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Provitamin A selection in African cassava

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## Leader



## Obituary



## Book Review



## Commentary

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**Cover caption**

A Knysna dwarf chameleon (*Bradypodion damaranum*). In their study, Adair and colleagues explored the efficacy of a minimally disruptive DNA sampling technique, buccal swabbing, as an alternative to tissue sampling via tail clipping, in dwarf chameleons (*Bradypodion* spp.).

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# Postgraduate students and publishing in academic journals

In common with other journals, the *South African Journal of Science* often receives submissions from authors who have recently completed a piece of postgraduate research and who are required to provide evidence either of having submitted an article to a journal or having published a journal article before they can graduate. In some cases, students are required to provide such proof before they are allowed even to submit their theses.

This practice raises important questions in a number of areas related to doctoral education. In South Africa, the *Qualification Standard for Doctoral Degrees*[1] draws on the concept of "graduate attributes" in standard setting. Attributes, or characteristics of the graduate, fall into two areas: knowledge attributes and skills attributes. If the Doctoral Standard is to count for anything, assessment of a candidate's readiness to be awarded the degree needs to include consideration of the attributes, not all of which are easily discernible in the thesis, although disciplinary differences will come into play here.

What is clear, however, is that the requirement to provide evidence of submission of an article or of the publication of an article in a journal, duplicates what is already likely to be evident in a thesis when, arguably, what is necessary are additional assessment requirements that will allow a candidate to demonstrate what is not so readily evident. A thesis in any knowledge area will provide evidence of the acquisition, on the part of the graduate, of "well informed relevant knowledge in the selected field" and of "expert, specialized and in-depth current knowledge of a specific area of research"[1(p.13)] but may not easily lend itself to a demonstration of the way in which "the specific area of research relates, or is relatable, to other fields of study", all of which are identified as "knowledge attributes" in the Doctoral Standard. A similar claim can be made of the "skills attributes", one of which involves the ability to "communicate research findings effectively to expert and non-expert audiences alike". While a journal article provides evidence of the ability to address expert audiences, another form of writing (in the form of, for example, an article in *The Conversation*) would be needed to be sure that the graduate is able to interact with non-experts.

Requirements to submit to, or publish in, a journal before a degree can be awarded also have important implications for supervision. The quality of supervision was a concern noted in the 2010 ASSAf report on the PhD[2] as, too, were issues related to the lack of sufficient supervisory capacity in universities and the concomitant overburdening of supervisors. If universities make the submission or publication of an article a requirement for the award of a degree, then, as with all other assessment, students need to be guided towards completing the assessment task. Theses and journal articles are different genres, not least because the readership is different. A journal article needs to take a 'slice' of a thesis and report on it succinctly. This means that supervisors not only need to develop students' capacity to write the thesis but also the genre of the article. Research on doctoral publication trends in South Africa[3] shows that fewer than half of all doctoral graduates from South African universities published from their theses within 10 years of their graduation. As many of these graduates working in universities will now be supervising, questions may well need to be asked about overall capacity within the system to provide guidance to doctoral candidates who need to write an article before they can graduate. Can a supervisor who has not published or who does not publish regularly provide the kind of guidance necessary to allow a student to do so?

In many articles submitted by postgraduate students, the lack of guidance that would be provided by a more experienced writer is immediately evident. Many submissions, for example, are effectively summaries of an entire thesis. As Tomaselli[4] points out, this then leaves journal editors and reviewers with the task not only of processing a submission but also, ideally, with that of providing the feedback that should have been the responsibility of the supervisor. Although many supervisors do not appear to offer any guidance to students required to write articles, submissions commonly still bear their names and the benefits derived from publication will still accrue to them.

The *Doctoral Degrees National Report*[5] recommends that universities should develop clear policies on their requirements regarding the publication of articles from theses and "should implement processes" to support them. These processes need to include ensuring that support for writing articles is provided by supervisors who are familiar with the genre of the article and the requirements and expectations of the readership of different journals. Arguably, the recommendation in the Report does not go far enough as it leaves the way open for claims to be made that an institution provides a generic writing course or a writing centre, despite the fact that the kind of expert guidance needed to be able to publish may very well be unavailable in either.

These issues are not unique to South Africa, and in fact our journal receives submissions from graduate students in other countries – students who also often appear poorly trained and supported in academic publishing but are required to produce publications. Tomaselli[4] has noted in the South African context that requirements for students to publish before they can graduate can be seen as 'milking' the DHET subsidy system. One of the purposes of the subsidy is to provide funding for teaching. Without careful thought being given to the role of the publication requirement in both assessment and teaching at postgraduate level, and without appropriate support within universities for graduate students, this claim may well have some merit.

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# George Ekama (1949–2023): A man of faith, humility and integrity and a world leader in wastewater treatment

Professor George Ekama, a distinguished leader in the field of wastewater treatment, passed away on 19 February 2023, at the age of 73. While his presence is sorely missed, his impact in the lives of colleagues and students, and in the field of wastewater treatment endures.

George was born on 17 June 1949, in Hilversum, the Netherlands. His family immigrated to South Africa in the 1950s, leaving the devastation of post-World War II Netherlands behind them for a new life in South Africa. Like his grandfather, father, and older brother, George was drawn to engineering. Unlike his family members though, he chose civil engineering and earned his bachelor's degree with honours from the University of Cape Town (UCT) in the early 1970s. He then worked on site for a few years – to pay back the bursary he had received as a student – for contractors building the container quay at the Cape Town harbour. He enrolled for evening classes at the same time, which is when he met Prof. Gerrit Marais, who was leading research on biological nutrient removal in wastewater treatment processes in his laboratory at UCT. How to remove nitrogen and phosphorus by biological means was a crucial question in sanitary and environmental engineering at the time as South Africa was faced with rampant algae growth in rivers, dams and lakes. Chemical nutrient removal was the norm, but unsuitable in the context of South Africa because of its impact on the salinity of surface water, already a problem from acid mine drainage. At the completion of his contract at the harbour, George joined Marais' group as a master's student to contribute to addressing the nationally and internationally significant question of biological nutrient removal from wastewater. He then upgraded to a PhD in Engineering which was awarded in 1978. George worked as a research officer at UCT from the late 1970s, funded by research grants from the Water Research Commission and others until he was promoted to Professor of Water Quality Engineering in 1991. He held this position until his formal retirement in 2017. However, for George, retirement meant less admin and therefore more time to spend on research, until a severe stroke in 2020 brought that to an end. Throughout his career, George researched, taught, supervised and published on the treatment of wastewater, both municipal and industrial, specialising in biological nutrient removal and wastewater treatment modelling. He became a leading figure in the international field.

George was a well-established world leader in the wastewater treatment field. His research was part of the original biological nutrient removal modelling research which developed at UCT in the 1980s and was incorporated into the famous IWA (International Water Association) Activated Sludge Models. These models have had a profound impact on biological nutrient removal research worldwide. George received numerous accolades for his work over the course of his career. In 2006, he received a National Research Foundation (NRF) A1-rating in recognition of excellent and impactful research in his field. He was one of very few environmental engineering professors listed on Thomson Reuter's 2002–2013 Highly Cited Researchers website. He was a major contributor to and editor of the IWA bestselling book *Biological Wastewater Treatment: Principles, Modelling and Design* (2008). This book has been a global success with the first edition translated into Spanish, Chinese, Russian and Arabic and a second edition published in 2020[1] now openly accessible. George was the recipient of the IWA Project Innovation Award in 2012. In 2013, he was awarded the South African Order of Mapungubwe Silver by the President for outstanding research and contribution to society. In 2017, the Academy of Science of South Africa (ASSAf) named him among the 53 scientists across all fields as 'Legends of South African Science'. And George was listed as a Water Research Commission 'Legend' in 2021. While George never sought credit, fame or accolades, his work was recognised as exceptional, both locally and internationally. He attributed his success to providence, going to work every day, working hard, and paying attention to detail.

He would be quick to add that he did not work alone – he worked as part of a team. At UCT he joined and later led the Water Research Group in the Department of Civil Engineering. Under his leadership the Water Research Group achieved global acclaim and recognition for excellent research. He co-authored over a hundred peer-reviewed journal articles during his career and was a contributor to and editor of books which continue to be read and cited across the world. Foremost among these are several publications forming the foundation of the IWA Activated Sludge Models.

He built strong international collaborations in research and teaching too. From the 1990s onwards, he regularly visited Hong Kong where he taught and supervised students at the Hong Kong University of Science and Technology. He developed strong ties with the Delft University of Technology and the UNESCO-IHE Delft Institute for Water Education where he taught specialist courses each year through the 2000s and 2010s. He spent sabbaticals at Virginia Tech in the USA and the University of Padua, Italy. He was a regular participant at international workshops, symposia and conferences.

George often described what he did as 'cleaning dirty water'. Wastewater treatment has long been recognised as crucial for public health. George recognised the significance of his research in our everyday lives, combined with the fact that not only how we use water but also how we treat wastewater has an enormous impact on the environment. George's enduring faith, commitment to serving society and stewarding the environment were the foundations of his work.

George was known for his intellectual depth, his wisdom, and his dedication to his students. Over the course of his career, George supervised numerous master's and doctoral students to graduation. His students are sought after by academic and industry employers, locally and internationally. He had a remarkable ability to see the


potential in others, and to encourage and support them in achieving their goals. Memorably, he was known to say that students are like a tube of toothpaste: you have to squeeze them to see what is inside. He was patient and tireless in his efforts to help his students and was always available to offer advice and reassurance. These same qualities shaped his approach to mentoring early career academics.

George was a keen marathon runner throughout his career. In running he applied the same discipline, perseverance and exceeding organisation which he demonstrated in his work, as well as his attention to detail and love of recordkeeping – recording and plotting his mileage in training, and graphing entire fields' finishing times on green graph paper. He completed numerous marathons around the Western Cape, a handful of Two Oceans ultramarathons, and one Comrades marathon. But he will likely be remembered by colleagues and former students for arriving on campus each day out of breath and sweating, brown backpack on, having run to Upper Campus from his home in Claremont. Later in his life the running became less frequent; instead, he drove up to campus on his much-cherished cherry-red Vespa.

Although a civil engineer by training, George became a foremost expert in a specialised area of biochemical reaction engineering. Remarkably, this was achieved without any systematic exposure to the discipline, as taught to chemical engineering students. Rather, it was the result of practical experience in the laboratory, in working with treatment plants, and in teaching. Equations 4–5 in Ekama and Brouckaert[2] provide a vivid glimpse into his approach to scientific discovery. They express an important stoichiometric relationship for organic material found in wastewater, in a particularly elegant and intuitively satisfying way. He had evidently built up the equations by considering many practical applications, because he did not know how to derive them from first principles. After explaining their practical significance and application, he made the following comment: "These rules reveal a remarkably consistent order – there is always much beauty when creation reveals its secrets." For him, scientific discovery was exploring a tiny corner of the mind of God, a concept which can provide inspiration to all scientists.

His life and work were celebrated at the 8th IWA Water Resource Recovery Modelling Seminar (WRRmod2022+), held in Stellenbosch, South Africa, in January 2023. In spite of the limitations caused by his stroke, George was present at the gala dinner, held in his honour, to receive acknowledgement and appreciation from some of his former students together with local colleagues and members of the international community of water and resource recovery modelling.

Professor George Ekama's impact on the field of wastewater treatment was monumental. He will be remembered as a man of faith, humility and integrity.

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# Vincent Clifford Moran (1937–2023): Internationally recognised entomologist and committed administrator

Cliff was born on 22 September 1937 in Harare, Zimbabwe (formerly Salisbury, Rhodesia) where he grew up, attending Prince Edward High School and attaining Cambridge 'A' levels. He then went on to study Entomology at Rhodes University in Makhanda (formerly Grahamstown), South Africa, where he graduated with a BSc (Honours) (1959), MSc (1962) and PhD (1967). In 1962 he married Peggy Maureen (née Fellowes). They had two sons, Derek (1967) and Peter (1970).

After completing his MSc, Cliff was appointed as a Lecturer in Zoology at Rhodes, with promotions to Senior Lecturer, Associate Professor and, in 1979, Professor of Entomology, a position he retained until 1985. He also served as Dean of the Faculty of Science at Rhodes from 1983 to 1985 and was elected to the Rhodes University Council from 1984 to 1985. In 1986 he moved to the University of Cape Town (UCT) where he served as Dean of the Faculty of Science, a permanent, full-time appointment he held until 1998. He was also elected to the University of Cape Town's Council from 1987 to 1995. He retired in 1999, becoming Professor Emeritus and a Research Fellow at UCT.

In his early career, Cliff excelled as a lecturer and inspired many young undergraduate and postgraduate students to pursue careers in entomology. He also became a diligent administrator and was widely appreciated for his fairness and untiring efforts to facilitate and improve the working environment for all. Cliff developed a keen enthusiasm for research, specialising in plant/insect relationships. Initially he worked on citrus pests, but he changed direction in 1974 when he teamed up with Dr Dave Annecke of the then Plant Protection Research Institute (PPRI) of the South African Department of Agriculture to become involved in research on biological control of invasive alien plants.

Cliff's role in the relationship was to bring university-based researchers on board, to complement those working at the 'coal face' at PPRI. Cliff tackled the task enthusiastically and the synergy flourished. A substantial team of entomologists and pathologists became established in facilities across the country, working on a diverse array of problematic plant species with some outstanding successes. The collaboration continues with researchers at several universities now actively involved, working closely with each other and with colleagues at the PPRI. Thanks to the leadership provided by Cliff and Dave in driving this initiative, South Africa became recognised as a world leader in the field of biological control. Cliff was especially pleased when his commitments to biological control turned full circle in 2017 with the formation of the Centre for Biological Control at Rhodes University where he had first become involved in this field of research.

Although Cliff was increasingly committed to administration as his career progressed, he never shied away from remaining active in research. He continued to supervise postgraduate students, leading to the award of 18 MSc and 18 PhD degrees. He particularly enjoyed being involved in projects which provided opportunities to spend time in the field where he could interact and socialise with students and colleagues. He and Dave Anneke wrote a book on insects and mites of cultivated plants in South Africa which remains a definitive work. He is an author on more than 100 research articles, most of which are published in international journals of repute (with citations of ca 5500, an *h*-index of 42, and i10-index of 93). Cliff was always convinced that research being undertaken in South Africa deserved to be exposed on the international stage. He insisted that findings should be published only in the most high-profile journals possible, and that MSc and PhD theses should be examined by overseas experts. He also took every opportunity to spend sabbaticals at the best universities he could get to, including Oxford University, Imperial College of London, Cornell University, University of Arizona, and Oregon State University. He befriended many overseas world leaders in the field of plant/insect relationships and persuaded several to spend time in South Africa working with colleagues and students here and providing an abundance of lasting benefits, particularly for research into biological control in South Africa.

His contributions to entomology were widely recognised both nationally and internationally. He was awarded an A-rating from the South African National Research Foundation from 1992 to 1996. Other national awards included the Botanical Society of South Africa's Denys Heesom Award (1990); Fellowship of the Royal Society of South Africa (1991); Life Fellowship of the University of Cape Town (1991); Founder Member of the Academy of Science of South Africa (1994); Honorary Life Membership of the Entomological Society of Southern Africa (1999); and Doctor of Science *honoris causa* (Rhodes University) (2005). Internationally he received fellowships from the Royal Entomological Society of London (1966), the Linnaean Society (1972), and the British Ecological Society (1984). He was also regularly invited as a plenary and keynote speaker at international conferences.

Cliff contributed in many ways to the advancement of entomology in South Africa and to other avenues of science. He served as editor of the *Journal of the Entomological Society of Southern Africa* (1972–1979) and as President of the Society (1981–1983). He also sat on several national science committees and was involved in organising national and international conferences, including as Chair of the IX International Symposium for the Biological Control of Weeds (Stellenbosch, 1996) and on the scientific programme committee of the International Congress of Entomology (Durban, 2008). In everything he became involved in, Cliff was always fully committed to the task at hand and ensured that any outcomes would be the best possible. There was no place in his life for half measures, or for accepting a position only to gain some limelight.

Cliff will be remembered by many as a diligent colleague, a fine tutor, a keen sportsman and a committed friend. He had boundless energy for everything he did. On the sports fields he excelled at squash, representing both South African Universities and Eastern Province. He was also a fine single-figure handicap golfer. He loved walking and spent many hours striding the countryside, exercising and gathering his thoughts. He never tired of visiting national parks and remote areas where he was seldom without a pair of binoculars, relishing the opportunity to observe animals, particularly birds, in their natural surroundings. Cliff also excelled at woodwork, having a well-equipped workshop in which he made, among other things, beautiful inlaid tables and very fine benches, both for his home and as generous gifts to appreciative friends. Listening to classical music was another pastime Cliff really relished, through both live performances and playing his extensive collection of vinyl records and CDs.

An integral part of Cliff's life was to settle down of an evening and reminisce over a beer or two. He was a raconteur of note, reciting tales of past experiences or making plans for what to do next. He lived life to the full – an enthusiasm that rubbed off and brought out the best in those who were lucky enough to spend time with him. With his passing on 30 August 2023, Cliff has left a great void and he will be very much missed for his many fine attributes.

Sincere condolences to Peggy, and to Derek and Peter and their families.

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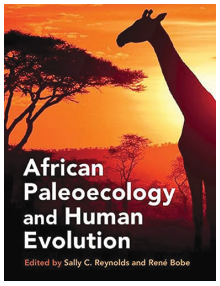


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# The next steps in human evolutionary palaeoecology

The science of human evolution has seen several milestone books over its history. Such books end up being highly influential and they normally take one of two forms. First, there are those that tackle a key research problem and bring together a wide range of researchers and opinions illustrating the agreements and disagreements. An example is the Mellars and Stringer book[1] on modern human origins. A second type are the encyclopedia or data-heavy books that summarise a generation or more of research and offer it up to the student and scientist in a single book. These often have two points of value to them. First, they compile data that are spread widely over sometimes many papers and books. Second, and sometimes inadvertently, they illustrate a weakness in the field, an impasse that requires a new approach or method.

*African Ecology and Human Evolution*[2], edited by F. Clark Howell and François Bourlière, is a landmark book of the second type, and *African Paleoecology and Human Evolution*, edited by Sally C. Reynolds and René Bobe, is a worthy successor. The former was a standard reference for 20 years and was a tour de force of presentation and synthesis. But it also illuminated the weak underbelly of palaeoanthropology – the lack of chronometric dating and insufficient climatic and environmental context. A revolution followed in both areas, and *African Paleoecology and Human Evolution* is the proof – our progress in dating has been fabulous and is getting better all the time, and we now have many proxies that provide both climatic and environmental contextual data from the sites themselves and from other valuable sources.

One of the most important of these other sources, and most used, are the long continuous palaeoclimatic and palaeoenvironmental records from ice cores, deep sea cores, and other palaeo-archives. They have become a go-to comparative data set, and these comparisons often have two forms. Researchers will often compare or slot-in their typically more discontinuous site-based records, or they will compare archaeological and palaeontological sequences to these records. This is seen repeatedly in *African Paleoecology and Human Evolution*, and, in my opinion, is a procedure that has reached its limits in returns.

The book opens with five chapters that introduce the volume and synthesise some time periods. It then follows a standard regional structure – Southern Africa, Eastern and Central Africa, and Northern Africa. Within these regions, experts, mostly people who were once or still are engaged in fieldwork and laboratory work, then provide summaries of the evidence for sites and localities (for example, Cooper's Cave and Gona) or small regions (for example, Dandiero Basic and Nachukui Formation). I count about 32 chapters of this type. I found each of these to be good to excellent, and I know that I will be regularly pulling this book off my shelf to consult with these chapters when I need some basic facts. A valuable exercise would be a graduate seminar that worked its way through every chapter just to make sure students specialising in African palaeoanthropology know the data.

For the most part, this book stops at the end of the Middle Pleistocene, and I found that curious and a little frustrating. The record for modern human origins, which is rich, is for the most part left out and you will need to go elsewhere to find it. But from the Pliocene through to the Middle Pleistocene, this book is a fantastic reference. The primary materials presented are the faunal assemblages and summary descriptions of those. Dating and geology are also regularly presented in tandem with the fauna. There is some archaeology presented but not a lot.

What does this volume reveal as areas of weakness and areas for growth? I see two. First, it shows us that, despite the title, there is very little palaeoecology happening. Ecology is the science of the interaction of organisms with their environment, which of course includes other animals. The vast majority of what is presented is palaeoenvironmental reconstruction and description of faunal communities. That is good, but it is not palaeoecology. What kinds of palaeoecological questions would I see as important? Something to remember is that our focus is human evolution, so our target is hominins and their interactions with the environment and other fauna. Here are some palaeoecology questions: What was the distribution of water, and how did hominins position themselves relative to it? Modern human hunter–gatherers always place their campsites near water – it structures much of their movement. What animals were the primary predators of hominins and how did hominins avoid them? The theory of landscape of fear shows us how important this question is. Do the faunal data show us what edible plants were available, and how these changed over time? We have become very good at assigning fossils to taxonomic groups and dating them, but we have yet to really make headway into understanding what these past ecologies were like.

Second, the book shows us that we have reached a glass ceiling with our current approach. Our current approach finds fossils, describes them, dates them, and then uses specific animals or groups to infer what the environment was around the site. If there is a sequence, then we might describe how that changes over time, juxtapose it against a long climate and environmental archive record, and look for correlations. We are still very much in an inductive phase of science, and most sciences eventually mature into more deductive forms where the data are used to test hypotheses. We now have the ability to begin to do this. Advances in our ability to create climate models, and environmental models from those, has exploded in the last 10 years and will become increasingly powerful. My recommendation, which we have argued for elsewhere[3], is that we start to nudge ourselves in that direction. In such a procedure, we would create formal models of climate and environment across regions, and then use our fragmented sequences to test and refine those models, thus creating palaeoscape models. Within those palaeoscapes, we can use computer simulations such as agent-based modelling to experiment with interactions between fauna and their environment, which of course moves us truly in the direction of palaeoecology.

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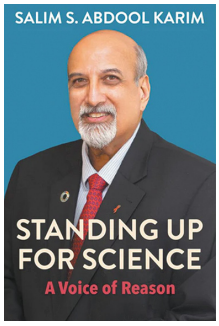


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# Shedding light on the vexed question of science and uncertainty

What is science and scientific work really like? Here, for the first time, is a South African book that sheds light on what is often obscured from public view, on the one hand, but also misrepresented in the minds of ordinary citizens, on the other hand. To many, science is reliable and objective, the outcome of rigorous investigation that, these days, comes with the mildly amusing epithet, *evidence-based* (how could it be otherwise?). Those unsmiling white men in white lab coats surrounded by test tubes and microscopes was the image of science and scientists that generations of children grew up with thanks to standard images represented in school textbooks, movies, and advertising.

Salim S. Abdool Karim's *Standing up for Science* pretty much destroys those facile images of how science does its work behind the scenes. Neither a scholarly work nor a popular science text, this thoughtful book owes its warmth and accessibility in large part to the charm and storytelling skills of the author. I remember Professor Abdool Karim coming to the University of the Free State during my tenure there as Vice-Chancellor to address my colleagues in the medical school. My brain started to play historical tricks on my mind in the moments before I introduced him. After all, the last famous Indian South African to come to the Orange Free State was Justice Ismail Mohammed who did his work in the Bloemfontein courts during the day but had to travel back and forth to Kimberley; the petty cruelty of the apartheid masters dictated that Indians could not sleep over in the province. That was in the past, but I still wondered how this accomplished scientist was going to win over the auditorium of serious looking white Afrikaans-speaking physicians in the audience. What happened next was masterful for the context. The speaker started by telling the medical school staff about how he got his famous nickname by which we all know him, Slim. An Afrikaans schoolteacher sarcastically asked him whether he thought he was clever and hence this lasting name, Slim Karim. From that moment on he had my colleagues eating out of his hand.

Slim brings this formidable talent to connect to a wary readership on pandemics by detailing what actually happened in the rarified atmosphere of the COVID-years. I learnt for the first time that there were famous scientists who, in Slim's telling, wanted to dislodge him from the leadership role he was called to perform on behalf of government. It was not a surprise, however, to read how often he disagreed with the authorities, including on the petty regulations that once again made some government leaders look ridiculous in the eyes of a knowing public; it was garlic in the AIDS days, then footwear under COVID. Slim writes with considerable restraint, but I have no doubt there must have been extreme frustration with officials high and low peddling such nonsense in the middle of an existential crisis.

Perhaps the most important scholarly contribution of the book is how it sheds light on the vexed question of science and uncertainty. The news coming in from China was not good; the explosive spread of the novel coronavirus across borders was frightening. Millions would surely die. We all look to medical science for certainty not ambiguity, for scientific truth not prevarication. But science is not like that. It would take time to isolate the virus, let alone develop responsive vaccines. Unsurprisingly, medical scientists of all stripes contradicted each other openly, some more certain than others but all of them working with incomplete knowledge. Some crazy ones even suggested herd immunity as a preferred policy posture, as if humans were heads of cattle. The conspiracy theorists joined the fray.

"Medicine's ground state is uncertainty," Atul Gawande once wrote, and this was painfully evident during the pandemic years. In such a crisis, governments understandably want to convey certainty and thereby offer public reassurance. But science is not like that, and scientists are seldom trained in the virtues of uncertainty and how to communicate that reality of science and scientific work, especially when lives are on the line. One can only hope that medical schools revisit and revise their curricula to include more substantive modules on science and uncertainty in times of crisis. This book would be a good recommendation for the reading list.

There are other gems in the book, including the way in which science in the Abdool Karim home quite literally became a family enterprise. Everyone contributed from their disciplinary perspective as the home resembled a veritable laboratory for churning through incoming data from all over the world. One thing I would have liked to see more of, however, is the contribution of Quarraisha Abdool Karim, a highly rated world scientist in her own right who has for decades worked alongside Slim in the course of their careers fighting AIDS.

Which brings me to a special moment in which I had the privilege to participate. As President of the Academy of Science of South Africa, I was asked to say a few words on the occasion of a global award for courage in science that would be shared by Slim and his friend and collaborator from the AIDS years, the famed American immunologist and adviser to presidents, Anthony Fauci. Both men had at various times been roundly lambasted by their respective governments for, well, standing up for science.

Now, right in front of me on the screen were two giants of medical science and I could not help but point out the obvious: that the success of the scientific enterprise, whether it be climate science or pandemics, depends on solidarities and friendships that cross the borders of race, ideology and geography. That too is a lesson about how science and scientists *should* work, and this superb book tells that story well.

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Undoing apartheid


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# *Undoing Apartheid*'s mechanisms of reproductive power

The arresting image on the cover of *Undoing Apartheid* – the sculpture "Read, man, Read!" by Chumisa Fihla – perfectly captures the provocative thesis of author Premesh Lalu. Namely, that some humans, engineered through technological bits and pieces, have come to find their sensory and material existence defined by the mechanising logic of petty apartheid. The sculpture thus evokes the gestuary or repertoire of petty apartheid: the intrusion into everyday existence of a mechanical form of life, one that lodges itself in circuits of sense and perception. Lalu's book offers a remarkable exploration of the subjectivity that came to accompany the broader, systemic features of apartheid in South Africa, and its continued reproduction in a post-apartheid world. To analyse the intersection of structure and subject, Lalu turns away from the grand apartheid familiar to most: the ideology of racial separation and the political acts of relocation and partition into ethnically defined homelands and racially defined Group Areas (p.27). Instead, he focuses attention on petty apartheid, or the system that controlled the minutia of everyday life. On his read, this daily life reflects more than the technical application of grand apartheid. It becomes a way to impel human beings to a "becoming mechanical", that is, to adapt increasingly mechanised forms of life that stifle desire and creativity. Despite changes to a legal code or political order, apartheid comes to persist in the reproduction of the everyday. As a result, apartheid becomes difficult to surpass or move beyond; differently put, "undoing apartheid" is a labour in itself, one to be held as distinct from efforts to supersede it. To read the world on new terms means infusing acts of interpretation with a desirous creativity that might interrupt the mechanical circuits laid down through petty apartheid.

*Undoing Apartheid* is organised around six chapters and covers a surprising amount of terrain. After a synoptic introduction, the second chapter takes seriously the "far-fetched mythic claims" (p.33) invoked to justify South African racist ideology through the origin stories told by Afrikaner nationalists, and considers how myth connected the technical system of slavery to that of industrialisation; simply debunking racial myths does not undo the economic rationales of efficiency that allowed racial domination to be quietly subsumed under industrialisation. Through a reading of Goethe's *Faust* against William Kentridge's *Faustus in Africa*, Chapter 3 traces the reconstitution of race – beset by conflicts between human, nature, and technology – as a collection of sense perceptions that consigns some to a mechanical existence able only to service (but never to be integrated into) the whole. Chapter 4 is the heart of the book, and narrates this psychological story as one that results in a subjectivity "whose sensory world has all but collapsed as a result of a sheer mechanization of life that strips individuals of desire, striving, and futurity" (p.103). Not only does a shrunken sensorium ease the meld between human and machine, it also transmutes perception into apperception by robbing people of the raw materials, that Freudian *Rohstoff*, that fuels invention, imagination, and fantasy. For there to be an "after" to apartheid, one that is an emancipation from not just the partitions of grand apartheid but also those of sense perception, requires undoing apartheid subjectivity at the juncture where senses and technology facilitate a relation to power.

Resisting a turn to affect theory, Lalu instead explores the aesthetic education offered through "stumbling" (p.150–): first the stumbling of a slapstick theatre that uncouples technology and mythic violence, and then the reworking of the division between sense and perception so as to imagine new futures and new experiences of freedom. "Undoing apartheid," concludes Lalu "thus requires setting to work on…crafting a workable concept of reconciliation, one capable of relinking sense and perception and staking a claim to truth content on its own terms" (p.187). It is work, insofar as it must slowly unravel the use of technology to maintain these links and to give these modern, neoliberal times the "meaning" of efficiency. Work, then, is deeply linked to political subjectivity rather than a Marxist class consciousness or an unalienated labour. It is the effort to render visible the divisions that sustain racial inequality, and the dependence on the immaterial labour of a racialised other (p.92).

Crucial to the iterability of apartheid is the double-take, or the uncanny doubling of its feelings, its patterns, and its reproductions (p.23, p.118). *Undoing Apartheid* is organised around a series of double-takes – the history of Athlone, at once a town in central Ireland, the name of the last British governor for South Africa, and a Cape Town district formed by the forced removals. Another is the repetition of the Trojan horse story, as voiced by Seamus Heaney in *The Cure at Troy* (and introducing the theme for each chapter), and its entwinement with the Trojan Horse massacre of 1985 in which anti-government students living in Athlone were ambushed by security forces. A third, already mentioned, is the reprisal of the Faust story. This panoply of figures serve as the mythic precursors for a racialised modernity that compresses the distance between the Global North and South, and that complicates any effort to separate this history or its archive. In contrast to those who distinguish the histories of the Global North and South – yet another division – Lalu does something refreshingly different with this history. He emphasises this uncanny as one that compresses time so as to conflate past, present, and future. Yet these doublings are not recuperative – with one an example of a flawed history, the other a modern update that "gets it right" – but rather an opportunity to glimpse how the fantastical becomes real, and how that reality might subsequently be dislodged. The turn to puppetry (Kentridge's *Faustus* is performed by Handspring Puppet Theater), shifts this intervention into the genealogy of race away from the register of mere representation and towards the register of labour: "The puppet is an uncanny prosthesis: one that conveys a sense of spirit, and that abides neither by received ideas of truth nor by premature declarations of reconciliation" (p.77). Static representation is replaced by dynamic process. Neither fully inanimate (the puppet master is all-too-visible) nor fully animate (it is, after all, a manipulated wooden object), the puppet challenges its viewers to reach "into a metaphysical core…of political transition" and to attend to the *process* of making and its interdependencies, rather than the *telos* of history's tragedy or redemption. The result is a gloriously complex approach to the making and unmaking of history in all its geographical and temporal sprawl.

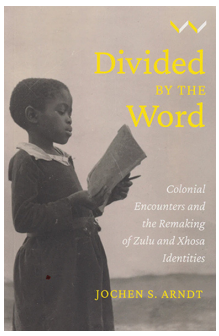
Lalu's account also gives his readers a provocative way of thinking change. Scholars have recently moved on from critiques of progress or enlightenment, and have instead sought to identify patterns key to racism's persistence and recurrence over time. Rather than striding confidently forward and past economic underdevelopment or failed nation states, Lalu asks his readers to linger on how they have *stumbled* – on how they have stumbled to move beyond the debunking of racist ideologies, or beyond a critique of "failed progress" – and to search out the time-ing that would let them catch themselves and recalibrate interpretations. The emphasis on time-ing is more than (yet another) historical turn. Lalu argues that historicisation can only go so far in undoing racism, as the historicising move presumes that racism is irrational (p.59). As a result, historicisation struggles to identify those rationales that made the reconstitution of race so, well, seemingly reasonable. Instead, the appeal to time-ing is an appeal to context and the material logics that organise particular experiences (p.64). What would it mean to abandon the search for the "best" form of governance or the "right" representations? How might we side-step existing determinate logics? Again, in raising questions about "what next?" many might luxuriate in affect, revel in the aesthetics of ambiguity, or seek to unmask through genealogy. Each of these responses, however, marks a turn *away* from relations of power, away from political agency, and away from the messy work of politics. In keeping an eye insistently on technologies of power, Lalu redirects us to the work of building something and in a way that places craft, formation, and productivity back in the hands of ordinary people. Rather than being beguiled by spectacle and unseeing to the puppet masters' manipulations, what would it mean to place the crafting of human beings (with their unsteady admixture of in/animation and in/dependence) in plain sight? How might people be taught or provoked to sense, perceive, and so act on different terms?

Such a framing of race as caught between the ambivalence of science and nature is an unusual way into a discussion of race and racial division. The Faustian wager is "rearticulated, so that colonialism is less a regressive accompaniment of global capitalism than a prognosis of that specific future of capital built on drives in which divisions between human and machine are rendered indistinct" (p.75). Lalu thus dispenses with theories of race that rely either on essential origins or the debunking of biological racism through reason that leads inevitably to progress. Instead, he reads late modernity on the terms of what Michelle Alexander has called "racism without race". Gesturing to a few landmark moments in late modernity's turn to technology, Lalu writes that "race is that excess of slavery transferred to a technological milieu, where this milieu operationalizes, distributes and controls its signification … this technological milieu is infused with mythic content in the colonial world" (p.54). Rather than rooting racism and race thinking in intention, genetics, ideology, or structure alone, Lalu instead gives us the causality of "consilience", or the unexpected convergence of physics and psychology, science and nature, and of affect and reason (p.7, p.97). This proposal is unexpected and cries for more elaboration. After all, Alexander's "racism without race" poses a thorny political problem: how can racism be named and called out, if racial outcomes cannot be clearly linked to racist logics or the intentions of obvious villains? Lalu's appeal to consilience similarly unsettles: if the unlikely convergence of faculties organises what is possible to think, then mobilising any political response requires first creating awareness of a problem. The sharp insight here is that such awareness can leave behind the tired categories of "identity politics", "authenticity", and "recognition" to instead concentrate on the materiality of the situation at hand, and to ask how and why certain divisions came to have organisational force.

Lalu's emphasis on the conjoinment of the sensorial and material offers a striking change from other approaches to thinking race that might rely either on affect theory, the critical ontology of Afro-pessimism, or phenomenology. For all that Lalu wants to "re-enchant the desire for post-apartheid freedom" (p.7), he does not propose a sensorial or affective turn to counter the mechanical and rote and he dismisses Afropessimist accounts of an ontology rooted irredeemably in racial difference as historically (and politically) conservative. To recall, this workable reconciliation "relinks sense and perception and stakes a claim to truth content on its own terms" (p.187). Yet this reconciliation is no rainbow coalition; Lalu dismisses such "rainbowism" in the very next sentence. "Reconciliation" speaks to the process of undoing the work of "consilience". It is a worlding that seems to take its inspiration from Canguilhem rather than Heidegger in that it aims to develop the capacity to tolerate variations in norms (rather than their integration into a cohesive whole).

The appeals to worlding and truth content sparks a desirous question: Beyond the practical work of undoing, what vision of freedom orients and sustains those who set it up? It certainly is not the transcendence of liberation, nor the inclusion of integration into liberal order. Lalu invokes the cinema as a way to illustrate the experience of an interval suspended between one present order and another yet to come. If petty apartheid allows for the mechanical repetition of apartheid's gestures and patterns of interaction, then what would it mean to interrupt it? How might memories be reconnected to different futures, and how might the unconscious differently attach to new symbolic forms? Although I am persuaded that this retooling must also be a reschooling, I'm left wanting more about the substance of this freedom. Such substance holds value because of who and how its mobilises, and how these unexpected collisions redirect technologies of power in unexpected ways. Lalu regrets that too often the intensity associated with the 1985 student movement becomes confused with a simplistic mobilisation of outrage as in #RhodesMustFall. Fanon and others have noted that racial division is an easy shortcut to political mobilisation, not just for colonisers but also for the colonised. He called for a renewed attention to deepening relationships and reciprocities between elites and masses as a path to the figuration of his own 'New Man'. I finished this book wondering in which relations of power Lalu's aesthetic education should be lodged, and oriented toward which normative and political ideals? What sites, and with what proximity to institutional power, might conjure new freedom dreams and give them the mass and corporeality they need to endure? Toward thinking through these questions, *Undoing Apartheid* offers an unexpected, unorthodox, and deeply rewarding read.

**BOOK TITLE:**
Divided by the word

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# Better together: Xhosa and Zulu – languages or dialects?

*Divided by the Word* is an important book. I wish it had been there when I started my career teaching Xhosa as a second language more than 30 years ago. Students identifying as Zulu would register for my beginner (and second- and third-year) courses in Xhosa, not attend a single lecture or tutorial, and pass with 100%. To me, they *were* Xhosa – or were they Xhosa Zulu?

Jochen Arndt answers this question with magnificent scholarship and clarity of argument: people only began to identify as Xhosa and Zulu once these separate **languages** were created in the mid-1800s. Before (and even some time after) the arrival of missionaries and settlers, people identified according to their clans, their *izibongo* (clan names) and social statuses. "Nguni speakers formed political communities that united people...from very different cultural and linguistic backgrounds", so what would make a difference to your social rank would be your personal genealogy, **not** your language (p.33–34). People did recognise differences in the way they spoke, but these were not mobilised as markers of identity and in fact did not matter that much: Arndt refers to these differences as "*soft* markers of *vague* regional identities" (p.37).

So how has it come to pass that conflicts arise in which the opposing parties are identified purely in terms of whether they speak Xhosa or Zulu (as happened on the Rand in the early 1990s)? And another question (not specifically stated in the book, but one that arises implicitly, and frequently confronts me as a teacher of African languages): Why are African language departments not teaching a general Nguni language course, instead of focusing on either Xhosa or Zulu?

Arndt answers these questions with meticulous attention to historical sources, which means that he has to bravely confront the term "Caffre" (with its notorious variants) and explain its meaning and role in unravelling complex realities. He acknowledges that because of its recent history the word "is rightfully considered hate speech" (p.38) but explains that at the beginning of European contact with the southern African region it referred to a **single language continuum** that included what we now know as the Nguni languages. In the course of his detective work, Arndt makes illuminating references to writings from explorers, naturalists and philologists. For example, in 1779, the Dutch explorer Robert Jacob Gordon observed that the "Caffres" north and east of the Cape Colony spoke "the same" language "but a different dialect" (p.50), while the geographer and linguist John Barrow acknowledged that "perhaps no nation on earth ... can provide so fine a race" (p.52). So what separated this language, this race? The answer can be found in the Bible.

Arndt's gripping narrative – honestly, sometimes it reads like a thriller! – interrogates the evidence: it was the late 18th and early 19th centuries that saw the dawn of the missionary enterprise and its concomitant need to make the message comprehensible to potential converts. The notion of a single language was strengthened when missionaries interacted with "people of European and African descent who migrated throughout this region and who used their skills in the speaking practices of the people from one end of the coastal belt to communicate successfully with the people from the other end" (p.57–58). The missionaries started to learn the languages of the people whose souls they wanted to win, but they still had to employ multilingual interpreters who were able to communicate with most people in the region because of language skills acquired during "trading, intermarriage, and political incorporation" (p.109).

Arndt notes that the communities that gave birth to these interpreters "practiced bilingualism and language mixing" and "did not demand that their members speak one specific language only and purely" (p.115). Inspired by their interpreters' and their own observations, Wesleyan missionaries began work on linguistic harmonisation to "transcend the differences between the speaking practices of the Xhosa, Thembu, Mpondo, Mfengu, Natal Africans, and Zulu" (p.117). This kind of work had already been done in England, "where the translation of the Bible had created a written language that unified a wide range of spoken dialects" (p.117).

However, in 1854, the American Board missionaries rejected the Wesleyan missionaries' proposal to work together to produce a translation of the Bible that would accommodate all Nguni speakers. The reasons behind this spurning of what would appear to be a logical solution to the problem of many dialects is that they had for some time "tied their identity to the 'Zulus' only" (p.125) and needed a way to distinguish "Zuluness" other than just culture (p.123). Their rejection of the proposal to create a Bible in a common language focused on unfamiliar words and differences in orthography which Arndt argues represented a "man-made obstacle that had little to do with people's actual speaking practices and their mutual intelligibility" (p.130).

In the rejection letter to the Wesleyan missionaries' proposal "to develop a single literary language for the coastal region", the writer comments that Zulu was superior "because it did not contract words" and because of "a smoothness and a beauty in the (King's) Zulu which they had not discovered in the other [dialect]" (p.146). After it was clear that the Wesleyans had failed in their attempt to translate the Bible using a single literary language, there were "separate standardizing projects that eventually culminated in the emergence of distinct Zulu and Xhosa literary languages and language-based identities" (p.171).

So the two languages, Xhosa and Zulu, were standardised, the Bible translated into both, and dictionaries for each created. Finally, the last nail in the coffin of a harmonised language was hammered in: mother-tongue education for the first years of schooling. This sounds like a pedagogically sound idea, but the fact that education only became compulsory for Africans in 1981 (whereas for white children it had been compulsory

since 1904) (p.187) meant that people had to rely on missionaries for their education and accept the language chosen by them as the standard. Understandably there would be a mismatch and "pupils began to consider their home dialects inferior variants" – a situation that continues to this day. I am frequently saddened by students who feel the need to tell me that "I don't have a proper mother-tongue" – meaning that they happen not to speak the standard Xhosa or Zulu that is taught at schools and universities.

In his concluding paragraph, Arndt takes us back to the Rand violence of the 1990s and argues that even though people associated the conflict with "South Africa's historical Zulu and Xhosa polities and clans, they were in fact modern identities – the products of the intense language mapping, standardization, and education activities European, American, and African-born actors had carried out since the early nineteenth century" (p.203).

Rarely have I felt as deeply grateful to an academic writer as I now do to Jochen Arndt. In *Divided by the Word* he has answered my questions with grace and insight – and immense scholarship. He has done a great job. *Wenze umsebenzi omkhulu.* (That last sentence is exactly the same in Xhosa and Zulu – of course!)

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# The long walk to STI policy coherence

**Significance:**

The 2022 Decadal Plan for Science, Technology and Innovation intends to shape innovation activities and their contribution to development out to 2031, serving as government master plan and pivoting the National System of Innovation to mitigate the socio-economic, health and environmental challenges of times that are volatile, uncertain, complex and ambiguous. But in trying to be all things to all people, the Decadal Plan avoids prioritisation, reading more as a Vision Statement than an Implementation Plan.

The 2022 Decadal Plan for Science, Technology and Innovation ('the Plan') intends to shape innovation activities and their contribution to development out to 2031. It has been produced in these 'worst of times' of rising global and domestic uncertainties, weak domestic growth, policy instability and strife. This Commentary has the task of analysing the substance and intent of the Plan, with its many voices and goals. Given the constraints facing its authors, one must commence with a statement of appreciation for their work. As Prussian military strategist Von Clausewitz observed, "The enemy of a good plan is the dream of a perfect plan."

Eliciting the underpinnings and philosophy of the Plan is no mean task. Recognising that bounded rationality applies to the process of the construction of the Plan[1], a process of deconstruction, drawing on radical structural analysis was employed for the task. Ardolan teaches that "the radical structuralist paradigm assumes that reality is objective and concrete, as it is rooted in the materialist view of natural and social world"[2]. To this add the cosmos of interests, explicit and implicit, that shape this reality. In South Africa the cosmos includes the corporatism of the Tripartite Alliance of the African National Congress (ANC), Congress of South African Trade Unions (COSATU) and the South African Communist Party (SACP) and combines with the voice of business in the form of ethnic and plural associations, lobby groups, academia, political parties, think tanks, civil society, and the dispossessed. This makes tight coordination of policy difficult to scope, let alone to achieve in practice.

## Context

The post-1994 government pushed an agenda of redress and modernisation, with the hybrid Department of Arts, Culture, Science and Technology issuing its White Paper on Science and Technology[3] that introduced the idea of a national system of innovation and motivated for new organisations. It argued the need for competitive research while reserving attention to the 'flagship' sciences of physics and astronomy and supporting Polanyi's notion of the 'Republic of Science'. In retrospect, the MeerKAT array is the ultimate commitment to such flagship science.

Upon its 2002 establishment, the standalone Department of Science and Technology (DST) set out the National Research and Development Strategy (NRDS) that inter alia moved the CSIR to DST, proposed a Foundation for Technological Innovation (today's Technology Innovation Agency), and scoped a new Strategic Management Model that abolished the Science Vote.[4] The NRDS declared four S&T missions along with 14 indicators and stretch targets for R&D inputs and scientific and innovation outputs. The ratio of gross expenditure on research and development (GERD) to GDP was to reach 1% by 2008, with government expenditure on R&D to double in current rands. The latter target was achieved, but GERD:GDP saturated at 0.92%. The 1% target was never attained.

The subsequent Ten-Year Innovation Plan 2008–2018 (TYIP)[5] was generated immediately before the Great Recession terminated the commodity super-cycle. In parallel, DST requested the Organisation for Economic Cooperation and Development (OECD) to conduct a review of innovation policy that found that policy downplayed the role of business, and that there was a design and engineering chasm and dangers associated with the fraying social fabric.[6] The TYIP proposed four Grand Challenges with links to the previous Missions, to be measured by some 50 indicators and targets, with GERD:GDP to reach 1.5% by 2018. This also proved a target too far.

The roll out of the TYIP coincided with the Mbeki Administration morphing into the Zuma Administration, on whose watch state capture intensified and innovation activities in the state-owned enterprises declined. From its 5.6% peak in 2006, economic growth entered a period of long decline with rising unemployment and dependency on the social wage, even though absolute poverty was reduced. Technical recessions occurred from 2007 onward with the 2020 COVID-19 pandemic driving steep losses. While mortality due to HIV/Aids declined through the roll out of the world's largest ARV programme, non-communicable disease morbidity grew.

DST gained the role of co-chair of the Economic Cluster and expanded its reach, working with the National Treasury to introduce an enhanced R&D tax incentive and with the Department of Trade and Industry to regulate intellectual property arising from publicly funded R&D. Come 2009, Minister Pandor asked a Ministerial Committee to assess the OECD recommendations and review the STI landscape.[7] On the global stage the BRICs (Brazil, Russia, India and China) surged, with South Africa joining the club in 2012. The same year saw the Paris Declaration on Climate Change; in 2013 the African Union announced Agenda 2063; in 2014 came the launch of the African Union STI Strategy for Africa 2024. A further influence came from the UN Sustainable Development Goals that were adopted in 2015.

These instruments and reviews form the policy background to the development of the White Paper on STI that entailed self-assessment, situational, performance and scientometric analysis, and the conduct of foresight studies in the hands of local and international consultants and stakeholders.[8-10] The White Paper on STI, in line with the 1996 White Paper, reiterated that

> *The newly elected government inherited a fractured society, a fiscally drained state and an unsustainable, resource-intensive economic growth path. The pre-1994 STI system*

> *was small, exclusive and in line with the agenda of the apartheid state, rather than oriented towards inclusive, sustainable economic development and social equity.*[11]

Business and consumer confidence have eroded, and emigration of the highly skilled has risen. The rebased GERD:GDP fell from 0.83% in 2017 to 0.62% in 2020, although aggregate scientific output was maintained and expenditure on basic research rose. Most critically, the full-time equivalent number of permanent and pensionable researchers rose by a mere 30% over the preceding two decades. The White Paper diagnosis might be re-phrased: "we remain a fractured society, with a fiscally drained state and an unsustainable, resource-intensive economic growth path. The STI system remains small, exclusive, and follows its own agenda."

The most comprehensive overview of the NRDS and TYIP is that conducted by CREST of Stellenbosch University for the National Advisory Council on Innovation (NACI).[12] Its main findings were that most of the organisational and financial proposals of the NRDS and TYIP were implemented, but the extent to which subsidiary strategies and interventions achieved their intended outcomes and impacts was more nuanced. A scoreboard of 60 strategic objectives was compiled and scored as follows: largely achieved (22), moderately achieved (21), poorly achieved (12) and unknown (5). The highest scores relate to support for framework conditions; the lowest scores to implementation of the Grand Challenges. The lesson for policymakers is that it is easier to conceptualise interventions than to drive them through to intended results. This is far from unique to South Africa.

## Philosophical underpinnings

The Decadal Plan was in the making from 2016, accelerated with the approval of White Paper 2, and then slowed as the pandemic irrupted. The virtual Cabinet meeting of 24 March 2021 approved the Plan, noting that

> *The policy responds to the rapid technological advancement and harnesses STI for the socio-economic development of the country. This Decadal Plan will serve as government master plan, which will incorporate other departments such as Departments of Agriculture, Land Reform and Rural Development; Mineral Resources and Energy; Health, and Trade, Industry and Competition. The implementation will be in collaboration with all the relevant departments.*

The Plan is expected to pivot the system to mitigate the socio-economic, health and environmental difficulties in times that are volatile, uncertain, complex and ambiguous.

All government policy instruments, legislation and regulation, involve innovation with intended and unintended consequences. Government initiates change, that once disseminated is an innovation, whether the change is internally driven or placed in the hands of agents. Various methodologies may guide these interventions, such as management by objectives, logical framework, results-based management, and more recently theory of change, that links intended impacts and the actions assembled to achieve them.[13] In the attempt to focus its policy intent, the Plan declares its theory of change. This behooves consideration of the implicit or explicit theory of change of preceding STI policy instruments.

The 1996 White Paper proposed the innovation system approach that rests on complex, self-organising behaviours, and the necessity of linkages among its actors[14], but its implicit theory of change is a linear model of innovation. The explicit theory of change of the NRDS is also a linear model of innovation coupled with the idea of the 'innovation chasm' between research and valorisation.[15] This idea is quite different from the concept that Moore[16] popularised. The NRDS ideas replicate in the TYIP and have then influenced DST, the Technology Innovation Agency (TIA), National Intellectual Property Management Office (NIPMO), NACI and related organisations.

The theory of change of the White Paper on STI is laid out in its §1.4. and may be summarised as follows: STI, in partnership between business, government, academia and civil society, through a coherent whole-of-society agenda can shape a different South Africa, if skills and funding, operating constraints, and appreciation of its value are addressed. This comprises a vision statement, not an actionable theory of change.

## The Intent

The Plan sees government as the guiding hand of the innovation and research system, hence the reference to a master plan across all government departments, in line with the doctrines of the industrial policy action plans and master plans of the Department of Trade and Industry.

At this point it is appropriate to refer to the sentinel recommendations of the 2012 Ministerial Review, namely first to establish a broad-based National Council on Science and Innovation (NCRI) to determine the priorities and set the agenda; second to transform the NACI into an arm's length Office for Science and Innovation Policy with the roles of measurement, foresight and M&E; third to establish sectoral innovation funds fed by resource rents that would support the research and innovation agenda of the NCRI; lastly to carry out a fit-for-purpose review of the public research organisations. To date, only the third recommendation has acquired traction.

In contrast, persuaded by the statism of government, the Plan commences by declaring what must be done, with joint agenda setting, prioritisation and M&E relegated to its final chapters, and this in contradiction with the theory of change of the White Paper on STI. While acknowledgement is given to Mazzucato's critique of neo-liberalism[17], her insights are appropriated to argue for statism, similarly the notions of Schot and Steinmuller[18] on Transformative Innovation Policy. In effect, the contract between the Department of Science and Innovation (speaking for government) and society is one that relegates the private sector to a secondary role, reflecting the ideologically driven mistrust between the Tripartite Alliance and business.

The contract between science and society is always subject to the political.[19] In the apartheid years, 'science walked on two legs' – the Republic of Science being one leg, and science for the warfare state the other.[20] It is averred that it still walks on two legs, with flagship science replacing the war machine, and the Republic of Science articulating the case for empirical evidence, as for example through the Academy of Science of South Africa (ASSAf) during the HIV/Aids debacle. It has since gained volume through research achievements in infectious diseases, radio astronomy and high-energy physics (witness the election of three of our scientists as Fellows of the Royal Society).

That noted, the Plan sets out to define STI priorities in agriculture, manufacturing, mining, health, and energy, and to harness prospects of the circular and digital economies. The Plan sets out to build a capable state, with economic inclusion, all the while addressing the Societal Grand Challenges of climate change and environmental sustainability, future-proofing education and skills, and the future of society. The Plan identifies nine megatrends: growing unemployment, megacities, climate change, COVID-19, migration, multi-polar world, growing inequality, new wars, and the hollowing out of nation states. A set of future priority domains further shapes the Plan, along with the associated measurement regime. These are important contributions to framing the shape of future interventions, but the mix lacks prioritisation and leaves the reader struggling to understand where the Plan is heading. To add to the confusion, and in its own words, "the Decadal Plan sees STI missions not as single projects, but as portfolios of actions involving grants, prizes, new forms of procurement and financial instruments". It is unsurprising then that the Decadal Plan lays out some 391 indicators to track its progress.

Last is the matter of strategic internationalisation. South Africa hosts five of the leading universities in Africa and retains the lead for the 'quality' of science outputs. This is to be expected as we remain a sub-metropole even though South Africa has slipped from producing the largest GDP on the continent to third place after Nigeria and Egypt. Until recently, South Africa acted as the higher education hub of sub-Saharan Africa, educating thousands of postgraduate scholars for the hinterland north

of the Limpopo. This, together with visible participation in Big Science and the flair of many trans-national corporations across the globe, rightfully persuades the authors of the Plan to think global. A crucial gap in the Plan is innovation in the 'third economy', that rests on the attainments of our transnational corporations such as Bidvest, Naspers, Derivco, Datatec and AECI. What could and should a Decadal Plan say to these corporations?

But there are contradictions – how can one conduct 'strategic internationalisation' while excluding international students and skills? Rising xenophobia has coloured politics and community life for more than a decade. Restrictions on the mobility of international staff and students will do serious injury to the Republic of Science. It is somewhat disingenuous to speak of open science when borders are closed to the highly skilled.

## Reprise

The politics of the Plan, especially the thinking that informing the Compacts, public procurement, "leakage" and "retaining Local Patents and Technologies" echoes themes of earlier techno-nationalism. Likewise the fixation with the merits of competitive advantage in the call for the beneficiation of locally abundant fluorspar, titanium and platinum. Beneficiation is fine in theory but comes with risk and externalities. So, for example, Sasol beneficiates coal into some 200 chemicals. The environmental damage is massive. Iscor beneficiated iron into steel, but Iscor is no more. The Hillside aluminium smelters at Richards Bay used the cheap electricity of the 1980s to beneficiate imported bauxite that provides the stock for today's aluminium building products industry. Respected economists Hausmann and Klinger[21] aver that the country might achieve a stronger growth path by focusing on manufacturing for exports – *vide* Sasol and Hillside. The disappointment in exploiting the appetite-suppressing properties of *Hoodia gordonii* notwithstanding, policymakers maintain the hope of identifying and exploiting medicinal plants to produce safe and responsible health innovations. The luck of nature does not a product innovator make.

In effect, the Plan tries to be all things to all people. It reflects a committee approach, is repetitive, and lacks a critical perspective on how business works. The drafters misinterpret both political and economic history.

On the positive side, the Plan has abandoned reference to the notion of the innovation chasm. Another positive that has since emerged, and this before finalisation of the Plan, is the realisation that the R&D Tax Incentive is not having its intended effect, so proposals for amendment are now being circulated for public comment. This is testimony to policy learning, and acceptance that experimentation and adjustment are absolute necessities. It is expected that the Decadal Plan will align itself with the goals of the NDP, recognising that a capable state rests on the impartial actions of people who demand the best of themselves, their peers, and service providers and who work with competitors to ensure a better life for all.

The challenge is to integrate innovation and industrial policy, to recognise that one size does not fit all, and to seek policy coherence rather than organisational coordination. Julius Nyerere understood the challenge of development, offering two gems of advice: "To plan is to choose" and "Your budget is your plan". Let us see the budget please.

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# Science and language, knowledge and power

**Significance:**

All scientific knowledge is encoded in socially constructed forms of communication with language being the primary mode. When language is understood as a socio-cultural practice and a resource for meaning-making, it has significant implications for how we understand knowledge-building in disciplines and the inherent power relationships that are created in the way we use language to construct different kinds of knowledge and position knowledge in the field. It also has implications for how we share and validate knowledge with and to others. If science is to be used for social justice, understanding science communication necessitates considerations of language, knowledge and power.

## Introduction

The Academy of Science of South Africa (ASSAf) invited us to be part of a panel in the World Science Forum (December 2022) addressing the topic 'Promoting social justice through accessibility of language in science'. This panel discussion was hosted by the *South African Journal of Science* (SAJS). The issue of language and scientific knowledge is not new to the journal, the impact of which is noted in their inclusive language policy that authors need to adhere to when disseminating their research. It is against this backdrop that we offer this Commentary to further the conversation about how language, knowledge, power, and social justice intersect and shape each other.

It is crucial that critical scholars in the arts, social and natural sciences pause to consider what role science broadly should play in social justice. For many scholars in the arts and social sciences, especially those who focus on the many evolving complexities of everyday life, politics, and economics, the pure and natural sciences may seem distant and inaccessible. In the same breath, the multiple and competing ontological and epistemological positionings (questions around what is real and how we come to know it) in the humanities and social sciences is often regarded as being incompatible with those working in the natural sciences. Yet, as the many storms of the Capitalocene[1] gather strength and intensity, playing out in multiple forms of human suffering, it is more crucial than ever that we seek ways to connect the findings of different kinds of scientific research with the project of social justice.

Given the dominance of the written text for disseminating scientific knowledge, questions around language are important to consider, irrespective of one's discipline. As Maton explains, however, ideas around the sociality of knowledge have been the site of contestation as many views offered misrepresent knowledge as "processes of knowing within the minds of the knower"[2]. Drawing on Alexander[3], he goes on to explain that this contributes to an "epistemological dilemma" whereby scholars are offered two polarising perspectives on which to draw: positivist absolutism (where knowledge becomes decontextualised, value-free, detached and certain) or constructivist relativism (where knowledge is seen to be only a social construct produced within cultural and historical conditions).[2] Writing from a social realist perspective that draws on the philosophy of Bhaskar's critical realism, we posit that the knowledge produced by science and its objects are real; however, social factors contribute to, and shape, its production. This position allows us to open up a conversation about language as a social practice and the implications this has on knowledge creation and dissemination without compromising the view that knowledge has intrinsic features which are real.

When adopting this view, it makes visible the responsibility of scientists to consider not only how they 'transmit' their knowledge and findings to the public, but also to be aware of how their ontological position impacts on the kind of knowledge they legitimate in these spaces and the inherent power dynamics that are created therein. Science itself cannot exist without some form of articulation of what is being found, how, why and what it means. When scientists see 'science communication' as an add-on or afterthought to the core work of whatever it is that they are researching, they are failing to acknowledge the social practices through which scientific knowledge is constructed and disseminated and the role language plays in this regard.

## Science and language

Drawing on Systemic Functional Linguistics (SFL)[4], Francis Christie's[5] work provides a useful distinction between language as a 'vehicle' for conveying pre-constructed thought and language as a *resource* for meaning making. SFL scholars argue that we make sense of, and construct understandings about our social and natural world through language. We also use language to transmit this meaning across contexts. If language is understood in this way it becomes possible to see how scientific knowledge, constructed through social discursive practices such as language, becomes laden with 'norms' and values of a discipline. Language becomes the signifier of what counts in a discipline – for example, the use of first person versus passive objectivity. In other words, the language we use is not arbitrary. It is determined by the disciplinary context in which we work and the value system that shapes that context. When we start to interrogate these discursive choices we can begin to appreciate that no scientific knowledge is neutral and that language plays a role in embedding power within knowledge practices.

## Language and knowledge

A number of critical social theories posit the political nature of knowledge and the power imbued in knowledge practices. For example, a Foucauldian approach to science requires a consideration of the relation between data, methodology and the exercise of authority. In short, studying how science is communicated requires us to consider the place of evidence in regimes of power.[6] Scientists should be encouraged to consider what forms of address,

relations of power, and values in relation to evidence are normative within their disciplines and fields, and how those in turn might show up in how they communicate the content and broader significance of their work. This critical reflexive work, although perhaps alien to many non-social scientists, can allow normative modes of knowledge-making to be questioned and deconstructed in ways that are supportive and constitutive of the collective project of creating a more just society.

A theory such as Legitimation Code Theory (LCT)[2] provides analytical tools for doing such critical reflexive work. Adopting a social realist perspective we offered at the beginning of this paper, LCT acknowledges the rational objectivity that knowledge does exist while at the same time recognising knowledge as a social phenomenon that is fallible rather than absolute or relative.[7] In this sense, it provides a realist way of thinking while at the same time maintaining the social character of knowledge. The framework offers multiple dimensions, each exploring one set of organising principles of dispositions, practices and fields. For example, the dimension of Specialisation explores practices in terms of knowledge-knower structures whose organising principles comprise relative strengths of *epistemic relations* and *social relations*. Epistemic relations relate to specialised knowledge, principles or procedures concerning specific objects of study. Social relations relate to the attributes of the actors involved in the knowledge production (such as race or gender, particular dispositions and/or identities).[2] While all knowledge practices necessarily have both sets of relations present at all times, by analytically separating these two sets of relations one can ask explicit questions of 'what' can be described as knowledge and 'who' can be a legitimate knower in any given field. Doing so reveals how different disciplines place emphasis on different relations. In other words, it is able to reveal the extent to which disciplines emphasise or value specialist skills or technical procedures for working with and constructing knowledge as opposed to highlighting significant authors or knowers in the field, specific perspectives, or particular kinds of dispositions (e.g. critical thinking).

To illustrate the implications of these different qualities on knowledge-building and knowledge dissemination, consider a crude comparison between the natural sciences and the humanities. Generally speaking, the natural sciences tend to foreground specific objects of knowledge and specialist procedures. For example, the use of the scientific method and the often structured manner of building claims is a technical process that scholars need to be highly trained to enact. Because of these explicit ways of working with and constructing knowledge there is often broad agreement in the disciplines about what counts as legitimate objects of knowledge and legitimate ways of working with that knowledge (the procedures involved). In Bernsteinian terms, this could be described as a "hierarchical knowledge structure"[8] as disciplines have some shared understandings of basic principles and premises on which individual disciplines are then built.

