Assessment of co-occurrence of cyanotoxins, toxic metals and anionic surfactants in irrigation water, agricultural soils and food crops

A Masters Research Thesis Submitted to the University of Venda, Faculty of Science, Engineering and Agriculture

Department of Geography and Environmental Sciences.

By

Sathekge Salphina Ntombikayise

Student number: 15015656

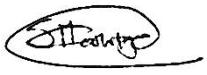
Supervisor: Prof Gitari W.M

Co-supervisor: Dr Mudzielwana R

Co-supervisor: Dr Ngure V

DECLARATION

I Sathekge Salphina Ntombikayise hereby declare that the dissertation for the masters degree project titled "**Assessment of co-occurrence of cyanotoxins, toxic metals and anionic surfactants in irrigation water and agricultural soils"** at the University of Venda, hereby submitted by me, has not been submitted previously for a degree at this or any other University, that it is my own work in design and in execution, and that all reference material contained therein has been duly acknowledged.

Signature:  Date: 02/02/2022

Abstract

Globally, the occurrence of cyanobacterial blooms in freshwater ecosystems has become a concern. Cyanobacteria produces secondary metabolites, known as cyanotoxins that cause acute and chronic poisoning in animals and humans. History of mining, industrial activities and poor maintenance of wastewater treatment infrastructure are the main causes of the hyper-eutrophic conditions affecting most dams in South Africa. The co-occurrence of multiple stressors in agricultural waters and soils potentially pose a human and animal risk if contaminated water and plants are ingested.

The study investigated the co-existence of cyanotoxins, anionic surfactants and metal species in irrigation water, agricultural soils and food crops and determine the health risks associated with consuming cyanotoxins contaminated plants in the Crocodile (West) Marico Water Management Area, which covers parts of Gauteng and Northwest Provinces. Lastly, the study assessed the applicability of passive sampling technology in monitoring of cyanotoxins using DIAON HP20 resins as an adsorbent. Water, food crops and soil samples were collected from Roodeplaat and Hartbeespoort dam sites in irrigation canals and cropping fields in June 2019, September 2019, February 2020, and March 2021. Seven sites were selected for sampling of water for cyanotoxins, anionic surfactants and toxic metals, while 4 farmland sites were selected for agricultural soils and food crops in Roodeplaat and Hartbeespoort sites. Physicochemical parameters of the irrigation water (pH, temperature, EC, TDS, DO), chlorophyll-a and dissolved nutrients were also monitored using Spectrophotometer and Spectro-Quant® Merck Pharo 100 with the photo-metric test kits from Merck, respectively. The levels of Microcystins (MCs), anionic surfactants, and metals were detected and quantified using the ELISA method, anionic surfactant portable photometer and inductively coupled plasma mass spectrometry (ICP- MS), respectively. The results are presented for each chapters below.

The results for chapter 1 revealed the co-existence of cyanotoxins, metal species and anionic surfactants in the irrigation water, and agricultural soils, across sampling sites, throughout sampling period. The microcystins in irrigation water ranged from 0.00 to 15.57 µg/L. Total anionic surfactants in irrigation water and agricultural soil ranged from 0.01 to 3.49 mg/L and 1.81 to 5.46 mg/kg, respectively. Among all the physicochemical parameters only pH ($p = 0.624$), TDS ($p = -0.466$), EC ($p = -0.445$), and turbidity ($p = 0.521$) correlated with MCs. Moreover, total anionic surfactant showed to have positive moderate relationship with levels of MCs in irrigation water ($p = 0.342$). Metal species in irrigation water were decreased in the following order: Al > Mn > Fe > B >

Zn > Ni > Cu > Pb > Cr > As and were all below the maximum DWAF acceptable limit, implying that the water was safe for irrigation use. Metal species in other soil sampling sites such as 16534.61 – 33285 mg/kg (Fe), 111.25 – 723.4 mg/kg (Cr),4.44 – 23.93 mg/kg (Pb), 0.80 – 9.70 mg/kg (As), 22.11 – 33.95 mg/kg (Cu), and 33.70 – 85.885 mg/kg (Ni) were above the maximum limit set by DEA, USEPA, and FAO/WHO for agricultural use. Thus, soils from Roodeplaat and Hartbeespoort farmland sites are contaminated by the mentioned metals.

The findings from the second chapter of results revealed the bio-accumulation of microcystins and metals in food crops. The estimated daily intake (EDI) for MCs in all food crops for both adults and children were below 0.04 µg/kg DW acceptable value set by World Health Organisation, implying that the crops were safe for human consumption by adult and children population. Metal species levels accumulated in plant samples collected from different sampling sites, showed that 0.21 to 10.80 mg/kg (Cr), 19.64 to 734.00 mg/kg (Fe), 5.45 to 76.80 mg/kg (Zn), 0.01 to 0.20 mg/kg (As), 0.96 to 60.40 mg/kg (Cu), and 0.10 to 0.70 mg/kg (Pb) were above the EU and FAO/WHO guideline standards. Spearman correlation between metals in plants and water showed that only Pb ($p = 0.874$) and As ($p = 0.809$) in irrigation water had a positive moderate association with metals in plants collected from the sampling sites. The estimated daily intake (EDI) of metals via consumption of the crops were found to be below the maximum tolerable daily intake (MTDI) proposed for each metal. The translocation factors (TF) showed that only Cu and Cd were rapidly transported to the plant's edible parts from the soil. Moreover, target hazard quotient (THQ) for each metal were below 1, indicating that consuming the food crops wont cause carcinogenic effect to the adult population, while hazard index (HI) for other sites was found to be >1 for crop plants, thus plants from these sites pose a health hazards to adult population. In addition, the target cancer risk (TCR) value for Cr and Ni in crops from other sampling sites were above the maximum threshold implying that there is a potential cancer risk to adult population over a long-term.

In addition, findings from the third chapter showed that SPATT was applicable in monitoring and detecting MCs across all sampling sites and sampling months. The MCs levels in grab and SPATT bags ranged from 0.14 to 13.03 µg/L and 0.99 to 2.28 ng/g resin throughout the sampling sites and months, respectively. Thus, showing the persistence of MCs in canals and farm dams of Roodeplaat and Hartbeespoort. A spearman correlation revealed that pH ($p = 0.776$), Turbidity ($p = 0.699$) and DO ($p = 0.829$) had a significant positive association with total toxins in grab samples, while total dissolved MCs in SPATT samples showed negative moderate relationship with TDS ($p = - 0.615$) and EC ($p = - 0.602$). Total toxin concentrations in SPATT bags and Grab samples did not show

any correlation this is because SPATT bags detect and collect microcystins within water column overtime, unlike point (Grab sampling), hence, there is no relationship between the two-sampling method. Overall results showed that SPATT bags with DIAON HP20 resin as an adsorbent proved to be applicable in monitoring and detecting microcystins in the irrigation water of Roodeplaat and Hartbeespoort sites.

Keywords: *Cyanobacteria, cyanotoxins, toxic metals, anionic surfactants, Irrigation water, agricultural soils, food crops, solid phase adsorption toxin tracking (SPATT)*

Acknowledgement

Above all, I thank our Almighty God, for being with me, guiding me, giving me wisdom and courage to complete my master's project.

My sincere gratitude goes to my supervisor, Professor Gitari W.M for his patience, guidance, assistance, motivation, and supervision throughout the project. I really appreciate you believing in me, and all that you have done for me.

I would also like to thank my co-supervisors, Dr Ngure V and Dr Mudzielwana for their guidance and assistance throughout the project.

I would also like to send my sincere gratitude to my mentor, Pindihama G.K, who has been with me from the start of the project till the end. I am so grateful for your mentor-ship, I have learnt a lot from you. Thank you for assisting me with field work, sample analysis. I am very grateful for a mentor like you.

I would also like to acknowledge people from the Hartbeespoort Irrigation Board, for making it possible for us to gain access to the sampling sites and showing us around, we really appreciate it.

I also like to send a special thank to the Department of Water Affairs Roodeplaat office for working with us, showing us around the area and assisting in gaining assess and collecting the samples from sampling sites. I would also like to acknowledge people from Agricultural Research Council (ARC), Department of Agriculture for allowing us to enter their premises and collect the samples.

A special thank to Mr Mulalo Mutoti for assisting us with the flow-cam and identification of the cyanobacterial species.

A big thank you to my research group (EnviReN) for assisting me with the work throughout the project via presentations, I really appreciate you.

I also want to thank, Water Research Commission (WRC), Sasol Inzalo Foundation and NRF for their financial support. I am also thankful to the University for funding this project through RPC funds.

Table of contents

Contents **Page**

DECLARATION i

Abstract ii

Table of contents vi

List of figures x

List of acronyms xiv

Chapter 1: Introduction 1

1.1Background information 1

1.2 Problem statement 3

1.3 Significance of the study 4

1.4 Objectives 4

1.4.1 Main objective 4

1.4.2 Specific objectives 5

1.5 Research Questions 5

References 6

Chapter 2: Literature review 11

2.2Eutrophication and harmful algae blooms 11

2.3 Cyanobacterial toxins (Cyanotoxins) 12

2.4 Environmental factors influencing cyanobacterial bloom 16

2.4.1 pH 16

2.4.2 TDS and EC 16

2.4.3 Nutrients (Nitrates and Phosphates) 16

2.4.4 Turbidity 17

2.4.5 Dissolved oxygen (DO) 18

2.4.6 Temperature 18

2.5Occurrence of cyanotoxins in terrestrial plants and agricultural soils 18

2.5.1 Implications of cyanotoxins on terrestrial plants 19

2.5.2 Implications of cyanotoxins on agricultural soils 20

2.5.3Accumulation of cyanotoxins in plants tissues and their potential transfer to humans 21

2.5.4Implications of cyanotoxins on human through consuming contaminated plants and drinking water 23

2.6Occurrence of metal species in agricultural soils 24

2.6.1 Implication of toxic metals on agricultural soils 25

2.6.2 Implications of toxic metals on plants 25

2.6.3 Implications of toxic metals on human health 28

2.7 Anionic surfactants and eutrophication 30

2.7.1 Effect of anionic surfactants on agricultural soils 31

2.7.2 Implications of anionic surfactants on terrestrial plants 31

2.8 Passive sampling technique (SPATT) as a device to monitor and detect toxins in aquatic environments. 32

2.8.1 DIAON HP20 as an adsorbent for toxins in aquatic environment 34

References 51

Chapter 3: Assessment of cyanotoxins, metals and anionic surfactants in irrigation water and agricultural soils 57

3.1 Abstract 57

3.2 Introduction 58

3.3 Materials and Methods 59

3.3.1 Introduction 59

3.4 Results and Discussions 65

3.4.1 Physicochemical water quality parameters 65

3.4.2 Water quality chemical parameters 69

3.4.3 Cyanotoxins and Anionic surfactants in irrigation water 72

3.4.4 Total levels of Anionic surfactants in agricultural soils 75

3.4.5 Metal species 76

3.4.6 Factors influencing *microcystins* levels and cyanobacterial blooms in the irrigation water 82

3.5 Conclusions 89

References 90

Chapter 4: Bioaccumulation of *microcystins* and metal species in vegetable crops and their potential human health risk 98

4.1 Abstract 99

4.2 Introduction 100

4.3 Materials and Methods 102

4.3.1 Introduction 102

4.4 Results and Discussions 107

4.4.1 Levels of *microcystins* in edible crops 107

4.4.2 Levels of metal species in vegetable, agricultural soils, and irrigation water samples ... 108

4.5 Conclusion 123

References 124

Chapter 5: Application of the solid phase adsorption toxin tracking (SPATT) technology for monitoring cyanotoxins in irrigation canals and farm dams of Roodeplaat and Hartbeespoort sites. 131

5.1 Abstract 131

5.2 Introduction 132

5.3 Materials and Methods 134

5.3.1 DIAON HP20 Resin and SPATT bags preparation and construction 134

5.3.2 Solid phase adsorption toxin tracking (SPATT) laboratory trials 135

5.4 SPATT bags monitoring sites 136

5.4.1 Field experiment and physicochemical parameters 136

5.5 Results and discussions 139

5.5.1 Laboratory trial results 139

5.5.3 Composition and identification of cyanobacteria in irrigation water 146

6.1 Introduction 162

6.2 Conclusions 162

6.3 Recommendations 163

6.4. Outputs 163

List of figures

Figure 2.1: The nutrient cycle within the aquatic ecosystem indicating the causes, and impacts of eutrophication (DWAF, 2002). 13

Figure 2.2 Cyanotoxins exposure routes, processes, and their impact on human and animals (Bouaicha and Corbel, 2016) 23

Figure 2.3 The ecotoxicological impacts of toxic metals in vegetable plants, and eventually their implication on human health (Rai et al., 2019). 29

Figure 2.4: Passive sampling process. (A) SPATT bag assembly, (B) SPATT bag activation in methanol, and (C) SPATT bag deployment in irrigation canals/Farm dams (Roue et al., 2018). 35

Figure 3.1: Map showing the selected irrigation canals/farm dams and agricultural field sampling sites of Roodeplaat and Hartbeespoort area 60

Figure 3.2: Total Concentrations of MCs in irrigation water collected from Hartbeespoort and Roodeplaat irrigation canals 73

Figure 3.3: Total concentrations of Anionic surfactants in irrigation water from Roodeplaat and Hartbeespoort irrigation canals 75

Figure 3.4: Mean levels of anionic surfactants in agricultural soils of Roodeplaat and Hartbeespoort agricultural farmlands 76

Figure 3.5: (A, B, C, D). Correlation matrix between MCs with pH, TDS, EC, and turbidity. 83

Figure 3.6: (A & B). Graphical representation of *microcystins* (MCs) means across sampling sites ($P < 0.0001$) and sampling periods ($P = 0.2815$). # * indicates statistical significance difference ... 84

Figure 3.7: Correlation matrix between Anionic surfactants levels, MCs levels ($P<0.05$) 88

Figure 5.1: Laboratory SPATT bags deployment trial set up (Bottle **A** with control sample with no SPATT bags inside; Bottle **B** and **C** water samples with SPATT bags inside. 135

Figure 5.2: A Map showing sampling locations for cyanotoxins monitoring using SPATT bags in Roodeplaat and Hartbeespoort irrigation canals and farm dams 136

Figure 5.3: SPATT bags deployment in the field 137

Figure 5.4: Variation of *microcystins* (µg/g resin) adsorbed by resin with exposure time of SPATT bags in field water during laboratory exposure ..... **Error! Bookmark not defined.**

Figure 5.5: The residual concentrations of *microcystins* (µg/L) after retrieval of SPATT bags over time 139

Figure 5.6 (A & B): The mean total MCs concentrations in Grab samples and SPATT bags collected from Roodeplaat and Hartbeespoort irrigation canals and farm dam's sites 143

List of tables

Table 2.1: Cyanobacterial toxins classification, their functions and mechanism of action. (Oberholster et al., 2008) 15

Table 3.1: Summary statistics of physical parameters of irrigation water for Roodeplaat and Hartbeespoort irrigation canals between Jun, Sep 2019 to Feb 2020 and Mar 2021 68

Table 3.2: Summary statistics of nutrients and chlorophyll-*a* recorded in the Hartbeespoort and Roodeplaat irrigation canals between June & September 2019, to February 2020. 70

Table 3.3: Summary statistics of metal species in irrigation water samples in the Roodeplaat and Hartbeespoort irrigation canals between Jun and Sep 2019, to Feb 2020. 78

Table 3.4: The mean concentrations of metal species in agricultural soils from Roodeplaat and Hartbeespoort cropping sites for Jun, Sep 2019, to Feb 2020 and March 2021 81

Table 3.5: Spearman correlation coefficient between *microcystins* levels and physical parameter in irrigation water 84

Table 3.6: Spearman correlation coefficients between Chlorophyll-*a* levels and physicochemical parameters in irrigation water 86

Table 3.7: Spearman correlation coefficient between MCs levels, nutrients, and chlorophyll-*a* in irrigation water. 87

Table 3.8: Spearman correlation coefficients between Anionic surfactants levels, MCs levels and Chlorophyll-*a* in irrigation water 88

Table 4.1: Total Concentration of accumulated *microcystins* (MCs µg/kg) and calculated EDI in food crops collected from agricultural fields in Roodeplaat and Hartbeespoort site in June, Sep 2019, and Feb 2020 108

Table 4.2: Metal concentration (mg/kg) (N = 14) in edible crop collected in Hartbeespoort and Roodeplaat agricultural field in February 2020 and March 2021 110

Table 4.3: Levels of metal species (mg/kg) in agricultural soils samples collected from Roodeplaat and Hartbeespoort farmlands were collected crops are grown. 112

Table 4.4: Levels of metal species (mg/L) in irrigation water samples collected from Roodeplaat and Hartbeespoort irrigation canals and farm dams 114

Table 4.5: Estimated daily intake (EDI mg/day/kg body weight, N=14) of metal species from food crop (Soybean) collected in February 2020 in Hartbeespoort agricultural sites. 117

Table 4.6: Translocation factors between vegetable crops and soils collected from Roodeplaat and Hartbeespoort farmland sites 118

Table 4.7: Target hazard quotient (THQ) and Health index (HI) due to consuming metal contaminated crops (Soybean) collected in Hartbeespoort agricultural sites in February 2020 and March 2021. 120

Table 4.8: Target hazard (TCR) due to consumption of metal contaminated crops (Soybean) collected in Hartbeespoort agricultural sites in February 2020 and March 2021. 122

Table 5.1: Physicochemical characteristics of irrigation water recorded from Roodeplaat and Hartbeespoort irrigation canals and farm dams 142

Table 5.2: Spearman correlation coefficient (r) describing relationship between total *microcystins* in Grab and SPATT samples with physicochemical parameters. 145

Table 5.3: The different composition of cyanobacterial toxins in irrigation canals and farm dams of Hartbeespoort sites. 147

Table 5.4: The different composition of cyanobacterial toxins in irrigation canals and farm dams in Roodeplaat sites 152

List of acronyms

AS : Anionic surfactants

DO : Dissolved oxygen

EC : Electronic Conductivity

EDI : Estimation of daily intake

ELISA : Enzyme- linked immunosorbent assay

HAB : Harmful algae blooms

MCs : Microcystins

SPATT : Solid Phase Adsorption Toxin Tracking

SPE : Solid Phase Extraction

TDI : Tolerable daily intake

TDS : Total dissolved solids

WHO : World Health Organization

FAO : Food and Agriculture Organization

USEPA : United States Environmental Protection Agency

THQ : Target Hazard Quotient

HI : Hazard Index

TF : Translocation Factor

TCR : Target Cancer Risk

Chapter 1: Introduction

1.1 Background information

Eutrophication is a process characterized by elevated nutrients such as phosphates and nitrates resulting from human actions such as intensive agricultural activities, discharge of treated and untreated sewage, domestic wastewater effluents and industrial effluents (Nyenje *et al.,* 2010; Machado *et al.,* 2017). Eutrophication of freshwater ecosystems is the most prevalent challenge worldwide (Xu *et al.,* 2010; Nyenje *et al.,* 2010). Human driven eutrophication results in water quality problems such as depletion in dissolved oxygen, impair water taste, decrease aesthetic value, increase turbidity and eventually rapid proliferation of cyanobacterial species, which in turn produce bio-active substances such as *microcystins*, *cylindrospermopsins*, *anatoxins*, and *saxitoxins* posing risk to aquatic ecosystem, animals, and human well-being (Matthews *et al.,* 2010; Nyenje *et al.,* 2010; Kozdeba *et al.,* 2014; Ndlela *et al.,* 2016; Dalu and Wasserman, 2018).

The occurrence of cyanobacterial blooms has particularly become a global concern in freshwater ecosystems (Cheung *et al.,* 2013; Beversdorf *et al.,* 2018) because cyanobacteria can produce secondary metabolites known as cyanotoxins (Spoof and Catherine, 2017). Cyanotoxins are responsible for acute and chronic poisoning of animals and humans, and have been classified into four groups; namely, *Hepatotoxins* (*Microcystins, nodularin's*), *Neurotoxins (anatoxin-a, anatoxin-a(s)* and *Cytotoxins* (*cylindrospermopsins*), *Dermatoxins (Lypopolysaccharide, Lyngbyatoxins and aplysiatoxin) (*Saqrane and Oudra, 2009; Davis *et al*., 2015). Cyanotoxins pose a threat to human health through indirect exposure, which includes consuming plants that are contaminated with cyanotoxins through irrigation water and fish that have accumulated the cyanotoxins in the water column, as well as direct exposure which, includes drinking contaminated water, and recreational activities, such as swimming in contaminated water bodies (EPA, 2014; Beversdorf *et al.,* 2018). These toxins can accumulate in plants tissues, animals, and are transferred to humans through the food chain (Purkayastha *et al*., 2010). Cyanotoxins do not only affect human health, but they also affect plant production, as they affect yield by hindering plant growth and development and altering the plant tissues (Purkayastha *et al*., 2010).

Among cyanotoxins, hepatotoxins are the most prevalent globally in freshwater ecosystems and they have received wide coverage in research. *Microcystins* are some of the hepatotoxins and commonly dispersed; about 80 congeners of MCs have been identified and are known to cause

significant harm on water quality, aquatic ecosystems, animals, and humans (Zhu *et al*., 2018). Due to their toxicity, the World Health Organization (WHO) has set a provincial guideline of 1µg/L for *microcystins* in drinking water (Meneely & Elliot, 2013). The tolerable daily intake (TDI) for *microcystins* in the diet is set at 0.04 µg/kg MC-LR bodyweight in food (EPA, 2014; Miller and Russell, 2017).

South Africa is a water scarce country and is positioned as the 30th driest country among other countries in the world (Bwapwa, 2018). Hence, water quality deterioration is a huge concern in South Africa. The country is semi-arid and gets up to 495 mm/year of precipitation which is half of the world average rainfall lower than 860 mm/year (DWA, 2013; Mudaly *et al.,* 2020). South African farmers rely on surface water to irrigate the vegetable crops, about 90% of vegetable crops rely on irrigation, and agriculture uses more than 62% of the countries fresh water (DWA, 2013; Bwapwa, 2018). The degradation of water quality in South Africa is a huge concern and it results into human activities such as mining and mineral handling (basically harmful metals), untreated and treated sewage effluents, domestic wastewater discharge, climate change, rapid population growth, intensive agriculture, urbanization, and industrial effluents, which adversely affect the water quality (Oberholster and Botha, 2014; Mudaly *et al.,* 2020). Hence, serious majors need to be taken to manage this essential scarce resource (Mudaly *et al.,* 2020). Cyanobacterial toxins are not the only concern which affects water quality and scarcity in water systems of South Africa. There are other water quality problems such as elevated levels of salts, water temperature, dissolved oxygen, pH, electrical conductivity (EC), pathogens, sewage spillage, nutrients such as such as phosphates and nitrates and turbidity (DWA, 2014; Edokpayi *et al.,* 2016; Bwapwa, 2018; Mudaly *et al.,* 2020).

Roodeplaat and Hartbeespoort dams have been classified as hyper-eutrophic and suffer severe cyanobacterial blooms (Van Ginkel, 2011; Matthews *et al.,* 2010; Mbiza, 2014; Lukhwareni and Van Dyk, 2018). Hartbeespoort dam is known for the occurrence of harmful cyanobacterial blooms of *microcystins* since 1950s (Oberholster and Botha, 2010; Ballot *et al.,* 2014). In 1976 to 1986, there were mortality of livestock and wild animals reported in Roodeplaat and Hartbeespoort dam shores and were linked to the harmful cyanobacterial blooms *microcystins* (Downing and Van Ginkel, 2004). Despite the severe growth of cyanobacterial blooms in the Hartbeespoort and Roodeplaat dams, there is lack of strategies to monitor, manage, and give early warning of cyanobacterial bloom formation (Pindihama and Gitari, 2019).

Hence, this study aims to determine the co-existence of cyanotoxins, toxic metals and anionic surfactants in irrigation water and agricultural soils and determine the health risks associated with consuming cyanotoxins contaminated plants in the Crocodile (West) Marico Water Management Area, which covers parts of Gauteng and Northwest Provinces. The area has a long history of mining, industrial effluent, and it is known to be hyper-eutrophic, in this manner cyanobacterial species associate with other pollutants such as metals, and anionic surfactants in the natural environment. The combination of these pollutants is very common in water bodies that are eutrophic, and edible terrestrial plants might be exposed through irrigation threatening food security and quality, and eventually posing human health risk via consumption of the contaminated food plants (Cao *et al.,* 2018; Jia *et al.,* 2018; Pindihama & Gitari, 2019). The study also aimed to evaluate the applicability of a passive sampling technology (SPATT) to monitor and detect cyanotoxins using DIAON HP20 resin as an adsorbent.

1.2 Problem statement

The occurrence of cyanobacterial toxins due to human induced eutrophication related effects are on the rise in recent decades. Anthropogenic activities like the rise in human population, agriculture intensification, fast economic development, and industrial activities alter the climatic conditions and composition of the aquatic ecosystems. Resultantly, rapid bloom of cyanobacterial species is experienced (Codd *et al.,* 2005; Bittencourt-Oliveira *et al.,* 2016; Lee *et al.,* 2017; Pindihama & Gitari, 2019). Persistent hyper-eutrophic conditions in Roodeplaat and Hartbeespoort dams are attributed to the long history of activities such as mining, industrial activities, and poor maintenance of wastewater treatment infrastructure. Roodeplaat and Hartbeespoort dams are part of the Crocodile West Marico Water Management Area. Also, the sampling sites chosen for this study receives water from the two dams for irrigation purposes. The water from the two dams is mainly used for irrigation purposes by farmers. Thus, irrigation water is exposed to multiple stressors contamination such as cyanotoxins, anionic surfactants and toxic metals which are common in eutrophic waters (Cao *et al.,* 2018; Jia *et al.,* 2018). Contaminated irrigation water transfers bio-accumulates pollutants in the crop's edible tissues. This poses a health risk to humans if such plants are consumed.

It is known that there is rapid proliferation of toxins producing cyanobacterial blooms in South Africa's water bodies. The existing monitoring and management methods of the blooms appear not effective (Pindihama and Gitari, 2019). For instance, there is a lack of early warning capabilities of

cyanotoxins contamination in fresh waters intended for irrigation or drinking. Moreover, the common cyanotoxins sampling technique relies on the traditional method of grab sampling with a lot of drawbacks. This method does not cater for variations in cyanotoxins concentration in water column over space and time (Davis and Hansen, 2013; Roue *et al.,* 2018). Additionally, traditional sampling method does not present the accurate profile of cyanotoxins due to spatial and time-based variation caused by hydro-logical and circulation effects (Zhao *et al.,* 2013). Also, the method provides a snapshot of cyanobacterial toxins present at one point at that time and may miss highest episodic peak of toxins (Wood *et al.,* 2011, Wood *et al.,* 2020). Also, the results are highly variable and unpredictable as algae cells and toxin are not evenly distributed in lakes during a bloom. This prompts the need for alternative methods of sampling cyanotoxins in aquatic ecosystems to give integrated reflection of cyanobacterial toxins in water bodies.

1.3 Significance of the study

Cyanotoxins do not exist in isolation in the natural environment. Rather, they interact with other pollutants, which might increase their toxicity or enhance their uptake by plants. The current study will provide the insights on the possible threats which might be posed to human health by irrigating food crops with water which might be contaminated with cyanotoxins, anionic surfactants and toxic metals. The study will also lead to the understanding of the fate of cyanobacterial cells and toxins during and after irrigation with water infested with cyanotoxins. The findings will help to develop a better understanding of the combined effects of cyanotoxins, anionic surfactants and metal species on terrestrial plant communities. The study will also seek to find an alternative and more reliable technique to monitor cyanotoxins in agricultural waters by investigating the applicability and use of a passive sampling technology (SPATT) to detect cyanotoxins using DIAON HP20 resin as an adsorbent. The proposed study is expected to contribute to the development of policies on utilizing water, which is contaminated by cyanotoxins, anionic surfactants and toxic metals and the acceptability of such plants for human consumption.

1.4 Objectives

1.4.1 Main objective

The aim of the study was to determine the prevalence of cyanotoxins in irrigation water and investigate their co-occurrence with anionic surfactants and toxic metals in irrigation water, agricultural soils, and agricultural produce.

1.4.2 Specific objectives

- To establish the presence and levels of cyanotoxins, anionic surfactants (AS), toxic metals, and other physicochemical parameters in irrigation water and agricultural soils.
- To determine the prevalence of cyanotoxins and toxic metals in food plants in the crocodile (west) and Marico Water Management Area and determine the potential human health risks.
- To evaluate the applicability of a passive sampling technology (SPATT) to detect cyanotoxins in monitoring and assessment programs using DIAON HP20 resin as an adsorbent.

1.5 Research Questions

- What are the levels of cyanotoxins, metal species, anionic surfactants, and physicochemical parameters in irrigation water and agricultural soils?
- What is the prevalence of cyanotoxins in irrigation water in the Crocodile (west) and Marico Water Management Area?
- Are cyanotoxins transferred into food plants when the plants are irrigated with cyanotoxins infested water, and could they be accumulating to levels that might pose a risk to human health?
- Can SPATT be used as an early warning tool to detect and monitor cyanotoxins in irrigation water in the study area?

References

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J. and Miles, C.O., 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Marine and Freshwater Research, 65(2), pp.175-189.

Beversdorf, L.J., Rude, K., Weirich, C.A., Bartlett, S.L., Seaman, M., Kozik, C., Biese, P., Gosz, T., Suha, M., Stempa, C. and Shaw, C., 2018. Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more than microcystin. Water research, 140, pp.280-290.

Bwapwa, J.K., 2018. Production of jet fuel from microalgae biomass cultivated in saline domestic wastewater (Doctoral dissertation).

Cao, Q., Steinman, A.D., Wan, X. and Xie, L., 2018. Bioaccumulation of microcystin congeners in soil-plant system and human health risk assessment: A field study from Lake Taihu region of China. Environmental Pollution, 240, pp.44-50.

Catherine, A., Bernard, C., Spoof, L. and Bruno, M., 2017. Microcystins and nodularins. Handbook of cyanobacterial monitoring and cyanotoxin analysis, pp.107-126.

Cheung, M.Y., Liang, S. and Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. Journal of Microbiology, 51(1), pp.1-10.

Codd, G.A., Lindsay, J., Young, F.M., Morrison, L.F. and Metcalf, J.S., 2005. Harmful cyanobacteria. In Harmful cyanobacteria (pp. 1-23). Springer, Dordrecht.

Dalu, T. and Wasserman, R.J., 2018. Cyanobacteria dynamics in a small tropical reservoir: Understanding spatio-temporal variability and influence of environmental variables. Science of the Total Environment, 643, pp.835-841.

Davis, S. and Hansen, C., 2013. Blue-Green Algae Toxin Monitoring and Response Project Final Project Report February 2013.

Davis, T.W., Bullerjahn, G.S., Tuttle, T., McKay, R.M. and Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during Planktothrix blooms in Sandusky Bay, Lake Erie. Environmental science & technology, 49(12), pp.7197-7207.

Department of Water Affairs. 2014. South Africa Yearbook 2013/2014 [online]. Available at: https://www.gcis.gov.za/sites/default/files/docs/resourcecentre/yearbook/2013-4Water_Affairs.pdf [accessed 30 June 2018].

do Carmo Bittencourt-Oliveira, M., Cordeiro-Araújo, M.K., Chia, M.A., de Toledo Arruda-Neto, J.D., de Oliveira, Ê.T. and dos Santos, F., 2016. Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners. Ecotoxicology and Environmental Safety, 128, pp.83-90.

Downing, T.G. and Van Ginkel, C.E., 2004. Cyanobacterial monitoring 1990-2000: Evaluation of SA data. Water Research Commission.

DWA., 2013. Classification of significant water resources in the Crocodile (West) and Marico water management area (WMA) and the Mokolo and Matlabas catchments: Limpopo WMA. Pretoria: Department of Water Affairs.

Edokpayi, J.N., Odiyo, J.O., Popoola, O.E. and Msagati, T.A., 2016. Assessment of trace metals contamination of surface water and sediment: a case study of Mvudi River, South Africa. Sustainability, 8(2), p.135.

EPA, N., (2014). Sydney Paten No. EPA 2014, 323.

Jia, Y., Chen, W., Zuo, Y., Lin, L. and Song, L., 2018. Heavy metal migration and risk transference associated with cyanobacterial blooms in eutrophic freshwater. Science of the Total Environment, 613, pp.1324-1330.

Kozdęba, M., Borowczyk, J., Zimoląg, E., Wasylewski, M., Dziga, D., Madeja, Z. and Drukala, J., 2014. Microcystin-LR affects properties of human epidermal skin cells crucial for regenerative processes. Toxicon, 80, pp.38-46.

Lee, S., Jiang, X., Manubolu, M., Riedl, K., Loraine, S.A., Martin, J.F. and Lee, J., 2017. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. Food Research International, 102, pp.234-245.

Lukhwareni, R. and van Dyk, C., 2018. Histology and ultrastructure of hepatic nodular alterations in Clarias gariepinus (Burchell, 1822). Journal of Fish Diseases, 41(12), pp.1859-1870.

Machado, J., Campos, A., Vasconcelos, V. and Freitas, M., 2017. Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: a review of their relevance for agricultural plant quality and public health. Environmental Research, 153, 191– 204.

Matthews, M.W., Bernard, S. and Winter, K., 2010. Remote sensing of cyanobacteria-dominant algal blooms and water quality parameters in Zeekoevlei, a small hypertrophic lake, using MERIS. Remote Sensing of Environment, 114(9), pp.2070-2087.

Mbiza, N.X., 2014. Investigation of the effectiveness of techniques deployed in controlling cyanobacterial growth in Rietvlei Dam, Roodeplaat Dam and Hartbeespoort Dam in Crocodile (West) and Marico Water Management Area (Doctoral dissertation).

Meneely, J.P. and Elliott, C.T. 2013. Microcystins: measuring human exposure and the impact on human health. Biomarkers, 18(8), 639–649.

Miller, A. and Russell, C., 2017. Food crops irrigated with cyanobacteria-contaminated water: an emerging public health issue in Canada. Environmental Health Review, 60(3), 58– 63.

Mudaly, L. and Van der Laan, M., 2020. Interactions between irrigated agriculture and surface water quality with a focus on phosphate and nitrate in the middle olifants catchment, South Africa. Sustainability, 12(11), p.4370.

Nyenje, P.M., Foppen, J.W., Uhlenbrook, S., Kulabako, R. and Muwanga, A., 2010. Eutrophication and nutrient release in urban areas of sub-Saharan Africa—a review. Science of the total environment, 408(3), pp.447-455.

Oberholster, P.J. and Botha, A.M., 2010. Use of remote sensing and molecular markers to detect toxic cyanobacterial hyperscum crust: A case study on Lake Hartbeespoort, South Africa. African Journal of Biotechnology, 9(51), pp.8791-8799.

Oberholster, P.J. and Botha, A.-M., 2014. Importance of water quality to the food industry in South Africa. Understanding the Food Energy Water Nexus. WWF-SA, South Africa.

Pindihama, GK. and Gitari, WM., 2019. Cyanobacterial toxins: an emerging threat in South African irrigation water. Water and Environment Journal 0: 1–11.

Purkayastha J, Kumar-Gogoi H and Singh L., 2010. Plant-Cyanobacteria interactions: phytotoxicity of cyanotoxins. Journal of Phytology 2(7): 07–15.

Roué, M., Darius, H.T. and Chinain, M., 2018. Solid phase adsorption toxin tracking (SPATT) technology for the monitoring of aquatic toxins: A review. Toxins, 10(4), p.167.

Saqrane, S. and Oudra, B., 2009. CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins, 1(2), pp.113-122.

Van Ginkel, C.E., 2011. Eutrophication: Present reality and future challenges for South Africa. Water SA, 37(5), pp.693-702.

Wood, S.A., Holland, P.T. and MacKenzie, L., 2011. Development of solid phase adsorption toxin tracking (SPATT) for monitoring anatoxin-a and homoanatoxin-a in river water. Chemosphere, 82(6), pp.888-894.

Wood, S.A., Kelly, L.T., Bouma-Gregson, K., Humbert, J.F., Laughinghouse IV, H.D., Lazorchak, J., McAllister, T.G., McQueen, A., Pokrzywinski, K., Puddick, J. and Quiblier, C., 2020. Toxic benthic freshwater cyanobacterial proliferations: challenges and solutions for enhancing knowledge and improving monitoring and mitigation. Freshwater Biology, 65(10), pp.1824-1842.

Xu, H., Paerl, H.W., Qin, B., Zhu, G. and Gaoa, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and oceanography, 55(1), pp.420-432.

Zhao, H., Qiu, J., Fan, H. and Li, A., 2013. Mechanism and application of solid phase adsorption toxin tracking for monitoring microcystins. Journal of Chromatography A, 1300, pp.159-164.

Zhu, J., Ren, X., Liu, H. and Liang, C., 2018. Effect of irrigation with microcystins-contaminated water on growth and fruit quality of Cucumis sativus L. and the health risk. Agricultural Water Management, 204, pp.91-99.

2.1 Introduction

This chapter elaborates more on the prevalence of co-existence of cyanotoxins, toxic metals and anionic surfactants in irrigation water, agricultural soils, and agricultural produce. It describes the effect of cyanotoxins on food plants and the health implications they might cause to humans upon consuming cyanotoxins contaminated plants. It elaborates more on the occurrence of anionic surfactants in irrigation water and agricultural soils and describes the presence and effect of toxic metals in irrigation water, agricultural soils, and food crops. Lastly, it elaborates more on the applicability of a passive sampling technology (SPATT) to monitor and detect cyanotoxins using DIAON HP20 resin as an adsorbent.

2.2 Eutrophication and harmful algae blooms

Eutrophication is a worldwide environmental problem, resulting in an increase in the production of cyanobacterial harmful algae (Xu *et al*., 2010). Eutrophication is a natural ecological process that results due to nutrients enrichment in the freshwater environment (Qin *et al.,* 2013). Anderson *et al*. (2002) defined eutrophication, as a process whereby there is an increase in nutrients input such as nitrogen and phosphorus in aquatic ecosystems resulting in cyanobacterial harmful algal blooms. The eutrophication process occurs naturally in aquatic ecosystems as a natural ageing of the aquatic ecosystem, through upwelling of water, and river runoff, but human activities such as application of fertilizers in agriculture, sewage discharge, animal wastewater discharge, and industrial discharges have accelerated this process in aquatic ecosystems, leading to the formation of cyanobacterial blooms (Xiao *et al*., 2017; Jiang *et al*., 2019; Van Meersschex, 2019).

The eutrophication process can occur naturally or through anthropogenic activities. The natural eutrophication process is caused by natural phenomenon such as influx of nutrients from rocks, sediments etc. The natural eutrophication cannot be reversible or controlled, but the process occurs at a slower rate compared to cultural eutrophication (Van Ginkel, 2011). The anthropogenic eutrophication is referred to as cultural eutrophication which is caused by human activities such as social and economic, and it can be controlled or lightened by minimizing and concoct measures to alleviate the effect of the activities which brings about cultural eutrophication (Lukhwareni and Van Dyk, 2018). The major concern regarding cultural eutrophication is the prevalence of toxic cyanobacteria in water column, resulting in death of livestock, domestic animals, and wildlife after

drinking the water contaminated heavily by cyanobacterial blooms (Chislock *et al.,* 2013; Wagenaar and Barnhoorn, 2018).

According to Lim and Lee (2017), the excessive introduction of nutrients such as nitrogen and phosphates leads to eutrophication of the freshwater ecosystem resulting in cyanobacterial blooms. The consequences of eutrophication on water quality includes algal toxin production, impair taste and odor of water, depletion of dissolved oxygen, decline in biodiversity and decrease in aesthetic value, increase in turbidity, disruption of flocculation and chlorination process from water treatment plants (Van Ginkel, 2011; Ndlela *et al.,* 2016; Dalu and Wasserman, 2018).

Cyanobacterial blooms are harmful in aquatic ecosystems because when the cell decays, it raptures and releases harmful cyanotoxins which are toxic to human beings, animals, and terrestrial plants (Cai *et al*., 2019). The decaying of the algae cells in aquatic ecosystems is a huge problem when the cell decays release undesirable odors, changes the taste of water and color, and cyanotoxins that are not easily eliminated from the aquatic environment (Qin *et al.,* 2010; EPA, 2014). Cyanobacterial harmful algae are common in eutrophic ecosystems due to an increase of nutrients such as nitrogen and phosphorus (Xie *et al*., 2003). Figure 2.1 underneath shows the nutrients cycle happening within the aquatic ecosystems, demonstrating the sources of excessive nutrients and their impacts in water bodies.

2.3 Cyanobacterial toxins (Cyanotoxins)

Cyanobacteria also known as blue-green algae are prokaryotic unicellular microorganisms, that are capable of photosynthesizing and forming harmful algal blooms (HABs) in eutrophic water bodies (Saqrane and Oudra, 2009). Cyanobacteria evolved 2.3 billion years ago because of their long evolutionary history, they can adapt in many different geographical regions, such as water (fresh, brackish), marine environment, and terrestrial environments and extreme environments such as salted soils, hot springs volcanic ash, snow, and cryoconites etc. (Codd *et al.,* 2005; O'Neil *et al.,* 2012; Vijay *et al.,* 2019; Gaysina *et al.,* 2019). These photosynthetic organisms occur naturally in aquatic ecosystems and play an important role of nitrogen fixing and nutrient cycling in the freshwater ecosystems (Cao *et al.,* 2016). Cyanobacteria are also used for sequestration of CO_2, and as a feed-stock for bio-fuel production, pharmaceutical probes, colorants, and fertilizers (Upendar *et al.,* 2018).

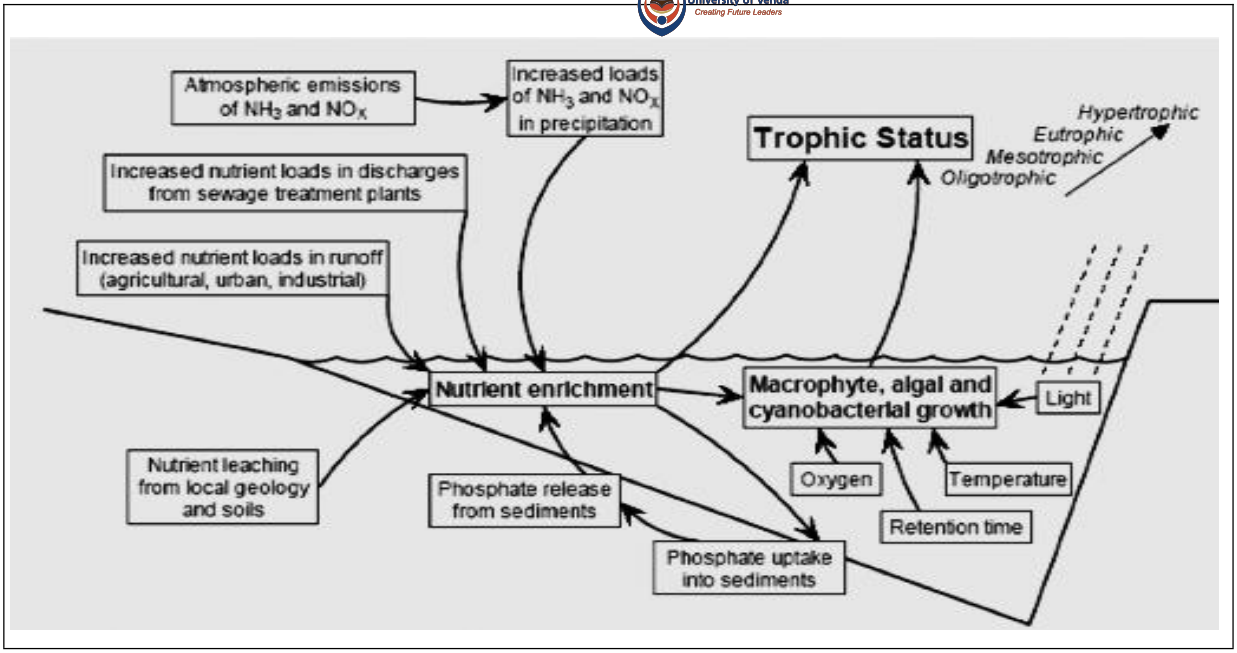


Figure 2.1: The nutrient cycle within the aquatic ecosystem indicating the causes, and impacts of eutrophication (DWAF, 2002).

Cyanobacterial species are present in aquatic environments in nature, however, human activities by means of point and non-point pollution sources such as urbanization, agriculture and industrial effluents have led to rapid multiplication of cyanobacterial harmful algal blooms (HAB) in aquatic ecosystems (Carr and Neary, 2008). This is a global concern because the blooms aggregate in surface waters forming a green scum that poses health risks to humans and animals (Merel *et al.,* 2013). The outbreak of cyanobacterial blooms in aquatic ecosystems under favorable conditions such as excessive nutrients levels, light intensity, rising water temperatures, stagnant or slow-moving water results in the production of toxins (cyanotoxins) such as *microcystins* into water column (Zhu *et al.,* 2018). The increase in cyanobacterial blooms in aquatic ecosystems is a huge problem because they can affect the aquatic ecosystems negatively, by altering the physicochemical characteristics of the water, such as the transparency, DO, modifying the interactions of aquatic organisms, and produce harmful cyanotoxins which can harm the health and development of aquatic organisms (Eisenhut *et al.,* 2008; Paerl & Huisman, 2009; Whitton & Potts, 2012; Lee *et al.,* 2017).

Cyanotoxins are large group of secondary metabolites that are produced by various cyanobacteria genera under eutrophic conditions, and consist of different chemical structures (Garget *et al.,* 2017; Huisman *et al.,* 2018; Oliver *et al.,* 2019). These secondary metabolites are formed by cyanobacteria forming blooms, whose rapid growth is controlled by both environmental factors and anthropogenic activities (Sanseverino *et al.,* 2016). The massive proliferation of cyanobacterial

blooms can be induced by a variety of physico-chemical parameters, biological factors, and the trophic state of the water bodies (Sanseverino *et al.,* 2016). Cyanotoxins are diverse group of bio-toxins and are responsible for acute and chronic poisoning of animals and humans. These secondary metabolites are classified into four groups, based on their biological effects. These include hepatotoxins (target the liver) (*microcystins, nodularin),* neurotoxins (target the nervous system) (*anatoxin-a, anatoxin-a(s),* and *cytotoxins* (target the liver and kidneys, spleens) *cylindrospermopsins, and* dermatoxins *(Lypopolysaccharide, Lyngbyatoxins and aplysiatoxin)* which cause skin irritant on contact (Saqrane and Oudra, 2009; Davis *et al*., 2015).

According to Machado *et al*. (2017), hepatotoxins are some of the cyanotoxins that are extensively spread worldwide in freshwater ecosystems. *Microcystins* are cyclic peptides and one of the hepatotoxins that are commonly dispersed and frequently encountered and produced by cyanobacteria in freshwater ecosystems. *Microcystins* concentration in surface water typically range from 1 to 100 µg/L, and values up to 10,000 µg/L are reported (Corbel *et al.,* 2014; Xue *et al.,* 2020). However, *microcystins* are not limited to eutrophic surface waters only, but they are also detected in terrestrial environments that were irrigated or flooded with water containing harmful algal blooms (Petrou *et al.,* 2020).

