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RAPID ASSESSMENT OF SOIL POLLUTION USING A FIELD PORTABLE X-RAY FLUORESCENCE SPECTROMETER

by

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DECLARATION

“I hereby declare that the thesis submitted for the degree Doctorate of Technology Chemistry, at the Tshwane University of Technology is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.”

J-N. OYOUROU

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ABSTRACT

The large number of abandoned and unattended mines in South Africa, combined with a long history of application of copper-based fungicides in horticulture, has raised concerns regarding metal pollution of the environment. The need for monitoring environmental pollution through the implementation of regular soil surveys and risk assessments necessitates the use of a rapid analytical technique, such as portable-X-ray fluorescence (pXRF) spectroscopy, in combination with conventional inductively coupled plasma-optical emission spectroscopy (ICP-OES).

In this study, pXRF spectroscopy was applied to screen soils from an abandoned mine contaminated with lead and zinc, and from mango orchards thought to be polluted with copper. Prior to field investigations, the reliability of the pXRF spectrometer for the *in situ* determination of the metals in soils was verified. After calibration of the spectrometer using appropriate certified reference materials, soil samples were analysed to determine their metal content and the results were compared to those obtained from the analysis of the same samples using ICP-OES and wavelength dispersive X-ray fluorescence (WDXRF) spectroscopy. The pXRF results correlated well with those from ICP-OES, as reflected by the coefficients of determination (r^2) of 0.8250; 0.9743 and 0.9899 for copper, zinc and lead, respectively. Quantitative data could be classified as quality level Q2 according to the USEPA for copper, zinc and lead, with reference to values obtained for the r^2, relative standard deviations (RSDs) and by applying inferential statistics. The metal concentrations determined using the pXRF spectrometer were also confirmed by an independent analysis of the samples using WD- XRF spectrometry.

Once the reliability of the pXRF spectrometer for field determination of lead, zinc and copper had been established, large scale screening was conducted to determine the spatial and depth distribution of metals in soils at the abandoned Edendale Mine (Mamelodi, Pretoria) and in mango and citrus orchards near Hoedspruit (Limpopo). Contamination indices were applied to establish the levels of pollution of the soils. As part of risk assessment, the mobility of the contaminants in soils was determined from the sequential extraction of representative soil samples. Risk assessment indices were applied to determine the ecological risk associated with the contamination.

The screening of soils at the old Edendale Mine using the pXRF revealed a maximum concentration of 7300 mg/kg for zinc and 21 000 mg/kg for lead. Of the 167 points sampled, 46% of the samples for zinc and 77% for lead contained more of these metals than the baseline concentrations (185 mg/kg for Zinc and 56 mg/kg for lead) set for South African agricultural soils (Department of National Health and Population Development). The geo-accumulation index indicated that 2% and 15% of the sampling points at the Edendale Mine were extremely polluted with zinc and lead, respectively. The high concentrations of lead present in the exchangeable, reducible, and oxidisable fractions of the soils following sequential extraction, indicated that the metal occurs in mobile forms. It was deduced that zinc was present in moderately mobile forms, since 61% of the total concentration was determined in the residual fraction. The risk assessment indices consistently indicated that lead in the soils poses a high ecological risk, but that zinc is associated only with a moderate risk.

A maximum copper concentration of 2600 mg/kg was measured in mango orchard soils using the pXRF spectrometer. Copper generally accumulated in the surface soil, while the concentration decreased with an increase of depth. This trend was ascribed to a high content of organic matter in the surface soil that confined the copper to the surface through binding mechanisms. Most of the soil samples analysed (64%) could be described as significantly to extremely contaminated with copper, resulting from the fungicides applied to the trees through spraying. Sequential extraction of selected orchard soil samples indicated that copper resided mainly in the oxidisable (32%) and reducible (28%) fractions. Only 14% of total copper was confined to the exchangeable fraction, reflecting limited mobility and therefore a moderate environmental risk for copper.

A phytoremediation trial was implemented in a contaminated mango orchard using two aromatic plant species i.e. *Rosmarinus officinalis* and *Ocimum labiatum*. After 15 months, plant samples were harvested. High copper concentrations were determined in the leaves of *R. officinalis* (73.6 to 236 mg/kg) and *O. labiatum* (333 to 675 mg/kg). Although the bioaccumulation and translocation factors for plant leaves were above 1, the plants did not qualify as phytoaccumulators. If these two aromatic species were to be interspersed between the mango trees, they could play an important role in mitigating the copper pollution. Such an intercropping practice could possibly benefit the orchard by attracting pollinators, yielding a commercial crop in the form of an essential oil, and perhaps even contributing to a decline in insect parasites, due to the repellent effect of emitted volatile organic compounds.

In conclusion, portable spectroscopy was successfully applied *in situ*, in combination with ICP-OES spectroscopy, to establish the levels of lead and copper pollution of soils that were found to be concomitant with extreme and moderate ecological risk at the abandoned Edendale Mine (Mamelodi), and mango orchards (Hoedspruit), respectively. The high levels of soil pollution are extremely concerning and remediating interventions should be initiated by local authority and stakeholders.

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LIST OF ABREVIATIONS

AAS	Atomic absorption spectroscopy
BAF	Bioaccumulation factor
BCR	Community Bureau of Reference
BRC	British Retail Council
CF	Contamination factor
CGS	Council for Geoscience
CRM	Certified reference material
CZT	Cadmium zinc tellurium
DC	Degree of contamination
DNA	Deoxyribonucleic acid
DTPA	Diethylene triamine penta-acetic acid
EDDS	Ethylene diamine disuccinic acid
EDTA	Ethylene diamine tetra-acetic acid
EF	Enrichment factor
ER	Potential ecological risk
GCF	Global contamination factor
GFAAS	Graphite furnace atomic absorption spectroscopy
GPS	Global positioning system
GRI	Global risk index
ICF	Individual contamination factor
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
IFOAM	International Federation of Organic Agriculture Movements

I_{geo}	Geo-accumulation index
LOD	Limit of detection
MPI	Metal pollution index
PGM	Platinum group metals
PI	Pollution index
PPC	Pretoria Portland Cement
PVA	Polyvinyl acetate
pXRF	Portable X-ray fluorescence
RAC	Risk assessment code
RSD	Relative standard deviation
SDD	Silicone drift detector
SSCS	Site specific calibration standard
TF	Translocation factor
UK	United Kingdom
USEPA	United States Environmental Protection Agency
WDXRF	Wavelength dispersive X-ray fluorescence
XRD	X-ray diffraction

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

South Africa's mineral reserves include gold, diamonds, vanadium, and the platinum group metals (PGM's). Although these minerals are usually confined to ores, they may become mobilised through natural processes, mining and industrial activities. Early geological processes that took place as a result of tectonic processes within the crustal layers of the earth have resulted in natural concentrations of some toxic elements being higher in the environment than the recommended or maximum acceptable values (Salminen & Gregorauskien, 2000). Statistical models have been used to establish the geochemical origins of elements, including iron, aluminium and arsenic, in polluted sediments (Kazantzis, 2000; Cram-Heydrich *et al*., 2010). Anthropegenic activities contribute extensively to high concentrations of potentially toxic elements in some areas. It is estimated that between 2000 and 5000 tons of thallium are annually mobilised and dispersed through industrial activities into the global environment (Kazantzis, 2000). Almost 30 years ago, the worldwide anthropogenic discharge of arsenic was approximated at 134 000 tons per year (Nriagu and Pacyna; 1988).

South Africa is one of the leading producers and exporters of fresh fruit[1]. In 2006, a total of 74 499 ha of deciduous fruits (apples, pears, apricots, peaches, nectarines, plums and prunes) were produced. At that stage, many of the orchards (41.1%) were more than 10-years old and were situated on 2226 farms, mainly in the Western Cape (Greeff & Kotze, 2007). Approximately 60 000 ha of citrus (oranges, soft citrus, grapefruit and lemons) are cultivated in South Africa, mostly in the Limpopo Province[2]. Pest infestations of orchards continuously cause damage to fruit, resulting in huge financial losses. The management of fungal infestation of orchards may require the application of copper-containing fungicides. Several copper-based formulation sprays, which may include copper sulfate, copper dihydrazine sulfate and copper oxychloride, are applied in orchards worldwide. The global use of copper-based fungicides was estimated to be 57 000 ton/year in 1994 (Lepp & Dickinson, 1994). The long term application of such chemicals results in the accumulation of copper and other potentially toxic elements in the

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[1] www.usda.mannlib.cornell.edu/usda/fas/citruswm/f. [Accessed]: 27/09/2016.

[2] www.cga.co.za. [Accessed]: 01/09/2016

soil. In South Africa, copper salts have been applied to protect mango and citrus orchards for decades, possibly leading to a build-up of the metal in orchard soils.

Potentially toxic elements, including aluminium, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, mercury, nickel, osmium, silver, thallium, tin and vanadium (Borůvka *et al*., 2005; Mireles *et al*., 2012), have been associated with a number of human health disorders. Exposure to lead causes brain and kidney damage, high blood pressure, anaemia, damage to the reproductive system in males and miscarriage in pregnant women (Abadin *et al*., 1997). Cadmium is associated with kidney dysfunction, hepatic injury and lung damage (John & Jeanne, 1994). High concentrations of mercury, lead and gold may lead to autoimmunity, characterised by non-recognition of the patient's own cells by the immune system (Nriagu, 1988). Excess ingestion of copper may result in acute kidney damage and hemolytic anaemia (Swaminathan, 2013).

Toxic elements are absorbed *via* various routes and accumulate in mammalian organs. Direct contamination occurs through inhalation of contaminated air, drinking of water and soil ingestion, while indirect exposure takes place through the food chain. Plants accumulate metals from soils, and animals ingest these plants. Humans, at the pinnacle of the food chain, are then exposed through contaminated food, both from plants and meat. Elevated concentrations of cadmium and lead were found in the kidneys and livers of birds residing in a polluted urban area of Rzeszow, Poland (Dżugan *et al*., 2012). Kidneys were found to be the major accumulators of toxic elements, since the levels of lead were several times higher in this organ than in the others. Analysis of water and fish tissues from the polluted Madivala Lake, Bangalore, India, revealed significant levels of lead, chromium, cadmium and nickel that corresponded to the levels of heavy metals in the water, raising concerns over the effects on human health (Begum *et al*., 2009).

The toxicity of metals is dependent on their ability to dissolve from the soil matrix and migrate into food crops, pasture and drinking water. A range of factors, including soil type, organic content and pH, microbial content and metal-metal interactions, play a role in the complex chemistry involved (Komárek *et al*., 2010; Duan *et al*., 2016). In several papers describing the determination of heavy metals in soils, the authors regarded the measurement of the extractable fraction, rather than the total metal load, to be more meaningful in assessing toxic potential (Meers *et al*., 2007; De Villiers *et al*., 2010).

The determination of toxic elements in contaminated soils is commonly carried out using inductively coupled plasma (ICP) spectrometry or atomic absorption spectrometry (AAS)

(Butler e*t al*., 2015). Tedious digestion steps are required prior to analysis with both these techniques. Field portable X-ray fluorescence (pXRF) spectrometers also yield valuable results for determining the presence of toxic elements in the field, although the values obtained may be elevated when compared to those obtained by ICP analysis (Shuttleworth *et al*. 2014). This may be explained by taking the inhomogeneity and moisture content of the samples into account (Argyraki *et al*., 1997). In most cases, high concentrations of the elements of interest can be determined successfully, but there may be inaccuracies when detecting low levels. For example, lead present at concentrations of 8810 mg/kg to 14 000 mg/kg were accurately determined in soil by Argyraki *et al.* (1997), but cadmium concentrations of 0.1-0.5 mg/kg in soil, could not be detected by Rose *et al*. (1979) when using a pXRF spectrometer. A similar comparative study conducted on soil in the vicinity of a copper smelter in Poland using a portable XRF, a laboratory-based XRF and an AAS, revealed good correlation between the instruments for the measurements obtained for lead, copper and zinc (Clark *et al.,* 1999). More recently, a study carried out by Rouillon and Taylor (2016) validated pXRF as a suitable alternative to ICP-OES for the determination of potentially toxic metals including chromium, copper, zinc, cadmium and lead, in contaminated soils.

The use of a field portable XRF for screening eliminates the tedious sample preparation steps needed for ICP and AAS. However, the greatest advantage is that the technique allows for the rapid and simultaneous on-site screening of soils for the presence of many elements. The instrument is capable of determining permissible levels of toxic heavy metals that are controlled by regulations.

1.2 PROBLEM STATEMENT

The mining industry is a serious source of pollution, since underground deposits are exposed during the extraction process, allowing easier infiltration of potentially toxic elements into the surrounding environment.

Extensive and long-term application of metal-based pesticides in orchards results in the build-up and accumulation of toxic elements in agricultural soils. These elements can also be leached into the surroundings, raising environmental concerns (Da Pelo *et al*., 2009). Toxic elements are, in many cases, taken up by plants, thereby entering the food chain. However, the risks to humans, animals and the environment associated with exposure to metal pollution, from both natural and anthropogenic sources, has not been extensively investigated in South Africa. The intensive and costly methods used for sampling and analysis, involving the transportation of solid samples to the laboratory and subsequent analysis by ICP (De Villiers *et al*., 2010), may be a contributing factor.

De Villiers *et al*. (2010) carried out a study to determine the levels of lead in South African soils. Only lead and cadmium were determined in the 1000 samples collected. Their large study area that stretched across the entire country, and the relatively small number of samples analysed (1000) suggests that sites with high levels of contamination may have been overlooked. The high lead and cadmium levels recorded in the Gauteng Province and in the Pietermaritzburg region of the KwaZulu Natal Province were attributed to anthropogenic sources. The obvious limitations of their study underline the need for a more comprehensive investigation to determine the extent of metal contamination of soils.

The Council of Geoscience (CGS), Pretoria, South Africa, used screening data to map the concentrations of various toxic elements present in South African surface soils. This map indicates the presence of high concentrations of certain elements at specific locations. A sampling interval of 1 km^2 was used. The mapping and sampling of the CGS was concentrated in the northern region of the country. Several areas containing high levels of lead in the soil were identified far away from anthropogenic sources. These naturally polluted areas were not sampled during the study of De Villiers *et al*. (2010). It is clear that more intensive screening is required in South Africa to ascertain the risk of exposure to toxic elements.

The South African fruit industry is constantly facing challenges with regard to adherence to stringent regulations and standards governing fruit exports to the European Union (EU). The EU countries continuously raise concerns pertaining to Good Agricultural Practice (GAP). Despite the long-term application of copper-based fungicides in orchards and vineyards, which might has led to the contamination of orchard soils, only a handful of investigations have been conducted to determine copper concentrations in orchards. This shortcoming can possibly be ascribed to the large number of orchards, and the tedious analytical techniques conventionally applied for the analysis. It is important for farmers to understand the consequences of continuous application of the copper-containing chemicals on soil quality, and eventually on fruit quality and yield. If very high concentrations are present, techniques for reducing the copper concentrations in orchard soils should be considered, such as phytoremediation.

The use of a pXRF allows large numbers of sites to be screened at low cost. It is a simple technique that enables the screening of polluted areas so that elements of concern can be identified. Selected soil samples can then be taken for more detailed analysis using ICP-OES and ICP-MS for verification purposes. It is necessary to distinguish between total element content and available species through the use of different extraction methods. Strong acid microwave digestion, such as described by the United States Environmental Protection Agency (USEPA) Method 3050 (USEPA, 1996), allows for the determination of the total concentration of individual elements, irrespective of their oxidation state. Diluted acids or a combination of organic solvents and mineral acids can be used for digestion when extractable individual species are to be determined (De Villiers *et al*., 2010). Results obtained using both methods should be compared to identify the most appropriate approach for assessing risk. Potential risks to humans and animals can only be fully evaluated if water and plant samples (grazing) are analysed to determine the levels of toxic elements present.

Although XRF has been widely used by many researchers (Argyraki *et al*., 1997; Clark *et al*., 1999) for elemental analysis of soils, the reliability of the technique must first be established by comparing measurements obtained by XRF to those obtained by ICP analysis. Despite the many advantages, pXRF has some limitations. These need to be better understood for future applications of the technique.

1.3 HYPOTHESIS

The use of pXRF spectrometry, in combination with ICP-OES, will allow areas of South Africa with high levels of toxic elements to be identified and monitored, so that the potential risk to humans and animals can be assessed.

1.4 AIMS AND OBJECTIVES

1.4.1 General aims

The focus of this study was to screen an abandoned lead mine as well as orchards in South Africa for the presence of potentially toxic elements with particular emphasis on zinc, lead and copper in the soils and sediments by means of a pXRF spectrometer. A further aim was to determine the levels of toxic elements in plant samples from contaminated areas using other spectroscopic techniques.

1.4.2 Specific objectives

The specific aims of the study were to:

- validate XRF results for field determination of several potentially toxic elements in soil;
- screen the soils of an abandoned mine and mango and citrus orchards to assess the levels of zinc, lead and copper present using pXRF;
- demonstrate the suitability of the pXRF for the screening of historically contaminated sites, as well as for sites with ongoing pollution;
- determine the total element concentrations in representative samples of soils from the contaminated areas;
- determine the total concentrations of elements identified in the soils from high-risk areas in plants obtained from those areas, in order to assess potential health hazards;
- determine bio-available element concentrations in samples from highly contaminated sites;
- analyse, using appropriate statistical methods, the data generated to establish the source of contamination and the risk to humans, animals and the environment;
- develop contour maps for the elements of interest for use by environmentalists; and to
- assess the ability of selected aromatic plants for the phytoremediation of contaminated sites.

A more detailed background to the study is provided in Chapter 2.

CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

Soil is defined a variable mixture of mineral, organic matter and water resulting from the weathering action of physical, chemical and biological processes on rocks (Manahan, 2010). As such, soils assume several functions, including the production of biomass necessary to support human and animal life (Blum, 2005). It also provides the physical basis and raw materials for human activities. Global industrialisation and economic development have contributed extensively to the pollution of soils and sediments in both developed and developing countries. Mining wastes resulting from the extraction of commodities (Weissenstein & Sinkala, 2011), spent gasoline from transportation vehicles (Harper *et al*., 2003; Monna *et al*., 2006), pesticides residues (Wightwick *et al*., 2008) and fertilisers used to increase crop production (Gao *et al*., 2016) contain residues of toxic elements that are deposited onto soils.

Trace elements including copper, zinc, iron, molybdenum, selenium and manganese are regarded as essential to microorganisms, plants, animals and humans (Swaminathan, 2013). In humans, they represent less than 0.01% of body weight, corresponding to a daily intake of below 100 mg/day in adults (Swaminathan, 2013). Essential elements play an important role in physiological processes at low concentrations. Deficiency in these minerals severely affects the health of organisms. These elements are involved in oxygen transport, metabolite catalysis (chromium, selenium, zinc, molybdenum and manganese), and hormonal effects (selenium and iodine) (Swaminathan, 2013). Zinc, for example, binds to protein to form a protein complex, which has been proved to be a tumour suppressor (Bao *et al*., 2014). Manganese plays a role as an enzyme cofactor in transduction of DNA and neurotransmitter biosynthesis (Fraga, 2005; Bao *et al.*, 2014). Nevertheless, these micronutrients become toxic to organisms when present at elevated concentrations (Fraga, 2005). On the other hand, trace elements such as lead, arsenic, mercury and cadmium have no beneficial effects for human or animal health and are toxic even at low levels (Nriagu, 1988).

Metal pollution of soils is a common phenomenon, which constitutes a threat to the sustainability of development, particularly in Third World countries. Soil pollution is often not visible, and usually harmful to the environment. It can often only be detected after a long period of time. However, soil and sediment contamination are “chemical time bombs”, which may cause severe environmental damage when tolerance limits are exceeded or after changes in environmental conditions (Wood, 1974; Stigliani *et al*., 1991; Hekstra, 1997). Unlike water and air pollution, for which the contamination can be reversed by dilution and self-purification, soil pollution is persistent and very difficult and expensive to reverse (Su *et al*., 2014). Moreover, metal contaminants are non-degradable, and complex pollution may occur as the soil and sediment becomes enriched with various toxic elements. The remediation of contaminated soils is difficult and the remediation cycle may take as long as one hundred to two hundred years (Wood, 1974).

2.2 SOURCES OF TOXIC ELEMENTS IN SOILS AND SEDIMENTS

2.2.1 Enrichment of soils and sediments from natural processes

The dispersion of toxic elements in the environment occurs through several processes including geogenesis, paedogenesis and man-made interventions. During hydrothermal processes, which lead to the formation of some rocks, minerals composed of major elements crystallised, following changes in temperature and pressure of the magma. Toxic elements were incorporated into the lattice of these minerals according to a diadochic substitution (Navrátil & Minařík, 2005). Toxic elements were then dispersed into the surface environment from physical and chemical weathering processes, which break down the parent rock, resulting in the formation of soil. Soil and sediment enrichment from these natural processes are referred to as being of geogenic, pedogenic or lithogenic origin, as opposed to pollution from anthropogenic origin, which refers to processes resulting from human intervention (Borůvka *et al*., 2005; Serelis *et al*., 2010; Wuana & Okieimen, 2011; Medunic *et al*., 2013). Other natural sources include enrichment of soils by toxic elements from volcanic eruptions, windblown dust, marine aerosols and forest fires (Nagajyoti *et al*., 2010). Soil and sediment contamination from geogenic sources have limited mobility and remain more strongly bound to soil and sediment solid particles than that from anthropogenic origin (Passos *et al*., 2011; Wuana & Okieimen, 2011). Generally, native plants growing on naturally polluted soils develop physiological mechanisms to adapt to the toxicity over time (Reichman, 2002). Tolerance to high metal concentrations in soils by endemic plants involve the evolution of coping mechanisms. These include metal exclusion that prevents pollutants from entering root cells, as well as metal absorption by roots and their accumulation in specific plant parts (Lasat, 2000). However, toxicity issues may arise when agricultural plants are introduced into contaminated media (Reichman, 2002).

2.2.2 Enrichment of soils and sediments from anthropogenic processes

Human activities are the principal contributors towards soil pollution by toxic elements. The anthropogenic sources of soil and sediment contamination emanate mostly from activities such as mining, industry, agriculture and transportation.

