OCCURRENCE OF GLYPHOSATE AND AMPA IN SOUTH AFRICAN SURFACE WATER RESOURCES

Report to the
Water Research Commission

by

S Horn, R Pieters, L Bredenhann, I Horak, W Malherbe

North-West University, Unit for Environmental Sciences and Management

WRC Report No. 3107/1/23
ISBN 978-0-6392-0565-6

December 2023

Obtainable from
Water Research Commission
Lynnwood Bridge Office Park, Bloukrans Building
4 Daventry Street
Lynnwood Manor
PRETORIA

This is the final report of WRC project no. C2020/2021-00195.

EXECUTIVE SUMMARY

BACKGROUND

The use of agrochemicals plays a crucial role in boosting crop production and reducing pests. South Africa is the greatest user of pesticides in sub-Saharan Africa and current-use pesticides – in contrast to the historically adopted persistent organochlorines, which are not biodegradable – are continuously employed in ever-growing amounts and can cause environmental contamination (Carvalho, 2017). One example of such a pesticide is the herbicide Roundup®, with the active ingredient glyphosate, N-(phosphonomethyl)glycine glyphosate (GLY), and its main metabolite, aminomethylphosphonic acid (AMPA).

Pesticides with glyphosate as their active ingredient are referred to as glyphosate-based herbicides (GBHs) and are broad-spectrum, non-selective, post-emergent herbicides used for nearly all agricultural systems including forestry, aquatic weed control (Horn et al., 2019; Meftaul et al., 2020; Saunders & Pezeshki, 2015; Szekacs & Darvas, 2012; Vera et al., 2010), as well as in commercial enterprises and domestic gardens. Glyphosate-based formulations represent the most globally applied herbicides and are approved for use in at least 130 countries (Mink et al., 2011). They are also the most used herbicides in South Africa (Gouse, 2014). The overall global application of glyphosate, for all purposes – both agricultural and non-agricultural – increased more than 12 times in two decades, from about 67 000 tonnes in 1995 to 826 000 tonnes in 2014 (Benbrook, 2016). The development of Roundup-Ready® crops has contributed to the high usage of Roundup® because farmers can spray larger quantities of glyphosate-containing herbicides, increase application rates, as well as spray them on the plants during the growing season while leaving the crops unharmed (Benbrook, 2012).

Glyphosate is claimed to be environmentally benign because it tightly binds to the soil, making it immobile with no soil activity and limited persistence (Reddy, 2001). There are however concerns whether this statement holds true as glyphosate may enter and pollute the rivers and dams where it may be harmful to aquatic organisms. Many countries across the world have quantified the levels of glyphosate in various water sources. However, no studies have ever been conducted to investigate the concentrations of glyphosate, and its main metabolite AMPA, in South African water sources except for the single study of Horn et al. (2019) which reported no levels when using enzyme-linked immunosorbent assays. There are also no local environmental guidelines for these compounds in the aquatic environment.

AIMS

The aims of the project were to:

1. Identify areas where large volumes of glyphosate-based herbicides are sprayed in South Africa and develop a usage map.
2. Determine the concentrations of glyphosate and AMPA in water, soil and sediment samples, at different sampling times during the planting season using analytical instrumentation.
3. Establish a method for analysis of glyphosate and AMPA at the North-West University in South Africa, which can be utilised for future monitoring; and
4. Conduct a desktop human health risk assessment about the hazards involved when exposed to glyphosate and AMPA in water resources.

METHODS

Soil, sediment and water samples were collected from agricultural areas where maize is cultivated and subsequently, glyphosate-based herbicides were sprayed. The sampling sites were chosen based on different agricultural practices from two maize-growing areas. Three non-agricultural areas were also

included to account for use of glyphosate-based-pesticides for household weed removal, and groundskeeping at a botanical garden and golf courses.

There were four sampling events where samples were collected between October 2020 and June 2021. These events were: i) before spraying (21-30 September 2020; 22 October 2020), ii) after herbicide application (16 November to 1 December 2020), iii) after a rain event (10-24 March 2021), and iv) after harvest (25 May to 8 June 2021) (end of the season). The samples were preserved until extraction and analysis commenced.

Various soil and sediment characteristics were determined such as soil texture (soil composition based on particle size), total organic carbon (%TOC) and cation exchange capacity (CEC). During sampling, water quality parameters such as pH, electrical conductivity (EC) and temperature were determined at each site. The concentration of nutrients and suspended solids in water samples was also determined.

The water, soil and sediment samples were extracted for glyphosate and AMPA. The concentrations of glyphosate in AMPA in the samples were determined by analysis of the extracts using ultra-high pressure liquid chromatograph (UHPLC) coupled to a mass spectrometer (MS/MS).

A human health risk assessment was done investigating both cancerous (CR) and non-cancerous (hazard quotient) due to exposure to glyphosate and AMPA levels in water sources via ingestion and dermal exposure routes.

RESULTS AND DISCUSSION

The data obtained from an international market research company Kynetec indicated that South Africa used 7 977 tonnes of glyphosate in 2017 compared to the 3 721 tonnes in 2009. The means that glyphosate-use more than doubled in 8 years in South Africa.

A method to analyse glyphosate and AMPA in water and sediment/soil sources was developed in collaboration with the National Metrology Institute of South Africa (NMISA). This method was deemed fit for purpose and was validated with satisfactory method statistics. The concentrations of glyphosate and AMPA were below the detection limit for all the water and soil/sediment samples. The limits of detection (LODs) for glyphosate and AMPA in water and soil ranged from 0.2-0.5 µg/ℓ. The LODs were comparable to what others have reported for glyphosate and AMPA in water and soil/sediment.

The majority of the water samples complied with the resource water quality objectives (RWQO) guidelines for nutrients of South Africa, except for ammonium and ortho-phosphates in almost all the samples that exceeded the RWQO guidelines for irrigation-use.

The soil characteristics indicated that the samples were of different soil textures and that there were differences in CEC, %TOC and pH between the different samples collected. These characteristics were determined to explain the sorption of glyphosate when looking at the concentration data, but due to all the samples being <LOD, these correlations could not be made.

The human health risk assessment revealed that, based on the calculations using the ½ LOD and the assumptions of exposure via ingestion and dermal, there were no risks identified.

CONCLUSIONS

- In South Africa in 2017 and 2019, an estimated 7 976 and 7 507 tonnes of GBHs were used respectively.
- The chemical analysis method for extraction and quantification for glyphosate and AMPA were validated and fit for purpose.

- No concentrations of glyphosate and AMPA were present in quantities above the limit of detection in the water, soil and sediment samples collected at the chosen sites in this study.
- No human health risks were identified as the CR and HQ did not exceed the acceptable risk level.
- This is the first South African report on chemical analysis of glyphosate and AMPA in water and soil/sediment collected at both agricultural and non-agricultural areas in intervals during the crop growing season.

RECOMMENDATIONS

- Considering the major input of glyphosate based on the use-data obtained in this study, further investigation is necessary since monitoring of glyphosate is not performed in South Africa.
- An optimised and more sensitive analytical method might contribute to finding glyphosate at quantifiable concentrations.
- Pesticide formulants contain inert ingredients (such as polyethoxythylene tallow amine (POEA) used in some GBH) and these are suspected/proven to be higher in toxicity than the actual active ingredient. Determining the levels of POEA in water will therefore be worthwhile.
- A similar study to this one would benefit from qualitative information from the farmers on the herbicide-use located close to sampling sites.
- Since the main path of degradation is microbial, microbial diversity analysis might contribute to explain the lack of concentrations.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project.

Reference Group	**Affiliation**
Mr HM du Plessis	*Consultant*
Prof SH Jooste	*Consultant*
Dr P Mensah	*Rhodes University / University of Cape Coast (Ghana)*
Ms P Moodley	*Golder Associates*
Ms D Muir	*Department of Environmental Affairs and Forestry*
Dr N Odume	*Rhodes University*
Mr E van der Walt	*The Agricultural Research Council*
Others	
Dr Caitlin Swiegelaar	*National Metrology Institute of South Africa*
Dr Laura Quinn	*National Metrology Institute of South Africa*
Dr Hannes Erasmus	*North-West University*

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

AMPA	aminomethylphosphonic acid
ASTER	Advanced Spaceborne Thermal Emission and Reflection Radiometer
AT	average lifetime
BM	body mass
BT	NWU Botanical Gardens
C1	Channel 1
C2	Channel 2
CDI	chronic daily intake
Cl^-	chloride
CR	cancer risk
Cw	concentration of herbicide residue
DEA	Department of Environmental Affairs
DGP	Downstream Potchefstroom Country Club
DMD	Downstream Middelburg Dam
DSD	Downstream Spitskop Dam
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EC	electrical conductivity
ED	exposure duration
EF	exposure frequency
FMOC-Cl	Fluorenylmethyloxycarbonyl chloride
GBH	Glyphosate based herbicide
GD	Grootdraai Dam
GG	Gimmie Gat
GIS	Geographic Information Systems
GLY	glyphosate
GM	Genetically modified
GT	Glyphosate tolerant
HDPE	high-density polyethylene
HI	health index
HLB	Hydrophilic-Lipophilic Balance
HPLC	High-performance liquid chromatography
HQ	Hazard quotient
HR	Harts River
IR	Ingestion rate
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LDPE	Low-density polyethylene

MD	Middelburg Dam
MIC	Main inflow channel
MM	Mooi River mall
MP	Mpumalanga
NASA	National Aeronautics Space Agency
NFEPA	National Freshwater Ecosystem Priority Areas
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NWU	North-West University
PO_4^{3-}	Ortho-phosphate
RfD	Reference dose
RWQO	Resource water quality objectives
SANBI	South African National Biodiversity Institute
SD	Spitskop Dam
SF	Slope factor (for carcinogenicity)
SO_4^{2-}	Sulphate
SS	Suspended solids
TEMP	Temperature
TH	Total hardness
TO	Taung outflow
TOC	Total organic carbon
TRE	Tuscany Ridge estate
TURB	Turbidity
UHPLC	Ultra-high performance liquid chromatography
USEPA	United States Environmental Protection Agency
V	Vaal River
VB	Vaal River Barrage
VIS	Vaalharts irrigation scheme
WWTP	Wastewater treatment plant
X	Life Water River
2D	Two dimensional

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Agricultural activities are a potential source of several chemicals that end up in the environment. These include the pharmaceuticals used for the treatment of livestock, the artificial fertilisers added to crops, and pesticide applications. Current-use pesticides, in contrast to the historically used persistent organochlorine pesticides, are made to be biodegradable, but the continuous use of high volumes causes contamination of the environment (Carvalho, 2017). One example of such a pesticide, is the herbicide Roundup®, with the active ingredient glyphosate ([N-(phosphonomethyl)glycine]). Roundup® is a broad-spectrum, non-selective, post-emergent herbicide used for weed and vegetation control. It is approved in 130 countries including South Africa and is the most-used herbicide in the world (Dai et al., 2016). It only has one mechanism of action that disrupts plant metabolism and is therefore deemed relatively safe for animals and humans. The development of Roundup-Ready® crops has contributed to the high usage of Roundup® because farmers can spray larger quantities of glyphosate-containing herbicides, increase application rates, as well as spray them on the plants during the growing season while leaving the crops unharmed (Benbrook, 2012). Global use of glyphosate-based herbicides has increased by a factor of more than 10 over the last 20 years (Shaner et al., 2012).

Glyphosate is a polar, non-volatile compound that is also highly water-soluble (more than 10 g/ℓ at 25°C) (Simonsen et al., 2008). Glyphosate is claimed to be environmentally benign because it tightly binds to the soil, making it immobile with no soil activity and limited persistence (Reddy, 2001). There are however concerns whether this statement holds true as glyphosate may enter and pollute the rivers and dams where it may be harmful to aquatic organisms. Many countries across the world have quantified the levels of glyphosate in various water sources. Skeff et al. (2015) reported concentrations of 0.028-1.7 µg/ℓ in the water from German Baltic estuaries. Glyphosate was also found in groundwater in Catalonia, Spain at a maximum concentration of 2.5 µg/ℓ and a mean concentration of 0.2 ug/ℓ (Sanchís et al., 2012). Van Stempvoort et al. (2016) reported glyphosate concentrations of 0.7 µg/ℓ in rural groundwater in the Nottawasaga River watershed, Canada. Smith et al. (1996) found 45 µg/ℓ glyphosate in well water seven weeks after spraying. This well is located at the Massey Drive substation, Newfoundland, Canada, where vegetation around the electric substation was sprayed with Roundup®. This station is built on a limestone bed that is highly permeable and could allow contaminants sprayed on the weeds to move from the surface to groundwater. Börjesson & Torstensson (2000) reported levels of glyphosate in groundwater ranging from 0.12 to 1.42 µg/ℓ due to its application to railway weed. In the USA, glyphosate has been detected in a stream and wastewater treatment plant (WWTP) effluent at a maximum concentration of 2.2 ug/ℓ (Kolpin et al. 2006). A very extensive study by Battaglin et al. (2014), in the USA, reported the following maximum levels in environmental matrices: streams: 73 µg/ℓ; groundwater: 2.03 µg/ℓ; ditches and drains: 427 µg/ℓ; large rivers: 3.08 µg/ℓ; soil water: 1 µg/ℓ; lakes, ponds, and wetlands: 301 µg/ℓ; precipitation: 2.5 µg/ℓ; soil and sediment: 476 µg/ℓ, and WWTP outfall: 0.3 µg/ℓ. It is evident that glyphosate ends up in the water sources. However, no studies have been completed to investigate the concentrations of glyphosate, and its main metabolite AMPA (aminomethylphosphonic acid), in South African water sources except for the single study by Horn et al. (2019) which reported no levels when using enzyme-linked immunosorbent assays.

It was estimated that South Africa used approximately 40 775 tonnes of glyphosate in 2012 (Gouse, 2014). In a water-scarce country such as South Africa, water contaminated with chemicals is of great concern as concentrations of chemicals increase as water decreases. Another issue to be highlighted is the fact that many residents are dependent on untreated surface and groundwater resources due to the lack of supply of treated drinking water (Dabrowski et al. 2014).

This study aimed to measure the concentrations of glyphosate and AMPA, in various water sources, soil and sediment in South Africa. These compounds are not monitored in South Africa and their persistence in the environment and the toxicity of glyphosate are still under scientific discussion worldwide. In addition, the aim was be to establish an analytical method to analyse glyphosate/AMPA in South Africa

1.2 PROJECT AIMS

The following were the aims of the project:

1. Identify areas where large volumes of glyphosate-based herbicides are sprayed in South Africa and develop a usage map
2. Determine the concentrations of glyphosate and AMPA in water, soil and sediment samples, at different sampling times during the planting season using analytical instrumentation.
3. Establish a method for analysis of glyphosate and AMPA at the North-West University in South Africa, which can be utilised for future monitoring.
4. Conduct a desktop human health risk assessment about the hazards involved when exposed to glyphosate and AMPA in water resources.

1.3 SCOPE AND LIMITATIONS

The study commenced in April 2020, amidst the COVID-19 pandemic. The analysis of glyphosate and AMPA was set to be done in Japan, but due to travel restrictions and entry restrictions set by Japan, the analysis was done elsewhere. NMISA was contacted and assisted in the development and analysis of glyphosate and AMPA within all of the environmental samples. The method was fit for purpose, but if international collaboration was possible at the time, it would have been worthwhile to find a laboratory which has developed a more sensitive method for the analysis of glyphosate and AMPA at environmental levels.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

South Africa is an agricultural-driven country and maize is grown on 3.8-4.8 million hectares (ha), which accounts for roughly 25% of the country's arable land. Maize is the second largest crop (by tonnage) produced in South Africa after sugar cane and the staple food for most of the population. Maize has an average yield of 10-20 million metric tonnes and South Africa produces enough maize per annum that there is no need for any maize imports. Maize is also used as a raw material to manufacture products such as paint, textiles, paper, medicine, and food.

South Africa relies on successful agriculture to meet the basic needs of its population for food security (Jury, 2002). Different maize crop cultivation practices are applied by farmers to ensure the best yield and reduce crop losses. Crop cultivation is not an exact science and cultivation methods applied to the crops may vary with each crop. Some farmers make use of conventional cultivation practices whereas others make use of a combination of conventional and conservational cultivation practices which comprises of reduced tillage, residue retention and crop rotation (Esser, 2017; Malobane et al., 2020). Some practices remain secret because of the competitive buyers' market. Many cultivation practices are communicated via word-of-mouth and are rarely documented.

Since South Africa is a water-scarce country, conventional practices are largely applied to increase the water infiltration in the soils via tillage and soil cover disturbance. These practices also include the use of fertilizers and pesticides (Lemanowicz et al., 2020). Pesticides are sprayed throughout the crop season, repeated every year, and continuously contaminate the soil and water sources.

2.2 GLYPHOSATE-BASED HERBICIDES IN SOUTH AFRICA

Most of the maize and soybean cultivated in South Africa are genetically modified (GM) to be herbicide-tolerant. In 2015/16 a record area of 687 000 ha of soybean was planted, with 90% that are glyphosate tolerant (GT) varieties (ACB, 2016). Of the 1.6 million ha of maize sown in the 2016/17 season, 89% contained an herbicide-tolerant gene (BFAPb, 2018). According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), 60% of GM maize varieties registered in South Africa are resistant to GLY and 50% of the soy is genetically modified (ISAAA, 2021). In view of these high numbers of herbicide-tolerant crops being planted, it is expected that they would lead to greater use of the herbicides to which they are tolerant, which include GBHs.

South African farmers mostly adopt conventional cultivation practices, which include tilling and ploughing. In 2008/09, only 7% of farmed land overall was under no-till cultivation (Derpsch et al., 2010). If this practice continues, we can assume that farming will still favour tilling. This is not ideal for a country with naturally low soil organic matter (Laker, 2004; Du Preez et al., 2011). Swanepoel et al. (2018) suggest that conservation agriculture should be adopted by South African grain farmers because of its perceived benefits, namely, improved soil health, increased crop yield and reduced input costs. But how would the expansion of conservation agriculture influence the behaviour of GLY and AMPA present in the South African soil? In a recent paper by Carretta et al. (2021), non-tilled soils in Italy were reported to demonstrate higher adsorption of GLY and AMPA than conventionally tilled soil, probably because the practice leads to a soil with higher carbon content, which thereby increased its capacity to adsorb the herbicide. The greater GLY content in the non-tilled soil in the Italian study lasted for the entire 182 days of the investigation. These authors reported that conservation agriculture, which is characterised by minimal soil disturbance, permanent soil cover and crop rotation, requires increased application of GBHs because the weeding effect of tilling is mostly absent and the cover crops are destroyed by having to use GBHs (Cassigneul et al., 2016). Thus, it seems that tilling and ploughing lead to degraded soil conditions and allow for quicker dissipation of GLY and AMPA compared with conservation practices.

Seven of the 78 GBHs registered for use in South Africa are indicated to be used in the ripening of sugar cane; however, the preferred agent for this purpose is fluazifop-*p*-butyl because of its fewer effects on the emerging ratoon (Van Heerden et al., 2014). Because fluazifop-*p*-butyl is preferred over GBHs for sugar cane ripening, its application would be an alternative to GLY and AMPA.

The reliance on glyphosate in agriculture has led to 55 weed species developing resistance to the chemical globally (Heap, 2021), so herbicides with different mechanisms of action must now be used. At least three weed species in South Africa have confirmed resistance to GLY. These are *Lolium rigidum* (rigid ryegrass), *Conyza bonariensis* (horse weed/hairy fleabane), and *Plantago lanceolata* (ribwort plantain/buckhorn plantain) (Pieterse, 2010; Heap, 2021), which occur mostly in vineyards and small-grain fields (such as those for barley). Mahajan et al. (2020) calculated yield loss due to weed interference in eight barley genotypes to range from 43% to as much as 78%. Increased weed resistance usually requires greater use of the herbicides to which weeds developed resistance (Benbrook, 2016).

South Africa has been controlling water hyacinth with herbicides since the early 1900s (Ashton et al., 1979). In 2005, Jadhav and co-authors reported that four GBHs were specifically used for water hyacinth control and, according to Agri-Intel (2021), 17 GBHs are registered for eradication of ‘aquatic weeds’. Despite this, there are no government-issued environmental water quality regulations despite guidelines proposed by Mensah et al. (2013). These authors derived water quality guidelines for environmental water using species sensitivity distribution concentrations for Roundup®. The short-term and long-term water quality criteria proposed were 0.250 (0.106-0.589) mg/ℓ and 0.002 (0.000-0.021) mg/ℓ, respectively.

The products derived from the crops cultivated with GBHs may be also be contaminated with GLY and AMPA (Fu et al., 2021). Soybean is an important source of protein in South Africa and maize is the primary staple (Viljoen et al., 2021); on average, approximately 500 g of maize meal is consumed daily by poor households (Payne, 2011). Other maize products prepared for human consumption include beer, such as umqombothi (traditional South African beer) (Hlangwani et al., 2020), ice cream and syrup (DAFF, 2003). Soy-based products include soy milk, soy sauce and curds in cheese (DAFF, 2003). Bøhn et al. (2014) reported that GT-soybean in the United States of America (USA), which was sprayed with GLY, contained high residues of glyphosate and AMPA. Wheat may be consumed as whole grains or flour; moreover, sunflower may be eaten as edible seeds, and in the form of margarine or salad dressing (DAFF, 2016). Viljoen et al. (2021) reported GLY in two-thirds of 81 commercially available food products during a 2015 survey of grocery stores in South Africa. These food items had maize and/or soybean as the primary ingredient. Fortunately, only one of the products exceeded the South African maximum residue limit of maize (2 mg/kg) (DALRRD, 2021) but their purchasers would likely consume more than one product made from crops treated with GBHs and the overall consumption might therefore exceed that of the maximum residue limit.

Several nations – including Thailand, Vietnam, Sri Lanka, countries of the Gulf Cooperation Council, St Vincent and the Grenadines, Bermuda, Austria, Belgium, the Czech Republic, Denmark, France, Italy, the Netherlands, and Colombia – as well as some regions in the USA have banned the use of GBHs

(Carlson Law Firm, 2021; Baum Hedlund Law, 2021). Germany is gradually reducing its use of GLY, which is expected to be phased out by 2024 (Reuters, 2021). There are also South African initiatives to join this ban (ACB, 2019a): in August 2021 the Cancer Association of South Africa officially joined the International Agency for Research on Cancer (IARC) when it published a fact sheet and position statement on glyphosate, accepting the classification of GLY as probably carcinogenic to humans (a group 2A carcinogen) (Herbst, 2021). Unfortunately, very little is known about the levels and distribution of GLY and AMPA in the South African environment.

2.3 LEVELS OF GLYPHOSATE AND AMPA IN WATER SOURCES GLOBALLY

Glyphosate and AMPA undergo mineralisation, immobilisation or leaching in the environment (Bai & Ogbourne, 2016). Mineralisation is considered the main degradation process, resulting in AMPA, the main metabolite of glyphosate, as well as methylphosphonic acid, glycine and sarcosine. AMPA is further mineralised to methylamine and phosphate, finally decomposing to CO_2 and NH_3. The mineralisation is driven by microbial activity, the success of which is determined by soil characteristics such as pH, the presence of Fe and Cu and organic carbon content. GLY is adsorbed by carbon particles in the soil, in which its half-life varies from mere hours to more than 110 days, depending on the nature of the soil (Bai & Ogbourne, 2016). Because of its chemical nature, GLY is strongly adsorbed to clay and organic matter, making it less available for microbial degradation. This may lead to its accumulation over time (Meftaul et al., 2020). Photodegradation and chemical decomposition play minor roles in GLY breakdown (Degenhardt et al., 2012; Feng et al., 2020; Nagy et al., 2020; Zhan et al., 2018).

To date, only one study has ever been conducted (by Horn et al. (2019)), to investigate the levels of GLY in South African water sources using an enzyme-linked immunosorbent assay test. Even though no GLY could be quantified (only detected), it was the first attempt to determine the levels of GLY in the South African aquatic environment. Glyphosate concentrations in water and soil sources have been reported in various countries across the world. MacLoughlin et al. (2020) studied the middle Carnaval-Stream basin of La Plata, Argentina. Here the GLY and AMPA were detected in 67% and 83% of the samples, occurring at respective maximum concentrations of 17 and 4.5 µg/ℓ in the soluble fraction and 35 620 and 19 586 µg/kg in the particulates. Skeff et al. (2018) reported concentrations of 0.028-1.7 µg/ℓ in the water from German Baltic estuaries and it was found in groundwater in Catalonia, Spain at a maximum concentration of 2.5 µg/ℓ and a mean concentration of 0.2 µg/ℓ. Van Stempvoort et al. (2016) reported GLY concentrations of 0.7 µg/ℓ in rural groundwater in the Nottawasaga River watershed, Canada. Okada et al. (2020) in Auckland, Australia, found that GLY and AMPA were detected in a large portion of urban surface water samples. The recurrence of detection of GLY was 77% in wetlands and 79% in urban streams. Also, the AMPA discovery was 91% in wetlands and 97% in urban steams, while it was just present at 6% of the rural stream (1.8 ± 2.2 µg/ℓ). An extensive study by Battaglin et al. (2014), in the USA, reported the following maximum levels in environmental matrices: streams: 73 µg/ℓ; groundwater: 2.03 µg/ℓ; ditches and drains: 427 µg/ℓ; large rivers: 3.08 µg/ℓ; soil water: 1 µg/ℓ; lakes, ponds, and wetlands: 301 µg/ℓ; precipitation: 2.5 µg/ℓ; soil and sediment: 476 µg/ℓ, and WWTP outfall: 0.3 µg/ℓ.

The half-life of glyphosate in soil and aquatic sources is 2-215 days and 2-91 days respectively (Grunewald et al., 2001; Vera et al., 2010; Battaglin et al., 2014). Different studies reported the half-life of glyphosate ranges from weeks to years (Nomura & Hilton, 1977; Feng & Thompson, 1990; Eker et al., 2006). The half-life of glyphosate was reported to be 45-60 days in soil, with residues being below 6-18% of the initial glyphosate after 360 days in a Canadian forest watershed (Feng & Thompson, 1990). Roundup-Ready® soybeans may still have high levels of glyphosate residues after two years in storage (Cuhra et al., 2016). Others report that glyphosate has a short environmental half-life due to microbial degradation (Duke & Powles, 2008). The half-life of glyphosate in Norwegian soil at temperatures varying between -5°C and +5°C was estimated to be between 15-28 months (Laitinen et al., 2009). Laitinen et al. (2009) only found 14% of glyphosate in the topsoil surface 30-50 min after application. After 38 days 25% glyphosate was left in the soil with the roots containing concentrations more than an order of magnitude higher than in the soil samples. Laitinen et al. (2006) concluded that the dissipation of glyphosate from the soil in their study was 11 months.

2.4 EFFECTS OF GLYPHOSATE AND AMPA ON NON-TARGET ORGANISMS

Glyphosate and GBHs have undergone extensive scrutiny due to concerns regarding their potential negative impacts on human and ecosystem well-being (Williams et al., 2008). Recent research has raised doubts about the safety of glyphosate and GBHs, challenging previous assumptions (Paganelli et al., 2010; Guilherme et al., 2012; Koller et al., 2012). The application of herbicides exposes not only the intended target species but also introduces residual active ingredients and surfactants into the soil and water, which could potentially pose health risks (Sihtmäe et al., 2013). Due to its specific mode of action, glyphosate is generally considered to have minimal toxicity to vertebrates and non-target organisms and is often promoted as environmentally friendly for these reasons (Sihtmäe et al., 2013).

Glyphosate-based herbicides have been found to exhibit chronic effects, including hepatorenal, teratogenic, and tumorigenic. These effects are thought to be linked to oxidative stress and disruptions in endocrine functions. Ongoing research is exploring their potential involvement in transgenerational, reproductive, and neurological disorders (Mesnage et al., 2015).

Numerous research findings suggest that the formulation of certain substances can be more toxic than the active ingredient itself, as demonstrated by Mesnage et al. (2013). One example of a surfactant is polyethoxylated tallowamine (POEA) in Roundup®. When herbicides are evaluated for registration, toxicity assessments typically focus solely on the active ingredient and primarily consider acute effects. Consequently, potential repercussions on non-target organisms resulting from chronic exposure to low concentrations, prevalent in environmental contexts, may have been disregarded.

Studies have reported moderate toxicity of Roundup® to certain aquatic organisms such as bluegill sunfish (*Lepomis macrochrirus*), common carp (*Cyprinus carpio*), and *Daphnia magna* (Cuhra et al., 2013). Furthermore, it is highly toxic to green algae (*Selenastrum capricornutum*), with an EC50 of 0.46 mg/ℓ after 72 hours of exposure. In contrast, the formulation is generally considered non-toxic to honeybees (*Apis mellifera*) and earthworms (*Eisenia foetida*). Glyphosate does cause slight dietary toxicity to bobwhite quail (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*).

The European Food Safety Authority (EFSA) categorized it as non-carcinogenic for humans. The Environmental Protection Agency (EPA) of the United States has however claimed that glyphosate and GBHs are safe to use following manufacturer's instructions. In addition, the World Health Organization (WHO) also concluded that glyphosate was not carcinogenic (Centner et al., 2019). On the other hand, the IARC classified glyphosate as "probably carcinogenic to humans" (Group 2A) in 2015, and it has also been associated with an elevated risk of non-Hodgkin's lymphoma (Weisenburger, 2021).

2.5 METHODS TO QUANTIFY GLYHPOSATE AND AMPA

Various methods can be used to extract and analyse glyphosate and AMPA. These methods include but are not limited to, capillary electrophoresis-mass spectrometry (CE-MS), gas chromatography-mass spectrometry (GC-MS), immunogenic assays, ion chromatography-mass spectrometry (IC-MS), liquid chromatography-mass spectrometry (LC-MS), colourimetric, nanosensor, and spectrophotometric analytical methods as well as ELISA (Kocadal et al., 2022). For further details on the methods available, Kosiken et al. (2016) has summarised the methods that were available from 2000-2015.

CHAPTER 3: MATERIALS AND METHODS

3.1 INTRODUCTION

Glyphosate-based herbicides are widely applied to crops and to some extent in other domestic weed control practices. Glyphosate is a polar, non-volatile and amphoteric compound that binds strongly to soil, but is also highly water-soluble (more than 10 g/ℓ at 25°C). The parent compound of glyphosate undergoes little or no metabolism in most plants, and it is readily translocated into metabolic sinks including plant roots. It is eventually released into the rhizosphere and subsequently to the soil (Kremer et al., 2005). Glyphosate is claimed to be environmentally benign because it tightly bounds to the soil making it immobile with no soil activity and limited persistence (Reddy, 2001), however, glyphosate and its metabolite AMPA have been detected in water sources elsewhere. In this study, the glyphosate and AMPA concentrations in water, soil and sediment samples were determined.

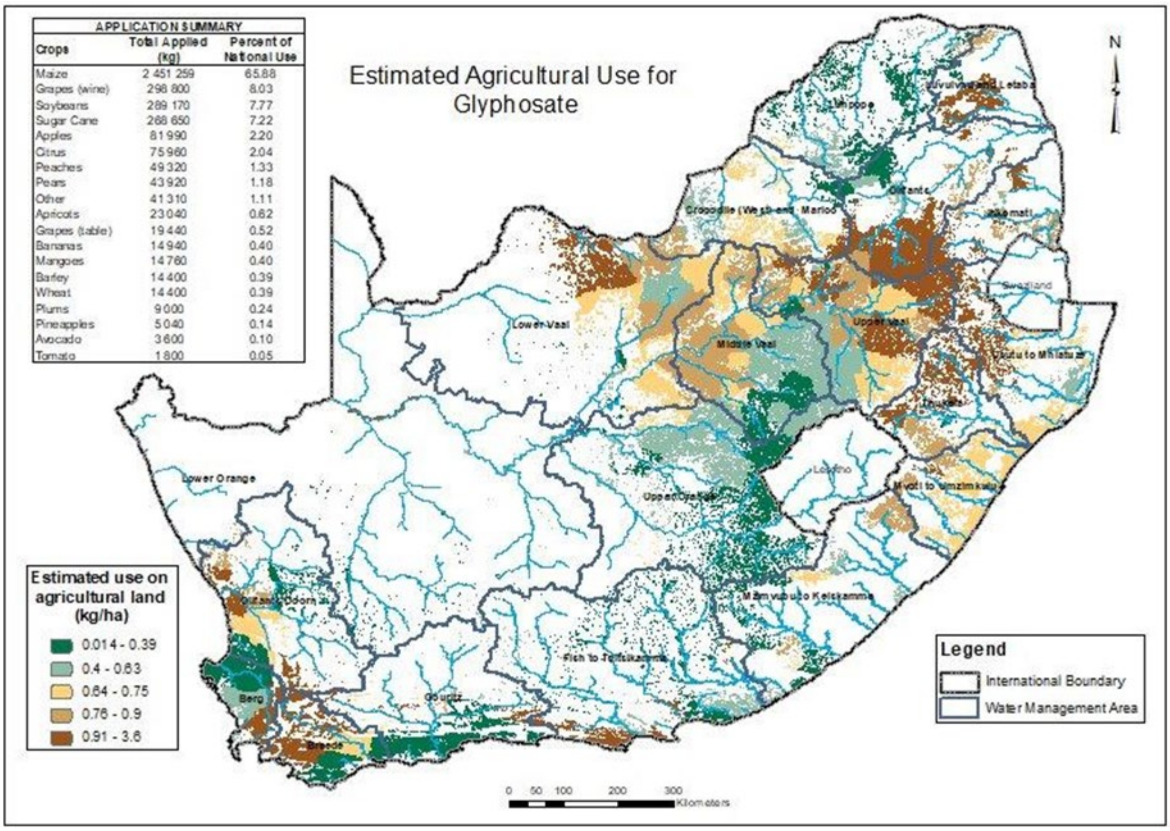
3.2 GLYPHOSATE-BASED HERBICIDE USE IN SOUTH AFRICA

In a country such as South Africa where the economy is dependent on agriculture and pesticides are widely used, there is no information (known to the authors) published on the levels of GLY and AMPA in the environment. The last published data on pesticide use was by Dabrowski, (2015), using data collected in 2009. This report stated that 3 700 tonnes of glyphosate (active ingredient) were used in South Africa. For this study, data on the volumes (tonnes) of GBHs applied to maize, soybean, wheat, and sunflower crops in 2017 were obtained from Kynetech. This information was obtained by conducting interviews with farmers asking what pesticides are applied, target crop sprayed, crop variety, crop seeding rate, and also the application rate.

The GLY usage data for South Africa was visualised by creating maps. The geospatial analysis was carried out as a desktop analysis by using a digital database from the North-West University (NWU). The database containing data of the NASA: ASTR 90m Digital Elevation Dataset; DEA: 2013-2014 SA National Land Cover Dataset; DWAF: Hydrology – Dams and SANBI: NFEPA River Network. Maps were created using Environmental Systems Research Institute's software, ArcGIS Desktop 10.5. The Kynetech Excel® file was exported into ArcGIS. The GBH tonnage amounts were added to the geo-referenced municipal boundary dataset to visualise the GBH applied to the various municipal boundaries. The various vector dataset coordinates (in decimal degrees) were projected with the WGS 1984 geographical coordinate system to indicate where the GBH applications are located. The volume of GBHs used in 2017 was divided into six intervals (Figure 3-1). The first interval depicts where there was no correspondence from the interviewed farmers and therefore was labelled as 'No data'. The remaining 5 intervals were established using the Jenks natural breaks classification which optimises the arrangement where the dataset values would naturally break. The Jenks natural break is one of the data classification tools in ArcGIS. This classification was used to minimise the average deviation from the class mean with also maximising the deviation from the means of the other groups (Weber et al., 2020).

3.3 SITE SELECTION

Agricultural sampling sites were chosen based on i) available data on the highest usage areas of glyphosate in South Africa based on data obtained from Kynetech and Dabrowski et al., 2015b; ii) types of crops cultivated making use of WRC report no TT 642/15 (Dabrowski et al., 2015a) (Figure 3-1). The non-agricultural sites were chosen based on accessibility and reports of glyphosate usage in these areas.