Microcystins (MCs) are produced by different cyanobacteria species such as *Oscillatoria, Aphanizomonon, Anabaena, Planktothrix and Anabaenopsis,* and about 80 congeners of MCs have been identified and are known to cause significant harm on water quality, aquatic ecosystems, animals, and humans (EPA, 2014). Microcystin-LR (MC-LR) is the most common congener and is prevalent in freshwater ecosystems. It is also the most poisonous MC variant compared to MC-RR and YR (Sanseverino *et al.,* 2017; Miller and Russel, 2017). *Microcystins* are reported to promote cancer development, and inhibit protein phosphatase 1 and 2A, also promote primary liver cancer in humans (Oberholster *et al.,* 2005). Example of cyanotoxins classification, their mode of action and toxicity mechanisms are presented in Table 2.1.

Table 2.1: Cyanobacterial toxins classification, their functions and mechanism of action. (Oberholster et al., 2008)

Toxin classification	**Primary target organ in mammals**	**Cyanobacteria taxon**	**Mechanism of toxicity**
1. Hepatotoxins			
Microcystins	Liver	*Microcystis, Oscillatoria, Nostoc, Anabaena*	Inhibition of protein phosphatase activity, hemorrhaging of liver,
Nodularin's	Liver	*Nodularia*	Inhibition of protein phosphatase activity, hemorrhaging of the liver
2. Cytotoxins			
cylindrospermopsins	Liver, kidney, spleen, intestine, heart, thymus	*Cylindrospermopsis*	Inhibition of protein synthesis
Neurotoxins	Nerve synapse	*Anabaena, Oscillatoria*	Blocking of post-synaptic depolarization
3. Anatoxins-a			
4. Dermatotoxins			
Aplysiatoxins	Skin	*Oscillatoria*	Protein kinase C activators, inflammatory activity
5. Irritant toxins			
Lipopolysaccharides	Any exposed tissue	All	Potential irritant and allergen

2.4 Environmental factors influencing cyanobacterial bloom

The development of cyanobacterial blooms is influenced by a complex relation between environmental factors (Harding and Paxton, 2001). The environmental factors which influence the rapid bloom of cyanobacteria include physicochemical parameters such as pH, TDS, EC, Temperature, Turbidity, nutrients (phosphates & Nitrates) and Dissolved oxygen.

2.4.1 pH

The pH refers to the amount of acid balance in an aqueous solution, and it is controlled by dissolved chemicals compounds and biochemical processes (Manjare *et al.,* 2010). The pH less than < 7 is considered acidic, while greater than > 7 is considered alkaline (Gorde *et al.,* 2013). The pH plays a vital role in the aquatic ecosystem as it controls or describe the acidity and alkalinity of water. Havens (2007) suggested that normal pH in water system range from 6.5 - 8.5. The pH can be an indicator of water that is chemically changing. Cyanobacteria are alkalopiles and grow effectively at pH ranging from 7.5 to 10 (Thajuddin and Subramanian, 2005). Cyanobacterial growth is suppressed at pH ranging from 4 to 5. The pH increases with increasing irradiance, resulting in dynamic alkaline environment.

2.4.2 TDS and EC

TDS is the measure of total solids in solution, while Electrical conductivity (EC) is the ratio between the current density and the electronic field, which estimates the amount of dissolved ionic matter in aqueous solution (Van Liere, and Walsby, 1982; Odiyo *et al.,* 2012; Ololo, 2013) Electrical conductivity involves the measure of ionic activity of a solution of its capacity to transmit current. Changes in TDS may result in changes of irradiance and salinity which directly influence growth and cyanotoxins production (Havens, 2007). Furthermore, EC is strongly associated with cyanobacteria dominance because high EC indicates persistent low flow conditions.

2.4.3 Nutrients (Nitrates and Phosphates)

Excessive nutrients in aquatic ecosystems results in mass reproduction of cyanobacteria and even the blooms of cyanobacteria (Hu *et al*., 2018). Nutrients are referred to chemical compounds which are essential for living organisms (Ololo, 2013). Cyanobacterial blooms in aquatic ecosystems are a result of elevated concentrations of nutrients specifically phosphates and nitrates in water column (du Plessis, 2007). On the other hand, Phosphorus (P) is an essential element for the growth of

cyanobacteria and plants, and it is released from the sediments, and rocks, with many studies investigating the effects of varying P concentrations (Gu *et al*., 2020). Phosphorus levels around 0.03 mg/L are sufficient for the growth of cyanobacteria. Total phosphorus concentration threshold of Microcystis-dominated blooms was determined to be below 0.05 mg L^{-1} (Xu *et al*., 2015).

Generally, phosphorus is considered the most important limiting nutrient in lakes and the one responsible for eutrophication (Schindler, 2012). The elevated temperatures of water bodies enhance nutrient loading by releasing phosphorus (P) and nitrogen (N) from the sediments (Jeppesen *et al*., 2009; Song *et al.,* 2015). When pH is above critical threshold, inorganic phosphorus desorbs from iron oxides at mineral surface. Elevation of pore water pH can promote sediment release of soluble reactive phosphorus, simultaneously supporting P demand, during cyanobacterial bloom in lakes water environments (Mur *et al*., 1999). A study by Hu *et al*. (2017) found that the water temperature and TP were significantly positively correlated. Beaver *et al.* (2018) found a strong positive relationship between high concentrations of Microcystis species and high nutrients levels nitrogen and phosphorus. Funari *et al.* (2017) and Cremona *et al*. (2018) highlighted that both nitrogen and phosphorus are the major causes of eutrophication and cyanobacterial blooms in water impoundments.

2.4.4 Turbidity

Turbidity refers to the cloudiness of water because of suspended particles such as clay, silts, organic particles such as plant debris, and organisms (Garracedo *et al*., 2017). Chaffin *et al.* (2018) highlighted that turbidity is associated with phytoplankton biomass and suspended solids from sediments. Turbidity is also one of the physical parameters to measure the quality of water (Chaffin *et al.,* 2018). Turbidity affects light penetration and water temperature and alters its spectral composition due to the nature of suspended particles (Ololo, 2013). *Microcystins* adapts to high lights intensities by reducing the chlorophyll content of the cells while at lower intensities and in darkness more chlorophyll is synthesized (Ololo*,* 2013). In turbid water algae species (such as *microcystis* which has gas vesicles), move under water to avoid high light intensity at the surface of water, and they float up when under light conditions are poor (Owuor *et al.,* 2007). The high levels of turbidity, caused by re-suspension of sediments by wind and fish, may suppress phytoplankton growth, resulting in a reduction in nutrient concentrations, phytoplankton biomass, and increase in cyanobacterial species (Jeppesen *et al.,* 2015; Medeiros *et al.,* 2015).

2.4.5 Dissolved oxygen (DO)

The amount of dissolved oxygen in water and the quality of water can be determined by the amount of oxygen in a water body (Mader *et al.,* 2017). Abundant oxygen levels in the surface water provides extra buoyancy for cyanobacteria. Depletion of oxygen caused by decaying algae may further induce heterotrophic conditions in riverine sections, resulting in a dysfunction of the whole aquatic ecosystem. Dissolved oxygen in water column increases because of phytoplankton photosynthesis, re-aeration, and decrease owing to phytoplankton respiration, while decrease in nitrate in water might be because phytoplankton up take the nutrients for growth (Ololo, 2013).

2.4.6 Temperature

Temperature influences water chemistry, and the rate of chemical reactions in water generally increases at higher temperatures (Robarts and Zohary, 1987; Manjare *et al.,* 2010). All the living organisms tolerate certain range of temperature to grow effectively. Increased droughts and temperatures are demonstrated to contribute to the dominance of cyanobacteria over other algae species (Van Liere and Walsby, 1982). Cyanobacteria exhibits optimum growth at high temperatures above 20° C and occur in large quantities in water bodies with low flow velocity and long residence time. Cyanobacteria are strongly associated with thermo stratification. Elevated temperature exacerbates massive cyanobacterial blooms in most of aquatic ecosystems favoring proliferation and dominance of cyanobacteria (Johnk *et al*., 2008). In a study by Berry *et al.* (2017) rising temperatures could induce the increased production of cyanotoxins. However, Paerl and Paul (2012) suggested that MCs grows slowly below 20˚ C but reaches maximum growth rate at approximately 30˚ C. When temperature of water increased to 25˚ C in July, the planktothrix occurs and when it has increased to 28˚ C in August, the microcystins MCs species became dominant (Zhang *et al.,* 2021). The optimum growth temperature for cyanotoxins is above 25˚ C, however, it depends between cyanobacterial species (Johnk *et al.,* 2008; Zhang *et al*., 2021).

2.5 Occurrence of cyanotoxins in terrestrial plants and agricultural soils

Main source of cyanotoxins in plants and agricultural soil is through irrigating edible plants with surface water that is enriched with cyanotoxins, and using cyanobacterial biomass, as organic fertilizers for plants to grow well (Machado *et al.,* 2017; Pindihama and Gitari, 2019). This practice could lead to high concentration of MCs accumulating in soil and plants (Chen *et al.,* 2012). Cyanotoxins are introduced to agricultural soils and plants through irrigation with

contaminated water (Saqrane and Oudra, 2009; [illegible] *et al.,* 2018). The usage of contaminated water to irrigate plants does not only pose problems to human health, but also causes a huge impact on the agricultural sector and the economy. According to Redouane *et al*. (2019), when MCs are introduced to the terrestrial plants and agricultural soils through irrigation or usage of cyanobacterial biomass as organic fertilizers, affects seed germination, prevent plant growth and development, and result in production losses. Corbel *et al.* (2014) highlighted that cyanotoxins such as MC-LR and *nodularin* are soluble in water and very persistent in the environment; therefore, plants and soil absorb the toxins and transport them from roots to shoots in crop seedlings. The contamination of vegetables via irrigating with cyanotoxins infested water, indicates the risks of metals, anionic surfactants and other pollutants transferred from lake to terrestrial system (Jia *et al.,* 2018).

2.5.1 Implications of cyanotoxins on terrestrial plants

Pindihama and Gitari (2019) highlighted that cyanotoxins do not kill plants; rather, they prevent the plants from growing, leading to loss of yield. Plants are capable of detoxifying pollutants, for instance they are able to detoxify cyanotoxins such as microcystins into nonpoisonous materials, but no studies have reported how long the plant takes to biodegrade cyanotoxins (Pindihama and Gitari, 2019). The uptake of cyanotoxins by agricultural plants has been reported by several studies to induce morphological and physiological changes that lead to a potential loss of productivity (Machado *et al.,* 2017). Cyanotoxins such as *microcystins* and *nodularin* are completely soluble in water; hence, they are easily absorbed by plants roots and transported to all parts of the plants; namely, stem, leaves, fruits and ultimately seeds, reduces chlorophyll content in plant cells, resulting in reduction of photosynthesis process (Purkayastha *et al*., 2010; Cao *et al.,* 2017).

When cyanotoxins are introduced to the terrestrial edible plants through irrigation, the toxins may affect the plants tissues, cells, and bio-molecules (Cheung *et al.,* 2013). A study by McElhiney et al. (2001) reported that MCs inhibited the growth of potatoes and the development of mustard seedlings by blocking photosynthesis in the leaves. While, Bittencourt-Oliveira *et al.* (2016) highlighted that MCs alter photosynthetic rates, gas exchange, and antioxidant enzyme activities of plants, as well as induce cellular damage of seedlings. Cyanotoxins in plants impair root development, inhibit seed germination, prevent plant growth, oxidative stress, lipid peroxidation, and reduces the total yield production of a plant (Prieto *et al.,* 2011). Cyanotoxins interfere with glutamine synthase activity and ferredoxin-glutamate synthase resulting in decrease in nitrogen

assimilation efficiency in both shoots and roots (Cao *et al.,* 2018). Jia *et al.* (2018) highlighted that cyanotoxins may affect the plant physiological disruption and abnormal developments which are irreversible. Cao *et al.* (2018) have highlighted that plant species react differently toward cyanotoxins.

Thus, for plants to be affected by cyanotoxins, it depends on the concentration of cyanotoxins, time of exposure, nature of toxicity of the congener, and chemical composition of agricultural soils. The use of cyanotoxins-contaminated water for agricultural purposes may therefore represent a threat to both food security and food safety and pose human health risks via ingesting the contaminated food crops (Machado *et al.,* 2017). Hence, a need to assess the bio-accumulation and presence of cyanotoxins in food crops irrigated with water containing cyanotoxins to prevent human health implications which might result from ingesting or consuming cyanotoxins contaminated plants.

2.5.2 Implications of cyanotoxins on agricultural soils

Cyanotoxins such as *microcystins* and *nodularin* are persistent in the environment. Therefore, they bio-accumulate in the soil-forming a reservoir of cyanotoxins and ending up-taken by plants roots, transporting them to a different part of the plants, stem, leaves and fruits (Pindihama & Gitari, 2019). However, cyanotoxins from soil may be eliminated either through absorption by plants or through biodegradation by the photochemical process via UV (Klitzke *et al*., 2011). Corbel *et al.* (2014) found that biodegradation or removal of cyanotoxins in agricultural soil ranges between 6 and 17.8 days. In agricultural soil with high organic content cyanotoxins, MCs degradation takes 9.9 to 17.8 days. Soil with higher microbial activity degrades MCs in 10 to 16 days. However, since there are other pollutants which are present in agricultural soils, such as toxic metals which might affect the diversity and abundance of microbial activity in soil, might affect the rapid rate of biodegradation of MCs in soils. Biodegradation of cyanotoxins in agricultural soils depends on the type of soil, the dose of cyanotoxins and soil parameters such as pH, organic content and type of fertilizers applied.

Cyanotoxins bio-accumulation in agricultural soils pose a negative effect on the structure of the bacterial community in agricultural soils (Cao *et al.,* 2017). Bouaicha and Corbel (2016) highlighted that presence of cyanotoxins in agricultural soil might change the structure and physiology of bacterial community in soil, resulting in edible plants taking up the cyanotoxins and transport them to different parts of the plant, such as stems, leaves, and flowers. Cyanotoxins in agricultural soils

lead to a decline in the roles that are played by the soil, such as carbon sink. Furthermore, nitrification rate may be altered because bacteria in the soil are reduced significantly by cyanotoxins (Cao *et al.,* 2017; Redouane *et al*., 2019). Cao *et al.* (2017) observed that two soils treated with >100 µg/L^{-1} *microcystins* (MCs) inhibited the nitrification of soil and reduced the abundance of ammonia-oxidizing bacteria in soils. Lee *et al*. (2017) highlighted those studies have been done on the accumulation of cyanotoxins on agricultural soils, but they have not been studied in detail.

2.5.3 Accumulation of cyanotoxins in plants tissues and their potential transfer to humans

Plants absorb cyanotoxins through many routes, via roots, which absorb or accumulate high concentration, and transport to all parts of the plants, which includes stem, and leave, flowers, fruits, seeds (Pindihama and Gitari, 2019). According to Wang *et al.* (2011); Zhu *et al.* (2015) and Bittencourt-Oliveira *et al.* (2016), irrigating crops with cyanotoxins contaminated surface water might lead to the accumulation of cyanotoxins into agricultural plant tissues and soils. Bio-accumulation of cyanotoxins in fresh produce may be an exposure route to humans, and this pathway should be considered a public health concern in areas where irrigation waters contain toxin-producing cyanobacteria (Bittencourt-Oliveira *et al.,* 2016; Lee *et al.,* 2017). Cyanotoxins have higher molecular mass (~ 1000 Da), which prevent them from penetrating through the cell membrane easily (Wang *et al.,* 2012; Pindihama and Gitari, 2019). However, since cyanotoxins co-exist with other pollutants in aquatic ecosystems such as linear alkylbenzene sulfonate, and toxic metals and others, they enhance the penetration of cyanotoxins into cell membrane of plants and living organisms.

Humans may be exposed to cyanotoxins through indirect and direct route, where direct route includes recreational activities in contaminated water bodies, and drinking water containing cyanotoxins, an indirect route that includes feeding on edible plants that are contaminated with cyanotoxins through irrigation process (Bouaicha and Corbel, 2016). The bio-accumulation of cyanotoxins is a huge concern because cyanotoxins such as microcystins accumulate in edible plants posing a health threat to humans and animals, and numerous studies have shown that MCs concentrations in edible plants can accumulate to levels beyond the world health organization (WHO) set guideline value of 0.04 µg kg (Cao *et al*., 2018).

Contamination of edible crop plants with cyanotoxins differs depending on the irrigation method used, and due to fact that some crops are highly susceptible to toxins, and there are those which can

tolerate the toxins and grow or develop better (Lee *et al.,* 2017; Zhu *et al.,* 2018). Lee *et al.* (2017) further highlighted that some of the crops bio-accumulate high concentrations of cyanotoxins in roots than leaves, for instance, radish and carrots were found to accumulate *microcystins* toxins in roots in a significant amount. Levizou *et al.* (2017) stated that edible plants accumulate cyanotoxins differently based on their developing stages, for example, lettuce seedlings were found to absorb the highest concentration of MCs toxins compared to when they are at the leave stage. Corbel *et al.* (2014) also supported that lettuce (*Lactuca sativa*) which is a salad vegetable resulted in a high accumulation concentration of cyanotoxins *microcystins* on the surface forming cell colonies, thus these could pose a significant threat since humans feed more on this salad.

A study by Redouane *et al*. (2019) also found that cucumber absorb a high concentration of toxins in roots in an early stage than when it is in the flowering stage. Zhu *et al.* (2018) also supported that cucumber roots accumulated higher concentrations of MCs at seedling stage, followed by flowering and fruiting stage. Hence, this shows that accumulation of cyanotoxins in plant tissues depend on the concentration of cyanotoxins in irrigation water and the amount of time the plant is exposed. The longer-term plants are exposed to the cyanotoxins, the higher the concentration of toxins they accumulate in plant tissues affecting physiological factors of plants (Zhu *et al*., 2018). Bittencourt-Oliveira *et al.* (2016) also found that lettuce bio-accumulated high levels of MC-LR and MC-RR at the highest exposure levels, and at the lowest level exposure, there were no detectable levels of microcystins in the food crop lettuce. They further concluded that bio-accumulation of MCs in lettuce varies according to the exposure concentrations of the toxins.

Several studies have investigated and reported the accumulation of cyanotoxins in palatable plants, amphibian species, for example, fish which people feed on might present medical conditions to people, however, the capacity of the cyanotoxins to enter food chain through consumable crops has not been studied thoroughly (Corbel *et al.,* 2014). Pindihama and Gitari, (2019) highlighted the need to give special consideration to the levels of toxins in irrigation water and the degree of them aggregating in palatable plants to be able to prevent transformation of toxins to food crops which meant for human consumption. Romero-Oliva *et al.* (2014) have likewise indicated that information about the accumulation of cyanotoxins MCs in plants species cultivated with contaminated water in the genuine field conditions is limited. Henceforth, the discoveries from this investigation will help in building up an improved comprehension of how the cyanotoxins enters natural way of life and in the end to people in the actual field conditions.

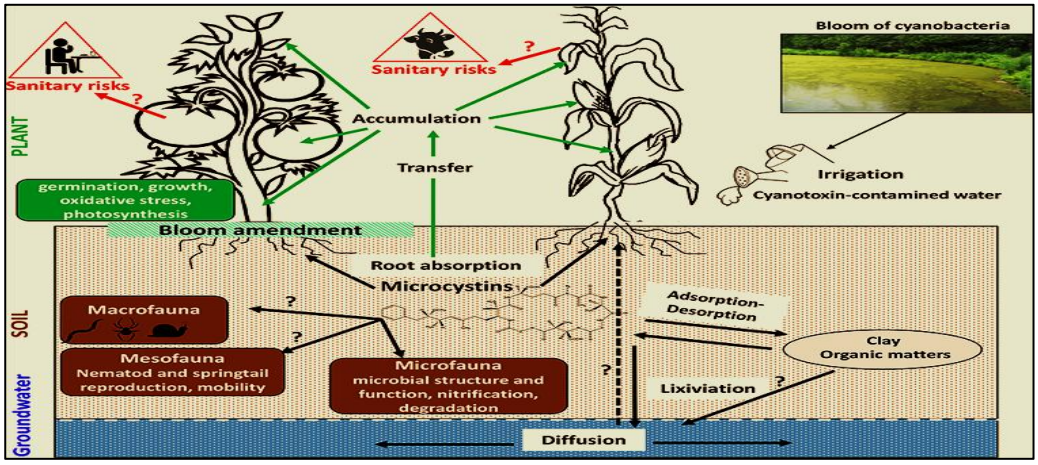


Figure 2.2 Cyanotoxins exposure routes, processes, and their impact on human and animals (Bouaicha and Corbel, 2016)

2.5.4 Implications of cyanotoxins on human through consuming contaminated plants and drinking water

Consumption of plants contaminated with cyanotoxins might result in human health risks overtime because the toxins may accumulate in the liver, kidneys and muscles. Exposure to contaminated drinking water has been reported to have resulted in symptoms such as fever, vomiting, weakness, liver problems, kidney failure, heart problems, brain, and skin damage and result in tumor growth (Lee *et al*., 2017). The most serious known episode associated with human exposure to MCs via drinking water occurred in Brazil where they used water containing microcystins for dialysis, and 131 patients experienced symptoms such as nausea and vomiting, 100 had acute liver failure, while 76 died (Carmichael *et al.,* 2001; Gaget *et al.,* 2017). Long-term exposure to cyanotoxins such as *microcystins* may result in acute and chronic health effects such as liver cancer (Zewde *et al*., 2018). Bittencourt-Oliveira *et al*. (2016) stated that cyanotoxins pose a negative impact on mammals, for example, cyanotoxins such as *microcystins* (MCs) can modify cytoskeletons of hepatocytes, induces intrahepatic hemorrhage, and causes hepatic insufficiency of liver tissues. Several studies used plants to investigate the presence of cyanotoxins and predict their health effects that they might pose to human if they consume contaminated plants and found that the most part of a plant to accumulate cyanotoxins is roots compared to other parts such as leaves, shoots, and fruits (Machado *et al*., 2017). Hence, the need to assess the transfer of cyanotoxins to food crops via irrigation, and the negative effect, they might cause to human health over long term exposure.

2.6 Occurrence of metal species in agricultural soils

Toxic metal is a general collective term applied to metallic elements which have high density of more than 6 g/cm^3 and are very poisonous in small concentration (Nagajyoti *et al*., 2010, Awodele *et al.,* 2013; Kohzadi et al., 2019). Toxic metals occur naturally in the soil in small quantities due to the weathering of bedrocks and volcanic eruptions, and they are reserved in the soil in the form that plants roots cannot absorb (Malan *et al*., 2015). However, due to human activities such as intensive agricultural, usage of fertilizers (organic and inorganic), agrochemicals, mining, industrial discharges, domestic effluent, sewage plant treatment, wastewater, they all have led to the increase in concentration of toxic metals in aquatic ecosystems and agricultural soils (Nagajyoti *et al*., 2010).

Toxic metals are classified into essential and non-essential. Essential metal species include iron, zinc, copper, cobalt, nickel, chromium, and manganese but they become harmful when they are in excessive concentrations inhibiting plant growth. Essential metals are micro-nutrients which play an essential function in metabolism and physiological activities of humans, plants, and animals, depending on their levels (Marschner, 2012; Rai *et al.,* 2019). Whereas, non-essential metal species include Pb, Hg, Cd which have no relevance biological function on plants, animals and humans, rather they are even poisonous at lower concentrations, even when they are below the acceptable guideline standard, they may cause significant harm on human health (Rai *et al.,* 2019; Okereafor *et al.,* 2020). Macron *et al*. (2010), state that the accumulation of toxic metals on plant tissues and animals depend upon the concentration of metals and the period the plants and soils were exposed. However, for toxic metals to accumulate in the soil and edible crops, it depends on the type of plant species and the soil, type; for example, clay, silty loam soil, or sandy soils (Khan *et al*., 2008).

Malan *et al.* (2015) highlighted that the use of wastewater to irrigate crops has led to high concentration of metals such as Cd (Cadmium), Cu (copper), Pb (Lead) and Zn (Zinc) in agricultural soils and terrestrial plants. According to Arora *et al.* (2008), toxic metals are a significant concern in the environment because they are non-biodegradable, meaning they are persistent in the environment and can become toxic even in small concentrations. Toxic metals such as Cd, Pb, Cu, and mercury are very dangerous in small concentration and are the major environmental pollutants in areas where anthropogenic activities have increased (Ullberg, 2015).

2.6.1 Implication of toxic metals on agricultural soils

The contamination of agricultural soils by metal species poses a significant risk to human health and the whole environment because soil plays a vital role in food mineral composition, and food safety (Toth *et al.,* 2016; Musa *et al.,* 2017). High concentration of metal species accumulation in agricultural soils is a major environmental constraint resulting in lower crop productivity, reduced food, and food safety (Maleki *et al.,* 2017). The sources of metal species in the agricultural oils are atmospheric deposition, livestock manure, irrigation with wastewater or polluted water, agrochemical such as metal-pesticides and herbicides, phosphate fertilizers, and sewage sludge as amendments (Elgallal *et al.,* 2016; Woldetsadik *et al.,* 2017; El-Kady and Abdel-Wahhab, 2018). High concentrations of toxic metal in soil are a serious problem because of their toxicity to soil microorganisms and impairment of ecosystem functions (Ding *et al.,* 2016).

The effect of metal species depends on the factors such as rate, exposure time, tolerance of the organisms, and the environmental conditions (Musa *et al.,* 2017). According to Musa *et al.* (2017), elevated concentration of metal species such as Fe, Pb, and Hg reduces the soil fertility and agricultural output produce. Beneficial soil insects specifically in agricultural soils such as invertebrates, and small and large mammals are all affected by the presence of high levels of toxic metals (Gall *et al.,* 2015; Bartrons and Peñuelas, 2017; Rai *et al.,* 2019). Gadd (2010) highlighted high levels of metal species in soil which reduce the biomass of microbes which play a vital role such as element bio-transformation, biogeochemical cycling, metals and mineral transformation, bio-weathering, and the formation of sediments. The reduction of microbes in soils due to high levels of metals species result in decrease in bacterial diversity in soil, slow down the rate of decomposition of organic matter, reduces soil respiration, and eventually leading to structural change of microbes (Giller *et al.,* 2009). Boshof *et al.* (2014) and Xie *et al.* (2016) stated that reduction in microbial diversity in soil may adversely affect nutrients uptake by plants and reduce the fertility of soil.

2.6.2 Implications of toxic metals on plants

Plants grown in a polluted agricultural soil can accumulate metal species at high concentrations and may serve as a main pathway for transferring metals into the food chain (Al-Othman *et al.,* 2016). Plants can absorb heavy metals and accumulate them in their tissues, thus, posing concern because they accumulate in plant leaves at very high concentrations, which in turn may be

consumed by humans or animals (Khan *et al.,* 2008). High concentrations of metal species can also affect the growth and yield of many crops.

Cadmium (Cd) decrease plant metabolic activity and induce oxidative damage. The Acceptable limit of Cd is about 100 mg/kg in agricultural soils (Salt *et al*., 1995). Edible crops that are grown in soils that contain high levels of cadmium, affect plants through preventing growth of plants, browning roots tips, uptake and transport of calcium, magnesium, potassium, water, phosphates, and reduces absorption of nitrates and its transport from roots to shoots, and ultimately the plant dies (Khan *et al.,* 2008; Guo *et al.,* 2008). Balestrasse *et al*. (2003) highlighted that cadmium was used in soybean plants, and nitrogen fixation and primary ammonia assimilation decreased in nodules of the beans.

Lead (Pb) is highly dispersed toxic metal and high concentration could pose significant impact on morphology, growth, chlorophyll, and photosynthetic processes inhibiting plant growth (Najeeb *et al.,* 2014). It prevents seed development of edible crops and photosynthesis process, inhibits seed development of crop vegetable such as *Pinus helipensis,* inhibits root elongation and leaf growth in *allium* species (Khan *et al.,* 2008). Nagajyoti *et al*. (2010) highlighted that low concentration of lead ranging from 0.005 ppm was found to influence lettuce and carrots reducing their growth, and water imbalance and preventing enzyme activities, thus, this proves that lead is poisonous even in small concentration to plants.

Chromium (Cr) is also one of the metal species which when in high concentrations can cause adverse effect on plants. Toxicity of Cr in plants when it has accumulated in high concentration includes, inhibition of plant growth and development, seed germination, decrease in plant biomass, leaf chlorosis, and affects photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation, and enzyme activity. (Nagarajan and Ganesh, 2014; Nematshahi *et al.,* 2012). Cr of greater than 100 mg/ L can cause adverse impact on plants such as reduction of morpho-physiology parameters, reduction in nutrients uptake and altering of biochemical processes (Nagarajan and Ganesh, 2014). Maleki *et al.* (2017) indicated that increase Cr accumulation in plant can cause a significant decrease in plant biomass, root and shoot length, and contents of proteins, sugars, chlorophyll and carotenoids.

Arsenic (As) is one of the non-essential metals, and it's very toxic even at low concentration on plants. High levels of As in plants can cause leaf necrosis and wilting, followed by root

discoloration and retardation of shoot growth, chlorosis, stunted growth, reduce seed germination and seedling height (Asati *et al.,* 2016; Edelstein and Ben-Hur, 2018).

Nickel (Ni) is an essential metal and is found in trace concentration in natural soils (Nagajyoti *et al.,* 2010). However due to anthropogenic activities, levels of nickel in soil have increased ranging from 200 to 26 000 mg/kg (Izosimova, 2005). Excessive nickel in soil may accumulate in plant resulting in alteration of physiological processes, spoil the nutrient balance, resulting in disorders of cell membrane functions chlorosis (Rahman *et al.,* 2005; Edelstein and Ben-Hur, 2018). Nagajyoti *et al.* (2010) indicated that exposure of wheat to high levels of nickel enhanced MDA concentration and resulted in decline in water content.

Cobalt (Co) metal occurs naturally from the earth crust and one of the essential metal species. Plants accumulate Co in traces from soil. A study by Li *et al.* (2009) reported that cobalt had an adverse effect on shoots growth and biomass of tomato, oil seed and barley, and it inhibited the concentration of iron (Fe), chlorophyll, protein, and catalase activity in leaves of cauliflower.

Manganese (Mn) excessive adsorbent of manganese in leaves by plants result in reduction of photosynthesis process. High levels of manganese in plants result in slow growth, decrease in chlorophyll content, inhibit synthesis of chlorophyll by blocking iron- processes, brown spotting on leaves, leaf browning and eventually death (Srivastava *et al.,* 2011; Asati *et al.,* 2016).

Iron (Fe) is also one of the essential micro-nutrient elements, and they play a vital role in plants such as chlorophyll, photosynthesis, and chloroplast development (Asati *et al.,* 2016). Although iron is essential, its excessive level in plants may cause adverse effect on plants. High levels of Fe in plants result in impairment of cellular structure which are irreversible, and damage membranes, DNA, and proteins (Arora *et al.,* 2002; de Dorlodot *et al.,* 2005). Nagajyoti *et al.* (2010) indicated that excessive iron reduced photosynthesis, yield, and increased oxidative stress in tobacco, canola and soybean.

Zinc (Zn) is one of the essential micro-nutrient elements. However, when it is in excessive concentration in soil, it may be toxic to plants by inhibiting plant metabolic function resulting in retarded growth (Asati *et al.,* 2016). High levels of zinc in plants limit the growth of roots and shoots, and cause chlorosis in younger leaves, and cause deficiency of Mn and Cu in plant shoots which are very essential for plant biological processes (Asati *et al.,* 2010).

Aluminium (Al) is one of the most abundant minerals in soil, phytotoxic elements which when is in high concentration in soil it may be absorbed by plants and result in reduction in growth and development (Schmitt *et al.,* 2016). Exposure of the plants to high levels of Al may result in significant impact such as cease in plant growth, deficiency in nutrients, inhibition in root elongation, reduced mitotic activity of roots etc. (Yang *et al.,* 2011; Schmitt *et al.,* 2016; Yan *et al.,* 2016).

Copper (Cu) is one of the essential micro-nutrients, which is very vital for the plant growth. However, this metal becomes highly toxic when it is in high concentrations above the threshold limit, resulting in plants bio-accumulating the micro element in high concentration resulting in toxicity effects such as generation of oxidative stress, leaf chlorosis and reactive oxygen species (Asati *et al.,* 2016). A study carried by Costa and Sharma (2016) on the physiology and biochemical behavior of rice treated with copper, found that copper reduced the root germination rate of rice, root growth, shoot length, biomass and photosynthesis content declined.

2.6.3 Implications of toxic metals on human health

The food chain (soil–plant–humans) is recognized as one of the major pathways for human exposure to metal species (Edelstein and Ben-Hur, 2018). The routes for the introduction of metal species into the human body include several routes such as direct inhalation of contaminated air, ingestion of contaminated water, and direct ingestion of soil and consumption of food plants grown in metal-contaminated soil (Bhagure and Mirgane, 2011; Al-Othman *et al.,* 2016). The accumulation of metal species in food plants is a huge concern because they can significantly affect plants, animals and human health. Several studies showed the potential risk of toxic metals to human health via ingesting plants contaminated with high levels of metals (Chauhan and Chauhan, 2014; Balkhair and Ashraf, 2016; Alghobar and Suresha, 2017).

Contaminated food crop by metal species has toxic effects on human health and can seriously deplete some essential nutrients in the body that are further responsible of reducing immunological defenses, intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Türkdoğan *et al.,* 2003; Arora *et al.,* 2008; Chaoua *et al.,* 2019*).* Ingestion of toxic metals through the uptake of contaminated plant or aquatic organisms such as fish by humans may result in different illnesses which disrupt the biochemical process within the body, such as depletion of essential nutrients,

decrease immunological defenses, intrauterine growth retardation, disabilities, and upper gastrointestinal cancer rates (Arora *et al*., 2008; Anhwange *et al.,* 2013).

Long-term exposure to humans through consuming plants contaminated by toxic chemicals may lead to the accumulation of metals in livers, and kidneys, ultimately resulting in the disturbance of biochemical processes such as nervous, kidneys, cardiovascular and bone disorders (Jarup, 2003). Metal species such as lead (Pb) is a potential carcinogen, and it can accumulate in the gray matter of the brain, damaging the neurons and their dendrites and synapses, and it also damages the red blood cells (Dzomba *et al.,* 2012; Kohzadi *et al.,* 2018). Toxic metals such as Cd, Pb, As, Hg, Zn, Cu and Al poisoning on humans include gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria, paralysis, vomiting and pneumonia (Jaishankar *et al.,* 2014). For this reason, it is essential to assess the accumulation of potentially toxic metals in edible plants to prevent human health risks by ingesting metal contaminated plants (Khan *et al.,* 2018). Figure 2.3 shows the ecotoxicological impact of metal species in vegetables crops, and their health implication they may pose to humans.

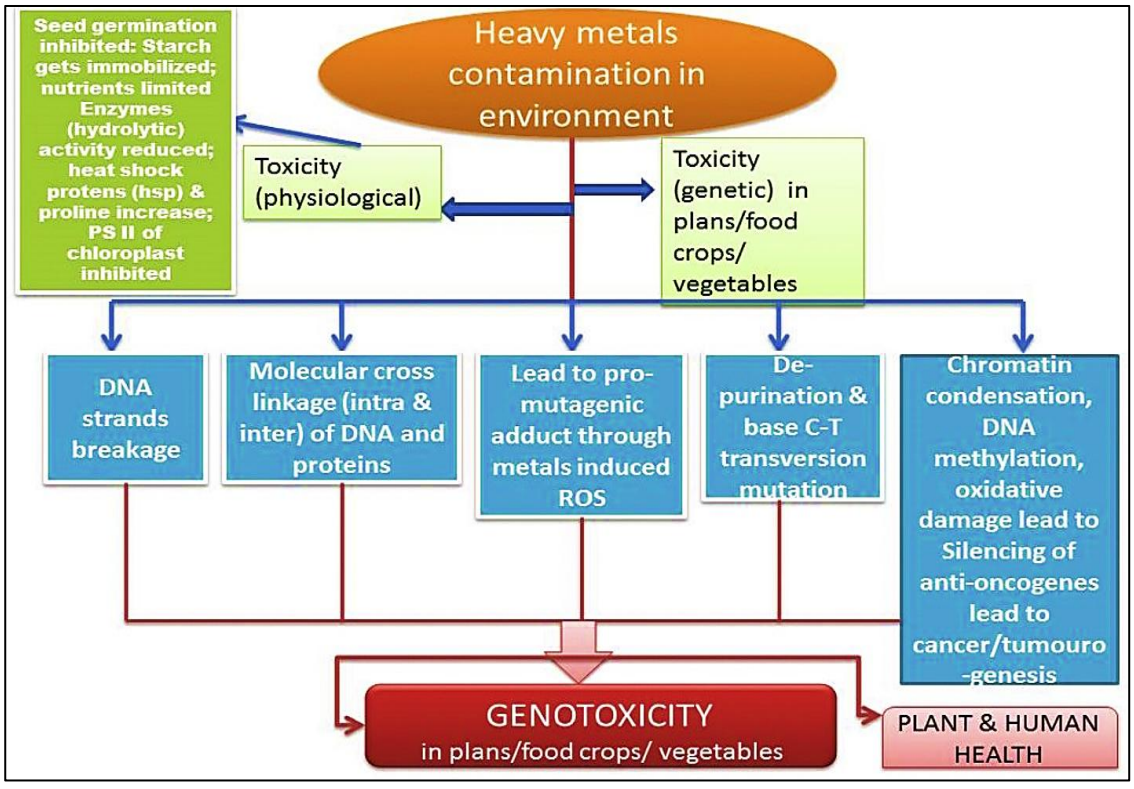


Figure 2.3 The ecotoxicological impacts of toxic metals in vegetable plants, and eventually their implication on human health (Rai et al., 2019).

2.7 Anionic surfactants and eutrophication

Anionic surfactants are (surface active agents) chemicals used to produce soaps and detergents (Nomura *et al*., 1998; Gordon, 2011; Zigolo *et al.,* 2020). The anionic surfactants linear alkylbenzene sulfonate are one of the most used surfactants which were introduced in the 1960s due to the fact that it is readily biodegradable and does not persist in the environment, replacing highly branched alkylbenzene sulfonate (LAB) which is not readily biodegradable (Eniola, 2007). A total of 18 million tons of surfactants are produced in the world every year (Cirelli et al., 2009; Ramprasad and Phillip, 2016). Anionic surfactants are amphipathic characterized by a hydrophilic (carboxyl, sulfate, sulfonates, phosphates) and hydrophobic (alkylphenyl ethers, alkylbenzenes) group (Cserhati *et al.,* 2002; Landeck *et al.,* 2020). Anionic surfactants may be used as emulsifiers, foamers, detergents such as (laundry powder, laundry liquids, dish washing products), solubilizers, wetting agent and used in pharmaceuticals (Anachkov *et al*., 2015). The occurrence of surfactants in an aquatic ecosystems is through discharge of treated and untreated wastewater, doing laundry activities alongside the river, sewage plant treatment discharges into the river, industrial and domestic waste, and urban wastewater (Eniola, 2007; Wang *et al.,* 2011). In South Africa especially urban areas, there are no proper treatment wastewater plants, hence the discharges end up directly into water sources introducing anionic surfactants (Quayle *et al.,* 2010). Anionic surfactants in aquatic ecosystem ranges between 0.001 and 20 mg/L (Wang *et al*., 2015). Low concentrations of anionic surfactants in aquatic ecosystems might result from elimination through rainfall, biodegradation and adsorption (Quayle *et al.,* 2010).

According to Nomura *et al*. (1998), the fact that there are no regulations to control the concentrations of surfactants in domestic wastewater, most of of surfactants end up in water bodies provoking the eutrophication processes. Wang *et al*. (2015) found that a high concentration of linear alkylbenzene sulfonate improved the growth production of MCs of one strain of *M. aeruginosa* in Lake Dianchi. Most of cyanotoxins, for example, MCs have large molecular mass weight (∼1000 Da) which makes it difficult to easily penetrate through the biological membranes and bio-accumulate (Wang *et al.,* 2012). The combined presence of anionic surfactants and cyanotoxins in the study area may affect the toxicity and accumulation of cyanotoxins in the crop plants. Surfactants because of their amphiphilic nature, may interact with inorganic and organic contaminants affecting their solubility and bio-availability (Cirelli *et al.,* 2009). According to Wang *et al*. (2012), the combination of surfactants and cyanotoxins is very common in the eutrophic water body because of the abundance of cyanobacterial blooms. Anionic surfactants such as linear

alkylbenzene sulfonate in eutrophic water bodies might be found in high concentrations because it favour aerobic conditions to degrade (Wang *et al*., 2015). However, dissolved oxygen in eutrophic water bodies is very low resulting in slow biodegradation of the anionic surfactants resulting in accumulation in soils and terrestrial plants via irrigation.

2.7.1 Effect of anionic surfactants on agricultural soils

Anionic surfactants are introduced into agricultural soils via several routes namely, irrigation with water contaminated with anionic surfactants, application of pesticides, amendment of soil by sewage sludge, irrigation with wastewater (Wang *et al.,* 2011). When surfactants enter the soil ecosystem, it may be adsorbed, affecting the physicochemical and biological properties of soils, the stability, and aggregates of soils (Rao & He, 2006; Cirelli *et al.,* 2009). The active anionic surfactants which are organic pollutants are readily degradable or broken down in soil, which is highly aerobic, with half-life ranging from 7 to 33 days (Wang *et al.,* 2007). Ekmekyapar and Celtikli, (2014) also found that half-life of linear alkylbenzene sulfonate in soil to be >30 days.

The presence of high levels of anionic surfactants in agricultural soils may pose a significant impact on food crops because surfactants such as linear alkylbenzene sulfonate are known to alter the cell membrane of organisms, enhancing the accumulation of other pollutants such as metals species and microcystins which might be present in the irrigation water. A study carried by Ekmekyapar and Celtikli, (2014) observed that the application of surfactant linear alkylbenzene sulfonate in soil enhanced the soil pH and electrical conductivity (EC), and the organic matter and cation exchange capacity of soil decreased with an increase in linear alkylbenzene sulfonate concentrations. Sanchez-Peinado *et al.* (2008) highlighted those high concentrations of anionic surfactants in agricultural soils inhibited the microbial activity, which might affect the soil from biodegrading pollutants such as cyanotoxins and metal species, resulting in pollutants being taken up by crops grown in the soil contaminated by anionic surfactants, posing health risks to human consuming the plants grown on the contaminated soil.

2.7.2 Implications of anionic surfactants on terrestrial plants

Anionic surfactants are introduced into terrestrial plants through irrigating with water contaminated with high concentrations of surfactants. Wang *et al.* (2015) demonstrated that anionic surfactants linear alkylbenzene sulfonate increases the effect of cyanotoxins toxicity on terrestrial plants. Wang *et al.* (2012) also highlighted that *Ruditapes philippinarum* accumulated a high concentration of

lead in the presence of linear alkylbenzene sulfonate compared to when it was exposed to lead only. Wang *et al.* (2011) observed that LAS enhanced the uptake of MC-LR by plants and its ecotoxicological effect. Anionic surfactants alter the permeability of cell membranes, enzymatic activity and tissue structure of terrestrial plants and aquatic species enhancing the accumulation of other pollutants such as *microcystis* and metal species. Anionic surfactant LAS also enhance *microcystins* accumulation in the plants, leading to reduction in quality and yield posing greater health risks to humans consuming contaminated plants (Wang *et al*., 2011; Wang *et al.,* 2012). Wang *et al.* (2011) showed that the combination of *microcystins* MC-LR and anionic surfactant LAS affected seed germination and seedling growth of lettuce *(Lactusa sativa L),* and the roots turned brown, and became dry in 3 days of exposure. A study by Wang et al. (2015) also reported that 20 mg/L of LAS surfactants inhibited the protein and carotenoid of a plant significantly after 6 to 12 days of exposure. The combination of MC-LR and LAS inhibited the seedling growth and increased the activities of superoxide dismutase and catalase (Wang *et al.,* 2011). Wang *et al,* (2011) further concluded that the interaction between *microcystins* MC-LR and LAS surfactants is synergistic.

2.8 Passive sampling technique (SPATT) as a device to monitor and detect toxins in aquatic environments.

The SPATT method was firstly used and introduced in the work of Mackenzie in 2004 to monitor contaminants and toxins (Zendong *et al.,* 2014). SPATT is widely used today because it is effective and provides a time-integrated warning of the presence of cyanotoxins in freshwater ecosystems (Howard *et al*., 2017). This method allows for the detection of toxic compounds directly in the water column and offers numerous advantages over current monitoring techniques (Roue *et al.,* 2018). Wood *et al.* (2008) and Roue *et al.* (2018) referred to SPATT as a technique that passively adsorbs dissolved cyanotoxins into a synthetic adsorbent which is porous inside a sachet or SPATT bag. Different types of SPATT bags are being used, such as sewn bags, PVC frames, embroidery disks (Zhao *et al*., 2013). SPATT technique involved dipping the SPATT bags containing synthetic porous resin inside the bag, into the water body to detect the dissolved cyanotoxins (Wood *et al.,* 2008). The SPATT method works by allowing passive adsorption of cyanotoxins onto the porous resin-filled inside the SPATT bag (Zendong *et al.,* 2014).

The benefit of the SPATT method is that the materials needed to produce SPATT bags are inexpensive, and it is a good tool to monitor and give early warning about the formation of cyanotoxins in aquatic ecosystems (Wood *et al*., 2008). SPATT innovation has been appeared to provide solid, delicate, and time-integrated sampling of different aquatic toxins, and has the potential to supply an early caution framework for both the event of harmful cyanobacteria, and bio-accumulation of toxins in food stuffs (Roue *et al.,* 2018). SPATT technology provide unique information on toxin dynamics, such as the origin of new toxins, environmental persistence, and varieties within the particular toxicity of producers (Mackenzie *et al.,* 2010). SPATT technology provides unique information on toxin dynamics such as the origin of new toxins, environmental persistence, and variations in the specific toxicity of producers (Mackenzie *et al.,* 2010). The disadvantage of the SPATT method is that the technique only monitors or detects dissolved toxins in aqueous solutions and the results obtained from SPATT cannot be converted easily to the concentration of cyanotoxins to be able to set standard guides.

According to Howard *et al.* (2017), SPATT is more reliable than the traditional grab-sampling method, because it gives reliable results, and detects cyanotoxins such as MCs where grab method is not able to detect. Traditional sampling methods for monitoring *microcystins* rely on collecting cyanobacteria biomass or water samples from sites (Kudela, 2011). These types of isolated samples do not present the accurate profile of cyanotoxins because of spatial and time-based variation because of hydro-logical and circulation effects (Zhao *et al.,* 2013). The traditional sampling practices provide only a snapshot of cyanotoxins present at one point at that time and may miss times of highest risk of cyanotoxins (Wood *et al.,* 2011). Mackenzie *et al.* (2004) also highlighted that the SPATT technique is a good tool to monitor cyanotoxins since it gives early warning on the formation of cyanobacterial toxins in aquatic ecosystems.