Mining of underground metal ores usually generate large amounts of wastes that often contain high concentrations of toxic metals. Open pit mining entails removing the surface soil covering the ore, which is then stored on-site as backfill material. The mine ore, consisting of solid materials containing low levels of the mineral of interest, is then removed and stored. Rocks containing high concentrations of minerals are removed, milled and processed, whereafter a mineral concentrate is prepared by mixing the finely crushed ore with water. The mineral concentrate is removed and further refined, whereas the water, containing fine particles, forms the tailing. The waste remaining after the removal of the ore, and the tailing, containing elevated concentrations of toxic elements, are dumped on-site and are a major cause of soil contamination. For example, soils and sediments from Kabwe and the Copperbelt areas in Zambia were found to be contaminated with heavy metals (Ikenaka *et al*., 2010). The source of the contamination was ascribed to copper mining operations in these areas. Contaminants migrate to surface water and sediment through run-off from the mine waste. Othmani *et al.* (2015) demonstrated that sediments collected downstream from the Oued Sidi Bou Said River in Tunisia were severely contaminated with cadmium, lead and zinc. The authors also confirmed that a historical mine tailing located upstream was the source of the contamination. Groundwater can also be contaminated by toxic elements leaching from soils and wastes. Gold mine wastes, for instance, contain large amounts of pyrite, which is oxidised by rainwater to form sulfuric acid. This acid percolates through the wastes and dissolves toxic elements to reach and contaminate the groundwater (Terence & McCarthy, 2011).

Industrial discharges may contain high concentrations of toxic elements. These waste products are released into the environment, resulting in soil and sediment contamination. The nature and level of contamination depends on the type of industry. In Malaysia, for example, arsenic pollution of the Juru River was attributed to effluents from chemical manufacturing of paper and printing, and from battery manufacturing (Al-Shami *et al*., 2010). The same river was found to be polluted with chromium, cadmium, zinc, copper, lead and mercury from electroplating, textile and leather tanning processes, and from fertiliser, pesticide, and insecticide production. Iron, lead, copper, zinc, chromium and nickel were released into an agricultural field from paper manufacturing in India (Devi *et al.*, 2011), while the same elements, resulting from food and pharmaceutical industries, contaminated the Sava River in Croatia (Radić *et al.*, 2009).

Agriculture also contributes to soil and sediment contamination arising from the application of fertilisers, pesticides and insecticides. Macronutrients, including nitrogen, phosphorus, potassium, calcium and magnesium are crucial for the growth of plants. Deficiency of these in soil is corrected by the use of fertilisers, which may contain residues of toxic elements, including cadmium and lead (Wuana & Okieimen, 2011). Extensive application of fertilisers in agriculture introduces cadmium and lead as soil contaminants. In the recent past, pesticide formulations contained mercury, zinc, arsenic, chromium, lead and manganese. Currently, the Bordeaux mixture, copper oxychloride and other copper-based fungicides, have been found to be sources of copper contamination of orchard soils (Zhou *et al.*, 2011; Silva *et al.*, 2012). Selected copper-containing fertlisers are extensively applied in orchards and vineyards since, these are acceptable in most countries for fungus control in organic crop production.

Globally, soils were found to be contaminated with lead, cadmium, zinc and nickel in the proximity of highways; the levels decreasing with distance from the road (Lagerwerff & Specht, 1970; Markus & McBratney, 2001; Harper *et al.*, 2003; Okonkwo & Maribe, 2004). The origin of the pollutants was ascribed to the emission of residues from fuel used in motor vehicles, as well as in car tyres. Leaded gasoline was phased out internationally in the 1980s, which consequently reduced lead contamination resulting from that source. Unleaded gasoline was made available in South Africa only in 1996. In 1991, Nriagu (1991) estimated that the consumption of leaded gasoline in South Africa was as high as 6×10^{10} L/year, corresponding to an annual lead consumption of between 1800 and 3600 metric tons. Large amounts of lead dispersed into the environment from gasoline, combined with the persistence of lead, may have contributed significantly to the contamination of roadside soils in the country.

Soils and sediments contaminated from anthropogenic sources present environmental risks due to the mobility and availability of the toxic elements, unlike contaminants resulting from natural origin (Passos *et al.*, 2010). Insoluble metal contaminants from anthropogenic origin are loosely attached to the soil solid phase and may leach into the groundwater or be absorbed by plants, soil organisms and microorganisms. Contaminated sediments, in turn, release the toxic elements into water and affect water quality.

2.3 IMPACT OF TOXIC ELEMENTS IN SOILS

2.3.1 Effect of soil contamination on soil quality

Flourishing soil biota symbolises a healthy soil that is favourable for plant growth. The presence of elevated concentrations of toxic elements in soil impacts on microorganisms and consequently impairs the soil quality and compromises biomass production. Zhou *et al.* (2011) demonstrated that elevated copper residues in citrus grove soils decreases bacterial biomass and diversity. Chander *et al.* (1995) reported as much as a 50% reduction in microbial biomass, as the heavy metal concentration in the soil increased. Soil enzymes are involved in the decomposition of soil organic matter, which is vital to microbial activities. High concentrations of toxic elements have been found to significantly decrease enzymatic activities in soils (Ash *et al*., 2012). The quality of the biomass produced deteriorates in the presence of high concentrations of toxic elements in soils. Khan *et al.* (2016) studied the effects of cadmium and lead on the absorption and bioaccumulation of macro- and micro-elements in potato, tomato and lettuce. The researchers found that soils contaminated with these metals contained fewer nutrients and that the nutritional composition of vegetables grown on these soils was negatively affected. Soils play an important role in converting organic forms of nutrients into minerals needed for plant growth. Plant micronutrients (nitrogen and phosphorous) are released into soils during the decomposition of living organisms after death by soil microorganisms (Murphy, 2014). Elevated concentrations of toxic elements destroy soil biota and negatively impacts their recycling capability.

2.3.2 Contamination of groundwater by toxic elements

Groundwater is the largest source of fresh water and the main source for many human activities. It can be contaminated by toxic elements originating from polluted soils (Ho *et al*., 2012). Contamination of the upper aquifer by chromium(VI), resulting from soils polluted by industrial wastes in Annaba (Algeria), was reported by Khérici-Bousnoubra *et al.* (2009). The chromium(VI) was found to originate from complex biochemical processes that occur during high and low precipitation, following the infiltration of chromates and sulfides from surface-contaminated soils (Khérici-Bousnoubra *et al.*, 2009). Bundschuh *et al.* (2012) described the history of poisoning of the Latin American people, which resulted from the intake of arsenic-contaminated groundwater. The main sources of contamination were attributed to primary (hydrothermal and sulfide ore deposits) and secondary (oxides and oxyhydroxide) mineralisation. Arsenic was then remobilised by dissolution of the oxides and oxyhydroxides under acidic conditions or desorption of oxyhydroxides under high pH and oxidising conditions.

2.3.3 Effects of soil contamination on plants

Plants are irrefutably the organisms most directly affected by changes in soil quality, since the soil provides the base and nutrients needed for growth. Toxic elements may be made available to plants, depending on the nature, concentration and oxidation state of the element, and the properties of the soil (Zitka *et al*., 2013). Although essential elements are absorbed by plants and participate in various vital physiological reactions, high levels of these elements may lead to phytotoxicity. On the other hand, even low concentrations of non-essential elements are phytotoxic. The phytotoxicity induced by toxic elements is characterised by the disruption of physiological processes, including the deactivation of enzymes and blockage of functional groups. Chromium poisoning, for example, is characterised by the chlorosis of leaves and stunted development of plant roots (Onder & Dursun, 2006; Zitka *et al.*, 2013). Copper in soil at 50 mg/kg was reported to affect citrus seeding, while 200 mg/kg resulted in withered wheat (Su *et al*., 2014). In extreme cases, high concentrations of toxic metals in soils leads to plant death. Poisoning from high concentrations of toxic elements in soils occurs in plants only when their defensive capacity is exceeded. Plants develop a number of tolerance mechanisms in response to stress resulting from high concentrations of toxic elements (Reichman, 2002). These tolerance mechanisms include the exclusion of toxic elements from the plant, and the compartmenting and complexing of toxic elements within plant cells (Reichman, 2002).

2.3.4 Effects of soil contamination on humans

The absorption of toxic elements in humans results in the development of various diseases, depending on the elements involved. Human diseases caused to by toxic elements have been reported (Nriagu, 1988) and are discussed in Chapter 1.

Exposure of humans to polluted soils occurs through both direct and indirect pathways. Toxic elements directly affect humans by oral intake, inhalation of dust generated from contaminated soils and absorption through the skin (Su *et al*., 2014). Children are the most affected *via* the hand-to-mouth pathway when exposed to contaminated soils (ATSDR, 2007). It has been estimated that between the ages of 1 and 6 years, they ingest an average mass of 100 mg of soil per day (USEPA, 2003). Dust inhaled from contaminated soils contains toxic elements, which puts human health at risk. Inhalation and ingestion of lead, for example, have the same physiological effects on the human body (Wuana & Okieimen, 2011). Damaged skin, and even death, following direct contact with contaminated soils has been reported (Su *et al.,* 2014). Skin contact was cited as being the major route of exposure to arsenic by 1800 farmers from the Heshan Village in China, between 1951 and 2012 (Su *et al*., 2014). The farmers suffered from chronic arsenic poisoning and 400 died, mostly from lung cancer. More than 100 hectares of land from this village were contaminated.

While human exposure to contaminated soils through direct pathways should not be neglected, the indirect exposure through consumption of crops grown on contaminated soils certainly poses perhaps the greatest threat to human health. It is well known that plants growing on contaminated soils may absorb toxic elements, which consequently affect human health upon their consumption (Teh *et al*., 2016). Plants absorb available toxic elements through their roots and the translocation and accumulation to the edible aboveground portions follow. Vegetables and fruits cultivated on contaminated soils with toxic elements have been reported to contain high concentrations of the polluting elements. Marwa *et al.* (2012) investigated the translocation and accumulation of toxic elements from contaminated soils in maize tissues in Tanzania. The authors found high concentrations of the toxic elements both in soils and maize tissue and concluded that the toxic metals translocated from the soil to accumulate in plant tissues. They advised people to cease consumption of the maize grown on the contaminated soils.

2.4 IMPACT OF CONTAMINATED SEDIMENTS ON THE ENVIRONMENT

Fluvial and alluvial sediments are important components of aquatic ecosystems of rivers. Contaminated sediments affect the quality of the overlying water and the development of aquatic organisms and microorganisms. The contamination sources are discharge from mining, industry and urban sewage into groundwater, rivers, lakes and oceans (Hanif *et al*., 2016). These toxic elements are deposited into sediments following a number of hydrodynamic and biochemical processes (Guo *et al*., 2010). Toxic elements do not remain permanently in the sediments, but can be released into the overlying water under various geochemical conditions. Komárek and Zeman (2004) demonstrated the dynamics of the release of copper, zinc, cadmium and mercury from sediment samples, which had been artificially contaminated. The metals were bound by sorption or precipitation to secondary minerals. The researchers found that the metal-binding to the sediment was not completely reversible. However, significant release of the metals into the infiltrated water occurred, depended on the type of metal, and when the sediment was exposed to extreme acidic reducing (cadmium, zinc and copper) or oxidising (mercury) conditions.

The release of toxic elements from contaminated sediments into an aquatic ecosystem is followed by their biomagnification in the food web. Humans are then exposed through the consumption of the contaminated aquatic resources (Solomon, 2008). Absorption of cadmium for example, reduces the growth of aquatic green plants; provokes skeletal deformity and disruption of kidney functioning in fish; and damages the liver and pancreas of marine crustaceans (Solomon, 2008). Besides the impact of sediment and water contamination on individual organisms, the structure of aquatic communities is also affected. Wu *et al.* (2016) investigated the ecological impact of severe metal contamination on Jinzhou Bay in China, and observed a reduction in the number of benthic organisms. Gyedu-Ababio *et al.* (1999) also reported the negative influence of high concentrations of toxic elements on the density and diversity of nematode communities in sediments from the Swartkops River (South Africa).

Sediment contamination also poses a threat to human health. The notorious Minamata disease was caused by acute mercury poisoning of fishermen, following the consumption of contaminated fish from the Minamata Bay in Japan (Solomon, 2008). In the bay, bacteria had transformed mercury oxides, discharged from a local industry, into the more toxic organic methylmercury (Solomon, 2008). The toxic compound had bioaccumulated and biomagnified in the aquatic ecosystem and humans were exposed through the consumption of fish.

2.5 REGULATIONS REGARDING METAL CONCENTRATIONS IN SOIL AND SEDIMENT

Governments worldwide have adopted regulatory policies and guidelines to prevent, manage and restrict the negative impacts of soil and sediment contamination on the environment and on human health. These policies and guidelines rely on the use of threshold values for soil and sediment as quality standards (Bachmann *et al*., 1999). Threshold values are concentrations, expressed in mg/kg, of the contaminant in dry weight of soil and sediment, or µg/L in soil and sediment eluate. They may, or may not, be legally binding for the prevention and management of soil contaminated sites. In the European Community, threshold values are regarded as guideline values for risk assessment of contaminated sites and are not legally binding, with the exception of Germany and Switzerland (Bachmann *et al*., 1999). In Europe, exceeding the threshold values generally requires remediation measures to be put in place, including site specific assessment, and restriction of the site to human activities (Bachmann *et al*., 1999). Threshold values have been referred to under various names (screening values, trigger values, intervention values, maximum permissible values, etc.), probably due to the translation from the original language to English, and have also served different purposes in different countries (Carlon *et al.*, 2007). These values are generally specified according to the land use, such as agricultural, natural, recreational, residential, industrial or commercial use (Carlon *et al.*, 2007).

Germany and the United Kingdom (UK) apply more complex classifications that take the soil-type (sandy, loam and clay) into account (Teh *et al.*, 2016). Threshold values and the decision to be taken when those values are exceeded, differ from one country to another, due to cultural differences and politics (Teh *et al.*, 2016). Italy, Poland and Lithuania have set a single generic threshold value for each toxic element, which in principle, leads to the remediation of the contaminated site. For these countries, the threshold values are named "limit value" or "maximum permissible value" (Carlon *et al*., 2007). Other countries have set two or more threshold values, known under different names and each representing a different level of contamination. In the Netherlands, the "target value" represents an absence of contamination, while the "intervention value" requires a site specific risk assessment. Obvious differences were observed among soil protection policies from the European Union countries. Nevertheless, soil screening values set as concentration thresholds, are available from these countries and generally correspond to negligible, intermediate and unacceptable risks (Carlon *et al.*, 2007). Most developed countries from Western Europe, Australia and North America have well established policies and regulatory frameworks to prevent and deal with soil and sediment contamination (IASS, 2012).

In developing countries, soil and sediment contamination are less of a priority. For this reason, only a few regulations and policies have been established. In some countries, regulations are completely absent (IASS, 2012). When they do exist, the regulations and policies have been adopted from international practices of developed countries (DEA, 2010). In South Africa, the "Framework for the Management of Contaminated Land" enforced by the National Environmental Management Waste Act 59 (2008), provides screening values for toxic elements, which may trigger a subsequent remediation programme when exceeded (DEA, 2010). This framework provides three soil screening values (SSV1, SSV2 and SSV3) that are based on the potential of the contaminant to directly affect human health or the environment, the probability that the soil contaminants will be released to other media, and their indirect impact on plant, animal and human health. The lowest screening values for residential soil in South Africa correspond to residential soils in informal settlements. These values are comparable to those established in developed countries (Canada, Australia and the Netherlands). Threshold values determined in Canada are available from the “Soil Quality Guideline for the Protection of the Environment and Human Health[3]”, which classifies soil contamination according to use. Australia has set threshold values representing standard pollutant limits. These values correspond to the ecological assessment levels. Threshold values for the Netherlands are target values set for unpolluted soils and sediments. The threshold values for these countries are compared to values for South Africa as presented in Table 2.1.

Table 2.1: Comparison of threshold values (mg/kg) for toxic elements in residential soils in South Africa to those from Canada, Australia and the Netherlands (DEA, 2010; Teh *et al*., 2016).

Elements	Countries			
	South Africa	Canada	Australia	Netherland
Arsenic	23	12	1	29
Cadmium	15	10	3	0.8
Chromium	6.5*	64	1*	100
Copper	1100	63	100	36
Lead	110	140	600	85
Mercury	0.93	6.6	20	0.3
Zinc	920	200	200	140

*Values correspond to chromium(VI)

South Africa is among the most conservative with respect to chromium and mercury, but the most tolerant towards copper and zinc when compared to the three other countries, probably because chromium(VI) and mercury are non-essential elements that are highly toxic, even at low doses. The relatively high levels set for copper and zinc may have been influenced by high natural background levels in certain regions of the country and the fact that these minerals are mined.

2.6 METHODS OF ANALYSIS FOR THE DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN SOILS AND SEDIMENTS

The determination of the screening values and the assessment of the ecological risks of contaminated lands and sediments involve scientific data collection. Various analytical techniques can be used for that purpose. Elemental analysis is commonly carried out using spectroscopic techniques, including AAS, ICPs and benchtop XRF. Some of the advantages and disadvantages of applying these techniques have been discussed in

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[3] www.esdat.net/Environmental%20Standards/Canada/SOIL/rev_soil_summary_tbl_7.0_e.pdf. [Accessed]: 04/08/2016.

Chapter 1. The pXRF spectrometer, utilised in this study, and the theory supporting this technology is described in the next section.

2.6.1 Theory of pXRF spectrometry

Measurement systems using XRF techniques are based on the radiation of specific X-ray photons from a source to induce the fluorescence of atoms in a sample, whereafter the resulting specific fluorescent energies emitted by the elements in the sample allow their quantification (USEPA, 2006; Hall *et al*., 2013). At the atomic level, electrons are distributed around the nucleus in shells represented by K, L, M, N, etc (Hall *et al*., 2013). Irradiation of a sample with X-rays may cause an inner shell electron, with the appropriate absorption energy, to be ejected. The filling of the vacancy left by the ejected electron with an outer shell electron (so that the atom returns to its ground state) causes the emission of photons with energy equivalent to the difference in energy between the two shells (Hall *et al*., 2013). Identification of the element in the sample is possible from the emitted energy, since the wavelength (λ) of emitted light is proportional to the square of the atomic number (Z) and is characteristic for each element, as described by Moseley's Law (Equation 2.1).

$$1/\lambda = K(Z - \alpha)^2 \qquad \text{Equation 2.1}$$

where K is a constant associated with series lines (K, L, M etc.) and α is a shielding constant related to the effective nuclear charge of the element.

Electrons in the K and L shells are more strongly bound to the atomic nucleus and consequently require high energies to be ejected, and are the most relevant in XRF spectrometry. The electron shells L, M, N, etc. are further divided into subshells, whereas shell K is mono-energetic. Transitions may occur from outer electrons to the vacant inner shell, or may be forbidden according to a rule of selection dictated by the quantum theory. When these transitions happen, they define X-ray emission energy lines referred to as Kα, Kβ, Lα, Lβ, etc. for each element (Hall *et al*., 2013). The Kα and Kβ lines are used for the analysis of low to medium Z elements, while high Z elements are analysed by applying L lines. The emissions from L lines of elements with small Z values are low and would not be transmitted in air. Even K lines of elements, from magnesium to calcium, need special adaptation to allow their determination by a Si(Pin) detector. For elements with high Z values, excitation potentials (absorption edge) for K lines are too high for the maximum kV setting of the instruments, so L lines are used for their analysis. A specific energy, known as the absorption edge, is required for the ejection of an electron from the K or L shells.

Irradiation of a sample by an X-ray source results in a photoelectric effect of an element only if the energy of the incident radiation is just above the absorption edge of the element in the sample. Qualitative analysis is possible, since the wavelength of the X-ray emitted is characteristic of a particular element. Quantitative analysis is possible from the collection of the number of X-rays emitted at a given wavelength (USEPA, 2006; Hall *et al*., 2013).

2.6.2 The pXRF spectrometer

The pXRF spectrometer comprises a radiation source, an X-ray detector and a pulse processing electronic system. Two types of excitation sources are used in these spectrometers, either an X-ray tube or a radioisotope (Markowicz, 2008). Sealed radioisotopes, including ^{55}Fe, ^{238}Pu, ^{109}Cd and ^{241}Am, were used as X-ray sources in earlier pXRF spectrometers to effectively excite elements. The capacity for multi-element analysis was developed by combining these radioisotopes. However, these radioisotopes have been replaced by miniaturised X-ray tubes to avoid problems associated with the use of radioisotopes. These include safety concerns, restriction of the power due to shielding of radiations and the limited life time of some radioisotopes, lack of optimisation, and absence of a continuum component in the source (Markowicz, 2008). Modern pXRF spectrometers are equipped with miniaturised tubes adapted to battery power. The X-rays are produced within anodes manufactured from rhodium, silver, tantalum, tungsten or chromium, after being struck by electrons accelerated through an electrostatic field. Anodes are selected in line with a particular application. However, some X-ray tubes are equipped with a filter that allows the modification of the spectrum of the emitted X-ray, according to the target analyte in the sample (Hall *et al*., 2013).

A detector converts X-rays emitted from samples into voltage pulses. The pulses are counted to provide the measurement of total X-ray flux (Hall *et al*., 2013). Early XRF spectrometers made use of a Si(Li) detector with a nitrogen cryostat cooling system (Markowicz, 2008). This cooling system was cumbersome and did not allow optimal portability of these devices. The Si(PIN) detectors, which are currently utilised in standard pXRF spectrometers, are smaller, offer better resolution and are cooled differently to the former detectors. An advancement of the pXRF technology was achieved by the discovery of semi-conductor detectors, which include the silicon drift detector (SDD), and the HgI_2 and CZT (cadmium-zinc-telluride) detectors with Peltier cooling. They offer a higher count rate than the Si(PIN) detector.

Electron charges are created in the detector crystal when collecting emitted X-rays from the sample. These charges are integrated by pulse processing electronics and converted to signals that form the X-ray spectrum. The signals are expressed as a function of the photon energy against the number of counts.

2.7 SPECIATION OF TOXIC ELEMENTS IN SOILS AND SEDIMENTS

The accumulation of toxic elements in soil is not necessarily an immediate threat to plants, animals or a risk to human health, unless they are mobile (Madrid *et al*., 2004). Toxic elements are normally strongly attached to soil particles through the formation of complexes with organic ligands, through precipitation, or by ion exchange; thereby reducing their mobility, bioavailability and leachability (Janoš *et al*., 2010). The release of these elements depends on physicochemical parameters including pH, electrical conductivity (EC), organic matter and clay content. To fully assess the environmental and human health risk associated with toxic element contamination, it is necessary to determine the mobility, rather than the concentration of the element in question.

