Determination of metal concentrations in water, soil, and sediment in the KwaZulu-Natal Province: Comparison of digestion methods, Environmental concerns, and human health risk assessment

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Determination of metal concentrations in water, soil, and sediment in the KwaZulu-Natal Province: Comparison of digestion methods, Environmental concerns, and human health risk assessment

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Declaration

I, Kavisha Naicker, declare that this research project is entirely my original work, except where stated otherwise and the other people's work included in the dissertation was acknowledged using citations. This dissertation has not been submitted for any other degree either here or other universities for examination.

(Signature of candidate) ……[redacted] ……………………

(Signature of Supervisor) ……[redacted] …………………………

(Date)…20/07/2022………………………

Abstract

Metals are environmental natural elements. However, due to the alteration of their geological process and biochemical equilibrium as a result of the metals wide usage for human purpose, they have become a source of environmental pollution which is a global problem. Thus, they need to be evaluated in various environmental compartments, and their human health and environmental toxicity be assessed. Therefore, in this work, a microwave, hotplate and ultrasonic - assisted digestion have been successfully optimised and applied to water, soil, and sediment samples, for the digestion of twelve metals. The inductively coupled plasma – optical emission spectroscopy (ICP-OES) was optimized using the typical analytical figures of merit (correlation coefficient (R^2), limit of quantification (LOQ), (limit of detection (LOD)) for metal quantification. The method validation of the mentioned digestion methods was performed by spiked recovery tests using the multi-element ICP standard containing the studied metals (Cd, Cr, Co, Cu, Ga, Li, Mn, Ni, Pb, Sr, Tl, Zn). The percentage recoveries obtained in water, soil and sediments were within the acceptable range of 70 – 120%. All digestion methods indicated a good degree of accuracy as per the calculated figures of merit. The average concentrations obtained ranged from 4.9 – 410.8 µg/L, 5.9 – 465.0 µg/L, 3.6 - 425.4 µg/L, 16.1 - 647 µg/L and 9.7 – 784 µg/L in tap water, river, influent, effluent, and sludge samples for all metal analytes studied, respectively. The influent, effluent and sludge samples were collected from wastewater treatment plants. All metals were below their maximum permissible limits except the Mn in all sludge samples and Pb in all tap water, Umhlathuzana River and Northern works influent samples. This indicates that the tap water from all the sampling areas is not safe for human consumption as Pb is one of the most toxic metals which could result to irreparable harm of the kidney, brain and nervous system in humans, animals. For soil and sediments, metal concentrations obtained ranged from 0.10 – 355.4 mg/kg and 1.50 – 308.3 mg/kg respectively. The environmental contamination was evaluated for the soil and sediments and revealed that heavy metal contamination was not severe however, Cu showed significant contamination. Lastly, agricultural soil metal concentrations ranged from 0.60 – 256.4 mg/kg, however, the metals were all lower than the maximum allowable limits in soil except for Cr. Even though the widely used and recommended method of digestion is microwave, hotplate-assisted digestion proved to be the inexpensive and accessible alternative for routine analysis. Even though the metal pollution was observed to be minor in this study, it is essential to continuously monitor the potential contamination dangers and human health risks in the future.

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"Do the best you can until you know better. Then when you know better, do better."
– Maya Angelou

Dedication

To my heavenly father, Preshotmin Arunageri Naicker and
my beautiful mother, Manimaglai Naicker.

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Glossary

ASTM	American Society for testing and materials
ADD	average daily intake
CR	cancer risk
CRM	certified reference material
CF	contamination factor
EF	enrichment factor
I_{GEO}	geo-accumulation index
HQ	hazard quotient
ICP-OES	inductively coupled plasma – optical emission spectroscopy
ICP-MS	inductively coupled plasma – mass spectrometry
LADD	lifetime average potential daily dose
LOD	limit of detection
LOQ	limit of quantification
MRL	maximum permissible limit
RI	overall biological hazard
PLI	pollution load index
PERI	potential ecological risk index
RfD	reference dosage
THI	total hazard index
TRM	total recoverable metals
TDM	total dissolved metals
US EPA	United States Environmental Protection Agency
UKZN	University of KwaZulu – Natal
WWTPs	wastewater treatment plants
WHO	World Health Organization

Chapter One

This chapter involves the introduction, followed by the rationale of the research and thereafter the aim and objectives required to achieve the purpose of the research.

1.1. Introduction

Water is a necessity for all living organisms and biological processes. However, water scarcity due to heavy metal contamination is one of the main challenges that freshwater sources experience (Bassioni *et al*, 2015). Apart from heavy metal contamination in water, it can also pose an indirect threat in solid matrices such as soil and sediments. The potential sources of metals in water and soil can be natural (lithogenic, geogenic, weathering etc.) or anthropogenic (industrial activities like manufacturing processes, chemical industries and smelting of metal, electroplating etc.), sewage discharge, urban and agricultural practices (the use of pesticides, fertilizers), etc (Jaishankar *et al*, 2014). Irrigation of agricultural lands with industrial wastewater has been found to transfer heavy metals to tons of grain each year (He *et al.*, 2015). This results in heavy metals being ran off into nearby rivers where they may accrue in aquatic life and enter the food cycle. Also, metal contamination from river water and metal leaching from water distribution systems may lead to their presence in drinking water and thus be consumed by human beings. This may result to severe human health effects especially under significant exposure to high concentration levels (Atlas *et al.*, 2017). The commonly found heavy metals in water include chromium, arsenic, lead, nickel, cadmium, copper, and zinc, all of which pose a possible risk to human health and the environment (Jiao *et al*, 2013). The health risks include alteration of the functioning of the brain, kidneys, and lungs while persistent exposure can result in cancer (Masindi and Muedi, 2018).

Anions may also threaten water sources thus it is vital to monitor their toxicity in the surrounding environment. Most anions in the environment are as a result of rainfall runoffs, sedimentation in freshwater sources and land pollutants. The commonly found anions include phosphates (PO_4^{3-}), nitrates (NO_3^-), sulphates (SO_4^{2-}) chlorides (Cl^-) and ammonia (NH_3^-). Nitrates and ammonia are generally present in many nitrogenous fertilizers used for agricultural activity and high concentrations of nitrates may result in methaemoglobinemia (WHO, 1984). The presence of increased phosphate levels in water sources can result in eutrophication and excessive algal bloom (Reeve, 2002). Chlorination, being a general water treatment process, may increase the presence of chloride ions in treated drinking water (Altundag *et al.*, 2019).

Moreover, it may result to the generation of problematic toxic by-products such as halogenated trihalomethanes and haloacetic acids resulting in cancers of vital human being organs (Gopal *et al*., 2006; Lehtonen *et al*., 2019). The human and animal health risks connected to the presence of metals and anions water bodies makes their assessment vital in order to ascertain if they are within the acceptable limits.

As a result of the environmental samples matrices complexity and the trace levels at which the metals are present, it is challenging to directly detect them using inductively coupled plasma and atomic absorption spectroscopy. Thus, for effective investigation of heavy metals concentration levels, it is crucial to follow appropriate processes from sampling to sample analysis to obtain reliable results (Jaishankar *et al*, 2014). Sample preparation is therefore considered to be the most important step as it allows sample homogeneity and analytes preconcentration. Acid digestion is one of the processes involved in sample preparation where the sample matrix is decomposed by adding a concentrated acid and heating (EPA 3005A). Nitric acid is commonly used for the digestion due to its strong oxidising ability even when used alone and thus minimise the possible error of incomplete digestion (Uddin *et al*., 2016). Acid digestion can be assisted by microwave, by direct heating on a hotplate or ultrasonic-assisted digestion. Microwave digestion technique is advantageous over open digestion like hotplate as it eliminates loss of volatile metals as well as analyst exposure to toxic acid fumes during digestion. Moreover, it allows multiple samples digestion at a shorter reaction time and consumes lower volumes of acid. However, hotplate digestion is not susceptible to problems related to pressure build up since it is done at atmospheric pressure (Lomonte *et al*., 2008). Furthermore, hotplate and ultrasonic digestion techniques are favoured due to low cost which makes them accessible in all laboratories. Once the sample has been prepared, the analytes are commonly analysed using spectroscopic methods and the widely employed technique for the analysis low concentrations of metal is inductively coupled plasma coupled (ICP) with optical emission spectroscopy (OES) or mass spectrometry (MS). The ICP-MS is the most sensitive, however, due to its high costs, it is not available in many laboratories and hence, the ICP-OES is commonly used. The ICP-OES technique is also preferred as it can monitor emission spectrum of each element at different wavelengths and can simultaneously quantify 60 or more elements (Reeve, 2002).

Even though heavy metal contamination exists in trace amounts, their monitoring is of increasing importance due to their toxic and accumulating ability in water bodies. Environmental contamination in soil can be determined by various calculations and mathematical expressions such as the pollution load index (PLI), contamination factor (CF), potential ecological risk index (*PERI*) and geo-accumulation index (I_{GEO}). The human health risk assessment can then be evaluated by the carcinogenic and non-carcinogenic risks, in which the cancer risk (CR) and hazard quotient (HQ) are calculated respectively, through three main exposure pathways in humans. These pathways included ingestion (via fresh produce), dermal contact and air inhalation (soil vapour). (Choi *et al*., 2018)

This work therefore focused on the assessment of metals in water, sediment and soil samples using ICP-OES. The Aquakem 250 is a discrete photometric analyzer used for the quantification of anion concentrations in the water samples. This instrument offers fast and reproducible results with very low carry over, can analyse a number of parameters per sample and consumes lower amount of reagent and sample. To the best of our knowledge, no work has been performed to assess the concentrations of the studied metals and anions in the selected study areas of KwaZulu-Natal. Also, the comparison of microwave and hotplate digestion methods was conducted for the first time to compare the total dissolved and recoverable metals concentrations in water samples (tap, river, and wastewater). Metal contamination in soil and sediment from selected sampling areas in KwaZulu-Natal involved three methods of digestion, primarily comparison between microwave and ultrasonic-assisted digestion for sediments and soil as well as seasonal variations effect. The Environmental contamination was then assessed from the concentrations obtained in soil as well as the human health risk based on the carcinogenic and non-carcinogenic risk.

1.2. Rationale of the project

Metal contamination, specifically, heavy metals are a main concern in the environment due to their toxicity and possible carcinogenic effects. Heavy metal contamination affects the human health and the environment. Their wide applications provide a medium for contamination in the environment through natural and anthropogenic processes. Metals can enter water sources and soil through various pathways such as rock erosion followed by runoff; earth’s crust degradation and weathering, sewage discharge, industrial activities (effluents and mining), as well as urban and agricultural runoffs. Heavy metals may accumulate on surface water and soil since they are strongly retained and can pose a potential health risk in humans, animals, and plants. Heavy metals can be toxic even at trace amounts in water sources and soil, however, based on their toxicity, accumulation in the human body can pose serious health risks. Most heavy metals are potentially carcinogenic and can effectively target various organs in the body leading to chronic disorders or temporary illnesses. Therefore, it is necessary to monitor their concentrations regularly. Previous studies have indicated that contaminated soil and crops have been above the regulatory standard therefore the need for continuous monitoring is required using accessible and easy methods for routine analysis of heavy metals. The commonly used digestion method for metals in environmental samples, is microwave assisted digestion. Despite the long list of advantages of this digestion method, there are drawbacks. The microwave digester is an expensive instrument that is not readily available in all laboratories, therefore alternative methods are required, that can ultimately allow for accurate quantification of metals.

1.3. Aim and objectives

To assess the metal concentrations in the KwaZulu-Natal Province in water, soil, and sediments, and to determine anions in water. Also, to assess metals toxicity in the environment and health risk in humans.

The objectives were:

- To calibrate the ICP-OES instrument for accurate determination of metal concentrations.
- To modify microwave, hotplate, and ultrasonic assisted digestion methods for effective digestion of metals from water, soil, and sediment samples prior to detection with ICP-OES.
- To validate the analytical methods to ensure high accuracy and precision of the measurements.
- To quantitatively assess if the metal concentrations in all samples are within the maximum concentrations present as per the stipulated guidelines in water, soil and sediments.
- To conduct environmental toxicity studies and human health risk evaluation that may be posed by the concentrations observed.

1.4. Research questions

- Are the metals present in the areas under study and what are their concentrations?
- What are the implications of the metal concentrations in the studied water, soil, and sediment samples?
- Are the metals in water associated to its presence in soil and sediment samples?
- What are the possible sources of the metals in water, soil, and sediments?
- Are the metal concentrations in influent wastewater lower than the effluent and their associated river water where the effluent is discharged?
- What are the possible health risks connected with heavy metal contamination?
- Which digestion method can be used as an inexpensive and easily accessible alternative to microwave-assisted digestion?

1.5. Research justification

Metal concentrations can inevitably increase in the environment, in the future, concurrently with an increase in urbanization, industrialization and other anthropogenic sources. This rapid growth has attracted significant interest from researchers and scientists. Metals, even in trace amounts can bioaccumulate and persist in the environment. Metals lack the ability to biodegrade in the environment thus substantiating their toxicity. The need for continuous monitoring in the environment for metal contamination is required. Acquiring accurate and reliable concentrations of metals, more specifically heavy metals in the surrounding environment is crucial in the implementation of the environmental protection acts. In addition, efficient analytical instrumentation for the assessment of the concentrations of metal in environmental samples are required with preferably low detection limits and minimal interferences, these include the ICP-OES, and ICP-MS. Sample digestion is crucial in releasing the metals in solution therefore the inexpensive, readily available, and efficient methods are required. Microwave-assisted digestion is a widely used and reliable method however, it is an expensive method for routine analysis. Alternative digestion methods include open heat (hotplate) and ultrasonic assisted, they are readily available and inexpensive when compared to microwave assisted digestion. In South Africa, there is insufficient research on trace metal concentrations in environmental samples, especially in the KwaZulu-Natal Province. There are few studies conducted on the comparison of digestion methods for metals determination, at the selected sampling sites, which indicates a degree of novelty in this work.

1.6. References

Atlas A, Usman K, Ur Rehman H, Khudadad S, Munawar A, Rab A, Khan MI, Gul M, Pervaiz M (2017). Analysis of heavy metals in river Kabul at Sardaryab Khyber Pakhtunkhwa, Pakistan. J Entomol Zool Stud 5: 14-16.

Altundag H, Agar S, Altıntıg E, Ates A, Sivrikaya S (2019). Use of ion chromatography method on the determination of some anions in the water collected from Sakarya, Turkey. J Chem Metrol 13:14-20. https://doi.org/10.25135/jcm.26.19.03.1221

Bassioni G, Ashraf K, Elhameed A (2015). Risk assessment using ICP-MS of heavy metals in groundwater in Upper Egypt. J Nat Resour Dev 65-70. https://doi.org/10.5027/jnrd.v5i0.09

Choi J and Jeon J (2018) A Geo-statistical Assessment of heavy metal pollution in the soil around a ship building yard in Busan, Korea. J Korean Soc 27:907-915. https://doi.org/10.7837/kosomes.2018.24.7.907

Gopal K, Tripathy S, Bersillon J, Dubey S (2007). Chlorination byproducts, their toxicodynamics and removal from drinking water. J Hazard Mater 140:1-6. https://doi.org/10.1016/j.jhazmat.2006.10.063

He, Z., 1 Shentu, J., Yang, X., Balighar, V.C., Zhang, T.4 & Stoffela, P.J (2015). Heavy Metal Contamination of Soils: Sources, Indicators, and Assessment, *Journal of Environmental Indicators,* 9:17-18, www.environmentalindicators.net.

Jaishankar M, Tseten T, Anbalagan N, Mathew B, Beeregowda K (2014). Toxicity, mechanism, and health effects of some heavy metals. Interdiscip Toxicol 7:60-72. https://doi.org/10.2478/intox-2014-0009

Jiao, F., & Gao, H. (2013). On-site solid-phase extraction and application to in situ preconcentration of heavy metals in surface water, *Environ Monit Assess*, 185, pp. 39-44.

Lehtonen J, Hassinen J, Honkanen R, Kumar A, Viskari H, Kettunen A *et al*. (2019). Effects of concentration on the water disinfection performance of silver containing nanocellulose-based composites. Sci Rep 9.https://doi.org/10.1038/s41598-019-56009-6

Lomonte C, Gregory D, Baker A, Kolev S (2008). Comparative study of hotplate wet digestion methods for the determination of mercury in biosolids. Chemosphere 72:1420-1424. https://doi.org/10.1016/j.chemosphere.2008.05.033

Masinda, V., & K. L. Muedi, K.L. (2018). Environmental Contamination by Heavy metals, IntechOpen, Chapter 7, pp. 116-133.

Reeve, R. (2001). *Environmental analysis*. New York: Wiley.

Uddin A, Khalid R, Alaama M, Abdualkader A, Kasmuri A, Abbas S (2016). Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. J Anal Sci Technol 7:6. https://doi.org/10.1186/s40543-016-0085-6

World Health Organization (1984). Guidelines for Drinking Water Quality, Volume I — Recommendations. Geneva, 1982. Acta Hydrochim Hydrobiol 12: 221-223. https://doi.org/10.1002/aheh.19840120217

Chapter Two

Introduction

In this chapter, observations made by researchers worldwide are discussed. The various metal sources, exposure pathways and their potential environmental and health effects are included.

2. Literature Survey

2.1. What are metals?

Metals can be defined as substances of high electrical conductivity, malleability, and lustre. They can willingly lose electrons and become cations (positively charged species). Heavy metals are metals and metalloids that have a density larger than 5g/cm^3 and considered to be toxic and dangerous to the environment that includes humans and aquatic organisms (Jaishankar *et al.*, 2014). There are 23 heavy metals known although some are more toxic than others. The commonly found heavy metals in water and soil include chromium, arsenic, lead, nickel, cadmium, copper, and zinc, all of which have the ability to pose environmental and human health risks (Jaishankar *et al.*, 2014).

2.2. Heavy metal sources and effects of their toxicity

Heavy metals may affect the quality of air, water, and soil as well as the human, plants, and animals' health in the environment. Their environmental sources are natural and anthropogenic. Natural processes include soil erosion followed by runoff, degradation, and weathering of the earth's crust. Anthropogenic activities have proven to be the main source of pollution, for example, agricultural activity where pesticides and fertilizers are employed, urban and agricultural runoff, disposal of industrial effluents and sewage wastes (Atlas *et al.*, 2017). Heavy metals are introduced into water sources through various pathways and can pose a health risk in humans (Jiao & Gao, 2013). Figure 2.1 indicates the potential sinks and sources of heavy metals.

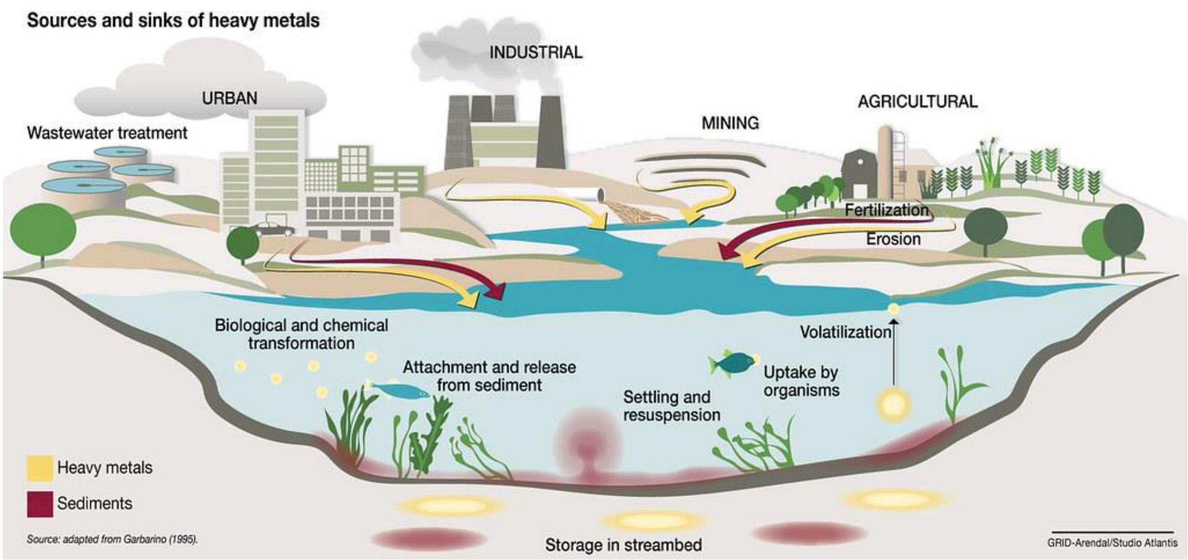


Figure 2.1: The potential sources of heavy metals (Garbarino,1995)

The metals mainly from urban, industrial, mining and agricultural activities enter into water sources generally in the insoluble form (Reeve, 2002). Processes such as solubilisation, volatilization and uptake by organisms promote the transfer of metals in and around the environment including in humans. Solubilization is a process whereby a substance dissolves in an aqueous medium. The pH of the water can strongly be associated with the solubility of metals and in high pH waters, metals become more soluble (Reeve, 2002). Solubilization is assisted by the formation of complexes with organic matter. Acids originating from acid rain or humic and fulvic which are produced as a result of organic decay, can also aid in solubilizing metals in water. Volatilization is a process where the dissolved metals get vaporized and enters the atmosphere where they can be inhaled along with oxygen. Resuspension allows for the metals to be trapped in sediment where it can be consumed by aquatic organism or ultimately penetrate into groundwater sources. The uptake of metal ions by organisms is via the food chain where sediments containing metals are consumed by smaller organisms (phytoplankton and filter feeders). The metals accumulate in their tissues and ultimately in humans due to consumption (Garbarino,1995; Reeve, 2002).

Soil is the main biosphere component and also considered major sinks for metals introduced into the environment. The accumulation of metals in soil, more specifically, in agricultural soil, is possibly from the application of chemical fertilizers, live-stock manure, inorganic pesticides, untreated or partially treated wastewater and sludge (Salem *et al*., 2020). Wastewater treatment plants (WWTPs) receive municipal, agricultural and industrial wastewater daily which potentially contains traces of heavy metals as shown in Figure 2.2. Due to the WWTPs continuously receiving loads of wastewater, this makes WWTPs one of the major sources of pollution by heavy metals. Furthermore, this indicates the potential for continued transfer of heavy metals to agricultural soils as effluent water is used for irrigation purpose, thus metals may be transferred to crops and eventually be consumed by human beings. Also, the release of treated wastewater effluent into the receiving rivers may continuously introduce them to the rivers.

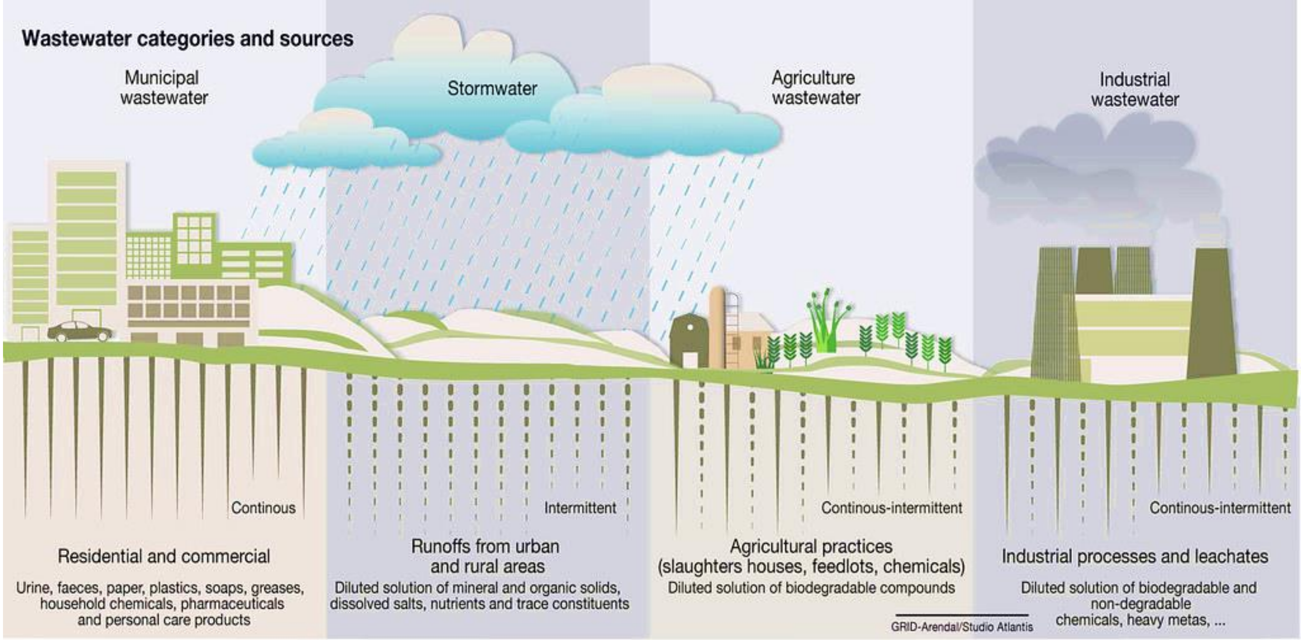


Figure 2.2: Sources of contamination in different wastewater categories (Garbarino, 1995))

Heavy metals enter the environment and water bodies naturally and unnaturally. Each heavy metal is toxic to the environment and its effects on the human body can be detrimental. Heavy metal toxicity can have acute or chronic effects. Figure 2.3 summarizes the heavy metals effects on human health and their target organs.

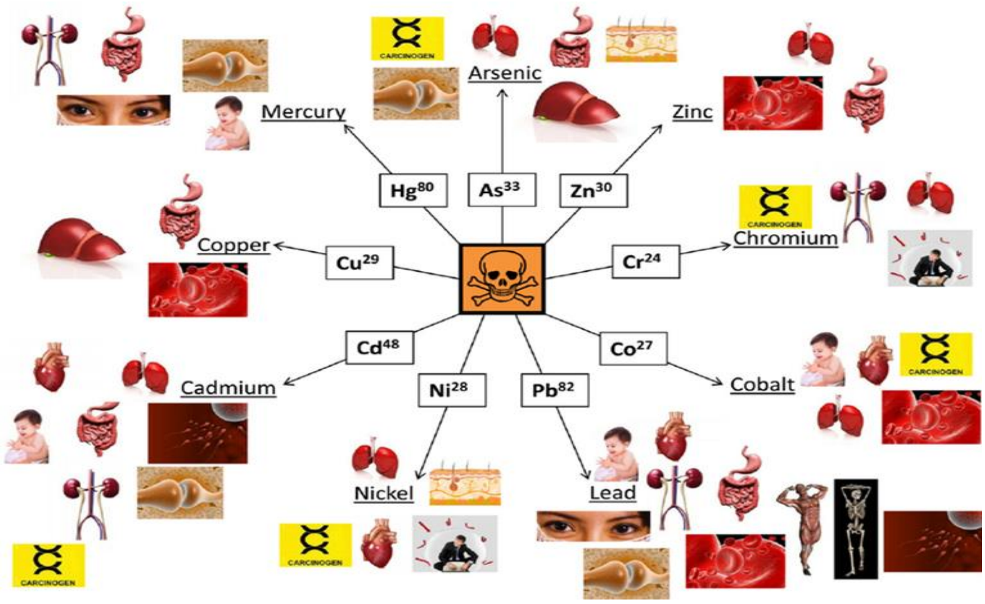


Figure 2.3: Heavy metal target organs in the human body (de Namor *et al*., 2012)

Lead (Pb) is a highly toxic heavy metal, and the environmental contamination is just one of the many concerns associated with it. Some sources of lead are largely due to industrial processes, food, and smoking, drinking lead contaminated waters and combustion of petroleum-based compounds. Lead contamination can create problems for the physiological processes in plants. Plants present in an environment with high lead concentrations can prove to be detrimental since it increases the generation of oxygen species that are reactive resulting in lipid membrane damage which ultimately affects photosynthetic and chlorophyll processes. It also inhibits the entire plant growth. Lead poisoning in humans is extremely common and it is largely associated with the central nervous system and gastrointestinal tract. Persistent exposure to lead can result in mental retardation, muscular weakness, and brain and kidney damage (Jaishankar *et al*., 2014).

Chromium (Cr) naturally occurs in rocks, animals, plants and soil. It is essentially present in water sediments and water sources. Chromium is used in protective metal coating; wood preservatives, metal alloys and thus can make its way into the water sources. Chromium can exist in different oxidation states, for instance, divalent, trivalent, tetravalent, and hexavalent. The most common oxidation states for chromium, is chromium (III) and chromium (VI). Chromium (VI) compounds can travel into the body much faster than chromium (III) and is reported to be highly toxic and carcinogenic. Chromium has no known physiological effect, but it is a carcinogen. Chromium is beneficial to humans, however, hexavalent chromium (Cr (VI)) can cause skin irritation and persistent exposure could lead to dermatitis and skin ulceration. Chromium compounds can cause DNA related problems such as chromosomal aberrations and can affect the replication and transcription processes (Jaishankar *et al*., 2014).

Cadmium (Cd) occurs naturally in soils, rocks, coal, and mineral fertilizers however, it is also a by-product in zinc production. Cadmium compounds are group I carcinogens as per the International Agency for Research on Cancer. Cadmium is present in the environment due to natural processes including weathering of rocks, volcanic eruptions, streamflow and also through human activities like mining, smelting, burning of municipal wastes and in the production of fertilizers. Cadmium can be extremely toxic to the kidneys and can cause bone mineralization as a result of bone damage or renal dysfunction. Consumption of large amounts of cadmium can result in stomach irritation, for example, experience symptoms such as vomiting and diarrhoea. It can cause acute disorders such as hypertension, liver damage, and bone degeneration. Consumption of lead and cadmium contaminated waters have proven to cause renal failure in humans. Lead poisoning can lead to permanent harm on the central nervous system, brain, and kidneys (Jaishankar *et al*., 2014).

Copper (Cu) and Zinc (Zn) are important metals for all living organisms however, the consumption of water containing their high concentrations can pose a threat to human beings, where it can result in nausea, abdominal pains, vomiting, diarrhoea etc, which is common especially in children. These heavy metals can also cause changes to DNA which can result in cancer. When copper enters the human body, in excessive limits, it can cause gastrointestinal problems as well as other disorders such as headaches, liver damage and emphysema (Atlas *et al*., 2017; Jaishankar *et al*., 2014). Nickel pollution in water is seen to cause hair loss in humans who consume it (Jaishankar *et al*., 2014).

Heavy metal toxicity in humans is dependent on the type/nature and the quantity ingested into the body. Despite assisting plants and animals to perform biological processes, their coordination ability and cationic form, allows them to complex with proteins in the body. The resulting complexes can be harmful as they contain amine ($-NH_2$), thiol (-SH) and carboxylic (-COOH) functional groups. The coordination between these groups and the cationic heavy metals tend to change the nature and structure of the protein thus destroying the molecule resulting in the inability to perform its designated function. This also destroys the catalytic ability of enzymes resulting in the production of radicals which can oxidize biological macromolecules in the body (Yayintas *et al*., 2007).

Maximum permissible limits are specified to govern the pollution in a certain area and these limits can be defined for a specific country or globally. Metals can potentially be toxic if they are present at a concentration above maximum allowable limits (Table 2.1) water and soil matrices (WHO, 2001, 2008) as well as guidelines established by the South African Nations Standards (SANS).

Table 2.1: Concentrations of metals in water and soil as per World Health Organization (WHO) permissible limits (WHO, 2001, 2008).

Metal analyte	**MRL values in water sources (µg/L), (WHO, 2008)**	**MRL values in soil (mg/kg), (WHO, 2001)**	**SANS 241 guidelines (SANS, 2015) (µg/L)**
Cadmium (Cd)	5.00	0.80	3
Cobalt (Co)	100	40	500
Chromium (Cr)	100	100	50
Copper (Cu)	2000	36	2000
Gallium (Ga)	-	-	-
Lithium (Li)	-	-	-
Manganese (Mn)	400	-	400
Nickel (Ni)	70	35	70
Lead (Pb)	50	85	10
Strontium (Sr)	4000	-	-
Thallium (Tl)	2.00	-	-
Zinc (Zn)	5000	50	5

2.3. Exposure pathways of humans to heavy metals

Humans are exposed to trace amounts of heavy metals by means of contaminated air, water, and food. Exposure pathways in human for heavy metal contamination include inhalation through the mouth and nose, ingestion via the mouth and skin absorption as a result of skin contact. Heavy metals can reach water sources through natural processes such as precipitation and runoff. Heavy rains and water flow can transport these heavy metals into freshwater sources. Aquatic flora and fauna are exposed to these heavy metals which enter and accumulate in their tissue. When these organisms are consumed by predators, the heavy metals are transferred to animals and ultimately consumed by humans (Sankhla *et al*., 2016). This is an example of bioaccumulation of heavy metals in the ecosystem. Bioaccumulation simply describes how contaminants enter the food cycle due to an increase of the contaminant in the biological system (Reeve, 2002; Sankhla *et al*., 2016).

Plants take up heavy metals through the process of adsorption and binding. Trace amounts of heavy metals may be toxic to plants however lead, cadmium and mercury can be toxic. Plants are considered to be good environmental quality indicators, for example, if these metals are present in excess in different matrices such as water, soil/sediment, and air, it will respond directly (Sankhla *et al*., 2016).

Aquatic organisms are to some extent, a food source for predators. Studies have shown that aquatic organisms such as plankton and fish, contribute to the transfer of heavy metals to other and food cycle higher trophic levels though ingestion. This ultimately results in heavy metal contamination in humans which lead to health risks. Ingestion may be the most common pathway however skin contact and inhalation can be as dangerous. Inhalation of heavy metals can be harmful; a study conducted on heavy metal assessment on heavy and radioactive metals in dust indicated that heavy metals are transported in dust particles and when inhaled, it can be exposed to humans. Everyday products such as pharmaceutical drugs, batteries, personal care products etc can also contain heavy metals and skin contact is inevitable. Heavy metals are transferred into the body through dermal exposure and absorption thus making skin contact another pathway for human exposure to heavy metals (Jaishankar *et al*., 2014; Reeve, 2002; Sankhla *et al*., 2016).

These toxic heavy metals have different exposure pathways that are more probable than others. Lead sources include factory chimneys, smoking and emission from vehicle exhausts. These sources allow lead to enter the human body through inhalation. Lead can also be absorbed by the skin since it is present in interior paints, plumbing pipes, storage batteries etc. Lead is also taken up by plants, fixed in soils and flow in water sources, therefore exposure is generally by water and food ingestion (Mohod & Dhote, 2013; Ebenebe *et al*., 2017). Water sources are the main contamination contributor for arsenic. These water sources can be contaminated with arsenic through pesticides, natural minerals, and inappropriate arsenic deposits. Arsenic's exposure to humans is mainly through ingestion of water and animals that have bio-accumulated arsenic. Cadmium is primarily exposed to human via inhalation and ingestion. Tobacco smoke exposure leads to humans though inhalation (Jaishankar *et al*., 2014; Mohod &Dhote, 2013; Sankhla *et al*., 2016). Cadmium is present in soils and sediments; plants consume and absorb them slowly. Cadmium accumulates and ultimately is consumed by humans. The least prevalent pathway is skin contact however cadmium exists in rechargeable batteries, special alloys and as impurity in detergents (Sankhla *et al*., 2016).

Mercury is largely found in water sources and is absorbed by microorganisms to form methyl mercury which is exposed to humans through consumption. Mercury is also used in instruments such as thermometers, pyrometers, barometers etc, when in contact; mercury can be absorbed into the skin. Mercury vapour can be inhaled by humans thus causing asthma, bronchitis, and other respiratory problems (Sankhla *et al*., 2016). The two most common pathways for chromium contamination in humans are through ingestion and skin contact. Chromium (VI) is highly soluble in water and therefore toxic to all organisms who consume these waters. Chromium is also used in paints, metallurgy and wood preservatives and can therefore be absorbed by the skin through skin contact. The main source of metal contamination for all organisms is water therefore ingestion is the most common pathway for metal contamination in humans and other living organisms (Mohod & Dhote, 2013; Sankhla *et al*., 2016).

2.4. Occurrence of metals in South Africa

South Africa is a semi-arid region in which water shortage is a major problem. Heavy metal contamination in water can threaten South Africa's water sources hence it is vital to comprehensively study and monitor water sources for pollution. Numerous studies on heavy metal contamination in rivers and other sources were conducted in the South Africa, mainly in the Limpopo and Gauteng province however, other provinces require heavy metal studies to assess and monitor water resources in the country.

A study conducted by Addo-Bediako *et al*, assessed the concentrations of heavy metal in water and sediments from five sites along the Steelpoort River system in the Limpopo Province. The water samples were acid treated and thereafter analysed using ICP-OES. The concentrations for the heavy metals were 20 - 60 μg/L for chromium, 10 - 12 μg/L for copper, 270 - 660 μg/L for manganese, 10 - 20 μg/L for nickel and 20 - 70 μg/L for zinc. Cadmium was not detected which could mean that it was not present in water, or it was lower than the limits of detection. Some of the concentrations were within the allowable limit range as per Department of Water Affairs and Forestry (DWAF, 1996) whilst Cr, Cu and Zn exceeded the guidelines (Addo-Bediako *et al.*, 2018).

Olujimi and co-workers assessed the variability of heavy metals in river water that receives effluents from industrial and domestic activity in the Western Cape. The analysis of the water samples was conducted using ICP-MS. The concentrations obtained were 0.56 – 23.78 μg/L for arsenic and 0.09 – 14.78 μg/L for cadmium and these concentrations fell in the range that is safe for human consumption. However, they were above the maximum concentration that is safe for aquatic life protection. Lead was found to range from 4.18 – 86.73 μg/L and mercury was 0.1 – 8.09 μg/L, which is larger than both the human consumption and aquatic life protection limits. It was observed that upstream and downstream were more contaminated than the discharge point of the river systems (Olujimi *et al.*, 2015).