Howard *et al.* (2017) deployed SPATT method to assess the prevalence of cyanotoxins in water bodies comparing to the grab sampling, and found that *Microcystis* were occurring more using SPATT, whereas the grab method detected nothing in aquatic ecosystem. Howard *et al.* (2017) concluded that SPATT method provides complete view of cyanotoxins in water bodies. Roué *et al*., (2018) highlighted that SPATT techniques its good due to fact that it is capable of monitoring or observing diversities of toxins in aquatic ecosystems. Pindihama and Gitari, (2020) highlighted a study carried by Wood *et al.,* (2008) which demonstrated the ability of SPATT to adsorb and monitor lipophilic toxins in the aquatic ecosystems. However, no literature has stated the specific time required for deployment of SPATT in the field, the duration depends on the type of monitoring

program undertaken, whether the monitoring program is for long term or short-term period (Roué *et al*., 2018).

2.8.1 DIAON HP20 as an adsorbent for toxins in aquatic environment

The DIAON HP20 is referred to an adsorbent which is highly porous non-polar and non-ionic styrene-divinylbenzene adsorbent resin, with a spherical particle size of 0.5 mm diameter (Latip *et al.,* 2000; Vidoca *et al.,* 2020). Latip *et al.* (2000) indicated that HP20 resin have been used extensively in food industries and pharmaceutical. Today, Diaon HP20 aromatic resin has proven to be effective in monitoring different types of marine and fresh water biotoxins, such as *hydrophilic phycotoxins, domoic acid, saxitoxins, microcystins and anatoxins* (Lane *et al.,* 2010; Wood *et al.,* 2011; Kudela, 2011). Most of the studies used different adsorbent materials such as DIAON HP20, SP207, SP207SS, Sepalbeads1 SP850, Sepalbeads1 SP825L, Amberlite XAD4, Dowex-Optipore L-493, For adsorption of hydrophilic toxins, the ethylene glycol methacrylate phosphate-based polymer for *saxitoxins* and Amberlite XAD761 for domoic acid has been used to monitor toxins (Turrel *et al.,* 2007; Cailluad *et al.,* 2011, Mashile and Nomngongo, 2017). Among all these, HP20 resin was found to be the most effective and widely used due to its excellent retention, and desorption of cyanotoxins such as *microcystins* LR-YR-LA and RR (Kudela, 2011; Zhao *et al.,* 2013). The pore size distribution of the HP20 resin enhances the toxins adsorption capacity of the adsorbent making it more effective adsorbent for toxins (Li *et al.,* 2011; Fan et al., 2014). Mackenzie (2010) further highlighted that the deployment of SPATT with HP20 resin provides robust monitoring of cyanotoxins such as *microcystins* because passive sampling SPATT bags monitor and detect cyanotoxins within water column overtime.

Figure 2.4: Passive sampling process. (A) SPATT bag assembly, (B) SPATT bag activation in methanol, and (C) SPATT bag deployment in irrigation canals/Farm dams (Roue et al., 2018).

References

, M.A. and Suresha, S., 2017. Evaluation of metal accumulation in soil and tomatoes irrigated with sewage water from Mysore city, Karnataka, India. *Journal of the Saudi Society of Agricultural Sciences*, 16(1), pp.49-59.

Al-Othman, Z.A., Ali, R., Al-Othman, A.M., Ali, J. and Habila, M.A., 2016. Assessment of toxic metals in wheat crops grown on selected soils, irrigated by different water sources. *Arabian Journal of Chemistry*, 9, pp. S1555-S1562.

Anachkov, S.E., Tcholakova, S., Dimitrova, D.T., Denkov, N.D., Subrahmaniam, N. and Bhunia, P., 2015. Adsorption of linear alkyl benzene sulfonates on oil–water interface: Effects of Na+, Mg2+ and Ca2+ ions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 466, pp.18-27.

Anderson, D.M., P.M. Glibert, and J.M. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25 (4b): 704–726.

Anhwange, B.A., Agbaji, E.B., Gimba, C.E. and Ajibola, V.O., 2013. Seasonal variations in trace metals contents of some vegetables grown on irrigated farmlands along the Bank of River Benue within Makurdi Metropolis. *Journal of Natural Sciences Research*, 3(2), pp.74-81.

Arora, A., Sairam, R.K. and Srivastava, G.C., 2002. Oxidative stress and antioxidative system in plants. Current science, pp.1227-1238.

Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N., 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. Food chemistry, 111(4), pp.811-815.

Asati, A., Pichhode, M. and Nikhil, K., 2016. Effect of heavy metals on plants: an overview. *International Journal of Application or Innovation in Engineering & Management*, 5(3), pp.56-66.

Awodele, O., Popoola, T.D., Amadi, K.C., Coker, H.A.B. and Akintonwa, A., 2013. Traditional medicinal plants in Nigeria—Remedies or risks. *Journal of Ethnopharmacology*, 150(2), pp.614-618.

Balestrasse, K.B., Benavides, M.P., Gallego, S.M. and Tomaro, M.L., 2003. Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. Functional plant biology, 30(1), pp.57-64.

Balkhair, K.S. and Ashraf, M.A., 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi Journal of Biological Sciences*, 23(1), pp. S32-S44.

Bartrons, M. and Peñuelas, J., 2017. Pharmaceuticals and personal-care products in plants. Trends in plant science, 22(3), pp.194-203.

Beaver, J.R., Tausz, C.E., Scotese, K.C., Pollard, A.I. and Mitchell, R.M., 2018. Environmental factors influencing the quantitative distribution of microcystin and common potentially toxigenic cyanobacteria in US lakes and reservoirs. Harmful Algae, 78, pp.118-128.

Berry, M.A., Davis, T.W., Cory, R.M., Duhaime, M.B., Johengen, T.H., Kling, G.W., Marino, J.A., Den Uyl, P.A., Gossiaux, D., Dick, G.J. and Denef, V.J., 2017. Cyanobacterial harmful algal blooms are a biological disturbance to Western Lake Erie bacterial communities. Environmental microbiology, 19(3), pp.1149-1162.

Bhagure, G.R. and Mirgane, S.R., 2011. Heavy metal concentrations in groundwaters and soils of Thane Region of Maharashtra, India. Environmental monitoring and assessment, 173(1), pp.643-652.

Boshoff, M., De Jonge, M., Dardenne, F., Blust, R. and Bervoets, L., 2014. The impact of metal pollution on soil faunal and microbial activity in two grassland ecosystems. Environmental research, 134, pp.169-180.

Bouaïcha, N. and Corbel, S. (2016) Cyanobacterial Toxins Emerging Contaminants in Soils: A Review of Sources, Fate and Impacts on Ecosystems, Plants and Animal and Human Health Cyanobacterial Toxins Emerging Contaminants in Soils: A Review of Sources, Fate and Impacts on Ecosystems, Plants. In: M. Larramendy (Ed.), InTech, pp. 105– 126.

Bwapwa, J.K., Anandraj, A. and Trois, C., 2018. Microalgae processing for jet fuel production. Biofuels, Bioproducts and Biorefining, 12(4), pp.522-535.

Cai, F., Li, X., Yang, Y., Jia, N., Huo, D. and Li, R., 2019. Compactonostoc shennongjiaensis gen. & sp. Nov. (Nostocales, Cyanobacteria) from a wet rocky wall in China. Phycologia, pp.1-11.

Caillaud, A., de la Iglesia, P., Barber, E., Eixarch, H., Mohammad-Noor, N., Yasumoto, T. and Diogene, J., 2011. Monitoring of dissolved ciguatoxin and maitotoxin using solid-phase adsorption toxin tracking devices: Application to Gambierdiscus pacificus in culture. Harmful Algae, 10(5), pp.433-446.

Cao, Q., Alan, D., Steinman, A.D., Wan, X. and Xie, L. (2018) Bioaccumulation of microcystin congeners in soil-plant system and human health risk assessment: a field study from Lake Taihu region of China. Environmental Pollution, 240, 44– 50.

Cao, Q., Steinman, A.D., Su, X. and Xie, L., 2017. Effects of microcystins contamination on soil enzyme activities and microbial community in two typical lakeside soils. Environmental Pollution, 231, pp.134-142.

Cao, X., Wang, Y., He, J., Luo, X. and Zheng, Z., 2016. Phosphorus mobility among sediments, water and cyanobacteria enhanced by cyanobacteria blooms in eutrophic Lake Dianchi. Environmental Pollution, 219, pp.580-587.

Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Human and ecological risk assessment: An International Journal, 7(5), pp.1393-1407.

Carr, G.M. and Neary, J.P., 2008. Water quality for ecosystem and human health. UNEP/Earthprint.

Carracedo, G., Serramito-Blanco, M., Martin-Gil, A., Wang, Z., Rodriguez-Pomar, C. and Pintor, J., 2017. Post-lens tear turbidity and visual quality after scleral lens wear. Clinical and Experimental Optometry, 100(6), pp.577-582.

Chaffin, J.D., Kane, D.D., Stanislawczyk, K. and Parker, E.M., 2018. Accuracy of data buoys for measurement of cyanobacteria, chlorophyll, and turbidity in a large lake (Lake Erie, North

America): implications for estimation of cyanobacterial bloom parameters from water quality sonde measurements. Environmental science and pollution research, 25(25), pp.25175-25189.

Chaoua, S., Boussaa, S., El Gharmali, A. and Boumezzough, A., 2019. Impact of irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in Morocco. Journal of the Saudi Society of Agricultural Sciences, 18(4), pp.429-436.

Chauhan, G. and Chauhan, U.K., 2014. Human health risk assessment of heavy metals via dietary intake of vegetables grown in wastewater irrigated area of Rewa, India. International journal of scientific and research publications, 4(9), pp.1-9.

Chen, W., Jia, Y., Li, E., Zhao, S., Zhou, Q., Liu, L. and Song, L., 2012. Soil-based treatments of mechanically collected cyanobacterial blooms from Lake Taihu: efficiencies and potential risks. Environmental science & technology, 46(24), pp.13370-13376.

Cheung, M.Y., Liang, S. and Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. Journal of Microbiology, 51(1), pp.1-10.

Chislock, M.F., Doster, E., Zitomer, R.A. and Wilson, A.E., 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nature Education Knowledge, 4(4), p.10.

Cirelli, A.F., Ojeda, C., Castro, M.J. and Salgot, M., 2009. Surfactants in sludge-amended agricultural soils: a review. Organic Farming, Pest Control and Remediation of Soil Pollutants, pp.227-251.

Codd, G.A., Morrison, L.F. and Metcalf, J.S., 2005. Cyanobacterial toxins: risk management for health protection. Toxicology and applied pharmacology, 203(3), pp.264-272.

Corbel, S., Bouaïcha, N. and Mougin, C., 2014. Dynamics of the toxic cyanobacterial microcystin-leucine-arginine peptide in agricultural soil. Environmental chemistry letters, 12(4), pp.535-541.

Corbel, S., Mougin, C., Nélieu, S., Delarue, G. and Bouaïcha, N., 2016. Evaluation of the transfer and the accumulation of microcystins in tomato (Solanum lycopersicum cultivar MicroTom) tissues

using a cyanobacterial extract containing microcystins and the radiolabeled microcystin-LR (14C-MC-LR). Science of the Total Environment, 541, pp.1052-1058.

Cremona, F., Tuvikene, L., Haberman, J., Noges, P. and Noges, T., 2018. Factors controlling the three-decade long rise in cyanobacteria biomass in a eutrophic shallow lake. Science of The Total Environment, 621, pp.352-359.

Cserháti, T., Forgács, E. and Oros, G., 2002. Biological activity and environmental impact of anionic surfactants. Environment international, 28(5), pp.337-348.

Da Costa, M.V.J. and Sharma, P.K., 2016. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa. Photosynthetica, 54(1), pp.110-119.

Dalu, T. and Wasserman, R.J., 2018. Cyanobacteria dynamics in a small tropical reservoir: Understanding spatio-temporal variability and influence of environmental variables. Science of the total environment, 643, pp.835-841.

Davis, T.W., Bullerjahn, G.S., Tuttle, T., McKay, R.M. and Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during Planktothrix blooms in Sandusky Bay, Lake Erie. Environmental science & technology, 49(12), pp.7197-7207.

De Dorlodot, S., Lutts, S. and Bertin, P., 2005. Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice. Journal of plant nutrition, 28(1), pp.1-20.

del Mar Sánchez-Peinado, M., González-López, J., Rodelas, B., Galera, V., Pozo, C. and Martínez-Toledo, M.V., 2008. Effect of linear alkylbenzene sulfonates on the growth of aerobic heterotrophic cultivable bacteria isolated from an agricultural soil. Ecotoxicology, 17(6), pp.549-557.

Ding, Y., Ye, Y., Jiang, Z., Wang, Y. and Zhu, C., 2016. MicroRNA390 is involved in cadmium tolerance and accumulation in rice. Frontiers in plant science, 7, p.235.

do Carmo Bittencourt-Oliveira, M., Cordeiro-Araújo, M.K., Chia, M.A., de Toledo Arruda-Neto, J.D., de Oliveira, Ê.T. and dos Santos, F., 2016. Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners. Ecotoxicology and environmental safety, 128, pp.83-90.

Du Plessis, D., 2007. Impacts of cage aquaculture on the farm dam ecosystem and its use as a multipurpose resource: Implications for irrigation (Doctoral dissertation, Stellenbosch: University of Stellenbosch).

DWAF (Department of Water Affairs and Forestry, South Africa) (2002) National Eutrophication Monitoring Programme. Implementation Manual. Compiled by K Murray, M du Preez and CE van Ginkel. Department of Water Affairs and Forestry, Pretoria. South Africa.

Dzomba, P., Chayamiti, T. and Togarepi, E., 2012. Heavy metal content of selected raw medicinal plant materials: implication for patient health.

Edelstein, M. and Ben-Hur, M., 2018. Heavy metals and metalloids: Sources, risks, and strategies to reduce their accumulation in horticultural crops. Scientia Horticulturae, 234, pp.431-444.

Ekmekyapar, F. and Celtikli, D.O., 2014. Effects of linear alkylbenzene sulfonate on agricultural soil and its degradation. Fresenius Environmental Bulletin, 23(12 A), pp.3188-3192.

Elgallal, M., Fletcher, L. and Evans, B., 2016. Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: A review. Agricultural Water Management, 177, pp.419-431.

El-Kady, A.A. and Abdel-Wahhab, M.A., 2018. Occurrence of trace metals in foodstuffs and their health impact. Trends in food science & technology, 75, pp.36-45.

Eniola, K.I.T., 2007. Response of resident bacteria in a tropical detergent effluent-polluted stream to linear alkylbenzene sulfonate (LAS). African Journal of Aquatic Science, 32(2), pp.159-163.

EPA, N., 2014. Sydney Paten No. EPA 2014, 32[illegible]Fan, L., Sun, G., Qiu, J., Ma, Q., Hess, P. and Li, A., 2014. Effect of seawater salinity on pore-size distribution on a poly (styrene)-based HP20 resin and its adsorption of diarrhetic shellfish toxins. Journal of Chromatography A, 1373, pp.1-8.

Funari, E., Manganelli, M., Buratti, F.M. and Testai, E., 2017. Cyanobacteria blooms in water: Italian guidelines to assess and manage the risk associated to bathing and recreational activities. Science of the Total Environment, 598, pp.867-880.

Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology, 156(3), pp.609-643.

Gaget, V., Lau, M., Sendall, B., Froscio, S. and Humpage, A.R., 2017. Cyanotoxins: Which detection technique for an optimum risk assessment? Water research, 118, pp.227-238.

Gall, J.E., Boyd, R.S. and Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a review. Environmental monitoring and assessment, 187(4), pp.1-21.

Gaysina, L.A., Saraf, A. and Singh, P., 2019. Cyanobacteria in diverse habitats. In Cyanobacteria (pp. 1-28). Academic Press.

Giller, K.E., Witter, E. and McGrath, S.P., 2009. Heavy metals and soil microbes. Soil Biology and Biochemistry, 41(10), pp.2031-2037.

Gorde, S.P. and Jadhav, M.V., 2013. Assessment of water quality parameters: a review. J Eng Res Appl, 3(6), pp.2029-2035.

Gordon, A.K., 2011. Assessing the effect of a laundry detergent ingredient (LAS) on organisms of a rural South African river (Doctoral dissertation, Rhodes University).

Gu, P., Li, Q., Zhang, H., Luo, X., Zhang, W., Zheng, Z. and Luo, X., 2020. Effects of Cyanobacteria on Phosphorus Cycling and Other Aquatic Organisms in Simulated Eutrophic Ecosystems. Water, 12(8), p.2265.

Guo, X., Zhang, S. and Shan, X.Q., 2008. Adsorption of metal ions on lignin. Journal of hazardous materials, 151(1), pp.134-142.

Harding, W.R. and Paxton, B.R., 2001. Cyanobacteria in South Africa: a review. Pretoria: Water Research Commission.

Havens K.E., 2007. Cyanobacteria blooms: effects on aquatic ecosystems. In: Hudnell KH (ed). Cyanobacterial Harmful Algal Blooms: State of the Science and Research, vol. 619. Springer: New York, pp 675–732

Howard, M., Nagoda, C., Kudela, R., Hayashi, K., Tatters, A., Caron, D., Busse, L., Brown, J., Sutula, M. and Stein, E., 2017. Microcystin prevalence throughout lentic waterbodies in coastal Southern California. Toxins, 9(7), p.231.

Hu, A., Ju, F., Hou, L., Li, J., Yang, X., Wang, H., Mulla, S.I., Sun, Q., Bürgmann, H. and Yu, C.P., 2017. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. Environmental microbiology, 19(12), pp.4993-5009.

Hu, X., Zhang, R., Ye, J., Wu, X., Zhang, Y. and Wu, C., 2018. Monitoring and research of microcystins and environmental factors in a typical artificial freshwater aquaculture pond. Environmental Science and Pollution Research, 25(6), pp.5921-5933.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M. and Visser, P.M., 2018. Cyanobacterial blooms. Nature Reviews Microbiology, 16(8), pp.471-483.

Izosimova, A., 2005. Modelling the interaction between calcium and nickel in the soil-plant system. Bundesforschungsanstalt für Landwirtschaft (FAL).

Izosimova, A., 2005. Modelling the interaction between calcium and nickel in the soil-plant system. Bundesforschungsanstalt für Landwirtschaft (FAL).

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary toxicology, 7(2), p.60.

Järup, L., 2003. Hazards of heavy metal contamination. British medical bulletin, 68(1), pp.167-182.

Jeppesen, E., Brucet, S., Naselli-Flores, L., Papastergiadou, E., Stefanidis, K., Noges, T., Noges, P., Attayde, J.L., Zohary, T., Coppens, J. and Bucak, T., 2015. Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity. Hydrobiologia, 750(1), pp.201-227.

Jeppesen, E., Kronvang, B., Meerhoff, M., Søndergaard, M., Hansen, K.M., Andersen, H.E., Lauridsen, T.L., Liboriussen, L., Beklioglu, M., Özen, A. and Olesen, J.E., 2009. Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. Journal of environmental quality, 38(5), pp.1930-1941.

Jia, Y., Chen, W., Zuo, Y., Lin, L. and Song, L., 2018. Heavy metal migration and risk transference associated with cyanobacterial blooms in eutrophic freshwater. Science of the total environment, 613, pp.1324-1330.

Jiang, Z., Gao, Y., Chen, Y., Du, P., Zhu, X., Liao, Y., Liu, X. and Zeng, J., 2019. Spatial heterogeneity of phytoplankton community shaped by a combination of anthropogenic and natural forcings in a long narrow bay in the East China Sea. Estuarine, Coastal and Shelf Science, 217, pp.250-261.

Joehnk, K.D., Huisman, J.E.F., Sharples, J., Sommeijer, B.E.N., Visser, P.M. and Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Global change biology, 14(3), pp.495-512.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z. and Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environmental pollution, 152(3), pp.686-692.

Khan, Z.I., Ugulu, I., Sahira, S., Ahmad, K., Ashfaq, A., Mehmood, N. and Dogan, Y., 2018. Determination of toxic metals in fruits of Abelmoschus esculentus grown in contaminated soils with different irrigation sources by spectroscopic method. International Journal of Environmental Research, 12(4), pp.503-511.

Klitzke, S., Beusch, C. and Fastner, J., 2011. Sorption of the cyanobacterial toxins cylindrospermopsin and anatoxin-a to sediments. Water research, 45(3), pp.1338-1346.

Kohzadi, S., Shahmoradi, B., Ghaderi, E., Loqmani, H. and Maleki, A., 2019. Concentration, source, and potential human health risk of heavy metals in the commonly consumed medicinal plants. Biological trace element research, 187(1), pp.41-50.

Kudela, R.M., 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae, 11, pp.117-125.

Landeck, L., Baden, L.A. and John, S.M., 2020. Detergents. Kanerva's occupational dermatology, pp.1131-1143.

Lane, J.Q., Roddam, C.M., Langlois, G.W. and Kudela, R.M., 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods, 8(11), pp.645-660.

Latip, R.A., Baharin, B.S., Man, Y.C. and Rahman, R.A., 2000. Evaluation of different types of synthetic adsorbents for carotene extraction from crude palm oil. Journal of the American Oil Chemists' Society, 77(12), pp.1277-1282.

Lee, S., Jiang, X., Manubolu, M., Riedl, K., Ludsin, S.A., Martin, J.F., et al. (2017) Fresh produce and their soils accumulate cyanotoxins from irrigation water: implications for public health and food security. Food Research International, 102, 234–245.

Levizou, E., Statiris, G., Papadimitriou, T., Laspidou, C.S. and Kormas, K.A., 2017. Lettuce facing microcystins-rich irrigation water at different developmental stages: Effects on plant performance and microcystins bioaccumulation. Ecotoxicology and environmental safety, 143, pp.193-200.

Li, A., Ma, F., Song, X. and Yu, R., 2011. Dynamic adsorption of diarrhetic shellfish poisoning (DSP) toxins in passive sampling relates to pore size distribution of aromatic adsorbent. Journal of Chromatography A, 1218(11), pp.1437-1442.

Li, H.F., Gray, C., Mico, C., Zhao, F.J. and McGrath, S.P., 2009. Phytotoxicity and bioavailability of cobalt to plants in a range of soils. Chemosphere, 75(7), pp.979-986.

Liere, L.V. and Walsby, A.E., 1982. Interactions of cyanoibacteria with light. Botanical monographs.

Lim, J.H., and C.W. Lee. 2017. Effects of eutrophication on diatom abundance, biovolume and diversity in tropical coastal waters. Environmental Monitoring and Assessment 189: 432–442.

Lukhwareni, R. and van Dyk, C., 2018. Histology and ultrastructure of hepatic nodular alterations in Clarias gariepinus (Burchell, 1822). Journal of fish diseases, 41(12), pp.1859-1870.

Machado, J., Campos, A., Vasconcelos, V. and Freitas, M. (2017) Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: a review of their relevance for agricultural plant quality and public health. Environmental Research, 153, 191– 204.

Mackenzie, L., Beuzenberg, V., Holland, P., McNabb, P. and Selwood, A., 2004. Solid phase adsorption toxin tracking (SPATT): a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves. Toxicon, 44(8), pp.901-918.

MacKenzie, L.A., 2010. In situ passive solid-phase adsorption of micro-algal biotoxins as a monitoring tool. Current Opinion in Biotechnology, 21(3), pp.326-331.

Mader, M., Schmidt, C., van Geldern, R. and Barth, J.A., 2017. Dissolved oxygen in water and its stable isotope effects: A review. Chemical Geology, 473, pp.10-21.

Malan, M., Müller, F., Cyster, L., Raitt, L. and Aalbers, J., 2015. Heavy metals in the irrigation water, soils and vegetables in the Philippi horticultural area in the Western Cape Province of South Africa. Environmental monitoring and assessment, 187(1), p.4085.

Maleki, M., Ghorbanpour, M. and Kariman, K., 2017. Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. Plant Gene, 11, pp.247-254.

Manjare, S.A., Vhanalakar, S.A. and Muley, D.V., 2010. Analysis of water quality using physicochemical parameters Tamdalge tank in Kolhapur district, Maharashtra. International Journal of Advanced Biotechnology and Research, 1(2), pp.115-119.

Marcon, A.E., de Morais Ferreira, D., de Moura, M.D.F.V., da Costa Campos, T.F., do Amaral, V.S., Agnez-Lima, L.F. and de Medeiros, S.R.B., 2010. Genotoxic analysis in aquatic environment under influence of cyanobacteria, metal and radioactivity. Chemosphere, 81(6), pp.773-780.

Marschner, P., 2012. Rhizosphere biology. In Marschner's mineral nutrition of higher plants (pp. 369-388). Academic Press.

Mashile, G.P. and Nomngongo, P.N., 2017. Recent application of solid phase-based techniques for extraction and preconcentration of cyanotoxins in environmental matrices. Critical reviews in analytical chemistry, 47(2), pp.119-126.

McElhiney, J., Lawton, L.A. and Leifert, C., 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. Toxicon, 39(9), pp.1411-1420.

Medeiros, P.M., Seidel, M., Ward, N.D., Carpenter, E.J., Gomes, H.R., Niggemann, J., Krusche, A.V., Richey, J.E., Yager, P.L. and Dittmar, T., 2015. Fate of the Amazon River dissolved organic matter in the tropical Atlantic Ocean. Global Biogeochemical Cycles, 29(5), pp.677-690.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E. and Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environment international, 59, pp.303-327.

Miller, A. and Russell, C., 2017. Food crops irrigated with cyanobacteria-contaminated water: an emerging public health issue in Canada. *Environmental Health Review*, 60(3), pp.58-63.

Mur, R., Skulberg, O.M. and Utkilen, H., 1999. Cyanobacteria in the Environment.

Musa, J.J., Mustapha, H.I., Bala, J.D., Ibrahim, Y., Akos, M.P., Daniel, E.S., Oguche, F.M. and Kuti, I.A., 2017. Heavy metals in agricultural soils in Nigeria: A review. *Arid Zone Journal of Engineering, Technology and Environment*, 13(5), p.593.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8(3), pp.199-216.

Nagarajan, M., 2014. Effect of chromium on growth, biochemicals and nutrient accumulation of paddy (Oryza sativa L.). International letters of natural sciences, 18.

Najeeb, U., Ahmad, W., Zia, M.H., Zaffar, M. and Zhou, W., 2017. Enhancing the lead phytostabilization in wetland plant Juncus effusus L. through somaclonal manipulation and EDTA enrichment. *Arabian Journal of Chemistry*, 10, pp.S3310-S3317.

Ndlela, L.L., Oberholster, P.J., Van Wyk, J.H. and Cheng, P.H., 2016. An overview of cyanobacterial bloom occurrences and research in Africa over the last decade. Harmful Algae, 60, pp.11-26.

Nematshahi, N., Lahouti, M. and Ganjeali, A., 2012. Accumulation of chromium and its effect on growth of (Allium cepa cv. Hybrid). *European Journal of Experimental Biology*, 2(4), pp.969-974.

Nomura, Y., Ikebukuro, K., Yokoyama, K., Takeuchi, T., Arikawa, Y., Ohno, S. and Karube, I., 1998. Application of a linear alkylbenzene sulfonate biosensor to river water monitoring. Biosensors and Bioelectronics, 13(9), pp.1047-1053.

O'Neil, J.M., Davis, T.W., Burford, M.A. and Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful algae, 14, pp.313-334.

Oberholster, P.J. and Ashton, P.J., 2008. State of the nation report: An overview of the current status of water quality and eutrophication in South African rivers and reservoirs. Parliamentary Grant Deliverable. Pretoria: Council for Scientific and Industrial Research (CSIR).

Oberholster, P.J., Botha, A.M. and Cloete, T.E., 2005. An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact and detection by molecular marker tools. Biokemistri, 17(2), pp.57-71.

Odiyo, J.O., Chimuka, L., Mamali, M.A. and Fatoki, O.S., 2012. Trophic status of Vondo and Albasini Dams; impacts on aquatic ecosystems and drinking water. *International Journal of Environmental Science and Technology*, 9(2), pp.203-218.

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T. and Mavumengwana, V., 2020. Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. *International Journal of Environmental Research and Public Health*, 17(7), p.2204.

Oliver, S., Corburn, J. and Ribeiro, H., 2019. Challenges regarding water quality of eutrophic reservoirs in urban landscapes: a mapping literature review. *International Journal of Environmental Research and Public Health*, 16(1), p.40.

Ololo, G., 2013. A limnological study of factors affecting algal biodiversity in the Hartbeespoort Dam (Doctoral dissertation, University of Johannesburg).

Owuor, K., Okonkwo, J., Van Ginkel, C. and Scott, W., 2007. Environmental Factors Affecting the Persistence of Toxic Phytoplankton in the Hartebeespoort Dam (Doctoral dissertation, Tshwane University of Technology).

Paerl, H.W. and Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental microbiology reports, 1(1), pp.27-37.

Paerl, H.W. and Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. *Water research*, 46(5), pp.1349-1363.

Petrou, M., Karas, P.A., Vasileiadis, S., Zafiriadis, I., Papadimitriou, T., Levizou, E., Kormas, K. and Karpouzas, D.G., 2020. Irrigation of radish (Raphanus sativus L.) with microcystin-enriched water holds low risk for plants and their associated rhizopheric and epiphytic microbiome. Environmental Pollution, 266, p.115208.

Pindihama, G.K. and Gitari, M.W., (2019). Cyanobacterial toxins: an emerging threat in South African irrigation water. Water and Environment Journal.

Prieto, A., Campos, A., Cameán, A. and Vasconcelos, V., 2011. Effects on growth and oxidative stress status of rice plants (Oryza sativa) exposed to two extracts of toxin-producing cyanobacteria (Aphanizomenon ovalisporum and Microcystis aeruginosa). Ecotoxicology and environmental safety, 74(7), pp.1973-1980.

Purkayastha, J., Gogoi, H.K. and S ingh, L. (2010) Plant-cyanobacteria interaction: phytotoxicity of cyanotoxins. *Journal of Phytology*, 2, 7– 15.

Qin, B., Gao, G., Zhu, G., Zhang, Y., Song, Y., Tang, X., Xu, H. and Deng, J., 2013. Lake eutrophication and its ecosystem response. *Chinese science Bulletin*, 58(9), pp. 961-970.

Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H.W. and Carmichael, W.W., 2010. A drinking water crisis in Lake Taihu, China: linkage to climatic variability and lake management. Environmental management, 45(1), pp.105-112

Quayle, LM, Dickens, CWS, Graham, M, Simpson, D, Goliger, A, Dickens, JK, Freese, S & Blignaut, J. 2010. Investigation of the Positive and negative onsequences associated with the introduction of zerophosphate detergents into South Africa. WRC Report No. IT 446/10. Water Research Commission, Pretoria, South Africa.

Rahman, H., Sabreen, S., Alam, S. and Kawai, S., 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. Journal of Plant Nutrition, 28(3), pp.393-404.

Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F. and Kim, K.H., 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment international, 125, pp.365-385.

Ramprasad, C. and Philip, L., 2016. Surfactants and personal care products removal in pilot scale horizontal and vertical flow constructed wetlands while treating greywater. *Chemical Engineering Journal*, 284, pp.458-468.

Rao, P. and He, M., 2006. Adsorption of anionic and nonionic surfactant mixtures from synthetic detergents on soils. *Chemosphere*, 63(7), pp.1214-1221.

Rao, P. and He, M., 2006. Adsorption of anionic and nonionic surfactant mixtures from synthetic detergents on soils. *Chemosphere*, 63(7), pp.1214-1221.

Redouane, E.M., El, S.A.Z., El, F.K., Oufdou, K., Oudra, B., Lahrouni, M., Campos, A. and Vasconcelos, V., 2019. Mode of action and fate of microcystins in the complex soil-plant ecosystems. *Chemosphere*, 225, pp.270-281.

References

Robarts, R.D. and Zohary, T., 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, 21(3), pp.391-399.

Romero-Oliva, C.S., Contardo-Jara, V., Block, T. and Pflugmacher, S., 2014. Accumulation of microcystin congeners in different aquatic plants and crops–A case study from lake Amatitlán, Guatemala. *Ecotoxicology and environmental safety*, 102, pp.121-128.

Roué, M., Darius, H. and Chinain, M., 2018. Solid Phase Adsorption Toxin Tracking (SPATT) Technology for the Monitoring of Aquatic Toxins: A Review. Toxins, 10(4), p.167.

Rundberget, T., Gustad, E., Samdal, I.A., Sandvik, M. and Miles, C.O., 2009. A convenient and cost-effective method for monitoring marine algal toxins with passive samplers. Toxicon, 53(5), pp.543-550.

Salt, D.E., Prince, R.C., Pickering, I.J. and Raskin, I., 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant physiology, 109(4), pp.1427-1433.

Sanseverino, I., António, D.C., Loos, R. and Lettieri, T., 2017. Cyanotoxins: methods and approaches for their analysis and detection. Centre, JR, Ed.

Sanseverino, I., Conduto, D., Pozzoli, L., Dobricic, S. and Lettieri, T., 2016. Algal bloom and its economic impact. European Commission, Joint Research Centre Institute for Environment and Sustainability.

Saqrane, S. and Oudra, B., (2009). CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins, 1(2), pp.113-122.

Schindler, D.W., 2012. The dilemma of controlling cultural eutrophication of lakes. Proceedings of the Royal Society B: Biological Sciences, 279(1746), pp.4322-4333.

Schmitt, M., Watanabe, T. and Jansen, S., 2016. The effects of aluminium on plant growth in a temperate and deciduous aluminium accumulating species. AoB Plants, 8.

Song, K., Xenopoulos, M.A., Marsalek, J. and Frost, P.C., 2015. The fingerprints of urban nutrients: dynamics of phosphorus speciation in water flowing through developed landscapes. Biogeochemistry, 125(1), pp.1-10.

Srivastava, S. and Dubey, R.S., 2011. Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. Plant Growth Regulation, 64(1), pp.1-16.

Thajuddin, N. and Subramanian, G., 2005. Cyanobacterial biodiversity and potential applications in biotechnology. Current science, pp.47-57.

Tóth, G., Hermann, T., Da Silva, M.R. and Montanarella, L., 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environment international, 88, pp.299-309.

Türkdoğan, M.K., Kilicel, F., Kara, K., Tuncer, I. and Uygan, I., 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environmental toxicology and pharmacology, 13(3), pp.175-179.

Turrell, E., Stobo, L., Lacaze, J.P., Bresnan, E. and Gowland, D., 2007, June. Development of anearly warning system'for harmful algal blooms using solid-phase adsorption toxin tracking (SPATT). In Oceans 2007-Europe (pp. 1-6). IEEE.

Ullberg, M., 2015. Temporal water quality study of the heavily human-impacted Likangala River, Zomba, Malawi.

Upendar, G., Singh, S., Chakrabarty, J., Ghanta, K.C., Dutta, S. and Dutta, A., 2018. Sequestration of carbon dioxide and production of biomolecules using cyanobacteria. Journal of environmental management, 218, pp.234-244.

Van Ginkel, C.E., 2011. Eutrophication: Present reality and future challenges for South Africa. Water SA, 37(5), pp.693-702.

Van Liere, L. and WALSBY, E., 1982. WITH LIGHT. The biology of cyanobacteria, 19, p.9.

Van Meerssche, E. and Pinckney, J.L., 2019. Nutrient Loading Impacts on Estuarine Phytoplankton Size and Community Composition: Community-Based Indicators of Eutrophication. Estuaries and Coasts, 42(2), pp.504-512.

Vidoca, L.P., de Almeida, E.S., Cardoso, M.F., Otavio, L., Valadares, L.F. and Monteiro, S., 2020. Extraction of carotene from crude hybrid palm oil using polymeric resin. Journal of Food Engineering, 278, p.109944.

Vijay, D., Akhtar, M.K. and Hess, W.R., 2019. Genetic and metabolic advances in the engineering of cyanobacteria. Current opinion in biotechnology, 59, pp.150-156.

Vos, A.T. and Roos, J.C., 2005. Causes and consequences of algal blooms in Loch Logan, an urban impoundment. Water Sa, 31(3), pp.385-392.

Wagenaar, G.M. and Barnhoorn, I.E.J., 2018. Health and chemical burdens of fish species from polluted and hyper-eutrophic freshwater ecosystems in South Africa. African Journal of Aquatic Science, 43(3), pp.271-280.

Wang, C.M., Xie, Z.C., Song, L.R., Xiao, B.D., Li, G.B. and Li, L., 2011. Dianchi Lake macroinvertebrate community succession trends and retrogressive analysis.

Wang, X., Yan, F., Li, Z., Zhang, L., Zhao, S., An, J. and Yu, J., 2007. Synthesis and surface properties of several nonionic–anionic surfactants with straight chain alkyl-benzyl hydrophobic group. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 302(1-3), pp.532-539.

Wang, Z., Xiao, B., Song, L., Wang, C. and Zhang, J. (2012) Responses and toxin bioaccumulation in duckweed (Lemna minor) under microcystin-LR, linear alkybenzene sulfonate.

Wang, Z., Zhang, J., Song, L., Li, E., Wang, X. and Xiao, B., 2015. Effects of linear alkylbenzene sulfonate on the growth and toxin production of Microcystis aeruginosa isolated from Lake Dianchi. Environmental Science and Pollution Research, 22(7), pp.5491-5499.

Whitton, B.A. and Potts, M., 2012. Introduction to the cyanobacteria. In Ecology of Cyanobacteria II (pp. 1-13). Springer, Dordrecht.

Woldetsadik, D., Drechsel, P., Keraita, B., Itanna, F. and Gebrekidan, H., 2017. Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa, Ethiopia. International Journal of Food Contamination, 4(1), pp.1-13.

Wood, S.A., Holland, P.T. and MacKenzie, L., 2011. Development of solid phase adsorption toxin tracking (SPATT) for monitoring anatoxin-a and homoanatoxin-a in river water. Chemosphere, 82(6), pp.888-894.

Wood, S.A., Holland, P.T., Selwood. A.I. & Mackenzie, L.A. (2008). Development of Solid Phase Adsorption Toxin Tracking Technology (SPATT) for Monitoring Anatoxins. Prepared for Hawkes Bay Regional Council. Cawthron Report No. 1528. 13p.

Xiao, X., He, J., Huang, H., Miller, T.R., Christakos, G., Reichwaldt, E.S., Ghadouani, A., Lin, S., Xu, X. and Shi, J., 2017. A novel single-parameter approach for forecasting algal blooms. Water research, 108, pp.222-231.

Xie, L., Xie, P., Li, S., Tang, H. and Liu, H., 2003. The low TN: TP ratio, a cause or a result of Microcystis blooms? Water Research, 37(9), pp.2073-2080.

Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L. and Fu, J., 2016. Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. Frontiers in plant science, 7, p.755.

Xu, H., Paerl, H.W., Qin, B., Zhu, G. and Gaoa, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography, 55(1), pp.420-432.

Xu, H., Paerl, H.W., Qin, B., Zhu, G., Hall, N.S. and Wu, Y., 2015. Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. Environmental science & technology, 49(2), pp.1051-1059.

Xue, Q., Steinman, A.D., Xie, L., Yao, L., Su, X., Cao, Q., Zhao, Y. and Cai, Y., 2020. Seasonal variation and potential risk assessment of microcystins in the sediments of Lake Taihu, China. Environmental Pollution, 259, p.113884.

Yang, Z. and Chu, C., 2011. Towards understanding plant response to heavy metal stress. Abiotic stress in plants–Mechanisms and adaptations, 10, p.24204.

Yu, H., Zhu, L. and Zhou, W., 2007. Enhanced desorption and biodegradation of phenanthrene in soil–water systems with the presence of anionic–nonionic mixed surfactants. Journal of Hazardous Materials, 142(1-2), pp.354-361.

Zendong, Z., Herrenknecht, C., Abadie, E., Brissard, C., Tixier, C., Mondeguer, F., Séchet, V., Amzil, Z. and Hess, P., 2014. Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins. Toxicon, 91, pp.57-68.

Zewde, T.W., Johansen, J.A., Kifle, D., Demissie, T.B., Hansen, J.H. and Tadesse, Z., 2018. Concentrations of microcystins in the muscle and liver tissues of fish species from Koka reservoir, Ethiopia: A potential threat to public health. Toxicon, 153, pp.85-95.

Zhang, Z., Fan, X., Peijnenburg, W.J.G.M., Zhang, M., Sun, L., Zhai, Y., Yu, Q., Wu, J., Lu, T. and Qian, H., 2021. Alteration of dominant cyanobacteria in different bloom periods caused by abiotic factors and species interactions. *Journal of Environmental Sciences*, 99, pp.1-9.

Zhao, H., Qiu, J., Fan, H. and Li, A., 2013. Mechanism and application of solid phase adsorption toxin tracking for monitoring microcystins. *Journal of Chromatography A*, 1300, pp.159-164.

Zhu, J., Ren, X., Liu, H. and Liang, C., 2018. Effect of irrigation with microcystins-contaminated water on growth and fruit quality of Cucumis sativus L. and the health risk. *Agricultural Water Management*, 204, pp.91-99.

Zhu, W., Li, M., Dai, X. and Xiao, M., 2015. Differences in vertical distribution of Microcystis morphospecies composition in a shallow hypertrophic lake (Lake Taihu, China). *Environmental Earth Sciences*, 73(9), pp.5721-5730.28(2).

Zígolo, M.A., Irazusta, V.P. and Rajal, V.B., 2020. Correlation between initial biodegradability determined by docking studies and structure of alkylbenzene sulfonates: A new tool for intelligent design of environmentally friendly anionic surfactants. *Science of The Total Environment*, 728, p.138731.

Chapter 3: Assessment of cyanotoxins, metals and anionic surfactants in irrigation water and agricultural soils

3.1 Abstract

Higher concentration of cyanotoxins, metal species, and anionic surfactants in irrigation water and agricultural soils poses a human health risk through contamination of food crops. This study evaluated the co-existence of cyanotoxins, metal species, and anionic surfactants in irrigation canals and farm dams. Water and soil samples were collected from Roodeplaat and Hartbeespoort dam sites in irrigation canals and cropping fields from June to September 2019 and February 2020 to March 2021. *Microcystins* (MCs), anionic surfactants, and metals concentrations were detected and quantified using ELISA method, Hanna (HI96769) Anionic surfactant portable photometer and inductively coupled plasma mass spectrometry (ICP- MS), respectively. The data was processed using Microsoft excel 2013, Graph Pad Instat 3 and IBM SPSS version 26 statistical packages. MCs levels in irrigation water ranged from 0.00 to 15.57 µg/L. The levels of anionic surfactants in water and agricultural soils ranged from 0.01 to 3.49 mg/L and 1.81 to 5.46 mg/kg, respectively. The metal species in irrigation water decreased in the following order: Al > Mn > Fe > B > Zn > Ni > Cu > Pb > Cr > As and were all below the DWAF guidelines standard set for irrigation water. The mean concentration of metal species in agricultural soils decreased in the following order Fe > Mn > Cr > Ni > Zn > Cu > Pb > As > Hg > Cd. Only Fe (16534.61 - 33285); Cr (111.25 - 723.4); Ni (33.70 - 85.85); Cu (22.11 - 33.95); Pb (4.44 - 23.93); and As (0.80 - 9.70) mg/kg concentrations, were above the DEA, USEPA and FAO/WHO guideline value for agricultural soils. Microcystins (MCs) level were positively correlated with pH ($p = 0.624$ and turbidity ($p = 0.521$); negatively related to TDS ($p = -0.466$) and EC ($p = -0.445$) for all physicochemical parameters. Moreover, mean MCs concentrations significantly varied across the sampling sites ($P < 0.05$), but did not vary across sampling months ($P > 0.05$). The study concludes that there is co-existence of cyanotoxins (MCs), metals and anionic surfactants in irrigation water and agricultural soils. High toxic metals observed in the soil samples prompt the need for further research in other agricultural sites in and around the study area to propose viable solutions to mitigate the sources. Intensive monitoring of microcystins and physicochemical parameters in irrigation water is also recommended.

Keywords: *Cyanotoxins (Microcystins), toxic metals, Anionic surfactants, irrigation water, physicochemical parameters*

3.2 Introduction

The occurrence of cyanobacterial blooms in aquatic ecosystems because of eutrophication threatens water security and has become a challenge worldwide and in South Africa. This leads to increased growth and dominance of cyanobacterial blooms resulting in production of cyanotoxins (toxic metabolites) that threatens aquatic ecosystems, animals, and human health (Matthews *et al.,* 2010; Liu *et al.,* 2019). South Africa (SA) is a water-scarce country; hence water management is of importance (Turton, 2016). Reportedly, eutrophication has been on the rise in the last few decades due to intensification of agricultural and industrial activities as well as the changing global climate which are the major causes to this phenomenon (Meneely and Elliott, 2013; Machado *et al.,* 2017).

Cyanotoxins are responsible for acute and chronic poisoning of animals and humans (Saqrane & Oudra, 2009). The cyanotoxins also alter plant tissues, affecting plant production, and inhibiting plant growth (Purkayastha *et al.,* 2010). Humans are exposed to cyanotoxins indirectly or directly. For example, indirect exposure includes consuming cyanotoxins contaminated plants and fishes. Direct exposure includes using contaminated water for drinking and direct contact through recreational activities like swimming (Wood, 2016).

South African farmers rely on surface water for crop irrigation (Duhain, 2011). However, surface water are reservoirs for multiple pollutants due to anthropogenic activities. In South Africa, cyanobacterial toxins are not the only concern which affects water quality and scarcity, but there are other problems such as elevated levels of salts, water temperature, dissolved oxygen, pH, electrical conductivity (EC), pathogens, sewage spillage, nutrients such as such as phosphates, nitrates and turbidity, metals, and anionic surfactants (DWA, 2014; Edokpayi *et al.,* 2016; Bwapwa, 2018). *Microcystins* (MCs) concentration in water ecosystems on average range from no detection to 100 g/L, or greater. This depends on environmental parameters such as pH, nutrients, and water temperature (Xiang *et al.,* 2019). In South Africa, MCs concentration ranges between 10 000 and 18 000 µg/L in reservoirs and rivers above the 1.0 µg/L World Health Organisation (WHO) recommended level (Turton, 2016). Harmful cyanobacterial toxins such as microcystins are known in Hartbeespoort dam since 1950s (Orberhoster and Botha, 2010; Ballot *et al.,* 2014). Livestock and wild animals' mortality were reported between 1976 to 1986 in Roodeplaat and Hartbeespoort dam shores which was linked to MCs (Downing and Van Ginkel, 2004).