One method of evaluating the mobility of a pollutant in the environment is to determine the pseudo-total elemental content. This concentration gives an indication of the overall amount of mobile and potentially mobile elements in contaminated soils and sediments and can be used to evaluate the long term maximum leachable concentration of toxic elements (Rao *et al*., 2008). The pseudo-total element content is determined following the digestion of soils and sediments using strong acids (hydrochloric or nitric acids) or *aqua regia* (nitric acid + hydrochloric acid) (Ure, 1996; Rao *et al*., 2008). These acids do not completely dissolve soil and sediment, but extract only the leachable elements, leaving elements that are strongly adsorbed onto the silicates.

Numerous other methods have been developed for determining the mobility of specific elements in soils and sediments. These methods characterise the phase or chemical state in which the elements occur and can be classified as either single or sequential extraction procedures (Rauret *et al*., 1999; Sahuquillo, 2003). They are based on chemical extractions and are designed to establish the availability and mobility of the pollutants, as well as to estimate their vertical and horizontal migration from the soil profile to ground and surface water. Although the speciation methods are based on the same principles, different procedures have been developed by researchers (Rao *et al*., 2008).

Although single extraction procedures are mostly applied in soil fertility studies, to predict the uptake of major nutrients and to diagnose the deficiency or excess of essential elements in soils (Rauret *et al.*, 1999), they are also used to extract pollutants from contaminated soils and sediments. These methods target and isolate a particular phase of the contaminant. Five single phases could be distinguished after the application of appropriate extracting solvents (Rauret *et al.*, 1999; Sahuquillo, 2003). Briefly, this procedure is as follows:

- A soil solution is obtained following centrifugation, after extractions with any of the following extractants: cold or hot water, calcium chloride, sodium nitrate or potassium nitrate solutions. This phase contains mobile and available species.
- The exchangeable phase contains elements that are not strongly adsorbed to the soil surface. Cations from the extracting solution (Ca^{2+}, Mg^{2+} or NH_4^+) are exchanged with toxic elements that are weakly attached to the soil surface. Reagents used to extract the exchangeable fraction include solutions of sodium nitrate, potassium nitrate, calcium chloride and ammonium acetate. The latter solution at 1 M and at pH 7 offers the benefit of stabilising the cation exchanged, by forming a stable complex with the acetate anion (Rauret *et al.*, 1999).
- The carbonate phase is obtained by sodium acetate buffered to pH 5 and acetic acid solutions of 0.1, 0.5 or 1 M. Elements removed in this phase are loosely bound to soil carbonates.
- The iron and manganese phase contains elements released from manganese and iron oxides. High to low concentrations of hydroxylamine favour the extraction of elements attached to manganese and iron oxides, respectively. A mixture of sodium dithionate, sodium citrate and sodium bicarbonate, buffered to pH 7.3, is also applied for this extraction, but contamination with zinc sometimes occurs.
- The organic fraction contains metals released from humic material to which they were attached. The dispersion is done by using ethylene diamine tetra-acetic acid (EDTA), diethylene triamine penta-acetic acid (DTPA), but preferably sodium pyrophosphate. Other solvents, such as sodium hypochlorite and hydrogen peroxide (pH 3), are also used.

Single extraction procedures may lack specificity. Acetic acid for example, which is used to extract the exchangeable fraction, partially attacks the carbonate and silicate phases (Rapin & Foerstner, 1983). Harmonised and operationally defined single extraction methods were validated for EDTA, DTPA and acetic acid, in contrast to calcium chloride, sodium nitrate and ammonium nitrate. The protocol for the validated methods describes in detail the steps to prepare the extracting solvent, as well as the rigorous extraction procedure.

In contrast to single extractions, sequential extractions involve a number of extracting agents in several consecutive steps. Sequential extraction protocols consisting of two to seven steps have been reported (Maiz *et al*., 2000; Baeyens *et al*., 2003; Ponce de Leon *et al*., 2003; Hanzhou *et al*., 2009; Krasnodebska-Ostrega *et al*., 2009). The most widely used protocol comprises three steps, derived from the work published by Tessier *et al.* (1979). Various solvents and procedures for extraction of toxic elements from soil have been implemented by different laboratories in a number of countries. Consequently, results of the analysis of soils and sediments are difficult to compare. Standardised methods for sequential extraction have been proposed under the sponsorship of the Community Bureau of References (BCR) (Rauret *et al*., 1999). The validation of the method involved cross-laboratory studies with the recommendation to strictly adhere to the defined protocol. The BCR sequential extraction procedure establishes four soil fractions, the exchangeable, reducible, oxidisable and residual fractions, by making use if 0.11 M acetic acid, 0.1 M ammonium hydroxylchloride or 8.8 M hydrogen peroxide, followed by 1 M ammonium acetate and *aqua regia* digestion, respectively. Details of the protocol are provided in Section 4.2.8.

2.8 ASSESSMENT OF THE CONTAMINATION LEVEL AND ECOLOGICAL RISK OF POLLUTED SOILS AND SEDIMENTS

The level of contamination and resulting ecological risk of soils and sediments are assessed using several indices. These are derived from the total concentration and the speciation of toxic elements in soils and sediments (Zhao *et al*., 2012). Indices most commonly used to determine the contamination levels of soils and sediments include the geo-accumulation index (I_{geo}), enrichment factor (EF), contamination factor (CF) and pollution index (PI). These indices are calculated according to mathematical expressions, taking the total concentration of the contaminant into account. The formulae for these indices are available in published literature (Guo *et al.*, 2010; Zhao *et al.*, 2012; Teh *et al.*, 2016). The values obtained vary from zero, indicating an absence of pollution, to above 40, corresponding to extreme pollution (Zhao *et al.*, 2012). The disadvantage of using these indices is that they consider only one contaminant and ignore the contribution of other toxic elements that are present. The interactive effect of pollutants can be addressed by applying, for example, the metal pollution index (MPI) and degree of contamination (DC) (Zhao *et al.*, 2012).

The potential ecological risk factor (ER) and the risk index (RI), which are based on total concentration, are used to determine the ecological risk of soils and sediments following multi-element contamination (Hakanson, 1980). Rather than using total metal concentration, the ecological risk of contamination should be assessed from the speciation of the contaminant in soils and sediments. The speciation of toxic elements is determined from sequential extraction schemes. For example, the BCR sequential extraction segregates the total concentration of metal contaminants into exchangeable, reducible, oxidisable and residual fractions. The percentage of total concentration of an element in the exchangeable fraction determines the ecological risk level, which ranges from no risk to very high risk according to the risk assessment code (RAC) (Zhu *et al*., 2012). The individual contamination factor (ICF), the global contamination factor (GCF), as well as the global risk index (GRI), used to evaluate the ecological risk associated with toxic element contamination, are also based on the speciation of the toxic elements in soils and sediments.

2.9 REMEDIATION OF CONTAMINATED SOILS

Soils contaminated with toxic elements impact on food production, food quality and safety, and pose a serious risk to the environment and human health (Leštan *et al*., 2008; Abdullahi, 2015). Globally, countries have implemented regulations to prevent and manage soil and sediment contamination. The remediation of contaminated soils and sediments is aimed at either stabilising or removing the contaminants from the soil until an acceptable level has been reached. Several techniques are available for remediation and their selection is based on knowledge of the source, as well as the chemistry and the potential risk of the toxic elements in the contaminated soils and sediments (Abdullahi, 2015). Although different names are used by authors in the literature to classify the various remediation techniques, they have the same meaning in most cases (Wuana & Okieimen, 2011; Yao *et al*., 2012; Abdullahi, 2015). Yao *et al.* (2012) classified remediation techniques for soil contaminated with toxic elements into three categories, i.e. physical, chemical and biological remediation.

2.9.1 Physical remediation

Physical remediation of soils contaminated with toxic elements consists of soil replacement and thermal desorption. Soil replacement is aimed at diluting the contaminant by partially or completely replacing the contaminated soil with clean soil (Yao *et al.*, 2012). Thermal desorption is applied to soils contaminated with volatile toxic elements, such as arsenic and mercury. In this technique, the toxic element is removed by heating the soil using microwave, steam or infrared radiation and the volatilsed species are then collected.

2.9.2 Chemical remediation

Soil remediation using chemical means consists of leaching, fixation, electrokinetic remediation and vitrification (Yao *et al.*, 2012). Leaching involves the washing out of contaminants from the soil using fresh water, reagents, fluids or gases. The movement of the contaminants from the soil to the leachate is realised through ion exchange, precipitation, adsorption or chelation (Yao *et al.*, 2012). Phosphoric acid (9.4%) was found to efficiently remove arsenic within six hours from artificially contaminated soils (Tokunaga & Hakuta, 2002). Diverse extractants, including Na_2EDTA, ethylenediaminedisuccinic acid (EDDS), and EDTA combined with cyclodextrin, were found to effectively remove toxic elements from contaminated soils (Yao *et al.*, 2012). A chemical fixation technique can be used to restrict the mobility of toxic elements by adding reagents or materials (clay, metallic oxides and biomaterial) to the contaminated soil (Yao *et al.*, 2012). Hodson and Valsami-Jones (2000) demonstrated that metal contaminants in soil could be immobilised using bone meal ($Ca_{10}(PO_4)_6OH$), whereby the bioavailability of the metals was reduced through the formation of metal phosphates. Materials, including bentonite and attapulgite clay were found to effectively reduce the mobility of cadmium in contaminated soils (Yao *et al.*, 2012).

Electrokinetic remediation involves the removal of metal contaminants from soils by creating an electric field through the application of a potential difference across the soil. However, this technology is relatively new and needs constant improvement due to its low efficiency. Applying high temperatures (1400 - 2000 °C) to contaminated soils results in the immobilisation of the contaminants by vitrification. During the process, gaseous and pyrolysis products are collected for further treatment, while metal contaminants are sequestrated in vitreous rock after cooling of the melt. This process is highly effective, but is expensive due to the energy requirements.

2.9.3 Biological remediation

This technique includes animal remediation, biological remediation and phytoremediation. Animal remediation makes use of lower organisms, such as earthworms, to reduce soil contamination, since they are able to absorb, degrade and migrate heavy metals (Yao *et al.*, 2012). A reduction in the extent of lead and copper contamination of soils was measured after introducing earthworms and a combination of earthworms and straw mulching (Wang *et al.,* 2007; Kou *et al*., 2008).

The use of microorganisms for the remediation of soils contaminated with toxic elements is termed biological remediation (Yao *et al*., 2012). Although microbes cannot degrade or destroy metal contaminants in soils, they are responsible for extracellular complexation, precipitation, oxidation and reduction reactions, as well as intracellular accumulation of toxic elements. This technology was successfully applied in industry to extract low grade ore and to treat waste products, sewage sludge and contaminated soil (Bosecker, 2001). The hazardous effects of toxic elements in soil were mitigated following the introduction of vascular arbuscular mycorrhizae, which are symbiotic fungi (Abdel-Aziz *et al*., 1997). The capacity of specialised microbes for biological remediation is limited and may result in secondary pollution. Several factors, including pH, oxygen, moisture and temperature, influence the success of biological remediation and are not always optimal for remediation of contaminated environments.

Phytoremediation is a relatively recent technology that is gaining popularity within the scientific community (Ziarati *et al*., 2014). This technology has been praised for its cost effectiveness compared to that of physical and chemical remediation. It involves the use of higher plants to immobilise or absorb toxic contaminants from soils. Plants that naturally establish on polluted areas may contribute extensively to the reduction in the concentration of the potentially harzardous elements.

CHAPTER 3
EVALUATION OF pXRF SPECTROMETRY FOR THE DETERMINATION OF METAL CONTAMINANTS IN SOILS AND SEDIMENTS

3.1 BACKGROUND AND MOTIVATION

Assessments of environmental elemental pollution is a costly and time consuming process when using laboratory techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma and benchtop XRF spectroscopy. The pXRF is gaining popularity amongst the scientific community and is a good candidate for the rapid screening of toxic elements in soil based on its simplicity, relatively low cost, non-destructive analysis capability, multi-element analysis capability and real time analysis (Shefsky, 1997a, 1997b; Kilbride *et al*., 2006; USEPA, 2006; Higueras *et al*., 2012; Schneider *et al*., 2015).

These advantages of the instrument over the conventional laboratory techniques have been proven by several researchers from many countries. Higueras *et al.* (2012) determined toxic metals in soil samples from a remote old mining district in the Alcudian Valley of Spain. The XRF instrument used in the determination of total toxic metals (iron, copper, manganese, cadmium zinc and lead) in contaminated soils in the UK demonstrated very good linearity when compared to the ICP-OES method following *aqua regia* microwave digestion (Kilbride *et al.,* 2006). Similar results were also obtained when comparing pXRF to AAS in the determination of lead and zinc concentrations in soil samples collected from the Silvermines area in Ireland (Radu & Diamond, 2009). High coefficients of determination (r^2 = 0.963 and 0.988) were found for the plot of the XRF and ICP-OES, and XRF and ICP-MS results for both zinc and lead in sediments collected from the Hudson River in the (USA) (Kenna *et al*., 2011). Hu *et al.* (2014) investigated the accuracy of the method for the determination of arsenic, copper, zinc and lead present in agricultural soils in China. Although poor agreement was obtained for these elements when comparing the concentrations obtained from an ICP-MS, significant improvement was achieved after the application of preparation steps to the soil samples.

Researchers admit that the quality of data generated by the instrument is influenced by several factors including analysis time, particle size distribution, humidity of the sample and the roughness of the soil surface (Argyraki *et al*., 1997; Shefsky, 1997a). It has been demonstrated in the determination of arsenic that 37% of the response was lost when the soil contained 20% water (Parsons *et al*., 2013). These researchers concluded that the measurement time should be adjusted according to the specific element being determined and argued that the analyst may in some cases be forced to compromise between the precision level of the study and the measurement time of the analysis. However, they found no significant differences in the concentrations of arsenic when soil samples with different particle sizes were analysed. Results from a study conducted by Kilbride *et al.* (2006) indicated consistency between the pXRF and ICP-OES results from the determination of copper, iron, lead and zinc in soil with a particle size below 2 mm. Correlation between pXRF and AAS results was generally improved for lead and zinc when the soil particle size was reduced from < 250 µm to < 125 µm (Clark *et al*., 1999). The coefficients of determination were 0.924 and 0.937 for lead and 0.997 and 0.776 for zinc in the soil sample for particle sizes of < 250 and <125 µm, respectively.

To evaluate the ability of the pXRF spectrometer to produce analytical results of acceptable quality, concentrations measured using the spectrometer were compared to those obtained from standard analytical techniques, such as graphite furnace atomic absorption spectroscopy (GFAAS), AAS, WDXRF (wavelength dispersive X-ray fluorescence spectrometry), XRD (X-ray diffraction spectrometry) and ICP-OES. In most comparative studies, the coefficient of determination (r^2) and the Pearson correlation coefficient (r) value were used to indicate the degree of correlation between the pXRF and the laboratory-based technique. The US Environmental Protection Agency (USEPA Method 6200) established a guideline to assess data quality for the field portable spectrometer when compared to the other accepted analytical techniques (USEPA, 2007). For guidance, the agency indicates that a coefficient of determination (r^2) equal or greater than 0.7 is only acceptable for screening level data, whereas a value of 0.9 and above, at a 99% confidence interval, meets the definitive level data criteria.

Most of the current pXRF spectrometers are preloaded with a choice of two default calibrations for geological applications: i.e. mining and soil modes (Hall, 2013). These modes apply two pre-installed calibration techniques (default settings), the fundamental parameter and Compton normalisation calibrations. The mining mode is based on the use of fundamental parameter calibration, which involves determining empirical mathematical formulae to correct for several factors that influence the results of the measurement, such as absorption and enhancement effects. With the fundamental parameter calibration, the analyst takes direct measurements, without the need for further calibration steps (USEPA, 2007; Hall *et al*., 2013). However, soil particle size distribution and matrix effects are the main limitations of this calibration technique. Where significant bias is observed within results from measurements of a certified reference material (CRM), an adjustment of the fundamental parameter calibration may be necessary. In the case of the Niton XL 3t

GOLDD^{TM}+, used in this study, a calibration curve was constructed by plotting the certified concentrations of a set of CRMs against the concentrations of the same CRMs (using the pXRF spectrometer). Thereafter, the slope and intercept values extracted from the equation of the calibration curve were integrated with the instrument software to adjust the fundamental calibration.

Besides the two proprietary calibration techniques, an empirical calibration can be established by the user to correct for matrix effects, particle size distribution and inter-element influences, to obtain more accurate results. This calibration technique involves analysing pure elemental standards (CRMs) to set the channel limit for each element for spectral deconvolution. This is followed by the analysis of well characterised site-specific calibration standards (SSCS), to yield the spectrum and intensity of each analyte in SSCS (USEPA, 2007). The concentration of each analyte in SSCS is then incorporated into the instrument software to develop a regression equation of these concentrations against the corresponding intensities (USEPA, 2007).

This chapter describes the preliminary work conducted to validate the use of the pXRF spectrometer as a convenient tool for the investigation of copper, zinc and lead contamination in soils and sediments. Once the evaluation was successful, the technique was implemented for on-site screening of soils and sediments from an abandoned mine site, as well as for mango and citrus orchards to determine metal contaminants.

Pelletized soil samples originating from Duikersdal (Prieska, Northern Cape Province, South Africa) were analysed to determine the levels of arsenic, chromium, copper, nickel, lead, rubidium, vanadium and zinc, using fundamental parameter and Compton normalisation calibrations of a pXRF spectrometer. The results obtained from this analysis were compared to data obtained from WDXRF analysis done by the Council of Geoscience in Pretoria to determine the most suitable calibration method for the determination of these elements in soil.

A preliminary *in situ* analysis of soils and sediments using the pXRF spectrometer was conducted at the abandoned Edendale Mine (Mamelodi, Pretoria), thought to be contaminated by zinc and lead. Field analysis of soils were also done in mango and citrus orchards suspected of copper contamination, using the pXRF. Soil samples from the sites, which were analysed *in situ*, were collected and processed according to two sample preparation methods (dried and sieved, and pelletized samples) and were analysed using pXRF and WDXRF spectrometers, as well as an ICP-OES. Data from the pXRF analysis of the processed soil and sediment samples were compared to those taken in the field and analysed in the laboratory. Results from field analysis and from the processed samples using the pXRF spectrometer were compared to those obtained with the ICP-OES and WDXRF. These comparisons were aimed at establishing the reliability of pXRF spectrometry for field screening of soils and sediments for the determination of metal contaminants. The outcomes of this preliminary study provided a basis for the decision to use the technique for large scale screening of soils and sediments.

3.2 MATERIALS AND METHODS

3.2.1 Comparison of pXRF and WDXRF for determination of elements in soil

Soil samples (n=592) were collected in the area of Duikersdal (Prieska, Northern Cape Province, South Africa) in a 1 km^2 grid as part of a geological survey conducted by the Council of Geoscience (Pretoria, South Africa). Samples were crushed and sieved (< 75 µm). A 12 g `portion of each sieved soil sample was mixed with 3 g of wax and pressed (25 ton) using a hydraulic press. The pellets generated were analysed using a PANalytical Magic-X Fast simultaneous WDXRF fluorescence spectrometer, equipped with a rhodium tube.

The soil pelletized samples were further submitted to elemental analysis using a portable energy dispersive spectrometer (Thermo Scientific Niton XL3t GOLDD^{TM}+; Tewksbury, USA). The portable spectrometer is equipped with a 50 kV X-ray tube (Ag anode), delivering a high X-ray flux to optimise the excitation of elements. Four energy filters are used to select the level of energy necessary to excite elements with atomic numbers (Z) < 17, those with 19 ≤ Z ≤ 24, as well as higher Z value elements so that they can be determined by the geometrically optimised large area drift detector (referred to as GOLDD^{TM}) that promotes high sensitivity. To analyse the soil pelletized samples, the device was mounted onto its stand and used in the laboratory (Figure 3.1). Each of the two internal calibration modes (soil and mining modes) were tested. The fundamental parameter, calibration mining mode (copper/zinc) or soil mode, were individually selected. The analysis time was set at 180 s, corresponding to 45 s for each of the filters to excite the full range of elements. Contamination of the X-ray window leading to drift of the instrument was checked using the standard reference, RCRA, as instructed by the manufacturer. The specifications of the spectrometer are summarised in Table 3.1.

The results from the analysis were correlated with data obtained using WDXRF spectrometry by applying linear regression analysis.

Table 3.1: Specifications for the Niton XL 3t GOLDD^{TM}+ spectrometer as adapted from the manufacturer's website[4]

Tube	Ag anode (6-50 kV, 0-200 μA max)
Detector	Geometrically optimised large area drift detector (GOLDD^{TM}) proprietary detector with 180 000 throughput cps
Resolution	< 185 eV; 60,000 cps; 4 μs shaping time
System Electronics	533 MHz ARM 11 CPU 300 MHz dedicated DSP 80 MHz ASICS DSP for signal processing 4096 channel MCA 32 MB internal system memory/128 MB internal user storage
Standard Analytical	Range up to 30 elements from Mg to U
Optional Light Elements	Ultra-low light element detection *via* He purge
Data Storage	Internal >10,000 readings with spectra
Data Transfer	USB, Bluetooth, and RS-232 serial communication
Mode (Varies by application)	Alloy modes: Metal alloy, Electronics alloy, Precious metals Bulk modes: Soil, Mining, TestAll™ Plastic modes: RoHS Plastics, Toy & Consumer Goods Plastics, TestAll, Painted Products Custom Modes: Upon request (based on application feasibility)

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[4] www.niton.com/docs/literature/Niton_XL3t_GOLDD_Spec_Sheet.pdf. [Accessed]: 15/02/2016.

Figure 3.1: The Niton XL 3t GOLDD^{TM}+ spectrometer used in this study for the analysis of pelletized samples in the laboratory

3.2.2 *In situ* analysis of soils and sediments from contaminated sites

The pXRF spectrometer was carried in a waistbelt when taken to the field. Two lithium batteries provided by the manufacturer allowed extended working hours without the need for charging. At the measurement point, the soil surface was cleared by removing dead leaves and twigs, stones and any extraneous materials, and the surface soil was loosened and levelled (Figure 3.2). The probe of the spectrometer was placed in contact with the soil and a measurement was taken by pressing the trigger, which was released after the analysis time was reached. These samples are referred to as “unprepared” samples in this study. A total of 34 measurements were randomly taken on soils at an abandoned mine (Edendale Mine) suspected of lead and zinc contamination and 15 measurements were taken from mango and citrus orchards in Limpopo suspected of copper contamination. Sediment samples (n=22) were collected from a tributary of the Edendale Spruit, flowing through the abandoned Edendale Mine. The water was drained from the samples and the wet samples were analysed. These *in situ* sediment samples are referred to as “wet” samples in this study.