APPLICATION SUMMARY

Crops	Total Applied (kg)	Percent of National Use
Maize	2 451 259	65.88
Grapes (wine)	298 800	8.03
Soybeans	289 170	7.77
Sugar Cane	268 650	7.22
Apples	81 990	2.20
Citrus	75 960	2.04
Peaches	49 320	1.33
Pears	43 920	1.18
Other	41 310	1.11
Apricots	23 040	0.62
Grapes (table)	19 440	0.52
Bananas	14 940	0.40
Mangoes	14 760	0.40
Barley	14 400	0.39
Wheat	14 400	0.39
Plums	9 000	0.24
Pineapples	5 040	0.14
Avocado	3 600	0.10
Tomato	1 800	0.05

Figure 3-1 Map of South Africa indicating the estimated use of glyphosate (kg/ha) (Dabrowski et al. 2015a)

3.3.1 Vaalharts Irrigation scheme, Northern Cape Province (irrigated and low rainfall region)

The Vaalharts irrigation scheme (VIS) is located on the border of the North West and Northern Cape Provinces. The sites in the VIS consisted of channels, a wetland and dams. In this area, man-made canals bring water from the Harts River (HR) to assist with irrigation practices. Sites were selected in the channels, in the HR and inside impoundments in the system. The ten sites in the VIS (Figure 3-2) were: Taung outflow, HR1, Channel 1, HR2, Channel 2, Main Inflow Channel, HR3, HR4, Spitskop Dam, and Downstream Spitskop Dam. The cultivated agricultural areas consisted of mostly pecan nut trees, soybean, maize and wheat.

3.3.2 Upper Vaal and Olifants River catchments (Mpumalanga Province) (non-irrigated and higher rainfall region)

The sites from the second sampling area, the Mpumalanga area, (MPA), are located in the Mpumalanga Province, stretching from Middelburg in the north, southwards along the Klein-Olifants-, Life Water- and

Vaal Rivers to Standerton (Figure 3-3). There were 15 sites (Middelburg Dam, Downstream Middelburg Dam, MP4, MP6, Pan 1, MP10, X1, X2, X3, V9, V11, V12, V13, Grootdraai and V15). The sites downstream of Middelburg Dam (OMD) to Pan 1, along the Klein-Olifants River are surrounded by coal mines (Figure 3-3). The majority of the sites are surrounded by cultivated croplands of maize, soybean, and wheat.

3.3.3 Non-agricultural sites (North West and Gauteng Provinces)

The non-agricultural sites are located in the Vaal River (Vanderbijlpark, Gauteng) and Mooi River (Potchefstroom, North West) (Figure 3-5). There were six sites (Vaal Barrage, Potchefstroom Country Club, Mooiriver Mall, Tuscany Ridge Estate, Gimmie Gat, North-West University (NWU) Botanical Gardens).

Site descriptions and soil types of each sampling points were summarised in Table 3-1.

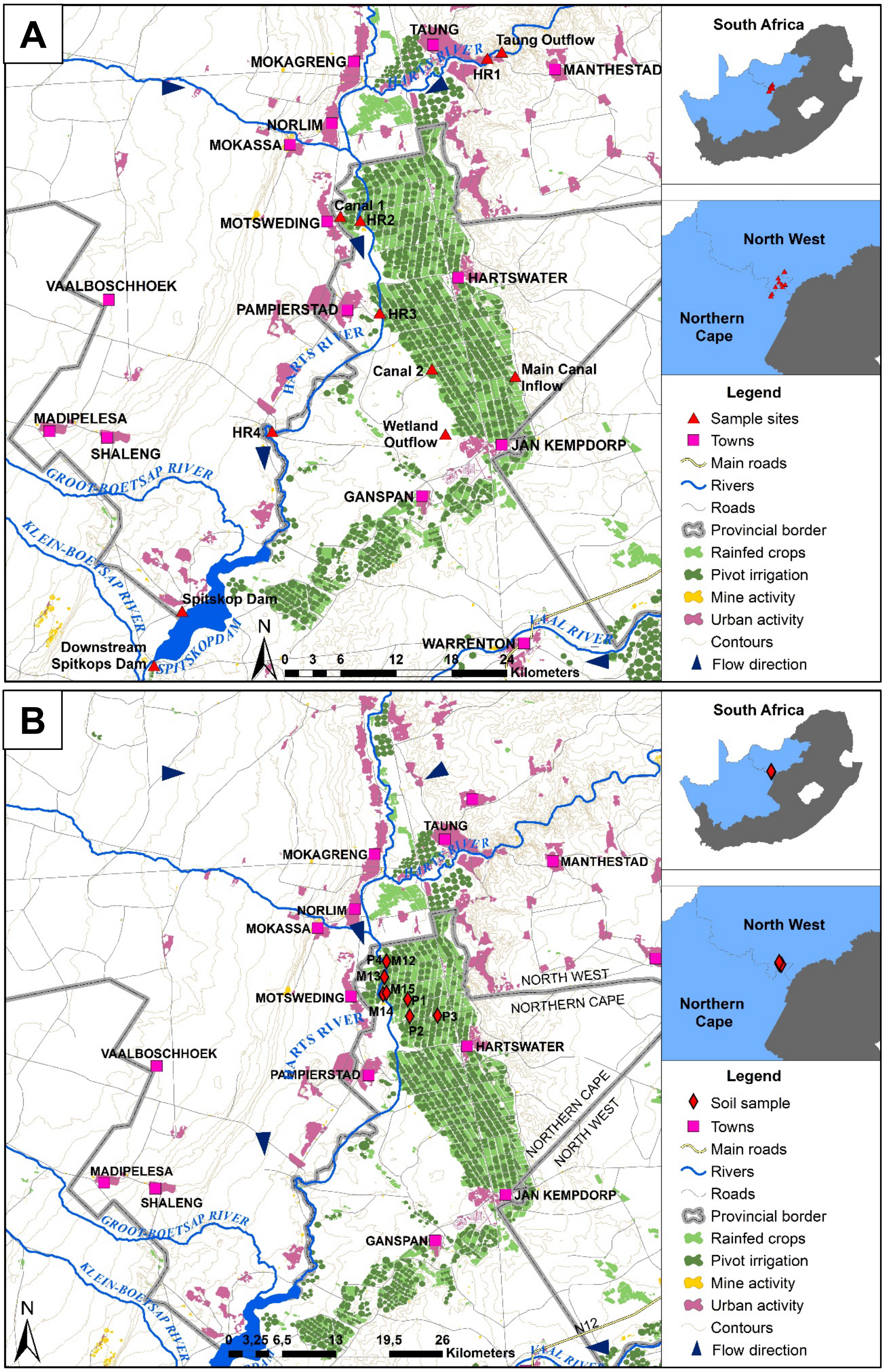


Figure 3-2 Map of sites located in the Vaalharts irrigation scheme (A water/sediment; B soil)

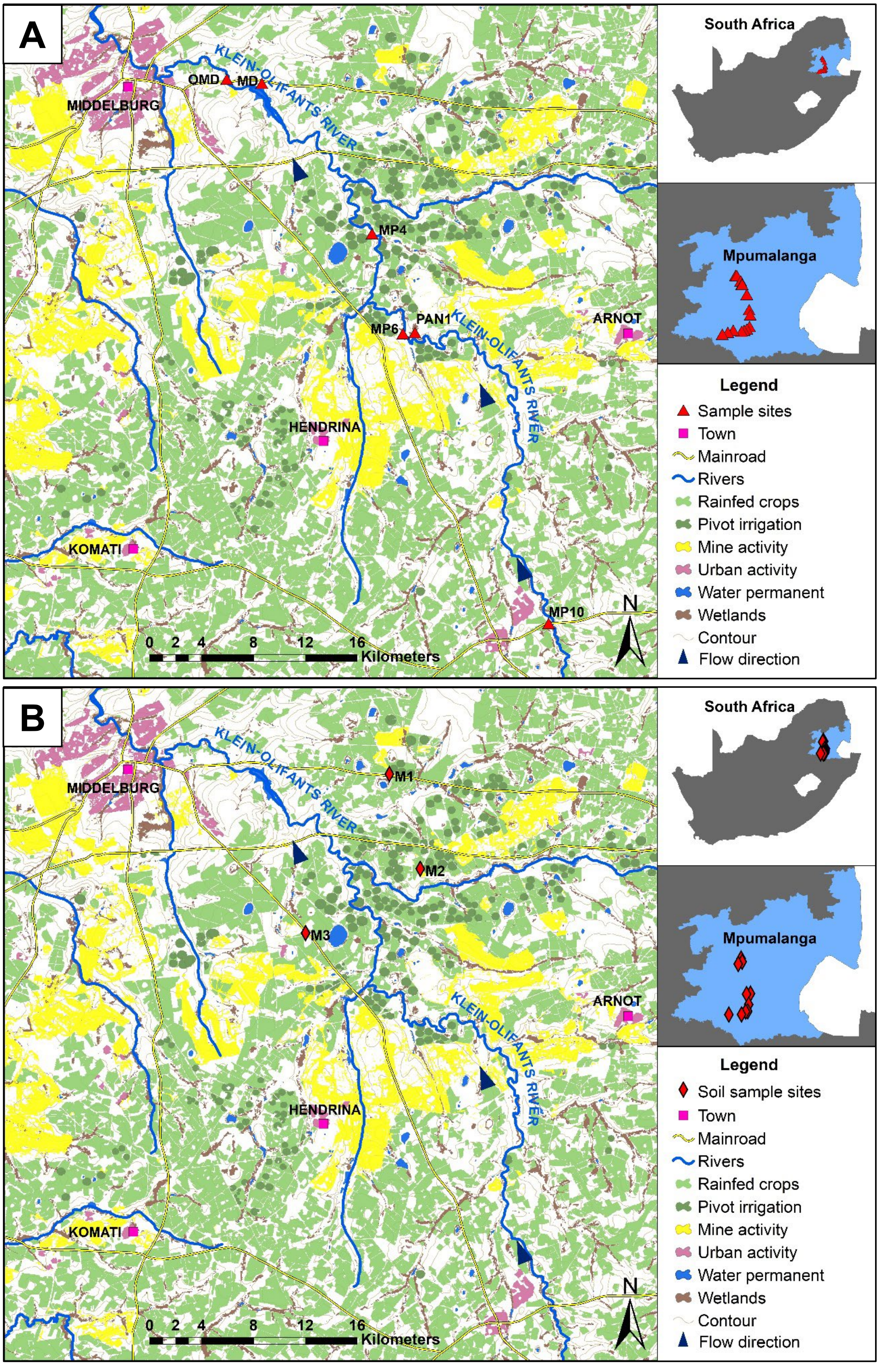


Figure 3-3 Map of the sites located in the Klein Olifants River (A water/sediment; B soil).

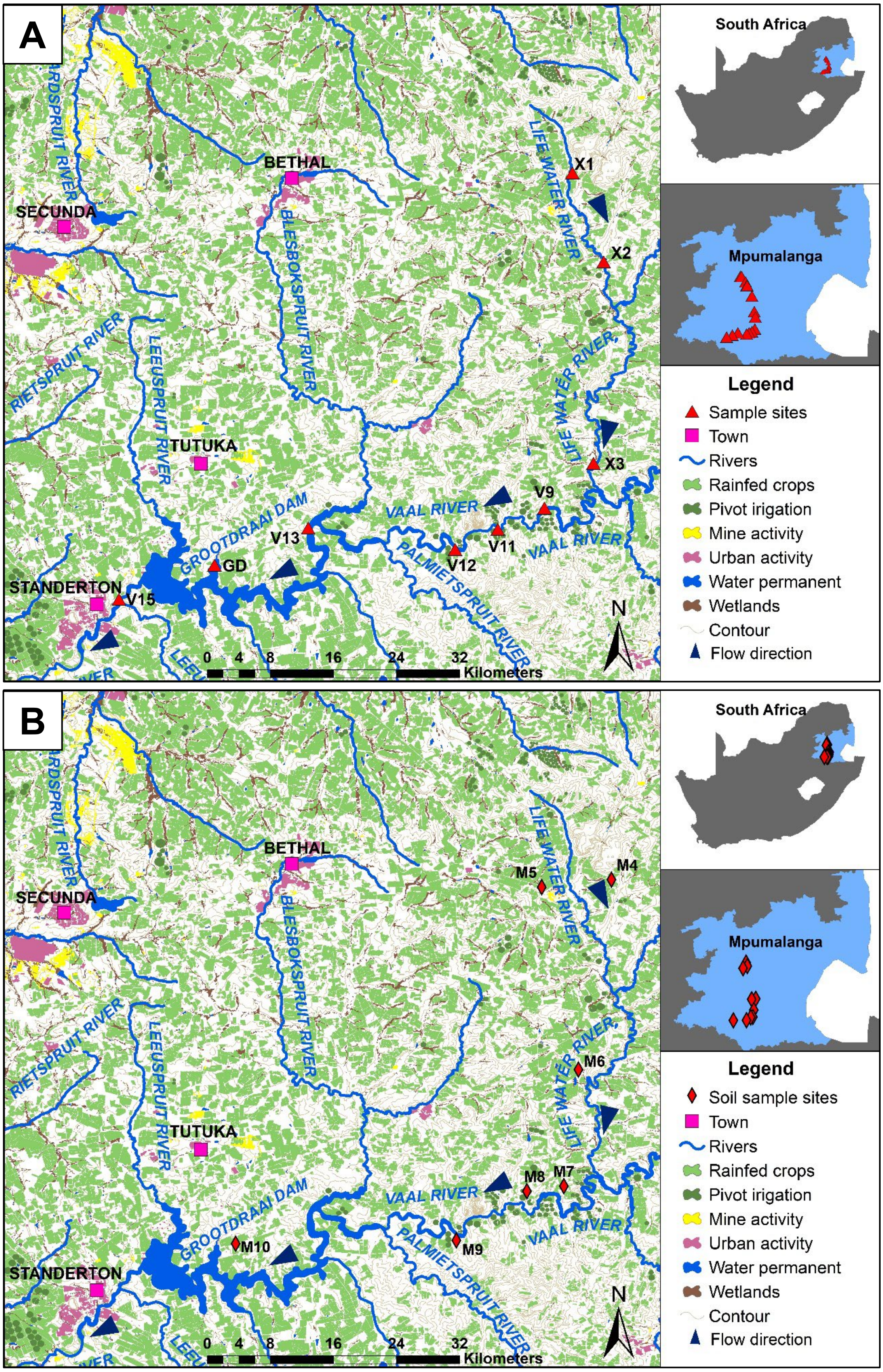


Figure 3-4 Map of the sites located in the Upper Vaal catchment (A water/sediment; B soil).

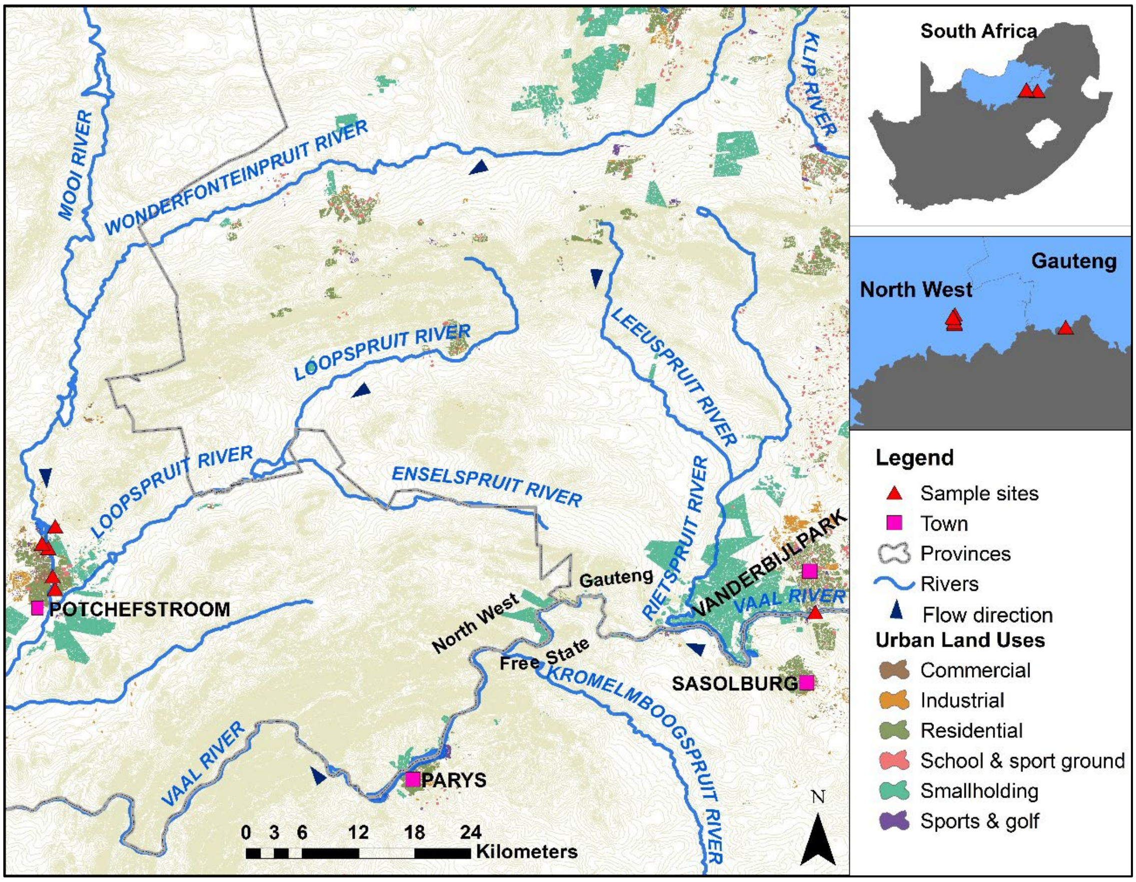


Figure 3-5 Map of non-agricultural sampling points

Table 3-1 Individual site descriptions of all three sample areas

Site	Abbreviation	Coordinates	Description	Sample collected
Klein Olifants River and Upper Vaal catchment sites				
Downstream Middelburg Dam	OMD	25°46'10.3"S 29°31'44.7"E	Rocky topography, personal products and waste dumping, the riverbank was at a 20-30° angle.	Water & sediment
Middelburg Dam	MD	25°46'20.7"S 29°33'12.6"E	Topography is flat with grass fields.	Water & sediment
Mpumalanga 4	MP 4	25°52'36.5"S 29°37'47.7"E	Flat topography, maize croplands with pivot points visible but not close to the site, surrounded with grass vegetation.	Water & sediment
Mpumalanga 6	MP6	25°56'45.9"S 29°39'04.8"E	Surrounded by grass fields.	Water & sediment
Pan 1	Pan 1	25°56'43.3"S 29°39'34.7"E	Croplands visible surrounding the site and cattle grazing in the distance (approximately 1 km) but not at the immediate site, close to the Arnot power station.	Water & sediment
Mpumalanga 10	MP10	26°08'49.7"S 29°45'08.5"E	Flat topography, grazing cattle, croplands visible.	Water & sediment
Life Water River 1	X1	26°26'54.5"S 29°47'50.4"E	Grazing cattle, maize croplands, flat topography.	Water & sediment
Life Water River 2	X2	26°33'03.9"S 29°49'05.2"E	Maize croplands were visible with grazing cattle, flat topography	Water & sediment
Life Water River 3	X3	26°46'51.0"S 29°48'23.5"E	Multiple maize croplands visible around the site, water monitoring station was observed and topography consists of bedrock, flat topography.	Water & sediment
Vaal River 9	V9	26°49'55.0"S 29°45'01.8"E	Hilly topography, steep riverbank (30-45° inclination), cattle grazing.	Water & sediment

Site	Abbreviation	Coordinates	Description	Sample collected
Vaal River 11	V11	26°51'22.4"S 29°41'50.3"E	Steep topography next to main road at an approximate 30°. Under a bridge. Riverbank was flat	Water & sediment
Vaal River 12	V12	26°52'44.5"S 29°38'55.2"E	Riverbank is steep (estimate of 60-90°) while above the bank is flat, maize croplands around the river, pivot points visible.	Water & sediment
Vaal River 13	V13	26°51'17.2"S 29°28'52.1"E	Topography has small to medium hills. Consists mainly of grass vegetation.	Water & sediment
Grootdraai	GD	26°53'49.6"S 29°22'27.1"E	Rocky topography, mainly grass fields. Lots of algae in the water.	Water & sediment
Vaal River 15	V15	26°56'09.7"S 29°15'54.7"E	Hilly topography at river site, pivot points, crops. Area consists mainly of grass vegetation. Water works were observed.	Water & sediment
Vaalharts irrigation scheme sites				
Taung Outflow	TO	27°32'5.3"S 24°50'19.7"E	Low bridge, sediment movement observed. Near informal settlement (estimate of 5-10 km). Grazing cattle and donkeys. No observable crops.	Water
Harts River 1	HR1	27°32'27.2"S 24°49'29.2"E	Directly under a bridge. Next to an informal settlement (1 km). Grazing cattle, pigs. Smells like urine. Filamentous agal growth.	Water & sediment
Channel 1	C1	27°41'40.1"S 24°40'55"E	Opposite of a maize field.	Water & sediment
Harts River 2	HR2	27°41'46.4"S 24°40'36.5"E	The following was observed: grazing cattle, small informal settlement, pivots on maize croplands and a natural wetland close-by. .	Water & sediment
Channel 2	C2	27°50'34"S 24°46'15.3"E	Surrounded by pecan orchard.	Water & sediment
Main Inflow Channel	MIC	27°50'59.7"S 24°51'7"E	Surrounded by grass fields.	Water
Harts River 3	HR3	27°47'13.7"S 24°42'18.1"E	Algal growths. Grass fields. No visible agricultural activities.	Water & sediment
Harts River 4	HR4	27°54'12.7"S 24°36'56.6"E	Surrounded by grass fields and tree vegetation.	Water & sediment
Spitskop Dam	SD	28°4'41.3"S 24°31'41.6"E	No observed crop activities. Presence of grazing wild animals. Filamentous agal growth.	Water & sediment
Downstream Spitskop Dam	DSD	28°7'51.6"S 24°30'3.6"E	Under a bridge. Surrounded by grass fields.	Water & sediment
Non-agricultural sampling sites				
Vaal River Barrage	VB	26°44'51.5"S 27°50'14.8"E	Site at a golf course and private residence. Landscaping activities. Algal growth. Flat topography.	Water & sediment
Downstream Potchefstroom Country Club	DGP	26°43'30.7"S 27°06'27.3"E	At a bridge. The golf course is upstream and downstream is surrounded by various vegetation.	Water & sediment
Mooi River Mall	MM	26°42'47.7"S 27°06'19.5"E	Site running beneath a mall. Various Landscaping activities observed. Flat river bank.	Water & sediment
Tuscany Ridge Estate	TRE	26°39'54.9"S 27°06'27.8"E	Pond in a residential estate. Landscaping activities observed. Site has filamentous algal growth and reeds.	Water & sediment
Gimmie Gat	GG	26°41'09.8"S 27°06'03.6"E	Surrounded by residential properties on one side and various vegetation on the other. Rocky topography.	Water & sediment
NWU Botanical Gardens	BT	26°40'54.1"S 27°05'42.8"E	Landscaping activities observed. Still standing pond with floating leaves.	Water

3.4 SAMPLING

Glyphosate is a pseudo-persistent compound in environmental water sources because it is constantly introduced. This is especially true during the planting season as farmers can spray large quantities where Roundup-ready® crops are planted. Roundup® is used as a pre-and post-emergent herbicide. Therefore, sampling times took place throughout the season to monitor the levels before spraying, after spraying, during the season and after the harvest (end of planting season).

3.4.1 Sampling times

Water, sediment and soil samples were collected from October 2020 to June 2021 in 4 events: 1) before spraying (21-30 September 2020; 22 October 2020), 2) after herbicide application (16 November to 1 December 2020), 3) after a rain event (10-24 March 2021) and 4) after harvest (25 May to 8 June 2021) (end of the season). These timeframes were chosen to assess and establish the baseline and concentrations of glyphosate-based herbicides and AMPA in the environment.

3.4.2 Sampling procedure

Glyphosate is known to strongly bind to soil, but it is also hydrophilic and therefore water and sediment were sampled. Water and sediment samples were collected in pre-cleaned (rinsed with ethanol) high-density polyethylene (HDPE) bottles (Nalgene™ 2104-0008, Sigma-Aldrich) for glyphosate and AMPA quantification (Kylin, 2013). For nutrient analysis, water samples were collected in low-density polyethylene (LDPE) bottles. All samples were transported at 4°C to the laboratory and frozen until extraction commenced.

For this study, the water samples were preserved, and the following three quality control actions were taken:

1. To limit microbial and photodegradation of glyphosate in water, the water samples were filtered to remove microbes, covered with foil and frozen at -20°C.
2. Different versions of the water samples were kept if needed for analysis. These include the whole sample, filtrate and filters.
3. Quality control samples (procedural blanks and spiked samples) were included and handled in the same way as the samples throughout the project. Procedural blanks control for contamination during the processing. The spiked samples contain a known concentration of glyphosate to control for the potential breakdown in the samples (Please note that these quality control samples do not refer to analytical method validation samples.)

Sediment was collected from the upper 10 cm and mixed well to make a composite sample. This was stored in pre-cleaned (rinsed with ethanol) polyethylene containers and the equipment was rinsed and cleaned after each sample.

3.4.3 *In-situ* field sampling

Water quality variables such as pH, electrical conductivity (EC) and temperature were determined at each site with a multimeter (ExStik II EC500, Extech Instruments). The sampling bottle was rinsed once with water at the site. The bottle was then filled with water and the parameters were determined by submerging the probes in the water sample. The probes were rinsed with deionised water between the sample sites.

3.4.4 Nutrient analysis of water samples

The nutrients and other chemical water quality variables of the unfiltered water samples were determined using the appropriate test kits following the manufacturer's instructions and using a spectrophotometer (Spectroquant® Pharo 300, Merck). The various water quality test kits were: ammonium (NH_4^+-N, 114752), chloride (Cl^-, 114897), nitrate (NO_3-N, 109713), nitrite (NO_2-N, 114776), sulphate (SO_4^{2-}, 114791), ortho-phosphate (PO_4^{3-}-P, 114848) and total hardness (TH, 100961). The suspended solids (SS) concentration (mg/ℓ) in water samples were determined by filtering 100 mℓ of water and trapping the SS on a cellulose nitrate filter (0.45 µm, Sartorius Stedim Biotech). The membrane filter was oven-dried overnight at 37°C (Mohamad-Zainal et al., 2021).

3.4.5 Soil and sediment characteristics

Sediment samples were thawed and dried in an oven at 35°C overnight. Of the dried sediment, 30 g of each sample was heated in a crucible at 600°C for six hours in a muffle furnace (L 40/11, Nabertherm). The percentage total organic carbon (%TOC) was calculated based on mass loss of after incineration (Gerber *et al.*, 2015). The %TOC was measured to evaluate to what extent compounds would adsorb to the carbon fraction (La Cecilia & Maggi, 2018). The soil texture based on particle size was determined by using the Endecott dry sieving system. A 20 g soil/sediment sample from each site was sieved by manually shaking the sieves with various mesh grid sizes for 5 minutes. The particle size categories are described as follow: mud (<65 µm), fine sand (212-65 µm), medium sand (500-212 µm, coarse sand (2 000-500 µm), very coarse sand (4 000-2 000 µm) and gravel (>4 000 µm). The soil composition was determined by the percentage of each particle size of the total soil sample (Gerber *et al.*, 2015).

3.4.6 Soil cation exchange capacity

The measure of a soil's ability to retain positively charged ions (cations) like calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) is called the cation exchange capacity (CEC). This capacity is determined by the number of negatively charged sites on the soil surface that can electrostatically attract and hold cations. Soils with higher CEC values can maintain sufficient levels of these nutrients because the electrostatically retained cations can be easily exchanged with other cations in the soil solution. However, it is worth noting that a higher CEC does not necessarily make soil more fertile since acidic cations like hydrogen (H^+) and aluminium (Al^{3+}) can also occupy the soil's CEC sites. The method by Gillman and Sumpter (1986) was used to determine CEC. This method determines the CEC at the pH and ionic strength of the soil. Understanding how glyphosate and AMPA interact with soil matrix, influence nutrient availability, affect pH, and impact environmental fate is critical for understanding the potential risks associated with their presence in soil.

First, the mass of a 50 mℓ centrifuge tube was measured to the nearest milligram (Shimadszu AY220). Next, 2 g of soil was added to the tube, along with 20 mℓ of 0.1 M $BaCl{\cdot}2H_2O$ extraction solution. The tube was then placed on a Labcon mechanical shaker and shaken for two hours. Following the shaking step, the pH was measured and recorded with a CRISON BasiC20. Afterward, the tube was centrifuged at a relative centrifugal force (rcf) of 10 000 *g* for 10 minutes and the resulting supernatant was carefully removed. Subsequently, 20 mℓ of 2 mM $BaCl_2{\cdot}2H_2O$ equilibrating solution was added to the tube and capped. The tube was briefly vortexed to disperse the soil pellet and then shaken on a mechanical shaker for one hour. Repeat the equilibrating solution and mechanical shaker for one hour steps twice more. But before the third centrifugation, obtain a slurry pH and record the measurement. After the third decantion of 2 mM $BaCl_2{\cdot}2H_2O$, add 10 mℓ of 5 mM $MgSO_4$ and shake gently for 1 hour.

Determine the conductivity of the 1.5 mM $MgSO_4$ solution (it should be 300 µS). If the conductivity of the sample solution is not 1.5x this value, add 100 µℓ increments of 0.1 M $MgSO_4$ (an adjusting solution) until it is (take note of the amount of 0.1 M $MgSO_4$ increments added). After the adjustments, the sample solution's pH was re-evaluated, and if it varied by more than 0.1 units from the previous reading, it was adjusted by lowering the pH by using 50 mM H_2SO_4. The sample solution was then diluted with distilled water until its conductivity approached that of a 1.5 mM $MgSO_4$ standard solution. Afterwards, the centrifuge tube was allowed to dry, and the mass of the tube and its contents were recorded.

The CEC was finally calculated as milli-equivalents/100 g (meq/100 g) as represented in Equation1-4. The data points collected included the mass of the total solution (g) by Equation 2, the amount of magnesium in the solution (Equation 2), the total magnesium added (Equation 3) and finally the CEC (Equation 4). The monitoring and capturing of the pH and EC were also to ensure the stability for reactions to occur.

$$\boldsymbol{A}: Total\ solution\ (g) = final\ tube\ mass\ (g) - tube\ tare\ mass\ (g) - 2\ g\ (mass\ of\ soil\ used)$$ Equation 1

$$\boldsymbol{B}: Mg\ in\ solution\ (meq) = \boldsymbol{A} \times 0.003\ meq/m\ell[1.5\text{ mM MgSO4}]$$ Equation 2

$$\boldsymbol{C}: Total\ Mg\ added\ (meq) = 0.1\ meq\ [10\text{ mL of }5\text{ mM MgSO4}] + m\ell\ added \times 0.2\ meq/m\ell[0.1\ M\ MgSO4]$$ Equation 3

$$\boldsymbol{CEC}\ (meq/100\ g) = (\boldsymbol{C} - \boldsymbol{B}) \times 50[\text{convert 2 g soil to 100 g soil}]$$ Equation 4

3.5 CHEMICAL ANALYSIS

3.5.1 Introduction

The need for analysis of glyphosate and AMPA is highlighted in previous chapters, hence, the aim of this chapter is to report on a validated method for extraction and analysis of glyphosate and AMPA from water, sediment and soil matrices. Glyphosate and AMPA are present in the environment in low concentrations and dictating that the target compounds in the environmental matrices should ideally be concentrated to ensure the analytical technique is capable of detecting the compounds. Water samples were evaporated and soil was extracted and evaporated and both types of extracts were analysed using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Compounds of interest were glyphosate, its metabolite AMPA and glufosinate. which fall into the class of highly polar pesticides and their chemical characteristics can be found in Table 3-2. Glufosinate was the internal standard (IS).

3.5.2 Chemicals and materials

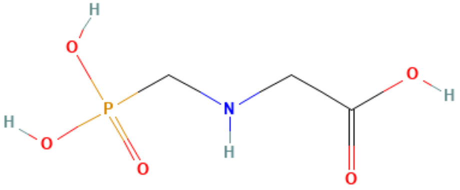
Analytical reference standards glyphosate, ≥98% purity (CAS# 1071-83-6) and AMPA, ≥99% purity (CAS# 1066-51-9) were obtained from Fluka (Sigma, Germany). Glufosinate (CAS# 51276-47-2) acted as internal standard and was purchased from Dr Ehrenstorfer GmbH, LGC Ltd. Methanol of spectrometry grade (Romil) were purchased from MicroSep, South Africa. Formic acid was obtained as 98% solutions from Fluka (Sigma, Germany). Acetonitrile (ACN) (MS-grade) (Honeywell Burdick & Jackson) were purchased from Anatech, South Africa. Diatomaceous earth was obtained from Anatech. Deionised water (18.2 MΩ cm) was obtained from an in-house ELGA PURELAB Ultra water purification system. Individual stock solutions for glyphosate, AMPA and glufosinate were prepared using a gravimetrical method by weighing 10 mg compound and dissolving it in 10 g (9.27 mℓ) of 10% acetonitrile (10:90 ACN:H_2O) resulting in 1 mg/g. Stock solutions were prepared in plastic 15 mℓ tubes and covered with foil to prevent UV degradation. These stock solutions were used to prepare standard mix solutions to be used to prepare the calibration curve and also spike the samples for recovery determination. During stock solution preparation an MS Excel spreadsheet was used to determine amount of volume/g of solvent to be added to prepare desired concentration.

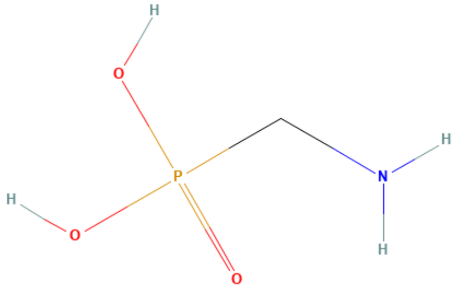
3.5.3 Extraction of glyphosate and AMPA from water

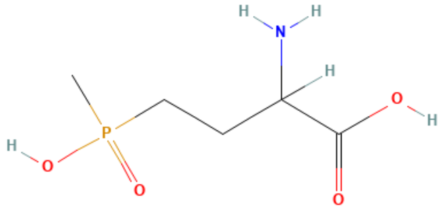
The extraction procedure of glyphosate and AMPA from water was adapted from the EURL-SRM QuPPe method for highly polar pesticides in fruit and vegetables (Anastassiades et al., 2023). After thawing the water samples, the bottles were inverted 200 times to ensure thorough mixing. Subsequently, 15 mℓ of a sample was aliquoted to a clearly labelled 50 mℓ centrifuge tube and covered with foil. Each water sample was weighed (gravimetric accuracy) and 250 µℓ internal standard (2.1 µg/g) was added to each sample and weighed again. The mixture was vortexed to ensure optimal equilibration. A volume of 1 mℓ methanol was added, and the samples were again vortexed for 2 minutes. Water was evaporated (Genevac, Fischer Scientific) until 5 mℓ was left. One millilitre was filtered with a 0.22 µm polytetrafluoroethylene (PTFE) filter (Pall Acrodisc®, Separations) and the extract was subjected to UHPLC-MS/MS for analysis.

Table 3-2 Chemical characteristics of the target compounds and internal standard (From Pubchem at https://pubchem.ncbi.nlm.nih.gov/)

Compound	CAS#	Molecular formula	Molecular mass (g mol^{-1})	Structure	Dissociation constants	Water solubility

Glyphosate	1071-83-6	$C_3H_8NO_5P$	169.07		pKa1 = 2.0;
					pKa2 = 2.6;
					pKa3 = 5.6;
					pKa4 = 10.6
						10 mg/mℓ

Aminomethyl-phosphonic acid (AMPA)	1066-51-9	CH_6NO_3P	111.037		pK1: 2.35; pK2: 5.9; pK3: 10.8	50 mg/mℓ

Glufosinate (Internal standard)	51276-47-2	$C_5H_{12}NO_4P$	181.128		pKa1 = <2;
					pKa2 = 2.9;
					pKa3 = 9.8
						1 370 mg/mℓ

3.5.4 Extraction of glyphosate and AMPA from soil and sediment

Multiple extraction methods were evaluated to achieve optimal recovery (APPENDIX A; method development report from NMISA) of glyphosate and AMPA from soil and sediment samples. In this report the method using an accelerated solvent extraction (ASE) method delivered an optimal recovery of 60% which was used to extract the samples with. The soil samples were dried at room temperature in the dark and ground into a fine powder with mortar and pestle to create a homogenous sample matrix. A subsample of 10 g sediment/soil was added to a labelled 50 mℓ centrifuge tube wrapped in foil to prevent exposure to light and the mass was noted. An equal amount (1:1) of diatomaceous earth was mixed with the soil sample. Two cellulose filters were placed at the bottom of a pre-cleaned and labelled ASE extraction cell (33 mℓ). The diatomaceous earth-soil/sediment mixture was added to the extraction cell and spiked with 126 µℓ internal standard (1 µg/g). The remainder of the cell was filled with diatomaceous earth, and the top end cap was hand tightened onto the cell body. No cellulose filter was required on top. The cell volume must be fully used, and the ASE cell must be packed consistently (similar density) to ensure repeatable solvent volumes with extraction on the ASE. The amount of diatomaceous earth to mix with the sample depends on the size of the cell used. The extraction solvent used was methanol with 0.1% formic acid and 2% water (Schafer et al., 2008). The following ASE parameters were used: temperature at 110°C, static time as 6 min, 3 cycles, 80% purge volume and 120s purge time. The extract was evaporated, resuspended in 1 mℓ and filtered using a 0.22 µm PTFE syringe filter (Pall Acrodisc®, Separations) before subjected to UHPLC-MS/MS for analysis.