Metal ions, excessive nutrients, pathogens, and anionic surfactants also degrade water quality (DWA, 2014). Several studies have reported significant accumulation of MCs in edible irrigated plant tissues (Corbel *et al.,* 2016; Drobac *et al.,* 2017; Lee *et al.,* 2017). Use of water containing cyanotoxins, metals and anionic surfactants contaminated water for agricultural purpose may represent a substantial source of these pollutants in agricultural soils and food crops. Irrigating food crops with cyanotoxins, anionic surfactants and metal contaminated water, may result in plant tissues accumulating these pollutants which eventually pose human health risks when consumed (Wang *et al.,* 2011; Zhu *et al.,* 2015). In spite of freshwater pollution caused by mining and industrial activities historically, no study investigated the co-existence of cyanotoxins, anionic surfactants, and toxic metals in irrigation water and agricultural soils in Roodeplaat and Hartbeespoort dam sites. The study assessed levels and co-existences of cyanotoxins, anionic surfactants, and toxic metals in irrigation water and agricultural soils. The study further assessed the correlation between physicochemical parameters and cyanotoxins (*Microcystins*) levels.

3.3 Materials and Methods

3.3.1 Introduction

This part of the study investigated the occurrence of cyanotoxins, anionic surfactants and toxic metals in irrigation water and agricultural soils. Field work and laboratory analysis was conducted. The chapter details the procedure and protocols used to achieve the objectives of the study.

3.3.1.1 Study area description and the location of sampling points

The study was conducted in Hartbeespoort (-25.724722 S, 27.850 E) and Roodeplaat (- 25.622 S; 28.373 E) under Crocodile (West) Marico Water Management Area (MWA). The MWA services Gauteng and parts of Northwest province. Hartbeespoort is 35 km west of Pretoria, south of Magaliesberg mountain range valley and north of the Witwatersberg mountain range. Roodeplaat is 20 km northeast of Pretoria and lies at the confluence of the Pienaar's river, Moretela, and Edendale spruit (Conradie and Barnard, 2012). Both dams are situated in a temperate climate. The minimum and maximum surface water temperature of Hartbeespoort and Roodeplaat range from 14.4 to 25.7 ºC and 15.2 to 27.8 ºC, respectively (Mbiza, 2014). The Hartbeespoort and Roodeplaat dam distributes water via a long network of canals to farmlands. Both dams are considered hyper-eutrophic, and warm monomictic impoundment (Van Ginkel, 2005). Higher concentration of phosphates and nitrates present in these dams intensifies eutrophication. The unabated discharge of

treated and untreated effluents from the water care works to water bodies introduces phosphates and nitrates in significant amount. Roodeplaat receives only 2 treated effluents from Baviaanspoort and Zeekoegat water care works. Hartbeespoort receives treated effluent from ten wastewater treatment plants (Cukic and Venter, 2012; Mbiza, 2014). Agricultural, industrial, and mining activities are common human activities taking place around the two dam sites. The purpose of these two water impoundments, includes livestock watering, irrigation, domestic and industrial activities, recreational activities, and fishing. Figure 3.1 shows the study area, and the green legend represents the agricultural site where soil samples were collected.

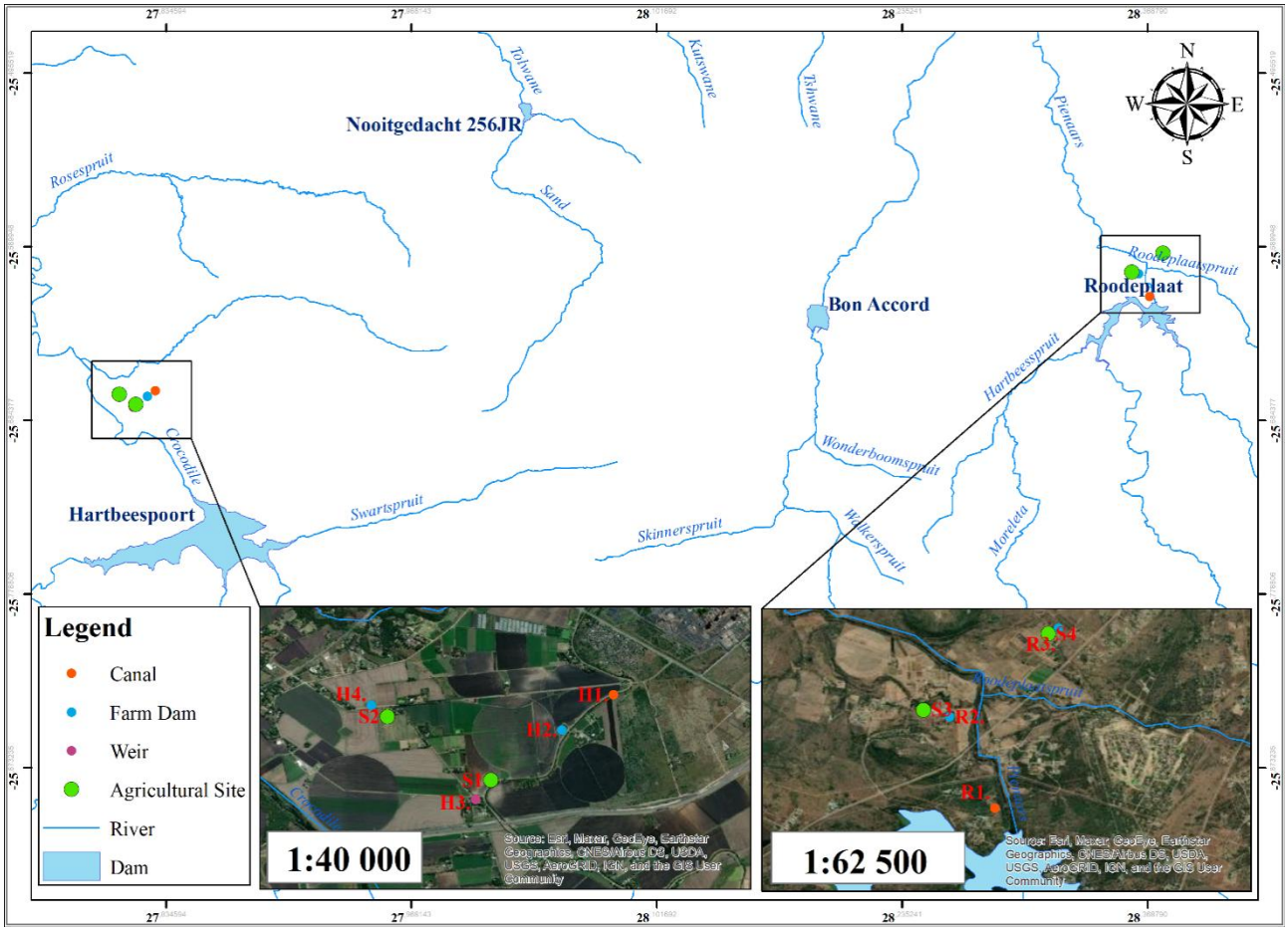


Figure 3.1: Map showing the selected irrigation canals/farm dams and agricultural field sampling sites of Roodeplaat and Hartbeespoort area

3.3.1.2 Sample collection

Water and soil samples were collected in June, September 2019, and February 2020 to March 2021. Water samples were collected from the irrigation canals and soil samples from cropping fields adjacent to the irrigation canals. A total of 4 cropping sites (S1, S2 in Hartbeespoort, and S3 & S4

in Roodeplaat) were selected. While total of 7 sampling sites (H1, H2, H3, H4 in Hartbeespoort), and R1, R2, R3 in for Roodeplaat) were selected for irrigation canals and farm dams. Schott amber bottles, and HDPE bottles were used to collect water samples for analysis of cyanotoxins, anionic surfactants, and metal species. The sampling containers were cleaned in three stages prior to field data collection. Firstly, they were washed with soap and rinsed with deionized water and left to dry off. Secondly, 10 % of hydrochloric acid (HCl) solution was used to wash before rinsing off with deionized water and left to dry. Thirdly, the sampling containers were rinsed again with 50 mL MeOH, then rinsed again with deionised water, and dried in the oven at temperature of 60ºC for 10 minutes.

For anionic surfactants, 30 mL of 40 % (v/v formalin) was added in each schott amber bottle to preserve water samples and prevent biodegradation of anionic surfactants by microorganisms. For total anionic surfactants in soil samples, grab samples from each chosen agricultural site were collected at a depth of 5 cm. The soil samples were then transferred into glass jars and preserved with 10 % formalin. Methanol washed aluminium foil was placed over the mouth of a glass jar and then sealed with a lead to prevent sample contamination. Water samples collected for metals analysis were acidified with 3 drops of nitric acid to prevent precipitation of the metal species due to ingress of carbon dioxide gas as well as microbial growth. For agricultural soil samples, a clean plastic shovel was used to collect soil at depth of 5 cm. The soil samples were transferred into a polyester bag for further analysis of metal species. All samples were collected in duplicates, and immediately after sampling, they were properly labelled and stored in the cooler box with ice for preservation and transported to the laboratory for further analysis.

3.3.1.3 Physical parameters of irrigation water

The physical parameters such as pH, TDS, EC, temperature, turbidity, and DO were measured using Jen-way pH/Cond meter (model 430) at each site. Turbidity was determined using TB200 portable turbidimeter model (#TB200-10), while dissolved oxygen (DO) was determined using the Rugged Dissolved Oxygen electrode (RDO) code: 087003 attached to a Thermo-scientific meter. The instruments were calibrated following the manufacturers' instruction prior to measurements.

3.3.1.4 Chemical analysis

The concentrations of nutrients, nitrates and phosphates were determined using Spectro-quant® Merck Pharo 100 model No: 07531-45 made in EU, and the photometric test kits from (Merck).

Toxic metal species in irrigation water and agricultural soil samples were determined using the inductively coupled plasma mass spectrometry (ICP-MS) (University of Stellenbosch Central Analytical Facility). Prior to analysis, water samples were filtered using 0.20 µm syringe filter and acidified with 3 drops of HNO_3 concentrated acid solution. For quality assurance, samples were analysed in duplicates to ensure accuracy of the analysis.

3.3.1.5 Chlorophyll-*a* analysis

Chlorophyll-*a* was used to estimate cyanobacterial biomass in the irrigation water. Chlorophyll-*a* concentration was determined according to the standard method adapted from Lawton *et al.* (1999). Briefly, 200 mL of water sample was filtered through a Whatman glass fiber filter membranes, 47 mm diameter to separate algal cell from water. The filter membrane with the algal cell was placed inside a 100 mL beaker with 2 mL of 90 % boiling ethanol. The samples were sonicated for 10 minutes to break down the algal cells using ultrasonic cleaner model 705, manufactured in South Africa. The ethanol supernatant was decanted into 50 mL centrifuge tube and then centrifuged for 10 minutes at 3000 rpm. The total chlorophyll-*a* was measured using a spectrophotometer (BGM Labtech, 601-1106, Germany). The difference in absorbance of the extracted chlorophyll-a were determined at 665 and 750 nm wavelength against the 90% ethanol blank. The samples were measured on a Spectrophotometer 665a and 750a wavelength before acidification and corrected from turbidity, and 665b and 750b after acidification and corrected from turbidity. The same samples were then acidified with a drop of 1 mol of hydrochloric acid (HCl) and were determined at same wavelength after 2 minutes. The total chlorophyll-a was determined according to the following formula provided by Lawton *et al.* (1999):

Correction for turbidity: absorbance 665*a* -750*a* = corrected 665*a* absorbance

665*b* – 750*b* = corrected 665*b* absorbance

$$Chlorophyll\text{-}a = \frac{29.62\ (665a - 665b)\ x\ Ve}{Vs\ x\ I}\ mg\ m^{-3}$$

$$Phaeophytin - a = \frac{20.73\ (665b\ x\ Ve)}{Vs\ x\ I}\ mg\ m^{-3}$$

$$Total\ Chlorophyll\text{-}a = Phaeophytin + Chlorophyll\text{-}a \quad (1)$$

Where:

Ve = volume of ethanol extract (mL)

Vs = volume of water sample (Litre)

I = path length of cuvette (cm)

3.3.1.6 Aqua regia digestion of agricultural soils for metal species analysis

Prior to the analysis of agricultural soil for metal species, samples were prepared as follows: soil samples were oven dried at 100 ºC for 48 hours to eliminate moisture and then milled to fine particles passing 250 µm sieve. Thereafter, the samples were digested using the aqua-regia method as described by Gaudino *et al.* (2007). Briefly, 10 g of finely milled soil sample was transferred into 250 mL beaker and 10 mL of deionized water added to hydrate the sample. A volume of 15 mL HNO_3 and 45 ml of HCl were added, then the mixture was placed on a hot plate for digestion at 100 ºC for 1 hour. Thereafter, samples were removed from the hot plate and allowed to cool to room temperature. The cooled residues were then transferred into a 100 mL volumetric flask and the deionized water was added to the 100 mL mark. Mixtures were then shaken vigorously for 1 minute, and then allowed to settle down for 30 minutes. Samples were then filtered through filter paper with 0.45 µm pore size, 0.125 mm diameter. The filtered samples were transferred into a 50 mL centrifuge tube for further analysis of metal species using inductively coupled plasma mass spectrometry (ICP-MS) at central analytical facilities in Stellenbosch University. The concentrations of metals obtained from the ICP-MS analysis were then used to calculate the total concentration of metals species in soil samples using the following equation:

$$\text{PPM} = \frac{C\,X\,V}{W} \qquad (2)$$

Where: C = concentration value from ICP-MS in (mg/L)

V = volume of the solution used in the analysis

W = weight of the soil sample (g)

3.3.1.7 Cyanotoxins (*Microcystins*) quantification

Microcystins levels in irrigation water samples were determined using a commercially available ELISA test kits supplied by Enviro-Logix (Kit Lot: 071499 Cat No: EP 022) and EUROFINS (Kit Lot No: 19I1120:PN 520011) following the manufactures instructions. This assay uses antibodies against *microcystin-LR*. All frozen water samples in amber Schott bottles were taken out of the freezer and were thawed to reach room temperature. Prior to analysis, 5 mL of each thawed sample was filtered using the 0.20 µm syringe filters. The filtered samples, and the antibody solution, enzyme conjugate, substrate solution and stop solution were deposited into the wells of test strips using the multi-channel pipette. After mixing, washing with the wash buffer solution, and incubating the *microcystins* solutions in the wells of the test strips, the micro-plate was placed into the micro-reader, and the absorbance were read within 15 minutes at 450 nm using Spectro-star Nano (BMG LABTECH, 601-1106, Germany). The concentration of microcystins was determined using the standard curves that were established based of the reference material provided with the kits. The samples were analysed in duplicates for quality assurance.

3.3.1.8 Determination of Anionic surfactants in water and soil

3.3.1.8.1 Extraction of anionic surfactants from soils

The overlying water in soil samples was removed through oven drying at 80˚C for 16 hours. The dried soil samples were taken from the oven and 10 g weighed into 50 mL centrifuge tubes. The soil samples were extracted with methanol in a sonication bath for 10 minutes. The samples were extracted 3 times (10 ml and 2 x 40 ml of Methanol) with the soil separated from the extract by means of a centrifuge step for 10 minutes at speed of (30 x 100 g) RPM 3000. The extracted eluent was dried using a nitrogen gas stream and the dried sample was re-suspended with 25 ml phosphate wash buffer to form the final extract for further analysis.

3.3.1.8.2 Quantification of total anionic surfactants from irrigation water and agricultural soils

Total anionic surfactants were determined using a Hanna HI96769 Anionic Surfactants portable photometer using the supplier's instructions and provided reagents. In brief, the water samples to be analysed were treated with chloroform and an excess amount of azure **A** reagent. In the presence of chloroform, the Azure A reacts with anionic surfactants and form a chloroform-soluble, blue-

coloured complex. Such complexes can be designated as azure **A** active substances (AAAS). The intensity of blue colour in the vigorously shaken and subsequently settled chloroform layer is proportional to the concentration of the azure A-surfactants complex. The blue colour of the azure A-surfactants complex can be measured calorimetrically by making spectrophotometric readings in the chloroform. The measurements were made using a Hanna HI96769 Anionic surfactant portable photometer. The total concentration of anionic surfactants in irrigation water and agricultural soils were determined.

3.4 Results and Discussions

3.4.1 Physicochemical water quality parameters

The physicochemical characteristics of the irrigation water samples from the Hartbeespoort and Roodeplaat irrigation canals/farm dams collected during June, September 2019 to February 2020 and March 2021 are summarized in Table 3.1, Appendix-Table A.

3.4.1.1 pH

The pH from selected irrigation canals / farm dam ranged from 6.3 to 10.59. This indicates water condition that is slightly neutral to strongly alkaline. The highest pH value of 10.59 was observed in February month at site R2, while the minimum 6.3 was observed in June month at site H1 (Table 3.1) (Appendix-Table A). The observed pH levels throughout the sampling months revealed that 43 % of the irrigation water samples exceeded the recommended standard guideline for irrigation water of between 6.5 to 8.4 (DWAF, 1996), whereas 57 % were below the threshold. The pH above 8.4 have significant impact on plants' growth, yield, and quality (Hopkinson & Harris, 2019). Edokpayi *et al.* (2014) reveal that alkaline pH influence accumulation of algae blooms in the water columns. In addition, Mbiza (2014) showed that cyanobacteria favour pH between 6 to 9. Anything, above or below this value, significantly decreases the cyanobacteria biomass. Throughout the sampling months, the pH in all sites ranged between 6 and 10. These conditions favour the cyanobacterial growth. The increase in pH from September to February month could be explained by the elevated photosynthetic activities. In these conditions, cyanobacterial bloom enhances the absorption of dissolved inorganic carbon (CO_2), resulting in low carbonic acid and high-water pH (Vos and Roos, 2005; Paerl and Paul, 2012). The elevated pH in aquatic water may result in cyanobacterial dominance in the aquatic ecosystem because of reduced competition among other

algae groups to utilize the dissolved carbon at higher pH (Dokulil and Teubner, 2000; Kozak *et al.,* 2019).

3.4.1.2 TDS and EC

The TDS and EC values ranged from 169.2 to 974.0 mg/L and 285.0 to 1545.0 µS/cm, respectively as shown in (Table 3.1). The highest TDS value of 974.0 mg/L was observed in June at site H4, whereas the lowest TDS value 169.20 mg/L, was observed in February at site R1 (Appendix-Table A). The highest EC value 1545 µS/cm was observed in June at site H4, and the lowest value 285 µS/cm in February at site R1. The observed TDS and EC levels revealed that 61% and 64 % water samples collected from irrigation canals/ farm dams' sites throughout sampling period were above the recommended value for irrigation water (DWAF, 1996). On the contrary, 39 % and 36 % were below the recommended limit. The low TDS and EC observed in September month (Spring) and February month (Summer) might be because of dilution factor from precipitation. The high levels of TDS and EC in June month (Winter) in the irrigation water samples might be because of irrigation runoff from the agricultural lands containing dissolved ionic matters. Thus, irrigation canals receive high concentrations of inorganic salts and minerals such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, calcium, magnesium, and potassium which might degrade the quality and health of the irrigated produce. Also, it may result in soil solidity (DWAF, 1996; Edokpayi *et al.,* 2014). Higher concentrations of EC reduce water available for plant up take even in wet soils (Mezgebe *et al.,* 2015). A pattern of high TDS and EC in June month (Winter), moderate in March month (Autumn), and low in September month (Spring), and February month (Summer) was observed (Appendix-Table A).

3.4.1.3 Temperature

The temperature values of the irrigation water samples ranged from 11.9 °C to 32.8 °C throughout the sampling months (Table 3.1). The highest temperature value of 32.8 °C was recorded in February month at site R2, whereas the lowest 11.9 °C was recorded in June winter month at site H2 (Appendix-Table A). A pattern of low temperature levels in June month, moderate in September and high in February was observed throughout the sampling months, and the variation in temperature could be due to seasonal variations. Studies show that temperature above 20 °C (O'Neil *et al.,* 2012) or 23 °C (Conradie & Barnard, 2012) increases the growth of cyanobacterial blooms and cyanobacteria in the water column species. Similarly, Wang *et al.* (2016) concluded that

temperature is directly proportional to the biomass of the *Microcystis* genus in water bodies. The temperature in two study sites were ideal for the growth of cyanobacterial blooms. There are no guideline standard of temperature for irrigation water.

3.4.1.4 Turbidity

The turbidity levels ranged between 0.77 to 588.9 NTU (Table 3.1). The highest turbidity level was recorded in February at site R2, while the lowest 0.77 NTU was recorded at site R1 in September month (Appendix-Table A). The high level of turbidity in site R2 in February month could be because of the presence of silt, suspended algal, clay, micro-algal, and fine organic matter suspended in the irrigation canals. The presence of silt and clay in all sites in February month could be because of agricultural activities, soil erosion from the cultivated land into the irrigation canals during heavy rainfall since the cropping field are adjacent to the canals. A pattern of high turbidity in February, moderate in March, and low in September was observed throughout the sampling period. There are no guideline standards for turbidity in South Africa for irrigation water use.

3.4.1.5 Dissolved oxygen

The dissolved oxygen of irrigation water samples ranged from 2.4 to 21.1 mg/L (Table 3.1). The highest dissolved oxygen level of 21.1 mg/L was recorded in September at site R3, while the lowest 2.4 mg/L was recorded in February at site H2 (Appendix-Table A). The high dissolved oxygen concentration in R3 might be because of micro-algae photosynthetic activities (Nezlin *et al.,* 2009). The low DO in site H2 in February might be because of high temperature and nitrates resulting in high chlorophyll-a, which indicate the high cyanobacterial biomass, which when dying consumes dissolved oxygen in water column. Vos and Roos (2005) indicated that the low DO in aquatic ecosystem might be because of growth of phytoplankton, when others die the organic matter is produced and become food for bacteria that decomposes it, resulting in depletion of dissolved oxygen in water ecosystems (Appendix- Table A).

Table 3.1: Summary statistics of physical parameters of irrigation water for Roodeplaat and Hartbeespoort irrigation canals between Jun, Sep 2019 to Feb 2020 and Mar 2021

Parameter	Points	R1	R2	R3	H1	H2	H3	H4	DWAF (1996b)
pH	Min	7.5	7.48	8.9	6.3	7.2	6.6	7.8	6.5-8.4
	Max	9.92	10.6	10.6	7.6	8.2	8.2	9.3	
	Mean ± SD	8.5 ±1.1	9.4 ±1.3	9.7±0.9	7.2 ± 0.6	8.0 ± 0.5	7.5 ± 0.7	8.8 ± 0.7	
TDS (mg/L)	Min	169.2	190.0	176.8	230.0	270.0	242.0	265.0	0-260.0
	Max	549.0	540.0	498.0	663.0	604.0	558.0	974.0	
	Mean ± SD	303.05 ±168.7	297.3 ±162.9	280.9±146.5	378 ± 197.5	434.5 ± 167.0	352.3 ± 148.7	453.5 ± 347.2	
EC (µS/cm)	Min	285.0	312.0	293.0	378.0	453.0	395.0	440.0	0-400.0
	Max	918.0	890.0	828.0	990.0	987.0	947.0	1545.0	
	Mean ± SD	506.3 ± 281.7	493.0 ± 267.1	466.8 ± 243.8	601.8 ± 275.1	713.5 ± 218.2	590.8 ± 256.1	732.5 ± 541.9	
TEMP (°C)	Min	15.1	16.0	15.0	14.1	11.9	15.8	16.4	n. a
	Max	23.3	32.8	29.9	23.0	27.3	22.6	29.3	
	Mean ± SD	18.9 ± 4.14	22.95 ± 7.08	21.8 ± 6.2	18.95 ± 4.94	20 ± 6.49	19.73 ± 3.43	21.93 ± 5.51	
Turbidity (NTU)	Min	0.8	3.8	8.3	0.8	12.9	0.9	11.0	n. a
	Max	8.6	588.9	57.6	2.5	75.8	3.0	50.3	
	Mean ± SD	4.6 ± 3.9	245.3 ± 305.6	29.6 ± 25.3	1.6 ± 0.9	37.0± 33.9	1.97 ± 1.47	32.3 ± 19.8	
DO (mg/L)	Min	7.9	8.3	15.5	3.6	2.4	3.0	8.9	
	Max	8.9	16.2	21.1	9.0	14.2	9.5	13.1	
	Mean ± SD	8.4 ± 0.7	12.2 ± 5.6	18.3 ± 3.9	6.3 ± 3.8	8.3 ± 2.0	6.3 ± 4.6	11.0 ± 3.0	

High temperatures observed in February month might be the reason behind the low levels of DO in water column because oxygen is more soluble in cold water than warm water (Horne and Goldman, 1994). According to CENR (2000), the dissolved oxygen less than 2 mg/L in the aquatic system is considered hypoxic, and with the results obtained from all sites the DO levels was above 2 mg/L. A pattern of high DO in September (Spring) and low in February (Summer) was observed at site (R1, R3, H1, H2, & H3), except for site R2 and H4 which had moderate DO in September and increased in February (Appendix-Table A). The reason behind moderate DO in site R2 and H4 in September might be because of low photosynthetic activities in spring, while the high DO observed in February might be because of summer rain, mixing water in the canals which resulted in an increase in dissolved oxygen in the water bodies (Ololo, 2013). There is no standard guideline for DO for irrigation water use.

3.4.2 Water quality chemical parameters

3.4.2.1 Nitrates

The total nitrates concentration shown in Table 3.2 ranged from 0.00 to 28.43 mg/L. The month of February had the highest nitrate value (27.50 mg/L) at site H2. The lower value (0.00 mg/L) was observed at sites R1, R3 and H2 in June sampling month (Appendix- Table A). Less than half of nitrates sampling sites (43%) were above the recommended levels (5 mg/L) for irrigation water (DWAF, 1996; FAO, 1985). The high level of nitrates observed in wet February might be due to fertilizer from irrigation runoff from the cropping sites. Nitrate could also be from plant and dead animal's decay. Shabalala *et al.* (2013) observed that a range of 2.5 to 10 mg/L of nitrate concentration induced eutrophication and resulted in algae and cyanobacterial blooms that favour Microcystis species. In all the sampling months, nitrates concentrations at other sites fell within 2.5 to 10 mg/L range, while other sites had nitrates levels which were above the mentioned range in February 2020. A pattern of low nitrates in June, moderate in September and February, and high in February was observed for all sites. Algae uptake of nitrates in winter for photosynthesis and growth, might explain the low levels of nitrates.

Table 3.2: Summary statistics of nutrients and chlorophyll-a recorded in the Hartbeespoort and Roodeplaat irrigation canals between June & September 2019, to February 2020.

Nutrients		**Sampling sites**						
		R1	**R2**	**R3**	**H1**	**H2**	**H3**	**H4**
Phosphates (mg/L)	Mean ± SD	1.0 ± 0.6	0.6 ± 0.5	0.5 ± 0.3	1.0 ± 0.2	0.4 ± 0.3	0.9 ± 0.2	0.4 ± 0.4
	MAX	1.7	1.5	0.9	1.1	0.7	1.1	0.9
	MIN	0.44	0.2	0.2	0.8	0.1	0.7	0.1
Nitrates (mg/L)	Mean ± SD	3.6 ± 3.1	3.2 ± 2.9	8.9 ± 3.3	5.4 ± 2.3	12.3 ± 10.9	4.3± 1.7	4.0 ± 2.3
	MAX	8.4	7.5	8.7	9.1	28.43	5.9	8.1
	MIN	0.0	0.4	0.0	2.7	0.0	2.1	0.9
Chlorophyll-*a* (µg/L)	Mean ± SD	58.01 ± 70.13	176.13 ± 237.86	250.81 ± 120.79	31.97 ± 39.72	265.47 ± 172.92	19.83 ± 16.23	422.61 ± 575.75
	MAX	208.2	672.27	373.90	115.20	441.41	46.35	1408.9
	MIN	0.00	1.78	109.72	5.92	10.37	0.00	1.48

SD: Standard deviation. (< 5 mg/L) Nitrates (DWAF, 1996); FAO, 1985 (0-2 mg/L) Phosphates', (DWAF, 2002) Chlorophyll-a (0<x<10 Oligotrophic); (10<x<20 mesotrophic); (20<x<30 Eutrophic); (> 30 hypertrophic); R (Roodeplaat samples); H (Hartbeespoort samples)

3.4.2.2 Phosphates

The total phosphates concentrations as shown in Table 3.2 ranged between 0.1 to 1.7 mg/L. September had the highest phosphate value (spring) at site R1, on the other hand, June had the lowest recorded value at site H2 (Appendix- Table A). Results show high, moderate, and low pattern of phosphate in September, June, as well as in February and March. The phosphate levels in all sampling months fell within the recommended FAO (1985) guideline of 0-2 mg/L for irrigation water. Low levels of phosphates in the wet season (February and March) are associated with phytoplankton and bacteria that uses dissolved phosphate for growth and photosynthesis. Moreover, the wet season accelerates phosphate adsorption to sediments (Balcioglu, 2019). At phosphates concentration levels of between 0.025 – 0.25 mg/L, the water body reaches the eutrophic level which supports toxic algae growth or formation. In this study, phosphates levels were above the DWAF (1996) value of 0.025 to 0.25 mg/L in all sites in the sampled months. Thus, sampling sites are described as eutrophic and possibility of toxic algae blooms.

3.4.2.3 Chlorophyll-*a*

Chlorophyll-*a* is used to estimate the algal biomass in water samples (Ramaraj *et al.,* 2013). South Africa like many other countries across the globe, have no regulations or policies on cyanotoxins in water intended for crop irrigation (Pindihama & Gitari, 2019). Chlorophyll-*a* was measured in this study to determine the trophic state and phytoplankton biomass. Chlorophyll-*a* ranged between 0.7 to 402.4 µg/L. The highest chlorophyll-*a* was observed in June at site H2, whereas low chlorophyll-a was observed at site R2 in September (Appendix-Table A). In June, 57% of the sampling sites fell within the hyper-eutrophic state. In September, 29 % of sampling sites fell within the hyper-eutrophic state, while 71 % were classified as oligotrophic. In February, all sites 100 % were within the hyper-trophic state > 30 µg/L. In March (2021), 86 % of sampling sites fell within the hyper-eutrophic state, while 14 % was classified as mesotrophic.

The low chlorophyll-*a* levels in September might be because of low photosynthetic activities in the aquatic ecosystem. Kansas (2011) highlighted that the concentration level of chlorophyll-*a* above 10 µg/L indicates the likelihood, of rapid growth of cyanobacterial blooms in the aquatic ecosystem. The high levels of chlorophyll-*a* in February might be due

to intensive photosynthetic activities. The levels of chlorophyll-*a* in all sampling sites throughout the sampling months were above the Target Water Quality Range (TWQR) value 0 - 1 µg/L for aquatic ecosystem health, except for site R1 in September 2019. High temperature and high concentration of nitrates in February, explains the reason behind the high levels of chlorophyll-*a* because organisms such as algal uptake the nutrients for photosynthesizing and growth resulting in an increase in chlorophyll-*a*. The high levels of Chlorophyll-*a* in February and March could explain high levels of microcystins in all sampling sites. As chlorophyll-*a* increases, so do cyanobacterial blooms and microcystins formation in water column (Appendix- Table A; Figure 3.2). The chlorophyll-*a* levels observed in Hartbeespoort sites (H1, H2, H3, and H4) were way much lower throughout the sampling months compared to the one reported by Ololo (2013) ranging between 0.14 µg/L to 8693µg/L. Thus, a decrease in the level of bloom formation in the dam.

3.4.3 Cyanotoxins and Anionic surfactants in irrigation water

3.4.3.1 Total concentrations of MCs in irrigation water

The total concentration of MCs in irrigation water samples ranged from 0.12 ± 0.00 to 15.57 ± 3.60 µg/L. The highest mean concentration of total MCs was recorded in March (autumn) at site R2, while the lowest was recorded at site H3, and H2 in June (winter) (Appendix-Table A). The decrease in MCs in September in all sampling sites could be because of the high pH which was above the optimal pH 7.5 – 9 for the growth of microcystins. The decrease in MCs production in September could be due to the decrease in chlorophyll-*a* observed in September (Appendix-Table A) which resulted in low cyanobacterial blooms, and low MCs production. The increase in concentration of MCs in February and March could be because of higher concentration of the alkaline pH which is favoured by cyanobacteria to strive and form bloom resulting in release of cyanotoxins in water column. The cyanobacterial blooms are formed rapidly in warmer temperatures than cold ones, and in February and March, the temperature was observed to be above 20°C which is favorable for the growth of *microcystis* resulting in release of *microcystins.*

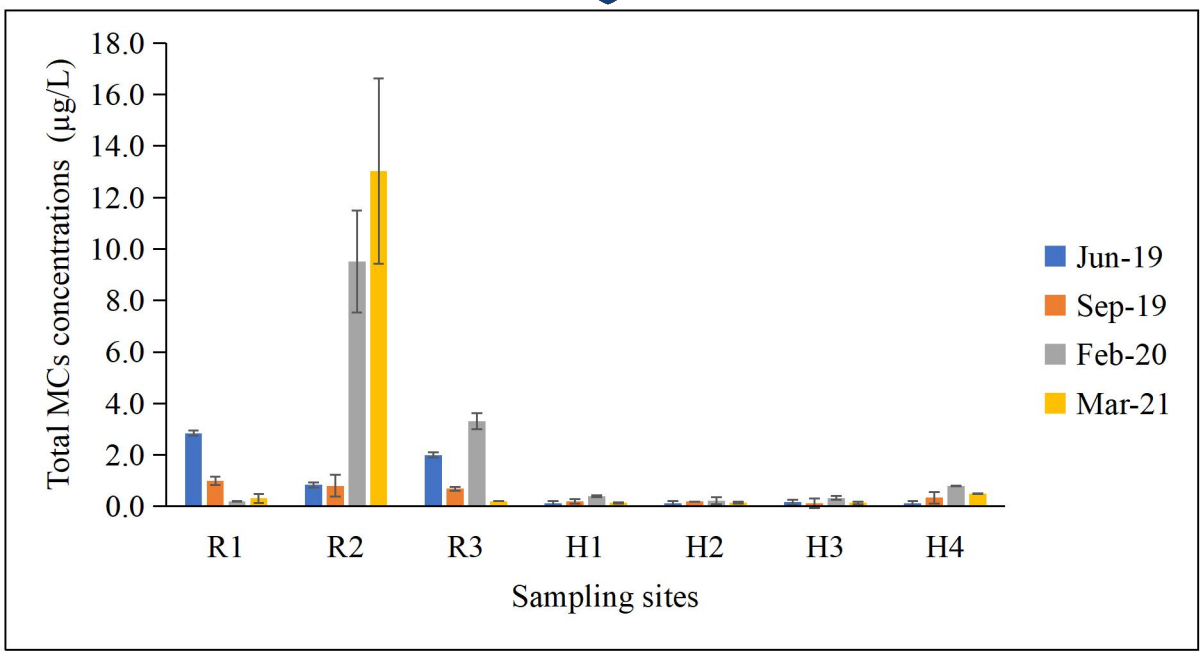


Figure 3.2: Total Concentrations of MCs in irrigation water collected from Hartbeespoort and Roodeplaat irrigation canals

These findings imply a risk to food crops irrigated with water containing *microcystins*. However, there are no guideline standards for cyanobacteria and their toxins in irrigation water. The concentrations of MCs reported from all sites were very low compared to the findings of previous studies (Van Ginkel, 2005; Conradie and Barnard, 2012). Previous studies have shown median concentrations of MCs of 580 µg/L and a maximum level of 14 400 µg/L, with the lowest consistently exceeding 10 µg/L (Van Ginkel, 2004; Turton, 2015). Mbiza (2014) found total MCs level at Roodeplaat dam and Hartbeespoort dam to be as high as 2.5 µg/L in wet season. In the current study, only sites R2 and R3 in the February (2019) and March (2021) sampling month, showed total MCs concentrations above the 2.5 µg/L reported by Mbiza (2014), which concur with the current study where high MCs levels were reported in the summer season. The low total concentration of MCs in Hartbeespoort sites throughout the sampling periods might be because of mechanical removal of algae from the main dam as part of the Hartbeespoort dam "me tsi a me" rehabilitation project by the department of water affairs (Mbiza, 2014; Carroll and Curtis, 2021).

3.4.3.2 Total level of Anionic surfactants in irrigation water

Artificial anionic surfactants are the active ingredients used in producing detergents with linear alkylbenzene sulfonate (LAS) being the primary anionic surfactants used in laundry detergents worldwide (Nomura *et al.,* 1998; Gordon, 2011). Figure 2 shows the total mean concentration of anionic surfactants measured in selected irrigation canals and farm dams from Hartbeespoort and Roodeplaat sites. The mean levels of anionic surfactants ranged from 0.01 ± 0.00 to 3.49 ± 0.00 mg/L. The highest mean level of anionic surfactant was recorded at site H4 3.49 mg/L in March sampling month, while the lowest concentration 0.01 mg/L was observed at site H3 in September month (Appendix-Table A).

The high concentration of anionic surfactants at site H4 in March, might come from domestic wastewater released into the main dams from the tributaries. The low mean concentration of anionic surfactants at site H3 might be due to moderate dissolved oxygen, which degrade the surfactants and low chlorophyll-*a* observed at the site. The low chlorophyll-*a* indicates low cyanobacterial biomass, resulting in moderate DO because organisms do not deplete oxygen in a rapid rate, hence, there are low levels of total anionic surfactants indicating that the site had a good aeration which allowed the organic pollutant to degrade.

Worldwide, there are no regulations governing the anionic surfactants concentrations in domestic wastewater and consequently, river water is polluted by high concentrations of surfactants (Wang *et al.,* 2012). After use, most of surfactants are ultimately discharged into aquatic ecosystems through treated or untreated wastewater. High levels of surfactants in aquatic ecosystems result in bloom of toxic cyanobacteria in the water column. Anionic surfactants are usually eliminated from water column via biodegradation and absorption.

The anionic surfactants degradation occurs very slowly under anoxic and anaerobic conditions; as a result, surfactants end up accumulating in the aquatic ecosystem. Wang *et al.* (2015) reported that the anionic surfactant linear alkylbenzene sulfonate concentration in surface waters normally vary between 0.001 and 20 mg/L, and the total anionic surfactants levels observed from the current study throughout the sampling months were within the range. Even though the surfactants level in all sampling sites seemed to be low. Wang *et al.* (2015) indicated that anionic surfactant linear alkylbenzene sulfonate levels as low as 0.02 to 1.0

mg/L may still cause significant impact in the aquatic ecosystems, such as damaging the cell membrane of organisms, enhancing bio-accumulation of other pollutants such as metals and cyanotoxins.

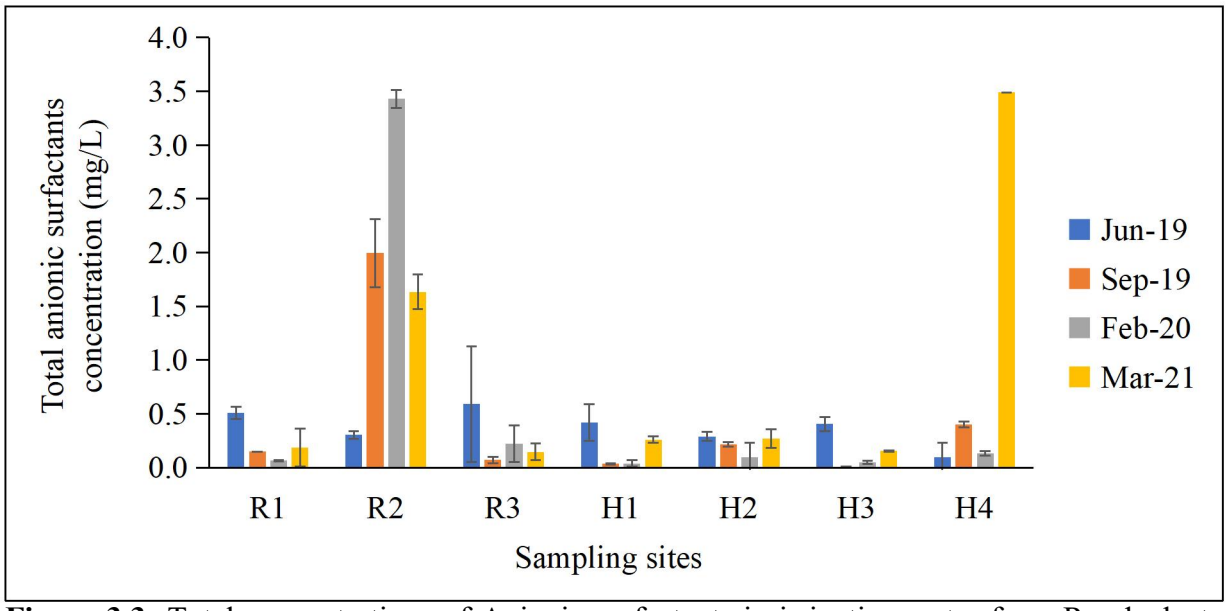


Figure 3.3: Total concentrations of Anionic surfactants in irrigation water from Roodeplaat and Hartbeespoort irrigation canals

3.4.4 Total levels of Anionic surfactants in agricultural soils

The total levels of anionic surfactants in agricultural soils are shown in Figure 3.4. The mean total anionic surfactants concentrations in agricultural soils ranged from 0.91 ± 0.44 to 8.73 ± 0.00 mg/kg. The highest anionic surfactant level 8.73 mg/kg was observed at site S2 in March month, while the lowest mean level 0.91 mg/kg was observed at site S2 in September month (Appendix-Table C). The presence of anionic surfactants in agricultural soils may have entered via irrigating with water infested with anionic surfactants, or application of pesticides. Boluda-Botella *et al.* (2010) found that the anionic surfactant linear alkylbenzene sulfonate sorption capacity levels were high in agricultural soils compared to commercial sand soils which have > 90% of particle size (0.100-0.315 mm). Such soils have high concentration of organic matter and fine particulate materials in agricultural soils, that enhance the sorption of anionic surfactant linear alkylbenzene sulfonate into soil. In this study, surfactants were present in agricultural soils. Like the current study, Ekmekyapar and Celtikil, (2014) found anionic surfactant linear alkylbenzene sulfonate ranging from 5.84 to 19.6 mg/kg in agricultural soils.

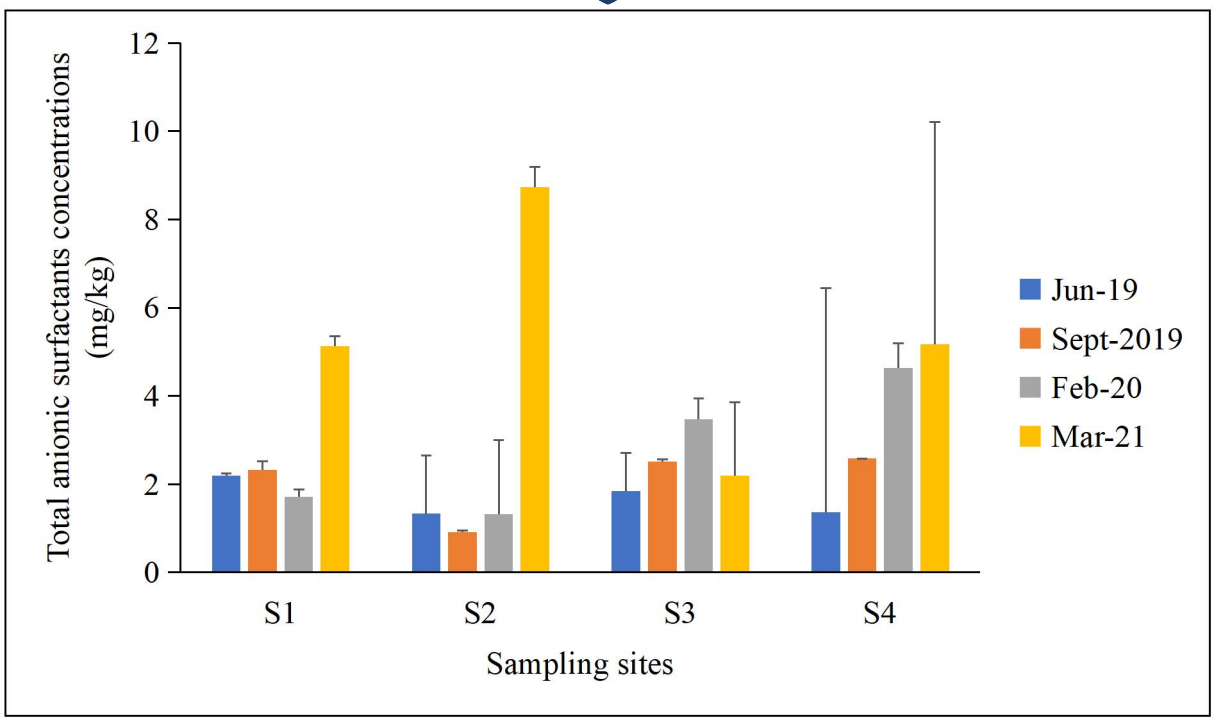


Figure 3.4: Mean levels of anionic surfactants in agricultural soils of Roodeplaat and Hartbeespoort agricultural farmlands

3.4.5 Metal species

3.4.5.1 Metal species in irrigation water

The result of total metal species concentrations in irrigation water are shown in Table 3.3. The mean concentration of boron in irrigation water was ranging between 0.042 to 0.050 mg/L (Table 3.3), with the highest value (0.072 mg/L) observed at site R2 in September. The mean concentration of Al was ranging between 0.108 to 0.581 mg/L, with the highest concentration 1.250 mg/L observed at site H4 in March, while Manganese mean concentration ranged from (0.075 to 0.518 mg/L), with site H3 having the highest level (1.240 mg/L) which was recorded in February in irrigation water. The mean concentration of Cr and Fe ranged from 0.001 to 0.004 mg/L, and 0.068 to 0.359 mg/L respectively. The highest level of Cr 0.010 mg/L was observed at site H3 in June, while highest level of Fe 0.824 mg/L was obtained at site R1 in February.

The mean concentration of Ni in water ranged between (0.004 to 0.007 mg/L), with site H4 having the highest concentration in March and Copper had a mean concentration ranging between 0.002 to 0.006 mg/L, with site H4 having the highest value (0.012 mg/L) in March,

while Zinc mean concentration ranged between 0.042 to 0.143 mg/L, with site R1 having the highest Zn levels 0.245 mg/L in March. Whereas the mean concentration of Pb ranged between 0.001 to 0.017 mg/L, with site H1 having the highest value 0.060 mg/L in June. In addition, mean concentration of arsenic in irrigation water samples were ranging between 0.001 to 0.002 mg/L with site R1 having the highest recorded value (0.006 mg/L) in March.

The results observed from the irrigation water samples showed that there is presence of both non-essential and essential metals in the water samples throughout the sampling period. The mean concentration values of all measured metal species were within the permissible limit prescribed by DWAF, (1996) and FAO (1985) guideline limit set for irrigation water use, except for manganese which exceeded the FAO guideline standard for irrigation water use. A pattern of high levels of metals in June 2019, moderate levels in September 2019, and low levels in February 2020 were observed. The low levels of metals in February in all sampling sites might be due to the dilution effect caused by precipitation to the irrigation water, or the metals have been adsorbed into sediments (Mohiuddin *et al.,* 2012; Islam *et al.,* 2015). However, the only concern is the pH of the irrigation water (as shown Table 3.1 and discussed in section 3.4.1.1 which is strongly alkaline and would make some of the metals to accumulate in the agricultural soils after continuous irrigation over the years. The results observed from this study for metal species in irrigation water implies that the water is safe for irrigation purposes.