Figure 3.2: Field analysis of soil using the pXRF spectrometer

3.2.3 *Ex situ* analysis of the soil and sediment samples collected from contaminated sites

Soil samples from the abandoned mine (n=34) and from the mango and citrus orchards (n=15), and sediment (n=22), which had been analysed in the field, were transported to the laboratory for treatment before being re-analysed using the pXRF. The samples were air-dried in the laboratory. Stones, twigs and leaves were removed from the dried samples, which were then transferred to a mortar and gently crushed using a pestle. Thereafter, the samples were sieved to pass through a 2 mm mesh. Those portions of the soil and sediment samples containing particles of a diameter below 2 mm were placed in a sample cup before pXRF measurements were made. Soil and sediment samples treated in this way are referred to as “dried and sieved” samples in this study.

Dried and sieved soil and sediment samples were pelletized. This treatment of the sediments and soils from the abandoned mine was done in the laboratory of Pretoria Portland Cement (PPC) in Germiston, Johannesburg, South Africa. The orchard soils were pressed at the Department of Geology, University of Pretoria using electrical and hydraulic presses. The preparation involved crushing the dried and sieved soil and sediment samples to particle sizes below 65 µm. A portion of approximately 20 g of each sample, to which a binding substance (1.2 g of paraffin wax or a few drops of polyvinyl acetate (PVA)) had been added, was transferred to a stainless steel tungsten carbide ring mill for grinding. Samples were crushed for one minute using a grinding machine (Retsch Disc Mills RS 200, Haan, Germany) operating at 950 rpm. Thereafter, the samples were transferred to pellet moulds, which were loaded into a press (Retsch Pellet Press PP 40, Haan, Germany). Samples prepared in this way are referred to as “pelletized samples” in this study.

Dried and sieved and pelletized samples were analysed using the pXRF spectrometer. The device was used as a laboratory analytical instrument by mounting it on a customised stand and connecting it to a laptop to which the NITON XL 3t software had been installed. The cupped samples or pelletized samples were placed on the appropriate platform. After placing the window of the analyser in contact with the sample, analysis was activated from the computer or by pressing the trigger.

3.2.4 Analysis of soil and sediment samples using ICP-OES following acid digestion

The soil and sediment samples from the abandoned mine and mango and orchards, which were previously analysed in the field using the pXRF spectrometer, were air dried in the laboratory, crushed using a mortar and pestle, sieved through a 2 mm sieve, and analysed again with the pXRF. Thereafter approximately 0.25 g portions of the dried and sieved soil, and sediment samples were weighed into microwave vessels. An acid mixture, consisting of 3.0 mL of nitric (Suprapur) and 2.0 mL of hydrofluoric acid 40% (m/v), which were purchased from Merck (Darmstadt, Germany), was added to each vessel. The samples were then placed into a microwave-assisted reaction system (MARS 5, CEM Corporation, Matthews, USA) and digested using the following programme: the temperature was ramped to 175 °C over 20 min and held at this temperature for 15 min. The microwave programme was run for a second time to ensure complete digestion of the samples. After allowing the samples to cool for 15 min, 2.0 mL of 7% boric acid (AR grade) (Merck, Modderfontein, South Africa) was added to each tube. The digests were transferred to 100 mL volumetric flasks and diluted to the calibration mark with ultra-pure deionised water. After filtration, using a 0.45 µm membrane filter, the copper, zinc and lead concentrations were determined in the digests using the ICP-OES spectrometer for comparison with pXRF results. Analyses of the digested soil and sediment samples were carried out using a Spectro Arcos FHS (Kleve, Germany) spectrometer.

The ICP-OES spectrometer is equipped with a peristatic pump, which delivers the liquid sample to a cross flow nebulizer, which atomises the fluid. The resulting mist is then conveyed to the plasma by argon gas flowing at 0.8 mL/min. Heating of the plasma (7000 K) takes place due to collisions of argon atoms accelerated in an electromagnetic field created by a radio frequency generator (1500 W). In the plasma, the sample breaks down into atoms and ions; electrons are excited and emit electromagnetic radiation when the electrons return to a lower energy state, with wavelengths that are characteristic of the elements. The emitted continuous radiation is separated into individual wavelengths using a dispersive system. A charge coupled device (CCD) quantifies the intensity of the distinct radiations by comparing the intensity to that of the calibration standards to determine their concentrations. The operating conditions of the spectrometer, which was used in this study is listed in Table 3.2.

Table 3.2: Optimum parameters for the ICP-OES used in this study

Parameter	**Setting**
RF Generator power (W)	1500
Plasma gas flow rate (L/min)	15.00
Auxiliary gas flow rate (L/min)	1.00
Nebuliser gas flow rate (L/min)	0.80
Sample uptake (mL/min)	1.60
Type of nebuliser	Cross flow
Type of spray chamber	Scott double pass

3.2.5 Independent analysis of the soil and sediment samples using a WDXRF

The pelletized soil and sediment samples from the Edendale Mine, as well as from the mango and citrus orchards, previously analysed using the pXRF, were sent to the laboratory of the Department of Geology of the University of Pretoria for independent analysis. A WDXRF spectrometer (ARL Perform'X Sequential XRF, Johannesburg, South Africa), operating with Uniquant software was used for the analyses. The results obtained were compared to those obtained using the pXRF when analysing unprepared, dried and sieved and pelletized samples.

3.2.6 Calibration of the spectrometers and method validation for the determination of copper, zinc and lead in contaminated soil and sediment samples

The internal mining mode calibration was adjusted from a calibration curve constructed from four replicate analyses of five CRMs i.e. NIST2709a and NIST2780 (National Institute of Standards and Technology, USA), TILL-4 (Natural Resources, Canada), AMIS0157 and AMIS015 (African Mineral Standards, South Africa). The results obtained were compared to the certified reference values, and calibration curves were generated for each of the elements of interest using Microsoft Excel (2013). The measured values of the reference materials (the y-axis of the calibration curve) were plotted as a function of the certified values of the reference materials (x-axis of the calibration curve).

The stock solution was obtained from 10 times dilution of a 1000.0 mg/L single element certified standard solutions (Spectroscan, Technolab AB, Sweden). Serial dilutions were made by transferring the required volume of the stock solution of the analyte into a 100 mL volumetric flask containing 3.0 mL nitric acid, 2.0 mL hydrofluoric acid (Suprapur; Merck, Darmstadt, Germany), and 2.0 mL of saturated boric acid (AR grade; Merck, Modderfontein, South Africa) and diluting to the graduation mark. To calibrate the ICP-OES instrument, six standard solutions with concentrations of 0.500, 1.00, 2.00, 5.00, 7.00; 10.0, 15.0 and 20.0 mg/L were prepared from 100.0 mg/L stock solutions of the elements. A blank solution was also included.

To validate the analytical methods, the soil CRMs containing low, medium and high concentrations of each analyte (RCRA, NIST2780, AMIS0153 and AMIS 0157) were analysed using both techniques (Table 3.3). Relative standard deviations (RSDs) calculated from seven replicate analyses of the CRMs permitted an evaluation of the precision of the analytical methods. The accuracy of each method was estimated from the percentage recovery, which was calculated from the certified values for the metals.

Table 3.3: Certified values for the CRMs used for the calibration and validation of the pXRF and ICP-OES methods

CRM	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
Till-4	237	70	50
NIST 2709a	33.9	103	17.3
NIST 2780	215.5	2570	5770
RCRA	-	500	-
AMIS0153	1814	87900	10191
AMIS 0157	600	30100	3227
AMIS0326	1215	-	-
AMIS0413	485	-	-

The limit of detection (LOD) for the pXRF method was statistically estimated as three times the standard deviation of seven replicate analyses of the reference material, RCRA (Bernick *et al.,*1995; Weindorf *et al*., 2008). For the ICP-OES, the LOD was obtained from regression analysis of the calibration data.

3.2.7 Statistical analyses

The paired t-test (MS Excel 2013 v.15.0) was applied to compare separately the concentrations of elements obtained from the unprepared, dried and sieved, and pelletized samples, following pXRF analysis. Linear regression analysis from Excel was also used to compare results from the pXRF for unprepared, wet, dried and sieved, as well as pelletized soil and sediment samples, with those obtained with the ICP-OES for the corresponding samples. Results were deemed significantly different at a 95% confidence level when p-values obtained from the paired t-test were less than 0.05. Linear regression is the statistical method most often used to evaluate the performance of the pXRF compared to conventional analytical instruments such as ICP-OES, ICP-MS, AAS and WDXRF (Shefsky, 1997a; Kalnicky & Singhvi, 2001; USEPA, 2007; Parsons *et al.*, 2013; Hu *et al.*, 2014). Linear regression was applied to evaluate the performance of the pXRF in comparison with the ICP-OES and WDXRF techniques.

3.3 RESULTS AND DISCUSSION

3.3.1 Comparison of results obtained from pXRF and WDXRF in the determination of elements in pelletized soil samples

Default calibrations (mining and soil modes) available on the pXRF spectrometer, were selected to analyse pelletized soil samples collected from Duikersdal and prepared by the Council of Geoscience to establish the mode that will yield the most accurate results for an individual metal using pXRF. The results of the analyses were correlated with those obtained using a WDXRF spectrometer. The suitability of the calibration modes with regard to the determination of the concentrations of major soil pollutants, including arsenic, chromium, copper, nickel, lead, rubidium, vanadium and zinc, was assessed by fitting the pXRF results to the data from the WDXRF (Figure 3.3).

An excellent fit was found for both calibration modes for rubidium and zinc. Good correlations were found between the pXRF in soil mode with the WDXRF for chromium, copper, nickel and vanadium. The concentrations of these elements were in the same ranges for the two spectrometers. However, poor correlations were found for chromium, nickel and vanadium in mining mode. For lead, results obtained using the mining mode correlated better with the WDXRF results than when the soil mode was applied. The two devices were of similar detection limit, regardless of the mode employed by the pXRF.

Results for lead concentrations in soil samples were lower with the soil mode than when measured using the mining mode. Generally, the results from the soil mode correlated better than those from the mining mode, probably because the concentrations of the elements in the soil samples were low, but perhaps also because of the nature of the soil matrix. The soil mode makes use of Compton ratioing to correct for matrix interferences, and only corrects for absorption effects, thus it’s application to trace elemental corrcentration. This calibration technique involves measuring the intensity of the Compton peak (backscatter peak), which is known to be inversely proportional to the mean mass attenuation coefficient of a sample (Kalnicky & Singhvi, 2001; Hall *et al*., 2013).

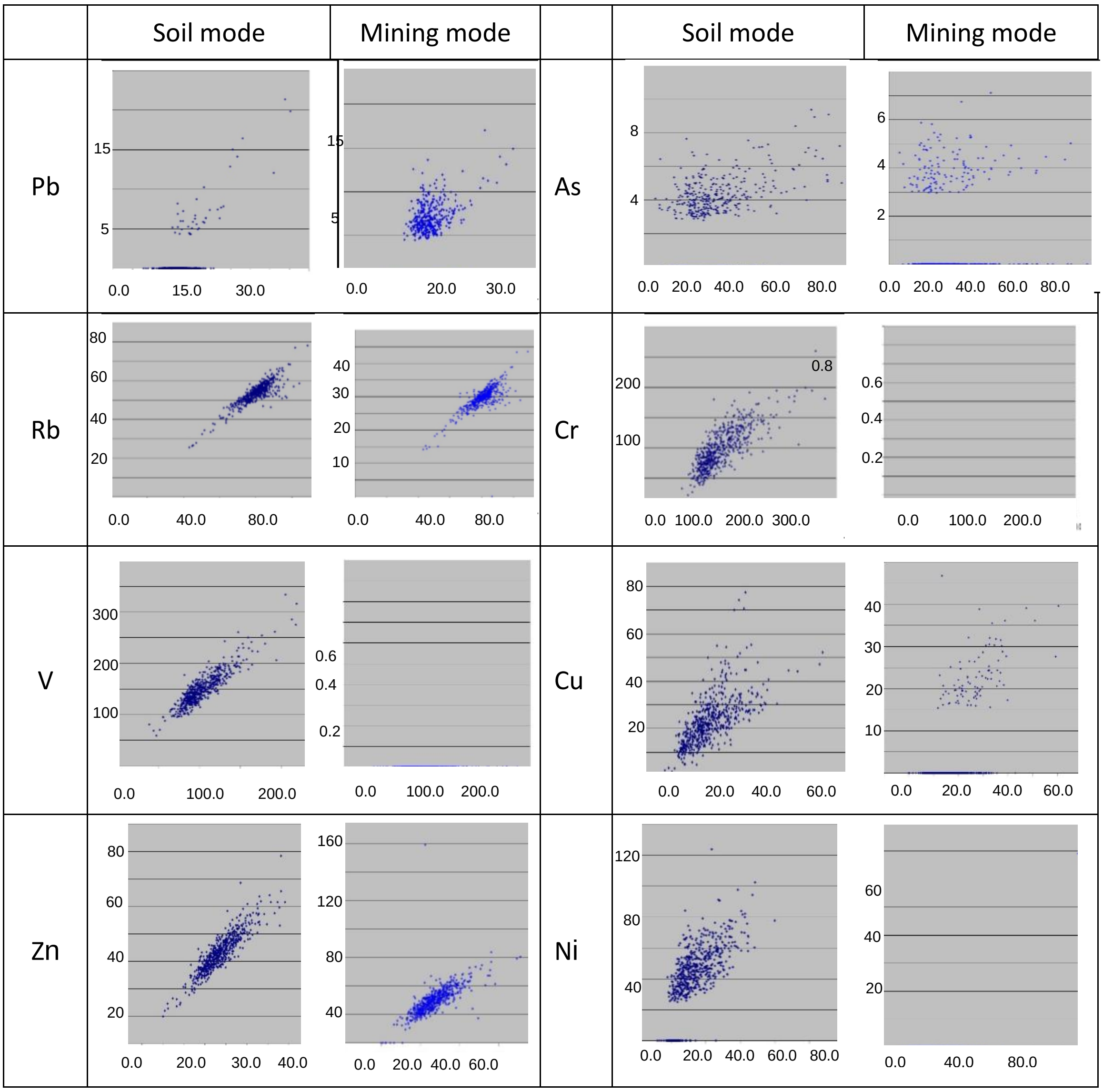


Figure 3.3: Correlation plots for arsenic, chromium, copper, nickel, lead, rubidium, vanadium and zinc, measured as mg/kg, using WDXRF (x-axis) and pXRF (y-axis) calibrated using soil or mining mode

Matrix correction is made by normalising the intensity of the fluorescence peak of the analyte to the intensity of the Compton peak of the sample, both simultaneously measured by the detector. This calibration is most suitable for the analysis of trace elements in soils, since soil matrices are predominantly composed of lightweight elements, which generate a higher intensity of the backscatter peak (USEPA, 2007; Hall *et al.*, 2013).

Soils from the survey contained relatively low concentrations of toxic metals, confined to a rich silicate matrix, which may explain the improved performance of the soil mode compared to the mining mode. Lead, even at relatively low concentrations, was better correlated in mining mode than when operated in the soil mode.

3.3.2 Effect of sample preparation method on the determination of copper, zinc and lead in contaminated orchard and mining soils

Soil and sediment samples from the Edendale Mine and the mango and citrus orchards, which were analysed *in situ* (unprepared), were processed (dried and sieved, and pelletized samples) and again analysed using the pXRF spectrometer. Concentrations of copper, zinc and lead were determined in the unprepared (field measurement), dried and sieved, and the pelletized soil samples using the pXRF spectrometer (Appendices 1, 2 and 3). Trends in the concentrations for each of these elements measured in the soils after applying the three sample preparation methods are presented in Figure 3.4. The highest copper level was found in orchard soil Sample O5, followed by O8, O4 and O11, whereas low levels for the metal were measured in Samples O7, O10, O12 and O13, regardless of the sample preparation method used. Copper concentrations measured in the dried and sieved samples were the highest, followed by the pellets and the unprepared soil samples for Samples O5 and O8.

Maximum and minimum zinc concentrations determined in the mine soil samples generally coincided for the three preparation methods. Two samples contained high concentrations of zinc i.e. Sample M22 (7300, 8800 and 8300 mg/kg) followed by Sample M15 (4400, 6100 and 6885 mg/kg) for unprepared, dried and sieved, and pelletized samples, respectively. Zinc concentration measurements were comparable in dried and sieved, and pelletized samples and both were higher than those measured in the unprepared samples.

The highest lead concentrations were found in Sample M12 (21000, 23137 and 24061 mg/kg), followed by Sample M22 (21200, 21000 and 23000 mg/kg) and Sample M1 (11000, 13000 and 14000 mg/kg) for the unprepared, dried and sieved, and pelletized sample, respectively. In these samples, the lead concentrations were always higher in the pelletized than in the dried and sieved, and unprepared soil samples, except in the cases of Samples M17 and M15, for which the lead levels measured were higher in dried and sieved, and in unprepared soil samples, respectively.

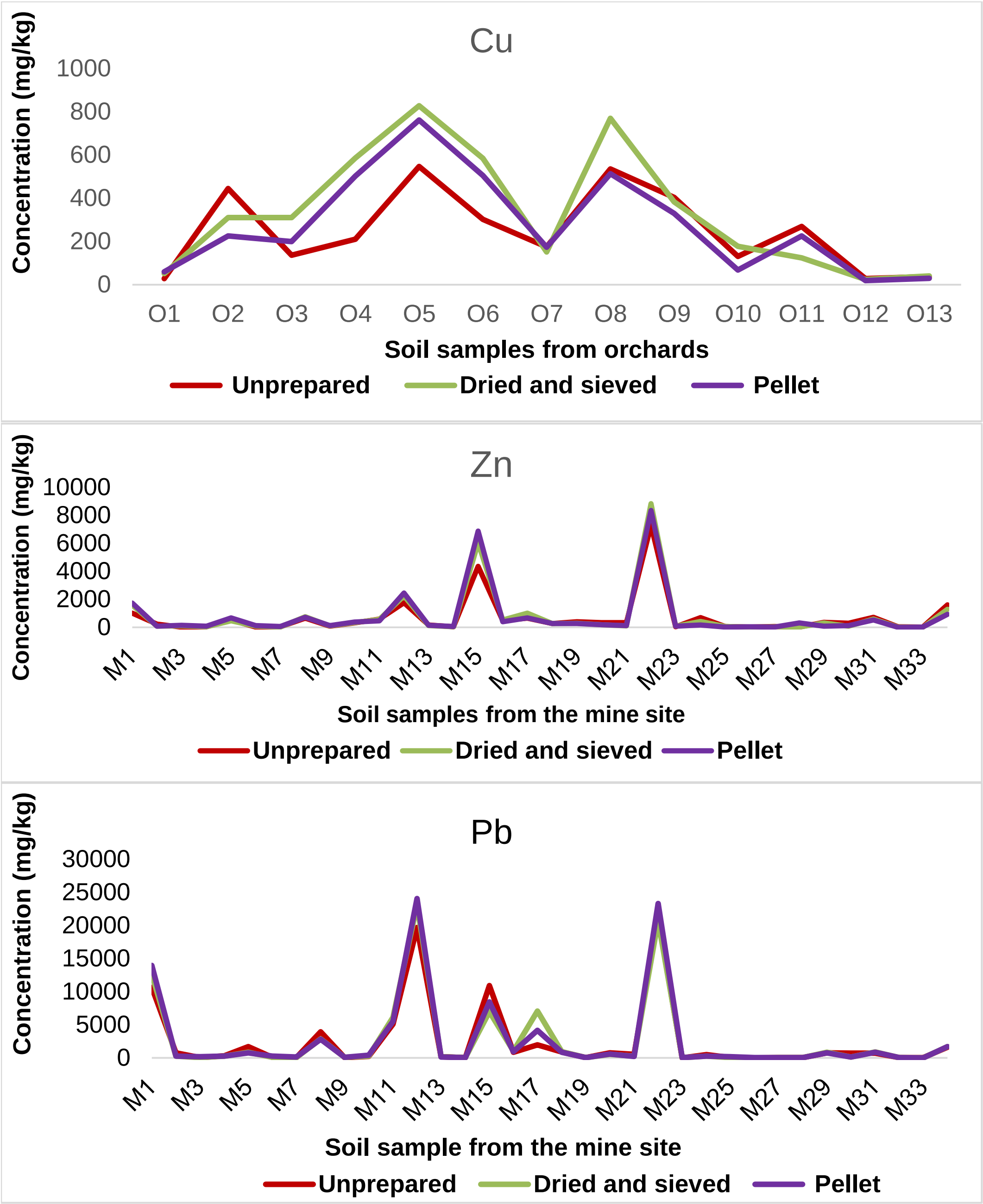


Figure 3.4: Metal concentrations in unprepared, dried and sieved, and pelletized contaminated soil samples as determined by pXRF for copper (Cu), zinc (Zn) and lead (Pb)

Statistical analyses were performed using Microsoft Excel software (2013). The paired t-test was applied to determine whether significant differences exist at a 95% confidence level between the concentrations of copper, zinc and lead measured in unprepared, dried and sieved, and pelletized soil samples, when determined using the pXRF spectrometer. A p-value < 0.05 indicated a significant difference. The average deviations of readings for copper, zinc and lead obtained between the sample preparation methods were calculated. Minimum and maximum deviations were determined, together with the percentages of samples with deviations below 20%, below 50%, and above 100%. Results of these statistical analyses are recorded in Table 3.4. To compare the results obtained from the three different methods of sample preparation, the values from the unprepared soil sample (U) were compared to those from the dried and sieved (DS) and pelletized (P) samples. Comparison of U with DS is indicated by U/DS, while that of U and P by U/P. In addition, the DS results were compared to those of P and are indicated by DS/P (Table 3.4). The same procedure was used later when comparing the unprepared wet sediment samples (W) with dried and sieved (DS) and pelletized (P) sediment samples.

Table 3.4: Results for the paired t-test to determine significant differences at the 95% confidence level for copper, zinc and lead concentrations obtained after applying three methods of sample preparation. The average deviation between the results obtained after applying different methods of sample preparation, minimum, maximum deviations and percentage of samples below 20% and 50%, and above 100% deviation are provided

	Soils from the Edendale mine						Soils from the mango and citrus orchards		
Element	Zinc			Lead			Copper		
Sample preparation	U/DS	U/P	DS/P	U/DS	U/P	DS/P	U/DS	U/P	DS/P
p-value	0.10	0.16	0.48	0.31	0.16	0.26	0.492	0.453	0.441
AD	33	89	129	39	68	50	56	43	28
Min	0.40	1.5	1.6	2.2	0.21	0.11	5.0	1.1	8.0
Max	240	113	145	257	315	439	178	138	82.3
< 20%	44	18	41	44	35	32.3	27	33	47
< 50%	77	50	62	82	62	62	53	73	67
> 100%	2.9	8.8	8.8	2.9	14.7	15	13	13	0

AD = average deviation
Min = minimum deviation
Max = maximum deviation
< 20% = percentage of samples below 20% deviation
< 50% = percentage of samples below 50% deviation
> 100% = percentage of samples above 100% deviation
U = unprepared soil sample
W = wet sediment sample
DS = dried and sieved sample
P = pelletized sample

The results for the paired t-tests indicate no significant difference ($p > 0.05$) at a 95% confidence level between U/DS, U/P and DS/P soil samples from the Edendale mine for zinc and lead concentrations measured using the pXRF spectrometer (Table 3.4). There were also no statistical differences between the copper levels after applying the various sample preparation methods to soil samples from the mango and citrus orchards, since the obtained p-values were above 0.05.

