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Mollusc collections at South African institutions

Ecological effects of clay mining by *Macrotermes* termites

Gold mining pollutants in the Klip River catchment, Johannesburg

Responses of South African value chain actors to plastic straw pollution

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Cover caption
Mollusc collections are among the oldest natural science collections in South Africa. In an article on page 38, Cole collates information about these diverse collections scattered across South Africa. Photo: Kevin Cole.

The value of error in complex times

The most recent editorial in this Journal[1] discussed, in part, the impact of fires on the environment and on the research collections at the University of Cape Town. As we finalise this issue, it is again a time of fires in South Africa – some literal, in the context of violence following the arrest of the former State President, and others metaphorical, in the context of the deadly third wave of COVID-19. At such difficult times, there is probably no correct way to act or even to comment editorially – we do not have the solutions for all the huge challenges the country faces. It would be easier, perhaps, to ignore the country's experiences of violence and pandemic and to focus on other matters in an editorial, but this silence is in itself a political choice. There is no way out for privileged people having to take some responsibility for the privileged voice that we have, especially in times like these. This is the case even if, by the time this editorial is read, other issues have come to seem more prominent than the current ones.

How do we explain, in a helpful way, what is happening in South Africa and more broadly at present? There are so many possible levels of explanation. We can start at a micro-level, talking about what is happening in the brains of people who perpetrate violence. We can point out the impact of years of assault on some of those brains – through under-nutrition, impoverished social circumstances, and the impact of trauma and substances. We can point to the literature on the strong relationship between social inequality and a range of poor social outcomes. We can take an historical view of the present, noting the impact of past and enduring injustice on current behaviour. We can look to the injustices of colonial and postcolonial history and to the increasing evidence of the deleterious effects of a range of forms of social exclusion and occupational deprivation. We can place this all in the context of species evolution, and beyond. All of these analyses have potential to shed light on our world; none is complete. As one of Africa's greatest writers, Chimamanda Ngozi Adichie, said in her TED talk dealing with issues of identity in literature (amongst other things), we need to consider 'the danger of a single story'.

Especially now, scientists, and especially social scientists, may be called upon, and may wish, to offer words of authority and decision – to provide the illusory comfort of a 'single story'. We know, however, that science is not about certainty but about debate, and about changing our minds as the evidence changes. It is never about having a single template into which everything can fit neatly. If we have such a template, then we never have to collect data again, as we know what the answer will always be.

Part of how we as scientists try to deal with multiplicity and the complexity of the real world is through multidisciplinarity – something very much to be celebrated, as we have suggested in this Journal before[2], and, indeed, at the heart of what we do and believe we should do. But there are challenges. In response to the most recent editorial[1], we were contacted by a conservation biologist who was concerned that it could be the view of the *South African Journal of Science* that fire is always a bad thing in relation to fynbos. As our interlocutor pointed out (and I did not make clear in the editorial), fire is in fact essential to the regeneration of Cape fynbos and renosterveld. The problem is the interface between humans and nature – there are issues here of poor management of invasive species, for example.

This issue, though, links to broader concerns about error, especially in an interdisciplinary journal where the Editor-in-Chief is not on top of all the sciences represented. We deal with this issue primarily, of course, by having Associate Editors who are subject experts; these Associate Editors generally appoint reviewers as familiar as possible with the specific areas researched by authors.

But it is in the nature of science that nobody can know everything about any subject, and that mistakes and errors are often what drives fields forward – some theorists talk of errors as leading edges for new growth. Good scientists should be able to make mistakes, and to change their minds as new evidence emerges. It is part of the function of a journal like this one, as we have suggested before[2], to expose readers to new ideas and to a multiplicity of perspectives. In practice, this important challenge forces us to think much more carefully, not just about views we may have and values we may hold, but about the strengths and weaknesses of our methods and the quality of our argumentation. For our authors, who come from a range of disciplines across the sciences (as broadly conceived of), and beyond, there is an extra burden which is often not carried when we write in discipline-specific journals. Our authors at their best make their methods and their argumentation accessible to people without discipline-specific skills and background knowledge. There is nothing more challenging or educational for a subject expert to make that expertise accessible to non-experts (in line with the old adage that the best way to learn is to teach); our authors have to address this challenge. There are issues at stake here not only of good science but also of good science communication. Multidisciplinarity is not about silos of knowledge where each group owns a territory; it is about the opportunities and risks of crossing boundaries. This takes time, commitment, and, indeed, courage.

As we confront the current, ongoing, and future challenges of our country, our continent, and our world, we thank our contributors for all they are already doing to take forward the agenda of the Journal – our vision remains 'To publish and promote the widest diversity of excellent South African research for the local and global academic community and inform policymakers and the public'. At times like these, it is clear that to reach this complex and multifaceted vision is easier said than done. Making mistakes and talking past one another is part of the process; to be part of enduring solutions we have to accept, and embrace error. The potential of what we have to offer lies not in easy pontification but in the constant recognition of, and struggle with, our limitations.

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Christof Heyns (1959–2021): Human rights lawyer, legal educator and activist

Professor Christoffel Hendrik (Christof) Heyns, who passed away suddenly and too early in March 2021, was an internationally renowned human rights lawyer, legal educator and activist.

He was one of the founders of the Centre for Human Rights in the Faculty of Law, University of Pretoria. The Centre, which in 2021 celebrates its 35th anniversary, was founded during the dark days of states of emergency in apartheid South Africa. Christof Heyns was its Director from 1999 to 2006. During the years of his leadership, the Centre became an academic department in the Faculty of Law at the University of Pretoria as well as an NGO promoting human rights across the African continent. Among his many initiatives while Director was the establishment of the African Human Rights Moot Court Competition, which also celebrates its 35th anniversary in 2021; the Nelson Mandela World Human Rights Moot Court Competition; and the National Schools Moot Competition (which was later extended to countries beyond South Africa, under the aegis of the Global Campus of Human Rights).

Christof also took the initiative towards establishing two master's programmes in the Centre that both had a profound impact in the region. The first is the Master's in Human Rights and Democratisation in Africa programme. This innovative programme, which has now been offered for over 20 years, has trained over 600 African human rights lawyers. Many of Africa's leading human rights lawyers are graduates of this programme. It is now also part of the Global Campus of Human Rights – a network of seven leading master's programmes across the world. During his tenure as Director, Christof also helped establish the LLM in International Trade and Investment Law in Africa because he recognised that, in order for Africa to experience sustainable and equitable development, it needed lawyers who have the knowledge and skills to be effective international trade and investment lawyers but who also understand how these areas of the law relate to human rights.

Christof stepped down as Director of the Centre to become Dean of the Faculty of Law at the University of Pretoria. He held this position from 2007 to 2010. As Dean of the Faculty, he insisted on a greater focus on postgraduate studies, and in particular doctoral studies, at the Faculty. He secured funding for full-time doctoral students, and made the Faculty a magnet for talented prospective students from across the African continent. After stepping down as Dean, he become the Founding co-Director of the Institute for International and Comparative Law in Africa at the University of Pretoria, with a thematic focus on 'freedom from violence' drawing a significant group of doctoral candidates.

In addition to his academic positions, Christof was also active in international organisations. He served as United Nations (UN) Special Rapporteur on extrajudicial, summary or arbitrary executions from 2010 to 2016; and was a member of the UN Human Rights Committee from 2017 to 2020. As Special Rapporteur, he drew attention to cutting-edge issues such as the use of force by private security providers in the law enforcement contexts; the use of drones and autonomous weapons in armed conflict or counter-terrorism operations; and the role of forensic science in protecting the right to life. During 2016, he chaired the UN Independent Investigation on Burundi.

As member of the Human Rights Committee, he was pivotal in the drafting of General Comment 37, the right of peaceful assembly (article 21 of the International Covenant on Civil and Political Rights). He also was a member of the Working Group on Death Penalty, Extra-Judicial, Summary or Arbitrary Killings and Enforced Disappearances in Africa of the African Commission on Human and Peoples' Rights. He had been leading discussions at the level of the Commission on how to curb the excessive use of police force in Africa.

Over many years and to generations of students, Christof was an inspiring teacher and mentor. He supervised a number of doctoral candidates who are in their own right contributing as South African legal academics: Bernard Bekink, Henk Botha, Willem Gravett, Magnus Killander, Wessel Le Roux, Frans Viljoen. Other supervisees of Christof include Thompson Chengeta, Waruguru Kaguongo and Zambian Judge Mumba Malila.

His academic interests were varied, and included expounding on the 'struggle theory' of human rights, and exploring the life and times of Jan Smuts. One of his abiding passions was to better track and understand the actual effect of international human rights on the real lives of people. This concern led him to devise a far-reaching study of the effect of the core UN human rights treaties in 20 UN member states, which culminated in the publication *The Impact of the United Nations Human Rights Treaties at the Domestic Level* (Heyns and Viljoen, Kluwer Law International; 2002). This work has been described as 'seminal'. Christof energised a follow-up study, involving 20 country-based researchers or teams, to track and analyse the changes in impact over the subsequent 20 years. At the time of his passing, Christof was in Stellenbosch on a sabbatical, preparing for publication of the results of this study.

Christof was also a great editor and collector of materials, with a view to make inaccessible documents available to a broader public. At a time when the African regional human rights system was largely unknown, he collected and published a number of volumes of texts and commentaries. In this way, he breathed life into an almost non-existent field of academic study. The collection *Compendium of Key Human Rights Documents of the African Union* edited by Heyns and Killander (Pretoria University Law Press; various editions) has served – and will still serve – as a source of reference to generations of students of African human rights law.

It was also his passion to see others publish, and he was involved in the founding of two such endeavours. First, he was a co-founding editor of the *African Human Rights Law Journal*, which has been published since 2001. Second, together with Faculty colleagues, he forged the Pretoria University Law Press (PULP) into being. Earlier in

2021, PULP published a landmark publication edited by Christof together with Philip Alston, Sarah Knuckey and Thomas Probert, entitled *Alston and Heyns on Unlawful Killings: A Compendium of the Jurisprudence of the United Nations Special Rapporteurs on Extrajudicial, Summary or Arbitrary Executions from 2004-2016.*

Christof was also an internationalist. He found great pleasure in regularly teaching at the University of Oxford and at the American University in Washington, D.C. He was a Humboldt Fellow at Heidelberg University, a Fulbright Scholar at Yale Law School and a Fulbright Fellow at Harvard Law School.

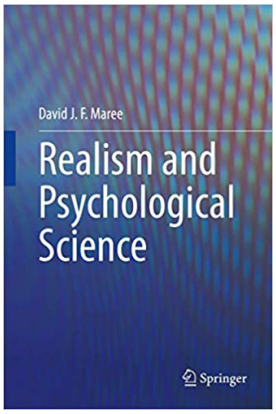
There were so many dimensions to Christof, each of which he inhabited so fully and so completely, whether it was rowing, being a father, a family man, a grandfather, playing the guitar, appreciating a good book or a piece of music, or working for human rights. He was a good and deeply moral man, integrity personified and warm-hearted. He had a quirky sense of humour, and was ready with a witticism for every occasion. Christof's instinctive warmth and genuine kindness stemmed from an abundant generosity and sense of humanity. His enthusiasm was boundless and infectious, leaving no one untouched whose life intersected with his.

Our heartfelt condolences and wishes of comfort and strength go to his wife Fearika, his children Willemien (and her husband Arné), Adam, and Renée, his mother, his first grandson Isak, other family, and all his friends and colleagues who had the privilege of walking some part of his path with him.

His personal ethic is captured in the phrase he often used at the Centre: 'Excellence with Ubuntu'. There is a Jewish legend that says that before each human being dies, an angel comes and asks the person what they have done to make the world they are about to leave a better place than the one they entered. We like to believe that Christof bought himself at least an extra hour of life as he described to the angel all the things he did to improve this world.

Photo: ICLA

BOOK TITLE:
Realism and psychological science



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Mythic images, realism and the shaping of psychological science

Professor David Maree takes us on a fascinating journey into realism and psychological science, beginning with the argument that, since the early 20th century, a narrow conceptualisation of science has been applied to discussions of psychology – a conceptualisation which, as Maree puts it, 'does not do justice to either psychology or science'. The relationship between 'science' and 'psychology' is one of the most debated features of contemporary psychological theory, research, and practice. For some, the alignment between psychology and science has been used to legitimise psychology alongside the 'hard' sciences. However, disagreement about the relationship between science and psychology has also spurred the development of many subfields of psychology that have questioned, to varying degrees, the extent of the relationship including whether psychology should be considered a science at all.

But what exactly do we mean by psychological science? Have scholars in psychology simplified ideas of science to such an extent that they have become unhelpful to knowledge production? What assumptions of psychological science can and should be interrogated? Are there ways in which we could reconceptualise science and psychology?

In chapter one, Professor Maree introduces how the 'mythic image of science' in psychology has influenced three central features of contemporary psychology, namely, methodology (quantitative versus qualitative methods), application (scientist–practitioner split) and metatheoretical opposition (constructionism and positivism). The three areas are carefully and deliberately selected because of their prominence in the discipline. Maree devotes a chapter to each of these topics (chapters two to four) and carefully interrogates some of the core assumptions of the 'mythic image science' and its manifestations by drawing on his extensive expertise in the history and philosophy of psychology.

A strength of the book for me is that it draws attention to a broader (beyond realism) point about the philosophical roots of psychology. Much of psychology is rooted in historical and philosophical thinking that is often glossed over or simplified. Few scholars and students engage with the complexity of those philosophical debates. With an interest in how we teach research methods in psychology, I am often struck by how superficially we cover the philosophical underpinnings of psychological research. This book reminds us of the rich philosophical and historical thinking that underlies psychology. It also reminds us that simplification may serve important political functions at key historical moments. For example, the mythic image of science was perhaps a necessary 'straw man' for some subfields to emerge and develop. It is, however, important to be aware of this and to recognise when this becomes unhelpful.

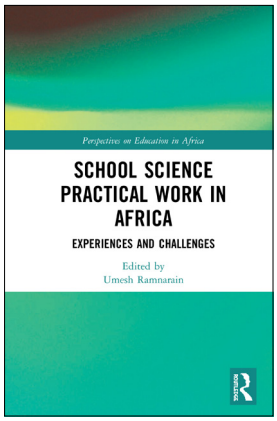
Of course, the main strength of the book is that it draws attention to the rich scholarship about realism itself. Chapter five delves into realism, its history, and variations. The chapter covers several important types of realism, including scientific, minimal scientific, critical, and situational realism. Importantly, it introduces the reader to scholars who write about the types of realist thought. I recognised some of the more well-known scholars but was happy to learn about the scholars I had not come across. I am not an expert in realism (those who are might have more to say about the chapter), but after reading this chapter I appreciated just how rich and complex the literature is. It certainly inspired me to relook at my engagements with realism.

The final chapter (six), aptly titled 'The realist image of science', invites us to re-imagine psychological science from the perspective of realism. Importantly, the chapter covers the question of 'so what?' Maree covers science as criticism, revisits the mythic image of science and discusses measurement, which is, of course, central to psychological science. Importantly, he refers to three studies that, while at face value differ significantly in paradigm and methodology (for example, experimental and qualitative), can be usefully framed by realism. The studies focus on personality, how infants count, and media coverage of the Marikana massacre. Interestingly, the latter lies outside of psychology, suggesting that this book may be useful to other social science and humanities disciplines too.

The book is interesting and engaging. It compels us to appreciate the complexity of realism and invites us to think differently about psychological science. Maree breaks down unhelpful dichotomies and demonstrates how realism may allow for a richer, perhaps even more liberating, engagement with psychological science. Even though some of the fundamental concepts of realism could have been explored in more depth, the book adequately demonstrates how the mythic image of science has hindered conceptualisations of psychological science and the potential for realism. Some of the arguments were difficult to follow, but this is probably a reflection of the fact that the material *is* complex, and that I too have engaged with it superficiall , until now. In addition, I would have liked to have read a more extensive application discussion about the three studies in chapter six, but this is perhaps a sign that the book stimulated sufficient interest for me to want to engage more

Very few publications have tackled the topic of realism and psychological science in South Africa, with the exception perhaps of Maree's previous works. The book is, therefore, timeous and relevant. It is well worth reading; and I recommend it to those who have broader interests in science, philosophy and psychology as well as to those with a more focused interest in realism and psychological research. It is also likely to be useful to those who teach psychological science and research methods.

BOOK TITLE:
School science practical work in Africa: Experiences and challenges



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A predictable picture of the state of practical work in science education in Africa

Reading this book, *School Science Practical Work in Africa*, reminded me of an invitation I had to write a chapter 'Teacher Education in Africa' for a special issue of the *Journal of Science Teacher Education* for each of the inhabited continents on the globe. One of the greatest challenges was how to face this daunting task given the paucity of accessible literature on the subject.[1] My co-author thus hit on the idea of getting in touch with many of his contacts on each continent requesting an account of teacher education in their country. From the resulting correspondence and a meta-analysis on our part, we were able to construct an article based on responses from role players in 12 African countries.[2]

A similar challenge faced the editor of this book and he responded to the challenge by making use of the many foreign nationals residing, working and studying in South Africa, as well as the networks that have been established through the Southern African Association for Research in Mathematics Science and Technology Education (SAARMSTE). The book consists of nine chapters written by 16 authors focusing on nine Anglophone African countries – five in the SADC region (South Africa, Zimbabwe, Zambia, Malawi and Namibia), three in East Africa (Kenya, Uganda and Tanzania) and one in West Africa (Nigeria).

The book is claimed to be of interest to academics, researchers, and postgraduate students in the fields of science education and educational policy. In fact, the chapters are a mixture of commentaries, literature reviews, single pieces of research and accounts of projects. One drawback of the book is that it is very expensive for local buyers, just under ZAR2000 for a hard copy or just under ZAR600 for the ebook.

Ramnarain's initial chapter is an account of inquiry-based learning in South Africa and its possible place in the South African curriculum. Asheela et al. report on an intervention with 21 Namibian in-service teachers to give them more confidence to do hands-on and minds-on practical work in schools, co-authored by master's degree supervisors, Ngcoza and Seery. Mvuru and Dudu follow with an account of inquiry-based practices in Zimbabwean schools. This is followed by an account by Upahi and Oyelekan on the role of practical work in the teaching of science in Nigerian schools. Next is a well-conceived empirical study by Nampota et al. looking at pedagogical orientations of Malawian science teachers towards practical work. Miheso follows with an account of enactment of practical work in Kenyan schools followed by Kibirige's empirical investigation into practical work in two elite schools in Uganda. Chabalengula and Mumba then provide a rigorous and well-written analysis of Zambian integrated science materials for science and engineering practices. Finally, Semali provides an account of i-SPACES – a project aimed at undertaking relevant practical projects in Tanzania, but which needs support from science, technology, society and environmental literature (e.g. Pedretti and Nazir[3]).

What emerges from the book is a predictable picture of the state of practical work in science education in Africa, characterised by a lack of resources sometimes illuminated by initiatives that make a difference. Despite the book's intention to decolonise, the literature is heavily laden with international references. As a reader I would have appreciated a closer look at the context of the education systems and their challenges in each of the countries. Although this book was largely written in the pre-COVID period, it would also have been interesting to assess the extent of Africa's adaptation to ICT in practical work.

As intimated in the introduction to this review, there are some issues regarding the references, some of which, in my view, could have been overcome. One problem is that the authors have cited many well-known overseas references on practical work, but have not always chosen the best of these references. For example, eight references by Millar are used, including one high-quality article that was used by six of the nine authors, suggesting that a common international literature review may have helped both the writers and readers. Of the other seven, only three can be judged to be of high quality. Another negative factor regarding referencing is several instances of the use of articles from predatory journals, a problem which should have been resolved by the publishers. Finally, I was surprised by the age of the references used to describe the current state of affairs of curricula and resources in each country, such as a reference to the latest curriculum in Nigeria dated 2009.

One flaw in the book is the lack of a common understanding of what is meant by practical work and how it relates to inquiry. Some articles claim to be about inquiry, but mostly deal with practical work. In my understanding, inquiry is far broader than practical work and may or may not include practical work. It would have been a good idea for the authors to agree on a common understanding before embarking on the book, and would have enabled the editor to clearly indicate the boundaries between what was to be included and excluded.

In general, however, this book is to be welcomed as an addition to the literature of African science education. Its worth will be measured by the extent to which it is cited in the literature. I encourage university libraries to buy the book.

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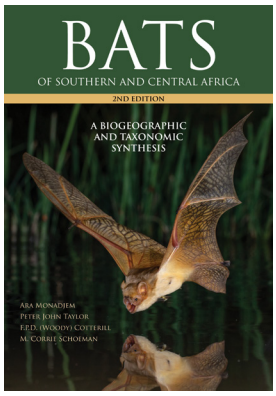
Bats of southern and central Africa: A biogeographic and taxonomic synthesis



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Latest compendium on bats of southern and central Africa

Public interest in bats has increased over the past few decades for a myriad of reasons. Bat-focused research and publications are escalating as bats face increasing threat from wind energy, habitat loss, white nose syndrome (a fungal disease that attacks the bare skin of hibernating bats and has killed millions of bats in North America in the recent past), persecution due to superstitions and misinformation on the spread of disease, and other factors. With a growing awareness of the important ecosystem services that bats provide, there is a rise in bat conservation efforts mediated by citizen science, and more public bat interest groups are being formed. For academics, conservationists, citizen scientists and others, an accurate and comprehensive reference book is essential for identifying bats, keeping abreast of the latest bat taxonomic changes, understanding bat biology and biogeography, and for informing expanding bat conservation efforts.

For the southern African region, the first bat-specific reference and field guidebook was brought out by Taylor[1] in 2000. Before this, general mammal reference books were used for obtaining information on bats. Taylor's[1] book was the start of a trend of southern African bat-specific books being published every decade. The first edition of *Bats of Southern and Central Africa: A Biogeographic and Taxonomic Synthesis*[2] was published in 2010, with this second edition being an update published in 2020. There was so much excitement within the scientific and amateur bat-orientated communities with the release of this second edition that it was, in fact, sold out a year before it was released! More copies are now available for purchase.

As a guest speaker during the last annual general meeting of the Gauteng and Northern Regions Bat Interest Group, lead author Ara Monadjem briefly summarised the updates of the second edition. Ara stated that the main aims of the book are to provide a way for people to identify differences between bat species through the use of identification matrices, and to obtain updated taxonomic information and distribution maps for the bats of southern and central Africa. The revised edition is larger in size (700 pages) and the hardcopy has a much stronger and field-friendly binding. Specific updates pertain, but are not limited to, the number of new species and distribution records presented, updated taxonomies, and additional and improved photographs and sonograms. It is important to note that information in the revised edition is current up until 2019 when the book was submitted to the publishers. The introductory chapters have been revised, with a very important update being changes in the conservation status of the now 125 described bat species for southern and central Africa (nine new species were added to the new book). Whilst bats are experiencing threats and some populations are declining, the significant changes in the conservation statuses reflect an altered vetting process by the International Union for Conservation of Nature, rather than definite changes in the conservation status of these species. The authors have done away with the modelled distribution areas that caused confusion for some users. A species' distribution is now displayed through the plotted locality points of all known museum specimens (as well as photographic distribution records for the more easily identifiable species, such as *Taphozous mauritianus* and *Eidolon helvum*). Many more sonograms are presented in the latest edition, and photographs of almost all bats and skulls are displayed. Besides photographs for the newly listed species, the quality of photographs has improved in some cases, especially within the family Vespertilionidae. Several species have received updated names, and more than 100 new references are included, with the reference list now exceeding over 700 sources of original work.

The book represents the most comprehensive account to date of the bats of southern and central Africa and is a valuable addition to the library of anyone with an interest in bats. I and my colleagues have used the first edition[2] extensively for bat work in South Africa, Zambia, Namibia and to some extent in the Democratic Republic of the Congo over the last decade (to the point where the binding has detached) and look forward to getting the same usage from this updated and stronger second edition in the southern and central African region. The authors can be commended for their efforts, and for many years of hard work in the area of bat research.

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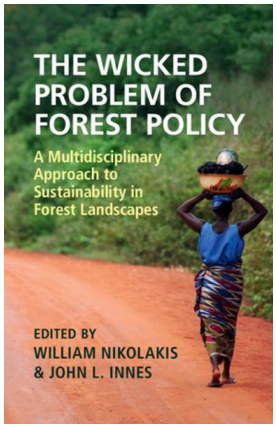
BOOK TITLE:
The wicked problem of forest policy: A multidisciplinary approach to sustainability in forest landscapes



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Confronting complexity in forest management

Wicked problems are a feature of the modern world, where more and more people compete for fewer and fewer resources, and where opinions on appropriate ways to deal with this differ widely. Wicked problems are characterised by a lack of consensus among stakeholders and the absence of stopping rules. Wicked problems have no right or wrong answers, and the implementation of proposed solutions often leads to new problems. The global COVID-19 pandemic has recently brought a wicked problem into sharp focus on a worldwide scale, as governments try to strike a balance between imposing restrictions that would slow the spread of the disease, while simultaneously avoiding economic collapse. Achieving the sustainable management of our environment is likewise fraught with wicked problems, not least of which are to be found in the forestry sector.

This book opens with a chapter on why forests matter. It explains that they matter at a local scale to people who live in or close to them, they matter nationally as they contribute a large percentage to the economies of many countries, and they matter globally because they house most of the world's biodiversity and they play a significant role in regulating the earth's climate. Developing effective policies for sustainably managing forests is a wicked problem because it matters to so many different people for so many different, often conflicting, reasons. The world's forests are also rapidly disappearing as humans exploit their many resources and convert the land to other uses. The process of trying to control this destruction, and steering the management of forests towards sustainable conservation and use, has proved to be a fertile breeding ground for wicked problems. This book provides an extensive review of these problems, the policy approaches that have been developed to address them, and how well they are working – or not.

By way of example (and there are many examples in this book), one could look at the issue of oil palm plantations in Sumatra and Borneo. The plantations have brought jobs and development, including health care and schools. But they come at the expense of the tropical forests that they replace, and these are home to abundant biodiversity and iconic species such as the orangutan. Local people tend to support the development, but it is anathema to conservation groups, which oppose it vehemently. Models have suggested that there could be a sustainable balance between the two, but a workable plan to implement this is being delayed by issues such as land tenure and indigenous rights. In the meantime, oil palm plantations continue to expand rapidly, with key decisions influenced by power relations rather than by democratic processes.

In an attempt to address problems like these, several ambitious and far-reaching schemes have been developed. Certification of forest products (for example by the Forestry Stewardship Council, or the Programme for Endorsement of Forest Certification) is now applied over more than 500 million hectares (but only 1.4% of this is in Africa). Although started with good intentions, certification schemes have failed, by and large, to achieve their goals. Payments for ecosystem services have perhaps been more successful, but only in situations in which they benefit local people, which is not always the case. A scheme labelled REDD+ (Reduced Emission from Deforestation and Degradation+) was introduced as a policy measure intended to mitigate global climate change. REDD+ has gone to great lengths to avoid problems of competing rights and widespread corruption, but in so doing has created a structure that is 'extremely complex and difficult to implement at local scales'. It has thus remained 'a great idea that has hardly been tried'. There appears to be broad consensus that devolution of decision-making about the management of forests to local governments and communities is the way to go, but in most cases this has been only partial, with central governments retaining key powers.

Forest management is also bedevilled by illegal activities, notably illegal logging. A chapter dealing with this issue notes that, of all forms of environmental crime (for example illegal fishing, wildlife poaching, or dumping of hazardous waste), forestry crimes have by far the largest impact on humanity. Illegal logging is directly responsible for up to 90% of all tropical deforestation, and is valued at over USD100 billion annually, yet the practice hardly features in international forest policy debates, where the subject appears to be taboo. Combined with widespread corruption in many forestry administrations, forestry crimes add a further complicating dimension to wicked problems in forest policy.

These and many other aspects are examined in detail in this book. There are five chapters devoted to 'tools to address wicked problems'. Most propose the devolution of meaningful decision-making to local levels, but note that established governments and corporations with vested interests continue to play powerful roles in determining the future of forests. An argument is put forward that corporate investment by the private sector may fare better in influen ing forest policy if such investment is done within a responsible framework. Currently though, this synthesis has shown that existing systems of regional, national and international government have essentially failed. Largely this is because of ongoing forestry crimes, deep-rooted corruption, and a reluctance on the part of governments and corporations to cede power to local actors. Wicked problems are by definition almost impossible to solve, but it would nonetheless be very informative for anyone involved in the environmental policy field to read this book. The treatment is detailed, and this provides the reader with a thorough background to the issues they will be facing, and potentially how they could address them.

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Ecological effects of clay mining by *Macrotermes* termites

The mounds of *Macrotermes* termites in sandy soils usually have a greater clay content than surrounding topsoils.[1] The origin of the clay used in the construction of the mounds has not been systematically investigated, but could be from the topsoil, subsoil or tens of metres into the regolith.[2] There are a range of potentially positive ecological effects of this clay mining by the termites. Erosion of the mound material (Figure 1) will increase the clay content of topsoils and consequently their nutrient status, water-holding capacity and nutrient-holding capacity. There is, however, a potentially negative effect of increased clay content in sandy topsoils, namely an increased tendency of the surface layer of the soil to seal during rain events, and to reduce infiltration of rainwater as a result.

Figure 1: A *Macrotermes* termite mound in northern Namibia. The direction of soil erosion from the mound is depicted by a black arrow.

Sealing of the soil surface during a rain event can, in many soils, have a large effect on infiltrability of water.[3-7] Such sealing is usually temporary. Raindrop impact disperses clay particles which block the soil pores within the top millimetre of soil.[3,6] This blockage can occur even in sandy soils with small amounts of dispersed clay.[7-9] When the soil dries after a sealing event, whether in a clayey or sandy soil, the clay particles shrink, the thin seal breaks apart, and the seal is no longer easily discernible in the field. Sealing is usually ephemeral, occurring during rain events, as opposed to crusting which may be evident at the timescale of decades.

Although a physical crust several centimetres thick will constrain infiltration, it is often the formation of a thin seal during a rain event at the soil surface that has a greater effect on infiltration [8,9] The ecological effects of this sealing process can be extreme, with seals of less than 0.1 mm thick reducing the rate of infiltration by a factor of 1800.[3] To investigate the likelihood of this potentially negative effect of sealing being increased as a result of clay mining by termites, we analysed a range of physico-chemical properties of seven termite mounds, constructed by *Macrotermes* species, as well as adjacent topsoils and subsoils in northern Namibia (Figure 2).

The results of these analyses are presented in Table 1. As expected, the mean clay content of mound samples was considerably greater than that of topsoils (23% vs 11%). Surprisingly, this greater clay content did not result in reduced infiltrabilit . Mean infiltrabil ty of mound samples and topsoils was ~180 mm/h and 115 mm/h, respectively. We attribute this result to the greater electrical conductivity, pH and exchangeable sodium percentage of the mound samples compared with the topsoil. These chemical changes in the soil would be expected to reduce the dispersibility of the clay and consequently reduce the tendency of the soil to seal.[6,8,9,10] This was borne out in the data, with mound samples and topsoils having similar amounts of water-dispersible clay (1–2%). Notably, the percentage of total clay dispersed was three times greater in topsoils than in samples from the top of the mounds (18% versus 6%).

In conclusion, our results show that the mining of clay by *Macrotermes* termites is unlikely to increase sealing and thereby reduce infiltrability of soils during rain events. This is because the mining is also associated with an increase in electrical conductivity, pH and exchangeable sodium percentage, all of which reduce the dispersibility of the clay.

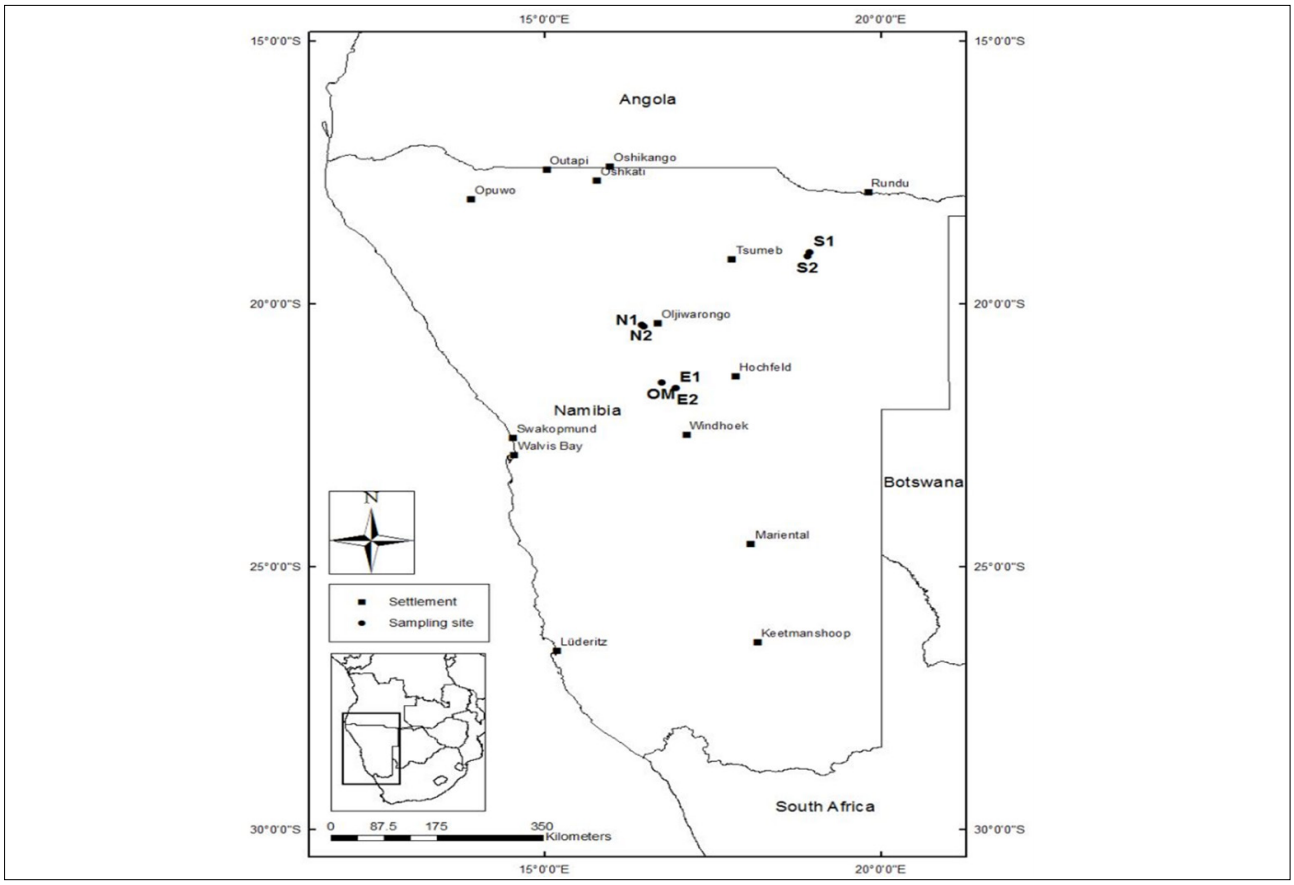

Figure 2: Location of sample sites in northern Namibia.

Table 1: Physical and chemical properties of the mound surface, mound interior, topsoils and subsoils. Values are presented as means and standard errors ($n=7$). Letters (a,b,c) indicate significant differences between values in a row ($p<0.05$). Statistical analyses were conducted as follows: linear mixed models using the 'lme4' package[11] in R[12]; sampling site was assigned as a random intercept to test the differences in soil properties within and not across sites; model residuals were inspected for normality and plotted against independent variables to test for linearity, ensuring no violation of linear mixed model assumptions; and Tukey's post-hoc tests were applied to identify significant differences between treatment means

		Mound		Topsoil	Subsoil
		Surface	Interior		
Soil texture[i]	Clay	23 (3.8) a	23 (3.9) a	11 (3.8) b	18 (3.3) ab
	Silt	8 (1.1) a	9 (1.4) a	11 (3.6) a	8 (1.6) a
	Clay + fine sil	28 (4.7) a	27 (4.6) a	17 (5.5) a	21 (4.3) a
	Sand	67 (5) a	66 (5) a	77 (7) b	73 (5) ab
Infiltrability (mm/h [ii]		176 (27) a	179 (38) a	115 (20) a	180 (25) a
Water-dispersible clay and fine silt (WD, %)[iii]	Clay	1.1 (0.3) a	2.5 (0.5) b	1.7 (0.4) ab	1.1 (0.2) a
	Clay + fine sil	7.3 (1.5) a	8.9 (1.4) a	6.5 (1.7) a	6.4 (1.1) a
WD clay as a percentage of total clay (%)		6.4 (3.4) a	13.2 (4.3) ab	18.2 (6.1) b	7.5 (1.9) a
WD clay + fine silt as a percentage of total clay + fine silt (%		30.5 (6.6) a	36.9 (5.9) ab	45.4 (5.4) b	36.9 (7.7) ab
EC (Sm^{-1})[iv]		164 (23) bc	234 (60) c	48 (13) a	73 (19) ab
pH (KCl)[iv]		6.7 (0.2) a	6.7 (0.2) a	5.6 (0.3) b	6.2 (0.4) ab
OC (%)[v]		0.36 (0.1) a	0.4 (0.1) a	0.5 (0.1) a	0.27 (0.1) a
Exchangeable cations ($Cmol_c/kg$)[vi]	Sum	19 (4) a	18 (4) a	8 (3) b	15 (4) a
	Ca	15 (3) a	15 (4) a	5 (2) b	13 (4) a
	Na	0.24 (0.02) a	0.3 (0.01) b	0.24 (0.02) a	0.23 (0.02) a
	Mg	2.4 (0.3) a	2.3 (0.3) a	1.5(0.3) c	1.9 (0.2) b
	K	0.7 (0.1) a	0.8 (0.1) a	0.7 (0.2) ab	0.4 (0.1) b
Exchangeable sodium percentage (%)[vii]		1.7 (0.4) a	2.3 (0.6) a	5.2 (1.2) b	2.9 (0.8) a

[i]Total calgon-dispersible particle size distribution[13]
[ii]Laboratory infiltration method[9]
[iii]Pipette method[13]
[iv]1:5 soil:water suspensions[14]
[v]Walkley-Black method[15]
[vi]Thomas[16]
[vii]ESP = Exchangeable {(Na)/(Ca + Mg + K + Na)} x 100

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The *Protection of Personal Information Act* and data de-identification

Data have become an exceptionally valuable resource. In light of the COVID-19 public health emergency, data sharing and the concept of open science has gathered momentum.[1] The advantages and disadvantages of open science notwithstanding, a pressing issue for the scientific community to consider – particularly in relation to health research – relates to the de-identification of data, and the impact of the *Protection of Personal Information Act 4 of 2013* (POPIA) on research activities in this context. For the purposes of this Commentary, 'health research' refers to scientific research designed to learn more about human health with a view to preventing, curing and treating diseases. This type of research invariably requires the use of personal information as defined in POPI .

On 23 September 2020, the Academy of Science of South Africa (ASSAf) announced that it would be embarking on a process to facilitate the development of a Code of Conduct for all scientific research activity with a view to submitting this Code to the Information Regulator for approval in July 2021.[2] Accordingly, the purpose of this Commentary is to: (1) discuss data de-identification and related concepts; (2) consider how data de-identification applies in the context of scientific practice in South Africa; and (3) consider relevant data de-identification principles in selected relevant foreign jurisdictions.

Background to POPIA

POPIA was the result of a painfully slow law reform process that was initiated in 2000 by the South African Law Reform Commission. The process operated under the name 'Project 124: Privacy and data protection', and, following an *issue paper* in 2003 (which announced an investigation into data protection, articulated the aim of the investigation, and pointed out solutions while also requesting comment), the project delivered a *discussion paper* in 2005 (which set out the South African Law Reform Commission's preliminary findings and recommendations and invited further comment). Thereafter, a *final report* was published in August 2009 – this report summarised the investigation, gave a detailed exposition of the applicable law, and set out draft law (known as a Bill) on protection of personal information.

POPIA was finally promulgated on 19 November 2013. Certain parts of the Act were made effective from 11 April 2014; however, the majority of the Act was effective from 1 July 2020. Critically, in terms of section 114 of POPIA, all parties have 12 months from the effective date to be fully compliant; 1 July 2021 is therefore the date by which all parties must be ready to comply with the Act.

POPIA will create a new data protection regime in South Africa, and, for the first time, the country will have a comprehensive data protection statute for all sectors – this will bring South Africa in line with many other developed nations where data protection laws are now the norm rather than the exception. The Act animates and gives effect to the right to privacy which is specifically protected by section 14 of South Africa's Constitution. Although the right is not absolute, it is now generally accepted that all persons in South Africa have a right to protection from unwanted collection and use of personal information.

However, this new regime should *not* represent a sea change for health research; treating data privately, securely and ethically should be something with which health researchers and scientists are familiar. For almost 20 years, the *National Health Act 61 of 2003* has regulated health records (see, in particular, Chapter 2 and sections 14–17 thereof which deal with confidentialit , access to records, and the protection of records). In addition, the *Health Professions Act 56 of 1974* establishes a Health Professions Council which has set out detailed ethical guidelines for good practice (see especially booklet 5 dealing with confidentiality). A thorough examination of these related provisions is beyond the scope of this Commentary, suffice to say: POPIA will not stand alone, and although it is now the point of departure when considering data protection in South Africa, depending on the context, it must be read together with other relevant legislation.

Data de-identification and POPIA

Personal information is widely defined in POPIA, and includes names, identity numbers, address information, online identifiers such as IP addresses, and, in the health research context, medical records of a patient, biometric data, and genomic data. Importantly, in terms of section 6 of POPIA, the Act will not apply to data 'de-identified to the extent that it cannot be re-identified again'. This principle, although expressed differently, is consistent with data protection legislation around the world – see, for example, Recital 26 of the European Union's Directive 95/46/EC (which is the General Data Protection Regulation also known as the GDPR)[3], and the American 'Privacy Rule' in relation to health information, articulated in section 164.514 of the *Health Insurance Portability and Accountability Act of 1996* (HIPAA).

Although the terms de-identification, anonymisation, and pseudonymisation are sometimes used interchangeably, there are subtle distinctions[4] in the meanings of these terms – and it should be noted that POPIA uses the term 'de-identification'. It is defined a

> *'**de-identify**', in relation to personal information of a data subject, means to delete any information that—*
>
> *(a) identifies the data subject;*
>
> *(b) can be used or manipulated by a reasonably foreseeable method to identify the data subject; or*

(c) can be linked by a reasonably foreseeable method to other information that identifies the data subject,

*and '**de-identified**' has a corresponding meaning*

As a result, de-identification in terms of POPIA is a process whereby a person takes steps to delete all personal information that can identify a data subject in the data set. In the context of health research, for data to be classified as de-identified, no person within the relevant research organisation must be able to identify the data subject by considering the data set itself, and by considering other information in conjunction therewith. Therefore, using a *reasonably foreseeable method*, a person should not be able to manipulate the data to identify a data subject – for example by changing or sorting columns and/or data, or by editing the characteristics or permissions of a file to reveal information that could identify a data subject. Further, using a *reasonably foreseeable method*, a person should not be able to use other data to link to the data set to identify a data subject; for example, by using other related or unrelated data that are available either publicly or to that person specificall . In addition, if the objective behind de-identification is to ensure that POPIA does not apply to the processing of that data, section 6 of the Act places a further condition on parties – namely, that the de-identified data *cannot be re-identified again*.

This raises two questions: What is a reasonably foreseeable method? And, what does this definition – read together with section 6 – mean practically? Generally speaking, it means that the typical researcher with the usual skills, expertise and knowledge of someone working in that field, should not be able to identify a data subject in the data set. (Note that in legal terms, when the term reasonable is used, the determination is achieved objectively.) Practically, when making this determination, one must consider the data being used, the characteristics of that data, as well as other data that are available to the researcher. One must also consider section 6 which stipulates that the data should not be able to be re-identified. With large swathes of data now available publicly via the Internet, and with increasing amounts of data being shared and available electronically in many different databases, this determination can be problematic. Where a principal investigator is in doubt, it is suggested that a final determination is made by an external expert with no links to the project (a person with no potential conflict of interest, and with the necessary skills to make the determination).

In a similar vein to de-identification, anonymisation is typically defined as a process in which personal information is removed from data so that a data subject cannot be identified The GDPR defines anonymous information in Recital 26 as 'information which does not relate to an identified or identifiable natural person or to personal data rendered anonymous in such a manner that the data subject is not or no longer identifiable'. This definition is exceedingly similar to de-identification in POPIA, and although it may appear redundant, there are some international academics and medical professionals[5] who are of the view that these terms have distinct meanings, and that in order to foster conceptual clarity, a clear distinction should be drawn between the two terms. Briefly put, the opinion is that although de-identification removes personal information, it is still possible to re-identify the data (although it should be difficult, time-consuming and improbable that the typical researcher would be able to re-establish the link between the data and the person). However, in contrast to de-identification, anonymisation is a process whereby a researcher can practically never identify a data subject. The data are stripped so that it is virtually impossible to identify a data subject – the data are anonymised to an irreversible extent. The key difference, according to this view, is that with de-identification the process may be reversed, whereas with anonymisation it is irreversible and virtually impossible to re-identify the data. If one accepts this distinction, which is admittedly subtle, in light of section 6 and the exclusions to the Act, arguably POPIA should have rather used the term 'anonymisation' instead of 'de-identific tion' (given that the Act requires that data cannot be re-identified again, this appears more consistent with anonymisation than with de-identificat on). Alternatively, section 6 of POPIA should have been crafted on a similar basis to section 164.514 (b) of HIPAA (where data is considered de-identified if certain information is removed, or if the chance of re-identific tion is very low and statistically improbable). That debate notwithstanding, the correct term in South Africa is currently 'de-identification', although some authors do refer to the terms 'de-identification' and 'anonymisation' interchangeabl .[6]

Another term that often features in the context of data protection is 'pseudonymisation'. Although this term is not used in POPIA, Article 4(5) of the GDPR defines pseudonymisation as a method by which personal data are processed such that the personal information can no longer be attributed to a data subject without the use of additional information, provided that the additional information is kept separately and subject to technical and organisational measures to ensure the data are not attributed to a data subject. Usually, this measure is taken as a step to ensure security of the data, to avoid bias, and to provide a level of integrity to the study. In these circumstances, someone in the organisation will have access to a master file or some other data that will facilitate the identification of the data subject if necessary (for example, the data subject may need to be identifi d quickly if an incidental finding is made, for audit purposes, or in the event of some medical emergency).

Scientific practice: POPIA will apply in most circumstances

In a South African context, other than the definition, POPIA does not contain any specific provision that deals with data de-identification directly. The term is mentioned in three sections of the Act (section 1, section 6, and section 14), but there is no specific guidance on how to achieve data de-identification, or any other detail in relation thereto. It is likely that after the Act has come into full effect in July 2021, the Information Regulator (the body responsible for enforcement, monitoring and education) will produce a guidance note on these issues, or that an industry Code of Conduct – such as the one being prepared by ASSAf – will articulate best practice and tips in relation thereto. For the time being, for analogous advice on techniques in relation to anonymisation, as well as useful case studies and practical examples, see the guidance set out by the United Kingdom's Information Commissioner's Offic [7]. For further practical insight, see further the Singaporean Personal Data Protection Commission[8].

If the goal is to ensure that POPIA need not apply to the data in question, as noted above, researchers must ensure that all personal information that can identify a data subject is removed, and that it cannot be re-identified by anyone in the organisation. By way of example, if a data set contains no actual names or identity numbers or other personal information of a group of persons with a rare disease, but does contain birth dates or physical addresses, it is probable that another researcher could identify an individual in the study by using other data sets, and, in this instance, one would need to consider further steps in order to classify the data as de-identified (such as removing exact birth dates by giving a range, or by removing physical addresses and providing province or post code information). As a result, before data can be re-purposed or published (assuming one does not wish to need to comply with POPIA), the data must be sufficiently bereft of personal information to be considered de-identified; further, it must not be reasonably possible to reverse the process and re-identify the data.

It appears that, given the ethical imperatives and objectives of medical studies (as well as audit requirements), the data will often not be de-identified because someone in the organisation will have the ability to identify a data subject. Consequently, researchers should be cautioned against operating under the belief that POPIA does not apply to them because an individual (or large parts of the team) cannot identify a data subject – if someone (even if only one person) in the organisation has the ability to identify a data subject (via access to a master file, or using some other technique to link the data) the data will not be regarded as de-identified, and POPIA will apply. In this instance, these techniques should rather be referred to as pseudonymisation, and viewed as one of the measures taken to ensure compliance with the eight conditions of POPIA.

A foreign perspective on data de-identification

The comparable US legislation (HIPAA's Privacy Rule) seeks to protect identifiable health information; so, although it is similar in many respects

to POPIA, it applies to a certain field only (see Section 164.514 (a)–(c)). In terms hereof, information will be de-identified if it is stripped of 18 specific identifiers, *or* if it is determined by a professional statistical analyst with appropriate knowledge and experience that the risk is very small that the information, on its own, or with other information, could be used to identify a data subject. I suggest that a good rule of thumb to achieve de-identification in South Africa would be to ensure that the 18 identifiers of an individual set out in HIPAA are removed from the data set; the elimination of the 18 identifiers is known as the safe harbour method, whereas the second avenue of achieving compliance is known as the expert determination method and relies on a statistician verifying that the risk of identification of a data subject is very low. The identifiers to be removed (adapted for South Africa) are: names, addresses (except city, province and post code), all elements of dates (except a year or dates in ranges), telephone numbers, fax numbers, email addresses, identity numbers, medical record numbers, medical aid details, account numbers, certificate/licence numbers, vehicle identifiers and serial numbers, device identifiers and serial numbers, URLs, IP addresses, biometric identifiers[9,10], photographs, any other unique identifying number, characteristic or code. Once removed, and assuming the data cannot be re-identified again, one can assume that the data are de identified.

In the United Kingdom, data protection is regulated by the *Data Protection Act of 2018*, read together with the United Kingdom GDPR. The legislation is very similar to that in South Africa, and as a general proposition, the system of law operates on a similar basis. The Information Commissioner's Office (akin to South Africa's Information Regulator) has published a code of practice on anonymisation, and of relevance for present purposes is the 'motivated intruder test' it sets out therein. The test involves assessing whether a 'motivated intruder' can identify the individual in the de-identified data – it is assumed that this person is reasonably competent, has access to all publicly available information, and would employ investigative techniques; however, the 'motivated intruder' is assumed to not have any specialist skills such as hacking, and to not resort to criminality such as burglary or unauthorised use of secured data. Therefore, in borderline cases, or where one is unsure of whether data are de-identified, applying this fictitious test can assist an information officer or research team to determine whether data are de-identified. The guide also provides some useful anonymisation techniques, case studies, and practical examples – although the terminology in this context is different (anonymisation instead of de-identification), I suggest that until the Information Regulator produces something similar, and in the absence of a Code of Conduct to provide further guidance, the guidance in this UK code of practice will assist local researchers by providing valuable insight and practical examples.

Conclusion

POPIA should not be feared. The legislation marks a watershed moment in South African law and will ensure that the country keeps abreast of foreign developments. Although there are no definitive interpretations or guidance notes on data de-identification, a similar approach is used around the world in a variety of jurisdictions. It is hoped that the Information Regulator produces a code of practice or guidance note, or alternatively, that a Code of Conduct for researchers explains these issues in more detail and clarifies some of the te minology and processes.

For now, as a short-term measure, I suggest that where doubt exists, South African researchers should: (1) look to the United Kingdom's Information Commissioner's code of practice on anonymisation; (2) alternatively to point 1 (or in addition thereto), review section 164.514 (b) of HIPAA for insight on how to interpret whether data have been de-identified; and (3) follow the GDPR definition of pseudonymisation

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Why POPIA does not apply to DNA

One man's trash is another man's treasure. This is especially true with human biological material. While one person may not care much about the fate of a biopsy sample taken from his or her body – and may just be focusing on the diagnosis – another person may see much research potential in the biopsy sample. In particular, geneticists may view the biopsy sample as a 'container' of genetic information. This gives rise to the question: How does our privacy law perceive human biological material? More specificall , does human biological material fall within the ambit of the *Protection of Personal Information Act 4 of 2013* (POPIA)? Thaldar and Townsend[1] analysed this question and answer it in the negative: POPIA does not apply to human biological material. Adams et al.[2] also answer the question in the negative, but express their uncertainty about 'the exact point at which biological samples become personal information'. Where does this leave DNA? In this Commentary, I analyse an important question for genetics researchers, namely whether POPIA applies to DNA.

Conceptual clarity

A good place to start the analysis is to distinguish the nature of biological material from that of genetic information. While biological material is a physical thing, genetic information is something incorporeal. Of course, information can be *contained* in a physical object, such as a memory stick – or in a string of DNA. However, although a physical thing can *contain* information, the two are conceptually distinct. In this light, consider the process entailed by genetics research. Typically, the following steps can be identified: (1) the sample is collected from research participants; (2) DNA is extracted from the sample and amplified; (3) the DNA is sequenced; (4) the genetic sequences are analysed, which may include collation with other information, etcetera. The output of step 2 is DNA. Although not visible to the naked eye, DNA is still within the realm of physical objects. In contrast, the output of step 3, namely a sequence of As, Cs, Gs and Ts, is something incorporeal: information. From the perspective of the legal protection of *information*, step 3 is a consequential step.

Analysis

POPIA's application provision, section 3, provides that POPIA applies to the processing of *personal information* that is *entered in a record* by or for a *responsible party*. In the following paragraphs, the analysis of whether POPIA applies to DNA is structured around these three italicised phrases, which builds on the analysis by Thaldar and Townsend[1].

'Personal information' is defined as information that relates to, inter alia, an identifiable, living, natural person. It includes biometric information, i.e. information that can be used in techniques such as DNA analysis to identify a person. Accordingly, any genetic information contained in DNA that is sufficiently unique to potentially identify a person would qualify as 'personal information'.

Next, it is worth highlighting that the concept 'responsible party' is a technical term in POPIA, which is defined as a public or private body or any other person which, alone or in conjunction with others, determines the purpose of and means for processing personal information. In the research context, this would typically be the principal investigator.

The phrase 'entered in a record' is crucial, as it qualifies the circumstances when personal information is regulated by POPIA. The word 'entered' implies a preceding action of entering information. This is important, as genetic information is *naturally present* in DNA, rather than being *entered* in such DNA by or for the principal investigator. Accordingly, genetic information that is present in DNA is beyond POPIA's scope of application. As POPIA does not apply to the genetic information that is naturally present in DNA, it follows that POPIA also does not apply to the physical DNA itself. It is only once the DNA has been sequenced and the genetic information has been *entered in a record*, for instance on a computer, that POPIA applies to the genetic information.

The conclusion that POPIA does not apply to DNA is subject to a caveat: My analysis of 'entered in a record' above is premised on the current state of biotechnology and may change as technology progresses. For example, synthetic DNA may be used as retrievable information storage.[3] In such a future scenario in which genetic information is *entered* in synthetic DNA, rather than *naturally occurring* in such DNA, POPIA will apply.

Excursus: DNA, computers and the cloud

Some ancillary issues related to the use of information technology in the process of DNA sequencing are worth considering: First, it is important to note that POPIA provides that the action of entering information into a record can be automated. This allows for computer-driven systems that sequence DNA and automatically record the genetic information.

Following on this observation, is the question: Would POPIA still apply if the computer-driven system that sequences DNA does not record the genetic information on any local hard drive, but only somewhere in the cloud (assuming that the cloud servers are not in South Africa)? I suggest the following answer: If the DNA sequencer is in South Africa, the answer would always be affi mative. The reason is as follows: Section 3(1)(b) provides that POPIA applies if the responsible party – i.e. the principal investigator – is domiciled in South Africa or makes use of *means* in South Africa. (There is an exception, namely where those means are used only to forward information through South Africa; this exception clearly does not apply in the present case.) Therefore, even if the responsible party is not domiciled in South Africa, and the genetic information is saved on the cloud rather than on any device in South Africa, as long as the DNA sequencer – the *means* – is in South Africa, POPIA will apply.

Concluding remarks

I must express my reservations about the formulation used by Adams et al.[2], referring to 'the *exact point* at which biological samples *become* personal information' (emphasis added). First, the physical DNA does not *become* a genetic sequence like a caterpillar that *becomes* a butterfl . After all, the genetic sequence was present in the DNA all along. Second, attempting to find an *exact point* may not always accord with contemporary research reality in the biosciences – especially not with genomics. For example, whole-genome sequencing using high-throughput sequencing technology is not an instantaneous event, but rather a gradual digital accumulation of genetic information over a period of hours. As such, thinking in terms of an 'exact point' may not be helpful. The crucial element that brings personal information within the regulatory ambit of POPIA is that it must be entered in a record. Accordingly, it would be conceptually and practically more accurate in the context of contemporary bioscience research to think in terms of *the process during which personal information from DNA is entered in a record*.

This Commentary's focus on genetic information from DNA should not lull one into thinking that POPIA only becomes applicable to a genetics research project broadly understood at the point of sequencing and digitally recording the genetic sequence. One should remember that there are other types of personal information that may also be relevant. Even prior to the time of biological sample collection, when personal information from research participants is collected as part of informed consent and eligibility screening, POPIA will apply to such information, and will therefore be relevant to a genetics research project. However, the genetic information locked up in a biological sample, and the sample itself – including DNA extracted from it – are all beyond POPIA's scope of application. It is only once the genetic information is sequenced and entered into a record that POPIA applies to it.

Lastly, one should remember that the law is multidimensional, and that POPIA is but one dimension of regulation that may be applicable to genetics research. Accordingly, even though DNA falls beyond POPIA's scope of application, DNA still exists within the broader legal universe that includes inter alia other statutes, case law, and the common law.

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Competing interests

I declare that there are no competing interests.

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The construction industry transformation and the digital divide: Bridging the gap

Professor Abimbola Olukemi Windapo is the recipient of the 2019/2020 NSTF-South32 Engineering Research Capacity Development Award for her research of construction business and management that confronts the problems of poor project and organisation performance from a practice perspective.

Numerous digital technologies aim to improve both the productivity and performance of construction professionals, projects and companies. However, they have not been completely adopted, and low productivity is evident. Maintaining technological skill levels in line with industry progress also presents a challenge to women, because crucial skill development in emerging technologies tends to be achieved by aspirational labour. Previous research suggests a possible transformation of the construction industry using digital technologies. However, a digital divide exists.

Introduction

The construction industry has not achieved the best possible performance, which is largely due to low productivity on construction sites.[1,2] This most often leads to time and budget overruns, which create a high-cost escalation for the projects.[3] The issue of low productivity is a problem worldwide, not just in developing countries; even developed countries suffer from a decline in productivity.[4] This is a major issue across the construction industry, and if the current trend continues, there will be a major negative effect on its health and viability.[1]

Howell and Higgins[5] hold a theory that organisations in the construction industry must be leaders in the identification, evaluation, and adoption of the latest technological innovations if they are to remain relevant and competitive. However, many companies do not fully adopt these innovative technologies.[6] The numerous innovative construction technologies available on the market aim to complement job functions and improve the performance of construction companies. However, in line with the Diffusion of Innovation theory, these technologies have not been adopted in the construction sector of many developing economies, such as Nigeria, and low productivity continues to affect them.[5]

Although previous research suggests that there are opportunities for the digital environment to transform the construction industry and make it more productive, scholars such as Oke et al.[7] note several barriers to adopting digital technologies. There is limited knowledge of how they could improve the productivity of projects and transform the construction industry. Here I examine construction's digital future and whether it can be proactively reconstructed, promoting gender equity in the construction industry, rather than creating a barrier, and how to bridge the digital divide. Recommendations are made to help professional builders, especially women in the construction industry, to take advantage of this digital environment to increase their effectiveness in technically dominated and male-dominated workplaces.