On the other end of the spectrum, if we consider the humanities, the emphasis is typically placed on cultivating particular ways of knowing and ways of interacting with knowledge rather than on specialist objects and technical procedures. For example, the emphasis is often on arguing for different viewpoints, engaging with particular authors in the field, aligning oneself with the work of particular scholars and distancing oneself from others. In such disciplines, the boundaries of what counts as legitimate knowledge and ways of coming to know that knowledge are much more fluid than that in the natural sciences. In Bernsteinian terms, this would represent a "horizontal knowledge structure"[8], where multiple theories, perspectives, value systems and knowledges compete for legitimacy alongside each other.

What is interesting to note in this broad comparison is that the natural sciences are not devoid of subjective aspects of knowledge-building (i.e. there are still 'knowers' involved) and the humanities are not devoid of specialist knowledge or procedures. Rather, both of these aspects are present but the language choices that are made when constructing the knowledge emphasise one set of relations over the other. For example, because the natural sciences espouse a shared understanding of principles of knowledge that rest on objectivity, subjective values and dispositions involved are typically downplayed or hidden from view. In this sense, the outputs of the scientist are commonly valued more highly than who the scientist is. This is one of the reasons why writing in the first person would be inappropriate in a natural science discipline – the focus is on the knowledge and the procedures, not the person.

## Knowledge and power

Critical reflexive work on how disciplinary knowledge is shaped by language is also important as it highlights the role of the *context* of knowledge-building: context determines what is appropriate and when. Returning to the comparison above, in the natural sciences where principles of accuracy, reproducibility, cross-cultural communication and validity are important, it would make sense, for example, to use very dense scientific names for objects and procedures. In this context, dense symbols and formulas may be necessary in order to communicate across social groups in ways that uphold the principles of knowledge. In the humanities, if the argument is the central function, where persuasion is key in order to convince a reader about a particular ideological position over another, then lexical choice and particular ways of building claims becomes imperative when considering language. Language, therefore, is not arbitrary: it is bound by and shaped by, and in turn shapes, disciplines and disciplinary knowledge.

What is interesting to note, however, is that when language is used to construct knowledge in particular ways, social power within the knowledge can be more or less visible. For example, scholars have argued that there is, at times, evidence of what Maton refers to as 'knower blindness' in the natural sciences (in contrast to 'knowledge blindness' that often results from constructivist relativism in the humanities and social sciences).[2] Blackie and Adendorff[9] have taken up this concept to describe how, in scientific research, the sociality of knowledge (such as the diversity the knower brings to the process of knowledge-building) is concealed from view. The authors argue that "[k]nower-blindness is not just an accident of the system, it is actively endorsed"[9]. This is because knower acknowledgement can, at times, threaten the principles for building 'objective' knowledge in particular disciplines. Yet as Blackie[10] argues, it is the social practice of science – that is, the act of practising scientists testing and refining theory – that enables disciplines to develop and grow in accuracy over time. A consequence of this interaction, however, is that social power shapes the development of science and the cultures in which the science occurs.[11]

Understanding that all forms of knowledge have social power, and how language plays a role in establishing and maintaining such power in societies, is important if we are to bring different kinds of knowledges (and scholars) together to solve wicked problems for social justice means. It also has implications for how we teach this knowledge to newcomers in the field. Again, drawing on LCT, this time incorporating the dimension of Semantics that considers how context dependent and complex knowledge is, scholars such as Ellery[12], Mouton and Archer[13] and Conana et al.[14] are doing important work on how access to knowledge practices and associated 'ways of being' are facilitated in higher education spaces. Such work problematises the notion that higher education is a meritocracy, and shows that some forms of knowledge, ways of knowing, and educational practices tend to 'match' the dispositions of some newcomers more so than others. They also draw attention to how curriculum design and pedagogy needs to be responsive to, and make explicit, not only the kinds of knowledge that are legitimated in different spaces, but also the associated literacy practices, dispositions and value systems that accompany different disciplinary areas. Without such awareness, the way in which access to disciplinary knowledge is facilitated has the power to include and exclude potential knowers.

The relationship between language, knowledge and power also has real implications for the way we communicate science, particularly across disciplinary contexts and into the public sphere. Our argument that scientific knowledge has both real and social qualities offers an invitation for researchers to reflect on the following questions: for whom does science work for or against, and with what purpose? When are scientists listened to or ignored, and why? Which scientists are taken seriously and by whom, and why? What forms of evidence are taken seriously by those in power, and which are not, and why? Which kinds of scientific evidence are amplified or silenced, and by which actors, in which contexts?

If science is to be used for social justice means, researchers need to be aware of the power imbued in their particular forms of knowledge and the impact this has on the sharing of ideas and working together to solve problems in our societies.

## Conclusion

When pausing to consider the importance of developing inclusivity in the broader project of science, knowledge dissemination is key. It is also important to remember that both communication and science have been, and can be, exercised as instruments of power (and indeed, resistance). Scientific communication, just like science, is not neutral, and is shaped by political economies of influence, bias, and unequal distributions of resources.[15] Just as all knowledge creation is an inherently complex social process, so too is communication always multidirectional, networked and not linear. Working for inclusivity and participation in relation to science creation and dissemination therefore requires interactive and dialogic modes of exchange: various forms of citizen science, listening as well as telling, and democratic and decolonial perspectives on what counts as knowledge.

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## Competing interests

We have no competing interests to declare.

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# Academic Publishing 101: The SAJS monthly Journal Writing and Peer Review Forum

**Significance:**

The *South African Journal of Science* monthly Journal Writing and Peer Review Forum is an open platform targeted at early career researchers who may be new to the publication and peer review process. This Commentary reflects on 10 frequently asked questions (FAQs) in the Forum and considers the collective response of the editorial team in helping to address each question in detail. The FAQs highlight some common concerns and uncertainties among new researchers, notably the issue of predatory publishing, turnaround time, and questions surrounding peer review.

## Background

To the uninitiated, the journey to publishing an academic journal article may seem intimidating. The publication process is often learnt through trial and error, and by following the advice of supervisors and mentors. Yet Castle and Keane[1] argue that "many academics and postgraduate students today, even in research-intensive universities...do not have access to the opportunities and experiences which could lead them to be productive and successful writers". High rejection rates may also discourage early career researchers from submitting their work for publication. As an intervention to help demystify the publication process, the editorial team at the *South African Journal of Science* (SAJS) has been running a monthly online Journal Writing and Peer Review Forum. The Forum provides an open platform for early career researchers to raise questions, share insights, and reflect on the publication and peer review process. There are currently 270 forum members, dominated by South African participants, but including researchers from at least six other African countries (Botswana, Ghana, Malawi, Mauritius, Nigeria and Tanzania) and from the United Kingdom. These meetings have highlighted several frequently asked questions (FAQs), which are summarised and addressed in this Commentary.

Before launching into these, it is worth bearing in mind certain aspects of the traditional publication process, which may come as a surprise to some. Firstly, the academic rigour of journal articles is upheld through the peer review process, which is intended to assess the quality and appropriateness of new submissions, and offer constructive criticism on how to further improve the work. Reviewing a manuscript is a time-consuming process, undertaken by specialists in the field, as a service to the academic community. This explains why the turnaround time for academic publication can often be painfully slow, because the process relies on finding reviewers who are willing to make time to review. A bizarre feature of the academic publishing process is that academics offer their research submissions freely, review freely, but often have to pay, via university library subscriptions, to access their own articles and those of the wider academic community. It is somewhat baffling that this system could persist for so long in academia. Encouragingly, there has been recent progress on this front, through the practice of making article preprints freely available online, the push for open access science, and other reforms. The SAJS is in a highly rare and fortunate position to offer fully open access publication with no article processing charges (APCs) or publication fees to authors. This is made possible through the sponsorship of the publisher, the Academy of Science of South Africa (ASSAf). I am providing this background to help early career researchers understand why the publishing process may at times seem frustratingly slow and cumbersome. Similarly, while peer reviews may occasionally seem harsh or overly critical, this response can be tempered with the appreciation that expert reviewers are taking the time to read and comment on your work, with the ultimate goal of improving its quality.

## Frequently asked questions

With this background in mind, below are 10 of the most common queries and concerns raised by the pool of participants in the monthly forum.

### *1. How do I choose a target journal?*

This question is one of the most asked questions in the Forum. If you have recently completed a research project or finished your postgraduate studies, and have discussed with your supervisor the possibility of publication, you need to then select a target journal, or create a shortlist of potential target journals.

A journal will likely desk reject manuscript submissions that do not align with the journal aims and scope, so it is worth spending some time selecting an appropriate journal for your work. When selecting a journal, it is easy to get caught up in the prestige of metrics such as journal impact factors or quantile rankings. According to SAJS Editor-in-Chief Prof. Leslie Swartz, start by asking yourself, "Who do I want to be in a conversation with?" Determining your target audience is key to identifying an appropriate target journal. This means that you must ask yourself whether your work is more appropriate for a multidisciplinary or a specialist audience. Also determine if your work stirs international interest or has more of a local focus. These criteria will help narrow the pool of potential journals. For further reading, Knight and Steinbach[2] provide a comprehensive overview of journal selection criteria.

Another approach to creating a shortlist is to consult the reference list of your own manuscript and compile a list of the journal names that keep appearing. For further inspiration, distil your study into keywords and use these as the subject of a Google Scholar search to see which journals are the top recent hits for your topic. From there you can look up the journal homepage, read the aims and scope of the journal, and assess its suitability. The aims and scope may include subtle clues as to the style of article that the journal seeks to publish. To obtain a more detailed

impression of the journal style read the current issue and older articles to get a feel for the style and tone. Once you have a shortlist of potential target journals, there are a few things you can check upfront:

- Is the target journal Department of Higher Education and Training (DHET) accredited? South African institutions receive a subsidy from the government for publications in DHET-accredited journals. Note, however, that the best way to advance your academic career is to contribute to the conversation within the academic literature, regardless of DHET accreditation status.
- Will publication in the target journal incur article processing charges (APCs) or colour page charges, and are these affordable to you? Note that many universities will pay or contribute towards page fees. This can be checked through your Research Office.
- Is the journal open access, hybrid, or paywalled? Open access journals help to promote broader readership, but unfortunately many will incur APCs.
- What are the article types that the journal will consider, for example, original research articles, review articles, or short communications? Also check associated word limits and figure and table limits for the article type you are intending to submit. Article types and length limits differ between journals and may not align with your idea.
- Another consideration is journal turnaround time. This can be determined by looking through recent issues to compare the date when each article was first submitted with the date of online publication.
- If metrics are a priority for you, you can look up the journal impact factor, which usually is available on the journal's website.

The above criteria will help you to compile a strong shortlist of suitable candidate journals. At this point, it may be worthwhile to revisit the journal homepages and look at recent issues to determine the appropriate journals on the shortlist. If you are still unsure, you also have the option to send the editor an email including the title and abstract of your manuscript to enquire whether it would be a good fit for the journal.

### *2. How do I go about submitting my manuscript?*

Visit the journal homepage and find the instructions or guidelines to authors. Read these guidelines carefully and prepare your manuscript according to the individual journal formatting and referencing requirements. The guidelines will also provide details on the basic submission process. Ensure that your co-authors are satisfied with the final manuscript and target journal before you submit. Be aware that in some fields you may be expected to nominate potential reviewers to evaluate your work. These should be subject specialists who can provide an unbiased assessment, and should not include recent collaborators. Here you may also list any opposed reviewers.

### *3. Can I send my manuscript to multiple journals to speed up the process?*

In most fields, it is considered unethical to send your manuscript to multiple journals at the same time because it wastes the time of the peer reviewers and editorial staff. During the submission process, it is standard practice to sign a compulsory declaration or publishing agreement which states that the work is not under consideration for publication elsewhere.

### *4. My manuscript has been in review for ages. What is an acceptable turnaround time and what are my options?*

Some journal submission systems will include a status to indicate whether the manuscript has gone out for peer review, so check for this where possible. While patiently waiting for a decision, it is useful to imagine the work going on behind the scenes. Editors face ongoing challenges in finding suitable reviewers and in getting the reviewers to respond to review invitations, and then in receiving the reviews within a reasonable timeframe. Usually the editor will need at least two independent peer review reports to make a decision, and securing these may require more peer review invitations. Further, consider that not all peer review reports will be considered acceptable by the editor. For example, reports which are offensive, or inherently biased will be excluded, forcing the editor to solicit further reports from a new reviewer. With these delays in mind, it is reasonable to send a polite follow-up email to the editor after 3 months. In some cases, the process may stretch on very much longer (for example, a year), and if after following up with the editor you are unsatisfied with the delays, you have the right to formally withdraw your manuscript from the journal at any stage in the process. Notably, keep in mind that long turnaround times are not uncommon and keep your expectations realistic. In some journals, a publication lead time of up to 2 years may be expected. Another important point to note is that the time spent in review is entirely unrelated to the quality of the manuscript or likelihood of acceptance for publication. In other words, do not think that a long review time will increase your chances of acceptance for publication.

### *5. How does the peer review process work?*

After submitting, the editor will assess your submission briefly to check that it is appropriate for the journal. If the manuscript is not appropriate, it will be desk rejected, usually within a few weeks. If it is appropriate, peer reviewers will be invited to assess the work. These peer reviewers are subject specialists with no conflicts of interest with the authors (such as past supervisors, collaborators, or researchers from the same institution). There are different peer review models, but the most prevalent are single- and double-anonymous review. In the case of single-anonymous review, the identity of the peer reviewers is concealed. In the case of double-anonymous review, the identities of both the peer reviewers and the authors are concealed. After receiving at least two independent peer reviews, the editor is usually in a position to make a decision regarding the outcome. In the case of contradictory review reports, the editor may choose to secure an additional review before deciding on the outcome. Possible outcomes include acceptance as is, acceptance with minor revision, major revision with another round of review, and rejection. In the case of major revision, the revised manuscript will go out for another round of review, ideally to the original reviewers where available, for reassessment. Importantly, the peer review process should not be viewed as a gatekeeping exercise, but rather as a constructive process designed to improve the quality of individual submissions.

### *6. My reviews have come back with major revisions. Where do I start?*

Remember that a key purpose of peer review is to improve the quality of manuscript submissions through constructive criticism. If the revisions seem overwhelming, compile a summary document or spreadsheet that lists each comment or criticism. You may find that both reviewers have requested related changes which can be combined. You will also quickly note which changes will be quick to fix, which will be challenging, and perhaps some comments that you can easily rebut or respond to. Note that polite rebuttal is certainly acceptable in the case of inappropriate comments or suggestions. Use this spreadsheet to guide your approach and document your progress in dealing with the revisions. When you are ready to resubmit your revised manuscript, this document can be used in your covering letter as a response to reviewers' comments. Use track changes when revising your manuscript, so that it is easier for the editors and reviewers to see exactly what changes were made.

### *7. What happens once my manuscript is accepted?*

Following acceptance, your manuscript will typically enter a copyediting phase and you will be sent proofs for final checking and correcting of minor errors and omissions, often with a rapid turnaround. Usually, the article will then be published for early access online with a unique Digital Object Identifier (DOI) number which allows for the article to be viewed and cited. The article is fully published once it appears within a specific issue of the journal and is assigned to a volume and issue. This often happens many months after online publication.

### *8. What is the role of supplementary information?*

Supplementary information is similar to an appendix in that you may include information which is relevant for reference purposes but not central to the main article. Text, figures and graphs included in supplementary information do not count towards the main manuscript word count or restrictions on the number of figures and tables.

### *9. I received an email invitation to publish my work in a journal. How do I know if this is a 'predatory journal'?*

As flattering as these invitations can be, it is best to remain vigilant unless you have verified that the journal is legitimate, as the vast majority of these requests are predatory or of dubious origin. Beall[3] used the term 'predatory' to refer to open access journals that publish substandard articles without sound editorial and peer review practices, for the purpose of financial gain via the author-pays publishing model (APCs). Tragically, there is evidence that most publications in predatory journals are sourced from Asian and African authors[4] and that predatory publishing practices remain prevalent in South African universities[5]. Aromataris and Stern[6] review the issue of accurately distinguishing predatory from legitimate journals, and point authors towards several online checklists such as Think.Check.Submit. (https://thinkchecksubmit.org/) and trusted guidelines such as those of the Committee on Publication Ethics (COPE)[7]. Research online details about the publisher or publishing group; this may be the easiest way to establish whether the publisher is associated with predatory publishing practices. If you need guidance, you can also refer to your librarian or research office for assistance in verifying the legitimacy of the publisher.

### *10. What developmental support does SAJS provide to early career researchers?*

SAJS hosts an annual Writing Workshop and an annual Peer Review Workshop, recordings of which are available on the SAJS YouTube channel. In addition, the monthly Journal Writing and Peer Review Forum, the subject of this Commentary, provides a smaller and more interactive opportunity to discuss and engage with the editorial team regarding any challenges you may be facing, or queries you may have. The workshops and forum meetings are conducted online and are open to all participants, regardless of the targeted journals for their work. SAJS also encourages peer review mentorship in which early career researchers can collaborate on the peer review process to gain experience. Finally, SAJS has an Associate Editor Mentorship Programme in which researchers can apply to participate and learn firsthand about the editorial process.

## Conclusion

Participation in the monthly Journal Writing and Peer Review Forum meetings has demonstrated that there is a real and pressing need for developmental support among early career researchers wanting to publish their research. This Commentary is a quick reference for those researchers who are unsure about the publication process, or who are contemplating submitting their first manuscript.

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# Distinguishing the genuine from the fake in South African universities: Scholarly awards, books and academic credibility

**Significance:**

Universities and their academics are faced with increasing pressure to contribute to knowledge. These pressures produce conditions for shortcuts and, worse, the production of unreliable research that has little value. This is the world of the fake, where universities are corrupted and their integrity undermined. We argue that the scholarly book that reflects deep thinking and major commitments of time, energy and resource is a counterweight to the temptations offered by short, quick and often shallow outputs processed in predatory journals and often rewarded by national and university publication incentives.

## Introduction

Jonathan Jansen's 2023 book *Corrupted: A Study of Chronic Dysfunction in South African Universities*[1] has ignited considerable debate around the conditions that render South African universities – regardless of their size, geography or historical genealogies – either dysfunctional or functional. This debate is not new, because, in the wake of #RhodesMustFall and #FeesMustFall, questions were asked about threats to the existence and purpose of universities.[2,3] In his recent book, however, Jansen points out that one of the most potent resources for a university to remain functional is academic credibility. There are values and taken-for-granted norms that underpin the academic project.[1(p.52,81)] An obvious point, surely. But not quite.

The moral economy of modern capitalism is closely tied to the idea of the fake. Fake news, fake goods, fake 'global collaboration', fake development. And fake academia. Fake academia looks like a commitment to academic credibility and the academic project – as a good fake should! – but it is in fact inimical to the kind of academic credibility that is one of the key foundations of a / the functional university. Jansen[1(p.132-133)] explains the workings of fake academia, where rent-seeking by both individuals and institutions extracts resources from university research income, particularly funding from the National Research Foundation (NRF) of South Africa. There is, for instance, a "subterranean economy of predatory journals"[4]. On the surface, publishing in journals identified as predatory appears quite legitimate, but it is really an academic corrupt practice of "enormous proportions"[5].

The commodification and privatisation of knowledge and the technology of the Internet have revolutionised the dissemination and consumption of research. Whereas half a century ago, research was produced and packaged in journals and books, printed on paper and distributed and sold to a relatively small academic market, the Internet made possible very rapid dissemination of knowledge to large, global audiences. In the process, market-opportunities opened up for publishing companies and for smaller enterprises to enter a competitive market where one could sell knowledge to huge audiences.

Academics were increasingly pressured to 'publish or perish' by universities in order to compensate for decreasing state funding and to compete in the new global university ranking system.[6] The incentives for academics to find outlets for their work thus grew, and so new outlets, often new journals operating from a variety of lucrative business models, were created to feed this market of desperate academics.[7] Academics now paid journals to publish their work. The quality assurance of peer review was undermined when journals claimed to abide by this practice but actually just paid lip-service to it. The production of journal articles has rocketed but many of them are of poor quality or of little academic merit or relevance. Thus was born the predatory or 'grey' journal, an animal that is not always easily or definitively identified.

In South Africa, the generous subsidies paid by the state for each 'accredited' journal article and other form of academic output have provided huge incentives to academics to publish anywhere that will take their work. In 2021, the unit value paid to a university for a research output like a journal article was ZAR123 635, an amount which had grown by 65% since 2006. When there is low institutional capacity to cultivate quality research in significant volume, and low institutional ability or appetite for screening out these bogus journals, some academics opt for the easiest route where acceptance rates for submissions are essentially dependent on paying the journal. While academics debate these practices[8,9], institutions may overlook these cynical publishing practices because they are benefitting from the state subsidy. Similarly, institutions are incentivised by the state to engage in industrial-scale graduation of doctoral students. The compendium of fake academia is weighty.[10-12]

Academics and universities are not compelled to endorse dodgy academic practices and there certainly are better ways of contributing to genuine knowledge-production and by so doing, maintaining universities as places of enquiry, debate and engagement rather than as mass-production factories. We argue that the scholarly book acts as a counterweight to quick and cynical practices. Its production takes time but it is a goal available to all scholars in all disciplines. The scholarly book offers an alternative, we would argue the supreme, form of research validation.

In this short piece we reflect on the production of one sort of scholarly output, the book. We ask specifically what the ethnographic life of the book tells us about how seriously a university takes questions of academic credibility and the robustness of an academic culture at a university. We also consider how individual academics approach the question of producing this most time- and energy-consuming of academic outputs in the light of the incentives offered for quicker easier forms of output. Moreover, we ask whether the emergence of a book from a university, the

kind of book we discuss below, may be interpreted as one, important, fragment of university functionality.

## Academy of Science's Humanities Book Awards

Thursday evening, 30 March 2023. The Vineyard Hotel, Newlands, Cape Town. A stunning view of Devil's Peak and the setting of the annual Humanities Book Awards Ceremony hosted by the Academy of Science of South Africa (ASSAf). Lesley Green's *Rock/Water/Life: Ecology and Humanities for a Decolonial South Africa*[13] is announced as the winner of the Established Research Writer Category. This is a story about the event, the book and the long journey of its production. It is also a cautionary tale, a polemic against the impulse for quick returns that fit university reporting cycles (usually annual) and fake academia.

Neil Roos, Chair of the ASSAf Humanities Review Panel and Dean of Humanities at the University of Fort Hare opened proceedings with an explanation of the origins of the book award and the importance of recognising scholarly books. In a way this marks the end of a journey, and not one enjoyed by every scholarly book that is produced. The process that we describe is largely hidden from view and one of which academic authors will be unaware when they begin their labours. Nevertheless we argue that it is part of a process that is critical to the life of the Humanities. In turn, the life of the Humanities is critical to the health of the country.

The adjudication panel which considered nominations for this year's ASSAf Humanities Book Award was quite diverse. All were South African scholars, from a variety of disciplines: literature, sociology, history, gender and queer studies. Some were seasoned, some mid-career. Some were from metropolitan universities, others from rural ones. Over the course of 6 months the panel deliberated over the books that were nominated for the two prizes awarded by the apex science organisation in the country, one for established authors and the other for emerging authors, broadly defined as scholars under 40 who would be eligible for an NRF Y-rating and whose submission was their first book, a 'dissertation book'.

The NRF rating system is a national system established in 1984 which ranks individual researchers in terms of their research output. The intention of the system was to promote national knowledge production. The system distinguishes between A-rated (internationally leading), B-rated (internationally recognised) and C-rated (established) researchers. It also has 'junior' ratings for early career researchers (having completed their doctorates in the previous 5 years) demonstrating potential: P-rated (likely to be international leaders and 35 years or younger) and Y-rated (likely to become established researchers and 40 years of age or younger).[14]

The panel had a formal set of criteria: is the book a scholarly book rather than, say a handbook or a textbook, has it been the subject of review by peers, and how was it received by these reviewers? The panel acknowledged that there were other criteria that were more elusive to codify, more difficult to reduce to an evaluation template. These included elements like the intellectual novelty of the book, whether it laid out new fields, whether it was written in a style of openness that invited dialogue and engagement with the book's major hypotheses. While the panel did consider whether a book might have a readership beyond its immediate field or discipline, it also recognised (and honoured) sheer scholarliness. One adjudicator pointed out that in one nominated book, not one sentence was frivolous, trite or superfluous; each one was 'heavy with meaning'. In other words, the search was for books that were scholarly, that pointed to new fields of enquiry in the humanities, that proposed new configurations of disciplines and knowledge, that changed how we conventionally receive fields and disciplines, or posed important new questions within these arenas.

Against these criteria the panel made two awards. The award for the emerging researcher was shared between B. Camminga's *Transgender Refugees and the Imagined South Africa*[15] and Dariusz Dziewanski's *Gang Entry and Exit in Cape Town*[16], while the main, open award for established researchers went to Lesley Green for *Rock/Water/Life: Ecology and Humanities for a Decolonial Africa*.

## The labour: Making excellence

Green's book consists of a series of case studies powered by the conceptual resources of progressive global scholarship which collectively show how the long shadows of inequality, racism and colonialism in South Africa have enabled environmental destruction. It argues that environmental research and governance can help to address the country's history of racial oppression and environmental exploitation by challenging some deeply entrenched antagonisms. As critic John Higgins points out, one of the book's most significant imprints lies in its mode of thinking, where it seeks to establish grounds for engagement between the politics of lived experience and those of an "over-confident and belligerent scientism"[17].

Lesley Green's book has many origins. We narrate one of them here. It is a story of how a university (the University of Cape Town, UCT) created the space for the writing of a scholarly book and how Green took that space.

In 2010 UCT's Research Office was funded by the Carnegie Corporation of New York to promote interdisciplinary research and Africa research collaboration. This was generous funding that was administered through a programme called the Programme for the Enhancement of Research Capacity (PERC). A founder of this programme was Professor Brenda Cooper, a well-published scholar of English Literature who had been based in the African Studies Centre at UCT. Her interest was, and remains, writing and the support of African voices.

In that year Robert Morrell became coordinator of PERC at UCT and set about implementing the vision of the programme. One of the first things he did was to create the position of Associate for the programme, a well-funded position with teaching buy-out. The first PERC Associate selected was Lesley Green.

Green at that time had already demonstrated that she was an outstanding intellectual visionary with a startling ability to raise funding. She had been awarded monies by the Andrew W. Mellon Foundation to host the Sawyer Seminar series ('Knowledges, Ways of Knowing and the Post-Colonial University') which brought scholars from around the world to Cape Town to develop a set of ideas that would contribute significantly to the launching of Environmental Humanities [with colleagues from political ecology (Frank Matose (Sociology)), ecocriticism (Ian Rijsdijk (Film Studies) and Hedley Twidle (Literature)) as well as environmental history (Lance van Sittert) and fine art (Virginia MacKenny)]. Over time the project has been built into a space where African and black environmentalism has found a strong voice, not least due to the nuanced integration of lived experience and African ecological thought. This ultimately led to the launching of Environmental Humanities, a funded interdisciplinary institute in the Humanities Faculty. Green's tenure as PERC Associate lasted 2 years (2010–2011) and featured a number of writing retreats, including one in 2011 titled 'Contested Ecologies: Multiple Natures and Democracies in the Global South' which brought together scholars from around the world: Eduardo Viveiros de Castro, Helen Verran, Mario Blaser, Harry Garuba, Elisio Macamo, Marisol de la Cadena, Laura Rival, Josh Cohen, Christopher Mabeza and Munyaradzi Mawere.

This early work was pioneering in the sense that Green was using both her experience of doing her PhD in the Amazon forests and her theoretical curiosity to draw into South African scholarship new strands of work, mostly European and South American, which were largely outside the ambit of national debate at that time. She steadily built this field – she had outputs – including her 2013 edited collection, *Contested Ecologies: Dialogues in the South on Nature and Knowledge*.[18] Good books emerge from bigger projects that involve more scholars than the singular author of a big book. These projects in turn draw on shifts, controversies, innovations and breakthroughs in a field or more than one field, even small, subterranean moves. Field- or discipline-building commonly involves conferences, workshops, and other scholarly interactions as well as the publication of essays or papers which test parts of a new set of propositions about a field or discipline.

Green's ability to gather together scholars from around the world was important for the way that, over time, she was able to address

central elements of the ASSAf criteria: how fields and disciplines are constructed. The key was also to pose important new questions within these arenas. It is by recognising the expanse of global scholarship and embracing its scholars (in this instance from the Global South) that major advances can be achieved and new fields such as Environmental Humanities energised. Indeed, the importance of such moves is now recognised in the work of Southern Theory and the geopolitical critique of knowledge-production.[19]

Green's journey is also a cautionary tale. This kind of work takes time, often a long time. Funder and university expectation is normally short term. Morrell was expected to demonstrate the success of PERC by referencing outputs. Yearly reports were expected to bulge with achievements, journal articles, conference attendances and so on. A funding cycle of 3 years was considered very generous. As a new audit culture emerged and settled itself in the corridors of university management, the focus on what could be counted in a unit of time (ever smaller) became the modus operandi.[20]

Carnegie's funding was generous and sponsored two more PERC Associates before it came to an end. The second was Professor Sophie Oldfield, a geographer working on issues of the city, housing, planning and belonging with an emphasis on theory and community making in the Global South. Her year-long tenure as PERC Associate was in 2012. During this time her major occupation was a monograph. That monograph will finally emerge this year as *High Stakes, High Hopes: Urban Theory in Partnership*.[21]

Universities and funders are not renowned for their patience. Yet this and scholarly persistence and courage on the part of an author is a key ingredient in the making of scholarly books. The rise of audit culture if anything inhibits creativity and strengthens impatience. Time is counted, money is accounted. Lesley Green's book makes a critical point. When we see things only as objects we become the slaves of a modernist legacy which is with us still, which fosters fakes in the elevation of things over people. Lesley's book highlights the importance of relationships, between people, between people and nature. It calls for a new respect and care for the environment and warns of the dangers of ignoring warnings about imminent crisis and the delicacy of life.

Green's award of the ASSAf prize is an affirmation of the genuine, a triumph of endeavour, commitment, curiosity, and scholarship. As Lesley strode to the stage to accept her award, she had a smile that lit up the room. A laugh deep from within her erupted. If we take delight in Green's triumph, we can also tip our hats to UCT for supporting her work and conclude the cautionary tale that despite the logics of the new audit-culture and funding and resource constraints, it is still possible for universities to support groundbreaking work and in so doing, elevate the genuine above the fake.

## Conclusion

We need big scholarly books to safeguard universities from the creep of commercialisation, from the temptations to take shortcuts and from the danger of becoming hollowed-out shells, closer to the fake version than the real one.

On the possibility, and indeed the value, of writing history on the basis of shards, or fragments of evidence, subalternist Sanjay Seth once commented that "we can learn big things from little things"[22]. We have suggested that if a university provides the ecology that both enables and supports the emergence of a book like *Rock/Water/Life*, with the necessary trial and error, slow and steady theory-testing and discipline-building, this is but one indicator that academic credibility and the academic project is intact at a university. There are similar markers in other reaches of the academy. Collectively these represent a riposte, a rebuke to the idea that the contemporary university, with its managerial and audit cultures and preoccupation with outputs, metrics and rankings, is necessarily built on the foundations of fake academia.

Funders are often critical, and unrecognised, supporters of research but they too are under pressure to deliver outputs. Can they be encouraged to take a longer-term view? Organisations like ASSAf should be lauded for their promotion of the scholarly book. The state and its higher education agency, the Department of Higher Education and Training, should be supported to reward and encourage the production of the scholarly book. Together, these supports should provide space for scholars to think big and write big. They will need time and, often, administrative and financial support to complete the arduous journey.

Recognising the scholarly book as the pinnacle of research achievement in the humanities is one way of ensuring that universities protect institutional research culture.

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We have no competing interests to declare.

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# Sutherland big telescope: Further opportunities for effective science engagement?

**Significance:**

The launch of the Southern African Large Telescope (SALT) in 2005, and the introduction of astronomy outreach to interface with the general public, signified a turning point in South African astronomy. We conducted a small study to begin to understand the perceptions of the community that hosts the research facility in Sutherland, in the Northern Cape Province, South Africa. There is an ongoing need for good stakeholder engagement for sustainability. Scientists need critically to consider and review engagement processes with remote communities, including those flanking astronomy infrastructures.

## Introduction

The South African Astronomical Observatory (SAAO) began its operations in Sutherland around the 1970s. The move to Sutherland came as a result of the light and air pollution which made it impossible for astronomy research to be done effectively in Observatory, Cape Town.[1] As a country trying to redefine itself in many aspects, including science, the South African government began building the Southern African Large Telescope – a 10-metre class telescope. The commission and launch of the Southern African Large Telescope (SALT) in 2005 marked a turning point in South African astronomy, when astronomy outreach was formalised by establishing the SALT Collateral Benefits Programme to interface with schools and the general public to justify the huge investment in astronomy.[2] The formalisation of astronomy outreach or astronomy engagement was buttressed by science and society advocacy dating back to the *1996 Science and Technology White Paper* asserting a resolution to advance a South African vision of an information society to serve the needs of the country instead of reflecting those of other countries.[3]

During the launch of SALT, the SAAO hosted an inclusive event with the Sutherland community at the rugby stadium in town. This closeness and consideration created hope and huge expectations. These expectations were partially met by a focus on astronomy outreach, a form of communication that was intensified and codified in a SALT Collateral Benefits Programme.[2] Concomitantly, attention was paid to edu-tourism, which was hoped would play a significant role in public understanding and engagement with science[4] and serve as economic fortification for the community, considering that tourism in South Africa and worldwide is deemed a catalyst for development linked to job creation[5]. From the community's perspective, the town of Sutherland would be visible for the first time, and there was indeed a tourism boom in the town with guest houses, and bed-and-breakfast establishments built in anticipation of the influx of tourists who would visit SALT and Sutherland as a centre of science and technology.

## What exactly is missing?

A recent very small study on perceptions of people in Sutherland about the Observatory[2] suggests that the surrounding community may feel there is a distance between them and SALT and that there is not much substantive involvement in the town beyond school outreach programmes. Although outreach efforts are visible and active, according to participants, there is a lack of career pathing despite the firm stance taken by the South African Department of Science and Innovation (DSI) on the wide-ranging categories focusing on the promotion of careers in science[6] and the National Research Foundation's (NRF's) mandate on the expansion of human and research capacity in the fields of science[7]. The Observatory thus far has trained and retained five young unemployed youth as tourist guides.[8] Though encouraging, this is insufficient to meet the broader mandate.

The launch of SALT occurred in the context of the Department of Arts, Culture, Science and Technology White Paper, the vision of which was to establish a conducive culture for knowledge advancement appreciation as a vital component of national development. On the occasion of the SALT launch in 2005, President Thabo Mbeki said:

> *The Observatory is a place dedicated to the pursuit of knowledge. Its sole purpose is the discovery of the unknown, and therefore the further liberation of humanity from blind action informed by superstition that derives from failure to fathom the regularities and imperatives of the infinite natural world.*[2]

Preceding both the SALT launch and the 1996 White Paper, was a year-long science engagement campaign to reignite South African society's interest in science.[3] Entrenched social segregation as a result of colonial and apartheid political systems, and dependence on science practices developed in the Global North, may all have contributed to a less than ideally locally inclusive approach to science communication.[9]

Against this backdrop, we were interested to hear the views of the Sutherland community on the SAAO, and we conducted a small-scale study to begin to address this question. Overall, participants reported an absence of community-based interventions and a lack of astronomy information.

Some of the themes that emerged from the data analysis highlight the perception participants had of the SAAO. It is important to stress that our sample was very small (seven people); nevertheless, the views expressed shed some light on possible expectations and hopes for better communication and an improved relationship.

## The problem of expectations

It appears that there were high expectations for the SAAO to provide substantial employment opportunities. Some non-professional positions were filled by Sutherland people during SALT's construction and afterwards as general workers. Given the very limited capacity created by the Observatory in anticipation of SALT, professional appointments would have been impossible. Some participants mentioned the importance of meaningful school outreach programme beneficiation through career-pathing so that in the future their community could have professionals employed by the Observatory: One participant said:

> *No, and most of the children really want to come back and work here at SALT but I think the last few years for three or more years they do not have mathematical teacher – its mos science and mathematics that must be your main subjects. A lot of them didn't have that or their grades wasn't that good. If SALT found something and they come and explain it maybe more of the children will be interested in astronomy.*

The education benefit plan of SALT was to jointly form an SAAO and Northern Cape Education mathematics and science academy to attract young people from the surrounding towns.[10] This did not fully materialise – in its place there is a mathematics teacher for both Sutherland schools. Hiring the teacher is a step towards addressing one of the strategic aims of the DSI science engagement strategy, which is to make science, technology, and engineering careers attractive to young people.[6] There are examples of astronomical observatories, in better resourced contexts, that have anticipated the need for technologically advanced skills that would meet their workforce demands, for instance, the Thirty Metre Telescope in Hawaii initiated a 1-million-dollar fund to upskill the workforce in preparation for their next-generation telescope commissioning.[11]

## Disconnection from the community?

In preparation for the SALT launch, the Observatory made use of Community Development Workers from the Karoo Hoogland Municipality, who were responsible for information dissemination within the community. After the launch, there was no communication until FOKUS – an environmental and current affairs television programme which airs every Sunday on SABC2 – visited Sutherland and asked the community members about their knowledge of SALT. In response, the Observatory had a regular tour bus in town bringing community members to SALT. Because the Observatory is a national facility, government officials sometimes visit SAAO. In these instances, the Observatory also invites community leaders to attend. A participant in our study commented: "No, no there was no chance that they recognise us. They just recognise us when there's dignitaries coming."

## The desire for broad science communication

"Everybody is curious, they just want to go to the Observatory to see how the process is with SALT."

Science often represents an important social good; therefore people ought to know about it. Some scholars argue that comprehension of science communication in a cultural context could help the public to be both analysts and practitioners.[12] During the interviews, participants spoke fondly about their pride in being located near a scientific institution like SAAO. They said that their proximity to the SAAO is a conversation starter whenever they are visiting family outside Sutherland. On the other hand, they expressed concern at the gaps in their own knowledge about astronomy. This, they said, makes it hard for them to engage meaningfully with tourists even though astro-tourism is the backbone of the unique selling feature of Sutherland.

The thriving tourism activity around the Observatory is evident in the numbers collected by SAAO. Sutherland receives 13 000 tourists per annum,[8] directly benefitting the many guesthouses and B&Bs that cater to the niche tourism product coined 'astro-tourism'. Astro-tourism is a fundamental cornerstone of a socio-economic strategy aimed at driving tourism-led destination economic growth[13], especially in the Northern Cape where astronomy infrastructure exists. Those who did not have the means to participate in the newly formed niche could benefit from a well-considered broad-based benefit focused on the tourism value chain.[14] At the macro-level, tourism value chain means individuals, organisations, and businesses that form collaborative intersections to co-create and co-deliver a sustained value to tourists.[15]

The Observatory is an entity integrated into national science programming and a facility of the NRF, whose responsibility and mandate is to advance the "transfer of technology and the implementation of research results and findings", part of which ideally should include making science knowledge broadly accessible.[7] The Observatory is obligated to follow the policies of its funder, the DSI, which recognises that a major goal of science engagement is critical engagement between science and society in order to enable society to engage meaningfully with policy development, including the ability to question controversial scientific projects.[6] The NRF and DSI mandates match science communication scholarship which underscores democratisation and public engagement.[16]

In order to engage substantively with the Science and Innovation Strategic Plan, which outlines the importance of revamping the general public's knowledge about science[17], the Observatory's approach to communication should ideally be built on an acknowledgement that science does not have a firm foothold within the South African society, given the historical and contextual factors mentioned above.[9] This requires strategic marshalling of communication as an influential foundation that sets up a stage from which relationships can be defined and disassembled.[18] Whatever practical actions the Observatory may take in this direction, what is needed is an ongoing engagement with the tools of science communication that consider cultural context so as to help the public to be both analysts and practitioners.

## Recommendations going forward

It is important to emphasise that our study was small; all recommendations must be tentative. Nevertheless, we hope this work will contribute to the debate in the field. There is an aspiration within the DSI policies and science engagement strategy to find ways to have a scientifically engaged society. Much has already been done, but in order to fulfil this mandate, there is a need for ongoing and reflective engagement between the SAAO and the community. Science communication and relationship building are ongoing processes rather than events; developing and maintaining a reputation for any enterprise requires ongoing work.[19] The DSI encourages using corporate communication departments within the national science institutions to collaboratively develop coordinated messaging with researchers, science engagement practitioners, and scientists.[20]

A particularly important feature of science engagement is with the youth. The science engagement strategy implementation plan from DSI recognises the importance of learner-focused intervention. As such, a policy framework specifically addressing skills development in science and technology in the communities in which astronomical observatories are located would be ideal. Currently, and understandably, the policy is very broad because it is meant for the whole country. The DSI clearly mentions in the implementation plan the importance of partnerships between willing universities and high schools. Given that Sutherland only has two schools that have recently acquired a mathematics teacher via SALT, the opportunity for such partnerships is ripe with possibilities to realise community aspirations of having "locally groomed astronomers, technologists, and engineers". This would also create a pipeline for Sutherland learners to easily join other programmes such as the National Astrophysics Space Science Programme whose origins were to improve astrophysics skills in the country (2016–2021). Science communication strategies represent an important investment in the future of science in South Africa.

## Competing interests

We have no competing interests to declare.

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# Reconsidering consent for biomedical research using human biological material and associated data

**Significance:**

Consent, in the context of biomedical research, biotechnology and the use of human biological material and associated data, is examined here, and it is demonstrated that informed and broad consent do not constitute valid consent. Dynamic consent is introduced as a viable alternative. New science requires new models of consent and this Commentary introduces such a model so that consent may become future-flexible.

## Introduction

Biomedical research is the area of research which studies treatment or prevention of disease, illness or death.[1] Biomedical research makes use of biotechnology and human biological material (HBM). HBM is material derived from living or deceased persons such as human tissue, blood, biofluids, cells or DNA. Biotechnology is technology which integrates natural and engineering sciences to produce or discover new medicines, treatments and therapies.[2]

The novelty of these medicines, treatments and therapies has given rise to regulatory difficulties and so, in this Commentary, consent in the context of the use of HBM and associated data is examined. Consent is indispensable[3], but obtaining consent in this context has become complicated, as will be discussed below. Many challenges have been identified in acclimating existing consent models to biomedical research, biotechnology and the use of HBM[4], such as a lack of applicability; the inability to meet the required consent elements of knowledge and understanding; difficulty in accommodating consent revocation; and insufficient processes for returning research findings to participants[5].

Although informed consent is deemed the golden standard, it does not allow future, secondary use of HBM and data in research. This is because informed consent requires the provision of information of what is known to be associated with that which is consented to – the scope of an intervention is determined. However, future uses, which are unknown, fall outside this information provision requirement, and so informed consent may be rendered invalid. To solve this problem, broad consent was developed as a solution. Broad consent, however, is also insufficient and might be seen as ethically problematic, as will be explained below.[6]

Here, consent in the context of biomedical research, biotechnology and the use of HBM is examined along with the shortcomings of informed and broad consent. A new model of consent – dynamic consent – is then introduced as the most appropriate consent model which allows unprecedented flexibility in consent, and so accommodates the possibility of future research.

## Informed consent

Consent as prerequisite to an intervention is based in recognising the unconditional worth of all humans, which is rooted in the principle of respect for autonomy.[7] Because this principle is the foundation for the right to make autonomous decisions, recognition has been given to specific autonomy-related rights such as bodily integrity.[8]

The informed consent model serves various purposes which include encouraging rational decision-making by allowing a person to come to a decision after considering and weighing the benefits and risks of a proposed intervention.[9] This means informed consent entails that a consenting person appreciate what they are consenting to.[10] As such, knowledge and appreciation on behalf of the consenting party is of primary importance in the process of consent, and so are seen as at least two of the essential elements establishing real, valid consent. The third is the provision of information and the last is that of acquiescence by acceptance. From this, the following requirements for valid informed consent may be identified: informed consent will only be valid where it is based on the provision of (1) appropriate information with corresponding acquisition of (2) knowledge and (3) understanding by the consenting person, followed by (4) acquiescence.[11]

Additionally, other requirements for validity have been identified by legal scholars and by development of the informed consent doctrine in case law.[12] Additional requirements most relevant to this discussion include:

1. The consenting person must have knowledge of the nature and extent of a proposed intervention. Also, there must be understanding and appreciation of these.
2. The consenting person must consent to the purpose, risks and dangers of an intervention.
3. The information provided must be comprehensive, extend to the whole intervention and must include the consequences thereof.

Due to the novelty of, fast advances in and possibility of future research using biomedical research, biotechnology and HBM, the foundations of informed consent have shifted, and it has become misaligned with the aims of research; consent must be finite while research is not.[5] With reference to the additional requirements for valid informed consent and in the context of biomedical research, biotechnology and HBM, this misalignment becomes

obvious, as all these requirements speak to the scope of the intervention, what is consented to, and so, the scope of the consent. As biomedical research, biotechnology and the use of HBM intend to create new medicines, treatments and therapies, the nature and extent of the research intervention is also new, meaning that any attempt at providing full information to the consenting party would not be comprehensive, not extend to the whole of the intervention nor include the consequences. The need to specify the purpose and scope of an intervention also inherently excludes future research which may not yet be fathomable. As this information cannot be provided, knowledge, understanding and appreciation are influenced negatively. Ultimately, the validity of informed consent in biomedical research, biotechnology and the use of HBM and associated data falls apart.

To complicate matters further, informed consent goes hand-in-hand with the duty of disclosure – the obligation of providing information to a consenting person. This duty requires that consent processes include an explanation of material aspects of an intervention which includes, among others, the aim of the intervention as well as the methods or techniques to be used. A research participant must be guaranteed that their material, donation or data will be used only in accordance with recognised standards, and they must be given the opportunity to ask questions and fully participate in the consent process. This allows a consenting person to identify information they might consider relevant in their decision-making.[13] Scholars have argued that the minimum standard of disclosure in research should be full disclosure, meaning that the participant must be informed that the proposed intervention entails research and be given detailed and comprehensive information on[14]:

1. the exact scope, nature, duration and purpose of the inquiry;
2. the scope, nature and consequences;
3. anticipated benefits and advantages for the person themselves, and society; and
4. any foreseeable risks, dangers and complications.

Again, the problematic application of informed consent in biomedical research, biotechnology and HBM and associated data, especially for future enquiries, becomes obvious as full disclosure is not possible. In order to attempt to accommodate the future uses and applications of biomedical research, biotechnology and HBM, broad consent has been advocated.

## Broad consent

Researchers use various methods and practices to obtain consent for research, and some concerns exist that certain HBM specimens may not be used due to the uncertainty and confusion regarding consent and that this would lead to a loss in public benefit. Broad consent is viewed by some as the best suited consent model for biomedical research, biotechnology and the use of HBM.[15]

Broad consent was introduced to solve a practical problem that arose through the rise of biobanking[16] and is essentially a strategy which accommodates future research and novel technologies using stored biological samples and data without having to renew consent.[16]

Broad consent in research, specifically biomedical research, biotechnology and the use of HBM, is often justified by relying on its potential benefits, the low risk involved and by questioning the centrality of informed consent.[6] As a result, broad consent coupled with oversight by ethics committees or review boards is seen as satisfactory.[17] This model encapsulates consent to various different conditions which require that a person other than the consenting person, normally the researcher, is permitted to make decisions regarding the donated HBM.[6]

Broad consent may be described as "consent for an unspecified range of future research, subject to substantive and/or procedural restrictions"[17]. This means it is less specific than consent for each individual use of HBM, but more specific than open-ended blanket consent with no limitations. A different definition of broad consent states that it is consent to a framework for future research of certain types, and it is not open blanket consent.[18]

Some supporters of broad consent have proposed that consent procedures should allow categories of research to which a participant may consent in general.[17] This means study-specific research descriptions would not be necessary in obtaining consent and that participants need only be given sufficient information to make a reasonably informed decision. The case of *Castell v De Greef*[19], which fully incorporated consent in South African law, held that in obtaining consent, material risks needed to be disclosed. To determine whether risk is material, the following test was developed: first, where a reasonable person in the position of the consenting party, if warned of the risks, is likely to attach significance thereto, or second, where a, *in casu*, medical practitioner is or should reasonably be aware that the consenting person, if warned of the risks, is likely to attach significance thereto.

In applying this reasonable person standard in determining the validity of consent, it may be argued that the information provided to a consenting person must be based on what a reasonable person would consider relevant in making their decision. Based on this, it is suggested that persons are willing to participate in research and to then give broad consent, but subject to certain exceptions or limitations.[17] In other words, broad consent may be problematic for those willing to participate in or donate material for certain studies but who are unwilling to participate in or donate to unspecified future research. It is suggested that broad consent is ill-equipped to deal with exceptions or limitations for which a reasonable person would have reservations as it is not the reasonable person who makes decisions regarding the future use of their material or data, but the researcher. What is significant to a research participant may not be significant to a researcher.

In investigating broad consent, it could be asked what exactly research participants are consenting to in biomedical research, biotechnology and the use of HBM. Are they consenting to the specifics of a study, or the wider nature thereof?[16] According to scholars, broad consent is not a decision based on information on the specific study, but rather a decision to let the researchers decide. This would mean that although broad consent decisions may be considered autonomous, they are not worthy of the same respect as informed consent because consent which is not fully informed, is ethically problematic.[6] As such, it is suggested that decision-making relates rather to identifying significant information than to processing as much information as possible. To make an autonomous decision, a person must therefore identify that which is likely to affect their willingness to participate in or donate to research, or not.[16] Such information is that which matters to the participant, for example, discovering they have a disposition to a terrible disease.[16] These are not matters which would be of the same significance to a researcher if the decision were left in their hands.

As mentioned, broad consent is consent to certain frameworks of information. This framework encompasses the aims, conditions of use and the governance of a research project. Where any of the components of the framework change, however, the framework's foundation alters and re-consent becomes necessary to lawfully continue using the participant's material or data.[16] A participant may therefore only be seen as informed where they have knowledge, understanding and acquiescence of the framework. The instant an activity is considered outside this consented-to framework, new consent must be sought.[16]

Arguments against broad consent hold that it is not in the best interest of the concerned participant's autonomy or in that of research as a whole.[20] On the other hand, it would seem that broad consent is ethically permissible, even optimal, where it includes initial consent, oversight and approval of future research activities and a process of ongoing provision of information to or communication with participants.[17] From this, especially the notion of ongoing provision of information and communication, however, it is suggested that such a manifestation of broad consent is more in line with dynamic consent discussed below, than the traditional understanding of broad consent. Further, these conditions indicate that broad consent as is, cannot be regarded as optimal as it necessitates a fundamental shift in the understanding of broad consent[12] as is indicative of the potential of, not preference of, dynamic consent as the model of consent in biomedical research, biotechnology and research using HBM and associated data.

## Dynamic consent

Few issues have been as controversial as biomedical research, biotechnology and the use of HBM for research, and one of the primary concerns relates to what the most appropriate and valid manner of obtaining consent would be.[21] Consent is so heavily relied on as a regulatory instrument that various consent models have been proposed as best suited.

In research, the necessity of consent is primarily based on the principle of respect for autonomy.[6] This means that true consent is not so much based on the provision of certain information, but on a deeper foundation on which persons are able to decide on the *amount* of information they receive and what they agree to.[22] This might then mean that research and those who participate in research are protected by providing participants with a flexible model of consent which accommodates different preferences. Such a model constitutes 'meta-consent' – a process which enables a person to design their consent.[18] In other words, a person is enabled to choose between different types of consent, such as informed or broad consent. Broad consent, as discussed, is problematic, and traditional informed consent, which does not accommodate future research not clearly described at the initiation of participation or donation, is invalid by definition, as it is not informed.[17] This means that even if a participant chooses informed consent, it may be invalid due to a lack of information.

In addressing the informational deficiencies of informed consent, it has been argued, however, that real consent does not depend on an overwhelming amount of information but rather on access to extendable, or flexible, information along with revocable consent and the right to veto certain activities.[23] Here, dynamic consent may offer a possible solution to the issue of consent.

Dynamic consent is a participant-centric initiative and may be described as a model of consent which requires a research participant to re-consent to every new study or change in research which involves them, their material or data.[24] Because biomedical research, biotechnology and the use of HBM constantly lead to, if not encourage, new avenues of inquiry and as such pose a deviation of proposed studies, it is suggested that dynamic consent is the format of consent most capable of accommodating the use of HBM and data due to the flexibility of the model.

Dynamic consent makes use of IT to enable continuous consent wherein the participant is kept abreast of new developments and potential studies using their material or data.[25] During online interactions, each participant must be sufficiently informed of the purpose and methods of a proposed study, the anticipated benefits and potential risks, and any other relevant aspects.[26] The participant must also be informed of their right to refuse to participate or to withdraw their consent at any time. After ensuring that the participant understands the information given, the researcher must attempt to obtain freely given consent.[26] Once consent has been obtained, the consented-to research activities may commence. Making use of the dynamic consent platform, the participant may then be updated on the use of their HBM and findings. Should secondary studies making use of this same participant's HBM or data arise, the participant is notified via the dynamic consent platform and again given the requisite information. The participant may then re-consent, revoke or withdraw or even change their preferences – consent to A, B and C but not X, Y and Z. The researchers must then adjust their actions accordingly. This continuous working of dynamic consent is illustrated in Figure 1.

Dynamic consent makes use of systems such as the Ensuring Consent and Revocation (EnCoRe) project and the 'CTRL' web-based application which provides real-time information on research projects as well as options regarding participation or donation, re-contact or revocation of given consent.[27]

EnCoRe is a participant-centric initiative IT system. It attempts to enable research participants to exercise the choice of granting or revoking consent over the use of their material or data as easy, intuitive and reliable as "turning a tap on and off"[28]. CTRL works in much the same fashion as EnCoRe and is a secure application that offers research participants the opportunity to engage with a study and update personal details and choices. Most importantly, it allows the participant to take the lead in making decisions regarding future use of their HBM or data.[29]

Although dynamic consent faces implementation challenges such as the digital divide, IT literacy and the cost of creating and maintaining such a system[12], it offers future-flexible consent by allowing for opt-in participation or donation and accommodates the preferences of the research participant.

## Conclusion

Consent in the context of biomedical research, biotechnology and the use of HBM was examined, and it is argued that informed and broad consent fall short of being truly valid. It has been shown here that for informed consent to be valid it must be based on the provision of appropriate information. However, due to the novelty, fast pace and possibility of future research using biomedical research, biotechnology and HBM, informed consent has become misaligned with the aims of research. The informational gap is complicated even more when considering the duty of disclosure.

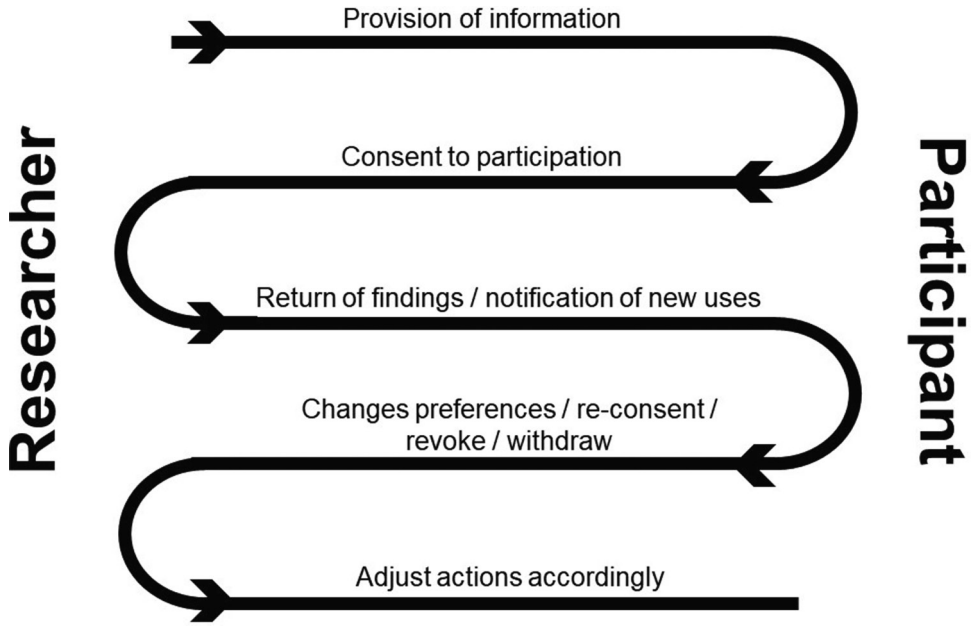


**Figure 1:** A schematic of dynamic consent.

It was further illustrated that broad consent encapsulates consent to various different frameworks but requires a person other than the consenting person to make decisions regarding the use of HBM. As such, it is not deemed truly valid as it does not accommodate the preferences of the research participant who wishes to exclude certain inquiries using their HBM or associated data.

Dynamic consent, which offers a flexible model of consent which is able to accommodate future research, was introduced, and it is here suggested that these new branches of science – biomedical research, biotechnology and the use of HBM – require a new model of consent.

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## Competing interests

I have no competing interests to declare.

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# Response to Brand et al. (2022) 'Data sharing governance in sub-Saharan Africa during public health emergencies'

**Significance:**

Various aspects of Brand et al.'s (S Afr J Sci. 2022;118(11/12), Art. #13892) overview of Africa's data protection legislation require clarification. Most pertinently, we provide the following clarifications:

- Ghanaian law does provide for cross-border data transfers; statements about the law being "inadequate" ought to be well substantiated.
- Nigerian law provides for adequacy decisions – not authorisations – in respect of cross-border data transfers.
- Kenyan law provides for an important exception relevant to public health emergencies.
- South African law currently requires, amongst others, prior authorisation from the Information Regulator for cross-border transfers of health data.
- South Africa does not yet have a code of conduct for research.

## Introduction

We start with some background. In 2021, Steytler and Thaldar published a review of recommendations for legal reform in South Africa relating to, inter alia, data sharing during public health emergencies.[1] These recommendations include, in respect of health data: (a) the creation of an African Data Corridor, (b) the adoption of open access research data, and (c) the development of data trusts, as suggested by the Organisation for Economic Co-operation and Development (OECD), and in respect of geospatial data used for health research: (d) an amendment to the *Space Affairs Act 84 of 1993*.[1] Recommendations (a) to (c) were based on the work of Townsend[2], and (d) on the work of Botes[3].

In their recent article in this journal, Brand et al.[4] add their voices to the discourse on improving data sharing governance during public health emergencies. We agree with the authors' recommendations, most pertinently the development of standard contractual clauses and data transfer agreement templates. These measures would indeed facilitate cross-border data transfer, as has been suggested in the South African (and African) context by Townsend[2].

However, we suggest that multiple aspects of Brand et al.'s overview of Africa's data protection legislation require clarification. In this article, we highlight the most salient of these aspects. Also, given the focus of Brand et al. on sub-Saharan Africa, we suggest that the authors' arguments could benefit significantly from being positioned within the relevant African Union (AU) policy framework.

## Legal aspects requiring clarification

### *Ghanaian law does provide for cross-border data transfers; statements about the law being "inadequate" ought to be well substantiated*

Brand et al.[4] state as follows regarding Ghana:

> *Ghana's data protection legislation does not contain any provisions pertaining to cross-border transfer of personal information and could thus be described as providing inadequate protection to data subjects in relation to the export of their personal data.*

This statement requires some analysis. The Ghanaian *Data Protection Act, 2012 (Act 843)* (DPA) defines the *processing* of information as including the "disclosure of the information or data by transmission, dissemination or other means available" (section 96 of the Ghanaian DPA), which would include the cross-border transfer of data out of Ghana. As such, we suggest that Brand et al.'s statement that Ghana's data protection legislation "does not contain any provisions pertaining to cross-border transfer"[4] be clarified. First, all the provisions of the Ghanaian DPA that govern the *processing* of information would apply to the cross-border transfer of data out of Ghana. These include, inter alia, compliance with Ghanaian law by foreign data processors (section 30(4) of the Ghanaian DPA). Moreover, the Ghanaian DPA provides for an extra layer of protection for sensitive data, which includes health data. Sensitive data may only be transferred outside of Ghana if: (1) there is consent, or (2) the transfer is necessary for medical purposes, which are defined to include 'health research' (section 37(6) and (7) of the Ghanaian DPA).

Brand et al. do not explain why, in their view, these protections afforded by the Ghanaian DPA are "inadequate"[4]. This is a strong claim, and clearly requires more substantiation. Can such substantiation be that Ghana is not included in other jurisdictions' adequacy lists? While the European Commission's adequacy list is well known[5], one should keep in mind that it does not include a single African country, and therefore does not provide grounds to single out Ghana

as being inadequate, as Brand et al. do. By contrast, Nigeria, Africa's largest economy[6], includes Ghana in its adequacy list (the South African Information Regulator has not yet issued a South African adequacy list). We suggest that Brand et al.'s statement that the Ghanaian DPA – in contrast with, for example, the South African, Nigerian, or Kenyan data protection statutes – is "inadequate" in respect of the protection that it affords to data subjects regarding the cross-border transfer of their personal data, clearly requires more substantiation.

### *Nigerian law provides for adequacy decisions – not authorisations – in respect of cross-border data transfers*

In Table 1 of their article, with regard to Nigeria, Brand et al. state that "cross-border transfer of personal data is subject to *authorisation* by the Attorney General or National Information Technology Development Agency (NITDA) based on an adequate level of protection"[4] (own emphasis). In our reading, this is not the case. To clarify, the role of NITDA and the Honourable Attorney General of the Federation is to make decisions regarding adequacy (regulation 2.11 of the *Nigeria Data Protection Regulation, 2019* (NDPR)), which is not the same as providing authorisation. These institutions have indeed developed a 'whitelist' of countries that are deemed adequate.[7] This means that any person seeking to transfer health data out of Nigeria to a whitelisted country can do so freely. By contrast, if a person seeks to transfer health data out of Nigeria to a country that is not whitelisted, then they must rely on any of the legal conditions, such as consent and public interest (regulation 2.12 of the NDPR). The recently signed *Data Protection Act 2023* also maintains a similar position to the NDPR, namely that the Nigerian Data Protection Commission is *only* to make decisions regarding adequacy and not to grant authorisations (section 42(4) of the Nigerian Data Protection Act). Therefore, it is clear that Nigerian law does not require authorisation for cross-border data transfers – whether it is to a whitelisted country or not.

### *Kenyan law provides for an important exception relevant to public health emergencies*

Brand et al. suggest that Kenya is amongst the countries that could be described as providing "stringent" data export protection to data subjects.[4] The authors define "stringent" protection as rules that[4]:

> *require notification of, or approval by, a relevant data protection authority, and/or special conditions (such as proof of appropriate safeguards with respect to the protection and security of personal data), as well as consent from the data subject.*

Although this description might apply to the *general* rules of Kenyan data protection law, we suggest that Brand et al. do not take adequate cognisance of an important exception to these rules in the context of public health emergencies. In terms of the Kenyan *Data Protection (General) Regulations, 2021*, if there is a "permitted health situation" or a "permitted general situation" that necessitates the cross-border sharing of health data, the legal requirements for prior authorisation from the Kenyan Data Commissioner and consent from data subjects are both waived. Accordingly, in this way, Kenyan law is designed to significantly relax its data protection rules in situations such as public health emergencies.

