental farm (Limpopo Province, South Africa) to determine the effect of three organic manures (cattle, poultry and their 1:1 combination, 20 t/ha) on sunflower (*Helianthus annuus* L.) performance, grain yield and selected soil properties under rainfed conditions. Poultry manure produced the highest final infiltration rate and cumulative infiltration followed by cattle manure, their combination and the control in that order. Total nitrogen, calcium, and zinc were significantly different between treatments in the first season while potassium, sodium, and zinc were significantly different in the second season. Manure combination and poultry manure produced the highest organic carbon and available phosphorus, respectively, in both seasons compared to other treatments. Organic manure application had a significant ($p<0.05$) effect on dry matter, plant height and stem girth at all growth stages in the second cropping season but only in the flower bud stage for both parameters in the first season. Manure application in the second season resulted in an increase in the grain yield compared to the first season, except after application of poultry manure whereafter the grain yield decreased significantly by 168% from the first cropping season. The application of organic manure had a significant effect on sunflower grain yield, dry matter, head dry matter, plant height and stem girth throughout all growing stages in the second cropping season with poultry manure producing the best values.

Significance:

- Application of the three organic manures served as a good source of organic amendments for improvement of plant nutrients and selected soil properties.
- Based on the results of this study, poultry manure can be recommended as the first choice among the manure used for local smallholder farmers, especially under evenly distributed rainfall.

Introduction

Sunflower (*Helianthus annuus* L.) is the most important oilseed crop in South Africa and the third largest grain crop after maize (*Zea mays*) and wheat (*Triticum eastivum*).[1] Its seed is used in the manufacture of sunflower oil and oilcake for animal feed. It is a widely adapted crop which grows well under both light-textured and well-drained heavy-textured soils.[2] Furthermore, it can be produced under irrigation as well as under hot and dry climate, making the Limpopo Province an ideal production area.[3] In South Africa, sunflower has been produced mainly in commercial farms.[4] Its production by smallholder farmers in Limpopo Province has been relatively low, accounting for only 526 tons by year 2002, which makes it the lowest among the main field crops.[3] Declining soil fertility coupled with low and erratic rainfall are some of the problems contributing to low agricultural production in this Province. The Limpopo Province is one of the driest in South Africa with an average annual rainfall ranging from 400 mm to 600 mm.[3] Most soils in the Province in general, and in Vhembe District in particular, are fragile and low in fertility that needs to be sustained through application of fertilisers.[5] However, research has shown that the majority of smallholder farmers in the District cannot afford chemical fertilizers. Nevertheless, manure is affordable and readily available for use as a soil amendment.[5]

Although the benefits of animal manure are well documented in the literature, effects on sunflower have not been tested under the dryland conditions of the Limpopo Province. It is well established that animal manures improve crop yields through organic matter addition which in turn improves soil physical, chemical and biological properties and reduces soil erosion.[6] Research elsewhere has shown that the application of 10 t/ha and 20 t/ha farmyard manure improved sunflower grain yield.[7] Recently Materechera[8] reported improved aggregate stability, reduced soil strength and bulk density and increased bambara nut (*Voandzeia subterranean* L.) growth and yield after applying 5 t/ha of cattle manure on a hard setting and crusting chromic Luvisol in South Africa. Dunjana[9] observed in clayey soils that infiltration rates were increased by 30% with 25 t/ha cattle manure applications over control, while no changes in steady state infiltration rates were observed with manure application on sandy soils after 6 years.

It has also been observed that soil pH, organic matter, nitrogen, available phosphorus, exchangeable potassium, calcium and magnesium increased relative to control levels after the application of 15 t/ha and 30 t/ha and 10 t/ha and 20 t/ha of cattle and poultry manure, respectively.[10] In contrast, Dikinya and Mufwanzala[11] reported that poultry manure application, irrespective of the application rate, did not change the pH or acidity of the Luvic Calcisol. However, a substantial pH increase or change in pH with increasing application rate of poultry manure was observed in the case of Ferralic Arenosol and Vertic Luvisols, whereas the amount of exchangeable bases increased with increasing application rate for all the soil types. Therefore, the benefit of animal manure on soil fertility and crop yield depends on its composition, the soil type and the specific crop.

In smallholder farming in South Africa, cattle, goats, sheep and chickens produce most of the animal manure.[12] The manure is collected, transported to the field and incorporated into the soil without composting[13], either because of a lack of knowledge or to reduce labour requirements[5,14]. Previous studies have indicated that less than 33% of smallholder farmers in the Vhembe District keep livestock (an average of 5 head of cattle, 10 goats and 8 chickens per household) and hence the quantity of manure was not enough.[5] Nevertheless, most farmers opted to mix the available manure to meet their manure requirements. A study by Aderinoye-Abdulwahab and Salami[14] reported that the most frequently used organic fertiliser by smallholder farmers is a combination of poultry and cattle manures (72.1%) as compared to poultry manure (29.8%) or cattle manure (25.9%) alone. Ibrahim and Fadni[15] reported that manure combination increased crop yield by 90% as compared to 70% and 50% of poultry and cattle manure, respectively. The practice of mixing different manure types has been reported elsewhere with similar result.[16-18]

Although the practice of mixing different organic manure types by smallholder farmers is common in most rural farming areas of South Africa, there has not been any agronomic evaluation of the practice. The aim of this study was to evaluate the application of three organic manures on sunflower performance and selected soil physical and chemical properties under rainfed conditions in the Limpopo Province. It was hypothesised that cattle manure, poultry manure and their combination would improve selected soil properties, leading to the improvement of sunflower performance and grain yield.

Materials and methods

Description of the study site

A field experiment was conducted during the 2013/2014 and 2014/2015 cropping seasons at the University of Venda experimental farm (22°58' S; 30°26' E) at Thohoyandou in the Limpopo Province of South Africa. The study site is 596 m above sea level and receives highly seasonal rainfall averaging 781 mm per annum. Most (85%) of the rainfall occurs between October and March (summer), coinciding with the highest evaporative demand, with an annual aridity index of 0.52, the area is considered borderline between semi-arid and sub-humid according to the UNESCO classification criterion.[19]

The soil at the experimental site has been described and is classified locally as Shortlands form[20] equivalent to Rhodic Ferralsol[21]. The soil is deep (>1200 mm), red (10YR3/3), moist clay with a weak angular and subangular blocky structure and well drained with a pH (H_2O) of 5.7.

Organic manure sources and application

The organic manure sources were cattle manure (obtained from a nearby smallholder farmer), poultry manure (obtained from the University of Venda broiler house), and the combination of the two manures at a ratio of 1:1 on dry weight basis (Table 2). The cattle manure was collected from the cattle pens from a depth of 0–10 cm using a spade and transported to the field. Poultry manure was collected from the broiler house into 50-kg bags and transported to the field. Both cattle and poultry manures were applied before composting them to mimic the local farming practice. Before applying the manure to the soil, it was homogenised by thoroughly mixing it and the larger particles were reduced manually. Manure was applied by evenly broadcasting and then thoroughly incorporated it into the experimental plots using a hand hoe to an approximate depth of 10 cm. Manure was applied 21 days before planting to allow sufficient time for it to react with the soil.[22,23] Each manure type and the combination was applied at a rate of 20 t/ha, except for control plots where no manure was applied.

Before application, the two organic manure sources were analysed to determine pH, organic matter content, total nitrogen, extractable phosphorus and zinc, exchangeable calcium, magnesium, sodium and potassium, and cation exchange capacity.

Land preparation and experimental layout

The experimental site was ploughed using a disc plough at the beginning of the first cropping season only. Ploughing was followed by manual seed-bed preparation and plot demarcation before planting. Land preparation for the second cropping season for each plot was done manually in order to retain the demarcation of the previous season. The field layout was a randomised complete block design totalling 16 plots with individual plots measuring 36 m^2 (6 x 6 m). The plots were separated by 1 m to avoid encroachment of manure, giving a total experimental site area of 841 m^2. Four treatments were applied (control, poultry manure, cattle manure and the 1:1 combination of manures) with four replicates of each.

A landrace sunflower seed collected from local smallholder farmers was planted. Two seeds were planted per hole at a 0.3-m intra-row spacing and 1-m inter-row spacing and at an approximate depth of 2.5 cm. Planting was done on 8 December 2013 and 28 November 2014 for the first and second cropping seasons, respectively. The seedlings were thinned to one plant per hole after 2 weeks of emergence. The plant density after thinning was approximately 33 333 plants/ha. Weeds were controlled manually.

Soil sampling and analysis

Each season before manure application and planting, three soil samples were randomly collected at a depth of 0–20 cm using a soil auger. Samples were bulked, dried, sieved (2 mm) and stored in a laboratory plastic bag for subsequent physical and chemical analyses. At the end of each cropping season, representative soil samples from each plot at the same depth were collected for analysis.

Bulk density was determined using the core method.[24] Particle size distribution analysis was carried out by Bouyoucos hydrometer method[25] using sodium hexametaphosphate (calgon) as the dispersant. Infiltration rate in each plot was determined using a double ring infiltrometer method following the procedure described in Bouwer[26]. The double ring infiltrometer consisted of two pairs of inner and outer rings, a driving plate, an impact absorbing hammer, measuring bridge and measuring rods with float. The inner ring measured 28 cm x 0.5 cm x 25 cm (diameter x thickness x height) while the outer ring measured 53 cm x 0.5 cm x 25 cm.

Soil pH was measured (in supernatant suspension of a 1:2.5 soil:water) using a pH/electrical conductivity/total dissolved solids multimeter probe.[27] Total nitrogen was determined by the micro-Kjeldahl method.[28] Available phosphorus was determined by the Bray 1 method.[29] Zinc was extracted with 0.1 M HCL and determined by atomic absorption spectrometry. Potassium, calcium, magnesium and sodium extractions were done using 1 M ammonium acetate at a pH of 7 and exchangeable cations were determined using atomic absorption spectrometry. Organic carbon was determined by the Walkley–Black procedure.[30]

Plant sampling and measurements

Plant samples were collected for above-ground dry matter at flower bud, flowering and maturity stages, for both growing seasons. Plants in the second outer rows were sampled over a row length of 1 m starting at 0.3 m from each row. Plant samples were partitioned into leaves, heads and stems, and thereafter dry matter was determined and expressed in kilograms per hectare. Plant height and stem girth at flower bud stage, flowering stage and grain maturity stage were measured from two marked plants (second and fourth plants) from each of the two central rows and an average was obtained. A total number of four plants per plot were used to determine the plant height and stem girth. The plant height was measured from the base of the plant to the tip of the top-most leaf.

At physiological maturity, two middle rows were harvested for yield component determination. All sunflower heads were then measured for head diameter (cm), head dry matter (g/head) and the weight of 100 seeds. Grain yield was determined after threshing. Seeds were dried at 65 °C in the oven for 24 h and seed weight adjusted to 13% moisture content.

Meteorological data were recorded in both cropping seasons by an automatic weather station located approximately 60 m from the experimental site. Precipitation was measured using three standard rain gauges installed on the experimental site and recorded as the average of three rain gauges.