Overview of digital innovation

According to Barrett[8], Construction 4.0 could potentially offer opportunities to resolve issues of gender equity in the industry. Barrett[8], however, notes that Construction 4.0 itself is a gendered concept that leans towards male professionals, because there has been no strategy offered to ensure that both women and men can fill anticipated skills shortages. This exacerbates the situation in which higher rates of men are attracted to and enter a digitally transformed construction industry, and in doing so, enable the bridging of the digital divide. Digital innovative technologies which may offer new opportunities to resolve issues of poor productivity and performance in the construction industry include 3D scanning, building information modelling/management, 3D printing, augmented/virtual reality, drone technology, the Internet of Things, big data analytics, machine learning and blockchain technology. These are explained in the following paragraphs.

The process of 3D scanning analyses a real-world object to collect data on its shape, and possibly its appearance. This type of scanner is used to model and build the structure. Laser scanning is used to create quick and accurate 3D models of existing buildings, to support the subsequent process of 3D scanning.

Building information modelling relies upon various tools, technologies, and contracts involving the generation and management of digital representations of buildings, and of the infrastructure's physical and functional characteristics.

Integrated building information modelling and 3D scanning involves converting laser scans into a Building Information Model. It thus collects and documents valuable information in a consistent building database that serves as an accurate source for engineering, design and construction.

3D printing is an automated, additive manufacturing process for producing three-dimensional solid objects from a digital model.

Augmented reality is an interactive experience of a real-world environment, where the objects in the real world are enhanced by computer-generated perceptual information, sometimes across multiple sensory modalities, including the following senses: visual, auditory, haptic, somato-sensory and olfactory.

Virtual reality is the use of computer technology to create a simulated environment. Unlike traditional user interfaces, virtual reality places the user inside an experience. Instead of viewing a screen in front of them, users experience themselves as immersed in, and interacting with 3D worlds.

Drone technology provides construction teams with an overhead view of job sites, materials, machinery and people. Contractors use autonomous flying machines to record videos that help optimise everything from grading plans and operations to identifying differences between as-designed and as-built site plans.

The Internet of Things is a system of interrelated computing devices, mechanical and digital machines, objects, animals, or people provided with unique identifi rs and the ability to transfer data over a network without requiring human-to-human or human-to-computer interaction. Internet devices can monitor and control the mechanical, electrical and electronic systems used in infrastructure and buildings in home automation and building automation systems.

Big Data Analytics is the process of collecting, organising and analysing large sets of data (called 'big data') obtained from text, audio, video, and images to discover patterns and other information. Analysts working with big data typically want the knowledge from analysing the data. Organisations in charge of assets analyse big data to find consumer patterns and trends, to make investment decisions.

Machine learning is an application of artificial intelligence that provides systems with the ability to learn from experience automatically, without being explicitly programmed. Machine learning focuses on developing computer programs that can access data and use it to learn for themselves. This iterative aspect is important; when models are exposed to new data, they can independently adapt, and produce reliable results.

Blockchain technology stores transactional records of the public, also known as the 'block', in several databases, known as the 'chain', in a network. This storage is referred to as a 'digital ledger'. Together with building information modelling, blockchain can create a single source of truth for all aspects of a construction project (see Figure 1). Such a model can become the trusted digital twin of an asset, supporting its design and construction and its operation and maintenance, through the life cycle.

Using innovative digital technologies

Low productivity on construction sites can lead to time overruns and excessive cost on a project.[3] Karim et al.[9] showed that the use of technology could improve both productivity and project performance. These results show that it is a priority to find effective innovative technology.

Traditional career structures are becoming less attractive and no longer the norm in the emerging digital environment.[8,10] Furthermore, the labour-intensive nature of construction is increasingly lessened by digitisation; automation and robotics increasingly perform physical tasks previously identified as only within men's ability. The new digital environments remove the physical visibility of distinctions between women and men, which reduces the likelihood of gender discrimination.

The use of innovative technologies has also been shown to positively impact companies' performance.[11] Pelser[12] found that innovation had a positive correlation with the company's performance, showing that when companies made positive use of the technologies, they could boost their competitiveness. Molenaar et al.[13] indicated that innovative technologies provided more collaboration and cohesiveness across the project team, which positively benefited cost, schedule, and quality performance measurements.

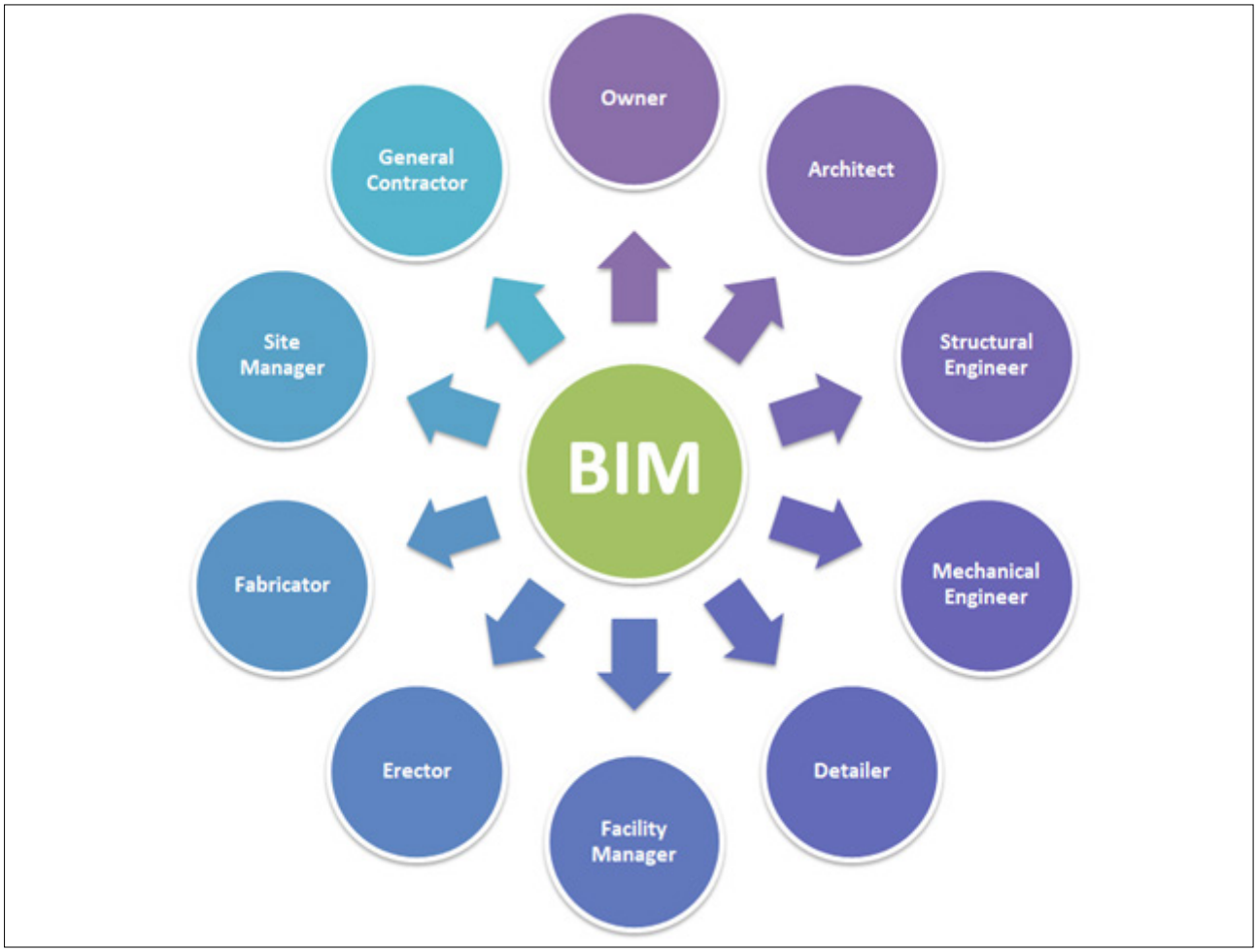

Figure 1: Smart contracts using blockchain technology.

The digital network has supported women to connect and network more flexibly with colleagues and clients. Previously, networking in the construction industry has seemed to exclude women and impose a barrier to career progression. The construction industry's digital access will widen participation in professional learning, enabling more opportunities for women in digital learning and networking.

Overview of barriers to the adoption of digital technologies

Mtya[14] found that digital technologies such as building information modelling are not widely adopted in the construction industry; although fi ms possess capabilities to use the technology, some barriers prevent them from using those capabilities and digitising. These barriers are outlined below:

Resistance by the consultant team

According to Oke et al.[7], the lack of cooperation within the professional team creates the most significant barrier to innovation. The strict standards across industries, and the unwillingness of professional councils to change, are a barrier to industry change.[15]

Training and upskilling of employees

The lack of structured training is another critical barrier to adopting innovative technology on projects.[16] Dupwa[17] states that significant investment in industry professionals' education in the use of innovative technologies is imperative to their successful adoption. The construction industry is extremely competitive, and a shift towards the use of innovative technologies has meant that company executives continually need to improve their staff's proficiency to improve overall performance.[18] A construction company's ability to adapt to new technology faster than its competitors is vital to a competitive advantage.[19] To meet the growing industry requirements for further adoption of innovative technologies on projects, fi ms are required to be more proficient in their use [20]

Costs incurred when adopting innovative technology

The high cost of training employees[7] combined with the high initial cost of implementing the technology[21] discourages companies from adopting the technology[22].

Current legislation

Mostafa et al.[23] identify the main barrier within developing countries as the current legislation – for example, legislation about drone technology. Many companies have not fully adopted innovative technologies on projects.[23,24] Additionally, data security, protection, and control of information are all barriers to adoption.[7]

Clients' lack of knowledge

Clients and governments lack an understanding of the positive impact that new technology can have on performance. This is a significant barrier to the implementation of innovative technology on construction projects.[24,25]

The way forward – bridging the digital and empowerment divides

Can construction's digital future be proactively reconstructed as an opportunity for change, promoting professional gender equity, rather than creating a barrier to it? In my appraisal of different types of innovative digital technology, the barriers to, and benefits of using it, several advantages of using innovative digital technology emerged, including high productivity, and project and company performance. These technologies have the added benefit of eliminating the necessity of women's physical visibility and allowing them to network easily. Digital technologies will empower women to overcome disadvantage and participate equally in the construction industry.[8] Therefore, the industry can be transformed to become more productive and representative, by using innovative digital technologies.

However, there are barriers to using innovative technologies in construction, which must be addressed to enable this transformation: resistance by the consultant team, the training and skills required, costs involved when adopting innovative technologies on projects, current legislation, and clients' lack of knowledge.

To bridge the digital divide and transform the construction industry, it is recommended that the project consultant team should develop relationships promoting the use of innovative technology; also that clients should be more exposed to the benefits of digital technology, despite the cost. Legislation should be updated regularly and should not lag behind industry regulations.

To meet these goals, the government and the construction sector must provide targeted investment in education and training. This should support individuals, especially women and girls, considering or beginning digital construction careers. Barrett[8] identifies a strategy of supporting businesses and organisations to remove the barriers that hinder women's career progression, by closing gender pay gaps and neutralising traditionally masculine cultures. The digital transformation should be paralleled by a cultural revolution, to fully embrace gender equity opportunities. Barrett[8] posits that this cultural shift should allow women to manage their diverse and fluctuating out-of-office commitments. It should also define alternative career and reward structures to encourage professionals to deliver their best work.

The construction industry can be transformed; and the digital divide can be closed if the government makes targeted investments in training and upskilling, and regularly updates legislation in step with new construction knowledge.

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South Africa suffers a second loss of the blue antelope (*Hippotragus leucophaeus*) as DNA analysis confirms that the sole specimen held in South African collections is sable (*H. niger*) material

The blue antelope, *Hippotragus leucophaeus* (Pallas, 1766), is the only African large mammal to suffer global extinction in historical times, around 1799/1800.[1] This species, endemic to a single habitat type in the eastern lowlands of South Africa's Cape Floristic Region, went extinct before any formal scientific studies could be made of living animals.[2] As a consequence, our information regarding the blue antelope is limited to frustratingly few anecdotal accounts from early naturalists[3], archaeological/fossil material[4,5] and a handful of museum specimens, mostly scattered in collections around Europe. To date, only one such museum specimen has been recognised as remaining in South Africa.[6] This is indeed a sad reflection of not only the loss of the species for our country, but also the fact that virtually all available specimens were taken out of the country. It is ironic that servicing the demand for specimens for foreign collections[7] may have contributed to the final death knell for the species[2]. Fortunately, modern museum ethics have evolved beyond such a cavalier approach to building collections, with internationally developed and agreed-upon standards of ethical practice now being applied.[8]

The opportunity for scientific study of the blue antelope has now become even more dire, as shown by a recently published genetic analysis of available material.[9] Hempel and colleagues[9] were able to physically sample 10 of the documented 16 blue antelope specimens (these vary from whole skin mounts to skull fragments) held in various museums. They subjected these samples to genetic analysis, which yielded a complete mitochondrial genome for the species, and phylogenetic analysis to confi m the species identity of the specimens. These analyses showed that only four of the ten sampled items could be confi med as representing the blue antelope. The six remaining specimens tested comprised material from congeners, either sable (*H. niger*) or roan (*H. equinus*) antelope. Thus, there are far fewer blue antelope specimens available for study than we thought. Among the victims of this genetic validation of samples is the sole specimen attributed to this species held in a South African collection. This specimen (SAM ZM 40759), a frontlet with horns (Figure 1) acquired by Iziko Museums (Cape Town) as recently as 1989[6], is now genetically classified as a sable antelop [9].

On a positive side, Hempel and colleagues[9] were able to test predictions[2,10] that the historically recorded blue antelope population was genetically depauperate. The mitochondrial sequences available from the four confi med specimens do indeed show low genetic diversity, comparable to that of other large mammals that have undergone major population declines, such as the European bison, *Bison bonasus.*[9]

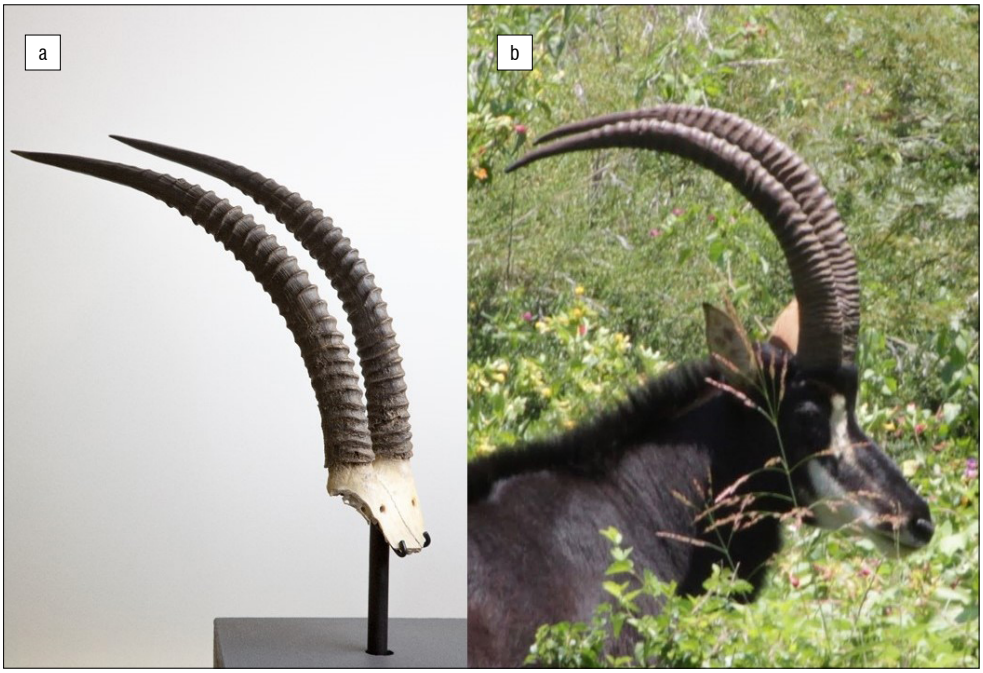

Figure 1: Images of (a) the Iziko Museum specimen (SAM ZM 40759) previously identified as a blue antelope and now shown to be a sable antelope (photo: Iziko Museums of South Africa/Nigel Pamplin) and (b) an adult sable antelope (photo: E. Le Roux), illustrating the annulated and back-swept horns characteristic of the hippotragine antelopes.

While not causally linked to the observed extinction, and based on a small sample, such low genetic diversity may reflect the predicted small population size and lack of metapopulation processes in the historical population[2], and suggests an inability to respond to changing environmental pressures[9]. This analysis highlights the deeper and enduring value of properly curated and accessible museum collections[11]; while we have lost the species, the study of museum specimens allows a (admittedly narrow) window of insight into the biology and ecology of such species. We therefore have a responsibility to protect and better resource our museums in the face of increasing societal neglect of these institutions and their collections.[11]

Of the six specimens that Hempel et al.[9] did not sample, two have already been suggested to be *H. niger* or *H. equinus*[9], leaving only four unvalidated specimens. Given that two of these are recognisable whole mounts, including the lectotype (held by the Rijksmuseum van Natuurlijke Historie, Leiden), there remains two specimens (comprising skeletal material) of untested identity. Whatever the outcome of further testing of these specimens, it appears that the global holding of blue antelope in natural history collections is likely to be no more than eight specimens. Clearly, there is much value in testing all the remaining specimens to confi m their genetic identity, and to add to our understanding of the genetic diversity of the blue antelope at the time its extinction was drawing near.

An interesting side note to Hempel et al.'s[9] study is the degree of confusion and lack of detail in the recorded provenance for the purported blue antelope specimens. Over the years, various authors[6,12], in grappling with the challenge of advancing our understanding of the blue antelope, have highlighted this confusion around the provenance of the available specimens. This situation is possibly best illustrated by the fact that the mounted specimen in the Muséum national d'Histoire naturelle (Paris) has been attributed to three different collectors. Only recently was Glenn[13] able to clear up this confusion and decisively show that this specimen was collected by Levaillant, presumably representing the blue antelope shot 'in early 1782 in the Soetmelks River Valley, between the current Genadendal and Riviersonderend'[13]. Hence, the legacy of the blue antelope is also marked by the poor quality of the records of the few specimens that are available to science.

The paucity of historically collected blue antelope specimens highlights the urgency to assess the validity of the available fossil material[4,5], which is similarly at risk of misidentification. This assessment could apply ancient DNA techniques that would not only assist in the validation of the fossil material, but would also extend our understanding of the genetic diversity of the blue antelope and possible hybridisation with the closely related and sometimes sympatric roan antelope, and explore patterns in genetic diversity over time.

The fact that all of the known blue antelope specimens available for scientific study are held in collections outside South Africa highlights the loss of biodiversity and intellectual property for South Africa during colonial times. As shown by Rookmaaker[7], much of the early interest in zoology in South Africa was driven by collectors motivated by the prospects of selling specimens to wealthy European individuals and institutions. South Africa now has strong legislation to manage the export and possible loss of biodiversity assets (*National Environmental Management Biodiversity Act No. 10 of 2004*). There is a clear need to maintain and enforce the regulations within this legislation, as also recently argued for live specimens of iconic species like rhinos.[14] Unfortunately, as the blue antelope saga shows, this legislation was clearly 250 years too late. This 'loss' of the South African specimen may, however, be an incentive for South Africa to initiate a conversation with the relevant institutions regarding the return of these exported blue antelope specimens.

Competing interests

There are no competing interests to declare.

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Fish farm effluent as a nutrient source for algae biomass cultivation

One of the challenges of microalgae biotechnology is the cost of growth media nutrients, with microalgae consuming enormous quantities of fertilisers, more than other oil crops. The traditional use of synthetic fertilisers in mass cultivation of microalgae is associated with rising prices of crude oil and competition from traditional agriculture. The fact that fish farm wastewater (FFW) nutrients are released in the form preferred by microalgae (NH_3 for nitrogen and PO_4^{-3} for phosphate), and the ability of microalgae to use nitrogen from different sources, can be exploited by using fish farm effluent rich in nutrients (nitrogen and phosphorus) in the cultivation of cheaper microalgae biomass for production of biodiesel. The cultivation of algae biomass in FFW will also serve as wastewater treatment. We reviewed the benefits and potential of fish effluent in algae cultivation for the production of biodiesel. Microalgae can utilise nutrients in FFW for different applications desirable for the production of biomass, including the accumulation of lipids, and produce a fuel with desirable properties. Also, treating wastewater and reducing demand for fresh water are advantageous. The high lipid content and comparable biodiesel properties of *Chlorella sorokiniana* and *Scenedesmus obliquus* make both species viable for FFW cultivation for biodiesel production.

Significance:

- The cost associated with microalgae growth media nutrients can be saved by using fish farm wastewater, which contains nutrients (nitrogen and phosphorus) suitable for microalgae cultivation.
- Fish farm wastewater has lower nutrient concentrations when compared to standard growth media suitable for higher lipid accumulation.
- Microalgae used as a biodiesel feedstock, cultivated in fish farm wastewater, has added benefits, including wastewater treatment.

Introduction

The ability of microalgae to adapt in a diverse environment is reflected in the patterns of lipids produced as well as their ability to synthesise various unusual compounds.[1] The kinetics of microalgae growth, lipid productivities, and the amount of biomass vary with the algal strain, culture, and physiological conditions.[2] Some species of microalgae, such as *Dunaliella Salina, Chlamydomonas reinhardtii, Chlorella,* and *Botryococcus braunii* can contain more than 60% lipid by dry cell weight.[3] However, microalgal species with high lipid accumulation (50–70% of dry cell weight) generally have a slow growth rate.[4] It is possible to find examples of microalgae that are fast growing and have a high lipid accumulation, e.g. *Nannochloropsis oculata* and *Chlorella vulgaris* (Table 1), and that have been used for biodiesel production.[5]

Increasing lipid production is possible through the cessation of cell division under environmental stress conditions. This switches from the synthesis of carbon dioxide (CO_2) to lipid production as energy storage and thereby increases the lipid content to 20–50% dry cell weight of mostly triacylglycerol.[2,6-9] Environmental stress conditions that can lead to lipid production include:

- low nitrogen concentration[10,11];
- low temperature[12];
- high light intensity[13]; and
- high ion concentrations[14].

Microalgae have a higher growth rate when compared to land-based plants[19-21] and can be harvested every few days[22]. Microalgae require less land than other oil crops[21,23,24] and can be grown on marginal lands not required for food cultivation[25,26]. Microalgae feedstock has a high lipid production advantage with 15–300 times more oil than plant-based biomass[27,28] (Table 2). Microalgae can make use of nutrients, especially nitrogen and phosphorus, from different sources of waste, including concentrated animal feed operations, industrial and municipal wastewater, and agricultural run-off.[7,29] This offers cost-savings from the purchase of exogenous nutrients such as sodium nitrate and potassium phosphate[30], reduces the use of fresh water[30,31] and provides the additional bioremediation benefits of wastewater treatment[7,29,32]. The production of microalgae biomass offers real opportunities for solving issues of CO_2 sequestration[32], and at the same time, generates economic value through the conversion of CO_2 into energy and chemical products[33], utilising about 1.83 kg of CO_2 for the production of 1 kg of microalgae biomass[18].

Table 1: The oil content of some microalgae

Microalga	Oil content (% dry wt)	Source
Achnanthes sp.	42.8–46.2	Doan et al.[15]
Ankistrodes falcatus	21.78–59.6	Singh et al.[16]
Chorella sorokiniana	26–39.1	Guldhe et al.[17]
Crypthecodinium cohnii	20	Chisti et al.[18]
Cryptomomas sp.	28–29.2	Doan et al.[15]
Cylindrotheca sp.	16–37	Chisti et al.[18]
Dunaliella primolecta	23	Chisti et al.[18]
Isochrysis sp.	25–33	Chisti et al.[18]
Monallanthus salina N	20	Chisti et al.[18]
Nannochloris sp.	20–35	Chisti et al.[18]
Nannochloropsis sp.	37.6–46.5	Doan et al.[15]
Neochloris oleoabundans	35–54	Chisti et al.[18]
Nitzschia sp.	45–47	Chisti et al.[18]
Phaeodactylum tricornutum	20–30	Chisti et al.[18]
Schizochytrium sp.	50–77	Chisti et al.[18]
Tetraselmis sueica	15–23	Chisti et al.[18]

Table 2: Average productivities of some common oilseed crops compared to those of microalgae

Oil source	Yield (L/m²/year)	Reference
Algae	4.7 to 14	Sheehan et al.[34]
Palm oil	0.54	Mata et al.[26]
Jatropha	0.19	Sazdanoff[35]
Rapeseed	0.12	Sazdanoff[35]
Sunflowe	0.09	Sazdanoff[35]
Soya	0.04	Sazdanoff[35]

Source: Griffiths et al.[36]

Microalgae growth requirements

The use of wastewater to efficiently grow microalgae is dependent on different variables, including the concentration of essential nutrients (such as nitrogen, phosphorus, organic carbon), temperature, pH of the medium, availability of light, CO_2, and oxygen.[37]

Nutrients

Microalgae require nutrients for growth, particularly carbon (in the form of CO_2), nitrogen and phosphorus[18,38] (Table 3). To provide these nutrients, different recipes for algae culture media exist (Table 4). The concentrations of nitrogen and phosphorus in the algae growth medium are considered fundamental factors affecting algae growth kinetics directly and are closely related to lipid accumulation and nutrient removal.[6] The main mechanism for nutrient removal by microalgae is by uptake into microalgae cells[6,39], while the rate of nutrient removal is directly affected by the microalgae population growth rate[6].

The usual carbon source for microalgae photosynthetic culture is CO_2, supplied either continuously or intermittently, from industrial exhaust gases, atmospheric CO_2, or chemically fixed CO_2 in the form of soluble carbonates, e.g. $NaCO_3$ and $NaHCO_3$.[30,40] The efficiency at which microalgae cells use carbon through photosynthesis is directly proportional to the microalgal biomass production rate.[41] The pH change in microalgae cultures is predominantly from the consumption of CO_2, while changes due to degradation of metabolites excreted or from the uptake of other nutrients are minimal.[42] Increasing the concentration of CO_2 can result in higher production of biomass and a decrease in pH which can cause harm to the microalgae physiology.[43]

The next most important element required for the nutrition of microalgae is nitrogen.[44] Nitrogen is directly involved with primary metabolism as it constitutes protein and nucleic acids.[40,45] The nitrogen content of the biomass can vary from 1% to above 10% (even within the same species) and depends on the type and availability of the nitrogen source.[46] Microalgae cultivation utilises a higher amount of chemical fertilisers (N-fertiliser), about 8–16 tons N/H, than other oil-bearing terrestrial plants.[47] The use of nutrients from wastewater, especially agricultural sources rich in inorganic pollutants (nitrogen and phosphorus), can be one alternative to traditional chemical fertiliser sources.[47]

Ammonia is the preferred form of nitrogen for micro-organisms.[46] Nutrients released from aquaculture are most suitable for the cultivation of algae as nitrogen is released as NH_3 and phosphorus as PO_4^{-3}.[48] On the other hand, microalgae species with a fast growth rate prefer the primary source of nitrogen in the form of ammonia over nitrate[49], although they can grow well with different sources of nitrogen[46,47,50].

Assimilation of either NH_4^+ or NO_3^- is related to the pH of the growth medium. The pH of the growth medium could drop during active algal growth when ammonia is used as the only nitrogen source. This is due to the release of H^+ ions. On the other hand, pH increases when nitrate is used as the only nitrogen source in the growth medium. At high pH, nitrate could be lost due to volatilisation. However, it is important to ensure an adequate supply of this important nutrient to achieve a high growth rate. Culture media are formulated to supply nutrients in excess to avoid nutrients becoming a limiting factor, except in specifi applications.[46]

Another important nutrient for microalgae growth is phosphorus, even though it forms less than 1% by mass.[46] According to Kumar et al.[40], phosphorus is the third most important nutrient for microalgae growth and is required in significantly excess supply because not all compounds of phosphorus are bioavailable, especially those combined with metal ions[18,46,51]. Microalgae store excess phosphorus in phosphate bodies, which they can use when phosphorus becomes limiting. The ratio of N:P in the growth medium is important, both in determining the growth potential and maintaining the dominance of cultured species in the culture.[46]

In Mostert and Grobbelaar's[52] study, nitrogen was supplied at concentrations between 25 mg/L and 5000 mg/L for *Scenedesmus* sp., *Chlorella* sp. and *Monoraphidium*, with a suggested optimal nitrogen concentration for maximum productivity of between 2 mg/L and 619 mg/L and a variation on phosphorus of between 0.98 mg/L and 179 mg/L. Studies with *Chlorella vulgaris* at different ammonia concentrations obtained algae growth at all concentrations of algae. Low algae growth was obtained at very high ammonia concentrations (above 750 mg/L) and very low ammonia concentrations (below 10 mg/L) while maximum cell density was obtained at nitrogen concentrations between 20 mg/L and 250 mg/L, with no difference in specific growth rates. The growth rate in the different ammonia media studied was comparable to growth in commercial Bristol medium in which nitrate was the nitrogen source.[53]

Table 3: List of nutrients required by algal cells for growth

Elements	Compounds
Sodium	Several inorganic salts, NaCl, Na_2SO_4, Na_3, PO_4
Potassium	Several inorganic salts, KCl, K_3PO_4, K_2SO_4
Calcium	Several inorganic salts, $CaCO_3$, Ca^{2-} (as chloride)
Hydrogen	H_2O, organic molecules, H_2S
Oxygen	O_2, H_2O, organic molecules
Sulfur	Several inorganic salts, $MgSO_4.7H_2O$, amino acids
Magnesium	Several inorganic salts, CO_2^{3-}, SO_4^{2-}, or Cl^- salts
Chlorine	As Na^+, Ca^{2+}, K^+ or NH_4^+ salts
Iron	$Fe(NH_4)_2SO_4$, $FeCl_3$, ferric citrate
Zinc	SO_4^{2-} or Cl^- salts
Manganese	SO_4^{2-} or Cl^- salts
Bromine	As Na^+, Ca^{2+}, K^+ or NH_4^+ salts
Silicon	$Na_3SiO_3.9H_2O$
Boron	H_3BO_3
Molybdenum	Na^+ or NH_4^+ molybate salts
Vanadium	$Na_3VO_4.16H_2O$
Strontium	SO_4^{2-} or Cl^- salts
Aluminium	SO_4^{2-} or Cl^- salts
Rubidium	SO_4^{2-} or Cl^- salts
Lithium	SO_4^{2-} or Cl^- salts
Copper	SO_4^{2-} or Cl^- salts
Cobalt	Vitamin B_{12}, SO_4^{2-} or Cl^- salts
Iodine	As Na^+, Ca^{2+}, K^+ or NH_4^+ salts
Selenium	Na_2SeO_3

Adapted from Grobbelaar[46]

Table 4: Recipe of some selected growth medium for different algae

Substrate*	BG11 (g)	Modified Allen's (g)	Bold's Basal (g)
$NaNO_3$	1.5	1.5	0.25
$K_2HPO_4.3H_2O$	0.04	0.039	0.075
KH_2PO_4			0.175
$MgSO_4.7H_2O$	0.075	0.075	0.075
$CaCl_2.2H_2O$	0.036	0.025	0.084
$Ca(NO_3)_2.4H_2O$		0.02	
$Na_2SiO_3.9H_2O$		0.058	
Citric acid	0.006	0.006	
Fe-Ammonium citrate	0.006		
$FeCl_3$		0.002	
$FeSO_4.7H_2O$			0.00498
EDTA, 2Na-Mg salt	0.001	0.001	0.005
Na_2CO_3	0.02	0.02	
NaCl			0.025
KOH	0.031		
H_3BO_4 (μg/L)	2.86	2.86	11.42
$MnCl_2.4H_2O$ (μg/L)	1.81	1.81	1.44
$ZnSO_4.7H_2O$ (μg/L)	0.222	0.222	8.82
$Na_2MoO_4.2H_2O$ (μg/L)	0.391	0.391	
$CuSO_4.5H_2O$ (μg/L)	0.079	0.079	1.57
$Co(NO_3)_2.6H_2O$ (μg/L)	0.0494	0.0494	0.049
MoO_3 (μg/L)			0.71
Adjusted pH	7.4	7.8	

Adapted from Grobbelaar[46]

**All concentrations are in g/L and quantities are for 1 litre of culture solution.*

Under nitrogen-rich conditions, rapid cell division and chlorophyll accumulation occur. Under depleted nitrogen conditions, no cell division occurs, but there is high lipid biomass accumulation for several more days, together with a rapid drop in chlorophyll.[34] At 2.5 mg/L nitrogen limitation, *Scenedesmus* sp. LX1 accumulated up to 30% lipids and up to 53% at phosphorus limitation of 0.1 mg/L.[6] Other studies have cultivated microalgae in different nitrogen and phosphorus concentrations. One example is Aslan and Kapdan[54] with 13.2–410 mg/L ammonia, 7.7–199 mg/L phosphorus and 25–200 mg/L urea.

Light

Light is an important requirement in microalgae growth, and should be delivered optimally to all microalgae cells within the culture. The highest photosynthetic efficiencies are realised at low light, as high light intensities not only cause inefficient use of absorbed light energy but also cause biochemical damage to photosynthetic machinery (photo-inhibition), as well as a reduction in dry weight.[3] Generally, the light intensity requirement of microalgae cultivation is lower than the light intensity needed for higher plants.[40] Microalgae photosynthesis and productivity is equal to the efficiency of light conversion when the only limiting factor is light.[55,56] Generally, specific growth increases with an increase in irradiance to a maximum point beyond which inhibition may occur due to any further increase.[55]

Temperature

One of the major factors controlling cellular, physiological, and morphological responses of microalgae is temperature. Generally, an increase in temperature increases the rate of metabolism, while a decrease in temperature decreases the growth of algae. Environmental parameters such as light intensity affect optimal temperature, with 20–25 °C reported as optimal for some species, and highest cell density occurring at 23 °C.[40]

Fish farm wastewater

Globally, aquaculture has been one of the food production sectors with rapid development and production growth, significant investment, and technical innovation.[57] The main pollutants of concern in fish farm wastewater (FFW) are particulate and dissolved nutrients (nitrogen and phosphorus), and specific inorganic and organic compounds.[58] The volume of waste discharged from aquaculture depends on the feeding regime, stocking density, and feeding rate, as these three factors determine the quantity of feed used.[59]

Nitrogen

Transformations of nitrogen are key biochemical processes in aquaculture systems, with protein as the major form of nitrogen in the fish feed.[45] In every ton of fish produced, approximately 132.5 kg nitrogen and 25.0 kg phosphorus are released to the environment.[59] Fish feed consumed is converted partially into fish biomass, egested as faeces or excreted through the gills as un-ionised ammonia, a major product of protein metabolism.[45,60,61]

Most ammonia produced in fish occurs in the liver and is voided through the epithelial surface and renal routes. Production of ammonia also occurs in the kidney, intestine, and muscle due to the presence of the amino acid deamination enzyme in the tissues.[62] Ammonia in fresh water is from excretion via passive NH_3 diffusion across the branchial epithelium. Next to the gill, this NH_3 subsequently gets trapped as NH_4^+ in an acidic boundary layer, which maintains the partial pressure gradient of blood-to-gill water NH_3.[63] Urea is produced through argininolysis or hepatic uricolysis and is excreted through the gills, kidneys, skin or faeces.

Nitrogen loading in fish farms can be generally grouped into three sources[59,61]:

1. feed wasted due to poor management and farm practice;
2. poor feed quality, leading to poor feed stability and rapid dissolution of fish feed in wate ; and

3. low absorption and retention of food ingested that can be due to poor food digestibility of fish metabolism

Large amounts of nitrogen in FFW are dissolved, with only 7–30% occurring in the form of particulates.[64] Nitrogen in aquaculture is predominantly excreted as ammonia[65] and only about 20–40% of total nitrogen is excreted as urea[62]. Ammonia nitrogen build-up is the second most limiting factor to an increased level of production in intensive aquaculture after dissolved oxygen.[66] Even at very low concentrations, ammonia – especially un-ionised (NH_3) ammonia – is toxic to fish, with maximum concentrations below 0.0125 mg/L seen as acceptable.[67]

In flo -through aquaculture systems, most of the total nitrogen in the system is produced as ammonia while recirculating systems with biofilters produce mostly nitrates.[68] As the most reactive nitrogenous species, the pelagic microbial community quickly take ups ammonia and produces other nitrogenous species such as nitrate.[69] The rate of ammonia reaction in water is rapid, having a half-life of fewer than 50 ms for interconversion of NH_4^+ to NH_3.[70] However, temperature, pH, and salinity of the water affect the relative proportion of the two forms of ammonia.[70] In natural water, ammonia exists as a component of pH and temperature-dependent equilibrium. Aqueous ammonia, an ionised form of ammonium (NH_4^+), is favoured within equilibrium pH (6.5 to 8.0), while a high pH >9 favours un-ionised form of ammonia (NH_3).[71]

Phosphorus

Phosphorus is a limiting nutrient in a freshwater ecosystem[72,73] and is excreted through urine in fishe [74]. Excretion of phosphorus, usually 60–86% of dietary phosphorus, is related to the source of origin, which different species use in different ways.[65] Water quality can be influenced by phosphorus from aquaculture[75], as elevated levels of phosphorus cause premature eutrophication[73]. Soluble phosphorus is not produced when feed with low phosphorus levels is consumed.[76] The particulate total phosphorus and particulate total nitrogen fractions of effluent from a salmonid farm range from 30% to 84% and 7% to 32%, respectively.[64,77,78]

Phosphorus is usually not lost in an aquatic environment but remains conserved in a series of fractions as a result of dissolution, adsorption, and precipitation.[79] This changes the form of phosphorus availability from dissolved orthophosphates to phosphorus attached to the suspended load.[80] This makes phosphorus a useful indicator of the environmental impact of fish effluent [81] Modern agriculture relies on non-renewable phosphate from rocks for phosphorus supply, which is estimated to run out in 50–100 years with the estimated increase in phosphorus use.[82] This makes it essential to recycle phosphorus in wastewater sources, manure, and even within production processes of biofuels to eliminate direct competition for phosphorus between algae cultivation and conventional agriculture.[83]

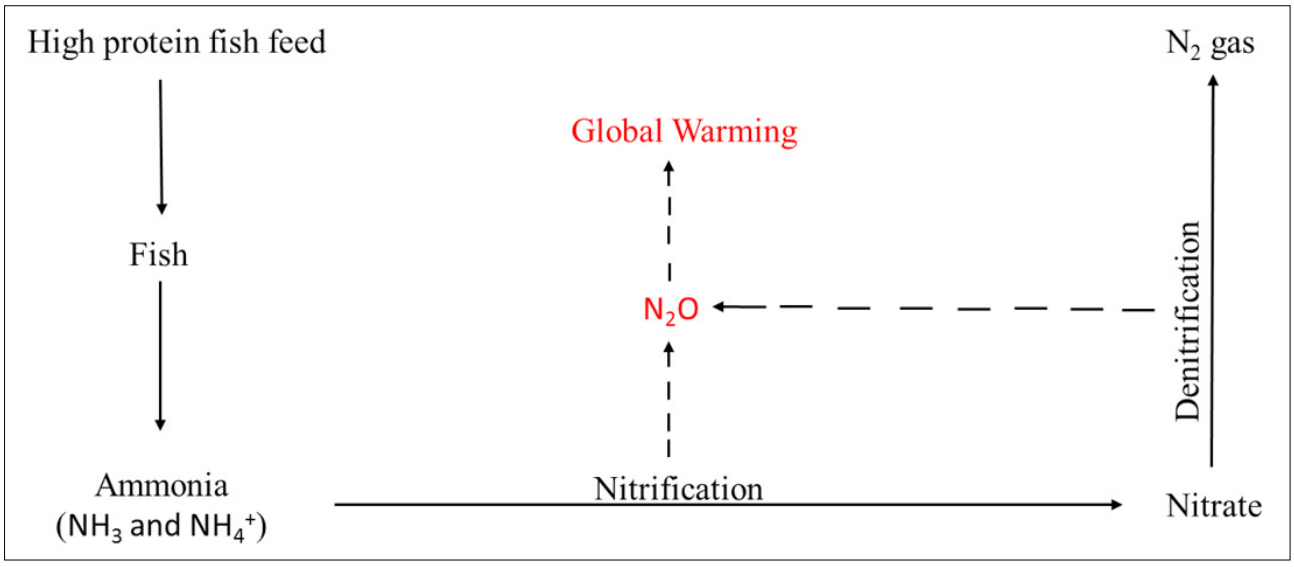
Impact of aquaculture discharge to the environment

Aquaculture's impact on the environment depends on feed type, stocking density, species, culture method, and farm practices.[84] The concentration or total amount of effluents released and the capacity of the environment to assimilate the particular constituent also affects the impact of aquaculture on the environment.[58] Nitrogen and phosphorus as major constituents of fish loading can affect the environment as a whole as well as the rearing of the fish [65] The introduction of organic and inorganic materials through feed for fishes has significantly impacted the nutrient and organic matter loading in coastal waters.

Rapidly growing intensive aquaculture systems would lead to various adverse effects on the environment. These effects might include:

- increased release of nutrients, which leads to eutrophication of coastal waters[85–88];
- shortage of drinking water resources as a result of release of toxic chemicals, including ammonia (NH_3) and nitrite (NO_2) from aquaculture, especially in intensive systems of fish cultur [89];
- reduction of wild-fish supplies which can affect the ecosystem through large input of wild-fish feed used in feeding carnivorous species, and also habitat modification for some aquaculture systems[90];
- competition for land and disturbance of wild ecosystems from escaped farmed fis [91];
- pollution from drug residues used in the prevention and treatment of diseases in aquaculture can lead to a change in biodiversity[92]; and
- environmental concerns from the use of chemicals (including antifoulants, vitamins) and the introduction of new genetic strains and pathogens. Cleaning of fouled cages can also add to the organic loading of the water.[84]

Microbial nitrificati n and denitrification are reactions common in aquaculture systems, which lead to the release of nitrous oxide (N_2O), a major greenhouse gas with 310 times more global warming potential than CO_2 over a lifespan of 100 years (Figure 1). Nitrous oxide destroys the ozone and has a lifespan of 114 years. It is estimated that aquaculture N_2O emissions will contribute roughly 5.72% anthropogenic N_2O-N emissions by 2030 if aquaculture maintains the current annual development rate of about 7.10%.[45]



Source: Reproduced with permission from Hu et al.[45]

Figure 1: Nitrous oxide (N_2O) emissions from aquaculture.

Benefits of utilising microalgae in FFW nutrient recovery

The use of algae for nutrient removal, especially nitrogen and phosphorus, has been demonstrated and has numerous advantages. These advantages include:

- low operating costs[30,54,89] by saving money for the purchase of exogenous nutrients such as potassium phosphorus and sodium nitrate;
- saving of freshwater resources[89];
- a suitable growth material with high tolerance[89];
- pollutant conversion and effluent conversion to clean wate [93];
- extra income when economic important species are used[87];
- increased productivity by eliminating pollutant nutrients[84]; and
- recycling nitrogen and phosphorus trapped in algae biomass as fertiliser avoids problems of sludge handling and oxygenated effluent discharge into the receiving water bod .[54]

The use of algae for nutrient removal is not environmentally dangerous as it follows the principles of the natural ecosystem and also does not lead to secondary pollutants as long as the biomass produced is reused.[94] Furthermore, the process is attractive for the treatment of secondary sludge as it has no carbon requirement for nitrogen and phosphorus removal.[54] Moreover, the use of wastewater from agricultural, industrial, and municipal activities can provide a sustainable and cost-effective means of cultivating algae for biofuels.[37] An alternative to synthetic fertiliser and eliminating the traditional use of synthetic fertilisers in the mass cultivation of algae is beneficial because of the rising prices of crude oil.[95] The use of residual nutrient and nutrient recycling can overcome the high cost of algae biomass production – a major drawback in algae biotechnology for biodiesel production.[96] Cultivation of microalgae also benefits the fish farmer by savings associated with the treatment of aquaculture wastewater before discharge, reducing demand for fresh water, and supplying algae biomass fish feed for the cultivation of fish [97]

Microalgae cultivation in FFW

Recently, studies using FFW have been carried out for different purposes.[97–106] Most of the studies[98–100,102,105] focused on the growth rate of algae in aquaculture wastewater, the rate of nutrient removal, the effect of aquaculture wastewater on algae composition, enhancing microalgae harvesting through bioflocculation by co-cultivation of microalgae with fungus and feed production. A few studies[97,101,103,104] determined the lipid content of the microalgae grown in FFW while fewer studies[97] went further to determine the fatty acid composition of the lipid accumulated. Enwereuzoh et al.[106] determined the quality of biodiesel from the FAME obtained from microalgae cultivated in FFW. However, most of the studies reviewed characterised the FFW used, determined biomass yield, and nutrient removal.

The characteristics of the FFW (Table 5) specific growth rate, biomass yield, biomass productivity, and lipid content (Table 6) are provided. All the studies utilising FFW for microalgae cultivation agree that FFW has sufficient nutrients to support microalgae cultivation. The concentration of nutrients in FFW were 0.48–433 mg/L for ammonia, 0.13–157 mg/L for nitrate, 0.14–28 mg/L for nitrite and 0.42–16.9 mg/L for phosphorus. These ranges are lower than concentrations obtained in standard growth media. For instance, the higher range of 157 mg/L obtained in FFW is only about 10% of the concentration of nitrate in both BG11 and Modified Allen's media and 62.8% in Bold's Basal standard media (Table 3). The lower biomass yield and productivity obtained in FFW when compared to the yield obtained in standard growth media have been attributed to the lower concentrations of nutrients in FFW.[100]

In this review, the highest biomass yield of 2.96 g/L and biomass productivity of 160.96 mg/L/d were obtained in *Ankistrodesmus falcatus* – cultivated in FFW with 5.32 mg/L ammonia, 40.67 mg/L nitrate and 8.82 mg/L phosphorus – are lower than the biomass yield and productivity obtained in the same species cultivated in standard growth media.[104] Biomass yield and productivity in the same study increased with increased supplementation of nutrients. These findings also confi m that nutrients in FFW support the growth of microalgae but are not sufficient for comparable biomass yield and productivity obtained with standard growth media. The high biomass productivity of *Ankistrodesmus falcatus* obtained in FFW cultivation may suggest that the species be included in future studies aimed at high biomass productivity with FFW. Most studies utilising FFW for cultivation have focused on *Scenedesmus* sp. and *Chlorella* sp.

Microalgae utilised nutrients in FFW for growth and accumulation of biochemical compounds and biomass production. The accumulation of more lipids by *Scenedesmus obliquus, Chlorella sorokiniana* and *Ankistrodesmus falcatus* cultivated in FFW[104] and in most species cultivated in FFW[97] when compared to the lipid content of the same species in standard growth media suggest that FFW is more desirable for cultivating microalgae for improved lipid content. The nutrient load of FFW reduced significantly after microalgae cultivation, indicating the suitability of the use of microalgae in the removal of nutrients in FFW. Nutrient removal efficiencies of up to 80% were recorded in studies in which nutrient removal was determined. In studies using *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Ankistrodesmus falcatus,* nutrient removal of 98.21% of ammonia, 80.85% of nitrate and 100% of phosphate was obtained.[104]

Additionally, FFW supported the accumulation of desirable fatty acid methyl esters in cultivated *Tetradesmus obliquus, Heterochlorella luteoviridis* and *Chlamydomonas reinhardtii*.[106] Better biodiesel properties were produced in *Chlamydomonas reinhardtii* cultivated in FFW than in standard growth media, and comparable biodiesel properties to those in standard growth media were produced in *Tetradesmus obliquus* and *Heterochlorella luteoviridis* in FFW. *Ankistrodesmus falcatus* had the highest biomass yield and productivity, but not the highest lipid content (25.2%), with *Chlorella sorokiniana* (31.85%) and *Scenedesmus obliquus* (30.85%) both accumulating more lipids. This makes *Chlorella sorokiniana* and *Scenedesmus obliquus* better producers of lipids, which is required for biodiesel production. Both species, when cultivated in FFW, have shown comparable biodiesel properties to the same species cultivated in standard growth media.

Conclusion

With an increasing world population and increased dependence on aquaculture for fish supplies, fish farm effluents are expected to grow. These effluents could provide nutrients for microalgae cultivation. Several studies have shown that the cultivation of microalgae in aquaculture wastewater is suitable for microalgae growth and biomass productivity coupled with efficient nutrient removal. The replacement of inorganic fertilisers with nutrient-rich fish farm effluent would eliminate the cost of purchasing fertiliser. This should lead to cheaper cultivation of microalgae biomass production for biodiesel production. When high costs – one of the major setbacks of algae biotechnology – are eliminated, the potential of microalgae biodiesel will be enhanced. Fish farm effluent nutrient recycling for microalgae cultivation for biodiesel production will at the same time eliminate numerous negative environmental effects associated with nutrient-rich effluent discharge to the environment, while also reducing the volume of water used.

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Competing interests

We have no competing interests to declare.

Authors' contributions

U.O.E.: Conceptualisation, methodology, data collection, data analysis, writing initial draft, writing revisions. K.G.H.: Conceptualisation, methodology, student supervision, project leadership. M.L.: Conceptualisation, methodology, student supervision, project leadership.

Table 5: Characteristics of aquaculture wastewater used for cultivating microalgae

Reference	NH_4 (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	P (mg/L)	pH	COD (mg/L)
Malibari et al.[97]	443	125.5	28.7	5.8	–	–
Gao et al.[98]	4.2	0.13	2	0.42	7.78	
Enwereuzoh et al.[106]	4.6	140	–	15	7.3	–
	2.3	70	–	7.5	7.3	–
Nasir et al.[99]	0.91	–	–	2.6	–	–
Egloff et al.[100]	–	67–157	–	–	–	–
Halfhide et al.[101]	–	2	–	16.9	6.94	238
Michels et al.[102]	0.48	40.7	0.14	4.96	7	115
Guerrero-Cabrera et al.[103]	24	–	–	10	7.5	–
Ansari et al.[104]	5.32	40.67	5.52	8.82	7.25	96
Guo et al.[105]	–	47.8	–	8.87	–	–
Range	**0.48–433**	**0.13–157**	**0.14–28**	**0.42–16.9**	**6.94–7.78**	**96–238**

Table 6: Species, initial algae cultivation concentration, specific growth rate, biomass yield, biomass productivity, and lipid content of some microalgae cultivated in aquaculture wastewater

Species	Initial concentration (g/L)	Specific growth rate (μ)	Biomass yield (g/L)	Biomass productivity (mg/L/d)	Lipid content (%)	Reference
Ankistrodesmus falcatus			2.96	160.79	25.2	Ansari et al.[104]
Chlorella sorokiniana			1.51	107.85	31.85	Ansari et al.[104]
Chlorella sp.		0.018	0.07	0.047	9.2	Guerrero-Cabrera et al.[103]
Chlorella sp.		0.18	0.058			Malibari et al.[97]
Chlorella vulgaris	0.025	0.17	0.044	7.3		Gao et al.[98]
C. vulgaris in membrane photo bioreactor	0.41			42.6		Gao et al.[98]
Monoraphidium sp.		0.013	0.162	19	10.4	Guerrero-Cabrera et al.[103]
Nannochloropsis sp.		0.16	0.073			Malibari et al.[97]
Scenedesmus obliquus	0.025	0.15	0.037	6.2		Gao et al.[98]
Scenedesmus obliquus			1.25	89.61	30.85	Ansari et al.[104]
Scenedesmus sp.		0.14	0.344	26	10.3	Guerrero-Cabrera et al.[103]

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What is the role of zooxanthellae during coral bleaching? Review of zooxanthellae and their response to environmental stress

Coral reefs are diverse and productive but sensitive ecosystems. Due to the impact of climate change, these organisms are in danger of dying out, mainly through the process of coral bleaching, which is the process by which zooxanthellae (algal endosymbionts) are expelled from their respective coral hosts, causing the coral to lose colour and become white. Coral bleaching has been linked to increases in sea surface temperatures as well as an increase in light intensity. We reviewed the different zooxanthellae taxa and their ecological traits, as well as the information available on the protective mechanisms present in zooxanthellae cells when they experience environmental stress conditions, such as temperature fluctuations, specifically concentrating on heat shock proteins and their response to antioxidant stress. The eight clades (A–H) previously recognised were reorganised into seven existing genera. Different zooxanthellae taxa exhibit different ecological traits such as their photosynthetic stress responses to light and temperature. Zooxanthellae have the ability to regulate the number and type of heat shock proteins (Hsps) they produce during a heat response. They can also regulate the host's respective Hsps. Antioxidant responses that can prevent coral hosts from expelling the zooxanthellae, can be found both within exposed coral tissue and the zooxanthellae cells. Despite the lower likelihood of bleaching in South African coral reefs, genetic engineering presents a useful tool to understand and adapt traits within zooxanthellae genotypes to help mitigate coral bleaching in the future.

Significance:

- Coral bleaching is the expulsion of zooxanthellae (algal symbionts) from the respective coral host, mainly due to elevated sea surface temperatures and light intensities, but numerous other factors, such as changes concerning salinity (ocean acidification), may also cause coral bleaching, although to a much lesser extent.
- A specific clade of zooxanthellae can be linked to their coral host's susceptibility to variation in oceanic temperatures, most probably by regulating both the host's respective heat shock proteins as well as their own.
- South African reefs have not experienced coral bleaching to the same degree as elsewhere in the world, mainly due to their unique reef topography and oceanic currents.
- Genetic bioengineering of zooxanthellae cells provides a plausible solution to save southern African coral reefs before it is too late.

Introduction

Coral reefs are one of the most diverse, productive and complex ecosystems on the planet, but due to global climate change, these phenomenal infrastructures are dying off, primarily through coral bleaching.[1] Coral bleaching is now five times more common than it was 40 years ago, and the global proportion of corals affected by bleaching per year has risen from 8% in the 1980s to 31% in 2016.[2] The western Indian Ocean has experienced bleaching events, which have been observed from 1983 up to 2016, with the most extreme event occurring during 1998. The bleaching events of 1998 and 2016 occurred as a result of abnormally high summer temperatures appearing concurrently with the El Niño Southern Oscillation. Numerous studies have been conducted on South African coral reefs since the 1998 El Niño, which had a devastating mass bleaching impact on other renowned coral reefs.[3] In 2005, a warm-water anomaly that affected much of the southern Indian Ocean resulted in moderately high coral bleaching on Madagascan, Mozambican and South African reefs.[4]

Coral bleaching can be described as the process by which zooxanthellae (algal symbionts) are expelled from the gastrodermal cavity (tissue) of the respective coral host. Thereafter coral whitening occurs, displaying the $CaCO_3$ skeletal structure of the coral which causes stress to the coral and it can then die. Most coral bleaching events are due to an increase in temperature above the normal stable temperature in which the holobiont survives. Scleractinian corals are reef-building corals that live in a mutualistic symbiotic relationship with single-celled zooxanthellae, referred to as dinoflagellates, belonging to the genus *Symbiodinium*.[5] The specific clade to which these resident *Symbiodinium* cells belong, can be linked to their host's susceptibility to variation in oceanic temperatures, thus variation in thermal tolerance is observed among individual colonies and host species.[6] In their study, LaJeneusse and co-workers[6] used molecular, morphological, physiological, and ecological information and proposed that these clades that were previously identified are equivalent to different genera in the family Symbiodiniaceae

Expulsion of zooxanthellae from the coral's internal tissues during coral bleaching is regulated by the rate of photo-inhibition and photo-damage to the zooxanthellae's thylakoid membranes' integrity and fluidit .[7] These regulating factors are also infl enced by the respective zooxanthellae clade/genus as well as the scleractinian coral host. Both

corals and zooxanthellae have many protective mechanisms against elevated temperatures and thermal stress, one of which is the induction of heat shock proteins (Hsps). According to Rosic and co-workers[8], numerous Hsps exist and are responsible for different stress response behaviours in the holobiont.

Coral bleaching is directly linked to elevated sea surface temperatures (SSTs) as well as an increase in light intensity. Downs et al.[9] suggested that elevated SSTs may result in an increased amount of reactive oxygen species (ROS) produced by the symbiont, which concurrently results in oxidative stress in both coral host and symbiont. During oxidative stress, ROS are produced in the endosymbiont's chloroplast, further producing hydrogen peroxide (H_2O_2). H_2O_2 can thereafter diffuse into the coral's cytoplasm and cause oxidative damage to the coral tissue. Damage is not only caused in the host's tissue but also within the thylakoid membranes of the endosymbiont.[10] Antioxidant response mechanisms are in place to prevent damage from increased levels of ROS such as antioxidant enzymes pathways including SOD (superoxide dismutase), ascorbate peroxidase and CAT (catalyse), which act together to inactivate superoxide radicals, neutralise hydrogen peroxide or inactive converted hydroxyl radicals (OH) and prevent coral hosts from expelling their vulnerable endosymbionts.[11]

Genetic editing has become a useful tool in understanding and manipulating variables and systems within the biological world. Several studies have suggested that environmental bioengineering can be an important consideration for future coral reef restoration strategies.[12,13] According to Levin et al.[12], zooxanthellae have the potential to be genetically edited, but genetic editing poses genomic challenges which should first be understood.

Our aim was firstly to review the different zooxanthellae genotypes and their traits concerning environmental stress. Secondly, we reviewed the information available on the protective mechanisms present in zooxanthellae cells when they experience temperature fluctuations, specifically concentrating on heat shock proteins and the antioxidant stress response within the coral and their zooxanthellae endosymbionts. Thirdly, we investigated the possibility of applying genetic modification to enhance the stress tolerance of zooxanthellae species.

Zooxanthellae genotypes

Within the taxonomic group (genus) *Symbiodinium,* different *Symbiodinium* genotypes (*Symbiodinium* cells with somewhat different genetic make-ups) exhibit different traits, such as their photosynthetic stress responses to light and temperature. Therefore, genetic factors that contribute to differences in stress tolerance between these *Symbiodinium* genotypes can also influence the coral's gene expression and its bleaching susceptibility.[12] It is therefore very important to understand these different genotypes better. Based on their genetic and physiological diversity, the *Symbiodinium* genotypes were firstly divided into eight clades (different groups of zooxanthellae that all evolved from a common ancestor) and classified from A to H, whereby only six of these clades are in a mutualistic relationship with corals.[14]

Recently, however, the organisms within these alphabetic clades were reviewed by LaJeunesse et al.[6] based on their genetic (phylogenetic), physiological, morphological, and ecological information. They further reorganised the zooxanthellae into seven existing genera within the family Symbiodiniaceae (a group of genera sharing a common attribute). This provided a more organised and systematic framework (Table 1). More genera may still be defined by other researchers. Therefore, for the rest of the review, the former clade designations will still be used.

Scleractinian corals (reef-building corals) increase their likelihood to resist thermal change by displacing their endosymbiont partners during temperature changes from a more thermal-sensitive zooxanthellae genotype to less thermal-sensitive zooxanthellae genotypes. This displacement of endosymbionts by the coral host is termed symbiont shuffling [15] Recent studies have shown that within most corals, clade C2 zooxanthellae (thermally sensitive) are displaced by clade D zooxanthellae (thermally tolerant) when subjected to elevated SSTs. The genus *Cladocopium* (corresponding to Clade C) as redefined by LaJeneusse et al.[6] is noted to be adapted to a wide range of temperatures and light intensities. According to Jones et al.[15], type C2 *Symbiodinium* is known to be thermally sensitive, and only actively photosynthesises at 27 °C; type C1, C13 and C15 are not able to function optimally above 33 °C. Therefore, even within a single clade, different types and strains exhibit different temperature tolerances.