For the sake of comprehensiveness, it should be mentioned that if health data are anonymised in terms of the Kenyan *Data Protection Act 24 of 2019*, this statute and its cross-border data transfer requirements would not apply to such data. (Note that Kenyan law uses the term *anonymise*. The corresponding – but not equivalent – term in South African law is *de-identify*.) However, we recognise that such anonymisation may be impossible or undesirable from a research perspective. In such cases, reliance can be placed on the exception discussed above.

### *South African law currently requires, amongst others, prior authorisation from the Information Regulator for cross-border transfers of health data*

Brand et al.'s description of South Africa's legal requirements for the cross-border sharing of personal information, as presented in Table 1 of their article, refers only to section 72 of the *Protection of Personal Information Act 4 of 2013* (POPIA).[4] However, if the relevant personal information is *health* information, it would additionally qualify as *special* personal information, and hence *also* trigger section 57(1)(*d*) of POPIA. This provision requires prior authorisation from the Information Regulator for transfers to a third party in a foreign country that does not provide an adequate level of data protection – except if a code of conduct has come into force for the relevant sector (section 57(3) of POPIA). Given that: (a) the Information Regulator has not yet issued a list of foreign countries that it deems to provide an adequate level of data protection, and (b) as there is not yet a code of conduct in force for research, section 57(1)(*d*) of POPIA would apply, and should, we suggest, have been included in Table 1 of Brand et al.'s article. (The issue of a code of conduct is addressed more fully below.)

Of course, similar to the case with Kenyan law discussed above, if health data are de-identified in terms of POPIA, POPIA would cease to apply, and there would be no legal requirement for the cross-border transfer of such data. Note, however, that de-identification in terms of POPIA requires that there must be *no reasonably foreseeable method* to re-identify the data (section 1 of POPIA). Such de-identification of health data may not always be possible or desirable from a research perspective. If health data are not de-identified, as contemplated in POPIA, any person intending to transfer such health data to a foreign country would need to comply with both sections 72 *and* section 57(1)(*d*) of POPIA.

### *South Africa does not yet have a code of conduct for research*

Brand et al. state that the Academy of Science of South Africa (ASSAf) "has developed a privacy Code of Conduct for Research"[4], and then proceed to refer to it as "The Code"[4]. For clarity, as of the date of writing this response, the *proposed* Code of Conduct for Research that was developed by ASSAf has been submitted to the Information Regulator, but is yet to be approved.[8] The Information Regulator may still request amendments. Only if, and when, the Information Regulator eventually approves the *proposed* Code of Conduct for Research will it have the legal status of a code of conduct.

## Developments in the African policy sphere

An important step towards data protection integration and collaboration within Africa was taken with the endorsement of the AU Data Policy Framework by the AU Executive Council in February 2022.[9] The AU Data Policy Framework makes detailed recommendations to guide African countries through the formulation of policy in their domestic context, as well as recommendations to strengthen cooperation among countries and promote intra-Africa flows of data.[9] However, Brand et al. seem to be under the impression that the AU Data Policy Framework is still under development. (The authors state that: "[T]he AU Commission is developing a data policy framework for Africa…."[4]) Consequently, Brand et al. present their recommendations without reference to the AU Data Policy Framework, and without acknowledgment that most of their recommendations have already been covered by the comprehensive recommendations made in the AU Data Policy Framework – a document that precedes the initial submission date of the authors' article by three months. Brand et al.'s work could have benefitted significantly from being positioned within the AU Data Policy Framework.

It is interesting to note the way in which the AU Data Policy Framework classifies cross-border data regimes. While Brand et al. describe a "stringent", or a "strict", and a "moderate" categorisation of cross-border data governance regimes[4], in contradistinction, the AU Data Policy Framework offers three stylised approaches to cross-border data governance, namely: (a) an 'open transfer', (b) a 'conditional transfer', and (c) a 'limited transfer' model.[9] This approach is drawn from the recent work of Ferracane and Van der Marel[10,11]. In a 'limited transfer' model, cross-border data flows are conditional upon governmental approval and localisation requirements for domestic storage or processing of data. Examples provided by the AU Data Policy Framework are that of China and Russia.[9] At the other end of the spectrum, an 'open transfer' model has relatively low a priori mandatory approval requirements and relies on voluntary standards. Between these two

models is the 'conditional transfer' model, which provides guidelines and mandatory regulatory safeguards which, once met, allow for the free transfer of data. Accordingly, it is our contention that South Africa would count as a 'conditional transfer' regime: that is, it is consensus-based, with established regulatory data safeguards and overarching regulatory guidance from data protection authorities or international agreements – not unlike the European Union's (EU) *General Data Protection Regulation, 2018* (GDPR) – rather than that of the stricter, 'limited transfer' model which is based on "strong national security and public data control imperatives"[9].

## Concluding notes

The topic of cross-border data sharing – especially the sharing of *health* data, and particularly during public health *emergencies* – should be a public policy development *priority*. Academic discourse can – and should – contribute constructively to this important process. It is in this spirit that we offer our response to Brand et al., and we invite the authors to engage with the entirety of our research group's past, present, and future research. Only through such a dialectic process can the academic discourse be clarified and improved.

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## Competing interests

We have no competing interests to declare.

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# A response to Thaldar et al. (2023): Data sharing governance in sub-Saharan Africa during public health emergencies

**Significance:**

We elucidate the misinterpretations raised by Thaldar et al. (S Afr J Sci. 2023;119(11/12), Art. #15722) on our previous publication in which we outlined the data sharing governance landscape in selected African countries.

We thank the SAJS for affording us the opportunity to respond to a commentary[1] on our article[2]. We will focus on the principal points as raised by Thaldar et al.[1]:

### *Ghanaian law does provide for cross-border data transfers; statements about the law being "inadequate" ought to be well substantiated*

Section 18(2) of Ghana's *Data Protection Act of 2012* (GDPA) contains no specific provisions on international transfer of data. The mere stipulation in this section that where a foreign data subject's personal data are sent to Ghana for processing, such processing should occur in accordance with the data protection legislation of the foreign jurisdiction, does not determine international data transfer requirements for data subjects in Ghana. Further, the GDPA does not specify data transfer governance prerequisites between Ghana and foreign jurisdictions. This compares unfavourably with legislation of jurisdictions such as South Africa, which explicitly governs the transfer of personal information outside the country (Section 27).[3] We affirm our position: the GDPA offers inadequate protection to its data subjects in relation to data transfers to foreign jurisdictions.

### *Nigerian law provides for adequacy decisions – not authorisations – in respect of cross-border data transfers*

At the time at which our manuscript[2] was submitted for publication (2022), Nigeria's Data Protection Regulation (DPR) of 2019 governed international data transfers in the country[4]. In 2023, the *Data Protection Act* was approved and promulgated in Nigeria, which repealed the Data Protection Regulation. In terms of the erstwhile DPR, specifically Reg. 2.11, international data transfer had to take place *under the supervision* of the Attorney General of Nigeria and *the National Information Technology Development Agency (NITDA)* had to determine whether the foreign country provided an adequate level of protection[4] (words italicised for emphasis). Authorisation for international data transfer was provided by way of a decision by NITDA.

### *Kenyan law provides for an important exception relevant to public health emergencies*

The Kenyan *Data Protection Act* is clear that public interest, which includes a public health emergency, could be a legitimate basis for the lawful transfer of personal data to another country (sec. 48 (c) (iii)).[5] Furthermore, the Kenyan *Data Protection Regulations, 2021*, provide strict rules for the international transfer of data that include "adequate data protection safeguards" as an important basis for allowing the transfer.[5] The fact that provision is made for exemptions in very specific situations, such as a public health emergency, does not weaken the legislative framework. On the contrary, the overall approach of the Kenyan legislature is to have a detailed strict regulatory framework which includes allowing for special cases such as public health emergencies.

### *South African law currently requires, amongst others, prior authorisation from the Information Regulator for cross-border transfers of health data*

Section 57 of the *Protection of Personal Information Act 4 of 2013* (POPIA) specifies that the responsible party must obtain prior authorisation from the Regulator, in terms of section 58, prior to any processing – if that responsible party plans to transfer special personal information (including health information of a data subject) to a third party in *a foreign country that does not provide an adequate level of protection for the processing of personal information*, as referred to in section 72[3] (words italicised for emphasis). No such prior authorisation from the Regulator is required if the foreign country is deemed to provide an "adequate level of protection". More significantly, sections 57 and 58 are not applicable if a code of conduct has been issued and has come into force in terms of Chapter 7 in a specific sector or sectors of society.

### *South Africa does not yet have a code of conduct for research*

ASSAf refers to its draft "Code of Conduct for Research" as "the Code"[6,7]. Reference to "the Code" in the manuscript is intended to be interpreted in this context.

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## Competing interests

We have no competing interests to declare.

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# Herbicide resistance cases in South Africa: A review of the current state of knowledge

Herbicides play a major role in weed management worldwide. However, herbicide resistance is a global challenge that threatens weed management and sustainable agriculture. In South Africa, over 36 years, ten weed species have evolved resistance to five modes of action. In this review, cases of herbicide resistance that were published in scientific journals, proceedings of congresses, theses or dissertations, and in the international survey of herbicide-resistant weeds, were included to give national and international scientists' perspectives on the current status of herbicide resistance in South Africa. Since the last review was published in 2010, there have been new insights and novel techniques to document the molecular mechanism of herbicide-resistant weeds. Most cases of herbicide resistance in South Africa involved monocot and dicot weeds which are problematic in various cropping systems such as *Lolium* spp. (annual ryegrass), *Phalaris* spp. (canary grass), *Avena* spp. (wild oats), and *Raphanus raphanistrum* L. (wild radish). Understanding the extent of herbicide resistance and the molecular mechanism involved in herbicide resistance is paramount to developing novel techniques to manage herbicide-resistant weeds.

**Significance:**

- Data presented in this review help raise awareness of the threat of herbicide resistance in South Africa.
- Herbicide resistance in South Africa continues to evolve steadily through a wide range of weed species and modes of action.

## Introduction

South Africa is a key player in global agriculture and is considered one of the biggest producers of grapefruit, maize, and pears. South Africa has a total area of 122 million hectares, of which 12–13% is cultivated. Approximately 1.3 million hectares are under smallholder agriculture and 14 million hectares are under commercial agriculture, which relies on a high degree of mechanisation and high herbicide use. Consequently, South Africa has 700 active ingredients registered for agricultural use. It has also been dubbed as the biggest consumer of pesticides in the African continent.[1,2] These herbicides are used to control weeds such as *Lolium* spp. (annual ryegrass), *Eleusine* spp. (goosegrass), *Phalaris* spp. (canary grass), *Avena* spp. (wild oats), *Conyza* spp. (horseweed), *Raphanus raphanistrum* L. (wild radish), *Chenopodium album* L. (lambsquarters), and *Amaranthus* spp. (pigweed), which are some of the most problematic weeds in various cropping fields.[3,4] Non-chemical options for weed management in South Africa are available and are preferred by upcoming farmers. However, commercial farmers generally prefer herbicides as they provide effective and timeous weed management.[5] This is because, among the chemicals used in agricultural systems, herbicides play a major role in crop protection.[6,7] The application of herbicides for weed control has been efficient and effective because of reduced costs and relieving the burden of mechanical weed control, which was highly labour intensive.[7] However, continuous use of the same herbicides has triggered a phenomenon commonly referred to as herbicide resistance.[6] Herbicide resistance has compelled farmers to reduce their overreliance on herbicides.[5]

Herbicide resistance has been defined as acquired heritable traits of weed species to flourish and reproduce after herbicide treatment. Genetic variability and reproductive biology are the most important factors controlling the evolution of resistance.[7] The evolution of herbicide-resistant weeds is one of the greatest threats to sustainable food production.[7] Herbicide usage has increased exponentially since the 1960s, and this is reflected by the new and unique cases of herbicide resistance.[8,9] It was predicted in 1957 that herbicide resistance will evolve just as insecticide resistance has.[10] Presently, there are 522 unique cases (species × site of action) of herbicide-resistant weeds globally, with 269 species (154 dicots and 115 monocots).[4] The first synthetic herbicide was developed in 1941. After 1941, new herbicides were continuously developed.[1] Although the number of herbicide-resistant weeds keeps on increasing, the modes of action have been decreasing.[11]

Since the last review was published in 2010, new cases of herbicide resistance have emerged. In this review, we provide an update on the current status of herbicide resistance in South Africa under various cropping systems and present recent findings on molecular mechanisms of herbicide resistance. All other cases of herbicide resistance and herbicide-related research reports are also included. We conclude by providing possible options to manage herbicide-resistant weeds.

## Herbicide resistance

Globally, herbicide-resistant cases have been increasing at an alarming rate. In 1957, there were only two cases reported. In 1972 four more cases were reported. In 1992, 140 cases were reported and in 2002, 275 cases. By 2012, 419 unique cases of herbicide resistance were reported. As of 2023, there are 519 unique cases of herbicide resistance. The bulk of these cases come from the USA (131), Australia (89), Canada (56), Brazil (47), and China (40). Countries with only one case each are Tunisia, Pakistan, Saudi Arabia, Kenya, and Lithuania. Most herbicide-resistant weeds occur in wheat (84), maize (65), rice (54), soybean (53), and roadsides (36). Most weed species

are resistant to acetolactate synthase (ALS), inhibitors of photosynthesis at PSII, inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), acetyl-CoA carboxylase (ACCase), and auxin mimics 172, 87, 58, 51 and 42. The species resistant to multiple sites of action are *Lolium rigidum* (12), *Poa annua* (12), *Amaranthus palmeri* (10), *Echinochloa crus-gali* (9), and *Eleusine indica* (8).[4] In the Iberian Peninsula (Spain and Portugal), the first case of herbicide resistance was reported in the early 1970s.[7] In New Zealand, the first case was reported in the early 1980s.[12] Countries such as Australia and the USA have significantly high numbers of herbicide resistance cases compared to countries in Asia and some countries in South America.[4,7]

Reports on herbicide resistance on the African continent are generally few. Although there are few cases from the African continent, South Africa is leading in herbicide-resistant cases in Africa.[4,7] Compared to other countries in the world, cases involving herbicide-resistant weeds in South Africa are evolving relatively slowly.[13] The majority of species in South Africa are resistant to ALS inhibitors, following the global trend. Ryegrass has developed resistance to several sites of action, also similar to the global trend. The evolution of herbicide-resistant cases in South Africa is shown in Figure 1. In 1986, only one case was reported. In 1993 two more cases were reported. By 2003, four unique cases were reported, and in 2022 one case (Figure 1). Currently, resistance in weed species has developed across five modes of action. Some of the earlier cases of herbicide resistance involved ACCase and ALS inhibitors. In 1996 cases of inhibitors of photosynthesis at photosystem II emerged, and by the early 2000s cases of EPSPS inhibitors were reported (Figure 1). It is, however, important to note that not all cases of herbicide resistance are reported on the international survey of herbicide-resistant weeds, even if such cases were published in peer-reviewed journals.[4,7]

## Herbicide resistance in various modes of action

### *Acetyl-CoA carboxylase*

The first case of herbicide resistance in South Africa was reported in 1986 in wild oats.[14] By 1993, Botes and Van Biljon reported multiple resistance in ryegrass to ACCase.[4] These findings were verified by Smit and De Villiers[15] and Kellerman[16]. In the study by Smit and De Villiers[15], seeds from ten localities were subjected to diclofop-methyl, clodinafop-propargyl, and tralkoxydim. Most of the populations showed high tolerance to the diclofop-methyl and clodinafop-propargyl, whilst six out of ten locations showed tolerance to tralkoxydim. Additional studies were conducted using ALS inhibitors (imazamox) as alternatives for ryegrass control. The results showed that the addition of imazamox resulted in sufficient control. This was attributed to the different modes of action. In 1999, *Lolium rigidum* Gaud (ryegrass) populations from the southern Cape region of South Africa were suspected to be resistant to cyclohexanediones. The putative-resistant populations were collected and then treated with diclofop-methyl (in increasing grams of active ingredient per hectare (g a.i./ha) of 177, 355, 710, and 1775) and tralkoxydim (125, 250, 500, and 1250 g a.i./ha). The results showed 100% mortality after diclofop-methyl treatments at application rates of up to 710 g a.i./ha; 1775 g a.i./ha resulted in 20% mortality in the ryegrass populations. All application rates of tralkoxydim resulted in acceptable control of the ryegrass populations. However, it was expected that the ryegrass populations would be resistant to both herbicides as they are both ACCase inhibitors.[17] Elsewhere, it has been shown that diclofop-methyl-resistant populations show cross-resistance to other ACCase herbicides, but not to tralkoxydim.[18] Wheat farmers in the Western Cape Province of South Africa also reported poor control of little-seeded canary grass. Four canary-grass-resistant populations were then collected and a susceptible population with no history of herbicide exposure was included as a control. The four populations were treated with diclofop-methyl, clodinafop-propargyl, and iodosulfuron. Diclofop-methyl was applied at rates between 45 and 2880 g a.i./ha. Iodosulfuron was applied at rates between 6 and 400 g a.i./ha. Clodinafop-propargyl was applied at 6 and 384 g a.i./ha. The $LD_{50}$ values were 594, 700, 225, and 2673 g a.i. relative to 184 g a.i./ha of the susceptible population. Thus, the resistance/susceptible ratios were found to be 3, 14, and 7. The $LD_{50}$ for clodinafop-propargyl was much higher at 79, 94, 29, and 280 g a.i./ha. However, for iodosulfuron, rates between 50 and 400 g a.i./ha resulted in 100% mortality of all the populations.[19] In 2001, Cairns reported multiple resistance to ACCase inhibitors in a ryegrass-resistant population.[4] The mechanism of resistance was investigated by Yu et al.[20] The populations were subjected to maximum doses of diclofop-methyl (4000 g/ha), xuazifop (200 g/ha), haloxyfop (208 g/ha), propaquizafop (200 g/ha), sethoxydim (400 g/ha) and tralkoxydim (608 g/ha). The ryegrass-resistant populations showed resistance to tralkoxydim, haloxyfop, diclofop, fluazifop, propaquizafop, and sethoxydim. Investigation into the mechanism revealed that resistance was due to insensitive ACCase. The inhibition of ACCase in ryegrass-resistant populations was found, by in vitro inhibition of ACCase activity assays, to be significantly insensitive. However, the study by Yu et al.[20] did not identify the mutation responsible.

### *Acetolactate synthase*

Acetolactate synthase (ALS) is an essential component of the biosynthetic pathway of the branched-chain amino acids (isoleucine, valine, and leucine). The ALS-inhibiting herbicides inhibit the production of acetolactate and acetohydroxybutyrate which results in chain amino acid starvation and thus cell death of susceptible populations. The ALS-inhibiting herbicides are broadly categorised into five groups: triazolopyrimidine (TP), imidazolinone (IMI), sulfonylurea (SU), pyrimidinyl thiobenzoate (PTB),

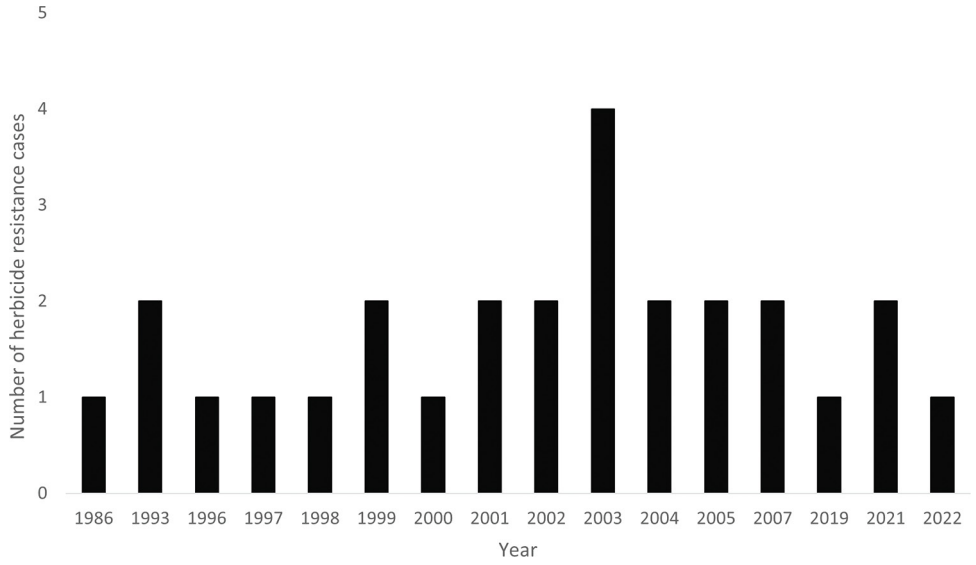


**Figure 1:** Cumulative total numbers of herbicide-resistant cases in South Africa over a 36-year period. Cases from the International Survey of Herbicide-Resistant Weeds database were also included.[7]

and sulfonylaminocarbonyl triazolinone (SCT). ALS-inhibiting herbicides are the herbicides of choice because of their broadspectrum and low mammalian toxicity.[21] In South Africa, ALS inhibitors are preferred in cereals.[22] The first case of ALS resistance was reported in wild oats.[14] The second case of ALS resistance was reported in ryegrass by Botes and Van Biljon in 1993.[4] Subsequently, wild radish was identified in the Western Cape Province to be problematic in wheat fields. The ALS inhibitors were applied in those fields for almost a decade. To document if resistance occurred in wild radish, the putative-resistant populations were treated with chlorsulfuron at rates of 1.4 and 90 g a.i./ha. The resistant population was found to be eight-fold more resistant than the susceptible population.[23] In 1999, Pieterse recorded resistance of canary grass to ALS in pastures and wheat. *Stellaria media* was also found to be resistant to chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl, and triasulfuron in cereal production areas.[4]

Using flucarbazone-sodium, Bester[24] tested resistance in wild oats from three sites in the Western Cape with high wild oats infestations. The authors applied the recommended rate, and twice and five times the recommended rate of flucarbazone-sodium. They reported contrasting results in the three sites. However, for the glasshouse studies, no mortality was recorded after subjecting wild oats to eight times the recommended rate. Investigations into the molecular mechanism found homozygous mutations of alanine to valine at position 205, the presence of tryptophan at position 574, and heterozygous mutations of proline at position 197 and serine at position 653. The other known case of resistance to ALS in South Africa is that of *Amaranthus* spp. to chlorimuron-ethyl.[25]

### *Inhibition of 5-enolpyruvylshikimate-3-phosphate synthase*

Glyphosate [N-(phosphonomethyl)-glycine] is the only inhibitor of EPSPS, which is an essential enzyme in the shikimate pathway. The shikimate pathway is a precursor of the synthesis of aromatic acids. Lack of these amino acids results in plant death.[26,27] The first case of glyphosate in South Africa was reported by Cairns and Eksteen in 2001.[4] By 2003, Cairns reported multiple resistance to glyphosate in ryegrass and plantago (*Plantago lanceolata* L.).[4] Eksteen[22] conducted confirmatory studies, then the mechanism of ryegrass resistance was confirmed by Yu et al.[20] Ndou et al.[28] documented the mechanism for resistance in plantago. In the first confirmatory studies by Eksteen[22], ryegrass populations were subjected to a dose-response trial using glyphosate at dosages of 0, 720, 1440, 2160, 2880, 3600, 4320, 5040, 5760, 6480, and 7200 g a.e./ha (grams of acid equivalent per hectare). The ryegrass populations showed a high survival rate after glyphosate application. Similar results were noted during the field trials. For the investigations into the mechanisms, ryegrass populations were sprayed with maximum doses of 7200 g a.i./ha glyphosate. The resistant populations from Tulbagh required greater than 3600 g a.e./ha, whereas the susceptible biotype required 450 g a.e./ha to result in significant mortality. Thus, the resistant population was 14 times more resistant than the susceptible population. The mechanism of glyphosate resistance in the resistant population was found to be due to a proline to alanine substitution at amino acid position 106 of the EPSPS gene, as well as reduced glyphosate translocation to young leaves after application of $^{14}$[C] glyphosate.[20] In another study, ryegrass biotypes from a town neighbouring Tulbagh were also found to be resistant to glyphosate. A novel proline to leucine mutation at EPSPS position 106 was found to confer resistance to the ryegrass biotypes.[29] The plantago populations reported by Cairns in 2003 were obtained from Robertson in 2019 and subjected to a dose-response trial. Glyphosate was applied at dosage rates of 0, 270, 540, 1080, 2160, 4320, 8640, and 17 280 g a.e./ha. The resistant population was found to be 43-fold more resistant to glyphosate. The $^{31}$P and $^{13}$C spectra showed reduced glyphosate translocation in the resistant population. Elevated results of shikimic acid were observed in the susceptible populations, pointing to target site resistance. Investigations into the target site resistance mechanism revealed a point mutation in the EPSPS gene, resulting in an amino acid substitution of proline to serine at position 106.[28]

Matshidze[30] tested possible glyphosate resistance in wild oats and found no indication of resistance in wild oats, although anecdotal evidence had indicated otherwise. In the former study, various populations of wild oats were subjected to 0, 270, 540, and 1080 g a.e./ha; the populations showed high mortality rates even at such low glyphosate dosages. *Conyza bonariensis* was reported to be glyphosate resistant by De Wet[31]. Populations from various locations in the Western Cape were collected and subjected to up to 2000 g a.e./ha of glyphosate. Populations showed high survival rates when treated with 400 g a.i./ha. However, 800 g a.i./ha resulted in 100% mortality of all the populations. The results of the study showed a resistance/susceptible ratio of less than 2. In 2013, there were also reports of poor control of *Conyza bonariensis* (L.) Cronquist with glyphosate in the Western and southern Cape.[32] Seeds of *C. bonariensis* were collected from 24 localities including vineyards, orchards, and wheat fields. The populations were treated with 225, 450, 900, 1800, and 3600 g a.e./ha of glyphosate. The results showed that approximately 40% of the 24 populations had a resistance/susceptible ratio of <10. The resistance/susceptible ratios ranged from 0.6 to 26.9 for the most susceptible and resistant populations, respectively. Investigation into the mechanism of resistance revealed an increase in shikimic acid levels in susceptible populations and a decrease in *Conyza*-resistant populations.[32] The accumulation of shikimic acid has long been used as an indicator of glyphosate activity (i.e. reduced amounts of shikimic acid suggest resistance to glyphosate).[26,33] The study by Okumu et al.[32] also showed that, in resistant populations, shikimic acid accumulation was higher in cold temperatures, suggesting higher sensitivity to glyphosate in cold temperatures in comparison to high temperatures.

*Eleusine indica* (L.) Gaertn, a grass weed, was investigated for glyphosate resistance. Glyphosate dosages applied were up to four times the recommended rate (900 g a.e./ha). The experiments were repeated twice. In all experiments, *E. indica* was found to be highly sensitive to glyphosate.[34] It does not appear as if *E. indica* is a major problem in South Africa. Elsewhere, it has been shown to be resistant to glyphosate.[35,36] More recently, *Amaranthus palmeri* was suspected to be resistant to glyphosate and other various modes of action. The resistant population was collected in cotton and maize fields. Dose-response experiments were conducted with glyphosate and other different modes of action at seven different rates. The highest rate was four times the recommended rate. The population was confirmed to be resistant to glyphosate. Decreased sensitivity was observed for other herbicides such as atrazine, mesotrione, S-metolachlor, and saflufenacil. On the contrary, other herbicides such as acetochlor, glufosinate ammonium, dicamba, isoxaflutole, 2,4-dinitrophenylhydrazine, diflufenican, and pyroxasulfone, and metribuzin resulted in acceptable control. The molecular mechanism of glyphosate resistance was found to be an increased copy number of the EPSPS enzyme coupled with serine mutation at position 653. However, the authors were unable to identify the mechanism involved in protoporphyrinogen oxidase inhibitors.[25] Recently, six *Conyza bonariensis* populations from the Western Cape and Free State Provinces were confirmed to be resistant to glyphosate. Sequencing of the EPSPS gene did not reveal any mutations. In fact, higher EPSPS gene expression was observed in the S biotype.[37]

### *Inhibition of photosynthesis at photosystem II*

Many herbicides inhibit photosynthesis at photosystem II (PS II), such as ametryne, chlorotoluron, diuron, and atrazine. Globally, atrazine is one of the most used herbicides that belongs to the triazines. Consumption of atrazine is estimated to be up to 90 000 tons globally. Around the world, atrazine is mostly used for grass and broadleaf weeds in maize sorghum, wheat, sugarcane, and canola cropping systems.[38] In South Africa, atrazine is also used in maize, sorghum, and sugarcane cropping systems.[39] However, there are very few cases of atrazine resistance. In the only known case, seeds from resistant and susceptible populations of *Amaranthus hybridus* were subjected to a dose-response trial at rates of atrazine of 1250, 2660, 5000, 7500, 10 000, 12 500, 15 000, 17 500, 20 000, 22 500 and 25 000 g a.i./ha. Results showed 100% survival at rates of 1250 and 25 000 g a.i./ha. The recommended rate of 1250–3000 g a.i./ha failed to control the resistant populations, whereas the lowest dosage of 1250 g a.i./ha controlled the susceptible populations.

Also, ten times the recommended rate failed to control *A. hybridus*. Cross-resistance was also apparent in these resistant populations; application of atrazine + cyanazine also resulted in poor control.[40] There have not been any new reports of atrazine resistance in South Africa and research seems to be declining, possibly because atrazine has been banned in the EU.[38]

### *Photosystem I electron diversion*

Herbicides that result in electron diversion in photosystem I (PS I) are the pyridiniums (paraquat and diquat). In South Africa, *C. bonariensis* was reported to be resistant to paraquat.[31] Six populations from various locations in the Western Cape were collected and subjected to 400 up to 2000 g a.i./ha. Two of the six C. *bonariensis* populations survived all the paraquat dosages. The two resistant populations showed a high survival rate of 80% and 87% at the highest paraquat dosage of 2000 g a.i./ha. In ryegrass, resistance to paraquat had already been reported in 2003 by Cairns[4]. The mechanism of resistance was confirmed by Yu et al.[20,41] and Eksteen[22]. Ryegrass seedlings were collected from four localities in the Western Cape and subjected to paraquat at 400 and 800 g a.i./ha. In another trial, paraquat was applied at dosages from 0 to 4000 g a.i./ha. Two of the localities showed 100% survival at 400 and 800 g a.i./ha. The other two populations gave high survival rates of 70% and 50% after paraquat application at a dosage of 4000 g a.i./ha.

For the second trial, varying responses amongst the populations were reported. Ryegrass populations were also placed in pots containing paraquat as a nutrient solution (20 g a.i./ha). The populations took up paraquat via the roots and some of the populations gave a high survival rate of 75% even after 7 days. The populations were then treated with $^{14}$[C] and imaged; reduced translocation was found to be the mechanism of paraquat resistance.[22] Yu et al.[41] also subjected the ryegrass populations from South Africa to a dose-response trial and investigated the mechanism that confers paraquat resistance. The $LD_{50}$ of the resistant populations was found to be 404 g a.i./ha – 14-fold greater than the susceptible population. The mechanism of resistance was also found to be reduced translocation after quantification for [$^{14}$C] in vivo and phosphor imaging. However, leaf uptake did not vary amongst the resistant and susceptible populations. Antioxidative enzymes superoxide dismutase and ascorbate peroxidase were similar amongst the resistant and susceptible populations, implying that there was no interaction between paraquat with PS I. The study also showed that the resistant populations required high paraquat dosages when kept in low temperatures relative to high temperatures of 30 °C.[41]

In a follow-up study, ryegrass populations were sprayed with doses of paraquat from 0 to 3200 g a.i./ha. The recommended rate achieved 100% mortality in susceptible populations. The resistant population was 32-fold more resistant to paraquat as compared to the susceptible population. Paraquat resistance in ryegrass populations was found to be due to reduced paraquat movement, assumed by the authors to be due to increased paraquat sequestration in young leaves.[20] More recently, plantago was also reported to be paraquat resistant. Populations from 22 vineyards and orchards in the Western Cape were collected and subjected to dose-response trials using paraquat at dosages of 0, 100, 400, 800, 1600, 3200, and 6400 g a.i./ha. The most resistant populations in the first experiment gave a resistance/susceptible ratio of 3. In the second experiment, the most resistant population gave a resistance/susceptible ratio of 9.[42,43] The mechanism of paraquat resistance in plantago has yet to be reported.

### *Inhibition of glutamine synthetase*

Herbicides that inhibit glutamine synthetase are bialaphos/bilanafos and glufosinate. After glyphosate and paraquat, glufosinate is the most popular herbicide in the world. Glufosinate is a fast-acting herbicide that targets glutamine synthetase which results in ammonia accumulation which causes reactive oxygen species and lipid peroxidation. There are limited cases of glufosinate resistance in South Africa. This is because the total area treated with glufosinate is far less in comparison to herbicides like glyphosate. Glufosinate has also been reported to be hydrophilic and has shown inconsistent results in the field and does not translocate well in plants.[44] Furthermore, it was found that the growth stage of resistant populations does not influence the efficacy of glufosinate.[45] Mucheri et al.[46] studied the responses of *Lolium* spp. to glufosinate ammonium application at different temperatures in South Africa. They applied glufosinate ammonium at 0, 300, 600, 900, and 1200 g a.i./ha and reported that 200 and 600 g a.i./ha was required to yield an $LD_{50}$ of ryegrass populations.

### *Auxin mimics*

Similar to glufosinate, there are very few reports of resistance to auxin mimics by various weed species in South Africa. There has been a report of resistance to synthetic auxin in wild radish.[5] Recently, various herbicides, such as carfentrazone-ethyl and glufosinate, two combined mixtures consisting of paraquat + diquat, terbuthylazine + S-metolachlor, and 2-methyl-4-chlorophenoxyacetic acid (MCPA), were screened to document alternative herbicides for the control of plantago. All herbicides managed to yield 100% mortality except for MCPA, suggesting possible tolerance to MCPA by plantago populations in the Western Cape Province of South Africa.[47] However, a proper MCPA dose-response trial will be necessary to determine resistance/susceptible ratios in these populations.

### *Pre-emergent herbicides*

In many cropping systems around the world, evolution of herbicide resistance has been mostly in post-emergent herbicides relative to pre-emergent herbicides.[48] Pyroxasulfone is a pre-emergent herbicide that inhibits lipid biosynthesis. Inheritance of evolved resistance to pyroxasulfone has already been reported.[49,50] In South Africa, there are no reports of pyroxasulfone resistance; the application of pyroxasulfone at 187.5 g/ha improved ryegrass control.[51] The aforementioned study was repeated under field conditions and similar results were reported. Due to the success of pyroxasulfone, other pre-emergent herbicides were explored: triasulfuron + prosulfocarb (30 and 45 g/ha) and triallate (3 and 4 L/ha) were applied. Similarly, the results showed that an increase in the dosage rate of the herbicides was very effective in weed control.[51]

Table 1 shows a summary of peer-reviewed herbicide resistance cases in South Africa. Possible solutions to manage herbicide-resistant weeds are in the supplementary material.

**Table 1:** Selected cases of herbicide resistance in South Africa

| Mode of action | References |
|---|---|
| Inhibition of acetyl-CoA carboxylase | 15 |
| Inhibition of acetyl-CoA carboxylase | 17 |
| Inhibition of acetyl-CoA carboxylase | 19 |
| PS I electron diversion, inhibition of EPSPS, and inhibition of acetyl-CoA carboxylase | 20 |
| Inhibition of acetolactate synthase | 23 |
| Inhibition of acetolactate synthase and inhibition of EPSPS | 25 |
| Inhibition of EPSPS | 28,29 |
| Inhibition of EPSPS | 32,37 |
| PS II photosynthesis inhibitors | 40 |
| PS I electron diversion | 41 |
| PS I electron diversion and inhibition of EPSPS | 42 |
| PS I electron diversion and inhibition of EPSPS | 43 |

## Conclusion

In South Africa, there are limited cases of herbicide-resistant weeds compared to other developed countries. This does not suggest that herbicide resistance cases in South Africa will not worsen or are unimportant. It is possible that other herbicide-resistant weeds have not been documented, as farmers in the Western Cape have reported other weeds which were not mentioned in this review and are currently not being controlled by several modes of action. It is also evident from this review that most resistant populations linked to herbicide resistance in South Africa were from the Western Cape Province. This finding is in agreement with those of Ferreira and Reinhardt[52] who reported that most proven cases of herbicide resistance in South Africa were documented from weeds that came from orchards, vineyards and wheat fields in the Western Cape.

There were reasonably more research articles on glyphosate and paraquat compared to any other herbicide in South Africa. This may be because glyphosate and paraquat are the most popular herbicides in the world.[53] Furthermore, glyphosate is the most used herbicide in South Africa.[54] A survey on herbicide usage in the winter rainfall area of South Africa also showed that growers in the Western Cape mostly use glyphosate and paraquat[55], further explaining why there are more cases involving glyphosate and paraquat. Although such surveys are essential, to date no survey has been conducted that encompasses the entire country. This is important because surveys that show the geographical distribution of herbicide-resistant weeds and herbicide usage can help shed light on the factors that contribute to herbicide resistance, which in turn will contribute to more precise herbicide management strategies.[10] Studies on physiological, genetic, biochemical, and molecular mechanisms give insights into the evolution of herbicide-resistant weeds. Such studies are very important because they encourage wiser usage of existing modes of action, and thus more sustainable ways to manage weeds and delay the onset of herbicide-resistant weeds.[3]

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

M.M.: Conceptualisation; writing – the initial draft; writing – revisions. V.N.: Conceptualisation; validation; writing – revisions.

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# Corporate tax avoidance: Is South African society negatively affected by chartered accountant CEOs?


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Corporate tax avoidance can impede governments' spending towards social and economic initiatives that can increase infrastructure development, economic growth, and equality, and reduce poverty. Yet, why some companies avoid more tax than others is not adequately understood, and, in particular, research regarding the influence of CEO-characteristics on tax avoidance, is lacking. This study is an empirical investigation into the influence of a CEO's tax knowledge and tax awareness, construed as a 'CEO effect', on corporate tax avoidance, using data from the 112 largest listed companies on the Johannesburg Securities Exchange between 2004 and 2018. We found that the CEO effect, not measured before, does not have an observable influence on the level of corporate tax avoidance. This finding assuages possible concerns that chartered accountants, and particularly chartered accountants in the top leadership positions in large companies, are more shareholder oriented, to the detriment of the interests of society, as suggested in the literature.

**Significance:**

Our findings suggest less influence of the CEO, as an upper-echelon member, on companies' behaviour, such as corporate tax avoidance, than other published studies have found. Moreover, the findings indicate that the tax knowledge and awareness construed as a CEO effect, does not influence corporate tax avoidance. In the main, the results provide little support for claims made by the South African Institute of Chartered Accountants, the chartered accountant's regulatory body, that chartered accountants can help companies to avoid tax to increase profits. This may sway society's view of the chartered accountant and their role in the South African economy.


## Introduction

An advert by the South African Institute of Chartered Accountants (SAICA), states that "the goal of a [chartered accountant] is to help businesses to profit from their extensive tax experience…to minimise the influence of tax on their profitability"[1]. Corporate tax avoidance enriches shareholders, but it harms governments and society when less funding is available for social causes. Despite much research on the topic, it is still unclear why some companies avoid more tax than others. Chief executive officer (CEO) characteristics have previously been offered as possible 'determinants' of tax avoidance. However, CEO tax knowledge and awareness, construed as a specific CEO characteristic, has not been explored in quantitative corporate tax avoidance literature.

The chartered accountant (CA) professional qualification is one of the most popular career choices for thousands of ambitious school leavers in South Africa, because the CA designation has become synonymous with prospects of extraordinary income-earning potential and societal status.[2] A 4-year university programme equips prospective CAs with integrated knowledge about the intricacies and relationships between taxation, accounting, corporate finance, and auditing, after which they start a 3-year internship at a SAICA-accredited firm. This is where their integrated skill sets are further developed before they can register as CAs. Many CAs later become CEOs of large, listed companies, which means that their power and ability extend beyond those of the 'average' CA. The CAs' integrated skill sets make them uniquely equipped to exploit corporate tax avoidance at the companies they lead. This argument is aligned with the claim in the SAICA advert. Furthermore, Terblanche[3] claims that the average CA is shareholder-oriented, which stems from the university curriculum, making them ignorant of social needs. There are arguments to the contrary. For example, CAs' training also exposes them to the increasing importance of social responsibility, and, for example, the King report, which advocates for 'responsible' tax behaviour.[4] Therefore, SAICA's advert highlights a contention which we investigated empirically in this paper.

Many company characteristics have been explored in quantitative research as determinants of tax avoidance. Variation in corporate social responsibility performance[5], debt levels, growth, profitability, company size, industry, and corporate governance[6], have all been considered, but the results are often inconclusive or contrary to theoretical expectations. Variation in the characteristics of corporate leadership is also sometimes investigated, described in the literature as 'CEO effects'. One study found that a CEO's educational background was not associated with tax avoidance, although personal idiosyncrasies were. However, the authors of that study did not specifically investigate a CEO's tax knowledge and tax awareness, because they argue that it is not measurable, and that CEOs are rarely tax experts.[7] Recent literature reviews still call for more research on the impact of CEO skills and knowledge on corporate tax avoidance[8,9], which is where our study makes its main contribution. A unique feature of the South African corporate landscape is that 30% of CEOs are CAs[10], which we use as a proxy for a CEO's tax knowledge and awareness. Our study is conceptualised from upper echelon theory and shareholder value maximisation theory, which postulate that the influence of top leadership permeates corporate behaviour and the company, perceived as a shareholder value maximising entity under control of the CEO, respectively.

We used data from the largest listed companies in South Africa, which we analysed statistically to determine the relationship between effective tax rates, our proxy for tax avoidance, and a CEO's tax knowledge. In contrast

with a more general and exploratory earlier study on South African corporate effective tax rates[11], in this study, we explored specific aspects regarding the relationship between CEO characteristics and corporate tax avoidance, as the following section explains as the gap where we make our contribution.

## Literature review and development of hypotheses

Quantitative research about corporate tax avoidance usually investigates the influence of corporate characteristics as 'determinants' of effective tax rates, as proxy for corporate tax avoidance. This proxy, although widely used in the literature as reported in the table below, is not perfect, nor does it differentiate between corporate tax avoidance and tax evasion, the latter being illegal. Table 1 summarises some of the corporate determinants investigated in the past. This table shows the mixed and inconclusive results pertaining to many cases.

**Table 1:** Company characteristics previously investigated as determinants of tax avoidance

| Characteristic | Coefficient sign |
|---|---|
| Intensity of the use of fixed assets | • Dyreng et al.[12] (+)<br>• Markle and Shackelford[13] (-)* |
| Corporate social responsibility | • Lanis and Richardson[14] (+)*<br>• Davis et al.[15] (-)* |
| Intensity of the use of tangible assets | • Davis et al.[15] (-)<br>• Dyreng et al.[12] (-)*<br>• Lanis and Richardson[14] (+) * |
| Leverage (debt levels) | • Davis et al.[15] (-)*<br>• Lanis and Richardson[14] (+)*<br>• Dyreng et al.[12] (-) *<br>• Gupta and Newberry[16] (-)*<br>• Markle and Shackelford[13] (-) * |
| Growth | • Davis et al.[15] (-)<br>• Lanis and Richardson[14] (+) |
| Profitability | • Davis et al.[15] (+)<br>• Lanis and Richardson[14] (+) *<br>• Gupta and Newberry[16] (+)* |
| Size | • Davis et al.[15] (-)*<br>• Dyreng et al.[12] (+) * (For domestic companies)<br>• Lanis and Richardson[14] (+)<br>• Rego[17] (+) *<br>• Gupta and Newberry[16] (^)<br>• Kim and Limpaphayom[18] (-)* |
| Industry | • Davis et al.[15] (Firm fixed effects used)<br>• Dyreng et al.[12] (Firm fixed effects used)<br>• Lanis and Richardson[14]<br>• Markle and Shackelford[13] |

*Coefficient sign (+ or -) indicated in brackets; *indicates coefficient significance; ^ indicates mixed results overall*

Beyond the influence of the corporate characteristics depicted above, the influence of various CEO characteristics on corporate tax avoidance has also been investigated. Olsen and Stekelberg[19] investigated the relationship between a CEO's personality and corporate tax avoidance, and found that companies with CEOs with narcissistic personalities are associated with more tax avoidance. Another study found that CEO compensation was not associated with corporate tax avoidance[20], while another[21] found a positive association. The CEO's background matters: CEOs with a military background are associated with less corporate tax avoidance.[22] Dyreng et al.[7] traced a specific person's trajectory as a CEO between different companies and report that similar corporate tax avoidance patterns follow the move. In addition, De Klerk and Mey[23] found that companies with a CA appointed as CEO, are associated with less earnings management, possibly suggesting that CAs are less aggressive in this regard.

We argue that a person with a combination of tax-related knowledge and in a position of absolute power, will have the propensity to effect tax avoidance at the company they lead. This is the basis of our hypotheses that there will be greater tax avoidance, on average, at those South African companies where CEOs are CAs. This is referred to as the 'CACEO effect' in this article.

### Hypothesis I

***$H_0$:*** *The CACEO effect between companies has no association with corporate tax avoidance.*

***$H_a$:*** *The CACEO effect between companies will be associated with more corporate tax avoidance.*

### Hypothesis II

***$H_0$:*** *The CACEO within company effect has no association with corporate tax avoidance.*

***$H_a$:*** *The CACEO within company effect is associated with more corporate tax avoidance.*

Hypothesis I focuses on the cross-sectional CACEO effect, while Hypothesis II focuses on the CACEO effect within individual companies as measured over the 15 years when chartered accountant CEOs were replaced by non-chartered accountant CEOs or vice versa.

## Data and methodology

The data in our sample were obtained from all companies listed on the Johannesburg Securities Exchange (JSE) with a market capitalisation exceeding ZAR4 billion on 31 December 2014. We excluded Real Estate Investment Trusts because they are subject to different tax regimes. Contrary to the approach in many papers, we retained banks and financial institutions in our sample, regardless of the fact that their business models are unique, because research suggests the important role that these companies play as facilitators of corporate tax avoidance.[24] Data were collected for the period from 2005 to 2018. A longer time series in the panel helps to allow for more variation in our variable of interest: whether a CEO is a CA or not. Financial data were obtained from Bloomberg, while SAICA's website was used to check whether CEOs are CAs. We did not identify chief financial officers (CFOs) who are CAs, because most CFOs of listed companies are CAs, which provides no variation that is necessary for regression techniques.

We limited our analysis to the larger companies listed on the JSE because we are interested in the tax behaviour of large companies. This resulted in 112 companies, from which we excluded 12 companies due to missing data, resulting in 100 companies.

We tested our hypotheses using multivariate regressions to determine the association between corporate tax avoidance and the CA status of the CEO whilst controlling for other variables. We performed pooled, fixed-effect, cross-sectional and quantile regressions. In the following sections we describe our variables, starting with the regressand, then the regressor, and, finally, the controls.

### *Effective tax rates as a measure of corporate tax avoidance*

The accounting effective tax rate (*AETR*) is our measure for corporate tax avoidance, calculated as the total expense according to the income statement, expressed as a percentage of pre-tax profit. In addition, we calculate the cash effective tax rate (*CETR*) as the total of taxes actually paid, expressed as a percentage of profit before tax, as the alternative proxy. These proxies do not capture all types of corporate tax avoidance; however, they are simple, and frequently used in the empirical literature.[7,25-27] Low effective tax rates provide evidence of corporate tax avoidance and vice versa. It is accepted that effective tax rates are carefully monitored and perhaps even managed. For example, Investec[28], with low effective tax rates, blames a significant drop in profit on a process of "effective tax rate normalisation", indicating that effective tax rates can be managed. Effective tax rates (*AETR* and *CETR*) are truncated between 0% and 100%.[5]

### *CEOs who are chartered accountants, as a measure of CEO tax knowledge*

Our variable of interest as indicator variable is called *CACEO*, which indicates whether the CEO has tax knowledge. We collected these data for the companies and years under observation, using biographical information on Bloomberg and information on corporate websites and companies' annual reports. The data were verified using SAICA's website. The homogeneous nature of the qualifications of CEOs in the South African landscape[29] makes it possible to operationalise tax knowledge attributable to a CEO in this way.

### *Control variables*

Table 2 lists and describes the control variables included in our regressions and the expected coefficient sign.

## Regression models

Models 1–2 below are pooled regression models, pooling the observations and disregarding the panel nature of the data. These pooled models combine the within-company and between-company effects. Models 3–4 are company fixed-effect models, run to investigate the within-company effect over the time series only. Models 5–6 present cross-sectional regressions to investigate the between-company effect over the cross-sections only. Models 1–6 investigate the effect of tax knowledgeable CEOs on the conditional average of tax avoidance. Perhaps the effect of a tax knowledgeable CEO is different for different levels of corporate tax avoidance and, therefore, we also perform a quantile regression to investigate this possibility.

Models 3 and 4 are performed to investigate Hypothesis II, and fixed-effect regressions are performed to assess the *CACEO* effect on tax avoidance within companies. Models 5 and 6 are cross-sectional regressions, aiming to place more emphasis on the between-company effect of *CACEO*.

**Models 1 and 2 (pooled regressions)**

$$\begin{aligned} AETR_{it} = {} & \beta_0 + \beta_1 CACEO_{it} + \beta_2 CAPINTENS_{it} \\ & + \beta_3 ESG_{it} + \beta_4 INTANGR_{it} \\ & + \beta_5 LEV_{it} + \beta_6 PTB_{it} + \beta_7 ROA_{it} \\ & + \beta_8 SIZE_{it} + \beta_9 STC_{it} + \beta_{10} PTC_{it} \\ & + \beta_{11-15} INDUSTRY\_DUMMIES_i + \varepsilon \end{aligned} \quad \text{(MODEL 1)}$$

$$\begin{aligned} CETR_{it} = {} & \beta_0 + \beta_1 CACEO_{it} + \beta_2 CAPINTENS_{it} \\ & + \beta_3 ESG_{it} + \beta_4 INTANGR_{it} \\ & + \beta_5 LEV_{it} + \beta_6 PTB_{it} + \beta_7 ROA_{it} \\ & + \beta_8 SIZE_{it} + \beta_9 STC_{it} + \beta_{10} PTC_{it} \\ & + \beta_{11-15} INDUSTRY\_DUMMIES_i + \varepsilon \end{aligned} \quad \text{(MODEL 2)}$$

**Models 3 and 4 (fixed-effect regressions)**

$$\begin{aligned} AETR_{it} = {} & \beta_0 + \beta_1 CACEO_{it} + \beta_2 CAPINTENS_{it} \\ & + \beta_3 ESG_{it} + \beta_4 INTANGR_{it} + \beta_5 LEV_{it} \\ & + \beta_6 PTB_{it} + \beta_7 ROA_{it} + \beta_8 SIZE_{it} \\ & + \beta_9 STC_{it} + \beta_{10} PTC_{it} + \varepsilon \end{aligned} \quad \text{(MODEL 3)}$$

**Table 2:** Control variables used in regression

| Variable abbreviation | Expected sign on coefficient | Variable description |
|---|---|---|
| *CAPINTENS* | (+/-) | Value of fixed assets as a percentage of total assets as an indicator of capital intensity of the company[12,15] |
| *ESG* | (+/-) | Bloomberg's disclosure performance score for Environmental, Social and Governance disclosure by companies[5] |
| *INTANGR* | (+/-) | Intangible assets as a percentage of total assets as an indicator of the intensive use of intangible assets[13] |
| *LEV* | (+/-) | Leverage, calculated as long-term debt, as a percentage of total assets[16] |
| *PTB* | (-) | Ratio of market value of equity to book value of equity, as an indicator of company growth[5] |
| *ROA* | (+/-) | Return on asset as the indicator for profitability, calculated as profit before tax, as a percentage of total assets[16] |
| *SIZE* | (+/-) | Natural log of total market capitalisation, as an indicator of company size[17] |
| *STC* | (+) | Dummy indicator to identify observations falling in a period before 2012 when tax legislation treated tax on dividends declared as a corporate tax |
| *PTC* | (+) | Dummy indicator to identify observations before 2008 when corporate tax rates were lower |
| *CGS* | (+/-) | Industry dummy variable for companies involved in consumer goods and consumer services sector[12,15] |
| *FIN* | (+/-) | Industry dummy variable for companies involved in financial services, including banks and insurance sectors[12,15] |
| *HTI* | (+/-) | Industry dummy variable for companies involved in health care, telecommunications, or IT sectors[12,15] |
| *INDSTR* | (+/-) | Industry dummy variable for companies involved in the industrial sectors[12,15] |
| *MMNR* | (+/-) | Industry dummy variable for companies involved in the minerals, mining, and natural resources sectors[12,15] |

$$CETR_{it} = \beta_0 + \beta_1 CACEO_{it} + \beta_2 CAPINTENS_{it} + \beta_3 ESG_{it} + \beta_4 INTANGR_{it} + \beta_5 LEV_{it} + \beta_6 PTB_{it} + \beta_7 ROA_{it} + \beta_8 SIZE_{it} + \beta_9 STC_{it} + \beta_{10} PTC_{it} + \varepsilon \quad \text{(MODEL 4)}$$

**Models 5 and 6 (cross-sectional regressions)**

$$AETR_{it} = \beta_0 + \beta_1 CACEO_i + \beta_2 CAPINTENS_i + \beta_3 ESG_i + \beta_4 INTANGR_i + \beta_5 LEV_i + \beta_6 PTB_i + \beta_7 ROA_i + \beta_8 SIZE_i + \beta_9 STC_i + \beta_{10} PTC_i + \beta_{11-15} INDUSTRY\_DUMMIES_i + \varepsilon \quad \text{(MODEL 5)}$$

$$CETR_{it} = \beta_0 + \beta_1 CACEO_i + \beta_2 CAPINTENS_i + \beta_3 ESG_i + \beta_4 INTANGR_i + \beta_5 LEV_i + \beta_6 PTB_i + \beta_7 ROA_i + \beta_8 SIZE_i + \beta_9 STC_i + \beta_{10} PTC_i + \beta_{11-15} INDUSTRY\_DUMMIES_i + \varepsilon \quad \text{(MODEL 6)}$$

The cross-sectional models above (Models 5 and 6) are extended to quantile regressions because of the possibility that CEO effects may vary for different levels of tax avoidance.

## Results

### *Descriptive statistics*

Table 3 presents descriptive statistics for the variables used in the statistical analyses.

Table 3 indicates that the *AETR* and *CETR*, the dependent variables in the regressions that follow, are both close to 28%, which is the current corporate tax rate in South Africa (at the time of publication of this paper), although *CETR* is somewhat higher. The mean of *CACEO*, the variable of interest being the proxy for a CEO with tax knowledge, is 31.4%, indicating that 31% of the observations had a chartered accountant as CEO (CEO attributed with tax knowledge). The average of *CAPINTENS* is 29.48%, while some companies appear much more capital intensive, with reference to the maximum of 89.5%. The average of the *ESG* variable is 41.49%, which seems low perhaps; however, the maximum indicates that some companies are more adept at the disclosure of Environmental, Social and Environmental aspects as based on the maximum score of 72.9%. The average use of intangible assets in business models in large listed South African companies seems moderately low at 9.32%, while the maximum score of 84.74% indicates that some companies are intensive users of intangible assets.

Figure 1 shows descriptive evidence that companies with CAs as CEOs should avoid more tax than other companies, especially relevant to the period from 2005 to 2014. However, in the next section, we subject these data to multivariate regression analyses to investigate the relationship between CACEO and ETRs.

### *Regression results: Pooled models*

The results of the pooled regression on *AETR* and *CETR* are presented in Table 4.

### *Regression results – Fixed-effect regression*

The results of the fixed-effect regression pertaining to Models 3 and 4 are supplied in Table 5. The fixed-effect regression is performed to assess the CEO effect within companies, as CEOs with different tax levels alternate through the years under observation.

**Regression results: Cross-sectional regression**

The results of the cross-sectional regression are supplied in Table 6.

**Table 3:** Descriptive statistics

| Variable | Obs | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| *AETR* | 1111 | 27.192 | 16.554 | 0 | 100 |
| *CETR* | 995 | 30.487 | 18.346 | 0.016 | 100 |
| *CACEO* | 1111 | 0.314 | 0.464 | 0 | 1 |
| *CAPINTENS* | 1111 | 29.485 | 25.014 | 0 | 89.537 |
| *ESG* | 1111 | 41.492 | 12.646 | 0.795 | 72.92 |
| *INTANGR* | 1111 | 9.366 | 12.843 | 0 | 84.742 |
| *LEV* | 1111 | 12.491 | 13.081 | 0 | 66.92 |
| *PTB* | 1111 | 3.005 | 2.726 | -5.99 | 23.527 |
| *ROA* | 1111 | 9.394 | 13.473 | -132.741 | 149.466 |
| *SIZE* | 1111 | 10.291 | 1.477 | 4.017 | 14.472 |
| *STC* | 1111 | 0.308 | 0.462 | 0 | 1 |
| *PTC* | 1111 | 0.093 | 0.29 | 0 | 1 |

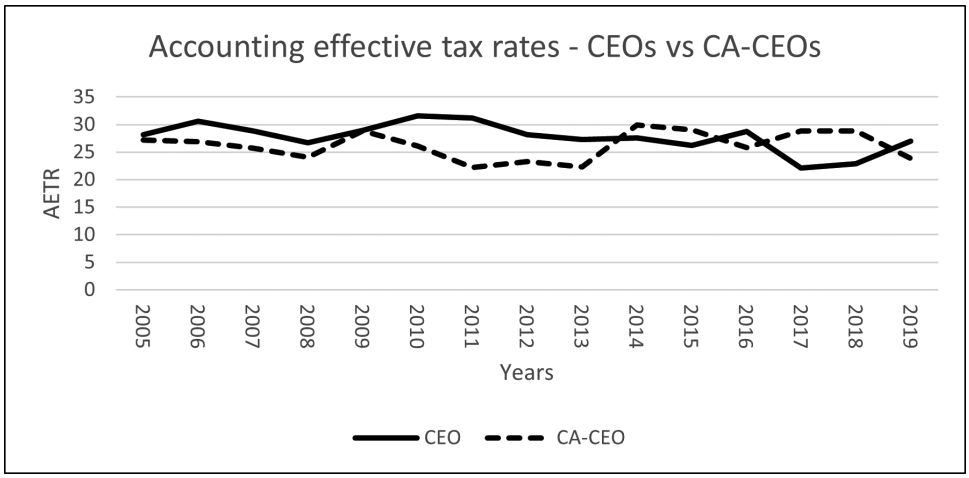


**Figure 1:** Average effective tax rates over 15 years: CEOs and CA-CEOs.

**Table 4:** Pooled regression results of the effect of CEO tax knowledge on corporate tax avoidance

| | Model 1 | Model 2 |
|---|---|---|
| **Variable** | **AETR** | **CETR** |
| *CACEO* | -0.538 | -1.421 |
| | (1.110) | (1.264) |
| *CAPINTENS* | 0.031 | -0.014 |
| | (0.032) | (0.036) |
| *ESG* | 0.032 | 0.229*** |
| | (0.052) | (0.059) |
| *INTANGR* | -0.039 | -0.029 |
| | (0.051) | (0.057) |
| *LEV* | -0.026 | -0.034 |
| | (0.048) | (0.056) |
| *PTB* | 0.158 | 0.632** |
| | (0.217) | (0.262) |
| *ROA* | 0.144*** | -0.460*** |
| | (0.044) | (0.069) |
| *SIZE* | -0.241 | -2.069*** |
| | (0.427) | (0.493) |
| *STC* | 2.498** | 1.480 |
| | (1.269) | (1.403) |
| *PTC* | -1.845 | 3.636 |
| | (1.971) | (2.320) |
| *CGS* | -2.397 | -6.388*** |
| | (1.930) | (2.219) |
| *FIN* | -0.918 | -7.859*** |
| | (2.526) | (2.944) |
| *o.HTI* | – | – |
| *INDSTR* | -1.299 | -6.300*** |
| | (2.046) | (2.347) |
| *MMNR* | -3.597 | -3.389 |
| | (2.372) | (2.766) |
| Constant | 27.718*** | 52.005*** |
| | (4.132) | (4.860) |
| Observations | 1111 | 995 |
| R-squared | 0.026 | 0.083 |

*Standard errors in parentheses ***p < 0.01, **p < 0.05, *p < 0.10*

*This table provides the pooled regression results for the effect of CACEO on corporate tax avoidance. AETR and CETR are alternative proxies for tax avoidance and CACEO indicates tax knowledge attributed to a CEO. Lower effective tax rates indicate corporate tax avoidance, and vice versa. The other variables are described in Table 2.*

**Table 5:** Fixed-effect regression results for the effect of a CEO's tax knowledge on corporate tax avoidance

| | Model 3 | Model 4 |
|---|---|---|
| **Variable** | ***AETR*** | ***CETR*** |
| *CACEO* | -0.709 | -3.008 |
| | (1.320) | (1.899) |
| *CAPINTENS* | 0.011 | 0.230** |
| | (0.157) | (0.112) |
| *ESG* | -0.092 | 0.134 |
| | (0.087) | (0.123) |
| *INTANGR* | -0.018 | -0.142 |
| | (0.090) | (0.108) |
| *LEV* | -0.037 | -0.001 |
| | (0.104) | (0.131) |
| *PTB* | -0.509 | -0.181 |
| | (0.352) | (0.633) |
| *ROA* | 0.140 | -0.719** |
| | (0.091) | (0.277) |
| *SIZE* | -0.908 | -4.688** |
| | (1.448) | (2.172) |
| *STC* | 0.851 | -0.872 |
| | (1.315) | (2.066) |
| *PTC* | -3.551* | 3.813 |
| | (1.869) | (2.470) |
| Constant | 41.166*** | 77.731*** |
| | (15.057) | (20.904) |
| Observations | 1111 | 995 |
| R-squared | 0.018 | 0.133 |
| Number of company groups | 100 | 100 |

*Robust standard errors in parentheses ***p < 0.01, **p < 0.05, *p < 0.1*

*This table provides the fixed-effect regression results for the effect of tax knowledge attributed to a CEO on corporate tax avoidance. AETR and CETR are alternative proxies for tax avoidance and CACEO indicates tax knowledge attributed to a CEO. Lower effective tax rates indicate corporate tax avoidance, and vice versa. The other variables are described in Table 2.*

### Results: Quantile regression

The results of the quantile regression performed on the cross-sectional effect between companies of effect of the CEO's tax knowledge at the different levels are not shown due to space considerations.