Table 1: Pre-cropping selected surface soil properties

Soil physical properties				Soil chemical properties								
Particle size (%)			Bulk density (g/cm^3)	pH (H_2O)	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (mg/kg)	Potassium (cmol (+)/kg)	Calcium (cmol (+)/kg)	Magnesium (cmol (+)/kg)	Sodium (cmol (+)/kg)	Zinc (mg/kg)
Sand	Silt	Clay										
22	18	60	1.12	5.7	1.57	0.081	1.63	0.54	6.82	2.41	0.12	2.60

Table 2: Chemical properties of the organic manures used

Organic manure sources	pH (H_2O)	Total carbon (%)	Total nitrogen (%)	Phosphorus (g/kg)	Potassium (g/kg)	Sodium (g/kg)	Calcium (g/kg)	Magnesium (g/kg)	Carbon:nitrogen ratio
Cattle manure	8.2	27	1.96	3.37	22.54	2.94	15.53	7.98	14:1
Poultry manure	7.0	31.9	1.61	9.68	11.21	2.18	90.59	6.58	20:1

Statistical analysis

Data collected were analysed using an analysis of variance for a randomised complete block design using IBM SPSS version 20.[31] Due to seasonal variability encountered during the two cropping seasons, particularly in terms of rainfall, further analysis was performed on two factors, namely cropping season and organic manure, and their interaction. The differences between the treatment means were separated using the least significant difference procedure.

Results

Pre-cropping selected surface soil properties

The soil was generally acidic (pH=5.7), dominated by clay with a low bulk density (1.12 g/cm^3), high in clay content (average of 60.5%), and had more calcium than other major nutrients, followed by magnesium, potassium and sodium, in that order (Table 1).

Chemical properties of the organic manure

Table 2 shows the results of the organic manure analysis. The pH of poultry manure was neutral, whereas cattle manure was slightly alkaline. Organic carbon content was higher in poultry manure than in cattle manure, whereas total nitrogen content was higher in cattle manure than in poultry manure (Table 2). Phosphorus content was about three-fold higher in poultry manure than in cattle manure. Potassium concentration was twice as high in cattle manure than in poultry manure. Calcium content of poultry manure was six times higher than that of cattle manure, while magnesium content of the two manures were similar (Table 2). The C/N ratio of poultry manure was higher than that of cattle manure (Table 2).

Climatic conditions

Ambient temperature was generally similar in both cropping seasons, while rainfall varied between the two seasons (Table 3). The total rainfall received in the first cropping season was about three-times higher than that in the second cropping season. Temperature was not different between the two cropping seasons, with maximum temperature slightly higher in the second cropping season. The mean maximum temperature (T_{max}) was about 30 °C while the mean minimum temperature (T_{min}) was about 20 °C during the cropping period (Table 3).

The highest rainfall received in the 2013/2014 season was in January (458 mm) when 37% (171 mm) of rainfall was received in 1 day. Unlike in January, rainfall in February was evenly distributed throughout the month with nearly the same number of rainy days between cropping seasons (Table 3). The month of March had a total rainfall of 192 mm with 99% (190.5 mm) of this rainfall occurring within the first 13 days of the month. Most of the rainfall in the second season occurred in December, with frequent rainfall occurring at the middle and towards the end of the month.

Table 3: Summary of monthly meteorological data during 2013/2014 and 2014/2015 cropping seasons

Month	Maximum temperature	Minimum temperature	Total rainfall (mm)	Rainy days
2013/2014				
December	26.74	18.59	269[a]	12.0
January	27.99	19.98	458	17.0
February	27.22	19.47	275	16.0
March	27.73	19.49	192[b]	12.0
Growing season	**27.42**	**19.38**	**1195**	**57.0**
2014/2015				
November	28.44	19.74	50[a]	3.0
December	30.49	18.99	313	16.0
January	31.20	19.40	56	12.0
February	32.94	19.22	16[b]	8.0
Growing season	**30.77**	**19.34**	**434.85**	**39.0**

[a]Rainfall received on or after planting; [b]rainfall received on or before harvest

Effect of organic manure on soil physical properties

Bulk density was not significantly different in the 2-year cropping period although the three manure treatments resulted in a slight decrease of less than 9.65% bulk density compared to the control (Table 4). Poultry manure produced the highest final infiltration rate (28.1 mm/h) and cumulative infiltration of all the treatments (Table 4). There was no significant increase between the addition of the combined manure and the control. The final infiltration rate after application of poultry manure and cattle manure increased by 67% and 43%, respectively, over that of the control.

Table 4: Effect of organic manure on surface soil physical properties

Treatment	Bulk density (g/cm^3)	Final infiltration rate (mm/h)	Cumulative infiltration (mm)
Control	1.14[a]	16.8[a]	101.3[a]
Cattle manure	1.07[a]	24.0[b]	151.2[b]
Poultry manure	1.03[a]	28.1[b]	185.8[b]
Combined manure	1.06[a]	17.4[a]	121.8[a]

Means in the same column followed by the same letter are not significantly different.

Effect of organic manure on surface soil chemical properties

Potassium and zinc were significantly different in the second cropping season (Table 5). A significant difference in pH was observed only in the second cropping season. Potassium was significantly different ($p<0.05$) among the treatments in the second cropping season, with the highest concentration obtained after the combination treatment.

Effect of organic manure on biomass and yield components

The highest dry matter yield was observed after application of poultry manure at the flower bud stage of the first (2571.4 kg/ha) and second (1141.7 kg/ha) cropping seasons (Table 6). In the flowering stage of the second cropping season, the highest value of dry matter (6127.4 kg/ha) was also observed under poultry manure treatment. Poultry manure increased ($p<0.05$) dry matter yield at flower bud stage by 1022.4 kg/ha and 617.1 kg/ha in the first and second cropping seasons, respectively, compared to the control.

Manure application had a significant effect ($p<0.05$) on plant height and stem girth in all growing stages in the second cropping season, whereas in the first cropping season, a significant effect was observed only in the flower bud stage for both parameters. Poultry manure produced the highest plant height in all growing stages for both cropping seasons. The manure combination (7.28 cm, 10.48 cm and 11.01 cm) and poultry manure (7.05 cm, 10.21 cm and 10.28 cm) produced the highest stem girth in all growing stages for the first and second cropping seasons, respectively.

The highest grain yield (3289.39 kg/ha) was recorded under poultry manure treatment in the first cropping season; this yield was six-fold higher than that of the control (Table 7). There was no statistically significant difference in yield after application of cattle manure and the combined manure (Table 7). Organic manure application in the second cropping season resulted in a higher grain yield compared to that of the

Table 5: Effect of organic manure on surface soil chemical properties for 2013/2014 and 2014/2015 cropping seasons

Treatment	pH (H_2O)	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (mg/kg)	Extractable cations (cmol(+)/kg)			Zinc (mg/kg)
					Potassium	Calcium	Magnesium	
First cropping season								
Control	5.91^{a}	1.71^{a}	0.045^{a}	7.59^{a}	0.4^{a}	6.743^{a}	2.162^{a}	1.90^{a}
Cattle manure	6.05^{a}	1.47^{a}	0.050^{a}	9.25^{a}	0.647^{a}	8.462^{a}	2.696^{a}	3.34^{a}
Poultry manure	5.94^{a}	1.92^{a}	0.042^{a}	31^{a}	0.643^{a}	5.548^{a}	1.937^{a}	10.98^{a}
Combined manure	5.97^{a}	1.96^{a}	0.037^{a}	8.49^{a}	0.572^{a}	5.629^{a}	2.119^{a}	5.64^{a}
Second cropping season								
Control	4.67^{a}	1.50^{a}	0.057^{a}	1.94^{a}	0.42^{a}	7.17^{a}	2.36^{a}	1.94^{b}
Cattle manure	5.88^{b}	2.00^{a}	0.065^{a}	2.67^{a}	1.07^{b}	8.72^{a}	2.98^{a}	3.51^{b}
Poultry manure	5.00^{ab}	1.75^{a}	0.057^{a}	30.27^{a}	0.98^{b}	6.29^{a}	2.23^{a}	10.38^{a}
Combined manure	4.26^{a}	2.18^{a}	0.078^{a}	29.40^{a}	1.42^{b}	7.50^{a}	3.10^{a}	10.63^{a}
Cropping season (CS)	$p<0.05$	ns	$p<0.05$	ns	$p<0.05$	ns	ns	ns
Organic manure (OM)	$p<0.05$	ns	ns	$p<0.05$	$p<0.05$	ns	ns	$p<0.05$
CS * OM	$p<0.05$	ns	ns	ns	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significant differently; ns, not significant (*p*<0.05).*

Table 6: Effect of organic manure on dry matter, plant height and stem girth determination

	Dry matter (kg/ha)			Plant height (cm)			Stem girth (cm)		
Treatment	Flower bud	Flowering	Maturity	Flower bud	Flowering	Maturity	Flower bud	Flowering	Maturing
First cropping season									
Control	1549.0^{b}	4285.1^{a}	5152.8^{a}	54.69^{a}	184.63^{a}	186.50^{a}	4.51^{b}	8.21^{a}	8.45^{a}
Cattle manure	2110.3^{a}	6969.5^{a}	7209.7^{a}	83.06^{b}	172.13^{a}	183.81^{a}	6.88^{a}	10.09^{a}	9.74^{a}
Poultry manure	2571.4^{a}	6515.0^{a}	6955.6^{a}	106.75^{c}	204.25^{a}	209.13^{a}	7.24^{a}	10.00^{a}	10.49^{a}
Combined manure	2485.4^{a}	7446.4^{a}	7275.5^{a}	90.88^{bc}	201.75^{a}	205.69^{a}	7.28^{a}	10.48^{a}	11.01^{a}
Second cropping season									
Control	524.6^{a}	2869.7^{b}	3416.1^{b}	39.31^{b}	150.86^{a}	152.75^{a}	4.00^{b}	6.19^{a}	6.25^{a}
Cattle manure	704.8^{b}	5395.7^{a}	8751.1^{a}	90.75^{a}	179.13^{b}	182.06^{b}	6.56^{a}	9.56^{b}	9.69^{b}
Poultry manure	1141.7^{c}	6127.4^{a}	8408.9^{a}	100.00^{a}	198.31^{c}	199.38^{c}	7.05^{a}	10.21^{b}	10.28^{b}
Combined manure	967.3^{d}	5914.5^{a}	9414.7^{a}	88.63^{a}	188.88^{d}	189.75^{d}	6.45^{a}	8.56^{c}	8.63^{c}
Cropping season (CS)	$p<0.05$	$p<0.05$	ns	ns	$p<0.05$	$p<0.05$	ns	ns	$p<0.05$
Organic manure (OM)	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
CS * OM	ns	ns	$p<0.05$	ns	ns	ns	ns	ns	ns

*Means in the same column followed by the same letter are not significantly different; ns, not significant (*p*<0.05).*

first cropping season, except after application of poultry manure which consequently decreased yield by 168% from the first cropping season.