Apart from surface water resources, groundwater is also an important source of water. Groundwater in Muledane Village in the Limpopo Province was analysed for heavy metal contamination to evaluate water quality and risk assessment for humans (Edokpayi *et al*, 2018). The analytical technique used for heavy metal determination was ICP-OES. It was concluded that 87.5% of the borehole water samples contained high concentrations of heavy metals (Cr, Fe, Mn, Cu, and Pb). The concentrations observed for each of these metals ranged from (5 - 150 μg/L) for Cr, (150 - 1860 μg/L) for Fe, (10 – 1220 μg/L) for Mn, (10 – 410 μg/L) for Cu and (2 - 26 μg/L) for Pb. These metals were observed to exceed the WHO and DWAF limits for water quality and consumption (Edokpayi *et al.*, 2018).

Heavy metal contamination is not specific to water; other matrices may also be prone to contamination. Soils are susceptible to heavy metal contamination largely through mining and mineral extraction. South Africa is known for its abundance in natural resources however, the extraction of these minerals is proven to be disastrous as it affects plant life negatively. A study by Gzik *et al*, involved heavy metal contamination in mining areas surrounding Pretoria and Johannesburg in the Gauteng Province. Soil samples were collected form sites near Rustenburg at different depths. Extraction of heavy metals from the soil samples was accomplished by using Aqua Regia. Thereafter the samples were treated fractionally with distilled water, sodium acetate and EDTA. The AAS and ICP-OES were used to analyse the sample extracts. It was found that the cadmium concentration (0.7 mg/kg) was the same at all sites. All soil samples had high concentrations of chromium (357-740 mg/kg) and nickel (146-593 mg/kg). Comparisons with the permissible limits showed that heavy metals are dangerous to soils, plant and animal life that are exposed to it (Gzik *et al*., 2003).

Heavy metal contamination is common in urban areas however, not much research has been conducted in small towns and rural areas where people are dependent on small scale farming as a source of income and food. A study conducted by Bvenura, and Afolayan in Eastern Cape focussed on heavy metal contamination of vegetables (cabbage, carrot, onion, spinach, and tomato) which were sampled randomly from small home gardens in three residential areas. The samples were washed, dried, and digested using sulfuric and salicylic acid. Thereafter, the samples were analysed using ICP-OES. The heavy metal concentrations in the vegetables were 0.01 - 1.12 mg/kg for cadmium, 0.92 - 9.29 mg/kg for copper, 0.04 - 373.38 mg/kg for manganese and 4.27-89.88 mg/kg for zinc. In all samples, lead was undetectable. It was concluded that heavy metal contamination in these vegetables do not pose a risk in human health (Bvenura & Afolayan., 2012).

As per the studies mentioned above, it can be seen that various matrices can be potential sources of heavy metal contamination; however, some can be less dangerous to the human health than others (Gzik *et al*., 2003; Bvenura & Afolayan., 2012).

2.5. Determination of heavy metals

Trace heavy metals can be analysed using inductively coupled plasma (ICP) and atomic absorption spectroscopy (AAS). The ICP could be coupled with optical emission spectroscopy (OES) or mass spectrometry (MS).

The AAS involves an external source of radiation to excite the gaseous atoms into their high energy states. Two common examples of this type of spectroscopy, is Flame Atomic Absorption Spectroscopy (FAAS) and Electrothermal Vaporization/Graphite furnace Atomic Absorption Spectroscopy (GFAAS). In FAAS, atomization (decomposition of analyte molecules into their gaseous atomic form) occurs as a result of the flame. It is a widely used technique however, chemical interferences can be an issue during the analysis. Examples of chemical interferences includes the formation of stable radicals in cold areas of the flame, formation of stable analyte compounds and ionization which results in loss of analyte.

However, there are methods that are used to overcome these interferences and thus allow accurate quantification. (Dean, 1997; Skoog *et al*., 1988).

Inductively coupled plasma optical emission spectroscopy (ICP-OES) can simultaneously quantify 60 or more elements. This technique involves the monitoring of emission spectrum of each element at different wavelengths where each element has its own characteristic wavelength at which they emit light. This technique can determine and quantify halogens, non-metals, metalloids, and metals (Skoog *et al*., 1988).

2.5.1. ICP Plasma origin and production

The ICP-OES contains induced plasma that plays an important role in atomization. Plasma is a superconductive gaseous mixture of cations and electrons which cannot be categorized as a gas or liquid. The changing magnetic field present in the ICP torch is responsible for the plasma production and sustenance. The coils surrounding the torch is made of copper and functions as a solenoid. The blue lines in Figure 2.4 are the magnetic field lines accompanied by arrows indicating the flow direction. The magnetic field lines are caused to change direction by the induced alternating current resulting to the production of another current (Nave, 2008).

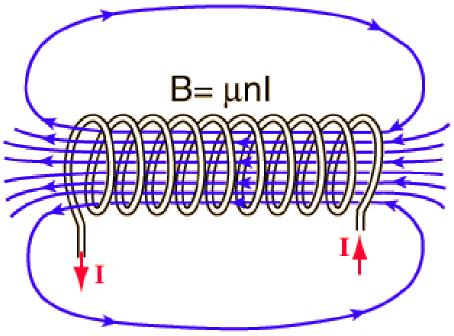


Figure 2.4: Diagram of a solenoid (Nave, 2008)

2.5.2. Plasma sustenance and sample introduction

Plasma is formed when an electrical pulse is released into a stream of flowing Argon gas thus forming Ar^+ ions and electrons. In order to sustain the plasma atomizer, argon atoms spin, collide and get ionized producing high-density stream of electrons. Thereafter the Argon ions and electrons are forced to move in a circular motion along the axis of the plasma and resistance to this circular motion results in the heating of the plasma to temperatures ranging from 8000-10000K. Since the plasma reaches high temperatures of 8000-10 000K, this essentially rules out chemical interferences. Spectral interferences arise from the different elements as there are more emission lines as compared to absorption lines. These interferences can be minimized by adjusting the position of the detector, in particular the axial viewing geometry. This involves the plasma torch being placed in a horizontal position where the detector is placed collinearly to the axis of the plasma torch (Dean, 1997; Skoog *et al*., 1988).

Once the plasma is initiated, it sustains itself and the 'extra' thermal energy is used to decompose, ionize, and atomize the sample analyte (Skoog *et al*., 1988). The plasma torch consists of three circular quartz tubes that contribute a total argon stream flow rate ranging between 11 – 17$L.min^{-1}$ (Figure 2.5). This flow rate, especially of argon gas is the main reason for this technique being expensive. Argon is a noble gas thus creating an inert environment for atomization of the analyte sample leading to low detection limits (ppb levels) and a wide linear dynamic range for quantification of the sample.

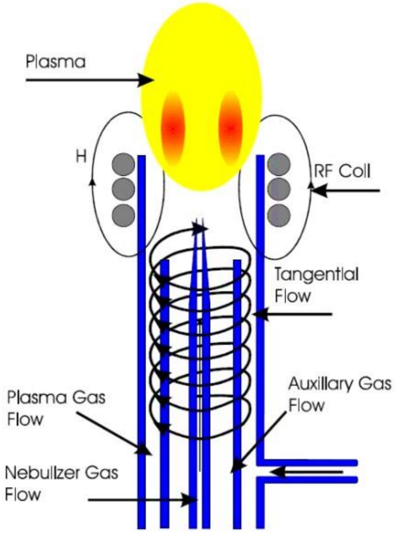


Figure 2.5: Schematic of an ICP plasma torch (Manning & Grow, 1997)

Sample introduction is the starting step in ICP analysis, and it involves the use of a peristaltic pump, sample capillary tube and most importantly, a nebuliser (Figure 2.6). The function of the nebuliser is to convert the liquid/aqueous sample into an aerosol (fine mist of liquid micro-droplets) with the use of a carrier gas (typically argon) and this process is called nebulisation. The most commonly used nebuliser is the pneumatic concentric nebuliser however there are others which include crossflow, high-solids, and ultrasonic nebulisers. The central capillary tube transports the argon gas together with the aerosol sample into the plasma torch. At this point, the sample atomization occurs in the presence of the heated plasma. The analytes/contaminants present in the aerosol decompose into its gaseous elemental form. At specific wavelengths, emission lines are produced by the radio-frequency ICP for each metal ion present in the aerosol sample using a polychromator. These emission lines are converted into comprehendible signals by processing the photocurrents by the photosensitive device and this is known as detection (Dean, 1997; Skoog *et al*., 1988). The schematic diagram of ICP-OES is shown in Figure 2.6

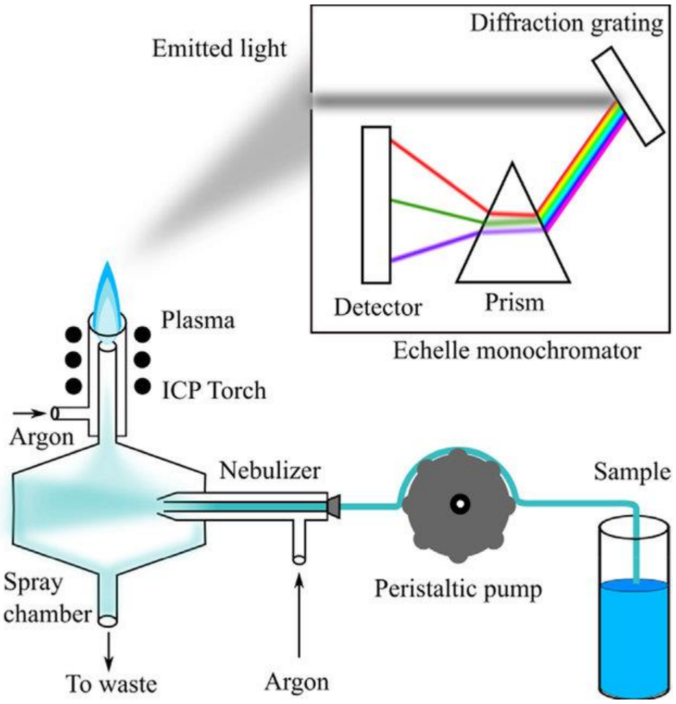


Figure 2.6: Schematic of operation of an ICP (Cherevko & Mayrhofer, 2017)

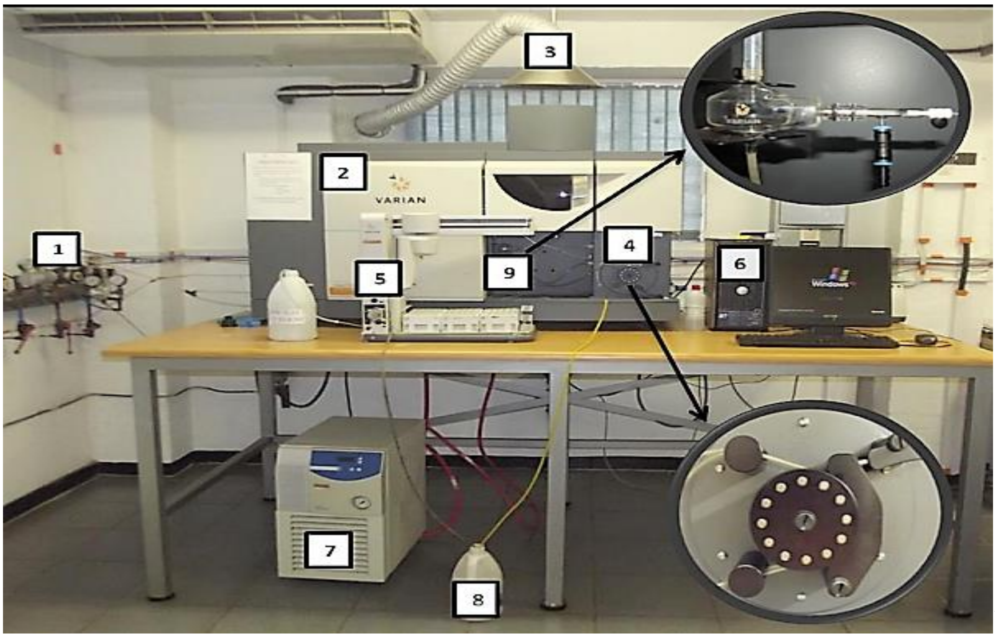


Figure 2.7: Varian 720-ES ICP-OES instrument used for analysis (Ngubane, 2016)

1 – Argon gas tap, **2** – ICP-OES instrument, **3** – Extractor fan, **4** – Peristaltic pump, **5** – Auto sampler, **6** – Computer, **7** – Cooling tower, **8** – Waste container, **9** – Nebulizer and spray chamber

ICP-MS is also another useful technique in the analysis of heavy metals in aqueous samples. ICP-MS uses inductively coupled plasma with mass spectrometry to identify and determine concentration of metals. The metals present in a sample are detected and determined sequentially and it is advantageous to trace metals as it can quantify low concentrations in a sample. The mass spectrometer is able to measure isotopic ratios of elements and the two important advantages of this, is that stable isotopes with changed isotopic ratio can be used for trace analysis and studies and stable isotopes can also allow for calibration of unknown samples without the need to prepare solutions for direct calibration and standard addition (Dean, 1997; Skoog *et al*., 1988).

2.6. Analysis of heavy metals in water

The presence of heavy metals in water sources can affect the wellbeing of all living organisms and while humans are unable to process and dispose of these heavy metals in their body, it is therefore essential to quantify and monitor the concentrations in water sources. Heavy metal contamination exists in trace amounts and can accumulate in water bodies and ultimately pose a serious risk in living organisms and the environment. Hence, it is crucial to follow appropriate processes from sampling to sample analysis and also, to develop and apply accurate method for their qualitative and quantitative analysis in environmental samples in order to obtain reliable results (Jaishankar *et al,* 2014). Figure 2.8 displays the representation of the important processes from sampling to analysis process (Mitra, 2003).

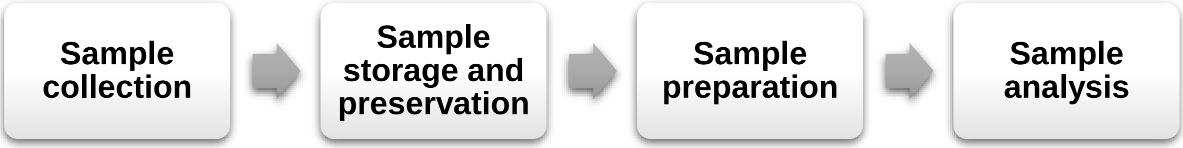


Figure 2.8: Simplified representation of the sampling to analysis process (Mitra, 2003)

The initial step is sample collection which requires a suitable sampling method to be chosen to ensure the collection of a representative sample. The next step involves sample storage and preservation which is done to ensure that the sample reaches the laboratory in its natural state. The use of appropriate container types is essential for preservation of the analytes in the samples. For the analysis of heavy metals in water, the samples should be collected in plastic bottle since metals tend to adsorb on glass thus making glass containers inappropriate. All storage vessels and containers should be thoroughly cleaned to prevent contamination. Sufficient water samples should be collected to allow for replication of analysis and to account for any loss of sample during transportation. Sample pre-treatment is considered the most important as it allows for the homogeneity of the sample. The removal of any solids present in the water samples through filtration is one way of homogenizing the sample. An acid should be added to the sample during storage to ensure that the metals remain in solution (Skoog *et al.*, 1988).

In sample analysis and preparation step, it becomes difficult to detect heavy metals in water samples since they are present in trace amounts/low concentrations, therefore the sample requires sample digestion prior to analysis (Mitra, 2003).

2.7. Sample digestion

This is a key step in the process of the sample analysis (Chao & Sanzobine, 1992). In general, if a sample is in the solid, then its dissolution is required (Hu, 2014). The primary role of the digestion of the sample is to transfer it to a phase appropriate for analytes to be analysed. The complexity of different sample matrices requires the appropriate sample digestion methods which will be compatible with the type of analysis conducted (Chao & Sanzobine, 1992). There are many factors that are required for the choice of digestion methods. These factors include the physical and chemical composition/properties of the studied analytes, sample size, the accuracy and precision required, the accessibility of laboratory apparatus required, economic aspects including reagent and labour consumption and lastly environmental safety (Hu, 2014). The use of strong mineral acids such as HCl, HNO_3, HF, H_2SO_4, H_3PO_4 and $HClO_4$ is common for the dissolution of the sample and the decomposition of different geologic samples, such as rocks, mineral ores etc, has been achieved through the use of proper acids combinations. Nitric acid (HNO_3) is one of the commonly employed as digestion acid and primary oxidant which is strong enough to solubilize metals from water and soils with an organic carbon content (Sastre *et al.*, 2002). The HNO_3 matrices are considered the best acid medium for ICP analysis. In addition, it does not hamper with many analysis and is commercially accessible with high purity. Along with the digesting acid, the use of digestion methods is crucial in the sample preparation process. The classical methods of microwave and wet acid digestion is widely used in the digestion of metals in environmental matrices (Sastre *et al.*, 2002). However, more improved methods such as ultrasonic digestion have shown promising results as convenient methods of digestion (Kazi *et al.*, 2008).

2.7.1. Microwave-assisted digestion

Microwave heating was commonly used for fast wet acid digestion of several biological samples (Abu-Samra *et al*., 1975). The discovery of microwave heating has allowed for the advancement of microwave technology used in the sample preparation such as extraction and digestion methods for different types of samples. The use of microwaves has expanded its horizon and application to synthetic chemistry, inorganic reactions, catalyst preparations etc (Chen *et al*., 2008). Microwave digestion originates from electromagnetic radiation with a commonly used frequency of 2450 MHz in order to produce heat (Hu *et al*., 2014). Microwave as a heat source is much more efficient when compared to other heating sources. The principle of microwave digestion is based on the irradiation of polar molecules and ions by microwave energy, in which they are energized through mechanisms such as ion conductance and dipole rotation (Neas & Collins, 1988; Gilman & Engelhart, 1989). The medium of digestion and sample molecules absorb the radiation which allows for the enhancement of the digestion process and the decomposition accomplishment of the desired sample. The dissolution rate can be increased due to internal specific sample particles heating (Nadkarni, 1984). Microwaves tend to only heat the liquid phase during digestion since the gaseous phase does not absorb microwave. Therefore, high temperatures are achievable with fairly low pressures in closed vessel microwave digestion. This is one of the main advantages of microwave heating, in addition, minimal contamination of sample, retain volatile analytes, rapidly heating and cooling in comparison to other conventional methods of digestion (Hu *et al*., 2014). Figure 2.9 shows a typical microwave digestor with the rotor and vessels.

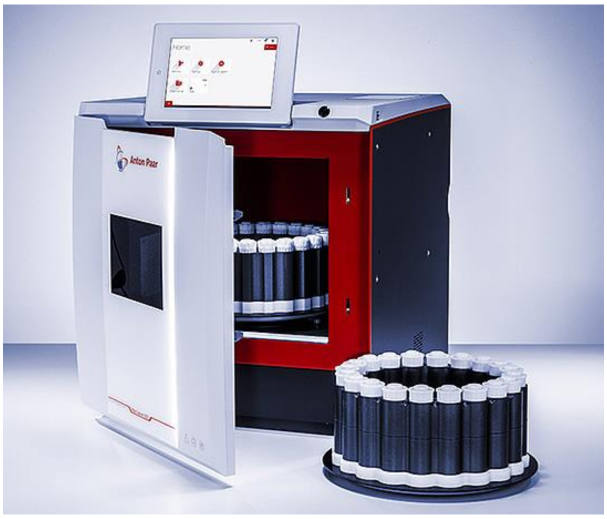


Figure 2.9: A typical microwave digester (Anton Paar, 2022)

2.7.2. Open heat/hotplate assisted digestion

Open heat acid digestion has been a conventional and common method used for organic and inorganic sample digestion in small scale laboratories. This method occurs in an acid medium using open containers/vessels with low pressure, which is generally placed on a hotplate (Figure 2.10). This type of digestion is highly recommended for routine analysis as a result of its variable parameter that can be changed with ease, such as time, temperature and, reagents addition (Hu *et al*., 2014). The maximum temperature obtained can be restricted by the heating medium or by the ambient boiling point of the digesting acid/acid mixture. Since this type of digestion is an open vessel, there is a possibility of contamination from the surroundings, large amounts of acid are generally used and there could be loss of volatile analytes. Due to these shortcomings, open vessel digestion is a more primitive method compared to the microwave, however, it is a readily accessible and inexpensive method for daily analysis. This digestion method is well-established and compatible for samples without refractory minerals. (Hu *et al*., 2014).

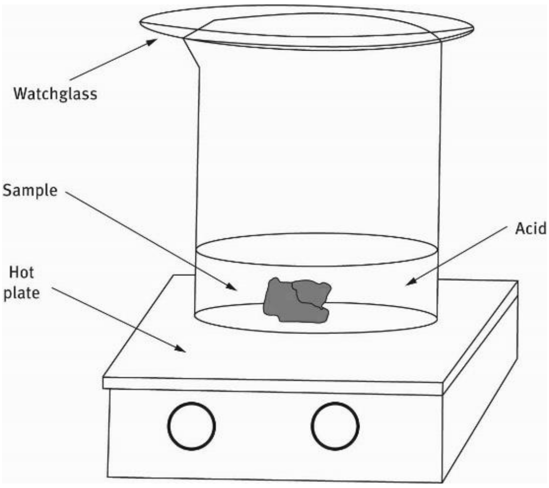


Figure 2.10: Typical illustration of open heat/hotplate assisted digestion (Matusiewicz, 2017)

2.7.3. Ultrasonic-assisted digestion

Ultrasonic digestion is recognized as an alternative for the pre-treatment of solid samples. This type of digestion makes use of ultrasounds which aids the dissolution, fusion and leaching processes (Afridi *et al*., 2006). The use of intense, high frequency ultrasonic energy allows for the mixing of the acid-sample mixture, inducing physical and chemical change (Kazi *et al*., 2008). This process allows for the formation and destruction of micro-bubbles in the acid-sample mixture, in addition, agitation also allows for the dissolution of the analytes (Mason, 1999). The ultrasonic-assisted digestion method is relatively simple and inexpensive. The solid sample is weighed and placed into the vessel thereafter the liquid phase is added, in this instance, the acid is added to the sample. The acid-sample mixture is submerged into the ultrasonic bath (Figure 2.11), exposed to ultrasonic irradiation under the specified conditions (temperature, frequency, and time). An ultrasonic bath can alternatively be used as the digesting vessel, this is not a recommended option since the ultrasonic bath walls can be corroded. Some of the factors that can affect the digestion using the ultrasonic bath can be the temperature of the medium, shape of the vessel, solvent properties etc (Priego-Capote & Luque de Castro, 2006). Advantages of ultrasonic assisted digestion include the gradual increase in the ultrasonic bath temperature to an equilibrium value, minimal contamination, inexpensive equipment. Due to the novelty the ultrasound-assisted digestion methods, it is quite often compared to other more conventional (open heat) or microwave-assisted digestion methods (Kazi *et al*., 2008).

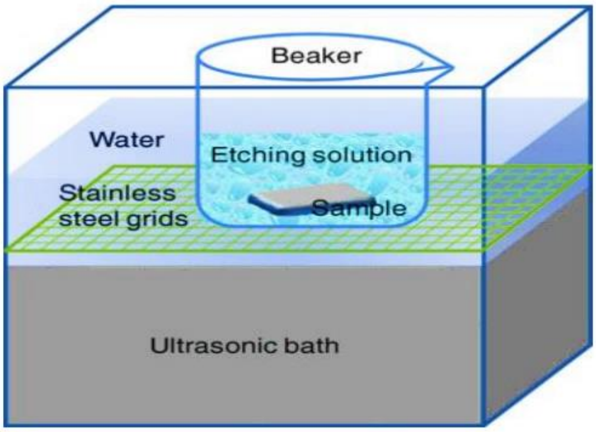


Figure 2.11: A typical ultrasonic assisted digestion diagram (González-Centeno *et al*., 2014)

2.8. Quality assurance

2.8.1. Analytical figures of merit

The analytical figures of merit are used for the validation of the instrument. The parameters used to validate the ICP-OES instrument are limit of quantification (LOQ), limit of detection (LOD), linearity, accuracy (%recoveries) and precision (%RSD). To ensure good quality of the results, quality measures need to be taken during the analysis, which improves the accuracy and precision of the data obtained and to identify applicable methods for analysis (Dean, 2002; Skoog *et al*., 1988).

To achieve good quality measurements, involves calibration of the instrument to attain a calibration curve with a linear calibration equation as given by equation 2.1.

$$\text{Signal Intensity} = k[C] + b \qquad (2.1)$$

Where k = Calibration sensitivity, C = Concentration of analyte, b = Y-intercept (can be S_{blank})

This involves preparation of a series of standard solutions, measurements of their signals and plotting of calibration curves and lastly calculation of the calibration constant using the slope (b) of the calibration curve. Preparation of the standard solutions may be considered one of the most important aspects of calibration. A series of standard solutions containing the target analytes (in this case, its heavy metals) are prepared using stock solutions with known concentrations of each analyte (metal ions). It is essential for the standard solutions to be in an increasing concentration and should cover a wide range that is within the expected range in real samples. In addition to the standard solutions, a sample blank is also prepared as it is important in identifying matrix interference. It is recommended to use distilled or ultrapure water in the preparation of the standards to avoid any interferences in analysis (Skoog *et al.*, 1988). An accurate linear calibration curve is important as it determines the results henceforth and this can lead to correct conclusions as well as ensuring that the aim is being achieved. Characteristics of an accurate linear calibration curve includes, high sensitivity, linear correlation coefficient (R^2) close to 1 and having a wide linear concentration range where the calibration constant remains constant. The method is considered to be calibration sensitive if the slope (b) of the calibration graphs is $\neq 0$.

In order to evaluate the accuracy of the instrumental method, the analysis can be conducted using a certified reference material (CRM). A CRM is used as original reference materials to validate the analysis and where the analysis concentrations agree with the values certified, the validity of the proposed method is approved. The precision of the measurements can be deduced if the results are reproducible and repeatable. Reproducibility refers to the closeness of data which is obtained under the exact same conditions whereas repeatability refers to the agreement between data under different conditions (Skoog *et al.*, 1988). Precision is commonly expressed as standard deviation, variance and, range for the results/measurements.

Assessment of the methods detection (LOD) and quantification limits (LOQ) is important as detection of the analyte in the sample can be difficult since analyte concentrations may be below the limit of detection for the method of analysis. The ICP-OES is known for its low detection limits and high sensitivity which results in trace analyte concentrations (ppb) to be quantified with ease (Skoog *et al.*, 1988). The limit LOD refers to the minimum detectable concentration of the analyte at a known statistical confidence level, it is normally the lowest concentration that can be accurately detected by the instrument. The LOQ refers to the lowest analyte concentration that can be accurately quantified (Armbuster and Pry, 2008). Their assessment was done by analysing blank samples (ultrapure water) 10 times followed by computing the standard deviation. The LOD and LOQ are calculated using equation 2.2 and 2.3 to assess the accuracy of the instrument, where k, is the slope of the calibration equation.

$$LOD = \frac{3S_{blank}}{k} \qquad (2.2)$$

$$LOQ = \frac{10S_{blank}}{k} \qquad (2.3)$$

2.9. Environmental contamination and toxicity studies for soil/sediments

Soil and sediments are considered sinks for heavy metal pollution and receives a significant amount each year, many of these toxic heavy metals originate from anthropogenic activity such as industrial processes, mining etc. Soil contamination has been reported in various countries and to a large extent due to anthropogenic processes (Liu *et al*., 2018; Wang *et al*., 2003). The environmental contamination and heavy metals toxicity are assessed by a wide variety of qualitative expressions. They have been developed and can be implemented in different countries to assess the heavy metal pollution and ecological risk in soil and sediment (Choi *et al*., 2018). The mathematically calculated parameters used to measure the heavy metal pollution are contamination factor (CF), enrichment factor (EF), metal correlation, geo-accumulation index (I_{GEO}) and pollution load index (PLI). The basic methods do not provide comprehensive and conclusive evidence of the toxicity for the overall heavy metal contamination. Therefore, the potential ecological risk index (*PERI*) is a widely used method to determine the potential risk of contamination since it considers the total concentration and the toxic response factors for each heavy metal. Most of these indices use the individual heavy metal concentration and background/baseline concentration (Liu *et al*., 2021). The background concentration of soils and sediments are heavy metal specific and aims to differentiate between the heavy metal concentration of the natural uncontaminated soil/sediment and the heavy metal concentration with anthropogenic influence in the study area (Choi *et al*., 2018). The concentration of heavy metals in soil/sediments present in the background value depended on geological factors and processes that form and occur in soil and sediments. It can allow for heavy metal concentrations being higher than the critical values, therefore making it difficult to determine natural background concentrations since geochemical composition and atmospheric deposition can contaminate the soil and sediments (Qing *et al*., 2015). However, there are country-specific background concentrations that can be used when quantifying these indices, hence in this study, South African background/baseline concentrations were obtained from literature (Herselman, 2005).

2.10. Human health risk assessment

Human health risk assessment of soil and sediments are used for quantification of non-carcinogenic and carcinogenic risks through three different pathways, namely ingestion, air inhalation and dermal contact (Qing *et al*., 2015). The mathematical expressions and the data required for the calculation of the non-carcinogenic and carcinogenic risks were based on the guidelines and exposure factors handbook of the US Environmental Protection Agency (US

EPA, 1986, 1989, 1997, 2001). For the non-carcinogenic risk, the average daily dosage (ADD) (mg/kg day) of potentially toxic heavy metals through ingestion (ADD_{ing}), dermal contact (ADD_{derm}) and inhalation (ADD_{inh}) are calculated for adults and children. The non-carcinogenic and carcinogenic effects are evaluated by the hazard quotient (HQ), hazard index (HI) and cancer risk (CR) (Wei *et al*., 2015; Chabukdhara & Nema, 2013). The HQ refers to the ratio of the average daily dosage and the relevant reference dose (RfD) for the mentioned exposure pathways (US EPA, 1989). The RfD refers to the maximum daily dosage of the heavy metal through the specified pathway in both children and adults. This stipulated maximum dosage will not lead to detrimental effects in humans during their lifespan (US EPA, 1989).

These ADD values can be categorized in manner in which, if they are less than the RfD and their corresponding HQ value is less than 1 ($HQ < 1$), non-carcinogenic risks are considered to have no detrimental health effects and if the ADD is greater than the RfD and the HQ value is greater than 1 ($HQ > 1$), these heavy metals can be potentially detrimental to human health (Qing *et al*., 2015, US EPA, 1989, 2001). The hazard index (HI) is quantified and refers to the sum of the HQ values and the averages of the total non-carcinogenic risks through the three exposure pathways for each heavy metal. HI values less than 1 ($HI < 1$) indicates no adverse non-carcinogenic effects occur whilst if $HI > 1$, these heavy metals can have adverse health risks in humans, ultimately the increase in HI values, increase the risk of non-carcinogenic effects. The carcinogenic or cancer risk is the probability of a person developing a type of cancer in their lifespan as a result of carcinogenic hazards by exposure of heavy metals (Li *et al*., 2014). Similarly, the lifetime average daily dose (LADD) is calculated for the three exposure pathways, the CR and HI values are also quantified using the slope factor (SF) and RfD. The SF represents the probability of developing cancer per unit exposure of mg/kg day along with their respective RfD values. The CR values less than 10^{-6} indicates negligible carcinogenic risk to human health and $CR > 1 \times 10^{-4}$ indicates a high risk of contracting cancer. The CR values in between the stipulated values mentioned, indicates acceptable risk to human health.

2.11. References

Abu-Samra, A., Morris, J., & Koirtyohann, S. (1975). Wet ashing of some biological samples in a microwave oven. *Analytical Chemistry, 47*(8), 1475-1477. doi: 10.1021/ac60358a013

Addo-Bediako A, Matlou K, Makushu E (2018). Heavy metal concentrations in water and sediment of the Steelpoort River, Olifants River system, South Africa. *African Journal of Aquatic Science*, 43:413-416. https://doi.org/10.2989/16085914.2018.1524745

Afridi HI, Kazi TG, Kazi GH & Jamali MK, Effect of Ultrasound agitation on the Release of Heavy Elements in Certified Reference Material of Human Hair (CRM BCR 397), J. AOAC Int. 89 (2006) 1410–1417

Microwave Digestion. (2022, May 10). Retrieved from https://www.anton-paar.com/corp-en/products/group/microwave-digestion/

Armbruster, D.A., & T. Pry, T. (2008). Limit of Blank, Limit of Detection and Limit of Quantitation, *The Clinical Biochemist Reviews* Vol. 29, pp. 1 – 4. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556583/pdf/cbr29_s_pgs49.pdf

Atlas A, Usman K, Ur Rehman H, Khudadad S, Munawar A, Rab A, Khan MI, Gul M, Pervaiz M (2017). Analysis of heavy metals in river Kabul at Sardaryab Khyber Pakhtunkhwa, Pakistan. *Journal of Entomology and Zoology Studies* 5: 14-16.

Bassioni G, Ashraf K, Elhameed A (2015). Risk assessment using ICP-MS of heavy metals in groundwater in Upper Egypt. *Journal of Natural Resources and Development* 65-70. https://doi.org/10.5027/jnrd.v5i0.09

Bhatti, S.S., Kumar, V., Singh, N., Sambyal, V., Singh, J., Katnori, J.K., & Nagpal, A.K., (2016). Physico-chemical properties and heavy metal contents of soils and Kharif crops of Punjab, India, *Procedia Environmental Science*. 35, 801 – 808.

Bvenura, C & Afolayan, AJ (2012). Heavy metal contamination of vegetables cultivated in home gardens in the Eastern Cape, S*outh African Journal of Botany*.

Chabukdhara, M., Nema, A. K., (2013). Heavy metals assessment in urban soil around industrial clusters in Ghaziabad, India: Probabilistic health risk approach. Ecotox. Environ. Safe. 87, 57-64. doi: https://doi.org/10.1016/j.ecoenv.2012.08.032

Chao, T., & Sanzolone, R. (1992). Decomposition techniques. *Journal Of Geochemical Exploration, 44*(1-3), 65-106. doi: 10.1016/0375-6742(92)90048-d

Chen, L., Song, D., Tian, Y., Ding, L., Yu, A., & Zhang, H. (2008). Application of on-line microwave sample-preparation techniques. *Trac Trends in Analytical Chemistry, 27*(2), 151-159. doi: 10.1016/j.trac.2008.01.003

Cherevko, S., & Mayrhofer, K.J.J. (2017). On-Line Inductively Coupled Plasma Spectrometry in Electrochemistry: Basic Principle and Applications. 10.1016/B978-0-12-409547-2.13292-5

Choi, J., & Jeon, S. (2018). A Geo-statistical Assessment of Heavy Metal Pollution n the Soil Around a Ship Building Yard in Busan, Korea. *Journal of the Korean Society of Marine Environment & Safety, 24*(7), 907-915. Doi: https://doi.org/10.7837/kosomes.2018.24.7.907

Collin, B., & Doelsch, E. (2010). Impact of high natural soilborne heavy metal concentrations on the mobility and phytoavailability of these elements for sugarcane. *Geoderma, 159*(3-4), 452-458. doi: 10.1016/j.geoderma.2010.09.013

Dean, J., Ando, D., & Metcalfe, E. (1997). *Atomic absorption and plasma spectroscopy*. Chichester: Published on behalf of ACOL (University of Greenwich) by J. Wiley.

Department of Water Affairs and Forestry, (1996). South African Water Quality. Guidelines. Aquatic Ecosystems, *Vol 7*.

Ebenebe PC, Shale K, Sedibe M, Tikilili P, & Achilonu MC (2017). South African Mine Effluents: Heavy metal pollution and impact on the ecosystem, *International Journal of Chemical Sciences*, *15*(1).

Edokpayi J, Odiyo J, Popoola E and Msagati T (2017) Evaluation of temporary seasonal variation of heavy metals and their potential ecological risk in Nzhelele River South Africa. *Open Chem Journal* 15:272-282. https://doi.org/10.1515/chem-2017-0033

Freeman, O., Ovie, O., & Omoregie, I. (2017). Effect of Climate Change through Temperature Increase on Heavy Metals Concentrations in Water and Sediment of Ekpan Creek, Delta State, Nigeria. *Advances In Research*, *10*(5), 1-16. https://doi.org/10.9734/air/2017/34754

Garbarino, J.R., Hayes, H.C., Roth, D.A., Antweiler, R.C., Brinton, T.I. and Taylor, H.E., (1995). Heavy Metals in the Mississippi River. Retrieved November 17, 2014, from http://pubs.usgs.gov/

Gilman, L.B., & Engelhart, W.G. (1989). Recent advances in microwave sample preparation, *Spectrosscopy,* 4(8), 14-21.

González-Centeno, M.R., Knoerzer, K., Sabarez, H., Simal, S., Rosseló & Fermenia, A. (2014). Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (*Vitis vinifera L.*) – A response surface approach, *Ultrasonics Sonochemistry, 21(6), 2176 – 2184.*

Gzik A, Kuehling M, Schneider I, & Tschochner B. (2003). Heavy Metal Contamination of Soils in a Mining Area in South Africa and its Impact on Some Biotic Systems, *Journal of Soils & Sediments*, 3.

Herselman E, Steyn C and Fey M (2005) Baseline concentration of Cd, Co, Cr, Cu, Pb, Ni and Zn in surface soils of South Africa. *South African Journal of Science* 101:509-512.

Hu, Z., & Qi, L. (2014). Sample Digestion Methods. *Treatise On Geochemistry,* 87-109. doi: 10.1016/b978-0-08-095975-7.01406-6

Jaishankar M, Tseten T, Anbalagan N, Mathew B, Beeregowda K (2014). Toxicity, mechanism, and health effects of some heavy metals. *Interdiscip Toxicol* 7:60-72. https://doi.org/10.2478/intox-2014-0009

Jiao, F., & Gao, H. (2013). On-site solid-phase extraction and application to in situ preconcentration of heavy metals in surface water, *Environ Monit Assess,* 185, pp. 39-44.