## Discussion

Pooled regression, fixed-effect regression, cross-sectional regression as well as quantile regression were performed to investigate the association between a tax-knowledgeable CEO and corporate tax avoidance. Our main hypothesis states that a tax knowledgeable CEO will be associated

**Table 6:** Regression results for cross-sectional regression

| | Model 5 | Model 6 |
|---|---|---|
| **Variable** | ***AETR*** | ***CETR*** |
| *CACEO* | 0.140 | -0.210 |
| | (2.405) | (3.370) |
| *CAPINTENS* | 0.031 | -0.054 |
| | (0.062) | (0.086) |
| *ESG* | 0.029 | 0.218 |
| | (0.122) | (0.171) |
| *INTANGR* | -0.062 | -0.041 |
| | (0.100) | (0.140) |
| *LEV* | -0.046 | -0.064 |
| | (0.107) | (0.151) |
| *PTB* | 0.678 | 1.058 |
| | (0.493) | (0.690) |
| *ROA* | 0.134 | -0.197 |
| | (0.120) | (0.168) |
| *SIZE* | -0.601 | -1.881 |
| | (0.922) | (1.292) |
| *STC* | 14.262 | 16.807 |
| | (12.539) | (17.572) |
| *PTC* | -1.171 | -11.022 |
| | (19.602) | (27.471) |
| *CGS* | -1.118 | 0.053 |
| | (3.786) | (5.306) |
| *FIN* | – | – |
| *HTI* | 1.826 | 7.408 |
| | (4.521) | (6.336) |
| *INDSTR* | 0.670 | 2.204 |
| | (3.771) | (5.284) |
| *MMNR* | -2.316 | 3.881 |
| | (4.453) | (6.241) |
| Constant | 25.337*** | 36.461*** |
| | (8.705) | (12.199) |
| Observations | 100 | 100 |
| R-squared | 0.136 | 0.129 |

*Standard errors in parentheses ***p < 0.01, **p < 0.05, *p < 0.1*

*This table provides the cross-sectional effect of a tax-knowledgeable CEO (CACEO-variable) on corporate tax avoidance (proxied by AETR and CETR respectively). CACEO, AETR, CETR, and the control variables are collapsed using the arithmetic mean for each company's observations over the 15-year period. The abbreviated variables are described in Table 2.*

with lower effective tax rates, meaning higher levels of corporate tax avoidance. Support of this hypothesis would be indicated in the form of a statistically negative coefficient on *CACEO*, the variable of interest. Neither the pooled regression results presented in Table 4, nor the regression results from the fixed-effect regressions presented in Table 5, nor the results of the cross-sectional regression in Table 6 indicate a statistically significant effect on the *CACEO* variable of interest. This suggests no evidence of the CACEO variable's influence on *AETR* or *CETR* as the measurement indicators for corporate tax avoidance. However, the coefficient signs on the *CACEO* explanatory variable are negative in the regression models, and often significantly so from an economical perspective with reference to the size of the parameter. For example, in the pooled models (Model 2, Table 4) *CETR* is associated with a −1.421 decline on average when a CEO is attributed with tax knowledge, while the fixed-effect regression models presented in Table 5 show a *CACEO* coefficient of −3.008. It should be noted that the regression models performed above analyse the association at the conditional mean between the explanatory variables and the dependent variables. Therefore, when we did not find a statistically significant coefficient in those regression models based on the conditional mean, we extended our analysis to quantile regression on the cross-sectional differences to assess whether the influence of the *CACEO* variable was not perhaps more significant at specific levels of *AETR* and *CETR.* The results of the quantile regressions performed of *AETR* & *CETR* respectively on *CACEO*, also indicate statistically insignificant coefficients on the *CACEO* variable. However, the positive coefficient sign on the *CACEO* variable changes to a negative sign at the 50th percentile for *CETR,* and the same happens for *AETR* where the positive sign of the *CACEO* coefficient changes to a negative one at the 40th percentile. This means that the *CACEO* effect is associated with increases in effective tax rates where tax avoidance levels are very low (suggesting corporate tax avoidance), but associated with higher levels of tax avoidance when effective tax rates trend higher, indicating that the CEO effect is picked up, although not significantly so. The changing coefficient on the CEO effect at different levels of *AETR* and *CETR* also indicates existence of a corporate governance mechanism: CEOs with tax knowledge manage effective tax rates upwards when they are too low, but manage them down when they are too high, considering that tax is an expense which reduces shareholder value. A look at the coefficients of other significant variables on the pooled regression results reported in Table 4 indicates a statistically significant positive association between *ESG* and *CETR,* providing some evidence that those companies with stronger corporate governance, social responsibility and sensitivity toward environmental impact pay more tax on average. This finding in the South African corporate context aligns with empirical evidence reported in the literature[14], meaning that companies with more corporate disclosure of corporate governance, environmental and social impact, pay more tax on average. This means that corporate behaviour related to corporate governance and social responsibility act in a complementary fashion with corporate tax behaviour, which can be seen in this context as an extension of responsible behaviour in other behavioural areas.

Company profitability, as measured by variable *ROA,* is statistically significant in terms of its association with both measures of effective tax rates, but not consistent in terms of the coefficient sign, which varies between the two proxies for corporate tax avoidance. *ROA* is positively associated with *AETR*, but negatively so with *CETR*. This may be explained by a possible tendency of companies to report higher accounting effective tax rates which are reported more conspicuously in financial statements, compared to *CETR* as another measure of corporate tax avoidance. *SIZE* is negatively, and statistically significantly so, associated with *CETR*, which means that larger companies, on average, pay less tax. This supports the theory that larger companies have more resources to develop tax avoidance strategies, which is also reported in the literature.[15,18] The statistically significant coefficient on *STC* is expected because secondary tax on companies increased effective tax rates as a corporate tax. Companies in the consumer goods and financial services industry, as well as industrial companies, indicated by the *CGS, FIN*, and *INDSTR* dummy variables respectively, pay significantly less tax on average as far as a negative coefficient is concerned. The fixed-effect regression results reported in Table 5 indicate no statistically significant effect on either *CACEO* variable of interest. This confirms the results of

the pooled regression. In this regard, a significant coefficient on the *CACEO* variable of interest would have indicated that alternation in the CEO's tax knowledge over the time period covered in this study is associated with variation in corporate tax avoidance. The same result is reported in Table 6, which presents the results of the cross-sectional regression. Overall, the results of these analyses do not provide statistically significant evidence to reject the null hypotheses stated before. This means that CEO tax knowledge based on this sample did not influence corporate tax avoidance.

## Conclusion, limitations, and opportunity for further research

We empirically investigated a CEO effect: the influence of tax-knowledgeable and tax-aware CEOs on corporate tax avoidance for large companies listed on the JSE. Our main hypothesis predicts that such CEOs would use their tax knowledge and complementary knowledge of financial management to effect and emphasise corporate tax avoidance to create shareholder value. The CEO effect is conceptualised from upper-echelon theory which attributes significant influence to members of top leadership on all corporate behaviour, including corporate tax behaviour. We used two different forms of effective tax rates to measure tax avoidance, while a CEO's tax knowledge is measured based on the CEO's status as a chartered accountant. The results of our analyses do not support our hypotheses, also indicating less support for the upper-echelon effect on corporate tax avoidance. The results indicate that corporate disclosure on aspects of corporate governance, social and environmental impacts extends to responsible corporate tax behaviour as well, because those companies are associated with less tax avoidance. Our results suggest little evidence for SAICA's claim that the appointment of chartered accountants could result in a reduction in corporate tax expenses. This result, however, bodes well for the reputation of the chartered accountancy profession, given the negative consequences of tax avoidance. Instead, the results of this study indicate evidence that chartered accountants do not use their specialist tax knowledge to enrich shareholders excessively.

From a tax avoidance literature perspective, this study contributes to research on the relationship between CEO characteristics and corporate tax avoidance, specifically regarding CEO skill sets as determinants of corporate tax avoidance. In this regard, it indicates that a CEO's tax knowledge does not necessarily influence corporate tax avoidance. Also, the study contributes to previous studies conceptualised from the upper-echelon effect. To this end, this study shows that the upper-echelon effect is not pervasively present in all aspects of corporate behaviour, in this case corporate tax behaviour.

Indeed, our proxy for a tax-knowledgeable CEO is not a perfect proxy, but others could improve on this attempt by using interviews, or by fine-combing other sources of textual data for biographical information, for example, to identify CEOs with other tax-related education. Our study was partly informed by SAICA's advert which differentiates on this aspect, which we can practically operationalise using publicly available data. Our findings cannot necessarily be extrapolated to all chartered accountants, as the role of CEO of large companies logically exceeds the power and ability associated with the average chartered accountant; however, it is the CEO effect that we investigated as our main focus. Last, effective tax rate, as used and reported in other seminal quantitative studies, is not a perfect proxy for tax avoidance, nor does it distinguish tax evasion from tax avoidance; however, we also did not aim to provide clarity on the difference between tax avoidance and tax evasion, which is becoming ever so grey.

CEOs of large companies are powerful; however, we argue that this power's influence on corporate tax avoidance cannot be reduced to a binary outcome, as far as CEOs could alter their behaviour based on changing priorities as circumstances inform.

## Competing interests

We have no competing interests to declare.

## Authors' contributions

P.v.d.S.: Conceptualisation; methodology; data collection; sample analysis; validation; writing – the initial draft; writing – revisions; project leadership; project management. P.d.J.: Conceptualisation; methodology; sample analysis; writing – the initial draft; writing – revisions; student supervision; funding acquisition.

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# Alcohol consumption patterns, suppliers and online alcohol marketing: Before and during COVID-19 alcohol bans

COVID-19-related alcohol sales bans and stay-at-home orders prompted the alcohol industry in South Africa to increase their online alcohol sales promotions. We investigated changes in alcohol-related behaviour and the drivers of illegal alcohol sales through a self-reported Facebook survey that ran from July to November 2020. Questions included socio-demographics and comparison of alcohol purchasing behaviour and intake during 2019 and 2020. Statistical tests were applied to find associations between illegal alcohol purchasing and alcohol-related behaviours. A total of 792 participants took part in the survey, 69.7% of whom were female. During lockdown periods, most participants (55.3%) bought alcohol illegally from illegal outlets or friends. Online alcohol-delivery marketing increased by 20 percentage points from 2019 to 2020, with participants stating that they saw a lot of advertisements per day and 80% of persons under 25 years were not asked to verify their age in 2020 upon delivery. Home-brewed beer and vodka intake increased in 2020 during the alcohol sales bans. Men from the Western Cape who engaged in daily or weekly heavy episodic drinking were more prone to purchase alcohol illegally. The Western Cape, which is South Africa's most prolific wine-producing region, had the highest odds of people buying alcohol illegally, with wine being found to be the most frequently bought alcohol online and consumed by these participants. There is a need for further research into the differences in alcohol-related behaviour affecting illegal alcohol purchasing according to income group, proximity to alcohol producers and underage alcohol sales and marketing through online applications.

**Significance:**

- Alcohol sales bans have the potential to reduce and stop the alcohol intake of moderate drinkers, but may make heavy episodic drinkers drink more than usual.
- During COVID-19 lockdown, illegal alcohol sales were taking place through unlicenced alcohol outlets and friends, and not through licenced online applications.
- Unlicenced alcohol outlets need to be addressed to prevent future illegal alcohol sales.
- Stricter regulations aimed at legal online alcohol sales applications should be put in place to prevent alcohol sales to minors and those who have already consumed too much alcohol.
- There should be a ban on marketing of addictive substances, such as alcohol.

## Introduction

South Africa implemented four alcohol sales bans of varying lengths, on both on- and off-premises sales, including the sale of alcohol online, during the COVID-19 pandemic in 2020 and 2021. These alcohol sales bans were an attempt to reduce the pressure on the intensive care units (ICU) in hospitals in the country. Given the impact of harmful alcohol use on the number of trauma-related injuries presenting to health facilities, these bans attempted to free up hospital space for COVID-19 patients by reducing the alcohol-related trauma burden.[1] As a result of these bans, less alcohol was consumed and there were significantly lower levels of alcohol-related injury and trauma during the sales bans.[2]

The most frequently consumed type of alcohol in South Africa is beer, followed by wine and then spirits, with approximately a third of all alcohol consumed in South Africa being sold from unlicenced vendors – in other words, illegal alcohol sales or home production.[3] South Africa has a history of selective prohibition (1948–1994), which allowed the illegal/unlicenced alcohol market to embed itself into some communities and is continuing to this day.[4] These illegal alcohol markets seemed to thrive during the COVID-19 pandemic, with 55% of surveyed South Africans reporting that they bought alcohol illegally during the alcohol sales bans in 2020.[5]

According to the World Health Organization's (WHO) Global Status Report on Harmful Use of Alcohol, 65.4% of people who drink alcohol in South Africa are heavy episodic drinkers (HED).[3] Research shows that both alcohol-outlet density and marketing frequency increase alcohol intake in those populations living in the most outlet-dense areas, and particularly influence the youth.[6,7] Increased ease of access to online alcohol purchasing and marketing have become the global norm[8], and may increase the availability of alcohol even more in many countries[9,10]. There are a large variety of negative health outcomes related to alcohol use and particularly HED.[3,11-13]

While people were ordered to stay at home during the COVID-19 pandemic, alcohol manufacturers around the world increased their digital marketing.[14] Online marketing may be seen by anyone using social media, including children

and people struggling with addiction, leading to the normalisation of alcohol consumption and increasing alcohol-related harm in future.[9,10] The WHO emphasises that the United Nations convention should uphold children's right to health, and protection from exploitation by alcohol manufacturers that invade digital social media spaces to market their products.[9] Women and youth in Africa are also being targeted by the alcohol industry.[13]

A national survey in South Africa found that significantly more HEDs than moderate drinkers reported drinking more alcohol during lockdown restrictions, while the majority of moderate drinkers drank less or stopped consuming alcohol completely.[5,15]

The purpose of our study was to explore and describe alcohol purchasing behaviours, and specifically alcohol purchasing changes between 2019 pre-COVID and 2020 during different periods of COVID-19 lockdown in South Africa, as well as to investigate possible drivers of illegal alcohol purchasing.

## Methods

### *Design*

A cross-sectional online survey was undertaken.

### *Procedure*

This self-reported survey was conducted online and is described in detail in a previous research paper.[5] The survey ran from 28 July 2020 to 28 November 2020 using the social media platform Facebook. The survey is provided in the Appendix in the Supplementary material and included questions on socio-demographics and alcohol purchasing behaviours before and during COVID-19.

### *Analysis*

Data cleaning and checking were done using Microsoft Excel (2018) and data were imported into the Statistical Package for the Social Sciences software (27.0 edition). Data were analysed in an iterative way by first doing basic descriptive analyses with selected variables and then searching for associations using chi-square tests for categorical data. After identifying variables that were significantly associated with the binary variable (yes/no) to the question of "Did you purchase alcohol illegally during the COVID-19 pandemic restrictions?", significant variables ($p<0.05$) were used to compile a standard multiple logistic regression in Statistical Software for Data Science (17th edition). The variables entered into the model to calculate the adjusted odds ratio (AOR) were the following: age category, sex, province, retired or not, frequency of consuming alcohol, frequency of HED episodes, purchasing more alcohol than usual during restrictions, having less money to purchase alcohol during restrictions, no longer purchasing alcohol during restrictions, and the ease of purchasing alcohol online compared to purchasing groceries. Multicollinearity was assessed by examining correlations between independent variables. No two predictors had a correlation of more than 0.5. Model fit was checked using an adaptation of Hosmer–Lemeshow's goodness-of-fit test, and all models indicated appropriate fit.

### *Ethics*

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Human Research Ethics Committee of the South African Medical Research Council (EC017-6/2020, 20 July 2020) and of the Biomedical Science Research Ethics Committee of the University of the Western Cape (BM21/5/11, 12 July 2021).

## Results

### *Socio-demographic description of participants in the survey*

A total of 792 participants took part in the survey, of whom 69.7% were female (Table 1). The majority of participants (55.3%) reported buying alcohol illegally during the COVID-19 alcohol sales bans in 2020 and were over the age of 55, of the white population group, working full time, and living in Gauteng or the Western Cape Provinces. Nearly 20% of participants stated they were able to work remotely, 16% said they had lost income and 13% reported being essential workers during the COVID-19 lockdown period. When comparing the proportions in each age category, there was a significantly higher frequency of people between the ages of 45 and 54 who purchased alcohol illegally, with the opposite being true for those who were 65 years and older.

**Table 1:** Comparison of the socio-demographics of people purchasing alcohol illegally and those who did not purchase alcohol illegally

| Variables | Total alcohol consumers *n*(%) 95% CI | Purchased illegally *n*(%) 95% CI | Did not purchase illegally *n*(%) 95% CI | *p*-value (chi²) |
|---|---|---|---|---|
| **Age** | | | | |
| 18–34 | 108(13.8) 11.5–16.4 | 67(15.5) 12.4–19.2 | 41(11.7) 8.6–15.4 | <0.001 |
| 35–44 | 144(18.4) 15.8–21.2 | 84(19.5) 16.0–23.4 | 60(17.1) 13.4–21.3 | |
| 45–54 | 162(20.7) 18.0–23.7 | 105(24.4) 20.5–28.6 | 57(16.2) 12.7–20.4 | |
| 55–64 | 229(29.3) 26.2–32.5 | 124(28.8) 24.6–33.2 | 105(29.9) 25.3–34.9 | |
| ≥65 | 139(17.8) 15.2–20.6 | 51(11.8) 9.0–15.1 | 88(25.1) 20.8–29.8 | |
| **Population group**[a] | | | | |
| Black African | 77(9.6) 7.7–11.8 | 36(8.1) 5.9–11.0 | 41(11.4) 8.4–15.0 | 0.344 |
| Coloured | 37(4.6) 3.3–6.2 | 18(4.1) 2.5–6.2 | 19(5.3) 3.3–8.0 | |
| White | 662(82.5) 79.8–85.1 | 374(84.6) 81.0–87.7 | 288(80.0) 75.6–83.9 | |
| Asian/other | 26(3.2) 2.2–4.6 | 14(3.2) 1.8–5.1 | 12(3.3) 1.8–5.6 | |
| **Sex** | | | | |
| Male | 240(30.3) 27.2–33.6 | 146(33.3) 29.0–37.8 | 94(26.6) 22.2–31.3 | 0.039 |
| Female | 552(69.7) 66.4–72.8 | 292(66.7) 62.2–71.0 | 260(73.4) 68.7–77.8 | |
| **Province** | | | | |
| Eastern Cape | 65(8.1) 6.3–10.1 | 29(6.5) 4.5–9.1 | 36(10.0) 7.2–13.4 | |
| Free State | 31(3.9) 2.7–5.3 | 20(4.5) 2.9–6.7 | 11(3.1) 1.6–5.2 | |

| Variables | Total alcohol consumers *n*(%) 95% CI | Purchased illegally *n*(%) 95% CI | Did not purchase illegally *n*(%) 95% CI | *p*-value (chi²) |
|---|---|---|---|---|
| Gauteng | 321(39.9) 36.5–43.3 | 181(40.7) 36.2–45.3 | 140(38.9) 34.0–44.0 | |
| KwaZulu-Natal | 121(15.0) 12.7–17.6 | 56(12.6) 9.7–15.9 | 65(18.1) 14.3–22.3 | |
| Mpumalanga | 32(4.0) 2.8–5.5 | 20(4.5) 2.9–6.7 | 12(3.3) 1.8–5.6 | |
| Limpopo | 27(3.4) 2.3–4.8 | 12(2.7) 1.5–4.5 | 15(4.2) 2.5–6.6 | 0.034 |
| Northern Cape | 10(1.2) 0.6–2.2 | 8(1.8) 0.9–3.4 | 2(0.6) 0.1–1.8 | |
| North West | 22(2.7) 1.8–4.0 | 10(2.2) 1.2–3.9 | 12(3.3) 1.8–5.6 | |
| Western Cape | 176(21.9) 19.1–24.8 | 109(24.5) 20.7–28.6 | 67(18.6) 14.9–22.9 | |
| **Are you any of the following: aged 70+, have a serious underlying medical condition or immunocompromised?** | | | | |
| Yes | 98(12.6) 10.4–15.1 | 46(10.7) 8.1–13.9 | 52(14.9) 11.5–18.9 | 0.081 |
| No | 680(87.4) 84.9–89.6 | 383(89.3) 86.1–91.9 | 297(85.1) 81.1–88.5 | |
| **Which COVID-19 pandemic restrictions are you currently in?**[b] | | | | |
| 1 Total restriction | 13(1.2) 0.7–1.9 | 5(0.8) 0.3–1.7 | 8(1.6) 0.8–3.0 | |
| 2 Very restricted | 134(12.0) 10.2–14.0 | 71(11.3) 9.0–14.0 | 63(12.8) 10.1–16.0 | |
| 3 Moderate restriction | 557(49.7) 46.8–52.7 | 317(50.5) 46.6–54.4 | 240(48.8) 44.4–53.2 | 0.156 |
| 4 Limited restriction | 357(31.9) 29.2–34.6 | 194(30.9) 27.4–34.6 | 163(33.1) 29.1–37.4 | |
| 5 Social distancing only | 30(2.7) 1.9–3.7 | 19(3.0) 1.9–4.6 | 11(2.2) 1.2–3.8 | |
| 6 No restriction | 29(2.6) 1.8–3.6 | 22(3.5) 2.3–5.2 | 7(1.4) 0.6–2.8 | |
| **What was your employment status before the COVID-19 pandemic (i.e. before the COVID-19 restrictions)? (yes)** | | | | |
| Working full-time | 408(36.3) 33.5–39.1 | 241(38.2) 34.5–42.0 | 167(33.8) 29.7–38.1 | 0.129 |
| Working part-time | 43(3.8) 2.8–5.1 | 27(4.3) 2.9–6.1 | 16(3.2) 1.9–5.1 | 0.367 |
| [Self-employed / contractor][c] | 137(12.2) 10.4–14.2 | 83(13.2) 10.7–16.0 | 54(10.9) 8.4–13.9 | 0.258 |
| [Student/homemaker/ unemployed/unable to work due to disability/ volunteer] | 93(8.3) 6.8–10.0 | 49(7.8) 5.9–10.0 | 44(8.9) 6.6–11.7 | 0.490 |
| Retired | 156(13.9) 11.9–16.0 | 61(9.7) 7.5–12.2 | 95(19.2) 15.9–22.9 | <0.001 |
| **How has your work status currently been impacted by the COVID-19 pandemic restrictions? (yes)** | | | | |
| I am considered an essential worker | 151(13.4) 11.5–15.5 | 93(14.7) 12.1–17.7 | 58(11.7) 9.1–14.8 | 0.143 |
| Able to work remotely | 206(18.3) 16.1–20.7 | 116(18.4) 15.5–21.5 | 90(18.2) 15.0–21.8 | 0.943 |
| I am unable to transition to work my usual role remotely | 31(2.8) 1.9–3.8 | 19(3.0) 1.9–4.6 | 12(2.4) 1.3–4.1 | 0.554 |
| I have lost income | 179(15.9) 13.9–18.1 | 110(17.4) 14.6–20.5 | 69(14.0) 11.1–17.2 | 0.115 |
| Change of employer or position | 59(5.2) 4.1–6.7 | 38(6.0) 4.4–8.1 | 21(4.3) 2.7–6.3 | 0.186 |

*[a]These terms originate from the apartheid era. They refer to demographic markers and do not signify inherent characteristics. They refer to people of European, African, Asian and mixed (African, European and/or Asian) ancestry, respectively. These markers were chosen for their historical significance. Their continued use in South Africa is important for monitoring improvements in health and socio-economic disparities, identifying vulnerable sections of the population, and planning effective prevention and intervention programmes.*

*[b]1 Stay at home – cannot leave house (or only for essentials with permission). 2 Stay at home – can leave for food/medical/exercise. Non-essential businesses/schools are closed. Public gatherings banned. Physical distancing required. 3 Stay at home – can interact with a few people outside your household. Some businesses are open. School may/may not be open. Public gatherings banned. Physical distancing required. 4 Most businesses, schools, workplaces open, gatherings of people allowed but size restricted, physical distancing required. 5 Physical distancing only required. 6 Life as normal.*

*[c]Square brackets indicate the variables that were combined into one variable.*

Women and people who were retired were significantly less prone to purchase alcohol illegally. People in the Western Cape Province had a significantly higher frequency of illegal alcohol purchasing, while participants from KwaZulu-Natal had a lower frequency of illegal alcohol purchasing.

### *Comparison of online alcohol purchasing behaviour*

The majority of both those who bought alcohol illegally during COVID-19 pandemic restrictions and those who did not, responded that they 'never' bought more alcohol online to continue drinking when their alcohol ran out, while a third (27%) said that they did buy alcohol online to continue drinking (Table 2). This was also true for HED and moderate drinkers. Unfortunately, too few respondents answered this question to determine a significant difference between the groups for this question.

Significantly more moderate drinkers than HED said they switched to buying alcohol online during the pandemic, while there was no difference found for buying alcohol online between illegal or non-illegal purchasers. Additionally, purchasing less alcohol than usual was only significantly higher for moderate drinkers, with no difference found between illegal and non-illegal purchasers. However, significantly more HED and illegal alcohol purchasers reported buying more alcohol than usual during alcohol sales restrictions, even though both HED and illegal alcohol purchasers also reported to have less money to purchase alcohol. Those who reported that they stopped purchasing alcohol during the pandemic restrictions were significantly more likely to be people who drank alcohol in moderation and those who did not buy alcohol illegally.

Significantly more participants who bought alcohol illegally said that it was 'more difficult' to buy alcohol online than it was to buy groceries online, while the majority of those who did not buy alcohol illegally did not notice any difference in difficulty. There was a similar trend seen for HED and moderate drinkers; however, these frequencies were found to be not significantly different.

### *Alcohol purchasing practices*

In relation to where respondents purchased alcohol between 2019 (before the COVID-19 pandemic) and 2020, we found an estimated 40, 32 and 29 percentage point decreases in alcohol being bought from liquor stores or alcohol shops, supermarkets, and on-licence types of shops, respectively, in 2020 (Figure 1). Purchasing alcohol from unlicenced/illegal outlets and from friends increased by 21 and 14 percentage points, respectively, while online/home delivery decreased by 2 percentage points between 2019 and 2020. People who stated that they did not buy any alcohol in 2020 went up by 13 percentage points, compared to 2019.

### *Types of alcohol purchased online*

There was an overall reduction in online purchasing of all types of alcohol between 2019 and 2020 (Figure 2). The type of alcohol purchased most frequently online did not differ from 2019 to 2020, with wine and sparkling wine being the most frequently bought alcohol online. Spirits were the second most frequently type of alcohol bought online, with beer third and ciders/alco-pops coming in fourth place.

**Table 2:** Comparison of online alcohol purchasing behaviour and household purchasing changes between those that consume alcohol who purchased alcohol illegally during COVID-19 restrictions and those who did not, as well as between heavy episodic drinkers (HED) and moderate drinkers

| Variables | Total alcohol consumers *n*(%) | Purchased illlegally *n*(%) | Did not purchase illegally *n*(%) | *p*-value (chi²) | Total alcohol consumers *n*(%) | HED *n*(%) | Moderate drinkers *n*(%) | *p*-value (chi²) |
|---|---|---|---|---|---|---|---|---|
| **If your alcohol has run out while you were in the middle of drinking, have you sometimes ordered more alcohol online to keep drinking during the COVID-19 pandemic restrictions?** | | | | | | | | |
| Often/sometimes | 40(26.7) | 20(29.4) | 20(24.4) | | 34(27.4) | 13(34.2) | 21(24.4) | |
| Never | 71(47.3) | 29(42.6) | 42(51.2) | 0.574 | 57(46.0) | 14(36.8) | 43(50.0) | 0.363 |
| Not applicable | 39(26.0) | 19(27.9) | 20(24.4) | | 33(26.6) | 11(28.9) | 22(25.6) | |
| **Have the COVID-19 pandemic restrictions affected your, or your household's, alcohol purchasing behaviour in the following ways? (yes)** | | | | | | | | |
| Switched to purchasing online | 274(24.4) | 144(22.8) | 130(26.3) | 0.175 | 200(26.4) | 80(21.8) | 120(35.8) | 0.006 |
| Purchasing more alcohol than usual during the restrictions | 384(34.1) | 250(39.6) | 134(27.1) | <0.001 | 288(38.0) | 178(48.5) | 110(28.1) | <0.001 |
| Less money to purchase alcohol | 444(39.5) | 292(46.3) | 152(30.8) | <0.001 | 298(39.3) | 164(44.7) | 134(34.3) | 0.003 |
| Purchasing less alcohol than usual during the restrictions | 501(44.5) | 282(44.7) | 219(44.3) | 0.904 | 348(45.9) | 143(39.0) | 205(52.4) | <0.001 |
| No longer purchasing alcohol | 260(23.1) | 59(9.4) | 201(40.7) | <0.001 | 143(18.9) | 46(12.5) | 97(24.8) | <0.001 |
| **During the COVID-19 pandemic restrictions, was/is it easier to get alcohol delivered online than fresh food/groceries?** | | | | | | | | |
| Easier | 72(23.7) | 51(28.3) | 21(16.9) | | 48(21.1) | 27(32.5) | 21(18.8) | |
| Haven't noticed any difference | 107(35.2) | 50(27.8) | 57(46.0) | 0.003 | 85(37.4) | 36(31.3) | 49(43.8) | 0.152 |
| More difficult | 125(41.1) | 79(43.9) | 46(37.1) | | 94(41.4) | 52(45.2) | 42(37.5) | |

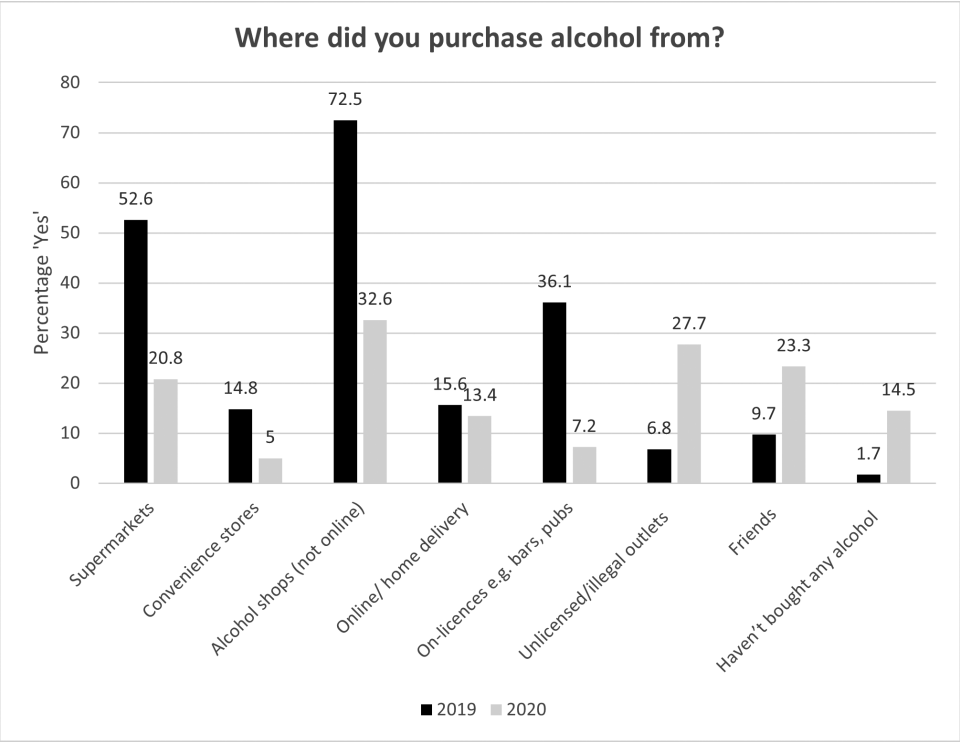


**Figure 1:** Comparison of alcohol purchasing venue/method between 2019 pre-COVID and 2020 during COVID (all respondents).

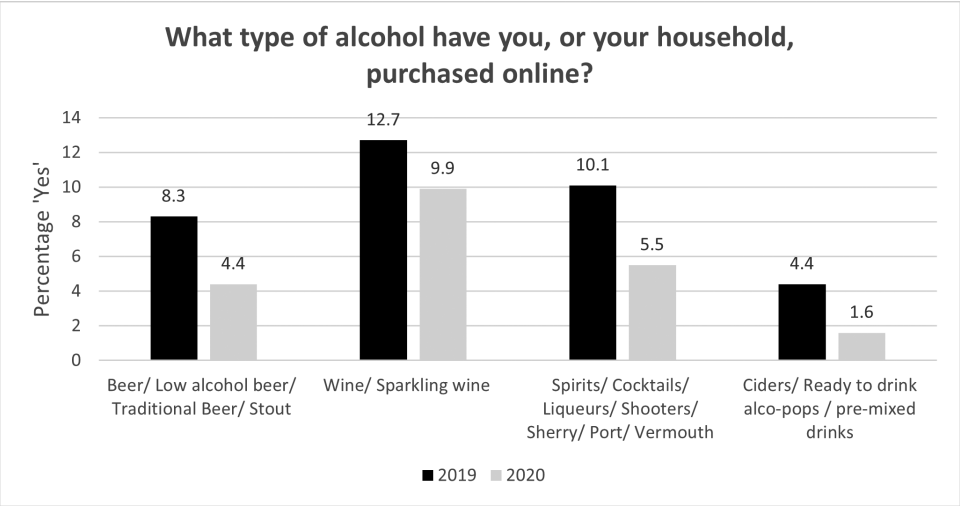


**Figure 2:** Comparison of types of alcohol purchased online between 2019 pre-COVID and 2020 during COVID (all respondents).

### *Types of alcoholic drinks consumed on a typical drinking occasion*

In 2019 wine was the most popular alcohol type consumed at 44%, followed by spirits at 35%, beer at 28% and ciders at 15% (Figure 3). All these alcohol types saw a reduction in consumption during the alcohol sales bans of 2020. However, home-brewed beer and home-brewed vodka increased by 5 and 2 percentage points, respectively.

### *Alcohol intake, online alcohol delivery and digital marketing of online delivery*

The frequency of drinking alcohol seemed to be similar between 2019 and 2020, with only slight decreases for the categories 'drinking more than once a day' and '1–6 times per week' in 2020 (Table 3). When asked how often respondents consumed more than six drinks on one occasion, responses indicated a decrease of 13 percentage points for 'weekly', and an increase of 18 percentage points for 'never', between 2019 and 2020. Having more than six drinks per occasion went up for the 'daily' category by 1 percentage point in 2020. Due to the reduction in excessive consumption of alcohol, the percentage of people classified as HED decreased by 5 percentage points.

Online alcohol delivery times seemed to slow down during 2020, with a 20 percentage point increase in respondents stating that it took more than 2 days for their alcohol to be delivered compared to 2019. Verifying age on delivery worsened, with 65% and 83% of people under 25 years stating that their identity document was not requested at the delivery of alcohol bought online in 2019 and 2020, respectively. There was a 20 percentage point increase in the proportion of respondents stating that they saw 'a lot of online alcohol delivery advertisements' in 2020 compared to 2019.

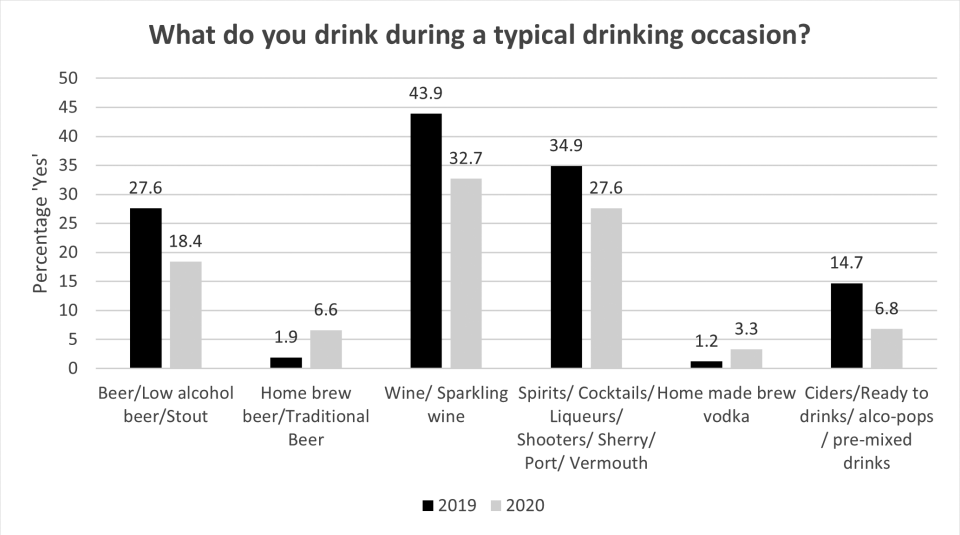


**Figure 3:** Comparison of types of alcoholic drinks that were consumed on a typical drinking occasion between 2019 pre-COVID and 2020 during-COVID (all respondents).

### *Predictors of illegal alcohol purchasing during the COVID-19 pandemic in 2020*

Multiple logistic regression analysis showed that, compared to 18–34 year olds, people who were 65 years and older had significantly lower odds of purchasing alcohol illegally (Table 4). Compared to men, women had significantly lower odds, and compared to the Eastern Cape Province, people in the Western Cape showed double the likelihood of buying alcohol illegally. Compared to retired people, those who were not retired also had a significantly higher likelihood of illegal alcohol purchasing. Compared to participants who reported that the COVID-19 pandemic restrictions affected their household's alcohol purchasing behaviour by no longer purchasing alcohol, those who continued to purchase alcohol were six times more likely to buy alcohol illegally.

There was a threshold of significantly reduced odds of purchasing alcohol illegally: as soon as someone drank only monthly, less than once a month, and less than four times a year, compared to people who drank daily, and when people reported drinking heavily monthly or less than monthly, compared to people who drank heavily daily.

Regarding purchasing behaviour, we found that the following factors significantly reduced the odds of purchasing alcohol illegally: participants who said that they bought less alcohol than usual, who said they had enough money to buy alcohol, and those who said that there was no difference in the difficulty between grocery shopping and alcohol shopping online.

## Discussion

During the COVID-19 pandemic lockdown restrictions and alcohol sales bans of 2020, it is evident that, even though alcohol consumption was reduced, more than half the participants of this survey reported buying alcohol illegally from friends or illegal/unlicenced on-site outlets. Even though 25.4% of participants switched to purchasing alcohol online during the COVID-19 restrictions, illegal alcohol sales were not taking place on licenced online alcohol applications. Factors that significantly increased the odds of people buying alcohol illegally were, in order of highest to lowest strength of the adjusted odds ratio: (1) being male, (2) buying more alcohol than usual during the restrictions, (3) not having enough money to buy alcohol, (4) drinking alcohol weekly or more often, (5) having HED episodes weekly or more often, and (6) having the perception that buying alcohol online is easier than buying groceries online.

Previous research on this data set found that HEDs were significantly more prone to buying alcohol illegally than were moderate drinkers.[5] HEDs and those who bought alcohol illegally were also significantly more prone to report buying more alcohol than usual during the COVID-19 pandemic restrictions, as well as report having less money to buy alcohol than moderate drinkers and those who did not buy alcohol illegally. This finding may be due to the addictive nature of alcohol, which leads people to spend their money on their addiction rather than on essential goods.[16]

We would expect those provinces with the highest frequency of HEDs to be the provinces with the highest frequency of illegal alcohol purchasing. However, this is not what we found. The finding that people residing in the Western Cape were significantly more likely to buy alcohol illegally, is in contradiction with the 2016 South African Demographic and Health Survey (SADHS), which reported that the highest frequency of male and female HEDs resided in Gauteng and the Northern Cape, respectively.[17] The finding that people residing in the Western Cape had twice the odds of buying alcohol illegally may be due to the small sample size of participants in the other provinces or that the Western Cape has an abundance of more than 2600 wine producers, producing more than 900 million litres of wine in 2021, making it easier to find alcohol being sold illegally during the alcohol sales bans.[18]

Our research found that wine and sparkling wine were the most frequently consumed and bought types of alcohol reported by participants. Strong liquor was consumed second most, while beer and cider were the third and fourth most frequently consumed, respectively. These trends stayed the same between 2019 and 2020. These alcohol statistics differ from the WHO statistics, which show that beer is sold most frequently in South Africa, then wine, and, thirdly, spirits.[3] This discrepancy could be due to the higher income level of respondents of this survey, which was only accessible to people with Internet, which is not freely available in South Africa. According to the Brand Mapp survey, wine is the preferred type of alcohol for older generations who earn more than ZAR10 000 per month (USD631) in South Africa and who are more likely to have Internet access.[19]

During the alcohol sales restrictions, there was a slight increase in the consumption of home-brewed types of alcoholic beverages, such as home-made beer and vodka. In South Africa, the price of pineapples increased during alcohol sales bans, due to them being used to brew alcohol at home.[20] When we asked participants where they purchased their alcohol, during the alcohol sales restrictions compared to the previous year, we found that illegal/unlicenced on-site sources and friends as a source both increased from the previous year. Online alcohol purchasing decreased by 2 percentage points from 2019, and even though marketing of online alcohol delivery agencies went up by 20 percentage points from 2019 to 2020, the majority of participants reported that online purchasing of alcohol was more difficult than buying groceries online. A total of 62% of participants said it took up to 2 days

**Table 3:** Comparison between 2019 pre-COVID and 2020 during COVID, of frequency of drinking, HED, online alcohol delivery times, age verification upon alcohol delivery and prevalence of digital marketing of online alcohol delivery advertisements noticed by participants

| Variables | 2019 *n*(%) | 2020 *n*(%) |
|---|---|---|
| **How often did you have a drink containing alcohol?**[a] | | |
| ≥1 times per day | 308(30.6) | 225(24.4) |
| 1–6 times a week | 544(54.0) | 440(47.8) |
| 1–4 times per month | 102(10.1) | 120(13.0) |
| Less than once a month | 25(2.5) | 52(5.6) |
| 1–3 times per year | 17(1.7) | 17(1.8) |
| Didn't drink | 11(1.1) | 67(7.3) |
| **How often did you have 6 or more drinks of alcohol on a single occasion?** | | |
| Daily | 95(10.4) | 87(11.5) |
| Weekly | 250(37.8) | 189(24.9) |
| Monthly | 146(16.0) | 91(12.0) |
| Less than monthly | 181(19.8) | 121(16.0) |
| Never | 240(17.4) | 270(35.6) |
| **Classified as HED**[b] | | |
| Yes | 491(53.8) | 367(48.4) |
| No | 421(46.2) | 391(51.6) |
| **How long on average did it take for your alcohol to be delivered?** | | |
| Less than 15 minutes | 10(5.3) | 1(0.7) |
| 15 minutes to an hour | 24(12.8) | 8(5.2) |
| 1 hour to 24 hours | 18(9.6) | 22(14.4) |
| Between 1 to 2 days | 56(29.8) | 27(17.6) |
| More than 2 days | 80(42.6) | 95(62.1) |
| **If you are under 25, have you been asked to show age ID when online alcohol has been delivered?** | | |
| Often | 6(23.1) | 3(17.6) |
| Sometimes | 3(11.5) | 0 |
| Never | 17(65.4) | 14(82.4) |
| **How many ads for online alcohol delivery did you notice?** | | |
| A lot of ads per day | 17(13.6) | 43(33.1) |
| Some ads per day | 28(22.4) | 24(18.5) |
| A few ads per day | 46(36.8) | 41(31.5) |
| No ads | 34(27.2) | 22(16.9) |

*[a]A drink is defined as a half-pint (340 mL) of beer containing 5% alcohol, or a shot (30 mL) of spirits containing 40% alcohol, or a small glass (120 mL) of wine containing 12% alcohol.*

*[b]HED, heavy episodic drinker. Based on the question "How often did you have 6 or more drinks of alcohol on a single occasion during 2019/the COVID-19 pandemic restrictions?" Those who answered anything more regular than monthly (including monthly) were classified HED while those who answered anything less frequently than monthly were classified as moderate drinkers.*

for alcohol to be delivered in 2020, which was similar to New Zealand.[10] A third of alcohol consumers reported buying more alcohol online when their alcohol was finished, presumably when alcohol sales restrictions were lifted. Even though there were other countries that also had alcohol sales bans during COVID-19, there were no other research papers that we could find that reported on where people illegally bought alcohol, or how many of them did so. A study from Thailand indicated that 5.8% of people saw illegal alcohol sales being conducted, while 15.7% of people drank socially during the 3-week (10 April – 1 May 2020) alcohol sales ban.[21]

The legal age for purchasing and drinking alcohol in South Africa is 18 years, and age has to be verified by alcohol trading companies, including

**Table 4:** Multiple logistic regression with illegal purchasing as the dependent variable

| Variables | AOR | 95%CI | *p*-value |
|---|---|---|---|
| **Age** | | | |
| 18–34 | (ref) | | |
| 35–44 | 0.86 | 0.51–1.43 | 0.553 |
| 45–54 | 1.13 | 0.68–1.87 | 0.642 |
| 55–64 | 0.72 | 0.45–1.15 | 0.173 |
| ≥65 | 0.35 | 0.21–0.60 | **0.000** |
| **Sex** | | | |
| Male | (ref) | | |
| Female | 0.72 | 0.53–0.98 | **0.039** |
| **Province** | | | |
| Eastern Cape | (ref) | | |
| Free State | 2.26 | 0.93–5.46 | 0.071 |
| Gauteng | 1.60 | 0.94–2.74 | 0.084 |
| KwaZulu-Natal | 1.07 | 0.58–1.96 | 0.828 |
| Mpumalanga | 2.07 | 0.87–4.92 | 0.100 |
| Limpopo | 0.99 | 0.40–2.45 | 0.988 |
| Northern Cape | 4.97 | 0.98–25.21 | 0.053 |
| North West | 1.03 | 0.39–2.73 | 0.945 |
| Western Cape | 2.02 | 1.14–3.59 | **0.017** |
| **Are you retired?** | | | |
| Yes | (ref) | | |
| No | 2.22 | 1.57–3.14 | **0.000** |
| **Frequency of drinking** | | | |
| ≥1 times per day | (ref) | | |
| 1–6 times a week | 0.96 | 0.68–1.35 | 0.800 |
| 1–4 times per month | 0.45 | 0.28–0.70 | **0.001** |
| Less than once a month | 0.26 | 0.14–0.49 | **0.000** |
| 1–3 times per year | 0.11 | 0.03–0.38 | **0.001** |
| **Heavy episodic drinking frequency of drinking occasions** | | | |
| Daily | (ref) | | |
| Weekly | 0.99 | 0.52–1.88 | 0.978 |
| Monthly | 0.42 | 0.21–0.83 | **0.012** |
| Less than monthly | 0.39 | 0.21–0.74 | **0.004** |
| **Purchasing more alcohol than usual during the restrictions** | | | |
| Yes | (ref) | | |
| No | 0.57 | 0.44–0.73 | **<0.001** |
| **Less money to purchase alcohol** | | | |
| Yes | (ref) | | |
| No | 0.52 | 0.40–0.66 | **<0.001** |
| **No longer purchasing alcohol** | | | |
| Yes | (ref) | | |
| No | 6.65 | 4.82–9.18 | **<0.001** |

| Variables | AOR | 95%CI | *p*-value |
|---|---|---|---|
| **Ease of buying alcohol online compared to groceries online** | | | |
| Easier | (ref) | | |
| No difference | 0.36 | 0.19–0.68 | **0.002** |
| More difficult | 0.71 | 0.38–1.32 | 0.277 |

*AOR, adjusted odds ratio*

for online alcohol sales, either upon ordering or delivery.[22] Our research found that age verification became even more careless in 2020 than it was in 2019, with 82% of participants under the age of 25 stating that their age was never verified when purchasing alcohol online. The lack of age verification was higher in South Africa than in other countries such as New Zealand, where it was found that 58% of people under the age of 25 reported that their age was not verified[10], 45% in the United States of America[23] and 36% in Australia[24]. There was no research on age verification through illegal alcohol sale avenues during the sales ban in South Africa. However, we can assume that it would have been even worse than what was found for legal online alcohol delivery applications.[4,25]

It has been well documented that early initiation of alcohol use is associated with risky or harmful alcohol use as an adult.[26] Added to this is the fact that online alcohol delivery increases the ease with which alcohol can be obtained, and the fact that an increase in alcohol outlet density and an increase in digital marketing of alcohol lead to an increase in alcohol use at a young age, which may lead to more alcohol-related harms within the household.[6,7,27-30] According to the international policy review done by Colbert et al.[29], steps should be put in place to strengthen online alcohol sales regulations.

Our findings demonstrate that the perception that it was easier to buy alcohol than groceries online, was associated with significantly increased odds of purchasing illegal alcohol. We could speculate that this finding is due to the perception that alcohol was easily accessible, even during alcohol sales bans, when legal and official online sales and deliveries were cancelled. However, illegal sales that were taking place via WhatsApp, Facebook, and other social messaging platforms, were probably continuing. By enforcing more robust regulations and fines on digital alcohol sales platforms, illegal alcohol sales may be reduced, and with it the perception of ease of online alcohol sales.[9, 31]

Nearly half of HEDs reported buying more alcohol than usual during the alcohol sales restrictions, while moderate drinkers were purchasing less alcohol than usual and even stopped buying alcohol. There was a reduction in HED classification of 5.4 percentage points between 2019 and 2020, with the largest reduction being shown in daily and weekly frequency of alcohol consumption. This finding highlights the fact that alcohol consumption behaviours reduced during the alcohol sales restrictions. However, those who wanted to buy alcohol managed to do so even though it was illegal. To reduce alcohol consumption, without having to implement alcohol sales bans, researchers have found that a minimum unit price of alcohol products of as low as ZAR10 (USD0.6) has the potential to save lives by reducing alcohol intake and harms associated with it.[32]

In the current study, we found that people who consumed alcohol daily or weekly, and had HED episodes daily or weekly, were around 60% more likely to buy alcohol illegally than those who consumed alcohol less frequently. This knowledge can be used to tailor interventions aimed at reducing illegal alcohol purchases, reducing consumption by those who are heavy drinkers, and ensuring online sales of alcohol are not allowed during times when physical outlets are closed.

A limitation of the current research is that our survey was placed on only one online digital platform, which excluded people from participating if they did not have Internet access, or were not on that social networking platform. There was a high response rate from older age groups and predominantly white population groups living in Gauteng and the Western Cape of South Africa, which limits the generalisability of the findings. Another limitation is that people may not have recalled exactly what they observed or did the year before when asked to compare 2019 to 2020.

## Conclusion

We found that alcohol sales bans increased alcohol purchasing by HEDs, even though they had less money to buy alcohol. However, overall, the alcohol sales ban led to a reduction in alcohol intake compared to the previous year. Illegal alcohol sales were not taking place through licenced online/home delivery applications, but rather through unlicenced/illegal outlets and friends. Retrospective research may shed light on where and how these illegal alcohol sales took place and to understand the phenomenon better. A third of alcohol consumers reported that they bought alcohol online to continue drinking when their alcohol ran out, indicating a need for online interventions that prevent high-intensity drinking.

To reduce heavy drinking, a multitude of interventions is needed simultaneously, including minimum unit pricing, stricter online regulations, especially preventing access and marketing of alcohol to minors, a ban on the marketing of alcohol overall, and a reduction in outlet density. The low levels of age verification for online alcohol sales indicate that immediate programmatic and enforcement of regulations, such as the inclusion and verification of an identification number when purchasing alcohol online, is needed in South Africa.

Whilst this study has yielded interesting results, it cannot be generalised to the entire population, but rather only to those who had Internet access and responded to an online survey.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

M.T.: Formal analysis; investigation; data curation; writing – original draft preparation; writing – review and editing; visualisation. R.S.: Writing – review and editing. M.L.: Conceptualisation; methodology; formal analysis; investigation; data curation; writing – original draft preparation; funding acquisition. C.P.: Conceptualisation; methodology; writing – review and editing; funding acquisition. P.P.W.: Conceptualisation; methodology; funding acquisition. N.H.: Conceptualisation; methodology; software; validation; investigation; resources; writing – original draft preparation; writing – review and editing; supervision; project administration; funding acquisition.

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# Radiocarbon-dated evidence for Late Pleistocene and Holocene coastal change at Yzerfontein, Western Cape, South Africa

We report radiocarbon dates obtained from on-shore marine and near-shore terrestrial deposits near Yzerfontein, on the West Coast of South Africa. These deposits include Late Pleistocene shell concretions from the southern end of 16 Mile Beach and a marine shell deposit inland of the coastal Rooipan (Red Pan); mid-Holocene coastal pan deposits exposed by modern storm erosion of the sandy 16 Mile Beach; and four Holocene storm beach deposits on a rocky shore to the south. We interpret the results in terms of local geomorphology constraints on sea-level fluctuations. The eastern margin of Rooipan is a >40 ka elevated beach deposit in a dune cordon that separates it from the adjacent Yzerfonteinpan. Both pans have gypsum deposits up to 2 m thick formed by repeated marine overwash. Saline pan deposits that are exposed intermittently on the beach are mid-Holocene and indicate a former westward extension of Rooipan. This is in contrast to storm beaches dating 8000–2600 cal BP at higher elevations on a rocky platform further south. This suggests that a dune barrier existed seaward of the present shoreline near Rooipan at this time. The coastal changes described here show that deposition and erosion can be affected significantly by the local palaeogeomorphology and cannot be ascribed solely to sea-level change.

**Significance:**

Mollusc shells from Yzerfontein, Western Cape Province, South Africa, show radiocarbon ages ranging from >40 000 years to a few decades before present. There is evidence for elevated sea levels between 8000 and 2600 years ago, and sea levels similar to the present in the last 2000 years. Neither the elevation of the deposits nor their ages conform to published sea-level change curves for the Western Cape coast. Inundation by rising sea levels in the Holocene was not spatially uniform. Former and present geomorphology have had a significant effect on deposition and preservation of indicators of sea-level change.

## Introduction

Yzerfontein Point (33° 20′ 46.9″ S; 18° 08′ 48.8″ E), situated 70 km north of Cape Town, forms a rocky promontory at the southern end of the log-spiral 16 Mile Beach (Figure 1) on the southwestern coast of South Africa. This section of coast is microtidal, but experiences a strong southwesterly swell and predominantly southerly winds that drive sand transport towards the north.[1] South of Yzerfontein Point there is a wave-cut platform at 6–12 m above mean sea level (asl), following the rugged coastline for 1 km south to Schaapen Island, another rocky promontory tidally separated from the mainland. North of Yzerfontein Point there is a pocket beach and a small rocky outcrop called Rooipan se Klippe (known to geologists as Gabbro Point). North of that a coastal dune cordon separates the sandy beach from Rooipan (Red Pan), a seasonally flooded saline pan named after the red, salt-tolerant vegetation surrounding it. Another larger pan, Yzerfonteinpan, is located some 1.5 km inland and is separated from Rooipan by a vegetated dune rising to 10 m asl. 16 Mile Beach extends north, unbroken, from Rooipan se Klippe to the Langebaan Peninsula.

In 2017, severe storm erosion of the coastal dune at the southern end of 16 Mile Beach caused significant destruction of coastal infrastructure (Figure 2), and focused attention on the vulnerability of the local coastal dune system.[2] This erosion also temporarily exposed dark, organic mud horizons containing numerous shells of the diminutive terrestrial snail *Tomichia ventricosa* in the beach seaward of the present coastal dunes (Figure 3). This anomaly stimulated further investigation of various onshore, near-coastal deposits in order to characterise local palaeoenvironmental change. We sampled molluscs and sediment from various near-shore deposits for radiocarbon dating in order to establish the sequence of depositional and erosional events leading to the configuration of the local modern shoreline. The main aim of this paper is to explain the apparently anomalous occurrence of pan deposits seaward of the modern dune cordon, and not to contribute to refinement of the local Holocene sea-level curve. For a comprehensive review of sea-level change in southern Africa since the Last Glacial Maximum, see Cooper et al.[3]

## Methods

Various locations on the wave-cut platform are conveniently named with concrete markers, and we use these names to identify our samples. We radiocarbon-dated disarticulated individual shell or shell fragments of *Choromytilus meridionalis* from elevated deposits on Schaapen Island (YZF12), Skuimgat (YZF13 & 52) and Spuitgat (YZF49–51) on the coastal wave-cut platform, and from the head of a narrow gulley at Duiwenes (YZF20 & 21). We also dated carbon-rich sediments and a composite of several *Tomichia ventricosa* shells from the strata temporarily exposed by modern storm erosion of 16 Mile Beach (YZF23 & 53); a composite of several modern *Tomichia ventricosa* shells from Rooipan for comparison; disarticulated single *Donax serra* shells from a semi-consolidated sand layer exposed by the storm erosion (YZF24), a shelly horizon within the present coastal dune cordon (YZF22) and an horizon on the inland dune margin of Rooipan (YZF07); and unidentified individual shell fragments from shelly concretion (coquina) from Rooipan se Klippe (YZF11). Figure 1 shows these locations.

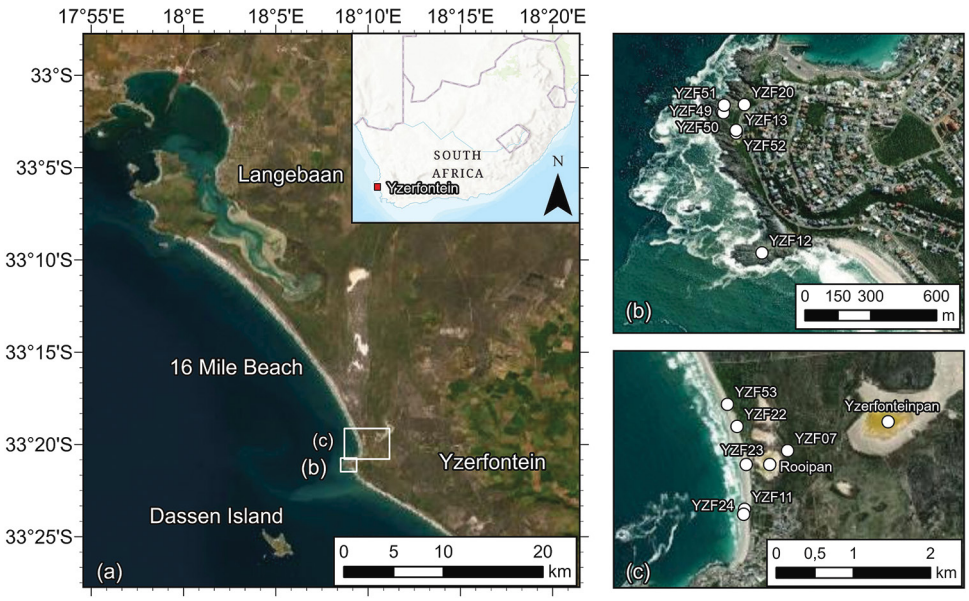


**Figure 1:** Map insert and aerial photographs showing the location of Yzerfontein in South Africa and the sampling locations of molluscs for radiocarbon dating.

**Figure 2:** Photograph taken on 27 June 2017 of the Strandkombuis restaurant on 16 Mile Beach at Yzerfontein, showing collapse due to cumulative storm damage earlier that month.

At each sampling location the elevation was measured using a Hemisphere S320 GNSS system. The transmitter was mounted on a measured pole and held in place until it received signals from a minimum of 50 satellites. Depending on the sites measured, the resolution ranged from 0.5 cm to 30 cm in the X, Y and Z dimensions. Elevation values reported are reduced relative to mean sea level.

Each sample was etched with acid before being crushed and reacted with hydrochloric acid to evolve carbon dioxide from the carbonate.[4] The carbon dioxide was distilled cryogenically, and subject to graphitisation using an iron catalyst in the presence of excess hydrogen.[5] The analysis was performed on the Tandem accelerator at iThemba LABS, Johannesburg, using coal as background and oxalic acid II as a standard. All calculations followed the protocol of Zoppi.[6]

Radiocarbon analysis of freshwater shells may be influenced by a hard-water effect[7], while results from marine shells must account for the marine reservoir effect (ΔR)[8]. These effects are time- and space-dependent functions of the aquatic environment that typically result in age determinations that are older than the true age. In order to account for the hard-water effect, we measured modern *Tomichia ventricosa* shells from Rooipan. Estimates of ΔR for the last 2600 years, and for the period 13 000–10 000 years ago are available, and we used the Western Cape estimate of 147 ± 86 years in the calibration.[9] The soil organic matter result was calibrated using the SHCal20 data set[10] and the marine shell samples were calibrated using the Marine20 data set[11], taking into consideration the difference between the modelled and measured local ΔR values[9]. The online calibration program CALIB ver 8.2 was used (http://calib.org/) for calibrations.[11]

## Results

Table 1 indicates the relative elevations and corresponding radiocarbon dates with their associated calibrations. Marine mollusc shell concretions (coquina) on and just north of Rooipan se Klippe yielded dates of 39 200 ± 2000 (iT-C-1971) and shells from consolidated beach sand from south

**Figure 3:** Mid-Holocene, dark organic mud horizons exposed temporarily in June 2017 by storm erosion of the beach deposits and the coastal dune at the southern end of 16 Mile Beach, Yzerfontein; capped by a yellow layer with polygonal desiccation cracks, on which the man is standing; overlain by two layers of semi-consolidated marine sand, with modern beach cobbles; in turn overlain by aeolian dune sand.

of Rooipan se Klippe yielded 50 500 ± 3400 (iT-C-2133). These dates are considered to be a minimum age estimate as shell assimilates post-depositional carbon over long periods[12], and after calibration these are interpreted as >40 700 cal BP and >50 000 cal BP, respectively. They probably represent the Late Pleistocene (Eemian) Velddrif Formation.[13,14]

We interpret a layer of marine shells exposed in a borrow pit on the inner dune margin of Rooipan (Figure 4) as a beach because of its cobbles, relatively pristine but disarticulated bivalves, and visual similarity to the current Yzerfontein beach. The radiocarbon date of 42 200 ± 2600 (iT-C-1973) on a *Donax serra* shell is also a minimum age estimate, and this calibrates to > 42 200 cal BP.

The terrestrial sediments exposed in 2017 on the beach consisted of a basal layer of finely laminated black mud about 50 cm thick, overlain by an approximately 50 cm thick layer of yellow mud, the upper surface of which had polygonal desiccation cracks. Above that was a double layer about 1 m thick of calcareous, semi-consolidated sand containing black mussel fragments, beneath 3 m of unconsolidated dune sand (Figure 3). The lower, muddy layers contained abundant shells of the pulmonate (air-breathing) snail *Tomichia ventricosa* (Figure 5), which inhabits coastal saline pans[15], and no other molluscs that would suggest a lagoon[16] or estuary[17]. A sample of these shells had a radiocarbon date of 5085 ± 45 (iT-C-1151) (5740–5890 cal BP), while one sample of the organic-rich mud yielded a date of 6370 ± 60 (iT-C-4686) (7170–7320 cal BP) and another that was associated with the *Tomichia ventricosa* shells yielded 4980 ± 60 (iT-C-922) (5600–5850 cal BP). The apparently greater age of the shells compared to the mud enclosing them is due to a hard-water effect on the shells. This is confirmed by the radiocarbon date of 235 ± 40 (iT-C-1968) obtained for modern *Tomichia ventricosa* collected from the surface of Rooipan. The hard-water effect is not subject to calibration. We interpret this sequence (Figure 3) as a mid-Holocene former saline pan, overlain by a sandy marine overwash deposit, capped by a late-Holocene to recent dune.