A two-tailed spearman correlation coefficient (r^2) was computed between metal elements levels in irrigation water samples. The results showed that there was a positive association between B, Cr, Ni, Zn, and Pb; Al, Ni and Cu; Cr, B, Fe, Cu and Pb; Fe, Cr, Cu, and Pb; Ni, B, Cu, and Al; Cu, Al, Cr, Fe, Ni, As, and Pb; Zn, B, and As, Pb, B, Cr, Fe and Cu in the irrigation water, respectively. The association between these metals in the water indicates that their origin might be from the same source.

Table 3.3: Summary statistics of metal species in irrigation water samples in the Roodeplaat and Hartbeespoort irrigation canals between Jun and Sep 2019, to Feb 2020.

Metal species	H1	H2	H3	H4	R1	R2	R3	FAO	DWAF (1996)
B	0.043±0.006	0.044±0.006	0.042±0.007	0.043±0.006	0.049±0.020	0.050±0.019	0.049±0.017	0.7	0.5-6.0
Al	0.108±0.168	0.332±0.359	0.420±0.592	0.442±0.600	0.581±0.948	0.455±0.479	0.224±0.209	5.0	5-20
Cr	0.001±0.002	0.003±0.003	0.004±0.007	0.001±0.001	0.003±0.005	0.001±0.001	0.002±0.001	n. a	0.1-1.0
Mn	0.492±0.430	0.139±0.053	0.518±0.435	0.173±0.103	0.393±0.233	0.235±0.127	0.075±0.028	0.2	0.02-10
Fe	0.151±0.183	0.068±0.054	0.252±0.443	0.131±0.099	0.359±0.575	0.099±0.756	0.108±0.064	n. a	5-20
Ni	0.004±0.002	0.005±0.001	0.005±0.003	0.007±0.006	0.005±0.002	0.005±0.001	0.004±0.002	0.2	0.2-2.0
Cu	0.003±0.004	0.004±0.003	0.006±0.005	0.006±0.005	0.005±0.006	0.006±0.004	0.002±0.001	0.2	0.2-0.5
Zn	0.076±0.032	0.063±0.035	0.049±0.031	0.048±0.020	0.143±0.095	0.067±0.039	0.042±0.045	2.0	1.0-5.0
As	0.001±0.000	0.002±0.000	0.001±0.000	0.002±0.003	0.002±0.001	0.002±0.001	0.001±0.000	0.1	0.1-2.0
Pb	0.017±0.035	0.001±0.002	0.003±0.005	0.003±0.004	0.002±0.003	0.001±0.002	0.001±0.003	5.0	0.2-2.0

3.4.5.2 Metal species in agricultural soils

The mean concentrations of metal species in agricultural soil are shown in Table 3.4. The mean levels of metal species in agricultural soils ranged from Cr (144.22 to 185.4 mg/kg); Ni (41.46 to 85.85 mg/kg); Cu (22.11 to 33.95 mg/kg); Zn (36.22 to 60.03 mg/kg); As (0.80 to 9.70 mg/kg); Cd (0.04 to 0.06 mg/kg); Pb (4.44 to 23.93 mg/kg); Fe (16534.61 to 28228.92 mg/kg); Mn (496.89 to 1804.58 mg/kg) and Hg (0.09 to 0.10 mg/kg) respectively. The mean concentration of metals in agricultural soils followed the decreasing sequence: Fe > Mn > Cr > Ni > Zn > Cu > Pb > As > Hg > Cd (Table 3.4). From the observed results, the mean concentration of Cr was above the DEA, (2010) and FAO/WHO (Chiroma *et al.,* 2014) guideline standard value set for agricultural soils in all cropping sites.

The mean level of Ni in agricultural soils site S1 and S2 were above the FAO/WHO (Chiroma *et al.,* 2014) guideline standard value for agricultural soils. Cu concentrations in agricultural soils in all sites were above the DEA, (2010) standard value but below FAO/WHO recommended value set for agricultural soil. The mean concentration of As and Pb were above the DEA (2010) recommended guideline value set for agricultural soils at site S4 and S3, while mean concentration of Fe was above the USEPA (Ahmad *et al.,* 2019) recommended value at site S4 and S3 shown in Table 3.4.

Metals such as As, Pb, Cd, and Hg are non-essential and even though they are in low concentrations, they are very toxic, due to non-biodegradability. Moreover, these metals build up in soil, eventually increase metal concentrations that end up being taken up by crops. The high concentration of toxic metals in agricultural soil might be because of agricultural activities which makes the toxic metals to be bio-available, usage of fertilizers and agrochemicals. In a related study, Che Nde and Mathuthu (2018) assessed potentially toxic elements in the upper crocodile catchment area which feeds the Hartbeespoort dam and found high concentration of Cr in the water and sediments which suggest an ecotoxicological risk of anthropogenic origin.

Correlation coefficient (r^2) was calculated between the metals in water-soil system to assess the source of metals in agricultural soil. The metals content in agricultural soils had non-significant correlation with levels of metals in irrigation water. However, a moderate negative

correlation of Manganese (Mn) was observed between metals in soil and water samples. Thus, implying that the levels of metals in soil might be from application of organic and inorganic fertilizers. Also, a two-tailed spearman correlation coefficient (r^2) was determined between metal elements levels in agricultural soil samples. The results showed that there was a positive association between Mn with Fe, Cu, Zn, As, Pb; Cu with Mn, Zn, As, Pb, and Zn with Mn, Cu, As, and Pb. While Fe had a positive association with Al and Mn, and negative relationship Ni. Ni had a negative association with As, Fe and Pb. In addition, As in soil showed to have positive association with Mn, Cu, Zn, Pb, and had negative association with Al and Ni. Also, Pb had a positive relationship with Mn, Cu, As, Zn, and a negative correlation with Al and Ni. These statistical results shows that the metal elements which have a significant association might be originating from the same source, thus a need to investigate their source in soil, since there was no significant relationship between metals in irrigation water and agricultural soils.

Table 3.4: The mean concentrations of metal species in agricultural soils from Roodeplaat and Hartbeespoort cropping sites for Jun, Sep 2019, to Feb 2020 and March 2021

Metal species	S1	S2	S4	S3	Controls	(DEA, 2010)	FAO/WHO (Chiroma et al., 2014)	EU/U SEPA (Ahmad et al., 2019)
Cr	185.4±50.37	149.78±78.08	144.91±63.10	144.22±76.19	250±98.99	6.5	100.0	
Mn	496.89±228.99	812.88±347.59	1804.58±1148.02	1263.41±890.67	625±304.06	n. a	n. a	2000.0
Fe	16534.61±10258.61	17075.82±11338.69	28228.92±14728.17	27139.54±14194.48	51335±17076.6	n. a	n. a	21000.0
Ni	85.31±11.43	85.85±16.95	44.46±21.46	41.46±23.91	76.94±50.81	91.0	50.0	
Cu	22.11±8.79	25.06±8.72	33.95±16.70	32.23±18.43	48.13±16.49	16.0	100.0	
As	0.80±0.33	2.83±3.21	9.43±4.41	9.70±4.31	6.02±3.03	5.8	20.0	
Zn	36.22±7.07	46.64±16.51	49.51±13.97	60.03±22.75	52.40±26.78	240.0	300.0	
Cd	0.044±0.01	0.06±0.02	0.04±0.02	0.04±0.02	0.03±0.01	7.5	3.0	
Hg	0.09±0.12	0.09±0.11	0.10±0.14	0.09±0.12	0.01±0.00	0.93	n. a	
Pb	4.44±3.08	6.41±2.41	23.93±10.62	20.52±13.20	11.23±0.400	20.0	100.0	

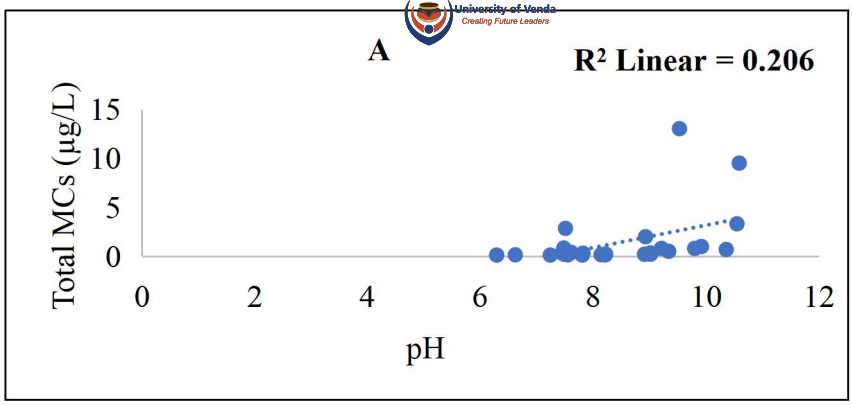
S1 & S2 (Hartbeespoort farmland sites); (S3 & S4 (Roodeplaat Farmland sites)

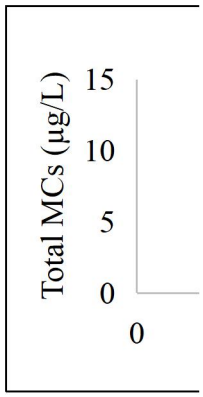
3.4.6 Factors influencing *microcystins* levels and cyanobacterial blooms in the irrigation water

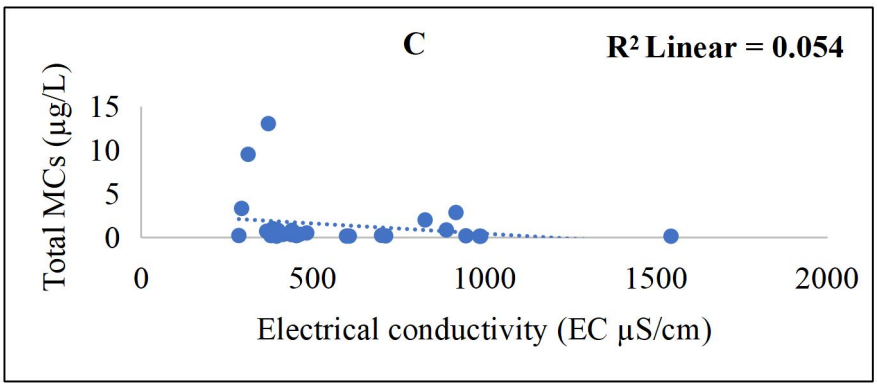
3.4.6.1 Physicochemical parameters influencing *microcystins* levels in irrigation water

The data for physical parameters, nutrients (phosphates and nitrates) and anionic surfactants were monitored in the irrigation water samples to identify which of this parameter influences or better predict the risk of cyanotoxins and cyanobacterial biomass in the irrigation water. A spearman (non-parametric) correlation matrix was done for physicochemical parameters and MCs in the irrigation water and the results are presented in (Table 3.5 and figure 6 (A, B, C). The findings showed that there was a strong positive correlation between *microcystins* and pH, moderate positive correlation between MCs and turbidity and negative correlation coefficient between MCs and TDS and EC, while other physical parameters such as Temperature, and DO did not have a correlation with microcystins. These findings contradicted with findings from Idroos and Manage (2014) which observed that water temperature had a strong positive relationship with *MC-LR* concentrations and pH was a moderate predictor of total *MC-LR* in Beira Lake, in the city of Colombo, Sri Lanka.

The current study showed no correlation between MCs levels and temperature, however, Dai *et al.* (2016) reported that an increase in temperature increased the growth rate of *Microcystis* species. Subbiah *et al.* (2019) found a direct correlation between MCs and anatoxin concentrations with turbidity in a reservoir in the southwest U.S. The significant difference between means of MCs across the sampling sites and sampling period were assessed by analysing the variance, with $P<0.05$ being considered significant. The results for the sampling sites revealed that P value was 0.0001 which shows significant difference between MCs concentrations across the sampling sites ($P<0.05$) (Figure 3.5 A). While the mean of MCs across the sampling period was evaluated and the results showed that P value was 0.2815, which showed that there was no significant difference between mean MCs concentrations across the sampling months ($p>0.05$) (Figure 3.5 B).







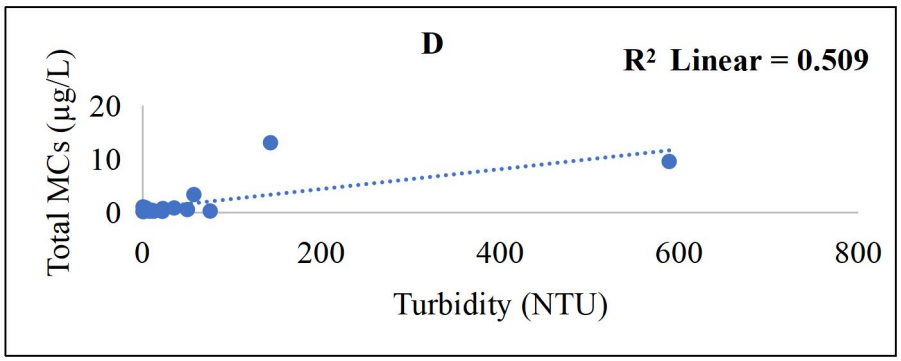


Figure 3.5: (A, B, C, D). Correlation matrix between MCs with pH, TDS, EC, and turbidity.

Table 3.5: Spearman correlation coefficient between *microcystins* levels and physical parameter in irrigation water

Physical parameters	Microcystins (MCs µg/L)
pH	
	0.624**
TDS (mg/L)	- 0.466*
EC (µS/cm)	- 0.445*
Temperature	0.220
Turbidity (NTU)	0.521*
DO (mg/L)	0.326

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at 0.05 level (2-tailed)

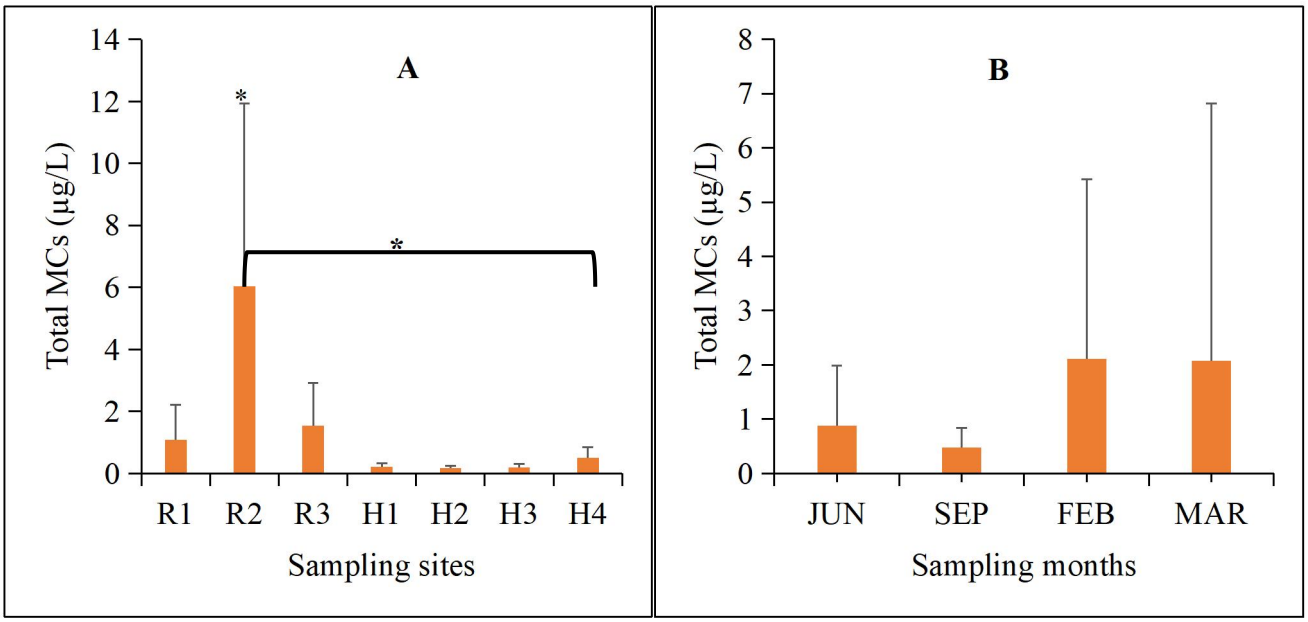


Figure 3.6: (A & B). Graphical representation of *microcystins* (MCs) means across sampling sites ($P < 0.0001$) and sampling periods ($P = 0.2815$). # * indicates statistical significance difference

3.4.6.2 Factors influencing cyanobacterial biomass in irrigation water

The spearman correlation was conducted to test the association between cyanobacterial biomass estimated by determining chlorophyll-*a* with physicochemical parameters. This was to determine which factor better predict biomass of cyanobacteria in the irrigation water (Table 3.6). A strong negative correlation (Inversely proportional relationship) was found between chlorophyll-*a* and dissolved phosphates, and this could be due to phytoplankton using the phosphates for their growth. A strong positive correlation between chlorophyll-*a* and turbidity was observed. Chlorophyll-*a* was not significantly associated with pH, TDS, EC, Temperature, DO and nitrates.

The finding from this current study is consistent to a study by Alcantara *et al.* (2011) which observed chlorophyll-*a* to be positively correlated with turbidity, while other parameters showed low correlation. Bbalali *et al.* (2013) also found a positive correlation between chlorophyll-*a* and nitrates, and no correlation between chlorophyll-*a* and dissolved phosphates (Figure 3.6). In lentic regions, Pan *et al.* (2009) found that total phosphorous was a major factor influencing chlorophyll-*a*. Higher nutrients levels do not necessarily translate into a large phytoplankton biomass under lotic conditions (Pan *et al.,* 2009). This probably explains the strong negative correlation between chlorophyll-*a* and phosphates in this current study since canals and farm dams are lotic in most cases. Also similar to this study, Balcioglu (2019) found an inversely correlation between chlorophyll-*a* and phosphates indicating that phytoplankton used the phosphates. These findings imply that pH is a better physical parameter predictor of MCs levels prevalence compared to other parameters.

Table 3.6: Spearman correlation coefficients between Chlorophyll-*a* levels and physicochemical parameters in irrigation water

	Chlorophyll-*a* (µg/L)
pH	0.227
TDS (mg/L)	0.025
EC (µs/cm)	0.030
Temperature (° C)	0.264
Turbidity (NTU)	0.777**
DO (mg/L)	0.187
Phosphates (mg/L)	-0.718**
Nitrates (mg/L)	0.152

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at 0.05 level (2-tailed)

3.4.6.3 Nutrients and chlorophyll-*a* as predictors of *microcystins* levels in irrigation water

The spearman correlation was conducted to test the association between chlorophyll-*a* and MCs levels, and nutrients with MCs levels, to see which one between nutrients and chlorophyll-*a* can better predict the microcystins levels in irrigation water. High concentrations of nutrients are known to favour the rapid growth of harmful cyanobacteria in the aquatic ecosystems, including MCs producing species *Microcystis* and *Anabaena*, which are very dominant in the two dams (Conradie and Barnard, 2012; Mbiza, 2014). The Spearman correlation showed no relationship between *Microcystins* levels with nitrates, phosphates, and chlorophyll-*a* (Table 4.7).

Table 3.7: Spearman correlation coefficient between MCs levels, nutrients, and chlorophyll-*a* in irrigation water.

	Microcystins (MCs µg/L)
Nitrate (mg/L)	-0.225
Phosphate (mg/L)	- 0.110
Chlorophyll-*a* (µg/L)	0.178

The finding of this study is consistent with Howard *et al.* (2017) who conducted a screening assessment survey of lakes, reservoirs, and coastal lagoons in US in 2013 and found that in depressional wetlands, chlorophyll-*a* was not a significant predictor of MCs concentrations. In addition, Howard *et al.* (2017) found no correlation between total nitrogen and phosphorus with *microcystins* levels. However, finding of this study contradicts with Kudela (2011) who found that chlorophyll-*a* was the best single predictor for toxin loads in Pinto Lake for both grab and SPATT samples. Kim *et al.* (2021) also found that MCs was significantly correlated with chlorophyll-*a* levels ($R^2 = 0.44$, $P < 0.05$).

3.4.6.4 Anionic surfactants concentration as predictor of MCs levels and cyanobacterial biomass in irrigation water

Anionic surfactants have been reported to be more common in eutrophic water bodies. Wang *et al.* (2012 and 2015) reported the co-existence of *microcystins Aeruginosa* and anionic surfactants linear alkylbenzene sulfonate (LAS) under hyper-eutrophic condition. Calculated spearman correlation between total anionic surfactants levels and MCs levels and chlorophyll-*a* are shown in (Table 8 and figure 9). A moderate positive significant correlation was found between MCs levels and total anionic surfactants concentrations in irrigation water, but no correlation with chlorophyll-*a* levels. Wang *et al.* (2015) reported that low LAS (< 10 mg/L) concentration improved the growth of *M. aeruginosa*, and similar levels were observed in this current study (Figure 3.3). These findings imply that anionic surfactants were a better predictor of MCs levels compared to chlorophyll-*a*.

Table 3.8: Spearman correlation coefficients between Anionic surfactants levels, MCs levels and Chlorophyll-*a* in irrigation water

	Anionic surfactants (mg/L)
Chlorophyll-*a* (µg/L)	0.216
Microcystins (MCs µg/L))	0.342*

*Correlation is significant at 0.05 level (2-tailed); **Correlation is significant at 0.01 level (2-tailed)

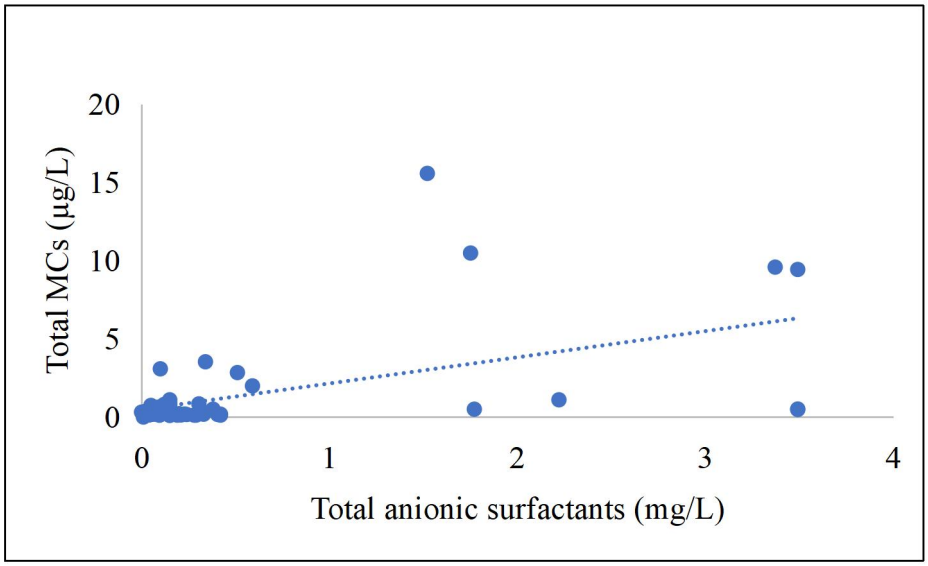


Figure 3.7: Correlation matrix between Anionic surfactants levels, MCs levels (P<0.05)

3.5 Conclusions

The current study concludes that there is a co-existence of cyanotoxins, toxic metals and anionic surfactants in irrigation water and agricultural soil samples of Roodeplaat and Hartbeespoort sites. This is a call for concern because anionic surfactants are known to damage the cell membrane of organisms allowing accumulation of pollutants such as metals and MCs via irrigation into food crops and thus potentially posing human health risks. Among all the physicochemical parameters only pH, TDS, EC, and turbidity had relationship with MCs. Metal species in irrigation water were below the maximum DWAF acceptable limit, implying that the water was safe for irrigation use. Metal species in other soil sampling sites such as Fe, Cr, Pb, As, Cu, and Ni were above the maximum limit set by DEA, USEPA, and FAO/WHO for agricultural use, thus implying that the soil from Roodeplaat and Hartbeespoort farmland sites are contaminated by the mentioned metals. The study recommends further studies to investigate the potential sources of the metals of concern and the potential health risks posed by irrigating food crops with such water in the study area, and constant, frequent, and intensive monitoring of *microcystins* for water meant for irrigating food crops.

References

Ahmad, K., Wajid, K., Khan, Z.I., Ugulu, I., Memoona, H., Sana, M., Nawaz, K., Malik, I.S., Bashir, H. and Sher, M., 2019. Evaluation of potential toxic metals accumulation in wheat irrigated with wastewater. Bulletin of environmental contamination and toxicology, 102(6), pp.822-828.

Alcântara, E., Novo E.M., Barbosa C.F., Bonnet M.P., Stech J., and Ometto J.P., 2011. Environmental factors associated with long-term changes in chlorophyll-a concentration in the Amazon floodplain. Biogeosciences Discussions 8: 3739–3770.

ANZECC., 2000. The Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand. Canberra, Australia. ISBN 0 9578245 2 1.

Balcıoğlu, E.B., 2019. Seasonal changes of LAS, phosphate, and chlorophyll-a concentrations in coastal surface water of the Prince Islands, Marmara Sea. Marine pollution bulletin, 138, pp.230-234.

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J. and Miles, C.O., 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Marine and Freshwater Research, 65(2), pp.175-189.

Bbalali S., Hoseini S.A., Ghorban I.R., and Kordi H., 2013. Relationships between Nutrients and Chlorophyll a Concentration in the International Alma Gol Wetland, Iran. J Aquac Res Development 4(3): 1-5.

Boluda-Botella, N., León, V.M., Cases, V., Gomis, V. and Prats, D., 2010. Fate of linear alkylbenzene sulfonate in agricultural soil columns during inflow of surfactant pulses. Journal of Hydrology, 395(3-4), pp.141-152.

Bwapwa, J.K., 2018. Production of jet fuel from microalgae biomass cultivated in saline domestic wastewater (Doctoral dissertation).

Carroll, A.S.D. and Curtis, C.J., 2021. Increasing nutrient influx trends and remediation options at Hartbeespoort Dam, South Africa: a mass-balance approach. Water SA, 47(2), pp.210-220.

Che Nde S., and Mathuthu M., 2018. Assessment of Potentially Toxic Elements as Non-Point Sources of Contamination in the Upper Crocodile Catchment Area, North-West Province, South-Africa. International Journal of Environmental Research and Public Health 15(576): 1-12.

Chiroma T.M, Ebewele R.O and Hymore K., 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey wastewater used for irrigation in Yola and Kano. Int. Ref. J. Eng. Sci 3: 1–9.

Conradie, K.R. and Barnard, S., 2012. The dynamics of toxic Microcystis strains and microcystin production in two hypertrofic South African reservoirs. Harmful Algae, 20, pp.1-10.

Corbel, S., Mougin, C., Nélieu, S., Delarue, G. and Bouaïcha, N., 2016. Evaluation of the transfer and the accumulation of microcystins in tomato (Solanum lycopersicum cultivar MicroTom) tissues using a cyanobacterial extract containing microcystins and the radiolabeled microcystin-LR (14C-MC-LR). Science of the Total Environment, 541, pp.1052-1058.

Cukic, E.Z. and Venter, P., 2012. Sediment removal and management: Hartbeespoort Dam remediation. Civil Engineering= Siviele Ingenieurswese, 2012(7), pp.42-47.

Dai, R., Wang, P., Jia, P., Zhang, Y., Chu, X. and Wang, Y., 2016. A review on factors affecting microcystins production by algae in aquatic environments. World Journal of Microbiology and Biotechnology, 32(3), p.51.

Dai, R., Wang, P., Jia, P., Zhang, Y., Chu, X. and Wang, Y., 2016. A review on factors affecting microcystins production by algae in aquatic environments. World Journal of Microbiology and Biotechnology, 32(3), p.51.

Department of environmental affairs. 2010. The Framework for the Management of Land, South Africa. [online]. Available at: http://sawic.environment.gov.za/documents/562.pdf [accessed 10 September 2019].

Dokulil, M.T. and Teubner, K., 2000. Cyanobacterial dominance in lakes. Hydrobiologia, 438(1), pp.1-12.

Downing, T.G. and Van Ginkel, C.E., 2004. Cyanobacterial monitoring 1990-2000: Evaluation of SA data. Water Research Commission.

Drobac, D., Tokodi, N., Kiprovski, B., Malenčić, D., Važić, T., Nybom, S., Meriluoto, J. and Svirčev, Z., 2017. Microcystin accumulation and potential effects on antioxidant capacity of leaves and fruits of Capsicum annuum. Journal of toxicology and environmental health, Part A, 80(3), pp.145-154.

Duhain, G.L.M.C., 2011. Occurrence of Cryptosporidium spp. in South African irrigation waters and survival of Cryptosporidium parvum during vegetable processing (Doctoral dissertation, University of Pretoria).

DWA (Department of Water Affairs). 2011. Directorate water resource planning systems: water quality planning. Resource directed management of water quality. Planning Level Review of Water Quality in South Africa. Sub-series No. WQP 2.0. Pretoria, South Africa.

DWA (Department of Water Affairs). 2014. South Africa Yearbook 2013/2014 [online]. Available at:https://www.gcis.gov.za/sites/default/files/docs/resourcecentre/yearbook/20134Water_Affairs.pdf [accessed 30 June 2018].

DWA (Department of Water Affairs). The Republic of South Africa. 2013. National water resource strategy second edition: Water for an equitable and sustainable future. Pretoria: Department of Water Affairs, Republic of South Africa.

DWAF (Department of Water Affairs and Forestry). 1988. Important announcement on implementation of the special phosphate standard in sensitive catchments. IMIESA (Johannesburg), 3 35.

DWAF (Department of Water Affairs and Forestry). 1996. Draft of South African water quality guidelines. Vol. 7. Aquatic Ecosystem. Department of Water Affairs and Forestry Pretoria.

Edokpayi, J., Odiyo, J., Msagati, T. and Popoola, E., 2015. A Novel Approach for the removal of lead (II) ion from wastewater using mucilaginous leaves of diceriocaryum eriocarpum plant. Sustainability, 7(10), pp.14026-14041.

Edokpayi, J.N., Odiyo, J.O. and Olasoji, S.O., 2014. Assessment of heavy metal contamination of Dzindi river, in Limpopo Province, South Africa. Int J Nat Sci Res, 2(10), pp.185-94.

Edokpayi, J.N., Odiyo, J.O., Popoola, O.E. and Msagati, T.A., 2016. Assessment of trace metals contamination of surface water and sediment: a case study of Mvudi River, South Africa. Sustainability, 8(2), p.135.

Ekmekyapar, F. and Celtikli, D.O., 2014. Effects of linear alkylbenzene sulfonate on agricultural soil and its degradation. Fresenius Environmental Bulletin, 23(12 A), pp.3188-3192.

Ekmekyapar, F. and Çeltikli, D.O., 2014. Effects of linear alkylbenzene sulfonate on agricultural soil and its degradation. Fresenius environmental bulletin, 23(12 a), pp.3188-3192.

FAO., 1985. Water Quality for Agriculture. Food and Agriculture Organization, Rome, Italy.

Gaudino S., Galas C., Belli M., Barbizzi S., De Zorzi P., Jaćimović, R., Jeran, Z., Pati., A and Sansone U., 2007. The role of different soil sample digestion methods on trace elements 570 analysis: a comparison of ICP-MS and INAA measurement results. Accreditation Qual. 571 Assur 12: 84-93. https://doi.org/10.1007/s00769-006-0238-1

Gordon, A.K., 2011. Assessing the effect of a laundry detergent ingredient (LAS) on organisms of a rural South African river (Doctoral dissertation, Rhodes University).

Hopkinson, Sampson, and Megan Harris. "Effect of pH on Hydroponically Grown Bush Beans (phaseolus vulgaris)." Int. J. Environ. Agric. Biotechnol. 4, no. 1 (2019): 142-145.

Horne, A.J. and Goldman, C.R., 1994. Limnology (Vol. 2). New York: McGraw-Hill.

Howard M.D.A., Nagoda C., Kudela R.M., KayashI K., Tatters A.O., Caron D.A., Brusse L., Brown J., Sutula M.A., and Stein E.D., 2017. Microcystin prevalence throughout lentic waterbodies in coastal Southern California. Toxins 9(231): 1-21.

Idroos S.F., and Manage P.M., 2014. Seasonal occurrence of Microcystin-LR with respect to physico-chemical aspects of Beira Lake water. International Journal of Multidisciplinary 1(2): 27-37.

Idroos, S.F. and Manage, P.M., 2014. Seasonal occurrence of Microcystin-LR with respect to physico-chemical aspects of Beira Lake water.

Islam, M.S., Ahmed, M.K., Habibullah-Al-Mamun, M. and Hoque, M.F., 2015. Preliminary assessment of heavy metal contamination in surface sediments from a river in Bangladesh. Environmental earth sciences, 73(4), pp.1837-1848.

Kansas department of health and environment (KDHE)., 2011. Water Quality Standards White Paper. Chlorophyll-A Criteria for Public Water Supply Lakes or Reservoirs. Kansas Department of Health and Environment, Bureau of Water. https://www.kdheks.gov/water/download/tech/Chlorophylla_final_Jan27.pdf

Kim, M., Kim, D., Kim, J., Hong, S. and Shin, K.H., 2021. Distribution of microcystins in environmental multimedia and their bioaccumulation characteristics in marine benthic organisms in the Geum River Estuary, South Korea. Science of The Total Environment, 757, p.143815.

Kozak, A., Celewicz-Gołdyn, S. and Kuczyńska-Kippen, N., 2019. Cyanobacteria in small water bodies: The effect of habitat and catchment area conditions. Science of The Total Environment, 646, pp.1578-1587.

Kudela R.M, 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae, 11. 117-125.

Lawton, L., B. Marsalek, J. Padisák, and I. Chorus. 1999. Determination of cyanobacteria in the laboratory, p. 347-367. In I. Chorus and J. Bartram (eds.), Toxic Cyanobacteria in Water. E & FN Spon, London, UK.

Lee, S., Jiang, X., Manubolu, M., Riedl, K., Ludsin, S.A., Martin, J.F. and Lee, J., 2017. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. Food Research International, 102, pp.234-245.

Liu, L., Chen, H., Liu, M., Yang, J.R., Xiao, P., Wilkinson, D.M. and Yang, J., 2019. Response of the eukaryotic plankton community to the cyanobacterial biomass cycle over 6 years in two subtropical reservoirs. The ISME journal, 13(9), pp.2196-2208.

Machado J., Campos A., Vasconcelos V., and Freitas M., 2017. Effects of microcystin-LR and cylindrospermopsins on plant-soil systems: a review of their relevance for agricultural plant quality and public health. Environmental Research, 153: 191–204.

Matthews M.W., Bernard S., and Winter K., 2010. Remote sensing of cyanobacteria-dominant algal blooms and water quality parameters in Zeekoevlei, a small hypertrophic lake, using MERIS. Remote Sensing of Environment 114(9): 2070–2087.

Mbiza, N.X., 2014. Investigation of the effectiveness of techniques deployed in controlling cyanobacterial growth in Rietvlei Dam, Roodeplaat Dam and Hartbeespoort Dam in Crocodile (West) and Marico Water Management Area (Doctoral dissertation).

Meneely J.P., and Elliott C.T., 2013. Microcystins: measuring human exposure and the impact on human health. Biomarkers, 18(8): 639–649.

Mezgebe K.., Gebrekidan A., Hadera A., and Weldegebriel Y., 2015. Assessment of Physico-Chemical Parameters of Tsaeda Agam River in Mekelle City, Tigray, Ethiopia. Bull. Chem. Soc. Ethiop 29(3): 377-385.

Miller A., and Russell C., 2017. Food crops irrigated with cyanobacteria-contaminated water: an emerging public health issue in Canada. Environmental Health Review 60(3): 58-63.

Miller, A. and Russell, C., 2017. Food crops irrigated with cyanobacteria-contaminated water: an emerging public health issue in Canada. Environmental Health Review, 60(3), pp.58-63.

Mohiuddin, K.M., Otomo, K., Ogawa, Y. and Shikazono, N., 2012. Seasonal and spatial distribution of trace elements in the water and sediments of the Tsurumi River in Japan. Environmental monitoring and assessment, 184(1), pp.265-279.

Nezlin, N.P., Kamer, K., Hyde, J. and Stein, E.D., 2009. Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California. Estuarine, Coastal and Shelf Science, 82(1), pp.139-151.

Nomura, Y., Ikebukuro, K., Yokoyama, K., Takeuchi, T., Arikawa, Y., Ohno, S. and Karube, I., 1998. Application of a linear alkylbenzene sulfonate biosensor to river water monitoring. Biosensors and Bioelectronics, 13(9), pp.1047-1053.

Nomura, Y., Ikebukuro, K., Yokoyama, K., Takeuchi, T., Arikawa, Y., Ohno, S. and Karube, I., 1998. Application of a linear alkylbenzene sulfonate biosensor to river water monitoring. Biosensors and Bioelectronics, 13(9), pp.1047-1053.

O'Neil, J.M., Davis, T.W., Burford, M.A. and Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful algae, 14, pp.313-334.

Oberholster P.J., and Botha A.-M., 2014. Importance of water quality to the food industry in South Africa. Understanding the Food-Energy-Water Nexus. WWF-SA, South Africa.

Oberholster PJ, Botha A.M., and Cloete T.E., 2005. An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact, and detection by molecular marker tools. Biokemistri, 17(2): 57-71.

Ololo, G., 2013. A limnological study of factors affecting algal biodiversity in the Hartbeespoort Dam (Doctoral dissertation, University of Johannesburg).

Owuor, K., Okonkwo; J., Van Ginkel, C.E. and Scot. W., 2007. Environmental factors affecting the persistence of toxic phytoplankton in the Hartbeespoort Dam. WRC Report No, 1401/331 of. Water research Commission, Pretoria.

Paerl, H.W. and Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water research, 46(5), pp.1349-1363.

Pan BZ, Wang HJ, Liang XM, and Wang HZ (2009) Factors influencing chlorophyll a concentration in the Yangtze-connected lakes. Fresenius Environmental Bulletin 18: 1894–1990.

Pindihama G.K., and Gitari W.M., 2019. Cyanobacterial toxins: an emerging threat in South African irrigation water. Water and Environment Journal 0: 1–11.

Purkayastha J., Kumar-Gogoi H., and Singh L., 2010. Plant-Cyanobacteria interactions: phytotoxicity of cyanotoxins. Journal of Phytology 2(7): 07–15.

Ramaraj R, Tsai DD-W., and Chen P.H., 2013. Chlorophyll is not accurate measurement for algal biomass. Chiang Mai J. Sci 40: 547-555.

Saqrane S., and Oudra B., 2009. CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins, 1(2), pp.113-122.

Shabalala A.N., Combrinck L., and McCrindle R., 2013. Effect of farming activities on seasonal variation of water quality of Bonsma Dam, KwaZulu-Natal. South African Journal of Science, 109(7-8), pp.01-07.

Subbiah S., Karnjanapiboonwong A., Maul J.D., Wang D., and Anderson T.A., 2019. Monitoring Cyanobacterial Toxins in a Large Reservoir: Relationships with Water Quality Parameters. PeerJ, 7, e7305. https://doi.org/10.7717/peerj.7305

Turton A., 2015. Sitting on the Horns of a Dilemma: Water as a Strategic Resource in South Africa. @ Liberty, Policy Bulletin of the Institute of Race Relations: 1–26 [online]. Available at: https://irr.org.za/reports/atLiberty/files/liberty-2013-sitting-on-the-horns-of-a-dilemma-2013-water-as-a-strategic-resource-in-south-africa [accessed 28 August 2018].

Turton A., 2016. Water Pollution and South Africa's poor. Published by the South African Institute of Race Relations. Johannesburg, South Africa. [Online] Available from: http://irr.org.za/reports-and-publications/occasional-reports/files/water-pollution-and-south-africas-poor [Accessed: 10th May 2017]

Van Ginkel, C.E., 2005. National Assessment Report: National Eutrophication Monitoring Programme 2004.

Vos, A.T. and Roos, J.C., 2005. Causes and consequences of algal blooms in Loch Logan, an urban impoundment. Water Sa, 31(3), pp.385-392.

Wang Z., Xiao B., Song L., Wang and Zhang J., 2012. Responses and toxin bioaccumulation in duckweed (Lemna minor) under microcystin-LR, linear alkylbenzene sulfonate and their joint stress. Journal of Hazardous Materials 229: 137-44.

Wang Z., Zhang J., Song L., LI E., Wang X., and Xiao B., 2015. Effects of linear alkylbenzene sulfonate on the growth and toxin production of Microcystis aeruginosa isolated from Lake Dianchi. Environ Sci Pollute Res 22:5491–5499.

Wang, C.M., Xie, Z.C., Song, L.R., Xiao, B.D., Li, G.B. and Li, L., 2011. Dianchi Lake macroinvertebrate community succession trends and retrogressive analysis.

Wang, X., Liu, W., Xin, C., Zheng, Y., Cheng, Y., Sun, S., Li, R., Zhu, X.G., Dai, S.Y., Rentzepis, P.M. and Yuan, J.S., 2016. Enhanced limonene production in cyanobacteria reveals photosynthesis limitations. Proceedings of the National Academy of Sciences, 113(50), pp.14225-14230.

Wood, R., 2016. Acute animal and human poisonings from cyanotoxin exposure—A review of the literature. Environment international, 91, pp.276-282.

Xiang, L., Li, Y.W., Liu, B.L., Zhao, H.M., Li, H., Cai, Q.Y., Mo, C.H., Wong, M.H. and Li, Q.X., 2019. High ecological and human health risks from microcystins in vegetable fields in southern China. Environment international, 133, p.105142.

Zhu, W., Li, M., Dai, X. and Xiao, M., 2015. Differences in vertical distribution of Microcystis morphospecies composition in a shallow hypertrophic lake (Lake Taihu, China). Environmental earth sciences, 73(9), pp.5721-5730.28(2).

Chapter 4: Bio-accumulation of *microcystins* and metal species in vegetable crops and their potential human health risk

4.1 Abstract

The current chapter assess levels of microcystins and toxic metals in food crops collected from Roodeplaat and Hartbeespoort agricultural field in June, Sep 2019, to Feb 2020 and March 2021 and further evaluates potential health risk associated with consumption of food crops infested with MCs and metal species. *Microcystins* (MCs) and metal species levels in food crops, agricultural soils, irrigation water were determined and quantified using the ELISA method and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), respectively. The *microcystins* (MCs) levels in food crops were found to be as follows; beetroot (0.002 µg/kg); cauliflower (0.006 µg/kg); onion leaves (0.017 µg/kg); onion bulb (0.019 µg/kg); wheat (0.013 µg/kg); soybean (S2) (0.047 µg/kg), and soybean (S3) (0.122 µg/kg), respectively. The estimated daily intake (EDI) of MCs accumulated in edible crops for adults and kids in all plants samples throughout the sampling months were below the 0.04 µg/kg DW established by world health organization (WHO). The levels of metal species ranged from 0.21 to 10.80 mg/kg (Cr); 19.64 to 734.00 mg/kg (Fe); 0.96 to 60.40 mg/kg (Cu); 5.45 to 76.80 mg/kg (Zn); 0.01 to 0.20 mg/kg (As) and 0.10 to 0.70 mg/kg (Pb) in edible, and exceeded the recommended value established by EU and FAO/WHO. The overall metal species accumulation in all collected plant samples followed the decreasing order: Al > Fe > Mn > Zn > Cu > Cr > Ni > Pb > As > Cd. The EDI value of metal species in edible crops due to consumption of the collected plants were below the maximum tolerable daily intake (MTDI). The target hazard quotient (THQ) determined based on each metal accumulated in food crops were less than 1, indicating no health risk via consuming the crops, except for Cu. The hazard index (HI) for crops collected from other sampling sites were > 1, indicating potential health implications via consuming the crops from the indicated sites. The results for target cancer risk (TCR) revealed that Ni and Cr in all crops exceeded the maximum threshold of 0.0001 mg/kg, suggesting that the plants might pose cancer risks to the adult population consuming the crops. The study recommends frequent monitoring of MCs and metal species accumulation in edible crops, to prevent potential health risks to consumers.

Keywords: *Agricultural vegetables, Health risks assessment, Microcystins (MCs), Toxic metals*

4.2 Introduction

The occurrence of cyanobacterial blooms has particularly become a global concern in freshwater ecosystems owing to human induced eutrophication (Cheung *et al.,* 2013; Machado *et al.,* 2017; Lee *et al.,* 2017). Challenges posed by eutrophication have been on the rise in the past few decades because of intensifying agriculture, industrial activities, and global climate change (Meneely and Elliott 2013; El Khalloufi *et al.,* 2016; Machado *et al.,* 2017). Among the impact of eutrophication is the increased growth and dominance of cyanobacterial blooms which produces secondary metabolites (cyanotoxins) which pose a threat to aquatic ecosystems, animals and human health (Matthews *et al.,* 2010; Buratti *et al.,* 2017; Zhang *et al.,* 2021).

Cyanotoxins are responsible for acute and chronic poisoning of human and animals, and they have been classified into 3 groups, namely, Hepatotoxins, Neurotoxins and Cytotoxins (Saqrane and Oudra, 2009; Buratti *et al.,* 2017; Gaget *et al.,* 2017; Huisman *et al.,* 2018). The most common cyanotoxins are *microcystins* (MCs) of which there are almost 200 different congeners identified (Fantanillo and Kohn, 2018; Altaner *et al.,* 2019). Microcystins (MCs) are commonly found in freshwater ecosystem worldwide, and because of their toxicity, in 1999, the World Health Organization (WHO) set a provisional guideline of 1 µg/L for *microcystin* (MC-LR) in drinking water (Meneely and Elliott, 2013; Cao *et al.,* 2018) and tolerable daily intake (TDI) of 0.04 µg/kg MC-LR bodyweight in food (EPA, 2014; Miller & Russell, 2017). The problem of eutrophication and cyanobacteria in South Africa reservoirs are well known and well documented (Mathew *et al.,* 2010; Department of water affairs (DWA, 2011; Turton, 2016). The levels of MCs in the range of 1000 and 18 000 µg/L have been reported in South African reservoirs and rivers (Turton, 2016). These levels are way above 1 µg/L recommended by WHO (1999).

South Africa is a water-scarce country, and most of the farmers rely on surface water for irrigation (DWA, 2013; Duhain, 2011). About 90% of vegetables crops in South Africa rely on irrigation, and agriculture uses about 62% of the countries water reserves (DWA, 2013). Deterioration of surface water quality due to cyanobacterial blooms and other pollutants such as toxic metals is a serious problem in South Africa water impoundments. Several studies have reported the toxic effect of MCs and *cylindrospermopsins* (CYN) on terrestrial plants including food crops (Corbel *et al.,* 2014; Bittencourt-Oliveira *et al.,* 2016; Levizou *et al.,* 2017; Lee *et al.,* 2017; Redouane *et al.,* 2019). Ever since, the use of surface water contaminated with cyanotoxins for agricultural purposes has been receiving a growing attention (Lee *et al.,* 2017). Use of water containing MCs and toxic

metals for irrigation may affect both plant crop yield and quality (Saqrane and Oudra, 2009; Sihlahla *et al.,* 2019). However, cyanotoxins toxicity on plants does not represent the actual environment owing to the interaction between other pollutants such as toxic metals etc (Wang *et al.,* 2012; Wang *et al.,* 2017; Wei *et al.,* 2020). Cao *et al.* (2018) highlighted that cyanotoxins and toxic metals in the natural environment commonly co-exist due to eutrophication.