Table 7: Grain yield, head diameter, head dry matter and 100-seed weight

Treatment	Grain yield (kg/ha)	Head diameter (cm)	Head dry matter (g/head)	100-seed weight (g)
First cropping season				
Control	582.47[a]	19.79[a]	27.38[a]	5.26[a]
Cattle manure	1173.20[b]	22.81[a]	42.63[b]	6.08[a]
Poultry manure	3289.39[c]	22.28[a]	49.27[c]	5.29[a]
Combined manure	1336.49[b]	20.53[a]	34.71[d]	5.55[a]
Second cropping season				
Control	968.35[a]	12.84[b]	12.06[b]	4.59[a]
Cattle manure	1646.14[b]	19.88[a]	27.32[a]	5.28[a]
Poultry manure	1227.75[a]	20.25[a]	29.28[a]	5.45[a]
Combined manure	1647.29[b]	20.50[a]	32.50[a]	5.24[a]
Cropping season (CS)	ns	$p < 0.05$	$p < 0.05$	ns
Organic manure (OM)	ns	$p < 0.05$	$p < 0.05$	ns
CS * OM	ns	$p < 0.05$	$p < 0.05$	ns

Means in the same column followed by the same letter are not significantly different; ns, not significant (p<0.05).

Cattle manure produced the highest head diameter (22.81 cm) in the first cropping season whereas the combined manure produced the highest value (20.50 cm) in the second cropping season. There was a significant effect ($p<0.05$) on head dry matter in both cropping seasons. Poultry manure (49.27 g/head) and the combined manure (32.50 g/head) produced the highest head dry matter values in the first and second cropping seasons, respectively. There was no statistical difference among the treatments for 100-seed weight.

Discussion

Effect of organic manure on soil physical properties

Manure application did not significantly lower soil bulk density in comparison with the control treatment (Table 4). The results are in contrast to the findings by Ojeniyi et al.[32] who observed a significant decrease in bulk density at 0–15 cm depth under 10 t/ha poultry manure application in a 3-year field experiment on an Alfisol. Lack of significant difference in bulk density among treatments could be attributed to the short duration of the experiment with organic manure being less effective in altering bulk density significantly. Slow alteration of bulk density after long-term application of organic manure was reported by Cebula[33] and Brar et al.[34] Tadesse et al.[35] reported no difference in bulk density at a soil depth of 0–20 cm after applying 7.5 t/ha and 15 t/ha of cattle manure for two cropping seasons, with 15 t/ha rates recording the lowest bulk density value on a Vertisol of 71.25% clay content. The lower values of bulk density for this study could be due to microorganisms burrowing in the soil and also large opened cracks which were observed during the cropping seasons. Ibrahim and Fadni[15] also reported no significant difference in bulk density after applying 10 t/ha of cattle manure, poultry manure and the combined manure on a sandy soil, with cattle manure recording the lowest bulk density followed by poultry manure then the combined manure in both 0–20 cm and 20–40 cm soil depths.

Poultry manure, followed by cattle manure, produced the highest infiltration rate until a steady state was reached. This result is shown by a high final infiltration rate of 28.1 mm/h (Table 4). The increase in infiltration rate under poultry manure application may be due to a relatively low bulk density brought about by poultry manure application (Table 4) and high organic carbon in poultry manure (Table 2). However, there was no significant difference in final infiltration rates between cattle manure and poultry manure treatments. Similar to the findings of this study, Mubarak et al.[36] observed that there was no significant difference in infiltration rate among treatments after 10 t/ha of cattle manure and poultry manure was applied on a sandy soil. The poultry manure treatment produced the highest infiltration value of 185.8 mm compared to the control (101.3 mm) (Table 4), which could be attributed to its low bulk density. Application of cattle manure and the combined manure produced final infiltrations of 151.2 mm and 120 mm, respectively. This may indicate an additive effect of cattle manure as found by Rasoulzadeh and Yaghoubi[37] who reported that by applying cattle manure (at 0, 30 t/ha and 60 t/ha) on a sandy clay loam for 9 months, cumulative infiltration increased with the increasing rate.

Effect of organic manure on soil chemical properties

The pH was significantly different between the treatments in the second cropping season only. The highest pH was recorded under cattle manure treatment for both cropping seasons. This finding could be attributed to the high pH of the cattle manure itself. Azeez and Van Averbeke[38] reported a significant increase in pH after the application of cattle and goat manures as compared to that after application of poultry manure.

The pH was not significantly different between the treatments in the first cropping season, which is in agreement with the findings of Dikinya and Mufwanzala[11] and Magagula et al.[39] who found that, after one cropping season, the application of cattle manure and poultry manure did not significantly change the pH of the soils, irrespective of the application rate. The pH in the second cropping season slightly decreased from that of the first cropping season – a finding similar to that of Roy and Kashem[40] who reported a gradual decrease in soil pH with an increase in application period.

In agreement with the results obtained in this study, Ibrahim and Fadni[15] recorded higher organic carbon after treatment with the combined manure than after cattle manure and poultry manure treatments. An increased organic carbon under the application of organic manure compared to control was observed by Okonwu and Mensah[41] and Roy and Kashem[40]. These authors further observed that organic manure application increased organic carbon[40,41], available phosphorus and extractable zinc[41]. The organic carbon, available phosphorus and zinc were found to be higher in poultry manure treatment than other treatments.[41]

Cattle manure produced the highest values of total nitrogen in both cropping seasons, which may be due to the fact that the cattle manure used had a higher total nitrogen than the poultry manure did (Table 2). Application of organic manure significantly improved available phosphorus in both cropping seasons. Poultry manure produced the highest phosphorus in both cropping seasons of all the treatments (Table 5), which may be because the poultry manure applied had more phosphorus than the cattle manure (Table 2). Ullah et al.[42] and Magagula et al.[39] also reported a higher phosphorus under poultry manure treatments at 5 t/ha and 20 t/ha, respectively. Similarly to our findings, Ullah et al.[42] reported that cattle manure produced higher availability of potassium in the soil than poultry manure.

Effect of organic manure on sunflower biomass and yield

Application of organic manure had a significant effect ($p<0.05$) on dry matter yield at all stages in the second cropping season but only at flower bud stage in the first growing season. An increase in dry matter yield under application of poultry manure at flower bud and flowering stages was reported by Helmy and Ramadan[16]. The dry matter yield produced in the first cropping season in this study was higher than that obtained in the second cropping season at flower bud and flowering stages (Table 6). This observation may be a result of the amount of rainfall received – more rainfall was received during the two growing stages in the first cropping season than in the second cropping season (Table 3). This observation may also be a result of late sampling in the first cropping season (45 and 75 days after planting) compared to 42 and 71 days after planting for the second growing season. In contrast, the dry matter yields in the second cropping season were higher than those of the first cropping season at maturity stage except for control treatment (Table 6). This decrease in the dry matter yield in the first cropping season at maturity stage may be due

to the excess rainfall experienced towards the end of the season that may have caused fungal diseases on plants as evidenced by plant wilt. Fungal disease is also shown by an average dry matter increase of 47.7% from flowering to maturity in the second cropping season in a period of 14 days, compared to 5.5% increases in the first cropping season in a period of 36 days. Adebayo et al.[43] observed that the values of growth parameters (dry matter weight, plant height and plant girth) obtained during a low rainfall season were generally higher than the values obtained during a high rainfall season as disease and pest infestation were very low during low rainfall.

Poultry manure produced the highest plant heights in both cropping seasons throughout all three growing stages (Table 6). A similar observation was reported by Adebayo et al.[43] The highest mean plant height (171.64 cm) of sunflower under 8 t/ha of poultry manure after 10 weeks of planting was reported by Wabekwa et al.[44] Poultry manure application also produced the highest sunflower stem girth in the second cropping season but only during flowering and maturity stages (Table 6). The manure combination (the combined manure) application produced the highest values of sunflower plant height and stem girth in the first cropping season throughout the three growing stages (Table 6). The highest values recorded after application of poultry manure could be attributed to the high amounts of primary (macro) nutrients (phosphorus and potassium) obtained (Table 2). These two primary nutrients are well known to be essential for improving the quality of grains, fruits and vegetables and for increasing water use efficiency, photosynthesis and disease resistance, and they also are essential for plant cell division and enlargement.

The application of organic manure had a significant effect ($p<0.05$) on the grain yield in both cropping seasons. Similarly to our findings, Munir et al.[45] recorded the highest grain yield under poultry manure application in the first cropping season. Cattle manure and the combined manure application produced statistically the same quantity of grain yield in both cropping seasons. Esmaeilian et al.[46] observed higher grain yield under cattle manure treatment, which yielded similar results to that after application of poultry manure. Rasool et al.[7] also observed a 15% increase in grain yield over control values after application of 20 t/ha cattle manure. Organic manure application in the second cropping season (low rainfall; Table 3) resulted in a higher grain yield than after the first cropping season, as was also previously reported by Adebayo et al.[43] However, poultry manure application in the second cropping season resulted in a significant decrease in grain yield by 2061.64 kg/ha (168%) from the first cropping season (Table 7). The decrease in yield after treatment with poultry manure was observed by a decrease in dry matter at the grain maturity stage of the second cropping season (Table 6). The significant decrease in grain yield observed under poultry manure application may be an indication that poultry manure has lower water retention capacity, as rainfall was lower in the second cropping season, therefore the crops may have experienced water stress, hence the lower grain yield. Similarly to our findings, Esmaeilian et al.[46] observed that the control treatment produced the lowest grain yield compared with cattle manure and poultry manure treatments.

Organic manure application had a significant effect ($p<0.05$) on head diameter in the second cropping season, in agreement with earlier findings by Esmaeilian et al.[46] The application of organic manure in the first cropping season produced the highest head diameter compared to that of the second cropping season for all treatments. Both cropping season and organic manure had a significant effect on head diameter. Organic manure contributed to a significant increase in head diameter over the control in the second cropping season compared to the first cropping season, supporting the findings of Wabekwa et al.[44] Cattle manure produced the highest head diameter (22.81 cm) in the first cropping season, as previously reported[46], whereas in the second cropping season, the combined manure produced the highest head diameter of 20.50 cm (Table 7). The lower values of head diameter and head dry matter recorded in the second growing season may be due to the low rainfall received in the second cropping season. The highest head dry matter value (49.27 g/head) was recorded by poultry manure application in the first cropping season compared to other treatments. In the second cropping season, the combined manure produced the highest value followed by poultry manure application. This implies that poultry manure application had an effect on head dry matter. There was an interaction between cropping season and organic manure. The 100-seed weight was not significantly affected by organic manure application in either cropping season, similarly to the results observed by Helmy and Ramadan[16].

Conclusions

Application of the three organic manures provided a good source of organic amendments for improvement of selected soil properties and plant nutrients. Application of poultry manure and cattle manure equally showed a significant influence on final infiltration rate and cumulative infiltration. Although not significant, organic manure application improved bulk density, with poultry manure treatment being the best among the treatments, followed by manure combination, cattle manure treatment and control, in that order. The manures produced a significant increase in pH, exchangeable potassium and zinc in the second cropping season. Exchangeable calcium increased by 20% and 14% under poultry manure treatment in the first and second cropping seasons, respectively. This increase may be attributable to a higher calcium content in poultry manure than cattle manure. Total nitrogen was not significantly affected by organic manure application.