To date, the zooxanthellae from clade D have been found to have exceptional thermal tolerance, thereby being a more thermally dominant clade.[15] Corals containing clade D zooxanthellae generally inhabit warmer tropical waters, and therefore require more protection against elevated SSTs. Furthermore, members of the newly proposed genus *Durusdinium* (corresponding to clade D), as defined by LaJeneusse et al.[6], are therefore also noted to be extremophiles, adapted to living in regions with large temperature variations. Clade D is often described as 'greedy' during a stress response, as these zooxanthellae retain a greater amount of their photosynthetically fixed carbon to mediate metabolic reactions within their cell structures against thermal fluctuations, thereby indirectly starving the host.[15]

Clade B zooxanthellae are normally found in cold-water corals and can tolerate a decrease in SSTs.[14] Clades A and F are not as commonly found within the coral species as are clades B, C and D, which might indicate that these types are more sensitive to thermal fluctuations or are specific to certain, not yet well-studied coral species.[14] The genus *Symbiodinium* (that corresponds to clade A) is most adapted to living in high light intensities or variable light conditions.[6]

Zooxanthellae and bleaching

According to both Downs et al.[9] and Carilli et al.[16], numerous protective and preventive mechanisms are in place within the zooxanthellae to decrease the effect of bleaching. These mechanisms include xanthophyll cycling, production of small heat shock proteins (sHsps), conformational change in lipid composition and the production of stress-stable enzyme complexes within the electron transport pathway (Figure 1).

Table 1: Recently redefined taxonomi designations of the well-known zooxanthellae clades into seven genera and their respective type species[6]

Former cladal designation	Redefined genus	Respective type species
Clade A (mostly free living)	*Symbiodinium* sensu stricto	*S. natans*
Clade B (mostly found in cold-water corals)	*Breviolum*	*B. minutum*
Clade C (mostly symbiotic and thermally sensitive)	*Cladocopium*	*C. goreaui*
Clade D (mostly symbiotic and thermally tolerant)	*Durusdinium*	*D. trenchii*
Clade E	*Effrenium*	*E. voratum*
Clade F (mostly free living)	*Fugacium*	*F. kawagutii*
Clade G	*Gerakladium*	*G. endoclionum*

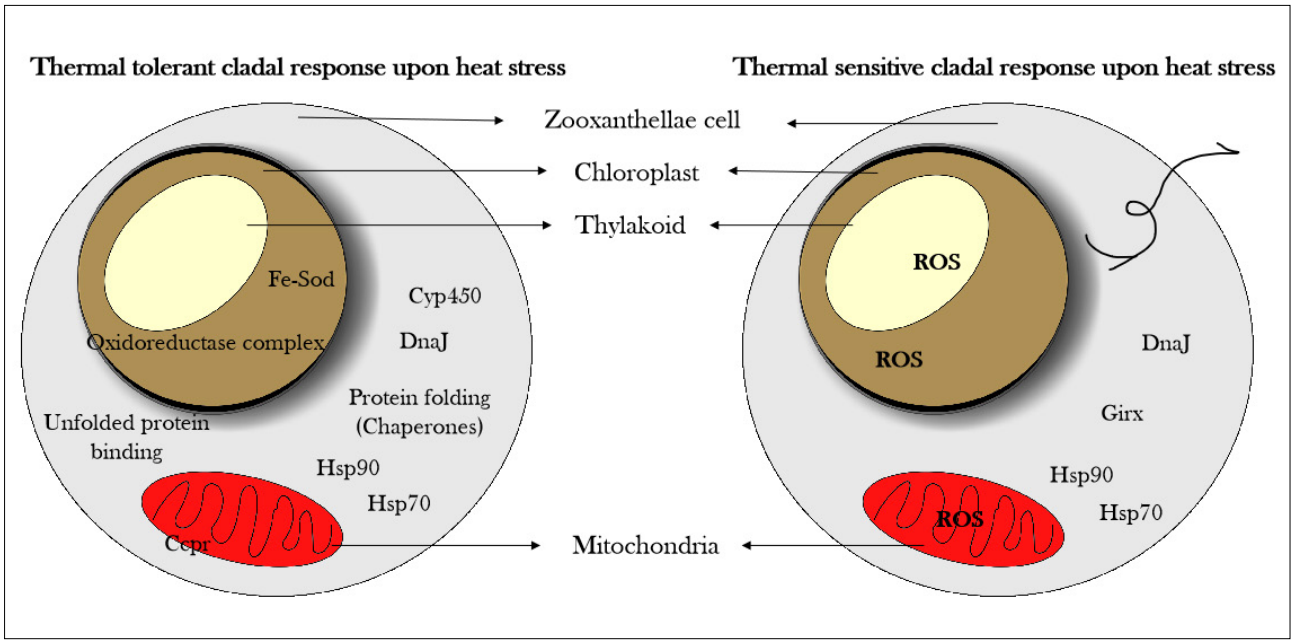

Figure 1: Protective mechanisms that are induced to actively counteract heat/thermal stress within both sensitive and tolerant zooxanthellae species according to Levin et al.[17]

Activated protective measures taken against induced thermal stress in thermal tolerant and thermal sensitive zooxanthellae endosymbionts, may be observed in Figure 1, as was discussed by Levin et al.[17] Within a thermal tolerant zooxanthellae clade, both Hsp70 and Hsp90, as well as protein folding and unfolding chaperones such as DnaJ, and antioxidant enzymes such as Fe-SOD, are expressed and activated within the zooxanthellae cell, each within its respective organelles (chloroplast and mitochondria). With this rapid response, the zooxanthellae cells mediate thermal stress and can ensure protection against oxidative stress within their cellular compartments. Within a thermal sensitive zooxanthellae clade, these mediating responses are not as effectively activated. Thus, some Hsps are expressed and activated, but ultimately the cell still produces ROS that can cause damage to the thylakoid membrane within the chloroplast, and subsequently damage within other compartments of the cell.

Numerous factors such as salinity, pollution, temperature, and pH, which are governed by CO_2 emissions, fisheries, and tourism, may have an impact on the distribution and biodiversity of zooxanthellae and their respective coral hosts. Unfortunately, these environmental factors may also contribute to coral degradation and ultimately coral bleaching events, and may result in both coral and zooxanthellae community structure and diversity changes. According to Chauka et al.[18], zooxanthellae diversity differs on account of many environmental aspects such as available light concentrations and light intensity tolerances, which may influence zooxanthellae distribution along environmental gradients due to photo-protection, acclimation, and their photosynthetic rates. In combination with symbiont shuffling, these changes are important cornerstones to understanding the adaptive capacity and nature of the entire coral holobiont and reef. Furthermore, Chauka et al.[18] further state that during their sampling, different symbiont types (clades) within colonies in the same localities were also observed and obtained, which suggests intracolonic variation amongst the zooxanthellae.

Photosynthetic dysfunction

The general consensus is that mass coral bleaching is due to the dysfunction of photosynthetic processes (such as a pronounced reduction in the activity of photosystem (PS) II and linear electron transport) in the algal endosymbiont as a result of the combined action of elevated temperature and light stress.

Takahashi et al.[7] and Smith et al.[19] described thermal bleaching as photo-inhibition of photosynthetic electron transport, such as the oxidation of plastoquinone by cytochrome complexes, reducing the excitation energy, and thereby suppressing the Calvin cycle and decreasing the rate at which photons are delivered to PSII within the zooxanthellae chloroplasts. Photodamage is due to the production of ROS in the thylakoid photosynthetic apparatus of the zooxanthellae, leading to oxidative stress within the holobiont (Figure 2).

Figure 2 demonstrates the process of oxidative coral bleaching and thermal stress response within both the zooxanthellae endosymbiont as well as its coral host. ROS are produced within the chloroplast of the zooxanthellae via several mechanisms associated with photosystem II and photosystem I catalysed electron transfer. During this reaction, hydrogen peroxide is generated within the zooxanthellae cell and accordingly diffuses from the zooxanthellae cell into the coral's cytoplasm. Once inside the coral cytosol, the hydrogen peroxide may either be neutralised by enzymatic and non-enzymatic antioxidant pathways or be converted into a more noxious ROS, referred to as the hydroxyl radical.

According to Gregoire et al.[20], chloroplast thylakoid membranes of *Symbiodinium* are exceptionally sensitive to thermal stress and can be irreversibly damaged. Bound to the thylakoid membrane are chlorophyll molecules which when damaged can result in the degradation of the entire photosynthetic apparatus and the loss of important photosynthetic products. Hill et al.[21] suggested that the PSII is the primary site of impact during coral bleaching and that the integrity and thermostability of the thylakoid membrane within the chloroplast of *Symbiodinium* is the major thermal-tolerant difference between species. The coral host may also influ nce the integrity and plasticity of the thylakoid membrane of *Symbiodinium* and therefore influence thermal acclimation and increase thermal tolerance of *Symbiodinium.*[20,22]

Thermal acclimation (a change in the lipid and protein composition) of the zooxanthellae thylakoid membranes during thermal fluctuations, has been demonstrated in species which inhabit warmer tropical regions and exhibit greater photosynthetic tolerance to elevated SST.[22] This is a strong indication that the zooxanthellae play a vital role in the survival of the coral host by exhibiting thermal tolerance independent of that of the coral host.[21]

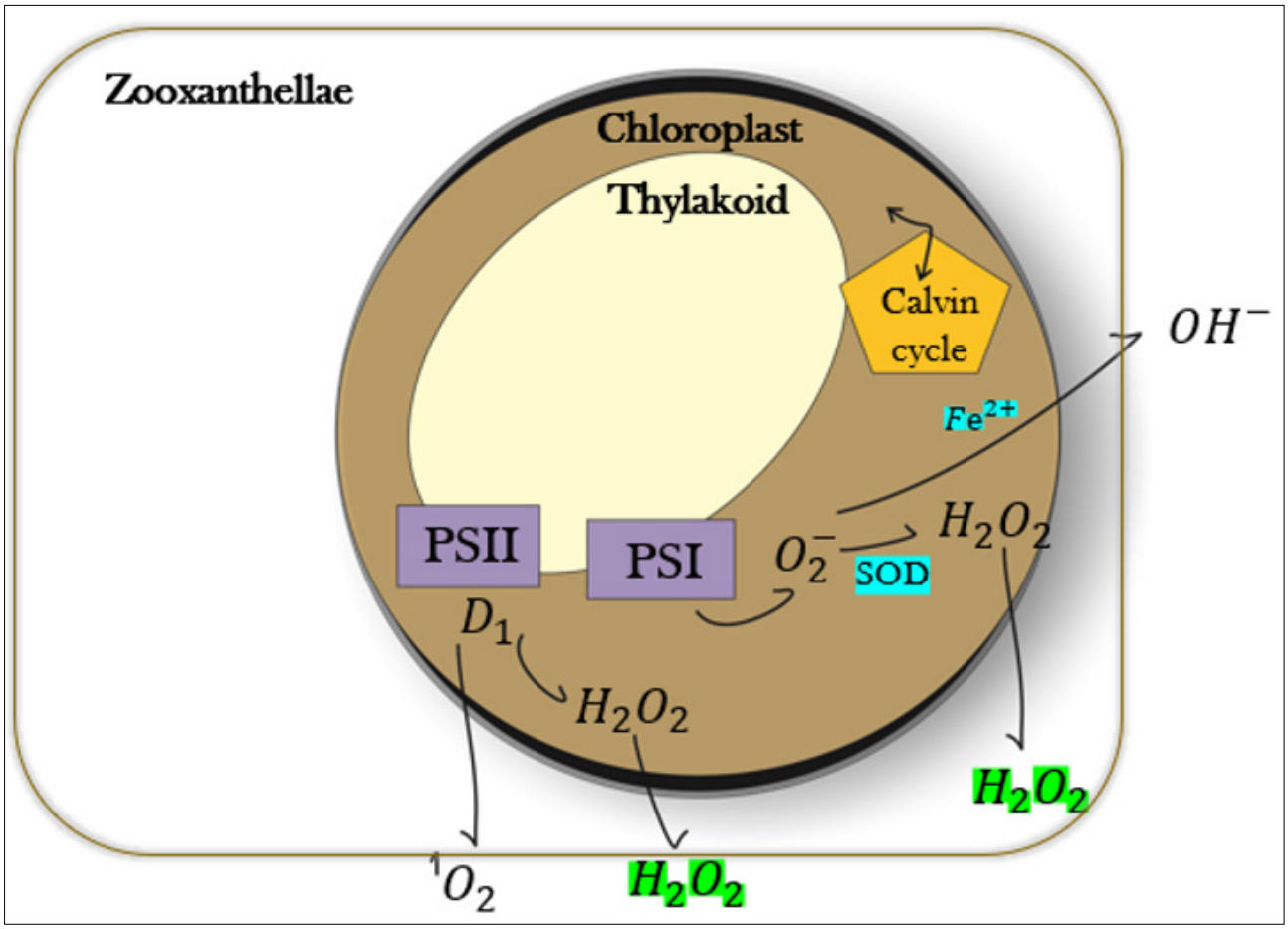

Figure 2: Schematic representation of the oxidative theory of coral bleaching within both the coral host and zooxanthellae endosymbiont, as described by Downs et al.[9] and Weis[25].

Function, activity, and effect of Hsps /chaperone proteins

According to Rosic and co-workers[8], Hsps are molecular chaperones that are responsible for protein folding and unfolding, aggregation, degradation and transport, thereby helping to regulate cellular reactions within a cell. Hsps are classified into families according to their molecular mass. Hsp70 and Hsp90 are two of the major cytosolic Hsps of *Symbiodinium* that contribute to the thermal stress response of the respective coral host. These Hsps are generally found in genotype C3 zooxanthellae.

Rosic et al.[8] conducted a study on these cytosolic Hsps and observed that Hsp70 can withstand temperature rises above 29 °C (leading to an increased *Hsp70* gene expression by up to 20%), but temperatures above 35 °C decreased *Hsp70* gene expression by 60%. In contrast, elevated temperatures caused a decrease in *Hsp90* gene expression. This may indicate that a reduction in the expression of Hsp90 inhibits a heat shock transcription factor (that regulates the expression of the heat shock proteins) and leads to the activation of heat-inducible genes and heat acclimation. This allows both the zooxanthellae and the coral to adapt to unforeseen temperature fluctuations. Symbiotic status did not control the expression of both Hsps genes; therefore, the initial thermal stress response is within the *Symbiodinium,* independent of the coral host.

Hsp90 operates as a dimer influenci g development and epigenetic changes. According to Rosic et al.[8], Hsp90 is represented in four forms: two cytosolic forms – an inducible alpha form as well as a constitutive beta form – and mitochondrial and endoplasmatic reticulum homologues. Induced Hsps are also present depending on the *Symbiodinium* genotype. As a result, Hsps can exhibit genetic variation among individuals of a species and therefore a difference in stress tolerance.

Feder and Hofmann[23] elaborated on the functions of different Hsps in zooxanthellae, and these are summarised in Table 2. Hsps mostly (1) interact with surrounding proteins and change their functions according to the cell's stress response, (2) recognise and bind to non-native proteins and (3) function as oligomers. Cytoplasmic Hsp70 and mitochondrial Hsp70 are responsible for maintaining peptides in an unfolded conformation, which allows these peptides to be transported through pores situated in the mitochondrial membrane. Hsp60 and Hsp10 then assist in the folding of unfolded imported proteins within the mitochondria.[23] Zooxanthellae are also able to modify and determine the amount of Hsps required for cell regulation and symbiosis.

According to Black et al.[24], the physiological and distributional differences between the clades can influence which Hsps the zooxanthellae contain and to what extent they will be expressed (thermal tolerance or sensitivity). This suggests that due to zooxanthellae's ability to determine the amount and type of Hsps present during a heat response, the zooxanthellae can regulate both the host's respective Hsps as well as their own.

Antioxidant response

The antioxidant and cellular stress capacities of both the zooxanthellae and the coral host also influence the rate of coral bleaching, especially on more threatened coral reefs exposed to oxidative stress due to environmental variability such as elevated SSTs, increased light intensity and mechanical damage. Oxidative stress can be defined as the imbalance in the pro-oxidant/antioxidant ratio favouring pro-oxidant and leading to oxidative damage/stress.[9] During mass coral bleaching, an increased amount of ROS is produced by the symbiont which concurrently results

in oxidative stress in both the coral host and the symbiont. ROS are produced in the symbiont's chloroplast, which is associated with electron transfers between PSI and PSII, producing hydrogen peroxide. Antioxidant response mechanisms are in place to counteract increased levels of ROS, but, unfortunately, algal-generated hydrogen peroxide can diffuse from the algal symbiont into the coral cytoplasm, which, once inside, can either be neutralised by enzymatic and non-enzymatic antioxidant pathways or be converted into a more noxious ROS.[9,25] This results in oxidative damage within the coral host and the coral host responds to the damage by expelling its symbiotic algal companion.[9,11]

Table 2: Heat shock proteins and their functions necessary for cellular regulation during thermal stress of the coral host (adjusted from Hill et al.[21])

Heat shock protein	Function
Hsp10	Protein folding in co-expression with Hsp60
Hsp27	Resistance to hydrogen peroxide, resistance to UV radiation, tolerance to hyperthermia, accelerated nuclear protein aggregation
Crystallin	Cellular tolerance to hyperthermia
Hsp60	Tolerance to hyperthermia
Hsp70	**Cellular:** tolerance to hyperthermia, recovery after translational and transcriptional inhibition after heat shock, regulation of heat-shock response, reduced protein denaturation upon elevated heat exposure, resistance to H_2O_2, tolerance of UV radiation, apoptosis, resistance to apoptosis **Tissue/organ:** reduction of hyperthermic damage **Organismal:** tolerance to hyperthermia, regulation of heat-shock response
Hsp72	Apoptosis, protection against heat-induced nuclear protein aggregation and thermal radio-sensitisation
Hsp90	Tolerance to hyperthermia, apoptosis
Hsp101	Tolerance to hyperthermia
Many Hsps	Recovery of cell proliferation and chromosome damage after heat shock, tolerance to hyperthermia
HSF (Heat shock factor)	Thermo-tolerance

Downs et al.[9] further states that antioxidant pathways such as the Asada–Halliwell pathway and xanthophyll cycling can be used to protect the coral host from oxidative damage by ROS. An individual can respond differently to each oxidative threat, depending primarily on higher or lower levels of antioxidant components within the individual species. Furthermore, specific Hsps can also protect both the coral host and its symbiont from oxidative stress through protecting glycolytic and electron-transport enzymes during photosynthesis and respiration. According to Downs et al.[9], heat stress can further increase the possibility of hydrogen peroxide mediated oxidative stress by deactivating numerous hydrogen peroxide neutralising pathways.

According to Higuchi et al.[10], antioxidant enzymes such as SOD, ascorbate peroxidase and CAT are responsible for detoxifying ROS. As a result, SOD, ascorbate peroxidase and CAT act together to inactivate superoxide radicals and hydrogen peroxide and prevent coral hosts from bleaching.[11] SOD is responsible for the dismutation of superoxide into oxygen and hydrogen peroxide, and CAT for the inactivation of hydrogen peroxide into water and oxygen.[10] It is important to mention that an increase in hydrogen peroxide not only induces antioxidant activity within the exposed coral tissue, but these antioxidant activities also occur within the zooxanthellae tissue in response to an increase in ROS.[11]

Genetic modification of *Symbiodinium* to enhance stress tolerance

Several studies have suggested that environmental bioengineering can be an important consideration for future coral reef restoration strategies.[12,13] The up-regulation of gene expression, which may mitigate thermal stress induction of any of the physiological aspects discussed earlier, can ensure stable coral–zooxanthellae symbiosis in the future. It presents a viable alternative strategy to preserve reefs amidst climate change.

However, even though zooxanthellae present promising candidates for genetic engineering, these dinoflagellates possess unusual biological features that have made gene editing within its genome difficult [12] They contain one of the largest nuclear genomes (1.5–112 Gbp) known, which exceeds that of the human haploid genome size. It is permanently condensed in liquid-crystalline chromosomes. Furthermore, trans-splicing of polycistronic mRNAs occurs and plastid genomes are divided up into mini-circles. So far, the successful transformation of *Symbiodinium* has been validated in only two cases.[26,27] In their study, Levin and co-workers[12] propose a tailored genetic engineering framework for zooxanthellae that might overcome these obstacles and prevent stress-induced reactions that lead to bleaching, such as by using the CRISPR/Cas9 system.

Is bleaching a threat to South African coral reefs?

According to Celliers and Schleyer[3], the South African coral reefs are located in a subtropical region and are the most southern coral reefs in the western Indian Ocean, and lack any topographical features, such as gullies and pinnacles, mainly due to less wave action and wave conformation within the increased depth. Sodwana Bay as well as all other South African reefs, form part of the high-latitude, sandstone reef structure and can be referred to as marginal reefs which are often falsely regarded as small and insignificant to the ecosystem when compared to massive reefs such as those on the Australian coast.[28] However, South African reefs associated with the continental margins have survived severe coral bleaching events in the past millennium that went unnoticed.

South African and African reefs have been surprisingly less impacted from coral bleaching events than other tropical reefs such as the Great Barrier Reef, off the Australian coast, for two obvious reasons. Firstly, bleaching levels are highest at the shallowest sites, due to increased light penetration and solar irradiance as well as localised heating and ultraviolet radiation. As the minimum depth of South African coral reefs is about 8 metres, bleaching conditions are less severe than for coral reefs closer to the surface. As a result, a negative correlation is observed between depth and bleaching percentage.[29]

Secondly, seasonal upwelling also contributes to a lessening in South African coral bleaching. The upwelling developing from the Benguela Current on the west coast of South Africa is responsible for the uplifting of cooler waters during the El Niño event in 1998 and reduced coral bleaching.[30] As a result, seasonal upwelling protects the coral reefs from severe bleaching by breaking the intense heat stratification within the water column and reducing the heat irradiance upon the coral reefs.[31]

Africa's southernmost coral reef assemblages occur in the South African Maputaland reef complexes. The region is generally predicted to become wetter and warmer, and the water more acidic as a result of climate change.[32] Temperature has been increasing regionally during the last century and a half. Because bleaching events have already become more frequent on the reefs of southern Africa, it is necessary to investigate how environmental stress leads to bleaching on these reefs.[33]

Within temperature anomalies and bleaching events such as the El Nino in 1998, the SST (<10 m) increased by 3–5°C, resulting in mortality of between 50% and 90% of coral in the central and western Indian Ocean.[34] Luckily, the occurrence of seasonal upwelling along South Africa's continental shelf and bank moderated these increases in SST, preventing the subsequent coral bleaching within the surrounding area.[28,29] Notably, depth has also played a considerable role in protecting South Africa's

most iconic and unique reefs from devastation.[29] However, these factors may make South African reefs susceptible to bleaching due to small changes in SST that might go unnoticed by the rest of the world.

Even with the advantage of depth, wave energy and seasonal upwelling, community concern should be raised, and early response and preventative measures should already be in place for possible future bleaching anomalies. Coral communities that have shown increased mortality generally grow within shallow areas with restricted water circulation and may after prolonged exposure or continuous bleaching events become more tolerant to thermal stress and increase acclimation towards increased SST in comparison with coral communities situated at increased depths.[29] As a result, with recurring and continuous climate change, bleaching events, and subsequent heating of sea temperature downward into the water column, deeper distributed coral reefs may be at risk of high mortality, further resulting in ecosystem dysfunction.[4] Upwelling events have also indicated lowered thermal thresholds amongst coral hosts and zooxanthellae, due to increased susceptibility of coral species to bleaching events which may occur within the future, uncontrolled and moderated by these upwellings.[33]

Coral mortality further creates opportunities for coral competitors such as algae and zoanthids to occupy the space made available by coral death and degradation. Fish communities shift from equally distributed and abundant herbivorous and corallivorous species to an increasing abundance of herbivorous fish communities which has further repercussions lower down and higher up in the trophic level.[35] Finally, beaches which are regulated by reefs that reduce water action will be increasingly eroded. All these effects which occur from the loss of functioning reef ecosystems have an enormous impact on the community's socio-economic status and recoveries, such as tourism, local and provincial fisheries, and prevention of storms.[36] Numerous other recovery and monitoring strategies that could be implemented to decrease effects before and after bleaching events have been reported and elaborated on.[36,37]

Even with monitoring and recovery strategies available, little attention has been paid to assess future bleaching occurrences, the role coral symbiosis with the harboured zooxanthellae plays during coral bleaching, and how these endosymbionts may play a role in preventing future bleaching events. Another pressing concern is the limited amount of research on how environmental stress induces bleaching on South Africa's high-latitude reefs and their potential resistance, adaptations, acclimations, and community diversity and change, from the perspectives of both coral species and zooxanthellae.

The world's carbon footprint is increasing annually, and it can be viewed by recent research monitoring temperature fluctuations and increases on the 2 Mile reef in Sodwana Bay during the years 2007, 2008, 2011, 2012 and 2013. Resistance against coral bleaching of coral communities decreased, therefore resulting in coral bleaching across the entire slope.[38] Increased percentage bleaching of 37.4 %, 17.4%, 23.8%, 33.6% and 38.8%, respectively, per these annual readings indicate decreased thermal thresholds and possible acclimation.[38] As a result, even if South Africa has deeper coral reefs, less wave destruction and less production of carbon products, future bleaching anomalies might be of too much risk to ignore in the present.

Sebastian et al.[33] investigated the bleaching response of corals as well as their *Symbiodinium* community in southern Mozambique and observed that the most dominant scleractinian coral species were *Stylophora pistillata*, *Montipora*, *Acropora*, *Pocillopora* spp., *Porites*, *Favites* and *Favia*. During their study, *Stylophora pistillata* and *Montipora* were the most susceptible to elevated temperatures. The *Symbiodinium* harboured by the scleractinian corals belonged mostly to clade C, of which two atypical C subclades were present in *Stylophora pistillata* and *Pocillopora*, which were more abundant in shallower sites. Clade D symbionts were also present but in lower amounts. *Pocillopora* harboured the most clade D symbionts in comparison to the other coral species. Neither *Symbiodinium* clade A nor B were detected in any of the abovementioned coral colonies. Because clade C is the more thermally sensitive symbiont, South African corals are therefore vulnerable to elevated SSTs, should they occur in the future.

Future research perspectives

Future research perspectives include extending research on the use of CRISPR/Cas9-based genome editing of zooxanthellae, fuelling the genetic modificatio within the zooxanthellae's complex genomes.[39] Gene drives can further be established to create reproducible inheritance systems of edited genetic material, thereby enhancing passage of a selected genotype to the offspring, both through sexual and asexual reproduction pathways, to spread the desired altered gene throughout the coral population.[39] Such studies and genomic procedures may also be considered to enhance the genomic stress responses of the coral itself. Physiological intervention may also be included in coral bleaching research needs, such as experimental research on pre-exposure to increase acclimation rate, adaptation opportunities, epigenetic modifications, and symbiont shuffling to enhance the stress tolerance of the coral host.[35] Genetic bioengineering and reproductive interventions, therefore, provide a plausible solution to increase genetic diversity within populations, increase acclimation and adaptation rates to a changing environment and permit the selection of traits that may improve coral resilience, persistence, and restoration strategies within future climate change.[35]

Conclusion

Coral reefs are vital to marine ecosystems and nutrient cycles but are unfortunately being increasingly damaged, mainly as a result of global warming. Elevated SST has led to coral bleaching, which is the disablement of the coral–algae symbiosis primarily due to photosynthetic dysfunction. There are, however, numerous protective measures to ensure that the coral tissue and the photosynthetic apparatus of the zooxanthellae are preserved and kept in a stable physiological condition during elevated temperatures. One of these measures is through Hsps, which play a vital role in the refolding of heat-stressed unfolded proteins, protecting stress-damaged proteins, transporting of transcribed proteins and inserting those proteins into organelles within both the zooxanthellae and the coral host. Antioxidant enzymes released by both symbionts and coral hosts also prevent coral bleaching through mitigating oxidative stress. Heterogeneous zooxanthellae genotypes can influence bleaching susceptibility towards thermal resilience upon temperature fluctuations due to their physiological and genetic diversity. As a result, coral bleaching is dependent on the Hsps expressed and the heterogeneous zooxanthellae genotypes present within the holobiont. South African reefs may have the ability to act as a refugee area for damaged coral reefs, but should still be actively monitored and researched to better predict future coral beaching occurrences and the effects they may have on the entire marine ecosystem. Genetic bioengineering within zooxanthellae endosymbionts has been suggested to be the turning point in coral bleaching, but numerous strategies are still to be explored in which this could be done successfully.

Competing interests

We have no competing interests to declare.

Authors' contributions

A.C. is a postgraduate student and was responsible for the literature review, writing the initial draft, and writing revisions. S.B. is the study leader and was responsible for the conceptualisation, funding acquisition and project management; and contributed to the writing of the manuscript.

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Mollusc collections at South African institutions: Development and current status

There are three major mollusc collections in South Africa and seven smaller, thematic collections. The KwaZulu-Natal Museum holds one of the largest collections in the southern hemisphere. Its strengths are marine molluscs of southern Africa and the southwestern Indian Ocean, and terrestrial molluscs of South Africa. Research on marine molluscs has led to revisionary papers across a wide range of gastropod families. The Iziko South African Museum contains the most comprehensive collections of Cephalopoda (octopus, squid and relatives) and Polyplacophora (chitons) for southern Africa. The East London Museum is a provincial museum of the Eastern Cape. Recent research focuses on terrestrial molluscs and the collection is growing to address the gap in knowledge of this element of biodiversity. Mollusc collections in South Africa date to about 1900 and are an invaluable resource of morphological and genetic diversity, with associated spatial and temporal data. The South African National Biodiversity Institute is encouraging discovery and documentation to address gaps in knowledge, particularly of invertebrates. Museums are supported with grants for surveys, systematic studies and data mobilisation. The Department of Science and Innovation is investing in collections as irreplaceable research infrastructure through the Natural Science Collections Facility, whereby 16 institutions, including those holding mollusc collections, are assisted to achieve common targets and coordinated outputs.

Significance:

- Mollusc collections are among the oldest natural science collections in South Africa, dating from just before 1900. They provide an invaluable resource of morphological and genetic diversity, with associated spatial and temporal data. They are spread across the country in three comprehensive and seven smaller, thematic collections and this paper puts together available information about these scattered and diverse collections. Each has its own strengths and specialisations, and together they cater to a variety of the country's identified research priorities. Although staff complements are small, mollusc collections are well curated and conserved, expanding, actively researched and associated data are available online or on request.

Introduction

Mollusca is the second largest animal phylum with approximately 85 000 described species worldwide and just under 4000 in South Africa (approx. 75% marine, 20% terrestrial and 5% fresh water). Their range of size and body form is unparalleled – from minute species visible only microscopically to large forms of several hundred kilograms, such as the giant clam and colossal squid. Their importance to humans is as wide-ranging as their physical diversity: marine species are important as food, utensils, adornment and even currency; freshwater snails serve as intermediate hosts of platyhelminth parasites of significance to human and livestock health; land snails are both friend and foe, but are also pertinent to land-use planning due to their narrow-range endemism.[1]

Mollusc collections are among the oldest natural science collections in South Africa and date to just before 1900; they are an invaluable resource of morphological and genetic diversity, with associated spatial and temporal data. They contribute material to address a variety of the country's identified research priorities. The taxonomy of many molluscan taxa is in need of revision, and a host of new species await description. An estimated 20–25% of the fauna remains to be described, based on numbers of described species[1,2], recently described taxa[3] (see also Appendix 1) and current research. In addition, as marine material in our collections continues to be studied, Indo-Pacific species not yet recorded from South Africa are added to the species list. Opportunities and challenges facing mollusc collections reflect issues pertaining to all natural science collections and need to be seen in the context of systematics and other elements of biodiversity science in South Africa. With a growing emphasis on cultural heritage, only two museums have staff dedicated to mollusc collections, research capacity to unlock their wealth of information is limited and the total number of staff responsible for the country's mollusc collections is 19 (Table 1).

There are three major and several smaller mollusc collections at museums and universities spread across the country (Table 1). (No attempt was made to include any private collections.) The three major collections cover all taxa and regions, and have different strengths and specialisations, while the smaller collections focus on particular themes (Table 2). The KwaZulu-Natal Museum is the African centre of malacological reference and expertise. Here I collate and discuss available information about all the collections, from their inception to the present.

Material and methods

Formal questionnaires were not sent to institutions, but lists of questions were emailed to Curators and Collections Managers (Table 1) and dialogue ensued.

Table 1: South African mollusc collections: locations, administering authorities and size ranges (catalogued lots)

Institution's name, city, province and abbreviation	State authority administering the institution	Size range	Department	Number of staff who are responsible for any aspect of the mollusc collection	Information about collection supplied by
KwaZulu-Natal Museum, Pietermaritzburg, KwaZulu-Natal (KZNM)	National Department of Sports, Arts and Culture	150 000	Malacology	3 (dedicated), 1 shared	Igor Muratov (Curator), Linda Davis (Collections Manager, retired), John Midgley (Assistant Director: Natural Sciences)
South African Museum, Cape Town, Western Cape (SAM)	National Department of Sports, Arts and Culture	30 000	Marine Biology	4 and 1 3-year contract post	Wayne Florence (Head of Marine Biology), Albe Bosman (Collections Manager)
East London Museum, East London, Eastern Cape (ELM)	Provincial Department of Sport, Recreation, Arts and Culture	23 000	Malacology	1 (dedicated)	
Durban Natural Science Museum, Durban, KwaZulu-Natal (DNSM)	eThekwini Municipality, Libraries and Heritage Department	5000	'Orphan'	0	David Allan (Curator of Ornithology)
Albany Museum, Makhanda/ Grahamstown, Eastern Cape (AMG)	Provincial Department of Sport, Recreation, Arts and Culture	3500	Freshwater Invertebrates	2	Helen James, Musa Mlambo (both Curators of Freshwater Invertebrates)
South African Institute for Aquatic Biodiversity, Makhanda, Eastern Cape (SAIAB)	National Department of Science and Innovation: National Research Foundation	1500	Aquatic Biodiversity	2 and 1 part-time volunteer	Marek Lipinski (voluntary Curator), Roger Bills (Senior Curator), Willem Coetzer (Biodiversity Information Manager), Nkosinathi Mazungula (Collections Manager)
Port Elizabeth Museum, Port Elizabeth, Eastern Cape (PEM)	Provincial Department of Sport, Recreation, Arts and Culture	1400	Marine Biology	2	Malcolm Smale (retired Curator of Marine Biology), Greg Hofmeyr (Curator Marine Mammals)
McGregor Museum, Kimberley, Northern Cape (MMK)	Provincial Department of Sport, Recreation, Arts and Culture	1250	Zoology	1	Beryl Wilson, Head of Zoology Department
Wits Life Sciences Museum, Johannesburg, Gauteng (WLSM)	University of the Witwatersrand	905	Zoology	2	James Harrison (Curator)
Bartolomeu Dias Museum, Mossel Bay, Western Cape (BDSM)	Provincial Department of Sport, Recreation, Arts and Culture	2000	Malacology	1	Amanda Human (Malacologist)

Table 2: Taxon and regional specialisations of South African mollusc collections

Institution	Strength and/or specialisation
KwaZulu-Natal Museum	Marine molluscs of southern Africa and southwest Indian Ocean
	Terrestrial molluscs of South Africa
Iziko South African Museum	Early dredgings of South African continental shelf and slope
	Surveys of entire South African coast (intertidal to deep)
	Cephalopoda (largest collection in southern hemisphere)
East London Museum	Molluscs of Eastern Cape Province
	Terrestrial molluscs of South Africa
Durban Natural Science Museum	Marine molluscs of eastern South Africa (KwaZulu-Natal and Eastern Cape)
	Historical record of Durban area
Albany Museum	Freshwater molluscs of southern Africa
South African Institute for Aquatic Biodiversity	Cephalopoda (south and west coast of South Africa)
Port Elizabeth Museum	Cephalopoda beaks (mainly Indian, Atlantic and Southern Oceans)
McGregor Museum	Terrestrial molluscs of South Africa (marine collection not examined)
Wits Life Sciences Museum	Marine molluscs of Port Alfred and Jeffreys Bay, Eastern Cape

Results

Tables 3 and 4 contain a summary of the numbers of catalogued lots, specimens, types, species per habitat and geographic coverage of each collection, while Supplementary tables 1 and 2 contain statistics about taxon coverage and preparations. Where information was not supplied or the required detail could not be extracted, the collection has not been included in the particular table or the field(s) has been le t blank.

KwaZulu-Natal Museum

The KwaZulu-Natal Museum (KZNM) is a national museum and holds the largest mollusc collection in Africa. This collection is one of the largest in the southern hemisphere, and in terms of its southern African holdings, is the largest in the world.[3] There are approximately 150 000 catalogued lots, 2400 primary types, including 521 holotypes.[4]

History – Establishment

Amateur collector Henry Clifden Burnup (1852–1928) served as honorary curator from about 1897. This date is regarded as the beginning of the scientific study of molluscs at the then Natal Museum, and in South Africa as a whole.[3] He built up the collection and much of it was identified by foremost authorities of the day. Burnup was the first resident to publish on South African molluscs[5], and produced five additional papers on terrestrial pulmonates.

Growth of the collection

The first professionally trained, salaried malacologist, A.C. van Bruggen, was appointed in 1962. He undertook collecting trips to distant areas of the country to augment the terrestrial mollusc collection and, after his departure to Leiden in 1966, continued to publish on South African snails. Richard Neil Kilburn (1942–2013) was appointed in 1969. Under his stewardship, the marine collection continued to grow steadily with the acquisition of several collections of regional importance including that of Rodney Wood (Mutare Museum) by exchange, and those of Clarice Connolly (largely South African), Kurt Grosch (northern Mozambique) and Eva Roscoe (Mozambique) by purchase.[6] Rationalisation of natural history collections in South Africa and institutional specialisation led to the acquisition of the historically important collections of the then Transvaal Museum (Ditsong) and the Albany Museum in 1978 and 1980, respectively, and the creation of the second malacology research post, filled by David Guy Herbe t in 1984.

Table 3: Numbers of catalogued lots, specimens, species and types in mollusc collections

Collection	Catalogued lots	Catalogued specimens	Approximate number of species	Primary types	Total types
KZNM	150 000	500 000†	13 500‡	2399	3753
SAM	28 487	64 915	6800	583	940
ELM	22 815	127 987	4800	1	208
DNSM	5147	§	2300	0	0
AMGS	3555	not available	220	0	0
SAIAB	1077	1077	140	3	21
PEM	1363		>81	0	0
BDSM	2000	2000		0	0
MMK	1250			At least 2	
WLSM	905	15 640	905	0	0

†Estimate based on 3-4 specimens per lot

‡10 000 marine, 3500 non-marine

§Only 1529 lots indicate the number of specimens

Table 4: Habitat and geographic coverage of molluscs in collections

Collection	Number of lots	Number of lots per habitat					Proportion South African (%)
		Marine	Non-marine	Terrestrial	Freshwater	Estuarine	
KZNM	±150 000	113 206	29 497	23 171			58%
SAM	30 889	not available	not available				72%
ELM	22 815	15 580		5915	456	103	70%
DNSM	5147	4844		135	51		75%
AMG	3555	0	3555		3551	4	86%
SAIAB	1077	1077	0				At least 65%
PEM	1363	1363	0				Mainly South African
MMK	±1250	825	411				
WLSM	905	±905					Mainly South African
BDSM	2000						

It was mainly through fieldwork, both shore and ship based, that the marine collection was built up to its present status by Kilburn and Herbert. The highly successful Natal Museum Dredging Programme was initiated in 1981 with the aim of sampling the little-known faunas of the continental shelf and upper slope.[3] Two of the most poorly investigated areas were targeted: the Transkei, which had never been dredged, followed by Zululand, and later the West Coast. For many known taxa, fresh material with bodies for anatomical investigation were preserved for the first time together with accurate depth, substratum and locality data. At least 27 malacologists in 12 countries worked on material from the Natal Museum Dredging Programme initially and described 164 new species and 18 supra-specific taxa.[3] Revisionary papers on a range of families have been ongoing since then, published in the *Annals of the Natal Museum*, renamed *African Invertebrates*.

Research on terrestrial molluscs was renewed by Herbert in the mid-1990s and became a primary focus of research. A field guide to the land snails and slugs of eastern South Africa[7] was published in 2004, the museum's centenary year.

Type holdings

The revision of types is ongoing[4,8,9] and these publications include good-quality colour photographs. Over 200 types were photographed on request in the last 10 years, obviating the need to loan material.

Current status of collection and staffing

The intention for the two malacology posts was to have one marine and one terrestrial malacologist. Igor Muratov, appointed in 2009, works on continental molluscan faunas of sub-Saharan Africa. Elodie Heyns-Veale, appointed in 2019, has begun working on dredged samples. The post of Collections Manager of Mollusca is currently vacant; Linda Davis was appointed in 1991 and retired in 2020.

Cataloguing, digitisation and imaging

Specimens entering the collection are written in a catalogue register and given a catalogue number, then entered onto Specify and integrated into the collection. Tissue samples are linked to the catalogued specimens from which they were taken.

Radula slides are given their own number and are entered in the catalogue register and on the label of the specimen from which the radula was removed. Egg masses are kept with the specimen.

The collection is fully databased and has recently been migrated to Specify, but is not yet available online. Scientists or members of the public are granted access to information in the database and/or photographs of specimens by individual request. Photographs in the numerous publications by current and previous malacologists are linked to specimens in the collection by the catalogue number. However, specimens in the collection database are not linked to photographs in publications.

South African Museum

History – Establishment

The Iziko South African Museum (SAM) was founded in 1825. In 1897, the Museum moved to its present building in the historic Company's Garden. The collection became established in 1896, although South African molluscs from the Cape of Good Hope had made their way overseas for over 300 years.[8] The SAM became the repository for large samples of all invertebrate taxa from early South African dredgings of the continental shelf and slope, beginning with the Government Fishery vessel, SS *Pieter Faure*, in 1897. Other sources of large quantities of molluscan and other marine invertebrate material were annual intertidal and shallow sub-tidal sampling of the entire coastline by the University of Cape Town Ecological Surveys, from the 1940s to about 1985, and Sea Fisheries Research Institute surveys which still take place under the banner of the Department of Environment, Forestry and Fisheries. The marine biology collections are focused on South Africa, extending into Angola, Mozambique and the Southern Ocean. The mollusc collection represents about one quarter of the total marine biology collection.

Growth of the collection

Among those individuals who made a significant contribution to the marine biology collections and research was Keppel Barnard (1887–1964), who was the first to deposit types in a South African institution. He was appointed in 1911 and retired in 1946 as Director (Florence W 2018, unpublished report). The first paper on South African marine molluscs by a resident was published in 1913 by Barnard[10]. A total of 593 mollusc collection records are attributed to him; he published 36 papers on molluscs and described 150 (valid) species.[11] Towards the end of his life, he mentored Brian Kensley (1944–2004) who also made an important contribution to the mollusc collections (over 550 lots) and to research. Other noteworthy contributors include Turton's collection of shells from Port Alfred during the 1920s[12], and Bill Liltved's contributions of over 900 lots.

The SAM contains the most comprehensive collection of southern African Polyplacophora and the wet collection of Cephalopoda is the largest in the southern hemisphere. Both assemblages date to about 1900. There are 17 specimens of giant squid (*Architeuthis dux*) – one of the largest collections in the world. The cephalopod collection is particularly important for Sepiidae and Ommastrephidae due to the work of Martina A. Roeleveld-Compagno (1943–2006). The southern African cephalopod fauna constitutes 20–30% of the world's species, so resolution of the many known systematic problems and undescribed taxa would contribute substantially to resolution at a global level.[2] Moreover, the sub-region includes at least 34 species of actual or potential commercial interest.[2] It is unfortunate that Roeleveld was not superseded, although Lipinski is working on Cephalopoda at the South African Institute for Aquatic Biodiversity (see below).

Nudibranchs are well represented and were one of Barnard's initial interests (see references in Gosliner[13]). Terence Gosliner added over 350 lots to the collection and continues to describe new species from South Africa.

The primary figure in terrestrial molluscan expertise was Matthew Connolly (1872–1947), a British soldier, sent to South Africa in 1900, and Henry Burnup became his mentor. In 1909, he presented a collection of South African land shells to the SAM and began publishing on the museum's non-marine mollusc collection. After World War I he became an honorary scientific worker in the then British Museum (Natural History). He soon became the foremost authority on southern African land and freshwater shells and published some 50 papers between 1910 and 1945. The majority of his holotypes are in the Natural History Museum. His most important publication was 'A monographic survey of South African non-marine Mollusca'[14] which remains the most complete reference work on the subject[4] and is still the only reference on several families. His publications on the non-marine Mollusca of other countries in southern Africa (Mozambique[15] and Namibia[16]) remain the only reference works on terrestrial molluscs for those countries. Very few terrestrial molluscs have been added to the collection since Connolly's time; exceptions are paratypes of Western Cape endemics described by Sirgel[17,18].

Type holdings

The mollusc collection contains 936 types and 583 primary types.

Current status of collection and staffing

Towards the end of the 1990s, the SAM became just one of 11 institutions under the Iziko Museums of South Africa, an agency of the Department of Sports, Arts and Culture, and curatorship posts began to be frozen (Florence W 2018, unpublished report). The Curator of Marine Invertebrates is Wayne Florence, a bryozoan specialist. After a hiatus in collections management staff for the marine invertebrate collections, the staff complement has increased recently (Table 1). For the first time in over a decade, research is being conducted on Mollusca (Polyplacophora), by means of a 3-year postdoctoral contract. Another positive development is a huge building project to expand and upgrade storage, and the mollusc collections are currently being transferred to the new facility after being in storage since 2011. Mollusc material continues

to be deposited at the SAM following large national programmes such as demersal trawl surveys of the Department of Environment, Forestry and Fisheries, South African Environmental Observation Network sampling, and the SeaKeys programme (see below). Material is identified to family and catalogued. The SAM has always been the repository of such material, but has not consistently had a curator of molluscs throughout its history. It may be strategic to consider depositing mollusc material at the KZNM where expertise exists.

Cataloguing, digitisation and imaging

Material entering the museum is given an accession number and immediately entered onto Specify and then catalogued (only digitally).

A total of 290 types have been imaged (about 1000 images to show characteristic features). Other images also exist for specimens in the collection, e.g. 162 photographs of live nudibranch species. Images are not linked to specimen records in Specify.

East London Museum

History – Establishment and growth

The East London Museum (ELM) is a province-aided museum under the Department of Sport, Recreation, Arts and Culture. The collection was started with shells from Marjorie Courtenay-Latimer's family collections in the 1930s, and for 30 years she was the curator. The museum began to specialise in marine molluscs of the Eastern Cape in the early 1960s when the first curator, Denis Kennelly, was appointed part-time.[19] He was followed in 1968 by Dick Kilburn who resigned when the post at the KZNM became vacant the following year. Kilburn did much to improve the scientific value of the collection, including starting the wet collection. During the next 20 years, the marine collection continued to grow under successive curators Maureen Latigan (local beached shells), Eva Roscoe (local and Mozambique species) and Sandra Muller (dredged and dived specimens).

Current status of collection and staffing

I was appointed in 1988 and am the first person to have served for more than just a few years. There are no other curatorial or technical staff in malacology or shared with other departments. There was an assistant for 10 years, Victor Mejane, who began his career at the ELM as a tourism student.

Recent research focuses on terrestrial molluscs – a previously neglected element of the biodiversity of the province. Collections-based research on terrestrial molluscs at the KZNM and ELM has produced several revisions and descriptions of 59 new species from South Africa (Appendix 1).

Type holdings

The ELM has a small type collection. Holotypes of species described by me are lodged at the KZNM with paratypes at ELM.

Cataloguing, digitisation and imaging

Specimens are catalogued in a written register and then entered onto Specify. Types are catalogued in a Type Register.

All catalogued lots have been digitised on Specify and this database was supplied to the South African National Biodiversity Institute (SANBI) so that the latter could make it publicly available together with other biodiversity data in South Africa (see below).

Photographs of specimens in publications or other associated photographs, e.g. habitats, are not linked to the specimens on Specify.

Durban Natural Science Museum

The shell collection of the Durban Natural Science Museum (DNSM) includes fine specimens donated by illustrious collectors. The DNSM collection contains the earliest date (1822) of a mollusc specimen in a South African collection. The first formal curator of the DNSM, J.F. Quekett, specialised in shells and he acquired many of the original specimens.[20] The collection is focused on the southeast coast of South Africa – a region where extensive habitat destruction has taken place. Some 25% of the specimens are from the eThekwini (Durban) area including Durban Bay, and provide a valuable historical record of the fauna of this highly modified region where most of the natural habitat has been destroyed.

Albany Museum

Freshwater molluscs form a small component of the National Collection of Freshwater Organisms housed at the Albany Museum. The oldest record was collected in 1905. Between 1950 and 1970, the National Institute for Water Research of the Council for Scientific and Industrial Research undertook surveys of many South African rivers. This large collection was identified by local and overseas scientists and added to the Albany Museum. The collection is growing through an active programme of research as well as donations and voucher specimens from river surveys. Specimens are catalogued in written registers and then entered onto Specify. Most (86%) of the collection is South African. There are approximately 500 records from other African countries.

A freshwater mollusc collection stemming from academic and student research projects is housed at the Unit for Environmental Sciences and Management at North-West University. Despite repeated emails to three people, no replies were received, so no details can be reported on. It would therefore seem unlikely that any member of the scientific community or public would be able to access the collection or its data.

South African Institute for Aquatic Biodiversity

The South African Institute for Aquatic Biodiversity (SAIAB), where the National Fish Collection is held, received a donation in 2012 of an estimated 10 000 cephalopod specimens (Bills R 2019, written communication, September 20) from Sea Fisheries Research Institute demersal surveys on the RS *Africana* and RS *Dr Fridtjof Nansen* along the south and west coasts of South Africa. This collection potentially has as many types as the collection at SAM (Lipinski M 2019, written communication, September 19), but the majority of the collection is still uncatalogued. The voluntary curator, Marek Lipinski, assembled the collection while working for the Sea Fisheries Research Institute, and now visits SAIAB specifically to identify specimens. Data are entered onto Specify by SAIAB staff. No students are being trained in cephalopod taxonomy.

Port Elizabeth Museum

The Port Elizabeth Museum (PEM), a provincial museum under the Department of Sport, Recreation, Arts and Culture, holds a cephalopod beak collection assembled between 1975 and 2015 from stomachs of predators including cetaceans, seals, and cartilaginous and teleost fishes. The main focus of the collection was to support prey identification of apex predators – a major theme of PEM research since the 1970s. The collection has been cited in over 32 publications by M.J. Smale and co-workers. A fish otolith collection was built up concurrently. The squid beak collection is mainly South African with occasional material from nearby regions. Beaks from >81 species are represented, which is c. 42% of the known fauna of southern African. The collection also has a large number of vouchers that are not identified to species level, but are available for research.

The staff complement at the PEM is dwindling, and the number of natural science staff has shrunk from nine to three in recent years due to vacant posts not being filled. The retired curator of the squid beak collection is willing to assist as a Curator Emeritus and is actively publishing on the collection.

McGregor Museum, Kimberley

There is a small collection of marine and non-marine molluscs with separate written registers which appear to go back about 100 years. The collection has been dormant for many decades and was known only from the registers until about 10 years ago when it was rediscovered in locked cabinets (Wilson BA 2020, written communication, September 30). It contains type material[14] and is a potentially valuable source of historic specimens and data.

Wits Life Sciences Museum

The Life Sciences Museum of the University of the Witwatersrand contains a collection of marine molluscs primarily from one source. The Edwin Knowles Jordan collection contains over 15 000 specimens of shells, representing 905 species, collected mainly at Port Alfred (about two thirds of the specimens) and Jeffreys Bay (about one third) over several decades around 1900. The collection also contains a few species from the former Transkei and a handful from Durban and from the Western Cape. Twenty-two species are labelled as rare.

Bartolomeu Dias Museum, Mossel Bay

This museum has a malacology department and collection originating from several small collections donated over the years and a limited amount of active collecting. The museum is well known for its extensive display of shells and aquariums of living specimens. The malacologist spends most of her time on management of the Shell Museum & Aquarium and research is not undertaken on the collection. There is a written register and a start has been made on an electronic register including photographs, but the majority of the specimens do not have provenance and are suitable for education only (Human A 2020, written communication, September 30).

Mollusc collections elsewhere in Africa

Collecting and study of natural history in Africa were historically conducted by overseas institutions. South Africa appears to be the only African country where some mollusc collections have dedicated staff and local collections are actively researched by local scientists who may deposit paratypes or other material in overseas institutions. The websites of the majority of museums outside South Africa do not supply any information about the museum's collections or staff. Information was gathered from Collections Managers (Table 5). No information about the possible existence of mollusc collections at museums in other southern African countries could be obtained, except for Mozambique which does have a collection of mainly marine specimens at the National Museum of Maputo (Table 5). There is a written register dating to the mid-1900s, but it does not have catalogue numbers, and an electronic database with added numbering and photographs is in progress (Vetina A 2020, written communication, October 5). A collection was started in Antananarivo, Madagascar, following terrestrial surveys at several localities across the country from the mid-1990s, spearheaded by Kenneth Emberton and Owen Griffiths [21]

The Global Taxonomic Initiative Africa Regional Workshop was held in South Africa in 2001 and represented 23 African countries.[22] All national representatives indicated that major biological collections were kept in their countries, although only a few were reported to be electronically databased.[23] Staffing was inadequate and the number of taxonomists practising locally was insufficient to address biodiversity issues.[23] Building capacity in order to change this was identified as a priorit .[22]

Several projects had some successes in skills development and staffing posts for several years (Seddon M 2019, written communication, October 23), but few have been sustainable for mollusc collections. One exception is the Darwin Initiative (http://www.darwininitiative.org.uk) which supported research by Christine Ngereza at the National Museum of Tanzania and she now has a full-time post. Her PhD was supported by the German Research Foundation.[24] A malacology post was supported at the National Museum of Kenya, but there has been no dedicated curator of molluscs for 8 years and funds for the post have been redirected. A small proportion of the collection (59 records of 24 species of freshwater gastropods and bivalves) was digitised through a European Union Global Biodiversity Information Facility (GBIF) funding grant (https://doi.org/10.15468/xt7aah). The land snail team working out of the Naturalis Biodiversity Centre, Netherlands, also incorporated training into their programmes in West Africa; De Winter has published extensively but has not deposited any specimens in West Africa because there has not been a collection into which to deposit them (De Winter A 2019, written communication, September 19).

Table 5: Mollusc collections in African countries outside South Africa

Country and city	Institution's name	Department / collection	Information about collection supplied by	Size (Mollusca)	Taxon coverage	Cataloguing	Condition and growth
Kenya, Nairobi	National Museum of Kenya	Invertebrates	Laban Njoroge, Collections Manager of Invertebrates	115 000 specimens	All families, African	No register, data on specimen labels	Good, approximately 10 specimens per annum
Tanzania, Dar es Salaam	National Museum of Tanzania	Invertebrates (mainly Mollusca)	Christine Ngereza, Curator of Invertebrates	25 670 lots	All families	Written register	Good, growing
Mozambique, Maputo	National Museum of Mozambique	Invertebrates	Alvaro Vetina, Curator of Invertebrates	"Small"	All families, Mozambique	Written register and electronic database	Good, growing
Morocco, Marrakech	L'Institut Scientifique de Rabat (ISR)	National Museum of Natural History	Dirk van Damme, University of Ghent		All families		
Morocco, Marrakech	Faculté de Sciences Semlalia	Hydrobiological Laboratory	Dirk van Damme		Stygobiont Hydrobiidae		
Morocco, Tetouan	Abdelmalek Essaadi University	Saoud Collection	Dirk van Damme		All families		
Egypt, Cairo	Egyptian Environmental Affairs Agency	National Biodiversity Unit	Dirk van Damme		Freshwater Mollusca		
Madagascar, Antananarivo	Botanical and Zoological Gardens, Tsimbazaza	Museum	Hajanirina Ramino, Curator	153 lots, 117 species	Terrestrial Mollusca	Written and typed	

Surveys of snails of many forests in Nigeria have been published, but the papers do not state where the collections have been deposited. Specimens from Omo Forest[25] were photographed at KZNM and deposited there (Muratov IV 2019, written communication, September 27).

In relation to its low diversity (366 species of freshwater gastropods[26] and 117 species of bivalves[27]), the freshwater mollusc fauna of the Afrotropical region has received disproportionate research attention, mainly by European and American scientists, but the majority of material is deposited in collections outside Africa. Some material has been deposited in collections in north Africa following local studies (Table 5), the most important being the collection at L'Institut Scientifique de Rabat, which includes historic material collected by Pallary, Pérès and Bédé (van Damme D 2020, written communication, March 4). There are also small collections at El Kala, Algeria and the University of Lubumbashi, Democratic Republic of the Congo (van Damme D 2020, written communication, March 4).

Discussion

Mollusc collections within the context of natural science collections in South Africa

Towards the end of the 1990s there was widespread concern that systematics was in decline in terms of capacity and resources.[28-30] Needs, priorities and actions for zoological, plant and marine systematics were formalised[31-33] and audits of herbaria and zoological collections were undertaken[34,35]. The South African Society of Systematic Biology was formed[36] and contributed to the development of the South African Biosystematics Initiative, funded by the then national Department of Science and Technology. KZNM malacologist Dai Herbert was at the forefront of this initiative. There was a call for a coordinated national body to provide focus and leadership for fundamental biodiversity research.[30]

The *National Environmental Management: Biodiversity Act, No. 10 of 2004* led to the establishment and functions of SANBI (http://www.info.gov.za/acts/2004/a10-04). The mandate of SANBI includes responsibilities to coordinate and promote the taxonomy of South Africa's biodiversity and facilitate access to biodiversity data (https://www.sanbi.org/biodiversity/). An assessment of the state and needs of biological collections and expertise was commissioned in 2008 by the National Research Foundation. While there were pockets of excellence, the collections were under-resourced and not used to their full potential, with many at risk of deterioration or even loss, and a relatively small proportion of the data from collections was accessible.[37,38]

Strategy documents for animal and plant taxonomy were released to address the taxonomic impediment to the sound management of biodiversity.[39-41] The Foundational Biodiversity Information Programme (FBIP) was initiated in 2013 to support integrated projects that generate and disseminate foundational biodiversity information. Over 144 small projects have been supported to date, including the migration of the malacology database of the ELM to Specify. Six large projects have been supported: five for terrestrial biodiversity and one for marine biodiversity. The latter, SeaKeys, has led to the publication of over 113 000 records on GBIF (containing fish and invertebrates) including several historic data sets dating to 1884 (Pauw L 2021, written communication, July 13). Further outcomes of SeaKeys pertaining to molluscs are a field guide to offshore marine invertebrates[42] and two Citizen Science projects, a Sea Slug Atlas and a Sea Shell Atlas. One of the two large terrestrial FBIP projects on forest fragmentation in the Eastern Cape included surveys of molluscs, incorporation in the ELM collection (282 catalogued lots) and 368 barcodes of 47 species. To date, 24 publications have emanated from this FBIP project, including records in the revision of an endemic mollusc genus.[43] Data from this and all the other projects will be published on the FBIP website and GBIF as soon as the data sets have been verified (auw L 2020, written communication, January 27).

Recent policies and programmes demonstrate that taxonomy has been re-energised and fi mly incorporated into the mainstream of science policy. There have been responses to the calls of 20 years ago for increased infrastructural support and funding for collections and systematics, including the training of more taxonomists and systematists. However, a concern expressed over a decade ago[23], and which remains a shortfall in capacity-building, is the emphasis placed on molecular phylogenetic analysis and the neglect of morphological taxonomy.

The Department of Science and Innovation is investing in collections through the Natural Science Collections Facility, one of thirteen South African Research Infrastructure Roadmap projects[44], in recognition that the natural science collections of the country are irreplaceable research infrastructure, spread across museums and other institutions (Hamer M 2016, unpublished report). The overall aim of the Natural Science Collections Facility is to ensure that collections and associated data are used for high-quality research and decision-making to address issues of national and global relevance. Participating institutions are assisted to achieve common targets and coordinated outputs including excellence in care of collections, data mobilisation, and collections-based research. All institutions (except Bartolomeu Dias Museum) holding mollusc collections are participants, and are therefore supported by the Natural Science Collections Facility.

The ecological and economic importance of specimen collections can only be fully assessed and harnessed if the data are accessible in meaningful and comparable ways and data mining is greatly enhanced by unifie collection portals such as GBIF[45] and iDigBio[46]. Currently, SANBI publishes data on behalf of several South African institutions to GBIF. Through the Natural Science Collections Facility and SANBI's National Biodiversity Information System, progress is being made towards upgrading and expanding collection databases and making these openly accessible in an integrated way, using Darwin Core[47] as the data standard (Table 6).