Toxic metals are metallic elements which have high density of more than 6 g/cm^3 and are very poisonous in small concentration (Nagajyoti *et al*., 2010; Awodele *et al.,* 2013; Kohzadi *et al.,* 2019). The metal species are classified into essential and non-essential. Essential metal species include iron, zinc, copper, cobalt, Nickel, Chromium, and manganese. These are micronutrients which play an essential function in metabolism and physiological activities of humans, plants, and animals, depending on their levels (Marschner, 2012; Rai *et al.,* 2019). Whereas, non-essential metal species include Pb, Hg, Cd and As which have no relevance biological function on plants, animals, and humans, rather they are even poisonous at lower concentrations (Rai *et al.,* 2019; Okereafor *et al.,* 2020). Consequently, irrigating crops with metal species contaminated water may result in crops bio-accumulating the metals, which is a huge concern because of the potential risk to human health (Al-Othman *et al.,* 2016). Few studies have been done on the combined effect of cyanotoxins and metals species on plants (Cao *et al.,* 2018; Jia *et al.,* 2018; Jia *et al.,* 2018; Cao *et al.,* 2020; Wei *et al.,* 2020).

The current study focuses on Roodeplaat and Hartbeespoort dam which are classified as hyper-eutrophic and suffer severe cyanobacterial blooms (Van Ginkel, 2005; Mbiza, 2014). Hartbeespoort dam is known for the occurrence of harmful cyanobacterial blooms since 1950s (Oberholster and Botha, 2010; Ballot *et al.,* 2014). However, cyanotoxins co-exist with other pollutants such as metal species etc, but studies on the co-existence of cyanotoxins and toxic metals on the aquatic ecosystems have not been thoroughly studied, and the combined effect of the pollutants on the terrestrial plants is not clear (Cao *et al.,* 2018; Wu, 2015). Major use of water from these two dams is for irrigation, and thus this might be a pathway of MCs and metal species to accumulate in plants via irrigation, eventually posing health risks to both human and animals. To the best of our knowledge no study has been done in the study area, concerning the combined bio-accumulation of cyanotoxins and metal species on terrestrial plants via irrigation water. Thus, the objective of this investigation is to determine the prevalence of cyanotoxins and metal species translocation and accumulation from irrigation water into food crops and to determine their potential human health risks.

4.3 Materials and Methods

4.3.1 Introduction

The aim of this part of the study was to determine the prevalence of cyanotoxins and toxic metals in food crops, and the potential human health risks. To achieve this objective, field work and laboratory analysis were conducted.

4.3.1.1 Plant material sampling

Vegetable samples were collected in June, Sep 2019, Feb 2020, and March 2021 in Roodeplaat and Hartbeespoort agricultural fields. A total of four (4) agricultural field sites were selected based on prior knowledge that farmers in those sites were using the water from the Hartbeespoort and Roodeplaat dams. The two (2) sites H1 & H4 were for Hartbeespoort site, while R1 & R2 for Roodeplaat cropping sites. Different types of vegetable samples were collected from chosen agricultural field based on their availability. The vegetable samples were collected in duplicates from each sampling site. After collection, the vegetable samples were rinsed off to remove soil residues and particles using field water, and dried using paper towel prior to storage. All samples were then labelled appropriately and stored inside a cooler box with ice and transported to laboratory for cyanotoxins extraction and metal species analysis.

In the laboratory, the plants were taken out from the freezer, thawed, and rinsed off with de-ionized water. The plants were then divided into different plant parts such as (leaves, stem, roots, shoots) etc, using sterile knife. The divided plant samples were then freeze dried for 48 hours. After freeze drying, the plant samples were then crushed into powder form using a blender (Phillips NL9206AD-4 Drachten made in China).

4.3.1.2 Cyanotoxins extraction from plant materials

The powdered plant samples were weighed using a weighing balance, then cyanotoxins (MCs) from the powdered plant samples were extracted using 50% methanol (50 methanol: 50 de-ionised water). The plants samples were extracted three times, first extraction with 10 mL of 50% methanol followed by 2 of 20 mL of 50% methanol, and sonicated for 10 minutes at (30% amplitude, 500 W, 20 KHz) using (Ultrasonic cleaner from Labotec (Model 705) ultrasonic cleaner and lastly centrifuged for 30 minutes at 4000 rpm (3200 x g) at room temperature using (HERMLE

Labortechnik GmbH, SN: 83170010, made Germany) centrifuge. After centrifuge, the supernatant was collected and transferred into 100 mL glass amber bottles. The extraction was repeated three times (10 mL, 20 mL, and 20 mL) and combined in to 100 mL amber bottles. The combined extracts were then purified using solid phase extraction (SPE) clean up method with Oasis HLB 3cc (60 mg) column (Waters Corporation Milford, Massachusetts, USA (Lot NO: 126B35029A, Part NO: WAT094226). The SPE column was conditioned with 6 mL of methanol, followed by 6 mL of ultra-pure water. The extracted samples (50 mL) were then passed through the column slowly and was then rinsed off with 6 mL of 20% methanol. The rinsed column was then eluted with 25 mL of 80% methanol. The final eluant was collected and was evaporated to dryness using water bath and gentle nitrogen gas stream. The dried samples were then stored in the freezer at -4 °C for further analysis.

4.3.1.3 Cyanotoxins analysis from edible plants materials

The dried samples were taken out from the freezer and were re-suspended with 2mL of phosphate wash buffer. Prior to analysis, the extracts were filtered using the 0.20 µm pore syringe filters. The filtered samples, and the antibody solution, enzyme conjugate, substrate solution and stop solution were deposited into the wells of test strips using the multi-channel pipette. After mixing, washing with the wash buffer solution, and incubating the extract solutions in the wells of the test strips for 90 minutes, the microplate was placed into the microreader, and the absorbance was read within 15 minutes at wavelength 450 nm to quantify the total *microcystins* concentrations using Spectro-star Nano (BMG LABTECH, 601-1106, made in Germany). The total concentrations of MCs were determined and quantified using the commercial ELISA test kits supplied by Enviro-Logix (Kit Lot: 071499 Cat No: EP 022) and EUROFINS (Kit Lot No: 19I1120:PN 520011) following the manufacturer's instructions. The ELISA test kit used was indirect competitive using b-amino acid 6E-ADDA as determinant for the quantitative analysis of all MCs analogs and *nodularins*. The total concentrations of MCs were calculated from the standard curve which was established with the values of standard solutions present in the test kit for each test. This assay uses antibodies against *microcystin-LR*. However, limitation of using ELISA for determining *microcystins* in plants samples is that it might create false-positives because of matrix effect derived from the plant materials (Levizou *et al.,* 2017).

4.3.1.4 Estimation of daily intake (EDI)

The levels of MCs in the food crops, were used to calculate the tolerable daily intake and to assess the human health risk. Health Risk Assessment associated with consuming contaminated plants was assessed by calculating the hazard index, which is the ratio between the estimated daily intake (EDI) and chronic tolerable daily intake (TDI) (Cao *et al.,* 2018). EDI was determined by taking 100 g as a representative of realistic meal portion per serving, with the human body weight of 60 kg for adults, and 25 kg for kids. The daily intake of cyanotoxins by humans through consuming contaminated plants was determined using the concentration of cyanotoxins recorded from plants materials in µg/kg, daily food intake and body weight of an individual. The EDI was calculated based on the following formula:

$$\mathbf{EDI} = \frac{C_{toxins} * D_{food\ intake}}{BW_{average}}\ \mu g/kg \qquad (1)$$

Where C is the concentration of cyanotoxins in the food crops in µg/kg, D is the total daily intake of food in kg/ person, and BW is average body weight in kg of humans (60 kg adults, 25 kg children).

4.3.1.5 Digestion of plant samples for metal species

Prior to the analysis of food crops for metal species, samples were prepared as follows: Food crops were freeze dried for 48 hours to eliminate moisture and then crushed into powder form using a food blender to obtain finer particles passing 250 µm sieve. Thereafter, samples were digested using the aqua-regia method. Briefly, 1 g of finely crushed sample was transferred into a 250-mL beaker and 5 mL of deionized water added to hydrate the sample. A 16-mL mixture of 65% nitric acid (HNO_3) and 37 % of hydrochloric acid (HCl) in the ratio (1:3 v/v) was added into the sample (Uddin *et al.,* 2016). The mixture was then placed onto a hot plate for digestion at 100 ºC for 90 minutes until white colored solution was formed. Thereafter, samples were removed from the hot plate and allowed to cool down to room temperature. The cooled supernatant was then transferred into a 100-mL volumetric flask and then deionized water was added to the 100-mL mark. Mixtures were then shaken vigorously for 1 minute, and then allowed to settle down for 30 minutes. Samples were then filtered using filter paper of 0.45 µm pore size, with 0.125 mm diameter. The concentrations of metal species in edible plant samples were determined using Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7900, Santa Clara, California), at central analytical

facilities in Stellenbosch University. The following equation was used to determine concentrations of toxic metals in edible parts of the plants:

$$PPM = \frac{C\,X\,V}{W} \quad (2)$$

Where C = concentration value from the ICP-MS (mg/L)

V = volume of the solution used for dilution after digestion, and

W = weight of the plant part used

For example, Boron (B) in edible part of soybean sample had a concentration value from ICP-MS of 0.70 mg/L, with volume of milli Q water being 100 mL, while mass of powdered edible plant part samples used was 1 g.

$$PPM = \frac{0.70\ x\ 0.1\ L}{0.001\ kg} = 70 \text{ mg/kg}$$

Therefore, there is 70 mg/kg of Boron (B) in the edible part of soybeans

4.3.1.6 Health risk assessment for metal species in vegetable samples

The health risk assessment for toxic metals in food crops were determined by calculating the Estimated daily intake (EDI), target hazard quotient (THQ), and target cancer risk (TCR) to evaluate the health risks posed to humans by consuming food contaminated with metal species. The EDI was determined following the equation adapted from Chen *et al.* (2011) and Gebeyehu and Bayissa (2020).

$$\text{EDI} = \frac{E_F * E_D * F_{IR} * C_M * C_f}{BW * T_A} \text{ x } 0.001 \quad (3)$$

Where E_f is exposure frequency (365 day/year); E_D is the exposure duration (65 years), equivalent to average lifetime (Woldetsadik *et al.,* 2017); F_{IR} is the average food (vegetable) consumption (240 g/person/day), which were obtained from the World Health Report (WHO, 2002); C_M is metal concentration (mg/kg^{-1} dry weight); C_f is concentration conversion factor for fresh vegetable weight to dry weight (0.085), BW is reference body weight of an adult, which is 70 kg (Woldetsadik *et al.,* 2017); T_A is the average exposure time (65 yrs x 365 days) , while 0.001 is a unit conversion factor.

Translocation Factor

Translocation factor (TF) was used to examine the uptake of metal species from soil and their bio-accumulation in plants using the following equation adapted from Chaoua *et al.* (2019):

$$TF = \frac{C_{plant}}{C_{soil}} \quad (4)$$

Where C_{plant} is the concentration of toxic metals in plant sample, while C_{soil} is the total concentration of metals in soils samples (mg/kg). A value > 1.0 indicate that the metal species are being rapidly transported from the roots to the plant tissues.

Target hazard quotient (THQ)

Target hazard quotient was determined to assess non-carcinogenic human health risks from consuming contaminated food crops by metal species. THQ was determined using the following equation adapted from Khan *et al.* (2008); Chen *et al.* (2011); Ezemonye *et al.* (2019) and Gebeyehu and Bayissa (2020).

$$\text{THQ} = \frac{EDI}{RfD} \quad (5)$$

Where EDI is the calculated estimated daily intake of edible plants, RfD is the oral reference dose (mg/kg/day) of metals. If the value of THQ is < 1, it is generally presumed to be safe for the risk of non-carcinogenic, however, when the THQ is > 1, it is supposed that there is a chance of carcinogenic health effects which might be posed over long-term when consuming the contaminated food crops by metal species.

Hazard index (HI)

The hazard index was determined by summing up the Target Hazard Quotient for various metal species using the following equation adapted from Kamunda *et al.,* (2016). If the HI value is > 1.0, it indicates potential health implications, and a serious chronic health implication might occur at HI value greater than > 10.0.

$$HI = \sum THQ \quad (6)$$

Target Cancer Risk (TCR)

The TCR was determined to assess the cancer risks which might be posed to humans by consuming food crops contaminated with metal species. The TCR was calculated following the equation adapted from Kamunda *et al*. (2016); Sharma *et al.* (2018) and Gebeyehu and Bayissa (2020).

$$CR = EDI * CPS_0 \quad (7)$$

$$TCR = \sum_{(n-1)}^{i} CR;\ i = 1,2,3,,,n \quad (8)$$

Where *CR* presents cancer risk over lifetime by ingestion of metal species, and *EDI* is the estimated daily metal intake of food crops in mg/day/kg body weight, CPSo is the oral cancer slope factor in (mg/kg/day $^{-1}$), and n is the number of metal species considered for cancer.

4.4 Results and Discussions

4.4.1 Levels of *microcystins* in edible crops

The results of levels of *microcystins* (MCs) in edible crops are presented in Table 4.1. The results for MCs accumulated in Beetroot, Corn flower, Onion leaves, Onion bulb, Wheat grains, Soybean H3, and Soybean plant H4 were found to be 0.002 µg/kg; 0.006 µg/kg; 0.017 µg/kg; 0.019 µg/kg; 0.013 µg/kg; 0.047 µg/kg and 0.122 µg/kg, respectively (Table 4.1). The high level of MCs accumulation was observed in February 2020, while the lowest was observed in June 2019. A pattern of low MCs accumulation in June, moderate in September and high in February was observed throughout the sampling period. The seasonal variation of MCs bio-accumulation might be because different plant species depend on exposure level of MCs, time, and the congener type that they are exposed to (Bittencourt-Oliveira *et al.,* 2016).

The levels of MCs accumulated in plants throughout the sampling periods were lower compared to the ones reported by Bittencourt-Oliveira *et al.,* (2016). The EDI values observed in food crops throughout the sampling period for adults and kids were below the 0.04 µg/kg standard set by world health organization (WHO, 2011), indicating that the plants do not pose any health risk to adults

and kids consuming the food crops (Table 4.1). The MCs levels, and EDI values observed in all food crops throughout the sampling months in this current study were way much lower than the data reported by Chen *et al.* (2012) and Zhu *et al.* (2018).

Table 4.1: Total Concentration of accumulated *microcystins* (MCs µg/kg) and calculated EDI (0.04 µg/kg) in food crops collected from agricultural fields in Roodeplaat and Hartbeespoort site in June, Sep 2019, and Feb 2020

	Sampling sites	Plant type	MCs (µg/kg)	EDI for Adults (µg/kg)	EDI for Children (µg/kg)
Roodeplaat					
June (2019)	S5	beetroot bulb	0.002	0.000003	0.000008
	S5	cauliflower	0.006	0.000009	0.000024
Sept (2019)	S5	Onion leaves	0.017	0.000026	0.000068
		Onion bulb	0.019	0.000029	0.000076
Hartbeespoort					
Sept (2019)	S3	wheat grains	0.013	0.00002	0.000052
Feb (2020)	S2	Soybean	0.047	0.000072	0.000188
	S3	Soybean	0.122	0.000187	0.000488

4.4.2 Levels of metal species in vegetable, agricultural soils, and irrigation water samples

4.4.2.1 Levels of metals species in vegetable samples

The concentrations of metal species in the edible part of the vegetable samples cultivated around Roodeplaat and Hartbeespoort farmland sites are presented in Table 4.2. The results revealed that the levels of aluminium (Al) ranged from 97.04 to 668.00 mg/kg, respectively. The vegetable

control plant sample collected from Rietvlei site had the highest level of Al compared to other plant samples.

The levels of Cr, Mn, and Fe in vegetable samples ranged from 0.21 to 10.80 mg/kg; 3.30 to 86.00 mg/kg; and 19.64 to 734.00 mg/kg, respectively. For Cr, all vegetable samples from chosen sampling sites had high concentrations above the FAO/WHO, (2007) and EU, (2006) permissible value for food crops, except for soybean plant sample collected at site S3. In addition, Fe was observed to be above the guideline value set by FAO/WHO, (2007) and EU, (2006) for medicinal plant (Serokolo) wild ginger collected from site S4, and soybean from site S1, and a control plant from Rietvlei site.

As can be seen from Table 4.2. Ni, Cu, and Zn concentrations in edible parts of the plant's samples ranged from 0.23 to 6.20 mg/kg; 0.96 to 60.40 mg/kg; and 5.45 to 76.80 mg/kg, respectively. Soybean plant sample collected from site S4 had high level of Zn which was above the FAO/WHO, (2007) and EU (2006) permissible guideline value set for food crops. While, for Cu only soybean from site S5 and control plant from Rietvlei were above the permissible limit guideline for food crops. Also, As, Cd, and Pb concentrations in edible part of the plant samples ranged from 0.01 to 0.20 mg/kg; 0.01 to 0.06 mg/kg; and 0.10 to 0.70 mg/kg, respectively. Lead (Pb) concentration in all plant's samples were above the FAO/WHO, (2007) and EU, (2006) permissible guideline value for food crops. The overall metal species accumulation in all collected plant samples followed the decreasing order: Al > Fe > Mn > Zn > Cu > Cr > Ni > Pb > As > Cd, respectively. Comparing the data obtained from the current study the toxic metals in plant samples such as Zn, Cu, Ni, Cr, Fe and Mn were way much higher than the one reported by Gebeyehu and Bayissa (2020) for levels of metal species accumulated in Tomato and Cabbage crops.

The accumulation of non-essential metals such as Pb, As, Cr, and Cd in all collected food crops calls for concern because these metals are highly toxic even at very low concentrations and might pose human health risks via consumption of the contaminated food crops over the long-term. Gidlow (2004) reported that high level of lead affects several human physiological systems, such as renal, neurological, and immunological functions. The metal species in the agricultural field might have been introduced via application of organic and inorganic fertilizers, and other agrochemical such as pesticides and herbicides.

Table 4.2: Metal concentration (mg/kg) (N = 14) in edible crop collected in Hartbeespoort and Roodeplaat agricultural field in February 2020 and March 2021

	S2	S3	S2	S4	S1	S5	Rietvlei (plant Controls)		
Metals	**Soybean**	**Soybean**	**Soybean**	**Wild ginger**	**Soybean**	**Soybean**		**FAO/WHO, 2007 (a)**	**EU, 2006 (b)**
Al	121.38	97.04	343.00	455.00	645.00	356.5	668.00	n. a	n. a
Cr	1.28[b]	0.21	4.10[b]	3.40[b]	4.70[b]	7.3[b]	10.80[a]	20.0	1.0
Mn	18.62	3.30	43.00	76.00	54.00	50.5	86.00	500	500.0
Fe	50.05	19.64	128.00	486.00[a]	477.00[a]	260.0	734.00[a]	450.0	n. a
Ni	1.74	0.23	5.50	2.00	9.20	3.95	7.80	68.0	n. a
Cu	3.50	0.96	10.50	8.10	13.10	51.7[ab]	60.40[ab]	40.0	20.0
Zn	10.92	5.45	30.90	76.80[ab]	37.90	23.25	37.40	60.0	50.0
As	0.02	0.01	0.10[ab]	0.20[ab]	0.10[ab]	0.1	0.20[ab]	0.5	0.2
Cd	0.03	0.01	0.01	0.06	0.02	0.03	0.01	0.2	0.2
Pb	0.38[a]	0.10[a]	0.20[a]	0.70[ab]	0.20[a]	0.45[ab]	0.70[ab]	0.3	0.43

[a] indicates values above FAO/WHO maximum guideline value for metals in food crops, [b] indicates values above EU, 2006 maximum guideline value for metals in food crops, [ab] indicates values above both FAO/WHO (2007) and EU, (2006) maximum guideline values for metals in food crops

4.4.2.2 Levels of metal species in agricultural soil samples

The levels of metal species in soil samples collected at sampling points where vegetable samples were collected were determined to evaluate the translocation factor of metals from the soil to the edible parts of the vegetable samples. The obtained data of metals in soil samples collected from Roodeplaat and Hartbeespoort farmland sites are presented in Table 4.3. Soil samples collected from all sampling sites had presence of all metal species analysed in this study. The mean concentration of metal species Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd and Pb ranged from 13110 to 42135 mg/kg; 87.75 to 723.40 mg/kg; 17064.53 to 34665 mg/kg; 39.8 to 166.30 mg/kg; 17.27 to 31.45 mg/kg; 29.26 to 86.5 mg/kg; 0.75 to 9.30 mg/kg, 0.04 to 0.06 mg/kg; and 3.45 to 19.5 mg/kg, respectively. The mean levels of As, Cr, Mn, Ni, Cu and Fe were found to be higher than the guideline limit value set by DEA, (2010), FAO/WHO and USEPA for agricultural soils (Ahmad *et al.,* 2019; DEA, 2010; Chiroma *et al.,* 2014). The obtained levels of metals in soil samples clearly indicate that the selected farmland sites agricultural soil is enriched with high levels of the analysed metals.

The concentrations of As, Pb, Cd, Zn, Fe, and Mn obtained from the soil samples in this study were found to be lower than the ones found in cabbage and tomato samples As (24.06 ± 0.05 mg/kg), Pb (35.80 ± 0.17 mg/kg), Cd (4.76 ± 0.15 mg/kg), Zn (93.66 ± 1.92 mg/kg), Fe (41410.00 ± 191.57 mg/kg) and Mn (1696.67 ± 15.27 mg/kg) by Gebeyehu and Bayissa, (2020). In addition, levels of nickel (Ni), copper (Cu) and (Cr) were found to be much higher than the reported one in soil samples were cabbage and tomatoes are grown in cabbage Ni(30.50 ± 0.81 mg/kg), Cu (25.50 ± 0.62 mg/kg), and Cr (35.93 ± 0.30 mg/kg) and tomatoes Ni (35.58 ± 0.56 mg/kg), Cu (25.96 ± 0.3 mg/kg) and Cr (36.23 ± 0.4 mg/kg) respectively by Gebeyehu and Bayissa, (2020).

The obtained results from the current study revealed that the soil in Roodeplaat and Hartbeespoort sites is contaminated with toxic metals such as As, Cu, Cr, Ni, Mn, and Fe as their content exceeded the guideline standard values for agricultural purposes.

Table 4.3: Levels of metal species (mg/kg) in agricultural soils samples collected from Roodeplaat and Hartbeespoort farmlands were collected crops are grown.

Metals	S1	S2	S2	S3	S4	S5	Controls	DEA (2010) **(a)**	FAO/WHO (Chiroma et al., 2014) **(b)**	USEPA (Ahmad et al., 2019) **(c)**
Al	30755.00±1944.54	36090.91	42135.00±530.33	30872.62	13110.00±98.99	13085.00±63.64	10622±14591.86	n. a	n. a	n. a
Cr	723.40±27.72[ab]	111.07[ab]	225±6.08[ab]	320.29[ab]	87.5±5.94[a]	87.75±2.62[a]	250±98.99[ab]	6.5	100	n. a
Mn	821.30±23.62	734.66	459.85±6.08	407.18	982±84.85	2715±569.93[C]	625±304.06	n. a	n. a	2000
Fe	33285.00±1322.29[c]	22240.13[c]	22870±113.14[c]	17064.53	35560±2743.57[c]	34665±3047.63[c]	51335±17076.63[c]	n. a	n. a	21000
Ni	166.30±6.36[ab]	76.59[b]	80.9±1.84[b]	101.50[ab]	29.6±2.12	39.8±4.81	76.94±50.81[b]	91	50	n. a
Cu	26.00±2.55[a]	17.27[a]	31.45±13.79[a]	17.90[a]	25.1±1.13[a]	29.85±3.46[a]	48.13±16.49[a]	16	100	n. a
Zn	59.35±2.19	37.19	37.25±0.78	29.26	86.5±8.20	46.9±0.42	52.40±26.78	240	300	n. a
As	1.15±0.07	1.18	0.75±0.07	9.30[a]	7.65±1.06[a]	6.15±2.90[a]	6.02±3.03[a]	5.8	20	n. a
Pb	6.8±0.71	7.62	3.45±0.07	5.45	8.25±1.06	19.5±2.97	11.23±0.400	20	100	n. a
Cd	0.06±0.01	0.046	0.05±0.00	0.041	0.05±0.01	0.05±0.01	0.03±0.01	7.5	3.0	n. a

[a] DEA (2010) indicates value above maximum guideline value, [b] FAO/WHO indicates values above the maximum guideline, [c] USEPA indicates values above the maximum guideline value, [ab] Values above maximum guideline standard of DEA (2010) and FAO/WHO (2019)

4.4.2.3 Levels of metals in water samples

The levels of metal species were determined from the irrigation water to evaluate the transfer of metals from the irrigation water to the irrigated crops. The results of mean concentrations of metals species in irrigation water samples collected from Roodeplaat and Hartbeespoort irrigation canals and farm dams are presented in Table 4.4. The mean levels of metal species in water samples ranged from (Al) 0.01 to 0.88 mg/L, (Cr) 0.00 to 0.002 mg/L, (Mn) 0.010 to 1.24 mg/L, (Fe) 0.04 to 1.13 mg/L, (Ni) 0.003 to 0.006 mg/L, (Cu) 0.001 to 0.004 mg/L, (Zn) 0.025 to 0.09 mg/L, (As) 0.001 to 0.003 mg/L, and (Pb) 0.000 to 0.001 mg/L. Metal species observed from this study in the irrigation water were all within the acceptable limit set by DWAFF (1996) and FAO/WHO (2008) for water meant for irrigation purpose.

The spearman correlation coefficient (r^2) between metals in irrigation water and plants samples were determined to evaluate if the metals in irrigation water are being transferred into crops via irrigation. Among all the measured metals only Lead (Pb) (r = 0.874*) and Arsenic (As) (r = 0.809*) had a moderate positive association with metals in irrigation water and plants. Thus, indicating that Pb and As in irrigation water are being transferred into food crops via irrigation, and eventually accumulate into food crops, resulting in human health risks through consuming the crops.

Table 4.4: Levels of metal species (mg/L) in irrigation water samples collected from Roodeplaat and Hartbeespoort irrigation canals and farm dams

Sampling sites	H1	H3	H3	H4	R2	R3	FAO (1985)	DWAFF (1996)
Al	0.02±0.01	0.01±0.00	0.010	0.293	0.88±0.09	0.45±0.26	5.0	5-20
Cr	0.001±0.001	0.00±0.00	0.001	0.002	0.001±0.001	0.00±0.00	n. a	0.1-1.0
Mn	0.81±0.02	0.81±0.06	1.24	0.37	0.21±0.002	0,010±0.002	0.2	0.02-10
Fe	0.07±0.001	0.04±0.03	0.12	1.13	0.13±0.005	0.05±0.02	n. a	5-20
Ni	0.004±0.00	0.003±0.00	0.004	0.006	0.005±0.001	0.005±0.002	0.2	0.2-2.0
Cu	0.001±0.00	0.001±0.00	0.001	0.001	0.01±0.001	0.004±0.001	0.2	0.2-0.5
Zn	0.09±0.03	0.03±0.01	0.012	0.025	0.06±0.04	0.01±.001	2.0	1.0-5.0
As	0.002±0.00	0.002±0.001	0.001	0.001	0.003±0.001	0.001±0.00	0.1	0.1-2.0
Pb	0.0001±0.000	0.0002±0.0002	0.000	0.000	0.001±0.0001	0.0002±0.0001	5.0	0.2-2.0

FAO and DWAFF (1996) maximum guideline values for irrigation water

4.4.2 Health Risk Analysis

4.4.2.1 Estimate daily intake of metal species

The estimated daily intake (EDI) was determined using the mean concentrations of each metal species in food crops and the results are presented in Table 4.5. The EDI values for Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, and Pb were found to be ranging from 0.028 to 0.19 mg/kg; 0.00006 to 0.002 mg/kg; 0.0009 to 0.02 mg/kg; 0.005 to 0.14 mg/kg; 0.00007 to 0.003 mg/kg; 0.0003 to 0.04 mg/kg; 0.0030 to 0.02 mg/kg; 0.000003 to 0.00006 mg/kg; 0.000003 to 0.00002; and 0.0003 to 0.0002 mg/kg, respectively. The EDI values obtained in each metal species in the food crop samples were below the maximum tolerable daily as indicated in Table 4.5.

The EDI for all collected plant samples across the sampling sites followed the decreasing order: Al > Fe > Mn > Cu > Zn > Cr > Ni > Pb > As > Cd, respectively. The EDI values of each metal obtained from the edible plants were lower than the data reported by Gebeyehu and Bayissa (2020) for tomato and cabbage.

Table 4.5: Estimated daily intake (EDI mg/day/kg body weight, N=14) of metal species from food crop (Soybean) collected in February 2020 in Hartbeespoort agricultural sites.

	S2	S3	S2	S4	S1	S5	
Metal species	Soybeans	Soybeans	Soybean	Serokolo (Siphonochilus aethiopicus)	Soybean	Soybean	MTDI
Al	0.035	0.028	0.10	0.13	0.19	0.10	n. a
Cr	0.0003	0.00006	0.001	0.0009	0.001	0.002	0.2
Mn	0.005	0.0009	0.01	0.02	0.02	0.01	2.0-5.0
Fe	0.015	0.005	0.04	0.14	0.14	0.08	15.0
Ni	0.0005	0.00007	0.002	0.0006	0.003	0.001	0.3
Cu	0.001	0.0003	0.003	0.002	0.04	0.02	2.5-3.0
Zn	0.003	0.0030	0.009	0.02	0.01	0.007	60.0
As	0.000006	0.000003	0.00003	0.00006	0.00003	0.00003	0.13
Cd	0.00001	0.000003	0.000003	0.00002	0.000006	0.000009	0.021
Pb	0.0001	0.00003	0.00006	0.0002	0.00006	0.0001	0.21
∑ EDI	0.06	0.04	0.16	0.31	0.40	0.22	

MTDI (Maximum Tolerable Daily Intake)

4.4.2.2 Translocation factor

The translocation factor was determined to evaluate the transfer of metal species from soil to edible parts of the food plants, as this acts as indirect entry route of toxic metals to the food chain. The translocation factor data for all plants species collected from the cultivated lands of Roodeplaat and Hartbeespoort sites is shown in Table 4.6. From the obtained data, the translocation factor for Cd TF= 1.33 in a Soybean plant collected from site S4 was > 1, indicating that the metal is rapidly transported to the edible part of the crop from the agricultural soil. Also, zinc (Zn) TF = 1.73 in a soybean plant collected from site S5 was > 1, indicating that zinc is rapidly transported to the edible parts of the plants from soil. From the data in Table 4, the transfer factors were decreasing in the following order of Cd > Cu > Zn > Pb > Ni > Mn > As > Al > Cr > Al for all collected food crops across sampling sites, throughout sampling period. The translocation factor values observed from this current study were lower than the one reported in the similar study carried by Gebeyehu and Bayissa (2020) in tomato and cabbage samples, except for Cd from sampling site S3 and S4, and Cu from sampling site S5 which were observed to be higher than the one reported by Gebeyehu and Bayissa.

Table 4.6: Translocation factors between vegetable crops and soils collected from Roodeplaat and Hartbeespoort farmland sites

Metal species	S2	S3	S2	S4	S1	S5
Al	0.03	0.003	0.008	0.03	0.002	0.03
Cr	0.01	0.001	0.02	0.04	0.006	0.08
Mn	0.03	0.008	0.09	0.08	0.07	0.02
Fe	0.02	0.001	0.000	0.01	0.01	0.01
Ni	0.02	0.05	0.07	0.07	0.06	0.10
Cu	0.20	0.05	0.33	0.32	0.50	**1.73**
Zn	0.29	0.19	0.83	0.09	0.64	0.27
As	0.02	0.001	0.13	0.03	0.09	0.02
Cd	0.6	0.25	0.2	**1.33**	0.36	0.67
Pb	0.05	0.02	0.06	0.89	0.03	0.02

4.4.2.3 Target hazard quotient

The target hazard quotient (THQ) and Health index (HI) was determined in this current study to evaluate the non-carcinogenic risks posed by consuming crops contaminated with metal species. The results for THQ and HI are indicated in Table 4.7. The results observed in this study for THQ showed that As, Pb, Cr, Cd, Zn, Fe, and Mn were all < 1 for all crop plants across the sampling sites, except for soybean plant collected from site S1 which had copper (Cu) THQ = 1.0 which indicates potential cancer health risks which might be posed to adult population via consuming the soybean from the chosen site over long term exposure. It is presumed that the THQ greater than 1 indicates significant cancer health risks to human consuming the contaminated plants. The results obtained from this study show the THQ for each metal species in all collected crops were all below 1 indicating that the food crop is safe for consumption and does not pose any cancer health risks to human, except for soybean from site S1. The THQ value of each metal in the current study were lower than the ones found in tomato and cabbage by Gebeyehu and Bayissa (2020). The Cu in soybean plant collected from sampling site S1 was higher than the THQ values which were found in tomato and cabbage (Gebeyehu and Bayissa, 2020).

The hazard index (HI) was also determined, and the obtained data is presented in Table 4.7. The results for HI for metals from all collected plants ranged from 0.08 to 2.01, with soybean plant collected from site S5 having the highest HI value of = 2.01 compared to other crop plants from other sampling sites. The HI values observed from this study were found to be lower than the HI values reported by Gebeyehu and Bayissa (2020) for cabbage and tomato plant samples. The HI values obtained from site S1, S4 and S5 were all > 1 indicating the potential health implications which might result to increased health risks to adult population via consuming the crops collected from the indicated sites.

Table 4.7: Target hazard quotient (THQ) and Health index (HI) due to consuming metal contaminated crops (Soybean) collected in Hartbeespoort agricultural sites in February 2020 and March 2021.

		S2	S3	S2	S4	S1	S5
Metal species	RfD (mg/kg/day)	Soybeans	Soybeans	Soybean	Serokolo (Siphonochilus aethiopicus)	Soybean	Soybean
Cr	0.003	0.1	0.02	0.33	0.3	0.33	0.67
Mn	0.14	0.04	0.01	0.07	0.14	0.14	0.57
Fe	0.7	0.02	0.01	0.06	0.2	0.2	0.11
Cu	0.04	0.03	0.01	0.075	0.05	**1.0**	0.5
Zn	0.3	0.01	0.01	0.03	0.07	0.03	0.02
As	0.0003	0.02	0.01	0.01	0.2	0.1	0.1
Cd	0.001	0.01	0.00	0.003	0.02	0.006	0.009
Pb	0.0035	0.03	0.01	0.02	0.06	0.02	0.03
Health Index =	∑ THQ	0.26	0.08	0.60	**1.04**	**1.83**	**2.01**

4.4.2.4 Target cancer risk

The target cancer risk (TCR) was also determined to assess the cancer risks posed by the accumulated metal species in crops. The results for TCR due to consumption of food crops contaminated with As, Pb, Cr and Cd are shown in Table 4.8. The TCR results for As, Pb, Cr, Cd and Ni in all plant species across the sampling sites ranged from 0.000005 to 0.00009 mg/kg; 0.0000003 to 0.000002 mg/kg; 0.00003 to 0.0005 mg/kg; 0.000001 to 0.00001 mg/kg; and 0.0001 to 0.005 mg/kg, respectively. The TCR value for Ni and Cr were observed to be above the maximum limit value for acceptable risk of developing cancer (0.0001 mg/kg) for plant species collected from site S2, S4, S1 and S5. Thus, indicating probability of cancer risks from chromium (Cr) and nickel (Ni) to the adult population via consuming the contaminated plants from the indicated sites.

The TCR value observed from this current study for As, Pb, and Cd in all sampling sites were lower than the one reported in tomato and cabbage, except for Ni and Cr from other sampling sites where the TCR values were almost the same as the one observed by Gebeyehu and Bayissa (2020) from Mojo area farmlands in Central rift valley of Ethiopia. They further, revealed that the implications of TCR values observed in the study revealed potential adverse cancer risk which might be induced by the mentioned metals. Metal species Cr and Ni are essential micro-nutrients, which are very vital in human functions, however when these metals are in high levels above the acceptable limit, they result in several human health problems. High levels of nickel in human may result in health problems such as kidney diseases, impairment of normal homeostasis of essential nutrients in human body such as Ca, Mn, Mg and Zn in different tissues, induces teratogenicity and carcinogenesis (Anke *et al.,* 2002; Torres *et al.,* 2009). While Cr is known to be carcinogenic to human when consumed over a long-term, it also results in kidney failure and reproduction problems (Achmad *et al.* 2017).

Table 4.8: Target hazard (TCR) due to consumption of metal contaminated crops (Soybean) collected in Hartbeespoort agricultural sites in February 2020 and March 2021.

Metal species	Oral cancer slope factor (CPS0)	S2	S3	S2	S4	S1	S5
As	1.5	0.000009	0.000005	0.00005	0.00009	0.00005	0.00005
Pb	0.0085	0.0000009	0.0000003	0.0000005	0.000002	0.0000005	0.0000009
Cr	0.5	**0.0002**	0.00003	**0.0005**	**0.0005**	**0.0005**	**0.001**
Cd	0.38	0.000004	0.000001	0.000001	0.00001	0.000002	0.000003
Ni	1.7	**0.0009**	0.0001	**0.003**	**0.001**	**0.005**	**0.002**

#: Values in bold font represent values above the maximum limit (0.0001 mg/kg

4.5 Conclusion

The study successfully investigated the prevalence of MCs and metal species in edible crops and evaluated their potential health risks due to consumption of the plants contaminated with both pollutants. The results for *microcystins* in food crops, revealed that plants accumulate MCs in their edible parts. The estimated daily intake (EDI) for MCs in all food crops for both adults and children were below 0.04 µg/kg DW acceptable value set by world health organization (WHO, 2011), suggesting that the plants may not pose any health risks to humans. The results for metal species levels accumulated in plants samples collected from different sampling sites, showed that Cr, Fe, Zn, As, Cu, and Pb were found to be above the EU (2006) and FAO/WHO (2007) guideline standard. The spearman correlation between metals in plants and water showed that only Pb and As in irrigation water had a positive moderate association with metals in plants collected from the sampling sites. The estimated daily intake (EDI) of metals via consumption of the crops was found to be below the maximum tolerable daily intake (MTDI) proposed for each metal suggesting that the collected crops across the sampling sites were safe for consumption by adults' population.

The translocation factor (TF) of metals from soil to edible parts of the plants, showed that only Cu and Cd from crops collected from site S5 and S4 had high TF value > 1, indicating that these metals are rapidly transported to the plant's edible parts from the soil. The target hazard quotient (THQ) of each metal were < 1, suggesting that consuming plants will not cause any carcinogenic effect on adult population., except for a plant collected from site S1 which had the THQ value of Cu which was above 1. The hazard index (HI) was found to above 1 for crop plants collected from site S1, S4 and S5, indicating that consuming the crops collected from the indicated sites might pose health implications to the adult population. The target cancer risk (TCR) value for Cr and Ni in crops collected from sampling sites S1, S2, S4 and S5 were above the maximum threshold value of 0.0001 mg/kg, suggesting potential cancer risk to adult population exposed to the crop over a long-term.

References

Achmad, R.T. and Auerkari, E.I., 2017. Effects of chromium on human body. Annual Research & Review in Biology, pp.1-8.

Al-Othman, Z.A., Ali, R., Al-Othman, A.M., Ali, J. and Habila, M.A., 2016. Assessment of toxic metals in wheat crops grown on selected soils, irrigated by different water sources. Arabian Journal of Chemistry, 9, pp. S1555-S1562.

Altaner, S., Puddick, J., Fessard, V., Feurstein, D., Zemskov, I., Wittmann, V. and Dietrich, D.R., 2019. Simultaneous detection of 14 microcystin congeners from tissue samples using UPLC-ESI-MS/MS and two different deuterated synthetic microcystins as internal standards. Toxins, 11(7), p.388.

Anke, M., Trüpschuch, A. and Gunstheimer, G., 2002. The Blological and Medical Importance of the Interactions between Nickel and Zinc, Magnesium and Manganese in Vivo. In Trace Elements in Man and Animals 10 (pp. 685-686). Springer, New York, NY.

Awodele, O., Popoola, T.D., Amadi, K.C., Coker, H.A.B. and Akintonwa, A., 2013. Traditional medicinal plants in Nigeria—Remedies or risks. Journal of ethnopharmacology, 150(2), pp.614-618.

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J. and Miles, C.O., 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Marine and Freshwater Research, 65(2), pp.175-189.

Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E. and Funari, E., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of toxicology, 91(3), pp.1049-1130.

Cao, Q., Alan, D., Steinman, A.D., Wan, X. and Xie, L., 2018. Bioaccumulation of microcystin congeners in soil-plant system and human health risk assessment: a field study from Lake Taihu region of China. Environmental Pollution, 240, 44– 50.

Cao, Q., Liu, W., Gu, Y., Xie, L., Jiang, W., Ga[illegible]Y. and Yang, L., 2020. Synergetic enhancement toxicity of copper, cadmium and microcystin-LR to the Ceratophyllum demersum L. Toxicon, 186, pp.151-159.

Chaoua, S., Boussaa, S., El Gharmali, A. and Boumezzough, A., 2019. Impact of irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in Morocco. Journal of the Saudi Society of Agricultural Sciences, 18(4), pp.429-436.

Chen C, Qian Y, Chen, Q. and Li C., 2011. Assessment of daily intake of toxic elements due to consumption of vegetables, fruits, meat, and seafood by inhabitants of Xiamen, China. J. Food Sci. 76: T181–T188.

Chen, J., Han, F.X., Wang, F., Zhang, H., and Shi, Z., 2012. Accumulation and phytotoxicity of microcystin-LR in rice (Oryza sativa). Ecotoxicol. Environ. Saf. 76, 193–199.

Cheung, M.Y., Liang, S. and Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. Journal of Microbiology, 51(1), pp.1-10.

Corbel, S., Mougin, C. and Bouaïcha, N., 2014. Cyanobacterial toxins: modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. Chemosphere, 96, 1– 15.

do Carmo Bittencourt-Oliveira, M., Cordeiro-Araújo, M.K., Chia, M.A., de Toledo Arruda-Neto, J.D., de Oliveira, Ê.T. and dos Santos, F., 2016. Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners. Ecotoxicology and environmental safety, 128, pp.83-90.

Duhain, G.L.M.C., 2011. Occurrence of Cryptosporidium spp. in South African irrigation waters and survival of Cryptosporidium parvum during vegetable processing (Doctoral dissertation, University of Pretoria).

DWA, 2013. Classification of significant water resources in the Crocodile (West) and Marico water management area (WMA) and the Mokolo and Matlabas catchments: Limpopo WMA. Pretoria: Department of Water Affairs.

DWAFF, 1996. South African water quality guidelines. 2nd ed. Volume 4: Agricultural Use: Irrigation, DWAF, Pretoria.

El Khalloufi, F., Oufdou, K., Bertrand, M., Lahrouni, M., Oudra, B., Ortet, P., Barakat, M., Heulin, T. and Achouak, W., 2016. Microbiote shift in the Medicago sativa rhizosphere in response to cyanotoxins extract exposure. Science of the Total Environment, 539, pp.135-142.

EPA, N., 2014. Sydney Paten No. EPA 2014, 323.

European Commission, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union, 364(365–324).

Ezemonye LI, Adebayo PO, Enuneku AA, Tongo I, and Ogbomida E., 2019. Potential health risk consequences of heavy metal concentrations in surface water, shrimp (Macrobrachium macrobrachion) and fish (Brycinus longipinnis) from Benin River, Nigeria. Toxicol. Reports. 6:1–9.

FAO, 1985. Water Quality for Agriculture. Paper No. 29 (Rev. 1) UNESCO, Publication, Rome, p. 96.

FAO/WHO, 2007. Joint FAO. WHO Food Standards Programme: Codex Alimentarius Commission. Report of the 28th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses.

Fontanillo, M. and Köhn, M., 2018. Microcystins: Synthesis and structure–activity relationship studies toward PP1 and PP2A. Bioorganic & medicinal chemistry, 26(6), pp.1118-1126.

Gaget, V., Lau, M., Sendall, B., Froscio, S. and Humpage, A.R., 2017. Cyanotoxins: which detection technique for an optimum risk assessment? Water research, 118, pp.227-238.

Gebeyehu, H.R. and Bayissa, L.D., 2020. Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. PloS one, 15(1), p.e0227883.

Gidlow, D. A., 2004. Lead toxicity. Occupational Medicine, 54, 76–81.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M. and Visser, P.M., 2018. Cyanobacterial blooms. Nature Reviews Microbiology, 16(8), pp.471-483.

Jia, Y., Chen, W., Zuo, Y., Lin, L. and Song, L., 2018. Heavy metal migration and risk transference associated with cyanobacterial blooms in eutrophic freshwater. Science of the total environment, 613, pp.1324-1330.

Kamunda C, Mathuthu M, and Madhuku M., 2016. Health risk assessment of heavy metals in soils from Witwatersrand gold mining basin, South Africa. Int. J. *Environ. Res. Public Health*. 13:663.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z. and Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental pollution,* 152(3), pp.686-692.

Kohzadi, S., Shahmoradi, B., Ghaderi, E., Loqmani, H. and Maleki, A., 2019. Concentration, source, and potential human health risk of heavy metals in the commonly consumed medicinal plants. *Biological trace element research,* 187(1), pp.41-50.

Lee, S., Jiang, X., Manubolu, M., Riedl, K., Ludsin, S.A., Martin, J.F. and Lee, J., 2017. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. *Food research international*, *102*, pp.234-245.

Levizou, E., Statiris, G., Papadimitriou, T., Laspidou, C.S. and Kormas, K.A., 2017. Lettuce facing microcystins-rich irrigation water at different developmental stages: Effects on plant performance and microcystins bioaccumulation. *Ecotoxicology and environmental safety*, 143, pp.193-200.

Machado, J., Campos, A., Vasconcelos, V. and Freitas, M., 2017. Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: a review of their relevance for agricultural plant quality and public health. *Environmental Research,* 153, 191– 204.

Marschner, H., 1974. Calcium nutrition of higher plants. NJAS wageningen journal of life sciences, 22(4), pp.275-282.

Matthews MW, Bernard S and Winter K., 2010. Remote sensing of cyanobacteria-dominant algal blooms and water quality parameters in Zeekoevlei, a small hypertrophic lake, using MERIS. Remote Sensing of Environment 114(9): 2070–2087.

Mbiza, N.X., 2014. Investigation of the effectiveness of techniques deployed in controlling cyanobacterial growth in Rietvlei Dam, Roodeplaat Dam and Hartbeespoort Dam in Crocodile (West) and Marico Water Management Area (Doctoral dissertation).