Application of organic manure significantly increased dry matter accumulation, plant height and stem girth in all growing stages in the second cropping season with poultry manure producing the highest values. In the first cropping season, a significant effect was observed in the flower bud stages only. Sunflower grain yield and head dry matter were significantly affected by manure application in both cropping seasons, with the highest value recorded under poultry manure treatment in the first cropping season and under manure combination in the second cropping season. Sunflower head diameter was significantly increased in the second cropping season.

Based on the results of this study, poultry manure is recommended as the first choice among these manures for local smallholder farmers, especially under evenly distributed rainfall. Long-term studies are needed in order to conclusively evaluate the effects of organic manures on soil properties, as recommended in the literature review. It should, however, be noted that the results obtained in this study are valid only for the specified soil and climatic conditions.

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Authors' contributions

M.J.M.: Conceptualisation, methodology, data collection, sample analysis, validation, data curation, writing – the initial draft, writing – revisions, project leadership, project management, funding acquisition. J.M.: Validation, methodology, data curation, writing – revisions, student supervision, project leadership. J.J.O.O.: Validation, data curation, writing – revisions, student supervision.

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First report of the isolation of entomopathogenic nematode *Steinernema australe* (Rhabditida: Steinernematidae) from South Africa

A survey was conducted in Walkerville, south of Johannesburg (Gauteng, South Africa) between 2012 and 2016 to ascertain the diversity of entomopathogenic nematodes in the area. Entomopathogenic nematodes are soil-dwelling microscopic worms with the ability to infect and kill insects, and thus serve as eco-friendly control agents for problem insects in agriculture. Steinernematids were recovered in 1 out of 80 soil samples from uncultivated grassland; soil was characterised as loamy. The entomopathogenic nematodes were identified using molecular and morphological techniques. The isolate was identified as *Steinernema australe*. This report is the first of *Steinernema australe* in South Africa. *S. australe* was first isolated worldwide from a soil sample obtained from the beach on Isla Magdalena – an island in the Pacific Ocean, 2 km from mainland Chile.

Significance:

- Entomopathogenic nematodes are only parasitic to insects and are therefore important in agriculture as they can serve as eco-friendly biopesticides to control problem insects without effects on the environment, humans and other animals, unlike chemical pesticides.

Introduction

Entomopathogenic nematodes are one of the most studied microscopic species of nematodes because of their potential to act as biological control agents.[1,2] Entomopathogenic nematodes are soil-dwelling obligate parasites of insects and have a symbiotic association with insect pathogenic bacteria.[3] Three genera of entomopathogenic nematodes have been identified thus far: *Heterorhabditis*, *Steinernema* and *Oscheius,* which are symbiotically associated with the insect pathogenic bacteria *Photorhabdus*, *Xenorhabdus* and *Serratia*, respectively.[4-6] Entomopathogenic nematodes are found in a variety of soil habitats and demonstrate considerable differences in terms of host range, infectivity, symptomatology, reproduction, distribution and conditions for survival.[7,8]

Entomopathogenic nematodes have been successfully applied as effective biological control agents against some significant lepidopteran, dipteran and coleopteran insects of commercial crops.[9] The effectiveness of entomopathogenic nematodes depends on both the nematodes' capability to locate, recognise and invade a host, and the virulence of the bacteria to susceptible hosts.[9]

Entomopathogenic nematodes infect vulnerable insect hosts while in the free-living, non-feeding infective juvenile arrested developmental stage. Once in the gut of the host, they regurgitate or release their associated pathogenic bacteria into the haemocoel, resulting in septicaemia or toxaemia in the host within 24–48 h.[4] Upon infection, *Steinernema* infective juveniles develop into either feeding males or amphimictic females.[10] The infective juveniles use the invaded insect cadaver as a source of nutrition and proliferate inside the insect host for 2–3 generations.[11] Once the entomopathogenic nematodes have depleted their resources, third-stage infective juveniles are produced and emerge from the cadaver, and remain in the soil while searching for a new host to infect (Figure 1).[8,12]

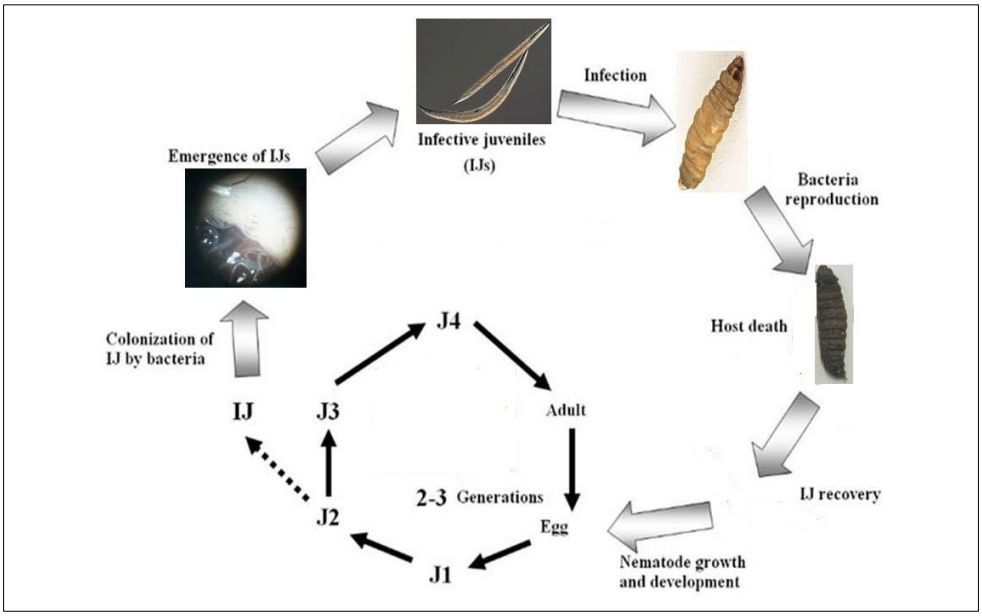


Figure 1: Life cycle of entomopathogenic nematodes.

With respect to ecological compatibility, the selection of specific species of entomopathogenic nematodes to be applied as biological controls depends on their geographical distribution patterns.[13] Many species of entomopathogenic nematodes are cosmopolitan in distribution and have been isolated from a diversity of edaphic habitats throughout the world and are able to survive under a range of environmental and climatic conditions.[14] Thus locally adapted species or isolates from native habitats need to be identified and their unique characteristics documented in order to evaluate their suitability as effective biological control agents against problematic insect pests within specific biogeographical regions. We aimed to isolate and identify entomopathogenic nematodes from South Africa to determine which, if any, nematodes were available in the area for potential use as biological control agents for insect pest control.

Material and methods

Soil sampling

Samples of soils with a sandy loam texture were collected from uncultivated grasslands near Walkerville, south of Johannesburg in the Gauteng Province of South Africa over a period extending from 2012 to 2016. Soil sampling was done during both autumn and winter months from various habitats and site locations. A total of 80 soil samples, comprising approximately 2 kg of soil, were kept in separate plastic containers and stored at 22–25 °C during transport to the laboratory. Sampling details and results are indicated in Table 1. A hand shovel was used to collect the soil in accordance with Kaya and Stock[15]; soil was collected to a depth of 15 cm.[11] Water was added to soil samples to give a moisture content not greater than 8% and the samples were then stored at room temperature overnight.[15] Soil samples were then baited with *Galleria mellonella* (Lepidoptera: Phyralidae), the greater wax moth.[16] A total of 10 *Galleria* larvae were placed on top of each container of soil and the container was inverted and then stored in an incubator at 25 °C. Observations were done daily to monitor for infected and dead larvae. The signs of infection in dead larvae were recorded and used for diagnosis of entomopathogenic nematode induced infection.

Figure 2: White trap showing infective juveniles emerging from the insect cadaver.

Collection of nematodes

Dead larvae were collected after 48–72 h and kept at room temperature on White traps to await the emergence of nematodes. Modified White traps as described by Kaya and Stock[15] were used for the isolation of infective juveniles from dead larvae suspected to be infected with entomopathogenic nematodes. White traps were placed on a bench at room temperature during warm summer days and, during cold winter days, a heater was used to keep the room temperature at 25 °C. Emergence of infective juveniles from infected cadavers into the water (Figure 2) was greatly favoured by room temperatures of 25–30 °C. Precautions were taken to ensure that the Whatman filter paper disc was not too moist so as to introduce excess water into the insect cadaver whilst on the trap, which would lead to the death of entomopathogenic nematodes before they could emerge. Surface-sterilised nematodes taken from White traps were used for molecular[17] and morphological identification[18].

Morphological identification

A total of 20 nematodes of first-generation males and infective juveniles were fixed with 3–4 mL of 100 ºC triethanolamine formaldehyde (TAF) and left for 24 h. TAF was then replaced with double-strength TAF and the nematodes were stored at 4 ºC to relax them for up to 1 h, whereafter 65 ºC TAF was added and the fixative was allowed to infiltrate for at least 24 h. Fixed nematodes were transferred to a Petri dish containing 0.5 mL Seinhorst I solution (20 parts 95% ethanol, 1 part glycerine and 79 parts water). A volume of 5 mL of 95% ethanol was placed into the watch glass containing the nematodes and the watch glass was placed in the desiccator. The desiccator was placed in an oven preheated to 35 ºC for 12 h. The watch glass with nematodes was then removed from the desiccator, filled with Seinhorst II solution (95 parts 95% ethanol, 5 parts glycerine) and placed in a glass Petri dish. The Petri dish was left partially open to allow for slow ethanol evaporation. The Petri dish containing the watch glass was placed in an oven preheated to 40 ºC for 3 h. Nematodes were then mounted on glass slides carefully to avoid crushing them before analysis under the microscope. Morphological analysis was done using an Olympus microscope at different magnifications. Morphometric measurements of the nematodes are presented in Table 2.

Molecular identification

The following primers were used for molecular identification: TW81 (F) 5'-GCGGATCCGTTTCCGTAGGTGAACCTGC-3'; AB28 (R) 5'-GCGGATCCATATGCTTAAGTTCAGCGGGT-3'. A polymerase chain reaction (PCR) was done using 25 μL PCR master mix, 22 μL nuclease-free water, 1 μL forward primer TW81, 1 μL reverse primer AB28 and 1 μL DNA (ng). Total reaction volume was 50 μL. Samples were mixed gently and placed under the following conditions to amplify the internal transcribed spacer (ITS) region found between the 18S and 28S rDNA: (1) initial denaturation before cycling: 94 °C for 5 min; 25 cycle amplification series; (2) denaturation at 95 °C for 60 s; (3) annealing at 64 °C for 60 s; (4) extension at 72 °C for 120 s; and (5) final extension after cycling: 72 °C for 10 min.