The present coastal dune cordon near the southern end of 16 Mile Beach contains several shelly layers, deposited either by storm surges or seasonal tidal overwash of low-lying portions, subsequently covered by aeolian sand. We sampled a *Donax serra* shell from a stratified layer about 1 m below the present dune crest and obtained a radiocarbon date of 615 ± 35 (iTh-C-2421) (280–480 cal BP). The present dune cordon is an active late-Holocene feature.

The radiocarbon dates from the storm beaches at elevations up to 11 masl on the rocky shore south of Yzerfontein Point ranged from mid-Holocene to modern. On the rocky promontory of Schaapen Island, at the head of a south-facing gulley there is a 1 m thick deposit of an unconsolidated shelly hash that is undergoing intermittent erosion by modern storm waves. The exposure is a low cliff, 2–3 masl, packed with shell fragments of a wide variety of species and sizes, and containing some pebbles and cobbles of the surrounding diorite rock (Figure 6). The deposit of comminuted and rounded shells is quite distinct in appearance and species composition from that of an archaeological midden, like the nearby one of Bakoond.[18] The Schaapen Island deposit was thought to be mid-Holocene storm beach with an assumed age of around 5000–4000 BP, like examples at similar elevations and ages recorded elsewhere along the west coast.[19] However, with radiocarbon dates of 1120 ± 60 (iT-C-1969) (310–520 cal BP) and 790 ± 45 (iT-C-1992) (0–180 cal BP) at the bottom and top, respectively, it clearly comprises late Holocene storm beach deposits.

The wave-cut platform about 6–12 masl immediately north of Schaapen Island has a mantle of water-worn mollusc shells of diverse species and sizes. There is no evidence of associated archaeological material and the water-worn nature and diversity of species and sizes suggests storm beach deposits. At Skuimgat we sampled a water-worn gastropod (possibly *Argobuccinum pustulosum*), a *Choromytilus meridionalis* shell, and a *Scutellasta granularis* shell from a dune mole-rat heap, and obtained early to mid-Holocene radiocarbon dates of 7980 ± 50 (iT-C-2419) (7990–8240 cal BP), 4190 ± 60 (iT-C-4187) (3730–4050 cal BP) and 3250 ± 45 (iT-C-4185) (2600–2870 cal BP), respectively. This we interpret as a mid-Holocene storm beach deposit containing a fraction of reworked older shells.

At Spuitgat there is an accumulation of mostly comminuted *Choromytilus meridionalis* shells to 11.3 masl. Again, there is no evidence that this is a hunter-gatherer midden, and close inspection showed distinct layers differentiated by the degree of bleaching of the shells. The white, lowermost layer dated to 1825 ± 40 (iT-C-4183) (950–1180 cal BP); a layer of semi-bleached (pink) shells dated to 630 ± 70 (iT-C-4373) ("recent"); and the uppermost unbleached (blue) layer yielded a result of 101.05 ± 0.87 percent modern carbon, which indicates the presence of bomb carbon and must post-date 1950 CE ("modern"). These are storm beach deposits on this exposed rocky promontory.

Duiwenes is a narrow, rocky gulley eroded into the 6–12 masl platform. At the head of the gulley there is a ±3-m-thick deposit of marine shells,

**Table 1:** Radiocarbon dates from onshore marine deposits near Yzerfontein

| Site number | Description | Latitude / longitude | Species / material | Elevation (masl) | Sample number | Radiocarbon age (years BP) | $\delta^{13}C$ (‰) | Calibration (years cal BP) |
|---|---|---|---|---|---|---|---|---|
| YZF12 | Schaapen Island (top) | S 33° 21′ 16.8″<br>E 18° 08′ 52.8″ | *Choromytilus meridionalis* | 5.9 | iT-C-1992 | 790 ± 45 | 0.0 | 0–180 |
| YZF12 | Schaapen Island (bottom) | S 33° 21′ 16.8″<br>E 18° 08′ 52.8″ | *Choromytilus meridionalis* | 5.1 | iT-C-1969 | 1120 ± 60 | 0.0 | 310–520 |
| YZF49 | Spuitgat shell grit (blue) | S 33° 20′ 56.0″<br>E 18° 08′ 46.9″ | *Choromytilus meridionalis* | 9.6 | iT-C-4186 | Post 1950 CE | 2.9 | Modern |
| YZF50 | Spuitgat shell grit (pink) | S 33° 20′ 56.1″<br>E 18° 08′ 47.1″ | *Choromytilus meridionalis* | 9.6 | iT-C-4373 | 630 ± 70 | -2.3 | Recent |
| YZF51 | Spuitgat shell grit (white) | S 33° 20′ 55.0″<br>E 18° 08′ 47.2″ | *Choromytilus meridionalis* | 11.3 | iT-C-4183 | 1825 ± 40 | 1.6 | 950–1180 |
| YZF52 | Skuimgat mole heap | S 33° 20′ 59.1″<br>E 18° 08′ 49.0″ | *Scutellastra granularis* | 10.9 | iT-C-4185 | 3250 ± 45 | 1.5 | 2600–2870 |
| YZF52 | Skuimgat mole heap | S 33° 20′ 59.1″<br>E 18° 08′ 49.0″ | *Choromytilus meridionalis* | 10.9 | iT-C-4187 | 4190 ± 60 | 2.3 | 3730–4050 |
| YZF13 | Skuimgat mole heap | S 33° 20′ 58.7″<br>E 18° 08′ 49.0″ | *Argobuccinum pustulosum* | 10.9 | iT-C-2419 | 7980 ± 50 | 0.0 | 7990–8240 |
| YZF20 | Duiwenes (top) | S 33° 20′ 54.9″<br>E 18° 08′ 50.2″ | *Choromytilus meridionalis* | 11.4 | iT-C-2418 | 4435 ± 50 | 1.1 | 4070–4380 |
| YZF21 | Duiwenes (bottom) | S 33° 20′ 54.9″<br>E 18° 08′ 50.2″ | *Choromytilus meridionalis* | 8.4 | iT-C-2434 | 4270 ± 50 | 1.1 | 3840–4140 |
| YZF22 | 1 m from crest of modern dune | S 33° 19′ 39.8″<br>E 18° 09′ 34.5″ | *Donax serra* | – | iT-C-2421 | 615 ± 35 | 0.8 | Recent |
| YZF23 | Modern shells from Rooipan surface | S 33° 19′ 51.5″<br>E 18° 09′ 42.2″ | *Tomichia ventricosa* | 1.0 | iT-C-1968 | 235 ± 40 | -4.5 | Modern |
| YZF23* | Black muddy sand in modern beach | S 33° 19′ 57.9″<br>E 18° 09′ 38.1″ | Soil organic matter | <1.4 | iT-C-922 | 4980 ± 60 | -25.6 | 5600–5730 |
| YZF23* | Shells from black muddy sand | S 33° 19′ 57.9″<br>E 18° 09′ 38.1″ | *Tomichia ventricosa* | <1.4 | iT-C-1151 | 5085 ± 45 | -4.5 | 5740–5890 |
| YZF53 | Black muddy sand in modern beach | S 33° 19′ 30.5″<br>E 18° 09′ 30.5″ | Soil organic matter | <1.4 | iT-C-4686 | 6370 ± 60 | 1.5 | 7170–7320 |
| YZF07 | Cobble layer of inner dune cordon | S 33° 19′ 50.0″<br>E 18° 09′ 55.7″ | *Donax serra* | 3.2 | iT-C-1973 | 42 200 ± 2600 | 0.8 | >42 200 |
| YZF11 | Rooipan se Klippe shell concretion | S 33° 20′ 14.6″<br>E 18° 09′ 37.7″ | Indeterminate | 1.3 | iT-C-1971 | 39 200 ± 2000 | -0.4 | >40 700 |
| YZF24 | Yellowish, semi-consolidated sand with *Donax* fragments | S 33° 20′ 16.7″<br>E 18° 09′ 37.3″ | Indeterminate | 0 | iT-C-2133 | 50 500 ± 3400 | 0.0 | >50 000 |

**This exposure was buried at the time of elevation measurement, and this result reflects a beach transect measurement from the same location.*

mostly *Choromytilus meridionalis*, currently being eroded by terrestrial drainage through the gulley. Radiocarbon dates of 4270 ± 50 (iT-C-2434) (3840–4140 cal BP) and 4435 ± 50 (iT-C-2418) (4070–4380 cal BP) from near the bottom and the top, respectively, show that this is a stack of mid-Holocene storm beaches.

## Discussion

The occurrence and age of these shell deposits must be viewed against other landscape features that also hold implications for sea-level change and sand supply. We highlight three features: the gypsum-rich pans

**Figure 4:** Layer of Late Pleistocene marine shells and rounded beach cobbles at 3 masl above clean white sand and below the brown soil horizon, exposed in a borrow pit in the dune on the inner margin of Rooipan (sampling location YZF07).

**Figure 5:** Mid-Holocene, dark muddy sediment containing abundant shells of the hypersaline terrestrial mollusc *Tomichia ventricosa*, exposed on 16 Mile Beach in February 2017 (sampling location YZF23).

immediately north of Yzerfontein (Figure 1); the evidence for an interplay between littoral dunes and saline pan features; and the presence of coeval storm beaches on the rocky promontories.

Yzerfonteinpan and Rooipan are relict Late Pleistocene features[15,20] with present surfaces at 1–2 masl. Rooipan is reported to have a basal layer of "sand or shelly limestone of the Late Pleistocene (Eemian/last interglacial) Velddrift Formation"[21]. This deposit indicates a former, Late Pleistocene, marine embayment of unknown extent, that probably also underlies the adjacent Yzerfonteinpan. Both pans have been mined for gypsum, from a layer up to about 2 m thick.[15,21] In Yzerfonteinpan this is intercalated with sand containing bleached *Choromytilus meridionalis* shell fragments, which indicate a Late Pleistocene, perhaps last interglacial (Eemian, 120–130 ka), age.[20] In undisturbed portions of Yzerfonteinpan, there are dark surficial muddy layers, in places containing burrows and shells of *Tomichia ventricosa*.[15,20] The surface of Rooipan has been more thoroughly disturbed by historical mining, and modern *Tomichia ventricosa* are found living directly on the exposed gypsiferous sand.

**Figure 6:** Eroded face of the Late Holocene storm beach at 2–3 masl on Schaapen Island (sampling location YZF12).

The gypsum deposits in both pans result from multiple, successive marine inundations over a coastal bar, followed by evaporation to a brine at least 3.35 times the salinity of sea water, to initiate precipitation of gypsum.[13] The succession of a marine embayment, followed by a pan with deposition behind an overwash bar, implies a falling sea-level, possibly linked to the waning Eemian interglacial. The Late Pleistocene beach deposit at 3 masl, preserved in the dune between the two pans, shows that Rooipan must be somewhat younger than Yzerfonteinpan, but that it developed under the same regime of intermittent inundation and evaporation of sea water behind an overwash bar somewhat further west. With a falling Late Pleistocene sea level, marine overwash ceased, and this bar too eventually was stranded. The cessation of marine overwash and gypsum deposition in both Yzerfonteinpan and the ancestral Rooipan was followed by seasonal inundation by terrestrial runoff, and the deposition of the fine muddy layers inhabited by *Tomichia ventricosa*.

We obtained a mid-Holocene date for the organic mud and *Tomichia ventricosa* shells from the pan deposits preserved beneath the present beach. This implies that the ancestral Rooipan must have been more extensive between 7200 cal BP and 5600 cal BP, with a sand barrier further west than the present dune cordon. We suggest that a substantial dune barrier formed seaward of the current coastline during a Late Pleistocene sea-level lowstand. This barrier protected the ancestral Rooipan from immediate inundation by the rising early Holocene higher sea level. The barrier must have been sufficiently robust and persistent to protect the ancestral Rooipan from the contemporaneous mid-Holocene storm surges recorded by the storm beaches on the exposed rocky platform to the south of Yzerfontein Point, which evidently was not protected by such a barrier.

Eventually the barrier that protected the mid-Holocene saline pan was breached and eroded, contributing its sand to the growing Holocene dune plume[22,23], the inner margin of which may form the present coastal barrier dune now protecting the residual Rooipan. Most of its former extent has been eroded by the sea, which has eroded the beach and dune margin by up to 80 m locally since 1936.[1]

Clearly, the storm beach deposits south of Yzerfontein Point, with various elevations above modern mean sea-level that do not correlate with their age, cannot be used as indicators of former Holocene sea levels, and the single mid-Holocene date for a saline pan just over 1 masl behind a now eroded barrier dune probably does not contribute significantly to refining the local Holocene sea-level curve.

There is little correspondence between published Holocene sea-level curves for the southern African west coast.[13,19,24-26] There appears to be consensus that rising sea level crossed 0 masl around 8000 years ago, with some higher indicators subsequently (Figure 7A)[16], but other than that the published data are inconsistent. Nevertheless, we note that Compton[16,25] has suggested a slight drop in sea-level with sub-aerial exposure of nearshore deposits at about 4500 BP. If this is the case, it may have contributed to protection of the ancestral Rooipan from mid-Holocene inundation.

The evidence presented here indicates that an open embayment existed north of Rooipan se Klippe/Gabbro Point at some point prior to 50 000 cal BP, which probably represents the Eemian sea-level highstand (Figure 7B). During the Last Glacial Maximum, lower sea level led to increased sand supply from the south, forming a dunefield to the west of the current beach. As sea level transgressed the modern level during the mid-Holocene, this dunefield resisted erosion sufficiently for an extensive ancestral Rooipan to form (Figure 7C). The dunefield that protected the ancestral Rooipan from the mid-Holocene sea-level highstand eventually breached, and overwash deposits accumulated on ancestral Rooipan. Ultimately, the beach eroded back to the modern shoreline, forming a littoral dunefield in the process (Figure 7D). The recent erosion of the southern end of 16 Mile Beach[2] is likely a process that has been ongoing through the latter half of the Holocene, with a return to the presumed Eemian shoreline being a possible endpoint.

## Conclusions

The southern parts of 16 Mile Beach have eroded recently[2], while further north coast-parallel dunes have prograded seaward in response to the marine regression since the mid-Holocene.[20] The implication is that palaeogeographic conditions were different between the southern and northern stretches of 16 Mile Beach, and the impact of sea-level change is determined by local sand supply and transport dynamics on these sandy shores. A more general implication is that local palaeoenvironmental reconstruction is crucial to interpreting local coastal change in response to changing sea level. Apparent sea-level change cannot simply be extrapolated from one area to another, even those along contiguous stretches of coastline.

It is anticipated that sea levels will rise over the coming decades in response to global climate change, and past transgressions provide a potential analogue for the impact that this may have on coastal morphology. Local sea-level rise is not a uniform 'tub filling', and our data show that local expression may be dependent on the existence of

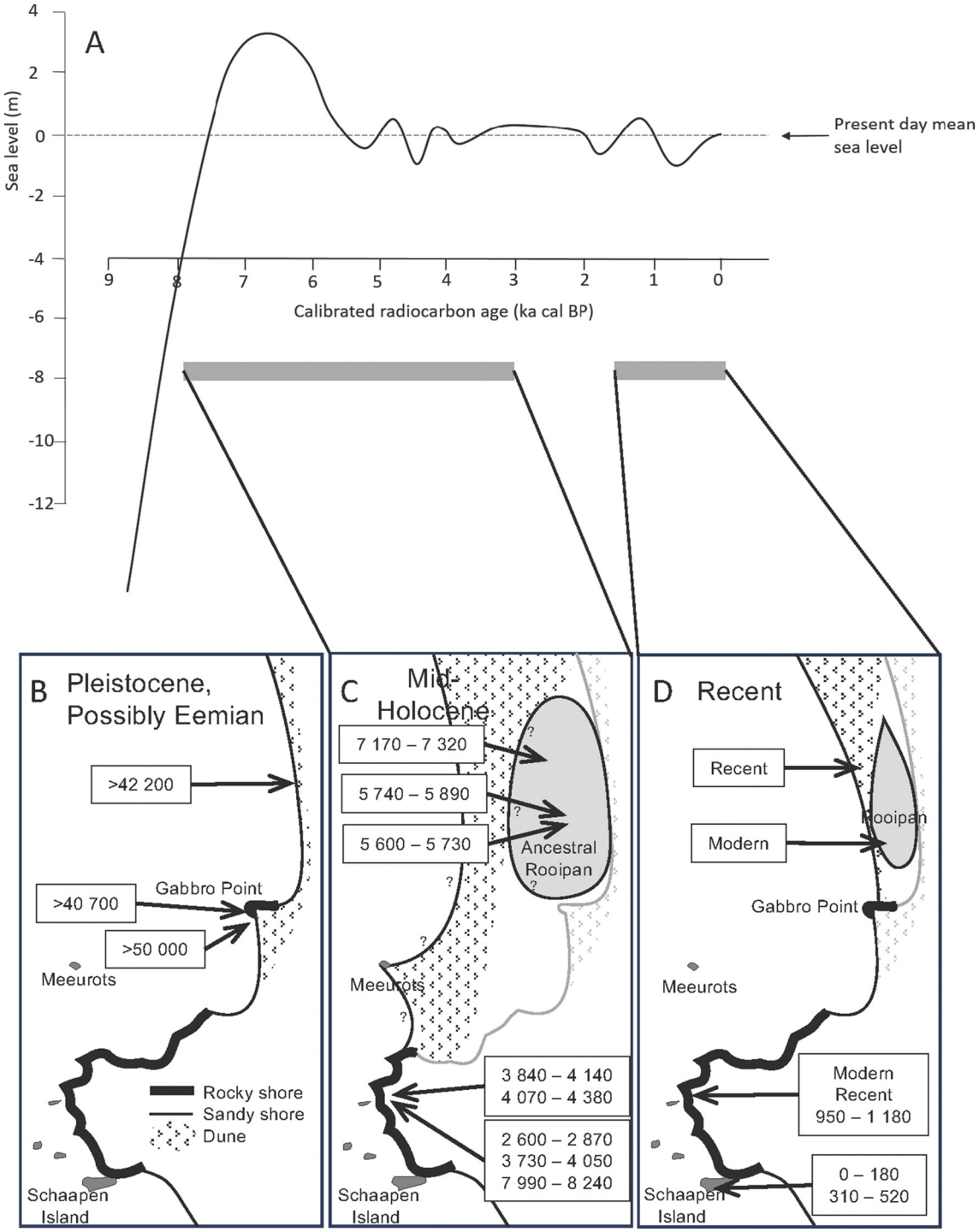


**Figure 7:** Summative diagram illustrating dating evidence for morphodynamic changes to the southern end of 16 Mile Beach in response to sea-level fluctuations. Dates are presented as cal BP ranges. (A) Holocene sea-level curve for the southwestern Cape coast based on data in Compton[16]. (B) Late Pleistocene open embayment north of Gabbro Point during a slightly higher than modern sea level. (C) During the Last Glacial Maximum, lower sea level led to the formation of a dune barrier that protected a larger ancestral Rooipan from erosion by the mid-Holocene transgression, while the rocky shelf to the south was exposed to storm surges. The exact location of the shoreline for this time cannot be determined precisely. (D) The barrier subsequently eroded with a littoral dune forming as the modern shoreline was established.

former barriers, variably resistant to erosion, that for a while may protect back-barrier pans and other landscape features vulnerable to subsequent inundation when these barriers have been breached. At the southern end of 16 Mile Beach the present dune barrier is eroding actively, its shoreward face having shifted some 30 m inland since the beginning of 2017.[2] This has obvious implications for infrastructural development on top of and immediately behind the present dune cordon. Any increase in storminess due to climate change may breach the lowest portion of this narrow dune barrier, which has been eroded beyond its former crest and is now only about 3 masl. The result may be intermittent flooding of Rooipan, with further damage to built infrastructure on its margins. The ultimate expression of this process may be the loss of the present dune cordon altogether, with the beach returning to the position of the Pleistocene inner dune cordon that marks the landward edge of Rooipan.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

S.W.: Conceptualisation; methodology; data collection; sample analysis; data analysis; writing – revisions. D.M.: Conceptualisation; methodology; data collection; data analysis; writing – initial draft; writing – revisions; project leadership; funding acquisition. M.E.: Data collection. H.C.: Data collection. S.W.: Sample analysis.

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# The pros and cons of buccal swabbing and tail clipping for monitoring reptilian biodiversity


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In biodiversity research, the retrieval of genetic material from organisms is a common and essential component for assessing genetic diversity. The welfare of the organism, however, needs to be balanced against the overall goal of the intended research. One sampling technique often applied to retrieve DNA material from small reptiles is the removal of a small portion of the distal end of the tail. While most squamate reptiles have tail autotomy, some species (e.g. many iguanid lizards and snakes) do not regenerate tail tissue. We therefore explored the efficacy of a minimally disruptive technique, buccal swabbing, as an alternative to tissue sampling via tail clipping, particularly for species without tail autotomy, using dwarf chameleons (*Bradypodion* spp.) as a case study. The two sampling techniques were compared to assess the efficacy of DNA retrieval. We also evaluated the financial implications of each technique. The results indicate that buccal swabs paired with a specialised DNA extraction kit offer a feasible (although expensive), once-off alternative to tissue sampling, but with no material left for biobanking. Deviations in swab type used and the DNA extraction process (i.e. using more affordable extraction procedures) resulted in poor DNA retrieval and unreadable sequences. This finding suggests that buccal swabbing can be a suitable alternative when finances are not constrained, an expensive extraction kit is available, and biobanking is not a concern. For researchers from low- to middle-income economies, this expensive alternative may hamper research progress by placing a financial obstacle in the way, and therefore the next best option is tissue sampling.

**Significance:**

This study provides guidance on the efficacy of buccal swabs as a viable alternative to tissue samples collected via tail clipping for DNA retrieval from small reptiles. The results indicate that swabs may be a feasible alternative to tissue samples when finances are not constrained. Deviations in buccal swabbing method (i.e. using more cost-effective alternatives) performed poorly in DNA retrieval and do not offer competitive alternatives to tissue samples. Although buccal swabs were shown to offer an alternative to tissue samples, the financial implications to research in low- to middle-income economies may hinder research goals unnecessarily.


## Introduction

Knowledge of the interactions between organisms, communities, and ecosystems is key to the implementation of successful biodiversity research; however, the active pursuit of this knowledge might have unintended consequences relating to the welfare of the studied organisms.[1] For example, the act of animal capture and handling can cause distress or even mortality in animals.[2] This dichotomy is undesirable, as biodiversity research and animal welfare are not diametrically opposed – both seek to guide mandates for the betterment of biodiversity protection and animal well-being, albeit in different ways.[3,4] A growing awareness of potential negative side effects from various sampling techniques and data collection methodologies, as well as the interplay between these two fields, has prompted the exploration of alternative, less disruptive methodologies for animal sampling used in conservation research.[5-9] These alternatives could be better used to apply the principles of 'Replacement, Reduction, Refinement' (also known as the 3Rs) when carrying out genetic sampling of non-primate, living animals.[10-12]

Customarily, the retrieval of multicellular organismal DNA involves the collection of tissue or blood samples from individuals, often by means that have differing levels of invasiveness (e.g. entire specimen collection and biopsy of organs, tissue biopsy with animal release, blood collection through venipuncture).[8,13,14] A common practice for collecting tissue samples from reptiles is to remove a small section (ca 1–3 mm) from the tip of the tail.[7,13] This approach may have little effect on squamates that have tail autotomy and regeneration as a predator defence mechanism.[15,16] Nevertheless, the loss of large portions of the tail probably has costs on the individual's survivorship and reproduction, so the proportion of the tail removed is usually minimised. In contrast, some species (e.g. snakes and many iguanid lizards) do not possess the ability to regenerate their tail. The permanent loss of caudal tissue therefore might be considered a lasting impairment.[7] The potential effects on survival or reproduction, however, are correlated to the amount of tissue lost, as well as body form and adaptive behaviour.[15] Therefore, the removal of a small proportion is typically deemed as meeting ethical guidelines relating to the 3Rs.

For species with tail autotomy, the rate of re-growth is also important for considering the ultimate costs to the individual. While the cost–benefit to the individual animal has been weighed[15,17-19], there has been less attention on the cost–benefit of invasive versus minimally disruptive methods for sampling of squamates with no tail autotomy. Clearly, research on wild populations of animals has important knowledge outcomes that affect how we protect and conserve the biodiversity of our planet. Therefore, we cannot afford to eschew foundational studies needed to gain this knowledge, but the balance between animal welfare and successful research needs to be put into perspective.

There are non-invasive alternatives for DNA collection, including retrieving DNA from excretions or exuviates (e.g. moults).[20-23] Although non-invasive sampling is ideal in terms of the 3Rs, it is not always achievable as organismal traces may be more difficult to locate than the organism in question, and the sample is most likely of lower quality

than tissue directly removed from a living animal.[24] Although some minimally disruptive techniques – such as extracting DNA from urine[25] or saliva[26] – could offer alternatives to DNA sampling, these methods also have drawbacks. The former could require prolonged containment periods until urine is produced. The latter could require a considerable amount of time spent handling the animal to gather the saliva. Thus, even minimally invasive methods have a degree of disruption that can cause stress.

The quality of these sample types varies widely and alterations to standard protocols are often needed to ensure adequate DNA retrieval.[22,23] One alternative method sometimes proposed for DNA sampling of large to moderately sized lizards is buccal swabbing.[13,14,27] This process includes insertion of a sterile swab into the mouth of the live animal, followed by moderate rotation of the swab to sample epithelial cells from the oral environment. This technique has led to successful DNA retrieval in lizards previously[28] and has been presented as a viable alternative to tissue sampling via tail clipping for DNA extraction[29]. The method is assumed to present few permanent side effects (with the initial stress from handling being the primary negative impact to the animal), as well as requiring minimal researcher training beyond animal handling. Therefore, swabbing is thought to be a less disruptive alternative to tail clipping in reptiles with nonautotomous tail-regeneration and studies have shown reliable retrieval of sufficiently high-quality DNA with the use of buccal swabs for some reptiles.[14,27,29]

In larger animals, buccal swabbing presumably causes no direct tissue damage and little distress.[14,29,30] In smaller animals, the tissue damage and/or stress levels from the handling during buccal swabbing are not known, but some bleeding of the buccal epithelium has been noted in amphibians[31], suggesting that in some cases there could be tissue damage despite the method being considered minimally disruptive. It appears that the efficacy of buccal swabbing is subject to the size of the buccal cavity of the organism relative to the size of the swab. In addition, buccal swabbing may cause less longer-term stress to an individual than clipping practices[32]; however, tail clipping offers a faster process with minimal handling time (measured in seconds), whereas buccal swabbing requires an extended handling time (measured in minutes). Additionally, the ability of the buccal cavity to house rich microbial diversity[33] raises concerns over the retrieval of high-quality host DNA, as microbial DNA may oversaturate the extractions.

Another factor that should be considered when evaluating the viability of a sampling technique is the cost post-sampling. This is especially pertinent in the field of biodiversity conservation because most global biodiversity is located in low- to middle-income countries where the lack of economic prosperity does not enable the prioritisation of conservation research.[34,35] Moreover, researchers in these countries typically face numerous financial barriers.[36] Thus, cost-effectiveness is a primary concern in the retrieval of DNA for studies conducted within these economically impoverished regions.

In the present study, we compared the efficacies of tail clipping and buccal swabbing using five species of small lizards in the genus *Bradypodion* (dwarf chameleon). Chameleons do not exhibit tail autotomy and regeneration; thus, DNA samples have historically been taken via a small tail clipping or from euthanised specimens. Given that the chameleon tail is functional in terms of locomotion[7,37,38], removal of a small portion of the tail might have an unintended effect on the individual, although direct investigation of this did not show diminished grip performance.[7] To assess whether buccal swabbing is a viable alternative to tail clipping for these small lizards, we first sought to investigate whether there is a trade-off between disruptiveness and DNA yield (both in terms of quantity and quality). We then sought to determine whether buccal swabs produced DNA of sufficient quality to allow for sequencing to species level, and, finally, we evaluated whether there is a trade-off between the cost of sampling and the yield of DNA.

## Method and materials

Five species (*Bradypodion damaranum, B. melanocephalum, B. setaroi, B. thamnobates*, and *B. ventrale*), with 10 individuals per species, were sampled in situ. Three different swab types and/or buffers were tested to investigate the impact of different cost options. The high-cost option included using sterile cotton FLOQswabs to collect buccal epithelial tissue from *B. melanocephalum, B. setaroi*, and *B. thamnobates*. These swabs were then stored in Zymo DNA/RNA Shield™ Collection Tubes. The moderate-cost option used sterile cotton FLOQswabs to collect buccal epithelial tissue from *B. ventrale*, which were then stored in Nucleic Acid Preservation (NAP) buffer. The low-cost option used sterilised cotton 'Q-tips' to collect buccal epithelial tissue from *B. damaranum* which were then stored in NAP buffer. Swabbing was achieved by gently coercing the chameleon to open its mouth after which the swab was rotated in the buccal cavity for approximately 1 min, occasionally longer. For each swabbed individual, a tail clip (ca 1–3 mm of distal tissue) was also taken using sterilised stainless-steel scissors and then preserved in NAP buffer. All samples were stored at −40 °C until DNA extraction. The use of different chameleon species for the different sampling protocols (swab type, preservation buffer) is not considered to be a confounding factor as there was no notable differences in species temperaments, as all sample collection was achieved with similar individual reactions. Moreover, all chameleons sampled were of similar size, and we were primarily interested in assessing the performance of the different swab and preservation types, as well as whether any of the techniques applied could identify samples to species level.

Total DNA was extracted from the 30 buccal swabs, sampled for the high-cost option using a Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit following the protocol provided in the user manual, with extended agitation time during the Bashing Bead (Zymo Research Corporation) step to accommodate the swabs. The Zymo kit was paired with the high-cost swabs, as means to evaluate the peak effectiveness for DNA extraction when FLOQswabs are combined with a specialised extraction kit. A second extraction was carried out for the 20 buccal swabs of moderate- and low-cost samples (10 FLOQswabs and 10 'Q-tips', respectively) with the use of a Qiagen DNeasy® Kit with an initial round of agitation via vortex, during tissue lysis, in order to accommodate the swabs in a similar manner to the Zymo kit extractions. The Qiagen kit was chosen for the moderate- and low-cost methods as a means to determine whether minimising costs through a non-specialised kit was a viable alternative for DNA retrieval. For all swab extractions, the entire sample was consumed during the extraction process. As a direct comparison between swabs and tail tissue, total DNA was also extracted from 50 tail clips using the same DNA Qiagen DNeasy® Kit following the manufacturer's protocol. For tail clips, approximately 2–4 mg of tail tissue was used, leaving all remaining tissue for DNA banking. Final elution volume for all samples was 50 μL.

Following DNA extraction, total nucleic acid concentrations (ng/μL) (both RNA and DNA) were quantified with the use of a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, USA), as well as measures of contaminant concentrations in the form of: $OD_{260/280}$ and $OD_{260/230}$.[39] Further quantification, specifically targeting dsDNA concentrations (ng/μL), were quantified with a Qubit 3 Fluorometer (Life Technologies) using a Qubit dsDNA HS Assay Kit (high sensitivity, 0.2–100 ng). The Qubit 3 allows for a higher specificity during quantification[40], compared to the NanoDrop One, and allowed for focused measurement of only the dsDNA in the extractions. These measures were then used to compare the effective DNA retrieval between tail tissue and buccal swabs through paired *t*-tests.[41]

To ensure extracted DNA from buccal swabs was representative of the host organism and not microbial saturation and to check the downstream use of the extracts, the *16S* mitochondrial gene was amplified for all sample types using primers 16Sa (5' CGC CTG TTT ATC AAA AAC AT 3') and 16Sb (5' CCG GTC TGA ACT CAG ATC ACG T 3').[42] Polymerase chain reaction (PCR) amplifications were completed in 25 μL reactions consisting of: 2.5 μL reaction buffer; 2.5 mM $MgCl_2$; 2 μM of each primer; 0.2 mM dNTP solution; 0.02 U/μL Taq Polymerase (SuperTherm); and 25–50 ng/μL of DNA template. PCR cycling conditions followed initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. All amplicons were then visualised on a 1% agarose gel with the use of SmartGlow™.

A subsample of 30 amplicon products (three per species per sample type) were Sanger sequenced at Macrogen Inc. (Amsterdam, the Netherlands) to confirm amplification of the target gene. All sequences were trimmed and aligned using Geneious R11 (https://www.geneious.com), and checked against the GenBank sequence database using the BLAST (Basic Local Alignment Search Tool: https://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm plugin in Geneious. The highest similarity scores for each sequence were taken as species identity. New DNA sequences generated for this study were deposited in GenBank (OR575523 – OR575547).

A cost analysis was generated based on the procedures and reagents used for each set of samples. Based on total cost, this was split into three independent options for buccal swabbing: the high-cost option; the moderate-cost option; and the low-cost option. The total cost for the processing of tail tissue was included for comparison. The costs were estimated over the different stages of the process: sample collection, extraction kit, DNA amplification, and sequencing – which were further subdivided into various reagents and processes. This allowed for a cross comparison between procedural cost and effectiveness of DNA retrieval.

All animal handling and sample collection was approved by both the University of the Witwatersrand (ethics no.: 2019/10/56/B) and the University of Johannesburg (ethics no.: 2019-10-10/van Vuuren_ Tolley). Research was carried out under permits from the relevant South African provinces: Gauteng (CPF6 000219), KwaZulu-Natal (OP2635/2020); Eastern Cape (RSH 24/2021); and Western Cape (CN44-59-11927).

## Results

Direct comparison of total nucleic acid concentration in extracted DNA solutions (i.e. using the NanoDrop One) showed, on average, higher yield and purity from tail tissue across all five species, regardless of sampling method (Table 1; Supplementary tables 1 and 2). The concentration of nucleic acid retrieval from buccal swab samples was only statistically lower from the nucleic acid retrieved from the tail clippings for one of the species from the high-cost sampling, *B. setaroi.* Furthermore, both the moderate- and low-cost swabbing options produced low levels of nucleic acid retrieval and purity (Table 1; Supplementary table 2).

Although these findings demonstrated the successful retrieval of nucleic acids, more focused quantifications of dsDNA concentrations were taken with a Qubit 3 Fluorometer to ensure the removal of any confounding variables (e.g. RNA, free-floating nucleotides), as NanoDrop One quantification is nonspecific in nucleic acid concentrations.[39] Averaged measures of dsDNA concentrations in the extracted samples showed similar product retrieval from the high-cost option and the tail tissue (Figure 1). Quantification of dsDNA concentrations from the moderate- and low-cost options, however, indicated poor dsDNA retrieval from all buccal swabs, whilst the corresponding tail tissue had high dsDNA retrieval (Figure 1).

Amplicon visualisation on agarose gels (Figures 2–4) showed that all tail tissue produced clear bands at the target region size for the *16S* gene. Amplification of the same region in the buccal swab samples was inconsistent. All swab samples were amplified for *B. melanocephalum* (S11–S20), and *B. thamnobates* (S21–S30). However, swab samples from *B. setaroi* showed inconsistencies in that samples S3 and S6 produced multiple bands, and samples S5 and S7 produced no bands (Figure 2). Most swab samples from *B. damaranum* and *B. ventral*e did not produce visible bands on the agarose gels (Figures 3 and 4).

For the sequenced samples, similarity searches using BLAST generated identifications for 25 of the 30 sequenced individuals (Table 2). Five swab samples (17%) were not successfully identified due to non-amplification or a low-quality DNA sequence, with one of these sequences originating from the high-cost samples. The BLAST identifications produced matches that confirmed field identification for five of the nine high-cost swab samples (Table 2). All samples from *B. melanocephalum* showed the best match with *B. thamnobates* sequences on GenBank; however, the *16S* gene does not provide sufficient resolution between these two closely related sister species to always provide appropriate species level similarity scores. In the sequenced swab samples originating from the moderate- and low-cost methods, confirmation of field identification was only produced for two samples (Table 2). The corresponding tail tissue samples, however, resulted in clear BLAST identifications for all six samples. The best match (highest similarity score with the highest coverage) for the identified *B. damaranum* sample was for GenBank

**Table 1:** Average nucleic acid concentrations (ng/μL) and average absorbance readings ($OD_{260/280}$; $OD_{260/230}$) of all extracted products measured on a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, USA) showing tail tissue samples (T1–T50) and buccal swab samples (S1–S50) grouped by species of *Bradypodion*. Included is the *p*-value of a paired *t*-test comparing the nucleic acid concentrations between sample types per species with significance ($p < 0.05$) shown by bold values.

| Sample type | Nucleic acid concentration (ng/μL) | $OD_{260/280}$ | $OD_{260/230}$ | *p*-value |
|---|---|---|---|---|
| ***Bradypodion setaroi*** | | | | |
| Tissue (T1 – T10) | 8.594 | 1.878 | 0.997 | **0.004** |
| Swab (S1 – S10) | 2.917 | 1.376 | 0.079 | |
| ***Bradypodion melanocephalum*** | | | | |
| Tissue (T11 – T20) | 8.857 | 1.882 | 1.087 | 0.214 |
| Swab (S11 – S20) | 6.355 | 1.424 | 0.082 | |
| ***Bradypodion thamnobates*** | | | | |
| Tissue (T21 – T30) | 19.582 | 1.841 | 1.138 | 0.051 |
| Swab (S21 – S30) | 6.585 | 1.393 | 0.098 | |
| ***Bradypodion ventrale*** | | | | |
| Tissue (T41 – T50) | 14.627 | 2.149 | 1.203 | **< 0.001** |
| Swab (S41 – S50) | 3.947 | 1.268 | 0.675 | |
| ***Bradypodion damaranum*** | | | | |
| Tissue (T31 – T40) | 6.958 | 1.901 | 0.410 | **0.001** |
| Swab (S31 – S40) | 2.980 | 1.489 | 0.513 | |

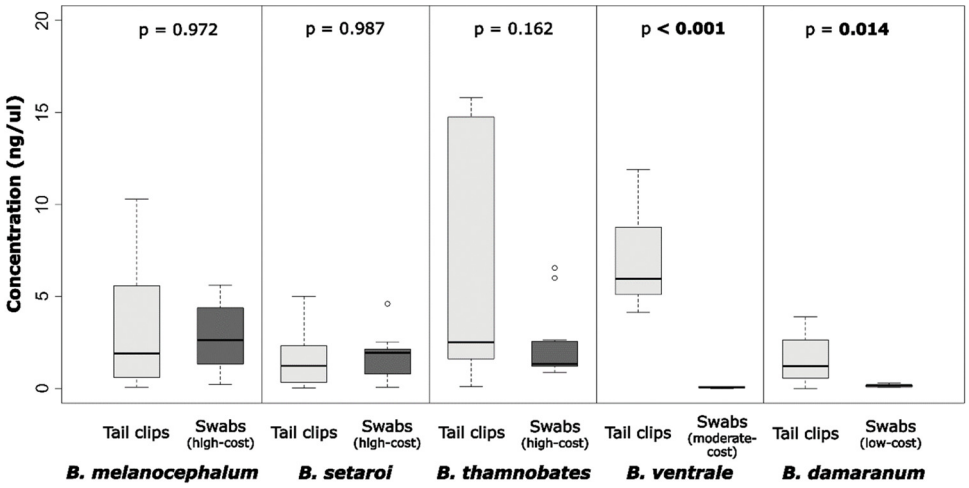


**Figure 1:** Box-and-whisker plots for dsDNA concentrations (ng/μL) of extracted products measured on a Qubit 3 Fluorometer (Life Technologies) with Qubit dsDNA HS Assay Kit (high sensitivity 0.2–100 ng) comparing tissue samples from tail clips [light grey] to buccal swab samples [dark grey] for each species (*Bradypodion melanocephalum, B. setaroi, B. thamnobates, B. ventrale*, and *B. damaranum*). Reflecting $n = 10$ for each sample type per species. Included are *p*-values displaying the significance of difference between sample type within each species, with significance ($p < 0.05$) shown by bold values.

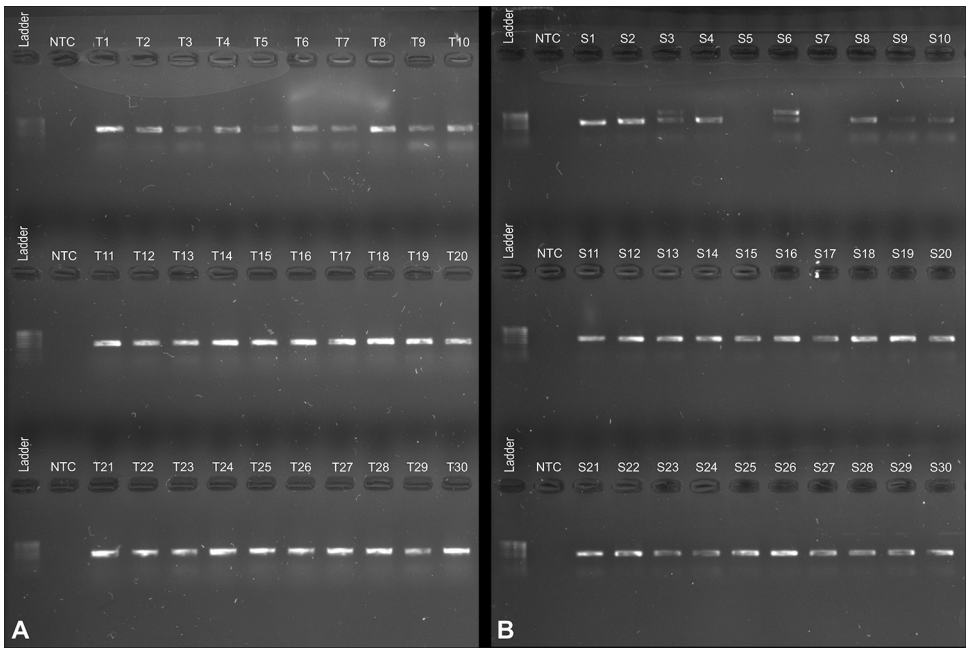


**Figure 2:** Visualised amplicon products for high-cost swab samples and corresponding tissue samples, with 1 kb ladder on 1% agarose gel visualised with SmartGlow™ for *Bradypodion setaroi* (T/S1–10), *B. melanocephalum* (T/S11–20), and *B. thamnobates* (T/S21–30): (A) gel image depicting tail tissue amplicon products (three no-template controls (NTC) and samples T1–T30); (B) gel image depicting buccal swab amplicon products (three no-template controls (NTC) and samples S1–S30).

accession AF121957 (i.e. *Chameleo dilepis*). We, however, consider the *C. dilepis* GenBank sequence to be erroneous as the sequence does not match any of the other *C. dilepis* on GenBank, only matching multiple *Bradypodion* sequences within a range of 100–90% similarity. Therefore, the second-best match (100% similarity, albeit with lower coverage) was taken as sequence identity for these samples, corresponding to GenBank accession MZ810539 (i.e. *Bradypodion damaranum*).

Estimations of the costs of the three variations in buccal swab sampling methods were generated in local currency (ZAR: South African rands) and converted to US dollars (USD) at an exchange rate as of 27 April 2023 of ZAR18.27 = USD1 (Table 3). These methods were: a high-cost option which made use of a Zymo collection FLOQswab, stored in DNA/RNA Shield™, extracted with a Zymo Quick-DNA™ Fecal/Soil Microbe Miniprep Kit amounting to cost of ZAR331.73 (USD18.15); a moderate-cost option which made use of a Zymo collection FLOQswab, stored in NAP buffer, and a Qiagen DNeasy® Kit for the DNA extractions, amounting to a cost of ZAR187.46 (USD10.26); and a low-cost option which made use of a sterilised cotton ear bud ('Q-tip') as the swab, stored in NAP buffer, and a Qiagen DNeasy® Kit for the DNA extractions, amounting to a cost of ZAR165.34 (USD9.05). Costs for the processing of tissue samples included: storage in NAP buffer, and DNA extraction with a Qiagen DNeasy® Kit, amounting to a cost of ZAR165.11 (USD9.04). All variations had the same estimated costs for PCR and sequencing.

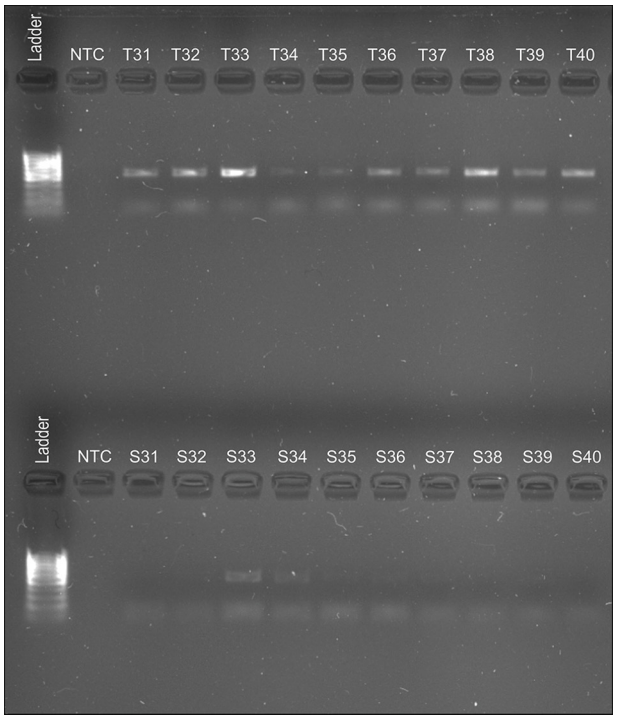


**Figure 3:** Visualised amplicon products for *Bradypodion damaranum* samples, representing the low-cost methodology, on 1% agarose gel visualised with SmartGlow™ showing 1 kb ladders, no-template controls (NTC), tissue samples (T31–T40), and buccal swab samples (S31–S40).

## Discussion

The main aim of this study was the exploration of the efficacy of buccal swabs as an alternative methodology for DNA retrieval compared to tail clippings using nonautotomous reptiles as a case study. The efficacy of buccal swabs as an alternative methodology was demonstrated with the successful retrieval of nucleic acids (Supplementary tables 1 and 2) and dsDNA material (Figure 1) for all examined methods. There was disparity, however, in the levels of dsDNA retrieval in the buccal swabbing methods, with the moderate- and low-cost swabbing options performing poorly in comparison to tail tissue. Only the use of high-cost FLOQswabs, with appropriate storage media and DNA extraction kits, are a comparable method of DNA retrieval to tail tissue. In juxtaposition to this, however, cheaper alternatives to FLOQswabs, and non-optimal extraction kits performed poorly in dsDNA retrieval from buccal swabs and do not present a suitable alternative to the use of tail tissue when high DNA yield is required.

Spectrophotometer readings using the Qubit instrument indicated that the extractions from the two swab samples (both *B. setaroi*) from the high-cost option with unsuccessful amplifications (S5 and S7; Figure 2) had less than 1 ng/μL of dsDNA in solution, suggesting suboptimal concentrations, with even less host mitochondrial DNA being available from that. This may have occurred as a result of suboptimal sampling which did not abrade the buccal epithelium sufficiently due to the small gape size of some individuals; however, this was not apparent in other individuals of this species. *Bradypodion* are small lizards with adult body sizes ranging from about 45 mm to 80 mm, species dependent.[43] The size of the swabs available may be too large to be accommodated by these relatively small species. In contrast, buccal swabs have been successfully used on *Chamaeleo*[14] which are much larger lizards ranging from 180 mm to 250 mm, species dependent[43]; suggesting that gape size (as related to body size) is a major factor in the successful retrieval of sufficient cellular material from the buccal cavity. This may indicate that a more intensive swabbing approach would need to be implemented

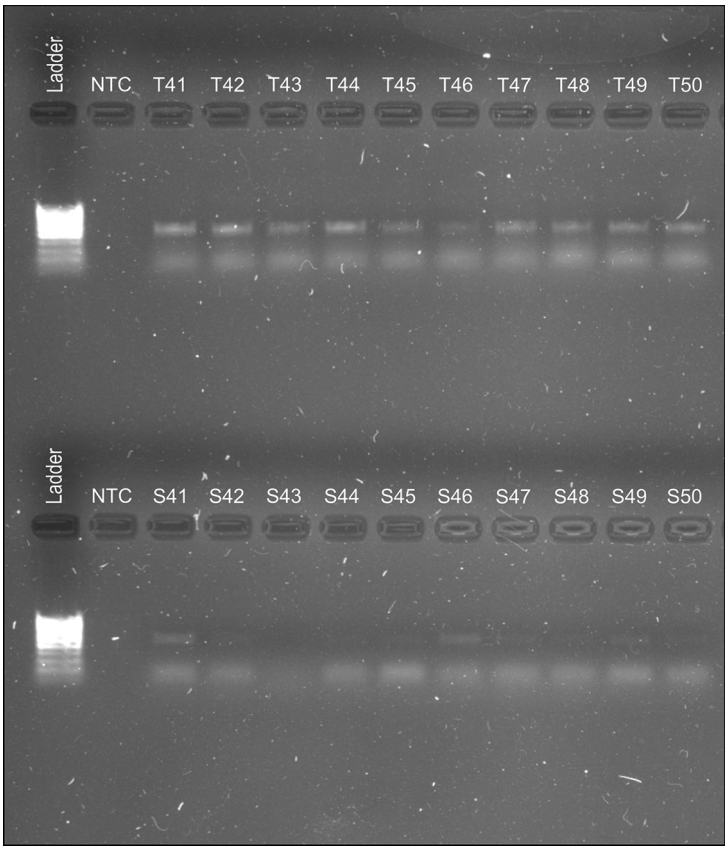


**Figure 4:** Visualised amplicon products for *Bradypodion ventrale* samples, representing the moderate-cost methodology, on 1% agarose gel visualised with SmartGlow™ showing: 1 kb ladders, no-template controls (NTC), tissue samples (T41–T50), and buccal swab samples (S41–S50).

**Table 2:** Similarity results for each sequence identified with the BLAST algorithm. Given are the *16S* GenBank accession numbers for each sample, query coverage, percentage similarity, species identity, and GenBank accession number of the most similar sequence. All samples are grouped by species.

| Sampled species | Sample | GenBank accession number | Query coverage (%) | Similarity (%) | Species identity of GenBank match | GenBank accession number |
|---|---|---|---|---|---|---|
| *Bradypodion setaroi* | T1 | OR575532 | 91.75 | 100.00 | *Bradypodion setaroi* | DQ234637 |
| | T2 | OR575533 | 88.82 | 100.00 | *Bradypodion setaroi* | DQ234637 |
| | T4 | OR575534 | 90.66 | 100.00 | *Bradypodion setaroi* | DQ234637 |
| | S1 | OR575539 | 87.35 | 100.00 | *Bradypodion setaroi* | DQ234637 |
| | S2 | OR575538 | 91.34 | 98.40 | *Bradypodion setaroi* | DQ234637 |
| | S4 | OR575540 | 89.98 | 100.00 | *Bradypodion setaroi* | DQ234637 |
| *Bradypodion melanocephalum* | T14 | OR575537 | 89.76 | 100.00 | *Bradypodion melanocephalum* | AY289825 |
| | T16 | OR575536 | 91.60 | 100.00 | *Bradypodion melanocephalum* | AY289825 |
| | T18 | OR575529 | 88.54 | 100.00 | *Bradypodion melanocephalum* | AY289825 |
| | S14 | OR575531 | 91.63 | 100.00 | *Bradypodion melanocephalum* | AY289825 |
| | S16 | OR575530 | 95.32 | 99.30 | *Bradypodion melanocephalum* | AY289825 |
| | S18 | No signal; poor sequence quality | | | | |
| *Bradypodion thamnobates* | T22 | OR575535 | 88.69 | 100.00 | *Bradypodion melanocephalum* | AY289814 |
| | T28 | OR575528 | 89.36 | 100.00 | *Bradypodion melanocephalum* | AY289814 |
| | T30 | OR575527 | 89.56 | 100.00 | *Bradypodion melanocephalum* | AY289814 |
| | S22 | OR575547 | 92.18 | 100.00 | *Bradypodion melanocephalum* | AY289814 |
| | S28 | OR575545 | 100.00 | 97.50 | *Bradypodion melanocephalum* | AY289814 |
| | S30 | OR575546 | 89.80 | 100.00 | *Bradypodion melanocephalum* | AY289814 |
| *Bradypodion ventrale* | T41 | OR575523 | 97.27 | 99.50 | *Bradypodion ventrale* | AY756645 |
| | T43 | OR575525 | 94.43 | 99.30 | *Bradypodion ventrale* | AY756645 |
| | T44 | OR575526 | 94.26 | 99.50 | *Bradypodion ventrale* | AY756645 |
| | S41 | No signal; poor sequence quality | | | | |
| | S43 | OR575524 | 98.06 | 90.10 | *Bradypodion ventrale* | DQ234636 |
| | S44 | No signal; poor sequence quality | | | | |
| *Bradypodion damaranum* | T31 | OR575544 | 96.37 | 99.80 | *Bradypodion damaranum* | MZ810539 |
| | T32 | OR575543 | 97.29 | 100.00 | *Bradypodion damaranum* | MZ810539 |
| | T33 | OR575542 | 94.89 | 100.00 | *Bradypodion damaranum* | MZ810539 |
| | S31 | No signal; poor sequence quality | | | | |
| | S32 | No signal; poor sequence quality | | | | |
| | S33 | OR575541 | 92.74 | 100.00 | *Bradypodion damaranum* | MZ810539 |

**First hit for* B. damaranum *samples T31, T32, T33, and S33 matched GenBank (AF121957); however, subsequent inquiry identified incorrect sequence identity for AF121957 on GenBank*

for consistent retrieval of high levels of host DNA; however, this could lead to long-lasting stress in the animals.[44]

A further two samples from *B. setaroi* (S3 and S6; Figure 2) produced multiple bands during amplification. This non-specific binding may be a consequence of unknown microorganismal DNA amplifying with the *16S* universal primers due to oversaturation in the extractions; however, it is unknown if this is due to non-optimal primer optimisation or unforeseen similarities in nucleotide bases between microbiota and the host. Nevertheless, this did not impede the amplification of the host gene region at the target length and could be removed before sequencing through PCR clean-up, making it minimally problematic.

The buccal swab samples from the moderate- and low-cost options had multiple unsuccessful amplifications based on the lack of visible bands in the 1% agarose gels (Figures 3 and 4). Furthermore, measures of nucleic acid retrieval and contamination ratios of the buccal swabs (Supplementary table 2) suggested poor DNA retrieval with high solution contamination in comparison to the respective tail tissue (Supplementary table 1). The likely cause for this discrepancy in the

**Table 3:** Relative cost, in South African rands (ZAR) and US dollars (USD) [at an exchange rate of ZAR18.27 = USD1 as of 27 April 2023], of sample processing for four options, namely: buccal swabbing low-cost, buccal swabbing moderate-cost, buccal swabbing high-cost, and tail tissue. Sample processing is divided into methodology stages. Lastly, accumulative cost for each methodology is shown.

| Method stage | Item/service | Cost per sample | Swabbing low cost | Swabbing moderate cost | Swabbing high cost | Tail tissue |
|---|---|---|---|---|---|---|
| Sample retrieval | NAP buffer with 2.0 mL Free Standing Screw Cap Tube | ZAR0.50 (USD0.03) | ✓ | ✓ | | ✓ |
| | Cotton 'Q-tips' | ZAR0.23 (USD0.01) | ✓ | | | |
| | Zymo Collection Swab | ZAR22.35 (USD1.22) | | ✓ | | |
| | DNA/RNA ShieldTM Collection Tube w/Swab | ZAR163.69 (USD8.96) | | | ✓ | |
| Extraction kit | Qiagen DNeasy® Kit (50) (with consumables) | ZAR74.61 (USD4.08) | ✓ | ✓ | | ✓ |
| | Zymo Quick DNATM Fecal/ Soil Microbe MiniPrep Kit | ZAR78.00 (USD4.27) | | | ✓ | |
| PCRs | Standard PCR (primers, reagents, and consumables) | ZAR10.00 (USD0.55) | ✓ | ✓ | ✓ | ✓ |
| Sequencing | Macrogen sequencing cost | ZAR80.00 (USD4.38) | ✓ | ✓ | ✓ | ✓ |
| Total cost | | | ZAR165.34 (USD9.05) | ZAR187.46 (USD10.26) | ZAR331.73 (USD18.15) | ZAR165.11 (USD9.04) |

low-cost option is due to the 'Q-tips' not acting as an amply abrasive medium for sampling epithelial tissue in the buccal cavity. This would suggest that 'Q-tips' are not a viable alternative to FLOQswabs for collection of epithelial tissue, likely as a consequence of the softer cotton structure causing sub-optimal abrasion resulting in minimal epithelial cell, and hence DNA, presence. This result indicates that FLOQswabs and 'Q-tips' paired with non-optimal extraction kits result in poor DNA retrieval.

The high similarity score for identified *B. damaranum* sample matched to GenBank accession AF121957 (i.e. *Chameleo dilepis*) was treated as erroneous. Therefore, the second-best match was taken as sequence identity for these samples, corresponding to GenBank accession MZ810539 (i.e. *Bradypodion damaranum*). The similarity of the *16S* gene sequence between *B. thamnobates* and *B. melanocephalum* was not considered problematic as there are limited *16S* sequence data for *B. thamnobates* available on GenBank for comparison, coupled to the similarity of gene sequences between these two species due to their recent divergence (ca 1.5 Mya; late Miocene[45]). Overall, the identification of species through BLAST searches of these sequences provides proof of concept for the present study (Table 2).

Due to the increased handling time needed for buccal swabbing (1 min or longer as compared to less than 10 s for tail clipping), sampled individuals may experience additional and undue stress[44]; however, no conspicuous indications of prolonged stress were observed after buccal swabbing. The intensity of stress responses, however, seems to vary between individuals[30-32], whereas tail clipping does cause some permanent external damage. Nevertheless, while it is difficult to assess and quantify pain or stress in non-human animals, obvious observable stress reactions (darkening of skin colour, excessive squirming) seem to be limited to the handling duration, or shortly thereafter. Markers of physiological stress were not measured during sampling; however, increased handling periods have been shown to cause greater physiological stress responses in reptilian species.[46-48]

A major drawback of the best performing buccal swab option is that it is far more expensive than the tail clipping method (Table 3). The low-cost buccal swabbing option, which made use of 'Q-tips', had a slightly higher cost, about ZAR0.23 (USD0.01), than the tail clipping method; however, it performed very poorly in the retrieval of high-quality DNA. The moderate-cost buccal swabbing option was ZAR22.35 (USD1.22) more expensive than the tail clipping method; however, the retrieval of DNA was also very poor from these swab samples. The high-cost buccal swabbing option was the only comparable option to tail tissue in terms of DNA retrieval and in terms of high-quality sequence reads; however, it amounted to more than twice the price of extracting DNA from tail tissue (Table 3). This makes the methodology highly problematic for implementation in many biodiverse regions as they are typically located in low- to middle-income countries[34,35] where research funding is often limited[36]. Thus, the implementation of buccal swabbing in preference to tail tissue for DNA retrieval could potentially hamper conservation research by increasing the per sample cost.

These costs, however, could become more justifiable if the swab DNA extracts are retained; as these might be used in the generation of multiple data types or data sets (e.g. host DNA, microbiome DNA, pathogen presence). This approach presumes there is adequate long-term storage for the DNA extracts (e.g. stable freezing at –40 °C with back-up systems in place) which might not be the case for research teams that have limited space and resources. Tissue samples should be less susceptible to degradation over the long term. Unfortunately, unlike tissue samples, buccal swabs have the disadvantage of being fully expended during the extraction process, meaning that swab biobanking is not possible. Biobanks are considered important resources[49-51] and can have crucial and unexpected uses for decades after the original collections were made. In cases where samples are difficult or expensive to acquire (e.g. remote localities, rare species) and might be useful in the future, the contributions to biobanking should also be considered when choosing the sampling method.

## Conclusion

Overall, our findings show that tail tissue performs better than buccal swabs for DNA retrieval in nonautotomous small reptiles; however, buccal swabbing can show comparable levels of DNA retrieval when used in conjunction with the more expensive, high-quality storage media and DNA extraction kits. Furthermore, buccal swabbing can be a suitable alternative to tail tissue in certain contexts where temporary stress is preferable to permanent tissue damage, such as species lacking a tail (e.g. fossorial species) or when biobanking is not a concern. Currently, financial costs severely hinder implementation of the above minimally disruptive sampling methodology in low- to middle-income economies. These costs can be justifiable if the swab DNA extracts are used in the generation of multiple data types or data sets (e.g. host DNA, microbiome DNA, pathogen presence); however, as swabs are fully expended during DNA retrieval, their potential benefit may be limited.

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## Data availability

The data are publicly available in GenBank; accession numbers are provided in Table 2. All tissue samples have been biobanked at the South African National Wildlife Biobank, Pretoria.

## Competing interests

We have no competing interests to declare.

## Authors' contributions

M.G.A.: Conceptualisation; methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft; project leadership; project management. J.J.F.: Methodology; data collection; sample analysis; writing – revisions. D.C.M.: Methodology; Data collection; sample analysis; writing – revisions. J.M.T.: Methodology; data collection; sample analysis; writing – revisions. J.M.d.S.: Conceptualisation; methodology; data collection; validation; writing – revisions; student supervision. K.A.T.: Conceptualisation; methodology; data collection; validation; writing – revisions; student supervision; funding acquisition. All authors read and approved the final manuscript.

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# SNP-based marker-assisted selection for high provitamin A content in African cassava genetic background

Vitamin A deficiency (VAD) contributes to significant levels of mortality and morbidity, particularly among children and women in Africa. Cassava is a major staple crop whose biofortification with beta-carotene can contribute to reducing the VAD prevalence in a cost-effective and sustainable approach. Developing high provitamin A content (pVAC) cassava varieties through the conventional approach is a laborious and slow process, partly due to the breeding bottlenecks caused by the biology of the crop. To complement the phenotypic screening for pVAC and increase selection efficiency as well as accuracy, we employed four Kompetitive Allele-Specific PCR (KASP) assays to predict the level of carotenoids in a cassava population developed from open-pollinated crosses. There was significant correlation ($r = 0.88$) between total carotenoid content (TCC) and root tissue colour score in the study population. Marker S1_24155522 at the phytoene synthase gene explained most of the phenotypic variation in TCC and root colour ($R^2 = 0.37$ and 0.55, respectively) among the genotypes evaluated in this study. The other markers did not individually account for much phenotypic variation in the trait in our study population. Three genotypes – namely UIC-17-679, UIC-17-1713, and UIC-17-2823 – had higher TCCs, ranging from 10.07 μg/g to 10.88 μg/g, than the national yellow check variety IITA-IBA-TMS070593 (9.20 μg/g). Marker PSY572/S124155522 is therefore recommended for routine use in marker-assisted selection for pVAC enhancement in African cassava germplasm.

**Significance:**

We evaluated the performance of the SNP markers associated with provitamin A content in a cassava population and draw relevant conclusions that will foster the application of these markers in different cassava improvement programmes with similar interests. Marker-assisted selection was sufficiently accurate for an early screening of individuals for carotenoid content, especially when thousands of genotypes are usually handled. This screening will reduce efficiently the challenges and burden attached to the use of sophisticated instruments for carotenoid quantification (e.g. HPLC and I-check) for the benefit of breeders and researchers in the field.