The calculated average deviations for copper concentrations varied from 28 to 56%, depending on the sample preparation mode. The largest deviations were observed between unprepared, and dried and sieved, samples (U/DS) for copper (Table 3.4). It was found that 27 to 47% of orchard soil samples deviated less than 20% for the sample preparation modes. A total of 58 to 73% deviated less than 50%. Although high deviations were observed between the sample preparation modes, only few samples were involved.

The average deviation range for zinc concentrations between the sample preparation modes for soils from the Edendale Mine was found to be between 33% and 129% (Table 3.4). The maximum and minimum deviation for zinc was found with U/DS. A range of 18 to 44% of the Edendale Mine soils produced a deviation of less the 20%, whereas 50 to 77% of samples displayed deviations below 50%. Although high deviations in zinc concentrations were obtained within each of the sample preparation modes, they represented only few samples from the Edendale Mine.

The average deviations of lead concentrations were between 39 and 68%, with maximum deviations of 439% found between DS/P. Eleven of the mine soil samples (32%), displayed deviations below 20% for the lead concentrations, irrespective of the mode of sample preparation. Deviations below 50% were reflected by 62 to 88% of the samples. Mine soil samples yielding deviations above 100% represented less than 15% of all the samples.

A large number of samples (50 to 82%) from all sites investigated was found to deviate by less than 50% and only 0 to 13% of the samples deviated by more than 100%. The deviations were generally lower between the processed samples, whereas they were higher with the unprepared and pelletized samples, and dried and sieved and pelletized samples. This finding is important for the field screening objective of our study where very high metal concentrations were expected. Although in some cases a 50% bias between the field and processed samples was obtained for these high concentrations, the results still reflect the degree of contamination of the site. The experiment also demonstrated that the pXRF spectrometer generally yielded higher readings for copper, zinc and lead in the pelletized and the dried and sieved samples than when the samples had not been processed in any way. These results were expected, since both preparation methods (drying and sieving, and pelletizing) involve the removal of extraneous components from the soil. These components have a dilution effect on the sample, resulting in lower concentrations of the analytes being recorded (Glanzman & Closs, 2007). In addition, a high water content of samples reduces the depth of penetration of the radiation (Jussi *et al*., 2005), also contributing to lower readings for moist samples. The removal of water from the soil samples consequently enhances the accessibility of soil matrix to the XRF radiation and results in higher readings for the contaminants (Kalnicky & Singhvi, 2001; Horta, *et al*., 2015). Jussi *et al.* (2005) reported that the removal of 30% moisture from their soil samples resulted in a doubling of the measured concentrations of zinc and lead.

Particle size also influences the results of soil analysis when using pXRF (Shefsky, 1997a, 1997b; Finkelshtein & Brjansky, 2009). Elemental concentrations measured in soil consisting of small particles are likely to be higher than when measured in soils with larger particles. Horta *et al.* (2015) reported higher values for lead, zinc and copper concentrations in soil when the particle size was reduced from 250 µm to less than 125 µm. In our experiment, the unprepared soil sample consisted of particles of more than 2 mm in diameter, while the dried and sieved soil samples contained particles smaller than 2 mm. In contrast, the particles of the pelletized sample were smaller than 65 µm. Maruyama *et al.* (2008) studied the effect of rocks crushed to 25-45, 45-90, 90-180, 180-250 and 250-500 µm on the X-ray fluorescence of aluminum, silicon, calcium, titanium and iron using Kα and Kβ lines. The authors concluded that the X-ray fluorescence from these elements decreased from smaller to larger particles, and this effect was more pronounced for lighter elements (Maruyama *et al*., 2008). The influence of particle size on X-ray fluorescence of elements irradiated by incident X-ray is well known and mathematical formulae have been proposed to correct for this effect (Criss, 1976; Cooper & Mustard, 1999; West, 2008). The net characteristic X-ray fluorescence emitted from a bulk sample is due to the absorption of the incident radiation, which depends on the composition and the size of individual particles of the sample (Criss, 1976).

The effect of sample particle size on the concentration (fluorescence) of an element in a sample can be easily understood considering the expressions of the absorption intensity (I) (West, 2008) and the attenuation factor (A) as proposed by Criss (1976);

$$I_i = AK_iC_i\rho X \qquad \text{Equation 3.1}$$

$$A = 1/(1 + ba)^2 \qquad \text{Equation 3.2}$$

where I_i is the beam intensity, K_i is a constant related to the absorption of the beam intensity I, C_i is the mass fraction of the element, ρ is the density of the absorber, X represents the finite thickness of the sample traversed, A is the attenuation factor, a is the particle diameter, and b is the coefficient of mass absorption of the particle (West, 2008).

From Equations 3.1 and 3.2 it can be deduced that the absorption intensity is proportional to the attenuation factor (A), which in turn, is inversely proportional to the diameter of the particle. Hence, a larger particle size results in a smaller attenuation factor, which consequently produces lower absorption of the incident radiation by the particle. On the other hand, smaller particle sizes result in a larger attenuation factor, thus producing greater absorption. Greater absorption of the incident radiation of an appropriate energy will allow more elements corresponding to that energy range to fluoresce. A large number of counts will be generated and collected by the detector resulting in a larger detector response.

Besides soil moisture, the presence of extraneous matter and soil particle size, the irregularity of the soil surface may be responsible for the differences in measured concentrations for the various samples prepared using different methods. The unprepared samples were analysed under field conditions. The surface was irregular compared to samples that were prepared by transferring the dried, ground and sieved soil sample into cups and levelling the surface. The pelletized soil samples offered the best analysis surface, since they had been finely crushed and pressed. The values for copper, zinc and lead obtained using pXRF in unprepared samples were also expected to increase when the samples underwent further preparation to pellets. Bonizzoni *et al.* (2006) analysed metal objects of irregular shape to determine the influence of metal shape on XRF results, and proposed a correction method based on the fundamental parameter calibration to improve the results obtained (Bonizzoni *et al*., 2006). Maruyama *et al.* (2008) simulated the roughness of lunar surface using 25 and 500 µm powdered rocks, which were analysed using XRF spectrometry. The researchers observed a 50% decrease in XRF intensity when compared to rocks characterised by a flat surface.

A similar study was carried out by Okada and Kuwada (1997) to evaluate the effect of soil surface irregularity on XRF analysis results using powdered silicon carbide and aluminum with particles of 5-150 µm diameters, which were mixed in different ratios. The results indicated that the intensities of incident and emitted X-rays were lower for a rougher surface. The variation in X-ray intensities observed was attributed to the variation of the incidence and emission angles of the radiation (Okada & Kuwada, 1997; Lin & Nittler, 2011). Gauvin and Lifshin (2000) also demonstrated that the intensity of the characteristic X-ray fluorescence emitted depends on the roughness of the sample surface. This finding was ascribed to the variation in the air gap between the sample and the analyser as the incident beam penetrates the sample, which in turn, affects the generation and absorption of the X-rays (Gauvin & Lifshin, 2000).

Reducing the matrix effect and irregularity of soil samples by homogenising soil samples and removing moisture generally contributes to the improvement of data quality in pXRF analysis (Horta *et al.*, 2015). These factors adequately explain the increase in the elemental concentrations from the unprepared to the pelletized soil samples observed in our study.

A study conducted in Hungary on the effect of sample preparation methods on measured zinc and lead concentrations in ground and underground contaminated soils revealed no significant differences between the results of the two types of samples (Tolner *et al*., 2015). It can also be concluded from our study that *in situ* screening of contaminated soils using the pXRF for the determination of copper, zinc and lead yields comparable results with those obtained when the soil samples are not processed.

3.3.3 Effect of sample preparation method on the determination of zinc and lead in contaminated sediments

Zinc and lead concentrations determined in sediment samples (Appendices 4 and 5) were plotted to evaluate differences obtained from the concentrations of samples prepared using the three methods (Figure 3.5). The highest and lowest concentrations were measured for zinc and lead in the same sediment sample, regardless of the sample preparation method employed.

The highest concentrations of zinc and lead were found in Sample S8, followed by Samples S6, S15 and then S10. For these samples, the measured values for zinc and lead were higher in dried and sieved samples than in the corresponding wet and pelletized samples.

The paired t-test, applied to results from the different sample preparation methods, yielded p-values below 0.05 (Table 3.5). This indicates that the concentrations of zinc and lead are significantly different when compared in W/DS, W/P and DS/P sediment samples

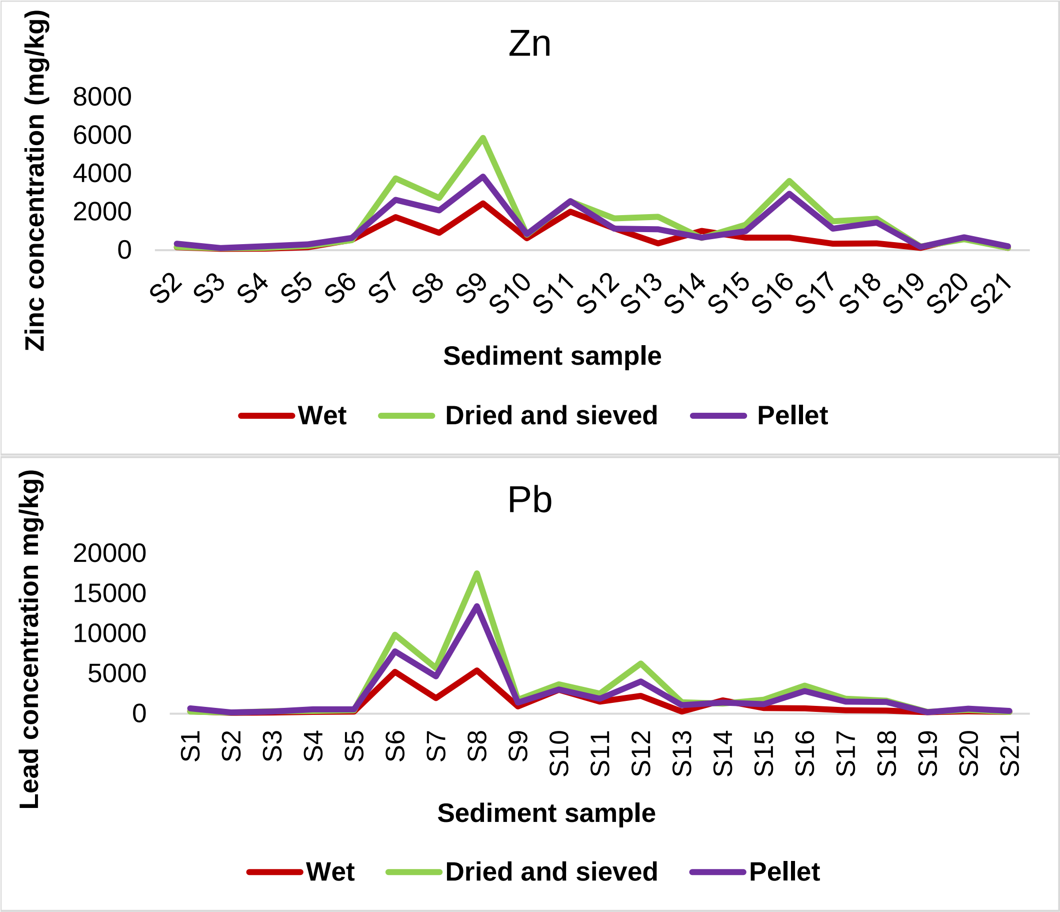


Figure 3.5: Metal concentrations measured in wet, dried and sieved, and pelletized contaminated sediment samples as determined by pXRF for zinc (Zn) and lead (Pb)

The average deviation of zinc concentrations were found to be between 29 and 123%. The minimum deviation was with DS/P and the maximum deviation with W/DS. The average deviations of lead concentration were found between 29 and 149%. Lead concentration deviations were more pronounced with W/DS, than with W/P and DS/P. Sediment samples with an average deviation below 20% for zinc and lead, for all sample preparation methods, was represented by 5 to 45% of the samples, while 45 to 85% of samples displayed an average deviation below 50% for zinc, and 30 to 90% for lead concentrations. A relatively large number of samples with a deviation above 100% were found for W/DS and W/P for zinc and for lead. However, very few samples displayed variation above 100% for lead and zinc for DS/P (Table 3.5).

In sediment samples, the presence of extraneous material and large and heterogeneous particle size distribution affected the measurements. However, the major interfering factor was probably, in the case of wet samples, the high moisture content (≥ 20% weight) of the sediment samples. The presence of water has been described as the most detrimental factor in the determination of elements in sediment using an energy dispersive XRF spectrometer (Glanzman & Closs, 2007).

Table 3.5: Results for the paired t-test to determine significant differences at the 95% confidence level for copper, zinc and lead concentrations obtained after applying three different methods of sample preparation. The average deviation between sample preparation modes, minimum, maximum deviations and percentage of samples below 20% and 50%, and above 100% deviation for sediment samples, are also indicated

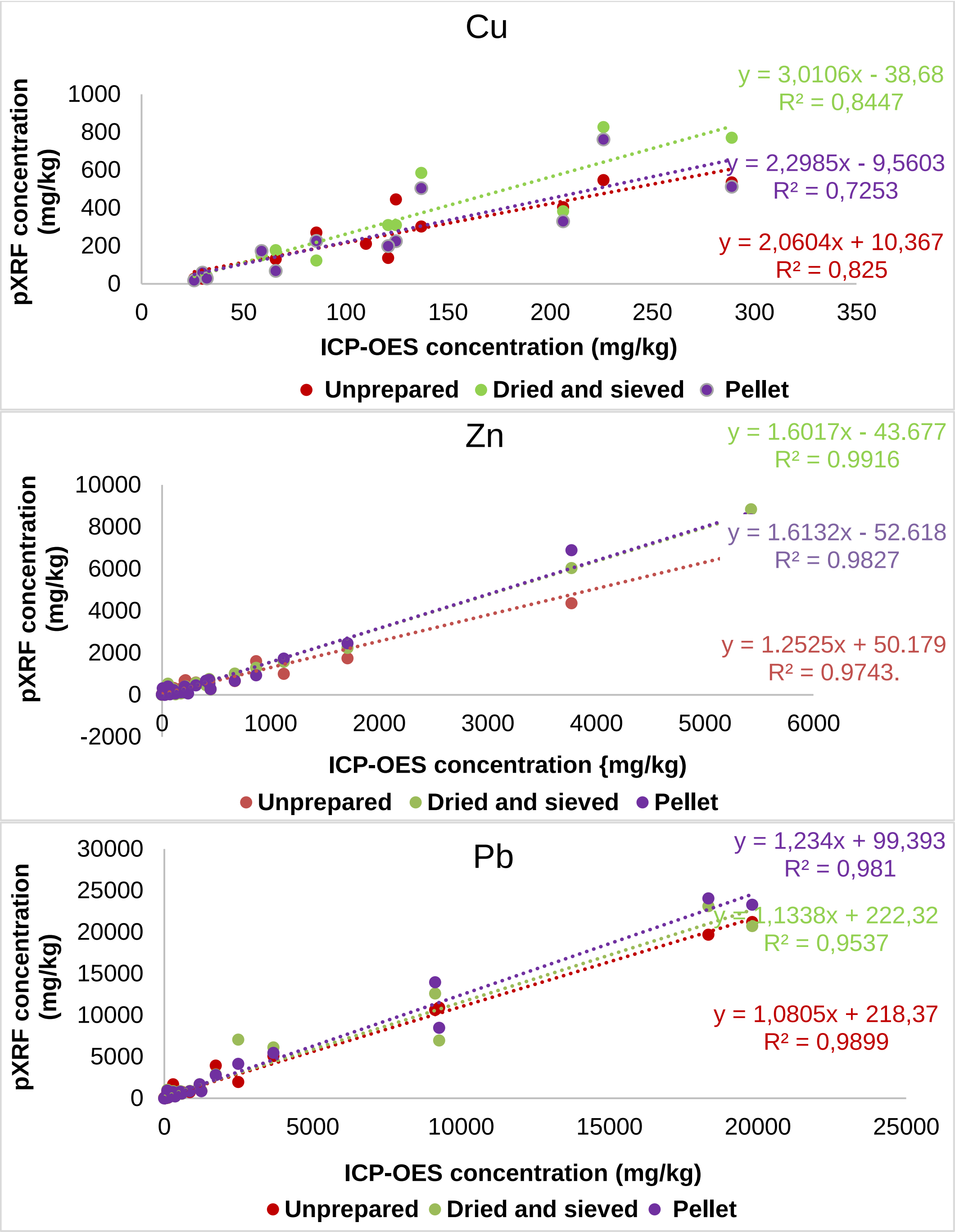
	Sediment samples					
Element	Zinc			Lead		
	W/DS	W/P	DS/P	W/DS	W/P	DS/P
p-value	0.002	0.001	0.013	0.01	0.01	002
AD	123	102	29	149	119	29
Min	2.2	1.1	0.31	18	1.9	3.6
Max	458	353	99	464	314	147
< 20%	25	15	35	5	10	45
< 50%	55	45	85	30	30	90
> 100%	40	30	0	30	35	5

AD = Average deviation
Min = minimum deviation
Max = maximum deviation
< 20% = percentage of samples below 20% deviation
< 50% = percentage of samples below 50% deviation
> 100%= percentage of samples above 100% deviation
W = wet sediment sample
DS = dried and sieved sample
P = pelletized sample

3.3.4 Comparison of the pXRF and ICP-OES results for the determination of copper, zinc and lead in soil samples

Mine and orchard soil samples, as well as sediment samples, all previously analysed in the field using the pXRF and again after the samples had been processed, were analysed using an ICP-OES. Results were compared by linear regression analysis and inferential statistics. Plots were constructed to compare the results obtained using the ICP-OES (x-axis) with pXRF (y-axis) for the three different methods of sample preparation i.e.

unprepared, dried and sieved, and pelletized soil samples (Figure 3.6). Perfect correlation



will yield a linear curve ($r^2 = 1$) that passes through the origin and has a slope of 1.

Figure 3.6: Correlation between metal concentrations measured in unprepared, dried and sieved, and pelletized soil samples measured by pXRF and by ICP-OES for copper (Cu), zinc (Zn) and lead (Pb)

The fitness of the two analytical results was assessed by comparing the coefficients of determination obtained with recommended values (USEPA, 2007). Coefficients of determination for unprepared, dried and sieved, and pelletized samples were 0.8250, 0.8447 and 0.7253; 0.9743, 0.9916 and 0.9827; and 0.9899, 0.9537 and 0.9810 for copper, zinc and lead, respectively. Good linearity was achieved for lead and zinc, irrespective of the type of preparation method used, since the associated coefficients of determination were all above 0.85. The USEPA (2007) accepts values of above 0.85 as indicative of good correlation. Pelletizing the samples did not substantially improve linearity for lead, and only a slight improvement in linearity was observed for zinc.

Linearity for copper was poor, compared to zinc and lead. The coefficients of determination were below 0.9 for the unprepared and dried and sieved samples, and below 0.8 for the pelletized samples. No improvement in linearity was obtained after sample preparation. Instead, linearity was the best for the unprepared samples, and became poorer as more processing was introduced and the samples were pelletized. Bonnard and Bour (2008) obtained a similar coefficient of determination (r^2 = 0.97) for lead when comparing pXRF and ICP-OES data obtained for soil samples that were sun-dried and then sieved through a 250 µm mesh (Bonnard & Bour, 2008). Schneider *et al.* (2015) reported a coefficient of determination > 0.78 when comparing copper, zinc and lead concentrations measured using an XRF spectrometer in air dried soil samples, ground to below 100 µm, with ICP-OES results of the same samples, following *aqua regia* digestion. McComb *et al.* (2014) applied a pXRF spectrometer to do on-site screening of zinc and copper in soils. The resulting concentrations were compared to those obtained using an ICP-OES after digestion of the corresponding samples. A relatively good correlation was found between the two techniques for zinc (r^2 = 0.76), whereas poor correlation was found for copper. These poor results were ascribed to the low concentrations of the element in the soils. Similar outcomes were also achieved by a research group in China (Wu *et al*., 2012), who concluded that the pXRF spectrometer is more reliable for the determination of lead and zinc than for copper (Wu *et al*., 2012). The poor performance of copper with regards to linearity has been attributed to the relatively low concentrations of the metal in the samples analysed (IAEA, 2005; McComb, *et al*., 2014). Researchers are in agreement that pXRF displays higher limits of detection than an ICP-OES (McComb *et al*., 2014). The determination of analyte concentrations that are near the LOD is problematic, regardless of the analytical technique applied (IAEA, 2005). This study revealed that results from the analysis of unprepared and contaminated soils using the pXRF is of good quality for the determination of lead (r^2 = 0.9889) and zinc

(r^2 = 0.9743), but less so for copper (r^2 = 0.8250). This conclusion is supported by the findings of similar research conducted in China. The coefficients of determination for lead, zinc and copper were reported as 0.925, 0.809 and 0.678, respectively, when comparing the field screening with ICP-MS results (Hu *et al.*, 2014).

Three data quality levels (Q1, Q2 or Q3) can be assigned to the pXRF results using inferential statistics, the RSD and the coefficient of determination (r^2) when benchmarking pXRF against laboratory-based techniques (Kilbride *et al*., 2006; USEPA, 2007; Margui *et al*., 2010; Parsons *et al*., 2013). The pXRF data is adequate only for qualitative screening (Q1), when $r^2 < 0.7$, RSD > 20% and the obtained p-value < 0.05. However, when $0.7 \leq r^2 \leq 1$; RSD < 20% and the p-value < 0.05, the pXRF data can be regarded as suitable for quantitative screening (Level Q2). The definitive data quality level (Q3) is reached when $0.85 \leq r^2 \leq 1$; RSD ≤ 10% and p-value > 0.05, namely when the slope = 1 and intercept =0. Quality level Q3 describes outstanding results from the pXRF instrument after pairwise comparison to a reference laboratory technique. Table 3.6 lists the r^2, RSD and inferential statistic results obtained for copper, zinc and lead for unprepared, dried and sieved pelletized samples.