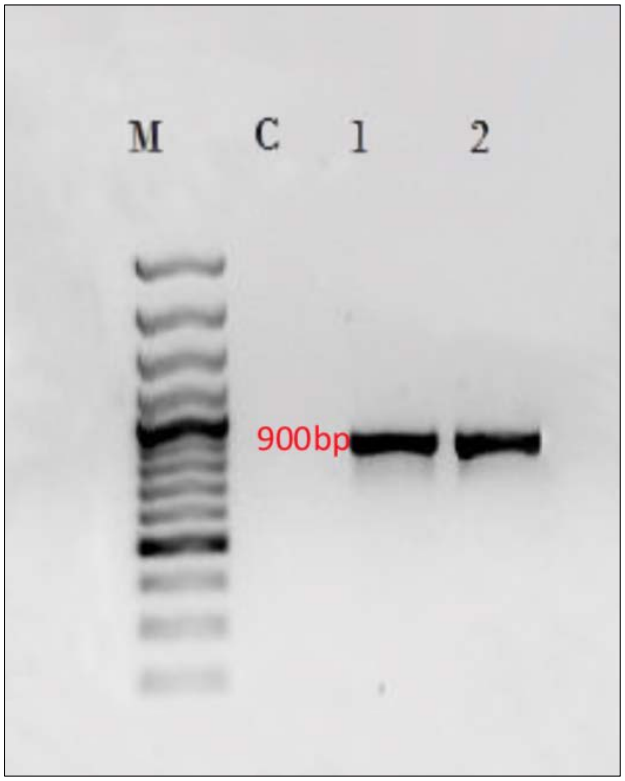
A 0.5% agarose gel was prepared in order to confirm the presence and quality of the extracted DNA. An amount of 0.25 g of agarose power was dissolved in 50 mL of 1 x TBE (Tris-borate-EDTA) buffer and 1 μL of ethidium bromide was added and the contents mixed gently. The gel was left to solidify with the well comb inserted. For each 10 μL sample, only 5 μL of the samples were loaded into the wells and separated on

Table 1: Locality, habitat and date of sampling and soil characteristics of samples

Sampling					Soil characteristics	
Location	**Coordinates: Positive site**	**Coordinates: Negative sites**	**Sampling dates**	**Habitat**	**Soil type**	**pH**
Walkerville, South Africa	26°28'12.1"S 27°56'33.0"E	26°28'12.8"S 27°56'33.2"E and 26°28'12.1"S 27°56'32.8"E	May 2012 August 2016	Uncultivated grassland	Sandy loam	6.1

the gel for 30 min at 90 V immersed in 1 x TBE with constant current. A 2% agarose gel with ethidium bromide was used for visualisation of the DNA (Figure 3).

Successful PCR products were sent to Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa) for sequencing. The nucleotide sequence alignment website (BLAST) of the US National Center for Biotechnology Information (NCBI) was used to identify the unknown species of nematodes. Phylogenetic analysis was done using sequences obtained from the NCBI's GenBank database. Sequences were aligned using MUSCLE on MEGA6 before generating phylogenetic trees.[19] The maximum-likelihood method based on the Tamura–Nei model was used to construct the trees.



M, 100 bp plus molecular weight marker; C, control lane (no DNA); 1, unknown; 2, replicate of unknown

Figure 3: 18S rDNA internal transcribed spacer (ITS) region PCR products resolved on a 2% agarose gel.

Animal handling

All relevant national regulations and institutional policies on the care and use of invertebrates in research were complied with.

Results

Steinernematids were recovered in 1 out of 80 (1.25%) soil samples obtained from uncultivated grassland in Walkerville, south of Johannesburg, South Africa. The soil positive for steinernematids was classified as sandy loam.

Morphometric analysis showed that the isolates obtained from infective juvenile and male nematodes belonged to the family Steinernematidae; the isolate (TEL) is conspecific with *Steinernema australe*.[20] Compared to that previously described for *S. australe,* the infective juveniles of TEL appear smaller in all measured characteristics – body length, tail length and pharynx length. *S. australe* TEL is characterised by the length of the male (1598 μm), which is slightly shorter than that previously described for *S. australe,*[20] which have a total body length of (1606 μm). The total body length of infective juveniles of *S. australe* TEL is 1301 μm, which is close to that of *S. australe* with a total body length of 1316 μm. *S. australe* TEL male spicule length is 70 μm and resembles *S. australe* spicule length of 72 μm. The *S. australe* TEL pharynx length is slightly smaller (181 μm) than the pharynx length of *S. australe* (186 μm). The male tail length of *S. australe* TEL is 27 μm, which approximates that of *S. australe* which has a tail length of 29 μm.

Table 2: Comparative morphometric data of *Steinernema australe* (South African isolate and 167 known species[20])

	***S. australe* TEL**	***S. australe* (*n*=167**
Male		
Body length	1598±30.9. (1382–1899)	1606±31.8 (1401–1937)
Tail length	27±0.8 (19–32)	29±0.9 (20–35)
Spicule length	70±1.1 (52–74)	72±1.2 (55–78)
Pharynx length	181±2.3 (158–198)	186±2.4 (167–205)
D%	68±1.2 (57–83)	71±1.3 (59–87)
E%	–	–
MUC	–	–
Infective juveniles		
Body length	1301±20.9 (1155–1480)	1316±21.4 (1162–1484)
Tail length	99±1.4 (90–111)	103±1.6 (92–114)
Spicule length	–	–
Pharynx length	163±2.7 (138–189)	169±2.9 (140–194)
D%	62±1.1 (56–74)	65±1.3 (57–78)
E%	104±1.8 (91–119)	107±1.8 (94–122)
MUC	–	–

All measurements represent mean and range in μm.

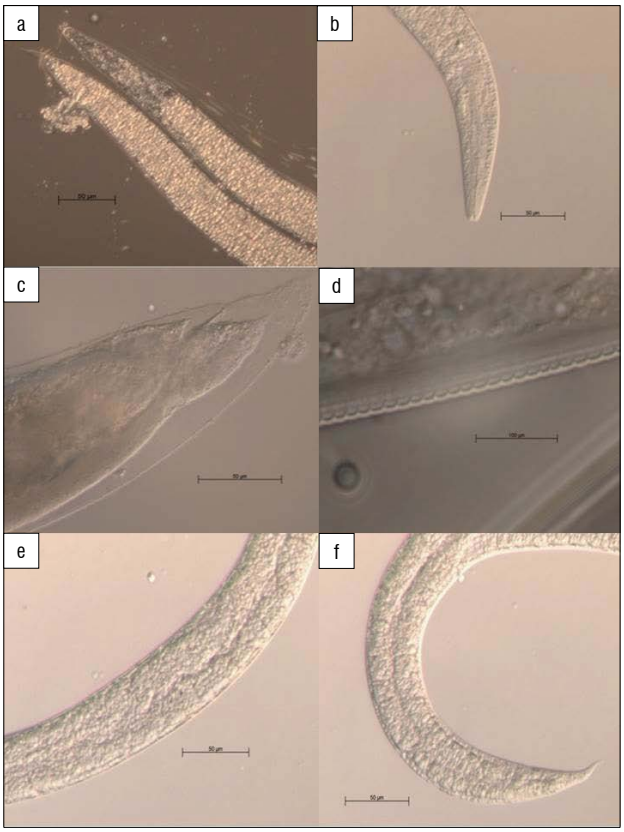


Figure 4: *Steinernema australe*: (a) male and female adult nematodes, (b) the head, highlighting the mouth, stylet, oesophagus and metacorpus, (c) tail of a female adult, (d) thick cuticle of an adult body, (e) intestines and (f) pointed female tail.

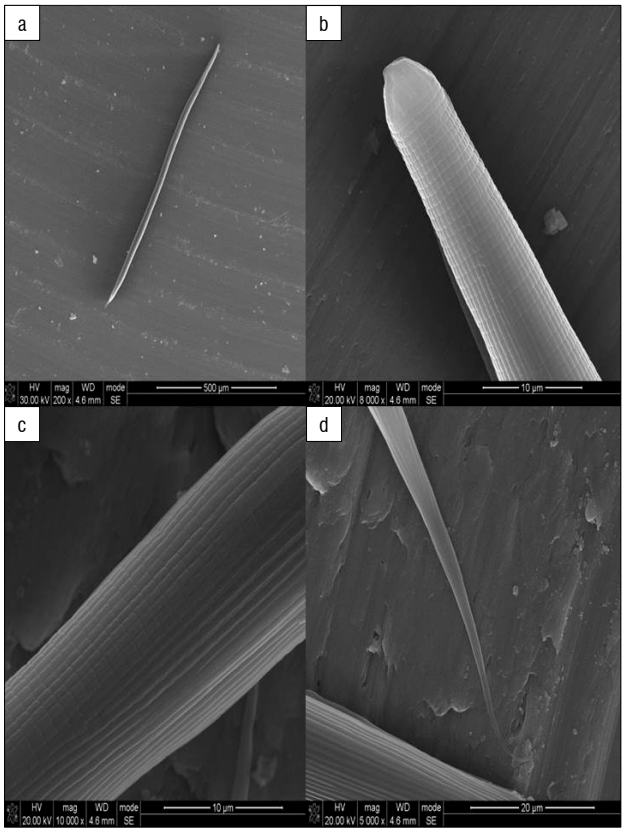


Figure 5: Scanning electron microscopy of *Steinernema australe*: (a) infective juveniles, (b) tessellate anterior region with the mouth and papillae, (c) radial symmetry and tessellate body and (d) pointed tail of a female.

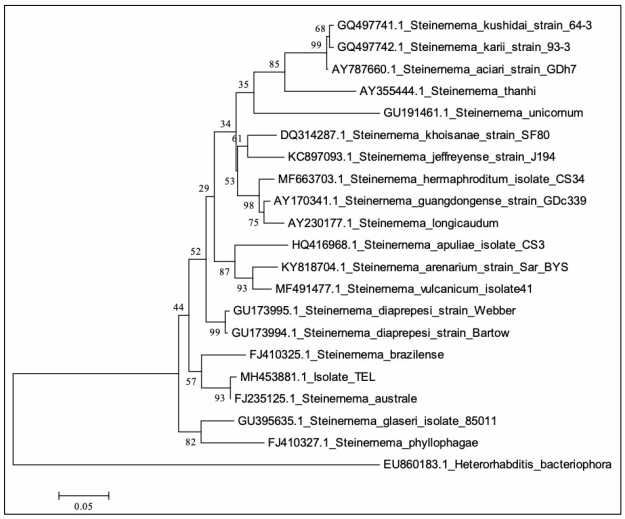


Figure 6: Evolutionary relationships of the new South African entomopathogenic nematode isolate (TEL). Sequences were aligned first using the MUSCLE alignment tool on MEGA6 software. The evolutionary history of the aligned sequences was centred on the analysis of the 18S rDNA internal transcribed spacer (ITS) region inferred using the maximum-likelihood method based on the Tamura–Nei model[19] in MEGA6. The bootstrap consensus tree was inferred from 1000 replications and the tree is drawn to scale, with branch lengths measured by the number of substitutions per site (next to the branches). The bar scale of 0.05 substitutes per nucleotide position.