## Introduction

Inadequate micronutrient intake is a major health challenge, particularly in developing countries.[1,2] This problem is exacerbated by a restricted diet that relies mostly on a single major staple crop for subsistence[3,4] or on a few crops lacking some essential nutrients. About 56% of reported childhood deaths in developing nations are due to nutritional deficiencies.[1] Vitamin A deficiency (VAD) causes night blindness and increases the risk of sickness and mortality in children and pregnant women.[5] Globally, more than 250 000 children become blind every year, of whom half die within 12 months of losing their sight.[5] In Nigeria, about 83% of children under five are vitamin A deficient.[6] Supplement-based strategies for treating vitamin A deficiencies have had limited success, in part due to the short-term nature of their interventions and the inability to purchase fortified foods.[4] A cost-effective approach to reducing the alarming rate of vitamin A deficiency in developing countries is through biofortification of staple crops.[7]

Cassava (*Manihot esculenta* Crantz) is an important staple crop that provides for the calorie needs of more than 800 million people in Africa, Asia and South America.[8,9] Unlike other staple crops, cassava's starchy roots are inexpensive, which encourages its widespread consumption and adoption, especially in Africa.[10,11] However, there is a reported correlation between consumption levels of white cassava storage roots and micronutrient deficiency, putting populations that rely on this crop at risk of concealed hunger.[12] Consequently, biofortification of cassava with provitamin A content (pVAC) would contribute, non-exclusively, to reducing the VAD prevalence in West Africa[4,13], and also play a crucial role in mitigating the rate of hidden hunger – a concept which refers to micronutrient deficiency with invisible symptoms in affected individuals[14,15].

Significant efforts have been undertaken to exploit the natural variability for carotenoid content in the existing cassava gene pool.[16-18] For instance, HarvestPlus programme and its partners have improved and released high vitamin A cassava in Nigeria in the past years. Between 2011 and 2014, two waves of yellow cassava varieties were released in Nigeria, with carotenoid contents ranging from 6 to 8 μg/g and 8 to 10 μg/g, respectively.[19] The existing pVAC cassava varieties can supply up to 40% of the recommended daily intake of vitamin A.[20] Nonetheless, there is a need to breed for higher pVAC cassava varieties as the breeding target fixed at 15 μg/g in yellow cassava genotypes has not yet been achieved in West Africa.[17,21]

The use of molecular markers to complement the conventional breeding approach is expected to increase and accelerate the genetic gain across environments and seasons[22,23] compared with conventional breeding which

depends on the environment[24] and relies upon identification of the traits at maturity[25]. Moreover, known phenotyping protocols used for carotenoid quantification, such as high-performance liquid chromatography (HPLC) and I-check, are sophisticated, time-consuming and expensive[21], thereby limiting the number of samples that can be handled within a short period without introducing errors at the early stage of selection when thousands of genotypes usually are handled.

Advances in molecular biology technology led to the discovery of single nucleotide polymorphism (SNP) markers which are prioritised in crop breeding due to their density in the whole genome and their need in advanced genotyping platforms.[26] The commonly used SNP genotyping platforms include GoldenGate, BeadXpress and Kompetitive Allele Specific PCR, the genotyping platform used in this study. Kompetitive Allele Specific PCR (KASP) is a simple and cost-effective genotyping system, convenient for studies that involve relatively few markers.[27]

Efficient biofortification of cassava storage roots using the available natural variation requires a prior understanding of the genetic architecture of the trait[28] and identification of quantitative trait loci (QTL)-linked markers for marker-assisted selection (MAS) implementation. A biparental QTL mapping using genotyping by sequencing was first conducted by Rabbi et al.[29] and enabled the identification of a major locus on chromosome 1 which explained 92.85% of the variation in root pulp colour. However, the QTL mapping method mentioned above is extremely dependent on the genetic diversity of the two parents, hence there is variation in QTL effects between populations.[30] Genome-wide association studies have been identified to overcome QTL mapping limitations whereby genomic regions responsible for phenotypic traits are identified by using a large size of diverse populations to retrieve significant associations narrowing down the candidate regions.[31]

Thus, using the genome-wide association studies approach, the same authors, Rabbi et al.[18], uncovered two major loci associated with increased pVAC on chromosome 1 at positions 24.1 and 30.5 Mbp. The two SNP-linked markers (S1_24121306 and S1_30543382) explained jointly 81% of total phenotypic variation in root colour score.[18] Other studies using independent populations have confirmed the association signals from the same genomic region[28,32,33] which contains phytoene synthase 2, a rate-limiting enzyme in the carotenoid biosynthesis pathway[34]. More recently, five additional QTLs on chromosomes 5 (S5_3387558), 8 (S8_4319215 and S8_25598183), 15 (S15_7659426), and 16 (S16_484011) were identified using a large genome-wide association studies panel of over 5000 cassava accessions.[33]

Four of the genome-wide uncovered markers (S1_24155522, S1_30543962, S5_3387558, and S8_25598183) linked to pVAC were recently converted to allele-specific PCR assays to facilitate their routine use in MAS.[35] We carried out MAS using these SNP markers on a pVAC breeding population developed at the University of Ibadan, Nigeria. We present the performance of the markers in predicting total carotenoid content and root colour score.

## Materials and methods

### *Plant materials and experimentation*

The study population was derived from five yellow cassava varieties (IITA-IBA-TMS011368, IITA-IBA-TMS011371, IITA-IBA-TMS011412, IITA-IBA-TMS070593 and IITA-TMS070539) released between 2011 and 2014 and used as female parents in open-pollinated crosses. Before this study, about 3200 half-sib seeds were generated and 89%, 62%, and 0.05% of the progenies were successively screened for different attributes such as cassava mosaic disease severity, plant architecture and root yield among other traits in seedling nursery, clonal evaluation, and preliminary yield stages, respectively.[23]

Sixty-five (65) cassava genotypes with yellow root tissues selected from preliminary yield trials and two check varieties (IITA-IBA-TMS070593 and IITA-IBA-TMS30555) were evaluated in this study for visual root tissue colour and lab-quantified total carotenoid content (TCC). The experiment was laid out in a randomised complete block design with two replications at the Teaching and Research Farm of the Department of Agronomy, University of Ibadan, Nigeria. To minimise the experimental error due to the relatively large experimental field size, genotypes were divided into two sets. Set 1 and 2 comprised 32 and 33 cassava genotypes, respectively, following the approach of Okechukwu and Dixon[36]. The check varieties were included in each set. The cassava cuttings were planted at a spacing of 1 m x 1 m with 20 plants representing each genotype in a 20-$m^2$ plot.

### *Carotenoid assessment in cassava storage roots*

The cassava genotypes were selected from preliminary yield trials based on their root colour score. However, to ascertain their actual carotene content, total carotenoid content was measured using iCheck™ (BioAnalyt GmbH, Germany; http://www.bioanalyt.com). The root colour was visually scored using a colour chart with a scale ranging from 1 to 8 where 1 = white; 2 = light cream, 3 = cream, 4 = light yellow, 5 = yellow, 6 = deep yellow, 7 = orange and 8 = pink.[37] At harvest (12 months after planting), two to three fully developed roots were selected for TCC quantification using the protocol developed by BioAnalyt laboratory and described by Jaramillo et al.[38] The selected cassava roots were properly washed, peeled, and rewashed to remove all impurities. Each root was divided into four vertically; two opposite quarters were chopped into small pieces (to ease the grinding process) and mixed thoroughly. A homogenised sample, 5 g in weight, was collected, macerated using a mortar and pestle, and mixed with 20 mL of distilled water. A final volume of 25 mL was obtained and transferred to a graduated tube. A slurry volume of 0.4 mL was extracted using a syringe and needle and injected into the reagent vial. The vial was allowed to stand for about 5 min to separate the liquid phase before taking and recording the iCheck™ reading.

TCC was calculated as described by Esuma et al.[39]:

$$TCC_{\mu g\, g-1} = \frac{V_s}{W_s} R_i \qquad \text{Equation 1}$$

where $V_s$ is the total slurry volume (25 mL), $W_s$ is the sample weight and $R_i$ stands for the iCheck reading. The iCheck™ carotene analysis procedure was conducted at the Crop Utilisation Laboratory of the Department of Agronomy, University of Ibadan.

### *DNA extraction and KASP genotyping*

The DNA was extracted from young freeze-dried cassava leaves following a modified Dellaporta et al.[40] procedure. The DNA quantity and quality were checked using a Nanodrop photometer (ND-8000, Thermo Fisher Scientific, USA) and DNA bands were viewed on agarose gel (1%). The KASP genotyping was performed as described by Codjia et al.[41] Four top SNPs (S1_24155522, S1_30543962, S5_3387558 and S8_25598183) coming from phytoene synthase 2 on chromosome 1 and new loci on chromosomes 1, 5 and 8 extracted from Rabbi et al.[33] were used for screening the cassava genotypes for pVAC. The pVAC-linked markers successfully converted to allele-specific PCR assay[35] enabled the molecular screening. Yellow variety IITA-IBA-TMS 07/0593 (one of the female parents) and white variety IITA-IBA-TMS 30555 were used as positive and negative controls, respectively, in the screening for carotene content. The KASP genotyping was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan.

### *Statistical analysis*

Data on TCC and root colour score were subjected to an analysis of variance (ANOVA) using the *Agricolae*[42] package in R software.[43] For the marker–trait association, the best linear unbiased estimator (BLUE) values for TCC and root colour were extracted from a fitted mixed model where blocks and replications were treated as random effects and genotypes, including controls, were regarded as fixed following an adapted mathematical formula for randomised complete block design

from Dixon[44]. Polymorphic information content (PIC) and favourable allele frequencies were calculated using Tassel[45] and R software jointly. The markers' effect on TCC and root colour was visually assessed using the *ggpubr* package.[46]

For marker predictive ability assessment, a multiple linear regression was computed using the *lm* function from the *lm4* package.[47] The traits (TCC or root colour) were considered as response variables and the markers as independent variables (predictors) according to the formula adapted from Uyanik and Güler[48]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \mathcal{E} \quad \text{Equation 2}$$

where $Y$ = the predicted value of the trait by the markers, $\beta_0$ = the constant intercept, $\beta_1, \beta_2, \ldots \beta_n$ = regression coefficient for each marker, $X_1, X_2 \ldots X_n$ = explanatory variables (markers) and $\mathcal{E}$ = residual value.

The best regression model was chosen through a stepwise regression method (both backward and forward) using the *Mass* package. For model validation, a bootstrapped sampling strategy was applied ($n = 10$) using the *tidymodels* package. The correlation matrix plot was performed using the *corrplot* package.[49]

## Results and discussion

### Variation for TCC among cassava genotypes

The analysis of variance revealed highly significant differences among the yellow cassava genotypes for TCC, but no significant difference among block components was observed (Table 1). The coefficient of variation (CV) was 26%. Genotypes UIC-17-679, UIC-17-1713, and UIC-17-2823 had TCCs ranging from 10.07 μg/g to 10.88 μg/g. These values are higher than the TCC value of 9.20 μg/g recorded in the yellow check variety IITA-TMS-070593 (Table 2).

### Frequency distribution and correlation between TCC and root colour score

Data on TCC followed a normal distribution (Figure 1). The TCC ranged from 2.43 μg/g to 10.88 μg/g with a mean of 5.88 μg/g. The highest TCC recorded in this study (10.88 μg/g) is similar to the maximum TCC (11 μg/g) reported by Esuma et al.[39] among the $F_1$ progenies of cassava using the iCheck™ carotene determination procedure. The root colour ranged from 1 to 4 with a mean of 3 in the panel of yellow cassava genotypes (Figure 1).

A highly significant correlation ($r = 0.88$; $p < 0.0001$) was observed between visual root colour score and TCC using iCheckTM (Figure 2), which is consistent with earlier submissions.[13,50,51] Screening large cassava populations becomes much more motivating due to the existing correlation between TCC and root colour score.[39] However, lab-based quantification of carotenoid content is still needed at later stages of selection in order to have more precise estimates, for example when recommending new genotypes for official registration and release.[52]

**Table 1:** Mean squares among cassava genotypes and other components for total carotenoid content

| Source of variation | df | Sum of square | Mean square | F-value | Pr(>F) |
|---|---|---|---|---|---|
| Genotype | 66 | 475.2 | 7.199 | 2.933 | 1.04E-05*** |
| Block | 1 | 0.0 | 0.008 | 0.003 | 0.954 |
| Residuals | 66 | 162.0 | 2.455 | | |
| Coefficient of variation | 26.25 | | | | |

***$p < 0.001$

**Table 2:** Cassava genotypes with the highest and lowest total carotenoid content (TCC)

| Genotypes | TCC | Groups | Standard deviation |
|---|---|---|---|
| **Highest TCC** | | | |
| UIC-17-679 | 10.88 | a | ±8.20 |
| UIC-17-1713 | 10.84 | a | ±2.97 |
| UIC-17-2823 | 10.07 | ab | ±0.58 |
| TMS07/0593 | 9.20 | abc | ±1.04 |
| UIC-17-2402 | 9.03 | abcd | ±1.98 |
| UIC-17-1632 | 8.39 | abcde | ±0.63 |
| UIC-17-1911 | 8.33 | abcde | ±0.66 |
| UIC-17-119 | 8.24 | abcdef | ±0.60 |
| UIC-17-707 | 8.00 | abcdefg | ±2.80 |
| UIC-17-26 | 7.98 | abcdefg | ±0.59 |
| **Lowest TCC** | | | |
| UIC-17-90 | 4.10 | opqrstuvw | ±0.87 |
| UIC-17-7 | 4.07 | opqrstuvw | ±0.63 |
| UIC-17-93 | 4.02 | pqrstuvw | ±0.35 |
| UIC-17-8 | 3.82 | qrstuvw | ±0.21 |
| UIC-17-646 | 3.75 | rstuvw | ±0.59 |
| UIC-17-1749 | 3.43 | stuvw | ±0.85 |
| UIC-17-1178 | 3.38 | stuvw | ±0.29 |
| UIC-17-2610 | 2.86 | tuvw | ±0.17 |
| UIC-17-1972 | 2.63 | uvw | ±0.42 |
| TMS30555 | 2.43 | vw | ±0.14 |

### Marker informativeness, technical metrics and genotypic population structure

The favourable allele frequencies for the SNP markers varied from 0.20 to 0.87 with an average of 0.49 (Table 3). The observed heterozygosity at the studied loci ranged from 0.19 to 0.89 with a mean of 0.51. The polymorphic information content (PIC) – which is a crucial metric for the informativeness of a marker – varied from 0.22 (marker S1_24155522) to 0.49 (marker S5_3387558) with an average of 0.38 (Table 3). The SNP markers S1_24155522, S1_30543962, S5_3387558, and S8_25598183 had call rates of 94%, 98.5%, 98.5% and 97%, respectively.

Approximately 90% of the study population were identified with favourable 'A' allele at marker S1_24155522 while about 3% of the genotypes did not have the favourable allele (Table 4). Furthermore, 72% were homozygotes fixed for the useful allele A whereas about 18% were found heterozygotes (CA) at phytoene synthase linked marker (Table 4).

### Effects of favourable and unfavourable alleles on TCC and root tissue colour

Exploratory analysis of genotype classes (AA, CA, CC) at SNP S1_24155522 versus TCC revealed a highly significant difference ($p < 0.0001$) (Figure 3). The individuals with two copies of favourable alleles (AA) at phytoene synthase locus had a TCC mean of 6.52 μg/g, whereas most of the genotypes (CC)

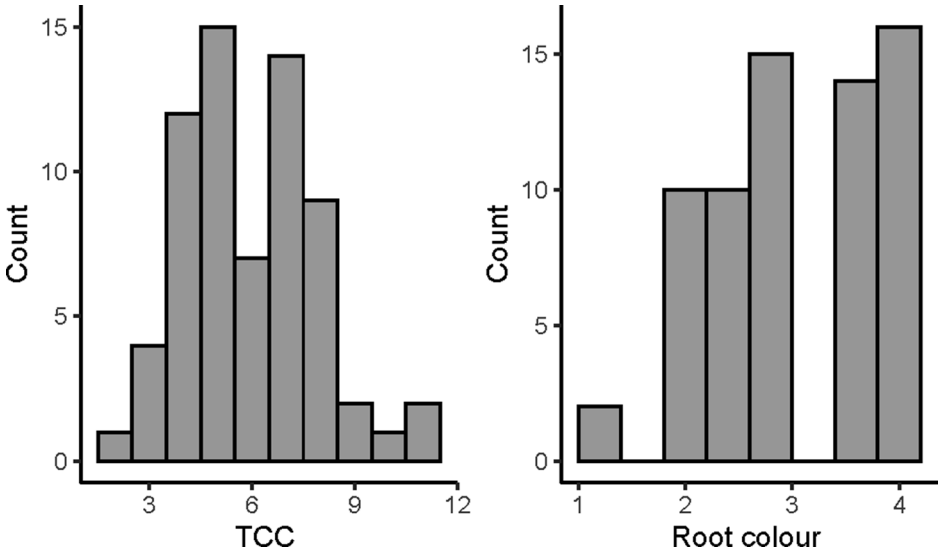


**Figure 1:** Frequency distribution of total carotenoid content (TCC) and root colour score among the yellow cassava genotypes.

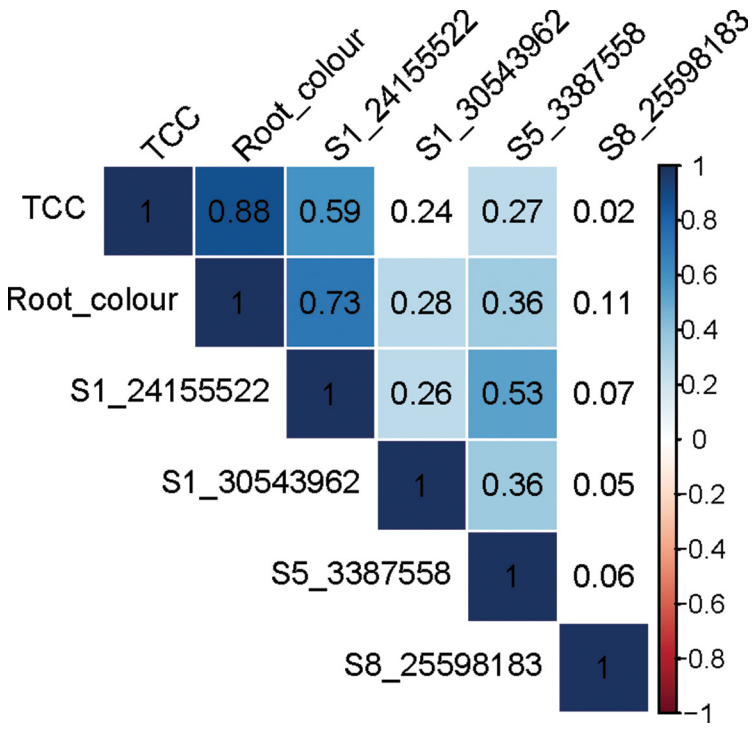


**Figure 2:** Correlation between markers and phenotypic traits. Blank spots are not statistically significant.

with the unfavourable allele had an average TCC of 2.54 μg/g. The majority of heterozygous genotypes (CA) had an intermediate level of TCC (4.25 μg/g). A *t*-test (pairwise comparison) revealed a significant difference at the 5% level at the other loci, except for marker S8_25598183 which was not significant in discriminating between genotypic classes for TCC trait (Figure 3). Similar observations were made for root colour scores at all loci, excluding S8_25598183 (Figure 4).

In a recent study conducted by Udoh et al.[13], the marker at PSY2 gene (S1_24155522) was significantly associated with root colour score and TCC. Phytoene synthase had previously been identified as a gene engaged in the initial step of cassava carotenoid biosynthesis.[18,34] Phytoene synthase is known for the accumulation of provitamin A carotenoid in cassava roots.[28,34] This study confirms the primary role of the PSY enzyme (Manes.01G124200), which converts geranylgeranyl diphosphate to phytoene.[18,34] Beta-carotene constitutes the main component of TCC in cassava[17,53], suggesting that the products of PSY2 are mostly channelled towards the lycopene beta cyclase (lcyB) part of the pathway.[54] Further research is needed to find markers that select for down-regulation of the activity of lycopene epsilon cyclase (lcyE), which will even boost the proportion of beta-carotene.[55]

Carotenoid accumulation in cassava is driven by an additive gene effect.[28] This observation is supported by the differential allele effect observed in the box-plot analysis for marker PSY_572/ S1_24155522 (Figures 3 and 4). Keller et al.[56] defined the additive effect as an allele

**Table 3:** Favourable allele frequency and informativeness of the SNP associated with provitamin A content in the study population

| SNP marker | Favourable allele | Alternate allele | Favourable allele frequency | Heterozygosity | PIC |
|---|---|---|---|---|---|
| S1_24155522 | A | C | 0.87 | 0.19 | 0.22 |
| S1_30543962 | G | A | 0.41 | 0.61 | 0.48 |
| S5_3387558 | T | C | 0.46 | 0.89 | 0.49 |
| S8_25598183 | T | G | 0.21 | 0.35 | 0.33 |

**Table 4:** Allelic and genotypic frequencies of the markers associated with provitamin A content in the cassava population

| Marker | Marker genotype | Counts | Genotype frequencies |
|---|---|---|---|
| S1_24155522 | AA | 48 | 0.72 |
| | CA | 12 | 0.18 |
| | CC | 2 | 0.03 |
| | Uncallable | 5 | 0.07 |
| S1_30543962 | GG | 7 | 0.10 |
| | GA | 40 | 0.60 |
| | AA | 19 | 0.28 |
| | Uncallable | 1 | 0.01 |
| S5_3387558 | TT | 0 | 0.00 |
| | TC | 60 | 0.90 |
| | CC | 6 | 0.09 |
| | Uncallable | 1 | 0.01 |
| S8_25598183 | TT | 2 | 0.03 |
| | TG | 23 | 0.34 |
| | GG | 40 | 0.60 |
| | Uncallable | 2 | 0.03 |

substitution effect at a locus that can explain half of the difference of homozygous individuals' performance as observed in this study at PSY2 gene (Figures 3 and 4). The additive nature of the provitamin A trait suggests that the accumulation of TCC in cassava storage roots would be improved through a recurrent selection allowing gene recombination within the breeding set.[16,28,51]

### *Prediction of TCC and root colour by markers using multiple linear regression model*

Individually, the marker at the phytoene synthase gene explained much of the phenotypic variation in TCC ($R^2 = 0.37$) while the other markers – S1_30543962, S5_3387558 and S8_25598183 – explained 11%, 7% and 5%, respectively. For root colour, each marker S1_24155522, S1_30543962, S5_3387558 and S8_25598183 accounted separately for 55%, 8%, 16% and 5%, respectively.

For markers predictive ability assessment, a multiple linear regression was computed with the four markers which explained 39% of the total variation in TCC in the present population (adjusted $R^2$=0.39; $p = 2.78E-05$) (Figure 5). On the other hand, these SNP markers together explained up to 54% of the total variation in visual root colour (adjusted $R^2$=0.54; $p = 3.901E-08$) (Figure 5). However, the best model chosen using a stepwise regression method returned a simple model only fitted with the most significant predictor at the phytoene synthase gene, indicating the elimination of other non-significant markers in the prediction model for TCC and root colour. The low phenotypic variation of TCC and root colour explained by the three markers S1_30543962, S5_3387558 and S8_25598183 suggests that the newly uncovered QTLs for pVAC have a minor effect on the trait, as previously reported by Ige et al.[35]

The best prediction model fitted with the most significant marker was validated using a bootstrap resampling strategy. The correlation coefficient between the predicted and the observed TCC values ranged from 0.36 to 0.80 with an average of 0.63 (Figure 6) whereas a correlation ranging from 0.66 to 0.89 with a mean of 0.75 was found between predicted and observed root colour score (Figure 7). These results confirm that marker S1_24155522 has sufficient predictive ability for usage in marker-assisted selection to enhance provitamin A content in African cassava storage roots.

### *Phenotypic and molecular improvement for provitamin A content*

In this study, the maximum TCC recorded in the cassava population was 10.88 μg/g – a value that, although higher than that of the national yellow control variety, still does not reach the breeding target (15 μg/g). The rapid-cycling recurrent selection approach is known to increase carotenoid accumulation in cassava storage roots, especially in breeding programmes targeting exclusively carotenoid content enhancement.[16] However, the method relies on precise carotenoid quantification using laboratory methods such as high-performance liquid chromatography (HPLC) which is costly, time consuming and labour intensive.[16,21] The use of SNP markers is expected to facilitate rapid population improvement and overcome the challenges associated with phenotype-based selection.[57]

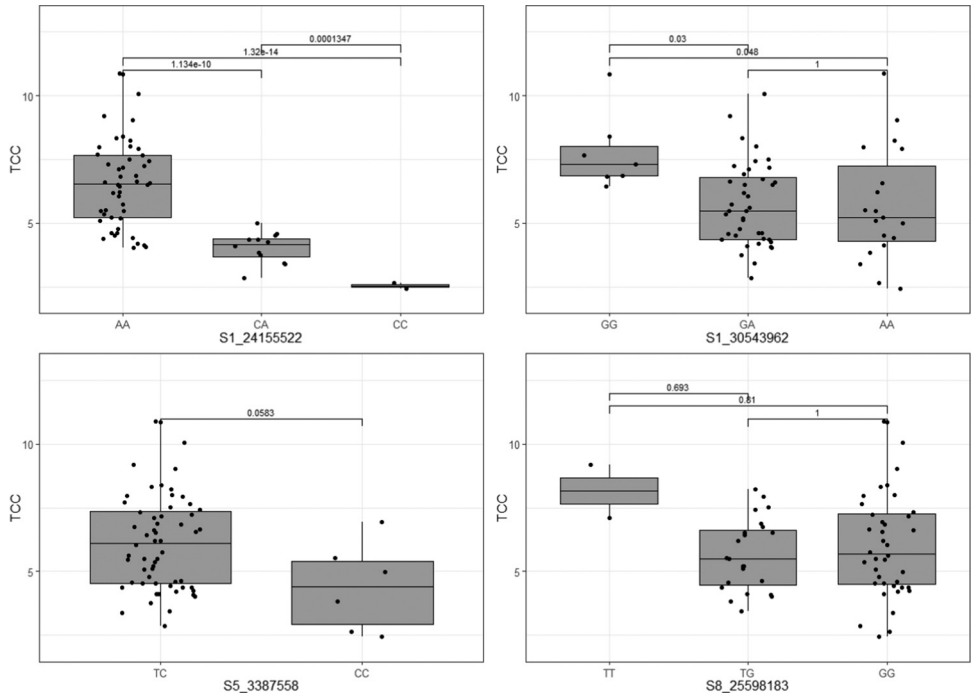


**Figure 3:** Effects of favourable and unfavourable alleles on total carotenoid content (TCC) in the evaluated yellow cassava population.

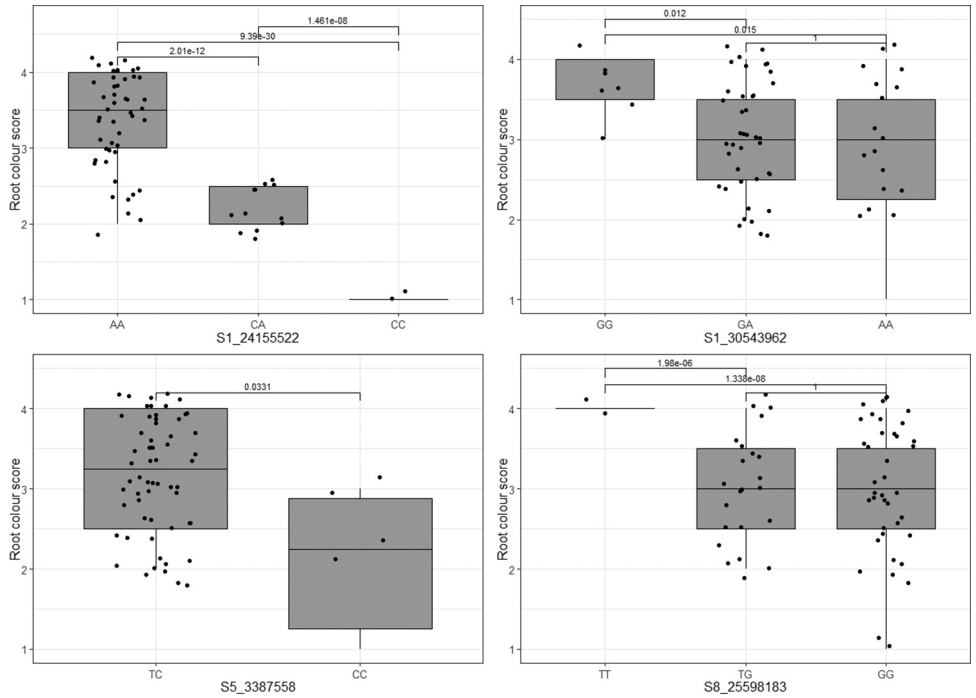


**Figure 4:** Effects of favourable and unfavourable alleles on root colour score in the study cassava population.

In our study, we used iCheck to quantify total carotenoid content rather than individual carotenoid components (alpha-carotene, all-trans beta-carotene, violaxanthin, lutein, 15-cis beta-carotene, 13-cis beta-carotene, 9-cis beta-carotene, and phytoene), a process that would require HPLC. In cassava, it is well established that more than 80% of TCC is total beta-carotene[32], a precursor of vitamin A known to be readily converted into retinol by the body[58] . Additionally, various studies have reported a high positive correlation between TCC and total beta-carotene in cassava storage roots.[16,32,59]

The accuracy of MAS found in this study for root colour score is 55%. A similar study using different cassava populations resulted in a higher accuracy of 80%.[35] The relatively lower prediction accuracy from our study could be explained by the small population size derived from five

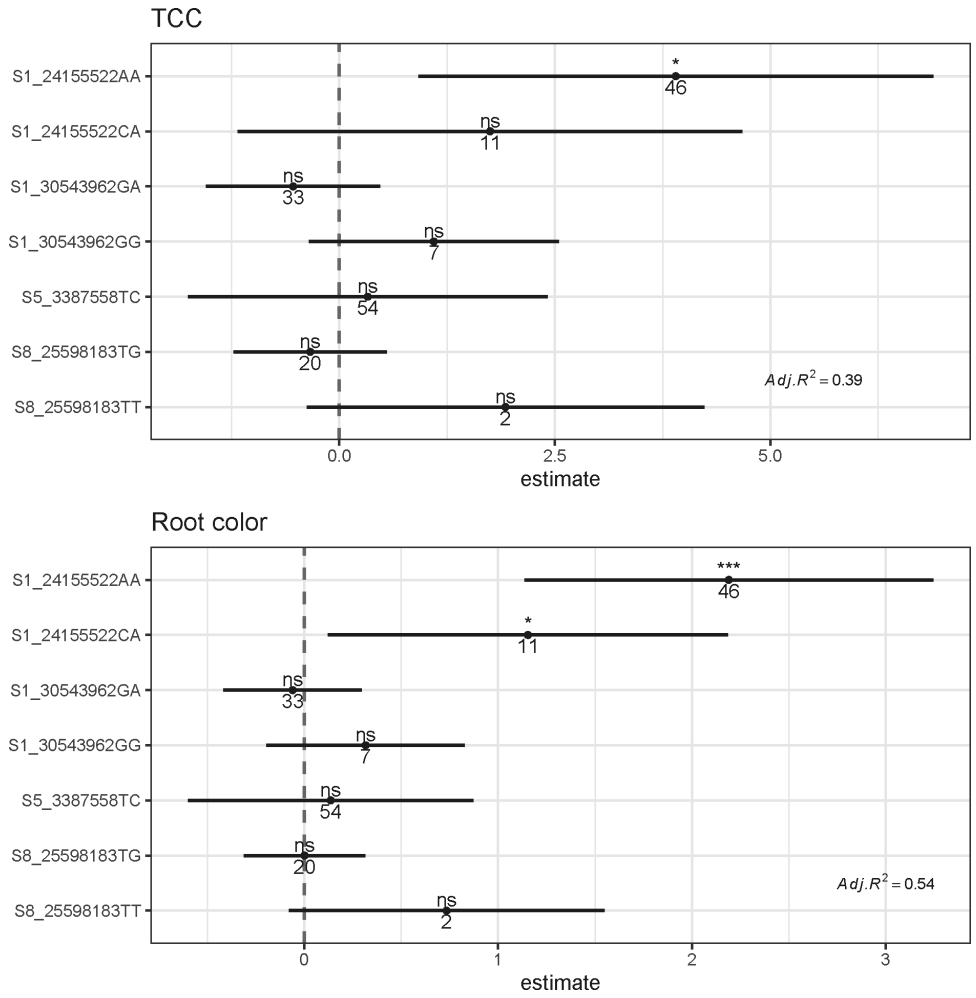


**Figure 5:** Estimates of the markers for the prediction of total carotenoid content (TCC) and root colour in the study population. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{ns}$ $p > 0.05$.

parents and the narrow range of variation in root pigmentation (between cream and yellow) after discarding white root clones at the early stages of selection.

Nevertheless, the observed accuracy from relatively few markers is in agreement with previous studies which have shown simple inheritance of carotenoid traits in cassava.[51,60] Further, the accuracy obtained from the present study is similar to that obtained from genome-wide predictions.[32,61] Ikeogu et al.[32] reported prediction accuracies up to 52% in a genomic selection with 594 cassava genotypes using the multiple trait carotenoids (individual TC components). Equally, Esuma et al.[61] reported an equivalent accuracy of 52% in root colour score while predicting carotenoids using genomic tools. This shows that MAS is sufficient for an early screening of individuals for carotenoid content. MAS is much more suitable for traits controlled by a few or major loci[62] as observed in this study with the major PSY2 gene known to be responsible for provitamin A accumulation and tagged by marker S1_24155522. The approach is particularly convenient when dealing with traits that are expensive to phenotype, such as carotenoid content, and which can only be evaluated at harvest. We demonstrate the suitability of carotenoid-content-linked SNPs for MAS in our breeding population. The present results open avenues for breeders to more comfortably fast-track the transfer of useful alleles of carotenoid genes into elite cassava breeding lines, thus contributing to the reduction of VAD prevalence, especially among pregnant women and children in developing countries.

## Conclusion

We carried out marker-assisted selection to increase provitamin A content in a cassava breeding population using four SNP markers and laboratory-quantified carotenoid content as well as root colour score. The marker accuracy evaluation revealed that the major *PSY2* locus on chromosome 1, tagged by S1_24155522 and driven by an additive gene effect, explained a relatively high phenotypic variation in TCC and root colour in the study population and could be deployed for MAS in cassava breeding programmes. The top three genotypes – UIC-17-679, UIC-17-1713, and UIC-17-2823 – with TCC greater than that of the national yellow check IITA-IBA-TMS070593 are recommended to be advanced to the next breeding stages for evaluation of other key traits such as root yield, dry matter, cyanide content, and root mealiness across multi-environments to satisfy farmers' and consumers' needs.

## Acknowledgements

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

E.D.C.: Conceptualisation; methodology; data collection; sample analysis; data analysis; data curation; writing – the initial draft; funding

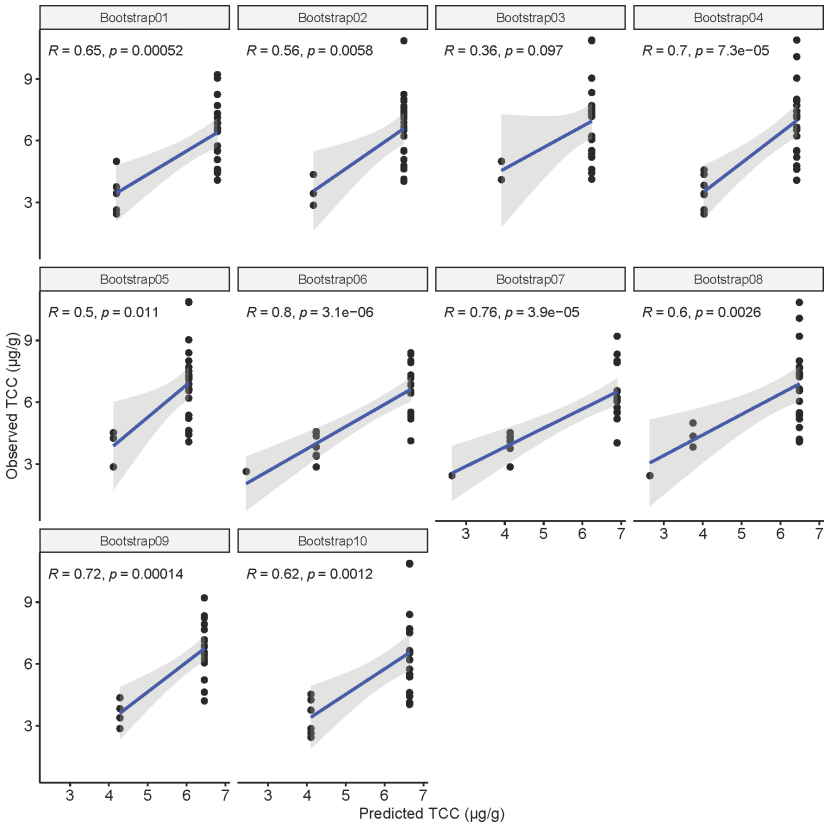


**Figure 6:** Scatter plot showing the predicted and observed total carotenoid content (TCC) values in the bootstrapped population.

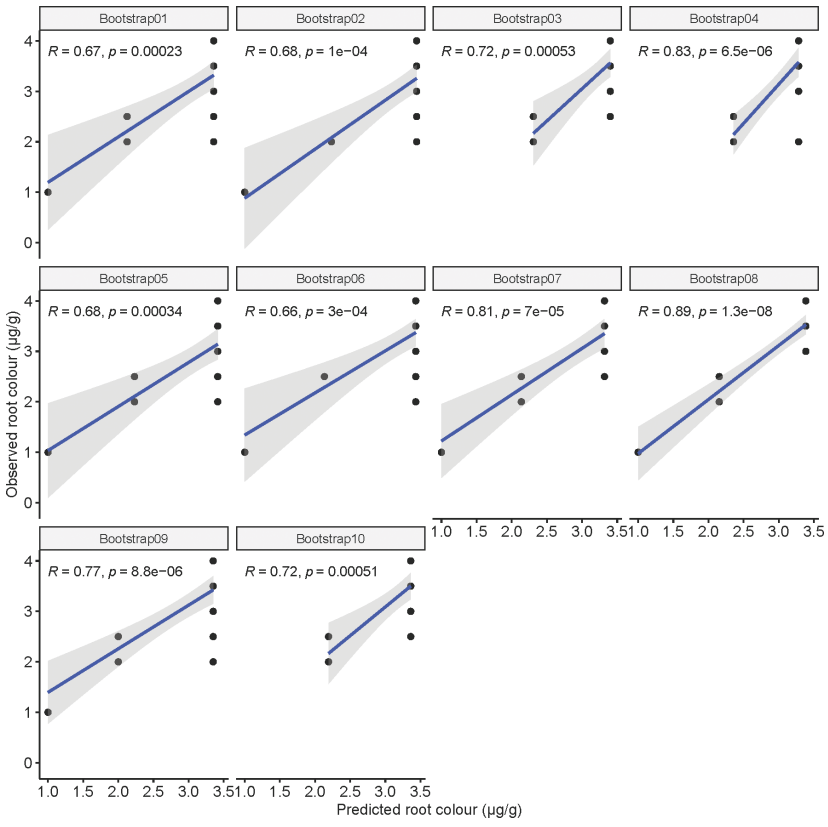


**Figure 7:** Scatter plot showing the predicted and observed root colour score on the bootstrapped population.

acquisition. B.O.: Conceptualisation; methodology; data curation; writing – revisions; student supervision; project management; development of the cassava population. I.Y.R.: Conceptualisation; methodology; writing – revisions; student supervision; project management; development of the molecular markers. C.E.U.: Data analysis.

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# A mesocosm study on the use of clay minerals to improve heavy metal phytoremediation capacity of vetiver grass (*Chrysopogon zizanioides* L. Roberty)

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Fast-paced global industrialisation due to population growth poses negative environmental implications, such as pollution by heavy metals. We assessed the application of vetiver grass assisted by clay minerals for the remediation of soil and water contaminated by multiple metals in a mesocosm study. The technique was tested previously in a greenhouse study that confirmed the effectiveness of 2.5% (w/w) attapulgite and 2.5% (w/v) bentonite to improve vetiver grass remediation of soil and water contaminated by multiple metals. At the end of the experiment, the total accumulation of Co, Cr, Cu, Ni and Zn by vetiver grass from the soil was 1.8, 38.1, 19.0, 7.2 and 55.4 mg/kg, respectively, while in water, the total metal accumulation of Al and Mn by vetiver grass was 4534.5 and 104.5 mg/kg, respectively. The results confirm the effectiveness of attapulgite and bentonite as amendments to improve the remediation potential of vetiver in soil and water under natural conditions. Metal accumulation was generally higher in the roots than in shoots. We found the removal efficiency in the soil to be in the order Zn > Cr > Cu > Ni > Co and Al > Mn in water. Results also demonstrated that heavy metal accumulation was even better under natural conditions than in the greenhouse study. For example, Zn accumulation increased from 0.4 mg/kg in the greenhouse study to 55.4 mg/kg in the outdoor study. This study validates the application of bentonite and attapulgite-assisted phytoremediation for heavy metal contaminated soil and water.

**Significance:**

- Heavy metal pollution of soil and water is very common in industrialised and mining areas.
- It is important to find cost-effective, eco-friendly and easy-to-apply methods of removing these heavy metals from soil and water, so as to provide a clean and safe environment for living organisms.
- Phytoremediation is the use of plants to remove pollutants from the environment and is a cost-effective, aesthetically pleasing and eco-friendly method.
- Attapulgite and bentonite (clay minerals) are effective in improving the phytoremediation capacity of a phytoremediation plant known as vetiver grass.

## Introduction

Rising global industrialisation and urbanisation consequentially increase the release of pollutants into the environment. While some pollutants are natural elements, anthropogenic activities can increase their environmental release, for instance, heavy metals. Naturally, heavy metals are commonly associated with bedrock, but due to anthropogenic activities, they have become a major class of environmental pollutants that adversely affect soil ecology and productivity and surface water and groundwater quality, thereby threatening biodiversity.[1] Most heavy metals are highly toxic to biota even at low concentrations.[2] Although some heavy metals are essential nutrients, they can be toxic when present at excessive concentrations.[3] For instance, Al and Mn can be more easily accumulated by living organisms from water than from other sources[4], and negatively affect many cell functions such as detoxification, brain function and metabolism, and can cause deoxyribonucleic acid (DNA) and tissue damage[4,5].

South Africa is rich in natural resources and has some of the world's largest reserves of gold, coal and platinum.[6] As a result of the exploitation of these resources, the legacy of mining has caused considerable heavy metal pollution, posing a risk to human and animal life. For example, health effects such as chest pain, wheezing, tuberculosis, diarrhoea, cough and itchy skin due to people's proximity to and contact with mine tailings have been reported in Gauteng, Mpumalanga, North West and Limpopo Provinces of South Africa.[7-9] Furthermore, animals from mining communities in the North West and Gauteng Provinces have high levels of heavy metals in their faeces and serum due to the mining in these areas.[10,11]

The KwaZulu-Natal, Mpumalanga, Limpopo and Free State Provinces hold the highest coal reserves in South Africa. Yearly, close to 65 Mt of waste is produced from coal processing; this waste contains high levels of sulfur and heavy metals which, upon releasee, pose an environmental risk.[12] Such risks include the disruption of soil and water ecosystems, the release of toxic metals into the food chain, and absorption through the skin.[3,13] Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn as environmental pollutants are commonly associated with coal.[14,15] In particular, heavy metals including Co, Cr, Cu, Ni, and Zn have been detected in the soil, while Al and Mn have been detected in water bodies surrounding a former coal mining environment located in Sasolburg in the Free State Province of South Africa.[14,16,17]

Considering the negative effects of heavy metals on the environment, several technologies have been identified for managing heavy metal polluted sites. Of these technologies, phytoremediation is a cost-effective and environmentally friendly option[18], and there is growing interest in the application and optimisation of phytoremediation[19,20]. Vetiver grass (*Chrysopogon zizanioides)* is a terrestrial plant that has been adapted for soil and water remediation purposes because it is easily propagated, with rapid growth and can survive in extreme climatic conditions. It has been

applied to wetlands, industrial wastewaters, mine tailings and agricultural soils.[21-23] A combination of two or three remediation options can result in more efficient outcomes[24], with combinations of amendments such as compost, red mud, clays, and biochar being applied for this purpose[19,25]. In addition, for a practical, real-life application of phytoremediation, mesocosm studies are essential to examine efficiency because mesocosms hold heightened environmental realism whilst allowing control of some environmental parameters.[26] Simply put, a mesocosm is an experimental setup in which some variables are controlled under natural conditions. Clay minerals are hydrous aluminosilicates that are naturally occurring, and possess high surface areas and cation exchange capacity, thus encouraging their application in many areas including adsorption and absorption of pollutants. Major examples of clay minerals include attapulgite, bentonite, montmorillonite, zeolite and kaolinite.[13] Previous studies by Otunola et al.[16,17] showed that attapulgite administered at 2.5% (w/w) was most effective to improve the phytoremediation capacity of vetiver grass in metals polluted soil, while bentonite administered at 2.5% (w/v) was best for water remediation.

In this study, we aimed to examine the efficiency of an optimised hybrid application of vetiver grass and clay minerals for remediation of soil and water contaminated with heavy metals (Al and Mn in water; and Co, Cr, Cu, Ni and Zn in soil) in a mesocosm setting as informed by success in previous greenhouse studies.[16,17] There is little documentation of mesocosm studies concerning assisted phytoremediation of soil and water[27-29]; therefore, this study contributes to the repository of available studies of phytoremediation in mesocosms, further encouraging its application.

## Materials and methods

### *Sample collection*

Soil and water samples were collected from a former coal mining area (26°50'50.4"S; 27°49'49.7"E) in Sasolburg, Free State Province, South Africa. The mining area is at a rehabilitation stage and several post-mining land uses have been implemented.[16,17] The general geology of Sasolburg comprises sandstone and shale, which have been intruded by dolerites in some localities. With a grazed grassland vegetation type, the area experiences summer rainfall and average temperatures of 21 °C during the summer season and 9 °C in winter.[14] A composite sampling method was employed to collect the soil samples from a depth of 20 cm using a shovel. The samples were stored in tightly sealed polypropylene bags and transported to the experimental site in Bloemfontein, Free State, South Africa (29°12'40.3''S; 26°20'42.4''E). The water samples were specifically collected from the Leeuspruit River, which flows through the mine boundaries. Water samples were collected in 25 L jerry cans following pre-rinsing with site water. Soil and water pH were measured before and after the experiment using a calibrated standard multi-parameter probe (YSI Incorporated, Model 85D, I.N058500, SN 09K 100684, Yellow Springs, Ohio, USA).[3031] The pH in the soil and water before the experiment was 6.2 and 6.6, respectively. Sampling was done in triplicate and samples were sent to the Central Analytical Facility, Stellenbosch University, South Africa for the determination of total heavy metal concentration using a Flexible Single Quadrupole Agilent 7900 Q ICP-MS.

### *Outdoor experiments*

Dead plant material and gravel were removed from the soil at the experimental site, then packed into plastic pots of 12 L capacity. Previous greenhouse experiments established that treatment AT2.5VT (attapulgite mixed with soil at 2.5% (w/w) + vetiver grass) and treatment BT2.5VT (bentonite mixed with water at 2.5% (w/w) + vetiver grass) were the most efficient of the tested hybrid treatments for metals contaminated soil and water[16,17]; therefore, only these two treatments were considered in this study as the aim was to test their performance under natural conditions.

Vetiver grass obtained from Hydromulch (Pty) Ltd, Johannesburg, South Africa, was used for both treatments. The vetiver grass was thoroughly washed using municipal tap water and then distilled water; after that, the grasses were trimmed to a shoot length of 30 cm and root length of 10 cm before being transplanted into the soil pots and watered with 500 mL of municipal tap water every two days in order to maintain the soil moisture content. For the water treatment, 8L plastic pots were filled with the water samples from the study area, and bentonite was added at 2.5% (w/v), after which vetiver grass of the same shoot and root lengths was transplanted into the water pots. The vetiver grass plants were placed over the water and pots which were maintained at the same level throughout the experiment i.e. refilled to the initial volume (8L) with water samples whenever the water levels became low due to transpiration, evaporation and/or plant uptake. Negative controls (no treatment) for soil and water were also set up. All the experimental pots were arranged in a randomised complete block design and maintained under natural sunlight, air, humidity and temperature (average 28 °C day and 10 °C night). Each treatment was done in triplicate (Figure 1).

### *Plant, soil and water sampling*

The experiment lasted for 21 days, after which the plants were harvested and thoroughly cleaned in deionised water. Fresh biomass, root and shoot lengths were measured. The plant parts were oven dried at 75 °C for 72 h, after which dry biomass was recorded. The dried plant parts were then milled and microwave digested following the US EPA procedure (Method 3052). Translocation factor (TF) is a plant's ability to transfer heavy metals from its roots (below ground parts) to its shoots (above ground parts).[17] Bioconcentration factor (BCF) is a plant's ability to remove heavy metals from substrates (soil or water) and accumulate the heavy metals within its roots and shoots. TF and BCF were determined at the end of the experiment.

**Figure 1:** Outdoor experimental setup for soil and water remediation.

### *Statistical analysis*

All data were subjected to statistical analysis and expressed as the mean ± standard deviation of three replicates and descriptive statistics were obtained. Using R software version 4.0.0 (2020)[32], a one-way analysis of variance (ANOVA) was carried out separately for metals accumulated in the roots and shoots (at $p < 0.05$) to compare the means of the accumulated metals and determine any statistically significant differences between the accumulated metals in each plant part. Tukey's post-hoc test was done to determine the treatments with significant differences.

## Results

### *pH and heavy metal content in soil and water before treatment*

The pH detected in the soil and water before the experiment was 6.2 and 6.6, respectively. The initial heavy metals in the soil before treatment were 39.4 ± 1.2 mg/kg Cr, 5.8 ± 0.5 mg/kg Co, 13.5 ± 0.1 mg/kg Ni, 9.1 ± 0.4 mg/kg Cu and 28.4 ± 4.3 mg/kg Zn. The initial (before treatment) heavy metal concentration in water was 0.05 ± 0.2 mg/L and 0.18 ± 0.3 mg/L for Al and Mn, respectively.

### *Biomass and heavy metal accumulation of plants*

The vetiver grass under the influence of clay amended contaminated soil and water was assessed by observing its physical changes and metal accumulation. The morphological properties of the plants in soil and water treatments are presented in Table 1.

For the treated water, Al uptake was 4177.7 ± 0.47 mg/L and 356.8 ± 0.22 mg/L in roots and shoots, respectively, while uptake of Mn was 68.2 ± 38.8 mg/L and 36.3 ± 3.2 mg/L in the roots and shoots of vetiver grass, respectively.

For the treated soil, the concentrations of heavy metals in plant parts at the end of the outdoor experiment are shown in Figure 2.

### *Translocation and bioconcentration factors*

For vetiver grass in the BT2.5VT water treatment, the translocation factor (TF) for Al was very low (0.09), while the TF for Mn was 0.53. The bioconcentration factor (BCF) was very high for both Al and Mn (Table 2).

In the AT2.5VT treatment for soil, vetiver showed a TF of 0.06–0.36 for the various heavy metals (Table 3). The BCF obtained for Cr, Co and Ni was <1, whereas BCF values observed for Cu and Zn were >1 (Table 3).

## Discussion

Outdoor mesocosm was used to mimic the real-life expected water and soil treatment conditions for vetiver grass in contaminated media. The pH observed in the soil and water were similar and close to neutral (6.2 and 6.6 for soil and water, respectively), thus encouraging moderate mobility of heavy metals. The final root and shoot lengths observed at the end of the experiment indicate that vetiver grass indeed is a fast-growing crop. An increase in shoot length of up to 4 cm was observed within 21 days, giving the plant more surface area for metal accumulation. This is because the rate of phytoremediation is proportional to the plant growth rate, where high biomass crops like vetiver are a very good option for phytoremediation. Vetiver grass uses a C4 photosynthetic pathway with higher rates of photosynthesis at high light intensities, supporting the better performance of vetiver in the outdoor experiment.[21] The presence of Cu in the soil may also have affected its biomass yield,

**Table 1:** Morphological properties of vetiver grass in water treated with vetiver + bentonite applied at 2.5% (w/v) (BT2.5VT) and soil treated with vetiver + attapulgite applied at 2.5% (w/w) (AT2.5VT)

| Property / treatment | Shoot length (cm) | Root length (cm) | Fresh shoot biomass (g) | Fresh root biomass (g) | Dry shoot biomass (g) | Dry root biomass (g) |
|---|---|---|---|---|---|---|
| BT2.5VT | 36.5 ± 2.1 | 16.5 ± 2.1 | 33.8 ± 17.3 | 23.7 ± 2.6 | 10.9 ± 5.1 | 8.2 ± 1.1 |
| AT2.5VT | 44.0 ± 4.2 | 15.5 ± 2.1 | 30.3 ± 5.8 | 17.8 ± 1.6 | 11.9 ± 2.6 | 6.7 ± 0.4 |

*Values are means ± SD, n = 3*

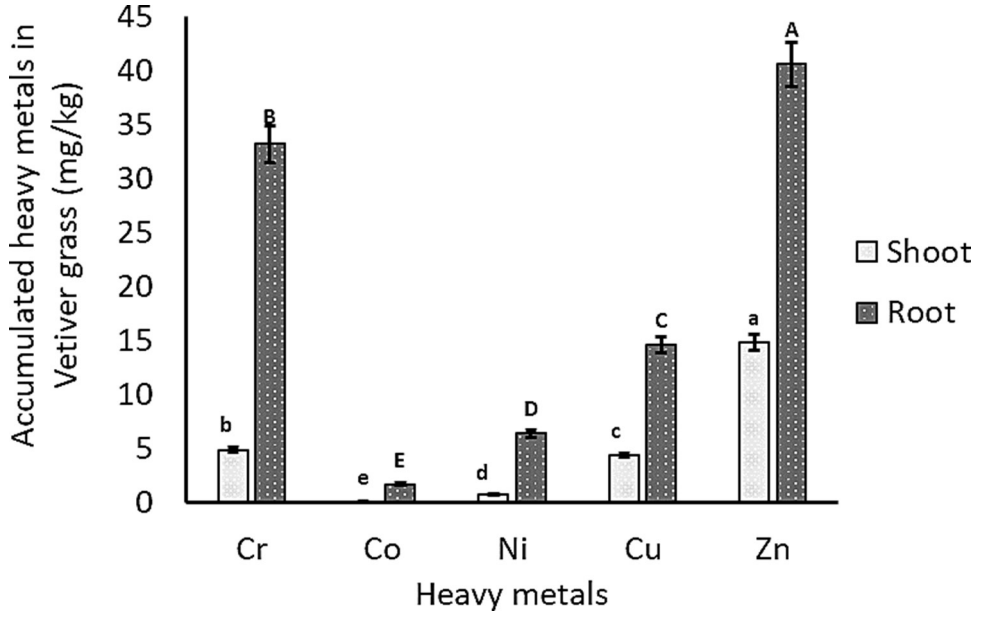


**Figure 2:** Concentrations of heavy metals in roots and shoots of vetiver grass in the soil treatment at the end of the experiment. Values are means (± SD; $n = 3$). Error bars represent per cent errors. Uppercase letters on top of the bars show statistically significant differences in root accumulation, while the lowercase letters show statistically significant differences in shoot accumulation.

**Table 2:** Root and shoot metals concentration, translocation factor (TF) and bioconcentration factor (BCF) observed for vetiver grass in the water treatment

| Heavy metal | Initial concentration[a] in water (mg/L) | BT2.5VTS (mg/kg) | BT2.5VTR (mg/kg) | TF | BCF |
|---|---|---|---|---|---|
| Al | 0.05 ± 0.2 | 356.80 ± 0.2 | 4177.70 ± 0.5 | 0.09 ± 0.4 | 90 690.00 ± 3.5 |
| Mn | 0.18 ± 0.3 | 36.31 ± 3.2 | 68.21 ± 38.8 | 0.53 ± 0.1 | 580.60 ± 1.7 |

*BT2.5VTS, metal concentration in shoots of vetiver; BT2.5VTR, metal concentration in roots of vetiver*

*[a]Before treatment*

*Values are mean ± SD, n = 3*

**Table 3:** Root and shoot metals concentration, translocation factor (TF) and bioconcentration factor (BCF) observed for vetiver grass in the soil treatment

| Heavy metal | Initial concentration[a] in soil (mg/kg) | AT2.5VTS (mg/kg) | AT2.5VTR (mg/kg) | TF | BCF |
|---|---|---|---|---|---|
| Cr | 39.4 ± 1.2 | 4.9 ± 0.4 | 33.2 ± 12.9 | 0.15 ± 0.0 | 0.96 ± 10.8 |
| Co | 5.8 ± 0.5 | 0.1 ± 0.0 | 1.7 ± 0.2 | 0.06 ± 0.2 | 0.31 ± 0.4 |
| Ni | 13.5 ± 0.1 | 0.8 ± 0.0 | 6.4 ± 0.8 | 0.13 ± 0.4 | 0.53 ± 0.8 |
| Cu | 9.1 ± 0.4 | 4.4 ± 0.1 | 14.6 ± 1.3 | 0.30 ± 0.1 | 2.08 ± 3.5 |
| Zn | 28.4 ± 4.3 | 14.8 ± 0.2 | 40.6 ± 12.4 | 0.36 ± 0.0 | 1.95 ± 2.9 |

*AT2.5VTS, metal concentration in shoots of vetiver; AT2.5VTR, metal concentration in roots of vetiver*

*[a]Before treatment*

*Values are mean ± SD, n = 3*

as Liu et al.[33] observed that Cu at high concentrations can enhance the growth and dry weight of vetiver grass.

The vetiver grass that was grown in the water treated with bentonite applied at 2.5% (w/v) (BT2.5VT treatment) showed root Al and Mn accumulation that was greater than shoot accumulation, but the higher the root uptake, the higher the shoot uptake as well. Al accumulation was 4177.7 ± 0.5 mg/L and 356.8 ± 0.2 mg/L in roots and shoots, respectively, while Mn was 68.2 ± 38.8 mg/L and 36.3 ± 3.2 mg/L in roots and shoots, respectively (Table 2). The higher root accumulation of both Al and Mn corresponds to findings of previous studies on the application of vetiver grass for metal removal.[19,34] The total (combined root and shoot) Mn accumulation in this experiment was 104.5 mg/L, which is 88% higher than Mn accumulation in a previous greenhouse experiment.[17] Likewise, the total amount of Al accumulated by vetiver in the mesocosm experiment was 4534.1 mg/L, while the total Al accumulation in the greenhouse experiment was only 371.8 mg/L.[17] It is evident that vetiver absorbed much higher amounts of Al and Mn in the outdoor mesocosm experiment than in the greenhouse experiment. This could be due to the lower initial concentrations of Al and Mn in the outdoor experiment. In the outdoor experiments, the Al and Mn concentrations were 0.05 mg/L and 0.18 mg/L, respectively, which was much lower than the concentrations in the greenhouse study – 5 mg/L and 1 mg/L, respectively. The natural conditions (such as temperature, sunlight, humidity and air) of the present mesocosm experiment may also account for the higher metal accumulation observed.[26] Different periods of sampling may also be a reason for the higher accumulation rates, because wet seasons increase the solubility of nutrients, thus increasing accumulation by plants. The rate of water evaporation from the plant leaves is also higher in the summer and spring seasons than in colder periods, and evaporating water serves as a pump for nutrients and heavy metals.[35]

The heavy metals accumulated by vetiver grass grown in the contaminated soil varied with metal type and initial concentration in the soil. After treatment, the order of abundance of heavy metals in the soil was Cr >Zn > Ni > Cu > Co. A similar order was observed for Zn and Cu by Kafil et al.[36] and there was a slight reduction of heavy metals in the untreated media. The order of accumulation in the roots of vetiver in this mesocosm experiment was Zn > Cr > Cu > Ni > Co, while for the greenhouse experiment, the order was Zn > Cr > Ni > Cu > Co, and the total amount of Zn was ~ 4 mg/kg, while the total Zn in this outdoor study was 55.4 mg/kg. Also, there was no shoot accumulation of Co and Zn in the greenhouse experiment reported by Otunola et al.[16], but these were found in concentrations of 0.1 mg/kg and 14.8 mg/kg, respectively, in the mesocosm study. A similar trend was observed in the root and shoot accumulation for all the heavy metals, whereby root accumulation was significantly greater than shoot accumulation (Table 4). It should also be noted that the same trend was observed for root and shoot metal accumulation in the previous greenhouse studies.[16,17]

In comparing the results of this outdoor experiment to previous greenhouse studies by Otunola et al.[16,17], as shown in Table 4, we found that in both the greenhouse and outdoor experiments, vetiver showed no signs of growth inhibition in the soil and water treatments. Also, the outdoor experiment yielded better results for both water and soil remediation. A reason for the better performance of vetiver in the outdoor experiment could be due to its exposure to natural conditions, including sunlight, air, humidity and the right temperatures for vetiver.[21] For soil remediation, the order of metals accumulation in the roots of vetiver grass showed a very similar trend to the observation from the greenhouse experiment.[16]

According to Gravand et al.[37], vetiver absorbed Ni (69.4 mg/kg), Mn (63.3 mg/kg) and Pb (282.5 mg/kg). After adding humic acid, Zn and Cu accumulation was promoted, although translocation was reduced due to low bioavailability.[22] Chelating agents and red mud have also been used to promote the growth and remediation capacity of vetiver grass.[38,39] These studies indicate that there was more root accumulation than shoot accumulation of heavy metals in vetiver grass. Vetiver mostly stores heavy metals in its roots or cell walls and heavy metals are likely to reduce water transport to shoots, thereby limiting the translocation of heavy metals. Coupled with the effects of clay minerals, metals sequestering in the roots and vacuoles may be responsible for reduced translocation. The positive side of metal sequestering and reduced translocation is that it limits translocation, so heavy metals will not damage photosynthetic organs.