Table 3.6: Evaluation of the pXRF data quality when compared to ICP-OES results for the determination of copper, zinc and lead in unprepared, dried and sieved, and pelletized soil samples

Copper						
	r^2	RSD	p-value	Slope	Intercept	Data quality
Unprepared	0.8250	5.9	0.22	2.0604	10.367	Q2
Dried and sieved	0.8447	1.5	0.018	2.9870	13.324	Q2
Pelletized	0.7253	3.2	0.131	2.2777	12.786	Q2
Zinc						
	r^2	RSD	p-value	Slope	Intercept	Data quality
Unprepared	0.9743	7.3	0.002	1.2525	50.179	Q2
Dried and sieved	0.9916	10	0.013	1.6017	43.677	Q2
Pelletized	0.829	8.8	0.018	1.6132	52.618	Q2
Lead						
	r^2	RSD	p-value	Slope	Intercept	Data quality
Unprepared	0.9899	12	0.000	1.0805	218.37	Q2
Dried and sieved	0.9537	2.2	0.002	1.1338	222.32	Q2
Pelletized	0.9810	6.0	0.010	1.234	99.393	Q2

Data quality level Q2 was achieved for copper, zinc and lead in the three methods of sample preparation in this study since the r^2-values were larger than 0.7, the RSDs lower than 20% and the data from the two spectrometers (pXRF and ICP-OES) were found to be statistically different at a 95% confidence level. Copper concentrations in unprepared samples were statistically similar, but the slope did not have a value of one (2.0604) and the intercept (13.367) was larger than zero. Consequently the data could not be regarded as Level Q3 quality.

Data obtained in our study using the pXRF is of high quality since it surpassed the qualitative screening level for all the metals determined for all three sample preparation modes. An equivalent data quality level (Q2) was achieved for zinc, lead and copper in unprepared, dried and sieved, and pelletized samples.

This finding suggests that a minimum degree of sample preparation is required to achieve acceptable and even high quality data for the determination of high concentrations of copper, zinc and lead in contaminated soils. Kilbride *et al.* (2006) similarly achieved quantitative level (Q2) data for zinc, and even reached the definitive data level (Q3) for copper and lead, when screening contaminated soils using the Niton XLt 700 series spectrometer. Sample processing may, in most cases, be unnecessary to achieve screening and quantitative quality data (Palmer *et al*., 2009).

3.3.5 Comparison of the pXRF and ICP-OES results for the determination of zinc and lead in sediment samples

Concentrations of zinc and lead determined in wet, dried and sieved, and pelletized sediment samples using the pXRF were compared to those obtained using an ICP-OES for the corresponding samples. Linear regression analysis was performed and the r^2-value was used to assess the fitness of the pXRF compared to the laboratory-based technique. Coefficients of determination for wet, dried and sieved and pelletized sediment samples of 0.6720, 0.9631 and 0.9408, and 0.8410, 0.9950 and 0.9958 were achieved for zinc and lead, respectively (Figure 3.7). Satisfactory linearity was found for dried and sieved, and pelletized sediment samples for both elements as reflected by values above 0.9 for the r^2. In contrast, relatively poor linearity was observed for the wet sediment samples for both zinc (0.6720) and lead (0.8410). The poor linearity observed was attributed to the high water content of the wet samples. The pXRF results are severely affected when the samples contain more than 20% moisture (Ge *et al*., 2005; Markowicz, 2008; Hall *et al.,* 2013; Parsons *et al.*, 2013). Analysis of the dried and sieved, and pelletized sediment samples (dried and homogenised) resulted in a minor matrix effect.

The *in situ* determination of zinc by the pXRF, for which the coefficient of determination was below 0.7, renders the technique unsuitable for field quantification of the metal. However, the technique can still be applied for qualitative screening. On the other hand, the quantitative determination of lead can be done *in situ*, since an $r^2 > 0.7$ was obtained for lead.

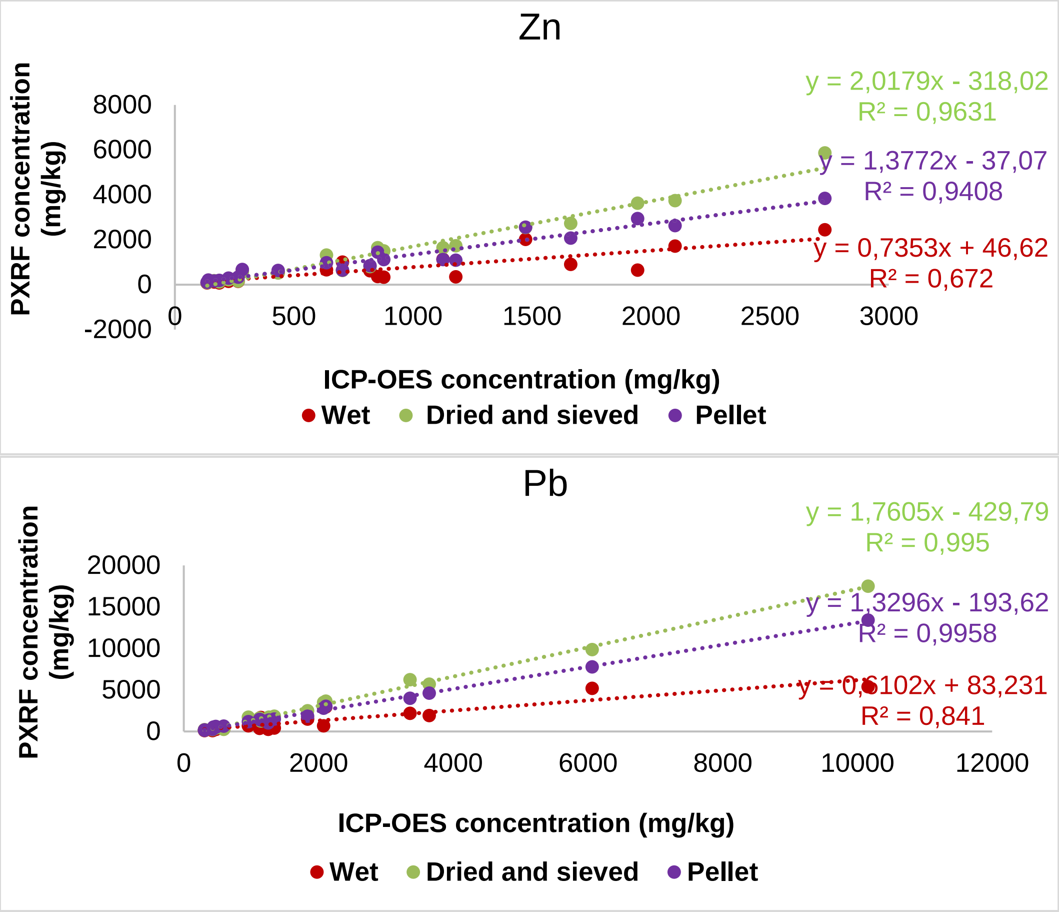


Figure 3.7: Correlation of metal concentrations in wet, dried and sieved, and pelletized sediment samples measured by pXRF and ICP-OES for zinc (Zn) and lead (Pb)

The pXRF data quality was also assessed using the r^2, RSD and the inferential statistic associated with the comparison of pXRF data with the corresponding ICP-OES results (Table 3.7). The RSDs obtained from replicate analysis (n=7), using the pXRF spectrometer, of wet, dried and sieved, and pelletized sediment samples for zinc and lead were below 10% in all cases, with the exception of the wet sediment determination of zinc (10.2%). Coefficients of determination for zinc and lead in the wet, dried and sieved, and pelletized samples were above 0.9, except in the cases of zinc (0.6720) and lead (0.8410) in wet sediment samples. Inferential analysis of the ICP-OES and pXRF data for the determination of zinc and lead indicated significant differences, since the calculated *p*-values for wet, dried and sieved, and pelletized samples were all less than 0.05 (Table 3.7).

Table 3.7: Evaluation of the data quality obtained using pXRF compared to using ICP-OES for the determination of zinc and lead in wet, dried and sieved, and pelletized sediment samples

Zinc						
	r^2	RSD	p-value	Slope	Intercept	Data quality
Wet	0.6720	10.2	0.031	0.7353	46.62	Q1
Dried and sieved	0.9631	3.1	0.002	2.0179	318.02	Q2
Pelletized	0.9408	3.5	0.001	1.3772	37.07	Q2

Lead						
	r^2	RSD	p-value	Slope	Intercept	Data quality
Wet	0.8410	9.7	0.006	0.6102	83.231	Q2
Dried and sieved	0.9950	0.2	0.010	1.7605	429.79	Q2
Pelletized	0.9958	2.6	0.012	1.3296	193.62	Q2

The data obtained for the measurement of zinc and lead in sediment reached quantitative data quality (Q2) for processed samples, but the quality of the wet samples can be classified as at qualitative screening level (Q1), only due to the r^2-value of 0.672. For lead, quantitative quality data Q2 was achieved with wet, dried and sieved, and pelletized sediment samples. These results indicate that qualitative screening of wet sediment can be done in the field for the determination of zinc, whereas quantitative analysis of the sediments sample can be achieved only after some processing of the samples. For lead, the field analysis of the wet sediment sample leads to quantitative screening data, as did the processed samples.

3.3.6 Independent analysis of soil pelletized samples for the determination of copper, zinc and lead using a WDXRF

Soil and sediment samples previously analysed by the pXRF in the field and in our laboratory were sent to the laboratory of the Department of Geology, University of Pretoria, for independent analyses using a WDXRF. Results from the two techniques for copper, zinc and lead were compared applying linear regression analysis (Figure 3.8).

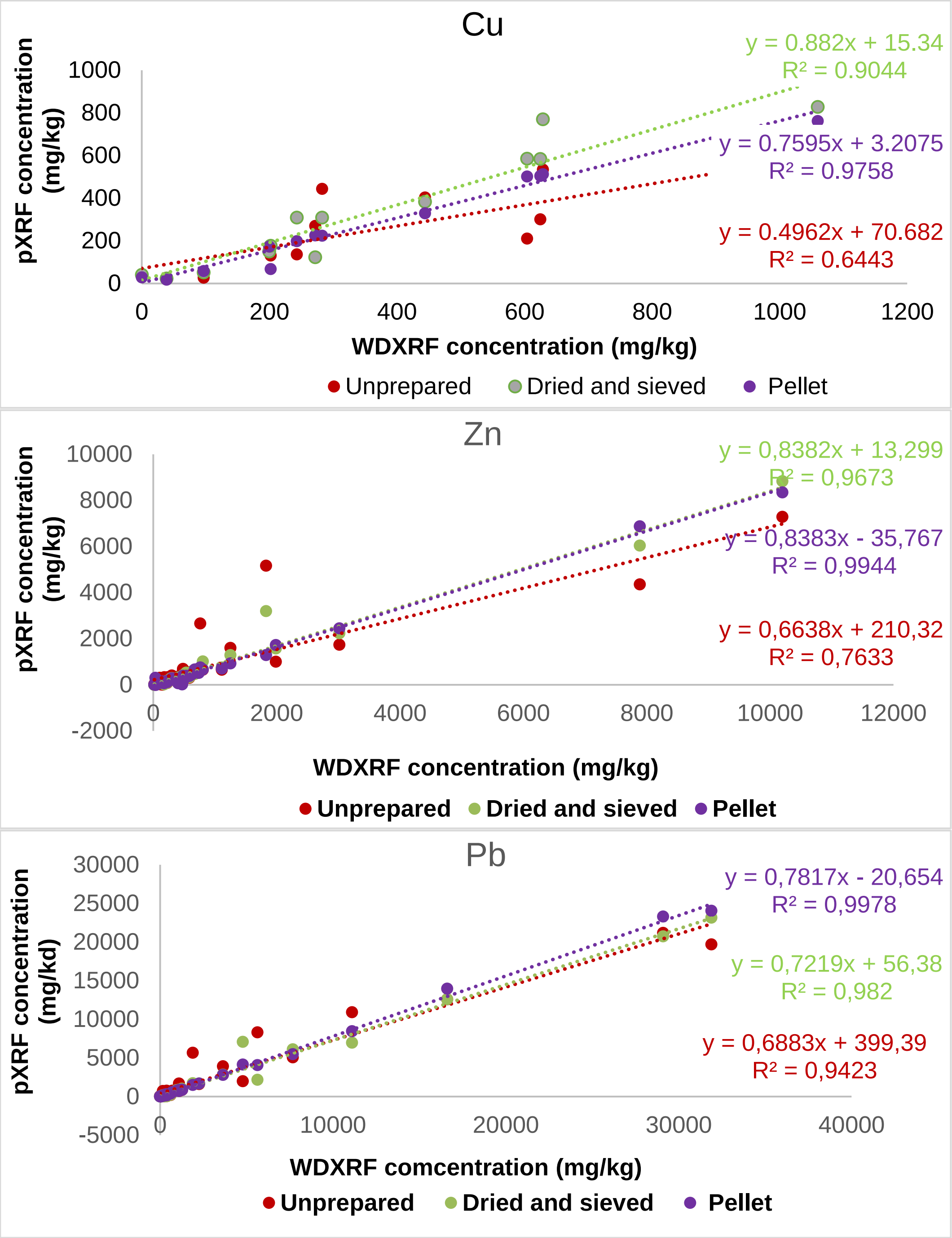


Figure 3.8: Correlation of metal concentrations measured in unprepared, dried and sieved, and pelletized soil samples by pXRF and WDXRF: copper (Cu), zinc (Zn) and lead (Pb)

For copper, the correlation plot displayed good linearity, since acceptable r^2 were achieved for dried and sieved ($r^2 = 0.9044$), and pelletized ($r^2 = 0.9758$) samples. The copper concentrations in unprepared soil samples did not correlate well with the WDXRF results ($r^2 = 0.6443$). For zinc concentrations in dried and sieved ($r^2 = 0.9673$), and pelletized samples ($r^2 = 0.9944$), the coefficients were all above 0.9, suggesting good correlation with the WDXRF results. Similarly, satisfactory linearity was achieved for lead, regardless of whether the sample was processed or not.

The r^2-values obtained for copper, zinc and lead associated with the three sample preparation modes are given in Table 3.8. Results for copper obtained using the two methods correlated the least, while those of lead correlated the most. This suggests that the pXRF is more suitable for the determination of lead than zinc or copper. An improvement in linearity was also observed in the results as the samples were increasingly processed (unprepared < dried and sieved < pelletized). The results obtained for copper and lead from the portable spectrometer were better correlated with the WDXRF results than with the ICP-OES results, since the r^2 obtained for this technique were generally higher than those obtained from the ICP-OES. This may be explained by the similarity of the two non-destructive XRF techniques compared to the destructive ICP technique, which requires sample dissolution.

Table 3.8: Coefficients of determination (r^2) for copper, zinc and lead concentrations measured by the pXRF spectrometer compared to the WDXRF results

	Cu	Zn	Pb
Unprepared	0.6443	0.7633	0.9423
Dried and sieved	0.9044	0.9673	0.9820
Pelletized	0.9758	0.9944	0.9978

3.3.7 Independent analysis of the sediment pelletized samples for the determination of zinc and lead using a WDXRF

Independent analysis using the WDXRF was carried out for sediment samples prepared according to three different preparation methods for the determination of zinc and lead. Results from the pXRF previously obtained were benchmarked against those from the WDXRF (Figure 3.9).

Coefficients of determination of 0.6404, 0.9009 and 0.9706 were achieved for Zn for wet, dried and sieved, and pelletized samples, respectively. For lead, r^2-values of 0.7182, 0.9458 and 0.9538 were obtained for each of the respective sample preparation methods. Excellent correlation was observed for zinc and lead when results from the pXRF were matched with the WDXRF results for dried and sieved, and pelletized sediment samples. Poor linearity was attained for the wet sediment samples, particularly for zinc ($r^2 = 0.6404$). Lead, with $r^2 = 0.7182$, met the requirements for quantitative screening. These results confirm the data obtained when comparing the results of pXRF and ICP-OES.

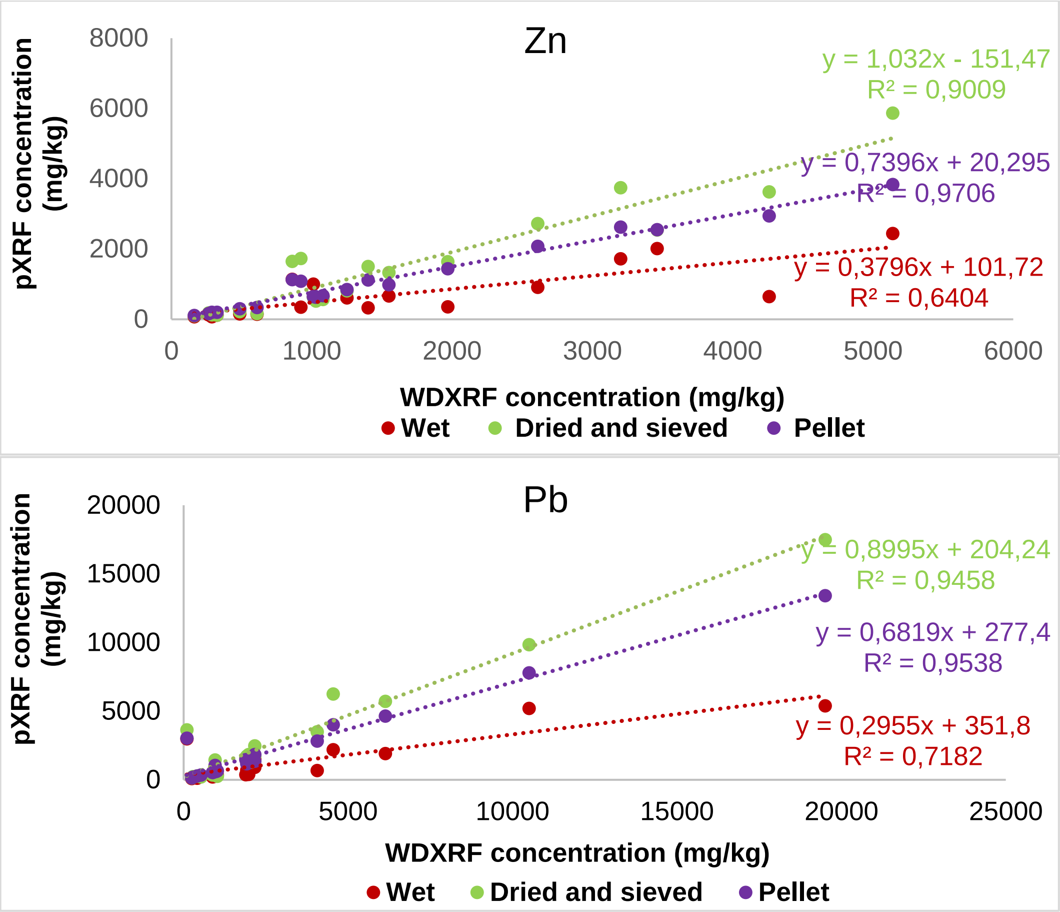


Figure 3.9: Correlation of metal concentrations determined in wet, dried and sieved and pelletized sediment samples by pXRF with the concentrations in the corresponding samples measured by WDXRF: zinc (Zn) and lead (Pb)

3.3.8 Calibration and method validation for the pXRF and ICP-OES for the determination of copper, zinc and lead in soils and sediments

3.3.8.1 The portable X-ray fluorescence spectrometer

The adjustment of the factory calibration of the copper/zinc mining mode (fundamental parameter) was necessary, since a bias was observed from the analysis of the CRMs. Calibration curves for zinc and lead were obtained by plotting the measurement values of the standards against their reference values on the Excel scatter chart (Figure 3.10). The curves were forced through zero (origin) to achieve satisfactory results. The slopes for copper, zinc and lead calibration curves were 1.1220, 1.0059 and 1.1005, respectively. These values were integrated into the instrument to correct for the bias. This is a type of standardsisation approach and generally excellent in correcting for particle size and mineralogical effects not compensated for with fundamental parameters or Compton ratio techniques. After integration, r^2-values of 0.9997, 1.0000 and 0.9954 for copper, zinc and lead, respectively, were reached, suggesting a good agreement between the measurements and the certified values.

The precision of the pXRF method for the determination of copper, zinc and lead in soil was assessed by seven replicates analyses of CRMs containing relatively low, medium and high concentrations of the metals. The RSDs for copper obtained with NIST2780 (215.5 mg/kg), AMIS 0157 (704.620 mg/kg) and AMIS0153 (2006 mg/kg) were 4.0%, 1.5% and 1.7%, respectively (Table 3.9). For zinc, these values were 4.9%, 0.40% and 0.49%, and were calculated after analysing NIST2709a, NIST2780 and AMIS0153, respectively (Table 3.10). Relative standard deviations of 1.0%, 0.67% and 0.86% were obtained for lead with RCRA, NIST 2780 and AMIS0153, respectively (Table 3.11). The RSDs were low in soils with high metal concentrations and relatively high in soils containing low concentrations. However, the RSDs for the three categories of soil CRMs were below 5.0%. The analytical methods for lead and zinc determination in soils were therefore deemed precise.

Means of the replicate measurements for copper, zinc and lead were compared to the reference values of the CRMs. The percentage recovery was calculated to evaluate the accuracy of the method. The percentage recovery for copper fluctuated between 90% and 99%. With zinc this value ranged from 94 to 110%, while for lead, the variation was between 100 and 110%. The high recovery for copper, zinc and lead measurements in soils demonstrated the accuracy of the pXRF method. The LOD was estimated as three times the standard deviation of replicate measurements (n=7) of analyte of low concentration in a reference material (Bernick *et al.*, 1995). The LODs were 23.7, 16.8 and 17.4 mg/kg for copper, zinc and lead, respectively.