3.5.5 Instrumental analysis

The analysis of glyphosate and AMPA were performed on a Waters ultra-high pressure liquid chromatograph (UHPLC) coupled to a mass spectrometer (MS/MS). The LC-MS/MS conditions were optimised for all compounds and included chromatographic separation, precursor and product ion identification for specific multiple reaction monitoring (MRM) transitions and associated cone voltage (CV) and collision energy (CE) for glyphosate, AMPA and glufosinate. All compounds were analysed in negative ion mode. The instrument parameters are summarised in Table 3-3 to Table 3-7. The data were acquired using MassLynx version 4.1 (Waters, San Jose, CA, USA).

Table 3-3 Liquid chromatography (LC) instrument parameters for analysis

Sample temperature	4°C
Column	Hypercarb analytical column (2.1 x 100 mm; 5 µm) (Anatech)
Column temperature	40°C
Injection volume	10 µL
Flow rate	0.2-0.4 mℓ/min (see table Hypercarbia 3-4)
Mobile phase A	1% acetic acid, 5% methanol in Milli-Q water
Mobile phase B	1% acetic acid in methanol
Run time	30 minutes

Table 3-4 Liquid chromatography (LC) mobile phase solvent gradient

Time (minutes)	Flow (mℓ/min)	% Mobile phase A	% Mobile phase B
Initial	0.2	100	0
10.00	0.2	70	30
11.00	0.4	70	30
18.00	0.4	70	30
19.00	0.4	10	90
22.00	0.4	10	90
22.10	0.2	100	0
30.00	0.2	100	0

Table 3-5 Liquid chromatography (LC) wash solvent composition

Seal wash	90% Milli-Q water:10% methanol
Purge solvent	90% Milli-Q water:10% methanol
Strong needle wash	5% Milli-Q water:5% isopropanol:90% methanol

Table 3-6 Mass spectrometer (MS) source conditions

Source temperature	150°C
Desolvation temperature	600°C
Desolvation gas flow	1 000 L/h
Source offset	70 V
Collision gas flow	0.15 mℓ/min

Table 3-7 Multiple reaction monitoring (MRM) transitions and mass spectrometer (MS) parameters

Analyte	RT (min)	Dwell time (ms)	Q1 (m/z)	Q3 (m/z)	Cone voltage (V)	Collision voltage (V)
Glyphosate	2.98	Auto	168	150, 124, 81, 63	25	18
AMPA	1.8	Auto	110	79, 63	35	13
Glufosinate	2.1	Auto	180	95, 85	30	16

Optimal separation of the glyphosate and AMPA standards was achieved (Figure 3-6 and Figure 3-7) based on instrumental parameters (Table 3-3 to Table 3-7). The glyphosate peak eluted at 2.98 min, AMPA at 1.8 min and glufosinate at 2.1 (peak not shown). The MRM parameters of additional highly polar pesticides (HPP)/metabolites of glyphosate were also analysed and their information can be found in Appendix A.

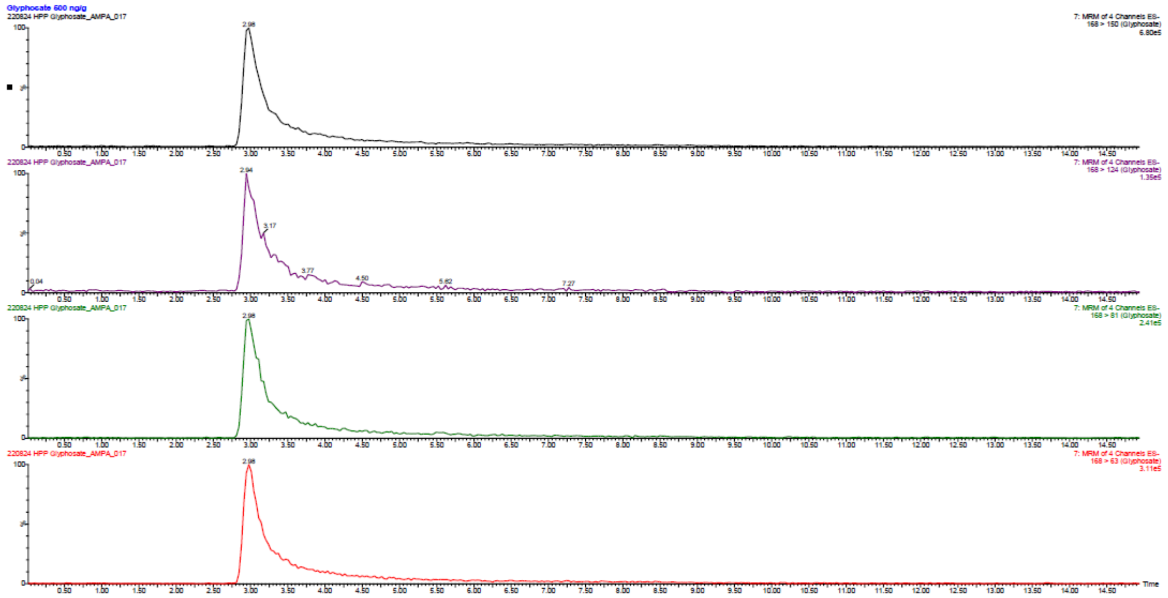


Figure 3-6 Multiple reaction monitoring (MRM) chromatogram for glyphosate

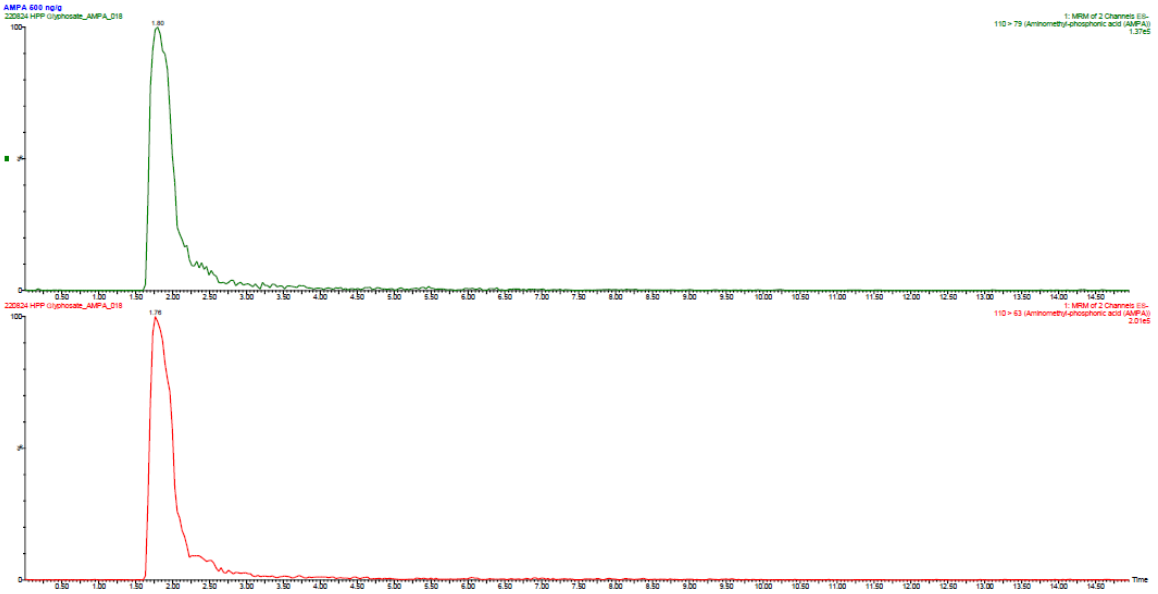


Figure 3-7 Multiple reaction monitoring (MRM) chromatogram for AMPA.

3.5.6 Method validation

The following section describes the quality control and quality assurance performed to complete the method validation for analysis of glyphosate and AMPA in water, soil and sediment.

3.5.6.1 Preparation of quality control samples

Quality control samples were prepared to determine the recovery % of target compounds from each type of matrix. These control samples were analysed together with each batch of samples. This enabled a way to evaluate degradation and correct for this deviation in the final data. Three samples of each matrix per batch were spiked with a known concentration prior to extraction, and three blank samples were extracted, but spiked only after extraction (Table 3-8). This is known as spike before (SB) and spike after (SA) quality control samples, respectively. Additionally, a matrix blank was also analysed to evaluate if there are any analytes of interest present in the matrix used for the spike samples as well as the external matrix matched calibration curve.

Table 3-8 Quality control sample preparation summary

Matrix			End concentration (ng/g)
		Spike before (SB)	
Water	Milli-Q® water	Three samples spiked, extracted, analysed	1 000
Soil	Blank soil	Three samples spiked, extracted, analysed	1 000
		Spike after (SA)	
Water	Milli-Q® water	Three samples extracted, spiked, analysed	1 000
Soil	Blank soil	Three samples extracted, spiked, analysed	1 000
		Blank	
Water	Milli-Q® water	No spike, extracted, analysed	0
Soil	Blank soil	No spike, extracted, analysed	0

3.5.6.2 Calibration curve

To account for matrix suppression and enhancement effects during quantification, an external matrix matched calibration curve (spiked post-extraction) was prepared for water and sediment/soil. The calibration concentration range (0, 0.002, 0.005, 0.01, 0.02, 0.06, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ug/g) was determined based on the expected levels of glyphosate and AMPA in the environment and the performance of the instrument. The calibration standards were not serially diluted but originated from different stocks. These standards were analysed in triplicate to assess the reportable range (Westgard, 2008). They were injected in order of increasing concentration, with blank injections in-between to prevent carry-over.

3.5.6.3 Limit of detection (LOD)/Limit of quantification (LOQ)

The sensitivity of an analytical method is defined as the increased response of the analyte linear to the analyte concentration (Whitmire et al., 2011). This is displayed with a calibration curve and the slope of the calibration curve. By using linear regression statistics, the uncertainties of the calibration curve can be used to calculate the limit of detection (LOD) and limit of quantification (LOQ) for the method from the external matrix matched calibration curve. By use of the y=mx+c model, LOD is calculated by 3*Sa/b and LOQ by 10*Sa/b; where Sa is the SD of the intercept (abundance) and b is the slope of the calibration curve. The LOD and LOQ was evaluated with gravimetric preparation of the calibration curve.

3.5.6.4 Data analysis

The concentration (conc) of glyphosate and AMPA in the samples was determined by using this formula:

$$X = ((\text{native}/\text{internal standard}) - c) / m) \times \text{IS conc}$$

Where X = calculated glyphosate or AMPA concentration; native = glyphosate or AMPA abundance; internal standard = glufosinate abundance; c = calibration curve y-intercept; m = slope of calibration curve; ISO conc = glufosinate concentration

3.5.6.5 Precision

Repeatability of the method was determined by analysing quality control (QC) samples. Precision was calculated using:

% RSD = (mean of SDEV of QCs/mean of QCs) x 100

For great repeatability, the % RSD should be lower than 15% (Schoeman et al., 2015). Intra-day precision was determined by six injections of the QC sample on the same day and inter-day precision was determined by six injections of a QC sample on another day.

3.5.6.6 Accuracy

Accuracy was determined by the mean-recovery of spiked compounds in the matrix to be analysed. The formula used was:

% Accuracy = (mean concentration obtained/true concentration) x 100.

3.5.6.7 Linearity

Linearity of the external matrix matched calibration curve was assessed by determining the R-square (R^2) value of the calibration curve. Good linearity is indicated with R^2 as close to one as possible (Miller and Miller, 2010).

3.6 HUMAN HEALTH RISK ASSESSMENT

3.6.1 Introduction

It is evident from the preceding sections that GLY ends up in the water resources and may be harmful to non-target organisms. In an attempt to predict the risks caused by GLY in the water sources, a human health risk assessment was conducted. The risk assessment process includes four steps: 1) hazard identification; 2) hazard characterisation; 3) exposure assessment and 4) risk characterisation (WHO, 2010). The hazard identification step identifies the ability of compounds/metals to cause various health effects. Hazard characterisation includes a qualitative and quantitative description of the properties of an agent having the potential to cause adverse health effects. The exposure assessment evaluates the concentration of a particular agent that reaches a target population. The final step is risk characterisation that summarises the finding to use for advice in decision-making.

3.6.2 Calculations

A human health risk assessment was done by using the well-established methods described by the United States Environmental Protection Agency (USEPA, (2020; Bhandari et al., 2020). The probability to develop health effects, both cancer risk (CR) and harmful non-cancerous effects (hazard quotient: HQ) were determined using the results concentrations determined for glyphosate and AMPA. Two exposures routes were explored in this human health risk assessment. Ingestion was considered as the main exposure route to glyphosate and AMPA via consumption and household-use of untreated surface water by residents living in areas where municipal treated drinking water is not always available. The second exposure route was via dermal absorption in the event where residents might bath in untreated surface water. The level of absorption of glyphosate and AMPA via dermal contact is however minimal. Dermal absorption is influenced by factors such as the type of glyphosate formulation, the concentration, the duration of skin contact, and the condition of the skin (broken or intact skin). The following equations were used to conduct the human health risk assessment.

The average daily dose/chronic daily intake (mg/kg-day) via ingestion was determined as follows: ing

$$CDI_{ing}=\frac{C_W \text{ x IR x EF x ED}}{\text{BM x AT}}$$

and

$$CDI_{der}=\frac{\text{Cw x SA x PC x ET x EF x ED x F}}{\text{BM x AT}}$$

where CDI_{ing} is the chronic daily intake (mg/kg-day) via ingestion and CDI_{der} the chronic daily intake via dermal absorption (mg/kg-day) (IRIS, 2023). The meaning of the abbreviations and are summarised in (Table 3-9).

Some values used in the calculations were selected arbitrarily since these were not based on any measurements. These include the number of times South Africans would take a bath per year, which was estimated to be everyday (EF in Table 3-9), and the time spent to bathe (ET in Table 3-9)

Table 3-9 Parameters used to determine the CDI for the South African scenario

Input parameters	Units	Value	Reference
Cw: concentration	mg/ℓ	quantified in this study	
BM: body mass	kg	adult: 66 kg	Pheiffer et al. (2018)
IR: ingestion rate	ℓ/day	1.5	Munene et al. (2023)
ET: duration in water	h/event	0.25 h	McDougal and Jurgens-Whitehead (2001)
EF: in water	events/year	365	
ED: exposure duration	year	adult: 70 years	
F: conversion factor	%	skin fraction in contact with water = 100	
AT: average time	days	365 x ED	
SA: skin surface area	m^2	Mostellar formula, mean SA adult mass and mean length of SA adult: 1.73 m^2	Global RPh (2017) NCD-RisC (2016) Puoane et al. (2002)
PC: dermal permeability coefficient (glyphosate and AMPA)	cm/h	2.40×10^{-5} & 5.21×10^{-6}	Wester et al (1996)

The cancer risk (CR) was calculated for ingestion and dermal exposure using the following formula:

$$CR=CDI \times SF$$

where, CR is the estimated cancer risk of contaminants from water, CDI is the estimated average daily intake (mg/kg-day) and SF is the carcinogenicity slope factor (mg/kg-day) (Table 3-10).

The non-cancer risk of glyphosate and AMPA via ingestion and dermal exposure, expressed as hazard quotient (HQ), was calculated by:

$$HQ=CDI\times RfD$$

where, CDI is the estimated average daily intake (mg/kg-day) and RfD is the reference dose (mg/kg-day) of glyphosate and AMPA.

For the compound specific slope factors (SF) and reference doses (RfD), refer to Table 3-10. The SF shows the 95-percentile upper-bound lifetime cancer risk from exposure to the carcinogen and the RfD is the safe dose that may be ingested with no adverse effect.

Exposed individuals were considered to be safe if the HQ < 1. A HQ exceeding 1 is not a statistical possibility of harm occurring, but instead, it is an indicator of whether (and by how much) an exposure concentration exceeds the reference concentration. A cancer risk calculated for ingestion which is less than 1×10^{-4} (1 in 10 000) is considered acceptable risk. Similarly, for dermal exposure a risk less than 1×10^{-6} (1 in 1 000 000) is considered negligible. Any CR values greater than these acceptable risks, means that cancer may develop due to exposures to glyphosate or AMPA based on the risk assessment under the assumptions made.

Table 3-10 Compound specific parameters used for cancer and non-cancer risk (Ferreira et al., 2023).

Formula abbreviation	**Glyphosate mg/kg-day**	**AMPA mg/kg-day**
SF: Carcinogenicity slope factor (oral and dermal)	6.3×10^{-4}	6.3×10^{-4}
RfD: Reference dose (oral and dermal)	1×10^{-1}	1×10^{-1}

4 RESULTS & DISCUSSION

4.1 INTRODUCTION

The water, soil; and sediment are subjected to various anthropogenic impacts that can influence the water, soil and sediment quality. The Department of Water and Sanitation (DWS) of South Africa National Water Act, (Act no. 36 of 1998) set parameters for surface water of South Africa, referred to as the resource water quality objectives (RWQO), to be used for irrigation purposes. These RWQO were based on the resource water quantity, quality, habitat and biota. The RWQO vary depending on in which water catchment the sample sites are located. These vary because of different lithologies and geologies in the areas together with the anthropogenic activities that may influence these different water quality parameters. The Harts River in the Vaalharts irrigation scheme is in the Lower Vaal water catchment (DWS, 2016a), the Klein Olifants River falls in the Olifants water catchment (DWS, 2016b), Life Water- and Vaal Rivers and non-agricultural sites are classified under the Upper Vaal water catchment (DWS, 2016c).

4.2 GLYPHOSATE-BASED-HERBICIDES USE MAP

The first aim of the project was to obtain updated data on the use of glyphosate-based herbicides in South Africa. The data bought from Kynetec was collected in 2017 and 2019 and visually presented in the form of maps (Figure 4-1 and Figure 4-2).

The 4-crop data visualisation (maize, wheat/barley, soybean, and sunflower) represents approximately 80% of the total GBH market in South Africa at an active ingredient level. Maize consists of 54% followed by soybean at approximately 15%. The share of non-agricultural (industrial and urban) GBH use is estimated to be between 2 and 2.5%. The highest GBH applications in 2017 and 2019 was recorded for the Free State Province.

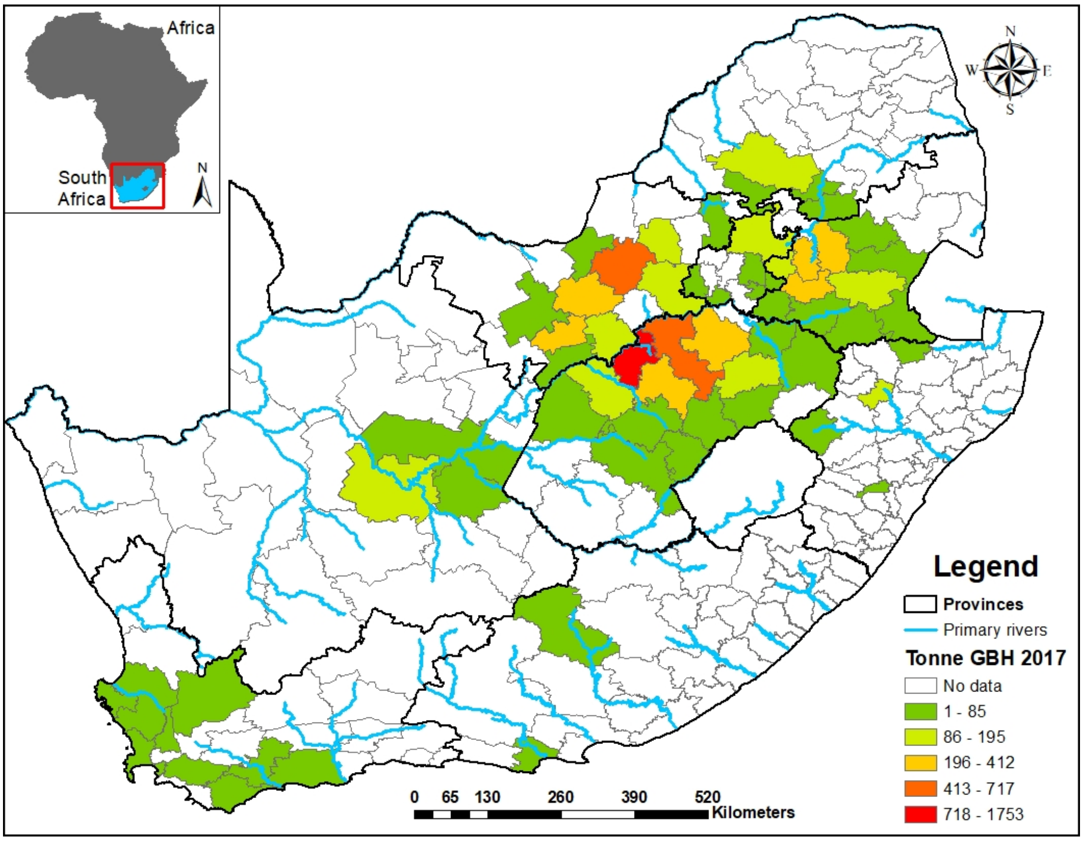


Figure 4-1 The application of glyphosate-based herbicides (GBHs) (tonnes) for the municipal districts of South Africa applied to maize, soybean, wheat, and sunflower for 2017.

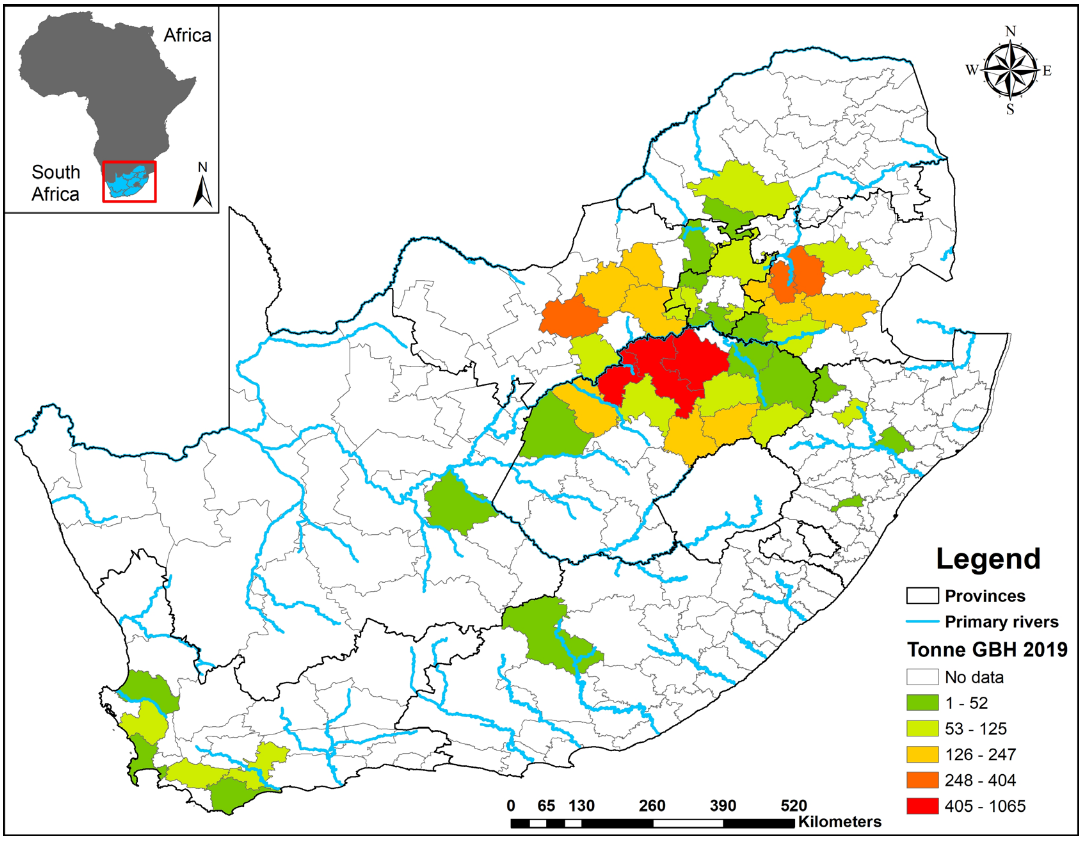


Figure 4-2 The application of glyphosate-based herbicides (GBHs) (tonnes) for the municipal districts of South Africa applied to maize, soybean, wheat, and sunflower for 2019.

4.3 PHYSICO-CHEMICAL PARAMETERS

4.3.1 Before spraying sampling-event

4.3.1.1 Vaalharts Irrigation Scheme (VIS)

Most water quality parameters for water from VIS did not exceed the RWQO for its catchment area (Table 4-1 Water quality results before spraying (September/October 2020) Vaalharts irrigation scheme in the Lower Vaal River Parameters such as the TEMP, SS, TURB, Cl^- and TH do not have an RWQO guideline level. The TEMP ranged from 18-23.5°C and the SS of the samples ranged 5-351 mg/ℓ, Cl^- had a range of 15-220 mg/ℓ and TH was 54-190 mg/ℓ. The TURB ranged from 2-40 FAU. However, NH_4^+ exceeded the RWQO at all VIS sites except MIC, ranging from 0.09-0.83 mg/ℓ with the highest being at HR2. The PO_4^{3-} exceeded the RWQO at all sites and ranged from 0.11-0.43 mg/ℓ (Table 4-1). Moreover, the NO_3^- also exceeded the RWQO with a range of 1.7-8.6 mg/ℓ with C2 having the highest value. The RWQO for the SO_4^{2-} is <200 mg/ℓ and C2, HR3, HR4 and SD exceeded the RWQO with a range of 205-248 mg/ℓ. The conductivity exceeded the RWQO at C2, HR3, HR4, SD and DSD 111.3-217 mS/m.

4.3.1.2 Upper Vaal and Olifants River catchments (Mpumalanga Province (MPA))

All of the water quality parameters (Table 4-2) were within the RWQO except for PO_4^{3-} and NH_4^+. The PO_4^{3-} levels exceeded the RWQO at all the sites (Table 4-2). The PO_4^{3-} ranged from 0.20-1.12 mg/ℓ and NH_4^+ was 0.13-3.44 mg/ℓ. The NH_4^+ levels at all sites, except MP10 and DMD, exceeded the RWQO guideline. The pH ranged from 6.41 to 9.43 at MP10, which exceeded the RWQO pH range of 6.5-8.5. As for the parameters with no RWQO, they ranged TEMP (13.7-26.9°C), SS (3-371 mg/ℓ), TURB (3-67 FAU), Cl^-(2-22 mg/ℓ) and TH (26-177 mg/ℓ). The conductivity at sites MP4, MP6 and X2 were higher than 111 mS/m but site PAN1 has a conductivity of 2 320 mS/m, 20 times higher than the limit.

4.3.1.3 Non-agricultural sites

The PO_4^{3-} ranged from 0.18-0.77 mg/ℓ and the NH_4^+ ranged from 0.13-4.24 mg/ℓ exceeding the RWQO at all the sites (Table 4-4). The pH at VB was lower than the RWQO at 6.06. All the other water quality parameters of the sites in Table 4-1 were all within the RWQO. These include the EC (0.64-1.04 mS/m), NO_2^- (0.02-0.30 mg/ℓ), and NO_3^- (0.80-2.10 mg/ℓ). For the parameters without an RWQO designation, the ranges were: TEMP (24.9-29.7°C), SS (3-52 mg/ℓ), TURB (4-16 FAU), Cl^- (32-79 mg/ℓ) and TH (137-228 mg/ℓ).

Table 4-1 Water quality results before spraying (September/October 2020) Vaalharts irrigation scheme in the Lower Vaal River catchment.

Water quality parameters	Symbol	RWQO	TO	HR1	C1	HR2	C2	MIC	HR3	HR4	SD	DSD
pH	pH	6.5-8.5	5.8	6.8	6.8	7.04	6.81	6.9	7.00	7.02	8.09	7.46
Temperature (°C)	TEMP	N/A	22.7	21.0	-	23.3	23.5	20.7	23.2	18.0	22.2	22.4
Conductivity (mS/m)	EC	<111	24.5	21.8	106.7	95.3	217	62.5	140	131	111.3	111.4
Suspended solids (mg/ℓ)	SS	N/A	71	15	26	5	43	12	28	10	14	351
Turbidity (FAU)	TURB	N/A	40	6	9	12	2	19	14	28	13	26
Ammonium (mg/ℓ)	NH_4^+	<0.07	0.10	0.09	0.18	0.83	0.30	0.07	0.32	0.21	0.25	0.29
Nitrite (mg/ℓ)	NO_2^-	<1	0.01	0.04	0.02	0.04	0.04	0.03	0.15	0.04	0.01	0.02
Nitrate (mg/ℓ)	NO_3^-	<1	1.80	1.70	5.20	3.90	8.60	2.10	4.70	7.90	2.20	3.30
Sulphate (mg/ℓ)	SO_4^{2-}	<200	13	16	189	180	248	114	226	228	205	198
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.025	0.23	0.11	0.21	0.14	0.40	0.29	0.25	0.43	0.22	0.27
Chloride (mg/ℓ)	Cl^-	N/A	15	19	68	83	220	42	118	136	96	86
Total hardness (mg/ℓ)	TH	N/A	54	54	180	153	190	87	101	176	169	175

RWQO = Resource water quality objectives, TO = Taung Overflow, HR1 = Harts River 1, C1 = Channel 1, HR2 = Harts River 2, C2 = Channel 2, MIC = Main inflow channel, HR3 = Harts River 3, HR4 = Harts River 4, SD = Spitskop Dam, DSD = Downstream Spitskop Dam. Grey highlighted values exceeded the RWQO.

Table 4-2 Water quality results before spraying (September/October 2020) in Mpumalanga in the Olifants River catchment.

Water quality parameters	Symbol	RWQO	MD	DMD	MP4	MP6	PAN1	MP10
pH	pH	6.5-8.5	6.75	6.41	7.36	7.06	8.27	9.43
Temperature (°C)	TEMP	N/A	20.6	19.7	22.2	23.3	26.9	20.4
EC	<111	85	105.4	316	260	2320	42.5	EC
Suspended solids (mg/ℓ)	SS	N/A	5	3	7	73	371	1
Turbidity (FAU)	TURB	N/A	7	3	12	3	94	11
Ammonium (mg/ℓ)	NH_4^+	<0.1	0.13	0.10	0.22	0.14	3.44	0.09
Nitrite (mg/ℓ)	NO_2^-	<4	0.01	0.01	0.01	0.01	0.00	0.01
Nitrate (mg/ℓ)	NO_3^-	<4	0.90	0.80	0.50	2.30	0.36	0.60
Sulphate (mg/ℓ)	SO_4^{2-}	<500	70	77	75	281	233	44
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.22	0.28	0.27	0.51	0.69	1.30
Chloride (mg/ℓ)	Cl^-	N/A	17	6	22	16	2	16
Total hardness (mg/ℓ)	TH	N/A	26	27	113	81	144	48

RWQO = Resource water quality objectives, MD = Middelburg Dam, DMD = Downstream Middelburg Dam, MP4 = Mpumalanga 4, MP6 = Mpumalanga 6, PAN1 = Pan 1, MP10 = Mpumalanga 10. Grey highlighted values exceeded the RWQO..

Table 4-3 Water quality results before spraying (September/October 2020) in Mpumalanga in the Upper Vaal River catchment.

Water quality parameters	Symbol	RWQO	X1	X2	X3	V9	V11	V12	V13	GD	V15
pH	pH	6.5-8.5	8.13	7.83	7.94	7.60	7.45	7.43	7.50	7.25	7.30
Temperature (°C)	TEMP	N/A	13.7	16.8	15.5	18.5	19.7	19.8	18.5	18.9	18.9
Conductivity (mS/m)	EC	<70	65.1	74.2	61	19.4	19.8	19.5	21.6	27.6	28.3
Suspended solids (mg/ℓ)	SS	N/A	2	9	34	53	67	63	72	34	29
Turbidity (FAU)	TURB	N/A	5	14	6	37	55	54	67	37	26
Ammonium (mg/ℓ)	NH_4^+	<0.073	0.30	0.16	1.02	0.19	0.13	0.19	0.16	0.11	0.33
Nitrite (mg/ℓ)	NO_2^-	<0.858	0.01	0.01	0.15	0.02	0.03	0.03	0.00	0.02	0.03
Nitrate (mg/ℓ)	NO_3^-	<0.858	1.23	1.00	2.50	1.10	1.20	0.50	1.30	1.00	1.00
Sulphate (mg/ℓ)	SO_4^{2-}	<500	52	62	61	37	38	46	44	56	60
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.02	0.20	0.99	1.12	0.22	0.23	0.98	0.59	0.60	0.90
Chloride (mg/ℓ)	Cl^-	N/A	21	15	19	12	10	18	10	12	12
Total hardness (mg/ℓ)	TH	N/A	151	177	104	36	35	39	36	44	46

RWQO = Resource water quality objectives, X1 = Life Water River, X2 = Life Water River, X3 = Life Water River, V9 = Vaal River 9, V11 = Vaal River 11, V12 = Vaal River 12, V13 = Vaal River 13, GD = Grootdraai, V15 = Vaal River 15. Grey highlighted values exceeded the RWQO.

Table 4-4 Water quality results of non-agricultural sites in the Upper Vaal River catchment in September/October 2020.

Water quality parameters	Symbol	RWQO	VB	DGP	MM	TRE	GG	BT
pH	pH	6.5-8.5	6.06	7.21	7.09	8.11	7.77	7.42
Temperature (°C)	TEMP	N/A	26.5	25.9	25.3	29.7	24.9	29.3
Conductivity (mS/m)	EC	<111	68	95.1	103.9	63.5	74.4	63.6
Suspended solids (mg/ℓ)	SS	N/A	43	3	14	52	38	7
Turbidity (FAU)	TURB	N/A	8	6	11	16	4	8
Ammonium (mg/ℓ)	NH_4^+	<0.073	4.24	0.13	0.14	0.27	0.31	0.23
Nitrite (mg/ℓ)	NO_2^-	<4	0.30	0.02	0.03	0.02	0.04	0.03
Nitrate (mg/ℓ)	NO_3^-	<4	2.10	0.80	1.00	1.00	0.70	1.50
Sulphate (mg/ℓ)	SO_4^{2-}	<500	103	143	163	124	124	84
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.77	0.56	0.72	0.27	0.20	0.18
Chloride (mg/ℓ)	Cl^-	N/A	44	48	79	32	41	48
Total hardness (mg/ℓ)	TH	N/A	137	143	228	143	179	161

RWQO = Resource water quality objectives, VB = Vaal Barrage, DGP = Downstream Golf course Potchefstroom, MM = Mooiriver Mall, TRE = Tuscany Ridge Estate, GG = Gimmie Gat, BT = Botanical Gardens. Grey highlighted values exceeded the RWQO.

4.3.2 Post herbicide application sampling-event

4.3.2.1 Vaalharts Irrigation Scheme (VIS)

The NO_3^- levels exceeded the RWQO at all the sites (Table 4-5), except for MIC which was only slightly below the RWQO. Sites SD and DSD had higher SO_4^{2-} levels of 204 and 215 mg/ℓ respectively, exceeding the RWQO (Table 4-5). These two sites are located in the catchment area of the Spitskop Dam before flowing into the Vaal River. The parameters with no RWQO limit showed a range for TEMP (24.5-33°C), SS (1-114 mg/ℓ), TURB (4-70 FAU), Cl^-(5-231 mg/ℓ) and TH (47-201 mg/ℓ). Sites C2, HR3, HR4, DSSD, and DSD exceeded the resource limit of the electrical conductivity.