Suelee et al.[34] observed that root length and density affected heavy metal accumulation by vetiver grass. Higher metal accumulation was achieved at higher root length and density and lower initial metal concentration.[34] In an experiment that tested the use of vetiver for industrial wastewater

**Table 4:** Comparison of heavy metals accumulated by the roots and shoots of vetiver grass in the present mesocosm study and previous greenhouse studies

| Heavy metal | Present mesocosm study | | Greenhouse studies[16,17] | |
|---|---|---|---|---|
| | Shoot (mg/kg) | Roots (mg/kg) | Shoot (mg/kg) | Roots (mg/kg) |
| Al | 356.80 ± 0.2 | 4177.70 ± 0.5 | 41.10 ± 0.3 | 330.70 ± 0.6 |
| Co | 0.10 ± 0.0 | 1.70 ± 0.2 | BDL | 1.39 ± 0.7 |
| Cr | 4.90 ± 0.4 | 33.20 ± 12.98 | 0.15 ± 0.0 | 2.79 ± 1.1 |
| Cu | 4.40 ± 0.1 | 14.60 ± 1.38 | 0.02 ± 0.0 | 1.67 ± 0.9 |
| Mn | 36.31 ± 3.2 | 68.21 ± 38.8 | 3.49 ± 0.9 | 14.40 ± 0.7 |
| Ni | 0.80 ± 0.0 | 6.40 ± 0.8 | 0.22 ± 0.1 | 1.38 ± 0.6 |
| Zn | 14.80 ± 0.2 | 40.61 ± 12.4 | BDL | 0.43 ± 0.2 |

*BDL, below detection limit*

treatment, it was found that vetiver behaved differently depending on the industry and wastewater type.[40] The study also revealed that Cu toxicity resulted in stunted growth, but organic fertiliser increased vetiver yield.[40] The addition of bentonite also reduced the bioavailable Ni in lime and wastewater.[20,41] This is typical of clay minerals, which reduce the bioavailable properties of heavy metals as they adsorb these metals onto their surfaces.[13,19]

Vetiver is also tolerant to Zn, absorbing up to 10 000 mg/kg within 30 days with a high translocation factor.[42] Ni is an essential trace element that improves crop yield, but its behaviour in plants is not yet well understood.[21] The concentration of Ni in the soil before treatment was 13.5 mg/kg, and a total of 7.2 mg/kg was accumulated within 21 days in the AT2.5VT treatment, which is a promising amount.

In evaluating a plant's ability for phytoremediation, after determining the amount of heavy metals taken up by the plant, the translocation factor (TF) and bioconcentration factor (BCF) should be calculated to examine the absorption and transfer of the metals. The TF is the ability of a plant to translocate metals from its roots to shoot and is calculated as the ratio of heavy metal concentration in the shoot to the concentration in its roots. TF values <1 indicate a plant is suitable for phytostabilisation or root storage of heavy metals, while TF values >1 indicate a plant's suitability for phytoextraction.[31] The BCF is the capability of plants to remove heavy metals in soil or water and accumulate them within their shoots and roots. This is expressed as the ratio of heavy metals in plants to that of the substrate.[31]

The observed TF values in this study correspond to the findings of Roongtanakiat[40] who observed TF values of 0.07 to 0.67 and indicated that the maturity of vetiver affects its ability to translocate heavy metals. The older vetiver gets, the less it can translocate heavy metals.[31] According to Roongtanakiat[40], vetiver demonstrated higher TF values for heavy metals in soil compared to water. The type and nature of the amendment applied can also increase or reduce translocation.[19] In this study, clay minerals were noted to increase root sequestration of heavy metals and reduce translocation. In the work of Danh et al.[21], a low TF was observed for As, Cd, Cr and Hg in vetiver, leading to only 16–30% translocation of heavy metals. Similar to the observation of Kafil et al.[36], BCF was 1.30 for Cu and 1.98 for Zn. Siyar et al.[43] found that, within 21 days, the phytoremediation potential of vetiver can be improved by electrokinetic energy. They also observed a BCF >1 in multiple metal contaminated sites.

Although the concentrations of Al and Mn at the start of the experiment were low (0.05 ± 0.2 and 0.18 ± 0.3 mg/L, respectively), Bokhari et al.[44] also observed that metal removal percentage was high (up to 80%) in *Lemna minor* L. despite a very low initial metal concentration. Aisien et al.[45] observed metal concentrations as high as 4870 mg/kg, 4150 mg/kg and 710 mg/kg for Zn, Pb and Cd, respectively, while a BCF of up to 1674 was recorded for Zn. Likewise, Rai et al.[46] recorded a BCF as high as 36 500 for Cd, confirming *Spirodella polyrrhiza* as an excellent hyperaccumulator for heavy metals.

As the observed BCFs are higher than 1 for most of the heavy metals in this study, while TF is <1, vetiver is recommended for phytostabilisation/rhizofiltration of Al, Co, Cu, Cr, Mn, Ni and Zn. Overall, the BCF and TF values differed by metal type and the treated media in this study.

## Conclusion

In the present study we have shown that vetiver grass has the potential to remediate heavy metal contaminated soil and water. There was a high BCF in the water experiment and for some metals in the soil experiment. BCF was <1 for Co, Cr and Ni but >1 for Cu, Zn, Al and Mn, while the translocation factor was <1 for all the heavy metals. The clay minerals restricted the translocation of some heavy metals from roots to shoots, which is considered an advantage because the adsorptive properties of these clays restricts the leaching of heavy metals from soil to water while controlling the amount of metals translocated to the shoots, thereby reducing metal toxicity in vetiver. Overall, the remediation levels achieved were higher than those obtained in greenhouse experiments, meaning that vetiver grass shows better performance in soil and water remediation in situ (outdoors under natural conditions). This demonstrates that attapulgite and bentonite are suitable for improving the phytoremediation capacity of vetiver for removing metal contaminants from soil and water. The results suggest that vetiver grass survives different concentrations of heavy metals in soil and water; and, in combination with clay minerals, it could be useful in a real-life scenario for the remediation of heavy metals contaminated soil and water.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

B.O.O.: Conceptualisation; methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft; writing – revisions; project management. M.P.A.: Conceptualisation; methodology; writing – revisions; student supervision; project leadership. M.T.: Conceptualisation; methodology; data collection; writing – revisions; student supervision; project leadership. O.O.O.: Conceptualisation; methodology; writing – revisions; student supervision; project leadership; funding acquisition.

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# Occurrence, quantification and removal of triclosan in wastewater of Umbogintwini Industrial Complex in KwaMakhutha, South Africa

We report on the detection of an organic pollutant mostly found in local streams and wastewater treatment plants, specifically on triclosan detected in the Umbogintwini Industrial Complex (UIC), located on the south coast of Durban, KwaZulu-Natal in South Africa. Triclosan was successfully extracted from effluent samples using molecularly imprinted membrane adsorbents (MIMs) before quantification and removal using high-performance liquid chromatography (HPLC). This was done through fabrication of a polyvinylidene fluoride polymer using selective microparticles and molecularly imprinted polymers by means of phase inversion and an immersion precipitation method which results in enhanced hydrophilicity and membrane performance. The optimisation of experimental parameters – i.e. contact time and sample size – was performed through different stages of analysis. The synthesised MIMs exhibited an outstanding adsorption efficiency of 97% for triclosan in relation to those of non-imprinted membranes (NIMs) and pristine membranes at 92% and 88%, respectively. The analytical method employed had limits of detection and quantification of 0.21 and 0.69 parts per billion (ppb or μg/L) in wastewater effluent, respectively. The obtained efficiency results show great potential for future use of membrane and molecular imprinting technology, and that MIMs can be adopted as adsorbents for water treatment. The fast and highly selective methodology presented in this work could also be employed for the examination of persistent organic pollutants in the future to combat water scarcity in South Africa.

**Significance:**

The key finding of this work is the incorporation of molecularly imprinted polymers with a membrane adsorbent to improve the performance of the membrane. An unexpected finding was the existence of pollutants like triclosan in water within the boundaries of the KwaMakhutha community, near the human settlement. Among the MIMs, NIMs and bare membranes, higher removal efficiencies were displayed by the synthesised MIMs against the discovered pollutants. This work could open doors for advanced research in the community.

## Introduction

Improvements in analytical technology have led to various transformation methods that enable the detection and quantification of unwanted pollutants in natural water bodies and wastewater treatment plants (WWTPs).[1] The presence of these pollutants in water, even at minimal traces, is of concern among stakeholders, such as drinking water regulators, the South African Department of Water Affairs (DWA), water suppliers and the public, due to the danger they pose to human health and aquatic organisms. Triclosan is an organic pollutant that has recently been detected in WWTPs; Table 1 shows the occurrence of triclosan in a number of regions.[2] Triclosan exposure via drinking water or flowing river water may have adverse effects on living organisms. Product proliferation and ready access to pharmaceuticals, coupled with an increasing human population, have significantly increased the deposition of these compounds into the environment.[3] Pharmaceutical and cosmetic industries are the biggest contributors to the discharge of toxic effluents, which indirectly leads to the proliferation of these drugs in water and may have an accumulative effect in any body they invade. One of the biggest anxieties is that the circulation of these pollutants in water can have an adverse impact on the health of civilians, especially in more vulnerable communities with many infants who are fed on baby formulas made with water from the tap.[2,4]

The presence of organic compounds in river water and wastewater treatment plants has captured attention due to the cost and time needed for treatment. In order to mitigate this crisis, a variety of steps is involved in which over 30 processes are primarily used.[5] For as long as people use chemicals for the treatment of ailments, in personal care products, medication and other cosmetics, trace levels of these substances are likely to be found in water. Table 2 shows the concentrations of triclosan present in various products used daily; the extent of their use indicates how these pollutants are extensively discharged and distributed unconsciously to the human body and further in the aquatic environment. A high content of drugs in uncontrolled discharges of treated wastewater to water bodies can be detected through analytical methods.[6] At present, just over 4000 drugs are listed for pharmaceutical use in the USA but only a few are included under monitoring programmes of water affairs. For some of these drugs, concentrations beyond levels of acceptance (±10 ng/L) have been detected in drinking water and, in all studies, indicate the source of water and the pre-treatment when it comes to wastewater.[7,8]

### *Target compound: Triclosan*

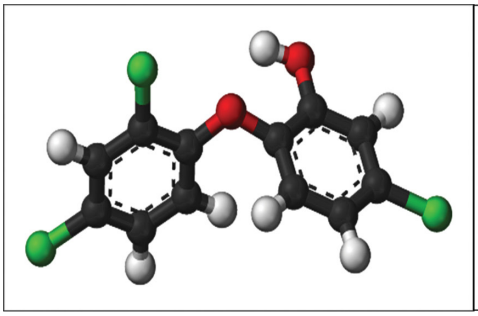
Triclosan can also be referred to by its IUPAC name 5-chloro-2-(2,4-dichlorophenoxy) phenol. The structure of triclosan is displayed in Figure 1. The physicochemical properties of triclosan are important to consider because of the wide use of triclosan in personal care products and other consumer products. This pollutant has been

**Table 1:** The presence of triclosan in environmental matrices

| Environmental matrices | Country | Detected concentration range | Reference |
|---|---|---|---|
| Wastewater treatment plant effluent (ng/L) | Australia | 23 – 434 | 9 |
| | Canada | 63 – 80 | 10 |
| | China | 22.6 – 122 | 11 |
| | | 30 – 1050 | 12 |
| | USA | 500 – 2700 | 13 |
| | | 190 | 14 |
| | Southern Africa | 431 | 4 |
| Hard water (i.e. tap) (ng/L) | China | 0.65 – 15.0 | 15 |
| | USA | <LOQ – 6.5 | 16 |

*Source: Mntambo[4]*

*LOQ, limit of quantification*



| | |
|---|---|
| Formula | $C_{12}H_7C_{l7}O_2$ |
| Molecular weight (g/mol) | 289.54 |
| Molecular width (nm) | 0.696 |
| Molecular height (nm) | 0.755 |
| Molecular length (nm) | 1.453 |
| Solubility in water (mg/L) | 11.9 |
| pKa | 7.82-8.16 |
| Log Kw | 5.15 |

*Values were obtained from the molecular modelling software, version 9.2.*

**Figure 1:** Chemical structure and properties of triclosan.

**Table 2:** Concentration percentage of triclosan in consumer products

| Type of triclosan-based product | Triclosan concentration (%) | Reference |
|---|---|---|
| **Oral-care products** | | |
| Toothpaste | 0.35 | 21 |
| Mouthwash | 0.04 | 22 |
| **Rinse-off products** | | |
| Dishwasher detergent | 0.12 | 7 |
| Skin cleanser | 0.32 | 7 |
| Liquid hand soap | 0.10 – 0.45 | 7 |
| **Leave-on skin products** | | |
| Facial moisturiser | 0.33 | 23 |
| Body lotion | 0.29 | 23 |
| Deodorant | 0.28 | 23 |

*Source: Dhillon et al.[3] under a CC-BY-4.0 licence*

found in alarming amounts in the monitoring of WWTPs, and normally finds its way to the environment through both treated effluent and pharmaceutical personal care products.[17,18] It is also likely to be detected between aquatic paths, sediments, and industrial water as it is not totally eradicated during the industrial treatment of wastewater.[6,19,20] These resultant traces of triclosan detected come from the breakdown of products shown in Table 2 – products which people use daily. It is also important to mention that triclosan is also found in many other consumer products, i.e. cosmetics, household cleaning products (for households), and is incorporated on the surface of medical devices, plastic materials, and textiles.

### *Molecularly imprinted polymers*

Molecular imprinting technology has emerged to be amongst the recently used techniques because of its specificity capabilities and imprinting of templates of organic components. These templates or specific components are called the target molecule. The imprinted target molecule is infused with a suitable functional monomer and a cross-linking agent. The resulting interaction controls the impact and the selectivity potential of the molecularly imprinted polymer (MIP) to yield maximum specificity and selectivity.[24] This technique brings a massive change for developing countries like South Africa, and the surrounding regions in Africa, that are water scarce. In these smart powders, selectivity is driven by the covalence and non-covalence interactions of the target molecule and monomer.[25] The MIPs are manufactured through a precipitation polymerisation method commonly referred to as bulk polymerisation. Before bulk polymerisation takes place, a self-assembly process happens between the functional monomer and the imprinted template, as Figure 2 shows.

### *Membrane technology coupled with molecular imprinting technology*

Membrane technology is a rapidly growing technique that exhibits tremendous advantages, like using a moderate amount of energy, requiring less chemical modification, good film-forming ability, flexibility, toughness, separation properties, and ease in integrating with other processes. Membranes are incorporated with MIPs to form molecularly imprinted membranes (MIMs) and non-imprinted membranes (NIMs).[27,28] The fabrication of these membranes leads to an improved hydrophilic

nature, mechanical behaviour, and thermal resistance, as well as improved anti-fouling ability. Membrane technology is efficiently applied in WWTPs because of its advantageous properties, such as its speed, ease, selectivity and flexibility.[29] Like any other matter, MIPs also have some limitations – during application on real water samples, they might require continual filtration of the aqueous sample after contact with MIPs, which can be endless and too much work for real water applications. Consequently, incorporating them into ultrafiltration membranes leads to efficiency and a feasible alternative.[30,31] The incorporation of MIPs into a polyvinylidene fluoride (PVDF) ultrafiltration membrane is likely to influence the membrane scaffold in terms of morphological impact, and flux and rejection performance of the entire resulting membrane adsorbent, which means that the effects of incorporation of any additive must be investigated thoroughly. In this paper, we focus on the performance and selectivity of MIMs in the removal of triclosan in wastewater. Figure 3 presents the membrane processes used in the industry for advanced treatment of wastewater.

### *Environmental exposure to triclosan*

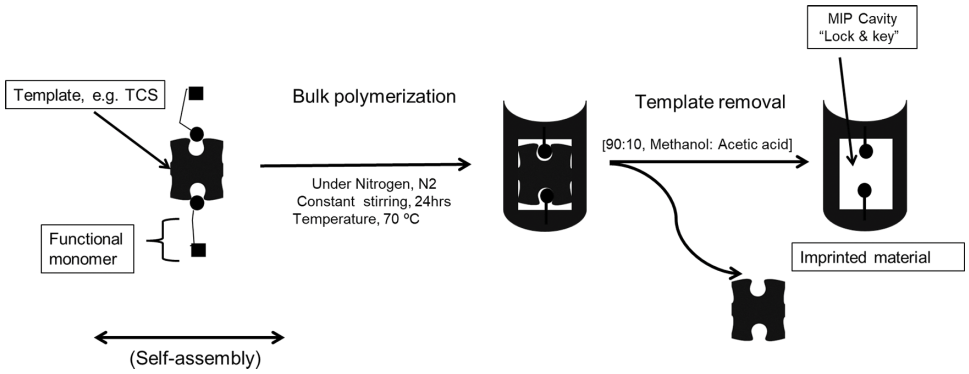
Triclosan is not completely eradicated during wastewater treatment processes, and this ongoing crisis subjects marine life and other water species to incessant exposure. Triclosan is said to accumulate and cause toxic effects within the tissues of these organisms. Unwanted significant traces of triclosan have been detected by researchers before. Mntambo[4] provides a summary of work conducted across the world. Filamentous algae and invertebrates were sampled downstream of a WWTP in Denton (Texas, USA) and triclosan levels of 99–150 ppb were found.[32] Levels of 0.75–10.0 ppb were detected in the plasma of pelagic fish in Detroit, USA[33]; 55–350 ppb in the muscles of freshwater snails in a stream 1 km north of a WWTP in Sweden[34]; 13 900–81 000 ppb in the bile of male bream at river sites in the Netherlands[35] and 0.25–3.41 ppb in muscles of male bream at river sites in Germany[35]; 0.12–0.27 in the plasma of bottlenose dolphins in an estuary in South Carolina[36]; and, lastly, a concentration of 9 ppb was found in the plasma of killer whales in the Vancouver Aquarium Marine Science Centre[37]. What could be concluded from these studies was that there is a bioaccumulation factor for triclosan of 1600; the heightened quantification was expected for all parent compounds with their methylated byproduct.

## Materials and methods

### *Analytical reagents and methods*

Polyvinylidene fluoride (PVDF) pellets and 1-methyl-2-pyrrolidinone (NMP) (99%) were purchased from Capital Lab Suppliers CC. A casting knife was acquired from Trilab. Irgasan (triclosan) (97%), 2-vinylpyridine (2-VP), ethylene glycol dimethacrylate (EGDMA), and 1,10-azobis-(cyclohexanecarbonitrile) (98%) (AIBN) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (ACN) (99.9%), methanol, toluene, formic acid as well as glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide pellets were acquired from Associated Chemical Enterprises (Johannesburg, South Africa). Ultrapure water was produced in the lab using reverse osmosis. AIBN had to be recrystallised before usage, and other chemicals were used without any further purification. Standards of triclosan (irgasan), ketoprofen, fenoprofen and gemfibrozil were purchased at Sigma-Aldrich, Germany. Nylon 0.45 μm filter paper was purchased from Millipore (Darmstadt, Germany).

The following physicochemical properties of the samples were measured immediately after the samples were collected: pH, conductivity, salinity, dissolved oxygen, and total dissolved solids, using a calibrated portable Bante 900P multi-parameter water quality meter purchased from Bante




*Source:* Based on Sharma et al.[26]; *TCS, triclosan*

**Figure 2:** Schematic diagram of the imprinting process for molecularly imprinted polymers (MIP).

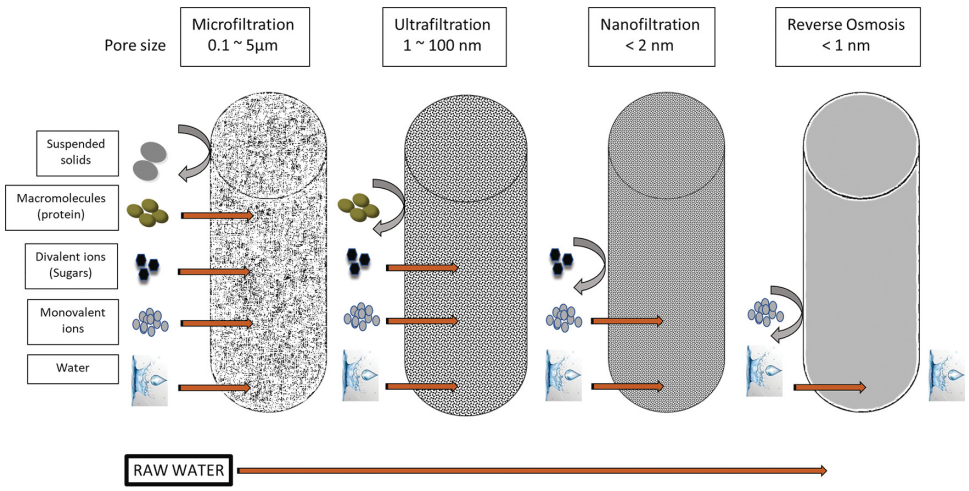



**Figure 3:** Membrane process for advanced treatment of wastewater.

Instruments in Shanghai, China. Calibration standards were provided by the Acacia Operations Services (AOS) laboratory.

### Chromatographic quantification

Quantification of triclosan was achieved using a liquid chromatography system from Shimadzu (Kyoto, Japan). The system is equipped with a degasser (model DGU-20A3), 20 µL sample loop, pump (model LC-20A), and UV/Vis detector. The column used was a Gemini (C18 110A, length 150 x 4.60 mm, ID 5 µm) shipped by Phenominex (CA, USA); the mobile phase mixture used was acetonitrile: 0.2% formic acid in water (80:20 v/v); at a flow rate of 1.0 mL/min and wavelength of triclosan at 254 nm. The chromatography was operated using Shimadzu LC solutions software for data processing.

## *Synthesis of molecularly imprinted membranes*

### Synthesis of MIPs and removal of template

The use of MIPs for screening or quantitative determination of pharmaceuticals in aqueous samples has been reported in other countries.[38] These smart materials are slowly getting recognition, even in developing countries like South Africa, and this work will further spotlight MIPs. Below is a brief description of how these MIPs can be synthesised in the laboratory.

The MIPs were synthesised using a *bulk polymerisation* process. This was done following the method of Dai et al.[39] with slight modification. The reaction mixture of template (1 mmol), functional monomer (3.8 mmol), cross-linker (20 mmol), initiator (30 mg), and porogenic mixture was added to a reaction flask. The mixture was then purged using nitrogen gas for the removal of oxygen, and the reaction flask sealed under nitrogen at 70 $^{o}$C and constantly stirred for 24 h. The MIP obtained was then ground and sieved into smaller particles. A control polymer, a non-imprinted polymer (NIP), was prepared in the same way, but no template was added. The template was then removed from the MIP with constant washing and centrifuging using a proper organic solvent (90:10 methanol:acetic acid).

### Synthesis of MIMs

The incorporation of microparticles to modify the PVDF polymeric membrane is vitally important in cultivating the resultant membrane's properties such as thermal stability, crystallinity, hydrophilicity, anti-fouling resistance and mechanical strength.[40]

When the MIP particles were prepared, they were disseminated accordingly with or in 83 wt% 1-methyl-2-pyrrolidone (NMP), after which 16.95 g PVDF pellets was slowly added while stirring. The polymer resin or mixture was allowed to mix for 8 h and then allowed to settle for 2 h at a constant temperature of 40 °C. The solution was then cast onto a clean glass (infected with NMP and wiped dry) using a casting knife with a blade height set at 50 µm. This was immediately submerged in a coagulation bath equipped with ice and deionised water for at least 15 min, and the anticipated membrane was formed. The resultant ultra-filtration membrane was stored in a refrigerator at 4 °C. Generally, the process was done twice, with the non-imprinted additive and without any additive, and at completion, three sets of polymeric membranes were obtained: MIMs, NIMs and bare membrane.

Thereafter, the MIMs were evaluated for screening of triclosan present in local rivers and WWTPs.[2,41] Table 2 shows different concentrations of triclosan detected in different geographical environments.

## *Landscape and sampling site*

The sampling site is located on the South Coast about 20 km from central Durban, South Africa. The landowners, Acacia Operations Services (AOS), requested an investigation to be undertaken within their territories as fish had been found dead in Kingsway Sea, and they suspected that the water bodies were contaminated. AOS now belongs to Umbogintwini Industrial Complex (UIC), an industrial park with over a century of history and consisting of nearly 210 hectares of landscape, including three nature reserves within the complex. This multi-user site gathers diverse, well-recognised entities, and many other subsidiaries of African Explosives and Chemical Industries (AECI). Figure 4 shows the sampling site.[4]

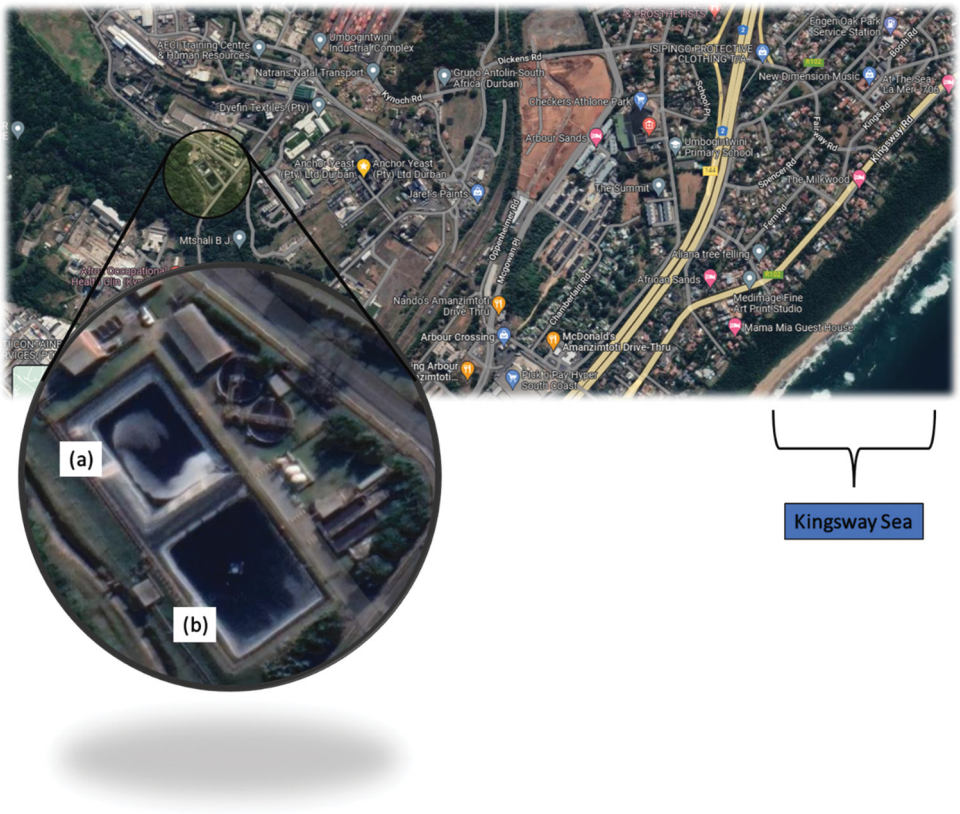



**Figure 4:** Satellite view of the sampling site and effluent treatment plant (ETP) [co-ordinates: 30°01'06.1"S 30°54'30.8"E]. Inset: (a) ETP process compartment and (b) ETP reservoir compartment.

Amongst many industries within the UIC, only AOS is licensed to dispatch wastewater into the Kingsway Sea – which means other industries would have to purge their daily effluents through the channel of the effluent treatment plant (ETP). Figure 4a displays where the treatment process occurs; and Figure 4b where wastewater is allowed to be discharged into the sea. AOS also has their established laboratory where wastewater testing takes place at daily, weekly, and monthly intervals.

### *Sampling and treated samples pre-analysis*

It is notable that the AOS laboratory had recently reported daily on undesirable changes regarding chemical oxygen demand (COD) from the ETP dam, which shows a possible high organic matter in treated water bodies. This crisis is now a worrisome issue to be monitored closely. Hereafter, this study plays a key part in examining traces of organic contaminants and quantifying triclosan in the ETP dam. During this investigation, variation in triclosan was scrutinised for 10 consecutive days (7–16 September 2017). September was selected primarily because it is the busiest month for the businesses in all industries in the complex due to steam and coal demand increasing ahead of the November/December shutdown.

Effluent treatment plant samples were picked up from AOS as composite samples, which means collected as a blend of samples grabbed in three separate intervals, that is, evening, midnight, and morning. The individual samples were then combined into one composite ETP sample. In addition, the grabbed ETP sample was a composite of all wastewater pipelines in the complex. Sample bottles were carefully rinsed with soap and deionised water, after which the composite sample was used to rinse the bottle a few times before a sample was taken. Because the samples were dirty, it was necessary to filter them before analysis.

It is important to analyse the samples for physicochemical properties – i.e. pH, conductivity, and suspended solids – immediately after sampling. Samples were filtered twice the following day. The pH for every collected sample was decreased to 3.0; subsequently, grabbed samples remained at 4 °C in the refrigerator until testing.

#### Sorption selectivity

The anticipated uptake efficacy of the MIMs for triclosan had to be examined using triclosan isomers, i.e. ketoprofen (KET), fenoprofen (FEN) and gemfibrozil (GEM). These isomers are frequently detected to coexist with triclosan in real waters. Consequently, various standard concentrations were used to confirm the unique selectivity of the synthesised MIMs.

Figure 5 confirms that the binding capacity of NIP for triclosan is lower than that of ketoprofen, fenoprofen and gemfibrozil; however, the binding capacity of triclosan on MIP is much higher than that of other pollutants (KET, FEN, and GEM). This confirms the existence of definite cavities or formed sites that favour the template or targeted pollutant whilst rejecting other interfering compounds. MIPs distributed on the membrane scaffold are capable of recognising only the targeted pollutant (imprinted) using their memory cavities through shape and size, and their unique relationship between template and the cavities or open sites. Therefore, all three competing compounds (KET, FEN, and GEM) are not able to bind as strongly as triclosan due to their different size and non-matching cavities or sites. Also, their substrate group is not able to bring about a specific binding coefficient in the same way as triclosan.[42] It is notable that the interaction capability taking place between template and cavities can be determined by the MIP's selectivity ability.[43]

Equation 1, called 'the Scatchard equation', is the calculation of maximum binding capacity ($Q_{max}$ ; mg/g) using the equilibrium dissociation constant of binding sites ($K_d$), the amount adsorbed by the adsorbent (Q; mg/g), and the concentration of the adsorbed triclosan ($C_{free;}$ ppb).

$$\frac{Q}{Cfree} = \frac{(Qmax - Q)}{Kd} \qquad \text{Equation 1}$$

## Results and discussion

### *Pre-analysis of physical and chemical parameters*

The physicochemical results are shown in Table 3. Most of the collected samples contained traces of soluble inorganic salts, which also means that there was negligible interference in the proposed analytical method. The salinity test depends on large quantities of inorganic soluble salts and organic compounds in a water body, as confirmed by Zhang et al.[44] in a 2012 study. The conductivity measurement (EC) of the 10 grabbed samples was found to be within the specific range of South African effluent which is ≤3500, with 1400 on Day 9 being the highest value obtained. Almost all suspended solids (SS) were ≤150 as a specified value for real samples and were immediately reported as non-conforming. We also noted all conforming values of suspended solids: 112%, 106%, 80%, 82% and 120% on Days 4,5,7,8 and 9, respectively. The testing laboratory also reported that these samples were sent to their subcontracted laboratories for further analysis and the results will be provided when available.

Subsequently, we found values for total dissolved solids (TDS) in the grabbed effluents that were within regulation, especially in relation to values previously reported, which were higher.[45] The highest value in this study was 710 mg/L on Day 9, whereas Anderson et al.[45] have previously reported a lowest value of 981 mg/L for TDS in a Canadian WWTP.

### *Enrichment of triclosan on imprinted membranes*

In order to assess the enactment of the polymers (by bulk polymerisation) and the synthesised imprinted membranes (through phase inversion by immersion precipitation), we had to conduct binding trials by spiking composite samples and enriching them with triclosan. The set binding procedure was done by first putting 50 mg of MIP in 10 mL spiked water and MIMs from 0.3 wt%. This was mixed until adsorption equilibrium was reached (at about 20 minutes with constant stirring at room temperature). The MIP or membrane was then separated with the aqueous solution through 15-min centrifuging at 3000 rpm and filtering through a 0.22 μm syringe filter. The next step was to inject into an HPLC chromatographic separator. Another important aspect was to consider studying different membrane types with their adjusted pH, enriched with 500 ppb triclosan spiked water. More so, this was done to express the existing relationship between the cavities of the MIP in MIMs and the target molecule (triclosan) and, of course, the influence of the pH of the aqueous solution (pH 3.00).

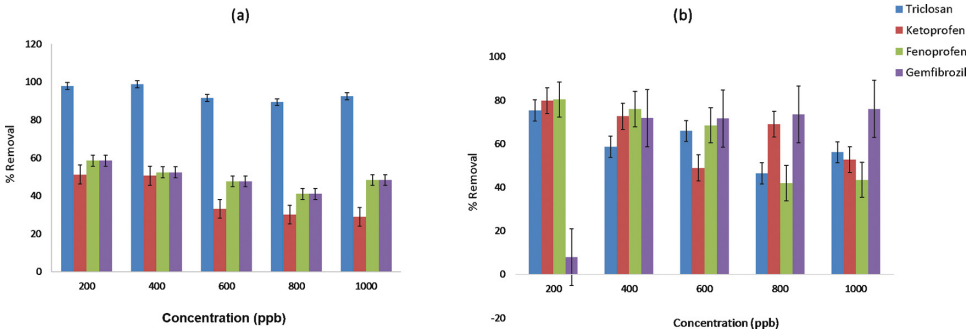



**Figure 5:** Removal efficiency for triclosan and competing compounds: (a) MIMs on different spiked deionised water and (b) NIMs on different spiked deionised water.

The aqueous solution was adjusted in order to populate the solution with hydrogen ions to form a H-H bond interaction as the MIP's monomer is negatively charged (containing a substrate of $N^-$); hence the pH of the spiked water plays a vital role in the attraction with the MIP cavities in MIMs. Hence, both the adsorption and removal efficacy are enhanced. It is noteworthy that differences in percentage removal efficiency are highly dependent on the pH and the additive used. The bare membrane also had a decent adsorption and removal efficiency, even if not as prominent as MIMs and NIMs because of the permeable nature and porous channels created during the synthesis of the membrane.[46]

### *Occurrence of triclosan in UIC wastewater effluent treatment plant*

The recorded concentrations of triclosan in this study show that triclosan is highly dependent on wastewater pre-treatment through processes such as the aeration process and pH adjustment in the ETP dam as displayed in Table 5. Aeration contributes a fair content of dissolved oxygen (DO) that is quantified in WWTP. Thereafter, the resulting triclosan detected in composite grabbed ETP samples confirms the variation on different monitored days. The content of triclosan quantified in the ETP dam samples from Day 1 to Day 10 was 35, 8, 6, 38, 22, 15, 44, 43, 55 and 18 parts per billion (ppb), respectively. On some days, triclosan in the ETP dam was driven by weather conditions. This was specifically observed for Days 2, 3 and 10 when rainfall was experienced (measured to be 3 mm, 5 mm, 6 mm, respectively [this report of rainfall was received from AOS laboratory]. Notably, on Day 7, the 13 September 2017, the triclosan concentration showed a rise. The reason for this undesirable increase was that one of the companies within the complex had done trial procedures on a weaving process – and it is important to mention that the grabbed sample was very dense in colour. Gracia-Lor et al.[47] suggested that this is likely to happen when matrix effects are heightened in the influents compared to the effluents in WWTPs, which leads to increased matrix suppression and, hence, heightened concentrations in effluents.

Table 4 presents recent data on the detection and quantification of triclosan from various wastewater treatment plants across the globe, including data from the current study.

### *Preliminary tests and method optimisation for removal of triclosan*

#### Influence of contact time, pH, adsorbent dosage and volume

***Optimisation A*** - The influence of contact time: optimum contact time for adsorption is vital; this was checked by stirring 40 mg adsorbent (synthesised MIMs, NIMs and bare) for 30 min at 5-min intervals with 15 mL of 500 μg/L triclosan spiked water.

**Table 3:** Physical and chemical properties of the samples from the effluent treatment plant (ETP) dam

| ETP Dam sample | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | pH | Conductivity (mS/m) | Suspended solids (%, m/m)[a] | Total dissolved solids (mg/L) | Salinity (psu) | Dissolved oxygen (%, m/m) |
| Day 1 | 8.09 | 695 | 410 | 603 | 3.94 | 1.90 |
| Day 2 | 8.06 | 744 | 340 | 605 | 3.77 | 0.38 |
| Day 3 | 8.12 | 586 | 160 | 598 | 4.88 | 0.13 |
| Day 4 | 7.67 | 418 | 112 | 515 | 4.13 | 0.45 |
| Day 5 | 7.82 | 482 | 106 | 495 | 3.08 | 0.65 |
| Day 6 | 7.54 | 733 | 290 | 674 | 2.88 | 0.38 |
| Day 7 | 7.93 | 738 | 80 | 599 | 5.18 | 0.69 |
| Day 8 | 7.96 | 1158 | 82 | 697 | 5.08 | 0.13 |
| Day 9 | 7.66 | 1400 | 120 | 710 | 4.93 | 0.27 |
| Day 10 | 8.09 | 835 | 204 | 605 | 3.88 | 0.22 |

*Source: Mntambo[4]*

*[a]Suspended solids (%, m/m) results were obtained from the ETP testing laboratory*

**Table 4:** Concentrations of triclosan in aquatic environments

| System type or medium | Sample description | Region or location | Triclosan concentration (μg/L) | Reference |
|---|---|---|---|---|
| **WWTP** | Effluent | South Africa | 5.5 – 55.1 | This study |
| **WWTP** | In-flowing wastewater | USA | 2.7 – 26.8 | 13 |
| | | Japan | 2.7 – 11.9 | 48 |
| **WWTP** | Treated water | USA | 0.03 – 2.7 | 13 |
| | | UK | 0.34 – 3.1 | 49 |
| **Sediment** | Fresh water | Switzerland | 53.0 | 50 |
| | | Spain | ND – 35.7 | 51 |
| | Marine | Spain | 0.27 – 130.7 | 52 |

*Source: Mntambo[4]*

***Optimisation B*** - The influence of pH: optimum adsorption pH was tested using MIMs or NIMs (synthesised adsorbent) in a pH range from 3 to 10. This procedure was done at the optimum contact time determined from Optimisation A. An adsorption was carried out at a pH of 3, 5, 7 and 10 by using 10 mL solution of 15 mL of 500 μg/L triclosan spiked water, with 40 mg adsorbent.

***Optimisation C*** – Another important aspect to be optimised was the sorption selectivity of the MIMs. This was assessed by introducing triclosan competing compounds (triclosan isomers). These isomers often co-exist with triclosan in effluent water; hence optimisation was carried out using a mixed standard of triclosan, KET, FEN and GEM. The current optimisation's results were obtained using Optimisation B conditions. Here, binding sites of the MIP in synthesised MIMs towards the target molecule (triclosan) were tested against competing isomers.

***Optimisation D*** – The general parameters and working conditions for triclosan removal and adsorption were established. Equilibration time was determined at the optimum initial pH and initial concentrations obtained from Optimisations A, B and C.

### *Removal of triclosan in UIC wastewater effluent treatment plant*

The total triclosan content in the UIC ETP dam is the composite of wastewaters for the entire UIC. Removal efficiencies were paralleled between the imprinted membranes and bare membrane. The outcome of these triclosan percentage removal efficiencies is listed in Table 5.

Table 5 shows that MIMs have higher adsorption capabilities for triclosan than NIMs and the bare membrane for most examined dates. The NIMs are very close, and this shows the effectiveness of PVDF membranes. Again, this is attributed to the strong binding sites stationed in the cavities of the MIP. It is notable that all membranes tested show comparable form in terms of the increase in removal capability. In addition, this denotes the reliability and efficiency of parameters that were initially optimised during instrumentation quantification, specifically: wavelength 254 nm, mobile phase composition (80%:20%), acetonitrile and 0.2% formic acid. Nevertheless, on NIMs and bare membranes, triclosan can be rinsed off easily as their membrane scaffolds are not 'lock and key' as with MIMs.

In some cases, abnormal concentrations of triclosan were observed, specifically on Day 7 compared to other monitored days. This notably high concentration secured a removal efficiency of around 62% using MIMs. This result can be associated with the great number of interferences in the sample – as it was dense and navy in colour. This is usually experienced in wastewater fields when monitoring drugs in wastewater.[53] In addition, the variation of triclosan traces in the composite ETP dam could be attributed to the fact that some industries do not operate daily; hence, their wastewater traces would have been ghosted in the composite ETP grabbed sample on non-operating days.

Removal efficiencies in WWTPs depend on various conditions, such as compound physicochemical properties and climatic conditions like heat intensity, cold weather and rain. Some treatment processes use activated sludge and the age of the activated sludge may have adverse effects.[54] It can then be concluded that removal efficiency is likely to show meaningful dissimilarities from one plant to another, and within a plant at different times. Hence, we monitored various days of busy industrial operation.

Table 6 presents the efficiency of various WWTPs in relation to the results obtained in this study. We can conclude that the imprinted membrane better reduces triclosan, compared to other plants globally.

### *Validation of the chromatography method*

The separation of triclosan was a reverse phase technique. Analytically, selective MIMs as a sorbent was validated based on sensitivity, accuracy, and precision. Limits of detection (LOD) and quantification (LOQ) were considered to monitor sensitivity of the method. LOD (3) and LOQ (10) are concentrations of signal-to-noise ratio. LOD, LOQ, recovery (%) and relative standard deviation (RSD; %) values ($n = 3$) for the spiked deionised water in the concentration range of 5 to 1000 μg/L were 0.21, 0.69, and 65±10% triclosan recovery at 5 μg/L (recovery (%) ± RSD (%)). In addition, 5, 50, and 1000 μg/L spiked water gave an impressive LOD and LOQ of 0.09 and 0.28, respectively, with 110±12%, 76±12%, and 66±5% recoveries, respectively.

The RSD stipulated in ± values communicates the correctness of the procedure used. A linearity ($R^2$) of 0.99 was accomplished for a calibration curve consisting of six ranges of standards (10 to 100 μg/L). Therefore, it can be concluded that the analytical method was accurate, hence the recovery was between 65% and 110%.

**Table 5:** Results on the removal of triclosan with different membranes on different monitored days [all analyses were conducted three times]

| Membrane type | % Removal efficiency | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
| **Bare membrane** | 62 | 30 | 41 | 61 | 56 | 65 | 44 | 96 | 31 | 65 |
| **MIMs** | 78 | 61 | 67 | 92 | 79 | 95 | 62 | 98 | 75 | 89 |
| **NIMs** | 81 | 53 | 63 | 86 | 72 | 90 | 60 | 100 | 70 | 88 |

*Source: Mntambo[4]*

**Table 6:** Previous findings on the removal efficiencies of triclosan from WWTPs against imprinted membranes

| State region or city | % Removal efficiency | Reference |
|---|---|---|
| Durban, South Africa | 62 – 100 | This study |
| Xiamen, China | >20 | 55 |
| Atlanta, USA | 50 – 100 | 56 |
| Gothenburg, Sweden | >90 | 56 |
| Washington DC, USA | 58 – 86 | 57 |
| Juliung, Tokyo | 45 – 65 | 55 |

*Source: Mntambo[4]*

## Conclusions

We studied the occurrence of triclosan as an organic pollutant in a local wastewater treatment plant based in the township of KwaMakhutha, UIC, KwaZulu-Natal in South Africa. The synthesised membrane provided a relatively good reflection of what needs to be done to mitigate or counteract the challenges of triclosan and other unwanted contaminants in WWTPs. The target compound was detected in South African WWTPs in almost similar traces to those in Europe and Asia – this indicates a global crisis to which researchers need to pay close attention. This study has shown a reduction in the poisonous pollutant contaminant discharged into Kingsway Sea of 62–95% using MIMs; this range is comparable to those of other global studies which indicated a wastewater challenge. The presence of this pollutant in the dam of Umbogintwini Industrial Complex indicates the ignorance of on-site companies with respect to contamination by influents during their daily operations. And these findings show that more research should be conducted

in all South African WWTPs, including rivers and dams. The performance of modified MIMs for treating wastewater through ultrafiltration was also investigated for different triclosan isomers. The prepared membranes displayed asymmetric membrane sorptivity and selectivity for triclosan, indicating that the MIMs produced are selective for triclosan. Water uptake regarding the MIMs confirms increased hydrophilicity when compared with a bare polyethersulfone membrane. The membrane surfaces incorporated with MIPs and the hydrophilic nature of the adsorbents (MIPs) also heightened the wettability, permeability and the anti-fouling ability of the membranes. A new type of polymeric blend membrane material based on MIMs has therefore been identified for treatment of industrial effluents and wastewater. A 0.3% MIM-blended membrane produced highly desirable results for the removal of triclosan in UIC treated effluent.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

S.A.M.: Conceptualisation; methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft; writing – revisions; project leadership; project management; funding acquisition. L.L.S.: Conceptualisation; validation; writing – revisions; student supervision; project leadership; funding acquisition. P.P.N.: Data curation; writing – revisions; student supervision.

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# Antibacterial activity of two actinomycetes species isolated from black sand in North Egypt

Increasingly high levels of multidrug-resistant (MDR) pathogens have necessitated the discovery of novel bioactive compounds. For this reason, two actinomycetes strains, *Streptomyces griseorubens* and *Streptomyces rochei*, were isolated for the first time from the black sand shores of Kafr El Sheikh in Egypt, which is home to several large fish farms. Isolates were identified via phenotypic, biochemical and 16S rRNA sequence protocols. Both strains exhibited powerful antimicrobial activity against three serious MDR pathogens: *Bacillus subtilis, Salmonella enteritidis* and *Pseudomonas aeruginosa*. The bioactive compounds of isolates' filtrates were identified using gas chromatography–mass spectroscopy (GC-MS). For *S. griseorubens*, the detectable antibacterial compounds were hexanoic acid, 2-ethyl-, 2-ethylhexyl ester, n-Decane, hexadecanoic acid methyl ester, benzene acetic acid, ricinolic acid, and ethylparaben, while *S. rochei* secretes heptadecane, 2,6-dimethyl-, benzene acetic acid, dibutyl phthalate, octacosane, hexacosane, and vitamin A aldehyde. These results strongly encourage the use of these eco-friendly isolates as a biocontrol against MDR pathogens that attack fish farms.

**Significance:**

*Streptomyces* spp. act as strong weapons for fighting multidrug resistance in pathogenic bacteria – one of the most important current threats to public health. They are additionally regarded as eco-friendly organisms that can be used as a biocontrol agent against infections that endanger fish farms.

## Introduction

Actinomycetes are prokaryotic organisms that are widely distributed in different habitats and are characterised by a high G+C ratio (>55%) in their DNA; due to their growth pattern, they more closely resemble fungi.[1] Among actinomycetes, *Streptomyces* is the most dominant genus in soil.[2] *Streptomyces* species are the main sources of biologically active compounds including antibiotics, anticancer agents, anthelmintics, antioxidants, and antifungals. As a result of the resistant behaviour of major bacterial pathogens against at least one antibiotic, finding new antibacterial compounds is a main aim of researchers.[3] As *Streptomyces* species are widely distributed and have been widely evaluated as potential sources of novel bioactive compounds, they are good weapons against multidrug resistance (MDR) in pathogenic bacteria.[4]

*Streptomyces* species produce around 7600 bioactive microbial metabolites, with rare actinomycetes producing an increasing share of novel compounds.[5] Both *Streptomyces griseorubens* and *Streptomyces rochei* show high application importance, where *S. rochei* has a high value due to its efficient activity against various human carcinoma cell lines[6], while *S. griseorubens* shows high biocontrol efficacy against *Fusarium* wilt disease of tomato.[7] Here, *S. griseorubens* and *S. rochei* were isolated from black sand soils and identified using modern techniques. Also, the fractionations of their filtrates by gas chromatography prove a promising source of antimicrobials against MDR pathogens.

## Materials and methods

### *Sampling and isolation*

Soil samples were collected from several locations at Mutubas shore (31°28'04.6"N; 30°24'09.2 "E), Kafr El-Sheikh Governorate. Samples were air dried at 35 °C for 34 h, crushed, and sieved via 2 mm pores. One gram of 0.1–2 μm sieved soil particles was suspended in 9 mL of sterile distilled water[8,9], followed by serial dilutions up to $10^{-4}$ dilutions. A volume of 50 μL from each dilution was spread on starch nitrate agar and incubated at 30 °C for 7 days.[10]

### *Morphological and biochemical characterisations*

The morphological features including texture, aerial mycelium, substrate mycelium, growth rate, and colour of colonies on the starch nitrate medium were investigated.[11] Biochemical features were performed using an API 20A kit (Biomerieux). API stripes were inoculated following the manufacturer's manual. Stripes were incubated at 30 °C for 24–48 h. After the incubation period, reagents were added to vials for 5–10 min followed by the stripes evaluation performed according to the manufacturer's instructions.

### *Molecular identification*

Pure isolates were cultured in SN broth medium at 30 °C with shaking for 7 days. Pellets were collected by centrifugation at 3000 rpm for 20 min. Genomic DNA was extracted using EZ-10 Spin Column Bacterial Genomic DNA Miniprep Kits. *Streptomyces* species were partially sequenced by the 16S region. The 16S rDNA regions were amplified using the universal forward primer 27F (5'-AGAGTTTGATC (AC) TGGCTCAG--3') and the reverse primer 1492R (5'-ACGG (CT) TACCTTGTTACGACTT-3'). The polymerase chain reactions (PCR) consisted of 4 μL of dNTPs (1.0 mM each, Roche, Penzberg, Germany), 2 μL of 10X buffer (Roche), 0.2 μL of each primer (0.5 μg), 0.2 μL of Taq polymerase (5 U/μL), 1 μL of 50 ng of template DNA, and sterile Milli-Q water in a final volume of 19.8 μL.

Amplification occurred through the following protocol: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s, and 72 °C for 7 min. A Qiagen PCR purification kit (Qiagen, Hilden, Germany) was used for purification of the obtained PCR fragments. The 16S rRNA genes of *Streptomyces* spp. were sequenced using the forward 27F primer and the Big Dye Terminator Cycle Sequencing kit v1.1. Produced DNA fragments were sequenced using the 3500xL Genetic Analyzer, Applied Biosystems, Foster City, California, USA. The resulting nucleotide sequences were aligned throughout the GenBank data, the BLAST-N program (Basic Local Alignment Search Tool – Nucleotides) from the website of the US National Center for Biotechnology Information (NCBI).[12]

### *Antimicrobial evaluation of Streptomyces species filtrates*

Three multidrug – resistant (MDR) pathogens – *Bacillus subtilis* (ATCC6633), *Salmonella enteritidis* (ATCC14028), and *Pseudomonas aeruginosa* (ATCC 10145) – were obtained from the strains bank at the Microbial Centre of Kafrelsheikh University, Egypt. A volume of 1 mL of either *S. griseorubens* or *S. rochei* suspension was inoculated in 50 mL of autoclaved SN broth medium cultured in a 250 mL Erlenmeyer flask. Cultures were incubated at 30 °C and 150 rpm for 7 days. After incubation, the supernatant was separated by centrifugation at 3000 rpm for 20 min.

For each of the pathogens, 1 ml of old culture broth (18 h) was swabbed separately on freshly prepared nutrient agar medium. In the separately inoculated plates, wells with a diameter of 5 mm were drilled with a sterile cork drill. Each well was injected with 100 µL of each *Streptomyces* sp. supernatant. The plates were incubated at 37 °C for 24 h. After the incubation period, the diameter of the zone of inhibition (mm) was measured.[13]

### *Extraction of antibacterial compounds*

Both *S. griseorubens* and *S. rochei* underwent fermentation. Each strain was sub-cultured separately for 5 days at 30 °C in starch nitrate broth that was used to inoculate 500 mL of fermentation broth and then incubated on a rotary shaker incubator (150 rpm) at 30 °C for 10 days. Filtration via Whatman no.1 filter paper separated the extracellular crude extract, which was then centrifuged at 8000 rpm for 15 min at 4 °C. Then the supernatant was aseptically transferred into 250 mL flasks and mixed with an equal volume 1:1 (v/v) of ethyl acetate. The mixture was agitated rapidly for 20 min before being stationary for another 15 min. By evaporation in a 40 °C oven, the aqueous and organic layers of crude extract were separated and concentrated to solvent-free content. The residue was vacuum dried, weighed, and dissolved in 1 mg/mL methanol. The antibacterial activity of dissolved substances was tested using the well diffusion method. Methanol was employed as a control against pathogens in each test.[14]

### *GC-MS Analysis*

Detection of active compounds present in the ethyl acetate extract of *Streptomyces* species that showed high bactericidal activity was performed using gas chromatography–mass spectroscopy (GC-MS) at the National Institute of Oceanography and Fisheries, Alexandria, Egypt. The GC-MS analysis was carried out using an Agilent 7890A GC instrument with an HP-5MS column (30 m x 250 m x 0.25 m film thickness) and an MS detector (Agilent 5975C). The oven temperature was set to 90 °C for 1 min, then increased to 300 °C at an 8 °C/min pace for 30 min. Helium was employed as the carrier gas at a flow rate of 1.5 mL/min. In the splitless mode, the sample injection volume was 1 L and the injector temperature was 290 °C. The mass spectrum was run at 70 eV with a mass range of 60–600 amu.[15]

### *Statistical analysis*

Data were recorded in triplicate. Statistical analysis was carried out using the SPSS program. Obtained data are shown as means and standard errors of the means.[16]

## Results

### *Identification of Streptomyces species*

#### Phenotypic and biochemical characteristics

*Streptomyces griseorubens* showed a grey colour for substrate and aerial mycelia. *S. rochei* also showed a grey colour for aerial and substrate mycelia, with no production of melanin pigment and positive reactions with a Gram stain for both. Both isolates showed positive reactions against glucose, mannitol, lactose, xylose, maltose, fructose, and rhamnose. Only *S. rochei* was positive against arabinose and only *S. griseorubens* was positive against mannose. Ureases and catalases were produced by *S. griseorubens* only (Table 1).

#### Molecular identification (16S rRNA sequence analysis)

Genome amplification of the isolates' DNA using PCR with the primer 16S rRNA gene resulted in DNA fragments appearing as sharp bands at approximately 300 bp (Figure 1). The obtained sequences were analysed through an online database (NCBI) and compared with other bacterial

**Table 1:** Biochemical characteristics of *Streptomyces griseorubens* and *S. rochei*

| Test | *S. rochei* | *S. griseorubens* | Test | *S. rochei* | *S. griseorubens* |
|---|---|---|---|---|---|
| **Glucose** | + | + | **Sorbitol** | _ | _ |
| **Mannitol** | + | + | **Rhamnose** | + | + |
| **Lactose** | + | + | **Trehalose** | _ | _ |
| **Saccharose** | _ | _ | **Fructose** | + | + |
| **Salicin** | _ | _ | **Galactose** | _ | _ |
| **Xylose** | + | + | **N-acetyl-β-glucosamindase** | _ | _ |
| **Maltose** | + | + | **Glycerol utilisation** | _ | _ |
| **Starch hydrolysis** | + | + | **Urease** | + | _ |
| **Arabinose** | + | _ | **Esculin hydrolysis** | + | + |
| **Cellobiose** | _ | _ | **Indole formation** | _ | _ |
| **Mannose** | _ | + | **Catalase** | + | _ |
| **Melezitose** | _ | _ | **Nitrate reduction** | _ | _ |
| **Raffinose** | _ | _ | | | |

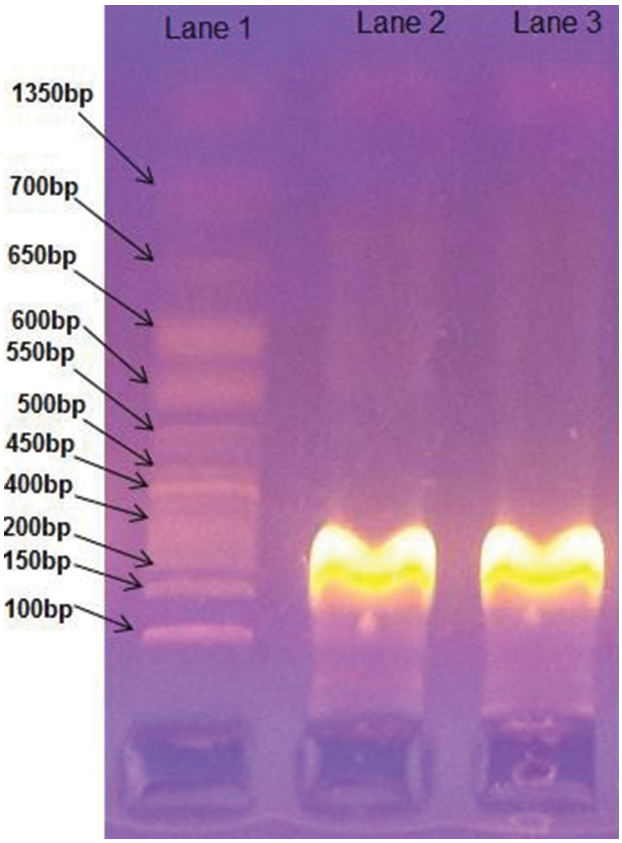


**Figure 1:** PCR amplified 16S rRNA gene. Lane 1: Molecular weight of SiZer™-1000 DNA marker; Lane 2: amplified DNA fragment of ~300 bp of *Streptomyces griseorubens;* Lane 3: amplified DNA fragment of ~300 bp of *S. rochei.*

isolates. The sequencing results showed that purified strains belong to the Phylum Actinobacteria, the Family Streptomycetaceae and the Genus *Streptomyces*. The data analysis confirmed isolates as *Streptomyces griseorubens* strain 173471 and *Streptomyces rochei* strain 1 (Table 2). A neighbour-joining tree was employed for both using MEGA-X software to show phylogenetic relationships between both of the *Streptomyces* strains and other *Streptomyces* neighbours (Figures 2 and 3).

### *Antimicrobial activity*

*Streptomyces griseorubens* filtrate exhibited significant antimicrobial activities against *Bacillus subtilis, Salmonella enteritidis* and *Pseudomonas aeruginosa,* with inhibition zone diameters of about 11 mm, 17 mm, and 20 mm, respectively, while that of *S. rochei* showed antimicrobial activities against *B. subtilis* and *S. enteritidis,* with inhibition zone diameters of about 15 mm and 17 mm, respectively (Table 3).

### *GC-MS Analysis*

Ethyl acetate extracts of *S. griseorubens* and *S. rochei* that showed high antibacterial activities were analysed by GC-MS. The mass spectrum of GC-MS was interpreted according to the US National Institute of Standards and Technology (NIST) database, by comparing unknown spectra with the known data stored in the NIST library. For *S.griseorubens,* observed data confirmed the presence of six active compounds with antibacterial activity (Figure 4). The GC-MS analysis of *S. rochei* also confirmed the presence of six antibacterial compounds (Figure 5). The secondary metabolites from *S. griseorubens* that showed antibacterial activities were hexanoic acid, 2-ethyl-, 2-ethylhexyl ester (3.14%), n-Decane (21%), hexadecenoic acid methyl ester (18%), benzene acetic acid (2.44%), ricinolic acid (8.17%) and ethylparaben (5.54%) (Table 4). For *S. rochei*, heptadecane, 2,6-dimethyl- (2.08%), benzene acetic acid (3.86%), dibutyl phthalate (17.01%), octacosane (8.95%), hexacosane (12.78%) and vitamin A aldehyde (4.82%) were detected (Table 5).

## Discussion

Two strains of *Streptomyces* were identified using the 16S rRNA sequence as well as traditional techniques, and their antibacterial potentiality was determined. The sequencing result of the PCR amplified 16S rRNA gene confirmed that isolate 1 belongs to Phylum Actinobacteria, Family Streptomycetaceae and Genus *Streptomyces*. The result revealed a 97.47% similarity with *Streptomyces griseorubens* strain 173471 according to NCBI GenBank and showed a similarity of about 97.29% with both *Streptomyces labedae* strain AJR1 and *Streptomyces albogriseolus* strain SY67903. According to Thirumurugan and Vijayakumar[17], *Streptomyces labedae* substrate mycelia appeared brown on SCA medium and showed positive results with catalase production and nitrate reduction, whereas *Streptomyces griseorubens* could not produce catalase or reduct nitrate. In addition, *Streptomyces griseorubens* had a grey colour for both aerial mycelia and substrate mycelia on the SNA medium and all ISP media, with no distinctive pigments.[18] According to El-Naggar et al.[19], *Streptomyces albogriseolus* showed grey aerial mycelia and yellow/brown substrate mycelia on the SNA medium, while *Streptomyces griseorubens* appeared grey in both aerial mycelia and substrate mycelia on the SNA medium. In addition, *Streptomyces albogriseolus* can utilise sucrose and raffinose while *Streptomyces griseorubens* cannot.

The sequencing result of the PCR amplified 16S rRNA gene revealed that isolate 2 belongs to Phylum Actinobacteria, Family Streptomycetaceae and Genus *Streptomyces*. It revealed a 97.97% similarity with *Streptomyces rochei* strain 1 according to NCBI GenBank and showed a similarity of about 97.87% with both *Streptomyces mutabilis* strain SAIG321 and *Streptomyces maritimus* strain UP1A-1.

According to *Bergey's Manual of Systemic Bacteriology*[18], *Streptomyces mutabilis* have white aerial mycelia on a starch nitrate agar medium while those of *Streptomyces rochei* are grey on SNA medium. In addition, *Streptomyces mutabilis* can utilise arabinose and sucrose while *Streptomyces rochei* cannot. According to Manikkam et al.[20], *Streptomyces maritimus* have light grey aerial mycelia on a starch nitrate medium, while those of *Streptomyces rochei* appear dark grey on SNA medium. In addition, *Streptomyces maritimus* cannot utilise xylose while *Streptomyces rochei* can.