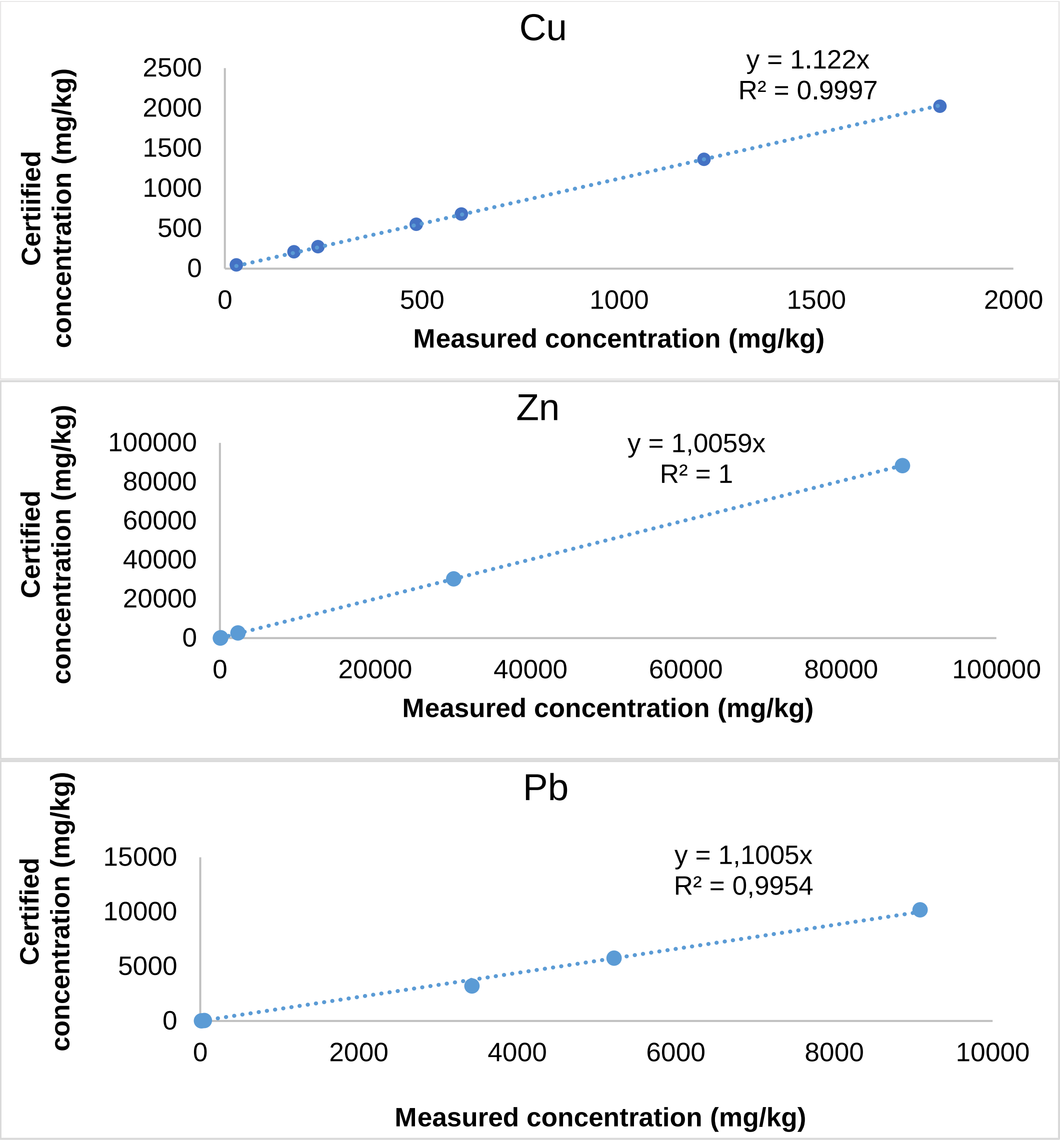


Figure 3.10: Calibration curves derived from pXRF data for copper (Cu), zinc (Zn) and lead (Pb)

Table 3.9: Precision and accuracy of the pXRF method for copper determination in soil

Standards	NIST2780	AMIS 0157	AMIS0153
Certified value	215.5	704.625	2006
Mean (mg/kg)	195.0	698.0	1993
SD (mg/kg)	7.9	11	33
RSD (%)	4.0	1.5	1.7
Recovery (%)	90	99	99

Table 3.10: Precision and accuracy of the pXRF method for zinc determination in soil

Standards	NIST 2709A	NIST 2780	AMIS0153

Certified value	103	2570	88400
Mean (mg/kg)	116	2420	86914
SD (mg/kg)	6	10	426
RSD (%)	4.9	0.40	0.49
Recovery (%)	110	94	98

Table 3.11: Precision and accuracy of the pXRF method for lead determination in soil

Standards	RCRA	NIST 2780	AMIS 0153
Certified value	500	5770	10191
Mean (mg/kg)	551	5750	10486
SD (mg/kg)	6	39	90
RSD (%)	1.0	0.67	0.86
Recovery (%)	110	99.7	103

3.3.8.2 The inductively coupled plasma-optical emission spectrometer

Calibration curves were constructed by plotting the concentrations of the metals against the intensity of the spectrometer's response. Coefficients of determination of 0.9997; 0.9997 and 0.9992 were determined for copper, zinc and lead, respectively, indicating close agreement between the concentrations and the instrument response (Figure 3.11). The LOD for each analyte was determined from the regression equation and were found to be 0.00358, 0.00275 and 0.0364 mg/kg for copper, zinc and lead, respectively.

The precision and accuracy of the ICP-OES method for the measurement of copper, zinc and lead was determined by replicate analysis (n=7) of the reference material AMIS0153 (Table 3.12). Relative standard deviations of 3, 5 and 6% were obtained for copper zinc and lead, respectively. The calculated percentage recoveries for the metals were found to be equal to 81, 94 and 105%, for copper, zinc and lead, respectively.

Table 3.12: Precision and accuracy of the ICP-OES method for the determination of copper, zinc and lead by the analysis of the reference material AMIS0153

Element	Certified concentration (mg/kg)	Mean concentration (mg/kg)	RSD (%)	Recovery (%)
Copper	1993	1633	3	81
Zinc	88400	82851	5	94
Lead	10191	10714	6	105

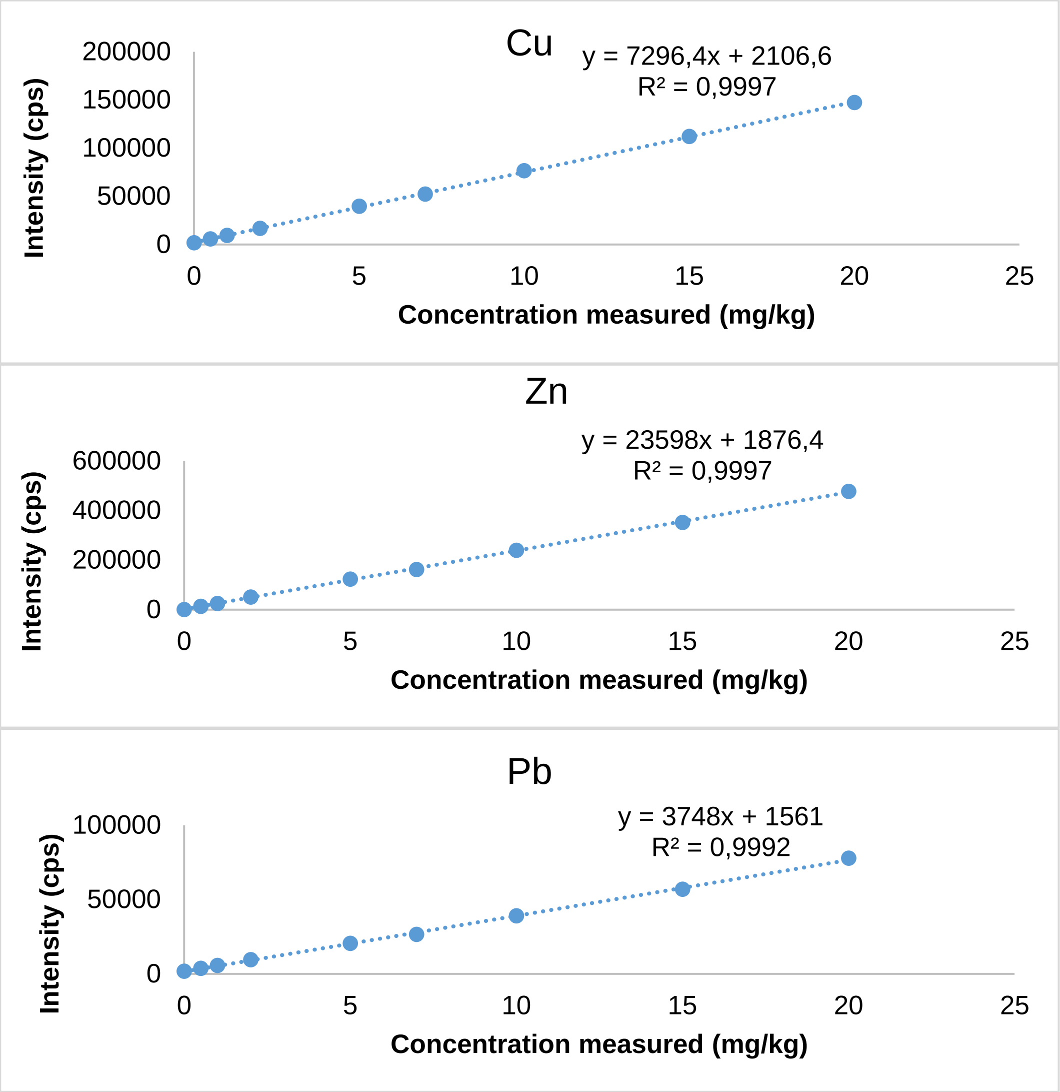


Figure 3.11: Calibration curves derived from ICP-OES data for copper (Cu), zinc (Zn) and lead (Pb)

3.4 CONCLUSIONS

This work was done to confirm the possibility of using (*in situ*) the Niton XL 3t GOLDD^{TM}+ pXRF spectrometer to screen for metal contamination in soils and sediments from the old Edendale Mine (Mamelodi, Pretoria) and soil samples from mango and citrus orchards. Currently, the use of pXRF in South Africa is limited to the valuation of scrap-metal. This study will provide impectus to applying the technique to geological samples and surveys.

- The study confirms the superiority of the Compton normalisation calibration over the fundamental parameter calibration for the determination of relatively low concentrations of metal contaminants in soil matrices dominated by high concentrations of low Z elements.
- No significant differences could be found between concentrations of copper, zinc and lead determined in unprepared, dried and sieved, and pelletized soil samples, using the spectrometer calibrated with the fundamental parameter. However, significant differences were found for sediment samples. Similar trends in metal concentrations were found for the three sample preparation modes investigated for soil and sediment samples. However, for most of the soil samples (50 to 82%) the deviations between the preparation methods were below 50%, regardless of the method applied. For sediments, 30 to 90% the samples deviated by less than 50%. Although very high deviation was observed, only a few samples were involved. The deviations were larger with U/P samples than with U/DS, and DS/P.
- When benchmarked against the ICP-OES, the pXRF results for unprepared samples correlated well and attained the coefficients of determination of 0.8250, 0.9743 and 0.9899 for copper, zinc and lead, respectively. The bias observed with zinc (r^2 = 0.6720) and lead (r^2 = 0.8410) for wet sediment samples, could be corrected by drying and sieving the samples. Using the r^2-values, RSDs and inferential statistics, quantitative data quality levels (Q2) were achieved for the metals in the analysis of contaminated soil samples. Quantitative data quality levels (Q2) were achieved for all the metals for the three sample preparation methods, with the exception of copper with the pelletized soil samples and for zinc in wet sediment samples. Concentrations obtained with the pXRF spectrometer were further confirmed by an analysis of the samples using an ARL PerformX Sequential XRF spectrometer.

CHAPTER 4
ASSESSMENT OF METAL CONTAMINATION AND ECOLOGICAL RISK OF SOILS AND SEDIMENTS FROM THE OLD EDENDALE MINE SITE

4.1 BACKGROUND AND MOTIVATION

Commercial mining in South African only really commenced after the first diamonds were found in the Orange River in 1867 and at Kimberley in 1871[5]. Intensive mining began with the discovery of the gold-bearing reef in Johannesburg in 1886. By 1913, South Africa had become the world's largest producer of gold with 280 tons produced in that year[6]. Many other minerals have been mined in South Africa, including coal, chromium, manganese, platinum, vanadium and palladium. These mineral resources have contributed to the growth of the economy, assisted in the development of infrastructure and provided employment.

The consequences of mining to the environment and human health are well documented (Nriagu, 1988; Mathee, 2014; Tóth *et al*., 2016). The mobilisation of toxic elements during the mining process, the improper disposal of mine waste, the use of controversial mining practices, the absence of proper regulation of the mining sector, and a disregard for the surroundings, have led to environmental issues. These include acid mine drainage, collapsing entry points and derelict mines that have been abandoned by their owners. Mining activities generate waste, mostly in the form of spent ore, which often contains high concentrations of toxic elements. Bioavailable toxic elements can contaminate crops growing in the area (Kalač, 2010) or eventually migrate and detrimentally affect groundwater (Rösner & van Schalkwyk, 2000). Moreover, sulfides, accumulated in mine waste, generate acid mine water (Rösner & van Schalkwyk, 2000). South Africa promulgated an act in 1991 (Act 50 of 1991) to force mining companies to comply with rules concerning mining practices, as well as their closure. The regulation forces mining companies to implement a rehabilitation programme for the mine site to ensure that the environment is in the same state as prior to mining.

According to reports, approximately 6000 abandoned mines are scattered around the country (SAI, 2009; van Schie, 2012). Most of these mines were closed prior to the act of 1991, escaping compulsory rehabilitation of the environment, which was later enforced by

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[5] www.thesolomon.co.za/heyday-of-diamond-mining.html. [Accessed]: 07/06/2016.

[6] www.miningartifacts.org/South-African-Mines.html. [Accessed]: 07/06/2016.

the act. The identification and screening of these mines is necessary before a remediation programme can be implemented. The Edendale lead mine belongs to this category of abandoned mines where the closure was not monitored. The mine was active for almost half a century (from 1890 to 1938) and produced lead, zinc and silver. Mobilisation and accumulation of the toxic metals, lead and zinc, through mining activities may have resulted in the pollution of soil, vegetation and water at the site (Wedepohl, 1995; Simón *et al*., 1999; Schramel *et al*., 2000; Zhuang *et al*., 2009). The consequences of human exposure to toxic elements, particularly lead and zinc, have been enumerated elsewhere (Nriagu, 1988; Wayne & Ming-Ho, 2005; Swaminathan, 2013).

Lead has no known physiological activity in living organisms. Human exposure to the element is associated with failure of the nervous, hematopoietic, skeletal, renal and endocrinal systems (West *et al*., 2007; Thompson, 2007). A report of the United States Agency for Toxic Substances and Disease Registry (ATSDR, 2007) indicates that lead poisoning is a cause of renal failure and hypertension.

Zinc, on the other hand, is an essential element, which contributes to the expression of deoxyribonucleic acid (DNA) by acting as a transcription cofactor (Swaminathan, 2013). The element also acts as an enzyme cofactor in cellular activities. The daily intake of zinc is estimated at between 8 and 11 mg. An excessive intake of zinc causes anaemia, hypercholesterolemia and diarrhoea (Swaminathan, 2013).

Gauteng is the smallest, but most inhabited, province of South Africa (GDHS, 2014). The population density was recorded as 675 people per square kilometre in 2014, and is increasing. This necessitates the constant development of new housing complexes (GDHS, 2014). A scarcity of space, combined with the rapid growth rate of the population, has led municipalities to extend townships without necessarily inspecting the area for metal pollution. Such investigations are necessary, since many old abandoned mines are situated near or in towns in Gauteng (Reeks, 2012).

Mining activities in Pretoria began around 1880, following the discovery of lead and silver deposits on surrounding farms (Reeks, 2012). Intensive prospecting, conducted in the regions, led to the establishment of more than thirty silver mines, of which the Albert, Willows and Edendale mines, and four Argent mines located in the northeast of Pretoria were the most successful (Reeks, 2012). These old mines were all closed well before the implementation of the regulatory act of 1991. The Edendale Mine, situated in the vicinity of Mamelodi, a township east of Pretoria, is one of these old mines. The geological formations of the area belong to the Silverton group of the Transvaal Supergroup from the Precambrian Era (Glass, 2006; Schröder *et al*., 2006). Geological formations in the region consist mainly of sedimentary rocks (carbonaceous shale and siltstone) that are overlain by Magaliesberg quartzite, currently covered by Cenozoic deposits (Glass, 2006). The Edendale Mine exploited lead, mineralised in a vein striking East to West with normal faults (Coetzee, 2001). The vein is about 2 m wide and 1170 m long, dipping 60° to 85° in a southerly direction (Kgwakgwe, 2001), where the galena (PbS) pockets were arbitrarily exploited and disseminated. The lead ore of the Edendale Mine is essentially composed of galena of a hydrothermal mineralisation, which co-occurs with other less abundant minerals, including sphalerite (ZnS) and chalcopyrite ($CuFeS_2$).

This investigation was inspired by the findings of a study of the area by Glass (2006), who concluded that the region was polluted with lead. However, the results from the study emanated from the analysis of a limited number of soil samples. The spatial distribution and mapping of the contaminants were not reported. In addition, no fractionation of the contaminated soils, an essential step for evaluating the mobilities of the contaminants, was not done. The screening of sediments from the Edendale Spruit, flowing through the old mining site, was also omitted.

The effectiveness of applying the pXRF spectrometry technique to the screening of soils and sediments that are highly contaminated with zinc and lead from the Edendale Mine site has been described previously (Chapter 3). Statistical analysis confirmed the similarity between data obtained in the field, and that found after the soil samples had been processed using the spectrometer in mining mode. Concentrations of lead and zinc determined using the pXRF spectrometer correlated well with values obtained using the ICP-OES and WDXRF spectrometer. Quantitative level data were achieved for the determination of the metals in soils. Concentrations of zinc and lead in sediment taken in the field were significantly lower (at the 95% confidence level) than those obtained from the analysis of the samples after minimal processing. Qualitative screening level data were achieved in the field, whereas quantitative data were obtained after minimal processing of the sediment samples. Based on these findings, the screening of soils and sediments at the abandoned Edendale Mine was implemented using pXRF spectrometry. The pXRF spectrometer (Niton XL 3t GOLLD+) was set in the mining mode, which had been calibrated using CRMs. The Edendale Mine area was screened to determine zinc and lead concentrations and to establish the spatial and vertical distribution of the contamination. Representative soil and sediment samples were fractionated to determine the mobility and the origin of the contaminants. The ecologic risk posed to the environment was evaluated from indices. Contamination of vegetation in the area was also investigated.

4.2 MATERIALS AND METHODS

4.2.1 Description of the old Edendale Mine site

The old Edendale Mine site is located in Pretoria East in the vicinity of Mamelodi (Figure 4.1). The mine consisted of two main working shafts, approximately 800 m apart, and separated by the R513. Shaft 1 is located to the north of the road, whereas Shaft 2 is to the south of the road. The area lies between latitude S25°41’00” and S25°41’15” and longitude E28°25’50” and E28°26’20”.

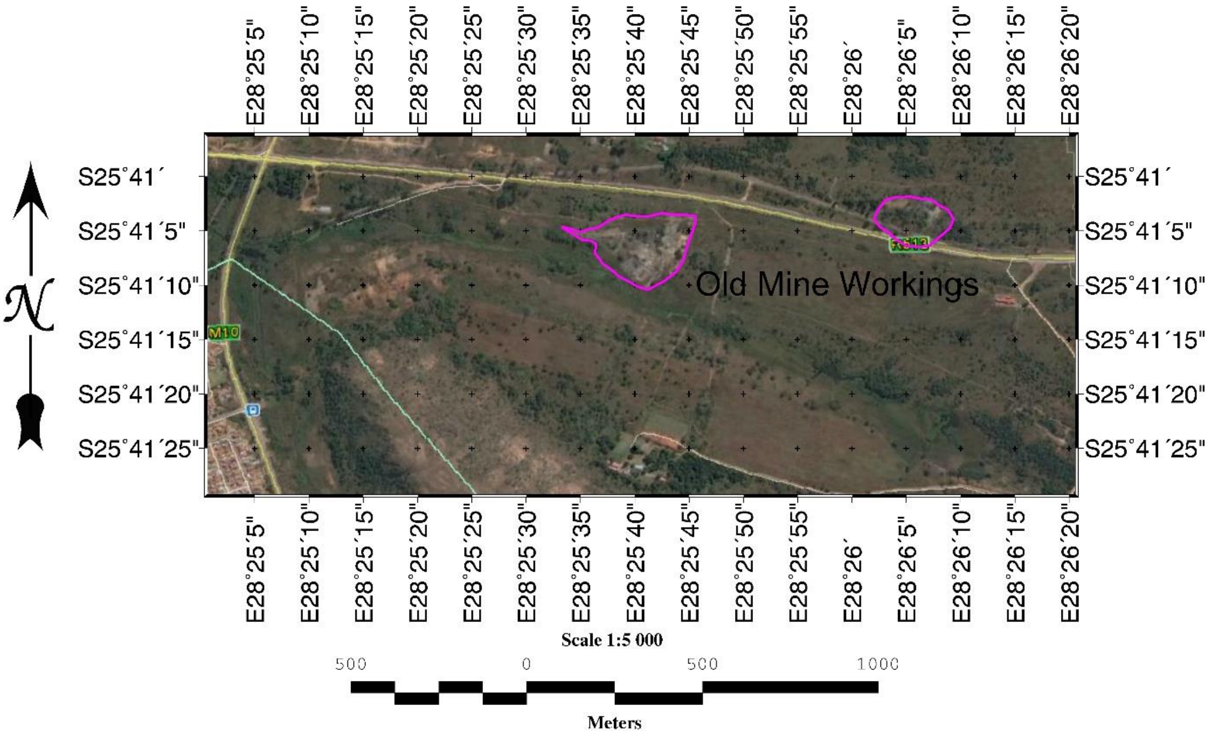


Figure 4.1: Google map of the Edendale Mine site indicating the two shafts

Evidence of the old mine can be seen in the form of abandoned buildings, heaps of rocks and water storage tanks (Figures 4.2 and 4.3). The area has an average rainfall of approximately 750 mm (Kgwakgwe, 2001). The vegetation consists of dispersed trees, shrubs and smaller plants that include acacia, *Lippia, Lantana*, weeds and grasses.

Figure 4.2: Rock heaps and abandoned buildings at the old Edendale Mine site

Figure 4.3: Abandoned buildings visible at the site of the old Edendale Mine

Sparse growth occurs on the surface of the former workings of the mine. A tributary of the Edendale Spruit flows from the northeast to the southwest at a distance of about 100 m from the second shaft. Various types of soils occur on the site. Sandy and rocky soils were found in the vicinity of Shaft 1, while in the proximity of Shaft 2, soils were mostly hard, dark and compact. Next to the river, soils were damp and darker. Waste rocks and slag heaps were left disseminated on the soil.