Kazi, T., Jamali M, Arian M, Afridi JN, Sarfraz R and Ansari R (2008). Evaluation of an ultrasonic acid digestion procedure for total heavy metals. *Journal of Hazardous Materials* 161:1391 - 1398. https://doi.org/10.1016/j.jhazmat.2008.04.103

Li H, Shi A, Li M, Zhang X (2013). Effect of pH, temperature, dissolved oxygen, and flow rate of overlying water on heavy metals release from storm sewer sediments. *Journal of Chemistry:*1-11. https://doi.org/10.1155/2013/434012

Liu, D., Wang, J., Yu, H., Gao, H., & Xu, W. (2021). Evaluating ecological risks and tracking potential factors influencing heavy metals in sediments in an urban river. *Environmental Sciences Europe, 33*(1). doi: 10.1186/s12302-021-00487-x

Manning, T. & Grow, W. (1997). Inductively coupled plasma-atomic emission spectroscopy, The Chemical Educator, http://www.mrl.ucsb.edu/mrl/centralfacilities/chemistry/icp.pdf (accessed April 2022)

Matusiewicz, H. (2017). Sample [reparation for Inorganic Trace Element Analysis. *Physical Science Reviews*, *2*(5), 20178001, https://doi.org/10.1515/psr-2017-8001

Mitra, S. (2003). *Sample preparation techniques in analytical chemistry*. New Jersey: John Wiley & Sons.

Mohod, C.V, & Dhote, J, (2013). Review of heavy metals in drinking water and their effect on human health, *International Journal of Innovative Research in Science, Engineering and Technology*, 2, 7.

Nadkarni, R. (1984). Applications of microwave oven sample dissolution in analysis. *Analytical Chemistry*, *56*(12), 2233-2237. doi: 10.1021/ac00276a056

Nave, C., & Nave, B. (1975). *Physics for the Health Sciences.* Philadelphia: J.B. Saunders.

Neas, E.D., & Collins, M.J. (1988). Microwave heating. Theoretical concepts and equipment design. In: Kingston HM and Jassie CB (eds) *Introduction to Microwave sample preparation, Theory and Practice*, Washington DC: American Chemical Society, 7 - 32

Ngubane, N.S, (2016). Investigating the standard method used by Umgeni Darvill Wastewater Works for the determination of pseudo total metal concentrations in their sewage sludge.

de Namor AFD, Gamouz AE, Frangie S, Martinez V, Valiente L & Webb OA (2012) *Journal of Hazardous Materials* 241-242, Pg 14 – 31, 2012.

Olujimi O, Fatoki O, Odendaal J, Oputu O (2015). Variability in heavy metal levels in river water receiving effluents in Cape Town, South Africa. *Research and Practices in Water Quality*. https://doi.org/10.5772/59077

Priego-Capote, F., & de Castro, L. (2007). Ultrasound-assisted digestion: A useful alternative in sample preparation. *Journal Of Biochemical and Biophysical Methods*, *70*(2), 299-310. doi: 10.1016/j.jbbm.2006.09.006

Qing, X., Yutong, Z., & Shenggao, L. (2015). Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. *Ecotoxicology And Environmental Safety*, *120*, 377-385. doi: 10.1016/j.ecoenv.2015.06.019

Rahmanian, N., Ali, S., Homayoonfard, M., Ali, N., Rehan, M., Sadef, Y., & Nizami, A. (2015). Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia. *Journal Of Chemistry*, *2015*, 1-10. https://doi.org/10.1155/2015/716125

Rai, P. K., Lee, S. S., Zhang, M., Tsang YF & Kim K, Heavy metals in food crops: Health risks, fate, mechanisms, and management, *Environmental International* 125, Pg 365 – 385, 2019.

Reeve, R. (2001). *Environmental analysis*. New York: Wiley.

Salem, M., Bedade, D., Al-Ethawi, L., & Al-waleed, S. (2020). Assessment of physiochemical properties and concentration of heavy metals in agricultural soils fertilized with chemical fertilizers. *Heliyon*, *6*(10), e05224. doi: 10.1016/j.heliyon.2020.e05224

Sankhla MS, Kumari M, Nandan M, Kumar R, & Agrawal, P (2016). Heavy Metals Contamination in Water and their Hazardous Effect on Human Health-A Review, *International Journal of Current Microbiology and Applied Sciences*, 5 (10), pp. 759-766, 2016.

South African National Standard (SANS) (2015). Drinking water 241. https://vinlab.com

Sastre. J.. Sahuquillo. A.. Vidal. M.. & Rauret. G. (2002). Determination of Cd. Cu. Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Analytica Chimica Acta*. 59-72

Skoog, D., West, D., Holler, F., & Crouch, S. (2014). *Fundamentals of analytical chemistry*. Belmont: Brooks/Cole, Cengage learning.

Timothy J. Mason. (1999). *Sonochemistry*. Oxford University Press. https://doi.org/10.1016/S0003-2670(02)00307-0

Tran, Quynh & Hsieh, Chi-Hsu & Yang, Tung-Yu & Tung, Hsin-hsin. (2019). Optimization of isopropyl alcohol degradation by microwave-induced catalytic oxidation process. *Journal of Water Reuse and Desalination*. 9. 10.2166/wrd.2019.015.

United States Environmental Protection Agency (1994). Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry, Method 200.7, Retrieved from https://www.epa.gov/sites/production/files/2015-08/documents/method_200-7_4-4_1994.pdf.

USEPA (US Environmental Protection Agency), 2001. Supplemental guidance for developing soil screening levels for superfund sites. OSWER9355.4-24. Office of Solid Waste and

Emergency Response. US Environmental Protection Agency. Washington, DC. http://www.epa.gov/superfund/superfund-soil-screening-guidance

USEPA, 2011. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F. https://www.nrc.gov/ML1400/ML14007A666.pdf

Wang, Q., Cui, Y., Liu, X., Dong, Y., & Christie, P. (2003). Soil Contamination and Plant Uptake of Heavy Metals at Polluted Sites in China. *Journal Of Environmental Science and Health, Part A*, *38*(5), 823-838. doi: 10.1081/ese-120018594

Wei, X., Gao, B., Wang, P., Zhou, H., & Lu, J. (2015). Pollution characteristics and health risk assessment of heavy metals in street dusts from different functional areas in Beijing, China. *Ecotoxicology And Environmental Safety*, *112*, 186-192. doi: 10.1016/j.ecoenv.2014.11.005

World Health Organization (2008). ‘WHO’ issues revised drinking water guidelines. 2008:7. https://doi.org/10.1016/s1365-6937(04)00477-0

World Health Organization (WHO) (2001) Barium and barium compounds. Geneva: World Health Organization. http://www.inchem.org/documents/ehc/ehc221.htm (accessed 9 October 2021)

Yayintas OT, Yilmaz S, Turkoglu M, Colakoglu, FA & Cakir F (2007). Seasonal variation of some heavy metal pollution with environmental and microbiological parameters in sub-basin of Kocabas Stream (Biga, Canakkale, Turkey) by ICP-AES, *Environ Monit Assess* 134, pp. 321–331, 2007.

It is important to note that this dissertation is presented in a form of publications. The experimental procedures are detailed in each of the written papers not presented in a separate chapter to avoid any repetition.

List of articles/papers submitted for possible publication

Paper 1 presented as Chapter 3: K. Naicker, P.N. Mahlambi and M.M. Mahlambi, *Assessment of metals and anions in tap, river, and wastewater: A hotplate and microwave assisted digestion method comparison* (submitted to the Journal of Metals, Materials, and Minerals)

Paper 2 presented as Chapter 4: K. Naicker, P.N. Mahlambi and M.M. Mahlambi, *Comparison of ultrasonic and microwave assisted digestion methods for the determination of heavy metals in soil and sediment: the effect of seasonal variations on metal concentrations and risk assessment* (ACCEPTED by Soil and Sediment Contamination: An International Journal)

Paper 3 presented as Chapter 5: K. Naicker, P.N. Mahlambi and M.M. Mahlambi, *Comparison of ultrasonic, hotplate and microwave assisted digestion methods for the assessment of metals in agricultural soil: Environmental contamination and human health risk* (submitted to the Journal of Environmental Quality)

Chapter Three

Assessment of metals and anions in tap, river, and wastewater: A hotplate and microwave assisted digestion method comparison

3.1. Abstract

The trace level determination of multiple heavy metals with high accuracy and precision is of paramount concern in analytical methodology. In the present study, the analysis of twelve metals (Cd, Cr, Co, Cu, Ga, Li, Mn, Ni, Pb, Sr, Tl, Zn) in tap, river, wastewater, and sludge samples were conducted using ICP-OES technique after digestion with hotplate and microwave assisted digestion. Both methods of digestion displayed a good degree of accuracy indicating their suitability for the analysis of the studied metals in water samples. From the optimization studies, 100 mL of HNO_3 was found to be the optimum sample volume and acid type for digestion. The average concentrations obtained for all metals studied ranged from 4.9 – 410.8 µg/L, 5.9 – 465.0 µg/L, 3.6 – 425.4 µg/L, 16.1 – 647 µg/L and 9.7 – 784 µg/L in tap water, river, influent, effluent, and sludge samples, respectively. All metals were below their maximum permissible limits except the Mn in all sludge samples and Pb in all tap water, from Umhlathuzana River and Northern works influent samples. The comparable recovery and metal concentrations were obtained by microwave and hotplate methods suggesting that hotplate can be used as a cheaper and effective digestion method for daily analysis. Common anions (PO_4^{3-} , Cl^-, NO_3^-, NH_3 and SO_4^{2-} **)** were analyzed and quantified in all water sources using Aquakem 250, the concentrations obtained ranged from 0.03 – 23.5 mg/L, 0.02 – 3064.67 mg/L and 0.32 – 175.67 mg/L for tap, river, and wastewater samples for all anions, respectively. The anions concentrations were found to be below the maximum acceptable limits indicating no possible health effect on human and aquatic life, except, Cl^- and SO_4^{2-} in Amanzimtoti and Northern River water.

3.2. Introduction

Water is vitally important to all living organisms and biological processes. However, water scarcity due to heavy metal contamination is one of the main challenges that all water sources experience (Bassioni *et al*., 2015). The contamination of water bodies by heavy metals, can naturally originate from weathering of minerals and rocks, or anthropogenic (industrial activities like manufacturing processes, chemical industries and smelting of metal, electroplating etc.), sewage discharge, urban and agricultural runoff (applied disease or insects control agents), etc (Jaishankar *et al*. 2014).

The treatment processes of the surface with heavy metals (As, Cd, Cu, Cr, Fe, Hg, Mn, Ni, Pb and Zn), and also the industrial products discharged in wastes after they have expired, are the main industrial sources of heavy metals emissions that end up in wastewater. Also, the effluents from household, business effluents (dental uses, car washes, etc.), traffic related emissions (tires, vehicle exhaust, brake linings, gasoline/oil leakage, asphalt wear, etc.) are the core urban inputs to sewage water are as they can be carried into the sewerage system along with the storm water. These can lead to an increased amount of heavy metals reaching the wastewater treatment plants. Thus, they are discharged to the nearby rivers with the treated effluent where they may accumulate in aquatic life and introduced into the food cycle. Also, metal pollution from river water and metal leaching from systems that distributes water may lead to the presence of heavy metals in drinking water and thus be consumed by human beings. This may result to severe human health effects especially under significant exposure to high concentration levels (Atlas *et al.* 2017). Moreover, the pollution of the environment by these heavy metals is a long-term and irreversible process (Olujimi *et al.*, 2012)

In wastewater treatment processes, large amounts of sludge are generated and when heavy metals in the wastewater influent undergo treatment, they become concentrated in the sludge. When sludge with heavy metals is used as manure on agricultural land or wastewater effluent is used for irrigation of agricultural crops, it may transfer metals to crops. This can negatively affect the crops productivity, the atmosphere quality resulting in the threat of animal health and human beings through the food chain (Yamgata *et al.*, 2010). Despite many European countries having a decrease in environmental pollution through implementation of legislature, improved treatment processes and eco-friendly industrial activities, the developing countries still struggle to control environmental pollution. Therefore, new, and efficient methods of treatment and consistent monitoring of water resources including wastewater are required (Olujimi *et al.*, 2012)

Environmental sample matrices can be complex thus requiring sample preparation in order to be homogenized and made suitable for instrumental analysis. In sample preparation, specifically digestion of metals with the addition of an acid or acid combination, a standard acid digestion method is required to evaluate the effectiveness of metal recovery to assess the methods accuracy and ability to completely digest the sample matrix (Jaishankar *et al.*, 2014). Acid digestions were previously performed in open systems by heating the solid waste with an appropriate acid or acid combination. However, with accessibility and improvements of technology, various other methods such as microwave-assisted digestion and ultrasonic digestion have become increasingly adopted due to their higher metal recovery rate. Microwave-assisted digestion have several advantages over the conventional hotplate digestion which include reduced digestion time and less contamination. Generally, an acid digestion reaction is dependent on many aspects which includes the acid type of used and its concentration, time of digestion, reaction conditions (temperature), and the metal form present in the solid waste matrix (Das & Ting, 2017). It is therefore important that suitable acid type and appropriate digestion time is applied for effective digestion of the sample matrix.

Along with heavy metals, anion contamination is another common environmental problem as they may also pose a threat to water sources if present in high concentrations. The commonly found anions in water sources include, chlorides, sulphates, phosphates, nitrates, ammonia etc. Anions like nitrates, commonly used in agricultural activity can lead to illnesses affecting the transportation of oxygen in the blood whilst excessive phosphates can cause environmental issues such as eutrophication and algal bloom. Chlorides are most common since chlorination is a common water treatment process which may increase the chloride ions present in water bodies (Altundag *et al.*, 2019). Despite chloride being an essential nutrient, its high levels on human consumption can lead to kidney disorders and increased blood pressure. In addition, it may lead to the production of problematic toxic by-products such as trihalomethanes and haloacetic acids resulting in cancers of vital organs (Gopal *et al.*, 2007, Lehtonen *et al.*, 2019).

The aim of this study was therefore to assess the heavy metal and anion contamination in tap, river, and wastewater treatment plants along with the rivers in which their effluents are discharged, using microwave-assisted and hotplate digestion. To the best of our knowledge, no reported work has been conducted to assess the concentration levels of the studied metals and anions in the selected study areas. Also, the comparison of microwave and hotplate digestion methods was conducted for the first time to compare the total dissolved and recoverable metals concentrations in the different water matrices.

3.3. Experimental

3.3.1. Sample storage and collection

Tap water samples were obtained from Richmond Crest, Mkhondeni, Woodlands, Boughton, Scottsville which are the suburbs in Pietermaritzburg area. River water samples were sampled along Msunduzi river at Bishopstowe, College Road, Camps Drift, Woodhouse and YMCA (Figure S3.1). The wastewater samples were obtained from three wastewater treatment plants (WWTPs) in the city of Durban, these include, Amanzimtoti, Umhlathuzana and Northern. In all WWTPs, water samples were taken in the influent (where water from domestic and companies' sources to be treated in WWTP comes in), in the effluent (where treated water from treatment plant discharged out into to the nearby or associated river) and liquid sludge (slurry material generated as a by-product of wastewater treatment processes). The Umhlathuzana WWTP receives influent from Marianridge and Shallcross which are then combined to one effluent after they been treated, and thereafter discharged into the Umhlathuzana River. Amanzimtoti WWTP discharge into Mbokodweni River, Northern WWTP discharge into Umgeni River. River water samples from the associated WWTPs were also collected to fully assess the metal concentrations and the efficiency of the WWTP. About 2.5 L of water sample was collected in polyethylene bottles at each site and immediately placed in cooler box. They were then transported to the laboratory and were filtered using 0.45 μm membrane filter consisting of biologically inert mixtures of cellulose acetate and cellulose nitrate (Merck, Darmstadt, Germany).

3.3.2. Reagents, reference materials and standards

Ultrapure water was employed in preparation of standard solutions for the ICP-OES instrument calibration and to rinse all glassware prior to use. The 55% v/v nitric acid (HNO_3), (Merck, Darmstadt, Germany) was used for cleaning glassware and to digest the water samples since it liberates the trace metal elements as the soluble nitrate salt. Standard solutions were prepared by appropriate dilutions of a stock standard of 1000 mg/L (Sigma Aldrich, South Africa). The standard reference material of trace elements in water (ULTRASPEC® Multi-Element Aqueous CRM) was employed for the accuracy evaluation of the method used for quantification of heavy metal in water samples.

3.3.3. Instrumentation

The sample digestion was performed using Multiwave 5000 (Anton Paar, Johannesburg). The Varian 720-ES ICP-OES (inductively coupled plasma-optical emission spectroscopy) was used for the determination of metals in water samples. The instrument operated at a frequency of 40MHz and RF power of 1.00kW, which consisted of a pneumatic concentric nebulizer with a flow rate of 0.75 L/min and pump rate of 15 rpm. The inert carrier gas used was argon (Ar). These conditions remain constant throughout the analysis. Table 3.2 showed the optimal wavelengths used for each metal element.

3.3.4. Sample preparation

The digestion of the samples was done using hotplate heating assisted digestion and microwave assisted digestion in order to determine the total recoverable and total dissolved metals in all the water samples. Spiked recovery tests were conducted for the optimization studies where the recoveries were calculated for all digestion methods.

3.3.4.1. Acid digestion by heating

The Environmental Protection Agency (EPA 3005A) digestion method was used to determine heavy metals in water. For total recoverable metals, a 100 mL water sample was transferred into a glass beaker. Thereafter, 5 mL of 55% v/v nitric acid was added, and the beaker was then heated on a hotplate to allow the contents to evaporate and reduce to around 20 mL. The beaker was removed from the hotplate and left to cool for 5 minutes, and another portion of 5 mL nitric acid was added and further heated for 15 minutes. The sample was then cooled and transferred into a 100 mL volumetric flask and filled up to the mark with ultrapure water. For determination of total dissolved metals, a 50 mL water sample was transferred into a 100 mL volumetric flask followed by addition of a 10 mL of 55% v/v nitric acid and made up to the mark with ultrapure water. No heating was required for the determination of total dissolved metals as evaporation alters the amount of the sample. The samples were then analyzed using ICP-OES.

3.3.4.2. Microwave assisted acid digestion for total recoverable and total dissolved metals

The American Society for Testing and Materials method (ASTM-D4309-18) was followed for the sample digestion to determine the total recoverable metals in water. To a 50 mL water sample, 5 mL of nitric acid was added and gently swirled. The sample-acid mixture was placed in a microwave vessel, tightly closed, and digested with the microwave. The microwave program involved heating to 170 ± 5 °C in 10 minutes and maintained for 10 minutes. For the determination of total dissolved metals, the EPA 3015A method was followed. The procedure was the same as that used for total recoverable method, however, the microwave program involved heating to 170 ± 5 °C in 20 minutes and maintained for 10 minutes. After the digestion process was complete, the vessels were removed from the microwave reaction, carefully opened in the fume hood, and transferred into ICP tubes for analysis.

3.4. Validation of methods for metal determination

The methods were validated based on spiked recovery tests in which the recoveries were calculated and expressed as a percentage. It assessed the accuracy of the digestion methods and was conducted using river water and wastewater spiked with 0.5 mg/L of a mixture of standards of metals.

3.5. Determination of anions in water samples

The Aquakem 250, discrete selective photometric analyzer was employed for the determination of anions (sulphates, chlorides, nitrates, phosphates, and ammonia) in river and wastewater samples.

Sulphate (SO_4^{2-}): barium chloride was used to precipitate the sulphate ions in a strongly acid medium. The turbidity resulted was then photometrically measured at 405 nm followed by comparison with appropriate calibration standard solution.

Chloride (Cl^-): mercury (II) thiocyanate was added to the sample which reacted with the chlorides present resulting to a formation of a soluble non-ionic compound. The iron (III) nitrate was then added to react with the released thiocyanate ions resulting to a formation of a red / brown iron (III) thiocyanate complex. This produced a stable colour and its intensity was spectrophotometrically measured at a wavelength of 480 nm which related to the concentration of chloride by means of a calibration curve.

Nitrate (NO_3^-): hydrazine sulphate was added to the sample under alkaline conditions where the nitrates present were reduced to nitrites. This was followed by the reaction of sulphanilamide and N-1-naphthylenediamine hydrochloride with the total nitrite ions under acidic conditions resulting to formation of a pink azo-dye. The absorbance connected to the total oxidised nitrogen concentration by means of a calibration curve was measured at 540 nm.

Phosphate (PO_4^{3-}): ammonium molybdate and antimony potassium tartrate (catalyst) were added into the sample under acidic condition to react with the orthophosphate ions resulting to a formation of a 12-molybdophosphoric acid complex. This was followed by a complex reduction using ascorbic acid to form a blue heteropoly compound. The absorbance which related to the phosphate concentration through calibration curve was spectrophotometrically measured at a wavelength of 880 nm.

Ammonia (NH_3^-): hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate reacted with ammonia present in the sample to form monochloramine. This reacted with salicylate ions in the presence of sodium nitroprusside around pH 12.6 forming a blue compound. The absorbance of this compound was measured spectrophotometrically at wavelength 660 nm and related to the ammonia concentration by a calibration curve.

3.6. Results and discussion

3.6.1. Optimization and validation studies

3.6.1.1. Effect of digestion acid type on the recovery of metals

An acid or acid-mixture choice is crucial in the digestion process since it is dependent on the nature of the metals that are digested and present in the environmental matrix. Concentrated hydrochloric and nitric acids were used as pure or in ratios of 3:1 and 1:3 to digest the water samples spiked with a concentration of 0.5 mg/L.

The percentage recoveries for the hotplate digestion ranged between (78 – 117%), (74 – 111%), (57 – 102%) and (88 – 116%) for 3:1 (HNO_3: HCl), 1:3 (HNO_3: HCl), HCl and HNO_3, respectively. The microwave assisted digestion yielded recoveries ranging between (62 – 95%), (67 – 111%), (66 – 113%) and (83 – 103%) for 3:1 (HNO_3: HCl), 1:3 (HNO_3: HCl), HCl and HNO_3 respectively (Table 3.1). The effectiveness of the digestion is governed by the acid used in the methods. The concentrated HNO_3 provided recoveries above 80% for all metals in both methods hence was chosen as the most suitable. This could be due to the high ability for nitric acid to extract a wide variety of metal salts, while hydrochloric acid is suitable for metals in the form of carbonates, phosphates, borates, sulfides, and some oxides (Reeve, 2002). Also, metals in the waste matrix tend to form soluble metal salts when subjected to oxidative acid digestion reactions (Das & Ting, 2017). These results disagree with those reported by Uddin and co-workers, where HNO_3 – HCl (1:3) showed to be the most appropriate acid combination in their study (Uddin *et al*., 2016).

3.6.1.2. Effect of sample volume on the recovery of heavy metals in hotplate digestion

The effect of sample volume was investigated using 25, 50, 100 mL of tap water sample. The results showed an increase in all metals recovery as the sample volume increased with recoveries ranging from 83-99% for 100 mL volume (Table 3.1). This could be due to increasing the sample volume use, which will increase the amount of metals available for digestion and ultimately the concentration recovered in the digestion solvent was increased. Also, the digestion process of high sample volume takes longer which might have improved the concentration of metals recovered, since digestion process is also influenced by time. The statistical analysis also confirmed that the mean recovery result for 100 mL is statistically different from 25- and 50-mL sample volume with the p-value ($p>4.2\text{x}10^{-7}$) for 25 mL vs 100 mL and ($p>3.7\text{x}10^{-6}$) for 50 mL vs 100 mL which are less than 0.05 (Table S3.1). The 100 mL sample was then taken as the optimum volume for further analysis.

Table 3.1: Microwave assisted and hotplate digestion (Total recoverable metals) recoveries for the use of different acids and acid combinations and sample volume optimization, n = 3

metal	**Acid optimization**								**Sample volume**		
	Microwave assisted digestion				**Hotplate digestion**				**25 mL**	**50 mL**	**100 mL**
	3:1	1:3	HCl	HNO_3	3:1	1:3	HCl	HNO_3			
Co	94	98	99	86	115	108	81	94	63	65	90
Cr	93	94	98	93	103	95	70	88	61	63	94
Cu	75	79	82	102	92	88	86	104	76	82	96
Cd	93	100	104	86	115	111	102	93	57	59	86
Ga	79	73	85	83	92	89	87	100	60	63	87
Ni	81	86	87	103	114	93	84	103	67	66	95
Pb	95	102	108	93	117	86	98	92	50	54	90
Sr	69	71	66	101	78	75	57	116	77	82	99
Tl	92	111	113	86	102	101	99	107	62	63	85
Zn	82	90	96	86	101	103	89	97	62	64	83

* 3:1 – (HNO_3: HCl) - (7.5: 2.5 mL), 1:3 - (HNO_3: HCl) - (2.5:7.5 mL)

3.6.1.3. Recovery of metals from different water matrices

The tap, river and wastewater matrices were spiked with a concentration of 0.50 mg/L metals mixture, digested, analysed and the percentage recoveries were calculated. It was observed that there was no trend in metals recoveries from all water samples which indicated that the recoveries are independent of the sample matrix. The recoveries were found to be within an acceptable range of 72 – 119% (Table 3.2). Total recoverable metal recoveries were higher than the total dissolved for both digestion methods. This is expected since the total recoverable determination takes into consideration both the suspended and dissolved metals concentrations. However, it was also observed for some metals that the total dissolved recoveries were higher, this could be due to the sample reduction step in the total recoverable determination where the analyte is lost via evaporation during the heating process (Sastre *et al*., 2018). The t-test conducted in the mean recoveries showed that they are not significantly different, and the p-values obtained for the microwave method were $p>0.139$, $p>0.201$ and $p>0.471$ for tap versus river, tap versus waste and river versus waste, respectively (Table S3.2) which was greater than 0.05.

Table 3.2: Effect of water matrix and spike concentration on the %recoveries for total recoverable metals (TR) and total dissolved metals (TD) in different water matrices, (n = 3)

Metal	**Effect of water matrix**											
	Tap water				**River water**				**Wastewater**			
	Heat		***Micro***		***Heat***		***Micro***		***Heat***		***Micro***	
	TR	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*
Cd	86	86	75	78	86	87	84	80	84	84	87	88
Co	88	90	75	84	87	90	85	75	85	87	88	88
Cr	93	94	93	99	92	96	104	100	88	90	92	92
Cu	101	96	76	105	93	98	96	91	91	72	95	95
Ga	88	87	86	91	85	88	90	75	79	89	83	84
Li	96	96	87	89	91	93	83	84	80	79	82	83
Mn	91	91	78	85	91	94	82	90	88	91	92	94
Ni	97	95	95	102	93	98	107	100	91	92	95	96
Pb	91	90	78	82	91	88	82	87	86	85	89	89
Sr	100	99	109	110	97	98	103	110	94	95	96	96
Tl	94	85	80	119	94	88	115	92	89	84	90	88
Zn	84	83	84	88	83	84	77	75	84	86	89	89
Effect of spike concentration												
	0.10				**0.50**				**1.00**			
Co	83	86	99	100	85	87	88	88	87	90	91	89
Cr	113	92	101	98	88	90	92	92	89	91	93	91
Cu	97	84	94	96	91	72	95	95	94	76	97	94
Cd	78	82	97	98	84	84	87	88	85	89	90	88
Mn	94	91	98	99	88	91	92	94	90	92	94	92
Ni	97	97	99	98	91	92	95	96	91	96	96	95
Pb	88	87	93	95	86	85	89	89	87	86	91	92
Zn	81	87	90	96	84	86	89	89	87	90	90	89

The effect of sample spiking concentration for the certified reference material recovery of total recoverable and total dissolved metals were investigated at 0.10, 0.50 and 1.00 mg/L spike levels. There was no specific trend observed in the percentage recoveries for the different spiking concentrations therefore it can be deduced that the recoveries are independent of sample spiking concentration (Table 3.2). Statistical t-tests conducted gave p-values obtained were greater than 0.05 which confirmed that the results were not significantly different. The obtained p-values for the influent were $p>0.525$ and $p>0.370$ for TR (0.10 ppm) vs TR (0.50 ppm) and TR (0.10 ppm) vs TR (1.00 ppm) for hotplate digestion (Table S3.3). The p-values for the influent were $p>0.536$ and $p>0.417$ for TR (0.10 ppm) vs TR (0.50 ppm) and TR (0.10 ppm) vs TR (1.00 ppm) for microwave assisted digestion (Table S3.4 and S3.5).

3.6.2. Physicochemical properties of water samples

The physicochemical properties of the water samples were measured prior the determination of metal concentrations. This included salinity, dissolved oxygen (DO), pH, total dissolved solids (TDS), conductivity and temperature (Table 3.3).

The measured temperature of tap and river water ranged between 17.4 - 22.6 °C and 17.1 - 23.2 °C while for wastewater, the temperature ranged between 13.2 – 24.0°C. Studies have shown that an increase in temperature can result in higher metals sorption by minerals, however, the average dissolved metal concentrations showed no dependence on temperature at 4 - 25 °C (Hu *et al.*, 2017). The study conducted by Li and co-workers discovered that Pb concentrations increased with increasing temperature (15 -35°C) however, no significant concentration variation was observed since the changes in concentrations were within 1µg/L. It was also observed that Cd was only detected at temperatures of 30 and 35 °C. The reason behind this discovery is the oxidizable fraction of the metal being transformed easily in chemical reactions which occurs when the temperature is increased (Li *et al.*, 2013).

The pH of the collected tap water was 6.2 – 6.9, which is within the WHO recommended range in drinking water (6.5 - 8.5). The acidic pH can result in the presence of metals (e.g., Fe, Mn, Cu, Pb and Zn) in drinking water due to leaching from plumbing systems (Rahmanian *et al.*, 2015). The pH in River water and wastewater samples was between 7.2 – 9.0 and 7.1 – 7.6 respectively. which is generally slightly basic, and this could be due to the presence of carbonates, bicarbonates and hydroxides originating from limestone found in the riverbed (Reeve, 2002). Metal ions can also be converted into slightly soluble forms which tends to adsorb on materials present in slightly basic water if there is high amount of dissolved oxygen. Dissolved oxygen (DO) is one of the most valuable requirements for all aquatic organisms as it allows them to perform metabolic processes (Freeman *et al.*, 2017). The DO levels ranged between 2.41 - 3.66 mg/L in tap water, 0.64 - 2.90 mg/L in river water and 0.33 – 2.61 mg/L in wastewater which were below the healthy water range of 6.5 – 8 mg/L stipulated by the US EPA guidelines. This indicated that these waters have a low DO level and can be detrimental to aquatic organisms. Oxygen is depleted in water when organic and or inorganic material reacts with oxygen. For example, Fe^{2+} can deplete oxygen via oxidation to form Fe^{3+} (Reeve, 2002) and the oxidation processes can be a possible explanation for the differences between the DO levels in tap and river water samples. Oxidation processes are used in water purification and treatment of drinking water, thus increasing the DO levels. Therefore, tap water is expected to have higher DO concentration levels compared to river water since microorganisms' consumption will significantly decrease the DO presence (Li *et al.*, 2013). Salinity is generally expressed in practical salinity units (psu) and accounts for the total salts concentration in water. The salinity in wastewater ranged between 0.29 – 0.64 psu and was found to be higher than in river water (0.10 - 0.15 psu) and tap water (0.19 – 0.44 psu). The conductivity in tap, river and wastewater samples ranged between 187 - 758 µS, 210 - 888 µS and 608 – 1312 µS respectively, which were below the maximum allowable limit of conductivity in water, which was 1000 µS as per the NDWQS guidelines (Rahmanian *et al.*, 2015).

Total dissolved solids (TDS) refer to the total concentration of dissolved matter consisting of inorganic salts and small amounts of organic matter (Rahmanian *et al.*, 2015). The TDS in tap river and wastewater samples were between 106 - 243 mg/L, 105 - 163 mg/L and 304 – 658 mg/L respectively, which were all below the acceptable limit of 1000 mg/L in drinking water (WHO, 2008). High concentration of dissolved solids is usually not considered a health hazard; however, it can produce hard water (presence of carbonates and bicarbonates) which can affect the physical properties of water. It can also indicate that harmful contaminants such as Fe, Mn, SO_4^{2-}, Br and As are possibly present in the water (Rahmanian *et al.*, 2015).

Table 3.3: The physical properties of the water samples

<table>
<tr><th></th><th>Sample</th><th colspan="2">DO (mg/L)</th><th colspan="2">Temp (°C)</th><th colspan="2">Salinity (psu)</th><th colspan="2">TDS (ppm)</th><th colspan="2">pH</th><th colspan="2">Conductivity (µS)</th></tr>
<tr><td rowspan="5">Tap water</td><td>Richmond Crest</td><td colspan="2">3.48</td><td colspan="2">17.4</td><td colspan="2">0.44</td><td colspan="2">168.8</td><td colspan="2">6.75</td><td colspan="2">421</td></tr>
<tr><td>Boughton</td><td colspan="2">2.89</td><td colspan="2">19.3</td><td colspan="2">0.35</td><td colspan="2">120.2</td><td colspan="2">6.87</td><td colspan="2">316</td></tr>
<tr><td>Woodlands</td><td colspan="2">2.85</td><td colspan="2">22.6</td><td colspan="2">0.28</td><td colspan="2">243</td><td colspan="2">7.39</td><td colspan="2">758</td></tr>
<tr><td>Mkhondeni</td><td colspan="2">3.66</td><td colspan="2">20.2</td><td colspan="2">0.19</td><td colspan="2">106</td><td colspan="2">7.34</td><td colspan="2">187</td></tr>
<tr><td>Scottsville</td><td colspan="2">2.41</td><td colspan="2">18.8</td><td colspan="2">0.33</td><td colspan="2">206.4</td><td colspan="2">6.21</td><td colspan="2">229</td></tr>
<tr><td rowspan="5">River water</td><td>Camps Drift</td><td colspan="2">2.66</td><td colspan="2">17.1</td><td colspan="2">0.15</td><td colspan="2">163</td><td colspan="2">8.01</td><td colspan="2">328</td></tr>
<tr><td>College Road</td><td colspan="2">2.90</td><td colspan="2">23.2</td><td colspan="2">0.10</td><td colspan="2">112</td><td colspan="2">9.01</td><td colspan="2">223</td></tr>
<tr><td>YMCA</td><td colspan="2">2.50</td><td colspan="2">17.5</td><td colspan="2">0.10</td><td colspan="2">108</td><td colspan="2">7.98</td><td colspan="2">212</td></tr>
<tr><td>Wood House</td><td colspan="2">1.39</td><td colspan="2">17.2</td><td colspan="2">0.10</td><td colspan="2">105</td><td colspan="2">7.59</td><td colspan="2">210</td></tr>
<tr><td>Bishopstowe</td><td colspan="2">1.29</td><td colspan="2">22.1</td><td colspan="2">0.15</td><td colspan="2">163</td><td colspan="2">7.25</td><td colspan="2">325</td></tr>
<tr><td rowspan="5">Wastewater</td><td></td><td>Inf</td><td>Eff</td><td>Inf</td><td>Eff</td><td>Inf</td><td>Eff</td><td>Inf</td><td>Eff</td><td>Inf</td><td>Eff</td><td>Inf</td><td>Eff</td></tr>
<tr><td>Amanzimtoti</td><td>0.39</td><td>1.56</td><td>14.0</td><td>18.5</td><td>0.59</td><td>0.46</td><td>623</td><td>475</td><td>7.12</td><td>7.81</td><td>1241</td><td>945</td></tr>
<tr><td>Northern</td><td>0.48</td><td>2.61</td><td>15.1</td><td>13.2</td><td>0.40</td><td>0.32</td><td>427</td><td>339</td><td>7.27</td><td>7.46</td><td>858</td><td>675</td></tr>
<tr><td>Umhlathuzana (MR)</td><td>0.33</td><td rowspan="2">2.30</td><td>17.6</td><td rowspan="2">24.0</td><td>0.64</td><td rowspan="2">0.34</td><td>658</td><td rowspan="2">352</td><td>7.23</td><td rowspan="2">7.62</td><td>1312</td><td rowspan="2">707</td></tr>
<tr><td>Umhlathuzana (SC)</td><td>0.56</td><td>14.9</td><td>0.29</td><td>304</td><td>7.05</td><td>608</td></tr>
</table>

3.6.3. Metal concentrations in water samples

3.6.3.1. Determination of metals from wastewater

In wastewater, there was no specific trend observed between total recoverable and total dissolved average metal concentrations (Table 3.4). The Co and Li concentration were found to be higher in influent samples, while they either below quantification or detection limit in the corresponding effluent samples indicating their partial removal by the WWTPs. The higher concentrations of Co and Li in the influent samples may be attributed to leaching of solid particles present during the treatment processes. The Mn was present in all sludge samples ranging between 192 – 785 µg/L where higher concentrations were observed for total recoverable Mn than total dissolved. The potential sources include through natural soil weathering, Mn fertilizers, industrial discharges and landfill leaching (Addo-Bediako *et al.*, 2018). This could be due to that liquid sludge contains some solid particles which can increase adsorption of Mn and since total recoverable determination includes dissolved and suspended metals high concentrations are expected (Addo-Bediako *et al.*, 2018). The Ni was only quantified at the Amanzimtoti WWTP influent for total recoverable and total dissolved (118.9 and 235 µg/L). However, it was below the quantification limit in the effluent and other samples thus an indication of efficient removal of Ni at the Amanzimtoti WWTP. A study conducted by Akan *et al* (2008), on heavy metals in the Jakara wastewater channel in Nigeria showed concentrations ranging between 11650 – 18450 µg/L for Ni which are significantly higher compared to those obtained in this work.