4.3.2.2 Upper Vaal and Olifants River catchments (Mpumalanga Province (MPA)

Ten out of the fifteen sites exceeded the NH_4^+ RWQO (Table 4-6). The NO_3^- exceeded the RWQO at most of the sites, except for MP6, V9 and GD (Table 4-6). The PO_4^{3-} at most of the sites also exceeded the RWQO (Table 4-6). The pH at MP4 and PAN1 were 8.59 and 8.65 (RWQO pH 6.5-8.5) respectively. The parameters with no RWQO limit showed a range for TEMP (23.1-30.9°C), SS (1-9436 mg/ℓ), TURB (2-81 FAU), Cl^-(7-243 mg/ℓ) and TH (53-322 mg/ℓ). The conductivity of PAN1 greatly exceeded the limit at 1656 mS/m.

4.3.2.3 Non-agricultural sites

The GG site had a high pH of 8.88 and a low pH of 6.05 was measured at DGP (Table 4-8). The NH_4^+ levels were higher than the RWQO limit at all non-agricultural sites and the highest levels were recorded at DGP. The PO_4^{3-} for sites GDP, MM, TRE and BT exceeded the RWQO (Table 4-8). Parameters within the respective RWQO limits showed the EC (0.46-1.69 mS/m), NO_2^- (1.3-4 mg/ℓ), NO_3^- (0.01-0.28 mg/ℓ) and SO_4^{2-} (59-166 mg/ℓ). The parameters with no RWQO limit showed a range for TEMP (23.8-28.7°C), SS (3-158 mg/ℓ), TURB (6-15 FAU), Cl^-(33-62 mg/ℓ) and TH (85-217 mg/ℓ).

Table 4-5 Water quality results post herbicide application (November 2020) in the Vaalharts irrigation scheme in the Lower Vaal River catchment.

Water quality parameters	Symbol	RWQO	TO	HR1	C1	HR2	C2	MIC	HR3	HR4	SD	DSD
pH	pH	6.5-8.5	7.94	8.38	7.73	7.53	7.74	7.99	7.75	7.84	8.18	8.18
Temperature (°C)	TEMP	N/A	26.7	28.1	33.0	31.5	30.2	26.5	28.3	26.0	24.5	24.7
Conductivity (mS/m)	EC	<111	33.1	27.6	77.8	101	226	66.6	194.2	168.2	123.1	120.4
Suspended solids (mg/ℓ)	SS	N/A	14	4	114	39	1	5	11	2	4	2
Turbidity (FAU)	TURB	N/A	9	17	70	31	4	34	33	25	42	55
Ammonium (mg/ℓ)	NH_4^+	<0.07	0.06	0.12	0.03	0.10	0.12	0.33	0.11	0.25	0.07	0.25
Nitrite (mg/ℓ)	NO_2^-	<1	0.00	0.01	0.08	0.03	0.05	0.01	0.24	0.02	0.00	0.01
Nitrate (mg/ℓ)	NO_3^-	<1	5.70	1.30	6.30	7.20	8.30	0.90	7.60	6.60	2.20	6.90
Sulphate (mg/ℓ)	SO_4^{2-}	<200	15	20	165	183	65	128	147	51	204	215
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.025	0.02	0.13	0.05	0.05	0.11	0.03	0.22	0.07	0.03	0.09
Chloride (mg/ℓ)	Cl^-	N/A	13	5	66	86	231	50	205	182	141	128
Total hardness (mg/ℓ)	TH	N/A	56	47	137	153	110	101	201	179	173	178

RWQO = Resource water quality objectives, TO = Taung Overflow, HR1 = Harts River 1, C1 = Channel 1, HR2 = Harts River 2, C2 = Channel 2, MIC = Main inflow channel, HR3 = Harts River 3, HR4 = Harts River 4, SD = Spitskop Dam, DSD = Downstream Spitskop Dam. Grey highlighted values exceeded the RWQO

Table 4-6 Water quality results post-herbicide application (November 2020) in Mpumalanga in the Olifants River catchment.

Water quality parameters	Symbol	RWQO	MD	DMD	MP4	MP6	PAN1	MP10
pH	pH	6.5-8.5	8.14	8.02	8.59	7.57	8.65	7.38
Temperature (°C)	TEMP	N/A	30.9	26.2	24.4	23.1	28.6	22.7
Conductivity (mS/m)	EC	<111	107.3	110.4	301.1	131.7	1656	40.7
Suspended solids (mg/ℓ)	SS	N/A	7	8	1	17	9436	13
Turbidity (FAU)	TURB	N/A	5	3	2	4	76	13
Ammonium (mg/ℓ)	NH_4^+	<0.1	0.06	0.14	0.15	0.12	4.21	0.08
Nitrite (mg/ℓ)	NO_2^-	<4	0.01	0.02	0.05	0.01	0.06	0.01
Nitrate (mg/ℓ)	NO_3^-	<4	24.9	4.5	21.7	2.70	12.6	25
Sulphate (mg/ℓ)	SO_4^{2-}	<500	195	136	217	243	231	36
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	4.16	0.04	2.24	0.04	4.59	0.05
Chloride (mg/ℓ)	Cl^-	N/A	16	17	22	18	243	38
Total hardness (mg/ℓ)	TH	N/A	221	244	239	322	230	104

RWQO = Resource water quality objectives, MD = Middelburg Dam, DMD = Downstream Middelburg Dam, MP4 = Mpumalanga 4, MP6 = Mpumalanga 6, PAN1 = Pan 1, MP10 = Mpumalanga 10. Grey highlighted values exceeded the RWQO.

Table 4-7 Water quality results post-herbicide application (November 2020) in Mpumalanga in the Upper Vaal River catchment.

Water quality parameters	Symbol	RWQO	X1	X2	X3	V9	V11	V12	V13	GD	V15
pH	pH	6.5-8.5	7.49	7.46	7.19	7.00	7.35	7.22	7.42	7.60	7.42
Temperature (°C)	TEMP	N/A	27.1	27.4	27.7	27.5	25.9	28.3	26.2	21.9	23.2
Conductivity (mS/m)	EC	<70	60.5	76.3	56.4	22.2	20.4	20.2	27.6	26.6	27
Suspended solids (mg/ℓ)	SS	N/A	9	14	22	77	3	68	104	157	11
Turbidity (FAU)	TURB	N/A	7	18	24	67	73	81	69	74	19
Ammonium (mg/ℓ)	NH_4^+	<0.073	0.14	0.09	0.86	0.18	0.10	0.47	0.04	0.00	0.04
Nitrite (mg/ℓ)	NO_2^-	<0.858	0.01	0.01	0.11	0.02	0.05	0.02	0.06	0.11	0.01
Nitrate (mg/ℓ)	NO_3^-	<0.858	23.4	21.1	5.50	2.00	6.10	6.5	23.8	3.00	24
Sulphate (mg/ℓ)	SO_4^{2-}	<500	56	60	85	48	53	44	59	59	62
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.02	0.04	0.07	0.65	0.08	0.08	0.18	0.05	0.15	0.06
Chloride (mg/ℓ)	Cl^-	N/A	28	30	31	7	10	14	8	7	8
Total hardness (mg/ℓ)	TH	N/A	197	234	119	53	52	53	69	65	67

RWQO = Resource water quality objectives, X1 = Life Water River, X2 = Life Water River, X3 = Life Water River, V9 = Vaal River 9, V11 = Vaal River 11, V12 = Vaal River 12, V13 = Vaal River 13, GD = Grootdraai, V15 = Vaal River 15. Grey highlighted values exceeded the RWQO

Table 4-8 Water quality results of the non-agricultural sites in the Upper Vaal River catchment in November 2020.

Water quality parameters	Symbol	RWQO	VB	DGP	MM	TRE	GG	BT
pH	pH	6.5-8.5	8.07	6.05	6.66	7.00	8.88	7.9
Temperature (°C)	TEMP	N/A	23.8	25.1	25.7	26.7	28.7	25.2
Conductivity (mS/m)	EC	<111	69	90.9	90.1	45.9	72	48.8
Suspended solids (mg/ℓ)	SS	N/A	158	4	29	19	39	3
Turbidity (FAU)	TURB	N/A	7	15	9	10	14	6
Ammonium (mg/ℓ)	NH_4^+	<0.073	0.15	2.94	0.41	0.13	0.14	0.13
Nitrite (mg/ℓ)	NO_2^-	<4	3.10	4.00	2.50	2.40	1.70	1.30
Nitrate (mg/ℓ)	NO_3^-	<4	0.03	0.28	0.05	0.06	0.01	0.01
Sulphate (mg/ℓ)	SO_4^{2-}	<500	106	152	152	166	88	59
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.09	0.84	0.77	1.16	0.10	0.14
Chloride (mg/ℓ)	Cl^-	N/A	33	54	62	60	44	35
Total hardness (mg/ℓ)	TH	N/A	154	118	217	204	85	132

RWQO = Resource water quality objectives, VB = Vaal Barrage, DGP = Downstream Golf course Potchefstroom, MM = Mooiriver Mall, TRE = Tuscany Ridge Estate, GG = Gimmie Gat, BT = Botanical Gardens. Grey highlighted values exceeded the RWQO

4.3.3 After rain sampling-event

4.3.3.1 Vaalharts Irrigation Scheme (VIS)

The pH at HR1 was lower than the RWQO guideline with a value of 6.1 (Table 4-9). The NH_4^+ at all the sites (except for C2) exceeded the RWQO ranging from 0.08-0.14 (Table 4-9). The NO_2^- at HR1 and SO_4^{2-} at C1 exceeded RQWO (Table 4-9). All the sites showed increased levels NO_3^- higher than the RWQO (NO_3^-<1 mg/ℓ), except for HR2. The PO_4^{3-} of all the sites exceeded the RWQO (Table 4-9). However, parameters that do not exceed the RWQO were the EC (0.21-1.59 mS/m). The parameters with no RWQO limit showed a range for TEMP (24.2-30.5°C), SS (97-399 mg/ℓ), TURB (25-67 FAU), Cl^-(14-24 mg/ℓ) and TH (5-301 mg/ℓ).

4.3.3.2 Upper Vaal and Olifants River catchments (Mpumalanga Province (MPA)

The levels of NH_4^+ exceeded the RWQO at sites MD, MP6, MP10, X1 and V13 and PO_4^{3-} exceeded the RWQO at sites DMD, MP6, MP10, X1, X2, X3, V9, V11, V12, V13, GD and V15 (Table 4-10). The parameters that did not exceed the RWQO were the EC (0.2-10.10 mS/m), NO_2^- (0.2-2.0 mg/ℓ), NO_3^- (0.2-2.0 mg/ℓ) and SO_4^{2-} (32-278 mg/ℓ). The parameters with no RWQO, the ranges were for TEMP (17.8-24°C), SS (5-405 mg/ℓ), TURB (6-43 FAU), Cl^-(14-25 mg/ℓ) and TH (5-288 mg/ℓ). PAN1 had a conductivity value of 1010 mS/m, ten times over the limit if 111 mS/m.

4.3.3.3 Non-agricultural sites

The pH at sites GG and BT had values of 8.66 and 8.45 respectively and exceeded the RWQO (Table 4-12). The NH_4^+ at Vaal Barrage (VB), DGP and MM exceeded the RWQO (Table 4-12) with values 1.15, 0.09 and 0.08 mg/ℓ respectively. As for the PO_4^{3-} all the sites exceeded the PO_4^{3-} (Table 4-12). The RWQO that were not exceeded, were the EC (0.59-0.78 mS/m), NO_2^- (0.01-0.35 mg/ℓ), NO_3^- (0.5-2 mg/ℓ) and SO_4^{2-} (43-133 mg/ℓ). As for the parameters with no RWQO, they ranged as follows: TEMP (19.5-22°C), SS (0-90 mg/ℓ), TURB (1-52 FAU), Cl^-(17-26 mg/ℓ) and TH (67-222 mg/ℓ).

Table 4-9 Water quality results after a rain event (March 2021) in the Vaalharts irrigation scheme in the Lower Vaal River catchment.

Water quality parameters	Symbol	RWQO	TO	HR1	C1	HR2	C2	MIC	HR3	HR4	SD	DSD
pH	pH	6.5-8.5	6.89	6.10	6.87	6.70	6.91	6.84	6.48	6.50	7.19	6.64
Temperature (°C)	TEMP	N/A	24.2	26.8	29.2	26.1	30.5	26.3	26.0	23.0	23.1	30.5
Conductivity (mS/m)	EC	<111	24.5	21	128.6	64.8	159.2	40.2	94.6	97.5	45.8	37.6
Suspended solids (mg/ℓ)	SS	N/A	75	56	83	155	164	215	155	399	97	153
Turbidity (FAU)	TURB	N/A	-	45	51	38	65	61	53	56	67	25
Ammonium (mg/ℓ)	NH_4^+	<0.07	0.09	0.25	0.16	0.41	0.06	0.08	0.16	0.13	0.18	0.14
Nitrite (mg/ℓ)	NO_2^-	<1	0.22	1.4	0.31	0.18	0.15	0.08	0.11	0.12	0.11	0.19
Nitrate (mg/ℓ)	NO_3^-	<1	1.10	14.00	10.00	0.60	3.40	1.20	1.80	1.90	1.00	4.10
Sulphate (mg/ℓ)	SO_4^{2-}	<200	44	46	202	78	177	90	130	150	63	67
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.025	0.13	2.60	0.31	0.05	0.04	0.05	0.06	0.08	0.06	0.08
Chloride (mg/ℓ)	Cl^-	N/A	17	20	14	24	24	21	18	21	22	23
Total hardness (mg/ℓ)	TH	N/A	5	5	301	101	151	5	29	114	211	78

RWQO = Resource water quality objectives, TO = Taung Overflow, HR1 = Harts River 1, C1 = Channel 1, HR2 = Harts River 2, C2 = Channel 2, MIC = Main inflow channel, HR3 = Harts River 3, HR4 = Harts River 4, SD = Spitskop Dam, DSD = Downstream Spitskop Dam. Grey highlighted values exceeded the RWQO

Table 4-10: Water quality results after a rain event (March 2021) in Mpumalanga in the Olifants River catchment.

Water quality parameters	Symbol	RWQO	MD	DMD	MP4	MP6	PAN1	MP10
pH	pH	6.5-8.5	7.86	8.02	7.87	7.95	7.30	7.60
Temperature (°C)	TEMP	N/A	21.1	20.7	21.5	20.6	17.8	18.5
Conductivity (mS/m)	EC	<111	77.3	108.4	191.2	117.5	1010	36.6
Suspended solids (mg/ℓ)	SS	N/A	405	5	13	5	32	35
Turbidity (FAU)	TURB	N/A	42	25	26	42	43	32
Ammonium (mg/ℓ)	NH_4^+	<0.1	0.13	0.04	0.04	0.13	0.05	0.12
Nitrite (mg/ℓ)	NO_2^-	<4	0.06	0.02	0.31	0.02	0.60	1.70
Nitrate (mg/ℓ)	NO_3^-	<4	0.80	0.70	0.90	1.10	0.60	1.70
Sulphate (mg/ℓ)	SO_4^{2-}	<500	197	257	158	237	278	42
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.08	0.34	0.05	0.66	0.08	0.12
Chloride (mg/ℓ)	Cl^-	N/A	18	18	20	19	22	18
Total hardness (mg/ℓ)	TH	N/A	180	228	188	164	202	10

RWQO = Resource water quality objectives, MD = Middelburg Dam, DMD = Downstream Middelburg Dam, MP4 = Mpumalanga 4, MP6 = Mpumalanga 6, PAN1 = Pan 1, MP10 = Mpumalanga 10. Grey highlighted values exceeded the RWQO.

Table 4-11: Water quality results after a rain event (March 2021) in Mpumalanga in the Upper Vaal River catchment.

Water quality parameters	Symbol	RWQO	X1	X2	X3	V9	V11	V12	V13	GD	V15
pH	pH	6.5-8.5	7.41	7.49	7.50	7.50	7.33	7.53	6.90	7.10	5.08
Temperature (°C)	TEMP	N/A	20.1	21.2	20.0	22.7	22.8	22.4	23.3	21.1	24.0
Conductivity (mS/m)	EC	<70	56.3	44.6	44.8	26.5	26.9	28.3	30.6	21.9	20.2
Suspended solids (mg/ℓ)	SS	N/A	105	60	129	98	94	110	44	77	109
Turbidity (FAU)	TURB	N/A	26	45	45	50	6	16	31	47	41
Ammonium (mg/ℓ)	NH_4^+	<0.073	0.41	0.08	0.06	0.09	0.05	0.09	0.25	0.05	0.08
Nitrite (mg/ℓ)	NO_2^-	<0.858	0.50	2.00	1.00	0.70	0.70	0.50	0.17	1.00	0.60
Nitrate (mg/ℓ)	NO_3^-	<0.858	0.5	2.00	1.00	0.70	0.70	0.50	0.70	1.00	0.60
Sulphate (mg/ℓ)	SO_4^{2-}	<500	54	50	58	37	38	48	79	47	32
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.02	0.45	0.35	0.39	0.31	1.26	0.20	0.19	0.65	0.28
Chloride (mg/ℓ)	Cl^-	N/A	25	17	19	14	18	17	15	15	14
Total hardness (mg/ℓ)	TH	N/A	73	80	100	44	38	51	5	18	7

RWQO = Resource water quality objectives, X1 = Life Water River, X2 = Life Water River, X3 = Life Water River, V9 = Vaal River 9, V11 = Vaal River 11, V12 = Vaal River 12, V13 = Vaal River 13, GD = Grootdraai, V15 = Vaal River 15. Grey highlighted values exceeded the RWQO

Table 4-12: Water quality results after a rain event of the non-agricultural sites in the Upper Vaal River catchment in March 2021.

Water quality parameters	Symbol	RWQO	VB	DGP	MM	TRE	GG	BT
pH	pH	6.5-8.5	7.85	7.97	8.15	8.46	8.66	8.54
Temperature (°C)	TEMP	N/A	22	20.9	21.4	21.1	21.6	19.5
Conductivity (mS/m)	EC	<111	70.3	77.5	72	71.4	71.4	59.4
Suspended solids (mg/ℓ)	SS	N/A	25	7	0	43	90	31
Turbidity (FAU)	TURB	N/A	48	46	1	52	49	8
Ammonium (mg/ℓ)	NH_4^+	<0.073	1.52	0.09	0.08	0.05	0.05	0.04
Nitrite (mg/ℓ)	NO_2^-	<4	0.02	0.03	0.02	0.35	0.03	0.01
Nitrate (mg/ℓ)	NO_3^-	<4	1.00	0.90	1.70	0.7	2.00	0.70
Sulphate (mg/ℓ)	SO_4^{2-}	<500	73	133	127	74	80	43
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.18	0.38	1.79	0.33	0.72	0.72
Chloride (mg/ℓ)	Cl^-	N/A	21	25	26	18	17	21
Total hardness (mg/ℓ)	TH	N/A	222	181	183	79	120	67

RWQO = Resource water quality objectives, VB = Vaal Barrage, DGP = Downstream Golf course Potchefstroom, MM = Mooiriver Mall, TRE = Tuscany Ridge Estate, GG = Gimmie Gat, BT = Botanical Gardens. Grey highlighted values exceeded the RWQO

4.3.4 After harvest/end of season sampling-event

4.3.4.1 Vaalharts Irrigation Scheme (VIS)

The pH values for MIC and SD were 8.71 and 8.56 respectively (Table 4-13). The NH_4^+ levels exceeded the RWQO at sites MIC, HR2 and HR4 (Table 4-13). There were also elevated levels of SO_4^{2-} at sites C2, HR3 and HR4 higher than the RWQO. The levels of PO_4^{3-} at all the sites (except at HR4) exceeded the RWQO. The NO_3^- exceeded the RWQO with a range of 1.10-4.80 mg/ℓ (RWQO NO_3^-<1). The parameters that did not exceed the RWQO were the EC (0.3-1.9 mS/m) and NO_2^- (0.2-0.29 mg/ℓ). The parameters with no RWQO, ranged TEMP (12.2-20.8°C), SS (4-150 mg/ℓ), TURB (1-33 FAU), Cl^-(22-252 mg/ℓ) and TH (65-291 mg/ℓ). The EC at C1, HR3 and HR4 was higher than the minimum, ranging 170.3-190.1 mS/m.

4.3.4.2 Upper Vaal and Olifants River catchments (Mpumalanga Province (MPA)

The pH at sites MD and DMD were 8.80 and 8.63 respectively (Table 4-14). At sites DMD and V12, the PO_4^{3-} (RWQO PO_4^{3-}<0.025) were 0.71 and 0.17 mg/ℓ respectively and exceeded the RWQO. The parameters that did not exceed the RWQO were the EC (0.21-11.46 mS/m), NO_2^- (0.01-0.06 mg/ℓ), NO_3^- (0.01-4 mg/ℓ) and SO_4^{2-} (38-238 mg/ℓ). As for the parameters with no RWQO, they ranged TEMP (6.1-17.7°C), SS (6-136 mg/ℓ), TURB (1-46 FAU), Cl^-(32-141 mg/ℓ) and TH (18-218 mg/ℓ). The MP4, MP6 and PAN1 had higher EC ranges at 262, 179.5 and 1146 mS/m respectively, exceeding the limit of the RWQO.

4.3.4.3 Non-agricultural sites

The pH at all the sites ranged from 8.98-9.46 and exceeded the RWQO (Table 4-16). Then the NH_4^+ exceeded the RWQO at VB and DGP with 0.09 and 1.79 mg/ℓ. Lastly, the PO_4^{3-} at sites DGP, MM and TRE exceeded the TWQO (RWQO PO_4^{3-}<0.125). The parameters that did not exceed the RWQO were the EC (0.55-0.79 mS/m), NO_2^- (0.01-0.19 mg/ℓ), NO_3^- (0.2-1.7 mg/ℓ) and SO_4^{2-} (89-111 mg/ℓ). As for the parameters with no RWQO, they ranged as follows: TEMP (10.9-13.8°C), SS (22-1333 mg/ℓ), TURB (9-45 FAU), Cl^-(66-112 mg/ℓ) and TH (32-181 mg/ℓ).

Table 4-13: Water quality results at the end of the maize growing season (May/June 2021) in the Vaalharts irrigation scheme in the Lower Vaal River catchment.

Water quality parameters	Symbol	RWQO	TO	HR1	C1	HR2	C2	MIC	HR3	HR4	SD	DSD
pH	pH	6.5-8.5	7.30	8.11	8.40	8.18	8.18	8.71	8.41	8.36	8.56	8.46
Temperature (°C)	TEMP	N/A	15.3	16.1	17.6	16.4	16.4	20.8	15.6	12.4	12.2	14.5
Conductivity (mS/m)	EC	<111	30	33.1	170.3	94.7	94.7	42.8	190.1	173	72.5	80.8
Suspended solids (mg/ℓ)	SS	N/A	23	4	150	73	94	61	94	91	51	119
Turbidity (FAU)	TURB	N/A	33	8	10	23	1	33	8	12	17	17
Ammonium (mg/ℓ)	NH_4^+	<0.07	0.05	0.05	0.05	0.09	0.05	0.14	0.07	0.09	0.05	0.05
Nitrite (mg/ℓ)	NO_2^-	<1	0.02	0.06	0.02	0.02	0.03	0.02	0.29	0.08	0.02	0.03
Nitrate (mg/ℓ)	NO_3^-	<1	1.50	1.10	2.10	2.30	4.80	1.30	2.00	2.20	1.50	1.40
Sulphate (mg/ℓ)	SO_4^{2-}	<200	26	4	291	145	297	60	212	200	70	80
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.025	0.09	0.03	0.06	0.03	0.03	0.06	0.08	0.02	0.08	0.04
Chloride (mg/ℓ)	Cl^-	N/A	32	22	190	121	231	41	252	211	159	83
Total hardness (mg/ℓ)	TH	N/A	65	67	265	235	287	79	291	277	145	159

RWQO = Resource water quality objectives, TO = Taung Overflow, HR1 = Harts River 1, C1 = Channel 1, HR2 = Harts River 2, C2 = Channel 2, MIC = Main inflow channel, HR3 = Harts River 3, HR4 = Harts River 4, SD = Spitskop Dam, DSD = Downstream Spitskop Dam. Grey highlighted values exceeded the RWQO

Table 4-14: Water quality results at the end of the maize growing season (May/June 2021) in Mpumalanga in the Olifants River catchment.

Water quality parameters	Symbol	RWQO	MD	DMD	MP4	MP6	PAN1	MP10
pH	pH	6.5-8.5	8.80	8.63	8.50	8.20	8.30	8.25
Temperature (°C)	TEMP	N/A	17.7	16.6	13.1	12.3	10.9	7.3
Conductivity (mS/m)	EC	<111	104.5	102.9	262	179.5	1146	39
Suspended solids (mg/ℓ)	SS	N/A	22	15	136	6	307	93
Turbidity (FAU)	TURB	N/A	14	40	1	3	46	10
Ammonium (mg/ℓ)	NH_4^+	<0.1	0.10	0.07	0.05	0.09	0.03	0.04
Nitrite (mg/ℓ)	NO_2^-	<4	0.05	0.01	0.01	0.06	0.01	0.02
Nitrate (mg/ℓ)	NO_3^-	<4	1.7	0.7	1.20	2.20	0.20	0.2
Sulphate (mg/ℓ)	SO_4^{2-}	<500	314	238	234	230	280	46
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.10	0.71	0.09	0.03	0.03	0.09
Chloride (mg/ℓ)	Cl^-	N/A	49	55	115	41	141	39
Total hardness (mg/ℓ)	TH	N/A	218	200	215	187	18	78

RWQO = Resource water quality objectives, MD = Middelburg Dam, DMD = Downstream Middelburg Dam, MP4 = Mpumalanga 4, MP6 = Mpumalanga 6, PAN1 = Pan 1, MP10 = Mpumalanga 10. Grey highlighted values exceeded the RWQO,.

Table 4-15: Water quality results at the end of the maize growing season (May/June 2021) in Mpumalanga in the Upper Vaal River catchment.

Water quality parameters	Symbol	RWQO	X1	X2	X3	V9	V11	V12	V13	GD	V15
pH	pH	6.5-8.5	8.22	8.18	7.82	8.40	8.42	8.09	8.02	7.85	7.25
Temperature (°C)	TEMP	N/A	6.1	12.7	12.1	18.1	13.2	11.4	13.0	12.9	12.0
Conductivity (mS/m)	EC	<111	58.6	54.7	51.9	39.8	41.6	42.9	42.8	20.9	22
Suspended solids (mg/ℓ)	SS	N/A	108	13	52	22	105	12	20	35	49
Turbidity (FAU)	TURB	N/A	10	25	22	8	31	10	23	27	25
Ammonium (mg/ℓ)	NH_4^+	<0.1	0.02	0.05	0.04	0.08	0.03	0.05	0.06	0.05	0.05
Nitrite (mg/ℓ)	NO_2^-	<4	0.03	0.02	0.03	0.04	0.01	0.01	0.01	0.01	0.03
Nitrate (mg/ℓ)	NO_3^-	<4	0.80	0.1	4	0.9	1.4	0.4	1.6	0.7	0.4
Sulphate (mg/ℓ)	SO_4^{2-}	<500	56	55	78	46	65	64	57	38	43
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.08	0.05	0.03	0.09	0.05	0.17	0.06	0.07	0.04
Chloride (mg/ℓ)	Cl^-	N/A	32	44	74	100	66	54	54	48	35
Total hardness (mg/ℓ)	TH	N/A	120	127	91	59	69	81	72	35	41

RWQO = Resource water quality objectives, X1 = Life Water River, X2 = Life Water River, X3 = Life Water River, V9 = Vaal River 9, V11 = Vaal River 11, V12 = Vaal River 12, V13 = Vaal River 13, GD = Grootdraai, V15 = Vaal River 15. Grey highlighted values exceeded the RWQO

Table 4-16: Water quality results at the non-agricultural sites in the Upper Vaal River catchment in May/June 2021.

Water quality parameters	Symbol	RWQO	VB	DGP	MM	TRE	GG	BT
pH	pH	6.5-8.5	9.20	8.98	9.44	9.23	9.46	9.18
Temperature (°C)	TEMP	N/A	12.8	13.8	13.3	12.4	13.8	10.9
Conductivity (mS/m)	EC	<111	71.1	73.3	78.8	77.1	55.3	58.8
Suspended solids (mg/ℓ)	SS	N/A	45	45	49	22	39	1333
Turbidity (FAU)	TURB	N/A	38	32	9	14	23	45
Ammonium (mg/ℓ)	NH_4^+	<0.073	0.09	1.79	0.05	0.05	0.05	0.05
Nitrite (mg/ℓ)	NO_2^-	<4	0.01	0.19	0.01	0.01	0.01	0.01
Nitrate (mg/ℓ)	NO_3^-	<4	0.20	1.30	0.60	1.00	0.90	1.70
Sulphate (mg/ℓ)	SO_4^{2-}	<500	101	111	111	111	103	89
Ortho-phosphate (mg/ℓ)	PO_4^{3-}	<0.125	0.06	0.91	0.13	0.13	0.10	0.08
Chloride (mg/ℓ)	Cl^-	N/A	66	98	101	71	84	112
Total hardness (mg/ℓ)	TH	N/A	181	112	176	70	32	31

RWQO = Resource water quality objectives, VB = Vaal Barrage, DGP = Downstream Golf course Potchefstroom, MM = Mooiriver Mall, TRE = Tuscany Ridge Estate, GG = Gimmie Gat, BT = Botanical Gardens. Grey highlighted values exceeded the RWQO

4.4 SOIL COMPOSITION

The majority of the sites were classified to mainly consist out of sand or silt (Table 4-20; Table 4-18; Table 4-19; Table 4-20). The total organic carbon content at most sites were <3% but some were as high as 10% (PAN1;Table 4-20: Soil particle size and total organic carbon (%TOC) after harvest season).

Most of the sites during the four sampling events in Tables 4.17-20 showed that there was a change in soil composition from one sampling event to the next: 15 samples were categorised as sandy to 12 that were regarded as silt (Table 4-17). At the end of the sampling period, only two sites were considered sandy while the rest were silt (Table 4-19).

Table 4-17: Soil particle size and total organic carbon (%TOC) before spraying sampling-event (September/October 2020).

	Sites	TOC (%)	Sand (%)	Silt (%)	Clay (%)
Vaalharts Irrigation Scheme	**TO**	0.7	13	58.5	25.4
	HR1	0.2	71.5	27.9	0.3
	C1	-	-	-	-
	HR2	0.3	28.2	70.1	1.2
	C2	-	-	-	-
	MIC	-	-	-	-
	HR3	0.4	50.6	42.8	6.2
	HR4	0.5	56	42.3	1.3
	SD	1.2	51.1	42.7	4.6
	DSD	1	91.8	7.9	0.2
Mpumalanga	**MD**	0.3	38.1	34.5	26.5
	DMD	0.3	69.5	29.8	0
	MP4	0.7	34	41.2	24.4
	MP6	0.5	11.1	70.6	17.4
	PAN1	2.8	95.4	3.7	0.8
	MP10	0.2	0	88	11.7
	X1	1	17.1	58.1	13.5
	X2	0.9	34.3	45.1	19.5
	X3	0.8	7.7	83.9	6.3
	V9	1.6	83.4	14.3	1.4
	V11	1.5	69.1	24.3	5.8
	V12	1.3	44.9	38.6	16.3
	V13	0.7	69.4	27.1	3.4
	GD	1.5	32.9	57.8	8.6
	V15	0.2	30.1	60.4	9
Non-agricultural	**VB**	1.1	95.5	2.6	1.5
	DGP	2.2	93.7	6.2	0
	MM	0.9	15	56.7	25.9
	TRE	1.2	51.3	38.7	8.9
	GG	0.3	11.6	74.9	12.9
	BT	-	-	-	-

Grey highlighted values represent the highest composition of the sand, silt or clay. "-" no data available due to inaccessibility of site.

Table 4-18: Soil particle size and total organic carbon (%TOC) post herbicide application (November 2020).

	Sites	TOC (%)	Sand (%)	Silt (%)	Clay (%)
Vaalharts Irrigation Scheme	TO	0.4	48.8	43.8	6.9
	HR1	0.8	6.4	82.4	9.8
	C1	0.2	9.3	66.7	22.8
	HR2	1	1.8	54.3	42.1
	C2	-	-	-	-
	MIC	-	-	-	-
	HR3	0.6	35.7	51.9	11.6
	HR4	1.3	27.1	65.3	6.3
	SD	2	3.1	47.9	46.4
	DSD	1.1	1.5	72.4	24.4
Mpumalanga	MD	0.4	20.1	34.5	44.8
	DMD	0.5	35.8	60.8	2.7
	MP4	0.5	16.2	55.9	26.3
	MP6	0.7	16.5	68	15
	PAN1	1.5	35	48.3	15.4
	MP10	0.4	10.2	82	7.1
	X1	0.3	4.7	75.1	19.7
	X2	1	70.7	18.9	9.8
	X3	0.8	2.9	74.3	22.1
	V9	0.5	17	71.7	10.6
	V11	1.1	63.2	30	6.5
	V12	2.4	99.8	0.4	0
	V13	2.3	99	0.7	0.1
	GD	1.7	76.7	18.9	3.8
	V15	0.4	40.7	53.7	4.6
Non-agricultural	VB	1.8	94.1	4.6	0.9
	DGP	3.4	76.1	19.6	3.8
	MM	2.5	37.3	37.5	24.1
	TRE	1.4	30.1	48.1	20.7
	GG	2.2	36.3	46.1	16.3
	BT	-	-	-	-

Grey highlighted values represent the highest composition of the sand, silt or clay. “-” no data available due to inaccessibility of site.

Table 4-19: Soil particle size and total organic carbon (%TOC) after the rain sampling-event (March 2021).

	Sites	TOC (%)	Sand (%)	Silt (%)	Clay (%)
Vaalharts Irrigation Scheme	**TO**	0.3	5.9	93.5	0.1
	HR1	0.2	28	71.6	0.3
	C1	-	-	-	-
	HR2	1.1	5.6	53.8	39.2
	C2	0.2	1.2	38.9	59.5
	MIC	-	-	-	-
	HR3	0.4	10.2	76.9	11.9
	HR4	2.1	0.9	61.1	36.5
	SD	1.7	4.8	41.7	50.1
	DSD	2.4	27.1	62.3	9.4
Mpumalanga	**MD**	0.9	47.4	46.8	5.1
	DMD	0.7	65.6	33.6	0.4
	MP4	-	-	-	-
	MP6	0.5	25.8	62.1	11.2
	PAN1	1.8	0.2	58	40.1
	MP10	0.3	5.8	78.4	10.5
	X1	0.2	16.4	70.1	12.8
	X2	2.1	11.5	66.9	20.3
	X3	0.5	5.8	83.6	9.2
	V9	1.2	80.1	17.2	2.3
	V11	1.6	37.9	48.6	11.5
	V12	1.7	83.8	13.8	2
	V13	0.9	8.2	59.6	30.4
	GD	0.7	9.2	87.4	3.2
	V15	0.2	0.4	75.2	30.2
Non-agricultural	**VB**	1.8	33.8	43.5	21.3
	DGP	3.5	44.6	44.7	11.7
	MM	3.1	26.3	44.6	24.7
	TRE	3.1	13.3	43.7	39.4
	GG	0.3	42.7	52.2	4.4
	BT	-	-	-	-

Grey highlighted values represent the highest composition of the sand, silt or clay. “-” no data available due to inaccessibility of site.

Table 4-20: Soil particle size and total organic carbon (%TOC) after harvest season (May/June 2021).

	Sites	TOC (%)	Sand (%)	Silt (%)	Clay (%)
Vaalharts Irrigation Scheme	**TO**	1.2	12.9	69.3	17.2
	HR1	1	3.5	54.6	41.8
	C1		-	-	-
	HR2	0.7	0.6	48.7	50.2
	C2	0.6	1.6	58.9	37.9
	MIC	0.2	18	55.4	24.7
	HR3		0	56.6	43.3
	HR4	1.4	3.1	66.5	26.4
	SD	9.1	12.9	67.6	19.1
	DSD	0.6	56.4	41.8	1.7
Mpumalanga	**MD**	0.8	53.5	41.6	4.8
	DMD	1.1	44.1	54.1	1.6
	MP4	0.4	2	49.7	42.5
	MP6	0.6	8.7	80.1	11.1
	PAN1	10.6	2.4	69.5	27.2
	MP10	0.2	0.9	81.2	17.3
	X1	0.8	2.3	67.3	29.7
	X2	1.7	7	72.9	18.6
	X3	1.7	2.7	85.9	11.2
	V9	0.1	5.8	90.9	2.2
	V11	0.3	44	52.8	2.7
	V12	5.3	6.7	80.3	12.4
	V13	0.4	2.9	33.7	61
	GD	6	6.3	75.1	18.3
	V15	0.1	1.7	76.8	21.3
Non-agricultural	**VB**	1.3	40.3	49.1	10.7
	DGP		-	-	-
	MM	6.4	2.5	60.6	36.6
	TRE	1	31.8	54.2	13.4
	GG	0.2	19.7	71.1	8.6
	BT		-	-	-

Grey highlighted values represent the highest composition of the sand, silt or clay. "-" no data available due to inaccessibility of site.