The morphometric data presented in Table 2 and Figures 4 and 5 are considered to be the most consistent for *S. australe* described by Adams and Nguyen[21]. Scanning electron microscopy provides an understanding of the surface structure of the nematodes (Figure 5).

Phylogenetic analysis of the studied isolate of *S. australe* and homologous sequence of the same genus from GenBank is presented in Figure 6. The phylogenetic tree reveals that the South African isolate is grouped with the strain *S. australe* and thus supports that the isolate identified in the study is *S. australe*. The sequences were deposited in GenBank under accession number 165 MH453881 (TEL).

Discussion

This is the first record of a specific species from the family Steinernematidae, namely *Steinernema australe,* found in South Africa, thus confirming the extent of its geographical occurrence in the southern hemisphere, for comparative purposes with previous studies. The prevalence of steinernematid isolation from soil samples in our study was 2%, which is similar to previous reports by Edgington et al.[20] *S. australe* was recovered from uncultivated grassland only, and all positive samples were from locations in close proximity.

Soil samples were collected between autumn and winter in South Africa and recovery of entomopathogenic nematodes from the positive site suggests that the nematodes are able to survive in both seasons. *S. australe* was found in sandy loam soil collected from uncultivated grassland on Road Number 6 in Walkerville whereas Road Number 7 and Boundary Road were negative sites, that is, no entomopathogenic nematodes were recovered from these sites, which were one block apart.

Our survey was conducted in Gauteng which is in the north of South Africa. In previous surveys conducted in various provinces in South Africa, a novel undescribed steinernematid was recovered only from the Free State and KwaZulu-Natal Provinces where humid subtropical environments predominate and the entomopathogenic nematodes reside in soil which has a high clay content and is less acidic.[22]

The effectiveness of entomopathogenic nematodes as biocontrol agents is affected by the type of soil in which they reside. Soil properties such as particle size, organic matter, texture and several other traits influence their foraging abilities and dispersal.[23]

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Authors' contributions

T.E.L.: Collected soil samples; undertook the research, species identification, experimental work as listed in the 'Materials and methods', and data analysis; interpreted the results; and wrote the manuscript. V.M.G.: Collected soil samples and edited the manuscript.

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Forgiveness moderates relations between psychological abuse and indicators of psychological distress among women in romantic relationships

Forgiveness frequently occurs in a relational context and is a key ingredient for restoring and maintaining intimate relationships. Yet, certain interpersonal dynamics that sometimes motivate forgiveness (e.g. abuse) have the potential to adversely affect well-being, especially when ongoing exploitation occurs. In this study, we examined the role of forgiveness in moderating relations between psychological abuse and indicators of psychological distress in a sample of community-based South African women currently in a heterosexual romantic relationship. Participants (n=515) completed measures of decisional and emotional forgiveness of their partner, psychological abuse committed by their current partner during the course of the relationship, and depression, anxiety, and stress. Latent profile analysis identified two subgroups characterised by differing levels of forgiveness: *partial forgiveness* (high decisional forgiveness and moderate emotional forgiveness) and *complete forgiveness* (high decisional and emotional forgiveness). Regression analyses revealed that the relations of psychological abuse with depression and stress, but not anxiety, were moderated by 'forgiveness of partner'. The complete forgiveness group scored lower on depression and stress when psychological abuse was lower, but higher on each outcome when psychological abuse was higher. The findings suggest that there may be conditions in which forgiveness of partner may promote or undermine the mental health of women who experience abuse perpetrated by their current partner.

Significance:

- Whereas women in continuing romantic relationships generally sought neither to avoid or seek revenge on their partners (i.e. decisional forgiveness), distinct subgroups were characterised by more or less reduction of negative emotions (i.e. emotional forgiveness).
- Within the context of continuing romantic relationships, the mental health benefits that ordinarily accompany more thorough processing of unforgiveness may be eroded when victims are exposed to severe levels of potentially ongoing psychological abuse.

Introduction

Forgiveness is a multifaceted process that involves (1) making a decision to relinquish negative behavioural intentions towards a transgressor and (2) replacing negative other-oriented emotions with positive other-oriented emotions.[1,2] An abundance of research supports the mental and physical health benefits of forgiveness[3], suggesting forgiveness should be encouraged. Yet, there are specific relational contexts in which the drawbacks of forgiveness for the forgiver may negate or outweigh its advantages. Research involving romantic partners (e.g. married couples) has highlighted the role of forgiveness in reinforcing negative partner behaviour.[4] Other studies have found increased problem severity among those who are more forgiving of partners who frequently engage in negative, hurtful behaviours.[5]

Intimate partner violence and forgiveness

One category of offence that may unduly exploit forgiveness within romantic relationships is intimate partner violence (IPV) – an umbrella term encapsulating physically, sexually and psychologically abusive behaviour committed by a current or former partner.[6] Evidence suggests that forgiveness offered by victims of IPV may contribute to the continuation of the victim–perpetrator abuse cycle. For example, victims who forgive their partner for IPV are more likely to minimise partner aggression[7] and return to their abusive partner after having previously left them[8]. These kinds of cognitive-behavioural responses represent mechanisms by which relationships with perpetrators may continue[9], thereby placing victims' well-being at risk.

Several studies have reported on relations between forgiveness for various forms of IPV and the physical and mental health of forgivers. Some findings identify forgiveness as a salubrious response that may buffer against maladjustment linked to IPV. In one study, Ysseldyk et al.[10] found that physical and psychological abuse moderated relations between forgiveness and depression in a cross-sectional sample of female undergraduate students. In particular, forgiveness yielded stronger negative associations with psychological symptoms at higher levels of abuse compared to lower levels of abuse. Other evidence suggests forgiveness may inadvertently contribute to an enduring pattern of IPV and undermine the physical and psychological health of victims. McNulty[9] investigated changes in psychological and physical aggression over a 4-year period in a sample of married couples. Findings revealed that psychological and physical aggression perpetrated by spouses tended to decline among partners who were less forgiving, but remained relatively stable for those partners who were more forgiving of their spouse. In another study focusing on mental and physical health symptom outcomes, Lahav et al.[11] found that the effect of forgiveness in protecting against distress among military spouses who experienced lower levels of partner abuse was absent at higher levels of abuse. Importantly, few studies have examined links between forgiveness and IPV in low- and middle-income regions (such as those in Africa) where prevalence estimates of IPV among women are typically high.[12] Prior studies in this area have also generally relied on measures of forgiveness that inadequately capture distinct decisional and emotional

components, which are conceptually unrelated processes.[13] In this study, we used a multidimensional approach to assess decisional and emotional forgiveness of a current, heterosexual romantic partner in a community sample of South African women.

Unique implications of decisional and emotional forgiveness

Deciding to forgive can trigger emotional forgiveness[14], but the process of emotional forgiveness is not necessarily predicated on or a byproduct of decisional forgiveness. A victim can make a decision to reduce negative behaviour toward a transgressor and perhaps act benevolently toward a transgressor, yet may still experience ongoing emotional unforgiveness (e.g. anger, disappointment, resentment).[1] Also, whereas the proxies of decisional forgiveness unfold at the interpersonal level (i.e. reduction of negative behavioural intentions towards the transgressor), emotional forgiveness is predominantly an intrapersonal process (i.e. reduction of negative emotions and possibly enhancement of positive emotions toward a transgressor).[15]

The salience of emotional and decisional forgiveness appears to vary by relationship context. When offences occur in close relationships, victims weigh relationship value cues relevant to the transgressor alongside cues associated with future risk of exploitation.[16] The behavioural proxies (e.g. avoiding the perpetrator, maintaining physical distance) that might accompany a decision not to forgive romantic partners who perpetrate IPV can help to protect victims from exposure to subsequent instances of abuse, but such benefits may not outweigh the potential implications (e.g. further disintegration of a valued relationship) of deciding to withhold forgiveness.[17] Victims who value the perceived benefits of the romantic relationship over the risk of future exploitation may make a decision to forgive their partner for IPV in an attempt to limit conflict, resolve relational disrepair, and restore the relationship back to pre-transgression levels of closeness.

Although withholding decisional forgiveness for IPV may serve an important role in promoting behaviours that safeguard against subsequent instances of abuse (e.g. physically distancing oneself from the transgressor), mental health benefits are usually derived by resolving emotional unforgiveness.[18] Some arrangement of positive emotions is needed for victims to neutralise emotional unforgiveness; victims' net final emotional valence towards offenders may be negative (partial forgiveness), neutral or positive (complete forgiveness).[14] In close and valued relationships, returning to a net positive valence towards an offender is considered a necessary part of rebuilding a healthy relationship between affected parties.[19] Reducing emotional unforgiveness beyond mere elimination of negative emotions may make forgiving a valued person more difficult[13], particularly as the victim attempts to absorb and make sense of being betrayed by a close person whom they trusted[20,21]. The efforts involved in reaching complete forgiveness may leave victims vulnerable to renewed, intensified psychological distress should they be taken advantage of again, as the distress evoked by recurring offences is likely to be compounded by victims' negative self-oriented responses (e.g. self-blame and diminished sense of self-respect) for opening themselves up to further emotional injury.[22,23] Drawing on longitudinal evidence indicating that subsequent IPV is associated with increased risk of internalising symptoms (e.g. depression) even after partialling out effects of prior IPV[24], recurring abuse in continuing romantic relationships has the potential to erode the mental health benefits that ordinarily accompany emotional forgiveness.

The present study

Women in continuing romantic relationships who tend to completely forgive their partners for offences involving abuse may be at risk of maintaining the cycle of abuse and their consequent psychological distress. To examine this proposition further, we applied a person-centred approach to identify unique combinations of emotional and decisional forgiveness of partner patterns among a community sample of South African women in a continuing heterosexual romantic relationship. We hypothesised that participants in each of the subgroups identified would tend to make decisions to behave differently toward their partner, yet would exhibit distinctions in processing of emotional forgiveness of their partner. We then examined whether the relations between psychological abuse and indicators of psychological distress were moderated by the forgiveness of partner profiles that emerged.

Method

Participants

A community-based sample (n=515) of South African women between 18 and 77 years of age (M_{age}=29.45, s.d.$_{age}$=10.06) participated in this study. The majority of the sample reported being in a non-cohabiting relationship (62.72%), with the remainder either in an unmarried, cohabiting relationship (8.74%), engaged to be married (6.21%), or married (21.55%); the relationship was unspecified for 0.78% of the sample. The race distribution of the sample was largely representative of the general population, consisting of those who identified as black/African (71.84%), coloured (6.60%), Indian (10.87%), white (9.71%), and 'other' (e.g. East Asian, 0.58% or unspecified, 0.39%). Regarding religious affiliation, participants identified their affiliation as Christianity (80.19%), Hinduism (3.30%), Islam (4.08%), atheism (5.63%) or 'other' (e.g. Buddhism, traditional African religion, 5.83%; unspecified, 0.97%).