The Pb, Sr and Zn concentrations were quantified in all waste and corresponding river water samples (where WWTPs discharge) however, they were found to be below the maximum permissible limits except for Pb. A study conducted by Akan and co-workers at the Jakara wastewater channel in Nigeria reported Pb concentrations ranging between 1230 – 2870 µg/L which are much higher when compared to this study (Akan *et al.*, 2008). The results indicate that wastewater treatment plants discharge these metals into the receiving rivers. The highest Pb concentration was found at the Northern River in both total recoverable and dissolved determination. Amanzimtoti WWTP had higher Zn concentrations in the effluent for total dissolved determination. This might be due to particulate Zn in the influent being transferred to the aqueous phase in aeration tanks used in the WWTPs. Also, higher amount of metals in the activated sludge may be transferred to the aqueous phase in aeration tanks which might have increased the amount of Zn present in the effluent (Yamagata *et al.*, 2010). The Co was only detected in Amanzimtoti influent and sludge at 37.4 µg/L and 28.6 µg/L, respectively as well as in Northern Works influent and sludge at 37.6 µg/L and 40.7 µg/L, respectively). The Li was only detected in Amanzimtoti influent and sludge at 13.9 µg/L and 20.3 µg/L, respectively and also in Northern Works sludge (17.6 µg/L) and Umhlathuzana influent (7.3 µg/L). Lastly, Cd, Cr, Cu, Co, Li, Ni and Tl were below detection or quantification limits.

Table 3.4: Average total recoverable (TR) and total dissolved (TD) metal concentrations (µg L^{-1}) obtained in wastewater, river water and sludge samples using microwave assisted method, n = 3

	Amanzimtoti								**Umhlathuzana**										**Northern Works**							
	TR				***TD***				***TR***					***TD***					***TR***				***TD***			
	Inf	***Eff***	***SG***	***RV***	***Inf***	***Eff***	***SG***	***RV***	***Inf 1***	***Inf 2***	***Eff***	***SG***	***RV***	***Inf 1***	***Inf 2***	***Eff***	***SG***	***RV***	***Inf***	***Eff***	***SG***	***RV***	***Inf***	***Eff***	***SG***	***RV***
Mn	95.8	100	396	bdl	115	87.0	363	bdl	107	Bql	101	200	bdl	102	Bql	bql	192	bql	bql	bql	785	bql	bql	bql	715	bql
Pb	bql	bql	59.1	45.8	40.4	18.2	bql	29.5	bdl	Bql	bdl	bdl	28.3	23.2	Bdl	40.3	bql	45.6	225	bdl	bdl	34.4	bdl	54.7	bql	63.9
Sr	233	98.5	117	465	96.5	59.0	88.2	462	56.9	35.4	30.6	78.1	69.2	61.4	30.0	38.6	87.6	70.9	50.1	41.6	83.7	437	82.6	81.9	92.1	416
Zn	302	72.4	58.6	16.0	79.0	151	21.9	15.7	37.3	34.3	129	49.1	56.7	114	93.6	62.5	90.9	129	125	49.2	102	332	223	138	9.7	339

* bdl – below detection limit, bql – below quantification limit, Inf – influent, eff – effluent, SG – sludge, RV – River, Inf 1 – Marian Ridge, Inf 2 – Shallcross

3.6.3.2. Determination of metals from tap and river water

The digestion of metals from tap and river water was conducted using hotplate and microwave assisted digestion. The average concentrations observed for total recoverable metals were much higher than that of the total dissolved metal concentrations in tap and river water (Table 3.5). This was expected since the total recoverable metal concentrations consider the soluble and insoluble metals (unfiltered samples) whilst the total dissolved metal concentration only considers the soluble metals as particulates (insoluble) are removed by filtration (US EPA, 1994). However, some metals were found to be higher for total dissolved compared to total recoverable especially using the microwave digestion. This could be due to that microwave makes use of a closed system digestion and apart from a considerable reduction in digestion time, it also results in minimal sample contamination and loss of volatile metals such as As, Hg and Cr (Sastre *et al*., 2002, Lomonte *et al*., 2008). In tap water, Li concentrations were approximately the same (4.9 – 5.5 µg/L) in all samples for both digestion methods. Nevertheless, in river water, it was only quantified in Woodhouse River water (34.5 µg/L) using the microwave digestion method. There is no maximum permissible limit set for Li in drinking water, however, the obtained values are below the oral reference dosage which is 700 µg/L, (US EPA 2003), and hence the analysed tap water can be assumed to be safe for consumption. The Li concentration has also been detected in tap water at 20-160 µg/L and 0.7-59.0 µg/L from Texas and Japan, respectively, and their maximum concentrations were higher than those obtained in this work (Ohgami *et al*., 2009). The highest concentrations obtained for Sr was 90.8 µg/L in Richmond Crest sample is lower that reported in drinking water from China (1690.0 µg/L) by Zhang *et al*. (2018).

The highest concentration of Zn (142.3 µg/L) was observed at Scottsville tap water sample. The presence of Zn (147.6 - 307.1 µg/L) in drinking water from Jordan has also been reported by Massadeh *et al*. (2020). Although its concentrations were higher than those reported in this work, they were still below the permissible limits. The presence of Zn and Li may be attributed to the inefficiency in water treatment processes employed to remove these particular metals from water distributed to household taps. The Pb was the only metal found in all tap water samples (59.2 - 155.1µg/L) that was present above the permissible limit of 50.0 µg/L. This indicates that the tap water from all the sampling areas is not safe for human consumption as Pb is one of the most toxic metals which could lead to permanent damage of the nervous system, brain and kidneys in humans and animals. The presence of Pb in tap water might be from plumbing systems in household with Pb containing solder, pipes, fittings, or service connections to homes (Mebrahtu *et al*. 2011). These findings agree with those obtained by Massadeh *et al*. (2020) where concentrations of Pb above permissible limits were observed in drinking water from Jordan (7.7 - 60.6 µg/L). These results indicate that the drinking water pollution by Pb is a prevalent global problem. The Cu and Co were not found in tap water samples, while Cr, Tl, Mn, Ni and Cd were either below detection or quantification limits. Even though, the concentration levels for all other heavy metals in this study are within the permissible limits, the high Pb concentration need to be continuously monitored to ensure that tap water is safe for human consumption.

In river water, Zn was present in most samples with its highest concentration in Woodhouse sample (58.4 µg/L) which is lower than the Zn concentrations (200.0 µg/L) obtained in the Steelpoort River in the Limpopo Province by Addo-Bediako *et al*. (2018). The increased concentration may be due to the sorption of Zn by hydrous metal oxides, clay minerals and organic material commonly found in river systems. However, it can be toxic to organisms when present in higher concentrations (Mebrahtu *et al*., 2011). The presence of Zn could also be due to pesticide and fertilizer contamination by means of agricultural runoff into the river systems (Oguzie *et al*., 2010). The Sr and Pb concentrations were detected in all samples and were below the permissible limits except for Pb in YMCA and College Road. The presence of Sr in water could be due to weathering of natural rocks as well as the direct discharge of wastewater into the rivers. The possible sources of Pb in river water could be the exhaust emissions from motor vehicles that can make its way into river systems resulting in the presence of Pb in high concentrations. This can have adverse effects on the surrounding environment such as inhibiting the growth in plants and affecting the central nervous system in humans upon consumption (Mebrahtu *et al*., 2011). The maximum concentration of Pb (51.8 µg/L) obtained in this work is lower than that reported in river water from Gauteng (86.73 µg/L) by Olujimi *et al*. (2018), however, they are both above the permissible limits. The concentrations of Cd, Cr, Mn, Ni, Ga, and Tl were found to be below the detection or quantification limits in river water samples.

In general, it was observed that the microwave assisted digestion was more sensitive as higher concentrations of metals were detected and quantified in all samples. Some metal concentrations were quantified using the microwave assisted method while they were either below the detection or quantification limits for the hotplate method thus suggesting that microwave digestion is more sensitive than hotplate digestion. Both digestion methods quantified the similar metals and statistical analysis showed that concentrations obtained by both hotplate and microwave digestion methods are not significantly different (Table S3.6). From the results obtained it can be concluded that hotplate can be recommended for daily routine analysis as it is a cheaper technique and hence can be accessible in any laboratory for use, since microwave-assisted digestion requires expensive instrumentation. The reason for different concentrations of heavy metals in water samples is that despite the estimation that applications are the same in nearly all countries, the consumption pattern may be different. For some applications which may have been phased out in some countries, may be significantly used in other countries which can result in the presence or absences of certain heavy metals in water systems (Olujimi *et al*., 2012, Akan *et al*., 2008).

Table 3.5: Average concentrations (µg/L) for total recoverable (TR), total dissolved (TD) metals in tap and river water from hotplate and microwave digestion methods and permissible limits (µg/L), (WHO, 2008; USEPA, 2003), n = 3

Tap water																				
Metal	**Hotplate Digestion**										**Microwave assisted digestion**									
	RC		**SV**		**MN**		**WL**		**BT**		**RC**		**SV**		**MN**		**WL**		**BT**	
	TR	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*
Li	5.4	5.1	5.0	5.0	5.0	5.0	5.0	5.4	5.0	4.9	5.3	5.3	4.9	4.9	5.1	4.9	5.0	5.0	5.0	5.0
Pb	69.3	78.6	59.2	62.5	79.2	61.1	116	82.1	75.0	62.0	84.7	62.0	74.1	58.2	155	78.9	64.0	58.6	72.0	91.6
Sr	39.1	90.8	33.4	17.2	38.9	17.2	36.4	19.4	37.8	18.2	46.5	32.1	41.1	33.9	40.1	34.8	37.1	33.1	36.9	36.3
Zn	23.5	12.5	114	58.9	37.0	19.8	50.2	121	65.5	20.0	31.5	11.5	143	118	111	28.5	46.8	28.3	33.7	40.4
River water																				
	BS		**CR**		**CD**		**WH**		**YMCA**		**BS**		**CR**		**CD**		**WH**		**YMCA**	
	TD	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*	*TD*	*TR*
Ga	bql	bql	bql	bql	bql	bql	bql	Bql	bql	bql	bdl	nql	bdl	nql	bdl	nql	bdl	nql	bdl	nql
Pb	30.1	36.4	30.6	30.6	30.1	32.5	43.7	30.9	34.4	52.3	27.8	32.4	51.7	51.8	27.1	40.4	42.6	35.3	42.4	27.0
Sr	38.9	73.3	40.5	69.1	65.2	120	42.7	77.2	39.7	76.0	70.3	66.2	86.7	72.1	115.3	131.4	75.4	131.8	70.3	71.4
Zn	15.5	5.9	bdl	9.7	6.8	bdl	bdl	17.2	5.9	13.4	32.2	8.8	48.7	11.4	9.0	23.9	12.0	58.4	12.2	bdl

bdl – Below detection limit, bql – Below quantification limit, BS-Bishopstowe, CD-Camps Drift, CR—College Road, WH-Woodhouse, RC-Richmond crest, SV-Scottsville, MN-Mkhondeni, WL-Woodlands, BT-Boughton

3.6.4. Removal efficiency of WWTPs

The removal efficiency (%) of heavy metals in WWTPs was calculated using equation 3.1:

$$\text{Removal efficiency} = \frac{C_{influent-}\ C_{effluent}}{C_{influent}} \times 100 \qquad (3.1)$$

where C_{influent} and C_{effluent} are the concentration obtained in the raw influent and final effluent, respectively.

The Cd and Li were also completely removed from the Amanzimtoti WWTP. The Mn was completely removed at Umhlathuzana, while -106 and 3.9% was removed at Amanzimtoti and Northern, respectively. The Pb removal was 25, -125 and 14.7%, while Sr was 37.6, 55.4 and -20.3%; and Zn was 07.9, -9.1 and -30.1% at Amanzimtoti, Umhlathuzana and Northern, respectively. The Zn showed negative removal in all WWTPs which indicated it high persistence within the wastewater treatment plants. A study conducted in Japan by Yamagata and co-workers revealed that Zn on adsorbed particulates could be easily removed however, there was difficulty in the removal of dissolved Zn in the influent during the activated sludge process at the WWTP (Yamagata *et al.*, 2010). Amanzimtoti showed to have better removal efficiency of most of the metals compared to Umhlathuzana and Northern Works. This may be due to the differences in water treatment processes employed at the wastewater treatment plants, despite the processes such as physical and biological treatment being similar, the difference may be due to the chemical processes employed to remove contaminants including heavy metals. These results indicate that the WWTPs contributes towards pollution of heavy metals on the river where they discharge their effluents.

3.6.5. Anion concentration

The common inorganic anions analysis in drinking, wastewater, surface waters, ground, as well as sludge is one of the most important analyses to ensure the reduction of pollutants discharge into water bodies and being consumed by humans and animals.

Phosphates were detected in all water samples (Table 3.6). The presence of phosphates in water is caused by the existence of essential nutrients, however, over-fertilization, agricultural and industrial resources tend to increase the phosphates in natural surface water which may result in eutrophication and excessive algal bloom (Altundag *et al.*, 2019). The concentration of phosphates was found to be lower in the effluent compared to the corresponding influent which could be due to the treatment processes applied in the plant and also adsorption on the sludge (El-Nahhal *et al.*, 2014). The highest phosphate concentration was found in sludge samples in Amanzimtoti WWTP (175 mg/L) possibly because of the solubility and pH effect as low pH values permits the adsorption of phosphate on sludge. Sludge samples had a pH of 7.36 ± 0.03 hence, phosphates were detected as this pH influenced them to strongly bind to the sludge. Also, phosphoric acid is a weak acid with three dissociations hence the phosphate levels observed in the sludge are those found in a neutral pH. However, more phosphates are expected to be found on more acidic pH (El-Nahhal *et al.*, 2014). The highest concentration of the phosphates in river water was observed in Amanzimtoti River (13.0 mg/L) which is higher compared to that of a study conducted in Sakarya River in Turkey which was 2.72 mg/L (Altundag *et al.*, 2019).

The chloride concentrations were found to be below the maximum acceptable values except in Amanzimtoti and Northern River water samples. Scottsville tap water contained the highest chloride level than all tap water samples (8.12 mg/L), however, they were below the maximum allowable limit. It is expected for chlorides to be present in tap water since chlorination is a common disinfection method used in water treatment. In river water, the highest concentration above maximum limit was found in Amanzimtoti (3064.54 mg/L) which is higher than 78.52 mg/L observed at the Sakarya River in Turkey (Altundag *et al.*, 2019). The chlorides concentration in the river water could be due natural sources such weathering of rocks and concentrations can increase because of evaporation. Despite chloride being an essential nutrient in humans, its high levels in drinking water can lead to complications in people who have kidney disorders and hypertension (Altundag *et al.*, 2019).

The highest nitrate concentration in river water was observed at Bishopstowe (6.42 mg/L), however, these results are lower than those observed by Altundag *et al.* (2019) in Sakarya River in Tukey (920 mg/L). Nitrates can reach surface waters from agricultural activity (fertilizers), oxidation of nitrogenous wastes, human and animal excreta. Scottsville tap water recorded the highest nitrates concentration in all tap water samples. Nitrates in tap water may be due to nitrite being formed chemically in distribution pipes by *Nitrosomonas* bacteria during the stagnation of nitrate-containing and oxygen poor drinking water in galvanized pipes. Another reason could be if chlorination (not well-controlled process) is used as a disinfectant thus increasing the nitrate concentration in the tap water (WHO 1984). The reduction of nitrate to nitrite gives rise to its toxicity in humans and high concentrations can cause methaemoglobin, that is the oxidation of haemoglobin (Hb) to methaemoglobin (metHb), thus be unable to transport oxygen to tissues and throughout the body (WHO, 1984). In wastewater, Amanzimtoti WWTP sludge had the highest concentration of nitrates (39.57 mg/L) while the influent had the lowest concentration at 0.32 mg/L. The Northern Works and Umhlathuzana also revealed high concentrations in the effluent compared to the influent, however, they were all below acceptable levels. The high concentration of nitrates in effluent compared to influent could be due to re-concentration of cations and anions which results in the conversion of ammonium hydroxide to nitrates in the presence of oxygen and nitrifying bacteria within the wastewater treatment plant (El-Nahhal *et al*., 2014).

Ammonia levels were found to be the highest in Richmond Crest tap water (23.51 mg/L), while the highest concentration of ammonia in river water was observed at Woodhouse (3.91 mg/L). Ammonia can have toxic effects on humans when consumed in large amounts and result in compromised capacity to detoxify. High levels of ammonia can also lead to toxic build up in internal tissues and blood in aquatic organisms. The main source for ammonia is human faeces which contain a high fraction of protein due to high consumption of protein (El-Nahhal *et al*., 2014). All WWTPs successfully removed the ammonia from the influent water resulting in low concentrations in the effluent and river water samples. The higher concentrations of ammonia in influent than effluent could be due to that of the organic fraction of nitrogen from fertilizers and other agricultural products containing ammonia (Altundag *et al*., 2019).

The highest concentrations of sulfates above permissible limits were observed in the river samples for both Amanzimtoti (437.80 mg/L) and Northern works (292.47 mg/L). Sulphate is most common anion after bicarbonate and chloride (Altundag *et al*., 2019). Sulphates occur naturally in minerals such as barite, epsomite and gypsum which can contribute to the sulphate content in drinking water. Other sources include fertilized agricultural lands and sewage treatment plants (Altundag *et al*., 2019). The high concentration of sulphates in the water samples may also be due to the high solubility of sulphates in river water which is not pH dependent thus increasing the concentration (El-Nahhal *et al*., 2014, Jing *et al*., 2013). Although, high sulphate concentrations can result in dehydration, it was reported that humans can adapt to high sulphate levels with time (WHO, 2004).

Table 3.6: Concentrations of anions (mg/L) obtained in all water samples

	Sample location	**Nutrient**				
		PO_4^{3-}	**Cl^-**	**NO_3^-**	**NH_3**	**SO_4^{2-}**
Tap water	Richmond Crest	3.00	0.50	0.03	23.51	n/a
	Mkhondeni	0.09	7.68	0.42	0.49	0.28
	Boughton	3.00	0.40	0.00	8.65	n/a
	Scottsville	0.08	8.12	0.71	0.26	0.25
River water	College Road	3.00	0.80	0.18	3.39	n/a
	Woodhouse	4.00	0.90	0.00	3.91	n/a
	YMCA	3.00	0.80	0.00	3.84	n/a
	Bishopstowe	1.00	47.17	6.42	0.24	39.15
	Camps Drift	0.26	40.53	3.81	0.34	16.01
	Mbokodweni	1.60	3064.54	3.20	0.02	437.80
	Umgeni	0.27	1454.78	2.17	0.30	292.47
	Umhlathuzana	13.00	2.8	1.74	3.60	-
Wastewater	Amanzimtoti infl	11.19	215.13	0.32	2.85	49.29
	Amanzimtoti eff	7.91	108.10	9.21	0.01	53.89
	Amanzimtoti sludge	175.67	105.62	39.57	158.44	35.72
	Northern infl	5.61	107.98	0.25	4.66	11.87
	Northern eff	3.43	103.25	32.77	0.08	59.01
	Northern sludge	126.26	96.40	3.79	44.05	66.39
	Umhlathuzana MR infl	78.00	3.4	0.00	148.29	-
	Umhlathuzana SC infl	22.00	2.3	1.72	139.44	-
	Umhlathuzana eff	24.00	3.5	2.26	9.94	-
Acceptable limits (US EPA, 2003), (WHO, 1984) & (WHO, 2004).		**-**	**250**	**45 or 50**	**-**	**250**

- = not analysed

3.7. Conclusion

The microwave-assisted and hotplate digestion methods followed by ICP-OES were successfully applied for the determination of heavy metals in tap, river, and wastewater samples. Most of the total recoverable metal concentrations were noted to be above the total dissolved which was anticipated. All metals were observed to be below the maximum limits permitted except for Pb. Regardless of both digestion methods proved to be reliable, hotplate digestion can be recommended for daily analysis as it is a more accessible and cheaper method. The anions were below the acceptable limits except for chlorides and sulphates in Amanzimtoti and Northern rivers. Despite the fact that natural occurrence and other sources plays a role towards metals and anions concentrations in the river, it was observed that WWTPs also contribute toward their pollution in the rivers. This indicated that treatments processes require improvement or perhaps new processes to be implemented to remove these heavy metals prior to discharge into river systems. The results obtained revealed pollution of the study area with anions and heavy metals, however, they are below the maximum limits except for Pb. This suggests the importance of continuous monitoring of the water resources to safeguard human health on consumption and ensure clean freshwater resources.

3.8. References

Addo-Bediako A, Matlou K, Makushu E (2018). Heavy metal concentrations in water and sediment of the Steelpoort River, Olifants River system, South Africa. *African Journal of Aquatic Science* 43:413-416. https://doi.org/10.2989/16085914.2018.1524745

Akan JC. Abdulrahman FI. Dimari GA. Ogugbuaja VO (2008). Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara wastewater channel in Kano Metropolis, Kano state, Nigeria. *European Journal of Scientific Research*. 23:122–133. https://www.eurojournals.com/ejsr.htm

Altundag H, Agar S, Altıntıg E, Ates A, Sivrikaya S (2019). Use of ion chromatography method on the determination of some anions in the water collected from Sakarya, Turkey. *Journal of Chemical Metrology* 13:14-20. https://doi.org/10.25135/jcm.26.19.03.1221

Atlas A, Usman K, Ur Rehman H, Khudadad S, Munawar A, Rab A, Khan MI, Gul M, Pervaiz M (2017). Analysis of heavy metals in river Kabul at Sardaryab Khyber Pakhtunkhwa, Pakistan. *Journal of Entomology and Zoological Studies.* 5: 14-16.

Bassioni G, Ashraf K, Elhameed A (2015). Risk assessment using ICP-MS of heavy metals in groundwater in Upper Egypt. *Journal of Natural Resources and Development*. 65-70. https://doi.org/10.5027/jnrd.v5i0.09

Das S. Ting YP. Evaluation of Wet Digestion Methods for Quantification of Metal Content in Electronic Scrap Material. *Resources*. 2017; 6:64. https://doi.org/10.3390/resources6040064

El-Nahhal IY. Al-Najar H. El-Nahhal Y (2014). Physicochemical Properties of Sewage Sludge from Gaza. *International Journal of Geoscience*. 5:586-594. http://dx.doi.org/10.4236/ijg.2014.56053

Freeman, O., Ovie, O., & Omoregie, I. (2017). Effect of Climate Change through Temperature Increase on Heavy Metals Concentrations in Water and Sediment of Ekpan Creek, Delta

State, Nigeria. *Advances In Research*, *10*(5), 1-16. https://doi.org/10.9734/air/2017/34754

Gopal K, Tripathy S, Bersillon J, Dubey S (2007). Chlorination byproducts, their toxicodynamics and removal from drinking water. *Journal of Hazardous Materials.* 140:1-6. https://doi.org/10.1016/j.jhazmat.2006.10.063

Jaishankar M, Tseten T, Anbalagan N, Mathew B, Beeregowda K (2014). Toxicity, mechanism, and health effects of some heavy metals. *Interdisciplinary Toxicology* 7:60-72. https://doi.org/10.2478/intox-2014-0009

Jing Z. Hu Y. Niu Q. Liu Y. Li YY. Wang XC (2013). UASB Performance and Electron Competition between Methane-Producing Archaea and Sulfate-Reducing Bacteria in Treating Sulfate-Rich Wastewater Containing Ethanol and Acetate. *Bioresources and Technology*. 137:349-357. http://dx.doi.org/10.1016/j.biortech.2013.03.137

Lehtonen J, Hassinen J, Honkanen R, Kumar A, Viskari H, Kettunen A *et al*. (2019). Effects of concentration on the water disinfection performance of silver containing nanocellulose-based composites. Sci Rep 9.https://doi.org/10.1038/s41598-019-56009-6

Li H, Shi A, Li M, Zhang X (2013). Effect of pH, temperature, dissolved oxygen, and flow rate of overlying water on heavy metals release from storm sewer sediments. *Journal of Chemistry :*1-11. https://doi.org/10.1155/2013/434012

Lomonte C, Gregory D, Baker A, Kolev S (2008). Comparative study of hotplate wet digestion methods for the determination of mercury in biosolids. *Chemosphere* 72:1420-1424. https://doi.org/10.1016/j.chemosphere.2008.05.033

Massadeh A, El-Rjoob A, Gharaibeh S (2020). Analysis of selected heavy metals in tap water by inductively coupled plasma-optical emission spectrometry after pre-concentration using chelex-100 ion exchange resin. *Water, Air, & Soil Pollution*, 231. https://doi.org/10.1007/s11270-020-04555-5

Mebrahtu G, Zerabruk S. (2011). Concentration and health implication of heavy metals in drinking water from urban areas of Tigray Region, Northern Ethiopia. *Momona Ethiopian Journal of Science 3*. https://doi.org/10.4314/mejs.v3i1.63689

Oguzie FA. Okhagbuzo GA (2010). Concentrations of heavy metals in effluents discharges downstream of Ikpoba river in Benin City, Nigeria. *African Journal Biotechnology*. 9:319–325. https://doi.org/10.5897/AJB09.1464

Olujimi O, Fatoki O, Odendaal J, Oputu O (2015). Variability in heavy metal levels in river water receiving effluents in Cape Town, South Africa. *Research and Practices in Water Quality*. https://doi.org/10.5772/59077

Olujimi OO. Fatoki OS. Odendaal J. Daso AP. Oputu O (2012). Preliminary Investigation into Occurrence and Removal of Arsenic, Cadmium, Mercury and Zinc in Wastewater Treatment Plants in Cape Town and Stellenbosch. *Polish Journal of Environmental Studies*. 21:1755–1765. https://www.researchgate.net/publication/285208687

Ohgami H, Terao T, Shiotsuki I, Ishii N, Iwata N (2009). Lithium levels in drinking water and risk of suicide. *British Journal Psychiatric* 194:464-465. https://doi.org/10.1192/bjp.bp.108.055798

Sastre J. Sahuquillo A. Vidal M. Rauret G (2002). Determination of Cd, Cu, Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Analytical Chim Acta*. 462:59-72. https://doi.org/10.1016/S0003-2670(02)00307-0

Rahmanian, N., Ali, S., Homayoonfard, M., Ali, N., Rehan, M., Sadef, Y., & Nizami, A. (2015). Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia. *Journal Of Chemistry*, *2015*, 1-10. https://doi.org/10.1155/2015/716125

Reeve, R. (2001). *Environmental analysis*. New York: Wiley.

Uddin A, Khalid R, Alaama M, Abdualkader A, Kasmuri A, Abbas S (2016). Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. *Journal of Analytical Science and Technology*. 7:6. https://doi.org/10.1186/s40543-016-0085-6

United States Environmental Protection Agency (1994). Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry, *Method 200.7*, Retrieved from https://www.epa.gov/sites/production/files/2015-08/documents/method_200-7_4-4_1994.pdf.

United States Environmental Protection Agency (2003). US EPA proposes drinking water rules, 40:10. https://doi.org/10.1016/s0015-1882(03)00713-4

World Health Organization (1984). Guidelines for Drinking Water Quality, Volume I — Recommendations. Geneva, 1982. *Acta Hydrochim Hydrobiology* 12: 221-223. https://doi.org/10.1002/aheh.19840120217

World Health Organization (2004). WHO issues revised drinking water guidelines, 2004: 4. https://doi.org/10.1016/s0958-2118(04)00256-3

World Health Organization (2008). 'WHO' issues revised drinking water guidelines. 2008:7. https://doi.org/10.1016/s1365-6937(04)00477-0

Yamagata H. Yoshizawa M. Minamiyama M (2010). Assessment of current status of zinc in wastewater treatment plants to set effluent standards for protecting aquatic organisms in Japan. *Environ Monit Assess*. 169: 67-73. https://doi.org/10.1007/s10661-009-1151-y/

Zhang H, Zhou X, Wang L, Wang W, Xu J (2018). Concentrations and potential health risks of strontium in drinking water from Xi'an, Northwest China. *Ecotoxicology and Environmental Safety*. 164:181-188. https://doi.org/10.1016/j.ecoenv.2018.08.01

Chapter Four

Comparison of ultrasonic and microwave assisted digestion methods for the determination of heavy metals in soil and sediment: the effect of seasonal variations on metal concentrations and risk assessment

4.1. Abstract

This study presents the analysis of twelve trace elements (Cd, Cr, Co, Cu, Ga, Li, Mn, Ni, Pb, Sr, Tl, Zn) in soil and sediment samples using microwave assisted and ultrasonic-assisted digestion prior to analysis with inductively coupled plasma optical emission spectroscopy. The recoveries obtained for all metals for the microwave assisted ranged between 81 – 101% and 80 – 98% while ultrasonic assisted recoveries were 80 – 108% and 79 – 103% for soil and sediment samples respectively. The metal concentrations obtained ranged from 0.10 – 355.4 mg/kg and 1.50 – 308.3 mg/kg in soil and sediments respectively. Most of the studied elements were below the maximum permissible limits in soil except for zinc. Both digestion methods revealed similar accuracy, indicating that both can be used for accurate determination of the target metals. However, ultrasonic assisted digestion can be recommended as an alternative method to the conventional microwave – assisted digestion since it can successfully digest without the use of extreme temperatures and pressures, and it requires inexpensive technique. Further geo-statistical analysis for heavy metal contamination in soil and sediment were assessed. The enrichment factor and geo-accumulation index ranged between 0.1 – 18.9 and -2.6 – 2.5 respectively. The potential ecological risk index showed the overall biological hazard to be the highest at Woodhouse soil classified as risk level C (strong pollution level), indicating a need for continuous monitoring of these metals.

4.2. Introduction

Heavy metal contamination is of serious concern due to its persistence in the environment (Kazi *et al.*, 2008). Heavy metals are generally produced from anthropogenic activities conducted in urban, agricultural, and industrial areas. This is due to the discharge of wastes containing heavy metals into aquatic environments in which they are transported and accumulate in surrounding soil and sediment and biomagnify through the food chain (Wu *et al.*, 2014). Natural processes and human activity also a play major role in the mobilization and redistribution of natural substances that contain heavy metals, within the environment. Heavy metal contamination has been associated with several human activities occurring at both the surrounding and outlying areas, these includes farmlands, game reserves, residential areas, natural weathering, and waste effluent discharges. As per the study conducted by Edokpayi and co-workers, Cu (566 mg/kg) and Zn (202 mg/kg) concentrations exceeded the CCME tolerable limits in the Nzhelele River sediments, therefore originating from wastewater effluents, agricultural and landfill run-offs (Edokpayi *et al*., 2017). It has been recognized that sediment and soil are considered sinks for heavy metals in aquatic environments and an indicator of heavy metals pollution in the surrounding water bodies (Wu *et al*., 2014). However, large amounts of heavy metals in soil and sediments can affect the health of plants and eventually humans and animals upon consumption. They can cause acute and chronic conditions such as stomach irritation, renal dysfunction, and more serious disease such as hypertension and cancer. The accumulation in river sediment can contaminate the surrounding aquatic systems, threatening the biological processes and aquatic organisms (Kazi *et al*., 2009; Edokpayi *et al*., 2017).

Soil and sediment are complex environmental samples and require destruction of sample matrix in order extract the analytes from the solid state into a solution prior to analysis. The conventional sample digestion methods such microwave-assisted and wet acid digestion have been used for the determination of metals (Kazi *et al*., 2008). These specific digestion methods require relatively high temperatures and pressures in order to promote complete dissolution of the contained metals from the solid sample matrix. One particular digestion method that has shown low digestion times, minimal waste produced and simplified sample treatment, is ultrasonic-assisted digestion. This method is advantageous since it heats the samples within the digestion medium with lower temperatures. Ultrasonic assisted digestion provides a source of high frequency ultrasonic energy to the sample along with an acid to initiate dissolution, agitate the sample and bring about chemical and physical change thus improves digestion process. Optimization of factors such as sonication time, temperature and solvent systems may improve the digestion efficiency of the ultrasonic assisted method as per the study conducted by Kazi and co-workers, it influenced the efficiency of digestion with shorter times and low solvent volumes when compared to the conventional wet acid digestion method of heavy metals (Kazi *et al*., 2009). Due to the novelty of ultrasonic assisted digestion, it is usually compared with other conventional methods, more specifically microwave assisted digestion (Kazi *et al*., 2009). Microwave assisted digestion is widely used and is an environmentally friendly, it significantly decreases the time required for metal extraction, has better controllable and selective heating when compared to ultrasonic assisted digestion (Knaislová *et al*., 2018). The restricting factors of the microwave assisted digestion is the accessibility and affordability of the instrumentation while ultrasonic baths are readily available and inexpensive to conduct routine analysis to monitor metal pollution in the study area.

The heavy metal contamination in soils and sediment can be assessed by geo-statistical evaluation which include enrichment factor, geo-accumulation index and potential ecological risk index. The severity of contamination is then classified based on the numerical values obtained and the potential anthropogenic sources can be identified (Choi & Jeon, 2018). The aim of this study was therefore to compare the efficiency of microwave assisted and ultrasonic assisted digestion methods for the quantitative and qualitative assessment of heavy metals in soil and sediments. The effect of seasonal variation on the heavy metal concentrations as well as geo statistical evaluation was conducted. To the best of our knowledge these studies were conducted for the first time on the soil and sediment from all the studied areas.

4.3. Experimental

4.3.1. Study area and sample collection

The selected study area is the city of Pietermaritzburg (provincial capital city) located in the KwaZulu-Natal Province (KZN) in South Africa (Figure 4.1). Soil and sediment were sampled along the Msunduzi River at Bishopstowe, Camps Drift and Woodhouse. Bishopstowe is situated in the lower region of the Msunduzi River nearby small-scale farming and residential areas while Camps Drift is located in the upper region near an aluminium company. Woodhouse is situated near a recreational golfing facility as well as food-processing and manufacturing companies which include Albany Bakery, Meadow Feeds, and Tiger Brands etc. Soil and sediment samples were collected in the dry (autumn) and wet (spring) seasons in order to assess the effect of seasonal variation on the concentrations. The samples were collected at a depth of 0-10 cm using a Dutch auger (Reliance laboratory, Germany). Portions of the surface soil samples were randomly obtained from different points in the sampling site and combined to make a representative sample of each site. They were then placed in polyethylene containers and transported to the laboratory where they air dried in a fume hood for 24 hours. Thereafter, they were then crushed, ground using a clean and dry mortar and pestle followed by sieving through a 400 μm sieve.

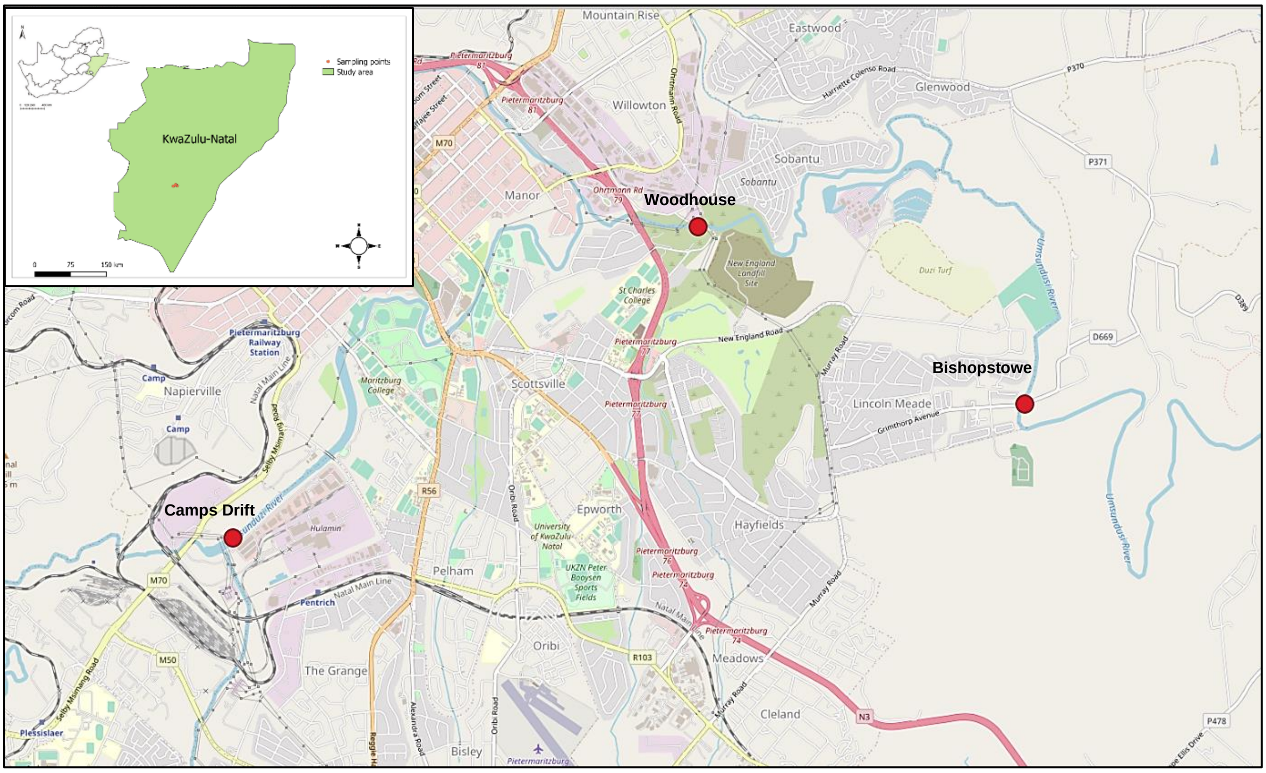


Figure 4.1: Geographical map showing the sampling points in the study area

Table 4.1: Geographical coordinates for the sampling locations (decimal degrees)

Sampling region	Sampling points	GPS coordinates
Pietermaritzburg (KZN)	Bishopstowe	30.447º - 29.618º
	Camps Drift	30.365º - 29.630º
	Woodhouse	30.413º - 29.602º

4.3.2. Reagents, reference materials and standards

Ultrapure water (Purelab, 18.2 MΩ.cm) was used in the preparation of all calibration standards and samples and to cleanse all glassware along with 5% v/v nitric acid. Nitric acid (55% HNO_3) (Sigma Aldrich, South Africa) was used in the preservation and digestion processes of the samples. The standard solutions were prepared using appropriate dilutions of a 1000 mg.L^{-1} ICP Multi-element standard (Sigma Aldrich, South Africa). The standard reference material of trace elements in a 5% v/v HNO_3 matrix (ULTRASPEC® Multi-Element Aqueous CRM) was employed to evaluate the accuracy and precision of the digestion method by means of spiked recovery tests for the extraction of trace metals in soil and sediment samples.