Table 6: Digitisation and data accessibility of mollusc collections (lots)

Collection	Catalogued lots	Digitised lots	% digitised lots georeferenced	% on GBIF	Data searchable online or by request
KZNM	150 000	143 887	100		Request
SAM	30 889	30 889	31	30	Request
ELM	22 815	22 815	100	90	Online
DNSM	5147	5147	0		Request
AMG	3555	3555	93	100	Online
SAIAB	1077	1077	69	100	Online
PEM	1363	1363	Not supplied		Request
MMK	1250	0	0	0	
WLSM	905	0	0	0	

Some museums are in the process of setting up their own Integrated Publishing Toolkit and will be publishing their records directly to GBIF, and are setting up online access to their records via their own websites. The Natural Science Collections Facility is developing an online Virtual Museum, with images of type specimens, specimen data and archival documents.

A major stumbling block to long-term security and effective use of collections is the fragmentation of governance, and the inappropriate placement of the natural science collections under national and provincial departments of sports, arts and culture (Table 1) which have no mandate for the curation of biological collections.[38,39] This could overturn the progress made over the past two decades to save and improve conditions for collections and taxonomic research. Because staff complements are small (Table 1), and replacement of staff who retire or resign is erratic, the loss of even a single staff member could leave a collection neglected and unused. Consolidation of collections at larger institutions has been proposed[38] and, where there is willingness, suggestions to send 'orphan' collections to institutions with a curator for that taxon. This may be strategic for a few of the smaller, but scientifically very important, mollusc collections.

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Competing interests

I have no competing interests to declare.

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Appendix 1: Recently described molluscan taxa

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Phytosanitary risk associated with illegal importation of pest-infested commodities to the South African agricultural sector

We evaluated the phytosanitary risk associated with illegal importation of pest-infested plant commodities into South Africa. Samples were collected from different South African ports of entry over 8 years (2011 to 2019) and data were analysed descriptively using Statistical Software Package. Pests were frequently detected on commodity species such as *Citrus* (18.31%), *Zea mays* (13.22%), *Phaseolus vulgaris* (12.88%), *Musa* spp. (9.15%) and *Fragaria ananassa* (5.08%). The highest number of pests intercepted occurred on fresh fruits (44.06%), followed by grains (26.44%) and vegetables (14.23%). The most intercepted organisms were *Callosobruchus rhodesianus* (7.79%), *Dysmicoccus brevipes* (7.11%), *Callosobruchus maculates* (6.10%) and *Phyllosticta citricarpa* (4.74%). The majority of intercepted organisms were non-quarantine organisms (70.50%), followed by pests of unknown status (17.28%), quarantine pests (10.84%) and potential quarantine pests (1.35%). *Phyllosticta citricarpa*, *Bactrocera dorsalis*, *Spodoptera frugiperda* and *Prostephanus truncatus* were the only quarantine pests intercepted in terms of South African regulatory status. The interception was mainly from southern African countries, particularly Mozambique, Zimbabwe and Eswatini. The findings present the level of phytosanitary risk associated with illegal importation and/or non-compliance in regard to plants and plant commodities from different countries through South African ports of entry. Crop production, biodiversity, food security, existing export markets, and access to new export markets could be threatened as importing countries may impose stringent phytosanitary measures to limit the chances of introduction and establishment of quarantine pests into their territories.

Significance

- Illegal importation of plant commodities may lead to the introduction, establishment and spread of pests that are of quarantine significance to South Africa
- Introduction of pest species such as *Phyllosticta citricarpa, Bactrocera dorsalis, Spodoptera frugiperda* and *Prostephanus truncatus* into South Africa could result in undesirable impacts on the ecosystem, agriculture, biodiversity and economy of the country.
- Access to new export markets of plant commodities could be threatened as importing countries may impose stringent phytosanitary measures to limit the chances of introduction and establishment of these quarantine pests into their territories.

Introduction

Introduction of alien and invasive species into regions outside of their native ranges can have undesirable effects on both ecosystem and agriculture.[1] Most countries, including South Africa, are currently facing threats from the introduction, spread and establishment of alien and invasive species. Various countries are struggling to prevent the influx of further alien and invasive species as the global economy expands and the movement of goods, services and people continues to grow.[2] A primary means by which alien and invasive species become established is through unintentional introductions associated with international trade.[3] These invasive alien species can have an exceedingly broad range of economic, environmental and social impacts.[4,5] International trade of plants and plant products is one of the major pathways for the introduction and spread of exotic pests. Some of these pests may affect agricultural production and/or limit access to international export markets. Phytosanitary inspections of plants and plant products at border ports are an important phytosanitary practice to determine the levels of compliance with phytosanitary requirements. To reduce phytosanitary risk, trading partners adhere to rigorous measures for import or export to avoid the introduction of alien and invasive species to new regions. Alien and invasive species are common stowaways on shipments of imported plants, plant products and other regulated articles.[6,7] Alien and invasive species may arrive on imports encompassing a variety of commodities, including agricultural produce, greenhouse and ornamental plants, nursery stock, cut flowers, wood products, stored products and packing materials.[8,9]

Global air transportation and road transportation greatly facilitate the unintended spread of pests, including invasive species, because of the large volumes of goods and people transported internationally[10] which also are gradually increasing every year[11]. Given the tremendous increases in global passenger travel, there is a need to better characterise the extent to which passenger baggage serves as an invasion pathway for alien and invasive species.[12]

In South Africa, the potential introduction of pests and diseases is administered through the *Agricultural Pests Act No. 36 of 1983* and its associated regulations[13], *National Environmental Management: Biodiversity Act No. 10 of 2004*[14], and Alien and Invasive Species Regulation (2014) as amended[14]. The Acts deal with prevention and control of the introduction and spread of pests. The *Agricultural Pests Act* compels imported controlled goods

to be declared and presented at ports of entry to the executive officer and/or authorised inspector who then inspects or samples the controlled goods as necessary. The inspection and sampling are done to determine the presence of regulated pests in a commodity. Regulated pests include both quarantine and non-quarantine pests.[15] The Directorate: Inspection Services and Directorate: Plant Health within the South African Department of Agriculture, Land Reform and Rural Development (DALRRD) are responsible for inspections, policy development and implementation. The inspectors from DALRRD ensure that imported controlled goods are free from non-indigenous organisms, including phytophagous insects, mites, molluscs, nematodes, plant pathogens and invasive weed species. The Directorate: Inspection Services conducts inspections of baggage carried by international travellers or passengers that arrives at South African ports and borders, primarily focusing on plant commodities which could likely harbour live plant pests. These inspections were conducted for 8 years (2011–2019). The Directorate: Plant Health publishes and maintains the database of intercepted pests.

The purpose of this study was to determine the trend associated with the interception of pests in passengers' baggage at South African borders and airports. We examined the origins of imported commodities, pests intercepted, status of intercepted organisms in South Africa and the likelihood of establishing in South Africa, as a critical effort to prevent negative impacts on the economy, ecosystems and agriculture through the possible introduction and spread of quarantine species.

Materials and methods

Sample collection and preparation

Plant commodities intended for import into South Africa and illegally moved were confiscated by the DALRRD. Samples ($n=341$) from various specimens were collected over a period of 8 years (2011–2019) from O.R. Tambo International Airport (Johannesburg) and nine border posts (Vioolsdrift, Nakop, Grobler's Bridge, Skilpad's Gate, Ramatlabama, Beit Bridge, Lebombo, Oshoek and Golela). The samples were then sent to the DALRRD Diagnostic Laboratories in Pretoria (Gauteng Province) and Stellenbosch (Western Cape Province) for pest identification. In cases in which identification could not be made with certainty, the specimens were sent to the Biosystematics Division of the Agricultural Research Council (Pretoria) for further identification to species level. Immature and damaged specimens were identified to only genus or family level.

Data analysis

Data and information regarding commodity species name, commodity type, pests detected, pests' quarantine status and country of origin were analysed descriptively using Statistical Software Package Version 2010 (Statsoft Inc., Tulsa, OK, USA).

Results

Pests were frequently detected on commodity species such as *Citrus* spp. (oranges, lemons and grapefruit; 18.31%), *Zea mays* L. (maize; 13.22%), *Phaseolus vulgaris* (common beans; 12.88%), *Musa* spp. (bananas and plantains; 9.15%) and *Fragaria ananassa* Duchesne (strawberries; 5.08%). The pests detected at less than 5% were on plant commodity species as listed in Table 1.

Illegally imported plant commodities included fresh fruits, grains, vegetables, cobs, tubers, bran, nuts, seeds, cut flowers, cuttings and oil cakes (Table 2).

Irrespective of the quarantine and regulatory status for South Africa, the most intercepted organisms were *Callosobruchus rhodesianus* (Pic) (cowpea weevil; 7.79%) from grains, *Dysmicoccus* brevipes (Cockerell, 1893) (pineapple mealybug; 7.11%) from pineapples, *Callosobruchus maculates* (F.) (cowpea weevil; 6.10%) from grains and *Phyllosticta citricarpa* (McAlpine) Aa (citrus black spot; 4.74%) from *Citrus* spp. (Table 3). The highest number of pests intercepted occurred on fresh fruits, followed by grains and vegetables. Interception of pests was less frequent on maize cobs, tubers, bran, seeds, nuts, cut flowers, bulbs, cuttings, oil cakes and meal.

Based on the risk, intercepted pests were divided into quarantine, potential quarantine, non-quarantine and uncategorised (N/A) pests. In terms of pest status in South Africa, most of the intercepted organisms were non-quarantine species (70.50%), pests of unknown status (17.28%), quarantine pests (10.84%) and potential quarantine pests (1.35%) (Figure 1). *Prostephanus truncatus* Horn (larger grain borer), *P. citricarpa*, *B. dorsalis* and *S. frugiperda* were the only quarantine pests intercepted from imported grains and fresh fruits (Table 2), but these pests have a high disaster risk to the South African agricultural sector as well as the economy. Pests of unknown status have a high potential to negatively affect agricultural production.

The highest frequency of pest interceptions occurred on commodities imported mainly from Mozambique (47%), followed by Zimbabwe (15%) and Eswatini (12%). The frequency of interceptions from Spain, France, Ukraine, Malawi, Ethiopia, Nigeria, Ghana, Mauritius, Zambia, USA and Israel was between 1% and 5%, while countries such as New Zealand, Angola, Kenya, DRC, Jordan, India, Russia and Namibia accounted for less than 1% (Table 3).

Discussion

The majority of pests intercepted were non-quarantine pests (Figure 1); however, these pests may also pose a risk in the agricultural sector by threatening food security and production. In terms of international and national prescripts, only regulated pests and/or quarantine pests are subject to phytosanitary measures.[16] This is because the introduction and spread of quarantine pests may destroy the agricultural and horticultural sectors. The introduction and spread of quarantine pests and pests of unknown status may ultimately affect the environment, economy, food security as well as export markets.[17] It could lead to agricultural production losses of 20–40%[18] and further increase the cost of operations and disease control.

In most cases, the entry and introduction of plant pests of economic importance from other countries into South Africa is through the movement of plants and plant products during international trade and/or movement of people with commodities. Most alien and invasive species are introduced and/or intercepted during international trade worldwide.[19]

It is thus important that plant pest interception is appropriately dealt with in accordance with phytosanitary measures and actions. In South Africa, according to the *Agricultural Pests Act,* ports of entry are prescribed under Regulations R.111 of 27 January 1984 and Government Notice R.1013 of 26 May 1989 as amended, wherein plant commodities are permitted to be imported.[13] This legislation requires all importers of plant products to comply with South Africa's import requirements and plant products are subject to inspection at the prescribed ports of entry.

In other countries, such as those in the European Union, alert lists on fruits have been established to manage the risk.[20] In South Africa, the majority of intercepted pests is found to be non-quarantine pests (Figure 1), and thus they are not subjected to phytosanitary measures. However, the majority of pests that have entered and been introduced into South Africa are pests of economic importance. This implies that the import procedures as well as the regulatory systems of South Africa need to be improved and strengthened.[21]

Although there are regulatory frameworks to regulate the importation of plant products in the Republic of South Africa, passengers still disregard the rules and manage to bypass the system with unauthorised commodities or by importing commodities that do not comply with South African import requirements. This study revealed that these activities normally happen at land borders (as opposed to the airport) where South Africa shares the borders with neighbouring countries such as Eswatini, Zimbabwe and Mozambique. The DALRRD should take decisive action in terms of plant health awareness as well as in enforcing the law at the ports of entry to ensure that a high level of compliance is realised. The *Agricultural Pests Act* requires that illegally imported plant products should be subject to confiscation and destruction, and offenders should be subject to Section 13 of the Act, which deals with 'offences and penalties'.

Table 1: Plant commodities imported into South Africa, 2011–2019 (n=341)

Commodity	Common name	Commodity type	Frequency of confiscated commodities (%)
Citrus spp.	Lemon/orange/lime/grapefruit	Fresh fruit	18.31
Zea mays L.	Maize	Grain/seed/corn	13.22
Phaseolus vulgaris L.	Bean	Grain/seed	12.88
Musa spp.	Banana/plantain	Fresh fruit	9.15
Fragaria ananassa Duch.	Strawberry	Fresh fruit	5.08
Ananas comosus L. Merr	Pineapple	Fresh fruit	4.41
Mangifera indica L.	Mango	Fresh fruit	4.41
Brassica oleracea L.	Cabbage	Vegetable	3.73
Lactua sativa L.	Lettuce	Vegetable	3.73
Spinacia oleracea L.	Spinach	Vegetable	3.73
Manihot esculenta Crantz	Cassava	Tuber	2.37
Gossypium sp.	Cotton	Grain/seed	1.69
Ipomoea batatas (L.)	Sweet potato	Tuber	1.36
Oryza sativa L.	Rice	Grain/seed	1.36
Pisum sativum L	Pea	Grain/seed	1.36
Sorghum bicolor L. Moench	Sorghum	Grain/seed	1.36
Vigna mungo L. Hepper	Blackgram	Grain/seed	1.36
Vitellaria paradoxa Gaertn.	Sheanut	Seed	1.36
Cucurbita sp.	Pumpkin/butternut	Vegetable	1.02
Prunus persica (L.) Batsch	Peach	Fruit	1.36
Rosa hybrida L.	Rose	Cutting	1.02
Abelmoschus esculentus (L.) Moench	Okra	Vegetable	0.68
Anacardium occidentale L.	Cashew nut	Seed	0.68
Annona senegalensis Pers.	Custard apple	Fruit	0.68
Dioscorea alata L.	Yam	Tuber	0.68
Vitis vinifera L.	Table grape	Fruit	0.68
Arachis hypogaea L.	Peanut	Grain/seed	0.34
Corchorus olitorius L.	Jute	Vegetable	0.34
Glycine max (L.) Merr	Soybean	Grain/seed	0.34
Nicotiana tabaccum L.	Tobacco	Leaf	0.34
Psidium guajava L.	Guava	Fruit	0.34
Saccharum officinarum L.	Sugarcane	Stem	0.34
Triticum aestivum L.	Wheat	Grain/seed	0.34

Table 2: Identity and quarantine status of intercepted organisms into South Africa, 2011–2019 (n=341)

Scientific name of pest	Order/sub-order: family	Common name	Quarantine status in South Africa	Interception (%)
Callosobruchus rhodesianus Pic.	Coleoptera: Bruchidae	Cowpea weevil	Non-quarantine pest	7.79
Dysmicoccus brevipes (Cockerell)	Hemiptera: Pseudococcidae	Pineapple mealybug	Non-quarantine pest	7.11
Callosobruchus maculatus Fab.	Coleoptera: Bruchidae	Cowpea weevil	Non-quarantine pest	6.10
Phyllosticta citricarpa (McAlpine) Aa	Botryosphaeriales: Botryosphaeriaceae	Citrus black spot	Quarantine pest	4.74
Chrysomphalus aonidum (Linnaeus, 1758)	Hemiptera: Diaspididae	Circular scale	Non-quarantine pest	3.05
Tribolium castaneum (Fabricius)	Coleoptera: Bostrichidae	Lesser grain borer	Non-quarantine pest	3.05
Helicoverpa armigera (Hübner, 1809)	Lepidoptera: Noctuidae	Cotton bollworm	Non-quarantine pest	2.71
Sitophilus oryzae (Linnaeus)	Coleoptera: Dryophthoridae	Greater grain weevil	Non-quarantine pest	2.71
Bactrocera dorsalis (Hendel, 1912)	Diptera: Tephritidae	Oriental fruit fl	Quarantine pest	2.37
Lagria sp.	Coleoptera: Tenebrionidae	Beetle	N/A	2.37
Sitotroga cerealella (Olivier)	Lepidoptera: Gelechiidae	Grain moth	Non-quarantine pest	2.37
Rhyzopertha dominica (Fabricius)	Coleoptera: Bostrichidae	Lesser grain borer	Non-quarantine pest	2.03
Plutella xylostella (Linnaeus)	Lepidoptera: Plutellidae	Diamondback moth	Non-quarantine pest	1.69
Ceratitis cosyra (Walker)	Diptera: Tephritidae	Mango fruit fl	Non-quarantine pest	1.35
Prostephanus truncatus (Horn)	Coleoptera: Bostrichidae	Larger grain borer	Quarantine pest	1.35
Parlatoria pergandii (Comstock, 1881)	Hemiptera: Diaspididae	Dictyospermum scale	Non-quarantine pest	1.35
Carpophilus dimidiatus (Fabricius, 1792)	Coleoptera: Nitidulidae	Cornsap beetle	Non-quarantine pest	1.01
Ceratitis capitata (Wiedemann)	Diptera: Tephritidae	Medfl	Non-quarantine pest	1.01
Frankliniella occidentalis (Pergande)	Thysanoptera: Thripidae	Western flower thrip	Non-quarantine pest	1.01
N/A	Hemiptera: Diaspididae	Scale insect	N/A	1.01
N/A	Lepidoptera: Arctiidae	Moth	N/A	1.01
N/A	Sarcotiformes: Acaridae	Predatory/fungi-feeding mite	Non-quarantine pest	1.01
Phenacoccus solenopsis (Tinsley)	Hemiptera: Pseudococcidae	Cotton mealybug	Non-quarantine pest	1.01
Piezotrachelus sp.	Coleoptera: Apioninae	Weevil	Non-quarantine pest	1.01
Planococcus citri (Risso, 1813)	Hemiptera: Pseudococcidae	Citrus mealybug	Non-quarantine pest	1.01
Sitophilus zeamais (Motschulsky)	Coleoptera: Dryophthoridae	Greater grain weevil	Non-quarantine pest	1.01
Spodoptera frugiperda (J.E. Smith)	Lepidoptera: Noctuidae	Fall armyworm	Quarantine pest	1.01
N/A	Hemiptera: Anthocoridae	Pirate bug	N/A	0.67
Aonidiella aurantii (Maskell)	Hemiptera: Diaspididae	Red scale	Non-quarantine pest	0.67
N/A	Hemiptera: Aphididae	Aphid	N/A	0.67
Brevipalpus californicus (Banks)	Prostigmata: Tenuipalpidae	False spider mite	Non-quarantine pest	0.67
Brevipalpus yothersi (Baker, 1949)	Prostigmata: Tenuipalpidae	False spider mite	Non-quarantine pest	0.67
Callosobruchus chinensis (Linnaeus, 1758)	Coleoptera: Bruchidae	Chinese bruchid	Non-quarantine pest	0.67
Chilo partellus (Swinhoe, 1885)	Lepidoptera: Crambidae	Spotted stem borer	Non-quarantine pest	0.67
Cryptolestes ferrugineus (Stephens)	Coleoptera: Cucujidae	Rusty grain beetle	Non-quarantine pest	0.67
Cryptolestes pusillus (Schönherr, 1817)	Coleoptera: Cucujidae	Flat grain beetle	Non-quarantine pest	0.67
Cylas sp.	Coleoptera: Apionidae	Sweet potato weevil	N/A	0.67
Ebertia sp.	Sarcoptiformes: Acaridae	Mite	N/A	0.67
Frankliniella schultzei (Trybom)	Thysanoptera: Thripidae	Common blossom thrips	Non-quarantine pest	0.67
Haplothrips gowdeyi (Franklin)	Thysanoptera: Phlaeothripidae	Black flower thrip	Non-quarantine pest	0.67
Hellula undalis (Hulst)	Lepidoptera: Crambidae	Cabbage webworm	Non-quarantine pest	0.67
N/A	Lepidoptera: Noctuidae	N/A	N/A	0.67
N/A	Diptera: Lonchaeidae	Lance fl	N/A	0.67
Neohydatothrips lepidus (Faure)	Thysanoptera: Thripidae	Thrips	Non-quarantine pest	0.67
Noctuid larva	Lepidoptera: Noctuidae	N/A	N/A	0.67
Oryzaephilus mercator (Fauvel, 1889)	Coleoptera: Silvanidae	Grain beetle	Non-quarantine pest	0.67
Sancassania oudemansi (Zachvatkin, 1937)	Sarcoptiformes: Acaridae	Mite	Non-quarantine pest	0.67
N/A	Diptera: Tephritidae	Fruit fly (tephritid la vae)	N/A	0.67
Thrips gowdeyi	Thysanoptera: Thripidae	Thrips	Non-quarantine pest	0.67
Thrips sp.	Thysanoptera: Thripidae	Thrips	N/A	0.67
Tuckerella cf. *murreensis*	Prostigmata: Tuckerellidae	Mite	Non-quarantine pest	0.67
Tuckerella ornata (Tucker)	Prostigmata: Tuckerellidae	Mite	Non-quarantine pest	0.67
Tyrophagus putrescentiae (Schrank)	Acari: Acaridae	Mite	Non-quarantine pest	0.67
N/A	Coleoptera: Curculionidae	Weevil	N/A	0.67

Table 2 continued

Scientific name of pest	Order/sub-order: family	Common name	Quarantine status in South Africa	Interception (%)
Aonidomytilus albus (Cockerell, 1893)	Hemiptera: Diaspididae	Tapioca scale	Non-quarantine pest	0.33
Aphis gossypii (Glover, 1877)	Hemiptera: Aphididae	Cotton aphid	Non-quarantine pest	0.33
Aphis sp.	Hemiptera: Aphididae	Aphid	N/A	0.33
Argopistoides octomaculata (Jacoby, 1892)	Coleoptera: Chrysomelidae	Beetle	Non-quarantine pest	0.33
Bagrada hilaris (Burmeister)	Hemiptera: Pentatomidae	Invasive stink bug	Non-quarantine pest	0.33
N/A	Prostigmata: Bdellidae	Predatory mite	N/A	0.33
N/A	NA	Beetle	N/A	0.33
Brevicoryne brassicae L.	Brassicales: Brassicaceae	Cabbage aphid	Non-quarantine pest	0.33
Brevipalpus sp.	Prostigmata: Tenuipalpidae	False spider mite	N/A	0.33
Bruchidius atrolineatus Pic.	Coleoptera: Bruchidae	African cowpea bruchid	Non-quarantine pest	0.33
Callosobruchus sp.	Coleoptera: Bruchidae	Weevil	N/A	0.33
Cenopalpus sp.	Prostigmata: Tenuipalpidae	Tenuipalpid mites	N/A	0.33
Ceratitis rosa (Karsch)	Diptera: Tephritidae	Natal fruit fl	Non-quarantine pest	0.33
N/A	Coleoptera: Bostrychidae	NA	N/A	0.33
N/A	Diptera: Tephritidae	Fruit fl	N/A	0.33
N/A	Diptera: Drosophilidae	Vinegar fl	N/A	0.33
Ferrisia virgata (Cockerell, 1893)	Hemiptera: Pseudococcidae	Striped mealybug	Non-quarantine pest	0.33
Hadromerus sp.	Coleoptera: Curculionidae	Bug	N/A	0.33
Lampides boeticus (Linnaeus)	Lepidoptera: Lycaenidae	Pea blue butterfl	Non-quarantine pest	0.33
Lasioderma serricorne (Fabricius, 1792)	Coleoptera: Anobiidae	Cigarette beetle	Non-quarantine pest	0.33
Lepidosaphes beckii (Newman, 1869)	Hemiptera: Diaspididae	Purple scale	Non-quarantine pest	0.33
Leucinodes orbonalis (Guenee)	Lepidoptera: Crambidae	Eggfruit and shoot borer	Non-quarantine pest	0.33
Lipaphis erysimi (Kaltenbach)	Hemiptera: Aphididae	Mustard aphid	Non-quarantine pest	0.33
Lipaphis pseudobrassicae (Davis, 1914)	Hemiptera: Aphididae	Turnip aphid	Non-quarantine pest	0.66
Myzus persicae (Sulzer)	Hemiptera: Aphididae	Green peach aphid	Non-quarantine pest	0.33
Nephus sp.	Coleoptera: Coccinellidae	Lady bird beetle	N/A	0.33
Nitidulid larva	Coleoptera: Nitidulidae	Sap beetle	N/A	0.33
Non-tephritid fl	N/A	N/A	N/A	0.33
Odonaspis saccharicaulis (Zehntner, 1897)	Hemiptera: Diaspididae	Paragrass scale	Non-quarantine pest	0.33
Penthimiola bella (Stål)	Hemiptera: Cicadellidae	Citrus leafhopper	Non-quarantine pest	0.33
Peregrinus maidis (Ashmead)	Hemiptera: Delphacidae	Corn planthopper	Non-quarantine pest	0.33
Phenacoccus madeirensis (Green)	Hemiptera: Pseudococcidae	Madeira mealybug	Non-quarantine pest	0.33
Phenacoccus sp.	Hemiptera: Pseudococcidae	Mealybug	N/A	0.33
Phytonemus pallidus (Banks)	Acari: Tarsonemidae	Cyclamen mite	Non-quarantine pest	0.33
NA	Acari: Phytoseiidae	N/A	Non-quarantine pest	0.33
Pseudococcus longispinus (Targioni Tozzetti)	Hemiptera: Pseudococcidae	Long-tailed mealybug	Non-quarantine pest	0.33
Saissetia sp.	Hemiptera: Coccidae	Scale insect	N/A	0.33
N/A	Sarcoptiformes: Acaridae	Mite	Non-quarantine pest	0.33
N/A	Hemiptera: Diaspididae	Scale insect	N/A	0.66
Scirtothrips aurantii	Thysanoptera: Thripidae	South African citrus thrips	Non-quarantine pest	0.33
Spoladea recurvalis (Fabricius, 1775)	Lepidoptera: Crambidae	Beet webworm moth	Non-quarantine pest	0.33
Stigmatonotum capucinum (Stål)	Heteroptera: Lygeidae	N/A	Non-quarantine pest	0.33
Tachinidae	Diptera: Tachinidae	N/A	N/A	0.33
Tarsonemus confusus (Ewing)	Prostigmata: Tarsonemidae	Tarsonemid mite	Non-quarantine pest	0.33
Tetranychus sp.	Prostigmata: Tenuipalpidae	Spider mite	N/A	0.33
Thaumatotibia leucotreta (Meyrick)	Lepidoptera: Tortricidae	False codling moth	Non-quarantine pest	0.33
N/A	Thysanoptera: Tubilifera	NA	N/A	0.33
N/A	Trombidiformes: Tydeidae	Mite (scavenger)	Non-quarantine pest	0.66
Tyrophagous sp.	Astigmata: Acaridae	Straw mite	Non-quarantine pest	0.33
Udea ferrugalis (Hübner)	Lepidoptera: Pyralidae	Rusty dot pearl	Non-quarantine pest	0.33
Ulotrichopus primulinus (Hampson, 1902)	Lepidoptera: Erebidae	Moth	Non-quarantine pest	0.33

N/A, not applicable (identified up to genus or family level to determine quarantine status in South Africa)

Table 3: Recorded pest interceptions into South Africa by country, 2011–2019 (n=341)

Country	Interception (%)
Mozambique	47.00
Zimbabwe	15.00
Eswatini	12.00
Spain	4.00
France	3.00
Ukraine	3.00
Malawi	2.37
Mauritius	1.99
Ethiopia	1.69
Nigeria	1.69
Ghana	1.35
Zambia	1.01
USA	1.00
Israel	1.00
New Zealand	0.90
Angola	0.67
Kenya	0.67
DRC	0.33
Jordan	0.33
India	0.33
Russia	0.33
Namibia	0.33

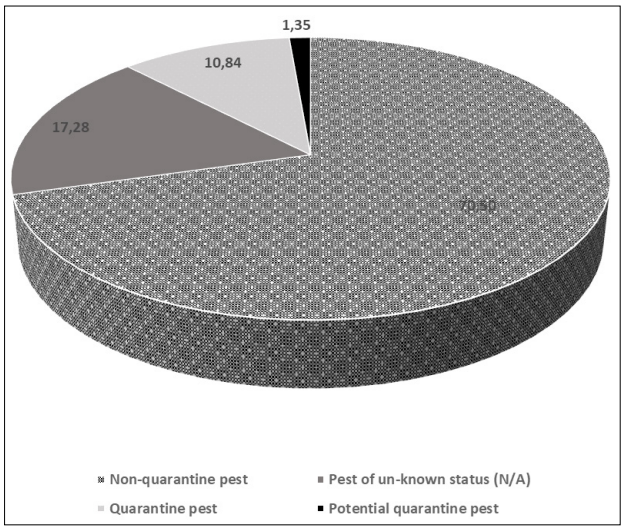

Figure 1: Categories of intercepted organisms (n=341) in South Africa, 2011–2019.

Currently, the DALRRD is undergoing a review of the *Agricultural Pests Act* in relation to phytosanitary measures so that there is better alignment with internationally prescribed export and import provisions. The approval of the proposed bill (Plant Health (Phytosanitary) Bill) could limit the introduction of exotic pests. The provisions of the proposed bill also provide for on-the-spot fines for non-compliance.[22] This approach is not new; the Australian government has imposed on-the-spot fines and/or penalties for non-compliance since 2009 under the *Plant Health Act, 2009* and this mechanism is working effectively to reduce smuggling and/or illegal imports of plant commodities.[23]

South African borders still lack relevant equipment such as scanners to detect undeclared plant products during movement of people crossing South African borders. Therefore, the majority of passengers with unauthorised plant products and/or fruits pass through undetected, as indicated by the high number of interceptions in this study. Currently, South Africa employs sniffer dogs in other ports of entry to detect plant commodities in passengers' luggage. However, these remain limited and need to be increased across all ports of entry in order to reduce the introduction of pests through undeclared and/or non-compliant plant commodities. The system may be further intensified at ports of entry with the establishment of the new South African Border Management Authority under the *Border Management Act, 2020 (Act No. 2 of 2020)*.

Based on our results, adequate measures are required at South Africa's land borders, particularly for imported fruits. The majority of illegal imports and pest interceptions occurred from Mozambique, Zimbabwe and Eswatini (Table 3), which thus require the most stringent measures to be put in place to prevent the introduction of pests. The challenge is not only in South Africa – all African borders are under enormous pressure due to the movement of people and goods across borders.[24] In contrast, in the USA, the highest number of pest interceptions was recorded at the airports, followed by the land border between the USA and Mexico.[25]

The results from this study suggest that imported fruits followed by grains are the commodities with the highest levels of interception of quarantine pests into South Africa from neighbouring countries. Fruits are also the major pathway of pests and disease worldwide.[26] The majority of countries, including South Africa, import fruits in large volumes. Insects and/or pests hide, grow and reproduce in those imported fruits, which are latter intercepted by the receiving country.[6] This implies that fresh produce (fruits and vegetables) is the major pathway for actionable pests in the USA[27] as well as in South Africa.

We have demonstrated a high phytosanitary risk associated with the illegal importation of plant commodities. Amongst the intercepted organisms, the quarantine pests were *B. dorsalis*, *S. frugiperda*, *P. citricarpa* and *P. truncatus,* which occur in South Africa but are currently under official control in terms of the *Agricultural Pests Act*. According to the International Plant Protection Convention, a quarantine pest is 'a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled'.

The discovery of *B. dorsalis* on the African continent during 2003 raised biosecurity concerns in South Africa where agriculture is of major socio-economic importance.[28] This is a pest species that has recently been introduced into eastern Africa and has subsequently made a rapid expansion across tropical Africa.[29] *B. dorsalis* is highly polyphagous[30,31], it has more than 40 known cultivated and wild hosts in Benin[32], and is expected to have a broad host range as exhibited by some other members in the *B. dorsalis* complex. Female individuals can lay an average of 1300 eggs during their lifetimes, depending on the host and climatic conditions. The species is multivoltine with an average life span of about 3 months.[33] Given the apparently rapid spread of *B. dorsalis* across Africa, and its impacts on local horticulture, the possibility of this species being introduced to, and establishing in and invading other regions of the world, should be prevented.[34] *B. dorsalis* is currently considered one of the major pests in Africa[28,35], displacing indigenous fruit flie [36]. This invasive species has major economic impacts, ranking among the most devastating pests of local agricultural products, particularly mango.[34]

Research in West and East Africa has demonstrated that it can become dominant in mango monocultures.[33]

Spodoptera frugiperda was also intercepted in this study. In Africa, the first detection of fall armyworm *S. frugiperda* was recorded in Nigeria at the beginning of 2016, from where it later moved to several western and central parts of the continent by April 2016.[37] *S. frugiperda* is widely distributed in North America, where it causes the most serious damage to about 80 different commercial crops, including maize, rice, sorghum, sugarcane, cabbage, beet, groundnut, soybean, alfalfa, onion, pasture grasses, millet, tomato, potato and cotton.[38] In Ethiopia, maize infestation of this insect pest was estimated to range from 24% to 39%, while in Kenya, the infestation was about 3854%.[39] Fall armyworm caused reductions in maize yields of 934 kg/ha and 1381 kg/ha in Ethiopia and Kenya, respectively.[39] Farmers in Ethiopia and Kenya observed a more than 82% increase in the spread of fall armyworm on their farms. The Ethiopian and Kenyan farmers reported fall armyworm as a serious insect pest, which caused a higher level of damage than the maize stalk borer.[39]

Another intercepted pest found in the current study was the citrus black spot pathogen, *P. citricarpa*, which is a fungal pathogen of citrus plants, specifically orange and lemon varieties.[40] In South Africa, citrus fruit exports to European and US markets are subjected to strict phytosanitary measures.[41] Internationally, *P. citricarpa* is considered a quarantine pathogen.[42] Citrus black spot occurs in citrus-growing regions with warm summer rainfall climates.[43]

The larger grain borer, *P. truncatus,* was also detected in the current study and is regarded as an economically important pest of stored commodities such as maize and dried cassava.[44] This pest is endemic to Mexico and Central America, although it has recently been introduced and become established on the African continent, where it has caused severe damage to stored maize. The first outbreaks were reported in the Eastern part of Africa.[45] *P. truncatus* became established in Togo in 1984, and gradually spread to Benin, Burkina Faso, Niger, Nigeria, Guinea Conakry and Guinea Bissau.[46] The economic impact of insect pest attacks on stored grain is associated with the amount of grain damaged or the percentage weight loss.[47] In Zimbabwe, it was first reported in Mashonaland West and Mashonaland Central Provinces, with potential distribution from the northern part of the country moving further inland towards the central and eastern parts, and neighbouring countries such as South Africa, Zambia and Mozambique.[48,49]

Conclusion

The risks posed by the illegal introduction of pests through a particular plant commodity should also be considered in a pest risk analysis, because even though the commodity itself may not pose a pest risk, it may harbour organisms that have pests. Therefore, illegal importation of plant and plant products into South Africa should undergo a high phytosanitary risk assessment. The introductions of alien and invasive pests into South Africa can have undesirable effects on the ecosystem, agriculture, biodiversity as well as the economy. The highest number of pest interceptions occurred on fresh fruits, grains and vegetables mainly from southern African countries, particularly Mozambique, Zimbabwe and Eswatini. Although the majority of intercepted pests were non-quarantine species, the consequences due to repeated interceptions of quarantine pests should be taken into consideration. The agricultural sector, particularly citrus, maize, fruit and vegetable industries in South Africa, could be under severe threat from the establishment and spread of quarantine pests such as *P. citricarpa, B. dorsalis, S. frugiperda* and *P. truncatus* to areas where these do not occur. In addition, consignments presented for import into South Africa must be subject to pre-import phytosanitary inspection. As an economic consequence, fresh produce farmers in areas free from these pests stand to spend more money in eradicating these pests if they are introduced and/or established. Many people stand to lose jobs as farmers will be reducing their workforce because of reduced profits. Crop production, biodiversity, food security, existing export markets, and access to new export markets could also be threatened as importing countries may impose stringent phytosanitary measures to limit the chances of introduction and establishment of these quarantine pests into their territories.

South Africa is a signatory member of the World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures and the International Plant Protection Convention. These international organisations acknowledge the sovereign right of Members to protect the life and health of plants within their territories by means of phytosanitary regulatory controls. The spread of quarantine pests to new areas where they have not previously occurred can lead to severe crop production losses and food insecurity.

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Competing interests

We have no competing interests to declare.

Authors' contribution

P.P.T.: Conceptualisation and development of the initial draft of the manuscript. L.R.N.: Conceptualisation and write-up. M.R.: Write-up of the article. R.A.M.: Data analysis and write-up. F.N.M.: Critique and validation of the article.

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Effect of glyphosate application time on yield parameters of South African glyphosate-resistant maize cultivars

Glyphosate is the most used herbicide in South Africa. Due to observations by some South African maize producers that the application of glyphosate to glyphosate-resistant (GR) maize cultivars resulted in reduced yield, we conducted an in-depth study under local conditions. Through field trials, over two seasons (2017/2018 and 2018/2019), we investigated whether the application time of glyphosate would impact maize yields negatively. Various yield parameters were measured subsequent to glyphosate application to the local GR maize cultivars DKC74-74BR, DKC78-79BR, KKS4581, KKS8408, BG5785BR, PAN6R-710BR, P1814R and P2880WBR. Four glyphosate products were included (Roundup PowerMax®; Slash Plus 540 SL; TouchdownForte® and Mamba™ DMA 480 SL), resulting in 32 cultivar x glyphosate product combinations. Each product was applied at V4, V4+V6, V6 and V8 growth stages together with an untreated control. Yield parameters measured (ears per plant, rows per ear, kernels per row, thousand kernel mass and yield) were expressed as a percentage of the control. The trials were planted as randomised complete block designs with three replicates. Limited response was observed with all the parameters investigated, with a significant negative yield response, greater than the untreated control, observed in only 3.1% of the cultivar x glyphosate product combinations evaluated. No clear trends or discernible and consistent impacts on yield and yield parameters could be established based on the application time of glyphosate (within label recommendations) across seasons. The findings contribute significantly to the knowledge base and current understanding of the international community and local producers alike regarding the effective use of glyphosate and generic variations thereof in crops of diverse genetic backgrounds.

Significance:

- Limited response in the yield parameters evaluated were obtained in response to the application time (V4, V4+V6, V6 and V8) of the four glyphosate products on eight GR maize cultivars tested ($p=0.1$).
- Inconsistent patterns or trends were detected in cases where significance was obtained, implying that it would not be possible to draw accurate conclusions or formulate recommendations.
- Application time of glyphosate did not result in a significant reduction in yield compared to the untreated control, in the majority of the cultivar x glyphosate product combinations investigated, confi ming that glyphosate application conducted within label specifications would not reduce yield, irrespective of the glyphosate product or genetic background of maize.

Introduction

Glyphosate, developed in 1964, was introduced to crop production during the mid-1970s as a broad-spectrum, non-selective, post-emergence herbicide.[1] Genetically modified (GM) crops, resistant to glyphosate and glufosinate, subsequently followed, with the first GM crops commercially cultivated in the 1990s.[2] Due to its rapid environmental degradation, minimal contamination of ground water, low costs as well as effective systematic action on most plants[2], glyphosate has since become one of the most widely used agrochemicals in modern agriculture[3]. Similar to the international trend, glyphosate is the most used herbicide in South Africa. Glyphosate is, however, aside from being a broad-spectrum herbicide, also a broad-spectrum chelator of macro- and micronutrients.[4] Due to this characteristic, glyphosate has been found to facilitate nutrient availability, access and/or absorption of some nutrients (Ca, Cu, Fe, K, Mg, Mn and Zn).[5] The immobility of nutrients would reduce their availability for processes such as photosynthesis, disease resistance and other essential functions in plants that in turn could potentially result in reduced yields.

The majority of herbicide-related research studies focus on weed control and the resultant maize grain yields, with few studies reporting on how herbicide applications may affect growth and development of herbicide-resistant maize plants in weed-free environments. Similarly, studies on the potential effects of glyphosate on plant health of glyphosate-resistant (GR) crops has mostly been focused on GR soybean.[6] Thelen and Penner[7], nonetheless, reported slight yield reductions in maize grain yields with the application of glyphosate under certain temporal high-yield environments. The authors speculated that under high-yielding conditions, injurious effects of glyphosate or glyphosate metabolites on GR maize may become measurable in terms of yield loss. In low-yield environments, yield limiting factors such as water stress may mask the less significant, subtler phytotoxic effects of glyphosate or glyphosate metabolites. Elmore et al.[8] concluded that, in a weed-free environment, glyphosate application had no effect on GR soybean yield and that yield suppression in GR soybean rather appears to be associated with the GR gene or its insertion process.[8,9]

Due to the expiration of the patent on glyphosate, producers have access to more glyphosate-containing herbicides.[10] All glyphosate products contain the same parent acid, but are sold as different salts of glyphosate with each formulation containing proprietary adjuvants that could influence product performance.[11] Studies to confi m the efficacy of such products concluded that there is little difference in product performance relating to glyphosate formulation and that a grower's choice among glyphosate products should be based on product cost, guarantees and other incentives from manufacturers.[11]

As glyphosate translocates from source tissue to sink tissue[12], developing sinks such as floral organs can be especially sensitive to glyphosate damage. Although glyphosate can infl ence reproductive development in GR and non-GR species, the impact on yield and fruit set varies greatly by crop, environment and timing of glyphosate applications. In most cases, the potential harmful effect of glyphosate is emphasised at growth stages that would be detrimental to the reproduction of the plant. Glasshouse and field studies have described several morphological abnormalities in affected flowers and balls of GR cotton.[13,14] Thomas et al.[15] demonstrated through glasshouse and field trials that maize pollen viability and overall quantity of pollen production were reduced when glyphosate was applied at growth stage V6 or later. Although both quantity and quality of pollen were compromised by glyphosate applied beyond the V6 stage, there was no significant effect on kernel set or yield using controlled pollinations. Pline-Srnic[16] speculated that sufficient pollen is produced by maize to ensure successful pollination even with reductions in viability and quantity, therefore preventing the manifestation of glyphosate effects on seed set. Glyphosate applications made near the time of pollen development in GR crops generally result in greater reproductive damage than early applications.[16] Locally, glyphosate application is recommended up until V8 leaf stage. Although several international studies have accordingly reported on the optimal time of glyphosate application[17], few investigated whether all applications applied before V8 would have an equal effect on crop yield in various genetic backgrounds of GM maize in weed-free environments.

Based on reduced yields reported by some local producers after glyphosate application, South African producers requested a study to establish whether the growth stage of glyphosate application could have a negative infl ence on yields of GM South African maize cultivars in weed-free environments. To achieve this objective, we conducted field trials over two consecutive seasons to establish whether yield parameters are affected by the growth stage of glyphosate application in various genetic backgrounds of South African GM maize.

Materials and methods

Eight randomised block design trials were planted during the 2017/2018 and 2018/2019 growing seasons, respectively, at the Agricultural Research Council's Grain Crops division in Potchefstroom, North West Province, South Africa (-26.743200°; 27.070775°). All eight trials were planted on 30 September 2017 for the 2017/2018 growing season and again on 23 October 2018 for the 2018/2019 growing season. Soil was prepared using standard seedbed preparations for a clay loam soil site (35% clay, 59% sand and 5% silt). Fertiliser with the formulation 3:2:1 was applied at 150 kg/ha, with LAN applied at V6 stage as top dressing at 150 kg/ha based on soil analyses. Each trial was planted to GR-maize cultivars that included DKC74-74BR, DKC78-79BR, KKS4581, KKS8408, BG5785BR, PAN6R-710BR, P1814R and P2880WBR. Plots consisted of eight 5-m rows with inter-row spacing of 0.9 m. The middle two rows of each plot were harvested. Plant density was approximately 34 000 plants/ha. Pre-emergence herbicides (Frontier® Optima and Gesaprim Super) were applied before onset of the trials after which weeds were removed from all plots by hand hoeing to prevent confounding effects of differential weed control. The treatment design was a strip-split with application at V4, V4+V6, V6 and V8 growth stages[18], respectively, as main plot factor and the four glyphosate products applied as sub-plot factor that were replicated in three blocks. The glyphosate products applied were (1) Roundup PowerMax® (540 g ae/L Monstanto, hereafter referred to as PowerMax), (2) Slash Plus 540 SL (540 g ae/L Villa Crop Protection, hereafter referred to as Slash), (3) TouchdownForte® (500 g ae/L Syngenta, hereafter referred to as Touchdown) and (4) Mamba™ DMA 480 SL (480 g ae/L Dow AgroScience, hereafter referred to as Mamba). An untreated control was included as the fi th treatment. Each product was applied at 2 L/ha, which is a common reference for glyphosate application amongst producers. PowerMax and Slash were accordingly applied at 1080 g ae/ha; Touchdown at 1000 g ae/ha and Mamba at 960 g ae/ha. The V4, V6 and V8 applications were conducted on 1, 12 and 20 December 2017 and on 21 and 28 November and 11 December in 2018. Ammonium sulfate (2%) was added as per label instruction. Herbicides were applied with a tractor sprayer, calibrated to deliver 200 L water/ha using flat fan nozzles. Supplementary irrigation was provided to all trials on a weekly basis as needed using overhead sprinklers. Daily weather data (maximum/minimum temperature and rainfall) were captured on site.

Parameters measured included ears per plant (EpP), rows per ear (RpE), kernels per row (KpR), thousand kernel mass (TKS) and yield (t/ha; 12.5% moisture). An average of five cobs per plot were randomly selected to determine RpE and KpR. From each cob selected, the number of kernels within two randomly selected rows were counted and the average number of kernels calculated to obtain KpR. All parameters were expressed as percentage of control.

For each cultivar x glyphosate product combination, the data over the two seasons were combined. Analysis of variance (ANOVA) was used to establish whether season and application time significantly impacted yield parameters in the respective combinations. Means were separated using Fisher's protected least signifi ant difference (LSD) if the F probability from the ANOVA was significant at the 10% ($p=0.1$) level of significance. The lower significance level used was due to limited significance in treatments in general, as well as to compensate for the variation in data generated. All the analyses were conducted using GenStat for Windows 18th edition.

Results

Weather data captured for the 2017/2018 and 2018/2019 planting seasons is presented in Table 1. The average temperatures achieved during October, November and December were 2.2 °C, 1.6 °C and 3.6 °C, respectively, higher during 2018/2019 than during 2017/2018. Total rainfalls recorded during the two seasons were similar. Higher early season rainfall (October to December 2017) was recorded for 2017/2018, compared to the corresponding period in 2018/2019. However, as all trials received supplementary irrigation, water was not a limiting factor. As all values were expressed as a percentage of the control, any value greater than the LSD of the relevant parameter assessed will also indicate that a specific treatment resulted in a significantly greater or lower measured effect than that of the untreated control. It was considered important to record such cases as an indication of whether the application of glyphosate would have a significant impact on the yield compared to that where no glyphosate was applied.

Ears per plant

Seasonal variation (year as main effect) significantly affected EpP with the application of PowerMax, to DKC74-74BR (Table 2), with an average reduction of 5.17% observed during 2018/2019 compared to the general increase of 1.67% noted in 2017/2018 (data not shown).

Application time (as main effect) significantly affected three cultivar x glyphosate product combinations (Table 2). Touchdown increased EpP by 19.5% in BG5785BR at V4, and by 23.1% in P2880WBR at V4+V6. Reductions in EpP detected at the various application times were never below 8% in either cultivar, and did not differ significantly from the untreated control (BG5785BR – $LSD_{(p=0.1)}$ = 15.1; P2880WBR – $LSD_{(p=0.1)}$ = 13.5). Slash applied at V4 reduced EpP by 13.3% in KKS8408, whilst an 11.5% increase was observed when applied at V8 ($LSD_{(P=0.1)}$ = 15.6) (Table 2).

Seven cultivar x glyphosate product combinations were significantly affected by the application time x year interaction. DKC74-74BR was significantly affected by Mamba and Touchdown with a significant increase in EpP observed (Mamba – 35.4%; Touchdown – 30.9%) when applied at V8 in 2017/2018. During the following season, reductions of 11.8% (Mamba) and 12.7% (Touchdown), respectively, were observed for the same application time. A similar effect is observed for KKS4851 (PowerMax). When applied to P1814R, PowerMax reduced EpP during the first season (2017/2018) by 35%, with an 11.1% increase observed for the same application time in the following season. Touchdown applied at V4+V6 reduced EpP of BG5785BR by 21.1% in the first season, but increased EpP by 13.4% in the second season at the same application time. Touchdown similarly resulted in a 10.5% reduction in EpP when applied to P2880WBR at V4 in the first season

Table 1: Temperature and rainfall data at Potchefstroom during the 2017/2018 and 2018/2019 planting seasons

		Temperature (ºC)					Rainfall (mm)	
		Maximum		Minimum				
		2017/2018	2018/2019	2017/2018	2018/2019		2017/2018	2018/2019
October	Average	26.4	28.6	11.4	11.4	Total	56.13	22.1
	Highest	32.4	34.4	17.1	18.7	Highest	23.88	11.68
	Lowest	18.0	17.2	5.1	5.8			
November	Average	29.1	30.7	12.7	14.0	Total	69.34	17.02
	Highest	34.5	36.8	17.3	23.4	Highest	18.54	10.16
	Lowest	17.1	23.4	4.5	4.3			
December	Average	29.3	32.9	15.7	16.2	Total	62.48	42.42
	Highest	33.4	38.8	19.1	20.7	Highest	13.72	12.19
	Lowest	15.8	21.9	10.3	11.2			
January	Average	31.0	31.5	16.1	16.7	Total	47.24	70.87
	Highest	36.6	36.2	20.3	19.5	Highest	12.45	25.65
	Lowest	24.4	21.2	9.3	13.5			
February	Average	27.7	28.7	15.6	15.6	Total	68.33	49.02
	Highest	31.5	33.3	17.7	19.0	Highest	14.99	8.64
	Lowest	20.5	22.0	11.8	11.4			
March	Average	27.5	30.2	14.6	15.1	Total	58.93	34.54
	Highest	31.1	33.9	19.2	19.0	Highest	21.84	22.35
	Lowest	17.6	20.4	10.2	11.3			
April	Average	25.3	23.8	11.1	11.7	Total	35.56	145.8
	Highest	29.0	28.7	16.1	17.0	Highest	10.67	35.31
	Lowest	19.7	14.4	5.6	5.4			
May	Average	22.8	24.3	4.9	5.8	Total	11.18	0.00
	Highest	26.4	28.1	12.1	11.2	Highest	9.91	0.00
	Lowest	16.4	20.7	1.3	0.6			
June	Average	21.6	21.5	1.5	1.1	Total	0.00	0.00
	Highest	25.6	25.0	4.6	7.5	Highest	0.00	0.00
	Lowest	17.5	17.7	-2.0	-3.6			
July	Average	19.3	21.8	1.1	-0.1	Total	5.08	0.40
	Highest	26.3	27.2	7.8	5.9	Highest	2.03	0.40
	Lowest	14.2	14.7	-6.0	-4.6			
						Total seasonal rainfall (mm)	414.27	382.17

In two of the seven cases in which the highest order interaction (application time x year) resulted in significant differences, the reduction observed was greater than the untreated control, suggesting that a significantly lower EpP was observed compared to when no product was applied. In both cases, the effect was observed for PowerMax (KKS8408 and P1814R) when applied at V8, but the seasons in which the effect was observed differed for the two cultivars. Based on these findings, it can be stated with 90% certainty ($p=0.1$) that the application time across two seasons (application x year interaction) affected EpP in 21.8% of the 32 cultivar x glyphosate product combinations investigated, but, that in only 6.3% of the cases, a significant decrease greater than the untreated control was achieved. The general effect observed was furthermore inconsistent between product, cultivar and season.

Kernels per row

Year as main effect significantly affected KpR in five of the cultivar x glyphosate product combinations tested. Lower KpR was achieved in 2018/2019 by DKC74-74BR (Slash and Touchdown), KKS4851 (PowerMax) and PAN6R-710BR (Touchdown) (data not shown). BG5785BR realised lower KpR in 2017/2018.

Application time (main effect) significantly affected KpR for Mamba applied to DKC78-79BR and P2880WBR, as well as PowerMax applied to KKS4851 (Table 3). Although Mamba reduced KpR of DKC78-79BR by 3% and 7.3% at V6 and V8, respectively, the reductions observed were not significantly greater than those for the untreated control. The 14.5% reduction observed in P2880WBR with the application of Mamba at V6, was signific ntly greater than the untreated control. PowerMax applied at V6 to KKS4851 significantly increased KpR by 14.1%

The application time x year interaction was only significant for the P1814R x Slash combination, in which a very variable response regarding application time across seasons was observed. A 16.1% increase in KpR was achieved at V4+V6 application during the first season (2017/2018), whilst no reductions significantly greater than the untreated control were observed for the remaining application times across seasons.

Seasonal differences subsequently resulted in significant differences in 15.6% of the cultivar x glyphosate product combinations evaluated, application time in 9.3%, and the application time x year interaction in 3.1% of the cultivar x glyphosate product combinations tested. The effect observed, similar to EpP, remains unpredictable regarding cultivar x glyphosate product combinations across seasons.

Rows per ear

Year as main effect significantly affected RpE in KKS4851 (PowerMax), PAN6R-740BR (Mamba) and P1814R (Touchdown) (data not shown).

Table 2: The effect of glyphosate application time on the number of ears produced per plant (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	8.6	-0.7	4.0	-0.4bc	-20.6c	-10.5	0.5	6.1	3.3	2.3ab	9.6a	5.9	-1.1	-11.7	-6.4	0.3	10.8	5.6	21.7	4.0	12.9	20.4	12.5	16.5
	V4+V6	-18.3	11.9	-3.2	-6.1bc	18.6ab	6.3	-16.0	10.2	-2.9	13.2a	-4.5ab	4.3	-5.1	4.5	-0.3	7.0	4.9	6.0	26.7	11.7	19.2	12.2	-0.8	5.7
	V6	11.5	4.2	7.9	-13.8bc	-13.5bc	-13.6	-17.5	-11.3	-14.4	-19.5b	-0.7ab	-10.1	-11.7	5.3	-3.2	5.4	-35.0	-14.8	-14.9	4.4	-5.3	8.1	-7.0	0.6
	V8	-0.9	-24.3	-12.6	35.4a	-11.8bc	11.8	16.1	-2.0	7.0	21.8a	-23.1b	-0.7	14.7	-6.3	4.2	-3.9	-16.2	-10.1	-10.9	-13.6	-12.3	0.2	-9.3	-4.6
LSD		*ns*			*AppxYr = 40.5*			*ns*			*AppxYr = 27.0*			*ns*			*ns*			*ns*			*ns*		
Roundup PowerMax	V4	-8.2	13.0	2.4	-21.2	-2.9	-12.1	10.8	5.2	8.0	4.8	-0.5	2.2	10.4a	-13bc	-1.3	8.2	-3.6	2.3	10.8a	-7.6abc	1.6	5.3	-5.8	-0.3
	V4+V6	-10.4	-12.7	-11.6	18.2	-2.1	8.0	40.8	24.7	32.8	-5.7	-5.7	-5.7	-0.5ab	8.5a	4.0	-5.3	6.0	0.4	-6.2abc	-7.4abc	-6.8	11.1	8.5	9.8
	V6	-8.0	3.8	-2.1	-3.9	-11.0	-7.5	-3.0	9.2	3.1	-0.7	23.0	11.1	2.7ab	3a	2.9	12.9	-19.9	-3.5	-21.5bc	0.4ab	-10.5	11.1	2.0	6.6
	V8	1.7	-35.5	-16.9	13.6	-4.7	4.5	-0.7	5.0	2.1	-3.5	-15.4	-9.4	8a	-22.9c	-7.4	28.7	-5.4	11.7	-35c	11.1a	-12.0	8.5	-6.9	0.8
LSD		*ns*			*Yr = 6.7*			*ns*			*ns*			*Appxyr = 16.1*			*ns*			*AppxYr = 28.6*			*ns*		
Slash Plus	V4	2.4	-19.8	-8.7	6.5	-6.1	0.2	-6.6	2.2	-2.2	2.7	-1.0	0.9	-15.8	-10.9	-13.3b	2.1	13.6	7.9	22.4	13.8	18.1	1.5	-11.4	-5.0
	V4+V6	-9.3	14.2	2.5	-5.6	-4.7	-5.2	49.9	-1.6	24.2	15.9	-14.6	0.6	-7.6	3.1	-2.3ab	31.4	15.1	23.3	-3.6	-17.6	-10.6	8.4	-7.2	0.6
	V6	10.0	-4.9	2.6	5.5	-4.8	0.4	23.1	-3.2	9.9	1.0	-4.1	-1.5	0.5	14.0	7.2a	0.0	1.1	0.6	-27.3	21.2	-3.0	5.0	13.2	9.1
	V8	-4.3	-22.2	-13.3	-10.0	-0.4	-5.2	-7.8	11.0	1.6	10.3	-11.0	-0.3	7.4	15.7	11.5a	23.0	13.2	18.1	-18.8	-10.6	-14.7	19.5	11.3	15.4
LSD		*ns*			*ns*			*ns*			*ns*			*App = 15.6*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	23.0a	16ab	19.5a	-20.4b	-9.3b	-14.8	-15.6	5.6	-5.0	21.9	-1.1	10.4	-12.7	-8.7	-10.7	-5.7	-23.9	-14.8	15.2	8.7	11.9	-10.5c	-2.4bc	-6.5c
	V4+V6	-21.1d	13.4ab	-3.8ab	7.2ab	6.8ab	7.0	26.8	-9.2	8.8	12.7	-2.6	5.0	-0.7	5.4	2.3	-4.8	23.4	9.3	-5.3	-3.3	-4.3	24.1a	22.2a	23.1a
	V6	5.2abc	-9.1cd	-1.9cd	-11b	-2.1b	-6.5	-9.7	-1.6	-5.6	-12.5	0.8	-5.9	-7.4	3.2	-2.1	13.1	-27.8	-7.4	-10.5	-2.3	-6.4	20.2ab	-3.3bc	8.5b
	V8	-3.9bcd	-11.2cd	-7.5cd	30.9a	-12.7b	9.1	-5.3	8.0	1.3	4.8	3.0	3.9	6.2	20.1	13.2	-9.4	14.5	2.6	-16.7	-0.2	-8.4	7.6abc	26.4a	17ab
LSD		*App = 15.1 AppxYr = 23.2*			*AppxYr = 30.5*			*ns*			*ns*			*ns*			*ns*			*ns*			*App = 13.5 AppxYr = 26.1*		

p = 0.1

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

Table 3: The effect of glyphosate application time on the number of kernels produced per row (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-14.5	-9.3	-11.9	-7.6	-6.9	-7.2	15.0	1.4	8.2a	6.3	-7.0	-0.4	4.1	-1.0	1.5	3.1	2.7	2.9	3.2	4.0	3.6	1.9	4.7	3.3b
	V4+V6	-16.2	-16.2	-16.2	0.4	-3.7	-1.6	8.7	4.1	6.4ab	5.5	-0.7	2.4	12.6	1.3	6.9	7.0	1.1	4.1	-8.7	8.3	-0.2	9.5	0.6	5.0b
	V6	-24.0	-1.2	-12.6	-4.4	-7.9	-6.2	-6.1	0.2	-3.0bc	-7.2	7.4	0.1	-5.1	-0.5	-2.8	-5.9	1.0	-2.5	-0.2	-13.2	-6.7	-24.1	-4.9	-14.5a
	V8	-20.3	-0.5	-10.4	23.9	-9.8	7.0	-1.0	-13.6	-7.3c	7.7	-4.7	1.5	2.1	-5.7	-1.8	-4.6	-3.1	-3.9	-1.3	2.7	0.7	-3.8	2.0	-0.9b
LSD		*ns*			*ns*			*App = 9.8*			*ns*			*ns*			*ns*			*ns*			*App = 12.0*		
Roundup PowerMax	V4	-19.1	-2.5	-10.8	-4.8	-1.8	-3.3	10.7	-2.9	3.9	1.6	-13.3	-5.8b	2.0	-1.3	0.3	16.5	0.2	8.4	1.9	0.9	1.4	-0.7	-0.1	-0.4
	V4+V6	-12.5	-9.2	-10.9	-2.7	3.4	0.3	-5.4	0.4	-2.5	10.0	-11.3	-0.6b	7.0	31.4	19.2	-14.6	3.4	-5.6	-14.9	1.2	-6.8	2.0	3.8	2.9
	V6	-20.3	-0.4	-10.4	5.8	-8.2	-1.2	2.5	3.1	2.8	16.3	12.0	14.1a	-13.2	-4.4	-8.8	-3.3	-1.0	-2.2	-1.9	1.5	-0.2	-0.9	0.6	-0.2
	V8	2.4	3.8	3.1	9.5	-1.8	3.8	14.8	-6.5	4.1	-1.1	-10.3	-5.7b	-8.7	-6.0	-7.3	-13.8	1.5	-6.2	5.1	2.2	3.6	-14.3	7.9	-3.2
LSD		*ns*			*ns*			*ns*			*Yr = 12.0* *App = 11.8*			*ns*			*ns*			*ns*			*ns*		
Slash Plus	V4	-10.4	-9.3	-9.9	19.9	-11.6	4.1	-11.8	-10.1	-11.0	-7.3	4.9	-1.2	-10.6	-9.7	-10.1	10.2	3.7	7.0	7.6abc	-5.4bc	1.1	14.7	8.7	11.7
	V4+V6	-8.8	-15.1	-12.0	6.3	-12.0	-2.8	-11.5	-4.6	-8.0	6.7	-0.8	2.9	6.0	-2.3	1.8	10.2	2.2	6.2	16.0a	4.7abc	10.3	11.2	4.8	8.0
	V6	-18.7	-1.3	-10.0	-10.8	-1.1	-5.9	-19.5	-10.1	-14.8	-4.1	3.3	-0.4	0.9	-0.7	0.1	0.3	0.6	0.5	-5.1bc	9.0ab	1.9	-6.8	4.3	-1.3
	V8	-15.8	-6.7	-11.3	2.0	-8.6	-3.3	8.1	-3.5	2.3	-4.5	0.6	-1.9	-5.1	-1.1	-3.1	-7.0	-2.8	-4.9	-6.3c	0.8bc	-2.7	-9.0	-0.3	-4.7
LSD		*ns*			*Yr = 11.0*			*ns*			*ns*			*ns*			*ns*			*AppxYr=14.3*			*ns*		
Touchdown Forte	V4	-5.9	-2.4	-4.2	1.2	3.1	2.1	5.9	5.5	5.7	6.8	0.3	3.6	6.9	-3.9	1.5	6.2	-3.4	1.4	7.2	3.4	5.3	-7.8	5.1	-1.4
	V4+V6	-10.8	-5.1	-8.0	-1.0	-2.8	-1.9	12.4	4.2	8.3	1.3	-2.3	-0.5	-6.0	1.3	-2.4	6.2	-2.9	1.7	-13.7	4.6	-4.6	10.5	7.4	9.0
	V6	-19.5	-3.3	-11.4	8.2	-14.4	-3.1	-12.7	-5.7	-9.2	7.2	0.7	3.9	-4.1	2.7	-0.7	-8.4	-3.5	-6.0	-3.6	1.2	-1.2	-4.2	9.7	2.8
	V8	-19.5	7.1	-6.2	26.9	-5.8	10.6	3.0	2.2	2.6	7.6	-4.0	1.8	-10.3	-1.1	-5.7	-2.2	-1.1	-1.7	-6.2	4.3	-0.9	-7.3	3.0	-2.2
LSD		*Yr = 8.5*			*Yr = 13.1*			*ns*			*ns*			*ns*			*Yr = 2.8*			*ns*			*ns*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

For both KKS4851 and P1814R, lower RpE was achieved in 2018/2019, whereas PAN6R-740BR had lower RpE in the first season (2017/2018).