4.5 CATION EXCHANGE CAPACITY

The cation exchange capacity (CEC) describes the portion of exchangeable cations in the soil that can neutralize its charge. The CEC therefore indicates the capability of the soil to retain nutrients or contaminants. The higher the CEC, the more compounds can be retained (Pessagno, Torres Sánchez & Dos Santos Afonso, 2008). We assessed the soil's CEC in an attempt to explain that if glyphosate was not detected, it could be due to sorption to soil particles and difficulty extracting glyphosate. The highest CEC were obtained for Maize 10>Spitskop Dam>Maize 5>Downstream Spitskop Dam (Figure 4-3).

The sorption of glyphosate to soil and sediment is controlled by cation exchange capacity, clay and total organic carbon content and pH (Dollinger et al., 2015). Based on this, these various soil characteristics were determined in this study. When assessing the results for the CEC, pH, %TOC and soil composition, differences can be seen between sites, but in light of the quantification results (to be discussed in the next section), correlations between concentrations and soil properties cannot be drawn. These parameters were not further discussed.

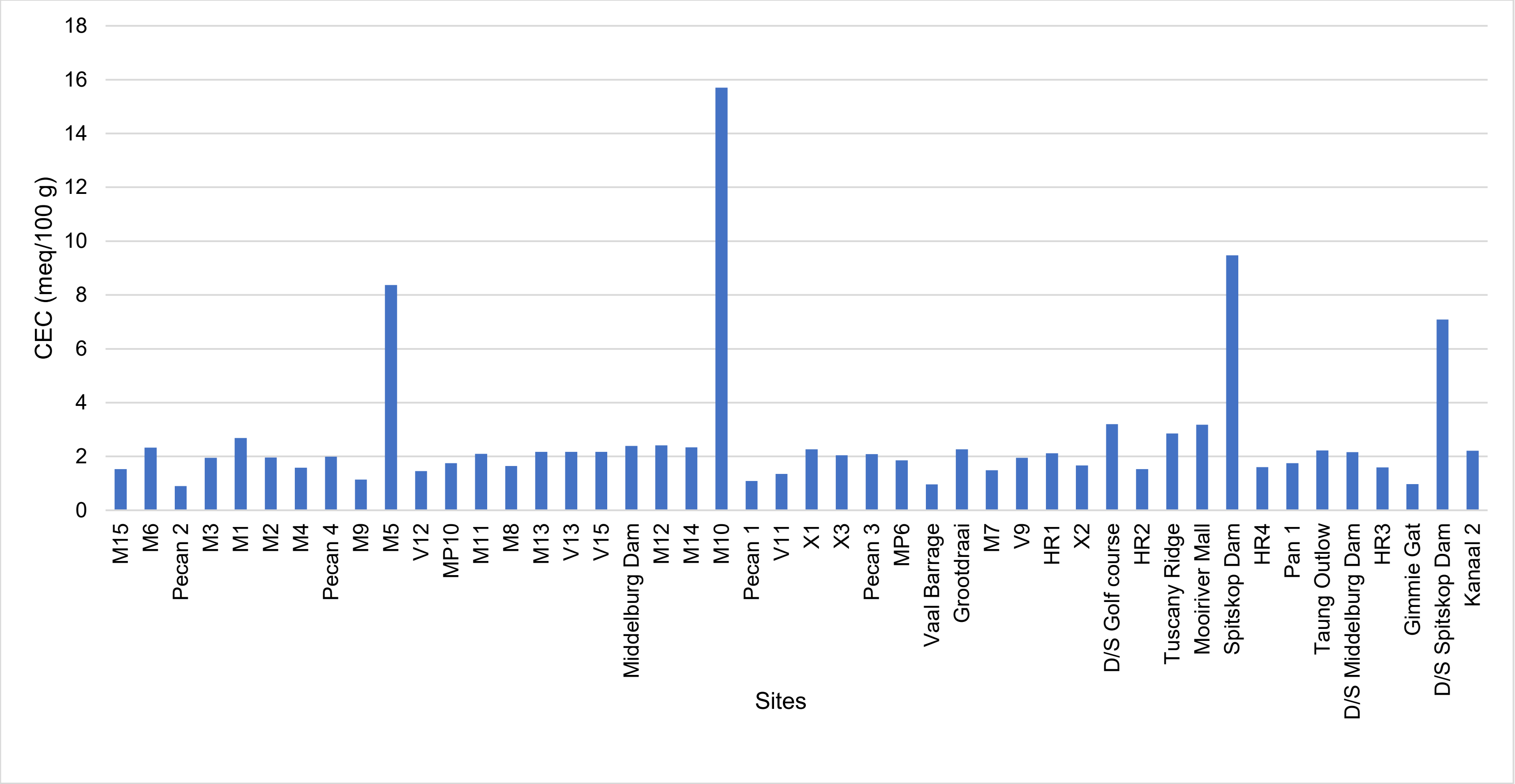


Figure 4-3 Cation exchange capacity (CEC) (meq/100 g) results.

4.6 QUANTIFICATION OF GLYPHOSATE AND AMPA IN SAMPLES

4.6.1 Method validation

All the samples and controls were analysed at NMISA based on the method as described in section 3.5. The use of HPLC with MS/MS for the quantification of the compounds present in environmental matrices may be subject to matrix interference by ion suppression or enhancement (Matuszewski et al., 2003). In this study, blank water and blank sediment were subjected to the chosen extraction process (section 3.5). Subsequently, the extracts containing the matrix (water, sediment or soil) were spiked with glyphosate and AMPA and analysed using liquid chromatography (section 3.5.5). The post-extraction method accounts for the potential matrix interference and evaluates the effect on chromatographic separation of glyphosate and AMPA (Figure 4-4-Figure 4-7). The peaks obtained for glyphosate and AMPA within the water and sediment matrices had a slight tailing effect, which have also been observed for glyphosate and AMPA analysed in other studies (Urlich and Ferguson, 2021). Considering the difficulty to analyse for glyphosate and AMPA within environmental matrices, these peaks were considered satisfactory. The peaks eluted at the same retention time and looked similar when multiple injections were performed. This confirmed the repeatability of the chromatography of this method.

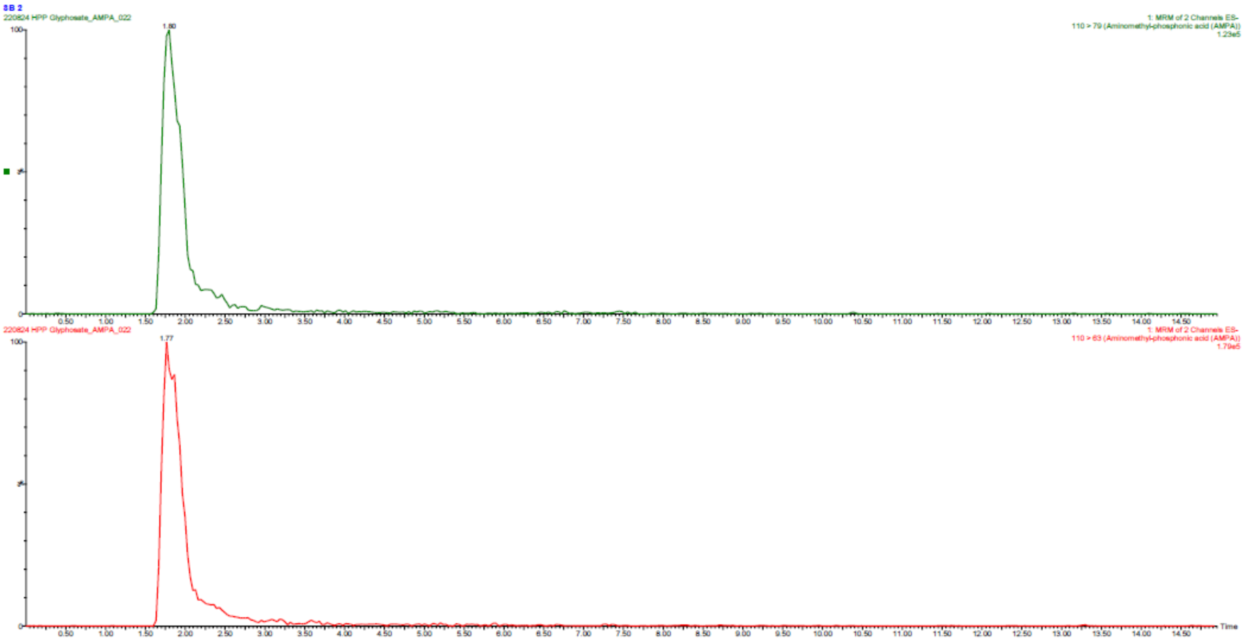


Figure 4-4 Multiple reaction monitoring (MRM) chromatogram for AMPA in spiked water extracted.

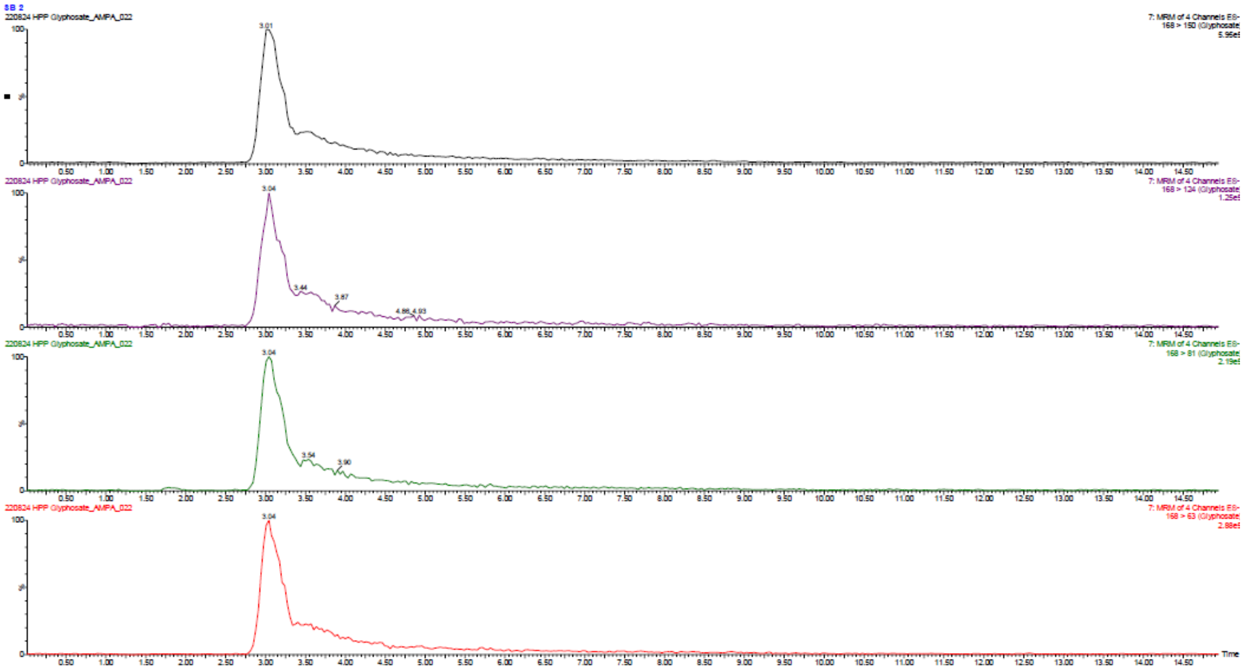


Figure 4-5 Multiple reaction monitoring (MRM) chromatogram for glyphosate in spiked water extracted.

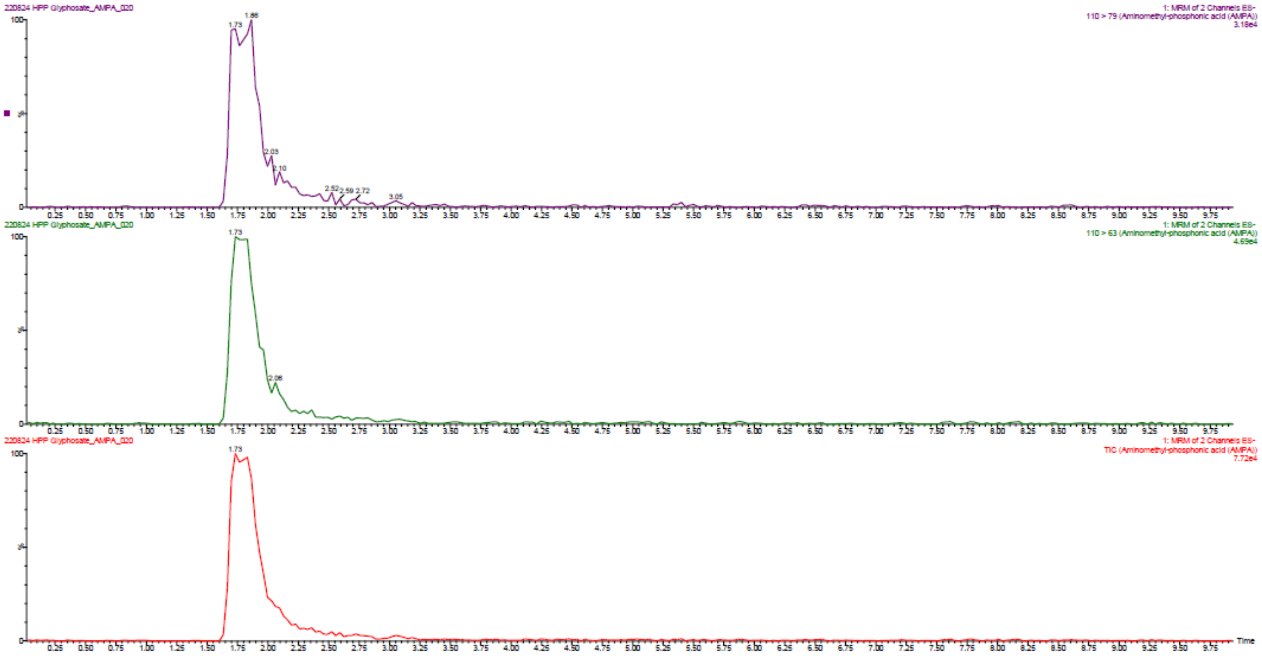


Figure 4-6 Multiple reaction monitoring (MRM) chromatogram for AMPA in spiked sediment extracted.

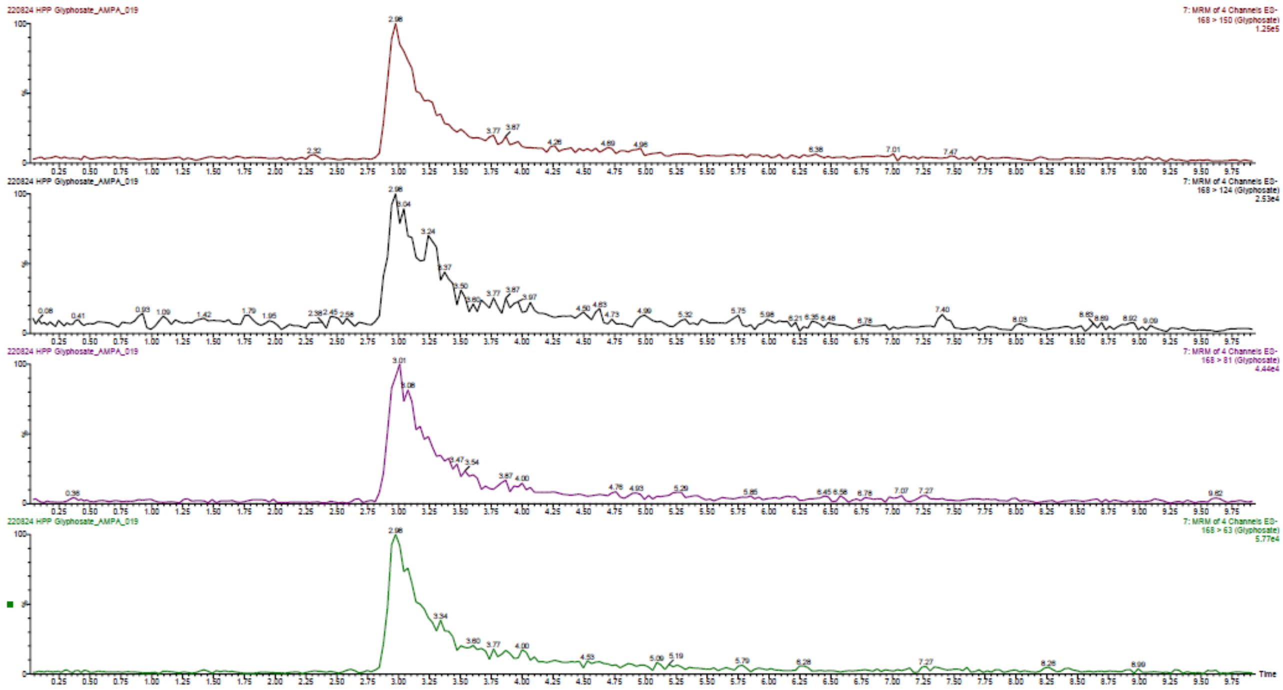


Figure 4-7 Multiple reaction monitoring (MRM) chromatogram for glyphosate in spiked sediment extracted.

The method validation parameters for linearity, accuracy and precision delivered acceptable results (Table 4-21 and Table 4-22). The R-square values were close to one indicating satisfactory linearity. The %RSD was below 15% which is acceptable for intra-and inter-day precision. The recovery (%Accuracy) for glyphosate and AMPA from water were 74% and 67% respectively. From sediment 76% and 60% of glyphosate and AMPA were extracted respectively. Even though these recoveries range from 60-75%, it is acceptable considering the difficulty of removing glyphosate bound to soil particles (Reddy, 2001). Other studies have succeeded in extracting high percentages (>80%) of glyphosate and AMPA from soil and water matrices (Hanke et al., 2008; Guo et al., 2019; Lin et al., 2022; Feltracco et al., 2022; Morimoto et al., 2023). These studies explored other technologies and analysis methods when compared to the current study. Although a few extraction methods were tested by NMISA (APPENDIX A), due to time and financial constraints an exhaustive list of methods was not tested.

Table 4-21 Method validation parameters for water analysis

Target compounds	R^2	LOD (µg/ℓ)	LOQ (µg/ℓ)	Inter-day precision (%RSD)	Intra-day precision (%RSD)	Accuracy (%)
Glyphosate	0.99	0.3	1.2	3.5	2.2	74
AMPA	0.99	0.2	1.1	6.5	4.2	67

R2= R square; LOD = limit of detection; LOQ= Limit of quantification; RSD = relative standard deviation.

Table 4-22 Method validation parameters for sediment and soil analysis

Target compounds	R^2	LOD (µg/ℓ)	LOQ (µg/ℓ)	Inter-day precision (%RSD)	Intra-day precision (%RSD)	Accuracy(%)
Glyphosate	0.99	0.5	1.7	4.6	3.2	76
AMPA	0.99	0.5	1.5	7.5	6.2	60

R2= R square; LOD = limit of detection; LOQ= Limit of quantification; RSD = relative standard deviation.

The LODs for the method used in this study for glyphosate and AMPA were comparable to those of previous studies for water (Toss et al. 2017; Guo et al., 2022; Morimoto et al., 2023) and sediment (Okada et al. 2019; Lin et al., 2022; Guo et al., 2022). In contrast to the LODs in this study, others have managed to obtain LOD of an order of magnitude lower (Hanke et al., 2008; Lin et al., 2022; Feltracco et al., 2022).

4.6.2 Concentration of glyphosate and AMPA in water, soil and sediment samples

No samples contained detectable levels of glyphosate or AMPA (Table 4-23-Table 4-28). Additionally, the samples were screened for the following compounds that are formed during glyphosate degradation: Phosphonic acid, fosetyl aluminium, ethephon, 3-methylphosphinico-propionic acid (MPPA), n-acetyl AMPA, n-acetyl glyphosate and n-acetyl glufosinate. None of the compounds screened for were present in the samples (results not shown).

In corroboration with the results of the current study, a study conducted by Ulrich and Ferguson (2021) also did not find any detectable levels of glyphosate and AMPA in surface water samples collected from rural streams of North-Carolina (USA) and Sri Lanka. But in contrast to our results, they did detect glyphosate and AMPA in water samples collected in non-agricultural areas and concluded that household use is a major contributor to the levels found in urban water sources (Ulrich and Ferguson, 2021). Glyphosate was also not detected in ground or surface water sampled in Mexico and analysed using an ELISA-based method (Reynoso et al., 2020).

The chemical characteristics of glyphosate and AMPA make them difficult to analyse for – especially in complex matrices. In this study, the extraction methods of glyphosate and AMPA were optimized and in combination with LC-MS/MS analysis, a reliable method was validated. Although, this method was fit for purpose for the study, there is a need to improve current quantification methods and lower sensitivity, to be able to detect glyphosate and AMPA at environmental levels (Romero-Natale et al., 2019; Stavra et al., 2020).

Table 4-23 Quantification results of glyphosate in the water samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Downstream Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 4	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 6	<LOD	<LOD	<LOD	<LOD
	Pan 1	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 10	<LOD	<LOD	<LOD	<LOD
	Life Water River 1	<LOD	<LOD	<LOD	<LOD
	Life Water River 2	<LOD	<LOD	<LOD	<LOD
	Life Water River 3	<LOD	<LOD	<LOD	<LOD
	Vaal River 9	<LOD	<LOD	<LOD	<LOD
	Vaal River 11	<LOD	<LOD	<LOD	<LOD
	Vaal River 12	<LOD	<LOD	<LOD	<LOD
	Vaal River 13	<LOD	<LOD	<LOD	<LOD
	Grootdraai	<LOD	<LOD	<LOD	<LOD
	Vaal River 15	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Taung Outflow	<LOD	<LOD	<LOD	<LOD
	Harts River 1	<LOD	<LOD	<LOD	<LOD
	Channel 1	<LOD	<LOD	<LOD	<LOD
	Harts River 2	<LOD	<LOD	<LOD	<LOD
	Channel 2	<LOD	<LOD	<LOD	<LOD
	Main Inflow Channel	<LOD	<LOD	<LOD	<LOD
	Harts River 3	<LOD	<LOD	<LOD	<LOD
	Harts River 4	<LOD	<LOD	<LOD	<LOD
	Spitskop Dam	<LOD	<LOD	<LOD	<LOD
	Downstream Spitskop Dam	<LOD	<LOD	<LOD	<LOD
Non-agricultural	Vaal River Barrage	<LOD	<LOD	<LOD	<LOD
	Downstream Potchefstroom Country Club	<LOD	<LOD	<LOD	<LOD
	Mooi River Mall	<LOD	<LOD	<LOD	<LOD
	Tuscany Ridge Estate	<LOD	<LOD	<LOD	<LOD
	Gimmie Gat	<LOD	<LOD	<LOD	<LOD
	NWU Botanical Gardens	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

Table 4-24 Quantification results of AMPA in the water samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Downstream Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 4	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 6	<LOD	<LOD	<LOD	<LOD
	Pan 1	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 10	<LOD	<LOD	<LOD	<LOD
	Life Water River 1	<LOD	<LOD	<LOD	<LOD
	Life Water River 2	<LOD	<LOD	<LOD	<LOD
	Life Water River 3	<LOD	<LOD	<LOD	<LOD
	Vaal River 9	<LOD	<LOD	<LOD	<LOD
	Vaal River 11	<LOD	<LOD	<LOD	<LOD
	Vaal River 12	<LOD	<LOD	<LOD	<LOD
	Vaal River 13	<LOD	<LOD	<LOD	<LOD
	Grootdraai	<LOD	<LOD	<LOD	<LOD
	Vaal River 15	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Taung Outflow	<LOD	<LOD	<LOD	<LOD
	Harts River 1	<LOD	<LOD	<LOD	<LOD
	Channel 1	<LOD	<LOD	<LOD	<LOD
	Harts River 2	<LOD	<LOD	<LOD	<LOD
	Channel 2	<LOD	<LOD	<LOD	<LOD
	Main Inflow Channel	<LOD	<LOD	<LOD	<LOD
	Harts River 3	<LOD	<LOD	<LOD	<LOD
	Harts River 4	<LOD	<LOD	<LOD	<LOD
	Spitskop Dam	<LOD	<LOD	<LOD	<LOD
	Downstream Spitskop Dam	<LOD	<LOD	<LOD	<LOD
Non-agricultural	Vaal River Barrage	<LOD	<LOD	<LOD	<LOD
	Downstream Potchefstroom Country Club	<LOD	<LOD	<LOD	<LOD
	Mooi River Mall	<LOD	<LOD	<LOD	<LOD
	Tuscany Ridge Estate	<LOD	<LOD	<LOD	<LOD
	Gimmie Gat	<LOD	<LOD	<LOD	<LOD
	NWU Botanical Gardens	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

Table 4-25 Quantification results of glyphosate in sediment samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Downstream Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 4	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 6	<LOD	<LOD	<LOD	<LOD
	Pan 1	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 10	<LOD	<LOD	<LOD	<LOD
	Life Water River 1	<LOD	<LOD	<LOD	<LOD
	Life Water River 2	<LOD	<LOD	<LOD	<LOD
	Life Water River 3	<LOD	<LOD	<LOD	<LOD
	Vaal River 9	<LOD	<LOD	<LOD	<LOD
	Vaal River 11	<LOD	<LOD	<LOD	<LOD
	Vaal River 12	<LOD	<LOD	<LOD	<LOD
	Vaal River 13	<LOD	<LOD	<LOD	<LOD
	Grootdraai	<LOD	<LOD	<LOD	<LOD
	Vaal River 15	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Taung Outflow	<LOD	<LOD	<LOD	<LOD
	Harts River 1	<LOD	<LOD	<LOD	<LOD
	Channel 1	<LOD	<LOD	<LOD	<LOD
	Harts River 2	<LOD	<LOD	<LOD	<LOD
	Channel 2	<LOD	<LOD	<LOD	<LOD
	Main Inflow Channel	<LOD	<LOD	<LOD	<LOD
	Harts River 3	<LOD	<LOD	<LOD	<LOD
	Harts River 4	<LOD	<LOD	<LOD	<LOD
	Spitskop Dam	<LOD	<LOD	<LOD	<LOD
	Downstream Spitskop Dam	<LOD	<LOD	<LOD	<LOD
Non-agricultural	Vaal River Barrage	<LOD	<LOD	<LOD	<LOD
	Downstream Potchefstroom Country Club	<LOD	<LOD	<LOD	<LOD
	Mooi River Mall	<LOD	<LOD	<LOD	<LOD
	Tuscany Ridge Estate	<LOD	<LOD	<LOD	<LOD
	Gimmie Gat	<LOD	<LOD	<LOD	<LOD
	NWU Botanical Gardens	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

Table 4-26 Quantification results of AMPA in sediment samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Downstream Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Middelburg Dam	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 4	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 6	<LOD	<LOD	<LOD	<LOD
	Pan 1	<LOD	<LOD	<LOD	<LOD
	Mpumalanga 10	<LOD	<LOD	<LOD	<LOD
	Life Water River 1	<LOD	<LOD	<LOD	<LOD
	Life Water River 2	<LOD	<LOD	<LOD	<LOD
	Life Water River 3	<LOD	<LOD	<LOD	<LOD
	Vaal River 9	<LOD	<LOD	<LOD	<LOD
	Vaal River 11	<LOD	<LOD	<LOD	<LOD
	Vaal River 12	<LOD	<LOD	<LOD	<LOD
	Vaal River 13	<LOD	<LOD	<LOD	<LOD
	Grootdraai	<LOD	<LOD	<LOD	<LOD
	Vaal River 15	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Taung Outflow	<LOD	<LOD	<LOD	<LOD
	Harts River 1	<LOD	<LOD	<LOD	<LOD
	Channel 1	<LOD	<LOD	<LOD	<LOD
	Harts River 2	<LOD	<LOD	<LOD	<LOD
	Channel 2	<LOD	<LOD	<LOD	<LOD
	Main Inflow Channel	<LOD	<LOD	<LOD	<LOD
	Harts River 3	<LOD	<LOD	<LOD	<LOD
	Harts River 4	<LOD	<LOD	<LOD	<LOD
	Spitskop Dam	<LOD	<LOD	<LOD	<LOD
	Downstream Spitskop Dam	<LOD	<LOD	<LOD	<LOD
Non-agricultural	Vaal River Barrage	<LOD	<LOD	<LOD	<LOD
	Downstream Potchefstroom Country Club	<LOD	<LOD	<LOD	<LOD
	Mooi River Mall	<LOD	<LOD	<LOD	<LOD
	Tuscany Ridge Estate	<LOD	<LOD	<LOD	<LOD
	Gimmie Gat	<LOD	<LOD	<LOD	<LOD
	NWU Botanical Gardens	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

Table 4-27 Quantification results of glyphosate in soil samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Maize 1	<LOD	<LOD	<LOD	<LOD
	Maize 2	<LOD	<LOD	<LOD	<LOD
	Maize 3	<LOD	<LOD	<LOD	<LOD
	Maize 4	<LOD	<LOD	<LOD	<LOD
	Maize 5	<LOD	<LOD	<LOD	<LOD
	Maize 6	<LOD	<LOD	<LOD	<LOD
	Maize 7	<LOD	<LOD	<LOD	<LOD
	Maize 8	<LOD	<LOD	<LOD	<LOD
	Maize 9	<LOD	<LOD	<LOD	<LOD
	Maize 10	<LOD	<LOD	<LOD	<LOD
	Maize 11	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Maize 12	<LOD	<LOD	<LOD	<LOD
	Maize 13	<LOD	<LOD	<LOD	<LOD
	Maize 14	<LOD	<LOD	<LOD	<LOD
	Maize 15	<LOD	<LOD	<LOD	<LOD
	Pecan 1	<LOD	<LOD	<LOD	<LOD
	Pecan 2	<LOD	<LOD	<LOD	<LOD
	Pecan 3	<LOD	<LOD	<LOD	<LOD
	Pecan 4	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

Table 4-28 Quantification results of AMPA in soil samples

	Sites	Before spraying	After herbicide application	After a rain event	After harvest
Mpumalanga	Maize 1	<LOD	<LOD	<LOD	<LOD
	Maize 2	<LOD	<LOD	<LOD	<LOD
	Maize 3	<LOD	<LOD	<LOD	<LOD
	Maize 4	<LOD	<LOD	<LOD	<LOD
	Maize 5	<LOD	<LOD	<LOD	<LOD
	Maize 6	<LOD	<LOD	<LOD	<LOD
	Maize 7	<LOD	<LOD	<LOD	<LOD
	Maize 8	<LOD	<LOD	<LOD	<LOD
	Maize 9	<LOD	<LOD	<LOD	<LOD
	Maize 10	<LOD	<LOD	<LOD	<LOD
	Maize 11	<LOD	<LOD	<LOD	<LOD
Vaalharts Irrigation Scheme	Maize 12	<LOD	<LOD	<LOD	<LOD
	Maize 13	<LOD	<LOD	<LOD	<LOD
	Maize 14	<LOD	<LOD	<LOD	<LOD
	Maize 15	<LOD	<LOD	<LOD	<LOD
	Pecan 1	<LOD	<LOD	<LOD	<LOD
	Pecan 2	<LOD	<LOD	<LOD	<LOD
	Pecan 3	<LOD	<LOD	<LOD	<LOD
	Pecan 4	<LOD	<LOD	<LOD	<LOD

LOD = limit of detection

4.7 HUMAN HEALTH RISK ASSESSMENT

4.7.1 Hazard identification

Glyphosate is the most used herbicide in the world. Its unique properties provide for a wide range of uses in agriculture but also in non-agricultural areas. Although glyphosate is well-known for tightly binding to soil, there have been reports on glyphosate detected in water sources. The evidence of glyphosate moving from soil to water is worrisome as exposure via water can pose risks to users of these untreated water sources. In this study data was obtained that indicated the estimated use of glyphosate-based herbicides in South Africa.

Considering this use data (section 4.4), the chemical characteristics and behaviour of glyphosate, and evidence of the health risks posed by glyphosate, a human health risk was conducted on glyphosate and its main metabolite, AMPA.

4.7.2 Hazard characterisation

In this study, the concentrations of glyphosate and AMPA were <LOD at all the sites sampled and in water, sediment and soil matrices. This might be due to the ability of an analytical instrument unable to detect compounds present in samples at very low concentrations. This is influenced by the extraction method, analysis method but also the technology of the analytical instrument. The LOD determined in this study compares to what other studies have reported for glyphosate and AMPA in water and soil.

When performing traditional human health risk assessments, the concentration of the target compound is used in a mathematical formula to determine the CR or HQ. If all, or the majority of the samples are <LOD, researchers have investigated other avenues to still conduct a human health risk assessment. A recent paper, Hwang et al. (2023), compared various models using LOD (method detection limits) to conduct a human health risk assessment of contaminated food. The study concluded that the use of half LOD is best suited for the type of risk assessment. Since the current study was a first investigation to determine the concentration of glyphosate and APMA collected from agricultural and non-agricultural areas in South Africa throughout the growing season, a decision was made to still conduct the human health risk assessment and predict risks based on the worst-case scenario by using the ½ LOD as the concentration.

4.7.3 Exposure assessment

The sampling sites from this study were located in agricultural and non-agricultural areas. The agricultural areas consist of croplands, grass fields and in some cases towns as well as rural informal settlements located close-by. The detailed descriptions can be found in section 3. In South Africa access to treated water is limited and residents are therefore forced to use the untreated (river) for household purposes. There are various informal settlements located next to the rivers where samples were collected. The use of untreated water was identified as a potential exposure route via ingestion and dermal absorption.

To locate informal settlements where residents potentially use the river water to drink, make food and for other household purposes, maps of informal settlements in the sampling areas were compiled (Figure 4-8 - Figure 4-10).

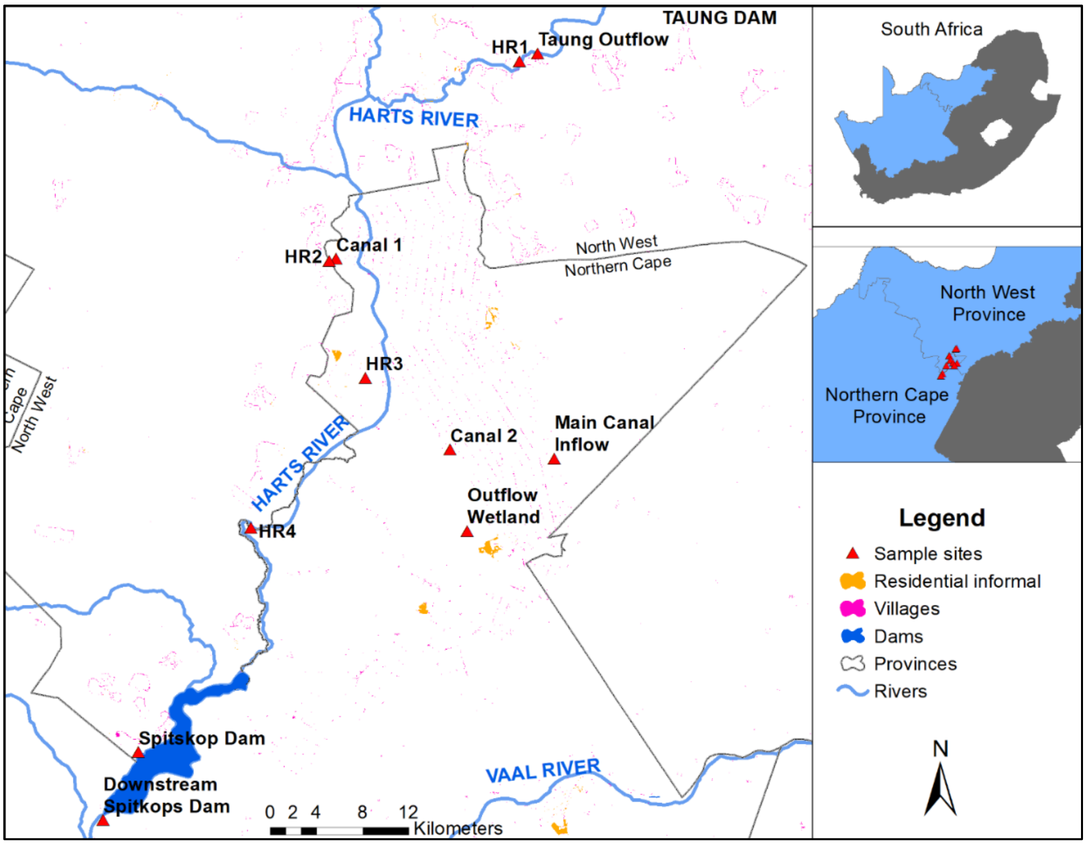


Figure 4-8 Map of informal settlements in the Vaalharts Irrigation Scheme.