The antibacterial compounds – hexanoic acid, 2-ethyl-, 2-ethylhexyl ester, n-Decane, hexadecanoic acid methyl ester, benzene acetic acid, ricinolic acid, and ethylparaben – observed in *Streptomyces griseorubens* were found to be strong antibacterial agents. Antibacterial action has been observed for hexanoic acid, 2-ethyl-, 2-ethylhexyl ester, which contains the short chain fatty acid (hexanoic acid). Short-chain fatty acids are commonly produced by healthy gut microbiota and serve as a defender against enteric infections. Furthermore, by lowering intracellular pH and diffusing across the bacterial membrane, they show a direct antibacterial effect against bacterial pathogens.[21] According to Al-Rubaye et al.[22], a *Streptomyces* sp. isolated from Tigris River sediments in Baghdad was found to generate short-chain fatty acids. N-decane is a straight-chain alkane that was extracted by the n-butanol of *Streptomyces* sp. Sp1 filtrate, which has been shown to have strong antibacterial activity and could be used as a biocontrol agent against *Vibrio anguillarum*.[23] The accumulation of such chemicals in the cell membrane can have a significant impact on its function and eventually lead to cell death.[24] Disruption of the electron transport chain and oxidative phosphorylation is linked to its mechanism of action. Inhibition of enzyme activity can also cause nutrient absorption failure, production of peroxidation and auto-oxidation degradation products, and eventually leads to bacterial cell lysis.[25]

Hexadecanoic acid is a saturated long-chain fatty acid that was observed in a *Nocardia* sp. filtrate, which was found to exhibit antibacterial action against methicillin-resistant *Staphylococcus aureus* (MRSA).[26] Benzene acetic acid is an organic compound containing a phenyl functional group and a carboxylic acid functional group. According to Al-Dhabi et al.[27], *Streptomyces* sp. Al-Dhabi-2 isolated from a harsh environment in Saudi Arabia was found to produce benzene acetic acid. It had substantial minimum inhibitory concentration (MIC) values of less than 39 μg/mL against *Bacillus cereus* and *Enterococcus faecalis*, and 78

**Table 2:** The sequence alignments of the 16S rRNA gene of *Streptomyces griseorubens* and *S. rochei* to the data available at NCBI (BLAST-N)

| ***Streptomyces griseorubens*** | | | | | | | ***Streptomyces rochei*** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Description** | **Max score** | **Total score** | **Query cover** | **E value** | **Percentage identity** | **Accession number** | **Description** | **Max score** | **Total score** | **Query cover** | **E value** | **Percentage identity** | **Accession number** |
| *Streptomyces griseorubens* strain 173471 16S ribosomal RNA gene, partial sequence | 946 | 946 | 40% | 0.0 | 97.47% | EU570629 | *Streptomyces rochei* strain 1 16S ribosomal RNA gene, partial sequence | 1705 | 1705 | 83% | 0.0 | 97.97% | MN589659 |
| *Streptomyces albogriseolus* strain SY67903 16S ribosomal RNA gene, partial sequence | 941 | 941 | 40% | 0.0 | 97.29% | MT611305 | *Streptomyces maritimus* strain UP1A-1 16S ribosomal RNA gene, partial sequence | 1701 | 1701 | 83% | 0.0 | 97.87% | MT627170 |
| *Streptomyces griseorubens* strain K5 16S ribosomal RNA gene, partial sequence | 941 | 941 | 40% | 0.0 | 97.29% | MT525003 | *Streptomyces rochei* strain STR1 16S ribosomal RNA gene, partial sequence | 1701 | 1701 | 83% | 0.0 | 97.87% | MT568582 |
| *Streptomyces labedae* strain AJR1 16S ribosomal RNA gene, partial sequence | 941 | 941 | 40% | 0.0 | 97.29% | MT463534 | *Streptomyces mutabilis* strain SAIG321 16S ribosomal RNA gene, partial sequence | 1701 | 1701 | 83% | 0.0 | 97.87% | MT355865 |
| *Streptomyces* sp. strain MJM15241 16S ribosomal RNA gene, partial sequence | 941 | 941 | 40% | 0.0 | 97.29% | MT393671 | *Streptomyces rochei* strain SA1019 16S ribosomal RNA gene, partial sequence | 1701 | 1701 | 83% | 0.0 | 97.87% | MT355863 |

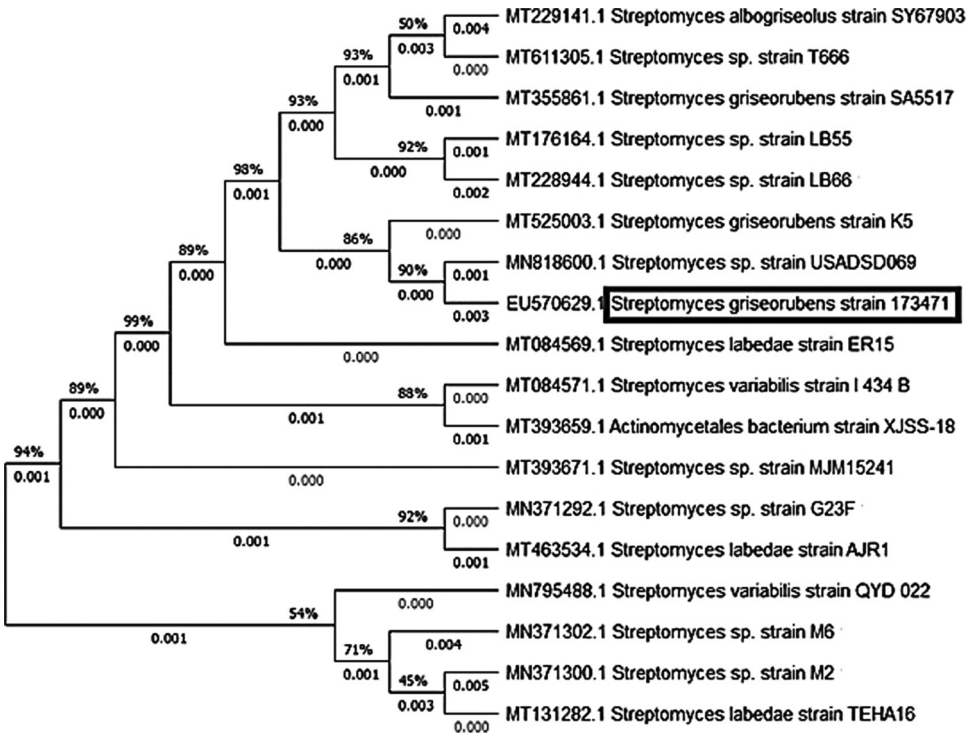


**Figure 2:** A phylogenetic tree illustrating similarity between *S. griseorubens* and other *Streptomyces* neighbours (constructed using MEGA-X software).

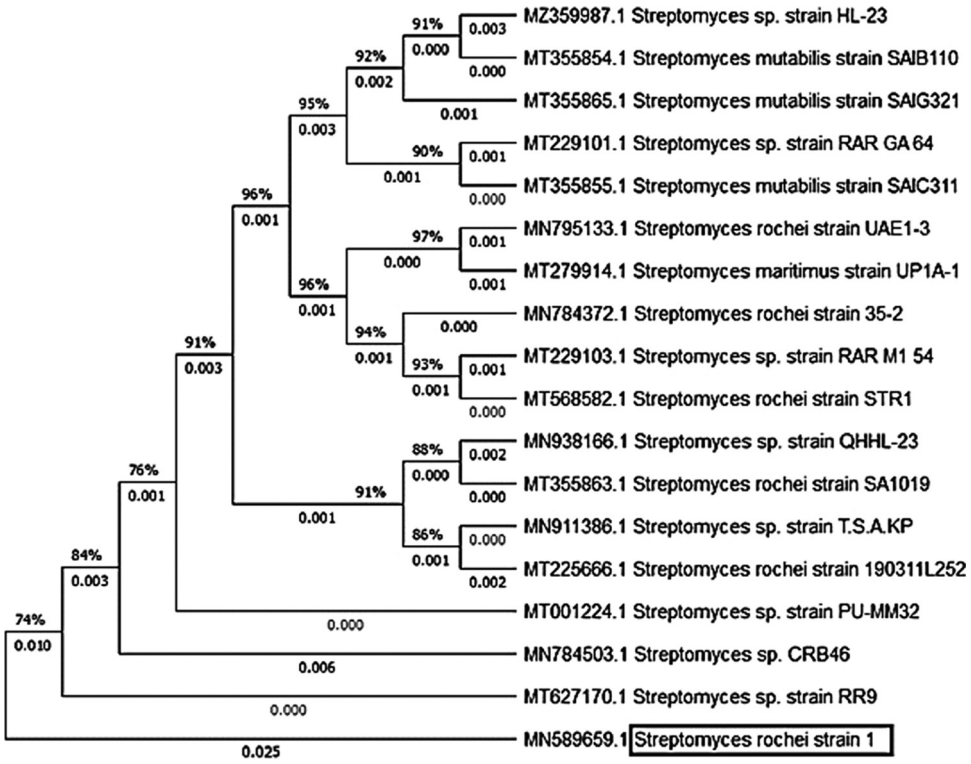


**Figure 3:** A phylogenetic tree illustrating similarity between *S. rochei* and other *Streptomyces* neighbours (constructed using MEGA-X software).

μg/mL against *Streptococcus agalactiae*. The harmful effect of benzene acetic acid is mostly mediated by acetic acid dissociation within the microbial cells, which results in lowering intracellular pH and metabolic disruption.[28] Ricinoleic acid [*R* (*Z*)-12-hydroxy-9-octadecanoic acid] is a fatty acid that is considered the major component of castor oil. The antibacterial activity of ricinoleic acid against *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been reported, with MICs of 2.68 μM and 2.60 μM, respectively.[29] The surfactant behaviour of ricinoleic acid moiety is due to the presence of long lateral hydrophobic methylene units that could disturb and inhibit the permeability of the bacterial cell membrane which consequently inhibits the growth of bacteria.[30] Ethylparaben is an ethyl ester of p-hydroxybenzoic acid. The GC analysis of a *Brevibacillus brevis* crude extract proved the presence of ethylparaben, which has a considerable bactericidal impact against *Escherichia coli*.[31] Although its

**Table 3:** Antimicrobial activities of *Streptomyces griseorubens* and *S. rochei* crude extracts against different multidrug-resistant pathogens

| Pathogen | Inhibition zone (mm) | |
|---|---|---|
| | ***S. griseorubens* crude extract** | ***S. rochei* crude extract** |
| ***Bacillus subtilis*** | 11±0.22 | 15±0.35 |
| ***Salmonella enteritidis*** | 17±0.29 | 17±0.24 |
| ***Pseudomonas aeruginosa*** | 20±0.33 | _ |

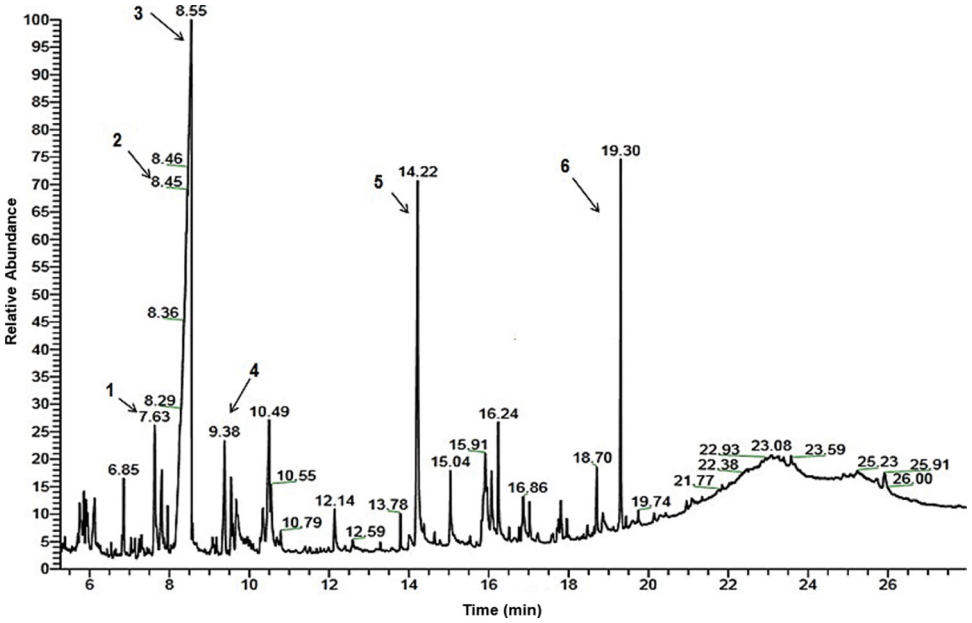


**Figure 4:** GC-MS chromatograph of extracellular filtrate of *Streptomyces griseorubens*.

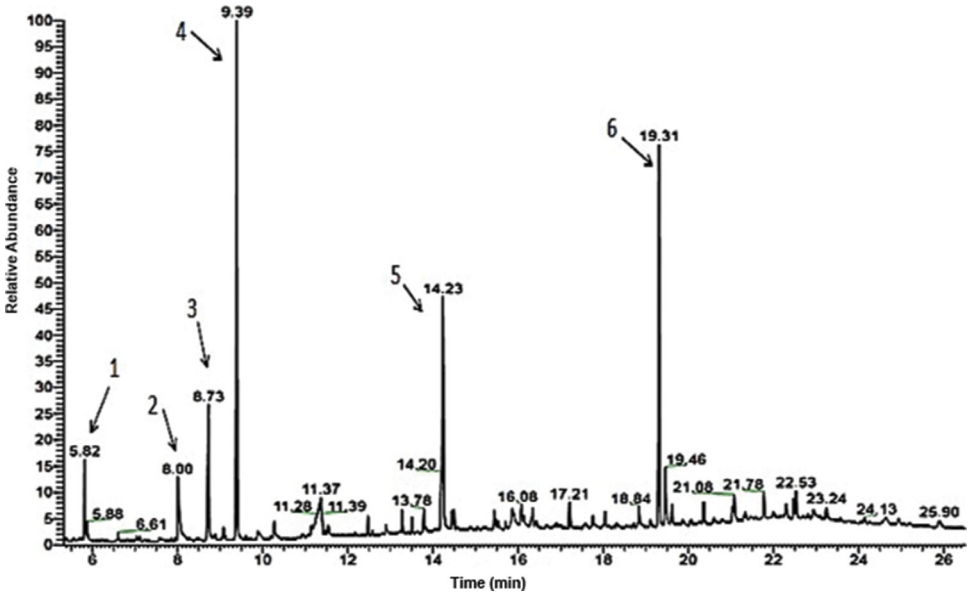


**Figure 5:** GC-MS chromatograph of extracellular extract of *Streptomyces rochei*.

mode of action is still unproven, one of the suggested modes of action of parabens is to disrupt osmotic gradients in bacteria by interacting with mechanosensitive channels.[32] *Streptomyces rochei* was detected to produce the antibacterials heptadecane, 2,6-dimethyl-, benzene acetic acid, dibutyl phthalate, octacosane, hexacosane, and vitamin A aldehyde.

Decane compounds have been shown to exhibit antibacterial action in several studies. Dodecane, n-Hexa decanoic acid, and 1-Octadecane were found in an ethyl acetate extract of *Streptomyces cavouresis* KUV39 by GC-MS.[33] Phthalic acid esters (PAEs) are a type of lipophilic compound that is commonly found in plants and microorganisms. Di-n-butyl phthalate, diethyl phthalate, and dimethyl phthalate are the

**Table 4:** Antibacterial compounds identified in the ethyl acetate extract of *Streptomyces griseorubens*

| N | Name of the compound | Molecular formula | Molecular weight (g/mol) | Retention time (min) | Peak area (%) | Activity |
|---|---|---|---|---|---|---|
| 1 | **Hexanoic acid, 2-ethyl-, 2-ethylhexyl ester** | $C_{14}H_{28}O_2$ | 228.37 | 7.63 | 3.14 | Antibacterial |
| 2 | **n-Decane** | $C_{10}H_{22}$ | 142.28 | 8.45 | 21 | Antibacterial |
| 3 | **Hexadecanoic acid, methyl ester** | $C_{17}H_{34}O_2$ | 270.45 | 8.55 | 18 | Antibacterial |
| 4 | **Benzene acetic acid** | $C_8H_8O_2$ | 136.14 | 9.38 | 2.44 | Antibacterial |
| 5 | **Ricinolic acid** | $C_{18}H_{34}O_3$ | 298.46 | 14.22 | 8.17 | Antibacterial |
| 6 | **Ethylparaben** | $C_9H_{10}O_3$ | 166.17 | 19.30 | 5.54 | Antibacterial |

**Table 5:** Antibacterial compounds identified in the ethyl acetate extract of *S. rochei*

| N | Name of the compound | Molecular formula | Molecular weight (g/mol) | Retention time (min) | Peak area (%) | Activity |
|---|---|---|---|---|---|---|
| 1 | **Heptadecane, 2,6-dimethyl-** | $C_{19}H_{40}$ | 268.5 | 5.82 | 2.08 | Antibacterial |
| 2 | **Benzene acetic acid** | $C_8H_8O_2$ | 136.14 | 8 | 3.86 | Antibacterial |
| 3 | **Vitamin A aldehyde** | $C_{20}H_{28}O$ | 284.43 | 8.73 | 4.82 | Antibacterial |
| 4 | **Dibutyl phthalate** | $C_{16}H_{22}O_4$ | 278.34 | 9.39 | 17.01 | Antibacterial |
| 5 | **Octacosane** | $C_{28}H_{58}$ | 394.77 | 14.23 | 8.95 | Antibacterial |
| 6 | **Hexacosane** | $C_{26}H_{54}$ | 366.71 | 19.31 | 12.78 | Antibacterial |

most common PAEs found in natural sources, and are reported to have antibacterial properties.[34] Using ultraviolet, Fourier transform infrared, and GC-MS analyses, the bioactive chemical dibutyl phthalate was reported to be generated by the soil actinomycete isolate *Streptomyces albidoflavus* 321.2, showing a considerable antibacterial effect against *E. coli* with an MIC of 53 μg/mL and *Bacillus subtilis* with an MIC of 84 μg/mL.[35] It was also isolated from a novel marine *Streptomyces* sp. that reduced *Colletotrichum fragariae* spore germination and mycelial growth.[36] Further studies are needed to understand the related antimicrobial mechanisms of PAEs. Hexacosane is a straight-chain alkane that is a volatile oil component and a plant metabolite. Hexacosane was discovered to be among the chemicals in the medicinal plant *Kielmeyera coriacea* that exhibits antibacterial activity against both aerobic and non-aerobic bacteria.[37] Octacosane is one of 17 chemicals found in *Elsholtzia ciliate* extracts that have antibacterial properties.[38] Its mode of action has been linked to accumulation in the cell membrane, which affects the function of the cell and finally leads to cell death.[21] Retinaldehyde is a stabilised form of vitamin A. According to Pechère et al.[39], vitamin A is thought to be anti-infectious. Although retinal resistance is not unique to infection, the mechanisms underlying these post-infective actions are largely unknown and are most likely related to its pleiotropic effects on immune function. Both in vivo and in vitro, retinaldehyde has been shown to have antibacterial action against *Propionibacterium acnes. P. acnes* No. CIP179 and CIP53119 have MICs of 4 mg/L, while *P. acnes* No. CIP53117 had a MIC of 8 mg/L.[40]

## Conclusion

Two strains, *Streptomyces griseorubens* and *Streptomyces rochei*, producing novel bioactive compounds were identified via 16S rRNA sequencing and traditional techniques. Antibacterial evaluation of the crude extract showed high efficiency against very serious MDR pathogens – *P. aeruginosa, S. enteritidis,* and *B. subtilis*. GC analysis of *S. griseorubens* revealed the presence of several strong antibacterial compounds: hexanoic acid, 2-ethyl-, 2-ethylhexyl ester, n-decane, hexadecanoic acid methyl ester, benzene acetic acid, ricinolic acid, and ethylparaben. Heptadecane, 2,6-dimethyl, benzene acetic acid, vitamin A aldehyde, dibutyl phthalate, octacosane, and hexacosane were detected in the *S. rochei* filtrate.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

B.M.A.: Methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft; writing – revisions. S.A.H.: Validation; data curation; writing – the initial draft; writing – revisions; student supervision. E.E-M.: Conceptualisation; data collection; data analysis; validation; data curation; writing – the initial draft; writing – revisions; student supervision; project leadership; project management.

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# Performance of leaf extract media in culturing mycorrhizal mushroom mycelium

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In-vitro culture of mycorrhizal mushroom (MM) species in southern Africa remains largely unexplored, particularly using tree-derived media. In this study, a *Julbernardia globiflora* [(Benth.) Troupin] leaf infusion was tested for its ability to promote MM mycelial growth. *Amanita loosii, Cantharellus miomboensis* and *Cantharellus heinemannianus* isolates were incubated at a pH of 2, 3, 4, 5, 6 or 7 and at 25 °C in six leaf extract agar (LEA) infusion concentrations of 150, 175, 200, 225 or 250 grams of leaves/L distilled water, with potato dextrose agar (PDA) as a standard. We determined mycelium growth rates for all treatment combinations. Mycelium growth rate was found to be optimal at a pH between 4 and 6 in all leaf infusion concentrations tested. Significant ($p<0.001$) linear regressions of *A. loosii* and *C. miomboensis* were found for pH only ($R^2=0.837$ and 0.8582, respectively) and a significant ($p<0.001$) regression was found for *C. heinemannianus* ($R^2=0.293$). *Amanita loosii* and *C. heinemannianus* had faster ($p<0.001$) growth in PDA than in LEA, while *C. miomboensis* had similar growth rates in the two media. Growth characteristics observed were attributed to acid phosphatase mediated physiological processes in mycelium for the different MM species with an optimum pH of 4–6. MM mycelia were white, mycelia for *A. loosii* and *C. miomboensis* were loose and for *C. heinemannianus* were thin filaments. LEA proved to be a potential alternative medium for culturing MM species.

**Significance:**

- A novel miombo tree extract medium was tested with three miombo mycorrhizal mushrooms.
- Our findings show the new medium to be a possible alternative to, but not as viable as, potato dextrose agar.
- The findings of his study widen the scope of use for the forest tree derived media and demonstrate the cultivability of miombo mycorrhizal mushroom species.
- Our findings improve the possibility of enhancing food security through culturing and possibly cultivating the less explored African mycorrhizal mushrooms.

## Introduction

Unlike for temperate habitats, the biology of edible mycorrhizal mushrooms (MMs) of the miombo biome is little understood and documented. Although mushroom consumption dates to 900 BCE and cultivation of saprotrophic species started around 1650 CE for *Agaricus bisporus* in China, in 600 CE for *Auricularia auricula-judae* and in 1100 CE for *Lentinula edodes*[1], MMs have largely eluded ex-situ cultivation. Over the millennia, saprotrophic mushroom cultivation has been modernised through specialised microbiology, hence their present significant contribution towards global food security.[2] The same effort for MM cultivation has been hampered by their requisite close association with woody plant hosts.[3] Although many temperate MMs have been successfully cultured in synthetic media containing a single carbon source[4], few tropical MM species have to date been tested.

Mushrooms reproduce and multiply sexually by releasing basidiospores which fuse and germinate into hyphae, which eventually form complex networks of vegetative mycelia and, subsequently, their fruiting bodies. Mushroom cultivation has avoided the use of basidiospores owing to non-reliability of the resultant product quality and trueness to type. Hence, modern mushroom cultivation involves two asexual phases of spawning materials, namely, primary spawn production and grain spawn running, giving a more farmer-usable material.[5] Mushroom primary spawning material has traditionally been grown in agar-based media to obtain their pure cultures. Although pure MM cultures of *Tuber* sp. (truffles) have been successfully used for infecting the roots of a variety of tree species[6], similar technology has not been attempted with edible miombo MM, thereby keeping them outside formal agriculture.

Several agar-based general media are used for fungal culture, including potato dextrose agar (PDA), malt extract agar, Czapek-Dox agar and yeast extract agar[7-9], but no new suitable media have been developed for tropical MM mushroom culture. PDA is, therefore, still the most widely used medium for mushroom spawn culture[10] for such mushrooms as *Pleurotus, Agaricus, Auricularia* and *Volvariella* species.[11,12] Gamborg, modified Melin-Norkrans, and Murashige and Skoog media have successfully been used on mycorrhizal *Phlebopus portentosus*, suggesting that suitable MM culture media development is possible.[13,14] However, these media have not completely succeeded for those MMs requiring more specific growing requirements[15] and thus the difficulty in obtaining viable in-vitro cultures.[16,17] In natural growing habitats, MM mycelia successfully grow under narrow pH ranges, for example, a pH of 4.0 for *P. portentosus* and pH 6.0 for *Coprinus phlyctidosporus*.[18] Media pH is critical in activation of most metabolic enzymes, including endoglucanase, cellobiohydrolase,[17,19,20] invertase, endoglucanase, and acid phosphatase.[21-23] Mycorrhizal mycelia have the capacity to release acid phosphatase, irrespective of available organic phosphorus (P) in the growing substrate, with general specificity to soil habitats pH.[23,24] Li et al.[25], however, found low substrate P to induce higher acid phosphatase activity, whereas Costa et al.[24] found organic phosphate to suppress synthesis of acid phosphatase in *Pisolithus microcarpus*, making it necessary to examine suitable media

pH for tropical MMs. In particular, pH was found to regulate extracellular proteases to release nitrogen (N) in amino acids for *Amanita muscaria*, an MM associated with pine,[20] making it necessary to find the right pH when developing culture media for tropical/subtropical MMs.

Concentration of macro- and micronutrients in culture media is critical in MM mycelium growth, some of which they naturally obtain from their woody hosts – their associated saprotrophic fungal partners.[26] As most MMs are incapable of using external sucrose as a carbon (C) source, preferring glucose and/or fructose,[27] standard media such as PDA are likely to favour MM mycelium growth only, but no balanced nutrients, as found in their native materials.[28-30] *Phlebopus portentosus* was successfully cultured in a medium with a C:N ratio of 10:1[18], unlike the optimum C:N ratio of 1:4 found for non-MM *Pleurotus tuber-regium*, suggesting that conventional media for non-MM species may not be suitable for MM culture. However, no similar studies have been documented for miombo MM, particularly *Amanita loosii, Cantharellus miomboensis* and *Cantharellus heinemannianus,* which are popular foods among communities in southern Africa.[31] The objectives of this study were to: (1) assess suitability of *Julbernardia globiflora* leaf extract in agar as an alternative medium for culturing MMs; (2) compare growth rates of the three MM species' mycelia when cultured in the leaf extract agar and PDA; and (3) develop a predictive model which relates MM mycelium growth rates with media pH and leaf extract concentration as independent variables; and hence, describe the growth form and appearance of mycelia of the three MM species as compared to *Pleurotus ostreatus*.

## Materials and methods

### Source of reagents and mushroom specimens

All experiments were conducted at Lupane State University (S19.15616˚, E029.79517˚) at an elevation of 862 m above sea level. Analytical grade PDA (Biolab, Merck 63725), Agar-agar (Philip Harris Education), 32% HCl (Merck), 99.8% NaOH (SkyLabs, South Africa), 90 mm plastic Petri dishes (Boxmore Plastics, South Africa) and sticky labels were obtained from Krain Laboratories (Bulawayo), and fresh *A. loosii, C. miomboensis* and *C. heinemannianus* sporocarps were harvested at Mtao Forest. Pure mycelial cultures were prepared in standard PDA by isolating 2 mm mushroom context disks in an aseptic method. Pure cultures were stored in the dark at 4 °C for 5 months.

### Source and preparation of leaf litter

Mature whole leaves of *J. globiflora* were harvested at Masenyane Village, Lupane, Zimbabwe, from two mature 20-year-old *J. globiflora* trees in winter. Fresh leaf samples were thoroughly mixed and sun dried on a metal sheet for 36 h to simulate field conditions. The samples were then oven dried for 24 h at 105 °C. A quantity of 1000 g of oven-dried leaves was soaked in 10 L distilled water for 72 h to leach out water-soluble compounds to simulate rainfall effects in mushroom habitats.[32] After decanting the water, leaves were oven dried in batches for 24 h each at 105 °C.

### Preparation and chemical analysis of leaf extract

Leaf extract preparation followed the protocol for PDA preparation by HiMedia, India (https://himedialabs.com/TD/GM043.pdf), with modifications in drying of the leaf litter, leaching it and oven drying before infusion. Oven-dried leached leaf portions of 150, 175, 200, 225 and 250 g were each immersed in 1000 mL distilled water at 96 °C for 30 min, giving different extract concentrations. Supernatants were transferred to 250 mL Erlenmeyer Academy flasks and pH (measured at 50 ± 2 °C) of each extract was determined using an electronic pH meter (Greisinger GMH 3500 Series) before adding 22 g/L agar-agar (determined by adjusting agar-agar concentration to attain media solidification from a preliminary test). The mixture was thoroughly stirred with a glass rod and autoclaved at 121 °C and 103.4214 kPa for 15 min in a Classic Prestige Medical autoclave. The autoclaved preparations were allowed to cool to 55 °C and pH was adjusted to 2, 3, 4, 5, 6 or 7[18,29] by addition of 1M HCl or 1M NaOH and thorough stirring with a glass rod[11,33,34]. Pre-run preparation for pH adjustment using 1M HCl or 1M NaOH was done with a dropping pipette to determine the required amounts for each targeted media pH. Standard buffer solutions for pH 1, 4, 6 and 9 were used to verify the adjusted media pH. The media were poured into plastic Petri dishes in 20 mL volumes and allowed to set, and the Petri plates were placed in a refrigerator at 4 °C for 24 h before inoculation. The positive controls were agar-agar plates while negative controls were blanks of leaf extract agar (LEA).

The atomic absorption spectrophotometer (AAS; Varian Spectr AA 200) was used to analyse the mineral content of the LEA and potato infusion in order to explain the difference in mycelium growth rates in the LEA medium. The potato extract was prepared using the standard protocol (HiMedia, 11 May 2017) with variation in filtering infusions through mutton cloth instead of cheese cloth. Table 1 gives the composition of the crude leaf extract and crude potato extracts analysed with AAS. Organic nitrogen analysis was done using the Kjeldahl method[35], while organic carbon was analysed using the Nelson and Sommers (1996) protocol[36-38].

Qualitative analyses showed that both the crude leaf and crude potato extracts contained reducing sugars, where crude potato extract contained starch with high levels of amino acids but no monosaccharides (Table 2).

### Inoculation and experimental design

Aseptic inoculation in the centre of the plates[39] was done using the tip of a flamed scalpel blade with pure cultures of the three mushroom species for all pH–concentration treatment combinations. Treatments were three MM species (*A. loosii, C. heinemannianus* and *C. miomboensis*), six levels of pH (pH 2, 3, 4, 5, 6 and 7), six levels of leaf infusion strengths (infusion dry mass g/L: 0, 150, 175, 200, 225 and 250) making 108 treatment combinations. Each treatment combination was done in triplicate. Two negative (uninoculated) controls were used for each pH versus concentration combination. After inoculation, all plates were sealed with parafilm (Bemis Flexible Packaging, Neenah, WI54956) and placed in a completely randomised design in an incubator (Genlab INC/150/DIG) at 25 °C.[18]

To compare mean growth rates in LEA at each MM determined optimum pH and leaf extract concentration($d$) with standard PDA for the three MMs, a completely randomised design was used with 15 replicates each and five negative controls, and they were incubated at 25 °C for 4 days taken by *C. heinemannianus* to complete growth coverage of the plate surface.

### Data collection and analysis

Simple linear regressions and a standard multiple regression were used to determine predictive ability of pH and $d$ (all controls were excluded in analyses) on MM mycelium growth rate at 3 days after inoculation (DAI) in SPSS Version 20 (IBM Corporation 1989, 2011). Variables of pH and $d$[40] were used to test their predictive ability on mycelium growth rates in a general multiple regression model:

$$\gamma = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon \qquad \text{Equation 1}$$

where $\gamma$ is the predicted variable (mycelium growth rate), $X_1$ is the pH variable, $X_2$ is $d$ in agar-agar, $\varepsilon$ is the error term (assumed to be zero), $\beta_0$ is the constant and $\beta_1$ and $\beta_2$ are the coefficients.

To determine the contribution of each of the two independent variables – pH and media concentration – on the response variable before selecting the regression model variables of mycelium growth rate for each mushroom species, daily growth curves were fitted to the data in SPSS 20.0 and the best-fit model was selected based on its significance ($p<0.05$), $R^2$ and F-value.

A *t*-test for independent samples was used to compare mean mycelium growth rates for each MM species when grown at an optimal LEA pH and extract concentration, and PDA at 3 DAI. The appearance of the mycelium and patterns of its growth were described for each species and growing media, and photographs were taken.

**Table 1:** Elemental composition of potato and *Julbernardia globiflora* leaf crude extracts (mg/L)

| Element | Substrate | |
|---|---|---|
| | Potato extract | Leaf extract |
| Phosphorus | 57.39 | 20.98 |
| Nitrogen (%) | 0.09 | 0.08 |
| Carbon (%) | 0.17 | 0.18 |
| Sulfur | 116.10 | 87.99 |
| Magnesium | 108.176 | 96.912 |
| Calcium | 33.67 | 337.95 |
| Iron | 0.346 | 8.2 |
| Nickel | 0.064 | 0.079 |
| Manganese | 0.179 | 7.02 |
| Lead | 0.27 | 1.200 |
| Copper | 0.190 | 0.253 |
| Cobalt | 0.036 | 0.004 |
| Zinc | 0.159 | 0.202 |

## Results

### *Curve fitting*

When curves were fitted to the data, the three fungal species showed varying responses to the explanatory variables, media pH and concentration in the leaf extract agar media (Figure 1a–d with the best fit lines highlighted in red, and $R^2$ and F values given on the caption notes).

#### Amanita loosii

For *A. loosii*, the fitted cubic model shows a pH of 6 to be optimum (Figure 1a) and no lag phase is found within the lower end of the pH range examined.

#### Cantharellus heinemannianus

Mycelium growth rate for *C. heinemannianus* was optimum at a pH of 6, with an anomalous decrease from pH 2 to pH 3 (Figure 1b).

For media concentration, the fitted quadratic curve for *C. heinemannianus* coincides with the cubic model, showing optimum media concentration to be about 215 g/L (Figure 1d). Beyond this concentration, the growth rate falls rather than levelling off.

#### Cantharellus miomboensis

Growth rate for *C. miomboensis* also showed a cubic model for pH with an optimum at pH 6 but with a plateau between pH 5 and pH 7, slowly tapering down beyond the examined pH range. This model clearly shows there was no growth below pH 2 (Figure 1c).

### *Regression results*

Using the models for best-fit curves in Figure 1a–d, a standard multiple regression was done to assess the ability of pH and medium concentration (*d*) to predict mycelium growth rate in millimetres per day determined at 3 DAI in the LEA medium for pH 2 to 7 and *d* of 150, 175, 200, 225 and 250 g/L for the three MM species *A. loosii, C. heinemannianus* and *C. miomboensis*. Preliminary checks showed the data complied with linearity, non-multicollinearity and homoscedasticity but failed normality tests for all available transformations.

A significant regression model (Equation 2) was found when *A. loosii* mycelium growth rate (mm/day) was regressed on pH: $F(1, 89) = 457.567$; $p<0.001$. The adjusted coefficient of determination ($R^2$) was 0.837. However, *d* was not a significant ($p > 0.05$) predictor for *A. loosii* mycelium growth rate.

**Table 2:** Qualitative analysis results of tests for starch, sugars and amino acids in leaf and potato extracts

| Test substance | Iodine ST test | Benedict's RS test | Barfoed's MS test | Ninhydrin PR test |
|---|---|---|---|---|
| Potato extract | ++ | + | - | +++++ |
| Leaf extract | – | ++ | + | + |
| Distilled water | – | – | – | – |
| Glucose | – | +++++ | ++ | – |

*- negative reaction; + positive reaction: the number of + signs shows the score relative to the magnitude of the positive result*

*ST, starch; RS, reducing sugar; MS, monosaccharides; PR, protein*

A significant regression model (Equation 3) was found when *C. heinemannianus* mycelium growth rate (mm/day) was regressed on pH and leaf extract concentration: $F(2, 88) = 18.025$; $p<0.001$ for pH and *d* and $R^2$ was 0.293.

A significant regression model (Equation 4) was also found when *C. miomboensis* mycelium growth rate (mm/day) was regressed on pH: $F(1, 89) = 533.781$; $p<0.001$ for pH with $R^2$ of 0.858. *d* was not a significant ($p>0.05$) predictor of *C. miomboensis* mycelium growth rate.

Equation 2 implies that for every unit increase in pH, mycelium growth rate increased by 2.897 mm/day within the specified pH limits.

$$\hat{Y} = 2.897\boldsymbol{X} - 0.685 \qquad \text{Equation 2}$$

where $\hat{Y}$ is mycelium growth rate in mm/day and $\boldsymbol{X}$ is pH with $2 \leq \boldsymbol{X} \leq 7$ for *A. loosii*, implying that for each unit increase in pH, mycelium growth rate increased by 2.897 mm/day, irrespective of *d* between 150 and 250 g/L.

Equation 3 implies that for every unit increase in pH, mycelium growth rate increased by 1.726 mm/day when *d* was kept constant, and mycelium growth rate increased by 0.042 mm/day for each unit increase in *d* when pH was kept constant. The model was true for the stated pH and *d* limits.

$$\hat{Y} = 6.05 + 1.726\boldsymbol{X}_1 + 0.042\boldsymbol{X}_2 \qquad \text{Equation 3}$$

where $\hat{Y}$ is mycelium growth rate in mm/day and $\boldsymbol{X}_1$ is pH with $2 \leq \boldsymbol{X}_1 \leq 7$ and $\boldsymbol{X}_2$ is *d* with 150 g/L$\leq \boldsymbol{X}_2 \leq$ 250g/L for *C. heinemannianus.*

Equation 4 shows that for every unit increase in pH, mycelium growth rate increased by 4.115 mm/day within the specified pH limits.

$$\hat{Y} = 4.115\boldsymbol{X} - 5.595 \qquad \text{Equation 4}$$

where $\hat{Y}$ is mycelium growth rate in mm/day and $\boldsymbol{X}$ is pH with $2 \leq \boldsymbol{X} \leq 7$ for *C. miomboensis*, that is, mycelium growth rate increased by 4.115 mm/day for a unit increase in pH irrespective of change in *d* between 150 and 250 g/L.

### *Comparative performance of A. loosii, C. miomboensis and C. heinemannianus when cultured in PDA versus LEA*

The *t*-test results of mycelium growth rate taken 3 DAI showed a higher growth rate ($p<0.001$) in PDA for *A. loosii* and *C. heinemannianus,* but similar growth rates in both media for *C. miomboensis* (Table 3). Optimum mycelium growth rates recorded were between pH 4 and pH 7.

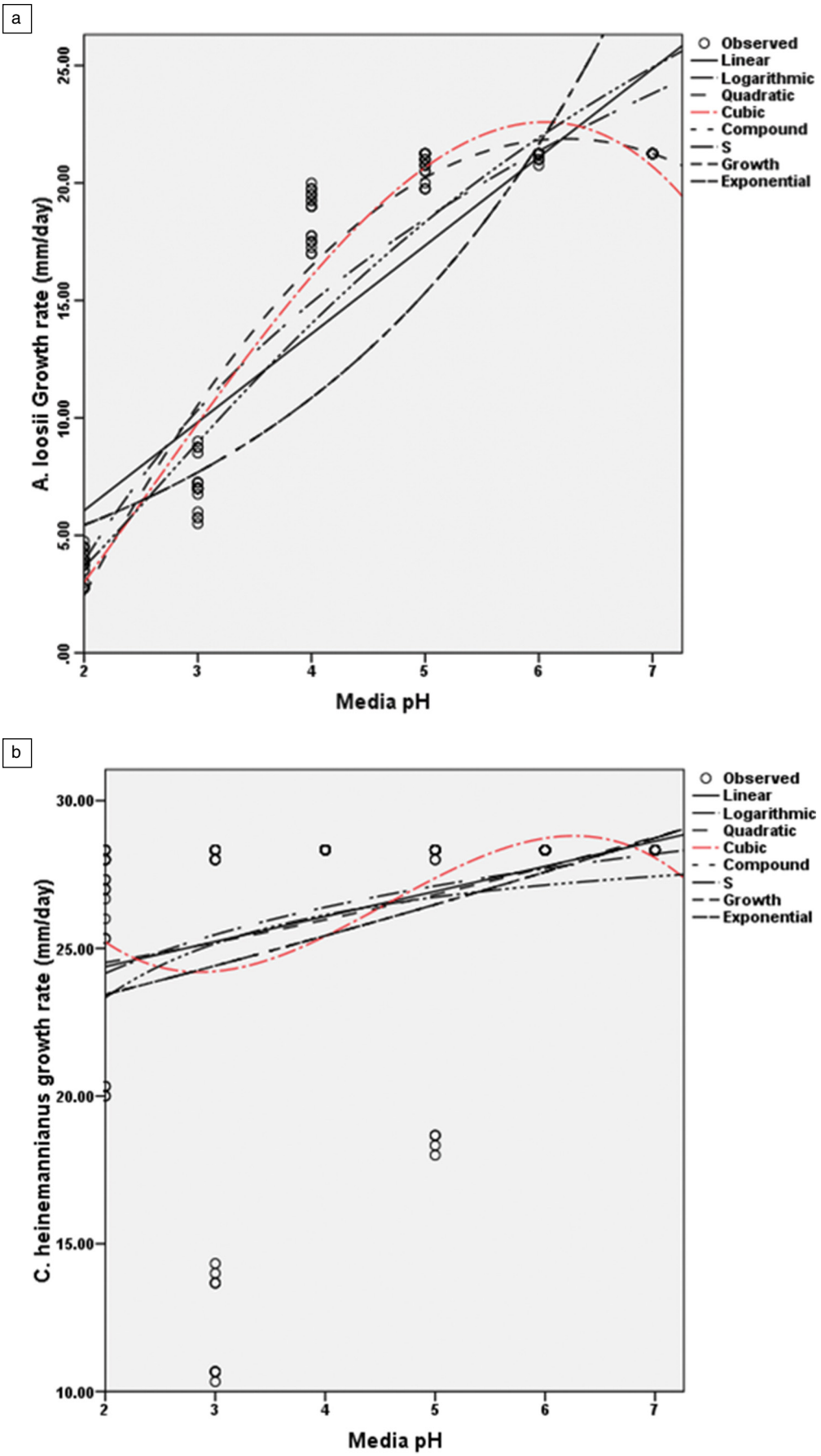


**Figure 1 continues...**

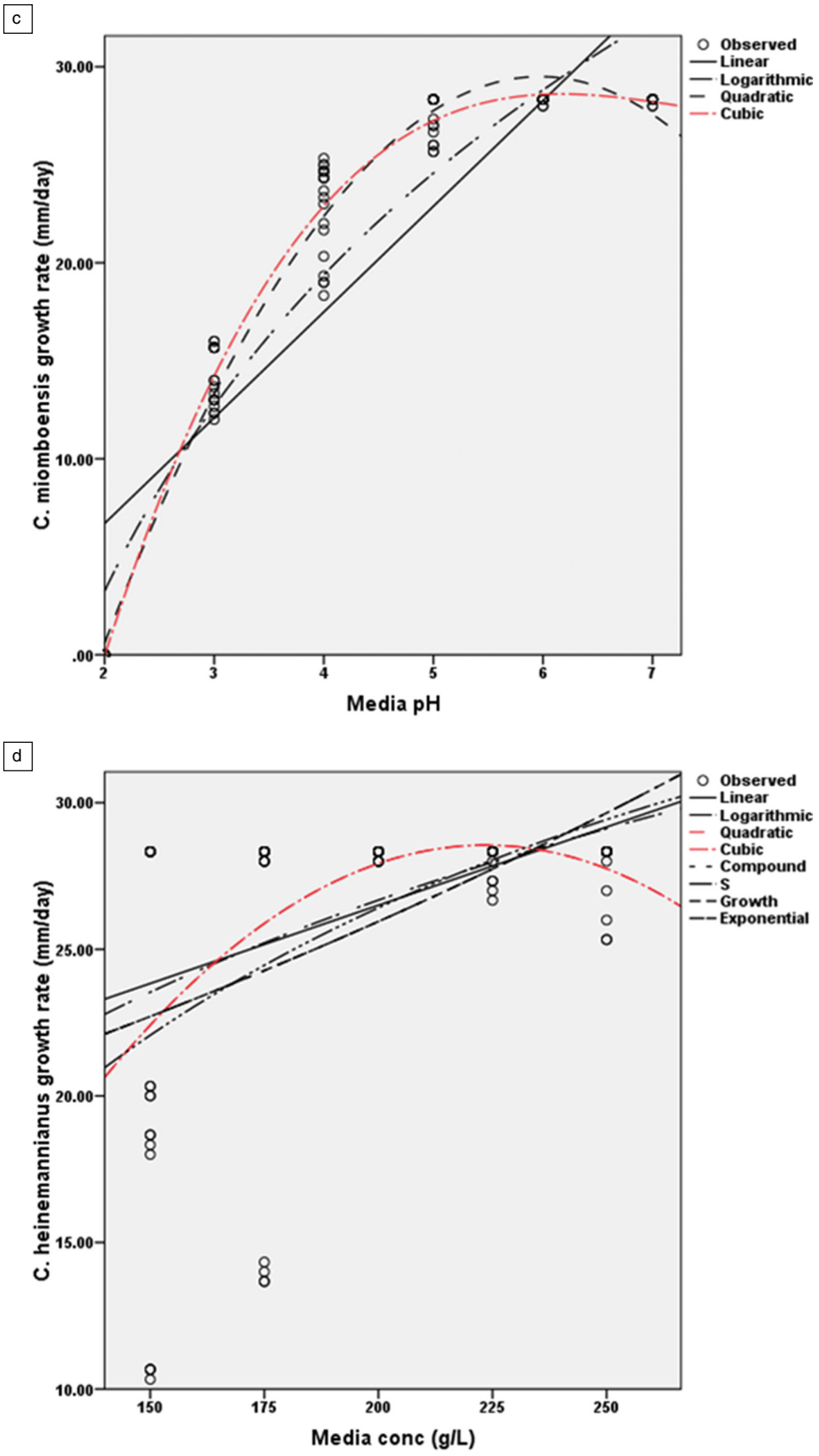


**Figure 1:** (a–c) Fitted curves for growth rate with media pH as the explanatory variable with the cubic curve as the best-fit significant ($p<0.05$): (a) *Amanita loosie* $R^2$=0.938 and F=585.934 (media concentration was not significant and was therefore omitted); (b) *Cantharellus heinemannianus* $R^2$=0.136 and F=6.084; (c) *Cantharellus miomboensis* $R^2$=0.987 and F=2854.139 (media concentration was not significant and was therefore omitted). (d) Fitted curves for growth rate for *Cantharellus heinemannianus* with media concentration as the explanatory variable with the quadratic curve as the best-fit significant ($p<0.05$) $R^2$=0.251 and F=19.563.

**Table 3:** *Amanita loosii, Cantharellus miomboensis* and *C. heinemannianus* mean mycelium growth rates in potato dextrose agar (PDA) versus leaf extract agar (LEA) in mm/day at 3 days after inoculation

| Media | *A. loosii* | *C. miomboensis* | *C. heinemannianus* |
|---|---|---|---|
| PDA | 18.934 | 14.267 | 28.330 |
| LEA | 15.578 | 14.360 | 16.983 |
| *n* | 15 | 15 | 15 |
| *t*-value | 6.051 | -0.136 | 36.600 |
| *p*-value | < 0.001 | 0.893 | < 0.001 |

The novel medium, LEA, was light brown compared to the light yellow colour of PDA after autoclaving (Figure 2). Mycelia patterns differed for the same MM mycelia when cultured in the two different media (Figure 3).

In the LEA medium, mycelium of *A. loosii* was found to be septate, branched and growing prolifically flat on the surface of the medium and submerged. Mycelia formed spherical spores on mycelia branches. *C. miomboensis* mycelium was septate and unbranched. Hyphae grew aerially away from the medium's surface and laterally beneath the surface of the medium. Scattered asexual spores were observed. *C. heinemannianus* mycelium was septate, bifurcate branched with hyphae growing both superficially and submerged, and surface hyphae grew vertically upwards in isolated clumps at pH 5 to pH 7 and grew prostrate at pH 2 and pH 3 (Figure 3). Its mycelium consisted of thick tufted filaments (Figure 3g). At pH extremes, *C. heinemannianus* mycelium seemed to be discontinuous between its edge and its central origin (Figure 3i) in both LEA and PDA media. For all MMs, mycelial strands were more discernible in LEA than in PDA (Figure 2).

## Discussion

We successfully cultured the investigated miombo MM species *A. loosii, C. miomboensis* and *C. heinemannianus* in the novel media of *J. globiflora* leaf extract with the inclusion of only the agar-agar fraction. Daily mycelium growth rates for the three mycorrhizal species were differentially influenced by pH to a minor extent as shown in Figure 1a–d, suggesting that they are intrinsically adapted to the same natural habitats. This was not surprising as, indeed, the source sporocarps were harvested from the same miombo woodlands. The optimum pH for growth found was to be a pH of 6 with *C. miomboensis* exhibiting a wider optimum range. In general, mycelium growth rate patterns were similar for *A. loosii* and *C. miomboensis,* but they differed from that of *C. heinemannianus*. This is because the latter was also significantly influenced by medium concentration, while the other two were not significantly influenced by medium concentration. However, regression analysis for growth rates of the three MM species gave varying growth performance under varying pH, while leaf extract concentration (*d*)

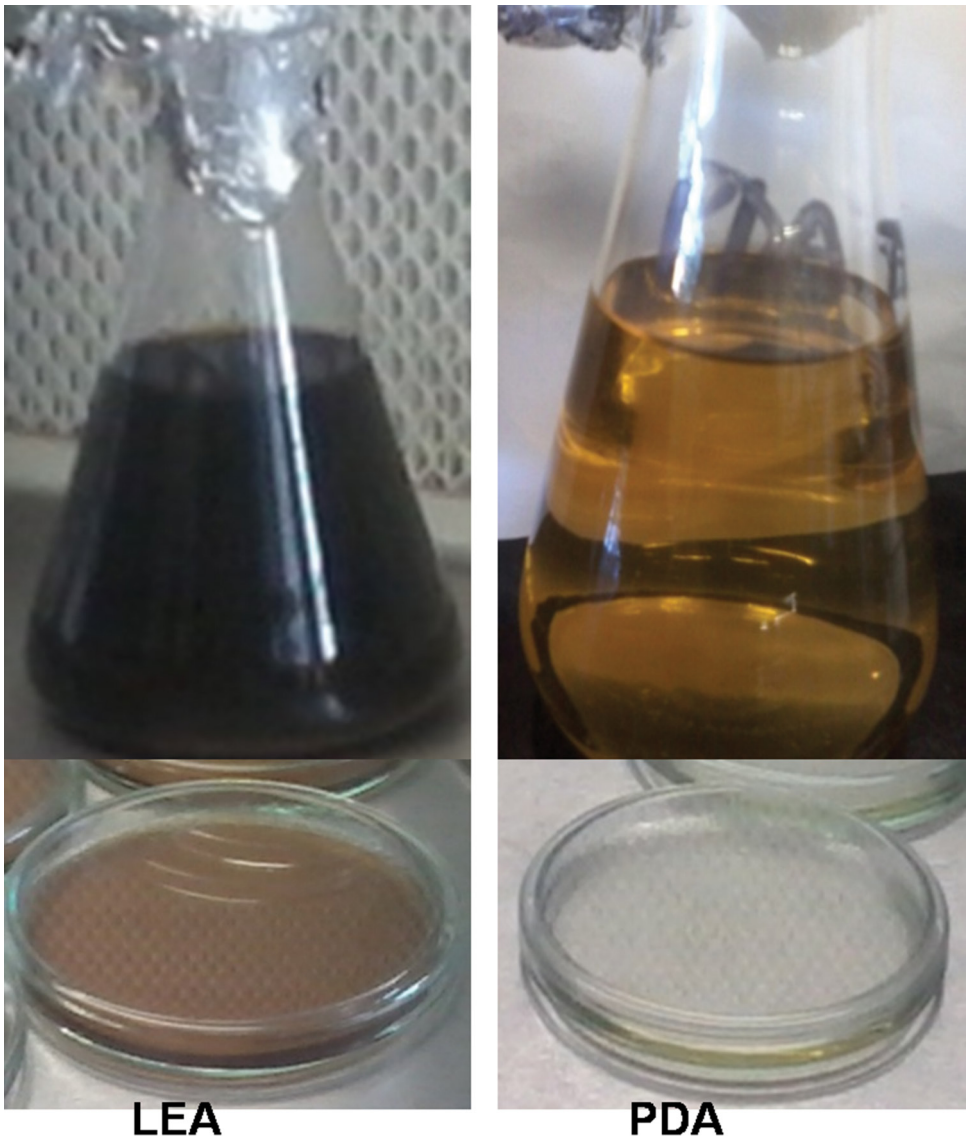


**Figure 2:** Contrasting appearance of leaf extract agar (LEA) and potato dextrose agar (PDA) in flask and Petri dish.

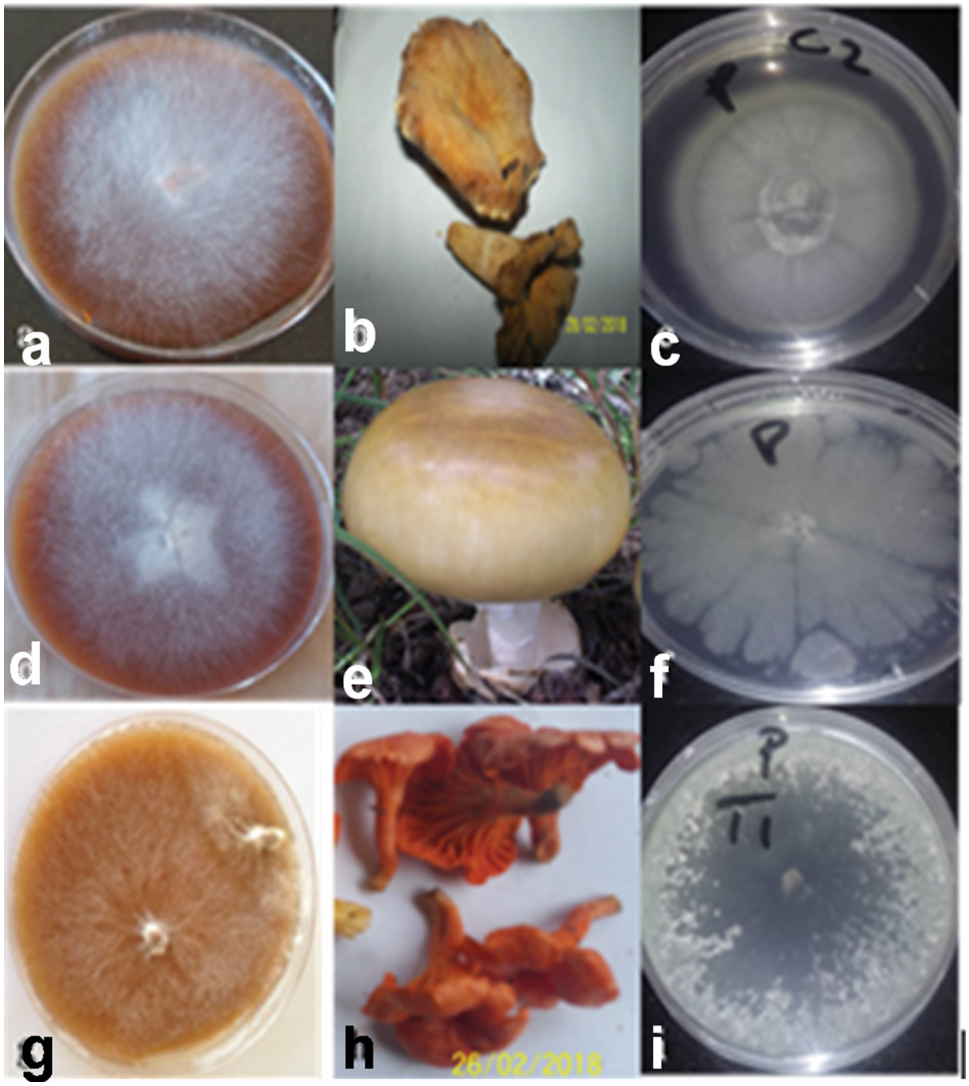


**Figure 3:** *Cantharellus miomboensis* in leaf extract agar (LEA) (a) and in potato dextrose agar (PDA) (c) and its sporocarps (b); *Amanita loosii* in LEA (d) and in PDA (f) and sporocarp (e); C. *heinemannianus* in LEA (g) and PDA (i) with sporocarps (h).

only influenced the mycelium growth rate of *C. heinemannianus* ($d > 0$ g/L) (Equation 3) for our novel media. We found $d$ to have no influence ($d > 0$ g/L) on mycelium growth rates of *A. loosii* and *C. miomboensis* (Equations 2 and 4). However, mycelium growth rates were higher in unmodified standard PDA (pH 5.4) for all MM mycelia, except for *C. miomboensis* which gave similar growth rates in both media, suggesting that LEA was a good alternative for *C. miomboensis* (Table 3). The high mycelium growth rates found in LEA media were attributed to the availability of all required nutrients as our analyses showed (Tables 1 and 2). The LEA content of monosaccharide-reducing sugar as a carbon source (Table 2), albeit in low concentration, could have greatly promoted the observed mycelium growth.

For MM species from elsewhere in temperate woodland biomes, several in-vitro culture successes were reported for *Tuber* sp.[6], *Tricholoma* sp.[41], *Cantharellus cibarius*[42], *Laccaria bicolor*[43], *Cantharellus tropicalis*[44] and *Boletus* sp.[45] using standard media under pH ranges of 4 to 7, suggesting that MM mycelium growth rates show strong pH dependence. We found optimum mycelium growth rates for *A. loosii, C. miomboensis* and *C. heinemannianus* to range between pH 4 and pH 6, which closely matched soil pH of 5.0 to 5.7 measured in their natural habitat soils in central Zimbabwe. The optimum pH of 4–6 that we found was similar to the findings of Li et al.[25], Adeoyo et al.[33], Obi et al.[46], Bedade et al.[47] and Daza et al.[48]

Mycorrhizal mushrooms thrive in phosphorus- and nitrogen-poor soils where they assist their host species to extract these nutrients from the soil.[49] MMs are able to meet their own phosphorus requirements, particularly from organic sources such as phosphate monoesters and diesters, with the largest amount in phytate form.[49-51] This is due to their ability to use released and/or surface-bound acid phosphatases of varying molecular species in the acidic pH range to mobilise organic phosphorus and carbon.[52,53] In particular, the optimum pH for acid phosphomonoesterase was reported to range from 4 to 6[54], that of phytase from pH 1.3 to 5.5[55], while that for phosphodiesterase was pH 3[54], which explains our pH optima observed for MM mycelium growth. In addition, the MM genera *Amanita* and *Suillus* were reported to have high efficiency in mobilising metabolic carbon and nitrogen from organic sources.[53] As the enzymes required for mobilising carbon from organic media, namely cellulase, xylanase and cellobiohydrolase, also have pH optima between 4 and 7[54], this further explains the mycelium growth optima we found.

Successful MM mycelium growth in LEA was also attributed to adequate iron, magnesium and calcium content (Table 1), which positively complement conditions for high activity of phosphatase and endoglucanase. Furthermore, the moderate content of phosphorus and nitrogen (Table 1) to support MM mycelium growth also accounted for the highly successful result we found for miombo MM species. Consistent with pH optima for phosphatase, phytase and carbon-liberating enzymes, therefore, under acidic pH 2 and 3, the studied MM species grew slowly (Table 3), irrespective of $d$ – a finding also in agreement with the findings of Obi et al.[46] Failure of *C. miomboensis* to grow at a pH of 2 suggests its inability to liberate its phosphorus and carbon requirements under such a

low pH; for example, phosphorus mobilisation from phytate.[55] However, *A. loosii* and *C. heinemannianus* managed to grow under such a low pH, suggesting their possible reliance on phosphodiesterase, which has a lower pH optimum than phosphomonoesterase, in phosphorus liberation from the media.[54] We hypothesise that these two MM species possess different metabolic enzyme systems as they were also found to develop sporocarps at different times of the season in their woodland habitats.[56] Hence further research, particularly for miombo MM species, needs to be conducted to test our hypothesis, also considering conditions other than pH and leaf extract concentrations used in the current study.

The MM species growth rate models for *A. loosii* and *C. miomboensis* could not use $\phi$ as a predictor where pH was the only important external growth factor, unlike that for *C. heinemannianus* (Equations 2, 3 and 4). Hence, apart from pH as a limiting growth factor, the models for *A. loosii* and *C. miomboensis* suggest that growth-limiting factors are more intrinsic than extrinsic in nature for these two MMs. Compared to growth of the latter two MM species, *C. heinemannianus* was generally more vigorous and not indifferent to substrate concentration, demonstrating its inherent intrinsic voracity to substrate utilisation (Table 3). When grown in PDA, *C. heinemannianus* also showed prolific growth, suggesting its successful adaptation in utilising different organic substrates through possession of more efficient carbon-metabolising enzyme systems.[57] The suppressed early growth for *A. loosii,* irrespective of pH level, indicated that environmental factors other than pH (such as temperature, food content/nutrient balance) and intrinsic factors were more important factors in regulating growth rates of its mycelium, and that the species may have completely lost its saprotrophic ability through co-evolution with woody host species.[58] This generally slow growth was also found when *A. loosii* was cultured in PDA. We found mycelium growth rates to be higher at higher pH values under pH 7 for the three MMs, with that of *C. miomboensis* being the highest (Equations 2 to 4). Growth rate models for *A. loosii* and *C. miomboensis* therefore suggest nutrient concentrations in the LEA medium to be non-limiting in minerals like iron, calcium, manganese and copper in supporting mycelium growth, even at the lowest concentration of 150 g/L (Table 1). The generally high mycelium growth rates of *C. heinemannianus* indicate the ability of this species to use phosphoesterases more efficiently in a wider pH range, which also explains the significant influence of $\phi$ observed for this species (Equation 3).

Results of a *t*-test to compare the efficacy of LEA against the standard PDA proved that LEA was generally inferior to PDA for *A. loosii* and *C. heinemannianus* but not for *C. miomboensis* (Table 2). Although the crude potato infusion extract had higher amino acid content (Table 2) than the leaf infusion extract used, the high carbon content in PDA owing to addition of 20 g/L dextrose accounted for PDA's superior performance. The surface hardness observed in LEA, due to resins and tannins, may also have retarded growth through restriction of air supply to the submerged mycelium. Hence, physical characteristics of LEA, including surface hardness, aeration, moisture retention and its organic nutritional content, need to be investigated further in developing these media for culturing MMs, particularly *A. loosii* and *C. heinemannianus*. The brown colour observed in the LEA was characteristic of phenolics, flavonoids and tannins, as found in most leaf infusions,[59] suggesting these compounds may be a contributing carbon source for mycelium growth in the cultures. Although the brown colour hampered visual observation of submerged mycelium, LEA revealed its potential as a discriminating morphological indicator (Figure 1) for MM species in this study.

## Conclusion

Our research widens the frontiers of culturable ectomycorrhizal mushrooms and the scope of MMs usable in in-vitro culture media. These media were able to discriminate mycelium characteristics, particularly between *C. heinemannianus* at varying pH, and complement existing media like PDA to discriminate MM morphological appearances, as demonstrated for *A. loosii* and *C. miomboensis*. Hence, use of both of these media can help future identification of the three species for future morphological studies. Our novel media proved a good substitute for PDA in culturing MM, particularly *C. miomboensis*. MM mycelium growth rate was also demonstrated to be strongly influenced by pH, with optimum pH being 6, and influenced to a lesser extent by media concentration, although more studies are necessary to establish the critical threshold concentration values. In attempting to in-vitro culture MM species, the extent to which they have lost saprotrophic ability must be understood so as to develop suitable optimum conditions different from those given by conventionally used general media for fungi. It is also clear that new media development without the need for costly additives such as glucose seems promising when materials from the mushroom's habitat are used. In addition to their ease of preparation, such cheaply sourced materials have the potential to replace conventionally used material in favour of those substrates that are more adaptable to the hard-to-culture MM, hence guiding microbiology into a future in which some of the new substrates can be used in identifying mushroom species on account of their different appearances in culture. To better understand the growth characteristics of subtropical mycorrhizal mushroom mycelium in culture, more detailed studies involving leaf or root extracts of their host woody species need to be explored to involve wider pH ranges and other environmental factors that simulate the mushrooms' natural habitats. From such studies, more predictive growth models can be developed.

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## Competing interests

We have no competing interests to declare.

## Authors' contributions

A.M.: Conceptualisation; methodology; data collection; sample analysis; data analysis; validation; data curation; writing – the initial draft. M.M.: Writing – revisions; student supervision; project leadership; project management.

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