Meneely, J.P. and Elliott, C.T., 2013. Microcystins: measuring human exposure and the impact on human health. Biomarkers, 18(8), 639–649.

Miller, A. and Russell, C., 2017. Food crops irrigated with cyanobacteria-contaminated water: an emerging public health issue in Canada. Environmental Health Review, 60(3), 58– 63.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M., 2010. Heavy metals, occurrence, and toxicity for plants: a review. Environmental chemistry letters, 8(3), pp.199-216.

Oberholster, P.J. and Botha, A.-M., 2014. Importance of water quality to the food industry in South Africa. Understanding the Food-Energy-Water Nexus. WWF-SA, South Africa.

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T. and Mavumengwana, V., 2020. Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. International journal of environmental research and public health, 17(7), p.2204.

Rai, P.K., Lee, S.S., Zhang, M., Tsang, Y.F. and Kim, K.H., 2019. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment international, 125, pp.365-385.

Redouane E. M., Zerrifi, S.E.A., El Khalloufi, F., Oufdou, K., Oudra, B., Lahrouni, M., Campos, A. and Vasconcelos, V., 2019. Mode of action and fate of microcystins in the complex soil-plant ecosystems. Chemosphere, 225, pp.270-281.

Saqrane, S. and Oudra, B., 2009. CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins, 1(2), pp.113-122.

Sharma S, Nagpal AK and Kaur I., 2018. Heavy metal contamination in soil, food crops and associated health risks for residents of Ropar wetland, Punjab, India and its environs. Food Chem. 255:15–22.

Sihlahla M, Mouri H, and Nomngongo P.N., 2019. Uptake of trace elements by vegetable plants grown on agricultural soils: Evaluation of trace metals accumulation and potential health risk. Journal of African Earth Sciences. 160: 103635.

Torres, F., das Gracas, M., Melo, M. and Tosti, A., 2009. Management of contact dermatitis due to nickel allergy: an update. Clinical, cosmetic and investigational dermatology: CCID, 2, p.39.

Turton, A., 2016. Water Pollution and South Africa's poor. Published by the South African Institute of Race Relations. Johannesburg, South Africa. [Online] Available from: http://irr.org.za/reports-and-publications/occasional-reports/files/water-pollution-and-south-africas-poor [Accessed: 10th May 2017].

Uddin, A.H., Khalid, R.S., Alaama, M., Abdualkader, A.M., Kasmuri, A. and Abbas, S.A., 2016. Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. Journal of analytical science and technology, 7(1), pp.1-7.

Wei, H., Wang, S., Xu, E.G., Liu, J., Li, X. and Wang, Z., 2020. Synergistic toxicity of microcystin-LR and Cu to zebrafish (Danio rerio). Science of The Total Environment, 713, p.136393.

WHO, 2002. Reducing risks, promoting healthy life, World Health Organization. https://apps.who.int/iris/handle/10665/42510 (accessed 30 October 2020).

WHO, 2011. Cyanobacterial toxins: microcystin-LR. Guidelines for Drinking-water Quality

Woldetsadik D, Drechsel P, Keraita B, Itanna F, and Gebrekidan H., 2017. Heavy metal accumulation and health risk assessment in wastewater-irrigated urban vegetable farming sites of Addis Ababa. Ethiopia. Int. J. Food Contam.pp; 4:9.

Zhang, Y., Husk, B.R., Duy, S.V., Dinh, Q.T., Sanchez, J.S., Sauvé, S. and Whalen, J.K., 2021. Quantitative screening for cyanotoxins in soil and groundwater of agricultural watersheds in Quebec, Canada. Chemosphere, p.129781.

Zheng N, Wang Q, Zhang X, Zheng D, Zhang Z, and Zhang S., 2007. Population health risk due to dietary intake of heavy metals in the industrial area of Huludao city, China. Sci. Total Environ. 387:96–104. https://doi.org/10.1016/j.scitotenv.2007.07.044 PMID: 17765948

Zhu, J., Ren, X., Liu, H. and Liang, C., 2018. Effect of irrigation with microcystins-contaminated water on growth and fruit quality of Cucumis sativus L. and the health risk. Agricultural Water Management, 204, pp.91-99.

Chapter 5: Application of the solid phase adsorption toxin tracking (SPATT) technology for monitoring cyanotoxins in irrigation canals and farm dams of Roodeplaat and Hartbeespoort sites.

5.1 Abstract

This study evaluated the applicability of the Solid Phase Adsorption Tracking Technology (SPATT) using the commercial resin DIAON HP20 as a passive sampling tool to monitor and detect cyanotoxins in irrigation canals and farm dams. SPATT bags were constructed using 100 µm nitex bolting cloth filled with DIAON HP20 resin. Prior to the field, SPATT bags saturation was assessed in the lab. From the obtained results SPATT bags showed to get saturated within 48 hours with toxins. Thus, this constant time (48 hours) which is 2 days, was employed in the field to deploy SPATT bags from February 2020 to March 2021, while the grab samples were also collected simultaneously upon deployment and retrieval of the SPATT bags using 100 mL amber bottles. *Microcystins* levels were analysed and quantified using the ELISA method. The composition of harmful cyanobacteria was identified using flow-cam. The MCs levels in grab and SPATT bags samples collected from Roodeplaat and Hartbeespoort sampling sites ranged from 0.14 to 13.03 µg/L and 0.99 to 2.28 ng/g resin throughout the sampling sites and months, respectively. A spearman correlation results revealed that among all measured physicochemical parameters pH (0.776), Turbidity (0.699) and DO (0.829) had a significant positive association with total toxins in grab samples, while total dissolved toxins in SPATT samples had a negative moderate relationship with TDS and EC. Total toxin concentrations in SPATT bags and Grab samples did not show any correlation this might be because SPATT bags detect *microcystins* in water column overtime. Among the identified cyanotoxins, *microcystis* species were the most dominant throughout the sampling sites. Overall results showed that SPATT bags with DIAON HP20 resin as an adsorbent proved to be applicable in monitoring and detecting *microcystins* in the irrigation water of Roodeplaat and Hartbeespoort sites.

Keywords: *Cyanobacteria, Microcystins, passive sampling (SPATT), Grab method*

5.2 Introduction

The occurrence of cyanobacterial blooms has specifically become a global concern in freshwater ecosystems because of anthropogenic activities namely, eutrophication and climate change (Cheung *et al.,* 2013; Machado et al., 2017; Lee *et al.,* 2017; Paerl, 2018; Beversdorf *et al.,* 2018). This is because cyanobacterial blooms can result in the production of secondary metabolites known as cyanotoxins (Catherine *et al.,* 2017). Cyanotoxins are responsible for acute and chronic poisoning of animals and humans, and have been classified into four groups; namely, Hepatotoxins (*Microcystins, nodularin's*), Neurotoxins *(anatoxin-a, anatoxin-a(s)* and Cytotoxins (*cylindrospermopsins*), Dermatoxins *(Lypopolysaccharide, Lyngbyatoxins and aplysiatoxin)* (Buratti *et al.,* 2017; Gaget *et al.,* 2017; Huisman *et al.,* 2018).

Among all known cyanotoxins, hepatotoxins are the foremost predominant in freshwater biological systems around the world, and they have received wide coverage in research (Harke *et al.,* 2016). The most common cyanotoxins are *microcystins* (MCs) which fall within the hepatotoxin group and almost 200 different congeners identified to date and are known to cause significant harm to water quality, aquatic ecosystems, animals, and humans health (Fantanillo and Kohn, 2018; Zhu *et al.,* 2018; Altaner *et al.,* 2019). MCs are produced by some bloom forming cyanobacteria, mainly *microcystis, anabaena, nostoc* and *Oscillatoria* in eutrophic water (Chen *et al.,* 2016; Cao *et al.,* 2018). Also, the presence of cyanobacterial toxins in irrigation waters might pose threats to human health via accumulating in food crops meant for human consumption. Thus, it is necessary to monitor presence and concentration of cyanotoxins in irrigation water. Additionally, cyanobacterial bloom in aquatic ecosystems can cause economic loss in tourism, and increment cost in drinking water treatment (Lee *et al.,* 2017). Due to their toxicity, the World Health Organization (WHO) set a provisional guideline value of 1 µg/L for *microcystin* (MC-LR) in drinking water (Meneely and Elliott, 2013; Cao *et al.,* 2018).

MCs can cause water quality impacts such as reducing water aesthetic, lower dissolved oxygen levels, cause taste odour problems, and promote liver cancer and tumor in humans and animals (Kudela, 2011). Deterioration of water quality due to eutrophication resulting in rapid growth of harmful cyanobacterial blooms in South Africa reservoirs has been well documented (Mathew *et al.,* 2010; DWA, 2011; Turton, 2016). The levels of MCs in the range of 1000 and 18 000 µg/L have been reported in South African reservoirs and rivers (Pindihama and Gitari, 2019; Turton,

2016). These levels are way above 1µg/L guideline value recommended by World Health Organization in 1998.

Despite the recognition rapid proliferation of toxin producing cyanobacterial blooms in South Africa's water bodies, the approaches to monitor and manage the blooms tend to be reactionary (Miller *et al.,* 2010; Pindihama and Gitari, 2019). Furthermore, there is a lack of early warning capabilities to cyanotoxins contamination in fresh waters intended for irrigation or drinking. To date, the most common sampling technique for cyanotoxins relies on the traditional sampling method, which has a lot of disadvantages. Cyanotoxins level in the water column vary over space and time and episodic peak events of high concentration of cyanotoxins may be missed (Kudela, 2011; Davis & Hansen, 2013). The disadvantage of grab sampling includes the fact that it gives a snapshot of the cyanotoxins levels in aquatic ecosystems and produces highly variable and unpredictable results due to the unequal distribution of algae cells and toxin in lakes during a bloom (Kudela, 2011). Furthermore, grab sampling may underestimate the presence of low levels of toxins in water column (Kudela, 2011; Davis and Hansen, 2013).

To overcome the drawbacks of the traditional method used to monitor cyanotoxins, several studies have used passive sampling techniques with different adsorbents as an alternative method to monitor cyanotoxins contaminants in aquatic ecosystems (Kudela, 2011; Mackenzie, 2010). Solid Phase Adsorption and Toxin Tracking (SPATT) technology was developed by Mackenzie *et al.* (2004) to determine dissolved bio-toxins levels in seawater, and it involves the passive accumulation of the toxins directly from the water-body through the deployment of absorptive resins (Lane *et al.,* 2010; Roue *et al.,* 2018). This method provides temporally and spatially integrated monitoring of the water column. SPATT can also be used to clarify toxin dynamics and environmental drivers, as well as train, validate and improve predictive models.

However, passive sampling using SPATT technology has also some drawbacks namely, lack of calibration, optimal deployment time, and it monitors only dissolved toxins (Roue *et al.,* 2018). Also, the total dissolved toxins concentrations found in SPATT bags device, cannot be converted into toxin concentrations in the real environment, unless the water flow parallel to the SPATT bag is measured (Kudela, 2011; Roue *et al.,* 2018). Several studies have developed and tested SPATT technology using a variety of resins (Lane *et al.,* 2010; McCarthy *et al.,* 2014; Zendong *et al.,* 2016; Kudela, 2017; Peacock *et al.,* 2018). Among all the resin used for SPATT samplers, DIAON HP20 resin has been found to be more versatile because it can be used to quantify *microcystins, anatoxins,*

saxitoxins, *domoic acid*, and *okadaic acid* in freshwater, brackish, and marine water (Miller *et al.,* 2010; Howard *et al.,* 2017; Kudela, 2017; Li *et al.,* 2016; Hattenrath-Lehmann *et al.,* 2018; Peacock *et al.,* 2018). Since its introduction more than a decade ago, several laboratory and field studies have been done to investigate the effectiveness of the SPATT technology. These studies concluded that passive samplers offer the ability to detect an extensive variety of toxins in the aquatic ecosystems (Roue *et al.,* 2018).

Roodeplaat and Hartbeespoort dams are some of the reservoirs in South Africa which are considered hyper-eutrophic and suffering from severe cyanobacterial blooms (Conradie and Barnard, 2012; Ballot *et al.,* 2013). No study has been conducted to investigate the effectiveness of SPATT bags with the DIAON HP20 as an adsorbent to monitor cyanotoxins production in irrigation canals and farm dams around Roodeplaat and Hartbeespoort irrigation schemes. Thus, the aim of the current study was to evaluate the applicability of a passive sampling technology (SPATT) to detect and monitor cyanotoxins in water intended for irrigation purposes using DIAON HP20 resin as an adsorbent.

5.3 Materials and Methods

5.3.1 DIAON HP20 Resin and SPATT bags preparation and construction

The adsorbent DIAON HP20 was purchased from Rochelle chemicals, South Africa. SPATT bags for both laboratory trials and field trials were constructed using the 100 µm nitex bolting cloth, which was sewn into sachets bags. The bolting cloth was sewn on 3 sides using a plastic sealer to close and form an open bag of 55 mm width. The SPATT bags for both laboratory trials and field experiments were filled with 3 g and 10 g (dry weight) of DIAON HP20 resin, respectively. The SPATT bags were then sealed on the fourth side forming a 55 x 55 mm dimension bag. The SPATT bags were activated by soaking each bag in 100% methanol for 48 hours. The methanol was rinsed off with deionised water by incubating the SPATT bags inside a beaker with 500 mL deionised water (Milli Q). The SPATT bags were then placed in Zip-lock bags with deionised water covering the resin to prevent it from drying out and stored in a cooler box with ice and transported to the field for deployment (Lane *et al.,* 2010; Kudela, 2011; Roue *et al.,* 2018).

5.3.2 Solid phase adsorption toxin tracking (SPATT) laboratory trials

The laboratory trial was carried to evaluate how long the DIAON HP20 resin inside SPATT bags takes to get saturated with dissolved microcystins. This was done in an attempt to assess the number of days to deploy the SPATT bags in the field. The laboratory SPATT bags with resin were incubated for 9 days. The experiment used three 1 L amber bottles. Wherein, one bottle was treated as control sample which had field water only with no SPATT samplers, while two 1 L amber bottles had field water with 4 SPATT bags containing 3 g dry weight of HP20 resin in each bottle. A shaker was used to agitate the samples for water flow to allow SPATT bags to passively adsorb the toxins in the water column. Individual SPATT samplers were retrieved daily from the bottles and rinsed off with deionized water and put inside a zip lock. Also, water sample treated as grab samples were collected daily when SPATT samplers were removed for monitoring toxin adsorption onto SPATT bags. All samples were stored at 4˚ C until toxin extraction.

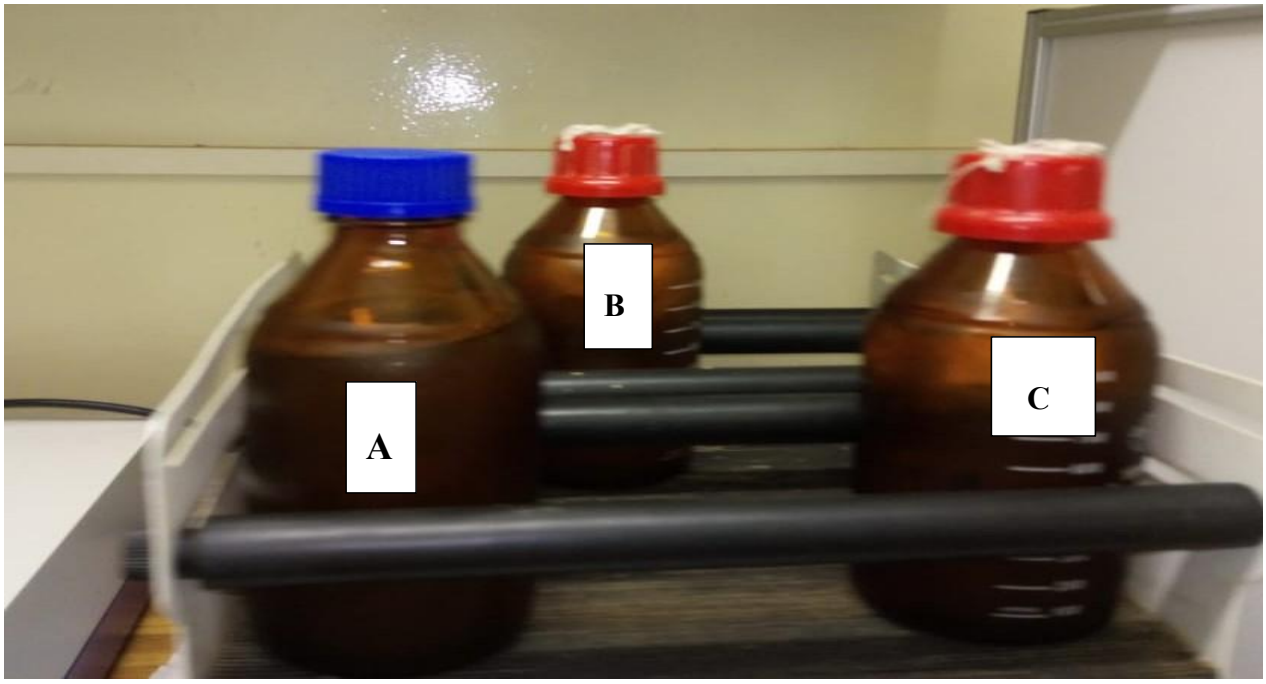


Figure 5.1: Laboratory SPATT bags deployment trial set up (Bottle **A** with control sample with no SPATT bags inside; Bottle **B** and **C** water samples with SPATT bags inside).

5.4 SPATT bags monitoring sites

Figure 5.2 shows the selected sampling sites for SPATT bags deployment for monitoring cyanotoxins over time. Total of 6 irrigation canal and farm dams were selected. Three sites (R1, R2, R3) for Roodeplaat and (H1, H2, H3) for Hartbeespoort sites. The selected sites were chosen because the water is being utilized for irrigating vegetable crops around the areas.

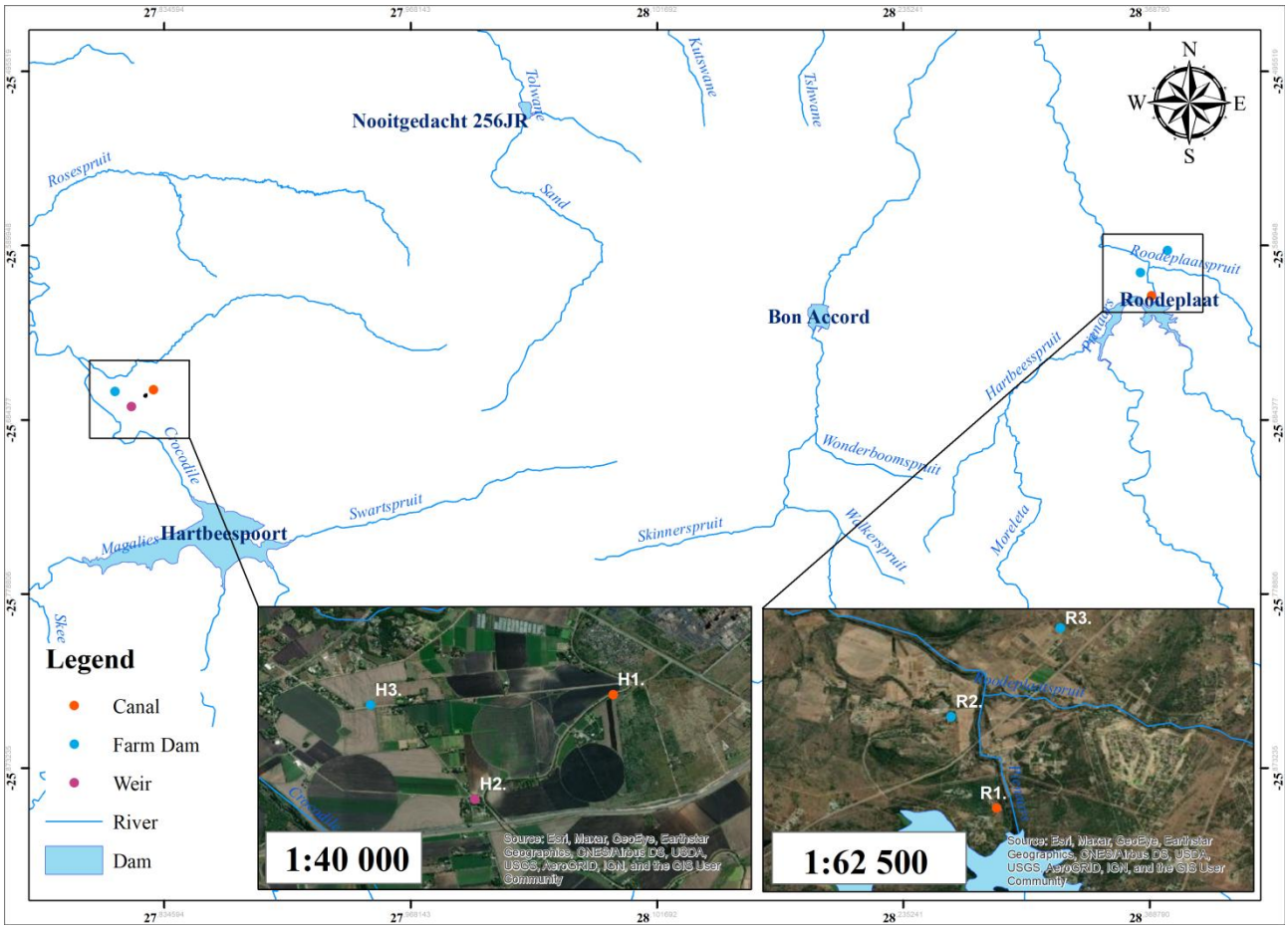


Figure 5.2: A Map showing sampling locations for cyanotoxins monitoring using SPATT bags in Roodeplaat and Hartbeespoort irrigation canals and farm dams

5.4.1 Field experiment and physicochemical parameters

5.4.1.1 Water quality parameters

The physical parameters such as pH, TDS, EC, temperature, turbidity, and DO were recorded in situ from the irrigation canals/farm dams at each site. The pH, EC, TDS, and Temperature were measured using Jen-way pH/Cond meter (model 430). Turbidity was determined using TB200 portable turbidimeter model (#TB200-10), while dissolved oxygen (DO) was determined using the Rugged Dissolved Oxygen electrode (RDO) code: 087003 attached to a Thermo-scientific meter

Manufactured in Singapore). The instruments were calibrated following the manufacturers' instruction prior to measurements. Water samples for nutrients, chlorophyll-*a* and for microcystins were collected at the end of SPATT bag deployment. The concentrations of nutrients, nitrates and phosphates were determined using Spectro-quant® Merck Pharo 100 model No: 07531-45 (Merck KGaA 64293 Darmstadt, EU), and the photometric test kits from (Merck company. Germany). Chlorophyll-*a* was used to estimate cyanobacterial biomass in water samples according to method adapted from Lawton *et al.* (1999).

5.4.1.2 Field deployment

SPATT bags were deployed for 2 days in Roodeplaat and Hartbeespoort irrigation canals/ farm dams in February 2020 and March 2021. The SPATT bags were clamped into plastic embroidery hoops and were protected by putting them inside a wire cage to prevent the sampler from being damaged by fish or other aquatic organisms and was secured with a rope at 2.5 m depth. The grab samples were collected using 100 mL amber bottles exactly where the SPATT bag was deployed. Upon retrieving, SPATT bags were unclamped from embroidery hoops and rinsed with field water to remove silt and debris. After retrieval, the samplers were labelled appropriately and stored in zip lock bags and stored in a cooler box with ice immediately and were transported to the laboratory and stored in a freezer - 4°C until retrieved for toxin extraction. Figure 5.3 below shows how the SPATT bags were deployed in the field.

Figure 5.3: SPATT bags deployment in the field

5.4.1.3 Toxin extraction from resin

For both laboratory and field SPATT samples were taken out from the freezer, thawed from ice completely before extraction. The SPATT bags were then cut open using a scissor, and the resin decanted into a 50 mL centrifuge tubes to extract toxins from the resin. The toxins extraction from SPATT bags were determined following the method described by Kudela (2011). First extraction, 10 mL of 50 % methanol was used, followed by two other extractions with 20 mL 50% of methanol. The extracted eluant was sonicated for 10 minutes and centrifuged at 4000 rpm (40 x g) for 30 minutes. The extracted supernatant was combined in 100 mL amber bottles. The combined extracts were evaporated to dryness at 50˚C using an electric water bath, under a stream of nitrogen gas. The dried samples were then re-suspended with 2 mL of phosphate wash buffer. Both grab samples and samplers eluant were analyzed for total microcystins using the commercial ELISA test kits supplied by EUROFINS (Kit Lot No: 19I1120:PN 520011) following the manufactures instructions. The total dissolved microcystins concentrations in SPATT bags were determined using the following formula:

$$\text{Total dissolved MCs (ng/g resin)} = \frac{(MCs\ conc\ \mu g/L - \text{extract})\ x\ (2\ mL\ extract - VOL)}{DW\ (g)} \quad (1)$$

Where MCs conc (µg/L) it is a total concentration of *microcystins* extracted from the SPATT bags resin, Extract volume is the amount of dissolvent (2 mL) of phosphate wash buffer which was converted to 0.002 L was used to re-suspend the dried samples, while Dw (g) is the dry weight of DIAON HP20 resin filled in SPATT bags. The total *microcystins* in SPATT bags (µg/kg) were then converted to (ng/g resin)

5.4.1.4 Statistical analysis

The obtained data were captured and processed using Microsoft excel, 2013. The results were compiled, and graphs were computed using excel. The statistical software Graph Pad Instat 3 was used to assess normality of the variance. After assessing the normality of all data, the Spearman rho correlation was determined to evaluate the association between environmental parameters such as (pH, TDS, EC, Temperature, DO, Turbidity, nitrate, phosphates, chlorophyll-*a*) with total *microcystins*. Also, between total *microcystins* concentrations in SPATT bags and grab samples using the statistical software IBM SPSS version 25 (IBM Corporation, Armonk, New York, USA), and the significance difference at the 95% level of confidence set at $P < 0.05$ for all the test.

5.5 Results and discussions

5.5.1 Laboratory trial results

5.5.1.1 Laboratory experimental results

Figure 5.4 shows the levels of MCs adsorbed by the SPATT bags over the duration of the experiment. The findings show that concentrations of toxins in the samplers were low in 24 hrs (0.025 µg/g), peaked in 48 hrs to (0.026 µg/g), and then decreased again in 72 hrs (0.025 µg/g resin). The levels of toxins decreased slightly in 72 hours, this might be because of fouling competes due to accumulation of unwanted materials on the surface of the SPATT bag preventing the resin to adsorb the toxins, or due to SPATT bag getting saturated (Davis and Hansen, 2013). The concentrations of toxins adsorbed by the resin in the SPATT bags samplers seems to reach a maximum at 48 hours with not much change as the resident time is increased to 72 hours.

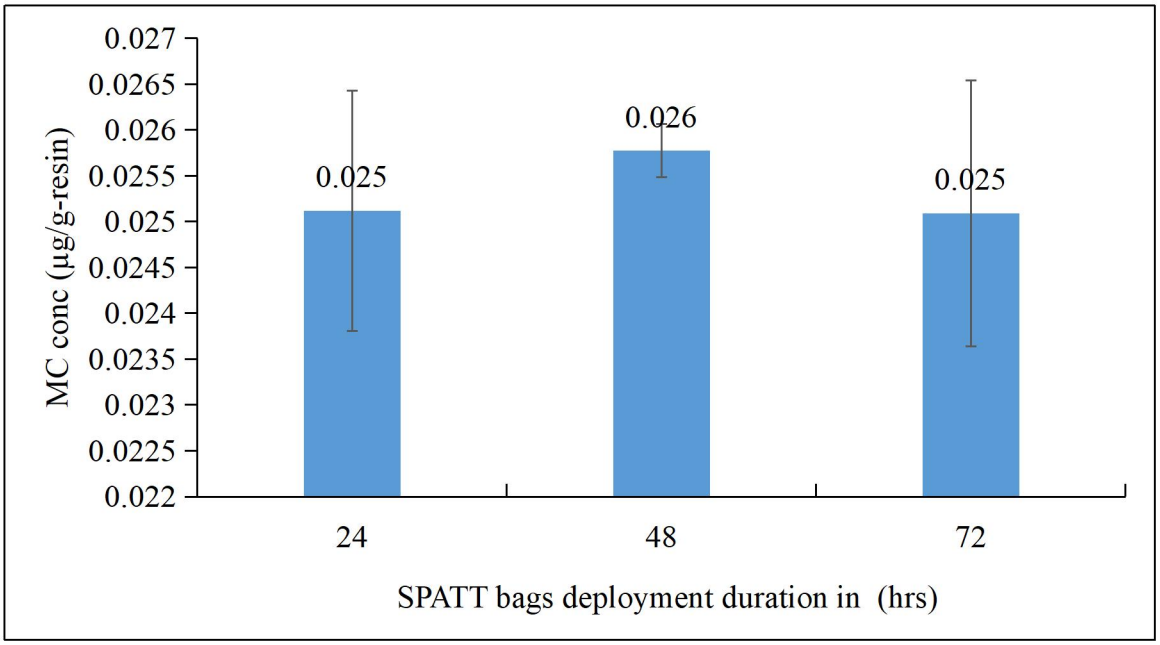


Figure 5.4: Variation of *microcystins* (µg/g) adsorbed by resin with exposure time of SPATT bags in field water during laboratory exposure.

Figure 5.5 shows the initial concentration of *microcystins* in the water sample before inserting the SPAATT bag and the residual concentration of MC after reinserting the SPAATT bag in Figure 5.4 into the water sample bottle. The initial concentration of water used in the laboratory tests prior to using the SPAATT bag was 8.98 µg / L. SPATT bags have been deployed and recovered over time. After removing the SPAATT bag from the water sample, the results showed that MC decreased significantly over time. The residual MC concentrations in SPATT bags ranged from 24 hours (0.28

µg / L) to 48 hours (0.12 µg / L) and 72 hours (0.10 µg / L), respectively. The *microcystins* residue was significantly lower than the initial concentration over time, suggesting that MC was adsorbed on the SPAATT bag and was significantly reduced.

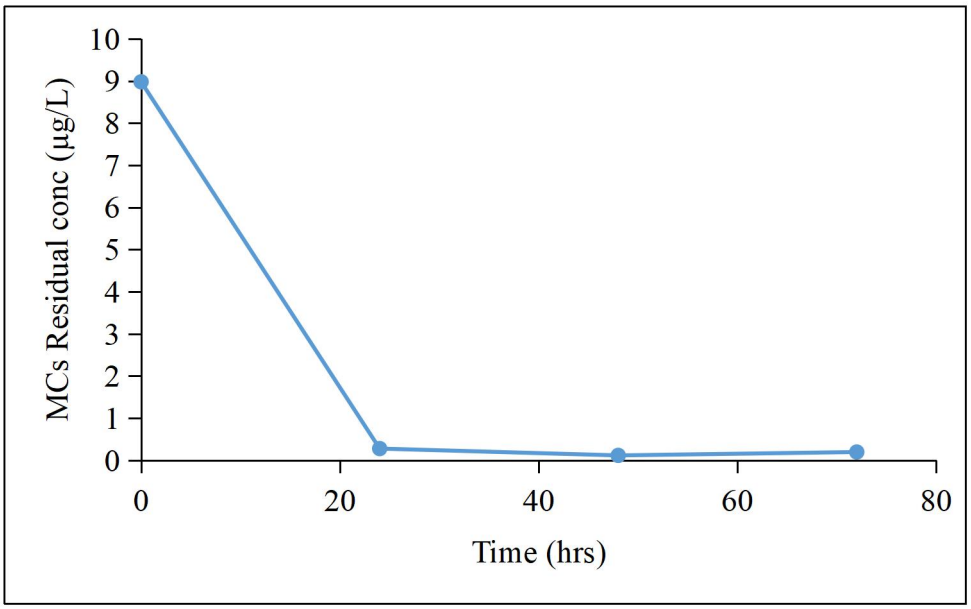


Figure 5.5: The residual concentrations of *microcystins (µg/L)* after retrieval of SPATT bags over time

5.5.2 SPATT field deployment and physicochemical variables

5.5.2.1 Physicochemical parameters

Table 5.1 shows the physicochemical characteristics of water sampled at each site of irrigation canals/farms dams where SPATT bag samplers were deployed in Roodeplaat and Hartbeespoort site. The pH of the water samples ranged from 7.04 ± 0.59 to 10.06 ± 0.75, indicating that the water was ranging from neutral to strong alkaline. pH recorded at site R2, R3 and H3 were above the DWAF, (1996) guideline value set for irrigation water use. Total dissolved solids (TDS) and electrical conductivity (EC) also varied between the sampling sites and sampling months and ranged from 202.4 ± 36.20 to 309.5 ± 67.78 mg/L, and 336.5 ± 61.52 to 519.5 ± 112.53 µS/cm, respectively. The total dissolved solids and electrical conductivity were above 0 - 260 mg/L and 0 - 400 µS/cm DWAF guideline value set for irrigation water at site H1, H2, and H3, respectively.

Temperature did not vary much among sampling sites and throughout sampling months and ranged from 22.4 ± 1.27 to 29.95 ± 5.59 ˚C. In addition, the concentration of biomass as chlorophyll-*a*

ranged from 31.17 ± 14.79 to 526.1 ± 65[illegible]0 µg/L. Kansas (2011) highlighted that the concentration level of chlorophyll-*a* above 10 µg/L indicates the likelihood, of rapid growth of cyanobacterial blooms in the aquatic ecosystem. The chlorophyll-*a* recorded in all sampling sites revealed that the irrigation canals were falling under hyper-eutrophic category (chlorophyll-*a* > 30 µg/L). Furthermore, the mean concentrations of nitrates, phosphates, turbidity, and dissolved oxygen ranged from 4.25 ± 1.91 to 6.47 ± 3.28 mg/L, 0.2 ± 0.03 to 1.01 ± 0.06 mg/L, 1.66 ± 1.23 to 366.03 ± 315.12 mg/L, and 3.03 to 16.2 mg/L, respectively. The phosphates levels in all sampling sites during the sampling months were above DWAF, (1996) value of 0.025 to 0.25 mg/L indicating that the sampling sites were eutrophic and there was likelihood of algae bloom growth.

Table 5.1: Physicochemical characteristics of irrigation water recorded from Roodeplaat and Hartbeespoort irrigation canals and farm dams

WaterQuality parameters	R1	R2	R3	H1	H2	H3	DWAF (1996) for irrigation water
pH	8.37 ± 0.77	10.06 ± 0.75	9.79 ± 1.08	7.58 ± 0.04	7.04 ± 0.59	9.28 ± 0.09	6.5-8.4
TDS (mg/L)	217.6 ± 68.45	206.5 ± 23.33	202.4 ± 36.20	309.5 ± 67.78	304.5 ± 85.56	277 ± 16.97	0-260
EC (µS/cm)	361.5 ± 108.19	341.5 ± 41.72	336.5 ± 61.52	519.5 ± 112.43	510.5 ± 136.47	461.5 ± 30.41	0.400
Temperature (°C)	22.4 ± 1.27	26.8 ± 8.49	29.95 ± 5.59	23.2 ± 0.28	22.6 ± 0.0	25.9 ± 4.81	n. a
Nitrates (mg/L)	6.15 ± 2.97	5.52 ± 3.23	6.16 ± 2.22	6.47 ± 3.28	4.25 ± 1.91	4.85 ± 2.98	< 5
Phosphates (mg/L)	0.55 ± 0.19	0.2 ± 0.03	0.29 ± 0.14	0.99 ± 0.10	1.01 ± 0.06	0.38 ± 0.12	n. a
Chlorophyll-*a* (µg/L)	49.01 ± 5.73	183.96 ± 39.44	326.41 ± 79.83	58.24 ± 53.06	31.17 ± 14.79	526.1± 655.80	n. a
Turbidity (NTU)	6.56 ± 2.91	366.03 ± 315.12	32.94 ± 34.80	1.66 ± 1.23	2.00 ± 1.42	42.9 ± 10.49	n. a
DO (mg/L)	7.89	16.2	15.51	3.64	3.03	13.08	n. a

Total mean *microcystins* concentrations detected and recovered from grab samples (µg/L) and SPATT bag resin (ng/g resin) in Roodeplaat and Hartbeespoort irrigation canals and farm dams collected in February 2020 and March 2021 are shown in Figure 5.6 (A & B). The total microcystins levels in Grab and SPATT samples ranged from 0.14 to 13.03 µg/l ad 0.99 to 2.28 ng/g resin shown in (Figure 5.6 (A & B), respectively. Both Grab and SPATT sampling method revealed presence of toxins MCs in all sampling sites of Roodeplaat and Hartbeespoort throughout the sampling months. Grab showed high levels of *microcystins* in February, which is a warm season, except for site R2 and R3 which the levels of MCs increased in March. Also, SPATT bags results revealed high MCs in February and decrease in MCs levels in March, except for site H2 and H4 which levels of of MCs increased in March.

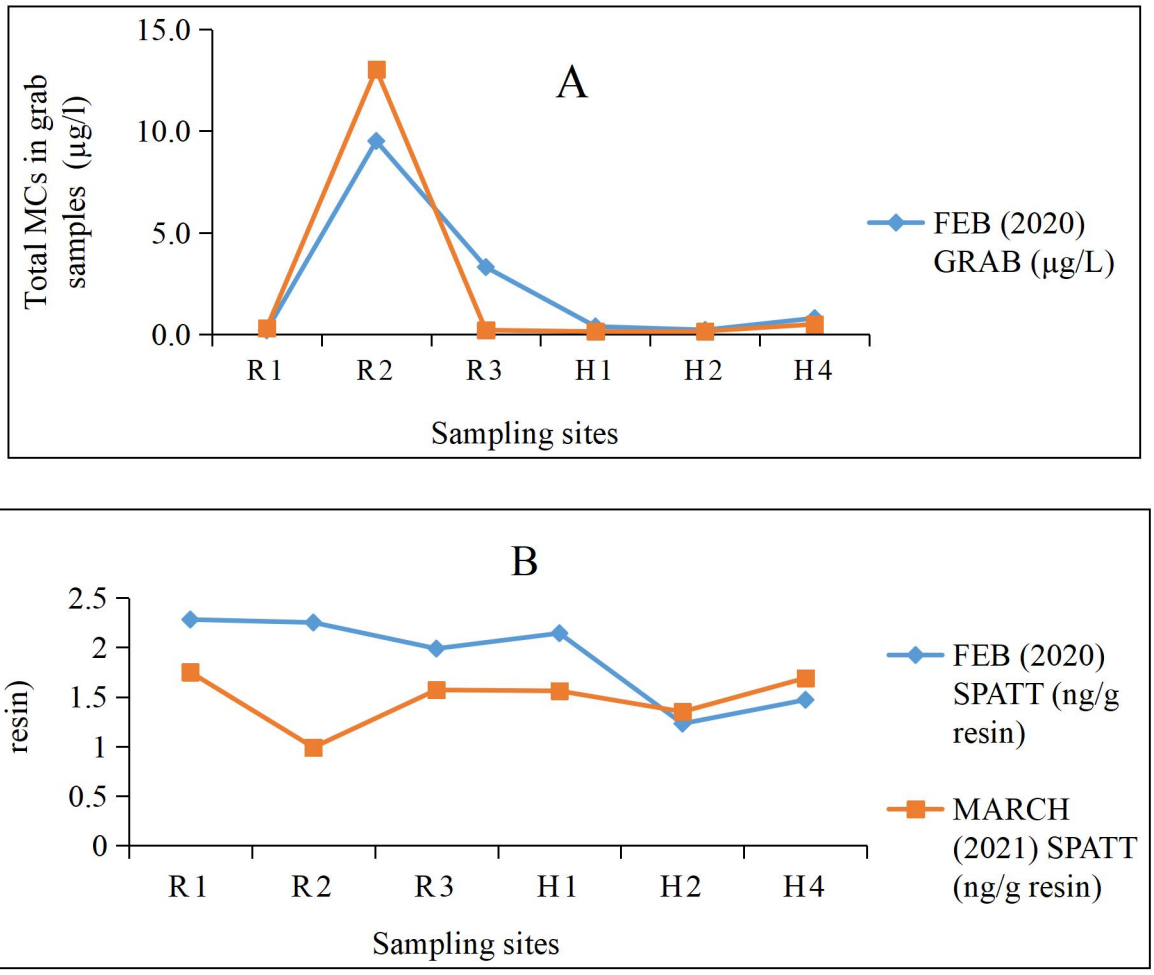


Figure 5.6 (A & B): The mean total MCs concentrations in Grab samples and SPATT bags collected from Roodeplaat and Hartbeespoort irrigation canals and farm dam's sites

The Grab samples showed lower levels of toxins because this sampling method can seriously underestimate concentrations of toxins in water column or miss the peak episodic event of the toxins in water since it gives a snapshot of toxins in water column at that point and that time (Appendix-Table E & F). SPATT bags detected MCs in the irrigation water over time, giving an integrated reflection of toxins over space and time in water bodies. This finding from this study is consistent with a study carried out by Davis and Hansen (2013) which observed that SPATT samplers could detect MCs even where grab samples could not detect. The Persistence of *microcystins* in both sites throughout the sampling periods is a cause for concern because the water is being used for irrigation purposes such as food crops, and this microcystins might accumulate in edible parts of the plants eventually posing health risks to humans via consuming the plants irrigated with cyanotoxins contaminated water.

5.5.2.2 Correlation between the environmental parameters and microcystins

The results for statistical analysis describing relationship between toxins in grab and SPATT samples with physicochemical variables are shown in Table 5.3**.** The Spearman correlation results revealed that there is no association between chlorophyll-*a* and total *microcystins* in Grab sample ($r = 0.208$, $P > 0.05$) and SPATT samples ($r = -0.441$, $P > 0.05$). These finding are not consistent with the result found by Kudela, (2011), where chlorophyll-*a* was found as the best predictor for toxins both Grab and SPATT samples in Pinto Lake. Study carried by Lehman *et al.,* (2010) also found a positive correlation between chlorophyll-*a* and *Microcystis* abundance and total *microcystins* for San Francisco Estuary.

Among all measured physicochemical parameters, only pH ($r = 0.776$ **) had strong positive relationship with *microcystins*, while turbidity ($r = 0.699$*) and dissolved oxygen ($r = 0.829$*) had moderate positive association with MCs levels in Grab samples. Thus, suggesting that all these 3 physicochemical parameters play a role in influencing the production of *microcystins* in water bodies. With regards to total dissolved toxins in SPATT samples, only total dissolved solids and electrical conductivity had a moderate negative association with the toxins detected in SPATT samplers, while other physicochemical parameters showed no association.

In addition, the correlation results between total dissolved MCs in SPATT samples and total *microcystins* in Grab samples showed that there is no association between the two-sampling method. These findings are not consistent with the study carried by Kudela (2011) were total *microcystins* in

Grab and SPATT samples were found to have a good correlation (Spearman r = 0.735, p < 0.001) in Pinto Lake.

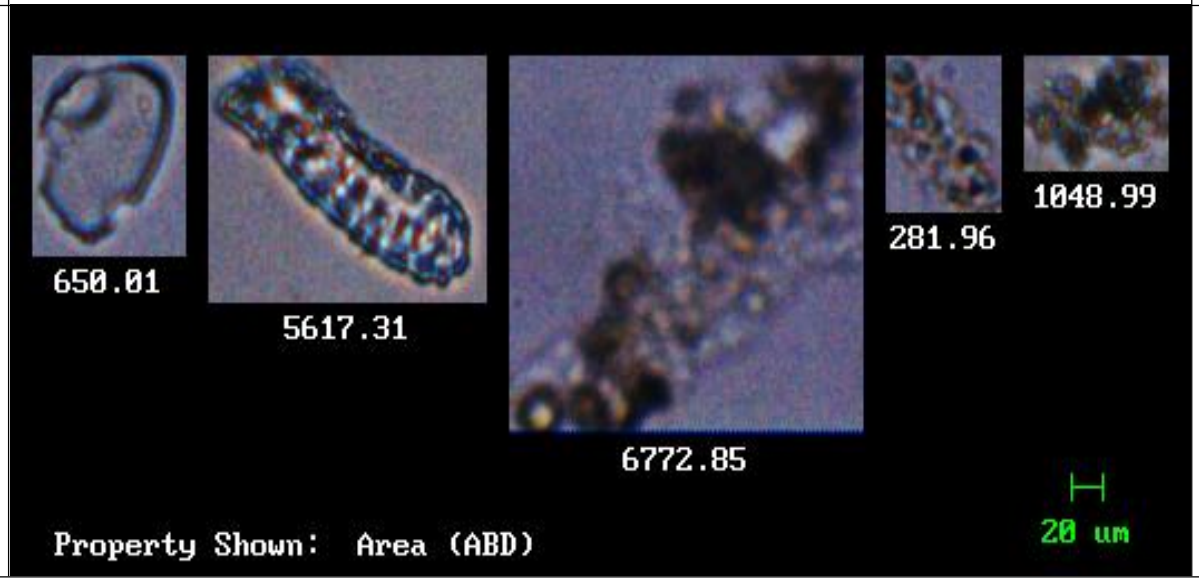
Table 5.2: Spearman correlation coefficient (r) describing relationship between total *microcystins* in Grab and SPATT samples with physicochemical parameters.