4.3.3. Instrumentation

The Multiwave 5000 (Anton Paar, Johannesburg) and ultrasonic bath (Science Tech, South Africa) were used for digestion of the soil and sediment samples. The Multiwave 5000 consisted of a closed vessel microwave system, with an SVT carousel rotor which can occupy a maximum of 20 digestion vessels. The temperature of the microwave exhibits a maximum temperature of 250°C and pressure of 100 bar. The Varian 720-ES ICP-OES (Varian, Australia) was used for determination of metals in water. The operating conditions of the ICP-OES instrument consisted of a frequency of 40MHz, RF power of 1.00kW and Argon as the inert carrier gas. A pump rate of 15rpm and a pneumatic concentric nebulizer with a flowrate of 0.75L/min was used.

4.3.4. Sample preparation

4.3.4.1. Microwave-assisted acid digestion for soil and sediment samples

The microwave assisted acid digestion process was adopted from the United States Environmental Protection Agency for the extraction/dissolution of trace metals in soil, sediments, sludge, and oils (US EPA 3051A). A 0.500g soil/sediment samples was weighed and transferred into a microwave vessel. A 10 mL of HNO_3 was added to the vessel under the fume hood to prevent an escape of toxic fumes from the acid and the vessel was sealed and placed into the microwave system. The temperature programme at which the digestion occurred was 175 ± 5℃ in approximately 5.5 ± 0.25 min and remain at 175 ± 5℃ for 4.5 minutes. The total digestion time was 10 minutes. After cooling, the contents were filtered using Whatman 70 mm filter paper, centrifuged at 3000 rpm. The filtrate was decanted from the centrifuge tube into a 100 mL volumetric flask and filled to the mark with ultrapure water. The aqueous samples were then transferred into ICP tubes for analysis.

4.3.4.2. Ultrasonic-assisted acid digestion for soil and sediment samples

The ultrasonic-assisted acid digestion method involved the weighing of 0.500g of the soil/sediment sample to a 100 mL Erlenmeyer flask followed by addition of a 5 mL portion of HNO_3. The flask along with the sample-acid mixture was placed in an ultrasonic bath at a temperature of 80°C for 22.5 minutes. This was followed by addition of another 5 mL portion of HNO_3 and further ultrasonicated for 22.5 minutes. The flask was left to cool for 5 minutes, and the contents were filtered (Whatman 70 mm) and centrifuged at 3000 rpm. Thereafter, the filtrate was transferred into a 100 mL volumetric flask and made up to the mark with ultrapure water and analysed with ICP-OES.

4.4. Analytical figures of merit for the determination of metals in soil and sediments

The performance of the analytical methods was assessed based on linearity, limits of detection (LOD), limits of quantification (LOQ) and percentage recovery tests. The mixture of heavy metals with concentration ranging from 0.05-10 mg/L were used to calibrate the ICP-OES and to assess linearity of the method. The intensities of ten blanks (n = 10) were measured. The LODs and LOQs for the species under study, were calculated as three and ten times the standard deviation (σ) of the average of ten individually prepared blank solutions. The assessment of the accuracy was conducted by spiking the soil and sediment samples with a certified reference material (CRM) containing metals, prior to employment of digestion methods. Thereafter, the percentage recoveries were calculated. Statistical t-tests were conducted to investigate any differences in the mean percentage recoveries.

4.5. Environmental contamination of heavy metals in soil and sediments

4.5.1. Enrichment factor (EF)

The enrichment factor (EF) is used to estimate the extent of contamination in soil and sediments and to differentiate between heavy metals originating from anthropogenic activities or natural sources. The enrichment factor is generally expressed as a ratio of the examined metal concentration in the sample (C_x) and background concentration of the examined metal (B_x) calculated from equation 4.1.

$$EF = \frac{C_x / C_{ref}}{B_x / B_{ref}} \qquad (4.1)$$

Where the background concentrations (mg/kg) for Co, Cr, Cu, P, Ni and Zn are 20.0, 71.9, 6.6, 6.6, 15.0 and 45.2 mg/kg for soil and 14.6, 71.3, 27.3, 31.9, 29.7 and 94.4 for sediment (Herselman, 2005).

The numerical results are indicated by different pollution levels and EF is classified into the five contamination categories as shown below.

- EF < 2: minimal
- 2 < EF < 5: moderate
- 5 < EF < 20: significant
- 20 < EF < 40: Very high
- EF > 40: extremely high

4.5.2. Geo-accumulation index (I_{GEO})

The index of geo-accumulation is used to grade the level of the metal contamination. It is calculated by the expression in equation 4.2 using the examined metal concentration in the sample represented by C_n and the background concentration of the examined metal by B_n.

$$I_{\geq 0} = \ ln \frac{C_n}{1.5\ B_n} \qquad (4.2)$$

The constant 1.5 is considered to be the natural variation in the sample and effect of anthropogenic sources. The geo-accumulation index is categorized into seven grades to compare their relative soil quality in Table 4.2.

Table 4.2: Grades of geo-accumulation index (Choi and Jeon, 2018)

Grade	Value	Soil quality
0	$I_{GEO} \ll 0$	Practically uncontaminated
1	$0< I_{GEO} <1$	Uncontaminated to moderately contaminated
2	$1< I_{GEO} <2$	Moderately contaminated
3	$2< I_{GEO} <3$	Moderately to heavily contaminated
4	$3< I_{GEO} <4$	Heavily contaminated
5	$4< I_{GEO} <5$	Heavily to extremely contaminated
6	$5< I_{GEO}$	Extremely contaminated

4.5.3. Potential ecological toxicity risk (PERI)

The potential ecological risk index (RI) is an indication of biological toxicity based on the degree of contamination (C_D), toxic response factor (T_R), while the potential ecological risk factor (E_R) is used to calculate the overall biological hazard (RI). The parameter is calculated using the equations below.

Contamination factor $(EC_f^i) = \frac{C_D^i}{C_R^i}$ (4.3)

where C_D^i refers to the heavy metal in the sample and C_R^i is the background concentration of the heavy metal as baseline.

Potential ecological risk factor $(E_R^i) = T_R^i \times C_f^i$ (4.4)

where T_R^i is the biological toxicity weight of a single element (Cd =30; Cr = 2; Co = 5; Cu = 5; Ni = 5; Pb = 5 and Zn =1) and C_f^i is the contamination level of a single element.

Overall biological hazard $(RI) = \sum_{i-1}^{m} E_R^i$ (4.5)

The calculations above are used to classify the degree of pollution along with their associated risk level in the Table 4.3 below.

Table 4.3: Potential ecological risk index as biological toxicity (Jiang *et al*. 2014)

E_R^i	**Pollution degree**	**RI**	**Risk level**	**Risk Degree**
$E_R^i < 30$	Slight	RI < 40	A	Slight
$30 \le E_R^i < 60$	Medium	40≤ RI < 80	B	Medium
$60 \le E_R^i < 120$	Strong	80 ≤ RI < 160	C	Strong
$120 \le E_R^i < 240$	Very strong	160 ≤ RI < 320	D	Very strong
$240 \le E_R^i$	Extremely strong	320 ≤ RI	-	-

4.6. Experimental

4.6.1. Analytical figures of merit for the digestion methods

The analytical method was validated by means of linearity, limit of detection (LOD), limit of quantification (LOQ). The calibration curves for all the metal analytes had R^2 values above 0.99 which indicated a good degree of linearity. The LOD and LOQ values were calculated from the calibration curves and converted from the standard mgL^{-1} to mg/kg, it ranged from 0.02 – 22.22 and 0.06 – 74.07 mg/kg (Table 4.4). The LOQ values for Ba, Co, Cd, Li, Sr, and Zn were low which allowed for accurate quantification of these metals in soil and sediments, especially in at trace levels. The LOQ values were relatively high for the other metals analyzed however concentrations below the quantification limit would be detected but not accurately quantified and thus be unreliable.

Table 4.4: The LOD, LOQ and Correlation coefficient (R^2) and the maximum permissible limits (MRL) for the metals in soil (WHO, 2001)

Metal analyte	**LOD (mg/kg)**	**LOQ (mg/kg)**	**R^2**	**MRL, mg/kg (WHO, 2001)**
Barium (Ba)	0.33	1.09	0.9998	-
Cobalt (Co)	0.44	1.45	0.9999	40
Chromium (Cr)	19.52	65.07	0.9995	100.0
Copper (Cu)	17.71	59.02	0.9999	36
Cadmium (Cd)	0.31	1.04	0.9999	0.8
Gallium (Ga)	17.07	56.89	0.9995	-
Lithium (Li)	0.02	0.06	0.9993	-
Nickel (Ni)	8.54	28.47	0.9999	35
Lead (Pb)	5.69	19.70	0.9997	85
Strontium (Sr)	0.64	2.12	0.9980	-
Thallium (Tl)	22.22	74.07	0.9995	-
Zinc (Zn)	1.32	4.42	0.9999	50.0

4.6.2. Ultrasonic digestion method optimization

4.6.2.1. Effect of single versus sequential digestion and the effect of digestion time on the recovery

This effect was investigated by digesting 0.5g spiked soil/sediment samples with one portion of 10 mL HNO_3 and ultrasonicating for 60 minutes. The sequential digestion was investigated by using two portions of 5 mL HNO_3 where 5 mL was initially added to a 0.5g spiked soil/sediment samples and ultrasonicated for 30 minutes. Then another 5 mL was added and further ultrasonicated for 30 minutes to make a total digestion time of 60 minutes. The percentage recoveries ranged between 71 – 86% and 71 – 82% for soil and sediment respectively using a single 10 mL HNO_3 addition. For two portions of HNO_3 the recoveries ranged between 75 – 111% and 76 – 109% for soil and sediment respectively (Table 4.5). This revealed that the successive addition of smaller volumes of acid allowed for more efficient digestion (Reeve 2002). A statistical t-test was conducted to investigate if the mean recoveries were significantly different. The p-values using 10 mL and two x 5 mL portion were $p < 0.031$ and $p < 0.027$ (Table S4.1) for soil and sediment respectively. Since the p-values are less than 0.05, it indicated that the mean recoveries are significantly different. Therefore, the two x 5 mL additions were selected at the preferred method of acid addition.

Sonication times of 15, 30, 45 and 60 minutes were assessed in order to determine the optimum time that will allow efficient digestion of all the metals in the soil sample (Table 4.5). The mentioned sonication times were chosen on the basis of incorporating various studies evaluating ultrasonic digestion. In a study conducted by Kazi *et al*. (2008) using ultrasonic acid digestion to evaluate the heavy metal content in solid samples (soil, sediment, and sludge), 25 minutes and 80°C were found to be optimum time and temperature. In studies conducted on ultrasonic extraction of metals (Cd, Cu, Pb and Mn) in biological samples, a sonication times of 18 min at 70°C (Mierzwa *et al*., 1997) were found to be optimum. On the contrary, in a study conducted by Minami and co-workers for quantitative determination of the same metals in powdered biological samples which made use of a similar sonication device, the optimum sonication was found to be 5 min at 40°C (Minami *et al*., 1996).

In the current study, the increase in sonication time showed an increase on metal recovery up to 45 minutes for most metals and they decreased at 60 minutes. This could be due to that ultrasonic agitation is known to initiate dissolution of the metal analytes from the solid samples however, excessive agitation can affect the extraction efficiency. Also, agitation exceeding the optimum time for digestion can possibly result in the agglomeration of the metals onto the soil particles (Ghafurian *et al*. 2019). Similarly, to a study conducted by Knaislová *et al* (2018) where the extraction of Cu, Ni and Co decreased for a long leaching time, this was possibly due to reverse precipitation reactions within the digestion flask. Statistical t-tests conducted gave p-values less than 0.05 for 15 and 30 minutes (Table S4.2) compared with 45 minutes therefore an indication of significant differences in their mean recoveries. The t-test conducted on the mean recoveries obtained at 45 minutes for both soil and sediment had a $p > 0.543$ which is greater than 0.05 indicating no significant difference (Table S4.3). Conclusively, 45 minutes was selected as the optimum sonication time for both soil and sediment as per the percentage recoveries obtained by the spiked recovery tests.

Table 4.5: Spiked recovery test (%) to assess the effect of single versus sequential digestion and digestion time, (n = 3).

Metal analyte	**Effect of single and successive digestion**				**Effect of digestion time**							
	60 min @ 80°C (10 mL HNO_3)		**2 x 30min @ 80°C (2 x 5 mL HNO_3)**		**15 minutes**		**30 minutes**		**45 minutes**		**60 minutes**	
	Soil	***sediment***	***soil***	***sediment***	***Soil***	***sediment***	***soil***	***sediment***	***soil***	***sediment***	***soil***	***sediment***
Ba	86	79	86	92	73	75	88	96	92	87	86	92
Co	78	77	82	81	77	75	81	81	84	83	82	81
Cr	79	81	81	83	75	75	81	87	89	86	81	83
Cu	77	82	111	109	82	88	87	92	82	87	111	109
Cd	72	71	76	76	74	70	76	77	85	89	76	76
Ga	76	77	81	76	78	72	84	77	90	87	81	76
Li	79	79	86	83	79	77	84	84	85	82	86	83
Ni	80	77	85	84	82	79	81	84	88	85	85	84
Pb	78	78	86	104	81	81	88	74	83	81	86	104
Sr	78	76	81	79	88	85	92	93	94	92	81	79
Tl	71	73	77	79	81	76	77	86	82	80	77	79
Zn	77	76	75	78	85	72	76	120	89	92	75	78

4.6.3. Validation of ultrasonic and microwave-assisted digestion

The two digestion methods were validated by spiking soil and sediment samples with a final concentration of 0.50 mg/L CRM containing all the metal analytes. The percentage recoveries obtained for the microwave assisted ranged between 81 – 101% and 80 – 98% while ultrasonic assisted recoveries were 80 – 108% and 79 – 103% for soil and sediment samples respectively (Table 4.4). The recoveries obtained were comparable for both ultrasonic and microwave assisted extraction, however for a well-established method, the interferences were greater than 20%. In the quantitative analysis, robust ICP-OES conditions were used, in which the optimum analysis conditions set automatically by the instrument. Also, they were described similar to the normal operating conditions as per a study conducted by Mermet and co-workers. These conditions as per the study were as follows, 1.0kW power, 0.85 l/min nebulizer flow rate with a sample uptake of 1 ml/min (Mermet *et al*., 1997), while the conditions used were, 1.0kW power and 0.75 l/min. In addition to the percentage recoveries, statistical t-tests approved that the mean recoveries are not significantly different with p-values $p < 0.635$ and $p < 0.718$ (Table S4.4) for soil and sediment respectively.

Table 4.6: %Recoveries for CRM spiked soil and sediment samples using both digestion methods, (n = 3)

Metal analyte	**Microwave-assisted digestion**		**Ultrasonic digestion**	
	soil	***sediment***	***soil***	***sediment***
Ba	101	98	88	96
Co	96	89	82	80
Cr	93	96	89	94
Cu	91	87	108	103
Cd	89	82	80	83
Ga	89	80	88	85
Li	92	81	90	86
Ni	88	84	87	85
Pb	81	80	96	93
Sr	85	88	89	86
Tl	89	90	80	81
Zn	82	84	83	79

4.6.4. Physicochemical properties of soil and sediment

The physicochemical properties of soil and sediment samples were measured and presented in Table (4.6). The measured temperature range of the soil and sediments were 21 – 24℃ with slight acidic to slightly basic pH range (6 – 8). The conductivity of the soil and sediment samples was found to be between 69 – 1060 µS, where the highest conductivity was observed in Bishopstowe sediment during Winter. This may be due to high salinity (0.4 psu), since in semi-arid to arid regions, low rainfall occurs or the use of saline irrigation water can allow soluble salts to accumulate and remain on the sediment surface. Conversely, (Salem *et al*., 2020). Organic matter plays a key role in the mobilization of heavy metals in soil and sediments, it can result in the decrease in available concentrations in soils due to complexation, adsorption, and precipitation (Collin & Doelsch, 2010). The organic matter of the soil and sediment samples were found to be between 2 – 7 and 4 - 5 respectively, with the lowest OM content at Woodhouse which may be attributed to the sandy texture of the soil. High temperature of soil increases and enhances the degradation of OM. It was reported that low organic matter in the soil is not favourable for HM absorption thus decreasing their presence in the soil and sediment samples (Bhatti *et al*., 2016).

Table 4.7: Physicochemical properties of soil and sediments

Type of samples	**Sampling points**	**pH**	**Temp (℃)**	**Salinity (psµ)**	**TDS (ppm)**	**Conductivity (µS)**	**D.O (mg/L)**	**Organic matter (OM)**
Soil`	College Road	6	23	0.2	176	351	8	6
	Woodhouse	7	23	0.1	115	230	2	2
	Bishopstowe	7	24	0.1	115	229	8	7
Sediment (Winter)	Woodhouse	8	22	0.1	75	206	10	5
	Bishopstowe	7	21	0.4	796	1060	10	4
Sediment (Spring)	Woodhouse	6	22	0.04	35	69	10	5
	Bishopstowe	8	23	0.04	39	78	8	4

4.6.5. The effect of seasonal variations on the metal concentrations

The effect of seasonal variation was investigated using soil and sediment samples collected during autumn (dry) and spring (wet) seasons in which rainfall occurrence varies distinctively. The Cd and Tl concentrations were undetected in soil and sediment samples for both digestion

methods (Table 4.7 and 4.8). In a study conducted on Jamaican soils, Cd concentrations between 0.2 - 409 mg/kg were reported in soils (Lalor *et al*. 1998) which are remarkably higher than the permissible limits of 0.8 mg/kg.
The Ba was detected in all soil and sediment samples at high concentrations which were also higher in spring than autumn season. There are no specified permissible limits for Ba however, concentration of 200 mg/kg could be moderately toxic while concentrations of 500 mg/kg could be considered toxic in plant life (Pais *et al*., 1998). Camp's Drift soil and sediment samples had Ba concentrations above 200 mg/kg but below 500 mg/kg, hence this is a concern in plants especially in edible plants as Ba traces might be transferred through the food chain to human and animals (Ong *et al*. 2013).

The Co, Ni and Pb concentrations in soil and sediment were found to be below their respective permissible limits in soil and sediments during autumn and spring seasons, which are 40, 35 and 85 mg/kg respectively. However, despite their relatively low concentrations, persistent contamination can be toxic to the environment. In a study conducted by Nazir *et al*. (2015) where the accumulation of heavy metals in soil, water and plants were assessed near Tanda Dam, Kohat. The Pb and Ni concentrations in soil ranged between 0.061 – 0.461 mg/kg and 0.086 – 1.031 mg/kg which were exceedingly lower compared to the concentrations obtained in this work. The Cr concentrations were found to be below the maximum permissible limit of 100 mg/kg in Woodhouse soil however, they were above the limits in Camps Drift and Bishopstowe sediment samples. Similarly, in a study conducted by Osmani *et al.* (2015) concentrations above limits were obtained for Ni, Co and Cr in metallurgical soils which were 610 mg/kg, 75 mg/kg, and 370 mg/kg respectively. These concentrations were also substantially higher compared to those obtained in this work. The presence of Ni in this study could originate from surrounding to metal fabrication industry, however, in general, Ni mining and electroplating and most commonly combustion of fossil fuels can be the potential sources in other studies. The Cr contamination can be caused by the leaching of Cr compounds by rainwater travelling through cracks in soil and roadways. Other potential sources could be chromium-laden dust transported via wind that settle onto soil and the transfer of Cr into soil used in surrounding wooden structures as a wood preservative (Osmani *et al*. 2015). The Co naturally occurs in Ni bearing laterites and Ni-Co sulphide deposits, therefore it is often extracted as a by-product of Ni and Cu. It was also discovered by Cobalt Development Institute that 48% of Co-production originated from Ni ores, which can account for the Co concentrations along with Ni and Cu present in soil and sediments (Osmani *et al*. 2015).

Furthermore, it was observed that the Co concentrations were lower in spring (rainy) using both methods of digestion, a similar trend was also observed in a study conducted by Oladeji and co-workers, where the least levels of Co were recorded in the rainy season (Oladeji *et al*. 2015). Autumn is a dry season in which there is little to no rainfall hence, reduction of pollutants concentrations through dilutions and transport to different areas away from point source is limited resulting in higher concentrations observed in this study for Co. However, in a study conducted by Olorunfemi and co-workers, they reported an increase in all metal concentrations during the wet season in both soil and edible plants. This indicated that water aids in the transfer of metals from the soil to the plants and could possibly increase the metal concentration in the surrounding area (Olorunfemi *et al*., 2018).

The Cu concentrations were below the quantification limit in Bishopstowe and Camps Drift samples. Woodhouse sediment sampled during autumn had Cu concentrations above the permissible limits of 36 mg/kg. Also, the difference in Cu concentrations could be due to the difference in absorption ability in soil (Ahmed *et al*. 2019). The variation in Cu concentrations in dry and wet seasons are evident at Woodhouse, with an increase in the dry season. In a study conducted by Mondol *et al* (2011), the variations in heavy metal concentrations were evaluated in plant and water sample around Tejgaon industrial are of Bangladesh and it was concluded that rainy (wet) seasons resulted in lower concentrations. This could be due to soil receding and the surrounding water bodies can be enriched with Cu present initially in the soil. The mobility of heavy metals is dependent on the physicochemical properties of the soil and in particular, it was reported that the mobility of Cu can be related to the transport of organic matter present in the soil. It was further discovered that Cu was highly adsorbed onto the organic material present in soil as opposed to the soil solids, consequently, resulting in higher Cu concentrations during the dry season (Altaher *et al*., 2011).

The Cu, Cr, Ga, and Li concentrations were higher using the microwave-assisted digestion as opposed to ultrasonic-assisted digestion. The microwave-assisted digestion method is a closed system digestion process thus resulting in minimal to no analyte loss from the sample through the evaporation process (Pecheyran *et al*. 1998). Moreover, due to heating occurring within the digestion medium, it is therefore more efficient than conventional heating. Microwave increases the temperature rapidly while ultrasonic gradually increases the temperature to an equilibrium temperature of 80°C (Kazi *et al*. 2008), which can have an effect on the extraction efficiency.

The Sr and Ga concentrations were present in all soil and sediment samples. The Ga concentrations ranged between 16.6 – 326.8 mg/kg and 33.1 – 280.7 mg/kg. The highest Ga concentrations were observed in Camps' Drift soil and sediment samples, which could be due to that Ga present in soil is dependent on soil location and soil properties such as soil type and mineral composition. The Ga commonly accompanies metals such as Al, Ge and Zn and is considered a volatile metal, its presence is associated with the occurrence of silty minerals. The high volatility for Ga could be the possible reason for very low concentrations obtained by ultrasonic compared to microwave assisted digestion method which could indicate that it evaporated to the surrounding during the digestion process. In a study conducted by Poledniok *et al*. (2012) a strong correlation was identified between Ga and Cd, Cu, Pb and Zn, indicating that the contents of Ga are closely associated with these heavy metals in soil and may potentially originate from the same or similar source.

The Zn was found to be above the maximum permissible limit of 50 mg/kg in all soil and sediment samples except in Bishopstowe soil. The Zn concentrations ranged between 17.8 -87 mg/kg and 31.8 – 118.7 mg/kg, which was similar to those obtained by Osmani and co-workers which was 86 mg/kg for soil. The Zn is commonly found in general household items such as batteries, paints, cosmetics, pharmaceuticals and galvanized iron and steel preventing corrosion (rusting) and this could be sourced from possible dumping of refuse on surrounding soil (Olurunfemi *et al*., 2018). A high organic content in soil tends to form chelates with Zn which can ultimately increase their solubility and availability to plants (Emurotu *et al*. 2017). The Zn concentrations fluctuated, which displayed no definite trend in both dry and wet seasons. The higher concentrations of metals obtained in this work and worldwide indicate a requirement for their consistent monitoring in soil and sediments.

Table 4.8: Average concentration (mg/kg) of metals in soil samples during Autumn and Spring using Microwave-assisted and Ultrasonic digestion, (n = 3)

Sample location	**Bishopstowe**				**Camps' Drift**				**Woodhouse**				MRL, mg/kg (WHO, 2001)
Season	***Autumn***		***Spring***		***Autumn***		***Spring***		***Autumn***		***Spring***		
Metal	*Micro*	*Ultra*	*Micro*	*Ultra*	*Micro*	*Ultra*	*Micro*	*Ultra*	*Micro*	*Ultra*	*Micro*	*Ultra*	
Ba	96.4	96.6	96.0	95.4	333.8	355.4	289.6	314.7	69.6	65.1	60.9	71.1	-
Co	4.4	5.4	2.3	3.7	40.5	31.2	32.8	27.8	4.9	3.9	3.3	3.4	40
Cr	nq	nq	Nq	17.5	112.6	nq	101.3	nq	nq	nq	nq	nq	100.0
Cu	nq	nq	Nq	12.1	nq	nq	nq	nq	106.2	92.4	124.5	nq	36
Cd	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.8
Ga	90.3	nq	79.9	16.6	326.8	93.4	276.5	76.5	91.4	nq	85.2	nq	-
Li	0.9	nd	0.1	nd	9.2	2.6	7.8	1.3	nd	nd	nd	nd	-
Ni	nq	nq	Nq	8.8	41.4	nq	35.3	nq	nq	nq	nq	nq	35
Pb	nq	nq	Nq	3.7	20.7	nq	nq	nq	20.3	24.6	31.7	nq	85
Sr	11.8	15.9	13.3	16.2	23.0	21.4	22.1	22.4	4.8	4.7	2.8	5.8	-
Tl	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-
Zn	26.2	17.8	49.0	32.3	81.0	43.5	87.0	47.6	77.9	63.1	66.4	51.2	50.0

* nd – not detected, nq – not quantified.

Table 4.9: Average concentration (mg/kg) of metals in sediment samples during Autumn and Spring using Microwave-assisted and Ultrasonic digestion, (n =3).

Sample location	**Bishopstowe**				**Camps' Drift**				**Woodhouse**				MRL, mg/kg (WHO, 2001)
Season	***Autumn***		***Spring***		***Autumn***		***Spring***		***Autumn***		***Spring***		
Metal	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	
Ba	107.0	100.0	114.1	99.1	271.9	308.3	133.7	120.9	64.9	58.5	92.6	81.2	-
Co	9.8	7.7	8.6	5.2	31.9	26.5	16.3	11.6	3.9	3.0	5.3	3.9	40
Cr	102.5	70.2	103.6	73.6	117.4	nq	80.8	nq	nq	nq	nq	nq	100.0
Cu	nq	nq	Nq	nq	nq	nq	nq	nq	88.2	64.8	nq	nq	36
Cd	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.8
Ga	140.9	nq	139.0	nq	280.7	79.2	163.7	nq	92.1	nq	113.0	nq	-
Li	2.6	nd	3.0	nd	8.3	1.5	4.1	nd	nd	nd	2.0	nd	-
Ni	nq	nq	Nq	nq	35.9	nq	nq	nq	nq	nq	nq	nq	35
Pb	nq	nq	Nq	nq	nq	nq	nq	nq	22.8	20.3	22.7	23.2	85
Sr	8.5	9.5	9.3	9.2	22.3	22.6	16.7	16.9	4.0	6.1	4.3	4.2	-
Tl	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-
Zn	109.1	82.8	118.7	88.4	73.9	44.9	51.6	31.8	69.6	52.2	92.2	70.8	50.0

*nd - not detected, nq - not quantified

4.6.5. Environmental evaluation of heavy metal contamination

4.6.5.1. Enrichment factor and geo-accumulation index

The enrichment factor (EF) and geo-accumulation index (I_{GEO}) were calculated based on the average concentrations of heavy metals obtained in soil and sediment samples and their background concentrations in the respective sample matrices (Table 4.9).

The enrichment factor along with the geo-accumulation index successfully categorized the degree of heavy metals (Co, Cr, Cu, Ni, Pb and Zn) in soil and sediments during the seasons of autumn and spring. The EF values obtained for Cr and Zn in all samples indicated minimal contamination potentially derived from natural processes and geogenic sources. The Co and Ni contamination at Bishopstowe and Woodhouse were minimal however at Camps' Drift, the ER values ($2 < EF < 5$) revealed moderate contamination. The EF values were below 20 ($5< EF <20$) in all soil and sediment samples consequently indicating no severe contamination (Table 7). In addition, moderate Pb contamination (2.6 – 4.8) was observed at Camps' Drift and Woodhouse soil samples. However, Cu contamination was significant in all soil samples with EF values between 1.8 – 18.9, similarly to a study conducted by Choi *et al*. (2018) where the enrichment factors obtained for Cu and Pb in that study ranged between 1.4 – 14.0 and 0.3 – 4.9 respectively concluded significant contamination in soil from Busan, Korea.

The index of geo-accumulation (I_{GEO}) showed a similar trend observed by the enrichment factor. An EF value greater than 5 and $I_{GEO} > 1$ is considered to be an environmental concern for heavy metal contamination (Choi *et al*. 2018). Further justification was provided for the EF values obtained for Cr and Zn, which revealed negative I_{GEO} values ($I_{GEO} < 0$) classified as grade 0 indicating practically uncontaminated. Co and Ni were classified as grade 1 (uncontaminated to moderately contaminated) with $0 < I_{GEO} < 1$ at the Bishopstowe and Woodhouse. Similarly, Pb at Camps' Drift and Woodhouse soil classified as grade 1. All soil samples were moderately to heavily contaminated with Cu, with Woodhouse being most contaminated with I_{GEO} values ranging between 1.6 – 2.5. The potential sources of Cu pollution in the soil could be due to surrounding manufacturing industries and presence of river systems nearby the soil contributing to Cu contamination (Choi *et al*. 2018). The effect of seasonal variation on contamination was insignificant since the contamination severity remained unchanged during autumn and spring. Despite the variation of concentrations observed during the dry and wet seasons, the concentrations were not severely different to indicate a difference in contamination.

Table 4.10: Enrichment factor and geo-accumulation index in soil and sediment samples

Sample location			**Bishopstowe**				**Camps' Drift**				**Woodhouse**			
Season			***Autumn***		***Spring***		***Autumn***		***Spring***		***Autumn***		***Spring***	
Metal			***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***	***Micro***	***Ultra***
Co	*Soil*	*EF*	0.2	0.3	0.1	0.2	2.0	1.6	1.6	1.4	0.2	0.2	0.2	0.2
		I_{GEO}	-1.9	-1.7	-2.6	-2.1	0.3	0.1	0.0	-0.1	-1.8	-2.0	-2.2	-2.2
	Sediment	*EF*	0.7	0.5	0.6	0.4	2.2	1.8	1.1	0.8	0.3	0.2	0.4	0.5
		I_{GEO}	-0.8	-1.0	-0.9	-1.4	0.4	0.2	-0.3	-0.6	-1.7	-2.0	-1.4	-1.7
Cr	*Soil*	*EF*	0.6	0.3	0.6	0.2	1.6	0.6	1.4	0.5	0.8	0.4	0.8	0.3
		I_{GEO}	-0.9	-1.6	-1.0	-1.8	0.0	-1.0	-0.1	-1.2	-0.6	-1.3	-0.6	-1.5
	Sediment	*EF*	1.4	1.0	1.5	1.0	1.6	0.5	1.1	0.5	0.9	0.4	0.9	0.4
		I_{GEO}	0.0	-0.4	0.0	-0.4	0.1	-1.1	0.3	-1.2	-0.6	-1.3	-0.5	-1.2
Cu	*Soil*	*EF*	3.3	2.7	2.1	1.8	7.0	4.0	6.3	5.9	16.1	14.0	18.9	7.6
		I_{GEO}	0.8	0.6	0.3	0.2	1.5	1.0	1.4	1.4	2.4	2.2	2.5	1.6
	Sediment	*EF*	0.9	0.9	1.0	0.8	1.5	1.0	1.2	0.8	3.2	2.4	1.2	1.2
		I_{GEO}	-0.5	-0.6	-0.4	-0.6	0.0	-0.4	-0.2	-0.6	0.8	0.5	-0.2	-0.3
Ni	*Soil*	*EF*	1.0	0.8	0.8	0.6	2.8	1.6	2.4	1.3	0.8	0.6	0.7	0.7
		I_{GEO}	-0.4	-0.7	-0.6	-0.9	0.6	0.1	0.5	-0.1	-0.6	-1.0	-0.7	-0.8
	Sediment	*EF*	0.5	0.3	0.6	0.3	1.1	0.6	0.7	0.3	0.4	0.2	0.5	0.2
		I_{GEO}	-1.0	-1.6	-1.0	-1.7	-0.3	-0.8	-0.8	-1.5	-1.5	-1.9	-1.1	-1.8
Pb	*Soil*	*EF*	0.8	0.9	0.5	0.6	3.1	2.9	2.6	2.6	3.1	3.7	4.8	2.6
		I_{GEO}	-0.7	-0.5	-1.2	-1.0	0.7	0.6	0.5	0.5	0.7	0.9	1.2	0.5
	Sediment	*EF*	0.5	0.6	0.5	0.6	0.5	0.6	0.3	0.3	0.8	0.7	0.8	0.8
		I_{GEO}	-1.1	-1.0	-1.1	-0.9	-1.1	-0.9	-1.5	-1.5	-0.7	-0.8	-0.7	-0.7
Zn	*Soil*	*EF*	0.6	0.4	1.1	0.7	1.8	1.0	1.9	1.1	1.7	1.4	1.5	1.1
		I_{GEO}	-1.0	-1.3	-0.3	-0.7	0.2	-0.4	0.2	-0.4	0.1	-0.1	0.0	-0.3
	Sediment	*EF*	1.2	0.9	1.3	0.9	0.8	0.5	0.5	0.3	0.7	0.6	1.0	0.8
		I_{GEO}	-0.3	-0.5	-0.2	-0.5	-0.7	-1.1	-1.0	-1.5	-0.7	-1.0	-0.4	-0.7

4.6.5.2. Potential ecological risk index (RI)

The potential ecological risk index was assessed by calculating the overall biological hazard (*RI*), (Table 5.10). In Figure 2, the RI values were represented graphically for soil and sediments during autumn and spring. In general, the RI values in all sediment samples were found in the slight grade (*RI*< 40), classified as risk level A. However, in soil samples, the pollution degree increased at each sampling location where Bishopstowe was classified as slight (RI< 40), Camps' Drift as medium level (40 < RI < 80) and Woodhouse as strong (80 < RI< 120) which showed the highest biological toxicity categorized as risk level C. Woodhouse sediment was categorized as the most contaminated with a strong pollution level. In a study conducted by Choi *et al*. (2018), it was reported that amongst the various heavy metals, the biological hazard of a single element of Cu was the largest which is similar to the findings of this work. There was no distinct trend observed for the overall biological hazard during autumn and spring similarly to the enrichment factor and geo-accumulation index (Table 4.10). Even though heavy metal contamination was localized at Woodhouse, steady monitoring of potential biological risks is required.

Table 4.11: Overall biological hazard (RI) for soil and sediment samples using microwave and ultrasonic assisted digestion

Sample location	**Season**	**Soil**		**Sediment**	
		Micro	**Ultra**	**Micro**	**Ultra**
Bishopstowe	Autumn	28.0	23.9	17.4	14.1
	Spring	19.8	17.0	17.6	13.3
Camps Drift	Autumn	79.4	52.2	30.9	21.9
	Spring	68.8	57.9	19.5	12.8
Woodhouse	Autumn	104.4	94.5	25.5	18.8
	Spring	125.8	57.0	16.9	13.9

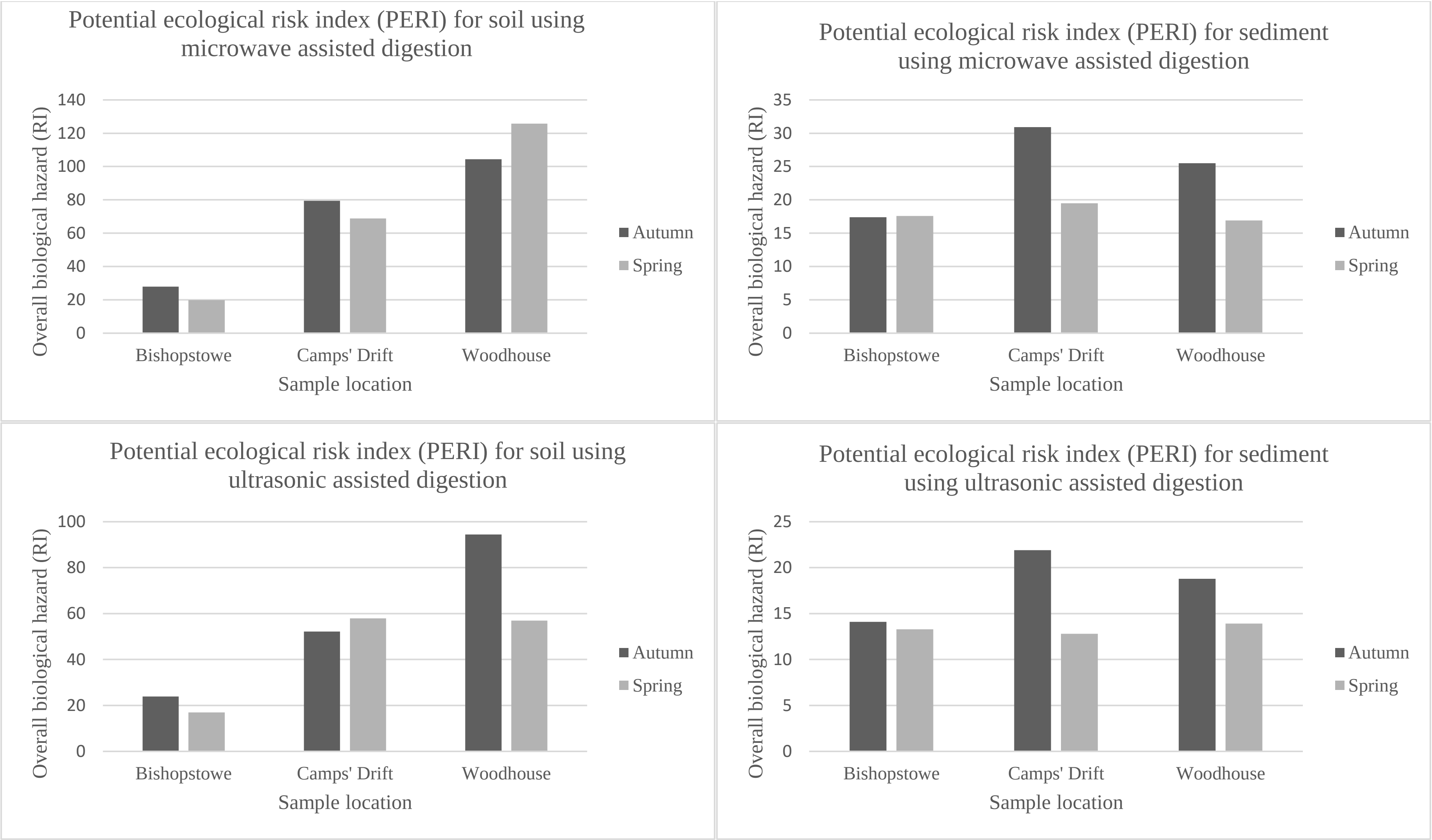


Figure 4.2: Potential ecological risk index for soil and sediment samples using microwave and ultrasonic assisted digestion

4.7. Conclusion

The ultrasonic and microwave assisted digestion followed by ICP-OES were successfully validated and applied for the determination of metals in soil and sediment. The Ba and Ga were the two most abundant metals present in high concentrations in all samples. The Co, Ni and Pb were below the permissible limits in all samples thus posing no serious environmental concerns. Both digestion methods gave acceptable recoveries during validation using the CRM, which were within the range of 80 – 120% indicating their good accuracy in digesting the metals of interest. However, the ultrasonic assisted digestion underestimated the concentrations as compared to the microwave. Some of the metals (Cu, Cr, Ga, and Li) had higher concentration using the microwave assisted digestion method making it a more sensitive digestion method. The ultrasonic assisted digestion can be used as an alternative method due to its availability and affordability as it gave acceptable recoveries of the metals. Certain metal concentrations were higher in autumn whilst others were higher in spring which highlighted the effect of seasonal variation on metal concentrations. Camp's Drift was conclusively found to be the most polluted location since most metal concentrations were amongst the highest observed. The effect of anthropogenic sources of heavy metals in soil and sediment was estimated by the evaluation of geo-statistical methods such as the enrichment factor, geo-accumulation index and potential ecological risk index. The heavy metal contamination in all samples were not severe however Cu showed significant contamination. Heavy metal pollution of soils has been reported to be worldwide problem and there is a possibility of accumulation in crops via the soil thus posing a serious health hazard in humans. Eventhough the metal contamination in this study was not severe, continuous monitoring of these soil and sediments are required for risk assessment and management.