Measures

Forgiveness

Participants completed adapted versions of the Decisional Forgiveness (DFS; i.e. an intent to act differently toward the transgressor) and Emotional Forgiveness Scales (EFS; i.e. an emotional change that involves reducing negative emotions and perhaps increasing positive emotions toward the transgressor).[25] Both scales consist of two subscales: DFS – prosocial intentions and inhibition of harmful intentions and EFS – presence of position emotion and absence of negative emotion. Because the subscales contain four items each, Worthington et al.[25] recommend collapsing the respective subscales for use as overall measures of decisional and emotional forgiveness. In this study, items were modified to obtain a general measure of participants' decisional (e.g. 'I act friendly towards him') and emotional (e.g. 'I'm bitter about what he has done to me') forgiveness of the person with whom they were in a romantic relationship at the time of data collection. Items were rated using a five-point response format (1 = Strongly disagree; 5 = Strongly agree). Respective items were added together to derive scale scores on the DFS (ω_t=0.77) and EFS (ω_t=0.87). Higher scores on each scale correspond with greater decisional and emotional forgiveness.

Psychological abuse

We administered the Psychological Maltreatment of Women Inventory (PMWI)[26], which is a measure of psychological abuse a woman has experienced during the course of her current romantic relationship. The PMWI consists of 58 items distributed across the dimensions of dominance-isolation and emotional-verbal abuse. In this study, participants rated the items with reference to the person with whom they were currently in a heterosexual relationship (e.g. 'He treated me like an inferior'). A five-point response format (1= Never; 5 = Very frequently) was used to rate each item. All items were summed for a global measure of psychological abuse (ω_t=0.97), with higher scores reflecting greater psychological abuse.

Psychological distress

Participants also completed the 42-item Depression, Anxiety, and Stress Scales.[27] The items are evenly distributed across the three subscales of depression (e.g. 'I felt down-hearted and blue'), anxiety (e.g. 'I was in a state of nervous tension') and stress (e.g. 'I tended to over-react to situations'). In this study, participants responded to each item by considering the extent to which each statement applied to them over the last month. Responses were provided using a four-point response format (0 = Did not apply to me at all; 3 = Applied to me very much, or most of the time). Subscale scores were calculated by summing respective depression (ω_t=0.96), anxiety (ω_t=0.94) and stress (ω_t=0.95) items. Higher scores on each subscale correspond with higher levels of psychological distress.

Procedure

Ethical permission to conduct this study was granted by the University of KwaZulu-Natal Humanities and Social Sciences Research Ethics Committee (reference number HSS/1722/016). Participants were recruited through online advertisement campaigns on a number of social media platforms (e.g. Facebook) and directed to a secure data collection site via a weblink. After voluntarily offering their informed consent, participants were presented with demographic items that contained several eligibility screening items. To be considered for inclusion in this study, participants had to be women of at least 18 years of age and in a heterosexual romantic relationship at the time of data collection. Those who did not meet either of these criteria were directed to a debriefing section to conclude participation, while eligible participants completed the measures in English.

Statistical approach

All statistical analyses were performed using R.[28] Missing data diagnostics did not reveal any item-level missing data needing replacement. Skewness and kurtosis values of the primary study variables ranged from good ($<|1|$) to acceptable ($<|2|$)[29], indicating that univariate normality could be assumed. Internal consistency of all measures was estimated using omega total (ω_t), a procedure that is robust to violations of tau equivalence.[30] Internal consistency for each measure was acceptable (i.e. >0.70).[31] Pearson correlations provided an indication of the bivariate associations among the primary study variables.

Latent profile analysis of emotional and decisional forgiveness

A latent profile analysis was used to test for the existence of forgiveness of partner subgroups based on unique combinations of decisional and emotional forgiveness. Models with one to six profiles were estimated to identify the model with the best fit. Along with the bootstrap (10 000 repetitions) likelihood ratio test, we report the log-likelihood value, the Akaike information criterion, and the Bayesian information criterion (BIC) and its sample-size adjusted variant. Information criteria were supplemented with values that estimate the precision of profile classification, including entropy and the mean posterior probabilities of profiles for each model. Statistically significant bootstrap likelihood ratio test p-values ($p<0.05$), lower BIC and sample-size adjusted BIC values, and models with entropy values closer to one and mean posterior probabilities ≥ 0.70 for all profiles were prioritised when making decisions about model fit.[32-34] The unique subgroups that emerged from the latent profile analysis formed the 'forgiveness of partner' variable.

Moderated regression of psychological abuse and forgiveness of partner

Separate multiple regression analyses were performed to examine whether 'forgiveness of partner' moderated relations between psychological abuse and indicators of psychological distress (i.e. depression, anxiety and stress). Main effects (i.e. psychological abuse and forgiveness of partner) and interaction effects (i.e. psychological abuse x forgiveness of partner) were specified for all models. The psychological abuse variable was mean centred before model estimation. Socio-demographic characteristics of age, relationship status, race and religious affiliation were included as covariates in each model. Visual inspection of quantile-quantile plots and residual plots produced via a Wallyplot technique[35] indicated that the residuals for each model appeared approximately normal and homoscedastic in distribution. Collinearity diagnostics did not reveal any multicollinearity issues (all variance inflation factor values ≤ 3.41).

Results

Descriptive statistics for the primary study variables and zero-order correlations among them are displayed in Table 1. Both decisional and emotional forgiveness evidenced negative relations with depression, anxiety, stress and psychological abuse, although effect sizes were generally larger for emotional forgiveness ($r=-0.30$ to -0.45, all $p<0.001$) than for decisional forgiveness ($r=-0.17$ to -0.27, all $p<0.001$). Psychological abuse associated positively with depression, anxiety and stress ($r=0.32$ to 0.45, all $p<0.001$).

Latent profile analysis of emotional and decisional forgiveness

Model fit indices for each model are reported in Table 2. Bootstrap likelihood ratio test p-values for models with two and six profiles both reached statistical significance. The two-profile solution yielded the lowest BIC and sample-size adjusted BIC values, and the highest entropy value. It was also the only solution in which the mean posterior assignment probabilities were ≥ 0.70 and the number of assigned cases was $\geq 5\%$ for all profiles. Overall, the two-profile solution yielded the best level of fit to the data. In Figure 1, we display the mean decisional and emotional forgiveness values for each subgroup.

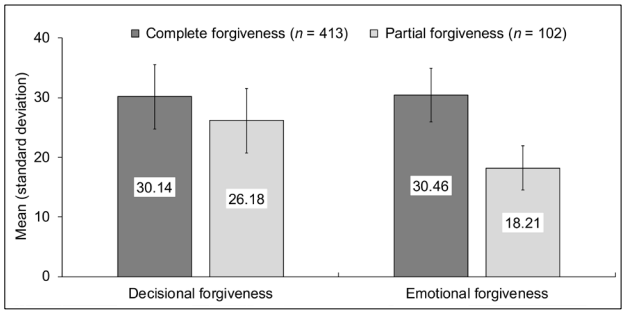

Figure 1: Profiles indicating decisional and emotional forgiveness of partner.

The subgroup of participants classified into profile one (80.19%) reported similar levels of decisional forgiveness (M=30.14, s.d.=5.36) to those grouped into profile two (M=26.18, s.d.=5.40). We applied a criterion value that corresponded with a net neutral level of emotional forgiveness

Table 1: Descriptive statistics, internal consistency estimates and zero-order correlations among primary study variables

	(1)	(2)	(3)	(4)	(5)	(6)
(1) Decisional forgiveness	0.77					
(2) Emotional forgiveness	0.46* [0.38, 0.52]	0.87				
(3) Psychological abuse	-0.17* [-0.25, -0.08]	-0.45* [-0.52, -0.38]	0.97			
(4) Depression	-0.21* [-0.29, -0.13]	-0.38* [-0.46, -0.31]	0.45* [0.38, 0.51]	0.96		
(5) Anxiety	-0.27* [-0.35, -0.18]	-0.30* [-0.38, -0.22]	0.35* [0.27, 0.42]	0.81* [0.78, 0.84]	0.94	
(6) Stress	-0.26* [-0.34, -0.18]	-0.30* [-0.37, -0.22]	0.32* [0.24, 0.39]	0.83* [0.80, 0.86]	0.86* [0.84, 0.88]	0.95
M (s.d.)	29.36 (5.59)	28.03 (6.52)	107.02 (40.75)	14.87 (12.09)	11.97 (10.32)	16.82 (10.92)
Range	11–40	8–40	58–274	0–42	0–42	0–42
Skewness	-0.23	-0.49	1.38	0.74	0.94	0.53
Kurtosis	-0.37	-0.02	1.69	-0.53	0.11	-0.61

*p<0.001
Omega total (ω_t) internal consistency estimates are presented along the diagonal.

Table 2: Fit indices for forgiveness of partner latent profile models

Model	LogLik	AIC	BIC	SABIC	BLRT	Entropy	nMPAP < 0.70	nP<5%
1-Profile	-3252.27	6514.54	6535.76	6519.89	–	–	–	–
2-Profile	**-3241.30**	**6498.60**	**6532.56**	**6507.16**	**<0.001**	**0.886**	**0**	**0**
3-Profile	-3240.53	6503.05	6549.74	6514.82	0.292	0.721	1	0
4-Profile	-3240.64	6509.29	6568.71	6524.27	0.854	0.607	4	0
5-Profile	-3238.18	6510.35	6582.51	6528.54	0.119	0.586	4	1
6-Profile	-3229.56	6499.12	6584.00	6520.52	0.008	0.661	3	0

LogLik, log-likelihood; AIC, Akaike information criterion; BIC, Bayesian information criterion; SABIC, sample-size adjusted Bayesian information criterion; BLRT, p*-value for bootstrap likelihood ratio test; nMPAP <0.70, number of mean posterior assignment probabilities below 0.70; nP<5%, number of profiles assigned fewer than 5% of cases; entries in boldface reflect selected model*

on the EFS (i.e. 24) to differentiate levels of emotional forgiveness. The emotional forgiveness of participants in profile one (M=30.46, s.d.=4.46) was consistent with complete forgiveness (i.e. neutral or net positive emotional forgiveness, ≥24), whereas emotional forgiveness of those included in profile two (M=18.21, s.d.=3.70) reflected partial forgiveness (i.e. net negative emotional forgiveness, <24). Based on these decisional and emotional forgiveness patterns, the subgroups were labelled *complete forgiveness* (profile one) and *partial forgiveness* (profile two).

Moderated regression of psychological abuse and forgiveness of partner

Results of the moderated regression analyses are reported in Table 3. Psychological abuse yielded positive relations with depression, anxiety and stress (all $p<0.001$). Forgiveness of partner was positively associated with depression ($p<0.001$) and stress ($p=0.029$), but not anxiety ($p=0.063$), such that the partial forgiveness group tended to report higher levels of depression and stress compared to those in the complete forgiveness group. Relations of psychological abuse with depression ($p=0.043$) and stress ($p=0.039$) were moderated by forgiveness of partner (see Figure 2), although no interaction effect was found for anxiety ($p=0.164$). Depression and stress were lower among participants in the complete forgiveness group at lower levels of psychological abuse, but were higher at more severe levels of psychological abuse.