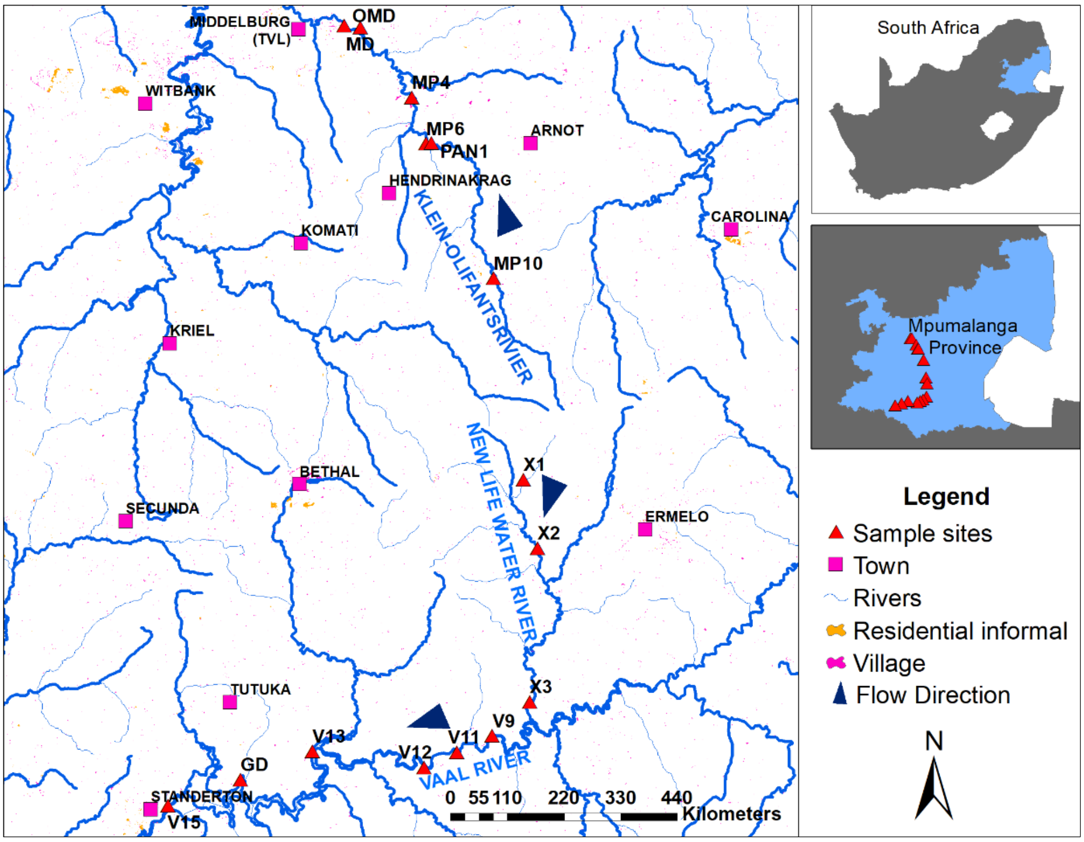


Figure 4-9 Map of informal settlements located in the Mpumalanga Province.

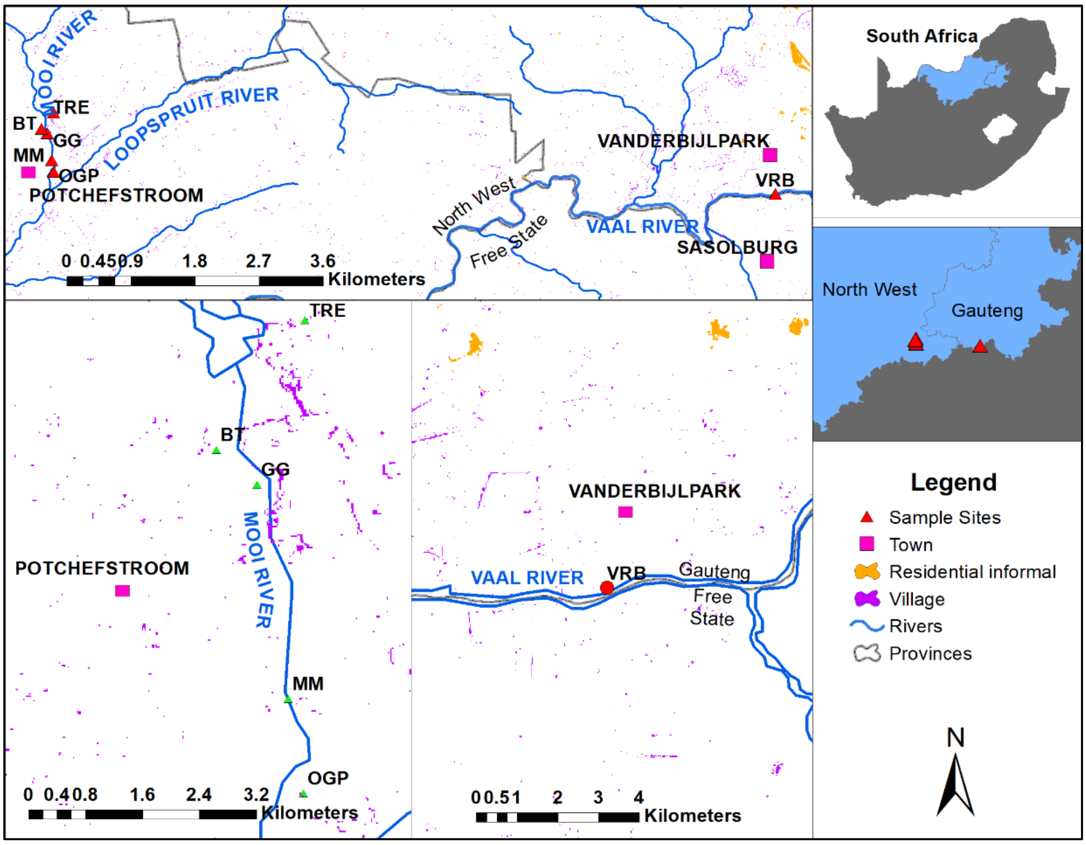


Figure 4-10 Map of informal settlements in the non-agricultural sampling area.

4.7.4 Risk characterisation

The probability of developing health effects, both non-cancerous harmful effects (hazard quotient; HR) and cancer risk (CR) were determined using the concentrations of ½ LOD of glyphosate and AMPA for the water analysis. Because all the concentrations of glyphosate and AMPA in the samples were <LOD and the LOD was a magnitude higher compared to other methods, this risk assessment was done as a baseline to predict possible risks to adults and children exposed to concentrations that were <LOD from a quantitative view but might pose risks. The CR and HQ were calculated as outlined in section 3, except for the modification in using ½ LOD and not reported concentrations for the target compounds as proposed.

Exposed individuals were considered to be safe if the HQ < 1. A HQ exceeding 1 is not a statistical possibility of harm occurring, but instead, it is an indicator of whether (and by how much) an exposure concentration exceeds the reference concentration. A cancer risk calculated for ingestion which is less than 1 x 10^{-4} (1 in 10 000) is considered acceptable risk. Similarly, for dermal exposure a risk less than 1 x 10^{-6} (1 in 1 000 000) is considered negligible. Any CR values greater than these acceptable risks, means that cancer may develop due to exposures to glyphosate or AMPA based on the risk assessment under the assumptions made.

Table 4-29 Cancer risk and hazard quotient determined for water sources

	Cancer risk		**Hazard quotient**	
	Ingestion	**Dermal**	**Ingestion**	**Dermal**
Glyphosate	1.05 x 10^{-9}	6.5 x 10^{-8}	1.5 x 10^{-5}	9.4 x 10^{-4}
AMPA	7.03 x 10^{-10}	9.7 x 10^{-8}	1.06 x 10^{-6}	1.4 x 10^{-4}

The results presented here are the CRs and HQ for glyphosate and AMPA since SFs and RfDs (both dermal and ingestion) are available (Table 4-29)Table 3-1. Overall, none of the CR or HQ results exceeded the level of acceptable hazard risk for adults.

5 CONCLUSIONS & RECOMMENDATIONS

5.1 CONCLUSIONS

- The number of glyphosate-based herbicides used in South Africa in 2017 and 2019 was estimated to be 7 976 and 7 507 tonnes respectively. This data was obtained from Kynetec and obtained through interviews with farmers and pesticide suppliers across South Africa.
- Ammonium and ortho-phosphates in almost all the samples exceeded the RWQO guidelines of South Africa for irrigation use.
- The chemical analysis method for extraction and quantification for glyphosate and AMPA were validated and fit for purpose. This was done in collaboration with the National Metrology Institute of South Africa (NMISA). Since the method is validated, this paid service is available to anyone for future analysis of glyphosate and AMPA in water, soil, and sediment.
- No concentrations of glyphosate and AMPA were present in quantities above the limit of detection in the water, soil and sediment samples collected at the chosen sites in this study. Even though samples were collected at different time points during the crop growing season of which one was after spraying and after a rain event.
- No human health risks were identified as the CR and HQ did not exceed the acceptable risks level. The human health risk assessment was conducted using half the limit of detection. This was done as a measure to identify potential risks in a worst-case scenario considering the limitations of the analytical method and instrumentation used. Exposure to the water sources was determined to be via ingestion and dermal contact.
- This is the first South African report on chemical analysis of glyphosate and AMPA in water and soil/sediment collected at both agricultural and non-agricultural areas in intervals during the crop growing season.

5.2 RECOMMENDATIONS

- Considering the major input of glyphosate based on the use data obtained in this study, further investigation is necessary since monitoring of glyphosate is not performed in South Africa.
- In addition to the work done in this project it is suggested that other sites should be included that coincide with the use maps obtained.
- An optimised and more sensitive analytical method might contribute to finding glyphosate at quantifiable concentrations.
- Pesticide formulants contain inert ingredients (such as POEA used in some GBH) and these are suspected/proven to be higher in toxicity than the actual active ingredient. Determining the levels of POEA in water will therefore be worthwhile.
- To obtain qualitative information from the farmers that are located close to sites on the herbicide use.
- Since the main path of degradation is microbial, microbial diversity analysis might contribute to explain lack of concentrations.

6 REFERENCES

ACB. AFRICAN CENTRE FOR BIODIVERSITY (2016) Genetically modified (GM) soya in South Africa: status quo report https://www.acbio.org.za/wp-content/uploads/2016/07/GM-Soya-Update-ACBio.pdf (Accessed 12 Nov 2021).

ACB. AFRICAN CENTRE FOR BIODIVERSITY (2019a) Africa must ban glyphosate now! https://www.acbio.org.za/sites/default/files/documents/Africa_must_ban_glyphosate_now_Aug2019.pdf (Accessed 10 Nov 2021).

ACB. AFRICAN CENTRE FOR BIODIVERSITY (2019b) More poisoning of South Africa's staple food given the go-ahead: 2,4-D gm maize varieties approved. https://www.acbio.org.za/more-poisoning-south-africas-staple-food-given-go-ahead-24-d-gm-maize-varieties-approved (Accessed 11 Nov 2021).

AGRI-INTEL (2021) Agricultural Intelligence. [Online], Available: https://www.agri-intel.com/ (Accessed 10 Nov 2021).

ANASTASSIADES, M., WACHTLER, A.K., KOLBERG, D.I., EICHHORN, E., MARKS, H., BENKENSTEIN, A., ZECHMANN, S., MACK, D., WILDGRUBE, C., BARTH, A., SIGALOV, I., GÖRLICH, S., DÖRK, D., CERCHIA, G., 2023. Quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC-or IC-MS/MS Measurement. Food of Plant Origin (QuPPe-PO-Method). URL https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN (Accessed 6 October 2022).

ASHTON PJ, SCOTT WE, STEYN DJ & WELLS RJ (1979) Chemical control programme against the water hyacinth Eichhornia crassipes (Mart.) Solms on Hartbeespoort Dam: historical and practical aspects. *South African Journal of Science* 75: 303-306.

BAI SH & Ogbourne SM (2016) Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environmental Science and Pollution Research* 23(19): 18988-19001.

BATTAGLIN WA, MEYER MT, KUIVILA KM & DIETZE JE (2014). Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *Journal of the American Water Resources Association* 50(2): 275-290.

BENBROOK CM (2012) Impacts of genetically engineered crops on pesticide use in the U.S. – The first sixteen years. *Environmental Sciences Europe* 24(24): 1-13.

BHANDARI G, ATREYA K, SCHEEPERS PT & GEISSEN V (2020) Concentration and distribution of pesticide residues in soil: Non-dietary human health risk assessment. *Chemosphere* 253, 126594.

BØHN T, CUHRA M, TRAAVIK T, SANDEN M, FAGAN J & PRIMICERIO R (2014) Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. *Food chemistry* 153: 207-15.

BÖRJESSON E & TORSTENSSON L (2000) New methods for determination of glyphosate and (aminomethyl)phosphonic acid in water and soil. *Journal of Chromatography A* 886(1-2): 207-216.

BAUM HEDLUND LAW (2021) Where is glyphosate banned? https://www.baumhedlundlaw.com/toxic-tort-law/monsanto-roundup-lawsuit/where-is-glyphosate-banned-/ (Accessed 10 Nov 2021).

CARRETTA L, CARDINALI A, ONOFRI A, MASIN R & ZANIN G (2021) Dynamics of glyphosate and aminomethylphosphonic acid in soil under conventional and conservation tillage. *International Journal of Environmental Research* 15(6):1037-1055.

CARLSON LAW FIRM (2021) Which Countries and the U.S. States are Banning Roundup? https://www.carlsonattorneys.com/news-and-update/banning-roundup (Accessed 10 Nov. 2021).

CARVALHO FP (2017) Pesticides, environment, and food safety. *Food and Energy Security* 6(2): 48-60.

CASSIGNEUL A, BENOIT P, BERGHEAUD V, DUMENY V, ETIEVANT V, GOUBARD Y, MAYLIN A, JUSTES E & ALLETTO L (2016) Fate of glyphosate and degradates in cover crop residues and underlying soil: A laboratory study. *Science of the Total Environment* 545: 582-90.

CENTNER TJ, RUSSELL L & MAYS M (2019) Viewing evidence of harm accompanying uses of glyphosate-based herbicides under US legal requirements. *Science of The Total Environment* 648: 609-617.

CUHRA M, TRAAVIK T & BØHN T (2013) Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna. *Ecotoxicology* 22(2): 251-262.

CUHRA M, BOHN T & CUHRA P (2016) Glyphosate: Too much of a good thing? *Frontiers in Environmental Science* 4(28): 1-14.

DABROWSKI JM, SHADUNG JM & WEPENER V (2014) Prioritizing agricultural pesticides used in South Africa based on their environmental mobility and potential human health effects. *Environment International* 62: 31-40.

DABROWSKI JM (2015a). Investigation of the contamination of water resources by agricultural chemicals and the impact of environmental health Vol 2: Prioritizing human health effects and mapping sources of agricultural pesticides used in South Africa. WRC Report no TT 642/15.

DABROWSKI JM (2015b) Development of pesticide use maps for South Africa. *South African Journal of Science* 111(1-2). https://dx.doi.org/10.17159/sajs.2015/20140091

DAFF. Department of Agriculture, Forestry and Fisheries, 2003. Maize production guidelines. [Online], Available: https://www.arc.agric.za/arc-gci/Fact Sheets Library/Maize Production.pdf (Accessed 04 Feb 2021).

DAFF. Department of Agriculture, Forestry and Fisheries. 2016. Wheat production guideline. Vol. 5. [Online], Available: https://www.dalrrd.gov.za/Portals/0/Brochures and Production guidelines/Wheat – Production Guideline.pdf (Accessed 04 Feb 2021).

DALRRD. Department of Agriculture, Land Reform and Rural Development. 2021. South African MRL lists. https://www.dalrrd.gov.za/Branches/Agricultural-Production-Health-Food-Safety/Food-Safety-Quality-Assurance/Maximum-Residue-Limits (Accessed 21 Dec 2021).

DAI P, HU P, TANG J, LI Y & LI C (2016) Effect of glyphosate on reproductive organs in male rat. *Acta Histochemica* 118(5): 519-526.

DERPSCH R, FRIEDRICH T, KASSAM A & HONGWEN L (2010) Current status of adoption of no-till farming in the world and some of its main benefits. *International Journal of Agricultural And Biological Engineering* 3 (1) 1-25.

DOLLINGER J, DAGÈS C & VOLTZ M (2015) Glyphosate sorption to soils and sediments predicted by pedotransfer functions. *Environmental Chemistry Letters* 13: 293-307.

DWS (DEPARTMENT OF WATER AND SANITATION). 2016a. Classes of Water Resources and Resource Quality Objectives for Catchments of the Lower Vaal in the National Water Act (No. 36 of 1998). Resource quality objectives.

DWS (DEPARTMENT OF WATER AND SANITATION). 2016b. Classes of Water Resources and Resource Quality Objectives for Catchments of the Olifants Catchment of the National Water Act (No. 36 of 1998). Resource quality objectives.

DWS (DEPARTMENT OF WATER AND SANITATION). 2016c. Classes of Water Resources and Resource Quality Objectives for Catchments of the Upper Vaal in the National Water Act (No. 36 of 1998). Resource quality objectives.

DU PREEZ CC, VAN HUYSSTEEN CW & MNKENI PN (2011). Land use and soil organic matter in South Africa 2: A review on the influence of arable crop production. *South African Journal of Science* 107(5): 1-8.

DUKE SO & POWLES SB (2008) Glyphosate: a once-in-a-century herbicide. *Pest Management Science* 64:319-325.

EU REFERENCE LABORATORIES FOR RESIDUES OF PESTICIDES – SINGLE RESIDUE METHODS (EURL-SRM) (2021) Quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC- or IC-MS/MS measurement. Version 12.

EKER S, OZTURK L, YAZICI A, ERENOGLU B, ROMHELD V & CAKMAK I (2006) Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus*) plants. *Journal of Agricultural and Food Chemistry* 54: 10019-10025.

ESSER KB (2017) Water infiltration and moisture in soils under conservation and conventional agriculture in Agro-ecological Zone IIa, Zambia. *Agronomy* 7(2).

FELTRACCO M, BARBARO E, MORABITO E, ZANGRANDO R, PIAZZA R, BARBANTE C & GAMBARO A (2022) Assessing glyphosate in water, marine particulate matter, and sediments in the Lagoon of Venice. *Environmental Science and Pollution Research:* 1-9.

FENG JC & THOMPSON DG (1990) Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. *Journal of Agricultural and Food Chemistry* 38(4): 1118-1125.

FERREIRA NG, DA SILVA KA, GUIMARÃES AT & DE OLIVEIRA CM (2023) Hotspots of soil pollution: possible glyphosate and aminomethylphosphonic acid risks on terrestrial ecosystems and human health. *Environment International*. Aug 12:108135.

FU H, GAO F, WANG X, TAN P, QIU S, SHI B & SHAN A (2021) Effects of glyphosate-based herbicide-contaminated diets on reproductive organ toxicity and hypothalamic-pituitary-ovarian axis hormones in weaned piglets. *Environmental Pollution* 272:115596.

GERBER R, SMIT NJ, VAN VUREN JHJ, NAKAYAMA SMM, YOHANNES YB, IKENAKA Y, ISHIZUKA M & WEPENER V (2015) Application of a Sediment Quality Index for the assessment and monitoring of metals and organochlorines in a premier conservation area. *Environmental Science and Pollution Research* 22: 19971-19989.

GILLMAN GP AND SUMPTER EA (1986) Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Australian Journal of Soil Research* 24: 61-66.

GLOBAL RPH The clinician's ultimate reference. Body surface area-BSA-multi-calculator (2017) https://globalrph.com/medcalcs/body-surface-area-multi-calc-multiple-analysis/#https://globalrph.com/medcalcs/body-surface-area-multi-calc-multiple-analysis/ (Accessed: 22 Sept 2023).

GOUSE M (2014) Assessing the value of glyphosate in the South African agricultural sector. Available at https://ageconsearch.umn.edu/record/206520/?ln=en Date of access: 18 September 2023.

GRUBER H, PAUL V, MEYER HHD & MÜLLER M (2012) Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt-maize MON810. *Transgenic Research* 21(1): 77-88.

GRUNEWALD K, SCHMIDT W, UNGER C & HANSCHMANN G (2001) Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). *Journal of Plant Nutrition and Soil Science* 164(1): 65-70.

GUILHERME S, SANTOS MA, BARROSO C, GAIVÃO I & PACHECO M (2012) Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (Anguilla anguilla): considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology* 21(5):1381-1390.

GUO H, GAO Y, GUO D, LIU W, WANG J, ZHENG J, ZHONG J & ZHAO Q (2019) Sensitive, rapid and non-derivatized determination of glyphosate, glufosinate, bialaphos and metabolites in surface water by LC-MS/MS. *SN Applied Sciences* 1: 1-8.

HANKE I, SINGER H & HOLLENDER J (2008) Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. *Analytical and Bioanalytical Chemistry 391*: 2265-2276.

HEAP I (2021) The International Herbicide-Resistant Weed Database. Available www.weedscience.org. Date accessed 12 Nov 2021.

HERBST MC (2021) Cancer Association of South Africa: Fact sheet and position statement on glyphosate. https://cansa.org.za/files/2021/08/Fact-Sheet-and-Position-Statement-on-Glyphosate-August-2021.pdf Date accessed 10 Nov 2021.

HLANGWANI E, ADEBIYI JA, DOORSAMY W & ADEBO OA (2020) Processing, characteristics and composition of umqombothi (a South African traditional beer). *Processes* 13;8(11): 1451.

HORN S, PIETERS R & BØHN T (2019) A first assessment of glyphosate, 2,4-D and Cry proteins in surface water of South Africa. *South African Journal of Science* 115(9-10): 1-7.

HWANG M, LEE SC, PARK JH, CHOI J & LEE HJ (2023) Statistical methods for handling non-detected results in food chemical monitoring data to improve food risk assessments. *Food Science & Nutrition.*

IRIS. INTEGRATED RISK INFORMATION SYSTEM (2023) https://www.epa.gov/iris. (Accessed: 11 July 2023).

ISAAA. INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS (2021) https://www.isaaa.org/gmapprovaldatabase/approvedeventsin/default.asp?CountryID=ZA. (Accessed 4 December 2021).

JADHAV A, HILL M & BYRNE M (2008) Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, Eichhornia crassipes (Mart.) Solms-Laubach. *Biological Control* 47, 154-158.

JURY MR (2002) Economic impacts of climate variability in South Africa and development of resource prediction models. *Journal of Applied Meteorology and Climatology* 41(1): 46-55.

KOCADAL K, ALKAS FB, BATTAL D & SAYGI S (2022) A review on advances and perspectives of glyphosate determination: challenges and opportunities. *Archives of Environmental Protection* 48(3): 89-98.

KOLLER VJ, FÜRHACKER M, NERSESYAN A, MIŠÍK M, EISENBAUER M & KNASMUELLER S (2012) Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Archives of Toxicology* 86(5): 805-813.

KOLPIN DW, THURMAN EM, LEE EA, MEYER MT, FURLONG ET & GLASSMEYER ST (2006) Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Science of the Total Environment* 354(2-3): 191-197.

KOSKINEN WC, MAREK LJ & HALL KE (2016) Analysis of glyphosate and aminomethylphosphonic acid in water, plant materials and soil. *Pest Management Science* 72(3): 423-32.

KREMER RJ, MEANS NE & KIM S. 2005. Glyphosate affects soybean root exudation and rhizosphere micro-organisms. *International Journal of Environmental Analytical Chemistry* 85(15): 1165-1174.

KYLIN H (2013) Time-integrated sampling of glyphosate in natural waters. *Chemosphere* 90(6):1821-1828.

LA CECILIA D & MAGGI F (2018) Analysis of glyphosate degradation in a soil microcosm. *Environmental Pollution* 233: 201-207.

LAKER MC (2004) Advances in soil erosion, soil conservation, land suitability evaluation and land use planning research in South Africa, 1978-2003. *South African Journal of Plant and Soil* 21(5): 345-368.

LAITINEN P, RÄMÖ S, NIKUNEN U, JAUHIAINEN L, SIIMES K & TURTOLA E (2009) Glyphosate and phosphorus leaching and residues in boreal sandy soil. *Plant and Soil* 323(1): 267-283.

LEMANOWICZ J, BARTKOWIAK A, LAMPARSKI R, WOJEWÓDZKI P, POBEREZNY J, WSZELACZYNSKA E & SZCZEPANEK M (2020) Physicochemical and enzymatic soil properties influenced by cropping of primary wheat under organic and conventional farming systems. *Agronomy* 10(11).

LIN JF, CHANG FC & SHEEN JF (2022) Determination of glyphosate, aminomethylphosphonic acid, and glufosinate in river water and sediments using microwave-assisted rapid derivatization and LC-MS/MS. *Environmental Science and Pollution Research* 29(30): 46282-46292.

MAC LOUGHLIN TM, PELUSO ML, APARICIO VC & MARINO DJG (2020) Contribution of soluble and particulate-matter fractions to the total glyphosate and AMPA load in water bodies associated with horticulture. *Science of The Total Environment* 703:134717.

MALLET C (2014) Analysis of Glyphosate, Glufosinate, and AMPA in Tap and Surface Water Using OpenArchitecture UPLC with 2D-LC Technology. Application Note. https://www.waters.com/webassets/cms/library/docs/720005169en.pdf (Accessed: 5 July 2020).

MALOBANE ME, NCIIZAH AD, MUDAU FN & WAKINDIKI IIC (2020) Tillage, Crop Rotation and Crop Residue Management Effects on Nutrient Availability in a Sweet Sorghum-Based Cropping System in Marginal Soils of South Africa. *Agronomy* 10(6):776.

MATUSZEWSKI BK, CONSTANZER ML & CHAVEZ-ENG CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC− MS/MS. *Analytical Chemistry* 75(13): 3019-3030.

MCDOUGAL JN, JURGENS-WHITEHEAD JL (2001) Short-Term Dermal Absorption and Penetration of Chemicals from Aqueous Solutions: Theory and Experiment. *Risk Analysis* 21(4): 719-26.

MEFTAUL IM, VENKATESWARLU K, ANNAMALAI P, PARVEN A, & MEGHARAJ M (2021) Glyphosate use in urban landscape soils: Fate, distribution, and potential human and environmental health risks. *Journal of Environmental Management* 292, 112786.

MENSAH PK, PALMER CG & MULLER WJ (2013) Derivation of South African water quality guidelines for Roundup® using species sensitivity distribution. Ecotoxicology and environmental safety 96: 24-31.

MESNAGE R, BERNAY B & SÉRALINI G (2013) Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology* 313, 122-128.

MESNAGE R, DEFARGE N, SPIROUX DE VENDÔMOIS J & SÉRALINI GE (2015) Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology* 84, 133-153.

MILLER JN & MILLER JC (2010) Statistics and chemometrics for analytical chemistry sixth ed. Prentice Hall, Harlow, England.

MOHAMAD-ZAINAL NSL, RAMLI N, ZOLKEFLI N, MUSTAPHA NA, HASSAN MA & MAEDA T (2021) Survivability of Alcaligenaceae and Chromatiaceae as palm oil mill effluent pollution bioindicators under fluctuations of temperature, pH and total suspended solid. *Journal of Bioscience and Bioengineering*132:174-182.

MORIMOTO T, ITO A & TANIMIZU M (2023) Simultaneous and sensitive analysis of glyphosate, glufosinate, and their metabolites in surface water by HPLC-ICP-MS/MS. *Analytical Sciences* 39 (7):1047-1052.

MUNENE EN, HASHIM NO & AMBUSSO WN (2023) Human health risk assessment of heavy metal concentration in surface water of Sosian river, Eldoret town, Uasin-Gishu County Kenya. *MethodsX* 11: 102298.

NCD-RIC (NCD RISK FACTOR COLLABORATION). 2016. A century of trends in adult human height. eLIFEe13410. DOI:10.7554/eLife.13410.

NOMURA NS & HILTON HW (1977) The adsorption and degradation of glyphosate in five Hawaiian sugarcane soils. *Weed Research* 17(2): 113-121.

OKADA E, COGGAN T, ANUMOL T, CLARKE B & ALLINSON G (2019) A simple and rapid direct injection method for the determination of glyphosate and AMPA in environmental water samples. *Analytical and Bioanalytical Chemistry* 411: 715-724.

OKADA E, ALLINSON M, BARRAL MP, CLARKE B & ALLINSON G (2020) Glyphosate and aminomethylphosphonic acid (AMPA) are commonly found in urban streams and wetlands of Melbourne, Australia. *Water Research* 168:115139.

PAGANELLI A, GNAZZO V, ACOSTA H, LO SL & CARRASCO E (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology 1586-1595.

PAYNE T (2011) The amazing whiteness of local staples – The Mail & Guardian. [Online], Available: https://mg.co.za/article/2011-11-11-the-amazing-whiteness-of-local-staples/ (Accessed 14 Sep 2021).

PESSAGNO RC, TORRES SÁNCHEZ RM & DOS SANTOS AFONSO M (2008) Glyphosate behavior at soil and mineral-water interfaces. *Environmental Pollution* 153(1): 53-59.

PHEIFFER W, WOLMARANS NJ, GERBER R, YOHANNES YB, IKENAKA Y, ISHIZUKA M, SMIT NJ, WEPENER V & PIETERS R (2018) Fish consumption from urban impoundments: What are the health risks associated with DDTs and other organochlorine pesticides in fish to township residents of a major inland city. *Science of the Total Environment* 628: 517-527.

PIETERSE PJ (2010) Herbicide resistance in weeds – a threat to effective chemical weed control in South Africa. *South African Journal of Plant and Soil* 27(1): 66-73.

PUOANE T, STEYN K, BRADSHAW D, LAUBSCHER R, FOURIE J, LAMBERT V & MBANANGA N (2002) Obesity in South Africa: The South African demographic and health survey. *Obesity Research* 10: 1038-1048.

REDDY KN (2001) Glyphosate-resistant soybean as a weed management tool: Opportunities and challenges. *Weed Biology and Management* 1(4): 193-202.

REYNOSO EC, PEÑA RD, REYES D, CHAVARIN-PINEDA Y, PALCHETTI I & TORRES E (2020) Determination of glyphosate in water from a rural locality in México and its implications for the population based on water consumption and use habits. *International Journal of Environmental Research and Public Health* 17(19): 7102.

REUTERS, 2021 German cabinet approves legislation to ban glyphosate from 2024. https://www.reuters.com/article/us-germany-farming-lawmaking/german-cabinet-approves-legislation-to-ban-glyphosate-from-2024-idUSKBN2AA1GF (Accessed 11 Nov 2021).

ROMERO-NATALE A, PALCHETTI I, AVELAR M, GONZÁLEZ-VERGARA E, GARATE-MORALES JL & TORRES E (2019) Spectrophotometric detection of glyphosate in water by complex formation between bis 5-phenyldipyrrinate of nickel (II) and glyphosate. *Water* 11(4): 719.

SANCHÍS J, KANTIANI L, LLORCA M, RUBIO F, GINEBREDA A, FRAILE J, GARRIDO T & FARRÉ M (2012) Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* 402(7): 2335-2345.

SCHÄFER RB, MUELLER R, BRACK W, WENZEL KD, STRECK G, RUCK W & LIESS M (2008) Determination of 10 particle-associated multiclass polar and semi-polar pesticides from small streams using accelerated solvent extraction. *Chemosphere* 70(11): 1952-1960.

SCHOEMAN C, MASHIANE M, DLAMINI M & OKONKWO OJ (2015) Quantification of Selected Antiretroviral Drugs in a Wastewater Treatment Works in South Africa Using GC-TOFMS. *Journal of Chromatography and Separation Techniques* https://dx.doi.org/10.4172/2157-7064.1000272].

SHANER DL, LINDENMEYER RB & OSTLIE MH (2012) What have the mechanisms of resistance to glyphosate taught us? *Pest Management Science* 68(1): 3-9.

SIMONSEN L, FOMSGAARD IS, SVENSMARK B & SPLIID NH (2008) Fate and availability of glyphosate and AMPA in agricultural soil. *Journal of Environmental Science and Health – Part B Pesticides, Food Contaminants, and Agricultural Wastes* 43(5): 365-375.

SIHTMÄE M, BLINOVA I, KÜNNIS-BERES K, KANARBIK L, HEINLAAN M & KAHRU A (2013) Ecotoxicological effects of different glyphosate formulations. *Applied Soil Ecology* 72: 215-224.

SKEFF W, NEUMANN C & SCHULZ-BULL DE (2015) Glyphosate and AMPA in the estuaries of the Baltic Sea method optimization and field study. *Marine Pollution Bulletin* 100(1): 577-585.

SMITH NJ, MARTIN RC & ST. CROIX RG (1996) Levels of the herbicide glyphosate in well water. *Bulletin of Environmental Contamination and Toxicology* 57(5): 759-765.

STAVRA E, PETROU PS, KOUKOUVINOS G, ECONOMOU A, GOUSTOURIDIS D, MISIAKOS K., RAPTIS I & KAKABAKOS SE (2020) Fast, sensitive and selective determination of herbicide glyphosate in water samples with a White Light Reflectance Spectroscopy immunosensor. *Talanta* 214: 120854.

SWANEPOEL CM, SWANEPOEL LH & SMITH HJ (2018) A review of conservation agriculture research in South Africa. *South African Journal of Plant and Soil* 35(4): 297-306.

TOSS V, LEITO I, YURCHENKO S, FREIBERG R & KRUVE A (2017) Determination of glyphosate in surface water with high organic matter content. *Environmental Science and Pollution Research* 24: 7880-7888.

ULRICH JC & FERGUSON PL (2021) Development of a sensitive direct injection LC-MS/MS method for the detection of glyphosate and aminomethylphosphonic acid (AMPA) in hard waters. *Analytical and Bioanalytical Chemistry* 413: 3763-3774.

USEPA (United States Environmental Protection Agency) (2020) United States Environmental Protection Agency, Risk Assessment. Regional Screening Levels (RSLs) – Generic Tables. November 2020, Washington DC. 20460. https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide#toxicity (Accessed 20 August 2021).

VAN HEERDEN PDR, EGGLESTON G & DONALDSON RA (2014) Ripening and post-harvest deterioration. In: Sugarcane physiology, biochemistry, and functional biology, Botha, F.C. and Moore, P.H. (Eds.), pp. 55-84, Wiley-Blackwell, USA, ISBN: 978-0-8138-2121-4.

VAN STEMPVOORT DR, SPOELSTRA J, SENGER ND, BROWN SJ, POST R AND STRUGER J (2016) Glyphosate residues in rural groundwater, Nottawasaga River watershed, Ontario, Canada. *Pest Management Science* 72(10):1862-1872.

VERA MS, LAGOMARSINO L, SYLVESTER M, PÉREZ GL, RODRÍGUEZ P, MUGNI H, SINISTRO R, FERRARO M, BONETTO C, ZAGARESE H & PIZARRO H (2010) New evidences of Roundup®(glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* 19(4): 710-721.

VILJOEN CD, KOORTZEN BJ, SREENIVASAN TANTUAN S (2021) Determining the presence of glyphosate and glyphosate-tolerant events in maize and soybean food products in South Africa. Food Additives & Contaminants: Part B 14(2): 91-97.

WEISENBURGER DD (2021) Perspective a review and update with perspective of evidence that the herbicide glyphosate (Roundup) is a cause of non-Hodgkin Lymphoma. *Clinical Lymphoma, Myeloma & Leukemia* 21: 621-630.

WHITMIRE M, AMMERMAN J, DE LISIO P, KILLMER J, KYLE D, MAINSTONE E, PORTER L and ZHANG T (2011) LC-MS/MS bioanalysis method development, validation, and sample analysis: Points to consider when conducting nonclinical and clinical studies in accordance with current regulatory guidances. *Journal of Analytical & Bioanalytical Techniques*, S4: 001.

WILLIAMS GM, KROES R & MUNRO IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology* 31(2 Pt 1): 117-65.

WEBER SJ, MISHRA DR, WILDE SB & KRAMER E (2020) Risks for cyanobacterial harmful algal blooms due to land management and climate interactions. *Science of the Total Environment* 703:134608.