4.8. References

Ahmed, M., Matsumoto, M., Ozaki, A., Thinh, N., & Kurosawa, K. (2019). Heavy Metal Contamination of Irrigation Water, Soil, and Vegetables and the Difference between Dry and Wet Seasons Near a Multi-Industry Zone in Bangladesh. *Water*, *11*(3), 583. doi: 10.3390/w11030583

Altaher, H., Dietrich, A., & Novak, J. (2011). Factors affecting copper sorption and mobility through A and B horizon soils from the Eastern shore of Virginia. *Yanbu Journal of Engineering And Science*, *2*(1). doi: 10.53370/001c.23749

Choi, J., & Jeon, S. (2018). A Geo-statistical Assessment of Heavy Metal Pollution in the Soil Around a Ship Building Yard in Busan, Korea. *Journal Of The Korean Society Of Marine Environment And Safety*, *24*(7), 907-915. doi: 10.7837/kosomes.2018.24.7.907

Edokpayi, J., Odiyo, J., Popoola, E., & Msagati, T. (2017). Evaluation of temporary seasonal variation of heavy metals and their potential ecological risk in Nzhelele River, South Africa. *Open Chemistry*, *15*(1), 272-282. doi: 10.1515/chem-2017-0033

Emurotu, J., & Onianwa, P. (2017). Bioaccumulation of heavy metals in soil and selected food crops cultivated in Kogi State, north central Nigeria. *Environmental Systems Research*, *6*(1). doi: 10.1186/s40068-017-0098-1

Ghafurian, M., Akbari, Z., Niazmand, H., Mehrkhah, R., Wongwises, S., & Mahian, O. (2020). Effect of sonication time on the evaporation rate of seawater containing a nanocomposite. *Ultrasonics Sonochemistry*, *61*, 104817. doi: 10.1016/j.ultsonch.2019.104817

Herselman, E., Steyn, C., & Fey, M. (2005). Baseline concentrations of Cd, Co, Cr, Cu, Pb, Ni and Zn in surface soils of South Africa. *South African Journal Of Science*, *101*(1), 509-512.

Jiang, X., Lu, W., Zhao, H., Yang, Q., & Yang, Z. (2014). Potential ecological risk assessment and prediction of soil heavy-metal pollution around coal gangue dump. *Natural Hazards And Earth System Sciences*, *14*(6), 1599-1610. doi: 10.5194/nhess-14-1599-2014

Kazi, T., Jamali, M., Arain, M., Afridi, H., Jalbani, N., Sarfraz, R., & Ansari, R. (2009). Evaluation of an ultrasonic acid digestion procedure for total heavy metals determination in environmental and biological samples. *Journal Of Hazardous Materials*, *161*(2-3), 1391-1398. doi: 10.1016/j.jhazmat.2008.04.103

Knaislová, A., Vu, H., & Dvořák, P. (2018). Microwave and Ultrasound Effect on Ammoniacal Leaching of Deep-Sea Nodules. *Minerals*, *8*(8), 351. doi: 10.3390/min8080351

Lalor, G., Raitray, R., Simpson, P., & Vutchkov, M. (1998). Heavy metals in Jamaica. Part 3: The distribution of cadmium in Jamaican soils. *Rev International Contamination Ambient*, *14*(1), 7-12.

Mermet, M., Marichy, M., & Mermet, J. (1987). Relationship between detection limits and mechanisms in inductively coupled plasma atomic emission spectrometry. *Journal Of Analytical Atomic Spectrometry*, *2*(6), 561. doi: 10.1039/ja9870200561

Mierzwa, J., Adeloju, S., & Dhindsa, H. (1997). Ultrasound Accelerated Solid-Liquid Extraction for the Determination of Selenium in Biological Samples by Electrothermal Atomization Atomic Absorption Spectrometry. *Analytical Sciences*, *13*(2), 189-193. doi: 10.2116/analsci.13.189

Minami, H., Honjyo, T., & Atsuya, I. (1996). A new solid-liquid extraction sampling technique for direct determination of trace elements in biological materials by graphite furnace atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *51*(2), 211-220. doi: 10.1016/0584-8547(95)01348-2

Mondol, M., Chamon, A., Faiz, B., & Elahi, S. (2011). Seasonal Variation of Heavy Metal Concentrations in Water and Plant Samples Around Tejgaon Industrial Area of Bangladesh. *Journal Of Bangladesh Academy Of Sciences*, *35*(1), 19-41. doi: 10.3329/jbas.v35i1.7968

Nazir, R., Khan, M., Masab, M., Rehman, H., Rauf, N., Shahab, S., & Shaheen, Z. (2015). Accumulation of Heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of Pharmaceutical Science*, *7*(1), 89-97.

Ogundele, DT., Adio A.A., & Oludele O.E. (2015). Heavy Metal Concentrations in Plants and Soil along Heavy Traffic Roads in North Central Nigeria. *Journal Of Environmental & Analytical Toxicology*, *05*(06). doi: 10.4172/2161-0525.1000334

Oladeji, S., & Saeed, M. (2015). Assessment of cobalt levels in wastewater, soil and vegetable samples grown along Kubanni stream channels in Zaria, Kaduna State, Nigeria. *African Journal Of Environmental Science And Technology*, *9*(10), 765-772. doi: 10.5897/ajest2015.1969

Olorunfemi, O., G. J., U., & I., O. (2018). Concentrations and Seasonal Variation in Heavy Metal Contamination of Leachates, Borehole Water, Soil and Edible Plants near Unengineered Dumpsites in Port Harcourt, Nigeria. *International Journal Of Scientific And Research Publications (IJSRP)*, *8*(9). doi: 10.29322/ijsrp.8.9.2018.p8169

Ong, G., Yap, C., Mahmood, M., Tan, S., & Hamzah, S. (2013). Barium levels in soils and Centella asiatica. *Trop Life Sci Res*, *24*(1), 55-70.

Osmani, M., Bani, A., & Hoxha, B. (2015). Heavy metals and Ni phytoextraction in the metallurgical area soils in Elbasan. *Albanian Journal Of Agricultural Science, 14*(1), 414-419.

Pais, I., & Jones, J. (1998). *The handbook of trace elements*. Boca Raton, Fla.: St. Lucie Press.

Połedniok, J., Kita, A., & Zerzucha, P. (2012). Spectrophotometric and Inductively Coupled Plasma–Optical Emission Spectroscopy Determination of Gallium in Natural Soils and Soils Polluted by Industry: Relationships between Elements. *Communications In Soil Science And Plant Analysis*, *43*(8), 1121-1135. doi: 10.1080/00103624.2012.662561

World Health Organization, W. (2001). Guidelines for heavy metals in soil. Retrieved 9 October 2021, from http://www.inchem.org/documents/ehc221.htm

Wu, B., Wang, G., Wu, J., Fu, Q., & Liu, C. (2014). Sources of Heavy Metals in Surface Sediments and an Ecological Risk Assessment from Two Adjacent Plateau Reservoirs. *Plos ONE*, *9*(7), e102101. doi: 10.1371/journal.pone.0102101

Chapter Five

Comparison of an ultrasonic, hotplate, and microwave-assisted digestion method for the assessment of metals in agricultural soil: Environmental contamination and human health risk

5.1. Abstract

This study compares microwave-assisted, hotplate and ultrasonic digestion methods to analyze twelve metals (Ba, Cd, Cr, Co, Cu, Ga, Li, Ni, Pb, Sr, Tl, and Zn) in agricultural soils before ICP-OES determination. The percentage recoveries for all methods were within the range of 74 – 112% for all three digestion methods, indicating that they can all be used to determine the target metals accurately. The hotplate method can be recommended as it does not require high pressure and temperature, which can degrade analytes, and it is easily accessible. On the hand, microwaves require an expensive instrument, thus its accessibility may be limited in other laboratories. At the same time, ultrasonic is susceptible to underestimating sample concentration due to incomplete digestion, especially for complex samples, as it uses lower temperatures. The metal concentrations obtained ranged from 0.60 – 256.4 mg/kg; however, all the metals were below the maximum permissible limits in soil except for Cr. The effect of anthropogenic activities on soil contamination was assessed by methods such as the contamination factor (CF), geo-accumulation index (I_{GEO}), ecological toxicity index (PERI) and metal correlation. The contamination factor and geo-accumulation index showed that the soil samples were mainly contaminated with Cu. The human health risk was assessed by calculating the non-carcinogenic (hazard quotient) and carcinogenic risk (cancer risk) in adults and children for main exposure pathways. Dermal contact was the primary exposure pathway in adults and children. Children were more susceptible to non-carcinogenic risks. Although metal contamination in this study was not severe, consideration and monitoring of potential pollution hazards and human health risks in the future around these agricultural soils are required.

5.2. Introduction

Heavy metals are present at trace levels in soils however, in agricultural soils, they can increase with an increase in pollutants (Arao *et al*., 2010). The presence of heavy metals in agricultural soils can be naturally or due to unnatural/anthropogenic sources. The natural sources consist of atmospheric emissions, circulation of continental dust and weathering metal-enriched rocks (Naveedullah *et al*., 2013). In agricultural soils, the anthropogenic sources comprised metal-enriched sewage sludges, irrigation water from wastewater treatment plants (WWTPs), livestock manure, application of metal-based pesticides, municipal wastes, and other agricultural activities. The heavy metal contamination in agricultural soils proves to be concerning since they can accumulate in crops through the soil, posing a significant threat to human health (Naveedullah *et al*., 2013). Chronic exposure of heavy metals over a long period of time can affect the human organs, such as the kidneys, liver, gastrointestinal tract and the central nervous system. Acute poisoning of these heavy metals is generally rare, however there is a possibility through dermal contact and ingestion (NRCS, 2000).

Agricultural soil is a complex environmental matrix consisting of organic matter, organic and inorganic compounds etc., which requires laborious sample pre-treatment. Sample digestion is one of the time-limiting steps in sample preparation. Several sample digestion methods can be employed to destroy the sample matrix (Kazi *et al*., 2008*)* where heat is used in a form of microwaves, hotplates, ultrasonic baths, etc. Microwave, hotplate and ultrasonic-assisted are the commonly used digestion methods in metal determination (Sastre *et al*., 2002); however, these digestion methods have their associated advantages and drawbacks. The microwave method is the most used digestion method; however, its availability can be limited as it requires an expensive instrument. The hotplate and ultrasonic bath can be cheaper alternatives; however, as they are open systems, sample contamination, loss of volatile analytes, acid fumes emission, and incomplete dissolution can hinder their applicability. Once the digestion is completed, the aqueous samples can be analyzed using spectroscopic techniques such as inductively coupled plasma–optical emission or mass spectrometry to quantify the metal concentrations (Sastre *et al*., 2002). Once the metals are quantified, the contamination studies are conducted specifically using heavy metal concentrations since they are persistent and toxic (Mussa *et al*., 2020).

In estimating heavy metal contamination due to anthropogenic activities, the contamination factor and geo-accumulation index can be calculated using mathematical expressions that include the metal and their respective baseline concentrations. The baseline/background concentrations of soil in South Africa are obtained from a study conducted by Herselman *et al*. (2005). The idea of background concentration is intended to indicate the natural heavy metal range before any contamination by human activity (anthropogenic sources). In addition, the potential ecological risk index can be calculated to assess the severity and risk levels of heavy metal contamination (Muzerengi, 2017).

The human health risks can be calculated and assessed according to the non – carcinogenic and carcinogenic risks in adults and children, where three main exposure pathways in adults and children are studied. These pathways included soil ingestion, dermal contact, and air inhalation. Children are not directly exposed when compared to agricultural workers; they are exposed to these agricultural lands, especially when conducting subsistence farming and during consumption. The hazard quotient (HQ) and carcinogenic risks (CR) are evaluated based on heavy metal presence in agricultural soil. Therefore, this study aimed to compare ultrasonic, hotplate and microwave digestion methods for metal determination in agricultural soils in KwaZulu-Natal and assess their environmental contamination level, potential ecological risk, and the human health risk of heavy metals in the soil. Few studies have been conducted in African countries on the occurrence and ecological risk assessment of heavy metals in the agricultural soils. This included a study conducted in Lake Chilwa catchment in Malawi, Southern Africa (Mussa *et al*., 2020). Also, Muzerengi (2017) assessed heavy metal toxicity where the enrichment factor, contamination factor, and geo-accumulation were calculated on soils near a gold mine in Limpopo, South Africa. However, to the best of our knowledge, the assessment of heavy metal concentration was done for the first time in the selected agricultural soils. In addition, no work has been conducted on the ecological and human health risks associated with heavy metals in KwaZulu-Natal agricultural soils.

5.3. Experimental

5.3.1. Study area and sample collection

This study was conducted in the KwaZulu-Natal Province in South Africa (Pietermaritzburg city). Pietermaritzburg is the provincial capital city with an estimated population of 900 000 residents. Soils were sampled at five agricultural lands (Curry Post, Cedara, Gilboa Farm, Richmond, and Umgeni Valley) as per Figure S5.1. Portions of surface soil samples (0-10 cm depth) were randomly collected at different points around each site using a Dutch auger (Reliance laboratory, Germany) and combined to make a representative sample of each site. The samples were stored in polyethylene containers and then transported to the laboratory, where they were air-dried in a fume hood to remove excess moisture. They were then crushed, ground using a clean and dry mortar and pestle and then sieved through a 400 μm sieve to fineness before acid digestion.

5.3.2. Reagents, reference materials and standards

The Purelab ultrapure water (18.2 MΩ.cm) was used to prepare all calibration standards and clean all glassware with dilute nitric acid. The 55% v/v nitric acid used in the preservation and digestion processes of the samples, 1000 mg/L ICP Multi-element standard and ULTRASPEC® Multi-Element Aqueous CRM in 5% v/v nitric acid were purchased from Sigma Aldrich (Johannesburg, South Africa). The standard reference material of trace elements was employed to evaluate the accuracy of the method used to determine metals in agricultural soil samples.

5.3.3. Instrumentation

The 720-ES ICP-OES instrument purchased from Varian (Johannesburg, South Africa) was used to determine metals in water. The instrument operated at a frequency of 40MHz, RF power of 1.00kW, a pneumatic concentric nebulizer was used at a flow rate of 0.75 L/min, and an inert carrier gas (Argon) was pumped at a rate of 15 rpm. The Multiwave 5000 microwave digester from Anton Paar (Johannesburg, South Africa), heating plate and ultrasonic bath from Science Tech (Durban, South Africa) were used to digest the agricultural soil samples. A centrifuge purchased at Shalom Laboratory (Durban, South Africa) was employed to separate and extract from the soil.

5.3.4. Sample preparation

5.3.4.1. Microwave-assisted acid digestion

The microwave-assisted acid digestion method was adopted from the United States Environmental Protection Agency (US EPA 3051A). A 0.500g soil sample was mixed with 10 mL of HNO_3 in a microwave vessel which was then sealed and placed into the microwave system. The microwave digestion was conducted at 175 ± 5°C in 5.5 ± 0.25 min. It remained at 175 ± 5°C for 4.5 minutes and the total digestion time was 10 minutes, followed by cooling of the vessels to the initial temperature. After cooling, the contents were filtered using Whatman 70mm filter paper, centrifuged at 2000 rpm and allowed to settle. The filtrate was decanted into a 100 mL volumetric flask and filled to a known volume with ultrapure water. The aqueous samples were then transferred into ICP tubes for analysis.

5.3.4.2. Hotplate assisted digestion

The hotplate assisted digestion, commonly classified as a wet digestion process, was adopted from the United States Environmental Protection Agency (US EPA 3050B). A 0.500g of soil was mixed with 5 mL HNO_3 in a 100 mL beaker to form a slurry; it was then covered with a watch glass and placed onto the hotplate where it was heated at 95 ±5 °C for 15 minutes without boiling. The contents were allowed to cool and a further 5 mL HNO_3 was added and placed on the hotplate at the same temperature, which generated brown fumes indicating the oxidation process. Once the brown fumes began to disappear, the watch glass was removed, and the contents were allowed to evaporate to approximately 5 mL. The total digestion time was 60 minutes. Once the digestion was complete, the samples were cooled, filtered, and centrifuged at 2000 rpm. The filtrate was transferred into a 100 mL volumetric flask and filled up to the mark with ultrapure water. The prepared samples were transferred into ICP tubes for analysis.

5.3.4.3. Ultrasonic-assisted acid digestion

For the ultrasonic-assisted acid digestion method, 0.500g of the soil sample was placed in a 100 mL Erlenmeyer flask, followed by the addition of 5 mL HNO_3. The flask and the sample-acid mixture were placed in an ultrasonic bath at a temperature of 80°C for 22.5 minutes. This was followed by the addition of another 5 mL and further ultrasonicated for 22.5 minutes to make a total digestion time of 45 minutes. The flask was left to cool for 5 minutes, and the digestate was filtered (Whatman 70mm) and centrifuged at 2000 – 3000 rpm and allowed to settle. Thereafter, the filtrate was transferred into a 100 mL volumetric flask, made up to the mark with ultrapure water, and analyzed with ICP-OES.

5.3.5. Validation of the analytical method for the determination of metals

The ultrasonic, hotplate and microwave-assisted digestion followed by ICP-OES analytical methods were validated in terms of linearity and percentage recovery test. The linearity was assessed over a concentration range of 0.05-10 mg/L. The accuracy of all digestion methods was evaluated as percentage recoveries by spiking the soil samples with a mixture of the metals of interest to make a final concentration of 0.50 mg/kg. The spiked samples were then digested using a hotplate, ultrasonic and microwave-assisted method followed by analysis with ICP-OES, and the percentage recoveries were calculated.

5.3.6. Statistical analysis

Statistical t-tests were conducted to investigate any differences in the mean percentage recoveries for all three digestion methods with a significance level set at $p < 0.05$ assuming unequal variances. The metal concentrations were assessed using metal correlation coefficients to identify the common and potential sources.

5.3.7. Environmental and human health risk assessment of agricultural soil

The qualitative evaluation of agricultural soil was used to evaluate the environmental contamination based on the calculation and classification of the contamination factor, pollution load, geo-accumulation, and potential ecological risk indices.

5.3.7.1. Contamination factor (CF) and pollution load index (PLI)

Contamination factor (CF) represents the impact of trace heavy metals in the examined soil and is calculated in equation 5.1.

$$CF = \frac{C_n}{C_{ref}} \qquad (5.1)$$

where C_n refers to the examined metal concentration in the studied soil and C_{ref} is the background concentration of the examined metal in the soil (Co, Cr, Cu, Pb, Ni and Zn are 20.0, 71.9, 6.6, 6.6, 15.0 and 45.2 mg/kg respectively). These background concentrations were obtained from Herselman (2005), in which the EPA Method 3050 was also employed as the digestion method for the quantified metal concentration in surface soil.

The pollution load index (*PLI*) represents the number of times the metal content exceeds the natural background concentration and indicates the overall metal toxicity in the studied sample. The pollution load index considers the contamination factor and number of metals at the sampling site to appropriately assess the degree of contamination using equation 5.2 (Muzerengi, 2017).

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots\dots \times CF_n)^{1/n} \qquad (5.2)$$

where n is the number of metals in this study ($n = 6$). $PLI < 1$ indicates that the site is free from contamination, $PLI = 1$ implies that contamination is at the background level and lastly, $PLI >$ 1indicates deterioration of soil at the site.

5.3.7.2. Index of geo-accumulation (I_{GEO})

Muller defined the geo-accumulation index (IGEO) in 1969 to assess soil contamination. It is calculated using the background and sample metal concentration in a mathematical relationship shown in equation 5.3

$$I_{GEO} = \log_2\left(\frac{C_n}{1.5 \times B_n}\right) \qquad (5.3)$$

Where C_n is the estimated concentration of each element in soil, B_n is the background concentration of the metal. The constant 1.5 accounts for the natural variation and effect of small anthropogenic sources in the soil sample. The soil quality is determined by the I_{GEO} values and classified into grades presented in Table 5.1(Choi & Jeon, 2018).

Table 5.1: Classification grades of geo-accumulation index

Grade	Value	Soil quality
0	$I_{GEO} \ll 0$	Practically uncontaminated
1	$0< I_{GEO} <1$	Uncontaminated to moderately contaminated
2	$1< I_{GEO} <2$	Moderately contaminated
3	$2< I_{GEO} <3$	Moderately to heavily contaminated
4	$3< I_{GEO} <4$	Heavily contaminated
5	$4< I_{GEO} <5$	Heavily to extremely contaminated
6	$5< I_{GEO}$	Extremely contaminated

5.3.7.3. Potential Ecological Risk Index (PERI)

The potential ecological risk index was proposed by Hakanson in 1980. It assesses the potential damage caused by heavy metal contamination, which combines the assessment of ecological risk and environmental toxicity. The PERI is calculated using equations 5.4-5.6

$$EC_f^i = {C_D^i}/{C_R^i} \qquad (5.4)$$

$$E_R^i = T_R^i \times C_f^i \qquad (5.5)$$

$$RI = \sum_{i-1}^{m} E_R^i \qquad (5.6)$$

Where C_D^i refers to the metal concentration in the soil sample, C_R^i is the background concentration of the metal. C_f^i is the contamination level of a single element, E_R^i is the biological hazard of a single metal, RI is the overall biological hazard and T_R^i is the biological toxicity weight of a single metal (Zn = 1, Cd = 30, Cr = 2, Pb, Ni, Co, Cu = 5). The RI values classify the risk levels of pollution as presented in Table 5.2.

Table 5.2: Classification of potential ecological risk index (PERI) as biological toxicity

E_R^i	**Pollution degree**	**RI**	**Risk level**	**Risk Degree**
$E_R^i < 30$	Slight	$RI < 40$	A	Slight
$30 \leq E_R^i < 60$	Medium	$40 \leq RI < 80$	B	Medium
$60 \leq E_R^i < 120$	Strong	$80 \leq RI < 160$	C	Strong
$120 \leq E_R^i < 240$	Very strong	$160 \leq RI < 320$	D	Very strong
$240 \leq E_R^i$	Extremely strong	$320 \leq RI$	-	-

5.3.8. Human health risk assessment

Health risk assessment bridges the gap between the level of heavy metal contamination in the environment and the possibility of humans' toxic effects. The non-carcinogenic and carcinogenic risks are assessed by quantifying the hazard quotient (HQ) and cancer risk (CR) for heavy metal exposure. There are three exposure pathways considered for agricultural soil: soil ingestion (directly consumed or via consumption of agricultural produce), dermal contact, and inhalation through soil vapour (Jiang *et al.*, 2016). The average daily intake (ADD) is calculated for the non-carcinogenic risk for all three exposure pathways using equation 5.7 – 5.9, and thereafter the hazard quotient component is obtained by dividing the ADD for each heavy metal by their corresponding reference dosage (RfD) value (equation 5.10) in adults (18+ years) and children (1 – 17 years). The lifetime average potential daily dose (LADD) for carcinogens is calculated (equation 5.11 – 5.13) are multiplied by their respective cancer slope factor (SF) to calculate the cancer risk for each pathway (equation 5.14).

Non-carcinogenic risk:

$$ADD_{ing} = \frac{C \times IR_S \times EF \times ED}{BW \times AT} \times 10^{-6} \qquad (5.7)$$

$$ADD_{dermal} = \frac{C \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \qquad (5.8)$$

$$ADD_{inh} = \frac{C \times IR_i \times EF \times ED}{PEF \times BW \times AT} \qquad (5.9)$$

$$HQ = \sum \frac{ADD_x}{RfD_x} \qquad (5.10)$$

Carcinogenic risk:

$$LADD_{ing} = \frac{C \times IR_S \times EF \times ED}{BW \times LT} \times 10^{-6} \qquad (5.11)$$

$$LADD_{dermal} = \frac{C \times SA \times AF \times ABS \times EF \times ED}{BW \times LT} \qquad (5.12)$$

$$LADD_{inh} = \frac{C \times IR_i \times EF \times ED}{PEF \times BW \times LT} \qquad (5.13)$$

$$CR = \sum (LADD_x \times SF_x) \qquad (5.14)$$

The abbreviations and numerical parameters for the health risk assessment calculations are presented in Table S5.2. Table S5.3 presents the reference dosage and slope factor obtained from the literature to calculate the hazard quotient and cancer risk, respectively.

5.4. Results and discussion

5.4.1. Validation of the analytical method

The calibration data and the optimum wavelengths and maximum permissible limits (MRL values) for the metals analyzed are presented in Table 5.3. The selection of optimum wavelengths has minimized the spectral interferences. The correlation coefficient for all metal analytes was greater than 0.99, indicating a good degree of linearity. The recoveries ranging from 74-112% were obtained, indicating good accuracy of the proposed digestion methods.

Statistical t-tests revealed no significant difference between the mean recoveries for all the three digestion methods since the p-values were above 0.05. The p-values were $p<0.89$ for microwave versus hotplate, $p<0.72$ for microwave versus ultrasonic and $p< 0.65$ for hotplate versus ultrasonic digestion methods (Table S5.1). The digestion methods can be applied to actual soil samples for metal quantification.

Table 5.3: Validation of the analytical instrument

Metal analyte	Wavelength (nm)	%Recoveries			R^2
		Micro	*HP*	*Ultra*	
Barium (Ba)	455.403	96	98	95	0.9998
Cobalt (Co)	228.615	84	81	85	0.9999
Chromium (Cr)	267.716	94	102	83	0.9995
Copper (Cu)	324.754	90	94	112	0.9999
Cadmium (Cd)	226.502	79	74	78	0.9999
Gallium (Ga)	287.423	100	101	99	0.9995
Lithium (Li)	670.783	98	86	83	0.9993
Nickel (Ni)	231.604	84	88	83	0.9999
Lead (Pb)	220.353	84	74	75	0.9997
Strontium (Sr)	407.771	81	82	97	0.9980
Thallium (Tl)	190.807	74	75	86	0.9995
Zinc (Zn)	213.857	78	81	83	0.9999

5.4.2. Physicochemical properties of the soil

The physicochemical properties of the agricultural soil were measured and presented in Table 5.4. Agricultural soils from Cedara and Gilboa Farm were at pH 4, which can be considered acidic. The lower pH in these agricultural soils could be due to the long-term use or application of nitrogen-containing fertilizers such as diammonium phosphate and urea. The release of H^+ ions from the plants' roots can also result in the acidic pH in the growing medium (Salem *et al.*, 2020). Heavy metals are made available to plants in acidic pH, considering that these soils are from agricultural lands (Salem *et al.*, 2020). In a study conducted by Li and co-worker, the release of Cd, Cr, Cu, Pb increased at low and high pH, however higher release rate was observed at low pH, therefore at acidic pH, metal concentration is expected to be low (Li *et al.*, 2013).

The salinity observed in the soil samples range between 0.02 – 0.2 psu, the sources of high salinity levels are generally present as alkali earth metal salts, for example, Ca and Mg salts, CO_3^{2-} and Cl^- ions in high concentrations (Reeve, 2002). The observation of salinity in these agricultural soils suggest the possibility for the presence of heavy metal in these soils. The electrical conductivity of soil aims to estimate the concentration of soluble salts. The conductivity of the soil samples was between 33 – 134 µS, where the lowest conductivity was observed at Cedara may be due to low salinity, which may have been washed during the irrigation process, the precipitation of salts and transportation into plants (Salem *et al*., 2020). As per this study, the influence of conductivity showed no effect on the metal concentrations, however, high salinity i.e., increased chloride ions can complex with metals which makes it difficult to transfer into water and remains in soil thus increasing the metal concentrations (Namieśnik & Rabajczyk, 2010).

Organic matter (OM) plays a vital role in the mobility of heavy metals in soil; it can decrease available concentrations in soils due to complexation, adsorption, and precipitation (Collin & Doelsch, 2010). The OM ranged between 10 – 19, with the lowest OM content at Richmond which may be attributed to the sandy texture of the soil. The temperature ranged between 21 – 29℃. The high temperature of soil increases and enhances the degradation of OM. Low organic matter in the soil is not favourable for heavy metal absorption, thus decreasing their presence in the soil samples (Bhatti *et al*., 2016).

Table 5.4: Physicochemical properties of agricultural soil.

Sampling points	**pH**	**Temp (℃)**	**Salinity (psμ)**	**TDS (ppm)**	**Conductivity (μS)**	**D.O (mg/L)**	**Organic matter (OM)**
Umgeni	7	29	0.03	57	57	2	11
Cedara	4	23	0.02	17	33	4	13
Curry Post	5	22	0.03	24	46	3	19
Richmond	5	23	0.04	47	65	2	10
Gilboa Farm	4	21	0.07	67	134	11	12

5.4.3. Metal concentrations in agricultural soils

The average metal concentrations decreased in the following order: Ga > Cr > Ba > Cu > Ni > Zn > Sr > Co > Pb > Li (Table 5.5). The Cd and Tl concentrations were undetected at all sampling sites, similarly to a study conducted on agricultural soil near Lake Chilwa in Malawi, Southern Africa, where Cd was also found to be undetected using the ICP-OES instrument (Mussa *et al.,* 2020)

The Ga concentrations were high in all samples, with the highest concentration observed at Cedara. This is a concern since Ga is considered one of the emerging contaminants which are non-essential and potentially toxic in living organisms since they are susceptible to soil contamination (Liu *et al*., 2021). The concentrations of Ga in Taiwan soils ranged from < 3 to 70 mg/kg, which is lower than the values obtained in this study (Liu *et al*., 2021). However, a maximum concentration of 437 mg/kg reported from Poland soil collected near a zinc refinery plant is higher than the maximum concentration observed in this study (Poledniok *et al*., 2012). The Ga occurs naturally in highly weathered soils and varies with different soil types (Liu *et al*., 2021).

The Ba concentrations were relatively high; even though there is no defined maximum permissible limit assigned for Ba presence in soil, Ba concentrations of 200 mg/kg can be moderately toxic, while concentration of 500 mg/kg is considered harmful to plant life (Pais *et al*., (1998)). At Umgeni, Ba concentrations were found to be relatively high compared to the other sampling sites, and despite it being lower than 200 mg/kg, Ba bioaccumulation in plants, specifically in edible plants, can result in its transfer via the food chain (Ong *et al*., 2013).

The Co, Ni, Pb and Zn were below their respective permissible limits in all soil samples. In a study conducted by Mussa and co-workers, Ni, Pb and Zn maximum concentrations were 43.18, 16.81 and 99.21 mg/kg, respectively, which are relatively higher than the maximum concentrations obtained in this study (27.5, 3.3 and 36.7 mg/kg, respectively). The possible sources for higher concentrations of these heavy metals could be the application of fertilizers, pesticides (lead and zinc arsenate used in vegetable gardens) and manure. However, the low concentrations obtained for Co and Zn suggest that they may be from natural sources such as parent material of soils and lithogenic sources (Mussa *et al*., 2020).

Umgeni was the most polluted since most metal concentrations observed were higher than the other sites. Umgeni soil had an alkaline pH along with high temperature, however, in many studies, the influence of temperature on metal concentrations is uncertain and quite complex to understand (Huang *et al*., 2017). Previous studies have shown that low pH can increase the release of metals from soils, conversely, it can be deduced that high pH will retain metals in soil thus increasing the concentration (Huang *et al*., *2017).* The Cr and Cu were above their maximum permissible limits. The possible sources of Cr and Cu in these agricultural soils could be due to their presence of farmyard manure, fertilizer or treated sludge, and wastewater effluent possibly used for irrigation. These heavy metals can accumulate in the soil and migrate into crops via plant-root respiration, ultimately into our bodies upon consumption (Mussa *et al*., 2020).

In general, the concentrations observed from the microwave and hotplate assisted digestion methods were comparable. These results suggest that even though the microwave is a widely used digestion method since it is a closed system process preventing loss of volatile analytes and sample contamination, the hotplate method can be used as a cheaper and accessible alternative (Sastre *et al*., 2002). The ultrasonic method underestimated the concentrations; despite the good recoveries achieved during the validation process. This was inconsistent with the data obtained by Kazi and co-workers, where the wet acid digestion and ultrasonic-assisted digestion resulted in similar metal concentrations in soil (Kazi *et al*., 2008). The inconsistency observed, maybe due to the application of the method to actual samples, specifically agricultural soil, which are complex and contains several other compounds. This may result in low total digestion due to the temperature restriction of the ultrasonic bath causing low desorption and degradation of the sample matrix, ultimately resulting in incomplete digestion (Kazi *et al.,* 2008).

Table 5.5: Average concentration (mg/kg) of metals in soil samples using the hotplate, ultrasonic, and microwave-assisted digestion methods and maximum allowable levels (MRL).

Metal	**Curry Post**			**Cedara**			**Gilboa Farm**			**Richmond**			**Umgeni**			**MRL mg/kg** (WHO, 2001)
	Micro	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	
Ba	28.8	26.3	18.6	55.1	51.5	25.7	18.1	29.5	26.4	62.0	59.5	30.9	117.1	108.9	84.9	-
Co	4.4	3.1	nd	11.4	10.5	1.6	9.9	17.5	12.4	4.1	4.0	nd	16.8	15.5	11.0	40
Cr	148.8	152.7	61.5	127.7	134.0	45.4	80.6	139.7	45.7	76.3	85.9	39.6	108.8	114.1	29.3	100.0
Cu	33.6	32.7	14.0	29.8	29.4	7.0	37.2	61.2	41.8	20.3	20.9	5.4	45.5	45.5	18.9	36
Cd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.8
Ga	246.5	218.0	49.8	256.4	240.5	65.1	139.0	226.8	81.2	185.4	178.2	35.5	158.7	157.9	27.9	-
Li	1.3	0.6	nd	1.1	0.5	nd	nd	nd	nd	1.1	0.6	nd	1.1	1.0	nd	-
Ni	13.8	15.7	5.9	20.6	21.7	4.5	18.3	27.5	12.8	12.1	14.8	7.0	32.5	34.1	15.6	35
Pb	Nd	nd	2.1	nd	2.7	3.3	nd	1.4	3.0	2.8	nd	2.9	nd	nd	nd	85
Sr	Nd	nd	nd	nd	nd	nd	nd	nd	nd	2.1	1.9	nd	23.8	23.1	18.5	-
Tl	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-
Zn	12.8	12.4	2.1	13.5	12.3	nd	18.7	20.7	5.2	9.0	8.9	Nd	36.7	35.8	12.8	100

*nd – not detected, Micro – microwave-assisted digestion, HP - hotplate assisted digestion, Ultra – ultrasonic-assisted digestion

5.4.4. Contamination factor and geo-accumulation index

The contamination factor and pollution load indexes for each site were calculated and presented in Table 5.6. The contamination factors (CF) ranged from 0.41 – 2.12. 0.82 – 9.27 and 0.30 – 2.27 for Cr, Cu and Ni, respectively, whilst the geo-accumulation index ranged from -1.30 – 0.35, -0.61 – 1.82 and -1.61 – 0.42 for Cr, Cu and Ni respectively. Based on CF values, the soil was contaminated with the studied heavy metals in the following order: Cu > Cr > Ni > Co > Pb > Zn. In a study conducted by Muzerengi (2017) on soil from the Limpopo Province, the CF value for Zn was also found to be the lowest where its presence can be derived predominantly due to natural processes or geogenic sources (Muzerengi, 2017).