Only the KKS4851 x Slash combination was significantly affected by the application time as main effect, with a 6.3% increase observed when glyphosate was applied at V6 (Table 4). None of the reductions observed in the remaining treatments were significantly lower than that of the untreated control ($LSD_{(p=0.1)}$ = 7.4).

The application time x year interaction significantly affected BG5785BR (Mamba), P1814R (Mamba) and DKC78-79BR (Touchdown). Of these, only Touchdown applied at V8 to DKC78-79BR during 2017/2018 resulted in a reduction in RpE (10.5%) which was significantly greater than the untreated control ($LSD_{(p=0.1)}$ = 7.7). A similar effect was not observed during the following season.

Seasonal variation was accordingly evident in 9.3% of the cultivar x glyphosate product combinations evaluated, application time in 3.1%, and the application time x year interaction in 9.3% of the cultivar x glyphosate product combinations tested. The observed effect of application time was furthermore unpredictable pertaining to the cultivar x glyphosate product combinations tested across seasons.

Thousand kernel mass

Seasonal variation (year as main effect) significantly influenced TKM obtained by PAN6R-710BR after the application of PowerMax and Slash, with lower TKM generally recorded for 2018/2019 (data not shown).

With application time as the main effect, significant differences were observed in 8 of the 32 cultivar x glyphosate product combinations (Table 5). PAN1814R generally yielded lower TKM with the application of Mamba, PowerMax and Slash at the later application times (V6 and/or V8). However, the reductions were not greater than that observed in the untreated control in any of these cases. A similar effect was observed in PAN6R-710BR, with the application of Touchdown at V8, reducing TKM by 6.1% (also not significantly different from the untreated control). KKS4851 was negatively affected with the application of Slash at V4, whilst the same product negatively affected TKM of KKS8408 at both V4 and V6 (not significantly different from the untreated control). Touchdown applied at V8 to DKC74-74BR resulted in a significant increase in TKM of 8.8% (Table 5). Of the eight cultivar x glyphosate product combinations which were significantly affected by application time, only the P2880BR x Slash combination resulted in significant differences in TKM which were greater than the untreated control ($LSD_{(p=0.1)}$=6.2%), with an 11%

Table 4: The effect of glyphosate application time on the number of rows produced per ear (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	9.7a	-0.3abcd	4.7	-4.2	2.7	-0.7	-5.5	1.0	-2.3	-6.3	-4.9	-5.6	3.5	-0.3	1.6	3.3	-1.9	0.7	-4.2bc	-2.4bc	-3.3	5.3	-0.1	2.6
	V4+V6	2.5ab	-6.1bd	-1.8	-7.0	0.9	-3.0	-5.6	-4.0	-4.8	-1.7	1.6	0.0	-0.8	7.6	3.4	-5.9	0.6	-2.7	9.6a	-1.6bc	4.0	0.4	8.2	4.3
	V6	-2.2bcd	2.3abc	0.0	-2.9	0.0	-1.4	-0.6	2.0	0.7	-10.9	-1.2	-6.0	-1.0	6.9	2.9	-5.4	-0.2	-2.8	-8.7c	0.1bc	-4.3	-13.7	3.8	-5.0
	V8	-2.2bcd	-0.3abce	-1.3	-2.6	0.9	-0.8	-5.6	0.0	-2.8	-0.6	-4.0	-2.3	3.8	2.7	3.2	-14.5	-1.9	-8.2	0.6b	-3.2bc	-1.3	-4.0	-1.3	-2.7
LSD		*AppxYr = 15.6*			*ns*			*ns*			*ns*			*ns*			*Yr = 3.91*			*AppxYr = 10.0*			*ns*		
Roundup PowerMax	V4	11.7	-1.2	5.3	2.5	-4.5	-1.0	-8.0	-4.0	-6.0	-1.2	-3.0	-2.1	8.4	-0.4	4.0	-5.5	-3.6	-4.6	0.2	-1.6	-0.7	0.6	2.9	1.8
	V4+V6	-7.0	9.8	1.4	-2.6	5.5	1.4	-5.6	-3.0	-4.3	-1.7	-0.2	-0.9	-1.0	0.6	-0.2	-5.9	0.7	-2.6	-4.0	0.9	-1.6	0.6	3.4	2.0
	V6	-7.0	0.1	-3.5	-2.6	-2.7	-2.6	4.4	-1.0	1.7	18.8	-4.9	7.0	4.0	-0.5	1.7	-1.0	1.5	0.3	-4.2	1.0	-1.6	0.6	-0.3	0.2
	V8	-2.5	2.3	-0.1	6.3	3.6	5.0	-0.6	-2.0	-1.3	4.0	-9.6	-2.8	-5.9	-0.3	-3.1	-5.9	-3.7	-4.8	5.4	-5.8	-0.2	-4.0	1.9	-1.1
LSD		*ns*			*ns*			*ns*			*Yr = 5.7*			*ns*			*ns*			*ns*			*ns*		
Slash Plus	V4	14.1	1.4	7.8	6.3	4.6	5.5	-3.1	5.0	1.0	-1.7	0.7	-0.5ab	-1.3	-2.5	-1.9	-2.6	-1.0	-1.8	7.0	0.1	3.5	5.3	3.0	4.2
	V4+V6	-1.9	-4.7	-3.3	11.9	0.9	6.4	-0.7	1.0	0.2	-6.3	-3.5	-4.9b	-1.2	5.6	2.2	-5.9	-0.2	-3.1	0.9	-2.4	-0.7	0.3	1.8	1.1
	V6	2.5	-2.1	0.2	2.5	1.8	2.1	-0.7	-3.0	-1.8	13.7	-1.2	6.3a	-3.2	6.8	1.8	3.8	-0.2	1.8	47.5	-2.4	22.6	-4.3	0.6	-1.9
	V8	2.2	-0.3	1.0	6.3	0.9	3.6	-0.6	-1.0	-0.8	-5.7	-3.1	-4.4b	3.8	2.6	3.2	-10.4	-4.5	-7.5	-3.7	2.6	-0.5	5.4	3.4	4.4
LSD		*ns*			*ns*			*ns*			*App = 7.4*			*ns*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	12.1	-8.3	1.9	-7.0	4.5	-1.2	4.4a	-2ab	1.2	-6.3	2.5	-1.9	3.9	-3.7	0.1	-5.6	0.7	-2.5	9.6	-0.7	4.5	0.6	2.1	1.4
	V4+V6	7.3	-2.1	2.6	9.4	2.8	6.1	-5.6bc	1ab	-2.3	3.4	3.6	3.5	-1.0	5.9	2.5	-1.1	-0.2	-0.7	-1.5	-3.2	-2.4	5.3	3.0	4.2
	V6	2.2	1.4	1.8	-7.6	-5.5	-6.5	-5.5bc	-2ab	-3.7	9.1	-0.2	4.4	3.9	4.8	4.4	-5.4	1.6	-1.9	0.2	1.8	1.0	0.6	0.6	0.6
	V8	2.5	-3.8	-0.7	-0.3	-0.9	-0.6	-10.5c	2ab	-4.3	-1.2	-0.2	-0.7	-1.0	-0.6	-0.8	-5.9	1.5	-2.2	9.8	-0.7	4.5	0.9	1.7	1.3
LSD		*ns*			*ns*			*AppxYr = 7.7*			*ns*			*ns*			*ns*			*Yr = 4.5*			*ns*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

reduction recorded at V6. As the application time x year interaction was also significa t for this specific cultivar x glyphosate product combination (Table 5), the interpretation of this result should also take seasonal variation into account.

The application time x year interaction was significant in 4 of the 32 cultivar x glyphosate product combinations (Table 5). TKM of P2880WBR was significantly affected with the application of Mamba, Slash and Touchdown (Table 5). The application of Slash to P2880WBR at V6 was, however, the only treatment combination which resulted in a reduction in TKM (20.3%; 2017/2018), which was significantly greater than the untreated control ($LSD_{(p=0.1)}=9.29$). The effect was not evident in the following season. Of the four cultivar x glyphosate product combinations affected by the highest order interaction, the DKC78-79BR x Mamba combination demonstrated the greatest variation in resultant TKM. Similar to what was observed for the aforementioned parameters, the effect of application time on TKM achieved was inconsistent across application times and seasons. The greatest reduction in TKM for the DKC78-79BR x Mamba combination was achieved at V4 of 2017/2018 (30.3%), followed by a 21.9% reduction at V8 of 2018/2019. The latter treatment indicated a 25% increase for the previous season (V8, 2017/2018).

Seasonal differences were subsequently evident in 6.3% of the cultivar x glyphosate product combinations evaluated. Although application time (as main effect) significantly affected 25% of the cultivar x glyphosate product combinations, a negative impact, which was greater than the untreated control, was observed in only 3.1% of the 32 combinations tested. A total of 12.5% of the cultivar x glyphosate product combinations tested were significantly affected by the application time x year interaction, with only 6.3% resulting in significantly reduced TKM compared to the untreated control. The effect observed was unpredictable regarding cultivar x glyphosate product combinations across seasons.

Yield

Of the five yield-related parameters investigated (EpP, KpR, RpE, TKM and yield), yield was characterised the most by large variations in yield response recorded within the same application time, and across the respective seasons. This contributed to a lower frequency of significant differences observed, regardless of apparent large percentage increases or decreases recorded at various application times, compared to the untreated control (Table 6). This observation is noteworthy and emphasises the need for a greater number of treatment replications for future field trials on glyphosate. Despite the large variation observed, significant differences were obtained, which, with regard to frequency, were somewhat consistent with the frequency of significance observed in the previous four yield-related parameters evaluated (EpP, KpR, RpE and TKM).

Seasonal differences were evident in 1 of the 32 cultivar x glyphosate product combinations (BG5785BR x Mamba), with greater yield reduction observed in 2017/2018 than in the following season.

Table 5: The effect of glyphosate application time on thousand kernel mass (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-7.0	6.5	-0.3	1.9	3.4	2.7	-30.3d	-2.1bc	-16.2	3.5	4.0	3.7	-6.8	2.0	-2.4	2.4	1.5	2.0	0.9	13.3	7.1a	-3.8b	-0.3ab	-2.0
	V4+V6	-8.1	7.1	-0.5	-3.6	-8.1	-5.9	-14.3bcd	2.5b	-5.9	2.0	7.7	4.8	-1.9	-9.1	-5.5	7.5	-1.6	3.0	22.8	11.2	17a	12.9a	-8.7b	2.1
	V6	1.9	-11.5	-4.8	1.1	2.2	1.7	0.6b	0.2bc	0.4	5.1	8.6	6.9	-0.4	-2.1	-1.3	3.8	4.3	4.1	-20.7	4.3	-8.2b	-8.5b	5ab	-1.7
	V8	-2.3	-11.0	-6.7	-0.2	-1.1	-0.6	25a	-21.9cd	1.5	-7.5	10.7	1.6	-9.6	-0.7	-5.2	-4.5	-3.5	-4.0	1.7	9.9	5.8ab	3.9ab	-1.4ab	1.2
LSD		*ns*			*ns*			*AppxYr = 21.8*			*ns*			*ns*			*ns*			*App = 14.25*			*AppxYr = 20.69*		
Roundup PowerMax	V4	7.0	14.5	10.5	2.6	0.5	1.5	14.7	0.1	7.4	5.2	6.0	5.6	-4.3	-0.5	-2.4	9.9	1.5	5.7	13.1	14.8	14a	7.1	6.0	6.6
	V4+V6	-4.0	2.0	-1.0	7.1	-0.5	3.3	-8.6	1.8	-3.4	1.7	2.3	2.0	-6.6	-1.8	-4.2	3.4	-12.8	-4.7	2.7	6.9	4.8ab	-11.6	6.0	-2.8
	V6	-3.0	-10.0	-6.5	8.2	1.9	5.1	-1.8	-8.0	-4.9	-3.5	3.9	0.2	-3.3	1.6	-0.9	0.6	-12.6	-6.0	-13.0	6.3	-3.4b	-2.4	2.9	0.3
	V8	-10.0	31.0	10.5	6.3	-3.2	1.6	7.3	-3.8	1.7	5.1	-4.1	0.5	-2.6	3.6	0.5	4.8	-1.0	1.9	-14.9	9.2	-2.8b	-3.1	1.7	-0.7
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*Yr = 5.15*			*App = 10.6*			*ns*		
Slash Plus	V4	-2.7	-4.0	-3.4	-16.1	-2.1	-9.1	-8.1	0.6	-3.8	-8.9	4.5	-2.2c	-17.4	1.7	-7.9b	1.8	-5.1	-1.7	8.7	12.1	10.4a	1.2a	5.2a	3.2a
	V4+V6	-4.3	-0.7	-2.5	0.4	4.3	2.3	-3.8	-0.2	-2.0	9.4	3.8	6.6ab	6.3	7.4	6.8a	4.6	-8.6	-2.0	14.7	13.0	13.8a	5.4a	2.7a	4.1a
	V6	-5.4	1.1	-2.2	-2.2	-2.4	-2.3	-3.6	-7.1	-5.4	1.6	2.0	1.8bc	-12.7	-2.7	-7.7b	11.3	-0.6	5.4	2.5	-1.9	0.3b	-20.3b	-1.6a	-11b
	V8	-17.1	30.7	6.8	-3.1	-1.7	-2.4	11.0	-14.5	-1.7	5.1	14.6	9.9a	-4.2	5.5	0.7ab	7.1	1.0	4.1	0.5	-1.9	-0.7b	6.2a	1.8a	4a
LSD		*ns*			*ns*			*ns*			*App = 7.1*			*App = 9.72*			*Yr = 8.4*			*App = 9.87*			*App = 6 .15* *AppxYr = 9.29*		
Touchdown Forte	V4	0.2	5.3	2.8	0.7	3.1	1.9b	-4.7	-4.0	-4.3	4.2	9.8	7.0	-3.4	0.9	-1.2	5.6	2.2	3.9a	4.4	1.6	3.0	0.53abc	3.49abc	2.0
	V4+V6	1.7	4.1	2.9	2.0	-0.9	0.6b	0.3	4.1	2.2	-2.7	0.5	-1.1	-13.1	-1.5	-7.3	5.5	2.2	3.9a	-7.4	6.3	-0.5	-0.78ac	7.91a	3.6
	V6	-13.0	-0.1	-6.6	1.4	-0.1	0.6b	1.9	2.5	2.2	-12.5	7.9	-2.3	-6.1	0.0	-3.0	16.8	-0.1	8.3a	10.6	4.9	7.8	5.64ab	-2.35bc	1.6
	V8	-4.3	4.9	0.3	5.4	12.1	8.8a	3.0	1.8	2.4	1.6	3.5	2.5	-5.3	-2.1	-3.7	-8.6	-3.6	-6.1b	2.7	-2.8	-0.1	1.53abc	1.26bc	1.4
LSD		*ns*			*App = 5.8*			*ns*			*ns*			*ns*			*App = 8.33*			*ns*			*AppxYr = 11.5*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

Three glyphosate product combinations were affected by application time as the main effect. PAN6R-710BR showed 20% and 8.5% yield reductions with the application of Mamba at V8 and V6, respectively ($LSD_{(p=0.1)}$=16.4). KKS4851 recorded 19% and 17.3% yield reductions with the application of PowerMax at V4+V6 and V8, respectively, whilst P2880WBR demonstrated a 12.3% yield reduction with the application of Touchdown at V4. The yields of both KKS4851 and P2880WBR were, however, significant y influenced by the application time x year interaction, and interpretation should take seasonal variation into account.

Yield was signific ntly influenced by the application time x year interaction in five cultivar x glyphosate product combinations, of which only one resulted in a yield which was significantly lower than that of the untreated control. The application of PowerMax to KKS4851 reduced yields by 28.1% and 27.1% when applied at V4+V6 and V8, respectively, during 2018/2019, which in both instances was greater than that of the untreated control ($LSD_{(p=0.1)}$=18.6). Yield reductions of 23.4% (V6) and 26% (V8) were observed in 2017/2018 and 2018/2019, respectively, with the application of Mamba to KKS4851 (but were not significantly greater than that of the untreated control). A 22.7% reduction was observed in KKS8408 with the same product, applied at V4 (but was not significantly greater than that of the untreated control). In P1814R, PowerMax reduced yield by 38.2% and 31.3% when applied at V6 and V8, respectively, during 2017/2018 (not greater than the untreated control). A similar effect was not observed in the following season.

Yield was accordingly significantly impacted by seasonal variation in 3.1% of the cultivar x glyphosate product combinations evaluated. A total of 9.3% of the cultivar x glyphosate product combinations were influenced by glyphosate application time alone and 15.6% by the application time x year interaction. However, significant yield reduction, which was greater than that of the untreated control, occurred in only 3.1% of the cultivar x glyphosate product combinations evaluated.

Discussion

Many research studies on maize and the effects of herbicides have focused on weed control and grain yields, with little focus on how herbicide applications may possibly affect growth and development of maize plants that have resistance to a particular herbicide (as tested in the absence of weeds).[6] Our objective in this study was to determine if label rate glyphosate, applied at different growth stages and in the absence of weed competition, alters the development of South African GR maize cultivars during the growing season in such a way that it would cause a reduction in yield.

The optimum growth in a maize crop occurs in climates with mid-summer temperatures of between 21 °C and 27 °C.[19] The average temperature ranges experienced in both seasons during this study – especially those in the months of November, December and January – were up to 5 °C above the documented and accepted norm of 27 °C. According to Thelen and Penner[7], sub-optimum growth conditions might result in additional

Table 6: The effect of glyphosate application time on yield (% of control) of eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-0.1	15.0	7.5	-9.9	-14.1	-12.0	5.2	11.8	8.5	-1.9ab	12.6a	5.3	14.7a	-22.7b	-4.0	12.3	7.3	9.8a	43.9	-2.1	20.9	13.8	2.8	8.3
	V4+V6	-40.9	26.5	-7.2	-33.8	14.6	-9.6	-3.9	4.0	0.0	17.8a	-5.6ab	6.1	0.1ab	2.7a	1.4	9.2	13.4	11.3a	42.5	24.8	33.7	3.3	-9.5	-3.1
	V6	0.4	3.1	1.8	-34.3	-4.9	-19.6	-14.6	-12.8	-13.7	-23.4b	10.1a	-6.7	-8.4ab	4.9a	-1.8	9.7	-26.7	-8.5b	-19.8	3.1	-8.4	8.6	0.7	4.7
	V8	-13.5	5.5	-4.0	45.3	-16.9	14.2	41.3	7.3	24.3	8.3a	-26b	-8.9	11a	-10.9ab	0.0	-21.5	-18.5	-20b	-6.9	-13.1	-10.0	-14.6	-18.3	-16.5
LSD		*Yr = 22.7*			*ns*			*ns*			*AppxYr = 32.6*			*AppxYr = 27.7*			*App = 16.4*			*ns*			*ns*		
Roundup PowerMax	V4	-9.0	30.2	10.6	-22.0	6.8	-7.6	-1.7	7.2	2.8	-2b	2.1b	0.1b	13.3	-13.2	0.0	31.1	-8.7	11.2	37.2a	-0.5abcd	18.3	-3.1	-9.8	-6.5
	V4+V6	-22.0	-23.2	-22.6	22.9	2.4	12.7	65.5	12.0	38.8	-9.8bc	-28.1c	-19.0c	15.7	3.3	9.5	-6.4	-1.4	-3.9	-6.3bcd	4.1abc	-1.1	-4.9	-8.0	-6.5
	V6	-35.5	6.4	-14.6	22.5	-3.8	9.3	17.4	0.9	9.2	0.0b	28.3a	14.1a	-8.4	4.8	-1.8	7.8	-23.1	-7.7	-31.3cd	12.4ab	-9.4	-5.2	-10.4	-7.8
	V8	-20.8	-2.7	-11.8	45.0	-2.5	21.2	1.0	-2.5	-0.8	-7.5b	-27.1c	-17.3c	-2.0	-16.0	-9.0	11.0	-3.5	3.8	-38.2d	8.8abc	-14.7	-7.6	-4.3	-6.0
LSD		*ns*			*ns*			*ns*			*App = 11.7 AppxYr = 18.6*			*ns*			*ns*			*AppxYr = 40.3*			*ns*		
Slash Plus	V4	-1.6	-40.0	-20.8	13.3	-8.3	2.5	-30.1	-8.4	-19.3	5.2	10.4	7.8	2.0	-13.6	-5.8	4.0	14.0	9.0	43.2	-4.1	19.6	-20.1	-18.0	-19.1
	V4+V6	-23.4	27.1	1.9	10.1	-2.3	3.9	71.7	3.2	37.5	7.3	-16.9	-4.8	-24.1	3.4	-10.3	6.0	8.4	7.2	12.9	11.5	12.2	4.9	-13.5	-4.3
	V6	-11.9	14.5	1.3	-3.3	-3.3	-3.3	13.3	-6.6	3.3	-16.2	5.5	-5.4	0.7	8.9	4.8	0.8	-4.1	-1.7	-14.7	17.5	1.4	-1.2	0.8	-0.2
	V8	-14.4	17.0	1.3	-4.9	6.9	1.0	-0.5	5.5	2.5	-2.4	-2.5	-2.5	2.6	11.8	7.2	8.5	9.2	8.9	-11.9	-16.9	-14.4	-8.9	-4.6	-6.8
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	5.2	11.0	8.1	-25.8	-3.2	-14.5	-26.0	3.4	-11.3	8.8	4.2	6.5	1.9	-20.7	-9.4	0.9	-27.6	-13.4	45.7	10.5	28.1	-22.8bd	-1.8abcd	-12.3c
	V4+V6	-26.2	11.1	-7.6	10.7	5.4	8.0	51.4	-14.3	18.6	-6.6	-4.2	-5.4	14.0	7.4	10.7	-1.0	15.3	7.2	-32.0	-1.7	-16.8	31.4a	7.1ab	19.3a
	V6	-35.7	-7.2	-21.5	-0.9	6.5	2.8	-14.5	-0.6	-7.5	-21.0	13.2	-3.9	-12.6	4.6	-4.0	7.9	-27.5	-9.8	-10.1	20.5	5.2	32.3a	-9.2abcd	11.6ab
	V8	-29.5	-5.7	-17.6	71.6	-0.7	35.4	4.0	19.2	11.6	-10.7	11.1	0.2	0.4	22.9	11.6	-17.9	9.0	-4.5	-12.5	6.4	-3.1	-1.2bc	6.8ab	2.8b
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*App = 12.6 AppxYr = 43.3*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

stressors on the plant and mask the possible effect that glyphosate might have on maize yield. Pline et al.[20] similarly reported greater sensitivity to glyphosate injury in GR soybean at higher temperatures, as warmer temperatures resulted in greater translocation of glyphosate to new meristematic areas within the plant. Despite the current study being conducted under conditions considered above the international norm for maize production[19], supplementary irrigation provided throughout the duration of the trials would have lessened the level of drought/heat stress experienced by the plants to some extent. Large variation in yield data generated within the same application time of various cultivar x product combinations nevertheless occurred, despite supplementary irrigation and the weed-free environment maintained throughout the season, suggesting that additional unknown external factors were at play. Our findings in this regard concur with that of the international community in that determining yield differences between glyphosate-treated and non-treated GR cultivars remains a challenge due to the influence of other environmental factors.[20] Future glyphosate yield related field studies would benefit greatly from more treatment replicates.

Investigating the possibility that glyphosate application at specific application times or growth stages would result in a more favourable yield response in GR cultivars (in the absence of weeds), it was necessary to assess whether any significant patterns became evident amongst the 32 cultivar x product combinations tested. Whether the glyphosate application time resulted in a response significantly greater or smaller than that of the untreated control, is accordingly not of importance here. A digestible manner in which the data could be approached is by first establishing the frequency at which significant differences occurred amongst the 32 cultivar x glyphosate product combinations. In this regard, application time as main effect resulted in significant differences observed in between 3.1% (RpE) and 25% (TKM) of the cultivar x glyphosate product combinations tested, depending on the yield-related parameter in question, whilst the application time x year interaction resulted in significant differences in 3.1% (KpR) to 21.8% (EpP) of the cultivar x glyphosate product combinations tested. From this result, it is evident that less than a quarter of the cultivar x glyphosate product combinations tested were affected in one way or the other by the application time of glyphosate, whilst in some cultivars, the effect, where present, was season dependent. Yield, being the most relevant parameter, was significantly affected by application time as main effect in 9.3% of the combinations tested, and by the application time x year interaction in 15.6% of the combinations tested. Focussing on the highest order interaction, no consistent pattern was evident across cultivar or product. A unique response was accordingly obtained by each of the cultivar x product combinations, which demonstrated significant differences, suggesting that it will be impossible to predict how any cultivar x product combination would react to glyphosate application at various growth stages.

The main concern of local producers is, however, whether the application of glyphosate itself, in the absence of weed pressure, results in a yield loss due to some form of genetic predisposition. In a recent local study, Odendaal[21] concluded that glyphosate application resulted in a reduction in plant height, dry mass and yield when applied at different growth stages of GR maize. The study, which evaluated two glyphosate products and five GR maize cultivars, also indicated that different GR maize cultivars showed significant variation in reaction to glyphosate applications, suggesting that some cultivars are more sensitive/tolerant to glyphosate than others. Seasonal variation was, similar to the current study, prominent within the field trials. In the current study, negative or positive values greater than the LSD represent instances in which a specific treatment resulted in a response which was either greater or poorer than that of the untreated control. In less than 6% of all cases where significance was observed in EpR, KpR, RpE, TKM or yield, a significant negative effect could be observed which was greater than that for the untreated control. For yield, only one cultivar x glyphosate product combination (KKS4851 x PowerMax) suffered yield losses which could be attributed to the application time of glyphosate. In this case, the effect was season dependent and was evident at the V4+V6 and V8 growth stages. Based on this observation, our findings from the current study concur with international research findings that the yield of GR maize cultivars is not significantly affected by the application of glyphosate or the application time thereof[8,13], under growing conditions in which water is not a limiting factor.

Conclusions

Whether the application of herbicides to transgenic, herbicide-resistance crops has a negative effect on crops, either directly or indirectly, has always been a controversial topic, with most of the controversy focussed on GR crops. Limited response was observed with the application of glyphosate at V4, V4+V6, V6 and V8 stages on eight South African GR maize hybrids evaluated with four different glyphosate products over two seasons. Only 3.1% of the cultivar x glyphosate product combinations evaluated showed a significant negative yield response which was greater than that of the untreated control. Based on the findings of the current study, we conclude that the application time of glyphosate (within label recommendations) did not affect yield consistently or sufficiently enough for it to be considered a threat to maize yields as evaluated with four glyphosate products and eight GM maize cultivars.

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Competing interests

We have no competing interests to declare.

Authors' contributions

M.C.: Project management, methodology, writing the first draft. P.T.M.: Data collection, writing revisions. L.M.: Data analyses, validation, writing revisions. A.E.J.S.d.T.: Project leadership, writing revisions, mentorship to M.C.

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Application of a soil quality triad in assessing ecological risk posed to croplands

Healthy soil ecosystems fulfil multiple functions (e.g. cycling nutrients and controlling pests), which play an important role in sustainable food production. However, the application of polluted irrigation water poses a major risk to soil quality (health) and warrants investigation to ultimately inform decision-making. We hypothesised that the standardised soil quality TRIAD approach (ISO 19204), which integrates the chemistry, ecology, and ecotoxicology lines of evidence, can be used as part of an ecological risk assessment of cropland soils. To investigate the applicability of this approach in an agricultural setting, we collected soils from croplands associated with the Hartbeespoort and Crocodile (West) irrigation schemes, which utilise water known to be heavily impacted by anthropogenic (metal, nutrient, and salt) pollution. Croplands associated with the Marico-Bosveld Irrigation Scheme served as the reference systems. Data from the three lines of evidence were scaled, weighted, and integrated. Moderate risk was evidenced for nutrient and salt content in most croplands associated with the Hartbeespoort Irrigation Scheme. However, either no or low risk was recorded for the ecology and ecotoxicology lines of evidence. Finally, the integrated risk assessment concluded that only low ecological risk was posed to soil quality, likely as a result of agricultural activities (e.g. tillage and fertiliser application) that deteriorated soils also at the reference system. This study shows important limitations in the application of ecological risk assessments in conventionally farmed soils, but still holds promise for organic and conservation systems.

Significance:

- A pollution linkage between irrigation water and cropland soils was evidenced, but presented only minimal risk to soil quality.
- Conventional agricultural practices (e.g. tillage) mask the risk posed by environmental pollution and impair the applicability of ecological risk assessments.
- It is possible that this approach can be applied in less disturbed crop production systems, for example in conservation (regenerative) and organic croplands.

Introduction

Agricultural output is required to double within the next 40 years in order to meet predicted global demands.[1] Producers and policymakers, as well as the general public, are increasingly aware of this need and the role that promoting soil quality (health) plays in sustainable agriculture.[2] However, soils in many agricultural systems are threatened by anthropogenic activities that result in environmental pollution.[3-5] Good examples of threatened systems are the Hartbeespoort and Crocodile West irrigation schemes, which utilise water from the heavily polluted Crocodile (West) River.[5,6] Therefore, where a pollution linkage exists, soil quality should be assessed and monitored in order to predict and mitigate the subsequent effects.[7]

Traditionally, soil quality assessments in agricultural systems were based on physico-chemical (abiotic) properties that influence crop yield and quality, while biotic attributes were mostly disregarded.[8] However, soils can be viewed as living ecosystems with the associated faunal assemblages fulfilling important functions including plant disease, insect and weed control; carbon transformation; nutrient cycling; and soil structure maintenance.[9-11] Assessing and monitoring soil quality thus requires a holistic approach that integrates both abiotic and biotic measurements. Although recently developed soil quality assessment frameworks for crop production (e.g. Soil Health Tool[8]) follow a more integrative approach, the aim of these frameworks is primarily to measure soil quality restoration and determine fertiliser needs. Therefore, these frameworks do not consider the ecological risk posed by anthropogenic pollution and also lack ecotoxicological perspective.

The TRIAD approach, in turn, incorporates the chemistry, ecology, and ecotoxicology lines of evidence (LOEs). Although this approach was originally developed as a sediment quality assessment[12], it has also been used to evaluate soil quality[13-15]. Yet, a framework for undertaking a soil quality TRIAD as part of an ecological risk assessment (ERA) was only standardised within the last few years.[7] According to ISO19204[7], each LOE is represented by one or multiple appropriate tests of which the data are scaled, for example between 0 (no effect) and 1 (maximum effect), and, if necessary, weighted (to reduce uncertainty). Finally, an integrated (combined) ecological risk number is calculated, which can be used as a decision support tool to inform policymakers on the necessity and urgency of mitigating ecological disturbance.

In this study, we applied the soil quality TRIAD approach and hypothesised that it could be used as part of an ERA of cropland soils. To our knowledge, this report is the first on the use of the standardised soil quality TRIAD approach, as part of an ERA, to evaluate the risk posed to cropland soils.

Material and methods

Site description

The study sites consisted of four croplands (HB1, HB2, HB3, and HB4) associated with the Hartbeespoort Irrigation Scheme (South Africa), which receive water via a canal system from the Hartbeespoort Dam, a major reservoir of the Crocodile (West) River System. An additional two croplands (CW5 and CW6), associated with the Crocodile (West) Irrigation Scheme (South Africa), which abstract water directly from the Crocodile (West) River downstream of the Hartbeespoort Dam, were also selected for investigation.

The Crocodile (West) River System is severely affected by pollutants (e.g. metals, nutrients, and salts) that originate from urban, industrial, and agricultural run-off, sewage effluent, as well as wastewater discharge.[6,16,17] According to Du Preez et al.[6], a cause for concern is the increase in salt and nutrient concentrations recorded from 2005 to 2015. Two croplands associated with the Marico-Bosveld (Ref 7 and Ref 8) Irrigation Scheme (South Africa) were selected as reference sites, as they receive water from the minimally impacted Marico River.[6,18]

Two sampling events were undertaken during March/April and September/October 2016 at all the listed study and reference sites. During the first sampling interval, the selected croplands were subjected to soybean crop production, while different crops (beetroot [*Beta vulgaris* L.], carrot [*Daucus carota* L.], maize [*Zea mays* L.], soybean [*Glycine max* L. Merrill], and wheat [*Triticum aestivum* L.]) were cultivated during the second sampling interval on the respective croplands. See Supplementary table 1 for further details.

Chemistry LOE: Sampling, processing, and analysis of soil

The stepwise execution of the soil quality TRIAD and ERA is schematically illustrated in Figure 1. For assessments associated with the chemistry LOE, 12 composite samples (consisting of five sub-samples each) of rhizosphere soils were collected per cropland. These sub-samples were collected following a diagonal sampling pattern[19] along 12 evenly spaced lines (one line per composite sample) extending from the centre to the edge of the irrigated croplands[5]. Using a clean hand shovel, soil was sampled up to a depth of 20 cm. These samples were transported and stored at -20 °C until further processing.

Soil samples were homogenised, dried at 40 °C for 48 h, and sieved (<2 mm). Subsequently, soil water (capillary water that occupies soil pores) was extracted using the saturated paste extraction method.[20] Although laborious and time consuming, this method is generally regarded as the most accurate measure of soil salinity under field conditions.[21,22] Extracted soil water samples were vacuum filtered with a 0.45-μm Sartorius CN sterile membrane, which allowed analysis of the dissolved fraction of metals, nutrients, and salts.

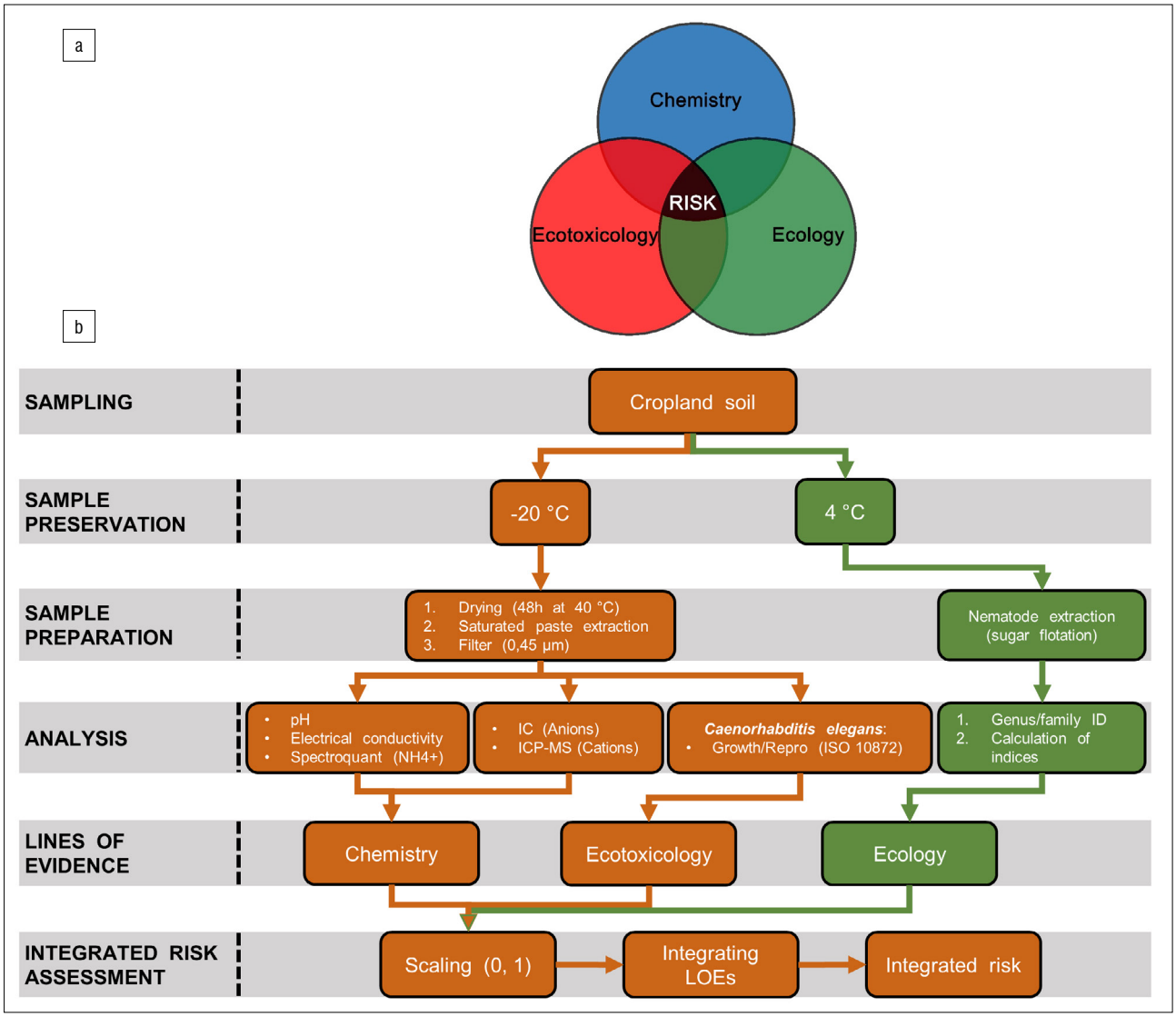

Figure 1: The (a) soil quality TRIAD approach and (b) its stepwise execution, which includes the integration of the chemistry, ecology, and ecotoxicology lines of evidence (LOEs) in order to calculate the ecological risk posed to cropland soils.

Electrical conductivity and pH were measured using WTW Cond 3210 and Mettler Toledo FE20 meters, respectively. Ion (calcium [Ca], magnesium [Mg], phosphorus [P], potassium [K], and sodium [Na]) and metal concentrations (see 'Scaling, weighting, and integration of TRIAD results' for a list of analysed metals) were measured using an Agilent 7500 CE series ICP-MS, while major anion (chloride [Cl], nitrate [NO_3], nitrite [NO_2], and sulfate [SO_4]) concentrations were quantified with a Metrohm 930 Compact IC Flex. A Pharo 300 Spectroquant was used to measure ammonium (NH_4) concentrations, while total alkalinity (pH < 8.2) was quantified by means of titration

Ecology LOE: Sampling, extraction, and analysis of nematode assemblages

For the characterisation of nematode assemblages (ecology LOE), rhizosphere soils were collected following the same methodology as described above. However, due to the typical heterogeneous distribution of nematodes in croplands, 20 composite samples (consisting of five sub-samples each) per cropland were analysed for this LOE. In total, 320 soil composite samples were collected and stored at 4 °C until further processing.

Soil samples were homogenised and nematodes were extracted from a 200-g representative aliquot using the decanting and sieving, sugar centrifugal flotation method.[23] Nematodes were stored in 10 mL filtered tap water at 6–8 °C and counted (within 2 weeks of extraction) using a Nikon Eclipse 50i light microscope (100× magnification). Family level occurrence and abundance data were generated in order to calculate the Maturity Index, used to classify soil ecosystems on a scale from 1 (disturbed/enriched) to 5 (mature/structured)[24], by applying the Nematode Indicator Joint Analysis (NINJA) web-based tool[25].

The Shannon Diversity Index was calculated as follows:

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i) \qquad \text{Equation 1}$$

where *pi* represents the proportion of the *i*-th taxa in a sample.[26] The inclusion of this index was considered appropriate as healthier soils typically present a higher diversity of biota.[27]

Ecotoxicology LOE: Measuring the toxicity of soil water samples

Because electrical conductivity serves as a measure of the concentration of dissolved ions[28], it was used as a proxy for salt and nutrient content. Subsequently, from each farmland, the sample with the highest electrical conductivity per sampling interval (Supplementary table 2) was selected for further investigation. *Caenorhabditis elegans* (sourced from the Caenorhabditis Genetics Centre, University of Minnesota, Twin Cities, United States of America) was used as the test organism, as this nematode species has been well established as a model organism for ecotoxicological studies.[29] Therefore, following ISO10872[30], the growth and reproduction of *C. elegans* was determined after exposure (96 h at 20 °C) to extracted soil pore water samples. Four replicates of each of the selected samples were tested. The negative control consisted of M9 medium, while a positive control (benzylcetyldimethylammonium chloride monohydrate [BAC-C16]) was included to ensure the validity of the test results.[30] The EC50 value of BAC-C16 was calculated (results not shown) as 16.94 mg/L. In order for the test to be valid, the percentage growth inhibition for the positive control should be between 20% and 80% when measured against the negative control.[30]

The growth and reproduction results were expressed as the percentage inhibition (against the negative control) as follows:

$$\%Inhibition = (100 - \frac{\bar{x}S}{\bar{x}C}) \times 100 \qquad \text{Equation 2}$$

where $\bar{x}S$ and $\bar{x}C$ represent the mean of the parameter for a cropland and the negative control, respectively. Furthermore, the data were tested for normality using the D'Agostino and Pearson omnibus test, after which the unpaired t-test (parametric data) or Mann–Whitney test (non-parametric data) was used to test for significant differences between the means. For parametric data with an unequal number of replicates, Welch's correction was applied. Significance for all univariate analyses was regarded at $p<0.05$ and performed using the Graphpad Prism 6 software package.

Scaling, weighting, and integration of TRIAD results

Based on the criteria listed in Table 1, scaling from 0 (no effect) to 1 (maximum effect) and weighting of results were first applied within each LOE, after which the integrated ecological risk was calculated. It should be noted that if any of the tests presented risk lower than the reference sites (averaged), a risk value of 0 was assigned.[14]

For the chemistry LOE, the concentrations of metals, nutrients, and salts were considered. Only metals (aluminium [Al], arsenic [As], chromium [Cr], copper [Cu], manganese [Mn], selenium [Se], uranium [U], and zinc [Zn]) for which a target water quality range (TWQR) is provided in the *South African Water Quality Guidelines: Aquatic Ecosystems*[31] were included in the assessment. These guidelines were used because no criteria exist for soil water extracted using the saturated paste method. Nonetheless, these guidelines have been developed by considering the toxic effect of dissolved metals on faunal assemblages[31] and therefore were deemed appropriate for use in this study[13]. However, to compensate for uncertainty associated with the use of these guidelines, scaled result values were weighted (see below). The concentration of each metal (averaged per cropland) was scaled as follows[13]:

Table 1: Ecological risk assessment analysis and criteria for each line of evidence

Line of evidence	Analysis	Criteria	Scaling (0–1)
Chemistry	Soil water (capillary water that occupies soil pores) content (dissolved)	**Metal content**: TWQR (Target water quality range as listed in the *South African Water Quality Guidelines: Aquatic Ecosystems*) and reference sites. **Nutrient and salt contents:** Reference sites	**Metals:** Ratio to TWQR value and background correct (reference sites) **Nutrient and salts:** Site hazard quotient calculation based on ratio-to-reference approach. Assignment of hazard classes to ranges on 0–1 scale
Ecotoxicology	*Caenorhabditis elegans*: growth and reproduction inhibition/stimulation	Reference sites	Integration using BKX ('bodemkwaliteitsindex') method with background correct (reference sites)
Ecology	Maturity Index	Nematode-specific index ranging from 1 (disturbed/enriched) to 5 (mature/structured)	Integration using BKX ('bodemkwaliteitsindex') method with background correct (reference sites)
	Shannon Diversity Index	Lower diversity = greater disturbance	

$$R_1 = 1 - [1/(1+[\frac{m}{TWQR}])] \quad \text{Equation 3}$$

$$R_2 = \frac{R_1 - R_{1ref}}{1 - R_{1ref}} \quad \text{Equation 4}$$

where m and ref represent the concentration of the metal at the study and reference sites, respectively, and TWQR the target water quality range for the specific metal. R_1 and R_2 denote the first and second step (i.e. result 1 and result 2) of the scaling approach, respectively.

The combined risk presented by the selected metals at each site was calculated as follows:

$$Risk = [1-([1-R_2]_1 \times [1-R_2]_2 \times [1-R_2]_3 \ldots [1-R_2]_3 \ldots [1-R_2]_n)^{\frac{1}{n}}] \times W \quad \text{Equation 5}$$

where n represents the number of metals and W the weighting factor (of 0.8), which accounts for the uncertainty associated with the use of the specified target water quality criteria [7]

However, the risk posed by nutrient (inorganic N [$NO_2 + NO_3 + NH_4$] and P) and salt (Cl, SO_4, Ca, K, Mg, and Na) ions were calculated differently as the South African Department of Water Affairs and Forestry[31] does not provide TWQR values for most salts. The combined risk was calculated based on the ratio-to-reference (RTF) method as implemented by Piva et al.[32] and Li et al.[33] as follows:

$$RTF = \frac{C_{site}}{C_{ref}} \times Z \quad \text{Equation 6}$$

where C_{site} and C_{ref} represent the concentration of the constituent (nutrient or salt ion) at the study and reference sites, respectively. Z represents the statistical significance (p-value) between the means of the study and reference sites as determined using an analysis of variance test. Z equals 1 [if $p<0.05$], $3.5 - (50 \times p)$ [if $0.05 \leq p \leq 0.06$], or $0.2 \times p^{-0.3257}$ [if $0.06 < p \leq 1$]. Analysis of variance tests were performed using the Graphpad Prism 6 software package. Thereafter, the hazard quotient (HQ) was calculated per site as follows:

$$HQ_{nutrients\ +\ salts} = (\%param_{RTF<1.3} \times 1) + (\%param_{1.3\leq RTF<2.6} \times 3) + (\%param_{2.6\leq RTF<6.5} \times 9) + (\%param_{6.5\leq RTF<13} \times 27) + (\%param_{RTF\geq 13} \times 81) \quad \text{Equation 7}$$

where $\%param_{RTF}$ is the percentage of RTF values within the specified range to the total number. Based on this assessment, each site's hazard level can be categorised in one of five classes, namely: Absent ($HQ=100$), Slight ($100<HQ<300$), Moderate ($300\leq HQ<900$), Major ($900\leq HQ<2700$), and Severe ($2700\leq HQ\leq 8100$).[33] However, in order to integrate these results into the ERA, each class was scaled by assigning an equal range between 0 and 1 as follows: Absent (0–0.2), Slight (0.21–0.4), Moderate (0.41–0.6), Major (0.61–0.8), and Severe (0.81–1). This was achieved by setting the limits of each HQ class to represent the limits of the corresponding scaled class and adjusting the values accordingly.

The risk results of the (1) metals and (2) nutrients and salt assessments were integrated into a single risk number (between 0 and 1) per site per sampling interval as follows:

$$Risk = 1-[(1-R_{metals}) \times (1-R_{nutrients+salts})]^{1/2} \quad \text{Equation 8}$$

The ecology (Maturity and Shannon Diversity indices) LOE was scaled using the BKX ('bodemkwaliteitsindex') method, as this allows results from different tests (within a LOE) to be integrated, while both lower and higher than reference values can be used.[13] The following equation was applied:

$$BKX = 1 - 10^{[(-\Sigma|logx_n|)/n]} \quad \text{Equation 9}$$

where x is the ratio between the study and reference sites and n is the number of results (toxicity endpoints).

The BKX method was also used for scaling the ecotoxicology (*C. elegans* growth and reproduction inhibition tests) LOE.

Finally, the integrated ecological risk number (between 0 and 1) for the chemistry, ecology, and ecotoxicology LOEs was calculated per site per sampling interval as follows:

$$Integrated\ ecological\ risk = 1 - [(1 - R_{chemistry}) \times (1 - R_{ecology}) \times (1 - R_{ecotoxicology})]^{1/3} \quad \text{Equation 10}$$

Equal weights (of 1) were assigned to risk numbers calculated for each LOE. Following Jensen et al.[13], each risk number was categorised as presenting either no, low, moderate, or high risk. Lastly, the standard deviation between the LOEs was calculated in order to evaluate the concordance between the three LOEs.[7,13]

Results and discussion

Soil quality TRIAD assessment

For the execution of the ERA, data for the ecotoxicology LOE were generated and are reported below, while data for the chemistry and ecology LOEs were sourced from Du Preez et al.[5] The latter authors found that the studied croplands, when compared against the reference sites, were irrigated with water that contained elevated salt and nutrient (inorganic N and P) concentrations, which influenced especially Na concentrations in the soil (chemistry LOE). Furthermore, nematode-specific and general community indices (ecology LOE) showed that the studied croplands, including the reference sites, presented disturbed soil ecosystems. (See Du Preez et al.[5] for a detailed discussion on the results.)

Results from the ecotoxicology LOE are presented as the percentage inhibition (against the negative control) of *C. elegans* growth and reproduction (Table 2). The percentage growth inhibition for the positive control, also measured against the negative control, was calculated as 55.3%; the tests were thus valid (i.e. ranging between 20% and 80% inhibition). Significant ($p<0.05$) inhibition of growth was observed for HB2 (5.8%), HB3 (5%), and HB4 (8.2%) during the first sampling interval. During the second sampling interval, significant ($p<0.05$) growth inhibition was observed for HB1 (9%) and HB4 (6.7%), while significant ($p<0.05$) stimulation was observed for HB3 (-7.7%). Reproduction of *C. elegans* was significantly ($p<0.05$) inhibited during the first sampling interval at all croplands associated with the Hartbeespoort Irrigation Scheme, as well as Ref 8 (19.5%). During the same sampling interval, *C. elegans* reproduction was significantly ($p<0.05$) stimulated for CW 6 (-44.4%) and Ref 7 (-31.6%). Furthermore, during the second sampling interval, HB1 (31.1%), CW6 (26.6%), and Ref 7 (38.4%) presented significant ($p<0.05$) reproduction inhibition, while HB3 (-37.7%) and CW5 (-42.1%) presented significant ($p<0.05$) reproduction stimulation.

The ecotoxicology results indicated that substantial variability occurred between the executed tests (growth and reproduction), as well as between sampling intervals. *Chaenorhabditis elegans* reproduction data also presented larger inhibition/stimulation ranges, when compared against growth, which indicates that reproduction was likely more sensitive. This supports findi gs by Höss et al.[34] who studied the response of *C. elegans* to contaminated soils and found reproduction to be the most sensitive parameter (compared to growth and fertility). Furthermore, the reproduction of target organisms is regarded as being more ecologically relevant than growth.[35] This represents one of the key advantages of *C. elegans* toxicity testing because intact (whole) individuals with different functioning physiological systems (e.g. digestive and reproductive) are exposed[36], resulting in the potential measurement of multiple physiological endpoints. The stimulation of *C. elegans* reproduction can be regarded as a toxic response (e.g. hormesis)[37] and was therefore included in the ERA.

Integrated ecological risk assessment

When considering the chemistry LOE, nutrient and salt contents presented moderate risk (Table 3) at most of the croplands (HB2, HB3 and HB4) associated with the Hartbeespoort Irrigation Scheme during the first sampling interval. However, for the remainder of the study sites, as well as during the second sampling interval, either no or low risk was evidenced for nutrient and salt contents. Metals presented only low risk (at some sites) during the first sampling interval and no risk during the second sampling interval.

The moderate risk evidenced for nutrient and salt contents at some croplands can lead to a negative impact on both the abiotic and biotic components of soil quality. From an abiotic perspective, elevated salt levels may cause salinity-induced water stress, which can have a negative impact on plant growth.[28] Furthermore, increased nutrient levels can result in excessive crop growth, while also resulting in algae and aquatic plants clogging irrigation infrastructure.[6,28] From a biotic perspective, increased soil salinity can inhibit microbial growth[38,39], while specific ions can present toxicity-induced effects[38,40]. Furthermore, increased nutrient levels, although potentially serving as a food source to soil communities, can alter food-web structures.[41-43]

For the ecology LOE, no risk was evidenced during both sampling intervals with the exception of low risk at HB4 (first sampling interval) and HB1 (second sampling interval). The ecotoxicology LOE presented no risk for all croplands during the first sampling interval and low risk at only HB3 and CW5 during the second sampling interval. It is clear that, although ecological disturbance and soil pore water toxicity were evidenced at most of the study sites, either no or low risk was calculated due to the reference sites also presenting ecological disturbance and soil pore water toxicity. Therefore, even though elevated salt and nutrient concentrations may pose a threat to soil quality, it was not evidenced in this study.

Table 2: Percentage inhibition (positive values) and stimulation (negative values) of sub-lethal toxicity endpoints (growth and reproduction) following exposure of *Chaenorhabditis elegans* to soil pore water from the studied croplands associated with the Hartbeespoort (HB), Crocodile (West) (CW), and reference (Ref) irrigation schemes. The percentage inhibition/stimulation per irrigation scheme is also provided. Values that differ significantly $p<0.05$) from the negative control (M9) are indicated with an asterisk.

	HB1	HB2	HB3	HB4	CW5	CW6	Ref 7	Ref 8
	First sampling interval							
Growth	0.7	5.8*	5*	8.2*	-2.6	-0.2	-4	0.3
Reproduction	14.5*	18.6*	11.2*	23.3*	7.9	-44.4*	-31.6*	19.5*
	Second sampling interval							
Growth	9.0*	-2.5	-7.7*	6.7*	-1.3	0.9	-0.2	1.5
Reproduction	31.1*	-11.2	-37.7*	25.8	-42.1*	26.6*	38.4*	-6.5

Table 3: Integrated ecological risk assessment of the chemistry, ecology, and ecotoxicology lines of evidence (LOEs) per cropland per sampling interval. The risk values associated with the chemistry LOE were integrated into a single value per cropland per sampling interval. Risk numbers are classified (and colour coded) as presenting either no, lo , moderate, or high risk according to Jensen et al.[13]

	First sampling interval						Second sampling interval					
	HB1	HB2	HB3	HB4	CW5	CW6	HB1	HB2	HB3	HB4	CW5	CW6
Chemistry (soil water) LOE												
Nutrients and salts in solution	0.34	0.65	0.51	0.54	0.36	0.49	0	0.26	0	0.49	0	0.24
Metals in solution	0.08	0.1	0.22	0.26	0.04	0.25	0	0.03	0.05	0.14	0	0.06
Ecology LOE												
Maturity and Shannon Diversity indices	0.17	0.13	0.1	0.21	0.09	0.19	0.22	0.13	0.02	0.14	0.09	0.06
Ecotoxicology LOE												
C. elegans: growth and reproduction	0.11	0.16	0.11	0.19	0.07	0.15	0.10	0.17	0.28	0.07	0.27	0.10
Chemistry LOE	0.22	0.44	0.38	0.41	0.22	0.38	0	0.15	0.02	0.34	0	0.15
Ecology LOE	0.17	0.13	0.1	0.21	0.09	0.19	0.22	0.13	0.02	0.14	0.09	0.06
Ecotoxicology LOE	0.11	0.16	0.11	0.19	0.07	0.15	0.10	0.17	0.28	0.07	0.27	0.10
Integrated ecological risk (IER)	0.17	0.25	0.21	0.28	0.13	0.25	0.11	0.15	0.12	0.19	0.13	0.11
Deviation	0.05	0.17	0.16	0.12	0.08	0.12	0.11	0.02	0.15	0.14	0.14	0.04
Risk indicators	0.00 ≤ IER ≤ 0.20			**no risk**			0.21 ≤ IER ≤ 0.50			**low risk**		
	0.51 ≤ IER ≤ 0.75			**moderate risk**			0.76 ≤ IER ≤ 1.00			**high risk**		

Ultimately, the integrated ERA evidenced only low risk during the first sampling interval, at HB2, HB3, HB4 and CW6, and no risk during the second sampling interval. This outcome can be regarded as valid as the standard deviation in ecological risk recorded between the three LOEs for the studied croplands was low (≤ 0.17) during both sampling intervals. This is indicative of low uncertainty relating to the execution of TRIAD tests and integration of the LOEs.[7] According to Mesman et al.[44], the maximum proposed deviation value is 0.4 – well above values evidenced during this study.