WESTER RC, QUAN D & MAIBACH HI (1996) In vitro percutaneous absorption of model compounds glyphosate and malathion from cotton fabric into and through human skin. *Food and Chemical Toxicology* 34(8): 731-735.

WESTGARD, JO (2008) Basic method validation: training in analytical quality management for healthcare laboratories. 3rd Edition. Madison, WI: Westgard QC. 51-176.

APPENDIX A: METHOD DEVELOPMENT REPORT

Measurement of Glyphosate and Aminomethylphosphonic acid (AMPA) in water and soil

17 November 2022

Scope and short description

- The purpose of this report is to provide feedback on the extraction and analysis method developed for highly polar pesticides (HPPs), Glyphosate and Aminomethylphosphonic acid (AMPA), in water and soil. Spiked samples were used to develop and evaluate the extraction and analysis method. The analysis and extraction methods were based and modified from the EURL-SRM QuPPe method for highly polar pesticides in fruit and vegetables (6.1) and an article utilising ASE extraction (6.2). An optimised water and soil method was achieved with acceptable recovery (60-120%).

Instrumental analysis

- The identification and quantification were performed on a Waters ultra-high pressure liquid chromatograph coupled to a mass spectrometer. The limit of detection and limit of quantification was evaluated with volumetric preparation of a calibration curve and could be improved with the application of gravimetric procedures. The achieved LOD/ LOQ is: Glyphosate – 0.5/ 1.8 ng/g; AMPA – 0.5/ 1.7 ng/g.

Table 1: Column and basic method specifications

C18 Column	Hypercarb 2.1 x 100 mm; 5 µm; 40℃
Sample Temp	Sample temperature: 4ºC
Injection volume	Injection volume: 10 uL
Mobile phase A	1% Acetic Acid, 5% Methanol in MilliQ water
Mobile phase B	1% Acetic Acid in Methanol
Run time	30 minutes

Table 2: Solvent gradient table

Time (min)	**Flow (mL/min)**	**% A**	**% B**	**% C**	**% D**	**Curve**
Initial	0.200	100	10	0	0	Initial
10.00	0.200	70	30	0	0	6
11.00	0.400	70	30	0	0	6
18.00	0.400	70	30	0	0	6
19.00	0.400	10	90	0	0	6
22.00	0.400	10	90	0	0	6
22.10	0.200	100	0	0	0	6
30.00	0.200	100	0	0	0	6

Table 3: Wash solvents composition

Seal wash	90% MilliQ water: 10% Methanol
Purge solvent	90% MilliQ water: 10% Methanol
Strong needle wash	5% MilliQ water: 5% Isopropanol: 90% Methanol

- LC-MS/MS conditions are optimised for all compounds and included chromatographic separation, precursor and product ion identification for specific MRM transitions and associated cone voltage (CV) and collision energy (CE) for selected HPPs. All compounds are analysed in negative ion mode. MRM transitions and MS parameters can either be obtained from literature or through experimental optimisation. A matrix-matched standard will also be run to ensure that no matrix specific interferences will influence quantification. The MRM transitions developed for the HPPs currently analysed by LC-MS/MS is summarised in **Table 4**.

Table 4: Optimal MS/MS parameters for the analysis of HPPs by LC-MS/MS

Compound	Precursor Ion (Da)	Product Ion (Da)	Cone voltage (CV)	Collision energy (CE)
Phosphonic acid	81	63	25	13
Phosphonic acid	81	79	25	11
Fosetyl aluminium	109	63	20	16
Fosetyl aluminium	109	81	20	10
Aminomethylphosphonic acid (AMPA)	110	63	35	13
Aminomethylphosphonic acid (AMPA)	110	79	35	14
Ethephon	143	79	15	7
Ethephon	143	107	15	7
Ethephon	145	107	15	7
3-Methylphosphinico-propionic acid (MPPA)	151	107	20	14
3-Methylphosphinico-propionic acid (MPPA)	151	133	20	11
N-Acetyl AMPA	152	63	30	15
N-Acetyl AMPA	152	110	20	17
Glyphosate	168	63	25	18
Glyphosate	168	81	25	18
Glyphosate	168	124	25	9
Glyphosate	168	150	25	9
Glufosinate	180	85	30	16
Glufosinate	180	95	30	16
N-Acetyl glyphosate	210	150	25	13
N-Acetyl glyphosate	210	192	25	9
N-Acetyl glufosinate (NAG)	222	69	30	15
N-Acetyl glufosinate (NAG)	222	136	30	20

Optimal separation of the two compounds of interest was achieved (**Figure 1 and 2**). The peak shape and separation within the spiked water (**Figure 5 and 6**) and sediment samples (**Figure 7 and 8**) were acceptable and met the recovery requirements.

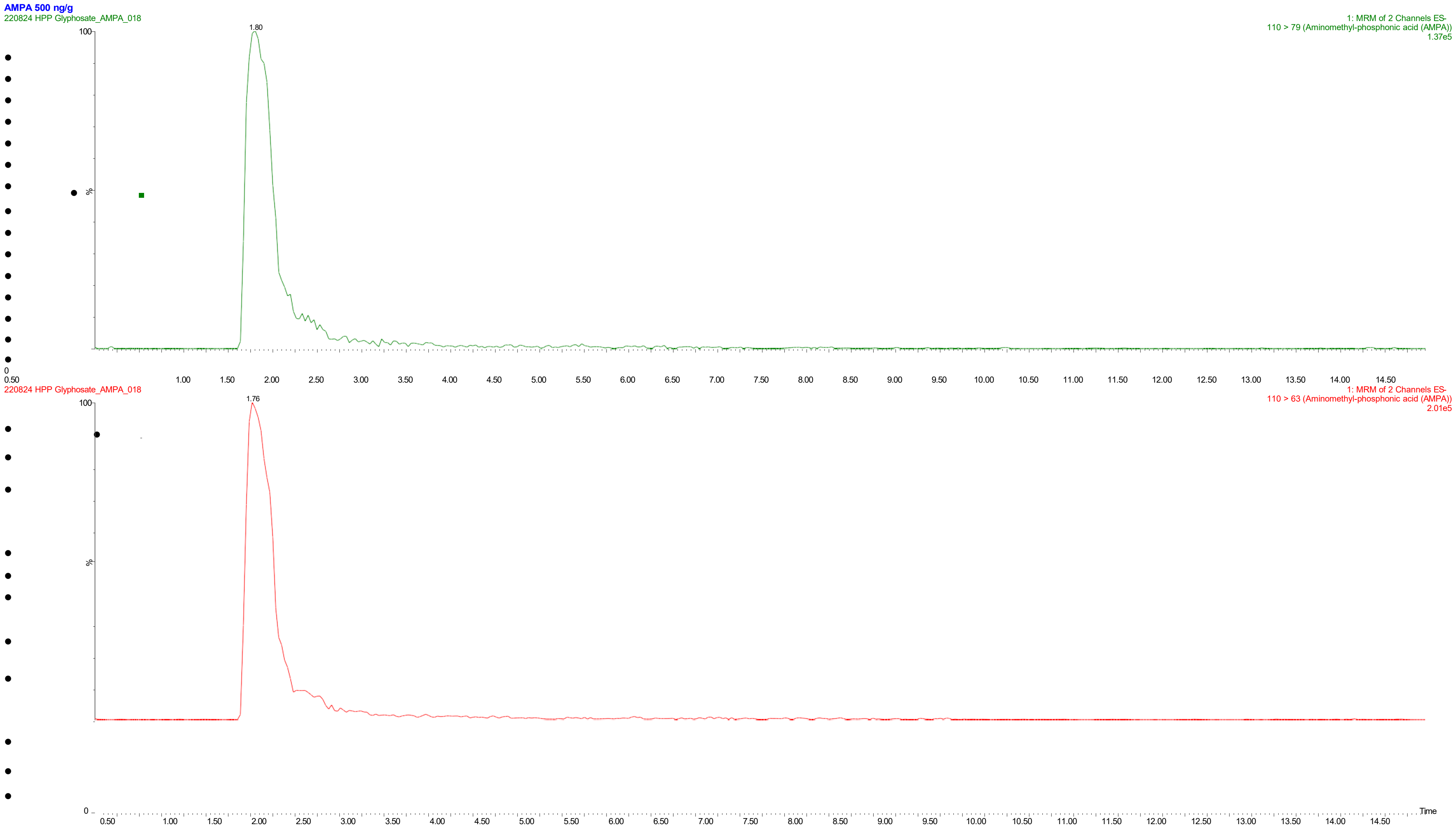


Figure 1: Optimal separation achieved for AMPA

- The AMPA is eluting at 1.5-2.5 minutes. The peak in the image is slightly overloaded leading to broad spread. However, this will be less pronounced in environmental samples as the concentration will be lower and matrix will be present.

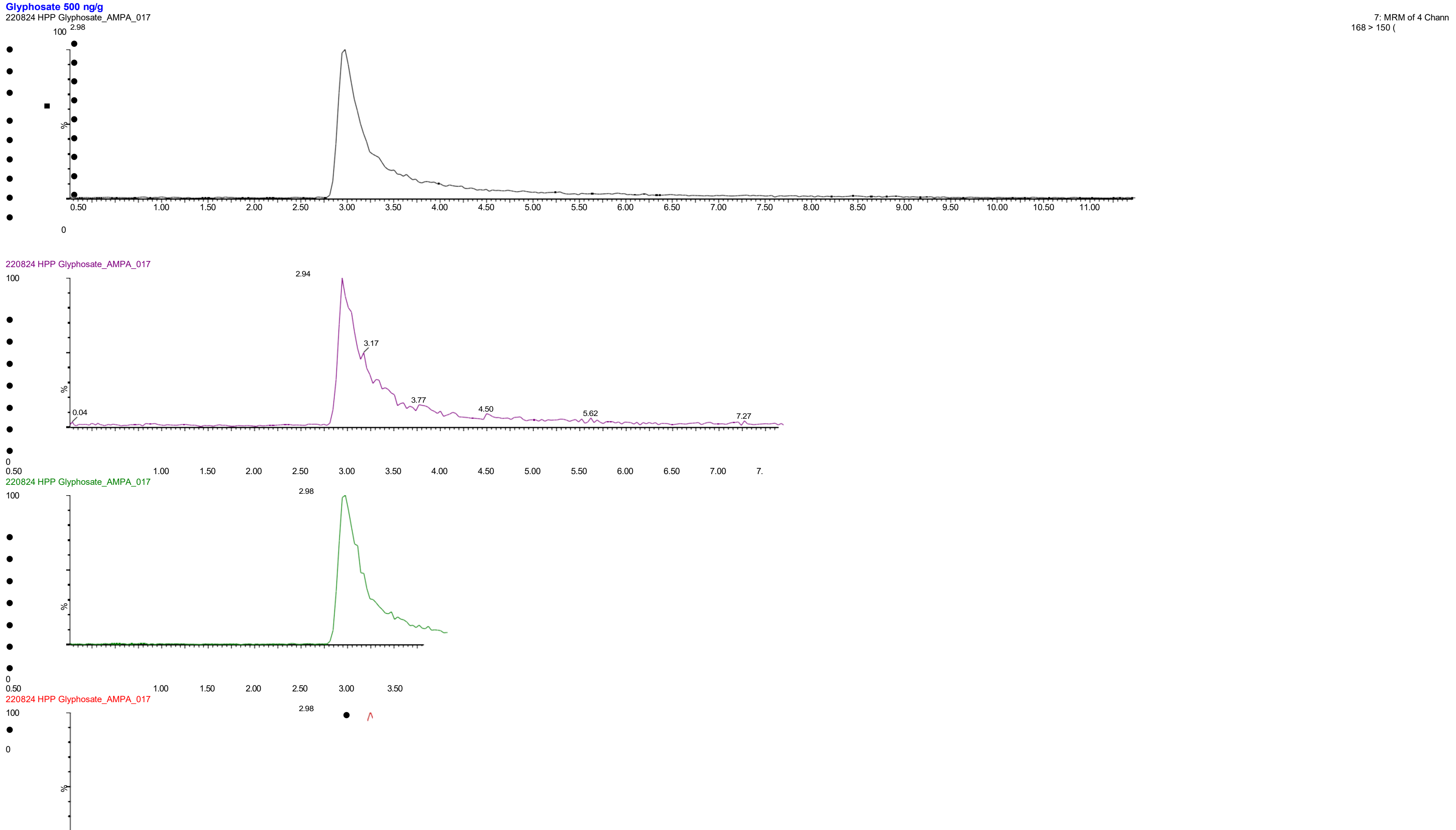
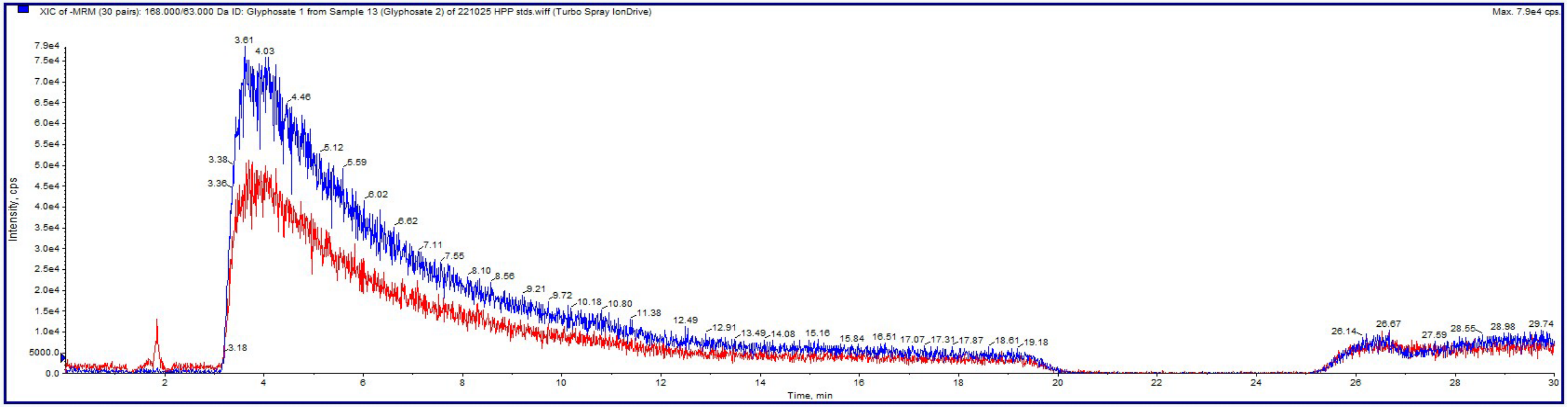


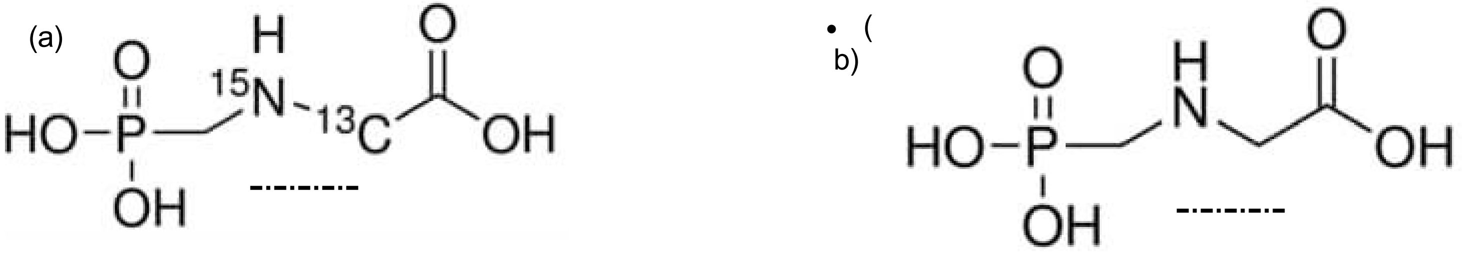
Figure 2: Optimal separation achieved for Glyphosate

- Glyphosate is eluting between 2.94 and 2.98 minutes. Peak tailing was observed, possibly due to chelation of glyphosate with metal ions in the LC system. This is a normal occurrence for Glyphosate and is acceptable. Literature has indicated that the tailing can be lessened by injecting 20 injections of matrix prior to analysing samples (6.1) and replacing the frit prior to each analysis batch.

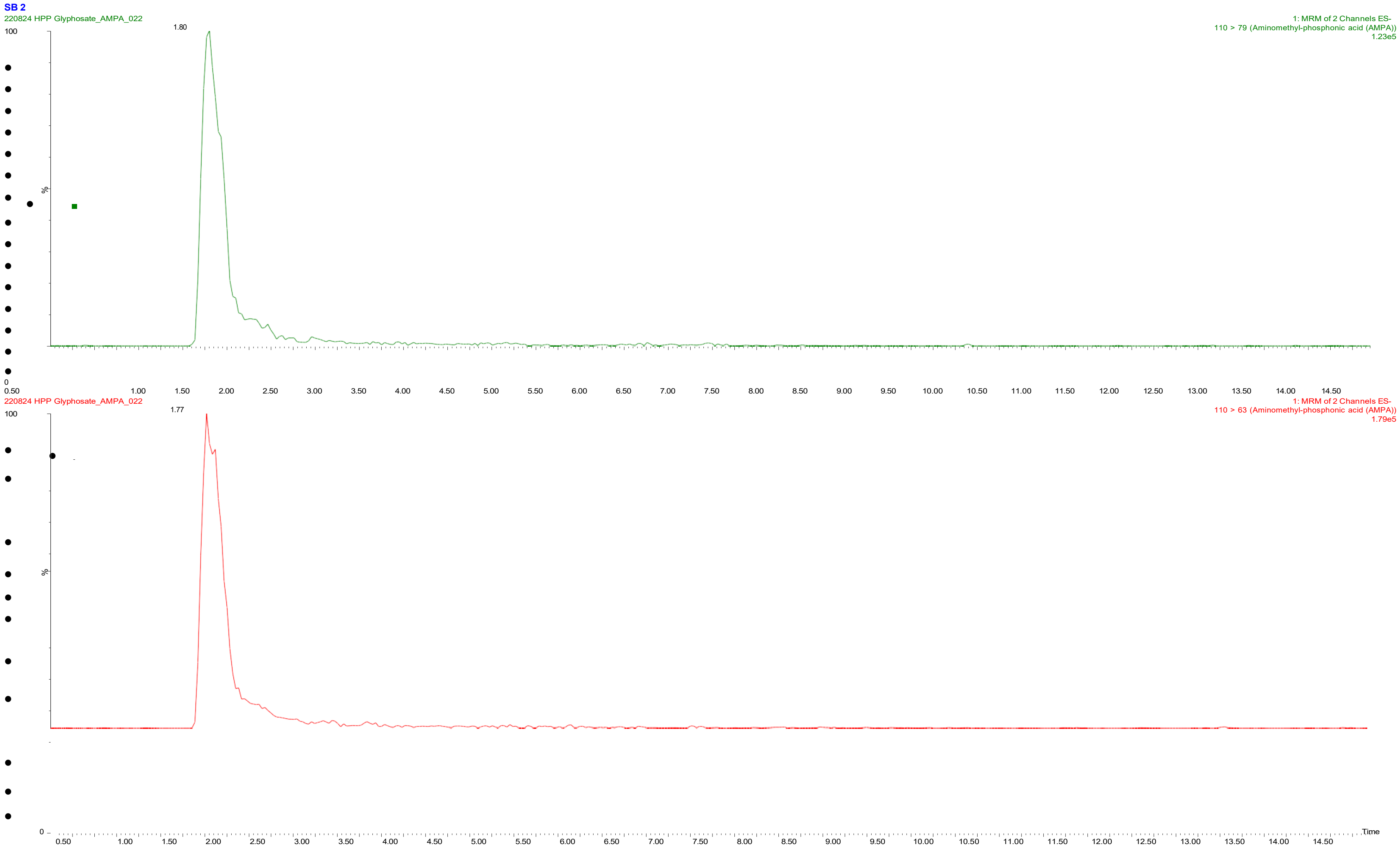
- The internal standard (Glyphosate-2-^{13}C) was evaluated to confirm if it was adequate to use during the extraction process. The standard was injected on the UHPLC coupled to the Sciex QTRAP MS/MS. Unfortunately, when energy is applied to the compound, the native of interest Glyphosate is formed and will interfere with the quantification of the compound of interest (**Figure 3**). Due to the positioning of the carbon label (**Figure 4**) during fragmentation both the isotope and native have fragment with identical masses. Unfortunately, the mass is the quantification mass and therefore there will be an interference during quantification. Other masses cannot be used for the quantification as the response is significantly lower in matrix and will not provide a constant mass fraction for quantification. Another HPP compound Glufosinate, can be evaluated as an alternative to this internal standard.



- **Figure 3:** Similar m/z values were achieved when analysing the Glyphosate-2-^{13}C compared to Glyphosate (m/z 168 > 63)



- **Figure 4:** Chemical structure of Glyphosate-2-^{13}C (a) and Glyphosate (b)



- **Figure 5**: Extracted MRM for AMPA in the spiked water extract
- Evaluation of the AMPA from water indicated no interference with the selected m/z values are detected after extraction. Optimal recovery was achieved and peak shape is adequate.

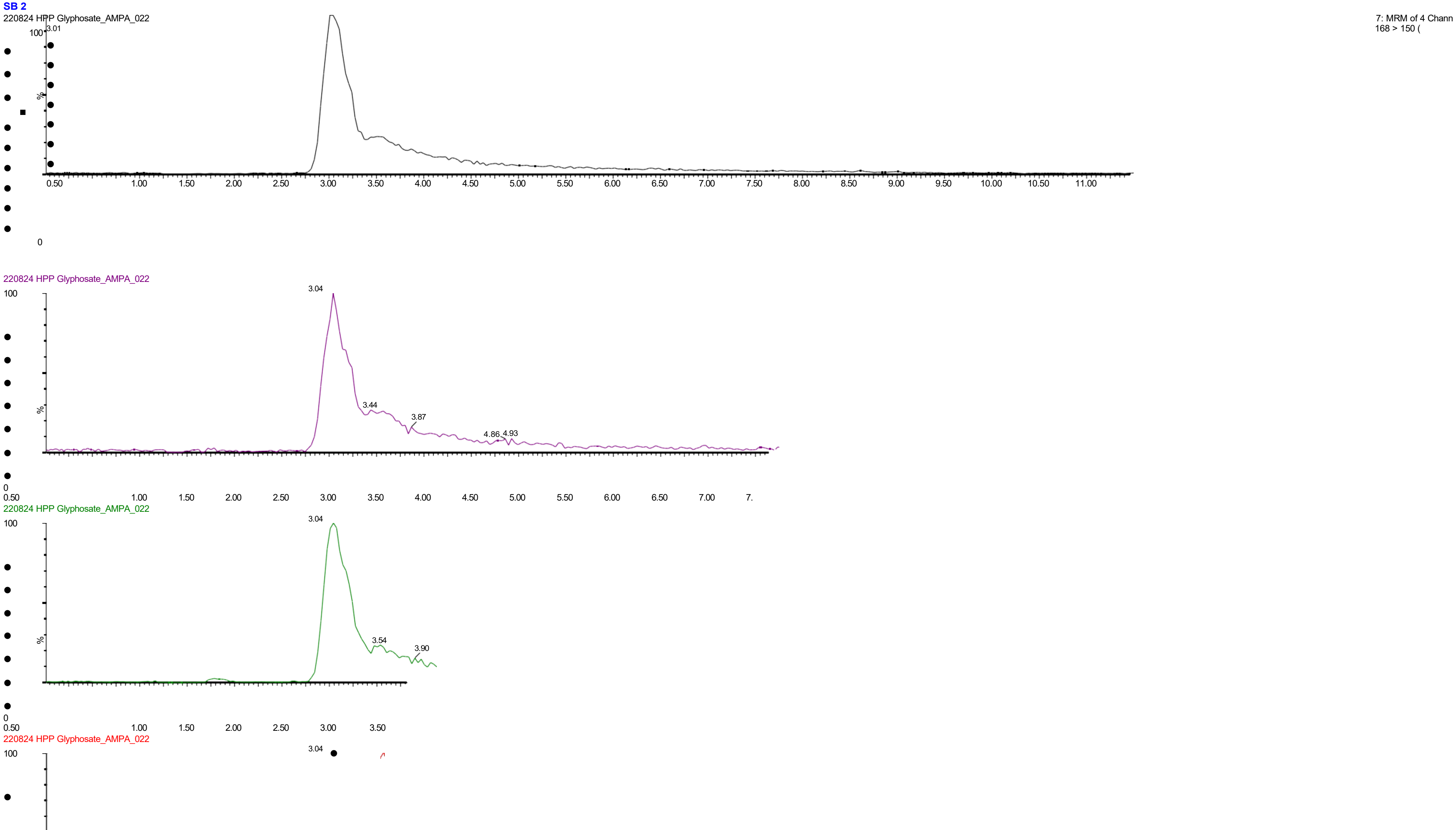
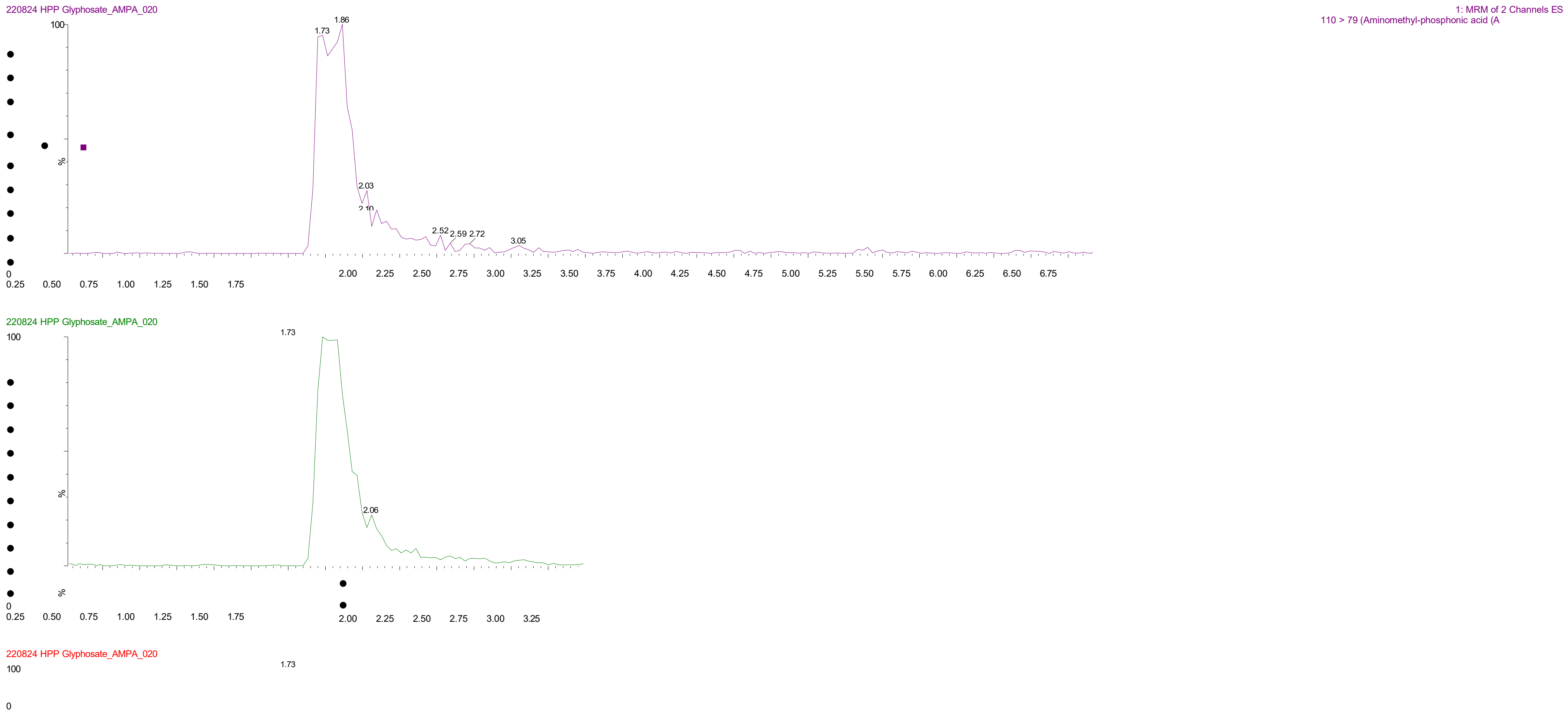


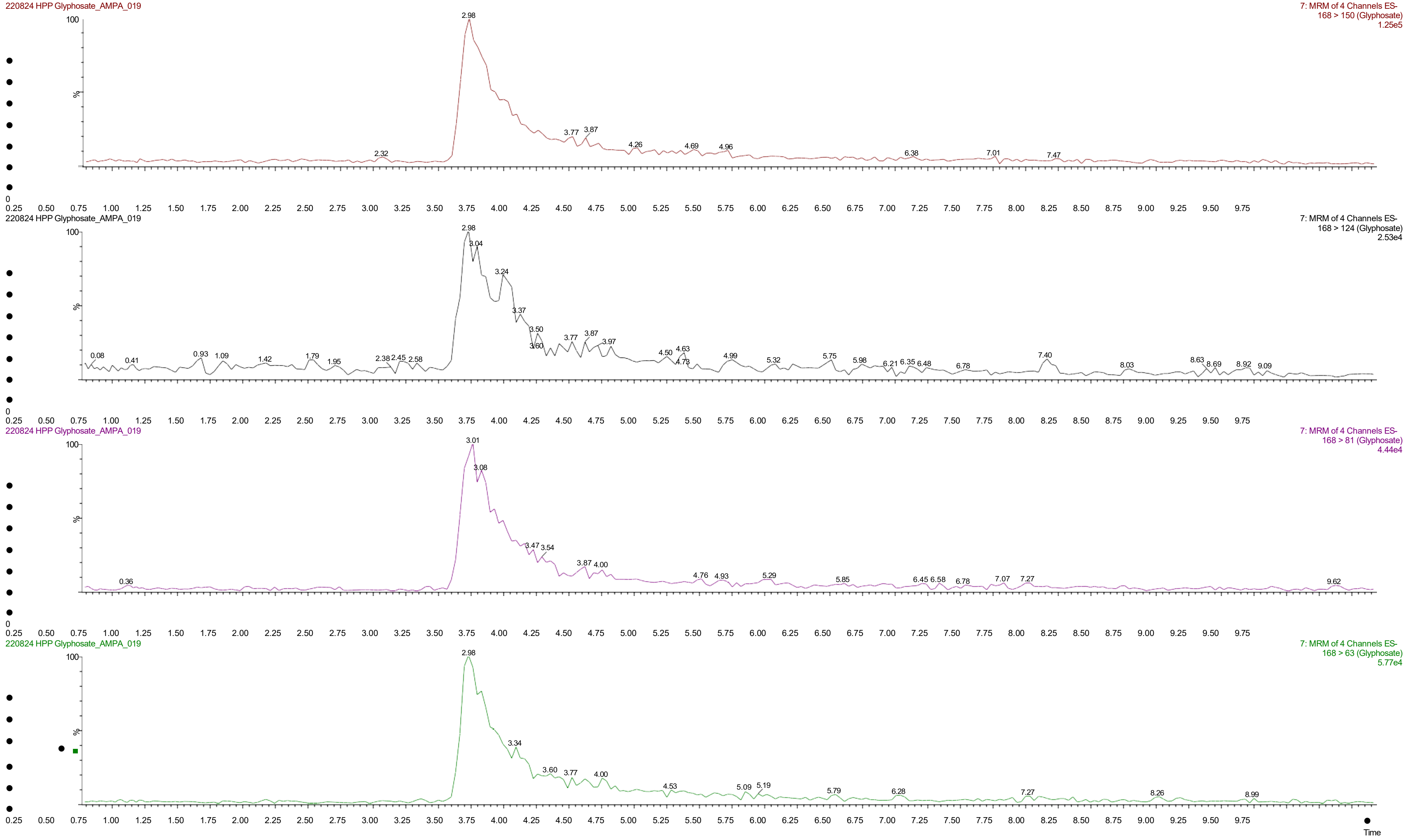
Figure 6: Extracted MRM for Glyphosate in the spiked water extract

Evaluation of the Glyphosate from water indicated no interference with the selected m/z values in the presence of matrix after extraction. Optimal recovery was achieved and the peak shape is adequate. Four mass transitions was evaluated to ensure no interference is present and to evaluate peak shape of minor m/z masses. Two transitions was selected after extraction evaluation for further analysis.



- **Figure 7**: Extracted MRM for AMPA in the spiked soil extract

- Evaluation of the AMPA from sediment indicated no interference with the selected m/z values detected in matrix after extraction. Acceptable recovery was achieved (60-120%). Peak splitting is present due to high concentration but this should not be a problem in the environmental samples as lower concentrations are expected.



- **Figure 8**: Extracted MRM for Glyphosate in the spiked soil extract

- Evaluation of the Glyphosate from sediment indicated no interference with the selected m/z values detected in matrix after extraction. Peak shape tailing and splitting is present and could be due to the presence of chelation during analysis. This will be prevented by ensure a clean frit is inserted before every analysis batch and matrix injected to ensure no active sites in the LC system.

Extraction of water

- The extraction procedure was adapted from the EURL-SRM QuPPe method for highly polar pesticides in fruit and vegetables. The samples were spiked at a known concentration with the compounds of interest and the recovery achieved evaluated. Optimal recovery between 80-110% was achieved with the selected method.

1.1 Clearly mark 50 mL centrifuge tubes with sample label and wrap with foil to prevent exposure to light
1.2 Bring sample to room temperature, vortex and weigh 10 mL into centrifuge tube
1.3 Add internal and/ or native standards if required. For the quality control sample, a concentration of 500 ng/g is advised. Vortex to ensure optimal equilibration.
1.4 Add 10 mL Acidified Methanol (1% Formic acid)
1.5 Vortex for 2 minutes
1.6 Ultrasonicate samples for 15 minutes
1.7 Centrifuge for 10 minutes at 6000 g, at 4 ℃
1.8 Filter the sample with a 0.22 µm PTFE filter and inject.

-

Extraction of soil

- Multiple extraction methods were evaluated to achieve optimal recovery. Three different techniques were evaluated: accelerated solvent extraction (ASE); Quick method for the analysis of numerous highly polar pesticides in food and plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPE-Method); and Potassium hydroxide (KOH) digestion (**Table 5**). Optimal recovery was only achieved with method 3 at 60%. Direct injection was done with each method where possible. In this case an aliquot from the extract is taken and injected on the LC prior to evaporation to evaluate if it is possible to detect any compounds. In most cases no pesticides could be detected, as the extract was too diluted.

- The extraction that provided the best results (between 58-63% recovery) is recommended. This the ASE extraction with Methanol, 0.1% Formic Acid, 2% Water (Method 3; **Table 5**). This method is detailed below.

1.9 Clearly mark 50 mL centrifuge tubes with sample label and wrap with foil to prevent exposure to light
1.10 Thoroughly mix dried sample and weigh 10 g into centrifuge tube
1.11 Mix hydromatrix/ diatomaceous earth with the sample. The cell volume must be fully used and packed consistently (similar density) to ensure repeatable solvent volumes with extraction on the ASE. Therefore, the amount of hydromatrix/ diatomaceous earth to mix with the sample depends on the size of the cell used.
1.12 Place two cellulose filters in a precleaned marked extraction cell and fill it hydromatrix/ diatomaceous earth to cover the filter
1.13 Transfer the sample to the extraction cell
1.14 Add the required internal and/ or native standard. For the quality control sample, a concentration of 1000 ng/g is advised.
1.15 Fill the remainder of the cell with hydromatrix/ diatomaceous earth, and hand tighten the top end cap onto the cell body. Note – no filter required on top is required.