The pollution load index and geo-accumulation index were greater than 1 in Gilboa farm and Umgeni, which resulted in environmental concerns due to heavy metal contamination. The PLI at Curry Post and Richmond were less than 1, which revealed that the sites were free from heavy metals contamination with negative geo-accumulation indices ($I_{GEO} \ll 0$), which is categorized as grade zero, indicating that the soil quality is practically uncontaminated. The CF values for Cr and Ni were greater than 2 at Curry post and Umgeni, indicating moderate contamination since these studied metals exceeded their respective background concentrations. It was also observed that no heavy metals in all sampling locations exceeded a CF value greater than 20. The highest CF value was obtained for Cu (9.27) at Gilboa farm, suggesting significant contamination. Pollution of Cu can be due to agricultural activities since Cu is used in fertilizers for crop production in the forms of copper sulphate and copper oxide (Alengebawy *et al.*, 2021).

5.4.5. Potential ecological risk index (PERI)

The potential ecological risk index (*PERI)* was calculated and presented in Table 5.7. It reflected the general contamination situation caused by the simultaneous presence of the six heavy metals. The RI values were classified in the slight risk level at Curry Post, Cedara and Richmond with ($RI < 40$), while at Gilboa Farm and Umgeni, the RI values were in the medium risk level with $40 \leq RI < 80$. Soils polluted by heavy metals can result in significant ecological risks. They can adversely affect the human health resulting from different interaction forms where very harmful heavy metals can be introduced through the food cycle. The heavy metals extreme accumulation in agricultural soils can cause an effect on the food safety and quality and further escalate severe diseases risk (liver and kidney damage, cancer, etc.) and have an influence on the surrounding ecosystems (Santos-Francés *et al.*, 2017). Although metal pollution was focused on a specific point, continuous monitoring and consideration of possible biological risks and are required.

Table 5.6: Contamination factor and geo-accumulation index for agricultural soil.

Metal	**Index**	**Curry Post**			**Cedara**			**Gilboa Farm**			**Richmond**			**Umgeni**		
		Micro	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***
Co	*CF*	0.22	0.16	-	0.57	0.53	0.08	0.50	0.88	0.62	0.21	0.20	-	0.84	0.78	0.55
	I_{GEO}	-1.96	-2.27	-	-0.97	-1.05	-2.93	-1.11	-0.54	-0.88	-1.99	-2.01	-	-0.58	-0.66	-1.00
Cr	*CF*	2.07	2.12	0.86	1.78	1.86	0.63	1.12	1.94	0.64	1.06	1.19	0.55	1.15	1.59	0.41
	I_{GEO}	0.32	0.35	-0.56	0.17	0.22	-0.87	-0.29	0.26	-0.86	-0.35	-0.23	-1.00	0.01	0.06	-1.30
Cu	*CF*	5.09	4.95	2.12	4.52	4.45	1.06	5.64	9.27	6.33	3.08	3.17	0.82	6.89	6.89	2.86
	I_{GEO}	1.22	1.19	0.35	1.10	1.09	-0.35	1.32	1.82	1.44	0.72	0.75	-0.61	1.53	1.53	0.65
Ni	*CF*	0.92	1.05	0.39	1.37	1.45	0.30	1.22	1.83	0.85	0.81	0.99	0.47	2.17	2.27	1.04
	I_{GEO}	-0.49	-0.36	-1.34	-0.09	-0.04	-1.61	-0.21	0.20	-0.56	-0.62	-0.42	-1.17	0.37	0.42	-0.37
Pb	*CF*	-	-	0.32	-	0.41	0.50	-	0.21	0.45	0.42	-	0.44	-	-	0.21
	I_{GEO}	-	-	-1.55	-	-1.30	-1.10	-	-1.96	-1.19	-1.26	-	-1.23	-	-	-1.96
Zn	*CF*	0.28	0.27	0.05	0.30	0.27	-	0.41	0.46	0.12	0.20	0.20	-	0.81	0.79	0.28
	I_{GEO}	-1.67	-1.70	-3.47	-1.61	-1.71	-	-1.29	-1.19	-2.57	-2.02	-2.03	-	-0.61	-0.64	-1.67
	PLI	0.90	0.86	0.40	1.13	0.94	0.38	1.10	1.19	0.69	0.60	0.68	0.55	1.73	1.72	0.59

Table 5.7: Potential ecological risk index for soil samples.

Metal	**Index**	**Curry Post**			**Cedara**			**Gilboa Farm**			**Richmond**			**Umgeni**		
		Micro	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***	***Micro***	***HP***	***Ultra***
Co	EC_f	0.22	0.16	-	0.57	0.53	0.08	0.50	0.88	0.62	0.21	0.20	-	0.84	0.78	0.55
	E^R	1.10	0.78	-	2.85	2.63	0.40	2.48	4.38	3.10	1.03	1.00	-	4.20	3.88	2.75
Cr	EC_f	2.07	2.12	0.86	1.78	1.86	0.63	1.12	1.94	0.64	1.06	1.19	0.55	1.15	1.59	0.41
	E^R	4.14	4.25	1.71	3.55	3.73	1.26	2.24	3.89	1.27	2.12	2.39	1.10	3.03	3.17	0.82
Cu	EC_f	5.09	4.95	2.12	4.52	4.45	1.06	5.64	9.27	6.33	3.08	3.17	0.82	6.89	6.89	2.86
	E^R	25.5	24.8	10.6	22.6	22.3	5.3	28.2	46.4	31.7	15.4	15.8	4.1	34.5	34.5	14.3
Ni	EC_f	0.92	1.05	0.39	1.37	1.45	0.30	1.22	1.83	0.85	0.81	0.99	0.47	2.17	2.27	1.04
	E^R	4.60	5.23	1.97	6.87	7.23	1.50	6.10	9.17	4.27	4.03	4.93	2.33	10.8	11.4	5.20
Pb	EC_f	-	-	0.32	-	0.41	0.50	-	0.21	0.45	0.42	-	0.44	-	-	0.21
	E^R	-	-	1.59	-	2.05	2.50	-	1.06	2.27	2.12	-	2.20	-	-	1.06
Zn	EC_f	0.28	0.27	0.05	0.30	0.27	-	0.41	0.46	0.12	0.20	0.20	-	0.81	0.79	0.28
	E^R	0.28	0.27	0.05	0.30	0.27	-	0.41	0.46	0.12	0.20	0.20	-	0.81	0.79	0.28
	RI	**35.6**	**35.3**	**15.9**	**36.2**	**38.2**	**11.0**	**39.4**	**65.4**	**42.7**	**21.9**	**24.3**	**9.73**	**53.3**	**53.7**	**24.4**

5.4.6. Metal correlation

The heavy metal correlation was assessed for the microwave method since it is the most common and widely used digestion method representing the metal concentrations observed in each soil sample. A number from -1 to +1, was used to express the relative correlation with -1 signifying that the relation is strongly negative while 1 signifying a strong positive relation. Furthermore, a heating map was employed for the expression of the correlation degree, using red being close to +1 and blue being close to -1 (Table 5.8). The correlation coefficients demonstrated that Zn displayed a synergistic (strongly positive) relationship with Co, Cu and Ni, thus owing to the possibility of a common source of these metals. The highly correlated heavy metals may potentially originate from the same source, specifically the use of agrochemicals such as manure, inorganic fertilizers, and pesticides (Mussa *et al*., 2020). The Cr displayed a strong negative (antagonistic) relationship with Co and Zn with R^2 values of -0.006 and -0.015, respectively, indicating that their concentrations are lower than Cr and potentially originated from natural sources.

Table 5.8: Heavy metal correlation in soil samples by statistical analysis using concentration from microwave digestion

Metal	**Co**	**Cr**	**Cu**	**Ni**	**Zn**
Co	1.000				
Cr	-0.006	1.000			
Cu	0.776	0.225	1.000		
Ni	0.972	0.068	0.818	1.000	
Zn	0.879	-0.015	0.891	0.948	1.000

5.4.7. Human health risk assessment

Five heavy metals (Cr, Cu, Ni, Pb and Zn) were considered in assessing human health risks due to their toxicity. The estimations of non-carcinogenic and carcinogenic health risks for the studied heavy metals in agricultural soil were calculated for adults (18+ years) and children (1 – 17 years) through the three major exposure pathways (Table 5.9 and 5.10).

The total hazard index (THI) values for adults and children were 1.63 and 8.69, respectively. Since the values were found to be greater than 1, it was deduced that people might experience non-carcinogenic effects; however, children are more prone when compared to adults. The HQ values followed the following order for the studied heavy metals: Cr > Ni > Cu > Pb > Zn. Amongst all the heavy metals, Cr in children had HQ values that exceeded 1. It was observed that the exposure pathways in both adults and children for the heavy metals decreased in the following order: dermal contact > soil ingestion > air inhalation. Dermal contact was the primary exposure pathway in both adults and children, where the contribution by dermal contact accounted for approximately 96.2% and 96.3% of their total hazard index, respectively. Generally, children will experience higher non-carcinogenic risk when compared to adults; hence, they are more prone to environmental contaminants. A study conducted by Jiang *et al*. (2017) made a similar observation, in which the physiological and behavioural characteristics such as high respiration rates per unit body weight and hand-to-mouth activities of children resulted in higher non-carcinogenic risks.

The carcinogenic risk was estimated for Cr, Ni and Pb since Cu and Zn are not considered carcinogenic (USEPA, 2016). Only two out of three pathways were considered since the slope factor for dermal contact was unavailable. The carcinogenic risk estimation of Ni and Pb was through only one pathway, inhalation and ingestion, respectively. The total carcinogenic risk (TCR) values were 2.71E-05 and 3.54E-05 for adults and children, respectively (Table 6.10). Soil ingestion was the primary pathway between the two considered. As per Fryer *et al*. (2006) study, the maximum tolerable risk of 1.00E-4 was higher than the calculated carcinogenic risks for both adults and children, while children showed higher carcinogenic risks similar to the non-carcinogenic trend. In the current study, the CR values for all examined heavy metals for adults and children are below the maximum tolerable risk (1.00E-6 - 1.00E-4), thus posing no significant health effect. The human health risk evaluated and the calculated HQ and CR values for hotplate and ultrasonic-assisted digestion are presented in Tables S5.4 – S5.7. The trends were consistent with that observed using microwave-assisted digestion methods. Even though no serious public health risk was identified in the study area, consistent estimation of human health risks is required.

Table 5.9: Estimation of non-carcinogenic (Hazard quotient, HQ) from heavy metals in agricultural soil using microwave-assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	1.51E-02	4.30E-01	9.32E-04	4.46E-01	8.20E-02	2.30E+00	9.61E-04	2.38E+00
	Cedara	1.30E-02	3.69E-01	8.00E-04	3.83E-01	7.04E-02	1.97E+00	8.25E-04	2.04E+00
	Gilboa	8.18E-03	2.33E-01	5.05E-04	2.42E-01	4.44E-02	1.24E+00	5.21E-04	1.28E+00
	Richmond	7.74E-03	2.21E-01	4.78E-04	2.29E-01	4.20E-02	1.18E+00	4.93E-04	1.22E+00
	Umgeni	1.10E-02	3.15E-01	6.81E-04	3.27E-01	6.00E-02	1.68E+00	7.03E-04	1.74E+00
Cu	Curry Post	2.56E-04	4.86E-05	-	3.05E-04	1.39E-03	2.59E-04	-	1.65E-03
	Cedara	2.27E-04	4.31E-05	-	2.70E-04	1.23E-03	2.30E-04	-	1.46E-03
	Gilboa	2.83E-04	5.38E-05	-	3.37E-04	1.54E-03	2.87E-04	-	1.83E-03
	Richmond	1.54E-04	2.94E-05	-	1.83E-04	8.39E-04	1.57E-04	-	9.96E-04
	Umgeni	3.46E-04	6.58E-05	-	4.12E-04	1.88E-03	3.51E-04	-	2.23E-03
Ni	Curry Post	2.10E-04	4.43E-05	2.75E-05	2.82E-04	1.14E-03	2.37E-04	2.83E-05	1.41E-03
	Cedara	3.14E-04	6.62E-05	4.10E-05	4.21E-04	1.70E-03	3.53E-04	4.23E-05	2.10E-03
	Gilboa	2.79E-04	5.88E-05	3.64E-05	3.74E-04	1.51E-03	3.14E-04	3.76E-05	1.86E-03
	Richmond	1.84E-04	3.89E-05	2.41E-05	2.47E-04	1.00E-03	2.07E-04	2.48E-05	1.23E-03
	Umgeni	4.95E-04	1.04E-04	6.47E-05	6.64E-04	2.69E-03	5.57E-04	6.67E-05	3.31E-03
Pb	Curry Post	nd	nd	-	0.00E+00	nd	nd	-	0.00E+00
	Cedara	nd	nd	-	0.00E+00	nd	nd	-	0.00E+00
	Gilboa	nd	nd	-	0.00E+00	nd	nd	-	0.00E+00
	Richmond	2.44E-04	9.25E-05	-	3.37E-04	1.32E-03	4.94E-04	-	1.81E-03
	Umgeni	nd	nd	-	0.00E+00	nd	nd	-	0.00E+00
Zn	Curry Post	1.30E-05	3.70E-06	-	1.67E-05	7.05E-05	1.98E-05	-	9.03E-05
	Cedara	1.37E-05	3.90E-06	-	1.76E-05	7.44E-05	2.08E-05	-	9.52E-05
	Gilboa	1.90E-05	5.41E-06	-	2.44E-05	1.03E-04	2.89E-05	-	1.32E-04
	Richmond	9.13E-06	2.60E-06	-	1.17E-05	4.96E-05	1.39E-05	-	6.35E-05
	Umgeni	3.72E-05	1.06E-05	-	4.78E-05	2.02E-04	5.66E-05	-	2.59E-04
Total metals		5.81E-02	1.57E+00	3.59E-03	1.63E+00	3.16E-01	8.37E+00	3.70E-03	8.69E+00

Table 5.10: Estimation of carcinogenic (CR) from heavy metals in agricultural soil using microwave-assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	7,10E-06	-	3,51E-07	7,45E-06	9,63E-06	-	9,05E-08	9,72E-06
	Cedara	6,09E-06	-	3,01E-07	6,39E-06	8,27E-06	-	7,76E-08	8,35E-06
	Gilboa	3,84E-06	-	1,90E-07	4,03E-06	5,22E-06	-	4,90E-08	5,27E-06
	Richmond	3,64E-06	-	1,80E-07	3,82E-06	4,94E-06	-	4,64E-08	4,99E-06
	Umgeni	5,19E-06	-	2,56E-07	5,45E-06	7,04E-06	-	6,61E-08	7,11E-06
Ni	Curry Post	-	-	6,35E-10	6,35E-10	-	-	1,64E-10	1,64E-10
	Cedara	-	-	9,48E-10	9,48E-10	-	-	2,44E-10	2,44E-10
	Gilboa	-	-	8,42E-10	8,42E-10	-	-	2,17E-10	2,17E-10
	Richmond	-	-	5,57E-10	5,57E-10	-	-	1,44E-10	1,44E-10
	Umgeni	-	-	1,50E-09	1,50E-09	-	-	3,86E-10	3,86E-10
Pb	Curry Post	nd	-	-	0,00E+00	nd	-	-	0,00E+00
	Cedara	nd	-	-	0,00E+00	nd	-	-	0,00E+00
	Gilboa	nd	-	-	0,00E+00	nd	-	-	0,00E+00
	Richmond	2,27E-09	-	-	2,27E-09	3,08E-09	-	-	3,08E-09
	Umgeni	nd	-	-	0,00E+00	nd	-	-	0,00E+00
Total metals		2,59E-05	-	1,28E-06	2,71E-05	3,51E-05	-	3,31E-07	3,54E-05

5.5. Conclusion

The hotplate, ultrasonic and microwave-assisted methods followed by ICP-OES were applied for metal determination in agricultural soil samples from different areas of KwaZulu-Natal. The percentage recoveries were within the acceptable range of 74 – 112%, indicating good accuracy for all methods. The metal concentrations decreased in the following order: Ga > Cr > Ba > Cu > Ni > Zn > Sr > Co > Pb > Li. The Ba and Cr were the two most abundant metals in all samples, while Cd was undetected in all samples. The Co, Ni, Pb and Zn were below whilst Cr and Zn were above their respective permissible limits in all soil samples. Umgeni soil was the most polluted since most metals had higher concentrations when compared to other sampling locations. Microwave and hotplate assisted digestion revealed similar metal concentrations, while ultrasonic-assisted digestion underestimated the concentrations. Despite the hotplate method being an open system method, it proved to be the most suitable substitute for microwave-assisted digestion compared to the ultrasonic method. This is due to convenience, accessibility and being a cost-effective method, which does not require expensive instrumentation as opposed to the microwave method. Umgeni and Gilboa Farm were the two most contaminated sites as per the calculated contamination factor, pollution load, geo-accumulation, and potential ecological risk indices.

The metal correlation revealed that Zn and Co, Cu and Ni possibly originate from the same source due to their synergistic relationship. The human health risk assessment showed that the exposure pathways for non-carcinogenic risk decreased in the following order: dermal contact > soil ingestion > air inhalation. Conclusively, it was observed that children experienced higher non-carcinogenic risk as opposed to adults since the THQ values were greater than 1. The CR values were below the maximum tolerable limit range of 1.00E-6 - 1.00E-4, presenting no serious health risk. Although heavy metal contamination was not severe at the selected sampling locations, the need for continuous assessment is required since these soils are used for agricultural activity.

5.6. References

Alengebawy, A., Abdelkhalek, S., Qureshi, S., & Wang, M. (2021). Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics, 9*(42), 1-33. DOI: https://doi.org/10.3390/toxics/9030042

Arao, T., Ishikawa, S., Murakami, M., Abe, K., Maejima, Y., & Makino, T. (2010). Heavy metal contamination of agricultural soil and countermeasures in Japan. *Paddy Water Environment, 8*, 247-257. DOI: https://doi.org/10.1007/s10333-010-0205-7

Bhatti, S.S., Kumar, V., Singh, N., Sambyal, V., Singh, J., Katnori, J.K., & Nagpal, A.K., (2016). Physico-chemical properties and heavy metal contents of soils and Kharif crops of Punjab, India, *Procedia Environmental Science*. 35, 801 – 808.

Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L., Men, Y., 2010. Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. *Journal of Environment Sciences (China)* 22, 1792-1799. https://doi.org/10.1016/s1001-0742(09)60321-1

Chabukdhara, M., Nema, A. K., (2013). Heavy metals assessment in urban soil around industrial clusters in Ghaziabad, India: Probabilistic health risk approach. *Ecotoxicity and Environmental Safety.* 87,57-64. DOI: https://doi.org/10.1016/j.ecoenv.2012.08.032

Chen, H., Teng, Y., Lu, S., Wang, Y., Wu, J., Wang, J. 2016. Source apportionment and health risk assessment of trace metals in surface soils of Beijing metropolitan, China. *Chemosphere 144*, 1002-1011. https://doi.org/10.1016/j.chemosphere/2015/09.081

Choi, J., & Jeon, S. (2018). A Geo-statistical Assessment of Heavy Metal Pollution n the Soil Around a Ship Building Yard in Busan, Korea. *Journal of the Korean Society of Marine Environment & Safety, 24*(7), 907-915. Doi: https://doi.org/10.7837/kosomes.2018.24.7.907

Collin, B., & Doelsch, E. (2010). Impact of high natural soilborne heavy metal concentrations on the mobility and phytoavailability of these elements for sugarcane. *Geoderma*, *159*(3-4), 452-458. DOI: 10.1016/j.geoderma.2010.09.013

Ferreira-Baptista, L., De Miguel, E., (2005). Geochemistry and risk assessment of street dust in Luanda, Angola: a tropical urban environment. *Atmosphere and Environment*. 39, 4501-4512. https://doi.org/10.1016/j.atmosenv.2005.03.026

Finley, B., Proctor, D., Scott, P., Harrington, N., Paustenbach, D., Price, P., (1994). Recommended distributions for exposure factors frequently used in health risk assessment. *Risk Analysis*. 14, 533-553. DOI: https://10.1111/j.1539-6924.1994.tb00269.x

Fryer, M., Collins, C., Ferrier, H., Colvile, R., & Nieuwenhuijsen, M. (2006). Human exposure modelling for chemical risk assessment: a review of current approach and research and policy implications. *Environmental Science and Policy, 9*, 261-274. DOI:10.1016/j.envsci.2005.11.011

Herselman, J., Steyn, C., & Fey, M. (2005). Baseline concentration of Cd, Co, Cr, Cu, Pb, Ni and Zn in surface soils of South Africa. *South African Journal of Science, 10,* 509-512. http://hdl.handle.net/10019.1/9660

Jiang, Y., Chao, S., Liu, J., Yang, Y., Chen, Y., Zhang, A., & Cao, H. (2016). Source apportionment and health risk assessment of heavy metals in soil for a township in Jiangsu Province, China. *Chemosphere, 168,* 1658 - 1668. http://dx.doi.org/10.1016/j.chemosphere/2016/11.088

Jiang, Y., Zeng, X., Fan, X., Chao, S., Zhu, M., Cao, H., (2015). Levels of arsenic pollution in daily foodstuffs and soils and its associated human health risk in a town in Jiangsu Province, China. *Ecotoxicity and Environmental Safety*. 122, 198-204. http://dx.doi.org/10.1016/j.ecoenv.

Kazi. T., Jamali. M.. Arian. M.. Afridi. J. N.. Sarfraz. R.. & Ansari. R. (2008). Evaluation of an ultrasonic acid digestion procedure for total heavy metals. *Journal of Hazardous Materials*(161). 1391 - 1398. DOI: http://dx.doi.org/10.1016/j.jhazmat.2008.04.103

Liu, Y., Shaheen, S., Rinklebe, J., & Hseu, Z. (2021). Pedogeochemical distribution of gallium, indium and thallium, their potential availability and associated risk in highly-weathered soil profiles of Taiwan. *Journal of environmental research*, 1-10. https://doi.org/10.1016/j.envres.2021.110994

MRPC, Ministry of Environmental Protection of the People's Republic of China, 2013. Exposure Factors Handbook of Chinese Population. *China Environmental Science Press*, Beijing, China.

Mussa, C., Biswick, T., Changadeya, W., Mapoma, H., & Junginger, A. (2010). Occurrence and ecological risk assessment of heavy metals in agricultural soils of Lake Chilwa catchment in Malawi, Southern Africa. *SN Applied Science, 2*(1910), 1-8. DOI: https://doi.org/10.1007/s42452-020-03718-7

Muzerengi, C. (2017). Enrichment and Geoaccumulation of Pb, Zn, As, Cd and Cr in soils near New Union Gold Mine, Limpopo Province. *Mine, Water and Circular Economy*, 720-727. https://www.imwa.info/docs/imwa_2017/IMWA2017_Muzerengi_720.pdf

Namieśnik, J., & Rabajczyk, A. (2010) The speciation and physico-chemical forms of metals in surface waters and sediments, *Chemical Speciation & Bioavailability*, *22*(1), 1-24, https://doi.org/10.3184/095422910X12632119406391

Naveedullah, Hashmi, M. Z., Yu, C., Shen, H., Duan, D., Shen, C., Lou, L., & Chen, Y. (2013). Risk assessment of heavy metals pollution in agricultural soils of siling reservoir watershed in Zhejiang Province, China. *BioMed research international, 2013,* 590306. https://doi.org/10.1155/2013/590306

NRCS, Natural Resources Conservation Service, United States Department of Agriculture, (2000). Heavy Metal Soil Contamination, *Soil Quality – Urban Technical Note, 3,* 1-7.

Ong. G.. Yap. C.. Mahmood. M.. Tan. S.. & Hamzah. S. (2013). Barium Levels in Soils and Centella asiatica. *Tropical Life Sciences Research. 24*(1). 55-70.

Pais I. Benton J and Jones Jr. (1998). *The handbook of trace elements*. Boca Raton: St Lucie Press. https://doi.org/10.2134/jeq1998.00472425002700040041

Poledniok. J.. Kita. A.. & Zerzucha. P. (2012). Spectrophotometric and Inductively Coupled Plasma–Optical Emission Spectroscopy Determination of Gallium in Natural Soils and

Soils Polluted by Industry: Relationships between Elements. *Communications in Soil Science and Plant Analysis. 43*(8). 1121-1135. https://doi.org/10.1080/2012/662561

Reeve, R. (2001). *Environmental analysis*. New York: Wiley.

Salem, M., Bedade, D., Al-Ethawi, L., & Al-waleed, S. (2020). Assessment of physicochemical properties and concentration of heavy metals in agricultural soils fertilized with chemical fertilizers. *Heliyon*, *6*(10), e05224. DOI: 10.1016/j.heliyon.2020.e05224

Santos-Francés, F., Martínez-Graña, A., Rojo, P., & Sánchez, A. (2017). Geochemical Background and Baseline Values Determination and Spatial Distribution of Heavy Metal Pollution in Soils of the Andes Mountain Range (Cajamarca-Huancavelica, Peru). *International Journal of Environmental Resources and Public Health, 14*(859), 1-22. https://doi.org/10.3390/ijerph14080859

Sastre. J.. Sahuquillo. A.. Vidal. M.. & Rauret. G. (2002). Determination of Cd. Cu. Pb and Zn in environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid extraction. *Analytica Chimica Acta*. 59-72 https://doi.org/10.1016/S0003-2670(02)00307-0

USDoE., 2011.The Risk Assessment Information System (RAIS). U.S. Department of Energy's Oak Ridge Operations Office (ORO). https://rais.ornl.gov (accessed 10 October 2021)

USEPA (US Environmental Protection Agency), 2001. Supplemental guidance for developing soil screening levels for superfund sites. OSWER9355.4-24. Office of Solid Waste and Emergency Response. US Environmental Protection Agency. Washington, DC. http://www.epa.gov/superfund/superfund-soil-screening-guidance

USEPA, 2011. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F. https://www.nrc.gov/ML1400/ML14007A666.pdf

USEPA, (2016). Integrated risk information system (IRIS). Retrieved from https://cfpub.epa.gov/ncea/iris/search/index.cfm?keyword=

World Health Organization (WHO). (2001). Geneva: World Health Organization. http://www.inchem.org/documents/ehc/ehc221.htm (accessed 9 October 2021)

Chapter Six

This chapter concludes the findings obtained from each chapter along with the recommendations for future research.

6.1. Conclusion

The US EPA acid digestion methods using microwave and open heat/hotplate were employed for the determination of metals in water, soil, and sediments in the Kwa-Zulu-Natal province. In addition, the ultrasonic -assisted digestion method was developed, in which sonication time and acid addition was investigated. A sonication time of 45 minutes and the sequential addition of HNO_3 was the optimum conditions for ultrasonic digestion. The evaluation of the analytical figures of merit (LOD, LOQ and R^2) of the ICP-OES revealed good accuracy of the instrument calibration, in terms of linearity, with R^2 values greater than 0.99. In all of the sub – studies, validation of digestions methods revealed an acceptable recovery range of 70 – 120%.

The successful application of microwave and hotplate assisted digestion methods in tap, river, and wastewater for the determination of metals revealed higher total recoverable metal concentrations as opposed to total dissolved metal concentrations. Despite both digestion methods being equally efficient, the hotplate digestion can be recommended as an inexpensive and accessible alternative for routine analysis of metal contamination. In general, all the metals studied in the water samples were below the maximum permissible limits except for Pb in tap water, reiterating the importance of continuous monitoring of the water resources to ensure clean water on consumption. Households can minimize contamination of Pb in tap water by installing "lead-free" plumbing fixtures, water filters can also reduce water contamination. In addition, anion concentrations were quantified in all water samples and found to be below the acceptable limits except for chlorides and sulphates in Amanzimtoti and Northern Rivers. Anions are generally naturally occurring however the high concentrations observed in wastewater associated rivers indicated that the treatment processes implemented, requires improvement.

The microwave and ultrasonic assisted digestion methods in soil and sediments sampled during Autumn and Spring revealed high concentrations of Ba and Ga, while Co, Ni and Pb which were found below the maximum permissible limits in all sampling sites thus posing no serious environmental risks. Despite the high recoveries obtained during the validation process, ultrasonic digestion underestimated the metal concentrations compared to microwave assisted digestion thus making it a more sensitive method. However, with more optimization for specified samples, the ultrasonic assisted digestion method can provide promising results in the future since the digestion method is a cheaper and available alternative compared to the microwave. The effect of seasonal variations was highlighted by the observed different metal concentrations during Autumn and Spring. Camps' Drift soil and sediment samples had higher metal concentrations during Autumn whilst Bishopstowe and Woodhouse had higher metal concentrations during Spring. Conclusively, Camps' Drift was found to be the most polluted location with an overall high total metal concentration. Anthropogenic effects were evaluated on the basis of enrichment factor, geo-accumulation, and potential ecological risk index categorization. Heavy metal contamination was not severe at all sampling locations however, Cu concentrations showed significant contamination thus requiring continuous monitoring.

Amongst the determination of metals in soils, agricultural soils from different areas of KwaZulu – Natal were assessed. The metal concentrations decreased in the following order: Ga > Cr > Ba > Cu > Ni > Zn > Sr > Co > Pb > Li. The Ba and Cr were the two most abundant metals in all the agricultural soils, with Cd remaining undetected. In addition, Cr and Zn were above their respective maximum permissible limits. As per the quantitative and qualitative (contamination factor, geo-accumulation index, pollution load index and potential ecological load index), Umgeni and Gilboa Farm were the two most contaminated agricultural sites. The human health risk assessment associated with heavy metals revealed exposure pathways decreasing in the following order: dermal contact > soil ingestion > air inhalation. Conclusively, it was observed that children experienced higher non-carcinogenic risk as opposed to adults since the THQ values were greater than 1. The CR values were below the maximum tolerable limit range thus presenting no serious carcinogenic risk. Although, heavy metal contamination was not severe in all sampling locations, continuous monitoring is required since these particular soils are used for agricultural activity.

Conclusively, the hotplate assisted digestion method proved to be the inexpensive and accessible alternative to microwave assisted digestion, successfully digesting, and extracting the metals from the water, soil, and sediment samples. The assessment of metals at the sampling sites provided significant data on the metal contamination and its potential effects on humans, animals, and their surroundings in the KwaZulu Natal Province. The need for continuous monitoring is required for pollution control and management of water resources and the surrounding environment.

6.2. Recommendations and future work

- The assessment of other potential contaminants originating from wastewater treatment plants, the monitoring of the efficiency of the treatment employed at the studied WWTPs.
- The continuous monitoring of water, soil, and sediment resources for the contamination of metals, in specific heavy metals in the KwaZulu-Natal Province and their associated environmental contamination and human health risk in water.
- The continuous monitoring of metals in different parts of the KwaZulu-Natal Province for a holistic overview of metal contamination in the province and in South Africa.
- The development of country specific maximum residual limits for metals in water, soil, and sediments from continuous monitoring of metal concentrations in South Africa.
- The further development for the ultrasonic – assisted digestion method for the efficient total metal digestion in complex solid samples (soil, and sediment).
- The development and application of adsorption techniques used for metals in water resources, in addition the use of environmentally friendly, accessible, and inexpensive adsorbents.

Appendix

Figure S3.1: A map of the sampling points for river water along the Msunduzi River

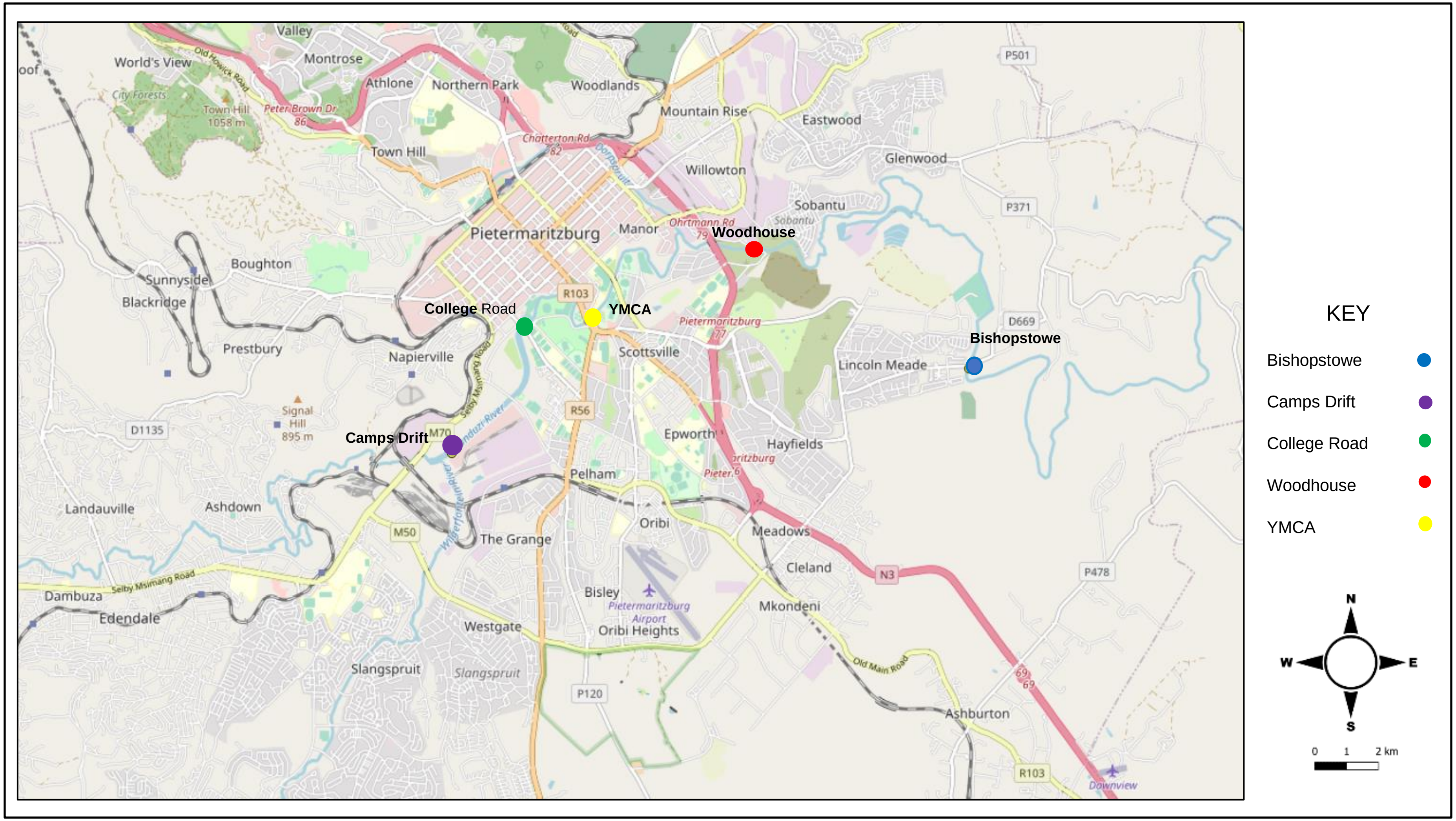


Table S3.1: Significance of results on the effect of sample volume on metal recoveries by hotplate method

t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	25 mL	*50 mL*		*25 mL*	*100 mL*		*50 mL*	*100 mL*
Mean	62,33333	64,91667	Mean	62,33333	91,58333	Mean	64,91667	91,58333
Variance	124,0606	140,2652	Variance	124,0606	29,7197	Variance	140,2652	29,7197
Observations	12	12	Observations	12	12	Observations	12	12
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	22		df	16		df	15	
t Stat	-0,55043		t Stat	-8,17083		t Stat	-7,08523	
P(T<=t) one-tail	0,293786		P(T<=t) one-tail	2,11E-07		P(T<=t) one-tail	1,86E-06	
t Critical one-tail	1,717144		t Critical one-tail	1,745884		t Critical one-tail	1,75305	
P(T<=t) two-tail	0,587571		P(T<=t) two-tail	4,21E-07		P(T<=t) two-tail	3,71E-06	
t Critical two-tail	2,073873		t Critical two-tail	2,119905		t Critical two-tail	2,13145	

Table S3.2: T-test assuming unequal variances for the three water matrices using hotplate and microwave assisted digestion

	HOTPLATE						*MICROWAVE*					
	TAP	*RIVER*	*TAP*	*WASTE*	*RIVER*	*WASTE*	*TAP*	*RIVER*	*TAP*	*WASTE*	*RIVER*	*WASTE*
Mean	93,000	90,636	93,000	86,818	90,636	86,818	85,545	93,091	85,545	90,091	93,091	90,091
Variance	27,800	16,855	27,800	21,364	16,855	21,364	104,673	158,091	104,673	21,291	158,091	21,291
Observations	11,000	11,000	11,000	11,000	11,000	11,000	11,000	11,000	11,000	11,000	11,000	11,000
Hypothesized Mean Difference	0,000		0,000		0,000		0,000		0,000		0,000	
Df	19,000		20,000		20,000		19,000		14,000		13,000	
t Stat	1,173		2,924		2,048		-1,544		-1,343		0,743	
P(T<=t) one-tail	0,128		0,004		0,027		0,070		0,100		0,235	
t Critical one-tail	1,729		1,725		1,725		1,729		1,761		1,771	
P(T<=t) two-tail	0,255		0,008		0,054		0,139		0,201		0,471	
t Critical two-tail	2,093		2,086		2,086		2,093		2,145		2,160	

Table S3.3: Significant difference of mean recoveries results on digestion of total recoverable metals in wastewater influent for the hotplate and microwave methods

	Hotplate						Microwave					
	H 0,10	*H0,50*	*H 0,10*	*H 1,0*	*H 0,50*	*H 1,0*	*M 0,1*	*M 0,50*	*M 0,1*	*M 1,0*	*M 0,5*	*M 1,0*
Mean	89,23077	87,07692	89,23077	85,69231	87,07692	85,69231	92,38462	90,46154	92,38462	89,23077	90,46154	89,23077
Variance	120,359	21,57692	120,359	74,23077	21,57692	74,23077	97,08974	23,60256	97,08974	92,19231	23,60256	92,19231
Observations	13	13	13	13	13	13	13	13	13	13	13	13
Hypothesized Mean Difference	0		0		0		0		0		0	
Df	16		23		18		18		24		18	
t Stat	0,651839		0,914589		0,510036		0,631144		0,826528		0,412386	
P(T<=t) one-tail	0,26188		0,184948		0,308112		0,26794		0,208323		0,342463	
t Critical one-tail	1,745884		1,713872		1,734064		1,734064		1,710882		1,734064	
P(T<=t) two-tail	0,523759		0,369896		0,616224		0,535879		0,416647		0,684927	
t Critical two-tail	2,119905		2,068658		2,100922		2,100922		2,063899		2,100922	