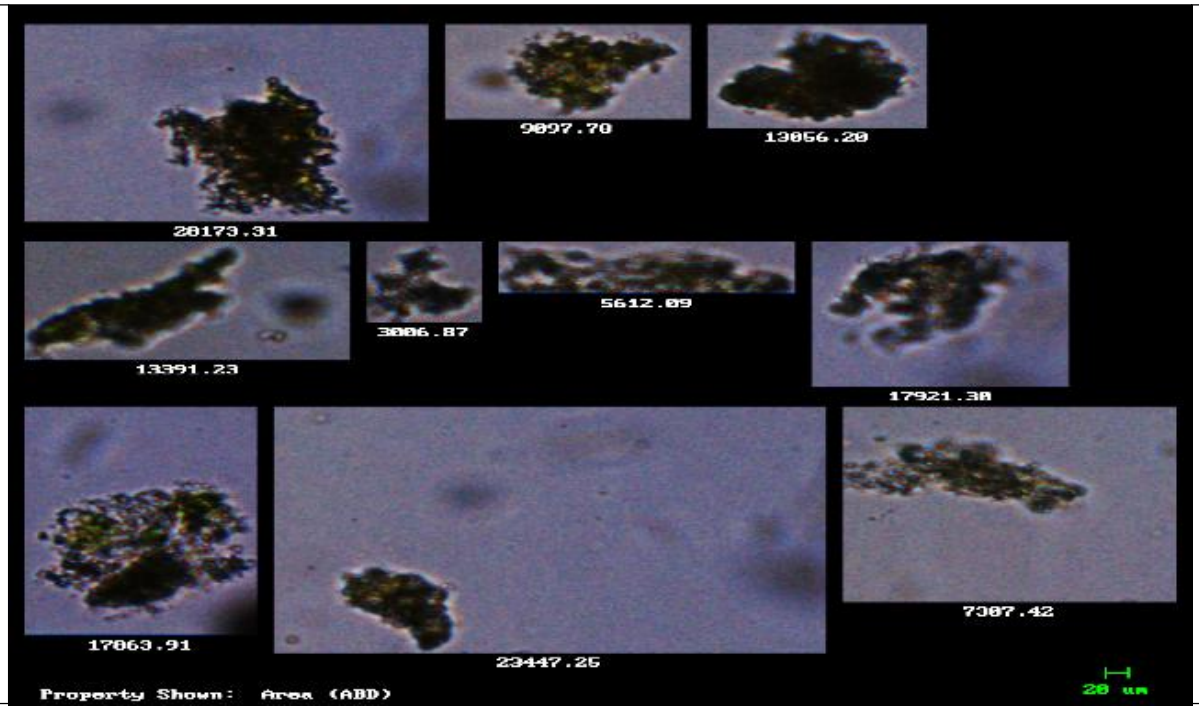
	SPATT (ng/g)		Grab (µg/L)	
Physicochemical variables	Correlation (r)	P-Value	Correlation	p-Value
pH	0.098	0.762	0.776**	0.003
TDS (mg/L)	-0.615*	0.033	-0.462	0.131
EC (µs/cm)	-0.602*	0.038	-0.462	0.130
Temperature (˚C)	0.308	0.331	0.133	0.681
Turbidity (NTU)	-0.196	0.542	0.699*	0.011
Microcystins SPATT (ng/g resin/day)			0.049	0.880
Microcystins Grab (µg/L)	0.049	0.880		
Nitrate (mg/L)	0.280	0.379	-0.231	0.471
Phosphate (mg/L)	-0.402	0.195	-0.049	0.879
Chlorophyll-*a* (µg/L)	-0.441	0.152	0.392	0.208
DO (mg/L)	0.371	0.468	0.829*	0.042

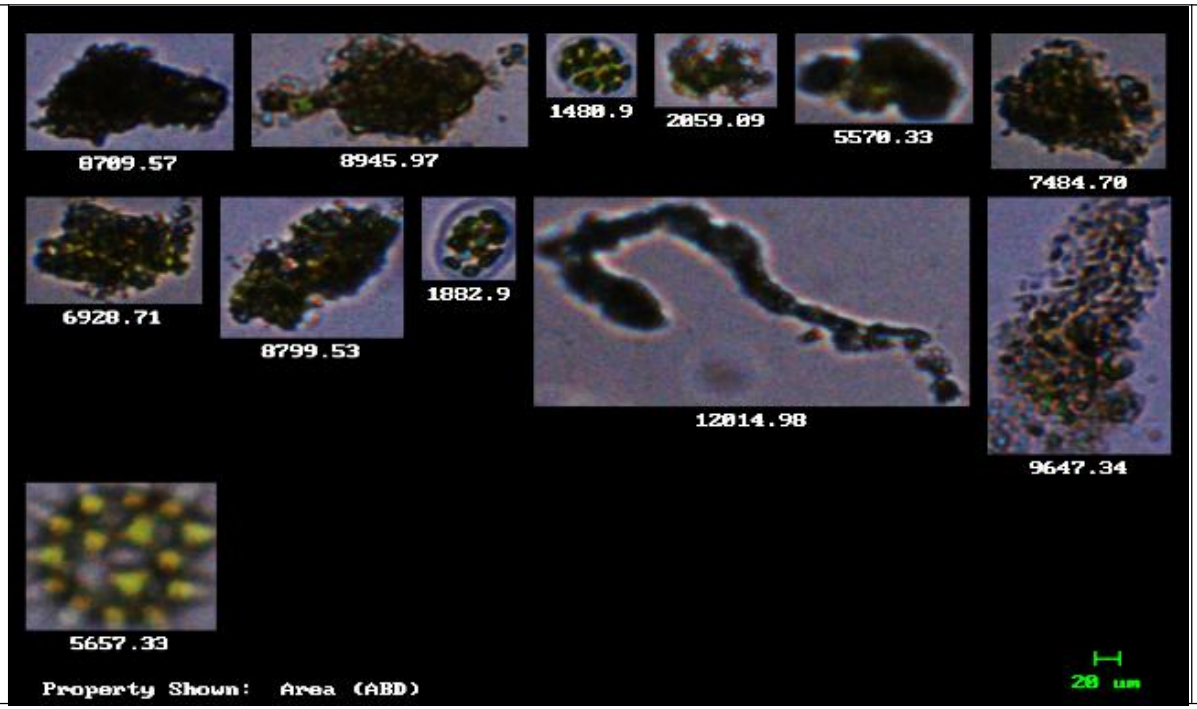
5.5.3 Composition and identification of cyanobacteria in irrigation water

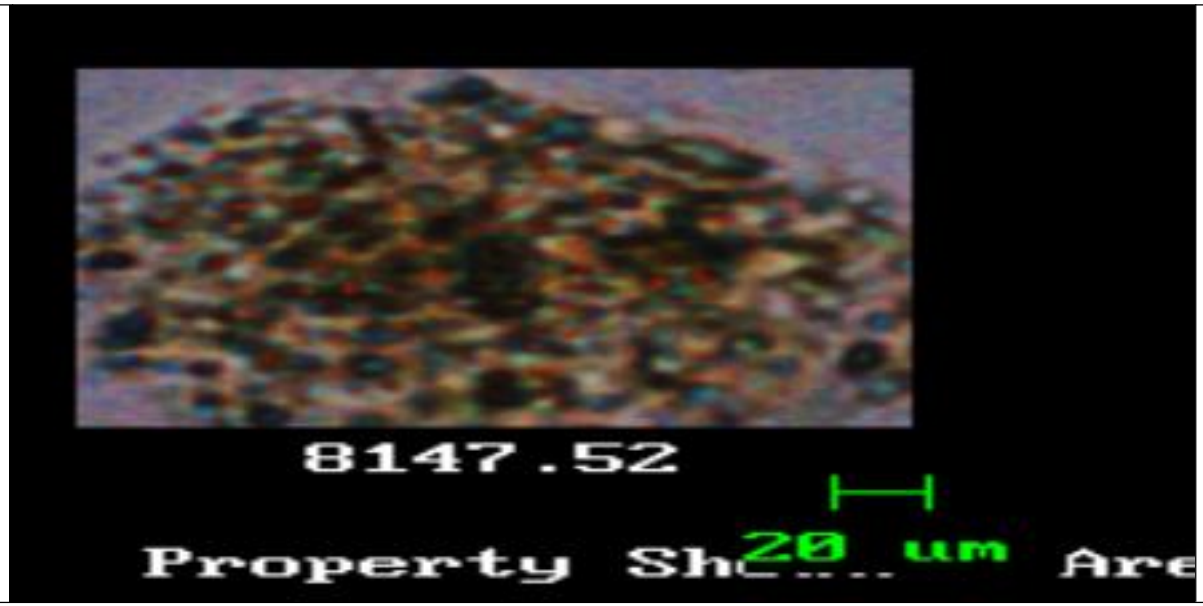
The composition of various cyanobacterial species in water collected from irrigation canals and farm dams in Hartbeespoort and Roodeplaat sites in March 2021 was determined. The various cyanobacteria species were identified in the irrigation water using a Benchtop Flow-Cam (Model US-IV). The following harmful cyanobacterial species were found across the sampling sites, namely: *microcystis*, *anabaena* and *oscillatoria* as shown in Tables 5.3 and 5.4, respectively. The *Microcystis genus* was the most abundant and the most frequently observed across all sampling sites, demonstrating that this specie is the most widespread in freshwater ecosystems. The results observed calls for concern because *microcystis* are the most common bloom forming, more poisonous and have potential to produce high levels of *microcystins* which accumulate in food crops via irrigation (Corbel *et al.,* 2016; Machado *et al.,* 2017; Preece *et al.,* 2017). Acute and chronic exposure to *microcystins* can promote tumor, and cause liver failure (Huo *et al.,* 2018; Greer *et al.,* 2018). The water collected and analyzed from the sampling sites are meant for irrigating food crops, thus posing human health risks via consuming crops which have accumulated the toxins.

Table 5.3: The different composition of harmful cyanobacteria in irrigation canals and farm dams of Hartbeespoort sites.

Sampling sites	Images of harmful cyanobacteria	Species	References
H1		(6772.85; 281.96; 1048.99) *Microcystis*; (5617.31) *Dinoflagelate*; (650.01) *Anabaena*	Huynh and Serediak, 2011. Tshifura, 2018

H2		*Microcystis*	Tshifura, 2018



H3		(8147.52) *Microcystis*	Tshifura, 2018

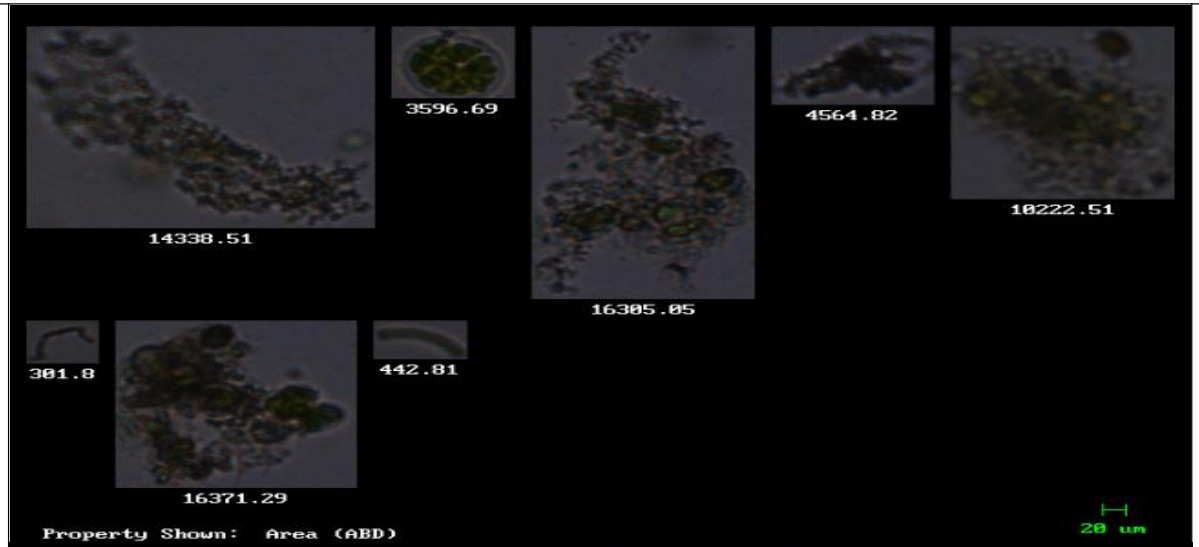
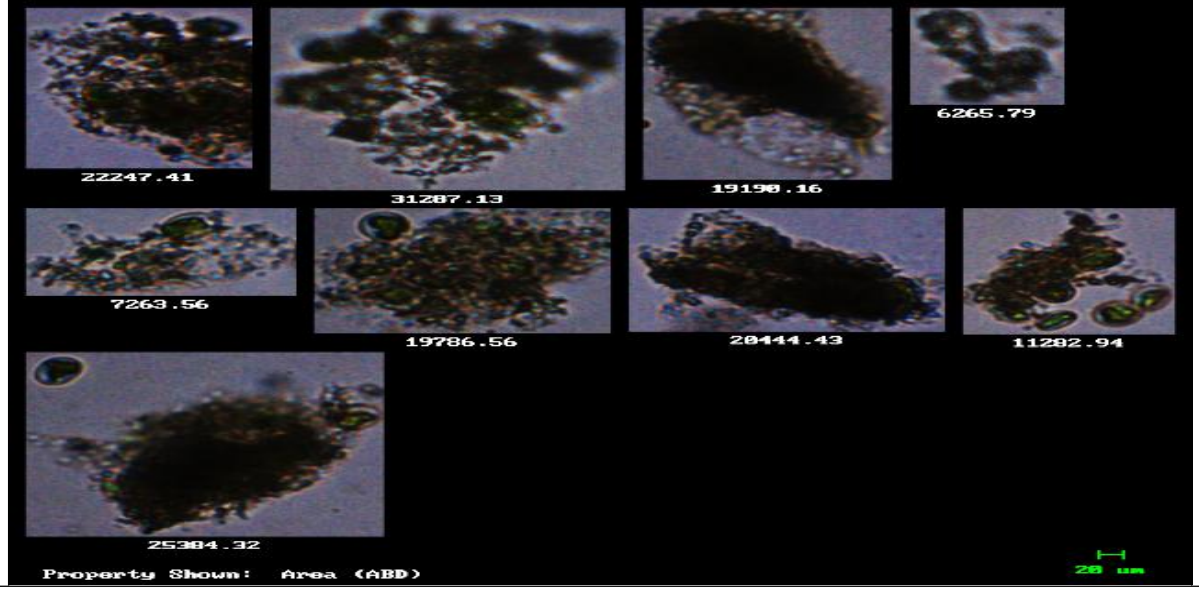
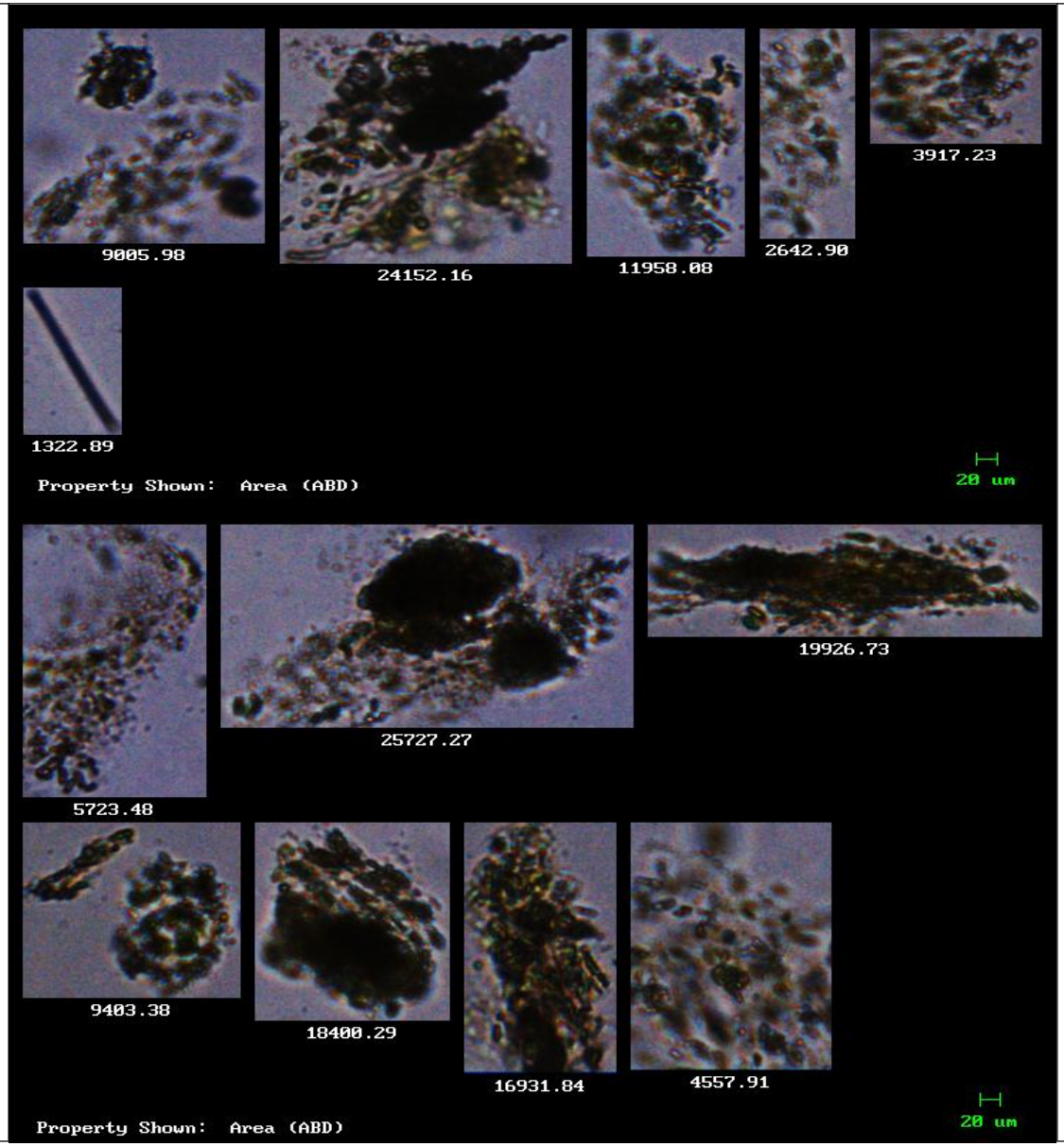
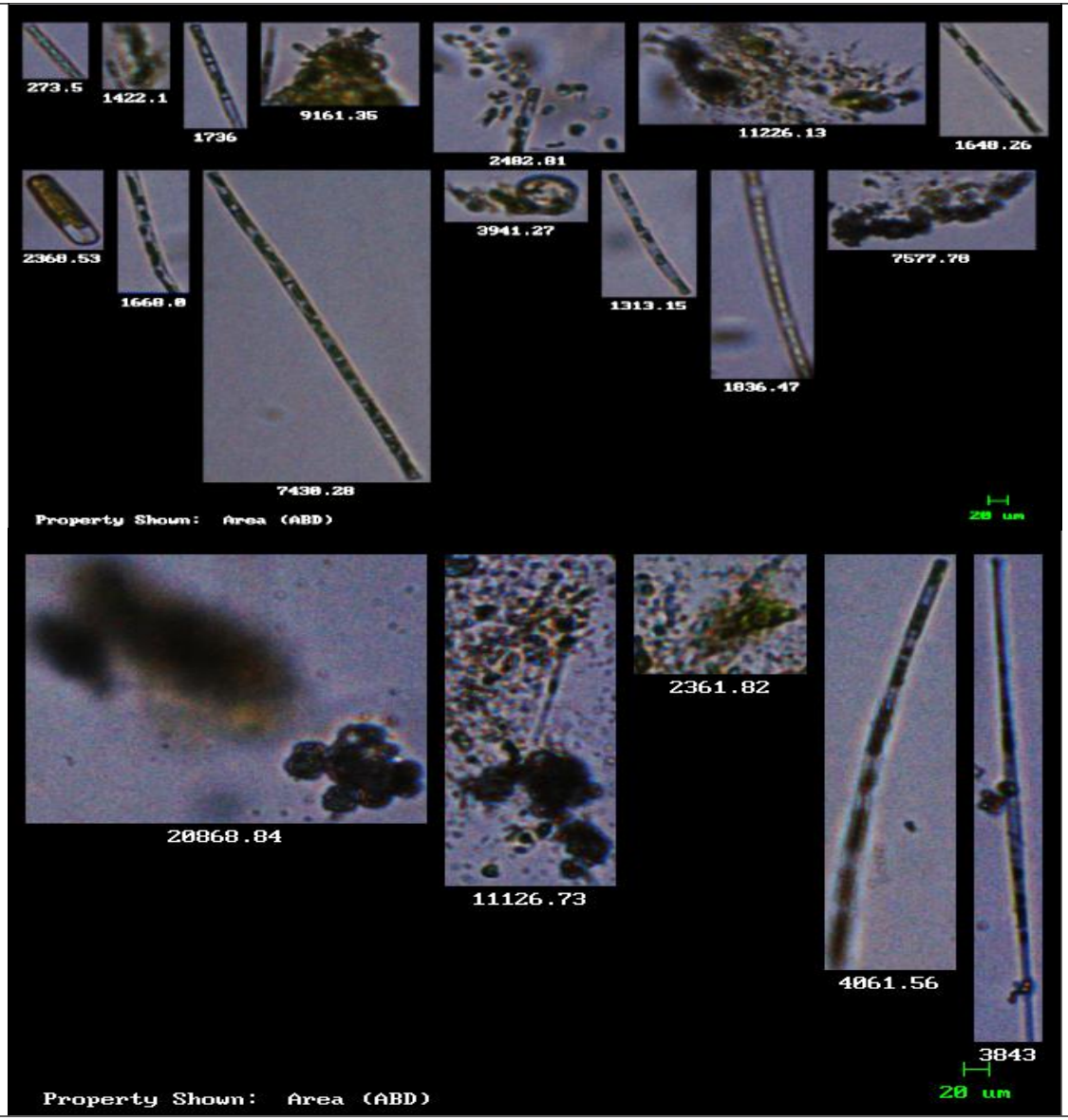
H4	 	*Microcystis*; (301.8) *Anabaena*; (442.81) *Oscillatoria*	Tshifura, 2018. Van Vureen *et al.,* 2006

Table 5.4: The different composition of harmful cyanobacteria in irrigation canals and farm dams in Roodeplaat sites

Sampling sites	Images of harmful cyanobacteria	Species	References
R1		(13701.48; 13206.07) *Microcystis*; (3003.47; 5439.92; 1765.34) *Anabaena*	Van Vureen *et al.,* 2006 Tshifura, 2018

R2		(9005.90; 24152.16; 11968.88; 2642.90; 9917.23; 5723.40; 25727.27; 19926.73; 9403.38; 18400.29; 16931.04; 4557.91) *Microcystis*; (1322.89) *Oscillatoria*	Tshifura, 2018

R3		(1422.1; 9161.35; 2482.81; 11226.13; 11126.73; 2361.82; 20068.84) *Microcystis*; (273.5; 1736; 1648.26; 1668.0; 7430.28; 3941.27; 1313.15; 1836.47; 4861.56; 3843) *Anabaena*; (2368.53) *Oscillatoria*	Van Vureen *et al.,* 2006 Tshifura, 2018

5.6 Conclusions

The laboratory experiment revealed that SPATT bags were saturated within 2 days (48 hours). The obtained results were used as a guide to deploy SPATT bags in the field. The SPATT bags proved to be applicable in monitoring and detecting microcystins in irrigation canals and farm dams of Roodeplaat and Hartbeespoort using DIAON HP20 resin as an adsorbent. The SPATT bags and Grab sampling methods revealed the presence of *microcystins* in all sampling sites throughout the sampling period suggesting that both sampling methods can be applied in monitoring and detecting *microcystins*. The different types of harmful cyanobacteria in irrigation water, such as *microcystis*, *anabaena* and *oscillatoria* were identified, with *microcystis genus* being the most dominant and widespread across all sampling sites. The persistence of *microcystins* within the study area is of great concern since they can be transferred into food crops via irrigation, and bio-accumulate, eventually posing major risks to human health through consumption of MCs contaminated crops.

References

Altaner, S., Puddick, J., Fessard, V., Feurstein, D., Zemskov, I., Wittmann, V. and Dietrich, D.R., 2019. Simultaneous detection of 14 microcystin congeners from tissue samples using UPLC-ESI-MS/MS and two different deuterated synthetic microcystins as internal standards. Toxins, 11(7), p.388.

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J. and Miles, C.O., 2013. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Marine and Freshwater Research, 65(2), pp.175-189.

Beversdorf, L.J., Rude, K., Weirich, C.A., Bartlett, S.L., Seaman, M., Kozik, C., Biese, P., Gosz, T., Suha, M., Stempa, C. and Shaw, C., 2018. Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more than microcystin. Water research, 140, pp.280-290.

Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E. and Funari, E., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of toxicology, 91(3), pp.1049-1130.

Cao, Q., Alan, D., Steinman, A.D., Wan, X. and Xie, L., 2018. Bioaccumulation of microcystin congeners in soil-plant system and human health risk assessment: a field study from Lake Taihu region of China. Environmental Pollution, 240, 44– 50.

Catherine, A., Bernard, C., Spoof, L. and Bruno, M., 2017. Microcystins and nodularins. Handbook of cyanobacterial monitoring and cyanotoxin analysis, 1, pp.107-126.

Chen, X., Jiang, H., Sun, X., Zhu, Y. and Yang, L., 2016. Nitrification and denitrification by algae-attached and free-living microorganisms during a cyanobacterial bloom in Lake Taihu, a shallow Eutrophic Lake in China. Biogeochemistry, 131(1), pp.135-146.

Cheung, M.Y., Liang, S. and Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. Journal of Microbiology, 51(1), pp.1-10.

Conradie, K.R. and Barnard, S., 2012. The dynamics of toxic Microcystis strains and microcystin production in two hypertrofic South African reservoirs. Harmful Algae, 20, pp.1-10.

Corbel, S., Mougin, C., Nélieu, S., Delarue, G. and Bouaïcha, N., 2016. Evaluation of the transfer and the accumulation of microcystins in tomato (Solanum lycopersicum cultivar MicroTom) tissues using a cyanobacterial extract containing microcystins and the radiolabeled microcystin-LR (14C-MC-LR). Science of the Total Environment, 541, pp.1052-1058.

Davis, S. and Hansen, C., 2013. Blue-Green Algae Toxin Monitoring and Response Project Final Project Report February 2013.

Department of Water Affairs. 2011. Directorate water resource planning systems: water quality planning. Resource directed management of water quality. Planning Level Review of Water Quality in South Africa. Sub-series No. WQP 2.0. Pretoria, South Africa.

DWAF. 1996. Draft of South African water quality guidelines. Vol. 7. Aquatic Ecosystem. Department of Water Affairs and Forestry Pretoria.

Fontanillo, M. and Köhn, M., 2018. Microcystins: Synthesis and structure–activity relationship studies toward PP1 and PP2A. Bioorganic & medicinal chemistry, 26(6), pp.1118-1126.

Gaget, V., Lau, M., Sendall, B., Froscio, S. and Humpage, A.R., 2017. Cyanotoxins: which detection technique for an optimum risk assessment? Water research, 118, pp.227-238.

Greer, B., Meneely, J.P. and Elliott, C.T., 2018. Uptake and accumulation of Microcystin-LR based on exposure through drinking water: An animal model assessing the human health risk. Scientific reports, 8(1), pp.1-10.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A. and Paerl, H.W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. Harmful algae, 54, pp.4-20.

Hattenrath-Lehmann, T.K., Lusty, M.W., Wallace, R.B., Haynes, B., Wang, Z., Broadwater, M., Deeds, J.R., Morton, S.L., Hastback, W., Porter, L. and Chytalo, K., 2018. Evaluation of rapid,

early warning approaches to track shellfish toxins associated with Dinophysis and Alexandrium blooms. Marine drugs, 16(1), p.28.

Howard M.D.A., Nagoda C., Kudela R.M., KayashI K., Tatters A.O., Caron D.A., Brusse L., Brown J., Sutula M.A., and Stein E.D., 2017. Microcystin prevalence throughout lentic waterbodies in coastal Southern California. Toxins 9(231): 1-21.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M. and Visser, P.M., 2018. Cyanobacterial blooms. Nature Reviews Microbiology, 16(8), pp.471-483.

Huo, D., Chen, Y., Zheng, T., Liu, X., Zhang, X., Yu, G., Qiao, Z. and Li, R., 2018. Characterization of Microcystis (Cyanobacteria) genotypes based on the internal transcribed spacer region of rRNA by next-generation sequencing. Frontiers in microbiology, 9, p.971.

Kansas department of health and environment (KDHE)., 2011. Water Quality Standards White Paper. Chlorophyll-*a* Criteria for Public Water Supply Lakes or Reservoirs. Kansas Department of Health and Environment, Bureau of Water. https://www.kdheks.gov/water/download/tech/Chlorophylla_final_Jan27.pdf

Kudela R.M, 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae, 11. 117-125.

Kudela, R.M., 2017. Passive sampling for freshwater and marine algal toxins. Comprehensive Analytical Chemistry. Elsevier Ltd, Amsterdam, pp.379-409.

Lane, J.Q., Roddam, C.M., Langlois, G.W. and Kudela, R.M., 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods, 8(11), pp.645-660.

Lawton, L., B. Marsalek, J. Padisák, and I. Chorus. 1999. Determination of cyanobacteria in the laboratory, p. 347-367. In I. Chorus and J. Bartram (eds.), Toxic Cyanobacteria in Water. E & FN Spon, London, UK.

Lee, S., Jiang, X., Manubolu, M., Riedl, K., Ludsin, S.A., Martin, J.F. and Lee, J., 2017. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. Food research international, 102, pp.234-245.

Lehman, P.W., Teh, S.J., Boyer, G.L., Nobriga, M.L., Bass, E. and Hogle, C., 2010. Initial impacts of Microcystis aeruginosa blooms on the aquatic food web in the San Francisco Estuary. Hydrobiologia, 637(1), pp.229-248.

Li, F.L., Li, Z.X., Guo, M.M., Wu, H.Y., Zhang, T.T. and Song, C.H., 2016. Investigation of diarrhetic shellfish toxins in Lingshan Bay, Yellow Sea, China, using solid-phase adsorption toxin tracking (SPATT). Food Additives & Contaminants: Part A, 33(8), pp.1367-1373.

Machado J., Campos A., Vasconcelos V., and Freitas M., 2017. Effects of microcystin-LR and cylindrospermopsin on plant-soil systems: a review of their relevance for agricultural plant quality and public health. Environmental Research, 153: 191–204.

Mackenzie, L., Beuzenberg, V., Holland, P., McNabb, P. and Selwood, A., 2004. Solid phase adsorption toxin tracking (SPATT): a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves. Toxicon, 44(8), pp.901-918.

Mackenzie, L.A., 2010. In situ passive solid-phase adsorption of micro-algal biotoxins as a monitoring tool. Current Opinion in Biotechnology, 21(3), pp.326-331.

Matthews M.W., Bernard S., and Winter K., 2010. Remote sensing of cyanobacteria-dominant algal blooms and water quality parameters in Zeekoevlei, a small hypertrophic lake, using MERIS. Remote Sensing of Environment 114(9): 2070–2087.

McCarthy, M., van Pelt, F.N., Bane, V., O'Halloran, J. and Furey, A., 2014. Application of passive (SPATT) and active sampling methods in the profiling and monitoring of marine biotoxins. Toxicon, 89, pp.77-86.

Meneely J.P., and Elliott C.T., 2013. Microcystins: measuring human exposure and the impact on human health. Biomarkers, 18(8): 639–649.

Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler, M., Miller, W.A., Toy-Choutka, S., Dominik, C. and Hardin, D., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. PLoS One, 5(9), p.e12576.

Paerl, H.W., Otten, T.G. and Kudela, R., 2018. Mitigating the expansion of harmful algal blooms across the freshwater-to-marine continuum.

Peacock, M.B., Gibble, C.M., Senn, D.B., Cloern, J.E. and Kudela, R.M., 2018. Blurred lines: Multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California. Harmful Algae, 73, pp.138-147.

Pindihama G.K., and Gitari W.M., 2019. Cyanobacterial toxins: an emerging threat in South African irrigation water. Water and Environment Journal 0: 1–11.

Preece, E.P., Hardy, F.J., Moore, B.C. and Bryan, M., 2017. A review of microcystin detections in estuarine and marine waters: environmental implications and human health risk. Harmful Algae, 61, pp.31-45.

Roué, M., Darius, H.T. and Chinain, M., 2018. Solid phase adsorption toxin tracking (SPATT) technology for the monitoring of aquatic toxins: A review. Toxins, 10(4), p.167.

Saqrane S., and Oudra B., 2009. CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins, 1(2), pp.113-122.

Serediak, N. and Huynh, M., 2011. Algae Identification Field Guide: An Illustrative Field Guide on Identifying Common Algae Found in the Canadian Prairies. Agriculture and Agri-Food Canada.

Tshifura, R.A. 2018. An Assessment of Algae and cyanotoxins in small-holder Aquaculture farms in Vhembe, South Africa. PhD thesis. University of Venda.

Turton A., 2016. Water Pollution and South Africa's poor. Published by the South African Institute of Race Relations. Johannesburg, South Africa. [Online] Available from: http://irr.org.za/reports-and-publications/occasional-reports/files/water-pollution-and-south-africas-poor [Accessed: 10th May 2017]

van Vuuren, S.J., Taylor, J. and van Ginkel, C., 2006. Freshwater algae.

Zendong, Z., Bertrand, S., Herrenknecht, C., Abadie, E., Jauzein, C., Lemée, R., Gouriou, J., Amzil, Z. and Hess, P., 2016. Passive sampling and high-resolution mass spectrometry for chemical profiling of French coastal areas with a focus on marine biotoxins. Environmental science & technology, 50(16), pp.8522-8529.

Zhu, J., Ren, X., Liu, H. and Liang, C., 2018. Effect of irrigation with microcystins-contaminated water on growth and fruit quality of Cucumis sativus L. and the health risk. Agricultural Water Management, 204, pp.91-99.

Chapter 6: Conclusions and Recommendations

6.1 Introduction

This chapter focuses on concluding and giving remarks for all the findings from the completed chapters. It also gives recommendations or solutions to what ought to be done going forward based on the findings from each chapter.

6.2 Conclusions

The study investigated the co-existence of cyanotoxins, metal species and anionic surfactants in irrigation water canals, farm dams, and agricultural soils in Roodeplaat and Hartbeespoort. Also, the relationship between microcystins levels with physicochemical parameters in irrigation water were investigated. It was revealed that there is co-existence of cyanotoxins, metal species and anionic surfactants in irrigation water canals, farm dams, and agricultural soils in all sites during the sampling period. It also emerged that only pH, turbidity, EC, and TDS had a relationship with concentrations of MCs levels in irrigation water. Furthermore, *microcystins* levels across the sampling month did not vary significantly statistically. However, MCs levels across the sampling sites varied significantly. The study also found that metal species levels in irrigation water were below the DWAF recommended threshold while in agricultural soils, metals like Cr, Ni, Cu, Pb, and As were above the guideline values set for agricultural soils.

The results showed that microcystins and metals accumulate in food crops through irrigation water and their calculated estimated daily intake (EDI) were below the World Health Organization (WHO) guideline value 0.04 µg/kg. Thus, plants in these sites are safe for consumption by adult and children population. For metals in food crops, only Cr, Fe, Cu, Zn, As, and Pb concentrations were above the EU and FAO/WHO threshold in food crops. However, the calculated EDI for each metal were all below the maximum tolerable daily intake (MTDI). Hence, food crops were safe for consumption by adult population. The THQ revealed no health risks for each metal which accumulated in the food crops, except for Cu from a plant collected from sampling site S1, S4 and S5. Thus, there is a potential health risks to adult population. The study also showed that SPATT method was applicable in detecting and monitoring microcystins in irrigation water. The identified cyanotoxins, showed the presence of *microcystis, anabaena* and *oscillatoria* in the irrigation water, with *microcystis* being the most dominant throughout the sampling sites. The presence of anionic surfactants, metals species and cyanotoxins in irrigation water and agricultural soils is a health hazard to humans and animals via consumption of contaminated plants irrigated with such water.

Hence, there is a need to monitor, manage and control the co-existence of cyanotoxins, metal species and anionic surfactants in irrigation water canals, farm dams and agricultural soils in Roodeplaat and Hartbeespoort.

6.3 Recommendations

Based on the findings the following recommendations can be made:

- This study is therefore recommending regular monitoring of *microcystins*, metals and physicochemical parameters in the irrigation water, and analysis of MCs ad metal species accumulation in edible crops, to prevent potential health risks to consumers.
- Studies looking at the bio-accumulation of *microcystins* in humans via consumption of infested MCs food crops
- Raise awareness among water users (farmers) about cyanotoxins, the potential impacts and risks they may have on irrigated crops and consumers.
- Educate water users (farmers) through awareness campaigns that help them to determine when an irrigation water source may be at risk of containing cyanotoxins
- Policy makers should come up with guideline standard for total *microcystins* in water meant for irrigating food crops to prevent bio-accumulation of MCs in food crops meant to be consumed by humans
- Further studies, on deployment of SPATT bags for longer period in different seasons for seasonal monitoring of MCs in irrigation water

6.4. Outputs

- The virtual poster presentation for AQUA=360: Water for all - Emerging issues & innovations, University of Exeter UK.
- Sathekge, SN. Pindihama, GK. Gitari, WM. 2021. Assessment of co-occurrence of cyanotoxins, metals, and anionic surfactants in irrigation water and agricultural soils. Poster session presented at: AQUA=360: Water for all emerging issues & innovations; 2021 31st of August to 2nd of September; University of Exeter United Kingdom

Appendices

Table A: Physicochemical parameters, chlorophyll-*a*, *microcystins* and anionic surfactants data recorded in Hartbeespoort and Roodeplaat irrigation canals.

		pH	TDS (mg/L)	EC (µs/cm)	Temp (°C)	Turbidity (NTU)	DO (mg/L)	Nitrates (mg/L)	Phosphates (mg/L)	Microcystins (µg/L)	Anionic surfactant (mg/L)	Chlorophyll-a (µg/L)
R1	Jun-19	7.51	549.00***	918.00***	15.10			ND	1.28	2.84**	0.51	208.20**
	Sep-19	9.92**	228.00	384.00	15.60	0.77	8.92	1.90	1.70	0.99	0.15	0.70
	Feb-20	8.91**	169.20	285.00	23.30	8.62	7.89	7.83	0.44	0.20	0.07	48.20**
	Mar-21	7.82	266**	438**	21.5	4.50		2.7	0.7	0.31	0.19	49.97**
R2	Jun-19	7.48	540.00***	890.00***	16.00			0.40	1.20	0.84	0.31	4.00
	Sep-19	9.80**	236.00	399.00	22.20	3.77	8.28	1.70	1.00	0.80	2.00	2.20
	Feb-20	10.59***	190.00	312.00	32.80	588.85	16.20	7.38	0.20	62.26**	3.43	171.84**
	Mar-21	9.53**	223	371	20.8	143.2		1.9	0.2	84.94**	1.64	440.24**
R3	Jun-19	8.93**	498.00***	828.00***	15.00			ND	0.50	1.99**	0.59	109.70**
	Sep-19	10.36***	221.00	366.00	20.20	22.91	21.08	1.05	0.90	0.69	0.07	146.60**
	Feb-20	10.55***	176.80	293.00	29.90	57.55	15.51	4.92	0.20	3.30**	0.22	302.67**
	Mar-21	9.02**	228	380	22.0	8.33		7.6	0.5	0.21	0.15	373.68**
H1	Jun-19	6.29	663.00***	990.00***	14.10			2.70	0.76	0.12	0.42	24.50
	Sep-19	7.49	230.00	378.00	15.30	1.41	8.98	5.10	1.05	0.20	0.04	6.70
	Feb-20	7.61	262.00***	440.00***	23.00	2.53	3.64	8.30	1.05	0.39	0.04	82.25**
	Mar-21	7.55	357**	599**	23.4	0.79		4.0	0.9	0.14	0.26	10.74

H2	Jun-19	7.24	604.00***	987.00***	11.90			ND	0.08	0.12	0.29	402.40**
	Sep-19	8.22	270.00***	453.00***	18.50	12.90	14.17	6.30	0.70	0.19	0.22	34.10**
	Feb-20	8.22	435.00***	701.00***	27.30	75.81	2.37	27.50	0.40	0.22	0.10	270.38**
	Mar-21	8.14	429**	713**	22.3	22.35		9.4	0.6	0.16	0.27	423.51**
H3	Jun-19	7.80	558.00***	947.00***	17.90			3.00	0.70	0.17	0.41	27.40
	Sep-19	8.21	242.00	395.00	15.80	0.93	9.53	5.80	1.10	0.13	0.01	1.50
	Feb-20	7.45	244.00	414.00**	22.60	3.01	3.03	5.30	1.00	0.33	0.05	38.35**
	Mar-21	6.62	365**	607**	22.6	0.99		2.3	0.9	0.14	0.16	17.70
H4	Jun-19	7.81	974.00***	1545.00***	16.40			0.05	0.90	0.12	0.10	160.50**
	Sep-19	9.01**	286.00***	462.00***	19.50	11.04	8.89	4.90	0.85	0.34	0.40	3.00
	Feb-20	9.21**	265.00***	440.00***	29.30	35.49	13.08	6.20	0.50	0.80	0.14	148.96**
	Mar-21	9.34**	289**	483**	22.5	50.32		6.2	0.2	0.50	3.49	1246.94**

#: ** indicate concentration levels above the guideline standard.

Table B: Metal species data (N = 49) recorded in Hartbeespoort and Roodeplaat irrigation canals (mg/L).

		B (mg/L)	Al (mg/L)	Mn (mg/L)	Ni (mg/L)	Cu (mg/L)	Zn (mg/L)	As (mg/L)	Pb (mg/L)	Fe (mg/L)	Cr (mg/L)
June (2019)	R1	0.065	1.022	0.355	0.010	0.010	0.170	0.004	0.004	0.826	0.007
	R2	0.060	0.023	0.370	0.005	0.004	0.053	0.001	0.003	0.141	0.002
	R3	0.052	0.082	0.045	0.003	0.003	0.026	0.001	0.004	0.186	0.003
	H1	0.045	0.060	0.160	0.004	0.010	0.065	0.002	0.060	0.371	0.004
	H2	0.040	0.050	0.085	0.004	0.006	0.006	0.002	0.006	0.065	0.002
	H3	0.055	0.728	0.129	0.007	0.008	0.050	0.002	0.010	0.692	0.01
	H4	0.045	0.025	0.120	0.004	0.010	0.050	0.001	0.008	0.225	0.003
September (2019)	R1	0.068	0.969	0.170	0.008	0.007	0.070	0.001	0.001	0.034	0.001
	R2	0.072	0.677	0.210	0.010	0.008	0.100	0.001	0.002	0.062	0.001
	R3	0.065	0.225	0.094	0.007	0.002	0.100	0.001	0.000	0.101	0.001
	H1	0.050	0.295	0.165	0.005	0.003	0.103	0.001	0.001	0.026	0.001
	H2	0.050	0.502	0.140	0.007	0.006	0.108	0.002	0.001	0.057	0.005
	H3	0.040	0.722	0.255	0.005	0.009	0.065	0.001	0.001	0.086	0.001
	H4	0.050	0.125	0.085	0.005	0.000	0.065	0.001	0.000	0.014	0.000
February (2020)	R1	0.022	0.054	0.332	0.004	0.001	0.022	0.001	0.000	0.067	0.001
	R2	0.029	0.022	0.060	0.004	0.002	0.043	0.002	0.000	0.018	0.001
	R3	0.035	0.049	0.047	0.002	0.001	0.023	0.001	0.000	0.071	0.001
	H1	0.041	0.012	1.166	0.004	0.001	0.017	0.001	0.000	0.121	0.001
	H2	0.040	0.318	0.232	0.006	0.002	0.031	0.002	0.000	0.175	0.009
	H3	0.038	0.010	1.240	0.004	0.001	0.012	0.001	0.000	0.119	0.001
	H4	0.035	0.293	0.367	0.006	0.001	0.025	0.001	0.000	0.126	0.002
March (2021)	R1	0.031	0.02	0.685	0.003	0.001	0.245	0.001	0.000	0.365	0.000
	R2	0.032	0.885	0.21	0.005	0.009	0.059	0.003	0.001	0.135	0.000

	R3	0.034	0.45	0.098	0.005	0.004	0.0095	0.001	0.000	0.055	0.000
	H1	0.036	0.02	0.81	0.002	0.001	0.09	0.002	0.000	0.07	0.000
	H2	0.039	0.45	0.15	0.004	0.004	0.07	0.002	0.001	0.03	0.000
	H3	0.036	0.015	0.808	0.003	0.000	0.03	0.002	0.000	0.045	0.000
	H4	0.038	1.25	0.215	0.014	0.012	0.045	0.005	0.001	0.155	0.000

Table C: Total anionic surfactants in agricultural soils (N = 32) data recorded in Roodeplaat and Hartbeespoort cropping sites (mg/kg).

Sampling sites	Jun (2019) (mg/kg)	Sept (2019) (mg/kg)	Feb (2020) (mg/kg)	March (2021) mg/kg
S1	2.19	2.33	1.72	5.13
S2	1.34	0.91	1.32	8.73
S3	1.84	2.52	3.48	2.2
S4	1.36	2.58	4.63	5.17

Table D. Levels of metal species (N = 56) in agricultural soils samples collected from Roodeplaat and Hartbeespoort Farmland sites (mg/kg)

Sampling sites	Sampling months	Cr (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
R1 (ARC)	Jun (2019)	125.79	32.98	25.75	43.59	11.46	0.04	25.17	0.3	1046.46	36110.39
	Sep (2019)	224.23	91.81	18.33	31.76	0.53	0.03	1.89	0.03		
	Feb (2020)	135.84	24.07	18.22	29.91	5.33	0.02	14.61	0.02	807.06	31660.97
	Mar 2021	87.5	29.6	25.1	86.5	7.65	0.045	8.25	0.018	982	35560
R2 (Agric)	Jun (2019)	128.5	32.46	24.43	42.87	10.22	003	23.26	0.27	983.88	35910.17
	Sep (2019)	94.37	71.74	23.16	64.27	329	0.06	6.52	0.05		
	FEB (2020)	158.88	26.88	20.15	33.33	5.75	0.02	16.2	0.02	881.06	41290.68
	Mar (2021)	89,75	39.8	29.85	46.9	4.45	0.045	19.5	0.026	2715	34665
H1 (Weir H3)	Jun (2019)	144.25	87.57	18.99	39.16	0.92	0.05	6.40	0.27	562.9	21997.07
	SEP (2019)	212.19	69.39	53.15	66.13	12.53	0.03	31.79	0.04		
	Feb (2020)	111.07	76.59	17.27	37.19	1.18	0.05	7.62	001	734.66	22240.13
	Mar (2021)	225.00	80.9	31.45	37.25	0.75	0.05	3.45	0.013	459.85	22870
H2 (H4 Farm)	Jun (2019)	145.52	88.80	19.12	38.65	0.81	0.06	7.83	0.26	655.38	23249.12
	SEP (2019)	220.85	70.99	54.15	65.79	13.43	0.06	32.20	0.04		
	Feb (2020)	320.29	101.50	17.90	29.26	9.30	0.04	5.45	0.02	407.18	17064.53
	March (2021)	124.2	89.2	36.5	45.7	1.15	0.06	5.35	0.009	1134	26195

Table E: Levels of *microcystins* detected from SPATT bags and GRAB sampling from Roodeplaat sites collected in February 2020

Roodeplaat sites	FEBRUARY, 2020		MARCH, 2021	
	GRAB	SPATT	GRAB	SPATT
R1A	0.19	2.222	0.18	1.73
R1B	0.21	2.334	0.44	1.77
R2A	9.58	2.36	10.48	1.79
R2B	9.43	2.148	15.57	0.19
R3A	3.08	1.976	0.2	1.73
R3B	3.53	1.998	0.21	1.41

and March 2021

Table F: Levels of *microcystins* detected from SPATT and Grab sampling method, collected from Hartbeespoort sites in Feb 2020, and Mar 2021

HARTBEESPOORT SITES	FEBRUARY, 2020		MARCH, 2021	
	GRAB	SPATT	GRAB	SPATT
H1A	0.36	2.148	0.12	1.37
H1B	0.42	2.136	0.16	1.75
H2A	0.31	0.724	0.18	1.56
H2B	0.12	1.73	0.13	1.14
H4A	0.8	1.348	0.51	1.78
H4B	0.79	1.584	0.48	1.6