- **Table 5**: Extraction methods evaluated for soil

#	Sample mass (g)	Extraction Solvent	After extraction	Outcome
Modified QuPPE method for dry foods				
1	5	Water and acidified Methanol (1% Formic acid) with additional freeze step	• Direct injection Evaporation to dryness and reconstitution in 5% Methanol	• None of the pesticides detected – likely due to the evaporation process
2	5	Acidified Methanol (1% Formic acid)	• Direct injection Evaporation to dryness and reconstitution in 5% Methanol	• None of the pesticides detected – likely due to the evaporation process
Extraction with potassium hydroxide (KOH)				
3	2	0.2 M KOH	Could not inject, further clean-up required by SPE	Requires additional clean-up
ASE				
The following ASE parameters were used for all extractions (6.2): Temperature 110℃, static time 6 min, cycles 3, purge volume 80%, purge time 120 s				
4	10	2:1 Ethyl acetate: Acetone	• Direct injection • Evaporation to dryness and reconstitution in 5% Methanol	• None of the pesticides could be detected • Loss of analyte was likely due to evaporation • The extract must be evaporated as ethyl acetate and acetone cannot be injected onto the column. These solvents will damage the column packaging material
5	10	Methanol	• Direct injection • Evaporation to dryness and reconstitution in 5% Methanol	• Low recoveries achieved (10-40%) • Likely due to the evaporation process • Due to the volume extraction, evaporation is required to make sure that the pesticides are within the quantification range.
6	10	Methanol, 0.1% Formic Acid, 2% Water	• Direct injection • Evaporation to 1 mL	• Recoveries between 58-63% achieved • Increase in recovery could be due to the addition of water. Water acting as an analyte protectant during evaporation. • For this method to work, it is crucial that extracts are not evaporated to dryness. The water phase will remain as the methanol will evaporate faster • This is the suggested method for extraction

Preparation of quality control samples

- Quality control samples will need to be prepared with each batch to ensure recovery and degradation can be evaluated to correct for this deviation in the final data. Three samples of each matrix per batch will be spiked with a known concentration prior to extraction, and three samples will be extracted, but only spiked after extraction (**Table 6**). This is known as spike before (SB) and spike after (SA) quality control samples. Additionally, a matrix blank will also be added to evaluate if any analytes of interest is present in the matrix used for the spike samples.

-
- **Table 6**: Quality control sample summary
-

Matrix	**Sample type**	**Samples**	**End concentration (ng/g)**
Spike before (SB)			
Water	MilliQ water	Three samples spiked as described in section 3.3	500
Soil	Any soil sample	Three samples spiked as described in section 4.6	1000
Spike after (SA)			
Water	MilliQ water	Three samples spiked after extraction prior to evaporation into 50 mL centrifuge tube	500
Soil	Any soil sample	Three samples spiked after extraction prior to evaporation 50 mL centrifuge tube	1000
Blank			
Water	MilliQ water	No spike, but follows the extraction procedure	0
Soil	Any soil sample	No spike, but follows the extraction procedure	0

Important notes

- A multitude of factors can influence the analysis and extraction of HPP. Precaution must be taken to prevent loss of analyte. Please note that the following factors must be taken into account.

1.16 Samples must be stored away from light and frozen to prevent breakdown.
1.17 Upon thawing samples should be extracted and analysed within 14 days
1.18 Ensure all standards and samples are not exposed directly to glass and stored in polypropylene or other suitable containers to prevent interaction with glass surface
1.19 Standards and samples must not be exposed to light
1.20 Standards must be made in 10% Acetonitrile in water to prevent breakdown and are not stable for longer than 2 weeks
1.21 Evaporation to dryness is not advised as this led to substantial loss of analytes.
1.22 Samples must be stored away from light at room temperature and analysed within 14 days. This was for fruit samples in a mixture of acidified Methanol and water. We would not advise longer than 7 days.

References

1.23 EU Reference Laboratories for Residues of Pesticides – Single Residue Methods (EURL-SRM). Quick method for the analysis of highly polar pesticides in food involving extraction with acidified methanol and LC- or IC-MS/MS measurement. Version 12, 2021.

1.24 Schafer *et al*., 2008. Determination of 10 particle-associated multiclass polar and semi-polar pesticides form small streams using accelerated solvent extraction. Chemosphere 70 (2008) 1952-1960.

APPENDIX B: CAPACITY BUILDING

STUDENT CAPACITY BUILDING

- Honours student: Monique Labuschagne (completed end of 2021)
- PhD student: Lohan Bredenhann (3rd year in 2023)
- PhD student: Ilzé Horak (graduated in June 2023)

Abstract

Although agrochemicals reduce pest-associated crop losses, these chemicals end up in non-target environments following rainfall and irrigation. When fractions of agrochemical mixtures are bioavailable, they pose a threat to human and environmental health. The aim of this study was to determine whether the water-soluble fraction of agricultural soils in South Africa elicit selected biological effects in vitro, as well as to determine which pesticides are likely causing the observed effects. Composite soil samples were collected in two maize growing regions – the Mpumalanga province and Vaalharts Valley – known for their extensive herbicide application. Water-soluble compounds were extracted from the soil using deionised water to mimic environmental conditions and obtain the bioavailable fraction. The H4IIE-luc reporter gene bioassay was performed to establish whether the soil-extracts contained aryl hydrocarbon receptor (AhR) ligands. This was followed by assessing (anti-)androgenic and glucocorticoid activity using the human breast carcinoma cell line MDA-kb2. The T47D-KBluc cell line was used to screen the water-soluble agrochemical residues for (anti-)oestrogenicity by evaluating binding to the oestrogen receptor. Oxidative stress responses (reactive oxygen species production, superoxide dismutase content, catalase activity, and lipid peroxidation), and non-neuronal acetylcholinesterase activity was also evaluated in vitro using the rat hepatoma H4IIE-luc and human duodenum HuTu-80 cell lines. Lastly, ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was used to quantify the following current-use pesticides in the soil: 2,4-dichlorophenoxy acetic acid (2,4-D), atrazine, dicamba, and imidacloprid. Results from the in vitro bioassays indicated that compounds present in the samples did not activate the AhR or androgen receptor (AR). However, at the concentrations evaluated in this study the soil-extracts from some sampling locations caused AR antagonism and (anti-)oestrogenicity, indicating endocrine disruptive effects. Although similar responses were observed in the HuTu-80 and H4IIE-luc cells following exposure to the bioavailable fraction for 24 hours (83 mg/mL), the rat hepatoma cell line was able to detoxify the xenobiotics present in the samples better. Furthermore, all four the target pesticides were quantifiable in at least one of the soil samples. Atrazine (89%) had the highest detection frequency, followed by dicamba (84%), 2,4-D (74%), and imidacloprid (32%). The combined use of in vitro bioassays and instrumental chemical analysis provided a holistic overview of the impact of agriculture on non-target organisms in the South African environment. Moreover, the findings of the present study contribute to the current understanding of the biological effects associated with the water-soluble fraction of agricultural soils, as well as the identity of the current-use pesticides likely causing these effects.

Keywords: endocrine disruption; mammalian tissue culture; oxidative stress; pesticides; so

INSTITUTIONAL CAPACITY BUILDING

- Collaboration with NMISA – Student will be exposed to analysis
- Collaboration between departments at NWU to set up method for analysis and increase

APPENDIX C: KNOWLEDGE DISSEMINATION

OUTPUTS FOR 2021

- Horak, I., Horn, S., Pieters, R. (2021). Agrochemicals in freshwater systems and their potential as endocrine disrupting chemicals: A South African context. Environmental Pollution 268: 115718.

Abstract

South Africa is the largest agrochemical user in sub-Saharan Africa, with over 3000 registered pesticide products. Although they reduce crop losses, these chemicals reach non-target aquatic environments via leaching, spray drift or run-off. In this review, attention is paid to legacy and current-use pesticides reported in literature for the freshwater environment of South Africa and to the extent these are linked to endocrine disruption. Although banned, residues of many legacy organochlorine pesticides (endosulfan and dichlorodiphenyltrichloroethane (DDT)) are still detected in South African watercourses and wildlife. Several current-use pesticides (triazine herbicides, glyphosate-based herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and chlorpyrifos) have also been reported. Agrochemicals can interfere with normal hormone function of non-target organism leading to various endocrine disrupting (ED) effects: intersex, reduced spermatogenesis, asymmetric urogenital papillae, testicular lesions and infertile eggs. Although studies investigating the occurrence of agrochemicals and/or ED effects in freshwater aquatic environments in South Africa have increased, few studies determined both the levels of agricultural pesticides present and associated ED effects. The majority of studies conducted are either laboratory-based employing in vitro or in vivo bioassays to determine ED effects of agrochemicals or studies that investigate environmental concentrations of pesticides. However, a combined approach of bioassays and chemical screening will provide a more comprehensive overview of agrochemical pollution of water systems in South Africa and the risks associated with long-term chronic exposure.

Keywords: agriculture; freshwater; organochlorine pesticides; DDT; oestrogenic activity; intersex.

- Horn, S., Chemical pollution of water resources in South Africa associated with feeding the world. Water Research Commission 5th Symposium. September 2021. Virtual.

- Bredenhann, L., Horn, S., Pieters, R. Glyphosate-based herbicide usage: South Africa's killer? SETAC Africa 10th Biennial Conference 20-22 September 2021. Virtual.

Abstract

Agriculture is a cornerstone of the South African economy and important for food security which is why herbicides are used to reduce crop losses. The most used herbicide worldwide is glyphosate-based herbicides (GBHs). The introduction of genetically modified crops that are glyphosate (GLY) resistant, resulted in the increased use of GBHs. Glyphosate is highly water-soluble and have been found in water and soil samples throughout the world. There are, however, no records on the concentrations of GLY and its metabolite, aminomethylphosphonic acid (AMPA) in the South African environment. This study used GBH application data of the four popular crops, maize, soybeans, wheat, and sunflower, to identify areas that are subjected to glyphosate contamination in South Africa. The crops are treated with GBHs with every planting cycle over millions of hectares and represent 80% of all GBH used in South Africa (15 484 tonnes). Distribution maps of the four crops together with the volume GBH applied in 2017 were created using geographic information systems. The data was bought from a market research company that surveyed pesticide-use by South African farmers. In 2017, the region with the highest GBH applications was the Free State province bordering the Vaal River (2 436 tonnes). The 4-crop data visualisation approximates to 80% of the total GBH market in South Africa at an active ingredient level. The most GBH was used on maize (54%) followed by soybean (15%). The share of GBH used in non-agricultural and urban areas is estimated to be between 2 and 2.5%. In areas where maize is rotated with soybeans, more GLY is applied within the same year and due to its half-life in soil (1-197 days), it may accumulate in those areas. Since it had been shown that GBHs cause a wide array of human disorders including Alzheimer's and Parkinson's diseases and cancers. With these potential effects, it is of great importance that we learn what the levels are in the South African environment to determine risks posed to human and environmental health.

Keywords: Glyphosate, crops, effects, concentration level

- Horak, I., Horn, S., Pieters, R. Biological effects of water soluble agrochemical mixtures on the H4IIE-luc rat hepatoma cell line. SETAC Africa 10th Biennial Conference 20-22 September 2021. Virtual.

Abstract

It is projected that by the year 2050 the global human population will exceed the 9 billion mark putting increased pressure on global food security. In crop-producing countries where poverty, hunger and health are important social issues, such as South Africa, the burden is even greater. Agrochemicals, such as pesticides, are widely used in the agricultural sector to reduce crop losses. Currently, South Africa is the largest pesticide user in sub-Saharan Africa, with more than 3 000 pesticide products registered for use. It is estimated that 10% of the overall applied pesticides reach non-target areas through runoff, leaching or spray drift. As a result, aquatic ecosystems and vulnerable human communities are exposed to these chemicals. One of the toxicological effects associated with pesticide exposure is endocrine disruption – whereby the chemicals act as agonists or antagonists of endogenous hormone receptors (such as the aryl hydrocarbon receptor, AhR). The aim of this study was to investigate the potential biological effects caused by water-soluble agrochemical residues extracted from soil that was sampled in maize growing regions of South Africa. An in vitro bioassay, using the H4IIE-luc rat hepatoma cell line, was performed to determine if the water-soluble agrochemical mixtures applied to crops can activate the mammalian AhR which is responsible for gene regulation in xenobiotic metabolism. The MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assay was also conducted to evaluate whether the water-soluble extracts from the soil are cytotoxic. Results indicated that compounds present in the water-soluble extracts of the soil samples did not activate the AhR. However, soil extracts from seven of the nineteen sample sites caused statistically significant ($p < 0.05$) cytotoxicity in the H4IIE-luc cells in a dose-dependent manner. In addition, the water-soluble extracts from two of the sites promoted cell proliferation ($p < 0.05$). The approaches followed in this study will aid in assessing the ecotoxicological risks posed by water-soluble agrochemicals which migrate to non-target areas and become bioavailable for aquatic biota.

Keywords: agriculture; endocrine disruption; cytotoxicity; pesticides.

- ENVIRA (UESM, NWU newspaper) popular science articles

Features

ENVIRA

Aquatic Ecosystem Health

The battle of chemical pollution associated with feeding the world

Suranie Horn & Rialet Pieters

Herbicides with different mechanisms of action are used to control weeds.

In 2012, French authors published on the tumour forming abilities of Roundup® (herbicide) and genetically modified (GM) maize in rats - which was retracted and republished (Séralini *et al.*, 2014). This incident is referred to as the Séralini-affair and the controversy surrounding the retraction red-flagged Roundup® for having harmful effects on animals and potentially humans too, despite the manufacturers claim. It is supposedly safe to use because it only targets plants (and bacteria). Roundup® is the most applied herbicide and used to reduce crop losses, feeding the growing global population. Research had been conducted world-wide to unravel whether this miracle chemical—both the formulation and its active ingredient, glyphosate—pose health risks. Other measures adopted to improve crop production include: GM crops (Bt maize) to kill insect pests by releasing Cry proteins; creating crops resistant to glyphosate's effects (Roundup® Ready crops), leading to even more glyphosate-based herbicides (GBHs) being sprayed; a variety of pesticides such as seed coating neonicotinoids and herbicides with different mechanisms of action. These compounds end up in the environment and occur in mixtures together with a multitude of other synthetic chemicals (Chang *et al.*, 2011). Complex mixtures are difficult to assess, as the constituents may react differently in one another's presence, making assessing their risks a challenging one.

Due to the lack of information about these compounds in the South African environment, a field trial was run, in which different maize cultivars were sprayed with different combinations of the two herbicides to create a mixture. At the end of the field trial, soil was extracted with rainwater to target specifically the water-soluble (bio-available) fraction. The unknown effects of these agricultural mixtures were determined by using effects based *in vitro* reporter-gene assays. The results showed that the environmental extracts that received both a pre- and post-emergent Roundup® application activated both the androgen (AR) and glucocorticoid receptors (GR) in the cells of the bio-assay. These responses exceeded the international drinking water trigger values specifically derived for such bio-assays for both the AR and the GR. Untimely and/or inappropriate binding to a hormonal receptor may interfere with their regulatory role, especially when it happens during a sensitive developmental foetal stage (Gauger *et al.*, 2004).

Beginning of the field trail with coloured markers to indicate the different maize varieties planted and herbicide combinations sprayed.

This study also revealed that at the end of the maize growing season, the Cry1Ab from GM maize, glyphosate, and 2,4-D were still detectable in the soil.

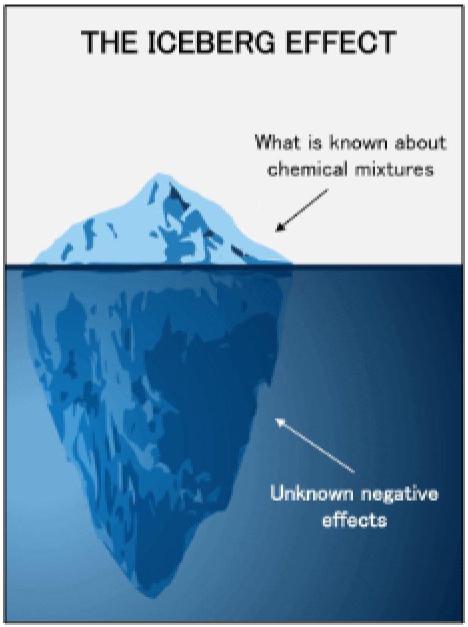
19

These chemicals are highly water-soluble and with run-off from irrigation or rainfall, move to water sources. Humans, dependent on river or other-than-piped water sources, might be exposed to these compounds through consumption and washing.

Dr Suranie Horn sampling river water for herbicides analysis.

There are more to unravel regarding exactly which chemicals in the environmental mixtures were responsible for the binding to the hormonal receptors, but these results act as a serious warning, prompting further investigation. Agricultural chemicals are present in the environment for prolonged periods and some exceed the drinking water guidelines. We, as toxicologists, are tasked to assess the risks associated with exposure to synthetic chemicals and help decide if the use of these outweigh their benefits. It is impossible to eliminate pesticides from agricultural activities in the short term, but they should be used with care and caution. We are currently conducting a study, funded by the Water Research Commission, in which we are the first research team to determine the concentrations of glyphosate and its main metabolite, AMPA, in South African water resources. Part of the outcomes will be to establish the analytical capacity at the NWU (USEM) to test for these compounds— enabling future monitoring studies.

Back to buttons



Environmental chemical mixtures may have far greater effects than anticipated.

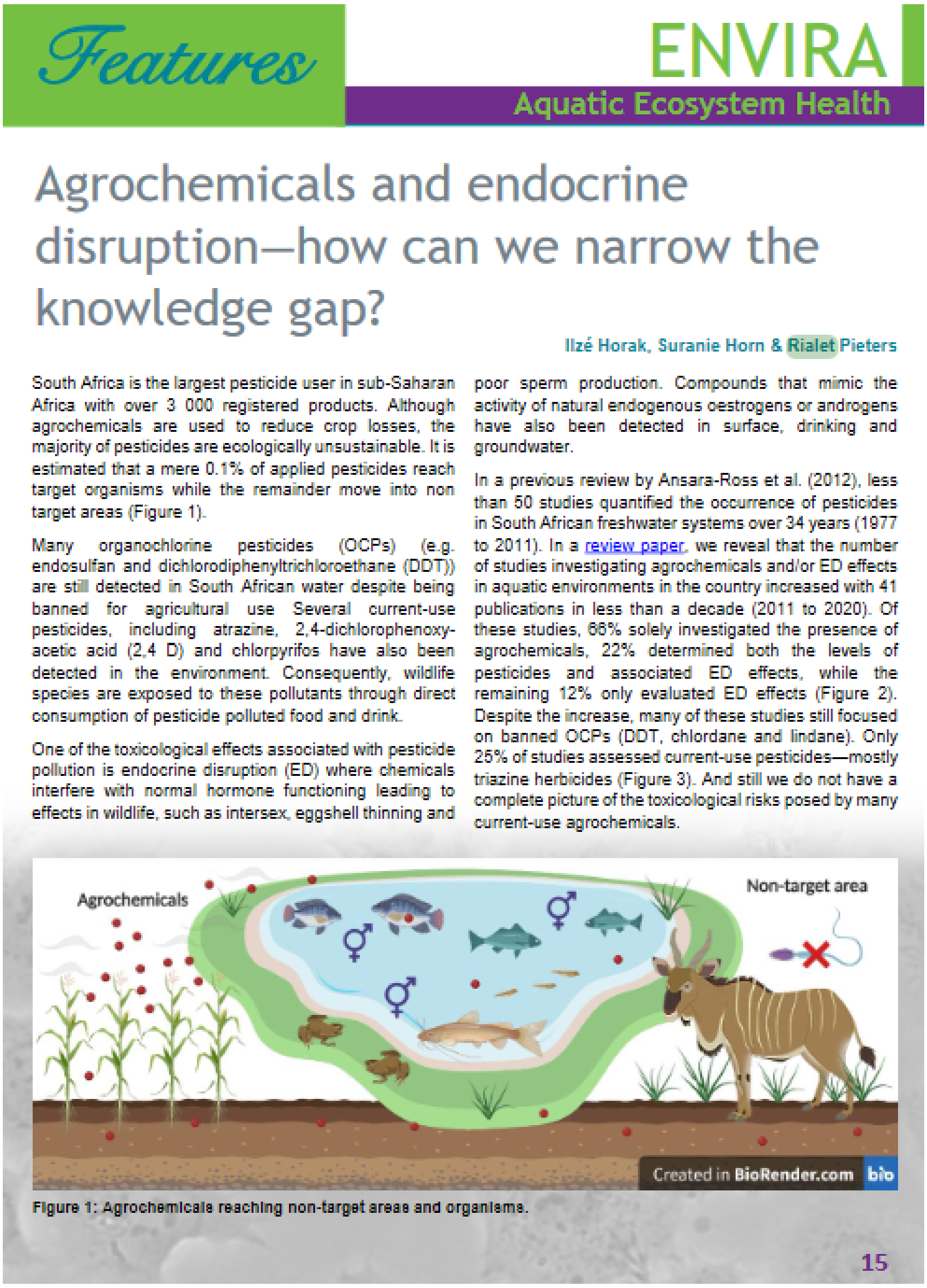
References

Chang, F.C., Simcik, M.F. and Capel, P.D. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environmental Toxicology and Chemistry*, 30(3), 548–555.

Gauger, K.J., Kato, Y., Haraguchi, K., Lehmler, H.J., Robertson, L.W., Bansal, R. and Zoeller, R.T. 2004. Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. *Environmental Health Perspectives*, 112(5), 516–523.

Séralini, G.E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., Hennequin, D. and de Vendômois, J.S. 2014. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Environmental Sciences Europe, 26(1) 14.

Photo credits:
Suranie Horn, Vickey-Luanne Harris



Features

ENVIRA

Aquatic Ecosystem Health

Agrochemicals and endocrine disruption—how can we narrow the knowledge gap?

Ilzé Horak, Suranie Horn & Rialet Pieters

South Africa is the largest pesticide user in sub-Saharan Africa with over 3 000 registered products. Although agrochemicals are used to reduce crop losses, the majority of pesticides are ecologically unsustainable. It is estimated that a mere 0.1% of applied pesticides reach target organisms while the remainder move into non target areas (Figure 1).

Many organochlorine pesticides (OCPs) (e.g. endosulfan and dichlorodiphenyltrichloroethane (DDT)) are still detected in South African water despite being banned for agricultural use Several current-use pesticides, including atrazine, 2,4-dichlorophenoxy-acetic acid (2,4 D) and chlorpyrifos have also been detected in the environment. Consequently, wildlife species are exposed to these pollutants through direct consumption of pesticide polluted food and drink.

One of the toxicological effects associated with pesticide pollution is endocrine disruption (ED) where chemicals interfere with normal hormone functioning leading to effects in wildlife, such as intersex, eggshell thinning and poor sperm production. Compounds that mimic the activity of natural endogenous oestrogens or androgens have also been detected in surface, drinking and groundwater.

In a previous review by Ansara-Ross et al. (2012), less than 50 studies quantified the occurrence of pesticides in South African freshwater systems over 34 years (1977 to 2011). In a review paper, we reveal that the number of studies investigating agrochemicals and/or ED effects in aquatic environments in the country increased with 41 publications in less than a decade (2011 to 2020). Of these studies, 66% solely investigated the presence of agrochemicals, 22% determined both the levels of pesticides and associated ED effects, while the remaining 12% only evaluated ED effects (Figure 2). Despite the increase, many of these studies still focused on banned OCPs (DDT, chlordane and lindane). Only 25% of studies assessed current-use pesticides—mostly triazine herbicides (Figure 3). And still we do not have a complete picture of the toxicological risks posed by many current-use agrochemicals.

Figure 1: Agrochemicals reaching non-target areas and organisms.

15

OUTPUTS FOR 2022

- Horak, I., Horn, S. Pieters, R. Endocrine disruptive effects of water-soluble agrochemicals extracted from maize and pecan soil. Society of Environmental Toxicology and Chemistry (SETAC) Europe 32nd Annual Meeting, 15-19 May 2022, Copenhagen, Denmark.

Abstract

Although beneficial in reducing crop losses, applied agrochemicals end up in non-target environments after irrigation and rainfall. When fractions of these chemical mixtures are accessible to living organisms for uptake across cellular membranes (bioavailable), they pose a threat to human and aquatic health. One of the toxicological effects associated with pesticide exposure is endocrine disruption – whereby the chemicals act as agonists and/or antagonists of endogenous nuclear receptors, including the mammalian aryl hydrocarbon receptor (AhR), androgen receptor (AR), and oestrogen receptor (ER). This study aimed to investigate the endocrine disrupting (ED) effects associated with the bioavailable fraction of agrochemical mixtures associated with maize soil in South Africa. Composite soil samples were collected in two maize growing regions – the Mpumalanga province and Vaalharts Valley – known for their extensive herbicide application. Water-soluble compounds were extracted from the soil using deionised water to mimic environmental conditions and obtain the bioavailable fraction. An in vitro H4IIE-luc reporter gene assay was performed to establish whether the soil-extracts contain AhR ligands. This was followed by assessing (anti-)androgenic and glucocorticoid activity using the human breast carcinoma cell line MDA-kb2. Lastly, the T47D-kbluc cell line was used to screen the water-soluble agrochemical residues for antagonistic oestrogenic activity by evaluating binding to the ER. The MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assay was run in parallel to the reporter gene bioassays to ensure that (i) the concentrations under investigation were not cytotoxic towards the respective cell lines and (ii) as quality control to prevent false negative reporter gene assay results. Results from the in vitro bioassays indicated that compounds present in the water-soluble extracts of the soil did not activate the AhR or AR. However, at the concentrations evaluated in this study the soil-extracts from some sampling locations caused AR and ER inhibition, indicating ED effects. The identity of the chemicals that likely caused the effects should be further investigated by applying chemical screening to the soil-extracts.

Keywords: agriculture; pesticide mixtures; bioavailable; androgenic activity.

- Bredenhann, L., Horn, S., Pieters, R. Potentially Sacrificing Human and Environmental Health for Food Security. Society of Environmental Toxicology and Chemistry (SETAC) North America 43rd Annual Meeting 2022. 13-17 November 2022.

Abstract

South Africa is the most prolific pesticide consumer in Sub-Saharan Africa, with glyphosate-based herbicides (GBHs) being the most widely used. In eight years, the use of these herbicides more than doubled, from 3721 tonnes in 2009 to 7977 tonnes in 2017. However, because of the perceived risk to non-target biota, many governments have banned the use of GBHs. Furthermore, the South African Cancer Association agrees with the WHO that glyphosate is a potential human carcinogen. However, there is little information available on the amounts of glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), in the South African environment. In addition, there are no environmental regulations for these chemicals in the aquatic environment. We looked at why GBH use is so high: the widespread use of herbicide-tolerant crops and crop-rotation practices, as well as their unintended consequences, such as contributing to an already polluted river system and promoting weed resistance. This research highlights for the first time the widespread use of GBHs in South Africa, where they were sprayed on four crops in 2017 – maize, soybean, wheat/barley, and sunflower – and we recommend regular monitoring for GLY and AMPA in the South African environment, despite the fact that there appears to be no evidence of risk to local human and animal health.

Keywords: Glyphosate, crop practices, effects, cancer

Potentially sacrificing human and environmental health for food security

Lohan Bredenhann, Rialet Pieters & Suranie Horn
Envirtox

South Africa produces 10–20 million metric tons of maize annually, all treated with agrochemicals, a critical component of food security. These agrochemicals inevitably end up in non-targeted areas of the environment and may pose health risks to humans and animals. Roundup® is one of the most commonly used herbicides in the world. It contains the active ingredient glyphosate (GLY) which inhibits an enzyme in the shikimate pathway found in plants, ultimately leading to plant death. Glyphosate-tolerant crops were developed, which meant that farmers could increase their glyphosate-use without harming the crops. Unfortunately, this also led to the surrounding weeds developing resistance against glyphosate. To fight resistance, pesticides with other mechanisms of action are now used in combination with glyphosate.

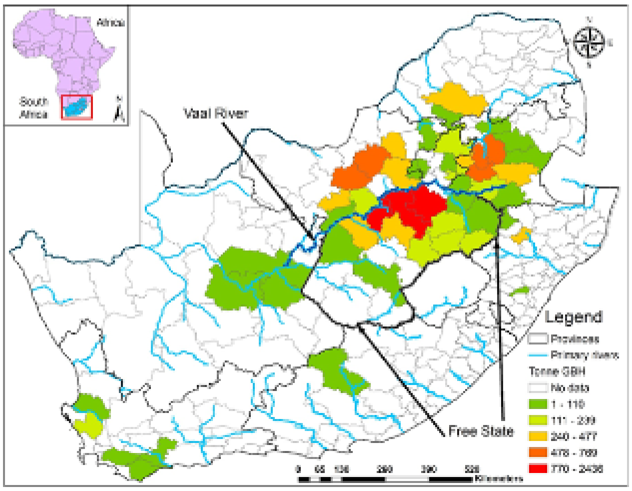
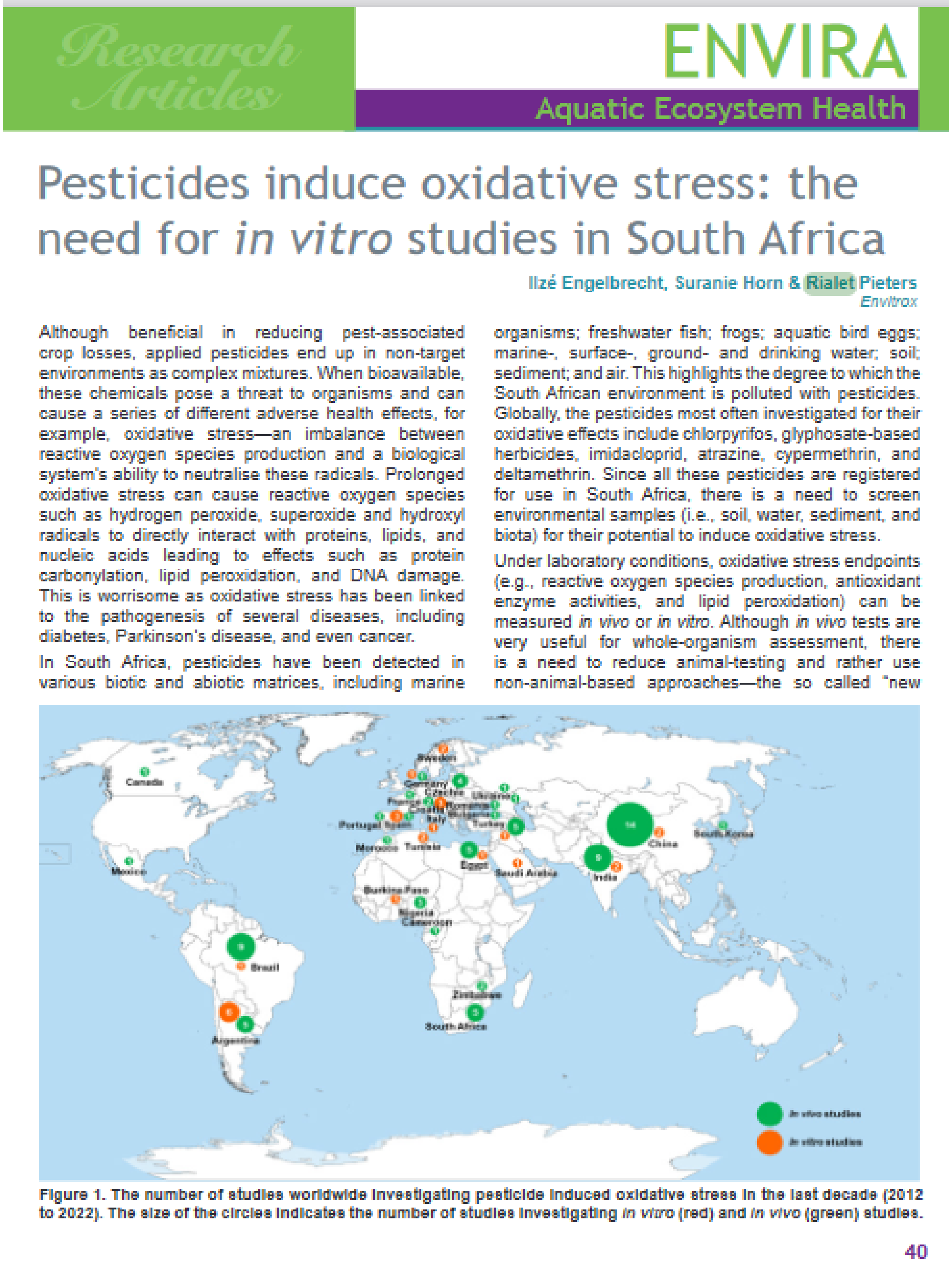


Figure 1: For the 2017 season, the application of glyphosate-based herbicides (GBHs) on maize, soybean, wheat, and sunflower crops in the municipal districts of South Africa (in tonnes a.i./ha). The WGS 1984 geographical coordinate system was used to project the vector data set coordinates (in decimal degrees) to show where the chemicals were used.

The last study published on general pesticide use in SA was in 2015, using data from 2009. In a recent study, data from 2017 showed the volumes of glyphosate-based herbicides (GBHs) applied to only four crops (i.e., maize, soybean, wheat and sunflower), which made up 80% of the total GBH applications. In 2009, the GBH application was approximated at 3 721 tonnes for all crops. However, in 2017 (eight years later), the amount had doubled to approximately 7 977 tonnes for use on only these four crops. The region with the highest GBH application (1 391–2 436 tonnes) was the Free State Province bordering the Vaal River between Vereeniging and Bloemhof (Figure 1). Most farmers in this region grow maize and/or soybeans and utilize conventional agricultural strategies when rotating their maize or soybean crops with a cover crop (e.g., replacing maize with legumes or soybean with wheat). Farmers spray herbicides on their cash crops before switching to another cover crop, which may also be sprayed with GBH, resulting in continuous herbicide use in these areas throughout the planting season.

Global GLY application for all uses—agricultural and non-agricultural—has increased more than 12 times in the last two decades, from 67 000 tonnes in 1995 to 826 000 tonnes in 2014. Studies done all over the world, e.g. Argentina, United States, Canada, Spain, Germany, Sri Lanka and New Zealand, have contributed to current knowledge that GLY and its main metabolite, AMPA, occur in the natural environment. Regrettably, there are no studies done on the GLY and AMPA concentration levels in SA, and subsequently no laboratory or agency in the country to analyse or monitor these chemicals in the environment and hence a lack of official regulatory guidelines for the levels of these harmful chemicals in soil or water. As a country we aim to encourage food security by reducing crop loss with GBHs, but to what extent will we be polluting our environment and consuming poisoned food, before any effective action is taken?

Back to buttons



Research Articles

ENVIRA

Aquatic Ecosystem Health

Pesticides induce oxidative stress: the need for *in vitro* studies in South Africa

Ilzé Engelbrecht, Suranie Horn & Rialet Pieters
Envitrox

Although beneficial in reducing pest-associated crop losses, applied pesticides end up in non-target environments as complex mixtures. When bioavailable, these chemicals pose a threat to organisms and can cause a series of different adverse health effects, for example, oxidative stress—an imbalance between reactive oxygen species production and a biological system's ability to neutralise these radicals. Prolonged oxidative stress can cause reactive oxygen species such as hydrogen peroxide, superoxide and hydroxyl radicals to directly interact with proteins, lipids, and nucleic acids leading to effects such as protein carbonylation, lipid peroxidation, and DNA damage. This is worrisome as oxidative stress has been linked to the pathogenesis of several diseases, including diabetes, Parkinson's disease, and even cancer.

In South Africa, pesticides have been detected in various biotic and abiotic matrices, including marine organisms; freshwater fish; frogs; aquatic bird eggs; marine-, surface-, ground- and drinking water; soil; sediment; and air. This highlights the degree to which the South African environment is polluted with pesticides. Globally, the pesticides most often investigated for their oxidative effects include chlorpyrifos, glyphosate-based herbicides, imidacloprid, atrazine, cypermethrin, and deltamethrin. Since all these pesticides are registered for use in South Africa, there is a need to screen environmental samples (i.e., soil, water, sediment, and biota) for their potential to induce oxidative stress.

Under laboratory conditions, oxidative stress endpoints (e.g., reactive oxygen species production, antioxidant enzyme activities, and lipid peroxidation) can be measured *in vivo* or *in vitro*. Although *in vivo* tests are very useful for whole-organism assessment, there is a need to reduce animal-testing and rather use non-animal-based approaches—the so called "new

Figure 1. The number of studies worldwide investigating pesticide induced oxidative stress in the last decade (2012 to 2022). The size of the circles indicates the number of studies investigating *in vitro* (red) and *in vivo* (green) studies.

40

OUTPUTS FOR 2023 (ENVISAGED)

- Two publications (Use manuscript + method, levels and risks in SA)
- Outcomes will also be presented at national and international conferences relevant to the topic
- A popular science article to reach public audience (maybe feature on *Omgewingspraatjies* – an environmental programme on a national radio station)

Horak, I., Horn, S. and Pieters, R., 2023. The benefit of using in vitro bioassays to screen agricultural samples for oxidative stress: South Africa's case. Journal of Environmental Science and Health, Part B, 1-15

Engelbrecht, I., Horn, S., Giesy, J.P. and Pieters, R., 2023. Determining superoxide dismutase content and catalase activity in mammalian cell lines. MethodsX, 102395.