Confounding influence of conventional agricultural activities

The ecological disturbance and soil pore water toxicity recorded at the reference sites are indicative of anthropogenic disturbance related to conventional farming practices. Du Preez et al.[5] showed that a strong, positive correlation existed at the study sites between soil inorganic N content, crop production (and the associated agricultural activities), and r-strategist nematodes, which are more tolerant to environmental disturbance than K-strategists.[24,45] This suggests that agricultural activities (e.g. tillage and fertiliser application) likely induced shifts in faunal community structures and impacted soil quality.[5] The physical disturbance or tilling of soils, as was performed at all the studied and reference sites at one or multiple points within a 2-year time period before sampling (from personal communications with farmers), negatively impacts soil quality.[46] Zhong et al.[47] reported that tillage not only influ nced soil structure, organic content, and the water retention capability of soils, but also the soil faunal community structure. With the application of nutrients (as fertilisers), Hu et al.[43] reported an increase in the abundance of especially bacterivore nematodes, while elevated levels of N and P may reduce soil biodiversity. Similarly, Sarathchandra et al.[48] reported a reduction in faunal diversity as a result of N application.

Considerations and recommendations

In the present study, the negative impact of agricultural practices associated with conventional farming masked the potential ecological risks posed to cropland soils. This represents an important limitation in the application of the soil quality TRIAD. Nonetheless, the soil quality TRIAD, as part of an ERA, has potential in agricultural systems that are less disturbed (e.g. conservation and/or organic systems) by agricultural activities.[2]

Furthermore, the lack of soil quality guidelines and target values specific for soil pore water extracted using the saturated paste method, creates some degree of uncertainty. Although weighting was applied in the present study to reduce this uncertainty, the development of such guidelines would be of benefit to environmental managers and/or researchers who aim to make use of the soil quality TRIAD approach. Alternatively, chemical extraction methods for which soil screening values are available can be considered.[13]

Conclusion

The standardisation of the soil quality TRIAD approach, as part of an ERA, provided a framework for the assessment and monitoring of agricultural systems threatened by anthropogenic pollution. Although we have demonstrated important limitations in the application of this framework in conventionally farmed croplands, it still holds promise for systems with minimal soil disturbance. It is therefore possible that this framework will be applicable in the assessment of conservation (regenerative) and organic crop production systems.

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Competing interests

We have no competing interests to declare.

Authors' contributions

All authors were involved in the conceptualisation of the study, while G.D.P. undertook field sampling and sample analysis. G.D.P. and V.W. were responsible for data analysis. The first draft of the manuscript was written by G.D.P. after which the remaining authors provided input. Finally, the manuscript was revised and approved for submission by all authors.

Data availability

The data generated and analysed during this study are available in the supplementary material and in previously published works as indicated. Any additional data will be made available by the corresponding author upon request.

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A simple approach for monitoring vegetation change using time series remote sensing analysis: A case study from the Thathe Vondo Area in Limpopo Province, South Africa

This study presents a simple approach of spatiotemporal change detection of vegetation cover based on analysis of time series remotely sensed images. The study was carried out at Thathe Vondo Area, which is characterised by episodic variation of vegetation gain and loss. This variation is attributable to timber and tea plantations and their production cycles, which periodically result in either vegetation gain or loss. The approach presented here was implemented on two ASTER images acquired in 2007 and 2017. It involved the combined use of band combination, unsupervised image classification and Normalised Difference Vegetation Index (NDVI) techniques. True colour composite (TCC) images for 2007 and 2017 were created from combination of bands 1, 2 and 3 in red, blue and green, respectively. The difference image of the TCC images was then generated to show the inconsistencies of vegetation cover between 2007 and 2017. For analytical simplicity and interpretability, the difference image was subjected to ISODATA unsupervised classification, which clustered pixels in the difference image into eight classes. Two ISODATA derived classes were interpreted as vegetation gain and one as vegetation loss. These classes were confi med as regions of vegetation gain and loss by NDVI values of 2007 and 2017. In addition, the polygons of vegetation gain and loss regions were created and superimposed over the TCC images to further demonstrate the spatiotemporal vegetation change in the area. The vegetation change statistics show vegetation gain and loss of 10.62% and 2.03%, respectively, implying a vegetation gain of 8.59% over the selected decade.

Significance:

- Vegetation change detection is essential in environmental monitoring and management of an area. This study presents a simple approach for assessing vegetation change over time. The approach involves change detection through the difference of spectral values of vegetation pixels of time series remotely sensed images.

Introduction

Time series remote sensing is an invaluable resource for dynamic monitoring of the environment over short and long time spans.[1] This is because of the ability of remote sensors to cover a large area in a short period of time as well as their capability to revisit and acquire data for the exact area, which optimises environmental monitoring of large areas based on time series image analysis.[2-4] In other words, as stated in Ghauri and Zaidi[5], 'remote sensing provides continuous monitoring and mapping, both spatial and temporal, as opposed to a limited frequency point measurement'. Furthermore, rugged and hilly terrains can be expensive and cumbersome to access for point measurements.[6] In addition to this, remotely sensed data of satellite platforms, such as the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) and Landsat, can be accessed at no cost as their data are readily available and accessible.

Remote sensing has become an integral part of environmental monitoring because of its flexibilit , efficienc , accessibility, and cost-effectiveness. Time series remote sensing analysis has been widely utilised in environmental monitoring and measurements of scale of land degradation[3,7-12], including monitoring of environmental improvement during and after mine rehabilitation processes[13-15]. The most typically used techniques for vegetation change detection include principal component analysis, minimum noise fraction, tasselled cap, Normalised Difference Vegetation Index (NDVI) and supervised image classification [2-4,16-18] Supervised image classification is the process of clustering pixels in an image into classes corresponding to user-defined training classes.[19] The accuracy of this classification method depends heavily on the quality of training sites and the spectral distinctness of the classes.

In this study, a simple approach for monitoring vegetation change using time series remote sensing analysis was proposed. The proposed approach involved the combined use of band combination, unsupervised image classification and NDVI.

Materials and methods

Figure 1 is a flow chart model illustrating the methodology of the proposed approach for monitoring vegetation using two time series images of the ASTER sensor.

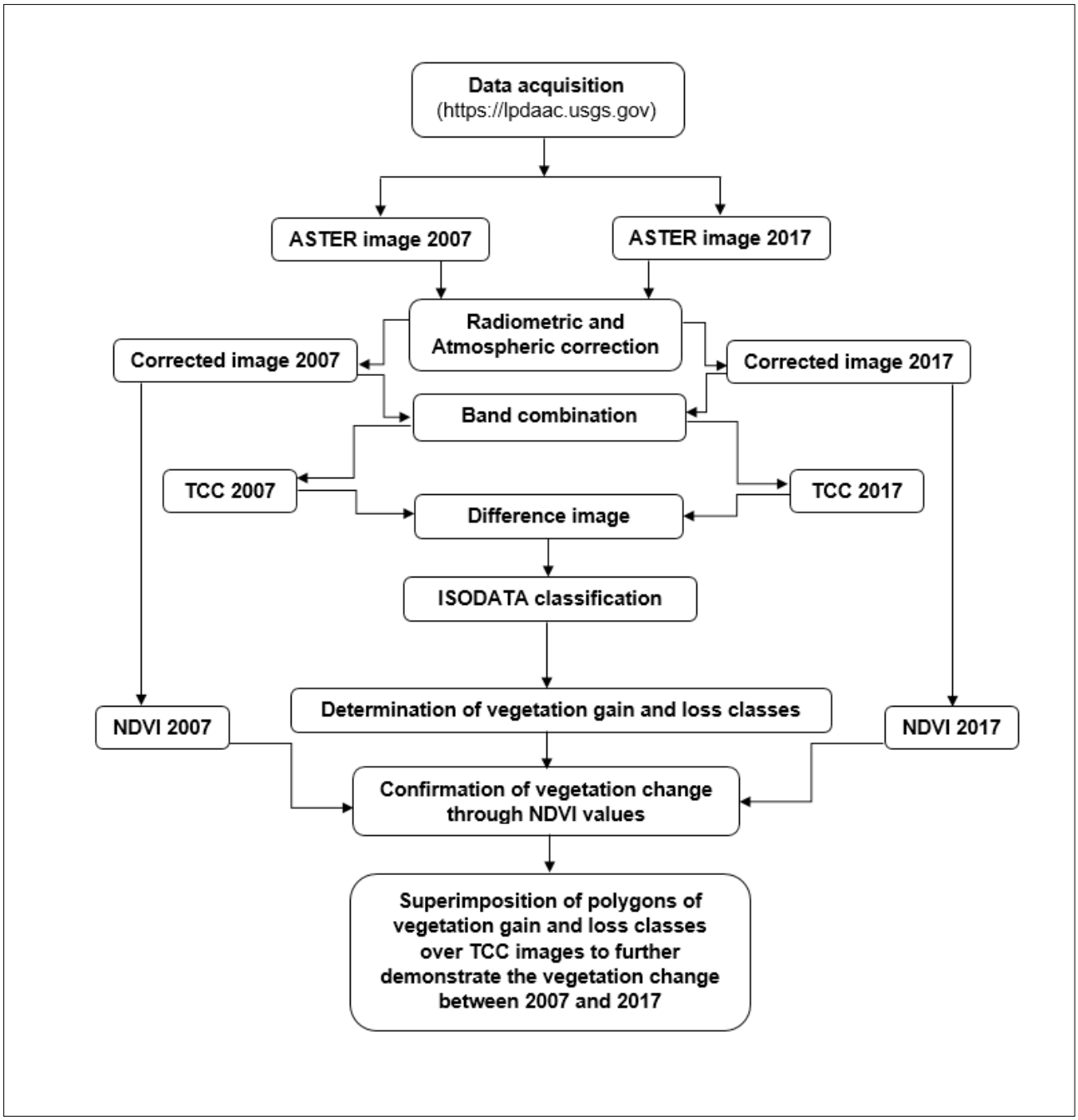

Figure 1: Flow chart of the methodology implemented in this study.

Study area

The area selected for this study was the Thathe Vondo Area, which is located about 13 km west of Thohoyandou town in the Limpopo Province of South Africa. The study area covered 150.84 km^2 (Figure 2). Thathe Vondo is unique as it is characterised by episodic variation in vegetation gain and loss. This variation is attributable to timber and tea plantations and their production cycles, which periodically result in vegetation gain or loss. In this regard, vegetation change in the area seems inevitable. Furthermore, this makes the study area ideal for assessing the proposed approach for monitoring vegetation change over time.

Remote sensing data sets

The remote sensing data selected for this study are ASTER L1T (Precision Terrain and Geometric Corrected Registered At-Sensor Radiance Product) scenes with Local Granule IDs of AST_L1T_00310262007081200 and AST_L1T_00310152017081915. The ASTER scenes were acquired in October 2007 and October 2017, respectively. These remote sensing data products were retrieved from the website (https://lpdaac.usgs.gov) maintained by the NASA Land Processes Distributed Active Archive Centre at the US Geological Survey / Earth Resources Observation and Science Centre in Sioux Falls, South Dakota, USA. ASTER covers a wide spectral region with 14 bands ranging from the visible to thermal infrared region. The spatial resolution varies with spectral region: 15 m in the visible and near infrared (VNIR), 30 m in the shortwave infrared, and 90 m in the thermal infrared. The VNIR bands (Table 1) were selected for monitoring vegetation gain and loss in the area. Vegetation has diagnostic spectral features in the VNIR spectral region of the electromagnetic spectrum. Furthermore, the VNIR region has better spatial resolution (15 m), which is the size of area represented by each pixel in an image. Spatial resolution has a significant impact on the spatial details of land-cover mapping.[19]

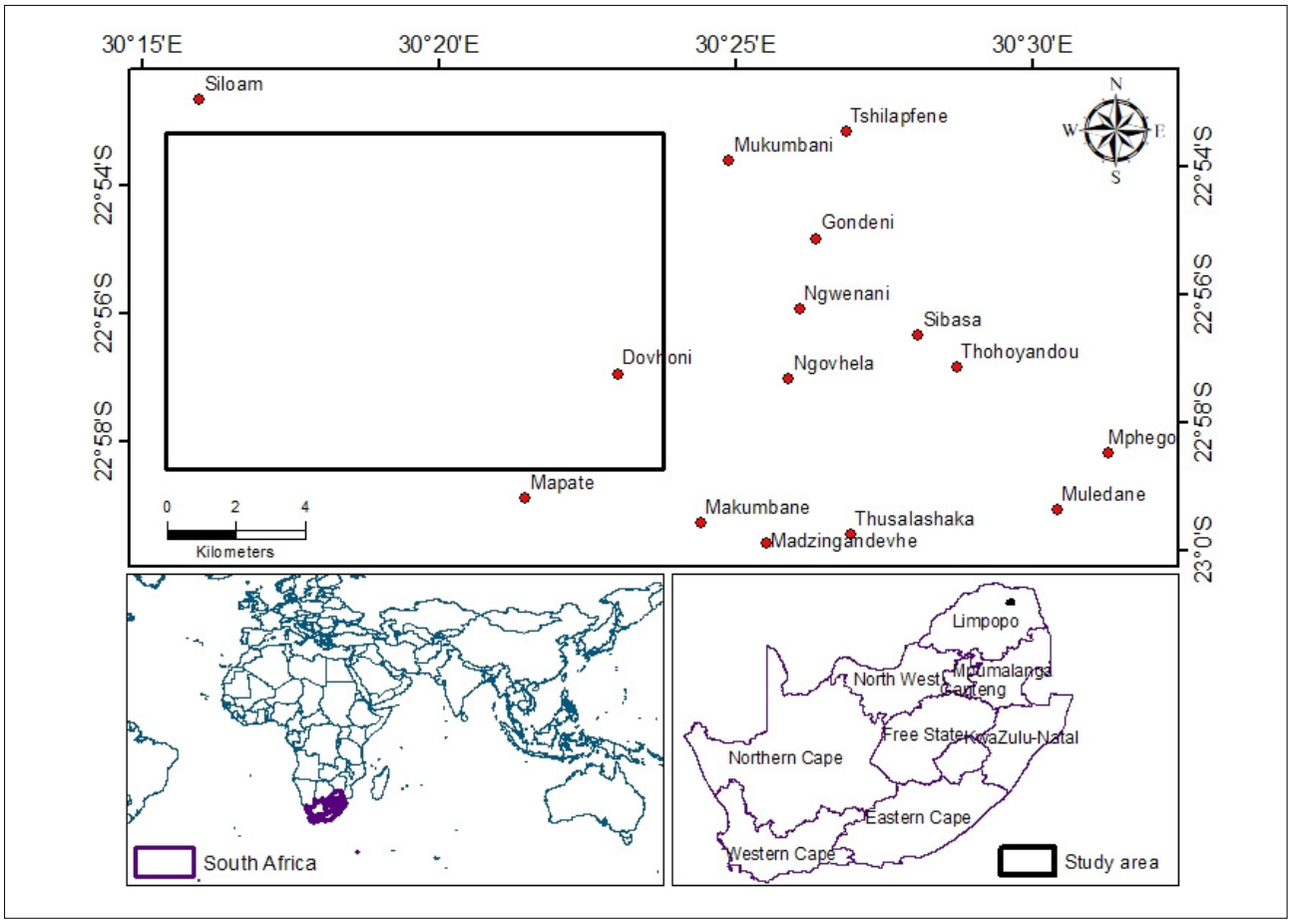

Figure 2: Locality map of the study area.

Table 1: Wavelength ranges, and spatial and radiometric resolutions of Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) bands

Spectral region	Band number	Wavelength range (μm)	Spatial resolution (m)
Visible and near-infrared	1 (green)	0.52–0.60	15
	2 (red)	0.63–0.69	
	3 (near infrared)	0.78–0.86	

Pre-processing and analysis of remote sensing data

The pre-processing and subsequent analysis of ASTER data were carried out using ENVI 5.0 and ArcGIS 10.3 software. The three VNIR bands of each ASTER scene were stacked together to build a new VNIR multiband image. The resultant multiband images of the two scenes were subset to cover the study area. Radiometric and atmospheric corrections of remotely sensed data in vegetation mapping have become a prerequisite. These corrections, particularly radiometric corrections, are essential when comparing multiple data sets over a certain period of time, as in the present study.[20] In addition to radiometric distortions, solar irradiance, atmospheric transmittance, instrument gain, topographic effects, and albedo effects from radiance data need to be removed to avoid interference with the quality of the results.[21] The dark object subtraction was applied to the two ASTER data sets in order to remove radiometric effects that may have affected the quality of the results. This calibration tool searches each band in an input data set for the darkest pixel value. Assuming that dark objects reflect no light, any value greater than zero must result from atmospheric scattering. The scattering is removed by subtracting this value from every pixel in the band.[21] Additionally, a log residuals calibration tool was applied to the radiometric corrected data sets to remove solar irradiance, atmospheric transmittance, instrument gain, topographic effects, and albedo effects from radiance data. The log residuals tool also converts radiance data to a reflectance image, which is useful in mapping vegetation cover.[21]

Band combination

Band combination involves combining and displaying three bands, where each band is assigned one of the primary colours (red, green or blue). The generation of true colour composite (TCC) images from band combination can increase distinction between features.[21] The TCC of ASTER images is created by combination of bands 1, 2 and 3 in red, blue and green, respectively. The advantage of a TCC image is that the features appear in their true colour form, which simplifies visual interpretability. For pixel-to-pixel-based comparison, the Image Analysis Tool from ArcGIS was used to create a difference image of the two time series images.

ISODATA classification

For best viewing and interpretation of the difference image, the ISODATA unsupervised image classification algorithm was applied to the difference image. The unsupervised image classification algorithm clusters pixels in an image based on statistics only, without any user-defined training classes.[22] The clustering is based on grouping pixels of spectral similarities into the same classes. This is achieved by calculating class means that are evenly distributed in the data space and then iteratively clustering the remaining pixels using minimum distance techniques. Each iteration recalculates means and reclassifies pixels with respect to the new means.[22]

Normalised Difference Vegetation Index

The NDVI technique is implemented using near infrared (NIR) and red bands.[23] The principle of applying the NDVI in vegetation mapping is that the vegetation is highly reflective and absorptive in NIR and visible red, respectively. The difference between these bands can be used to indicate the presence and greenness of vegetation. In other words, the NDVI is a biophysical parameter that is related to the photosynthetic property of vegetation. This parameter is capable of providing valuable information on the dynamic changes of vegetation cover, given that time series images are analysed. This makes the NDVI a good indicator of seasonally or periodically dynamic changes in vegetation condition. As a result, the NDVI has been widely used in monitoring the change in vegetation cover and vegetation health and productivity.[2-4,17,18,24] The NDVI is mathematically expressed as the difference between NIR and red channels divided by their sum of them[23]:

$$NDVI = \frac{NIR - Red}{NIR + Red} \quad \text{Equation 1}$$

where NIR and Red are the normalised reflectance values of NIR and red bands, respectively. The NDVI resulting values range from -1 to 1 and the common range for green and healthy vegetation is 0.2 to 1[23], while bare soil is typically represented by values <0. For pixel-to-pixel-based comparison, the Image Analysis Tool from ArcGIS was again used to generate the NDVI difference image of the two time series images.

Results and discussions

Figure 3 shows the TCC images for 2007 and 2017. The vegetation inconsistencies are evident in these images (Figure 3). The difference image of the TCC images is shown in Figure 4. As stated under the methodology, the difference image was subjected to ISODATA unsupervised classification for analytical simplicity and interpretability purposes. The ISODATA clustered pixels in the difference image into eight classes (Figure 5). To assign the ISODATA classes to vegetation gain and loss, the TCC image of 2017 was superimposed over the TCC image of 2007, and the Swap Layer Function from ArcGIS was used to carefully analyse and compare their dissimilarities. The Swap Layer Function involves superimposition of one image layer over another layer of similar geographical area, and cautiously swaps the superimposed layer to better illustrate the land-cover dissimilarities of the two image layers. Consequently, Classes 1 and 2 from the ISODATA image (Figure 5) were interpreted as vegetation gain and Class 8 as vegetation loss that occurred between 2007 and 2017 (Figure 6).

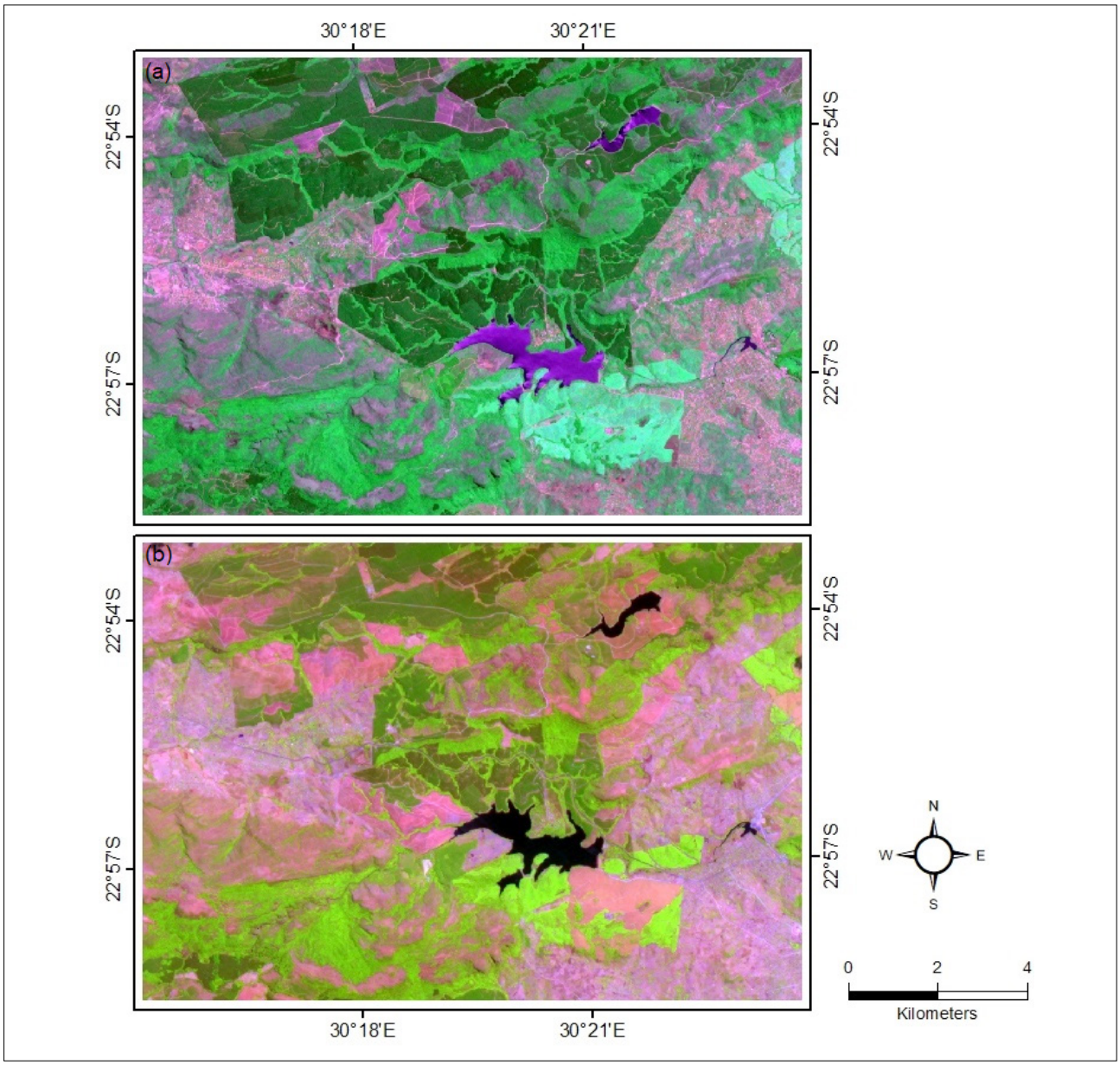

Figure 3: Advanced Spaceborne Thermal Emission and Reflection adiometer (ASTER) images of the study area acquired in (a) 2017 and (b) 2007.

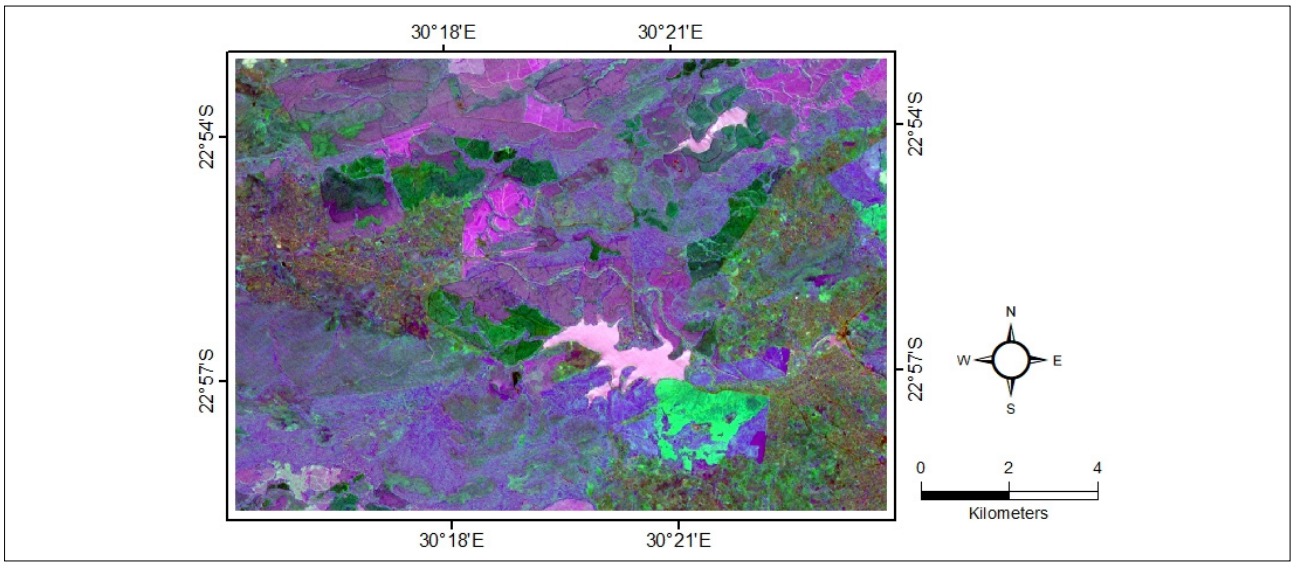

Figure 4: The difference image.

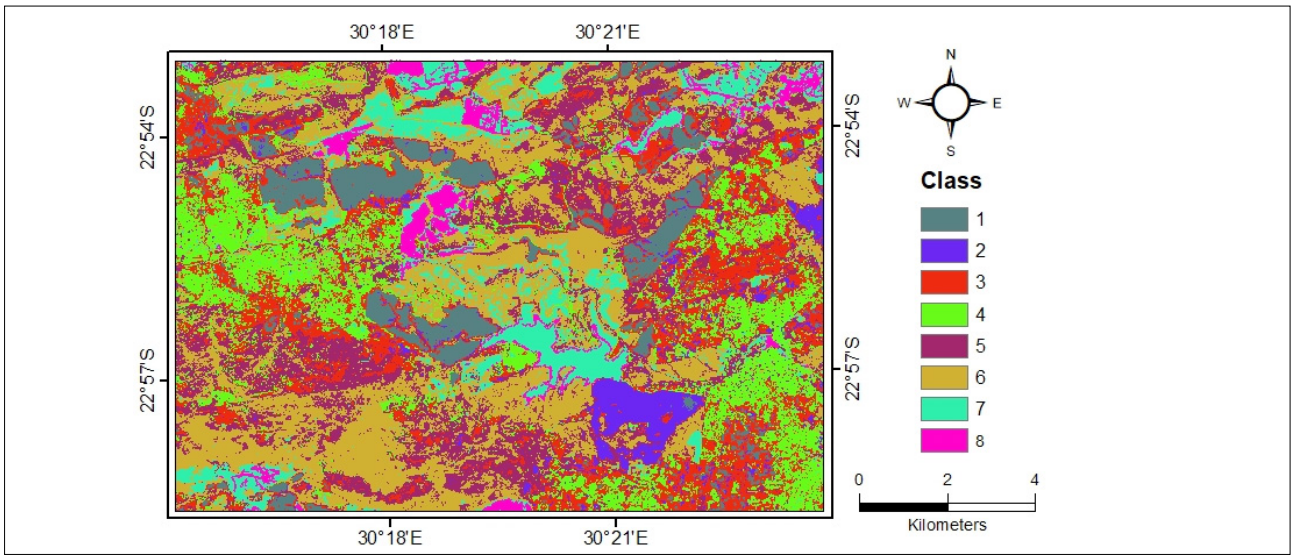

Figure 5: The ISODATA classification of the difference image

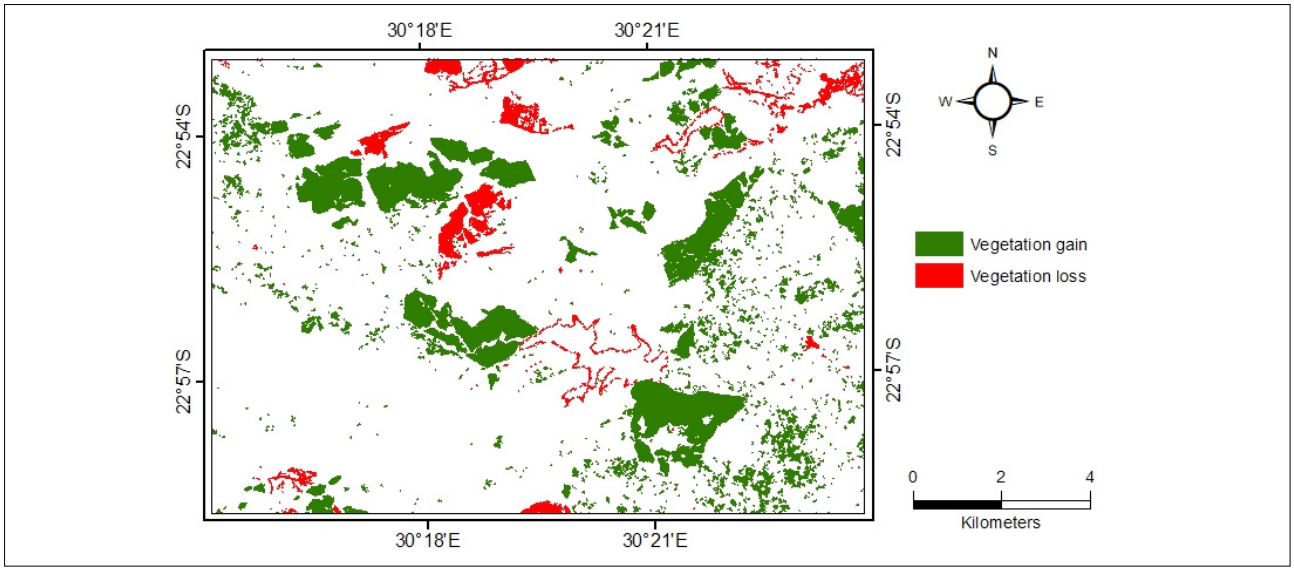

Figure 6: Vegetation gain vs vegetation loss.

The NDVI values for the two-time series images are shown in Figure 7. As previously stated, the principle behind the implementation of NDVI in vegetation mapping is that vegetation is highly reflective and absorptive in NIR and visible red, respectively. As a result, the difference between these bands can indicate the presence and greenness of vegetation.[23] The NDVI values of 2017 and 2007 range from -0.06 to 0.35 and -0.05 to 0.41, respectively (Figure 7). However, most pixels from 2017 are characterised by relatively higher NDVI values than pixels from the 2007 image. This implies that, by 2017 the area experienced vegetation gain and vegetation health improved. The NDVI difference image shows that the NDVI values increased to a maximum value of 0.21, which is considerably high and supports significant vegetation gain and productivity in the area (Figure 8). The NDVI values, of the difference image, which represent gain (0.07 to 021) and loss (-0.25 to -0.06) (Figure 8) correspond to vegetation gain and loss classes, respectively, in Figure 6.

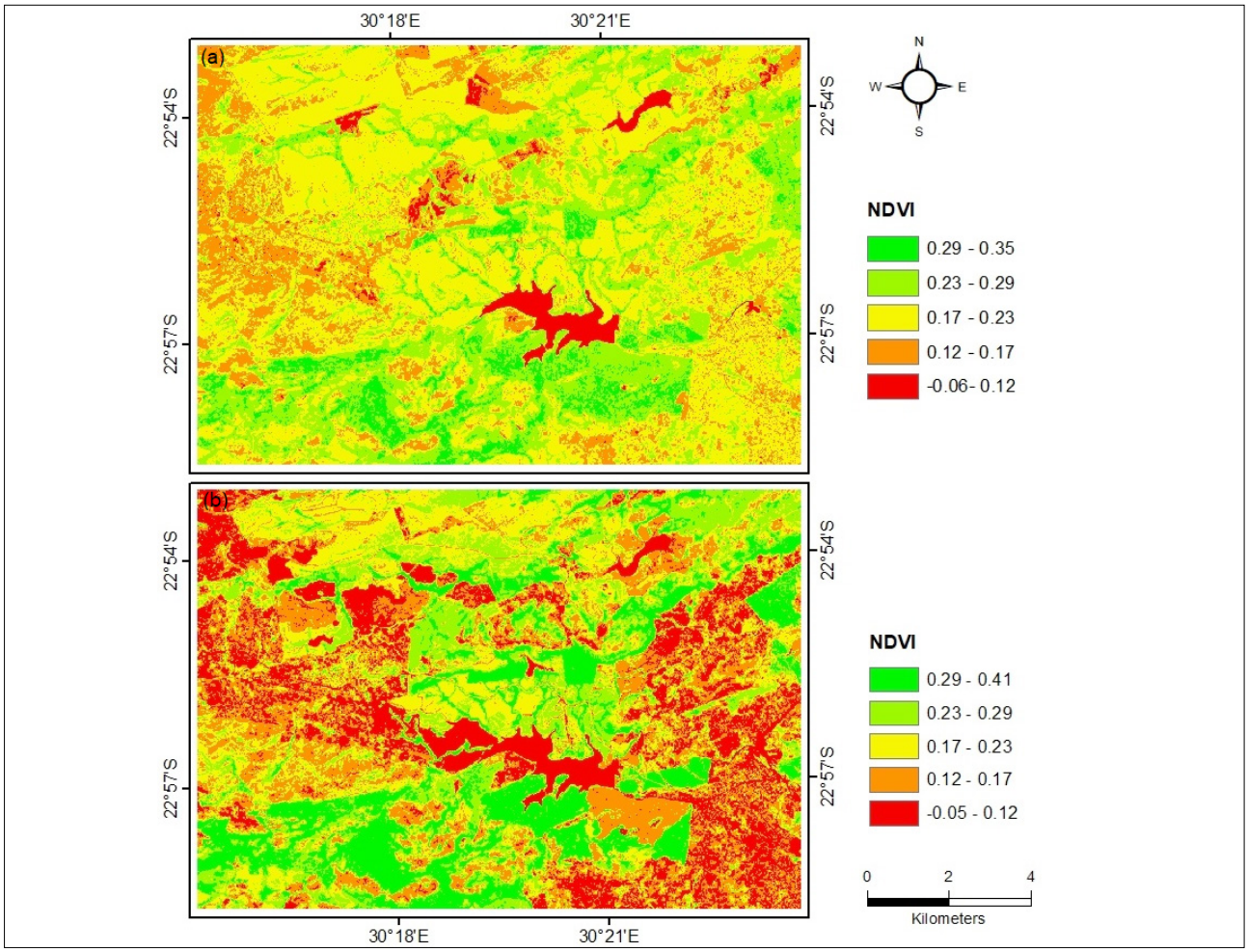

Figure 7: The Normalised Difference Vegetation Index (NDVI) values of (a) 2017 and (b) 2007 Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) images.

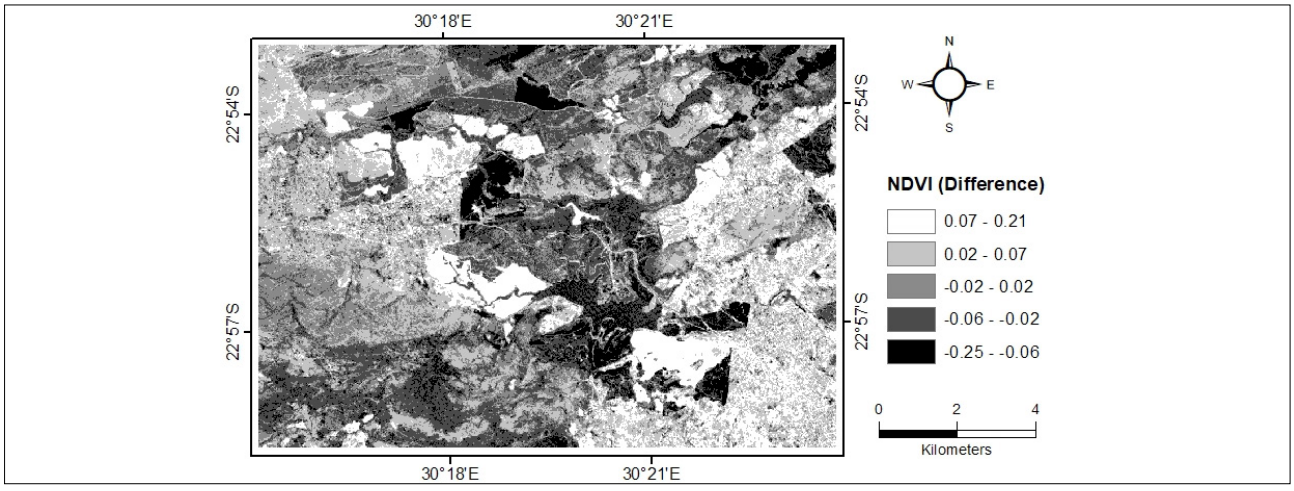

Figure 8: The Normalised Difference Vegetation Index (NDVI) difference image of the time series images.

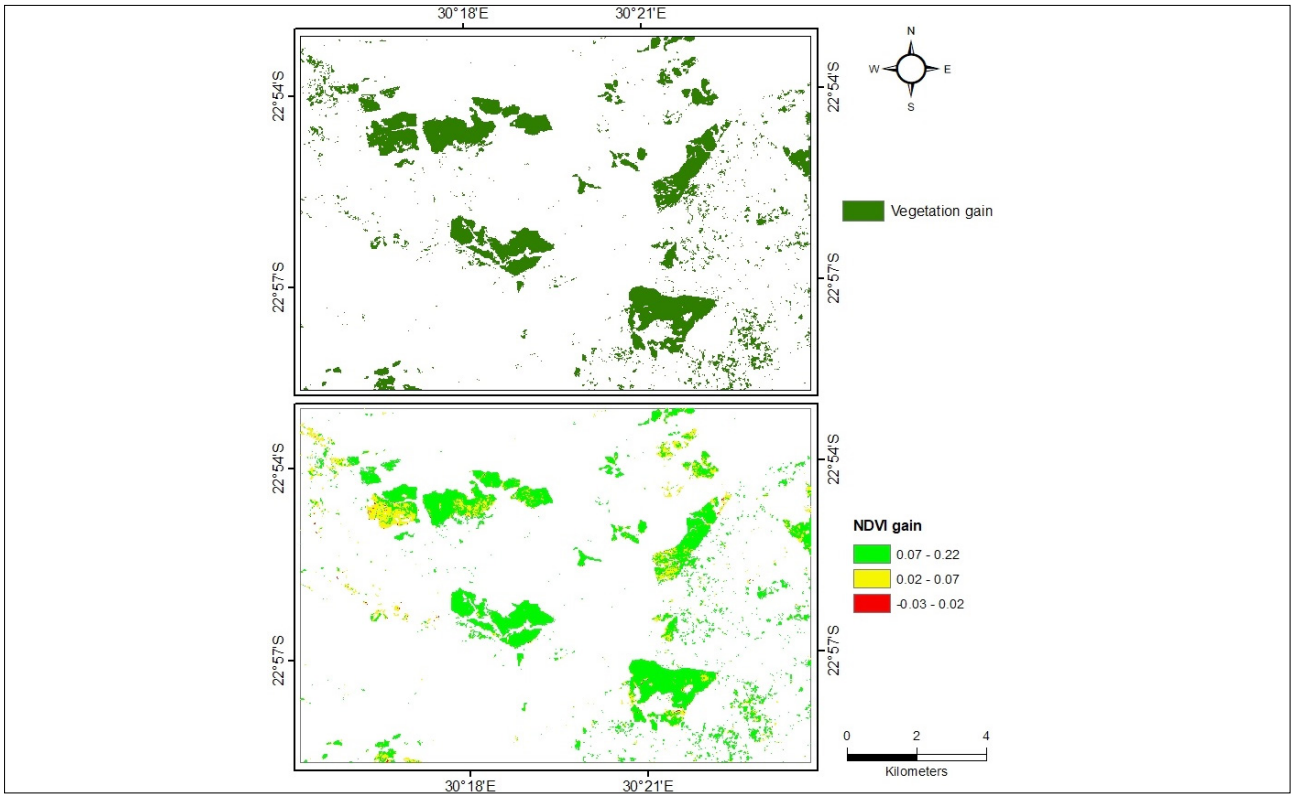

Figure 9: Vegetation gain vs Normalised Difference Vegetation Index (NDVI) gain.

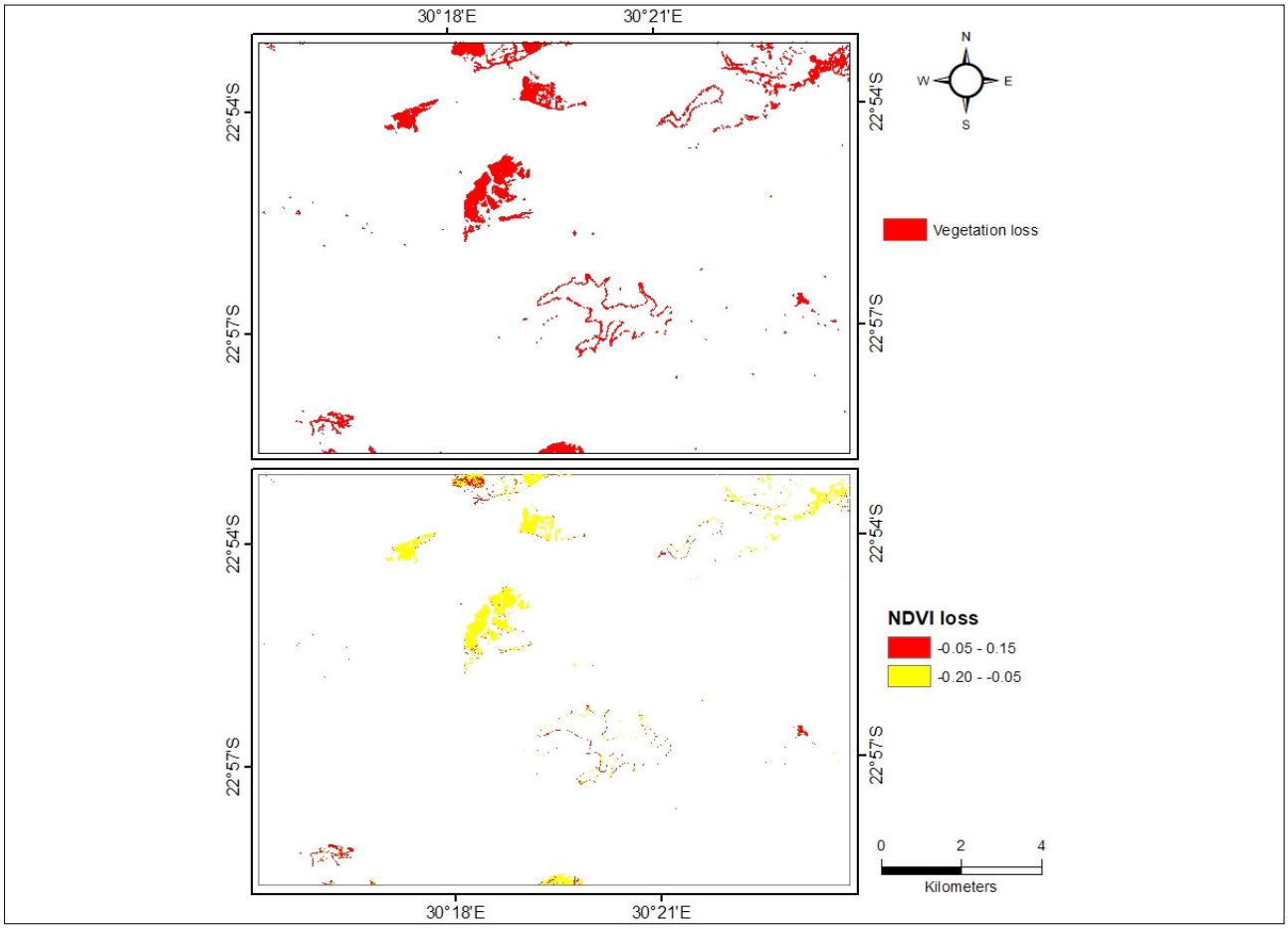

Figure 10: Vegetation loss vs Normalised Difference Vegetation Index (NDVI) loss.

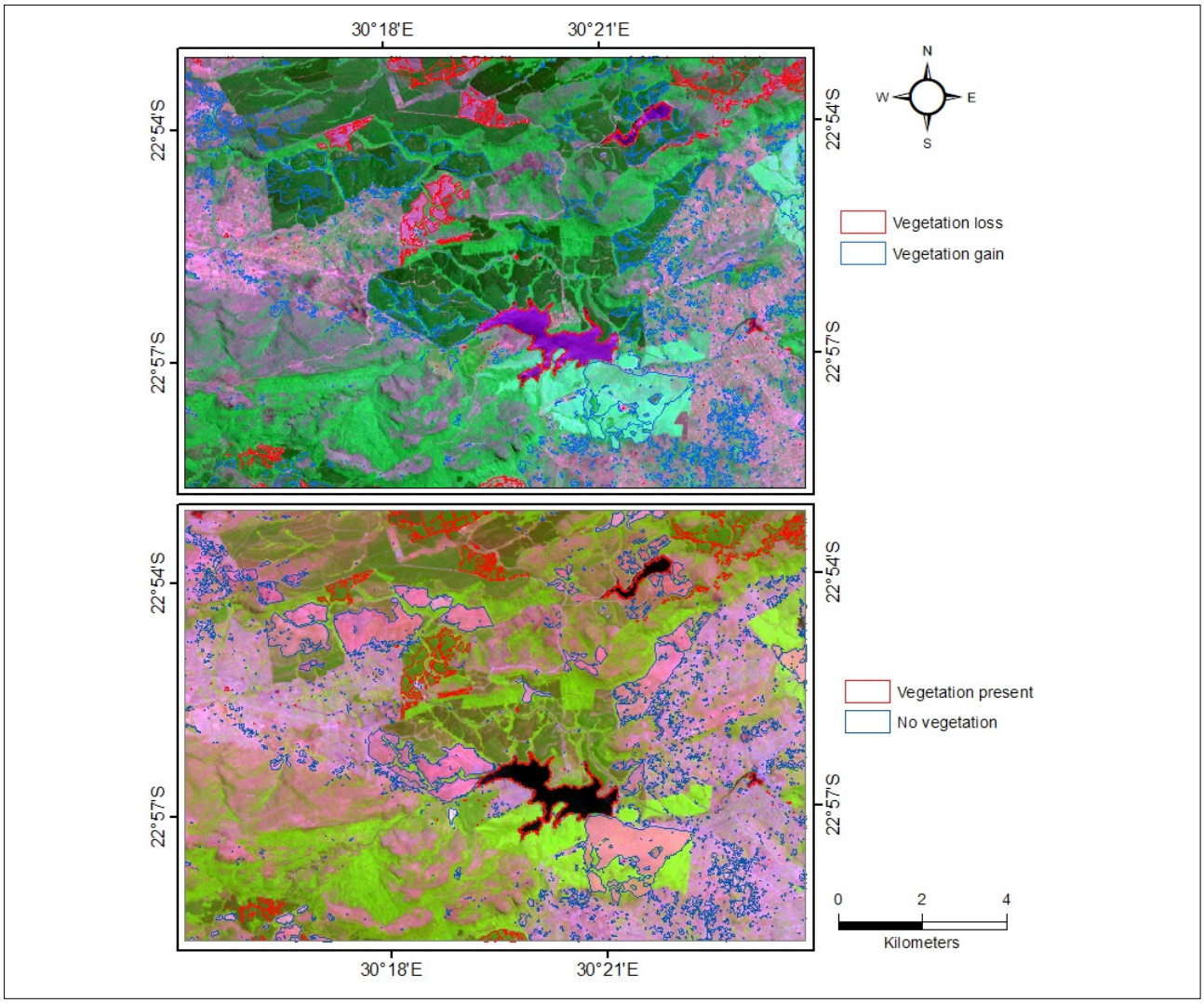

Figure 11: Vegetation gain and loss polygons superimposed over true colour composite images of 2017 (top) and 2007 (bottom).

The vegetation gain and loss classes were converted to polygons. These polygons were used to extract NDVI values from the two NDVI images shown in Figure 7. This was done for comparative simplicity of vegetation gain and loss classes and their respective NDVI values. The vegetation gain class is characterised by high NDVI values and vegetation loss by low NDVI values (Figures 9 and 10) related to barren land. Thus, the NDVI values confi med the ISODATA classes interpreted as vegetation gain and loss.

In addition, the vegetation gain and loss polygons were superimposed over the TCC images to further illustrate and verify the spatiotemporal vegetation change that occurred in the area from 2007 to 2017 (Figure 11). From Figure 11, the vegetation gain and loss regions are vividly pronounced from both images. The vegetation change statistics of the area between 2007 and 2017 are reported in Table 2. From October 2007 to October 2017, vegetation cover in the study area increased by 8.59%.

Table 2: Vegetation change statistics between 2007 and 2017

Item	Number of pixels	Percentage (%)
Total area	671 389	100
Vegetation gain	73 109	10.62
Vegetation loss	13 645	2.03
Vegetation difference = vegetation gain (10.62) – vegetation loss (2.03) = 8.59%		

Conclusions

This study presents a simple approach of spatiotemporal change detection of vegetation cover based on time series analysis of remotely sensed images. The approach involves pixel-to-pixel-based comparison by differencing of spectral values of time series images. This approach is complemented by confi mation of vegetation change through NDVI values, whereby the increase and decrease in NDVI values correspond to vegetation gain and loss, respectively. The approach is quick and simple but yet effective, and it can be applied at any region under any environmental conditions.

Competing interests

I have no competing interests to declare.

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Diverse trends in observed pan evaporation in South Africa suggest multiple interacting drivers

Planning for future water resource management in a warming climate is confounded when an expectation of increasing evaporation from open water surfaces with global warming is contradicted by observations of secular declines of pan evaporation. Decreasing pan evaporation has been observed globally – a trend which has been attributed variously to declines in wind run ('global stilling'), declines in radiation ('global dimming') and increases in ambient humidity. This contrast between expectation and observation is known as the 'evaporation paradox'. We evaluated trends in Symons pan evaporation from 154 pans across South Africa. Whilst 59 pans (38% of the 154) showed a statistically significant decrease in observed evaporation rates ($p \leq 0.05$), 30 (20%) showed an increase, and 65 (42%) showed no change. These results do not support simple attributions of trends to a common global cause. There is no spatially coherent pattern to trends across South Africa, suggesting that shifts in local drivers of evaporation confound expectations of secular trends due to global drivers. Changes in fetch conditions of the Symons pan installations may be implicated, whereby increasing tree density (through afforestation, alien plant invasion and woody thickening) increases surface friction, reducing wind run, and/or irrigation nearby, increasing local humidity. Correct attribution of the evaporation paradox to reduced wind run in South Africa must consider changing local conditions. Increased tree cover has been observed near a third of the South African Symons pans. Observed evaporation increases for one fi th of pans may implicate expected global drivers for pans where local fetch conditions have remained relatively constant.

Significance:

- Observed trends in Symons pan evaporation data for stations across South Africa comprise significant decreases (38% of stations), no change (42%) and significant increases (20%), with no clear geographic bias or coherency in the distribution of these trends.
- The observed diversity in trends appears to reflect local and global drivers, with land-cover changes emerging as a likely dominant local driver via friction-induced reductions in wind-run, possibly resolving the 'evaporation paradox'.
- Observed trends in pan evaporation data may only be of value in testing for the impact of global drivers, such as global warming or global stilling, if local effects are accounted for. Caution is urged when using pan evaporation data for water resource planning.
- Attribution of observed trends requires a case-by-case assessment of local to regional land-cover and land-use changes, in addition to global influences

Introduction

Routine estimation of atmospheric water demand and rates of evaporation from natural and agricultural land surfaces have been made for decades through the use of evaporating pans.[1] Despite their known limitations, evaporating pans continue to be used for estimating water use by land uses that include crop, plantation, pasture, natural vegetation[2,3], and open water and wetland surfaces[4-6]. Ease of use and simplicity in construction and maintenance favour their retention for these purposes[1], and the stationarity benefits conferred by a consistent methodology are advantageous for long-term trend analysis.

Many reports based on such long-term trends demonstrate declining pan evaporation around the world.[7-10] Extensive reviews of these changes are given by McVicar et al.[11] and Roderick et al.[12] who attribute them mainly to regional or global meteorological changes. In South Africa, Hoffman et al.[13] found consistent declines in an analysis of records from 20 selected meteorological monitoring stations in the Western Cape Province of South Africa, attributing these to the same regional changes.

Such declines are unexpected in the light of rising air temperatures due to global warming, which should result in increasing evaporation via increasing air vapour pressure deficit. This violation of expectation is known as the 'evaporation paradox'.[14] The decline in pan evaporation has widely been associated with 'global stilling', which is the purported global slowing of surface winds[12], and 'global dimming', which is a global decline in solar radiation at the earth's surface[12]. Other reasons for the evaporation paradox include increased cloudiness[15], or a complementary relationship between actual and potential evaporation, where increasing actual evapotranspiration from surrounding areas suppresses pan evaporation[16,17], and changes in ambient humidity[14].

However, questions remain about the attribution of the observed declines of pan evaporation.[11] This is particularly because declines are not observed universally. Several regions have been reported to show increases in pan evaporation, including parts of conterminous USA[17,18], Israel[18] and Australia[19].

We explore here trends in surface evaporation in South Africa, using data from Symons pan evaporation observations that span a longer monitoring period, over a larger region, and using more stations than provided in the analysis by Hoffman et al.[13] The Symons pan is the standard evaporation recording instrument at all major South African reservoirs and pans were installed widely around the country, from as early as the 1920s when the first major efforts in constructing large reservoirs began. As a result of mostly continuous observations, this valuable data set provides the basis for a trend analysis undertaken for a longer term than has been done before in South Africa, with a view to assessing potential drivers over a period of accelerating anthropogenic climate change.

Three alternative explanations could be advanced to account for evaporation trends and changes. Rising temperatures due to anthropogenic climate change, already identified in South Africa[20], should result in generally increasing evaporation rates in a spatially coherent pattern because of the established relationship between air temperature on evapotranspiration[2]. Alternatively, the impact of possible global dimming and/or global stilling would be to reduce evaporative demand in a regionally coherent pattern. However, a spatially incoherent mix of upward, downward and non-significant trends would indicate a likely role for local surface conditions controlling the planetary boundary conditions and energy partitioning, which may override or exacerbate the impacts of global drivers. In this study, we tested for the predominant trends in evaporation and their spatial coherence that are conditioned upon the prevalence of global vs local drivers of evaporation. We also tested the potential value of Symons pan data for attributing the impacts of these drivers.

Methods

Monthly Symons pan evaporation data were obtained from the open-access South African Department of Water and Sanitation's (DWS) hydrological database. The Symons pan is a square container measuring 1.83 m on each side, is 0.61 m deep and is set into the ground so that it has a rim of 0.076 m above ground level; the inside is painted black. This instrument is used commonly across southern Africa and is the standard evaporating pan used by the DWS, particularly at its large dams. The instrument has also been installed at irrigation scheme offices and at some wastewater treatment plants. Evaporation readings are collected daily and communicated to the central office of the DWS, where the data is curated on their Hydrological Information System. The records span a range of durations, with some beginning as early as the 1920s, some ending in 2018/2019, and others terminating earlier.

We selected appropriate recording stations to use in this analysis as follows. The evaporation records were initially scanned to determine the quality of the data. Generally, the station firstly needed to have 30+ years of record, assuming a period that gives sufficient time for any trends to develop in the data set. Those with a substantial quantity of missing data, or data considered unreliable (according to notes on the database), were discarded. All data are recorded with quality codes prepared by the DWS office responsible for maintaining the Hydrological Information System, ranging from good observed values through good monthly estimates to unreliable and lastly to missing data for some months. Some records are estimated, others are recorded as missing, or unaudited.

The remaining station records were then edited for continuity. Where a month of data was missing in a run of reliable records, missing values were interpolated based on the expected value for that month of the year based on adjacent values. We estimated that the error introduced into the data set in this way was no more than ±2% in each individual year.

Where several consecutive months were missing, the record was filled with a missing data flag (NA values). The data were not adjusted for homogeneity, which would possibly destroy signals of change and because cyclicity across numbers of instruments was detected in pre-analyses. The monthly records were then aggregated to annual values and a linear regression model fitted to the time series, with the slope of the regression determining the trend in the data. Years with missing data were dropped from the regression by specifying a high minimum threshold value for the data set before inclusion in the calculation. This threshold value varied according to the station and was set by observation in each case, and also by inspecting the raw data to check that those annual values excluded did indeed contain missing monthly data and were not merely unusually low values. The beginning and end years of each record were also excluded if they were incomplete.

We tested for normality of the residuals of the regressions. Two methods were used in conjunction: Q-Q plots gave a visual view of the closeness of the residuals to normality and the Shapiro–Wilk test was used as a robust statistical test, with a chosen alpha value of 0.05 ($p < 0.05$). Most of the records indicated near normality from the Q-Q plot. The Shapiro–Wilk test was more discerning and indicated a substantial number of regressions had non-normal residuals. Close inspection of the outputs revealed that the most likely reason for non-normality was the cyclic behaviour of evaporation quantities over the time series for each station. Because the cycles likely had definable external causes but did not affect the larger trends in the data, it was decided that linear regression was an appropriate and robust method for conducting the main trend analysis.

The results of the trend analysis were tested against the Standardised Precipitation Evapotranspiration Index (SPEI), which is discussed in detail by Beguería et al.[21] and Vicente-Serrano et al.[22] The SPEI can be considered an independent estimation of water demand by the atmosphere based on a climatic water balance which comprises both precipitation and evaporation. The conceptual justification for using the SPEI is that its variables are found to cluster according to the potential evapotranspiration radiation term and that the mass transfer term which integrates wind and humidity has little or no effect on the SPEI, according to Stagge et al.[23] Further support for this idea is obtained from Hobbins et al.[24] who discuss a complementary relationship between actual (E_a) and potential (E_p) evaporation, in which the amount of water in the environment is the controlling factor. As precipitation increases, the evaporative process becomes less water limited and more energy limited, and E_a and E_p converge. Indeed, supporting evidence for this approach is given in this paper, where a relationship between precipitation and pan evaporation is observed and reported as an explanation of cyclicity in pan evaporation. The precipitation values used in this comparison were obtained from the CRU TS 4.03 data[25] for 0.5° geographic grid intervals, representing regionalised monthly data, which were aggregated to annual values for the 0.5° grid interval in which the Vaal Dam is located and compared against the Symons pan located there.

The modelled SPEI would reflect a potential response to mainly the radiation term, and thus provide a test for the impact of global or regional dimming. Poor agreement between the pan data and the modelled SPEI trends would therefore indicate that factors other than dimming are driving trends in pan evaporation rates.

The SPEI 0.5° gridded data layers were obtained from Open Database in netCDF and calculated from monthly CRU TS 4.03 data using R code developed by Beguería et al.[26] The CRU TS variables include temperature, precipitation, diurnal temperature range and vapour. The estimates of the evapotranspiration component of the SPEI were calculated from these climatic variables using the FAO-56 Penman–Monteith equation of Allen et al.[2] at a sub-regional scale (quarter degree square) based on these interpolated climate variables[21].