Discussion

The purpose of this study was to (1) identify distinct emotional and decisional forgiveness of partner patterns among a sample of South African women in ongoing heterosexual romantic relationships and (2) examine whether relations between psychological abuse and psychological distress would be moderated by the forgiveness of partner profiles that emerged. The findings revealed that forgiveness of partner experiences varied based on unique combinations of decisional and emotional forgiveness, namely *partial forgiveness* (i.e. higher levels of decisional forgiveness and lower levels of emotional forgiveness) and *complete forgiveness* (i.e. higher levels of decisional and emotional forgiveness). The focus of this study was on psychological abuse perpetrated throughout the duration of the current romantic relationship, so it is possible that behavioural proxies of decisional forgiveness (e.g. reconciliation) have a role in preserving the ongoing status of close relationships. Although victims' processing of emotional forgiveness could still be ongoing, evidence of heterogeneity in processing of emotional forgiveness suggests that victims may not necessarily return

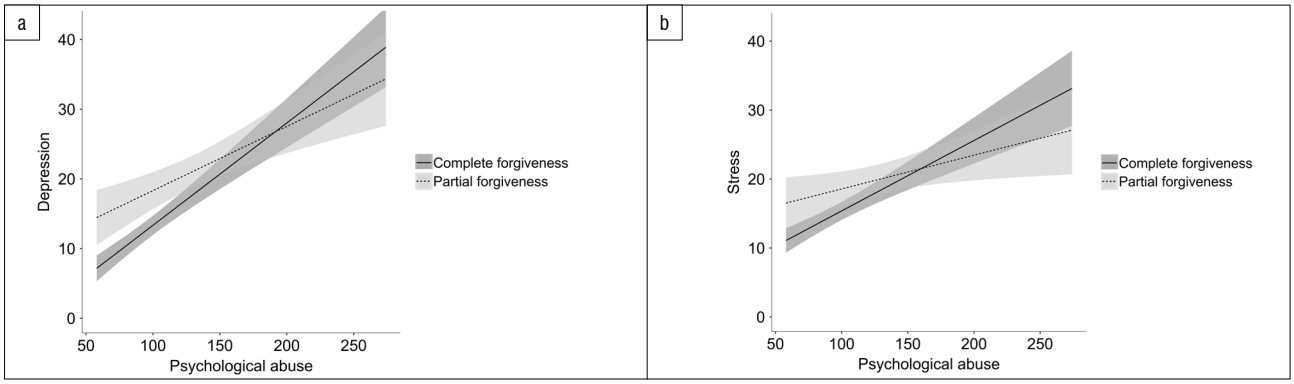

Figure 2: Relations of psychological abuse with (a) depression and (b) stress are moderated by forgiveness of partner.

Table 3: Summary statistics for multiple regression analyses

Predictors	DV = Depression		DV = Anxiety		DV = Stress	
	B (s.e.)	β [95% CI]	B (s.e.)	β [95% CI]	B (s.e.)	β [95% CI]
Psychological abuse	0.15** (0.02)	0.49 [0.39, 0.60]	0.10** (0.01)	0.39 [0.28, 0.51]	0.10** (0.02)	0.38 [0.27, 0.49]
Forgiveness of partner†	4.59* (1.35)	.15 [0.06, 0.24]	2.25 (1.21)	0.09 [-0.00, 0.18]	2.82* (1.28)	0.10 [0.01, 0.20]
Psychological abuse x Forgiveness of partner†	-0.05* (0.03)	-0.11 [-0.22, -0.00]	-0.03 (0.02)	-0.08 [-0.19, 0.03]	-0.05* (0.03)	-0.12 [-0.23, -0.01]
R^2	0.25		0.17		0.16	
F(df)	10.62** (15, 488)		6.65** (15, 488)		6.17** (15, 488)	

All models control for age, race, religious affiliation and marital status.
†Reference group = complete forgiveness
*p<0.05, **p<0.001

to a net neutral or positive emotional experience toward their current partner in the aftermath of an offence.

The unique forgiveness of partner patterns evidenced in this study might reflect differences in the function of victims' forgiveness. Strelan et al.[36] found that forgiveness of close transgressors was more likely to be experienced out of benefit to the self and the relationship than that of the transgressor, but that forgiveness for the sake of the relationship yielded the strongest associations with forgiveness and relationship closeness. Perhaps victims in the complete forgiveness group have a preference towards relationship-focused forgiveness of their partner in order to preserve the valued relationship. On the other hand, the primary focus of forgiveness for those in the partial forgiveness group could be the self. Although self-focused forgiveness may serve to protect the victim from further emotional injury, it may be detrimental to restoration of relational closeness.

Distinctions in forgiveness observed in the current study also align with the mixture of individualistic and collectivistic principles that permeate the ways in which forgiveness is experienced in South African culture.[37,38] At the expense of victims' own needs, collectivistic norms may emphasise the need for victims to forgive transgressors out of obedience to social expectations.[39] Collectivistic principles might explain the decisions of those included in the current sample to forgive their partner, but processing of emotional forgiveness may depend on the extent to which victims' intrapersonal needs are adequately met.

Our results also indicate that relations between psychological abuse and indicators of psychological distress were moderated by forgiveness of partner. In contrast to the partial forgiveness group, those in the complete forgiveness group were found to be at reduced risk of psychological distress at lower levels of psychological abuse, but at increased risk of distress at higher levels of abuse. These findings resonate with previous research that has identified divergent implications of forgiveness for the mental health of victims of abuse[11], particularly the notion that the protective effects of forgiveness may be eroded by abuse that occurs in continuing romantic relationships.

A useful perspective for understanding the pattern of findings in this study is need fulfilment in romantic relationships. We speculate that forgiveness (or lack thereof) for psychological abuse may promote or diminish victims' psychological well-being to the extent that forgiveness of partner contributes to the fulfilment of victims' psychological needs. In close relationships, forgiveness is thought to promote relationship-constructive behaviours (e.g. conciliatory actions) that increase the likelihood of restoring the severed relationship to pre-transgression levels of intimacy.[40] Victims may offer forgiveness in order to continue receiving the psychological benefits that accompany a valued relationship[16,36], but such attempts are likely to be unproductive if perpetrators' post-transgression actions are disagreeable (e.g. continued re-offending).

Drawing on several studies that have found victims' needs may be deprived when undeserved forgiveness is offered[23,41], women who tend to process emotional forgiveness of their partner more thoroughly (i.e. complete forgiveness) when abuse is higher might be at risk of increased psychological distress because of the incongruency between perpetrators' post-transgression attempts at relationship reconstruction and victims' efforts to resolve emotional unforgiveness. Conversely, partial emotional forgiveness of partner may undermine psychological well-being at lower levels of abuse via the effect emotional unforgiveness (e.g. anger, resentment) has on social-cognitive processes (i.e. lower cognitive interdependence)[42] that prolong relationship disintegration with the perpetrator. Unforgiveness could also have carryover effects on victims' needs to belong by reducing feelings of relatedness towards others more generally.[43] Given the cross-sectional nature of the data in this study, research using methodologies that monitor changes in outcomes following specific incidents of psychological abuse is needed to understand the conditions in which type and degree of forgiveness may promote or undermine fulfilment of psychological needs.

A substantive contribution of this study is the use of a two-dimensional approach to measuring forgiveness in relation to IPV in ongoing romantic relationships. Whereas prior studies have largely focused on *degree* of forgiveness, the findings of this study offer additional insight into the role of decisional and emotional components of forgiveness in promoting or undermining the mental health of women who experience varying degrees of psychological abuse from their current partners. Decisional and emotional aspects of forgiveness need to be considered together when making determinations about the appropriateness of forgiveness as a treatment modality for victims of IPV. Assessments that emphasise degree of forgiveness, whilst neglecting type of forgiveness, may limit therapeutic effectiveness.

Broadening the scope of previous research that has tended to focus on abuse that transpires in situations involving conflict[10], the present findings also highlight the importance of identifying effects of psychological abuse that may be perpetrated across a broader range of situations. As such, there is a need to contextualise forgiveness within a wide range of victim–partner interactions in which psychological abuse occurs. Use of measures that are sensitive to detecting covert forms of psychologically abusive partner behaviour may provide opportunities for enhancing the effectiveness of therapeutic efforts targeting forgiveness.

The current findings may help inform the clinical application of forgiveness for victims of IPV. Whereas Fincham et al.[44] suggest that forgiveness of close others typically involves more than mere reduction of negativity toward a transgressor and includes enhancement of positive other-oriented emotion, this expectation may be unrealistic when a close relationship is characterised by severe or persistent abuse. For this reason, alongside making a decision not to personally retaliate against an abusive partner, therapeutic gains may be enhanced if IPV survivors establish an adaptive level of emotional forgiveness that balances the emotional burden of unforgiveness with the potential for future exploitation that might occur upon reconciliation with an abusive partner. Exploration of the meaning of residual negative feelings toward an abuser in a safe and supportive environment might be beneficial if it reveals how forgiveness operates in tandem with other character strengths, such as having the wisdom to accurately assess the quality of an abusive relationship.

Limitations and future research directions

Alongside the strengths of this study, there are several methodological limitations. Use of a cross-sectional design prevents inferences about causality and directionality. Experimental and longitudinal studies are needed to understand how the processes and outcomes of decisional and emotional forgiveness (both individually and in combination) change over time in women who experience psychological abuse in continuing romantic relationships. The findings of this study should be interpreted together with our methodological choice to assess forgiveness without reference to a specific offence involving abuse. Transgression-specific variables (e.g. recency and frequency of abuse) and relationship dynamics (e.g. commitment) likely influence victims' experiences of state forgiveness in response to specific types of abuse.

Although the sample included in this study corresponded with the diverse sub-populations of South Africa, cross-cultural generalisability of the finding may be limited. Research is needed to identify cross-cultural distinctions in the consequences of forgiveness (and unforgiveness), given that conceptualisations and tolerance of IPV differ across societies, cultures and ethnic groups. For example, Rajan's[45] qualitative study involving a Tibetan group of victims, friends/relatives of victims, and perpetrators of physical partner abuse identified conditions in which abuse was perceived to be acceptable, or even justified. Along similar lines, based on evidence highlighting the role of third parties in the forgiveness process[46], it would be prudent to explore the relevance and impact of broader social influences (i.e. proximodistal social factors that are beyond the victim–perpetrator dyad) in facilitating or deterring forgiveness among victims of IPV.

Conclusion

In this study, we identified the existence of two unique forgiveness of partner patterns in a sample of South African women who were in a continuing heterosexual romantic relationship, each of which exhibited

distinct effects on relations between psychological abuse and indices of psychological distress. Notwithstanding the need for additional research in this area, the findings are consistent with a growing body of evidence that has identified circumstances in which the protective mechanism of forgiveness may be overwhelmed by IPV that occurs within the context of ongoing romantic relationships.

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Authors' contributions

All authors developed the study concept and contributed to the study design; R.G.C. coordinated data collection, performed the data analyses and drafted the manuscript. E.L.W., B.J.G. and R.C.G. provided critical revisions to the manuscript. All authors approved the final version of the manuscript for publication.

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