Table S3.4: Significant difference of mean recoveries results on digestion of total dissolved metals in wastewater influent for the hotplate and microwave methods

	Hotplate						Microwave					
	H 0,10	*H0,50*	*H 0,10*	*H 1,0*	*H 0,50*	*H 1,0*	*M 0,1*	*M 0,50*	*M 0,1*	*M 1,0*	*M 0,5*	*M 1,0*
Mean	86,38462	86,92308	86,38462	85,61538	86,92308	85,61538	91,61538	90,92308	91,61538	87,92308	90,92308	87,92308
Variance	45,08974	42,57692	45,08974	128,4231	42,57692	128,4231	107,2564	25,41026	107,2564	90,57692	25,41026	90,57692
Observations	13	13	13	13	13	13	13	13	13	13	13	13
Hypothesized Mean Difference	0		0		0		0		0		0	
Df	24		20		19		17		24		18	
t Stat	-0,20735		0,210554		0,360561		0,216715		0,946498		1,004357	
P(T<=t) one-tail	0,418742		0,417683		0,361202		0,415505		0,176664		0,164259	
t Critical one-tail	1,710882		1,724718		1,729133		1,739607		1,710882		1,734064	
P(T<=t) two-tail	0,837484		0,835366		0,722405		0,831009		0,353328		0,328518	
t Critical two-tail	2,063899		2,085963		2,093024		2,109816		2,063899		2,100922	

Table S3.5: Significant difference of mean concentration results on digestion of metals in tap water by hotplate and microwave methods tap water, RC

t-Test: Two-Sample Assuming Unequal Variances

	TR, heat	TD, heat
	5,4	*5,1*
Mean	43,96667	60,63333
Variance	542,1733	1774,823
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-0,59972	
P(T<=t) one-tail	0,295483	
t Critical one-tail	2,353363	
P(T<=t) two-tail	0,590967	
t Critical two-tail	3,182446	

t-Test: Two-Sample Assuming Unequal Variances

	TR, micro	TD, micro
	5,3	*5,3*
Mean	54,23333	35,2
Variance	752,4133	644,77
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0,88196	
P(T<=t) one-tail	0,213805	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,42761	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Unequal Variances

	TR, heat	TR, micro
	5,4	*5,3*
Mean	43,96667	54,23333
Variance	542,1733	752,4133
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-0,49422	
P(T<=t) one-tail	0,323529	
t Critical one-tail	2,131847	
P(T<=t) two-tail	0,647059	
t Critical two-tail	2,776445	

t-Test: Two-Sample Assuming Unequal Variances

	TD, heat	TD, micro
	5,1	*5,3*
Mean	60,63333	35,2
Variance	1774,823	644,77
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	0,895556	
P(T<=t) one-tail	0,21824	
t Critical one-tail	2,353363	
P(T<=t) two-tail	0,436481	
t Critical two-tail	3,182446	

tap water, SV

t-Test: Two-Sample Assuming Unequal Variances

	TR, heat	*TD, heat*
Mean	52,85	35,9
Variance	2141,05	847,02
Observations	4	4
Hypothesized Mean Difference	0	
df	5	
t Stat	0,620161	
P(T<=t) one-tail	0,28116	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,56232	
t Critical two-tail	2,570582	

t-Test: Two-Sample Assuming Unequal Variances

	TR, micro	*TD, micro*
Mean	65,825	53,65
Variance	3459,516	2292,31
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	0,321067	
P(T<=t) one-tail	0,379524	
t Critical one-tail	1,94318	
P(T<=t) two-tail	0,759048	
t Critical two-tail	2,446912	

t-Test: Two-Sample Assuming Unequal Variances

	TR, heat	*TR, micro*
Mean	52,85	65,825
Variance	2141,05	3459,516
Observations	4	4
Hypothesized Mean Difference	0	
df	6	
t Stat	-0,34675	
P(T<=t) one-tail	0,370307	
t Critical one-tail	1,94318	
P(T<=t) two-tail	0,740614	
t Critical two-tail	2,446912	

t-Test: Two-Sample Assuming Unequal Variances

	TD, heat	*TD, micro*
Mean	35,9	53,65
Variance	847,02	2292,31
Observations	4	4
Hypothesized Mean Difference	0	
df	5	
t Stat	-0,63359	
P(T<=t) one-tail	0,277097	
t Critical one-tail	2,015048	
P(T<=t) two-tail	0,554193	
t Critical two-tail	2,570582	

tap water, MN											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, heat*	*TD, heat*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	40,025	25,775	Mean	77,9	36,775	Mean	40,025	77,9	Mean	25,775	36,775
Variance	923,9492	596,2292	Variance	4601,36	954,3025	Variance	923,9492	4601,36	Variance	596,2292	954,3025
Observations	4	4	Observations	4	4	Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	4		df	4		df	6	
t Stat	0,730967		t Stat	1,103489		t Stat	-1,01907		t Stat	-0,5587	
P(T<=t) one-tail	0,246173		P(T<=t) one-tail	0,165867		P(T<=t) one-tail	0,182896		P(T<=t) one-tail	0,298291	
t Critical one-tail	1,94318		t Critical one-tail	2,131847		t Critical one-tail	2,131847		t Critical one-tail	1,94318	
P(T<=t) two-tail	0,492346		P(T<=t) two-tail	0,331735		P(T<=t) two-tail	0,365791		P(T<=t) two-tail	0,596582	
t Critical two-tail	2,446912		t Critical two-tail	2,776445		t Critical two-tail	2,776445		t Critical two-tail	2,446912	

tap water, WL											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	52	56,925	Mean	38,225	31,25	Mean	52	38,225	Mean	56,925	31,25
Variance	2200,987	2925,583	Variance	614,3492	483,07	Variance	2200,987	614,3492	Variance	2925,583	483,07
Observations	4	4	Observations	4	4	Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6		df	5		df	4	
t Stat	-0,13757		t Stat	0,421103		t Stat	0,519226		t Stat	0,879527	
P(T<=t) one-tail	0,447541		P(T<=t) one-tail	0,344174		P(T<=t) one-tail	0,31288		P(T<=t) one-tail	0,214391	
t Critical one-tail	1,94318		t Critical one-tail	1,94318		t Critical one-tail	2,015048		t Critical one-tail	2,131847	
P(T<=t) two-tail	0,895081		P(T<=t) two-tail	0,688348		P(T<=t) two-tail	0,62576		P(T<=t) two-tail	0,428782	
t Critical two-tail	2,446912		t Critical two-tail	2,446912		t Critical two-tail	2,570582		t Critical two-tail	2,776445	

tap water, BT											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	45,825	26,275	Mean	36,9	43,325	Mean	45,825	36,9	Mean	26,275	43,325
Variance	989,7892	612,5825	Variance	753,2867	1285,729	Variance	989,7892	753,2867	Variance	612,5825	1285,729
Observations	4	4	Observations	4	4	Observations	4	4	Observations	4	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	6		df	6		df	6		df	5	
t Stat	0,976776		t Stat	-0,28457		t Stat	0,427543		t Stat	-0,78266	
P(T<=t) one-tail	0,183201		P(T<=t) one-tail	0,392769		P(T<=t) one-tail	0,341951		P(T<=t) one-tail	0,234628	
t Critical one-tail	1,94318		t Critical one-tail	1,94318		t Critical one-tail	1,94318		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,366403		P(T<=t) two-tail	0,785538		P(T<=t) two-tail	0,683903		P(T<=t) two-tail	0,469257	
t Critical two-tail	2,446912		t Critical two-tail	2,446912		t Critical two-tail	2,446912		t Critical two-tail	2,570582	

Table S3.6: Significant difference of mean concentration results on digestion of metals in river water by hotplate and microwave methods

river water, BS											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	28,16667	38,53333	Mean	43,433333	35,8	Mean	28,16667	43,43333	Mean	38,53333	35,8
Variance	139,6933	1139,103	Variance	546,20333	832,36	Variance	139,6933	546,2033	Variance	1139,103	832,36
Observations	3	3	Observations	3	3	Observations	3	3	Observations	3	3
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	2		df	4		df	3		df	4	
t Stat	-0,50211		t Stat	0,3560914		t Stat	-1,00966		t Stat	0,106625	
P(T<=t) one-tail	0,332709		P(T<=t) one-tail	0,3698797		P(T<=t) one-tail	0,193513		P(T<=t) one-tail	0,46011	
t Critical one-tail	2,919986		t Critical one-tail	2,1318468		t Critical one-tail	2,353363		t Critical one-tail	2,131847	
P(T<=t) two-tail	0,665417		P(T<=t) two-tail	0,7397594		P(T<=t) two-tail	0,387027		P(T<=t) two-tail	0,92022	
t Critical two-tail	4,302653		t Critical two-tail	2,7764451		t Critical two-tail	3,182446		t Critical two-tail	2,776445	

river water, CD											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
							]				
	TR, heat	*TD, hear*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	35,55	49,85	Mean	62,366667	45,1	Mean	35,55	62,36667	Mean	49,85	45,1
Variance	49,005	741,125	Variance	446,33333	954,79	Variance	49,005	446,3333	Variance	741,125	954,79
Observations	2	2	Observations	3	3	Observations	2	3	Observations	2	3
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	1		df	4		df	3		df	3	
t Stat	-0,71945		t Stat	0,7989709		t Stat	-2,03718		t Stat	0,180983	
P(T<=t) one-tail	0,301482		P(T<=t) one-tail	0,23453		P(T<=t) one-tail	0,067205		P(T<=t) one-tail	0,433959	
t Critical one-tail	6,313752		t Critical one-tail	2,1318468		t Critical one-tail	2,353363		t Critical one-tail	2,353363	
P(T<=t) two-tail	0,602964		P(T<=t) two-tail	0,4690599		P(T<=t) two-tail	0,134411		P(T<=t) two-tail	0,867917	
t Critical two-tail	12,7062		t Critical two-tail	2,7764451		t Critical two-tail	3,182446		t Critical two-tail	3,182446	

river water, CR											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	47,65	76,5	Mean	42,75	55,675	Mean	47,65	42,75	Mean	76,5	55,675
Variance	616,005	3872	Variance	2394,47	2599,836	Variance	616,005	2394,47	Variance	3872	2599,836
Observations	2	2	Observations	4	4	Observations	2	4	Observations	2	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	1		df	6		df	4		df	2	
t Stat	-0,60902		t Stat	-0,365783		t Stat	0,162736		t Stat	0,409519	
P(T<=t) one-tail	0,325876		P(T<=t) one-tail	0,3635387		P(T<=t) one-tail	0,439308		P(T<=t) one-tail	0,360927	
t Critical one-tail	6,313752		t Critical one-tail	1,9431803		t Critical one-tail	2,131847		t Critical one-tail	2,919986	
P(T<=t) two-tail	0,651751		P(T<=t) two-tail	0,7270773		P(T<=t) two-tail	0,878617		P(T<=t) two-tail	0,721853	
t Critical two-tail	12,7062		t Critical two-tail	2,4469119		t Critical two-tail	2,776445		t Critical two-tail	4,302653	

river water, WH											
t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances			t-Test: Two-Sample Assuming Unequal Variances		
	TR, heat	*TD, heat*		*TR, micro*	*TD, micro*		*TR, heat*	*TR, micro*		*TD, heat*	*TD, micro*
Mean	43,2	41,76667	Mean	38,075	70,175	Mean	43,2	38,075	Mean	41,76667	70,175
Variance	0,5	988,5633	Variance	780,79583	1792,269	Variance	0,5	780,7958	Variance	988,5633	1792,269
Observations	2	3	Observations	4	4	Observations	2	4	Observations	3	4
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	2		df	5		df	3		df	5	
t Stat	0,07893		t Stat	-1,265638		t Stat	0,366587		t Stat	-1,01876	
P(T<=t) one-tail	0,472137		P(T<=t) one-tail	0,1307059		P(T<=t) one-tail	0,369128		P(T<=t) one-tail	0,177527	
t Critical one-tail	2,919986		t Critical one-tail	2,0150484		t Critical one-tail	2,353363		t Critical one-tail	2,015048	
P(T<=t) two-tail	0,944275		P(T<=t) two-tail	0,2614118		P(T<=t) two-tail	0,738256		P(T<=t) two-tail	0,355053	
t Critical two-tail	4,302653		t Critical two-tail	2,5705818		t Critical two-tail	3,182446		t Critical two-tail	2,570582	

Table S4.1: Significant difference of mean recoveries for the effect of digestion acid addition for ultrasonic assisted digestion of soil and sediment samples

	Soil (60 min with 10 mL HNO_3)	*Soil (2x30 min with 2x5 mL HNO_3)*	*Sedi 60 with 10 mL HNO_3*	*Sedi (2x30 min with 2x5 mL HNO_3)*
Mean	75.33333333	83.91666667	77.16666667	85.33333333
Variance	78.06060606	87.90151515	9.424242424	117.5151515
Observations	12	12	12	12
Hypothesized Mean Difference	0		0	
Df	22		13	
t Stat	-2.308033527		-2.510945245	
P(T<=t) one-tail	0.015390513		0.013022708	
t Critical one-tail	1.717144374		1.770933396	
P(T<=t) two-tail	0.030781026		0.026045415	
t Critical two-tail	2.073873068		2.160368656	

Table S4.2: Significant difference of mean recoveries for the comparison of 15 with 30, 45 and 60 minute sonication times for ultrasonic-assisted digestion

	Soil (15 min)	*Soil (30 min)*	*Sedi (15 min)*	*Sedi (30 min)*	*Soil (15 min)*	*Soil (60 min)*	*Sedi (15 min)*	*Sedi (60 min)*
Mean	82.91666667	79.58333333	87.58333333	77.08333333	82.91666667	83.91666667	87.58333333	85.33333333
Variance	26.81060606	20.08333333	150.0833333	28.81060606	26.81060606	87.90151515	150.0833333	117.5151515
Observations	12	12	12	12	12	12	12	12
Hypothesized Mean Difference	0		0		0		0	
Df	22		15		17		22	
t Stat	1.686207472		2.71945645		-0.32343422		0.476465342	
P(T<=t) one-tail	0.052943843		0.007914313		0.375155576		0.319220527	
t Critical one-tail	1.717144374		1.753050356		1.739606726		1.717144374	
P(T<=t) two-tail	0.105887686		0.015828625		0.750311152		0.638441054	
t Critical two-tail	2.073873068		2.131449546		2.109815578		2.073873068	
	Soil (15 min)	*Soil (45 min)*	*Sedi (15 min)*	*Sedi (45 min)*	*Soil (30 min)*	*Soil (45 min)*	*Sedi (30 min)*	*Sedi (45 min)*
Mean	82.91666667	86.91666667	87.58333333	85.91666667	79.58333333	86.91666667	77.08333333	85.91666667
Variance	26.81060606	15.90151515	150.0833333	15.53787879	20.08333333	15.90151515	28.81060606	15.53787879
Observations	12	12	12	12	12	12	12	12
Hypothesized Mean Difference	0		0		0		0	
Df	21		13		22		20	
t Stat	-2.120191281		0.448622853		-4.234793228		-4.594897721	
P(T<=t) one-tail	0.02303417		0.330545201		0.000170016		8.77069E-05	
t Critical one-tail	1.720742903		1.770933396		1.717144374		1.724718243	
P(T<=t) two-tail	0.04606834		0.661090401		0.000340032		0.000175414	
t Critical two-tail	2.079613845		2.160368656		2.073873068		2.085963447	

	Soil (30 min)	*Soil (60 min)*	*Sedi (30 min)*	*Sedi (60 min)*	*Soil (45 min)*	*Soil (60 min)*	*Sedi (45 min)*	*Sedi (60 min)*
Mean	79.58333333	83.91666667	77.08333333	85.33333333	83.08333333	83.91666667	82.83333333	85.33333333
Variance	20.08333333	87.90151515	28.81060606	117.5151515	38.81060606	87.90151515	18.15151515	117.5151515
Observations	12	12	12	12	12	12	12	12
Hypothesized Mean Difference	0		0		0		0	
Df	16		16		19		14	
t Stat	-1.444545777		-2.362567258		-0.256448579		-0.743522396	
P(T<=t) one-tail	0.083941962		0.015575457		0.40018037		0.234730219	
t Critical one-tail	1.745883676		1.745883676		1.729132812		1.761310136	
P(T<=t) two-tail	0.167883925		0.031150914		0.800360739		0.469460438	
t Critical two-tail	2.119905299		2.119905299		2.093024054		2.144786688	

Table S4.3: Significant difference of mean recoveries for the comparison of soil and sediment samples for 45 minute sonication time for ultrasonic-assisted digestion

	Soil (45 min)	*Sedi (45 min)*
Mean	86.91666667	85.91666667
Variance	15.90151515	15.53787879
Observations	12	12
Hypothesized Mean Difference	0	
Df	22	
t Stat	0.617808018	
P(T<=t) one-tail	0.271522461	
t Critical one-tail	1.717144374	
P(T<=t) two-tail	0.543044921	
t Critical two-tail	2.073873068	

Table S4.4: Significant difference of mean recoveries for CRM spiking for microwave-assisted and ultrasonic-assisted digestion methods

	Micro soil	*Ultra-soil*	*Micro Sediment*	*Ultra-Sediment*
Mean	89.66666667	88.33333333	86.58333333	87.58333333
Variance	31.51515152	59.87878788	35.53787879	53.90151515
Observations	12	12	12	12
Hypothesized Mean Difference	0		0	
Df	20		21	
t Stat	0.483137404		-0.366290959	
P(T<=t) one-tail	0.317122438		0.358906196	
t Critical one-tail	1.724718243		1.720742903	
P(T<=t) two-tail	0.634244876		0.717812392	
t Critical two-tail	2.085963447		2.079613845	

Figure S5.1: The map showing the sampling points of agricultural soils

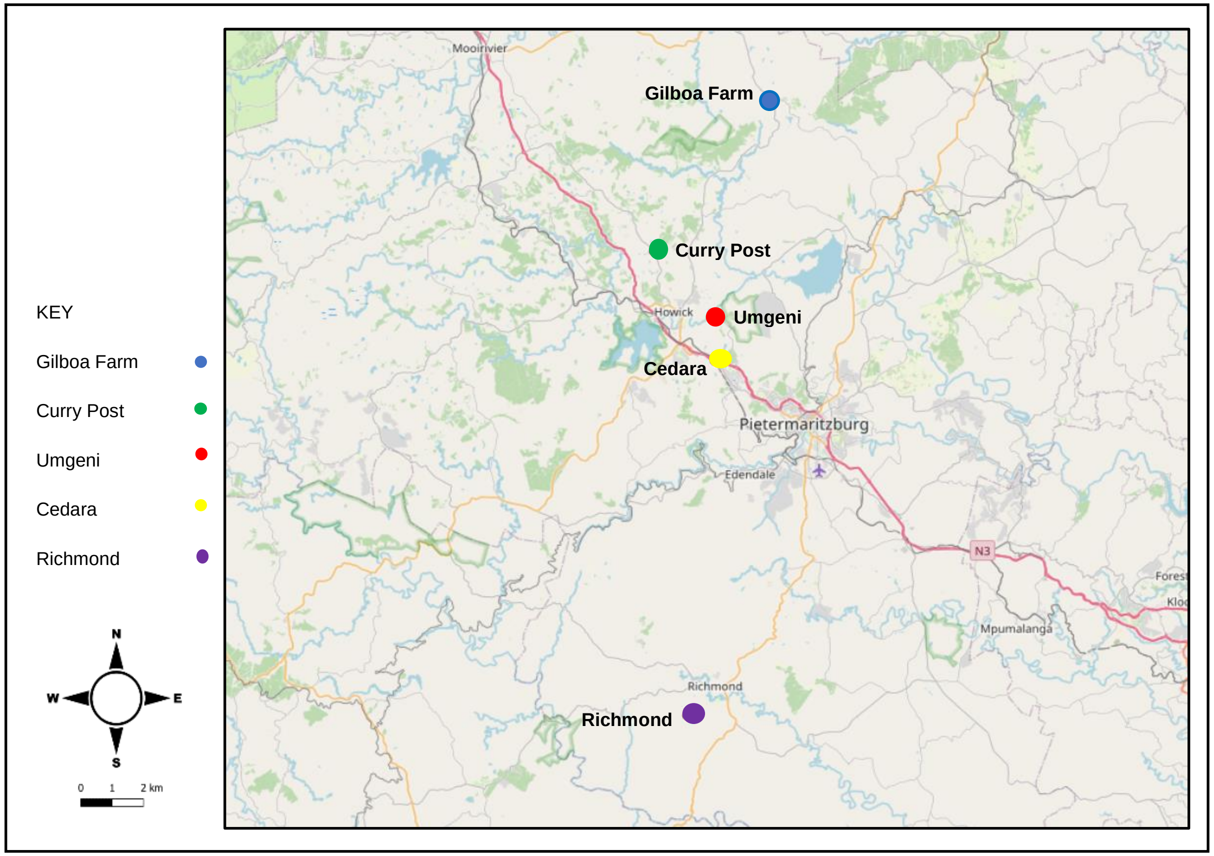
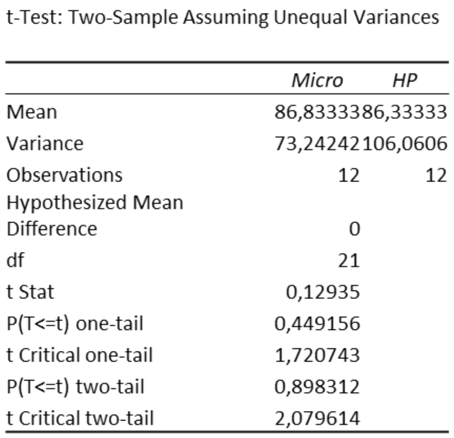
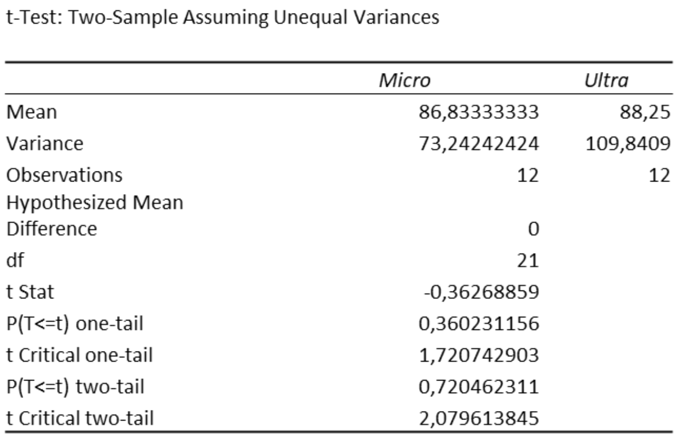


Table S5.1: Significant difference of mean recoveries for the effect of spiking concentration using all three digestion methods in soil samples



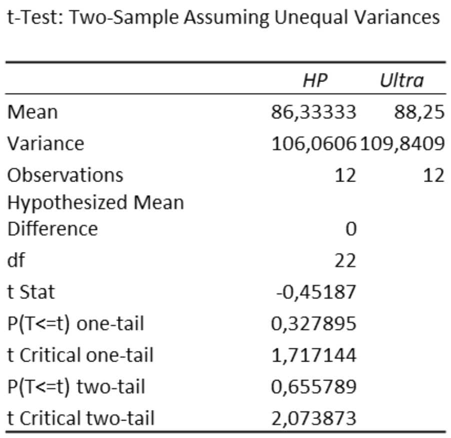
t-Test: Two-Sample Assuming Unequal Variances

	Micro	*HP*
Mean	86,83333	86,33333
Variance	73,24242	106,0606
Observations	12	12
Hypothesized Mean Difference	0	
df	21	
t Stat	0,12935	
P(T<=t) one-tail	0,449156	
t Critical one-tail	1,720743	
P(T<=t) two-tail	0,898312	
t Critical two-tail	2,079614	



t-Test: Two-Sample Assuming Unequal Variances

	Micro	*Ultra*
Mean	86,83333333	88,25
Variance	73,24242424	109,8409
Observations	12	12
Hypothesized Mean Difference	0	
df	21	
t Stat	-0,36268859	
P(T<=t) one-tail	0,360231156	
t Critical one-tail	1,720742903	
P(T<=t) two-tail	0,720462311	
t Critical two-tail	2,079613845	



t-Test: Two-Sample Assuming Unequal Variances

	HP	*Ultra*
Mean	86,33333	88,25
Variance	106,0606	109,8409
Observations	12	12
Hypothesized Mean Difference	0	
df	22	
t Stat	-0,45187	
P(T<=t) one-tail	0,327895	
t Critical one-tail	1,717144	
P(T<=t) two-tail	0,655789	
t Critical two-tail	2,073873	

Table S5.2: The parameters used in the human health risk assessment calculations for adults and children

Parameter	Description	Units	Value	References
IR_s	Ingestion rate of soil	mg/day	20 for adults and 50 for children	USEPA. 2011
IR_p	Ingestion rate of homegrown produce; resulted from questionnaire survey of local people	mg/day	Species specific	Cao *et al*. 2010; Jiang *et al*. 2015
IR_i	Inhalation rate of soil	m^3/day	16 for adults and 7.6 for children	MRPC. 2013; Chabukdhara & Nema. 2013
SA	Skin area available for soil contact	cm^2	5700 for adults and 2800 for children	USEPA. 2001
AF	Soil-to-skin adherence factor	kg/cm^2-day	2×10^{-7} for adults and 10^{-6} for children	Finley *et al*. 1994; US EPA. 2011
ABS	Absorption factor	unitless	0.001	Ferreira-Baptista and De Miguel. 2005
PEF	Particle emission factor	m^3/kg	1.36×10^9	USEPA. 2001
EF	Exposure frequency	day/year	350	USDoE. 2011
ED	Exposure duration	year	24 for adults and 6 for children	USEPA. 2011
BW	Body weight	kg	63 for adults and 29 for children	MRPC. 2013;
AT	Average time	day	ED × 365	USEPA. 2011
LT	Lifetime expressed in day	day	76.6 × 365	MRPC. 2013

Table S5.3: Values of RfD (mg/kg/day) and SF (per mg/kg/day) for seven heavy metals (Chen *et al*., 2016)

	Cr	Pb	Cu	Zn	Ni
RfD for ingestion	3.00E-03	3.50E-03	4.00E-02	3.00E-01	2.00E-02
RfD for dermal absorption	6.00E-05	5.25E-04	1.20E-02	6.00E-02	5.40E-03
RfD for inhalation	2.86E-05	-	-	-	9.00E-05
SF for ingestion	5.00E-01	8.50E-03	-	-	-
SF for dermal absorption	-	-	-	-	-
SF for inhalation	4.20E+01	-	-	-	8.40E-01

Table S5.4: Estimation of non-carcinogenic (HQ) from heavy metals in agricultural soil using hotplate assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	1,55E-02	4,42E-01	9,56E-04	4,58E-01	8,42E-02	2,36E+00	9,87E-04	2,45E+00
	Cedara	1,36E-02	3,88E-01	8,39E-04	4,02E-01	7,38E-02	2,07E+00	8,66E-04	2,14E+00
	Gilboa	1,42E-02	4,04E-01	8,75E-04	4,19E-01	7,70E-02	2,16E+00	9,03E-04	2,24E+00
	Richmond	8,69E-03	2,48E-01	5,36E-04	2,57E-01	4,72E-02	1,32E+00	5,53E-04	1,37E+00
	Umgeni	1,16E-02	3,30E-01	7,14E-04	3,42E-01	6,29E-02	1,76E+00	7,37E-04	1,82E+00
Cu	Curry Post	2,49E-04	4,73E-05	-	2,96E-04	1,35E-03	2,52E-04	-	1,60E-03
	Cedara	2,24E-04	4,25E-05	-	2,67E-04	1,22E-03	2,27E-04	-	1,45E-03
	Gilboa	4,66E-04	8,85E-05	-	5,55E-04	2,53E-03	4,72E-04	-	3,00E-03
	Richmond	1,59E-04	3,02E-05	-	1,89E-04	8,64E-04	1,61E-04	-	1,03E-03
	Umgeni	3,46E-04	6,58E-05	-	4,12E-04	1,88E-03	3,51E-04	-	2,23E-03
Ni	Curry Post	2,39E-04	5,04E-05	3,12E-05	3,21E-04	1,30E-03	2,69E-04	3,22E-05	1,60E-03
	Cedara	3,30E-04	6,97E-05	4,32E-05	4,43E-04	1,79E-03	3,72E-04	4,46E-05	2,21E-03
	Gilboa	4,19E-04	8,84E-05	5,47E-05	5,62E-04	2,27E-03	4,71E-04	5,65E-05	2,80E-03
	Richmond	2,25E-04	4,76E-05	2,94E-05	3,02E-04	1,22E-03	2,54E-04	3,04E-05	1,50E-03
	Umgeni	5,19E-04	1,10E-04	6,78E-05	6,97E-04	2,82E-03	5,85E-04	7,00E-05	3,48E-03
Pb	Curry Post	ND	ND	-	0,00E+00	ND	ND	-	0,00E+00
	Cedara	2,35E-04	8,92E-05	-	3,24E-04	1,28E-03	4,76E-04	-	1,76E-03
	Gilboa	1,22E-04	4,63E-05	-	1,68E-04	6,61E-04	2,47E-04	-	9,08E-04
	Richmond	ND	ND	-	0,00E+00	ND	ND	-	0,00E+00
	Umgeni	ND	ND	-	0,00E+00	ND	ND	-	0,00E+00
Zn	Curry Post	1,26E-05	3,59E-06	-	1,62E-05	6,83E-05	1,91E-05	-	8,74E-05
	Cedara	1,25E-05	3,56E-06	-	1,61E-05	6,78E-05	1,90E-05	-	8,68E-05
	Gilboa	2,10E-05	5,99E-06	-	2,70E-05	1,14E-04	3,19E-05	-	1,46E-04
	Richmond	9,03E-06	2,57E-06	-	1,16E-05	4,90E-05	1,37E-05	-	6,27E-05
	Umgeni	3,63E-05	1,04E-05	-	4,67E-05	1,97E-04	5,52E-05	-	2,52E-04
Total metals		6,72E-02	1,81E+00	4,15E-03	1,88E+00	3,65E-01	9,67E+00	4,28E-03	1,00E+01

Table S5.5: Estimation of carcinogenic (CR) from heavy metals in agricultural soil using hotplate assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	7,28E-06	-	3,60E-07	7,64E-06	9,89E-06	-	9,28E-08	9,98E-06
	Cedara	6,39E-06	-	3,16E-07	6,71E-06	8,68E-06	-	8,15E-08	8,76E-06
	Gilboa	6,66E-06	-	3,29E-07	6,99E-06	9,05E-06	-	8,49E-08	9,13E-06
	Richmond	4,08E-06	-	2,02E-07	4,28E-06	5,54E-06	-	5,20E-08	5,59E-06
	Umgeni	5,44E-06	-	2,69E-07	5,71E-06	7,39E-06	-	6,94E-08	7,46E-06
Ni	Curry Post	-	-	7,22E-10	7,22E-10	-	-	1,86E-10	1,86E-10
	Cedara	-	-	9,98E-10	9,98E-10	-	-	2,58E-10	2,58E-10
	Gilboa	-	-	1,27E-09	1,27E-09	-	-	3,26E-10	3,26E-10
	Richmond	-	-	6,81E-10	6,81E-10	-	-	1,76E-10	1,76E-10
	Umgeni	-	-	1,57E-09	1,57E-09	-	-	4,05E-10	4,05E-10
Pb	Curry Post	ND	-	-	0,00E+00	ND	-	-	0,00E+00
	Cedara	2,19E-09	-	-	2,19E-09	2,97E-09	-	-	2,97E-09
	Gilboa	1,13E-09	-	-	1,13E-09	1,54E-09	-	-	1,54E-09
	Richmond	ND	-	-	0,00E+00	ND	-	-	0,00E+00
	Umgeni	ND	-	-	0,00E+00	ND	-	-	0,00E+00
Total metals		2,99E-05	-	1,48E-06	3,13E-05	4,06E-05	-	3,82E-07	4,09E-05

Table S5.6: Estimation of non-carcinogenic (HQ) from heavy metals in agricultural soil using Ultrasonic assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	6,24E-03	1,78E-01	3,85E-04	1,85E-01	3,39E-02	9,49E-01	3,97E-04	9,83E-01
	Cedara	4,61E-03	1,31E-01	2,84E-04	1,36E-01	2,50E-02	7,01E-01	2,93E-04	7,26E-01
	Gilboa	4,64E-03	1,32E-01	2,86E-04	1,37E-01	2,52E-02	7,05E-01	2,95E-04	7,30E-01
	Richmond	4,02E-03	1,15E-01	2,48E-04	1,19E-01	2,18E-02	6,11E-01	2,56E-04	6,33E-01
	Umgeni	2,97E-03	8,47E-02	1,83E-04	8,79E-02	1,61E-02	4,52E-01	1,89E-04	4,68E-01
Cu	Curry Post	1,07E-04	2,02E-05	-	1,27E-04	5,79E-04	1,08E-04	-	6,87E-04
	Cedara	5,33E-05	1,01E-05	-	6,34E-05	2,89E-04	5,40E-05	-	3,43E-04
	Gilboa	3,18E-04	6,04E-05	-	3,78E-04	1,73E-03	3,23E-04	-	2,05E-03
	Richmond	4,11E-05	7,81E-06	-	4,89E-05	2,23E-04	4,17E-05	-	2,65E-04
	Umgeni	1,44E-04	2,73E-05	-	1,71E-04	7,81E-04	1,46E-04	-	9,27E-04
Ni	Curry Post	8,98E-05	1,90E-05	1,17E-05	1,21E-04	4,88E-04	1,01E-04	1,21E-05	6,01E-04
	Cedara	6,85E-05	1,45E-05	8,95E-06	9,20E-05	3,72E-04	7,72E-05	9,24E-06	4,58E-04
	Gilboa	1,95E-04	4,11E-05	2,55E-05	2,62E-04	1,06E-03	2,19E-04	2,63E-05	1,31E-03
	Richmond	1,07E-04	2,25E-05	1,39E-05	1,43E-04	5,79E-04	1,20E-04	1,44E-05	7,13E-04
	Umgeni	2,37E-04	5,01E-05	3,10E-05	3,18E-04	1,29E-03	2,67E-04	3,20E-05	1,59E-03
Pb	Curry Post	1,83E-04	6,94E-05	-	2,52E-04	9,92E-04	3,70E-04	-	1,36E-03
	Cedara	2,87E-04	1,09E-04	-	3,96E-04	1,56E-03	5,82E-04	-	2,14E-03
	Gilboa	2,61E-04	9,92E-05	-	3,60E-04	1,42E-03	5,29E-04	-	1,95E-03
	Richmond	2,52E-04	9,58E-05	-	3,48E-04	1,37E-03	5,11E-04	-	1,88E-03
	Umgeni	1,22E-04	4,63E-05	-	1,68E-04	6,61E-04	2,47E-04	-	9,08E-04
Zn	Curry Post	2,13E-06	6,07E-07	-	2,74E-06	1,16E-05	3,24E-06	-	1,48E-05
	Cedara	ND	ND	-	0,00E+00	ND	ND	-	0,00E+00
	Gilboa	5,28E-06	1,50E-06	-	6,78E-06	2,87E-05	8,02E-06	-	3,67E-05
	Richmond	ND	ND	-	0,00E+00	ND	ND	-	0,00E+00
	Umgeni	1,30E-05	3,70E-06	-	1,67E-05	7,05E-05	1,98E-05	-	9,03E-05
Total metals		2,50E-02	6,41E-01	1,48E-03	6,68E-01	1,36E-01	3,42E+00	1,52E-03	3,56E+00

Table S5.7: Estimation of carcinogenic (CR) from heavy metals in agricultural soil using ultrasonic assisted digestion

		Adults (Aged 18+)				**Children (Aged 1-17)**			
Heavy metal	**Sample locations**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**	**Soil ingestion**	**Dermal contact**	**Air inhalation**	**Total pathways**
Cr	Curry Post	2,93E-06	-	1,45E-07	3,08E-06	3,98E-06	-	3,74E-08	4,02E-06
	Cedara	2,17E-06	-	1,07E-07	2,28E-06	2,94E-06	-	2,76E-08	2,97E-06
	Gilboa	2,18E-06	-	1,08E-07	2,29E-06	2,96E-06	-	2,78E-08	2,99E-06
	Richmond	1,89E-06	-	9,33E-08	1,98E-06	2,56E-06	-	2,41E-08	2,58E-06
	Umgeni	1,40E-06	-	6,90E-08	1,47E-06	1,90E-06	-	1,78E-08	1,92E-06
Ni	Curry Post	-	-	2,71E-10	2,71E-10	-	-	7,00E-11	7,00E-11
	Cedara	-	-	2,07E-10	2,07E-10	-	-	5,34E-11	5,34E-11
	Gilboa	-	-	5,89E-10	5,89E-10	-	-	1,52E-10	1,52E-10
	Richmond	-	-	3,22E-10	3,22E-10	-	-	8,31E-11	8,31E-11
	Umgeni	-	-	7,18E-10	7,18E-10	-	-	1,85E-10	1,85E-10
Pb	Curry Post	1,70E-09	-	-	1,70E-09	2,31E-09	-	-	2,31E-09
	Cedara	2,68E-09	-	-	2,68E-09	3,63E-09	-	-	3,63E-09
	Gilboa	2,43E-09	-	-	2,43E-09	3,30E-09	-	-	3,30E-09
	Richmond	2,35E-09	-	-	2,35E-09	3,19E-09	-	-	3,19E-09
	Umgeni	1,13E-09	-	-	1,13E-09	1,54E-09	-	-	1,54E-09
Total metals		1,06E-05	-	5,24E-07	1,11E-05	1,44E-05	-	1,35E-07	1,45E-05