A linear trend analysis was applied to the SPEI estimates, followed by a correlation analysis that measured the strength of association between the slopes of pan evaporation regression trends and the slopes of SPEI regression trends.

Results

Results indicate that 20% of pans showed positive slopes (β) of evaporation with time, 42% of pans showed no change ($\beta = 0$), and 38% showed declines, all at the $p \leq 0.05$ significance level with a two-sided test (Table 1).

Table 1: Trends in Symons pan evaporation across South Africa, for a range of durations. All slopes (β) of evaporation with time $p \leq 0.05$ using a two-sided test.

	Increase	Decrease	No change
n = 154	30	59	65
%	20	38	42

Thus, a minority of stations showed increasing observed pan trends, with the balance split about equally between no changes and decreasing trends. The statistical distribution of the trends (slope term of the regression) is illustrated in the histogram in Figure 1, representing the changes in mm/annum. The steepest negative trends for three stations showed a slope or reduction in evaporation of -34 mm/a (Q3E001 at Halesvlakte/Halesowen, an agricultural training school near Craddock), -23 mm/a for B7E004 at Phalaborwa and -17 mm/a for H2E002 at RoodeElsberg Dam near the Hex River valley in the Western Cape mountains. In the instances of increases in pan evaporation, the largest are 15 mm/a for G2E015 at Simon's Town, 8 mm/a for C1E007 at Groootdraai Dam and 8 mm/a for A2E003 at Hatfield, the agricultural research unit of the University of Pretoria, in Pretoria.

The diversity of trend responses is shown in Figure 2a, 2b and 2c, which respectively illustrate examples of Symons pan trends of rising, no change and declining evaporation, at different locations around South Africa, at the $p \leq 0.05$ significance level. Finally, plotting trend directions spatially to test visually for coherence (Figure 3) showed that each trend category was about equally and randomly distributed across South Africa. These apparently random patterns imply that there are no regional or sub-regional controls on pan evaporation trends.

Statistics of correlation

A Pearson correlation coefficient was calculated as a measure of the strength of association between all observed Symons pan evaporation and the P-M estimates of evaporation used in the SPEI, as described in the Methods above. This gives an r_{xy} of -0.00517, indicating no appreciable correlation between the two data sets (Figure 4). Focusing on a narrower data set, by removing potential outliers (accepting only -10>trend<10), confi ms that a linear regression of the estimated values on observed values is also not significant at $p=0.05$, indicating that the observed values are not a response to regional climatic factors, in that neighbouring pans can show different trends and there are no spatially uniform effects such as decreasing wind speeds or increasing humidity at the larger scales (see Figure 3), or at most that such regional influences are obscured by stronger influences of local conditions (Figure 5).

A Pearson chi-square test of independence of categorical variables was performed to determine whether the observed pan trends are linked to the P-M estimates of regional drivers of evapotranspiration. There are three categories for trends in both observed and estimated data based on the significance determinations of the regression analyses for each station ($p=0.05$): (1) increase, (2) no change and (3) decrease. A cross-tabulation of these categorical variables is given in Table 2.

Our null hypothesis *H0* is that the trends are independent and the alternative hypothesis *H1* is that the trends are related. The Pearson chi-square then gives $X^2 = 4.3769$, df = 4 and p-value = 0.3221. With the $p>0.05$ signifi ance level, *H1* is rejected, further supporting the conclusion that the observed and estimated values of evaporation trends are not related, and likely driven by a variety of local drivers, that possibly interact with weaker global drivers.

Pan evaporation values show some cyclicity for many of the instrument records (see Figure 2a,b,c, for example). There is a moderate inverse relationship of pan evaporation with annual rainfall (Figure 6).

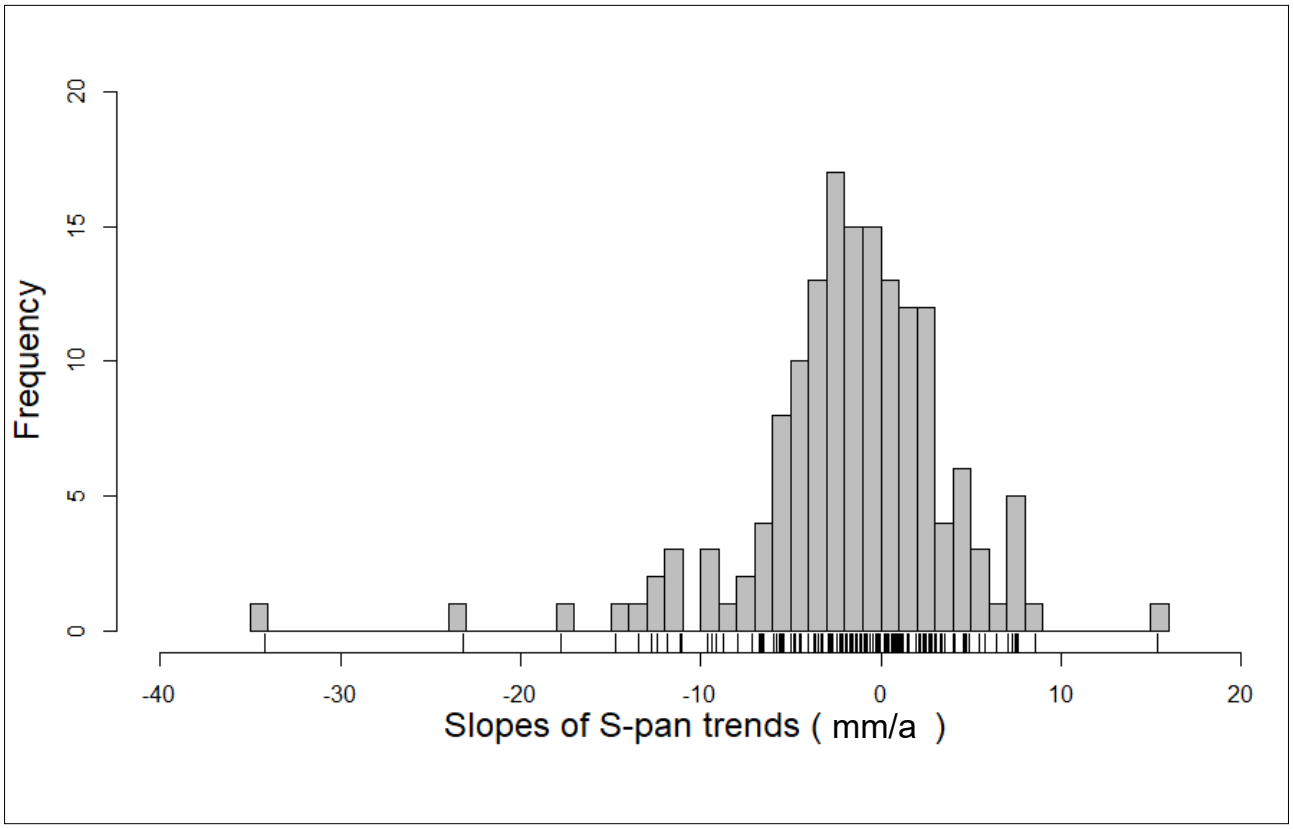


Figure 1: The distribution of changes in mm/a in S-pan evaporation for all results ($n=154$) as represented by the slopes in linear regression.

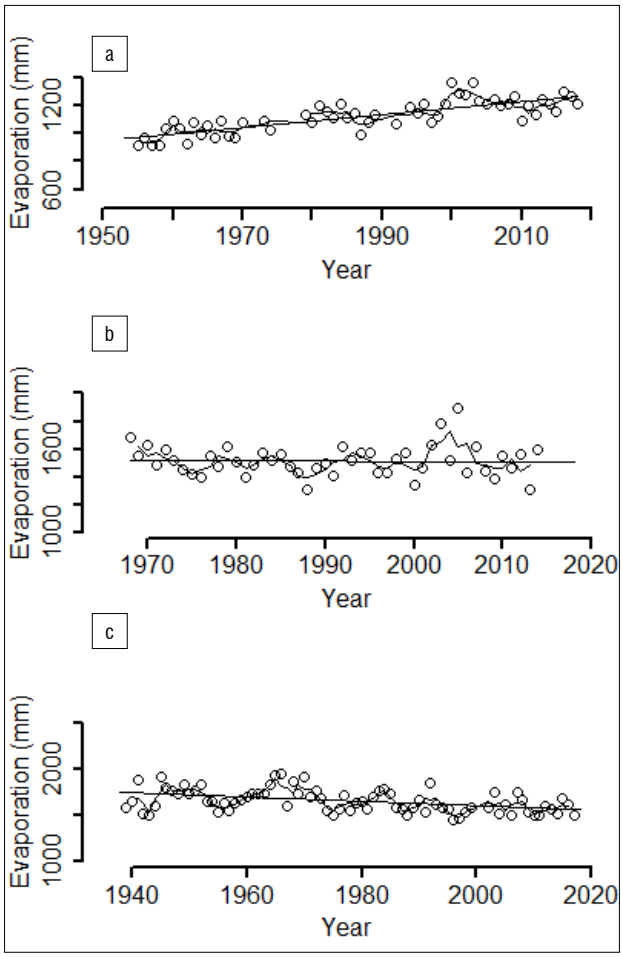


Figure 2: (a) A rising trend (β=4.6900) in Symons pan evaporation on Table Mountain, Western Cape, station G2E004 Tafelberg. (b) No significant trend (β=-0.0615), Bronkhorstspruit Dam, Gauteng Province, station B2E001. (c) A declining trend (β=-2.2166) Vaal Dam, Gauteng Province, station C1E001.

Discussion

The wide range of trends derived from the observed data do not accord with the hypothesis of a single dominant factor, such as global stilling or global dimming (or other large-scale influences) as the sole driver of evaporation. Our assessment is that trends in pan evaporation reflect the net result of multiple drivers which are a likely combination of local and regional to global phenomena.

We conclude from the results of the tests of correlation (Pearson coefficient, chi-square) that the observed longer-term trends of pan evaporation are unrelated to the regional atmospheric drivers of evaporation, assuming the P-M trend estimates are largely correct. At sub-decadal time scales, pan evaporation does correspond somewhat to annual variations in precipitation (Figure 6). During relatively wet years, pan evaporation declines and during relatively dry years, pan evaporation increases. This is likely a result of increased cloudiness and water in the environment supressing evaporation during the wet years. During the dry years, more energy is available in the environment and pan evaporation increases. This relationship supports the use of the SPEI as an independent test of the regional drivers of pan evaporation, and supports the hypothesis of a complementary relationship between E_a and E_p.[24]

Changes in local near-field conditions (fetch) that affect wind runs and humidity have a greater impact on advected energy that controls pan evaporation, while the two dominant global drivers of change (increasing temperature and decreasing wind speeds, if true), would tend to be counterbalancing. Global dimming is not accepted as cause for declining trends in evaporation because the observed direction of changes in surface solar radiation has generally been one of dimming in the 1950s to 1980s, brightening from the 1980s to 2000 with India still dimming, and after 2000, largely brightening but dimming in China/Mongolia and India.[27] The causes of these secular trends are the initial dimming with increased global production of aerosols, including sulfates, that came with industrialisation in the earlier parts of the 20th century, and then brightening as industrial processes reduced aerosol emissions.[27] We also do not accept local dimming because there is no evidence to suggest there are highly localised dimming causes.

The trends in the observed data in our results compare well to the range of trends found by others. Rayner[19] observed trends of -19 to 29 mm/a at different sites in Australia and related these to changes in wind run.

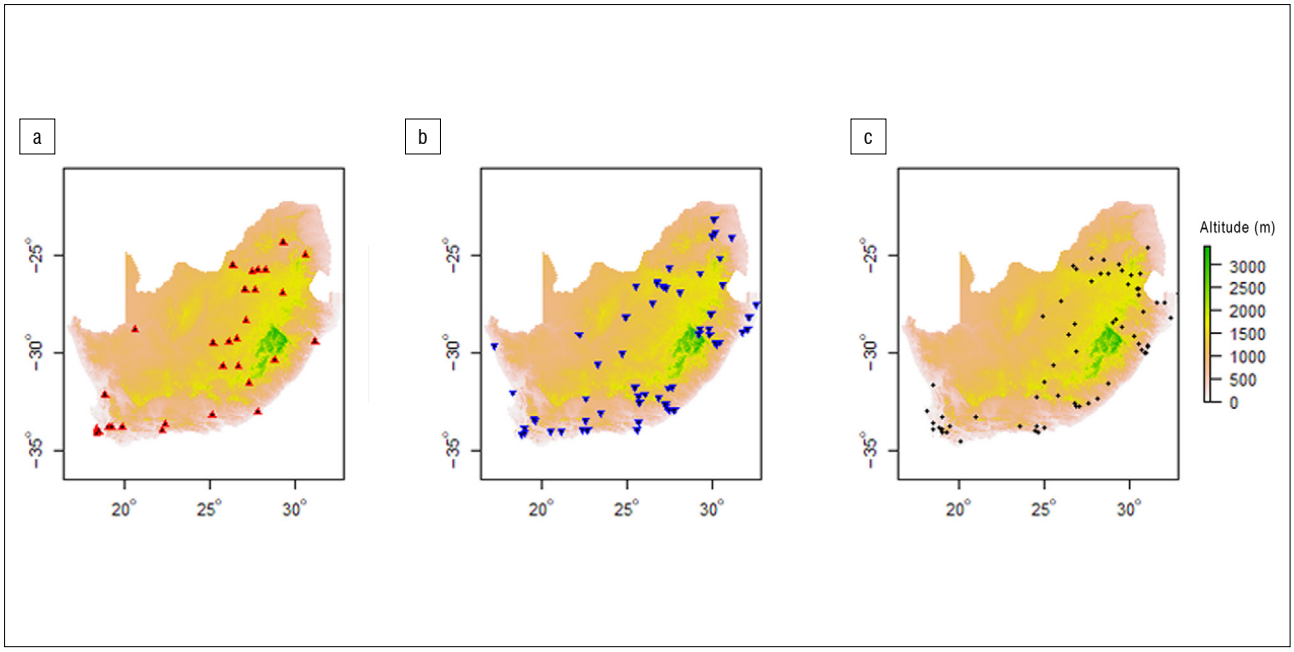


Figure 3: The spatial distribution of pan evaporation trends across South Africa, showing stations with (a) increasing, (b) decreasing, and (c) no trend.

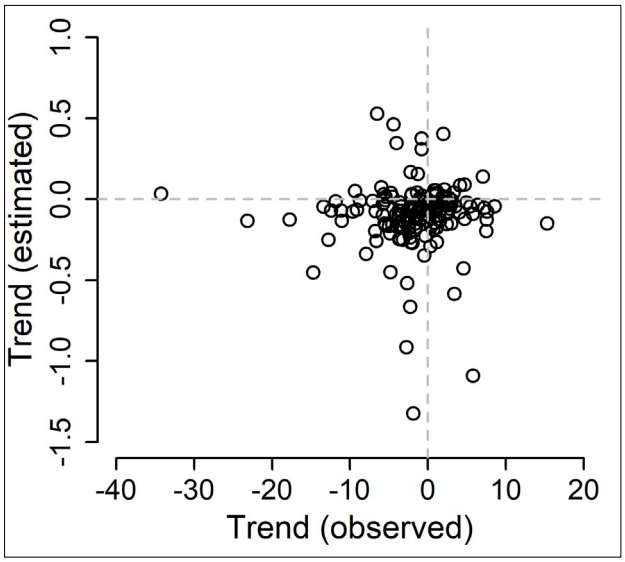


Figure 4: The slope coefficients (trend) in the regression equations of the Standardised Precipitation Evapotranspiration Index estimates plotted against the slope coefficients of the regression equations of the observed evaporation values (mm/a), $n=154$.

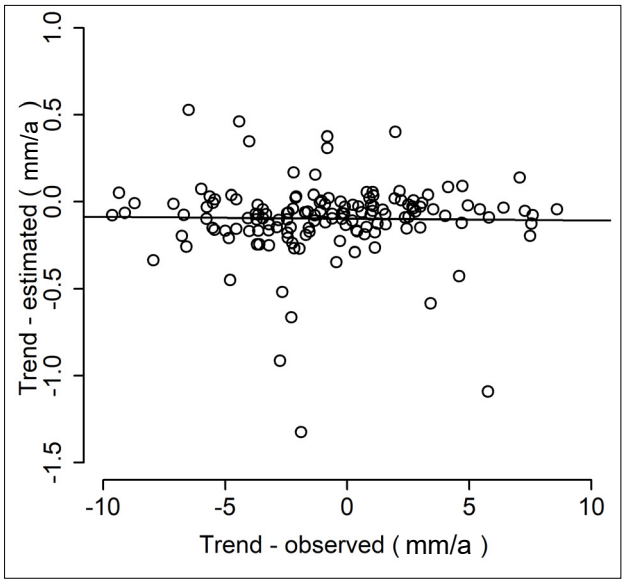


Figure 5: The slope coefficients (trend) in the regression equations of the Standardised Precipitation Evapotranspiration Index estimates plotted against the slope coefficients of the regression equations of the observed evaporation values with trend outliers removed (only -10 > observed mm/a < 10 accepted). The regression of observed on estimated (solid line) is not significant at $p=0.05$, $n=145$.

Table 2: A cross-tabulation of categorical variables of the observed against estimated evaporation trends

		Observed		
		Decrease	**No change**	**Increase**
Estimated	**Decrease**	3	6	2
	No change	53	58	29
	Increase	4	1	0

The causes of change in wind run could not be determined, but Rayner[19] noted that afforestation processes near Mt Gambier in South Australia could be a cause for wind run declines there. McVicar et al.[11], in their global assessment, reported a range of evaporation trends from -25.27 mm/a to 16.08 mm/a from a comprehensive list of studies and gave a number of possible reasons for declines in wind run, including the increase in surface roughness caused by increasing vegetation cover at local scales.

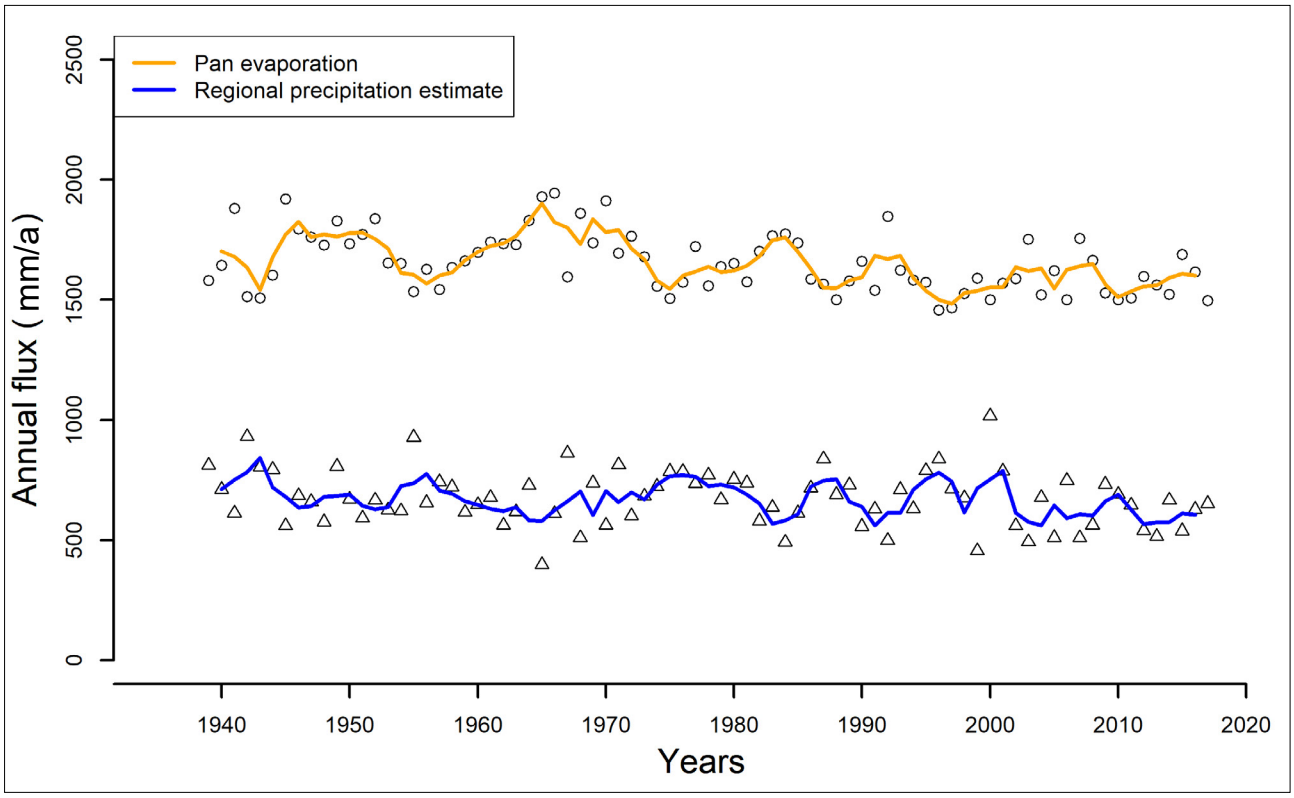
We propose that changes in fetch over the duration of observations are most likely drivers of change in pan evaporation rates and responsible for differences between the regional estimates and local pan evaporation. An inspection of selected evaporation monitoring sites showing the greatest decreasing and increasing trends, using Google Earth, supports this conjecture. For sites which could be visually located in the images, those showing steep or even moderate declines (see Figure 1) are usually associated with irrigation farming practices, have trees that have been planted as windbreaks or have grown unmanaged in the vicinity along fence lines and road edges, or have irrigated grass nearby (lawns surrounding buildings near where the instrument is positioned), or are located adjacent to unpaved roads (usually within 20–30 m) which possibly allows dust formation that coats the water surface of the nearby instrument. Those sites showing the strongest negative trends usually have a combination of these local environments, usually within 20–30 m of the instrument. This distance is much closer than the minimum fetch requirement of >150 m of short ground cover specified by Allen et al.[2] The static fetch requirements for using evaporating pans are not set, but various authors imply the need for minimum fetch distances from 100 m to 1000 m.[2,28,29] A changing surface within those ranges implies impacts on pan evaporation rates.

For those sites showing the strongest increases, the causes may be related to an increasing heat island effect, or are unknown at this stage. A full analysis of local land-cover changes at all stations is beyond the scope of this study and could be unfeasible for all stations. Further interrogation of this aspect using a more sophisticated approach for simulating trends in response to both local and global changes would be of great value for correct attribution of the diverse trends observed.

Global stilling as a phenomenon is not well supported by South African wind data. Kruger et al.[30] present data showing increases in gust strength at four sites in South Africa. Wright and Grab[31], analysing 20 years of wind data, note that of observed wind speed trends in the Western Cape with statistical significa ce (out of 14 recording sites), three showed declines and one an increase. These authors also note that 20 years is insuffic ent to establish conclusively the pattern of change in wind speeds. Jung et al.[32], in an analysis of global and national wind energy potential, specifically note South Africa as having a statistically significant increasing wind energy generation potential, implying that, at national scales, wind speeds are increasing.

The observation of declining wind speeds over terrestrial surfaces at specific locations[13] (local stilling) is difficult to reconcile with observed increasing trends in wind speeds over the ocean. Young et al.[33] and Young and Ribal[34] show increasing velocities over the Southern Ocean – trends which at least have effects on wind speeds along and near the coastal regions of South Africa. One explanation for locally declining wind speeds is the increasing density of trees in South African landscapes.[35-37] Woody encroachment – the spread of invasive alien trees across biomes and planted trees – likely causes lower surface wind speeds and is very likely a cause of changes in fetch conditions, possibly also accounting for attributions of global stilling for the evaporation declines postulated by others.

At more localised scales, we note that while all data sets used in the study by Hoffman et al.[13] showed declining pan evaporation in the fynbos region of the Western Cape, this study shows eight pans in the same region with statistically significant increases in evaporation over various time durations. Local influenc s on monitoring sites may be responsible for this discrepancy, and we urge care in over-interpreting observed trends without such consideration.



Data source: Regional precipitation estimates are from Harris et al.[25]

Figure 6: Annual Symons pan evaporation values (orange) for the Vaal Dam plotted against the CRU TS 4.03 precipitation data for the 0.5° gridded square for the same region.

We concur with McVicar et al.[11] that the problem of apparent declining evaporation rates has important implications for hydrological evaluations in those regions which are energy limited, but not those which are water limited. We point out that southern Africa, and especially the southwestern region of South Africa, are strongly water limited with respect to controls of water availability to meet evaporative demand (Figure 6).

Additional factors should also be considered. Within the terrestrial mid-latitudes in particular, the suppression of cloud formation (therefore leading to brightening) has been attributed to changes in land–atmosphere coupling triggered by atmospheric carbon dioxide (CO_2) enrichment.[38] Higher CO_2 concentrations reduce stomatal conductance, increasing energy partitioning into sensible heat and favouring higher free water evaporation, which is limited in availability.

Through our use of the Symons pan data, we have observed that the DWS evaporation database shows a marked decline in data quality, mostly from around the years 2004–2005 when substantial quantities of data are simply missing, or dispersion in the data increases, implying a loss of accuracy, which appears to occur from about 2000 onwards. In a number of cases, much of the evaporation record is unusable and had to be discarded from this analysis.

Conclusions

Our findings suggest there is no spatially or directionally coherent signal of declining pan evaporation over South Africa, as previous investigators have suggested based on smaller data sets.[13] There is sufficient evidence, as presented here, that pan evaporation not only has no statistically discernible trends in the majority of cases, but increases at some sites, including in the Western Cape Province where Hoffman et al.[13] showed decreases. We concur with authors who argue that changes in local conditions affect pan evaporation, and we reject the hypothesis that evaporation trends can be directly attributed to global influences alone.

Our analysis suggests that any signals from global drivers are overwhelmed by changes in local conditions. Changes in the fetch conditions around the evaporating pans, which include, by observation, increases in the number of trees and buildings which create resistance to air flows, irrigation practices leading to more humid air, and possibly dust from nearby unpaved roads, are likely responsible for most of the observed decreases in evaporation. Authors of global studies in which decreases in wind speed are observed, need to carefully consider changes over time in the fetch conditions upwind of the observing instrument. Increases in pan evaporation are likely to be attributable to increased wind runs at some sites – these changes could be caused by changing global circulation patterns or even the removal of objects which could have previously reduced the wind speeds near instruments. Thus, we conclude that the evaporation paradox is explained by a changing local environment that alters the wind run around evaporating pans.

Future research could usefully undertake further studies into the changing nature of the landscape with particular regard to fetch conditions around meteorological instruments. It would be useful to understand how conditions have changed around all of the pans, not only those which have showed decreasing trends in evaporation but also those which show increases. The evaporating pan remains an instrument of choice for its simplicity of operation; however, the findings here indicate the need for abundant caution when pan data are used for determining water budgets of reservoirs as well as water resource studies. Pan evaporation records in a large proportion of those examined prove not to be stationary. We conclude that attribution of observed trends likely requires case-by-case assessment of local to regional land-cover and land-use changes in addition to global influences. We also point to the obvious declines in data quality increasingly present in the evaporation component of the DWS's Hydrological Information System and suggest that attention urgently be given to rectify the situation to preserve the value of these long-term records.

Competing interests

We have no competing interests to declare.

Authors' contributions

R.A.C. described the fundamental problem, conceptualised part of the approach, collected the evaporation data, wrote the codes, performed the data analysis, and wrote substantial parts of the draft. G.F.M. conceptualised some of the methodological approaches, and undertook some of the writing, particularly the revisions of the draft. K.S. extracted the data for the independent test and undertook this, prepared the maps and contributed text to the draft.

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Gold mining's toxic legacy: Pollutant transport and accumulation in the Klip River catchment, Johannesburg

Waste from gold mines is considered to constitute the largest single source of waste pollution in South Africa and contributes significantly to acid mine drainage, which remains one of the country's most serious environmental and socio-economic issues. Run-off from the Central Rand Goldfield discharges into wetlands along the Klip River, which are known to be important sinks for toxic pollutants. The aim of this study was to examine the transport, migration and sequestration of metal pollutants in the upper Klip River catchment in further detail. Analyses reveal that the majority of pollutants are associated with contaminant plumes that emanate from mine dumps and enter the wetland via groundwater recharge. This water carries highly elevated concentrations of Co, Ni, Zn, U and rare earth elements, which are naturally sequestered within the wetland, largely through precipitation and adsorption. While surface run-off from mine dumps severely contaminates watercourses within the upper catchment, surface inputs are considered relatively minor contributors to the overall pollutant load entering the Klip River wetland, although aerosol fallout is an important source of Pb. The extensive accumulation of metals within the Klip River wetland reflects the contaminant legacy associated with gold mining on the Witwatersrand and highlights the vital role this natural system has played in trapping vast quantities of toxic pollutants and remediating downstream waters. Contaminant plumes associated with mine dumps will likely persist for decades; preventing further deterioration of the Klip River wetlands is thus critical for safeguarding water sources in the region.

Significance:

- Run-off and groundwater emanating from the Central Witwatersrand Basin is highly contaminated with toxic metals.
- The seepage of acidic water from mine waste dumps is considered the primary source of contamination.
- Significant quantities of pollutants are trapped naturally within the Klip River wetland
- Preventing further deterioration of the Klip River wetlands is critical for the protection of freshwater resources in the region.

Introduction

The Witwatersrand Basin is the world's largest gold resource; it has yielded more than one third of all the gold ever produced.[1] Although gold mining gave rise to the city of Johannesburg and formed the foundation of the country's economy for over a century, today it presents one of the largest threats to South African water resources and human health.[2,3] Gold mining is associated with numerous environmental and health issues, although acid mine drainage (AMD), which emanates both from underground workings and waste disposal sites, is undoubtedly the most serious of these.[4]

AMD arises from the oxidation of pyrite, ubiquitous in the Witwatersrand gold-bearing conglomerates, to form sulfuric acid. The acid produced mobilises constituents contained in the waste material and underground mine workings, releasing toxic metals such as cobalt, nickel, zinc and uranium. The major source of AMD on the Witwatersrand gold mines is associated with seepage from mine waste dumps, known as tailings storage facilities (TSFs). Waste from gold mines is considered to constitute the largest single source of waste pollution in South Africa.[5] There are more than 270 TSFs in the Witwatersrand Basin alone, most of which are unlined and pose serious threats to both groundwater and surface water quality. Although some TSFs have been partially or completely reclaimed, their chemical footprint continues to contaminate groundwater even after the waste material has been removed for reprocessing.[6] AMD also arises from the discharge of contaminated water from flooded defunct gold mines and discharge of partially treated water pumped from producing mines has also contributed to the problem in recent years.[2,7] Many of the gold mines on the Central and West Rand are situated beneath karst aquifers and must be constantly dewatered to prevent effluent rising to the su face and decanting from flooded mine voids.

The Central Rand, just south of Johannesburg, has been a prolific producer of gold for more than 100 years and is severely affected by the impact of mine tailings. Run-off from the Central Rand Goldfield discharges into wetlands along the Klip River, the principal drainage of the southern portion of Johannesburg. As a result, the Klip River wetland has been accumulating pollution since the founding of Johannesburg and previous studies have highlighted the extensive pollutant load sequestered within the wetland sediments.[8,9] While the lower reaches of the wetland are severely degraded, the upper reaches, which receive polluted water from old gold mines, are still reasonably intact and have a beneficial impact on the quality of water entering the Vaal River.[10] Given the economic and environmental value of the Klip River wetland, this study was undertaken to examine the transport, migration and sequestration of metal pollutants in the upper Klip River catchment in further detail. Firstly, we assessed the

significance of surface run-off and groundwater seepage as potential sources of pollutants by examining metal concentrations in sediment and water samples collected from the upper Klip River catchment. Secondly, we examined the ingress of metal contaminants and variability in their sequestration across the Klip River wetland through the detailed geochemical analysis of a series of sediment cores.

Study area

Regional setting

The Klip River basin is a sub-basin of the Vaal-Orange River system and represents the most significant drainage system for the southern Witwatersrand region (Figure 1a).[11] The Klip River and Klipspruit are the primary tributaries draining the Witwatersrand mining-industrial complex and discharge into the extensive wetland area further downstream.

Geology and geohydrology

The bedrock geology of the region is summarised in Figure 2. Gold is found in quartz pebble conglomerates, which are hosted in a sequence of siliceous quartzites of the Witwatersrand Supergroup. The Witwatersrand strata are conformably overlain by basaltic volcanic rocks of the Ventersdorp Supergroup, which in turn are overlain by dolomitic rocks of the Transvaal Supergroup. These rocks are karstic and host the Klip River wetlands. The wetlands are sustained predominantly by groundwater recharge from the underlying dolomite. During summer, this discharge is augmented by surface run-off from rainfall, with the Klip River and Klipspruit being the dominant fluvial inputs. The Klip River wetland drains into the Vaal River, which supplies approximately 23% of the South African population with potable drinking water.[12] Groundwater from underlying aquifers is also extracted for domestic consumption, and informal settlements use untreated river water for domestic and agricultural purposes.[13]

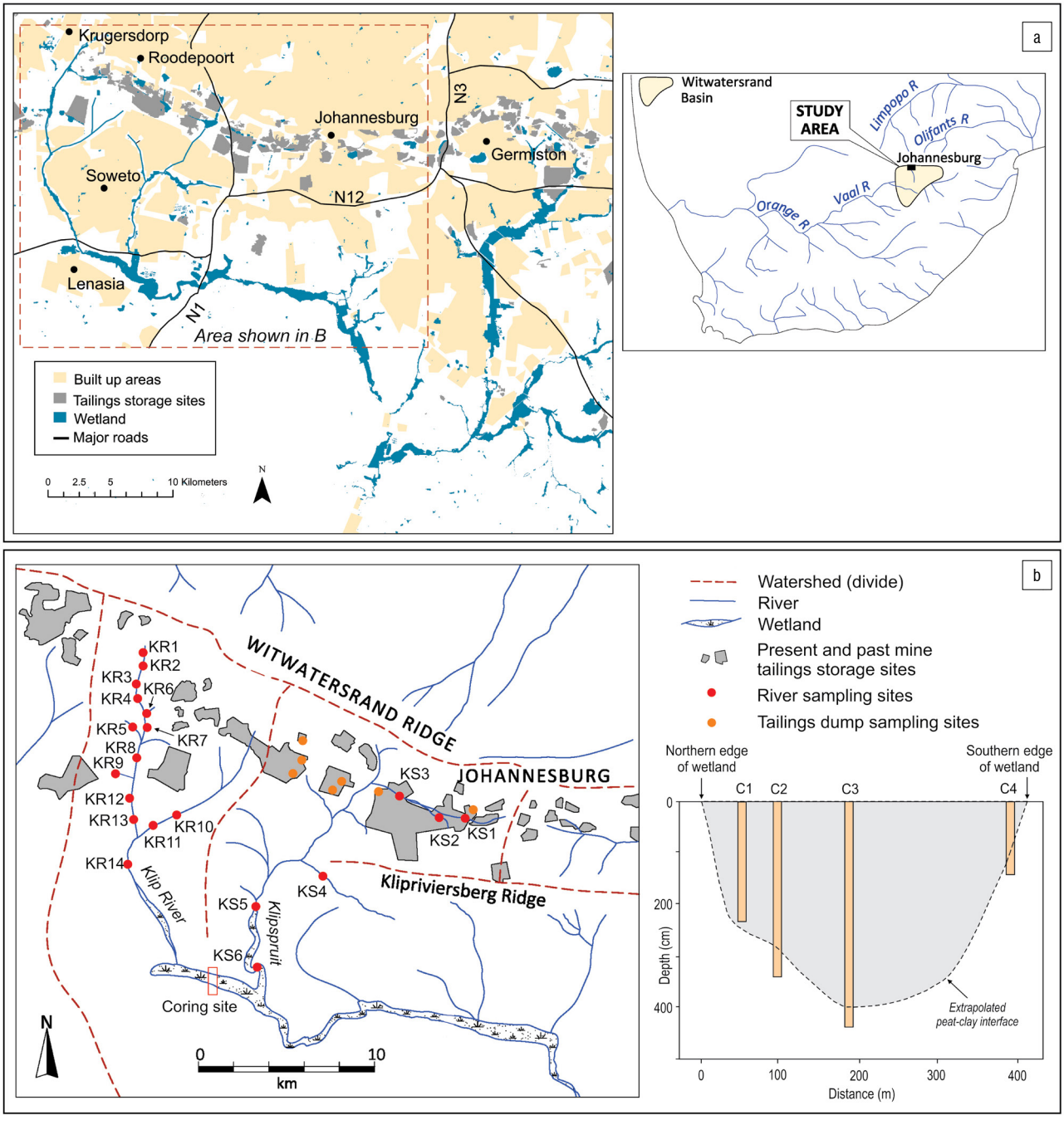

Figure 1: (a) Map of the study area showing the drainage network of the upper Klip River catchment in relation to mining activities and major urban centres in the Johannesburg area. The locations of water and sediment sampling sites along the Klip River (KR1–14) and Klipspruit (KS1–6) are also indicated. (b) Cross-sectional transect across the Klip River wetland showing the position of the four cores (C1–4) collected.

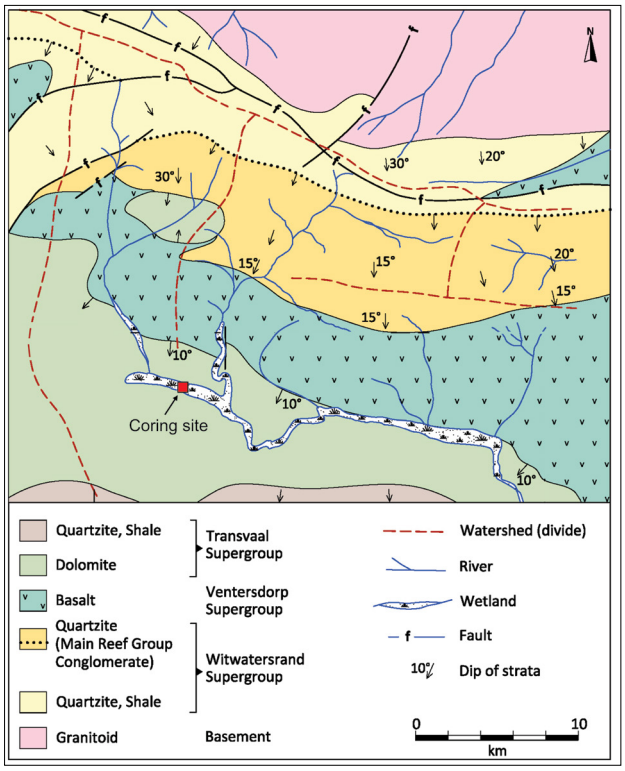

Figure 2: Simplified geology of the study area [15]

Pollutant sources

TSFs represent important sources of pollution within the Witwatersrand Basin and are typically associated with extensive acid plumes that contaminate the local groundwater. The formation of acid plumes is caused by the infiltration of rainfall through the TSFs, resulting in the discharge of contaminated water into nearby streams and rivers.[14] The Klip River wetland also receives run-off from several large informal settlements and industrial regions, as well as discharge from three sewerage treatment plants.

Methods

Sampling

Water (n=20) and surface sediment (n=15) samples were collected from the upper Klip River (KR1 – 14) and Klipspruit (KS1 – 6) tributaries during March 2017 (Figure 1). Sampling sites were established based on accessibility and in an attempt to capture downstream variations. The pH and electrical conductivity of samples were measured in field using a portable combination meter (Thermo Scientific Orion Star) calibrated against appropriate standard solutions. Water samples were immediately passed through 0.45-μm filters, acidified with 1% HNO_3 and then refrigerated. Sediment samples were placed in bags and stored at 4 °C. Surface material from several mine TSFs in the upper catchment was also sampled (Figure 1) in order to characterise the chemical composition of gold mine tailings.

A series of four sediment cores (C1–C4) was collected along a 350-m north-south orientated transect established in the upstream, western section of the Klip River wetland (Figure 1). This section of the wetland is characterised by intact reed-covered swamp dominated by *Phragmites australis* and is considered to have remained relatively undisturbed for the last ~80 years based on examination of historical aerial photography and maps.[10] Samples were collected to refusal depth using a Russian peat corer. Sub-samples were taken at 10-cm intervals and sealed in plastic bags for subsequent analysis.

Chemical analysis

Tributary and wetland sediment samples were air-dried, milled and combusted at 500 °C for 4 h to determine loss on ignition. Combusted sample powders (~25 mg) were microwave digested in 9 mL HF:HNO_3:HCl (6:3:1) using an Anton Paar Multiwave Go system.[16] Solutions were evaporated to dryness following the addition of perchloric acid (0.5 mL), before finally being diluted with 1% HNO_3 and spiked with Rh as internal standard. Metal and rare earth element (REE) concentrations were measured against external matrix-matched standards using an Agilent 7800x inductively coupled plasma mass spectrometry (ICP-MS). Procedural blanks were analysed with each digestion batch and used to correct final sample concentrations. Sediment standards PACS-2 and CGL-111 were used to assess the accuracy and reproducibility of the method, with recoveries ranging between 88% and 110% (n=6). Internal precision was typically <3% for all elements. Major elements were analysed by powder X-ray fluorescence using a Bruker Ranger S2, following calibration against a range of local and USGS (US Geological Survey) rock standards.

Metal and REE concentrations in water samples were measured by ICP-MS as reported above. Where possible, porewater was extracted from wetland core samples using direct centrifuge drainage.[17] The pH and metal content of porewater samples were determined using the procedures described above.

Enrichment factors

To account for variations in sample composition and mineralogy, metal concentrations are expressed as enrichment factors (EF). While the calculation of EF values is subject to several assumptions (see for example Reimann and Caritat[18]), we apply this normalisation in examining the spatial distribution in metal concentrations within the study area. Enrichment factors were calculated relative to upper continental crust (UCC) values[19] using Li as the normalising element (Equation 1). Lithium behaves conservatively in the environment and is not associated with mine waste.

$$EF = \frac{\left[\frac{C_{Metal}}{C_{Li}}\right]_{Sediment}}{\left[\frac{C_{Metal}}{C_{Li}}\right]_{UCC}} \qquad \text{Equation 1}$$

Calculated EF values were assessed and classified into seven categories: (1) <1: no enrichment; (2) 1–3: minor enrichment; (3) 3–5: moderate enrichment; (4) 5–10: moderately severe enrichment; (5) 10–25: severe enrichment; (6) 25–50: very severe enrichment; (7) >50: extremely severe enrichment.[20,21] Relationships between metal concentrations were examined using analysis of variance (ANOVA), with significance set at p<0.05.

Results

Surface sediment and water chemistry

Water samples collected from the upper Klip River catchment and Klipspruit revealed a high degree of chemical variability (Table 1). Highest trace metal concentrations were generally measured in water samples from the Klipspruit, which were typically characterised by high electrical conductivity and elevated levels of Fe and Al. Several other metals were detected in significant concentration in the Klipspruit, notably Co, Ni and Zn. The most contaminated sample was collected in the vicinity of TSFs located in the upper Klipspruit catchment (site KS3) and contained highly elevated concentrations of Fe (330 mg/L), Co (2.1 mg/L), Ni (2.7 mg/L), Zn (2.8 mg/L) and U (0.35 mg/L).

Metal concentrations measured in porewater samples from the Klip River wetland were extremely variable. Average concentrations exceeded those measured in both tributaries, with several porewater samples characterised by exceptionally high levels of Al, Fe, Co, Ni and Zn. Comparatively lower concentrations of Cu, Pb and U were detected. Maximum measured metal porewater concentrations exceeded those typically found in mine void water from the Central Witwatersrand Basin.

Most trace metals were present in highly elevated concentrations in sediments collected from the Klipspruit and upper Klip River catchment (Table 2). Sediments from the Klipspruit were characterised by considerably higher proportions of clay, while those from the Klip River tended to be more organic-rich. Normalised metal concentrations show Klip River sediments to be substantially more enriched than those of the Klipspruit.

Table 1: Chemistry data for surface water catchment samples and porewater samples from the Klip River wetland

	pH	Electrical conductivity (mS/m)	Al	Fe	Co	Ni	Zn	Cu	Pb	U
			mg/L							
Klip River (n = 14)										
Maximum	7.6	95	0.84	2.5	0.032	0.20	0.48	0.4	0.012	0.10
Minimum	4.3	15	<DL	<DL	0.0002	0.0012	<DL	<DL	<DL	<DL
Mean	6.7	52	0.091	0.30	0.0082	0.075	0.15	0.086	0.0028	0.020
s.d.	0.8	33	0.23	0.68	0.0088	0.064	0.12	0.10	0.0044	0.028
Klipspruit (n = 6)										
Maximum	7.8	379	60	330	2.1	2.7	2.8	0.35	0.0076	0.34
Minimum	6.5	15	<DL	0.012	0.0002	0.0044	0.073	<DL	<DL	<DL
Mean	7.1	112	8.9	47	0.33	0.46	0.66	0.10	0.0019	0.055
s.d.	0.6	150	22	124	0.77	1.0	0.99	0.14	0.0029	0.13
Wetland porewater (n = 94)										
Maximum	7.9	60	1000	804	8.9	47	30	14	1.4	8.1
Minimum	5.8	0.9	<DL	<DL	<DL	0.006	0.36	0.026	0.004	<DL
Mean	6.9	26	66	55	0.75	3.9	5.5	1.2	0.15	0.36
s.d.	0.4	5.6	155	118	1.4	7.3	6.6	1.9	0.27	1.1
DWAF[22]	6.5–8.4	<70	0.15	0.1	0.5	0.15	5	1	0.02	0.02
Central Basin void mine water[23] (n = 12)	3.0	397	120	40	4.7	10.6	9.1	0.33	0.028	0.61

DL, detection level

Table 2: Mean concentrations (mg/kg) and enrichment factors (EF) for metals in tributary surface sediments and material from tailings storage facilities (TSFs)

	Klip River (n = 10)		Klipspruit (n = 5)		TSFs (n = 7)	
	Mean±s.d.	EF	Mean±s.d.	EF	Mean±s.d.	EF
C_{org} (%)	18 ± 14	–	12 ± 12	–	0	–
Li	17 ± 9.6	–	108 ± 95	–	7.6 ± 3.9	–
Al (%)	3.6 ± 1.5	0.50	6.6 ± 3.3	0.20	3.4 ± 0.89	1.0
Fe (%)	5.4 ± 3.0	1.2	4.6 ± 3.2	0.50	2.9 ± 1.1	1.7
Co	104 ± 73	13	198 ± 304	2.8	13 ± 13	2.1
Ni	312 ± 234	11	328 ± 483	1.8	<DL	–
Zn	512 ± 445	13	700 ± 298	2.6	<DL	–
Cu	219 ± 218	13	114 ± 66	1.3	25 ± 27	2.8
Pb	79 ± 56	8.5	62 ± 54	1.8	33 ± 17	7.2
U	20 ± 21	11	23 ± 25	4.6	12 ± 12	13
∑REE	125 ± 54	–	304 ± 175	–	100 ± 19	–
$\sum REE_{PAAS}$	8.7 ± 3.9	–	15 ± 5.4	–	5.5 ± 1.3	–

DL, detection limit

Most metals were present in Klip River sediments at severe enrichment levels, while minor to moderate enrichment characterised sediments from the Klipspruit. Surface material from TSFs comprised predominantly (~95%) SiO_2 and Al_2O_3, but showed enrichment with respect to some trace metals, notably Pb and U. Co and Cu exhibited minor enrichment, while Ni and Zn were not detected in any of the TSF samples analysed.

Core characteristics and major chemical composition

The cores retrieved from the Klip River wetland ranged in length from 140 cm to 440 cm. An underlying clayey sand layer was intercepted at all sites, except site C1, and marked the depth at which point of refusal was reached. The upper sections of all cores were generally composed of organic-rich sediment (peat), with loss on ignition varying between 65% and 85% (Figure 3a). Little variation was observed in pH, both with depth and between individual coring sites.

The inorganic fraction of the peat was dominated by fine-grained material composed largely of SiO_2 (59±6.9 %) and Al_2O_3 (16±3.5 %), with cores closer to the edge of the wetland (C1 and C4) characterised by slightly higher SiO_2 and Al_2O_3 contents. Marked variations in Fe_2O_3 and CaO abundances were observed, both downcore and across the wetland (Figure 3b,c). Fe_2O_3 was typically enriched in the upper peat layers, with enrichment most prevalent in C2. Peat CaO concentrations ranged between 0.3% and 21%, with cores C2 and C3 characterised by particularly high concentrations. In contrast to Fe_2O_3, CaO concentrations were typically highest within the middle and lower sections of the peat profiles and were closely matched by variations in total sulfur, which ranged between 0.1% and 11%.

Trace metal profiles

Metal profiles showed substantial downcore variations, although enrichments occurred in different sections of the peat profiles. Co and Ni showed similar downcore trends, with highest enrichment typically found near the base of the peat sequences (Figure 4a,b). Overall, Ni was significantly more enriched than Co, with EF values varying between 0.2–68 and 0.2–87, respectively. However, both metals showed similar lateral trends, with enrichment in the peat generally decreasing from north (C1) to south (C4). Co and Ni concentrations were approximately 15 and 6 times lower in C4 than in C1. Zn was concentrated in the middle and lower sections of the peat profiles, where it was present at highly enriched levels (Figure 4c). Enrichment in Zn tended to increase towards the centre of the wetland, with EF values of up to 300 recorded for C3. Lowest Zn concentrations were measured in C4 (EF<10).

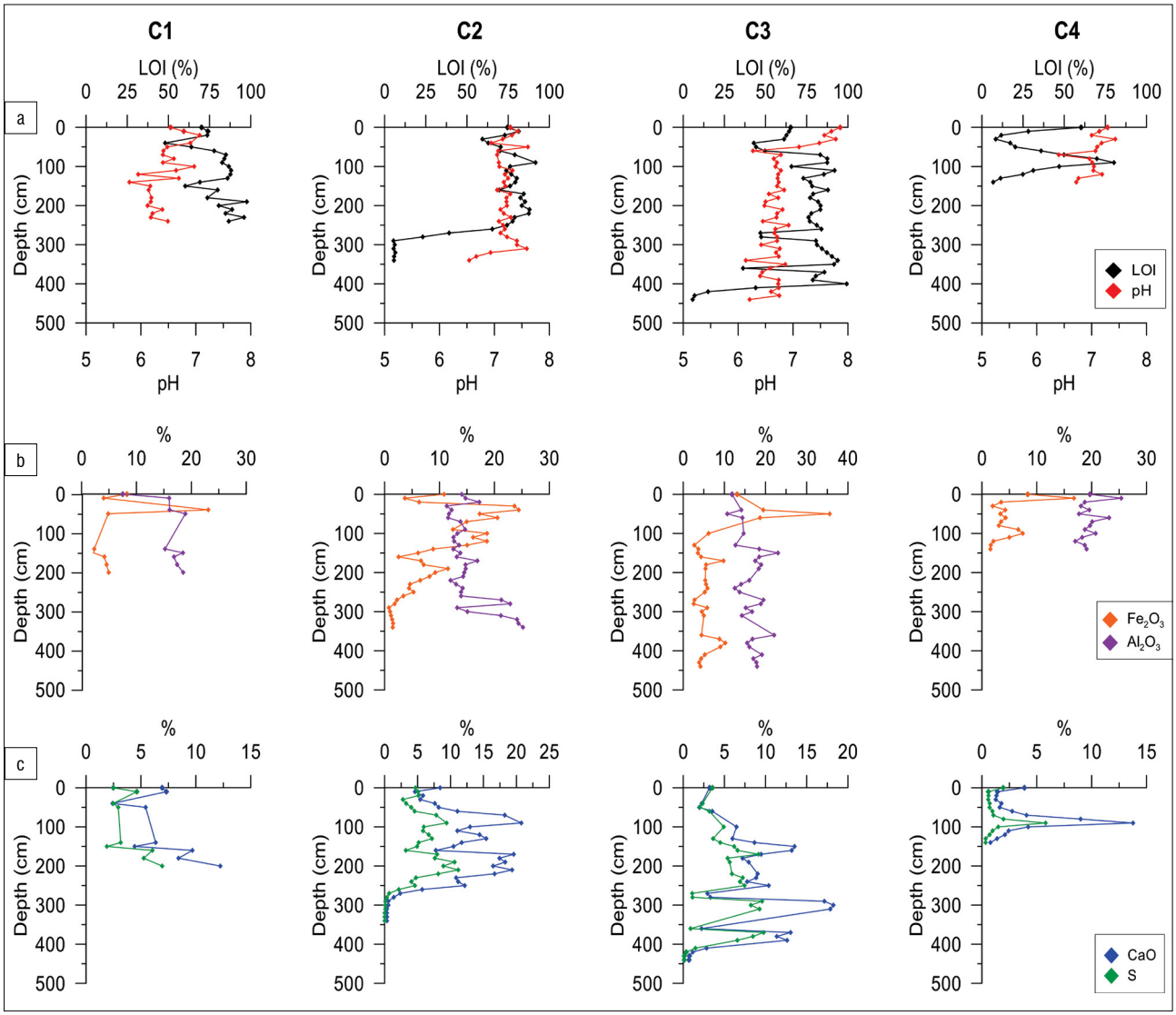

Figure 3: Downcore profiles showing variations in (a) sediment pH and loss on ignition (LOI), (b) Fe_2O_3 and Al_2O_3 concentrations, and (c) CaO and S concentrations at coring sites C1–4.

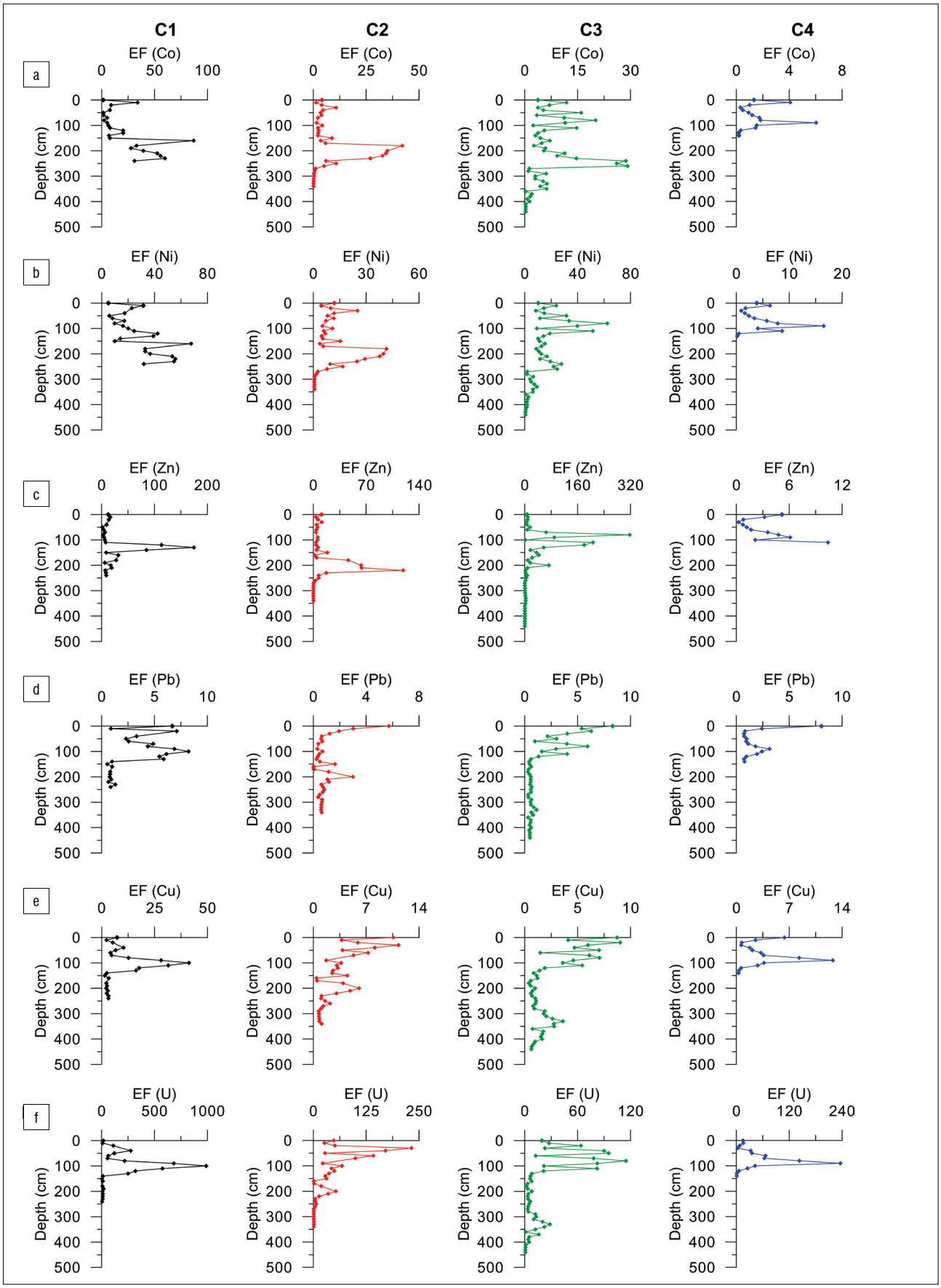

Figure 4: Downcore metal enrichment profiles for sites C1–4. Enrichment factor (EF) values calculated relative to upper continental c ust.[19]

In contrast to the sub-surface enrichments displayed by Co, Ni and Zn, Pb was found at highest concentrations within the upper (<100 cm) sections of the peat sequences (Figure 4d). At the surface, Pb enrichment typically ranged between 6 and 8, showing little lateral variation across the wetland. Cu and U exhibited similar downcore distributions, with highest enrichments typically confined to the upper 100 cm of the peat profiles (Figure 4e,f). Both metals were significantly enriched in C1, with pronounced decreases in concentrations towards the centre of the wetland.

Rare earth element patterns

The downcore distributions in ∑REE concentration were remarkably similar across all four profiles (Figure 5a). Highest concentrations were typically confined to a pronounced zone of enrichment between 50 cm and 150 cm. There were modest variations in ∑REE concentration across the wetland, although highest concentrations (~6000 mg/kg) were found in C1. Post-Archaean Australian Shale (PAAS)-normalised patterns indicate that REEs in the upper section of C1 are enriched three-fold relative to the other cores (Figure 5b). Enrichment in the middle REEs (MREEs) relative to both the light (LREEs) and heavy REEs (HREEs) is a characteristic across all coring sites.

Discussion

Pollution extent, sources and pathways

The Central Basin extends approximately 55 km, from Durban Roodepoort Deep in the west, where the Main Reef terminates against the Roodepoort Fault, to East Rand Proprietary Mines in the east (Figure 6). Mining operations commenced in 1886, and although the mines started off as separate entities, the underground workings eventually merged over time, forming a continuous mine void extending from the East Rand to the West Rand.[7] Large-scale mining began to curtail in the 1950s, with the closure of Durban Roodepoort Deep in 1999 and East Rand Proprietary Mines in 2008 bringing an end to deep-level operations in the basin. A long history of mining operations, together with the progressive closure of mines, has resulted in the wide-scale discharge of contaminants into the streams and watercourses draining the Central Basin.[9,10,14]

AMD issuing from gold mines on the Witwatersrand is characterised by high concentrations of Ni, Co, Zn and U.[23] These metal contaminants enter the river systems through run-off from TSFs as well as through decant of effluent from slimes dams and mine voids. The two streams sampled in this study, the Klip River and Klipspruit, drain the western portion of the Central Basin, where several large mining operations were located, including Durban Roodepoort Deep and Crown Mines (Figure 6). Samples collected from the Klip River and Klipspruit revealed widespread contamination of watercourses in the region. Metal concentrations often exceeded accepted maximum levels, particularly for Al, Fe, Ni and U. Higher concentrations measured in water samples from the Klipspruit compared to the Klip River likely reflect the degree to which the upper reaches of each tributary have been affected by mining. The Klipspruit drains a portion of the Central Basin that has been intensively mined out and which is characterised by the presence of several large TSFs. Interestingly, sediments from the Klip River revealed higher levels of metal enrichment compared to the Klipspruit. To some extent, this appears to reflect differences in the nature of the material analysed, with sediment from the Klip River characterised by substantially lower aluminium concentrations (reflecting a lower clay content) and higher amounts of organic carbon (averaging ~20%) compared to the Klipspruit. Effluent from industrial and residential sources, particularly around Roodepoort and Soweto, may also be a significant contributor to pollutant loads in the Klip River.

Contaminants associated with AMD may also enter the groundwater via seepage through TSFs or directly from flooded mine voids. TSFs on the Witwatersrand are known to be significant contributors to AMD[14,24] and often constitute the single largest source of mining-related water pollution[24]. Tailings material, which is dominated by unconsolidated silicate minerals, is prone to leaching and percolation of rainwater through the dumps, resulting in the formation of polluted groundwater plumes. The geochemical processes characterising Witwatersrand TSFs have been fairly well studied[25,26], with the mobility of metals within dumps largely controlled by a combination of pH and redox parameters. Impoundments are generally considered to consist of two key zones – an outer oxidation zone characterised by the presence of sulfate minerals (especially jarosite and gypsum) and secondary oxy-hydroxides, and an inner reduction zone.[26]

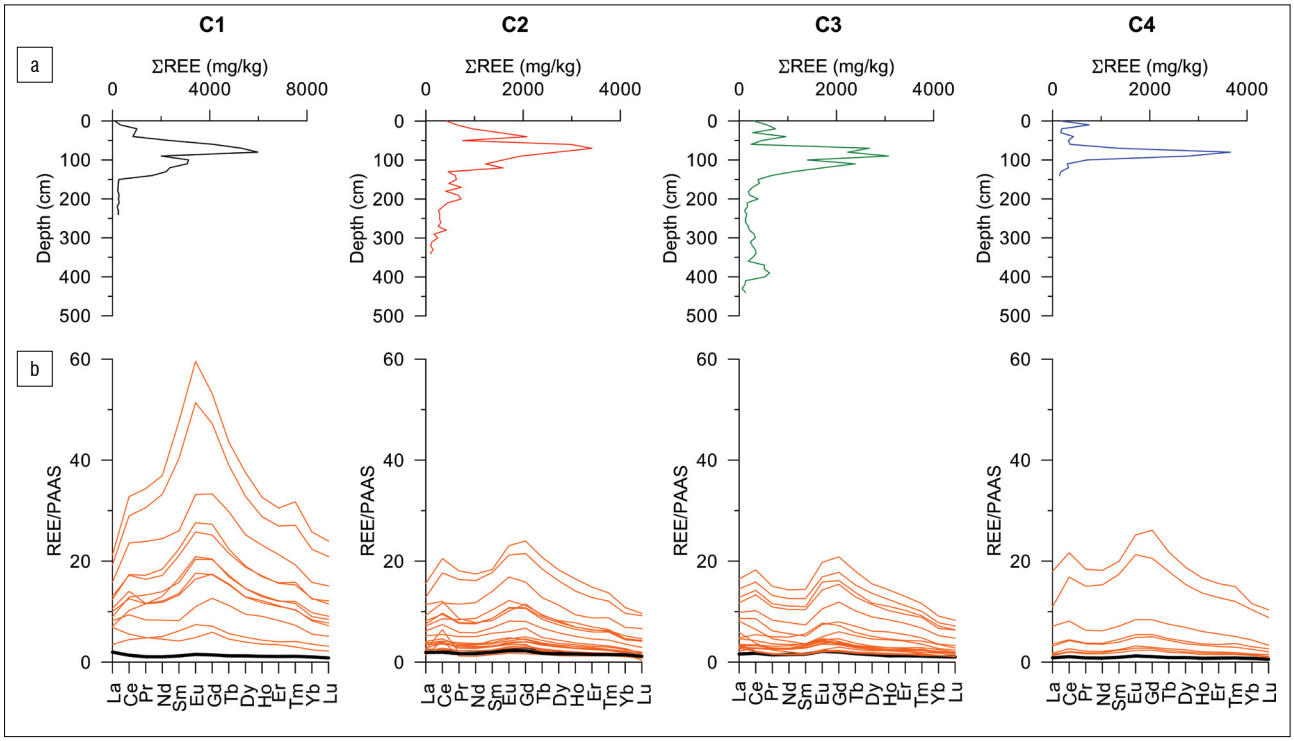

Figure 5: (a) Downcore total rare earth element (∑REE) concentration profiles for sites C1–4 and (b) Post-Archaean Australian Shale (PAAS)-normalised REE patterns. REE-enriched samples (REE/PAAS >1) are shown in orange, while the average of non-enriched samples is indicated in black.

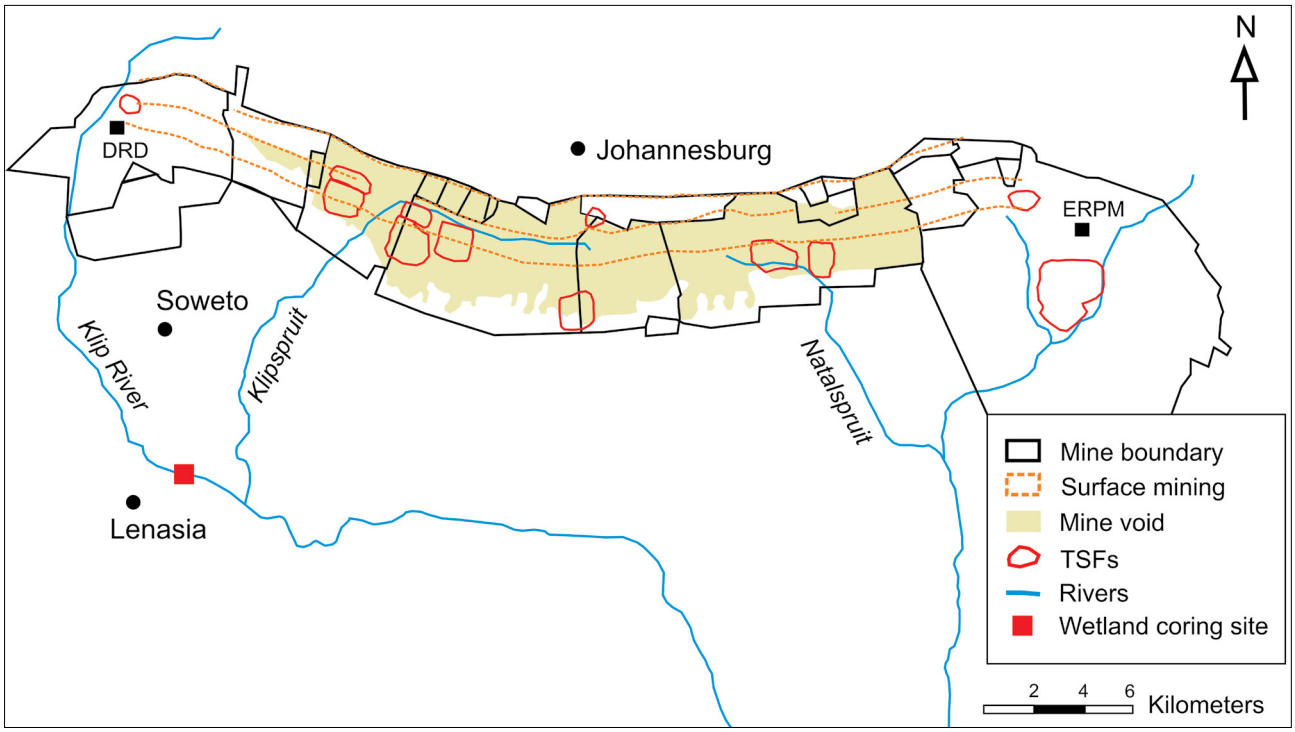

Figure 6: Map showing the extent of mining activities and areas of major water ingress into the Central Basin mine void.[23]

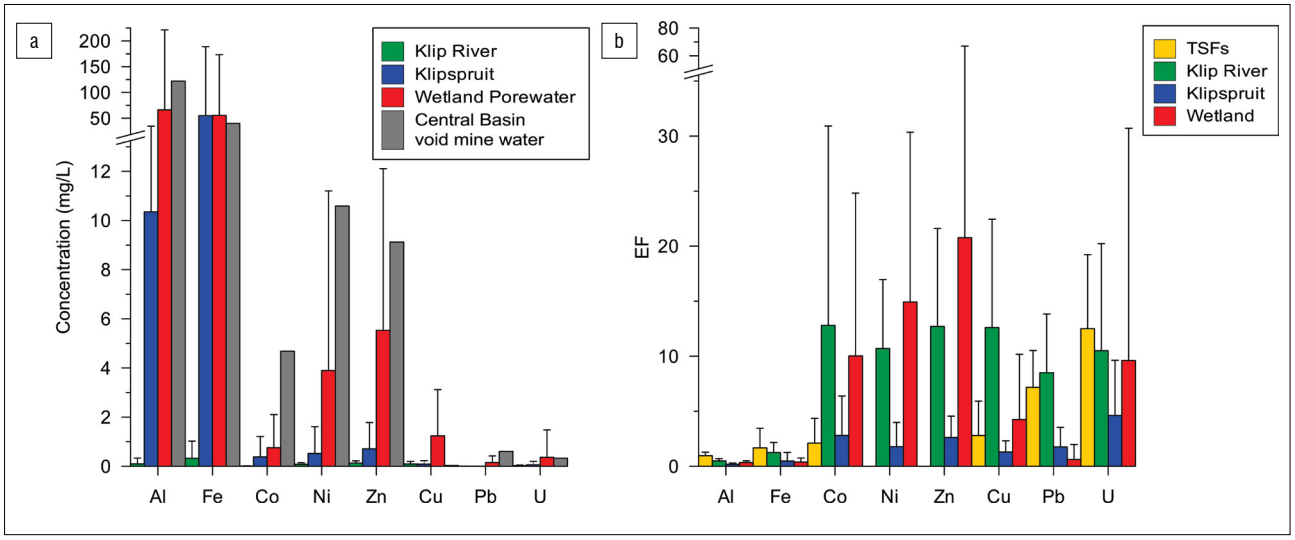

Figure 7: (a) Comparison between average metal concentrations measured in water from the Klip River, Klipspruit, Klip River wetland (this study) and the Central Basin mine void[23], and (b) average metal enrichment factors (EF) measured in material collected from tailings storage facilities (TSFs), Klip River, Klipspruit and wetland.

Pyrite on the Witwatersrand is associated with several trace metals found in relatively high concentration, notably Co (1006 mg/kg), Ni (1930 mg/kg) and Zn (90 mg/kg).[27] These metals are released during the oxidation process, which also results in the formation of sulfuric acid. Most metals – including Fe, Al, Ni and U – are highly soluble under acidic reducing conditions and are leached from TSFs into the groundwater.

Samples collected from several TSFs located in the Central Basin show that tailings surface material is characterised by several trace metals that are present in elevated concentrations, notably U and Pb (Figure 7). Elevated concentrations of U (up to 5.8%) are a prominent feature of Witwatersrand gold-bearing conglomerates.[25] Although originally mined as a by-product of the gold industry, most of the U extracted as part of the gold mining process was dumped in slimes dams on the Witwatersrand.[6] Uranium is found in the mineral form uraninite (UO_2), which reacts in acidic solutions to produce soluble U^{4+} ions. Mine dumps thus contain and leach considerable quantities of U, which accumulates in wetlands and river sediments in the region.[25,28] Pb is often present in the Witwatersrand conglomerates as galena (PbS), but it also found in association with pyrite.[27] Although galena may be oxidised under acidic conditions, Pb is not a notable feature of AMD on the Witwatersrand and has been reported in relatively low concentration (<1 mg/L) within shallow acidic groundwaters.[14] This is consistent with Pb enrichments measured in tailings material, suggesting that Pb is present in insoluble oxide and sulfate forms, or absorbed to Fe-(oxy)hydroxides or onto clay minerals, limiting the degree to which it is leached from TSFs. However, several other important metals were either not detected in tailings material (e.g. Ni and Zn) or showed only moderate enrichment (e.g. Al, Fe, Co and Cu). These metals are leached from the tailings under acidic conditions and form the major constituents in seepage from mine dumps on the Witwatersrand.

Acidic seepage from TSFs drains directly into underlying aquifers. Because the Witwatersrand Supergroup lies adjacent to the karstified dolomites of the Malmani Subgroup, seepage from TSFs results in widespread groundwater contamination in the area. Many tailings dumps and slimes dams along the Witwatersrand were purposefully positioned on dolomite to facilitate water drainage, resulting in drier, more stable impoundments.[7] Seepage of AMD directly into the karst system below not only results in the contamination of deeper groundwater, but also dissolution of the dolomite.

Neutralisation is likely an important process for metal sequestration and is a common process employed in wetlands constructed for AMD treatment.[29,30] The high concentrations of calcium (up to 20%) measured in peat from the Klip River wetland indicate the important role the underlying dolomite substrate likely plays in sequestering metals from groundwater flowing into the wetland. Sulfate salts would be expected to form when sulfuric acid reacts with the alkaline dolomite and the strong relationship between Ca and S abundances suggests the widespread precipitation of gypsum within the wetland. The high metal load carried in the acidic groundwater plumes oxidises and precipitates within the wetland under higher pH (>6) conditions, resulting in substantial peat enrichments, particularly with respect to Ni, Zn, Co and U (Figure 7b).

Variations in pollutant accumulation and sequestration processes

The contamination of both surface water and groundwater discharging from the Central Basin causes pollutants to enter and accumulate in different parts of the Klip River wetland. Contour plots show that metals strongly associated with AMD (Co, Ni and Zn; Figures 8a–c) enter the wetland along the northern edge via groundwater flow and accumulate predominantly within the deeper peat (typically below 1 m). The ingress of these contaminants into the wetland is associated with acidic groundwater plumes, which originate from the numerous TSFs that dominate the Central Rand. Although these contaminants enter the wetland via the same transport processes, their migration and sequestration within the peat is influenced by various physico-chemical factors. Co and Ni show similar distribution patterns (R^2=0.73), suggesting that these metals are sequestered via a common mechanism. Humphries et al.[9] assessed the partitioning of metals within Klip River sediments and found that Co and Ni were preferentially associated with the reducible (Fe-hydroxides) and oxidisable (organic matter/sulfides) fractions. Zn, which shows no correlation with Co and Ni, is instead predominantly (>50%) associated with Fe-hydroxides[9], which precipitate readily under wetland conditions (pH=6–8).

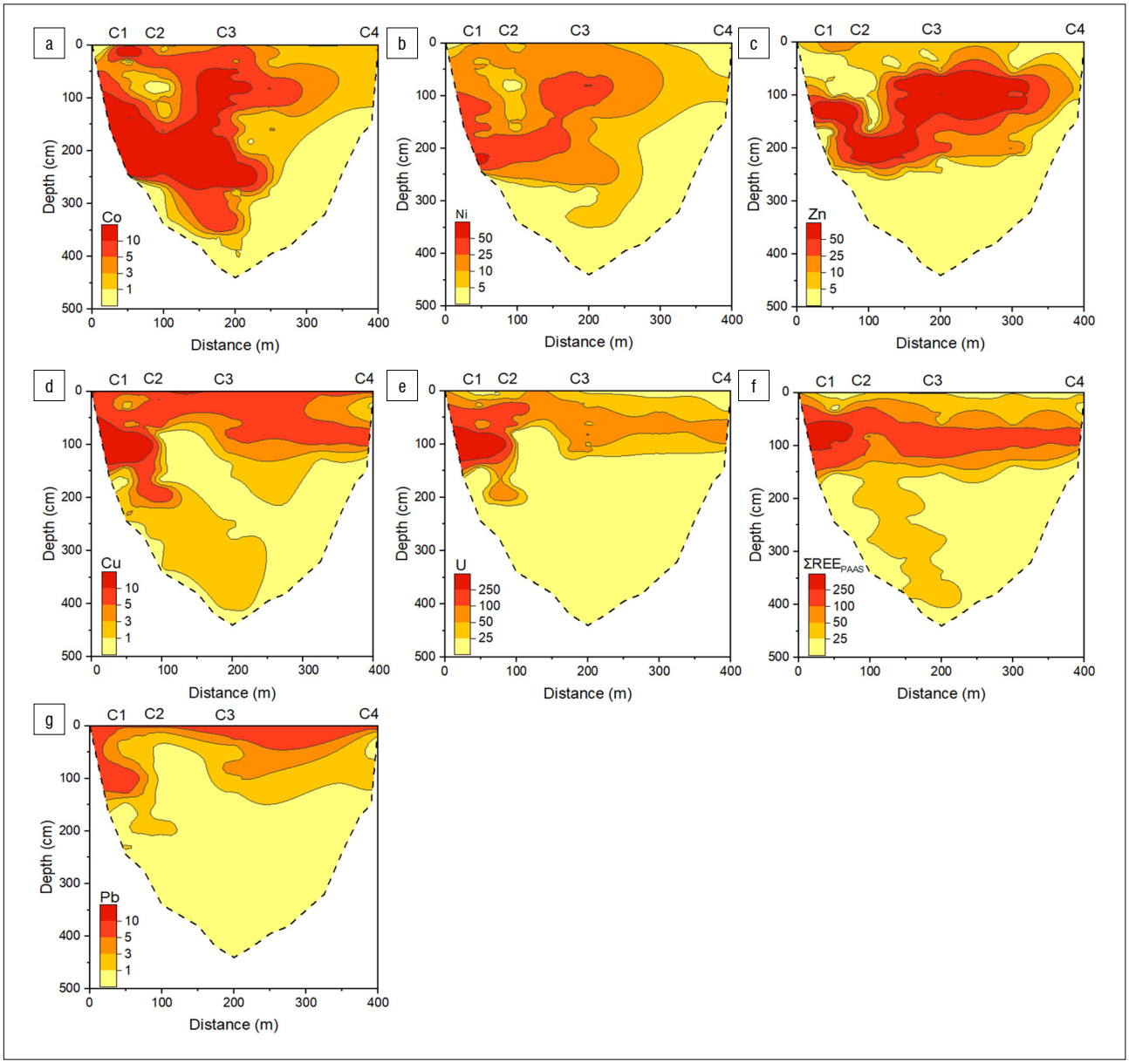

Figure 8: Contour maps illustrating the ingress of pollutants using the enrichment factors in the wetland.

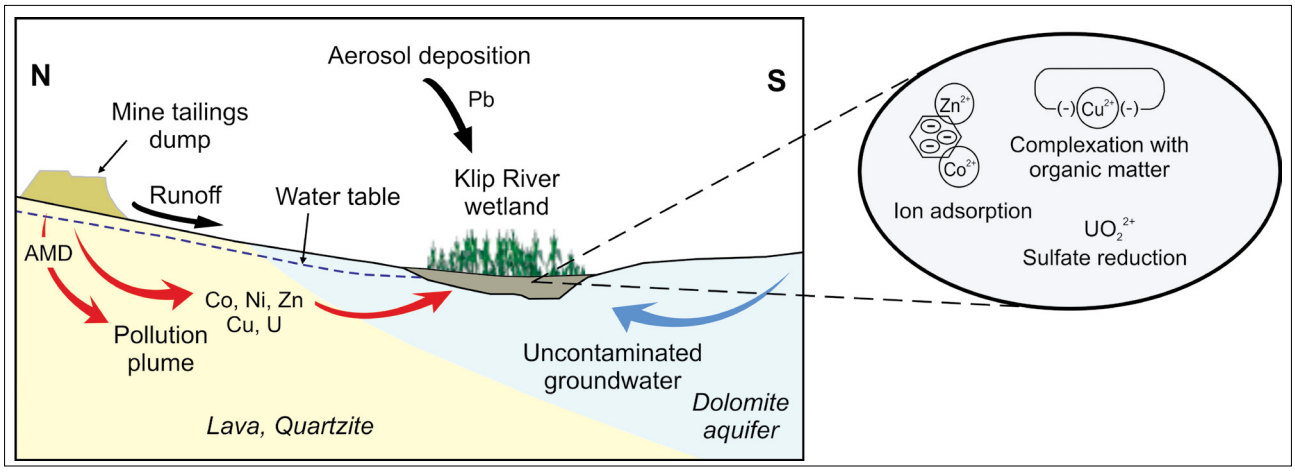

Figure 9: Conceptual hydrogeological model showing the main transport pathways of metal contaminants to the Klip River wetland (diagram not to scale).

U and Cu exhibit similar distributions (R^2=0.76), and although they enter the wetland via the same groundwater process, these metals tend to accumulate predominantly within the upper sediments of the wetland (Figures 8d,f). Cu is primarily associated with the oxidisable fraction[9] and it is likely that Cu and U are sequestered as sulfides or bound to organic matter. REEs appear to remain fairly mobile within the peat and migrate laterally across the wetland (Figure 8e). Their sequestration predominantly within the upper 1.5 m is likely associated with Fe-hydroxide adsorption.

Distribution patterns suggest that Pb (Figure 8g) is predominantly unrelated to the ingress of AMD-contaminated groundwater. Instead, localised surface enrichment within the wetland suggests that Pb contamination most likely originates from surface run-off and/or atmospheric fallout. Lead aerosol deposition may be related to historical combustion of Pb-gasoline or dust originating from TSFs[31] and other industrial activities.

Summary and conclusions

The results of this study reveal the Klip River wetland to be an important sink for pollutants originating from mining activities on the Witwatersrand. Pollutants enter and accumulate within the wetland via different processes, summarised in Figure 9. Most pollutants are likely associated with AMD plumes that emanate from TSFs and enter the wetland via groundwater recharge. This water transports highly elevated concentrations of Co, Ni, Zn and U, which are sequestered within the wetland sediment through a variety of precipitation and adsorption processes. While surface run-off from TSFs severely contaminates watercourses within the upper catchment of the Klip River wetland, surface inputs are considered relatively minor contributors to the overall pollutant load entering the Klip River wetland. Aerosol deposition is, however, likely an important source of Pb.

The extensive accumulation of metals within the Klip River wetland reflects the contaminant legacy associated with gold mining on the Witwatersrand that has spanned over a century. While the efflux of AMD is a particularly difficult and costly problem to control, this study highlights the effectiveness of natural wetlands in trapping vast quantities of toxic pollutants and remediating downstream waters. From this perspective, the Klip River wetlands are undoubtedly some of the most valuable natural assets in Johannesburg. However, wetlands have the potential to release trapped pollutants, particularly if the natural biogeochemical conditions favouring metal sequestration are disrupted. Preventing erosion and further deterioration of the Klip River wetlands is thus critical for the protection of freshwater resources in the region, particularly as the contaminant plumes associated with TSFs will likely persist for decades. Despite 130 years of mining, the Witwatersrand goldfield is still estimated to contain in the order of 30 000 tonnes of gold, representing almost 45% of the world's known gold deposit.[32] Although much of this gold is found at depth and in lower-grade narrow reefs, mining and metallurgical advancements could revitalise operations in the future. Under such circumstances, the role of the Klip River wetland in trapping toxic metals emanating from the Central Basin will be critical.

Acknowledgements

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Competing interests

We have no competing interests to declare.

Authors' contributions

M.S.H. and L.P. conceptualised the study. S.C. carried out the sample analysis, method validation and data interpretation. All authors were involved in sample collection, data interpretation and writing of the manuscript.

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Environmentalism or greenwashing? Responses of South African value chain actors to plastic straw marine pollution

The increasing global concern surrounding plastic marine pollution has placed a spotlight on the key items identified as major contributors. The subsequent public outcry has forced key value-chain actors – such as brand owners, retailers and restaurateurs – to be seen to be responding to the issue. However, are their responses motivated by a true desire for environmentalism or are actors engaging in greenwashing? In this case study on plastic straws, the brand owners and retailers interviewed are driven by a desire to meet consumer expectations. This desire has led to the substitution of plastic straws with glass, paper and polylactide alternatives. However, the broader environmental implications of the alternatives are rarely considered. This single-minded focus on marine pollution has the potential to result in inadvertent greenwashing as alternative products may result in more harm in other environmental compartments.

Significance:

- The increasing concern surrounding plastic pollution has placed a spotlight on key items, forcing plastic value-chain actors to respond.
- The broader environmental impacts of the interventions are rarely considered, resulting in the potential for adoption of products which may result in increased harm in other environmental compartments.

Introduction

The accumulation of plastic in the marine environment has been a global concern for many decades as it poses a threat to wildlife, humans and ecosystems. Impacts of plastic pollution on marine life have been well documented and include entanglement, smothering and ingestion.[1-5] Previous studies have found that 40–80% of macro- (>20 mm in diameter) marine debris is plastic, most of which is associated with food and beverage products such as bottles, lids/caps, bags, drinking straws and polystyrene fragments.[6-11]

The growing concern surrounding the impact of plastic pollution has led to the development of a myriad of policies at city, national and regional levels in an effort to mitigate the problem.[12] More specificall , an increasing number of policies have been developed which aim to address problematic products that have been identified as major contributors to marine litter. A notable example is the widespread response to the threat posed by plastic bag pollution which began in the early 1990s and has seen many countries implementing interventions which vary in range and scope.[13] Policy interventions range from taxes or levies on the sale of plastic bags, bans on thin and lightweight bags, and, in some cases, complete bans on the production, import, sale and use of plastic bags.[13,14] In recent years, a spotlight has been placed on single-use food-related plastic products including utensils and polystyrene containers. In 2018, Jamaica announced a ban on plastic bags, straws and polystyrene food containers, effective from January 2019.[15] However, the use of straws in medical facilities such as care homes and hospitals was exempted from the ban. Furthermore, bags with dimensions greater than 61 cm by 61 cm, and those necessary for maintaining public health and safety (e.g. packaging for raw meat, rice and baked goods) were also exempted. Dominica also announced bans on food-related plastic items in 2018, effective from January 2019, including straws, plates, utensils and polystyrene cups and containers.[16] More broadly, in the same year, the European Union approved a ban by 2021 on single-use plastics which had been identified as major contributors to marine pollution, including straws, cutlery, plates, polystyrene cups and cotton bud sticks.[17] Furthermore, India made a similar pledge to ban single-use plastics by 2022.[18] In this case, single-use plastics are defined as 'disposable plastics which are used only once and then thrown away by the user' and which are completely made of plastic (items such as juice cartons with plastic lids would not be included in the definition) [19]

As evidenced above, straws are one of the items which have been the subject of public outcry globally, with many consumer-led campaigns calling for material alternatives or the outright banning of plastic straws.[20-22] This has led to a multitude of responses from both companies and governments, in an effort to reduce the consumption of plastic straws and subsequent waste generation. Consequently, there has been an increasing popularity of alternative straw materials, both disposable and reusable, which are often touted as more 'environmentally friendly'.

Some major retailers and restaurant chains in South Africa have responded to the rhetoric surrounding plastic straws by choosing to replace them with alternatives (Figure 1). In June 2018, major retailers Pick n Pay and Woolworths announced a set of initiatives to combat plastic pollution to coincide with World Oceans Day and World Environment Day, respectively.[23,24] These initiatives included the phasing out of plastic straws from stores in favour of paper straws. Later that year, Famous Brands replaced plastic straws with paper straws in all of their franchises.[25] In October 2018, Coca-Cola Peninsula Beverages, which provides straws to resellers, announced the same shift.[26] In 2019, following the trends of the aforementioned countries, South Africa announced a proposal to ban straws, citing the ready availability of alternative materials.[27]

Whilst Ocean Basket was the first major franchise to respond to the straw issue[22], they are a good example of the complexity associated with such a decision. Initially, they resolved to eliminate all straws from their restaurants in January 2018.[28] However, as the year progressed, the franchise started offering paper straws and then announced their intention to start providing straws made from compostable maize starch.[29] Similarly, Famous Brands announced a shift to paper straws in October 2018, but then switched to polylactide (PLA) straws in July 2019.

Vince and Stoett[30] warn that the popularity of anti-plastic activism, particularly on social media, may lead to greenwashing by industry. Greenwashing refers to the misleading of consumers on the environmental benefits associated with a company's products, processes or practices.[21,31] This sentiment is echoed by Stafford and Jones[32] who suggest that an overemphasis on plastic pollution and mechanisms to address it may lead to corporate greenwashing through providing a distraction from greater environmental threats such as climate change.

The responses of consumer value chain actors to the public outcry against plastic straws and their underlying motivations are explored here, as well as the extent to which the broader environmental impacts associated with interventions were taken into consideration.

Methods

Primary data were sourced via semi-structured interviews with seven value chain actors: a brand owner, four retailers (which all had in-house brands) and two restaurateurs (Table 1). The interviewees all held senior positions in their fi ms and played an active role in decision-making. Selection of participants was based on their market share and their role in bringing straws to market and/or directly to consumers. However, accessibility to value chain actors was a limitation, as not all identified actors were willing to participate.

Interview questions were developed based on whether or not the value chain actor had developed a strategy to address the growing concern surrounding straws as a major contributor to plastic pollution. The questions were open ended, allowing for the interviewer to ask probing questions to elicit further information and explore different avenues that arose. Furthermore, the interview protocol allowed for the interviewer to move back and forth between questions based on the participant's responses. The questionnaires are presented in the supplementary material.

Interviews were conducted face-to-face or via electronic communication, depending on the participant's preference. Interview duration was approximately 1 h; the interviews were recorded and later transcribed.

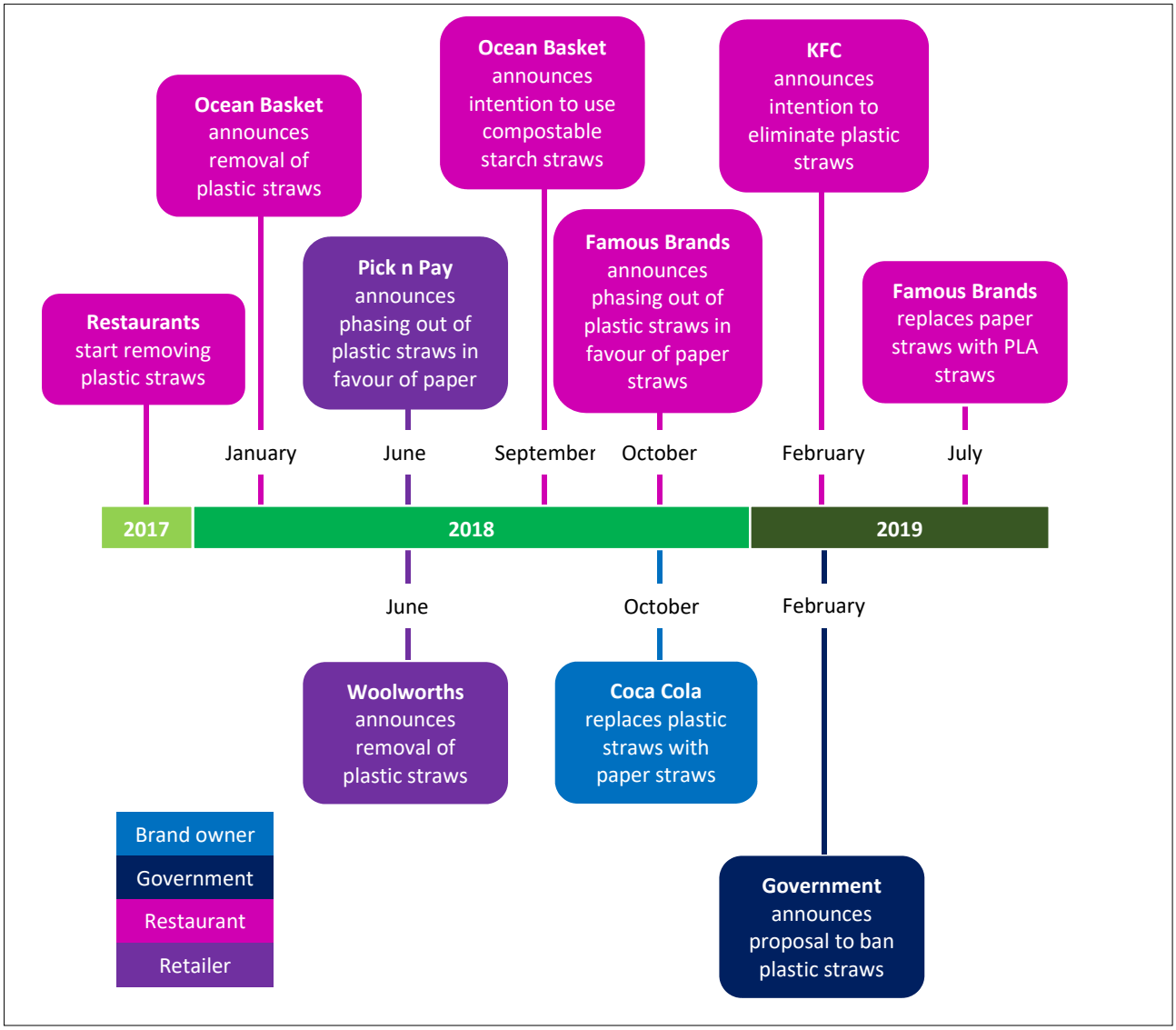

Figure 1: Timeline of responses of value-chain actors in South Africa to plastic straw pollution.

The study was approved by the University of Cape Town's Engineering and Built Environment Ethics in Research Committee prior to commencement. To maintain anonymity, participant identities are presented in an anonymised form which excludes participant position.

Results and discussion

Value chain actor responses

Of the seven value chain actors consulted, six had shifted away from plastic straws to alternative materials (Table 1). Paper was the most popular alternative amongst retailers, with Retailers A, B and D all replacing plastic with paper straws. Retailer B worked in partnership with their supplier, Brand Owner C, which had led to them deciding on paper straws. In addition, Brand Owner C was a supplier for Retailer D that had also reached out to the supplier to discuss a replacement. Unlike other retailers, Retailer C operates a decentralised model with different locations being operated by individual owners. Thus, there was no official stance on straws, with owners being given the freedom to offer any alternative or to continuing using plastic straws. Restaurateur A replaced plastic straws with PLA straws, whereas Restaurateur B used a combination of paper and glass straws for takeaway and sit-down beverages, respectively.

Table 1: Responses of the value-chain actors interviewed regarding plastic straw alternatives

Participant	Response
Retailer A	Paper straws
Retailer B	Paper straws
Retailer C	No official response; responses le t to individual store owners
Retailer D	Paper straws
Brand Owner C	Paper straws
Restaurateur A	PLA straws
Restaurateur B	Paper and glass straws

Value chain actor motivations

Amongst retailers, a major motivating factor was the rising unpopularity of straws amongst consumers, due to their high visibility as a contributor to plastic pollution. This was similar to a finding by Haddock-Fraser and Tourelle[33] who analysed corporate environmental activities and found that consumer reputation was a key motivator for companies to undertake environmental activities. Furthermore, as plastic straws currently have readily available material alternatives, they presented a relatively easy opportunity for retailers to be viewed as environmentally responsible to their consumer base.

> *Everybody just saw it as a quick win!* – Retailer D

This is in line with a suggestion by Stafford and Jones[32] that the visibility associated with plastic pollution creates an opportunity for 'environmental branding' of individuals and corporations through the publicising of interventions such as product substitution or clean-up activities. In addition, retailers were motivated by a desire to maintain their competitiveness amongst consumers.

Unlike retailers, the restaurateurs cited that they were motivated by their own personal convictions and a desire to reduce their contribution to plastic pollution. Brand Owner C took an extended producer responsibility approach whereby they viewed it as their responsibility to provide an alternative:

> *We romanticised the straw and that is why we need to now take responsibility of shifting the consumers' choices away from plastic straws.*

Thus, they viewed their decision to switch to paper straws as a way of providing a product that would be less detrimental to the marine environment under current consumer practices (i.e. littering).

Considerations and challenges

When selecting an alternative, a number of factors were taken into consideration. Cost was cited as a major factor by all actors. In the case of retailers, they had the advantage of economies of scale due to the large quantities they require, which reduced the unit price. In addition, due to the size of their organisations, they were more financially capable of absorbing the extra cost. Restaurateur A cited cost as a major inhibitor to the adoption of reusable straws due to the likelihood of theft by patrons. This was also cited as an issue by Restaurateur B, in addition to breakage of glass straws necessitating replacement. However, they were able to overcome this by partnering with a local glass straw manufacturer to supply their straws, reducing the overall cost of the straws.

The functionality of the straw was a concern to participants, particularly in the case of paper straws. More specificall , the structural integrity of paper straws when immersed in beverages for an extended period of time was of concern, with Retailer A citing that they had received consumer complaints in this regard, necessitating an internal review of locally available paper straws. Restaurateur A also cited this aspect as a consideration in their decision against adopting paper straws as they were perceived as likely to 'disintegrate' in frozen beverages, which are popular in their establishment. However, Retailer B acknowledged that it was a trade-off between straw quality and cost.

Hygiene was cited as a concern by Retailer A, when considering reusable straws such as glass or steel. The retailer viewed reusable straws as taking food safety out of their hands whilst still leaving them vulnerable to liability. They used the example of a consumer potentially improperly cleaning a straw bought from the retailer, getting sick from the poor hygiene and blaming the retailer. Restaurateur B also cited hygiene as a concern, whereby glass was seen as more favourable than steel as it was possible to visually inspect the interior for cleanliness.

When it came to the broader environmental impacts associated with straw alternatives, participants did not express consideration of any impacts beyond the potential marine pollution impact. Thus, they selected material alternatives which they perceived to have a low risk of marine pollution impacts through the consideration of their biodegradability. This single-mindedness has the potential to distract from other environmental issues and result in environmental trade-offs being made unknowingly.

As shown in Table 1, paper straws were a popular alternative due to the material's biodegradability in different environments.[34] From a life-cycle assessment perspective, previous studies comparing plastic vs paper packaging (particularly shopping bags) have commonly found the former to be more favourable in terms of climate change emissions.[35-37] A comparative study of single and reusable straws conducted by Zanghelini et al.[38] in Brazil found that plastic straws had lower climate change emissions in comparison to paper. However, a similar comparative study conducted in South Africa by Chitaka et al.[39] found that paper straws had lower climate change emissions than plastic. This was attributed to the use of coal as a primary feedstock during polypropylene plastic production.[39] Furthermore, paper was found to have the least potential impacts across the majority of categories including freshwater and marine ecotoxicity, particulate matter formation, terrestrial acidification and freshwater eutrophication. However, it is important to note that the broader environmental impacts associated with paper straws were not a consideration for the value chain actors interviewed.

Bio-based plastics (i.e. plastics either partly or wholly derived from biomass), such as PLA, have been rising in popularity as an alternative to traditional plastics. Retailers expressed concern in this regard, citing the amount of misinformation surrounding them, and in particular, the marketing of compostable plastics as biodegradable which gave the impression that they were biodegradable in all environments. This was evidenced in the case of Restaurateur A who cited the perceived biodegradability of PLA in all environments – which is how they had been marketed – as a major motivating factor in their decision. According to

a study conducted by Greene[34], PLA did not meet the ASTM D 6691 test requirements to be deemed marine biodegradable. Furthermore, Chamas et al.[40] found similar polymer surface degradation rates between PLA and polypropylene. The adoption of PLA straws demonstrates the potential for inadvertent greenwashing in the fight against marine pollution

Conclusions and recommendations

Value chain actors are under increasing pressure to be viewed as taking action against plastic pollution, including consumer demands and competitive pressure. Although the majority of value chain actors interviewed selected an option which was deemed favourable from a life-cycle perspective (i.e. paper), this was merely coincidental. For larger organisations (retailers and brand owners), the choice of alternative materials was a business decision to find a cost-effective way to respond to consumers' concerns surrounding plastic marine pollution. Whilst smaller value chain actors (restaurateurs) expressed a personal desire to reduce marine pollution, they were more vulnerable to false marketing regarding the environmental impacts associated with a product. This single-minded focus on plastic pollution without the consideration of broader environmental impacts, can potentially lead to inadvertent or deliberate greenwashing.

When developing strategies or interventions to mitigate plastic pollution, it is important to take into consideration the broader environmental implications. This is particularly important in the case of material substitution, as demonstrated by the case of straws. To ensure that value chain actors do not engage in inadvertent greenwashing, their decisions should be based on robust scientific evidence to ensure that their solutions address the problem they are trying to mitigate – in this case marine pollution – *and* that they are not engaging in burden shifting to other environmental compartments.

Future research is required to investigate the challenges, barriers and drivers for the adoption of environmental assessment tools such as life-cycle assessment in decision-making processes. This will provide insights into how a more holistic perspective to strategy development can be facilitated.

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Competing interests

I have no competing interests to declare.

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Implications of new AMS dates for the Khami Period in the Mapungubwe Landscape

After the abandonment of Mapungubwe, the Limpopo Valley was reoccupied first by Sotho people, making *Icon* pottery, and then by Kalanga speakers making *Khami* pottery. The senior Kalanga chief, in this case Twamamba, was based at Machemma about 60 km to the south, while several petty chiefs administered various portions of the valley itself. Because of fluctuating rainfall, the occupations of both Sotho and Kalanga people occurred in pulses during higher rainfall periods. New AMS dates place one site in the Icon Period, eight sites in *Pulse 1* (AD 1400–1480) and eight sites or components in *Pulse 2* (AD 1520–1590). Kalanga people occupied the best agricultural land near the Limpopo floodplains and Sotho people lived on the plateau to the south. The two groups thus shared the landscape, but not the resources equally. The ceramic record documents this unequal interaction. This interaction, facilitated by male and female initiation schools on the ethnic boundary, helped to create Venda as a language and macro-cultural entity.

Significance:

- Interaction between Sotho (Icon) and Kalanga (Khami) over some 200 years led to the creation of Venda.
- New radiocarbon dates relate to Tshivenda origins, the language spoken by Venda today.
- Initiation schools in the Limpopo Valley provide a model for interaction in the rest of Venda.

By AD 1320, Mapungubwe people had abandoned the Limpopo Valley and the landscape remained unoccupied by agriculturalists for some 80 years until early Sotho people took up residence.[1,2] With a probable origin in East Africa[3], *Icon* pottery marks the first appearance of Sotho speakers in southern Africa. Shortly afterwards, Kalanga speakers (Western Shona) who made *Khami* pottery moved south from Zimbabwe and reoccupied the valley: it was their ancestors who had lived at Mapungubwe.

The Khami Period

Khami chiefdoms can be ranked according to a hierarchy in which each chiefly level was the apex of a pyramid of lower courts.[4] Between AD 1400 and 1450, Khami[5] near Bulawayo (Figure 1) became the large capital of an independent Torwa state in an area known as Butua[6]. States with such large capitals encompassed some 90 000 km^2 and multiple smaller chiefdoms. In the early 15th century, Khami expanded its control into Botswana[7], southern Zimbabwe[8] and across the Limpopo. The senior chief for the Limpopo settlements was based at Machemma[9], about 60 km to the south of the Limpopo. A paramount chief at this time did not exist in South Africa nor in Botswana, and so, the provincial capital must have been in Zimbabwe.

Because Shona and Venda people use natural features to mark political boundaries[10], the Shashi and Limpopo rivers most likely separated the southwest (Botswana) and southern (South Africa) districts from those in Zimbabwe. Interaction between early Sotho and Kalanga appears to have occurred only in the southern district and further southeast. Kalanga people withdrew during the dry period around AD 1500 and then returned to the Limpopo, and to Machemma, when rainfall improved.[10]

Sotho and Kalanga interaction in the Mapungubwe Landscape

Interaction between Sotho and Kalanga peoples is of special interest because it led to the creation of Venda as a language and macro-cultural entity. Venda people are archaeologically and anthropologically important because they have continued the essence of the pre-colonial Zimbabwe Culture – class distinction and sacred leadership – distinguishing them further from Sotho- and Nguni-speaking communities.[11]

The Tshivenda language is known to be a unique blend of Shona (both Kalanga and Karanga) and Sotho.[12] Previous research near the Soutpansberg shows that the creation of Tshivenda parallels the creation of *Letaba* (Venda) ceramics from a blend of *Icon* (Sotho) and *Khami* (Kalanga) pottery.[13]

It is not possible to use ceramic data alone to identify which, if any, present-day Sotho and Kalanga dialects were spoken 600 years ago. Broad correspondences between *Icon* and Sotho, and *Khami* and Kalanga, are nevertheless sufficient for our Limpopo research

Foot surveys in the Limpopo Valley have recently yielded some 398 sites with *Icon* (95) and *Khami* (285) pottery, plus some with both (18) (Figure 2): Khami people occupied the best agricultural land, demonstrating their political dominance. The sites belonged to two main pulses: *Pulse 1* in the 15th century and *Pulse 2* in the mid-16th century. Isotopic analyses of the rings of four baobab trees (with ±5-year ring accuracy) show that these two pulses experienced relatively high and sustained rainfall separated by dry conditions inimical to farming.[14]

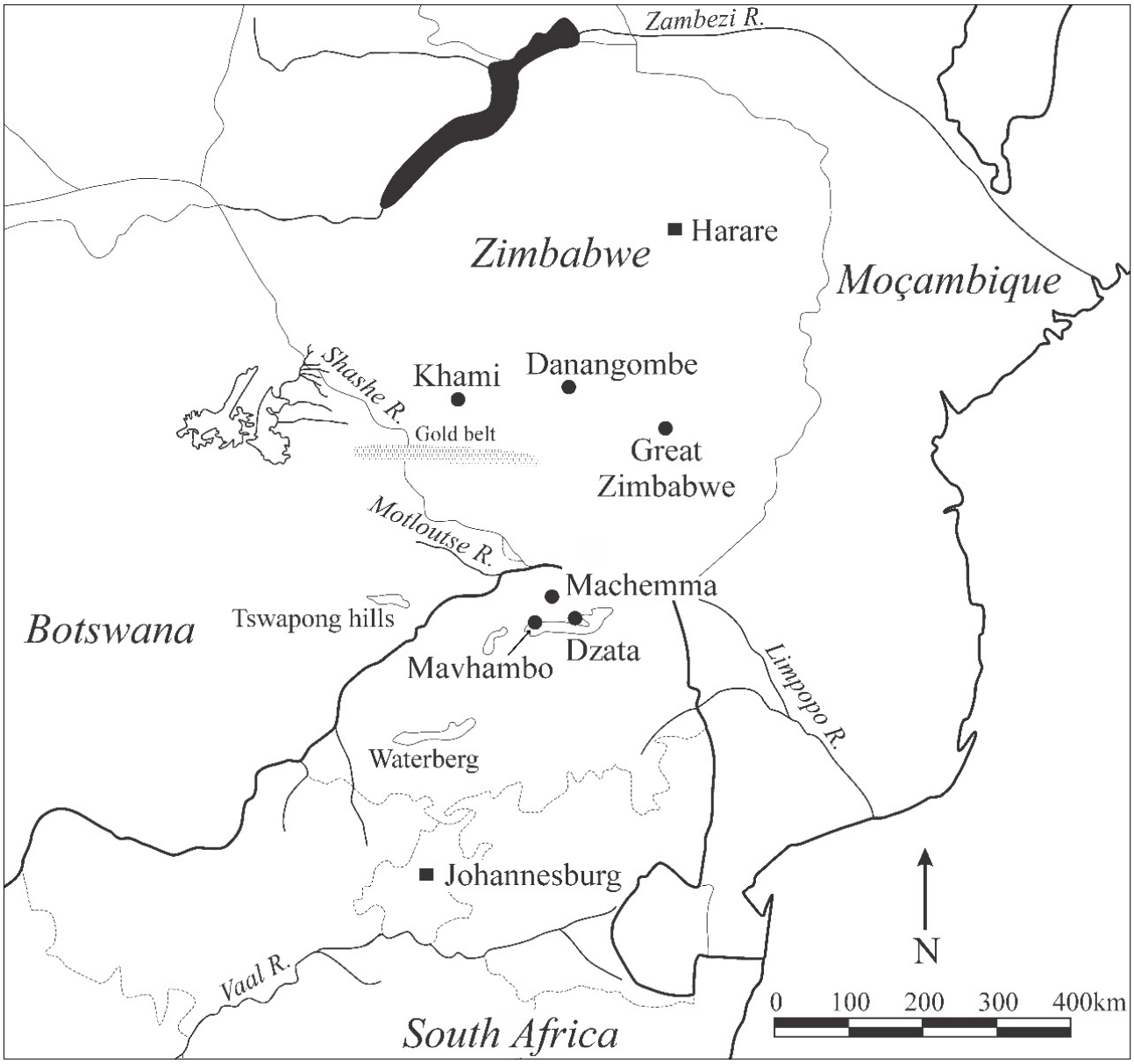

Figure 1: Important Khami-Period sites mentioned in the text.

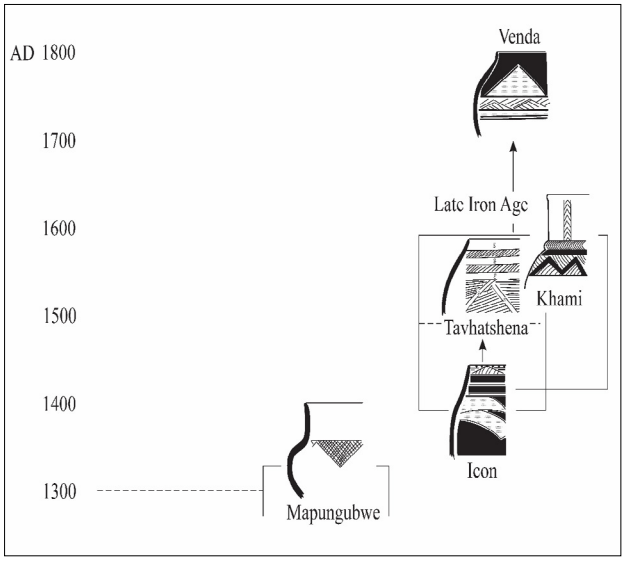

Figure 2: Ceramic sequence associated with Venda origins.

New dates

We have recently processed dung samples from 16 new sites or components related to Venda origins. They were collected from below the surface level inside central cattle kraals: eight in *Pulse 1* and eight in *Pulse 2* (Table 1). We use the southern hemisphere data set SHCal20 at one sigma accuracy, following Calib 8.10 (available online at http://calib.org/calib/calib.html). We use one sigma errors rather than two because each dung sample derives from a short, discrete dating event limited by our tight ceramic and baobab sequences. Our sequence confi ms that recorded by Loubser[13]: a few Icon sites date to the beginning of the 15th century (e.g. Edmondsberg site AD 161); then sites with *Khami* pottery, or *Khami* with *Icon*, date to *Pulse 1* (e.g. Machemma DC1); and *Khami* and *Tavhatshena* date to *Pulse 2* (e.g. DS 32). *Tavhatshena* pottery is similar to *Icon* (and difficult to distinguish), but with the addition of *Khami* motifs. Indeed, *Icon* and *Khami* motifs occasionally occur together on the same vessel surface. The third and final step in the creation of Venda ceramics, *Letaba*, is not present in the Limpopo Valley until the 19th century, after it evolved elsewhere. This third step is not part of the Limpopo sequence because it was too dry for farmers to live there after *Pulse 2*.

Table 1: New AMS dates for the Khami Period in the Mapungubwe Landscape: SHCal20 from Calib 8.10

Site	Ceramics	Lab no.	$\delta^{13}C$	BP ± 1σ	SHCal20 1σ
***Pulse 2* [AD 1520–1590] BP 300–390**					
DS 32 (VI/G) (V/B/UF)	Khami + Tavhatshena	IT-C-1087 IT-C-1497	-24.2 -24.9	300±33 380±37	1512-1546 1544-1625
Edmondsberg AD161a	Icon/Tavhatshena	IT-C-683	-13.8	310±49	1569-1585
Kongo AD531	Icon/Tavhatshena	IT-C-682	-14.4	310±49	1569-1585
Samaria AB354	Khami	IT-C-2053	-14.8	320±26	1511-1547 1564-1571
KK 110 (IV/K/2)	Khami	IT-C-1499	-24.1	320±39	1509-1551 1558-1582
Glen Avon AD433	Icon/Tavhatshena	IT-C-2059	-12.3	350±31	1508-1587
Breslau AB603	Khami	IT-C-2044	-15.1	380±31	1546-1625
Breslau AB605	Khami	IT-C-2035	-14.1	390±27	1550-1559
***Pulse 1* [AD 1400–1480] BP 420–550**					
Kilsyth HM AD268	Khami	IT-C-2046	-13.1	420±27	1457-1501
Greefswald AB16	Khami	IT-C-2057	-15.7	430±33	1454-1501
Collins AD642	Khami	IT-C-565	-16.2	440±31	1451-1498
Samaria AB161	Khami + Icon	IT-C-2054	-5.8	460±27	1443-1485
Horizon 104 BC5	Icon	IT-C-2036	-16.5	460±27	1443-1485
Venetia BCP	Icon	IT-C-2055 IT-C-2061	-16.2 -16.2	470±27 500±27	1438-1462 1432-1453
AD533B	Icon	IT-C-2048	-11.5	480±27	1436-1459
DS 32 midden II/A/4	Khami + Icon	IT-C-528	-23.6	530±32	1418-1445
Icon					
Edmondsberg AD161	Icon	IT-C-638	-13.8	630±62	1381-1415

Initiation schools

Loubser's[13] research suggested that the Soutpansberg was the boundary between Shona speakers to the north and Sotho speakers to the south. Our survey shows that the initial interaction took place in more confined localities, such as the Mapungubwe Landscape. The channel for this interaction was probably through initiation schools. Such schools as *domba* inculcate various aspects of culture, including history, worldview and proper moral behaviour[15-18], and both royal and commoner youths attend. It is therefore significant that the remains of these schools stretch along the boundary between Khami and Icon settlements (e.g. Faure AD 2 and Kilsyth AD 268) and nowhere else in the Mapungubwe area. They are not located in the centre as researchers once thought.[10] The new dates show that these schools date to both *Pulse 1* and *Pulse 2*, when *Icon* changed to *Tavhatshena*. In addition, male circumcision sites occur on the eastern boundary associated with the Khami-Period palace called Haddon.[9,19] Both types of schools were therefore present in the valley at the same time, and over the years, hundreds of youth would have attended both. These schools provide a model for interpreting Tshivenda origins in the rest of Venda.

End of Khami

In AD 1644, the Khami capital was destroyed by a combined force of Portuguese and a disgruntled faction of the Torwa state[20], and for the next 40 years, no single leader controlled the former kingdom. A few chiefs controlled some areas in Botswana[21], but none appears to have been based in in the Limpopo Valley, perhaps because this Interregnum Phase was too dry. The third step in the evolution of Tshivenda took place at this time, when Butua lacked strong leadership.

Strong leadership was reinstated when the Rozvi established a new capital at Danangombe[22] (formerly Dhlo Dhlo) in the 1680s[20]. This new state did not expand to the south but instead defeated the Portuguese to the east and northeast. When the famous Rozvi leader, Changamire Dombolakonachingwano, died in 1696 after defeating the Portuguese, at least three sons competed for kingship. One unsuccessful son went to the Hwange area to give rise to the Nanzwa[23] and another crossed the Limpopo to establish the Singo capital at Dzata in present-day Venda[24]. Some researchers[25] consider the Singo as 'true Venda', but as Loubser's[13] archaeological research shows, Venda language and identity had evolved before the Singo established Dzata. Once there, the Singo forged a new state, incorporating the Khami-Period chiefdoms that extended east from Machemma. *Letaba* pottery and Venda walling at the top of the Machemma sequence documents this change.[10] Somewhat later, the Tshivhula dynasty of the Twamamba built a new headquarters at Mavhambo at the base of the Soutpansberg (Figure 3).[13] The Tshivhula claim that their territory once extended from Machemma to the Limpopo.[26] Because each chiefdom level is two to three times the size of the level below, Machemma would have controlled about 10 000 km^2, corresponding to the area claimed by the Tshivhula. It is therefore reasonable to conclude that the Khami Period in the Mapungubwe Landscape marks the arrival of the Tshivhula dynasty, or at least the Twamamba.[10] Originally, they would have spoken Kalanga, but by the 17th century, they had become Venda and independent of the Khami state.

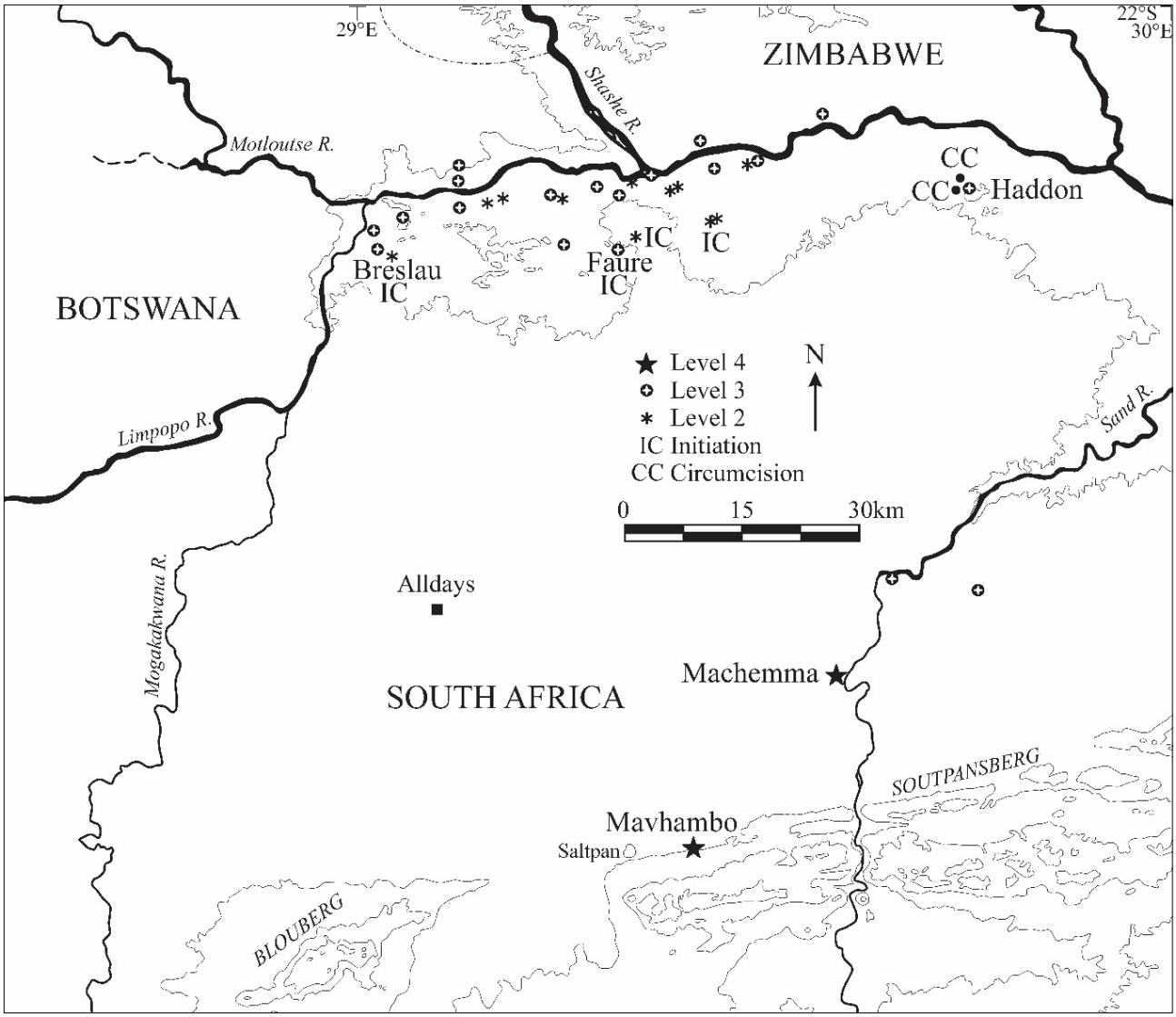

Figure 3: Location of Machemma and Mavhambo in relation to the Limpopo Valley.

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Competing interests

We have no competing interests to declare.

Author's contributions

T.N.H. directed the fieldwork and S.W. the AMS dating. Both authors helped prepare the manuscript.

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