4.2.2 *In situ* screening of soils and sediments for toxic elements using the pXRF spectrometer

Topsoils (n=136) were analysed to determine their zinc and lead concentrations at 50 m intervals, from where the background concentration (50 mg/kg) increased or was reached (Figue 4.4). At each point of analysis, the soil was cleared by removing vegetation, debris and stones. The soil was then loosened and the surface flattened before measurements were taken (Shefsky, 1997; Hu *et al*., 2014). The front probe window of the instrument was placed in contact with the soil and the trigger depressed for the duration of the measurement (Figure 4.5).

Figure 4.4: The Edendale Mine site surroundings and the sampling design

Figure 4.5: Measurement of metal concentrations in soil using the portable X-ray fluorescence spectrometer

Measurements were automatically stored in the instrument and later exported to Microsoft Excel (2013) for processing. The global positioning system (GPS) coordinates for each sample were simultaneously recorded by a receiver in the pXRF instrument. Topsoil samples (0-20 cm depth) were collected using an auger or a spade, depending on the nature of the soil. Soil samples were stored in plastic Ziploc® bags for further analysis in the laboratory.

Sediment samples (n = 22) were collected from the bed of the Edendale Spruit over a distance of 1000 m, at 50 m intervals. At each sampling point, the sediment was cleared of plants and other organic matter. Samples were taken from 0 to 20 cm depth from an area of approximately 1 m^2 around the GPS position recorded. A spade was used to remove the samples, where after the water was drained from the sediment sample before transferring it to plastic bags. A portion of each sample was transferred to a sample cup, the surface was flattened and measurements was taken using the pXRF spectrometer.

4.2.3 Mapping the spatial distribution of toxic elements in soil

The spatial distribution of zinc and lead in the study area was done using software developed in-house. The software, employing minimum curvature geostatistics (a form of next neighbour) is used in conjunction with MicroStation tools that have graphics capability. A data grid was prepared using the USGS software and the maps were produced using ER-Mapper© 2011 software (v 11.0). After construction, zinc and lead contamination maps were overlaid onto the Google map of the area. Sample concentrations and the corresponding GPS coordinates were uploaded. Metre and mg/kg were used for distance and concentration units, respectively. Gradient colour from blue to intense red was used to symbolise an increasing concentration of the element.

4.2.4 Assessment of soil and sediment contamination at the old Edendale Mine

Representative soil (n=36) and sediment (n=22) samples were selected to evaluate zinc and lead pollution of the area. The assessment was based on total concentrations of the potentially toxic elements obtained from field measurements. The pollution level of the soils and sediments was evaluated using the geo-accumulation index as proposed by Muller (1969). The index is calculated using the binary logarithm of the ratio of the concentration of the contaminant measured and the background concentration.

The mathematical formula is as follows:

$$I_{geo} = \log_2 C_n / 1.5B_n \qquad \text{Equation 4.1}$$

where C is the concentration of the element measured in the samples, B is the background concentration of the element, n the element under consideration and 1.5 is a background correction factor.

A concentration of 185 mg/kg for zinc and 56 g/kg for lead were used as background concentrations. These are the permissible concentrations for the two elements in agricultural soil in South Africa (Herselman *et al.*, 2005). The calculated I_{geo} values categorise seven levels of contamination ranging from unpolluted to extremely polluted as indicated in Table 4.1.

Table 4.1: Pollution classes obtained from the I_{geo} values (Muller, 1969)

Class	I_{geo} value	Pollution level
1	< 0	Unpolluted
2	0 – 1	unpolluted to moderately polluted
3	1 – 2	moderately polluted
4	2 – 3	moderately to strongly polluted
5	> 3	strongly polluted
6	3 – 4	strongly to extremely polluted
7	> 4	extremely polluted

4.2.5 Analysis of contaminated soils at different depths

Drill samples of soils from highly contaminated areas of the old Edendale Mine were collected and the metal contaminants were determined on-site using the pXRF spectrometer. The drill samples were obtained using a Stihl drilling machine (Germany; Figure 4.6). Two drill bits of 1 m can be joined and attached to the machine.

Measurements were taken at the soil surface, as well as at 0.5, 1 and 1.5 m depths, where possible. Drill samples were collected from three sites, the first was near Shaft 1 (S 25.68416; E 28.43537), while the second and the third were from the vicinity of Shaft 2 at the GPS coordinates (S 25.69694; E 28.42872) and (S 25.68572; E 28.42838), respectively.

Figure 4.6: Drilling machine with a 1 m bit for collection of soils at different depths

4.2.6 Distribution of contaminants according to soil particle sizes

The concentrations of the contaminants according to soil particle distribution size was determined. A portion (300 g) of air dried soil was oven dried at 50 °C for 24 h and placed on the first of a set of sieves with diminishing apertures, after stones, debris and soil particles larger than 5 mm had been removed. The sieves were sealed and vibrated at 1 Hz for 3 min, thus separating the samples into eight fractions containing particles with diameters of 5.0, 2.0, 1.0, 0.50, 0.25, 0.15, 0.075 and 0.040 mm. Metal concentrations were determined in each fraction using the pXRF spectrometer. Fractions F1, F2, F3. F4, F5, F6, F7 and F8 represented soil with diameters of between 5.0-2.0, 2.0-1.0, 1.0-0.50, 0.5.0-0.25, 0.25-0.15, 0.15-0.075, 0.075-0.040 and < 0.04 mm, respectively. Each soil fraction was weighed and the mass percentage of the element of interest determined from the bulk sample.

4.2.7 Determination of pH and electrical conductivity of the soils

The pH and the electrical conductivity (EC) of soils and sediments were determined using a combined pH and EC meter (Martini Instruments, MI 805, Romania). The instrument was calibrated for pH using three buffer solutions (pH 4.00, 7.00 and 10.0), whereas a solution of 1415 µS/cm was used to calibrate the device for EC measurements. Soil samples were suspended in water with a ratio of 1:5 (v/v) and shaken for 30 min

(Blakemore & Daly, 1987). The suspension was allowed to settle before triplicate measurements were done by introducing the electrode into the supernatant.

4.2.8 Speciation of metals in soils and sediments from the Edendale Mine

The mobility and the bioavailability of contaminants present in soils and sediments were evaluated using a sequential extraction method established by the Bureau Community of Reference (BCR) of the European Commission (EC) (Rauret, 1998; Zemberyova *et al*., 2006). The three-step procedure applied to 1.00 g of sample consisted of:

Step 1: Shaking overnight at room temperature (20 °C) in 40 mL 0.11 M acetic acid to leach the exchangeable and acid soluble fractions.

Step 2: The residue from Step 1 was shaken overnight at room temperature (20 °C) in 40 mL 0.1 M hydroxylammonium chloride (NH_2OH, HCl) to extract the reducible fraction.

Step 3: The residue from Step 2 was digested for 1 h with 10 mL 8.8 M hydrogen peroxide (H_2O_2) at 85 °C in a water bath and the process repeated before the addition of 50 mL 1.0 M ammonium acetate, adjusted to pH 2 with nitric acid. This step yields the oxidisable fraction.

Step 4: The residue from Step 3 was microwave digested and concentrations of zinc and lead determined in all the fractions using the ICP-OES. This process allowed the residual fraction to be measured.

From the screening, three highly contaminated centres (C1, C2 and C3) were identified at the following GPS coordinates at Edendale Mine: C1 (S 25.68 403°, E 28.43 544°) near Shaft 1, the second, C2 (S 25.68 470°, E 28.42 690°), is situated at Shaft 2 and the third highly contaminated centre C3 (S 25.68 420, E 28.42 190°) is located southeast of Shaft 2. Three soil samples, containing low, moderate and high lead concentrations, were selected from each contamination centre for BCR sequential extraction (Table 4.2). The soil samples were extracted in triplicate. Enrichment of soils and sediments with metal contaminants is generally established in comparison with a reference element, which is invariably present at high concentration in the studied area and consequently minimises the effect of variability towards the contaminant (Iqbal & Shah, 2011; Saleem *et al*., 2015). Iron, which was found consistently at high concentrations in soils and sediments at the abandoned Edendale Mine, was used as a reference element for comparison with zinc and lead (suspected contaminants). The results of the extraction are presented as percentage of the total concentration of the elements in each fraction.

Table 4.2: Soil samples of low, moderate and high contamination, selected from the three centres of contamination for BCR sequential extraction

	Low	Moderate	High
Centre 1 (C1)	S1-13	S1-14	S1-1
Centre 2 (C2)	S2-43	S2-19	S2-3
Centre 3 (C3)	S2-55	S2-31	S2-29

4.2.9 Ecological risk assessment of metals in soils and sediments

The ecological risk of iron, zinc and lead at the old Edendale Mine was assessed from the results of the fractionation of the metal in soils and sediments. The risk assessment code (Muriithi *et al*., 2016), the individual contamination factor (ICF) and global contamination factor (GCF) were applied to determine the level of risk posed to the environment by the element. This code assigns a scale to the percentage of metal in the exchangeable fraction obtained from the BCR sequential extraction (Saleem *et al*., 2015) (Table 4.3). The exchangeable fraction contains loosely bound metals of anthropogenic origin (Saleem *et al*., 2015).

The ICF for an element was obtained by dividing the sum of concentrations of the element in the fractions (F1, F2 and F3) by the total concentration in the residue (F4), while the GCF was obtained by summing the ICF values for the metals (Ikem *et al*., 2003). The ICF and GCF were interpreted as suggested by Zhao *et al.* (2012). Values of ICF < 1 and GCF < 6 indicate low risk; 1 < ICF < 3 and 6 < GCF < 12 indicate moderate risk, 3 < ICF < 6 and 12 < GCF < 24 indicate considerable risk and ICF > 6 and GCF > 24 indicate contamination (Zhao *et al*., 2012).

Table 4.3: Modified degree of ecological risk using the risk assessment code (RAC) (Muriithi *et al.*, 2016), individual contamination factor (ICF) and global contamination factor (GCF) (Zhao *et al*., 2012)

Degree	RAC	ICF	GCF
Low	< 10%	< 1	< 6
Moderate	10 - 30%	1 -3	6 – 12
Considerable	30 - 50%	3 – 6	12 – 24
High	> 50%	> 6	> 24

4.2.10 Contamination of vegetation at the Edendale Mine site

The contamination of plants growing on the mine site was investigated. Three sites of high contamination, as found by using the pXRF, were selected for sampling. A non-contaminated site was used as control. In each of the selected sites, the most representative plants species were harvested in an area with a 10 m diameter. The concentrations of the potentially toxic elements in the plant parts (roots and leaves) were determined using the ICP-OES. Fresh leaves and roots of each plant species were separated and washed in running water for 5 min and rinsed three times using double deionised water (Ataabadi *et al*., 2012). All plant parts were dried in an oven at 70 °C for 48 h, whereafter they were pulverised. A mass of 0.250 g of the plant material was microwave digested with 5 mL of nitric acid (65% m/m). The digests were transferred to 50 mL volumetric flasks and diluted with deionised water.

4.3 RESULTS AND DISCUSSION

4.3.1 Introduction

The old Edendale Mine and surroundings are currently subjected to various human activities. Houses that form part of the Mamelodi township are located less than 300 m from the southeast boundary of the site**.** A housing development programme is ongoing southwest of the mine site. Farms and a school that rely on borehole water are adjacent to the site. The people living in the proximity of the old mine could be at serious risk if the soils are polluted. Direct contamination may occur through inhalation or skin contact with the dust from the polluted soils. In addition, toxic elements may leach or drain into groundwater, which could pose a risk to human and animal health.

Evidence of the land being used for agriculture was found on the mine site. Small stands of maize and vegetables were being cultivated at the time of sampling. The land was also used as pasture for cattle. Plants are capable of taking up toxic elements from soil. These toxic elements can be accumulated in roots, twigs and/or leaves, as well as in seeds and fruit (Peralta-Videa *et al*., 2009; Álvarez-Ayuso *et al*., 2012; Gallego *et al*., 2012). Indirect contamination may occur from the consumption of contaminated cereals and vegetables. Grazing animals may be exposed to toxic metals in contaminated pastures and consumption of the meat from such animals may be a health risk.

A tributary of the Edendale Spruit flows from the northwest to the southeast through the area and drains into the Roodeplaat Dam, a major water reservoir servicing formal and informal settlements, industries and agriculture (DWAF, 2000). Contamination of fresh water and water resources may occur by the release of toxic elements from the underlying polluted sediments (Yu *et al*., 2011). Risk to humans may also occur by direct consumption of polluted water or by eating fish from the dam (Taweel *et al*., 2013).

4.3.2 Field analysis of soils and sediments using the pXRF

The old Edendale Mine area was screened to identify and quantify all elemental constituents present in the soils. However, zinc and lead that co-occur in the geological formations of the area and in the ore were expected to be present in high concentrations in the soils. The versatile pXRF spectrometer, equipped with a 50 keV X-ray tube that delivers a high X-ray flux, was used for this project, because it is capable of simultaneously detecting and quantifying most of the potentially toxic elements in soils. The large drift detector (LDD), with high count capability, made it possible to quantify elevated contaminant levels in the soils. Mining mode was selected from the default calibrations available from the analyser. This mode makes use of a fundamental calibration technique, which is based on the use of an algorithm to predict the detector's response to known parameters, such as measurement conditions and inter-element interferences. It offers a wide concentration range of analysis (USEPA, 2007; Radu & Diamond, 2009).

Although the concentrations of all the potentially toxic elements were determined in the soils using the pXRF, of the potentially toxic elements, only zinc and lead were present at very high concentrations. Moreover, only concentrations of these elements higher than the LODs established for the spectrometer (zinc = 16.8 mg/kg and lead = 17.4 mg/kg; Chapter 3, Section 3.3.8.1) have been reported. The average concentration of zinc in the soils (n=167) analysed was 480 mg/kg (Figure 4.7). The lowest and highest concentrations of zinc were present in soil samples S1-8 (18 mg/kg) and S2-52 (7300 mg/kg). The average concentration for lead was substantially higher (1100 mg/kg). The lowest lead concentration was 22 mg/kg (Soil S2-36), whereas Soil S2-52, which contained the most zinc, also contained the most lead (21 000 mg/kg).

The measured concentrations of zinc and lead in soils at the old Edendale Mine are up to six times higher for zinc and 375 times for lead than the highest permissible concentrations in South African agricultural soils (185 mg/kg for zinc and 56 mg/kg for lead) (Herselman *et al*., 2005). Of the 167 sampling points analysed, 46% of the zinc and 77% of the lead values were higher than the respective baseline values. A lead threshold value of 7600 mg/kg for waste destined for landfill was published in the South African Government Gazette (13 August 2013), as an amendment to the Waste Classification and Management Regulations: Waste Act (Act No. 59 of 2008) (Bomo, 2013). In the current study, 3% of the samples analysed contained two to three times more lead than this threshold value. However, none of the samples exceeded the threshold value of 640 000 mg/kg established for zinc.

Zinc and lead concentrations at the mine were substantially higher than the threshold target and intervention values for soil quality standards (140 and 720 mg/kg) and (85 and 530 mg/kg), respectively, set by countries from the European Economic Community (EEC) (Rösner & van Schalkwyk, 2000). The EEC has also established screening values for metals that pose an unacceptable risk to human health. Belgium, probably the most tolerant in this regard, recommends a maximum of 700 and 2500 mg/kg for lead in soils used for residential and industrial purposes, respectively (Carlon *et al.*, 2007). These levels are exceeded by those from the Edendale Mine.

Research in Congo-Brazzaville yielded similar findings. Concentrations of lead in soil of an abandoned lead ore treatment plant reached 11 800 mg/kg (Matini *et al*., 2011). In Poland, the concentration of lead reached 13 600 mg/kg in soil at the historical Leszczyna and Chelmiec Mine (Karczewska *et al*., 2006). An even worse scenario was described for a battery manufacturing plant in Nigeria, where the levels of lead ranged from 243 to 126 000 mg/kg in the surrounding soil (Adie & Osibanjo, 2009). The elevated concentrations of zinc and lead in soil at the Edendale Mine site were attributed to the disposal of mine waste or to the accumulation of the lead ore on and in the soil (Glass, 2006).

Concentrations of zinc and lead in sediment samples collected from the floor of the Edendale Spruit were measured on-site using the pXRF, after the surplus water was drained. The average concentrations in the sediments were 750 mg/kg for zinc and 1200 mg/kg for lead (Figure 4.8). Zinc minimum and maximum concentrations were 77 mg/kg (Sed3) and 2400 mg/kg (Sed8). The minimum concentration of lead was 107 mg/kg (Sed2), while the maximum concentration was 5400 mg/kg (Sed8). The concentrations of the two elements were low in the sediments located up-stream from the mine (Sed1, Sed2, Sed3, Sed4 and Sed5), but increased from Sed1 to Sed5. The highest concentration was present in Sed 6 and Sed 8 and decreased downstream from Sed10 to Sed22.

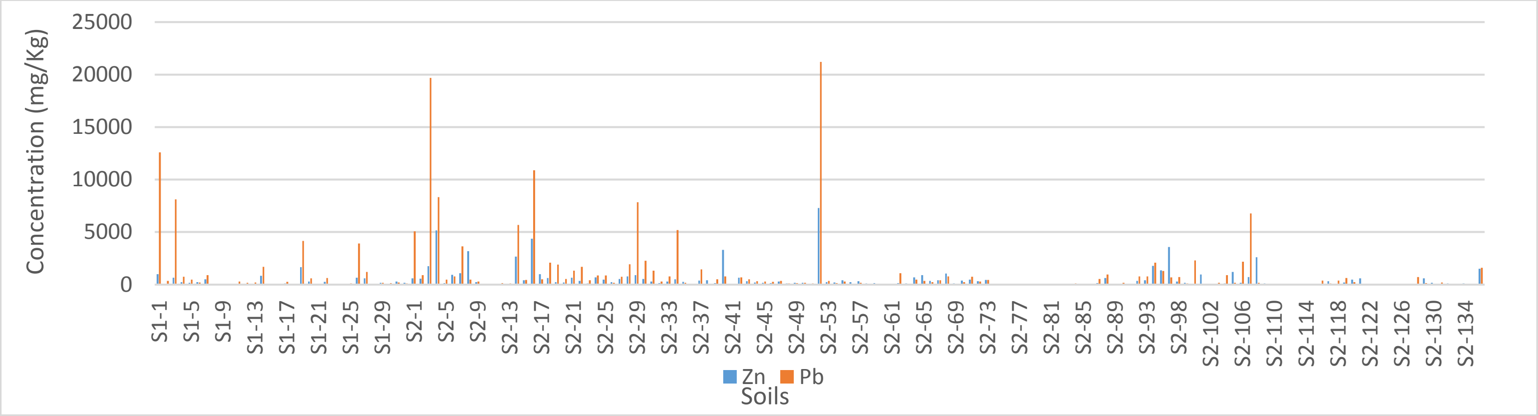


Figure 4.7: Zinc and lead concentrations (mg/kg) in soils from the Edendale Mine, measured according to the sampling grid

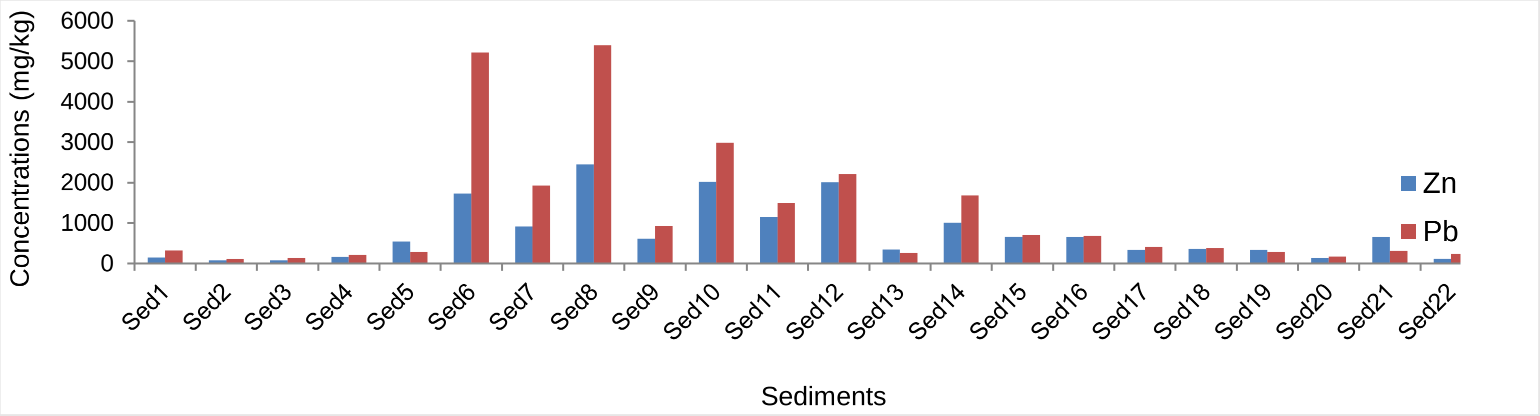


Figure 4.8: Concentrations of zinc and lead in sediments from the Edendale Spruit

The use of a pXRF, *in situ*, for the determination of toxic metals in sediment has been reported by several researchers. Stallard *et al.* (1995) completed a survey of marine sediment in San Diego Bay using a Spectrace 9000 portable XRF spectrometer, while on board a small vessel. Lead levels in peat from wetlands in the vicinity of Manchester and Sheffield (England) were determined *in situ* using a Niton XL3t 900 XRF (Shuttleworth *et al*., 2014).

Zinc and lead concentrations in sediments from the Edendale Spruit were higher than the threshold effet level of the Canadian Sediment Quality Guidelines (SQGs) (zinc = 410 mg/kg; lead = 218 mg/kg) CCME, 2001). They also surpassed the effect range medium reported by Long *et al*. (1992) (zinc = 315 mg/kg; lead = 91.3 mg/kg). Above these values, adverse effects are likely to occur. Maximum concentrations of zinc and lead found in sediments from the Edendale Spruit were higher than those reported for sediments from other South African rivers. Maximum levels of 0.49 mg/kg for zinc and 0.067 mg/kg for lead were found in sediments from the Tyume River in the Eastern Cape Province (Awofolu *et al*., 2005).

Jackson *et al*. (2009) found maximum concentrations of 270 mg/kg for zinc and 275 mg/kg for lead in sediments from the Plankenburg River in the Western Cape. The highest concentrations found in sediments collected from the Vaal River in the Northern Cape Province were 53.8 mg/kg for zinc and 10.1 mg/kg for lead (Pheiffer *et al*., 2014). Edokpayi *et al*. (2016) obtained maximum concentrations of 1524 mg/kg for zinc and 8.37 mg/kg for lead in sediments from the Mvudi River in Limpopo Province.

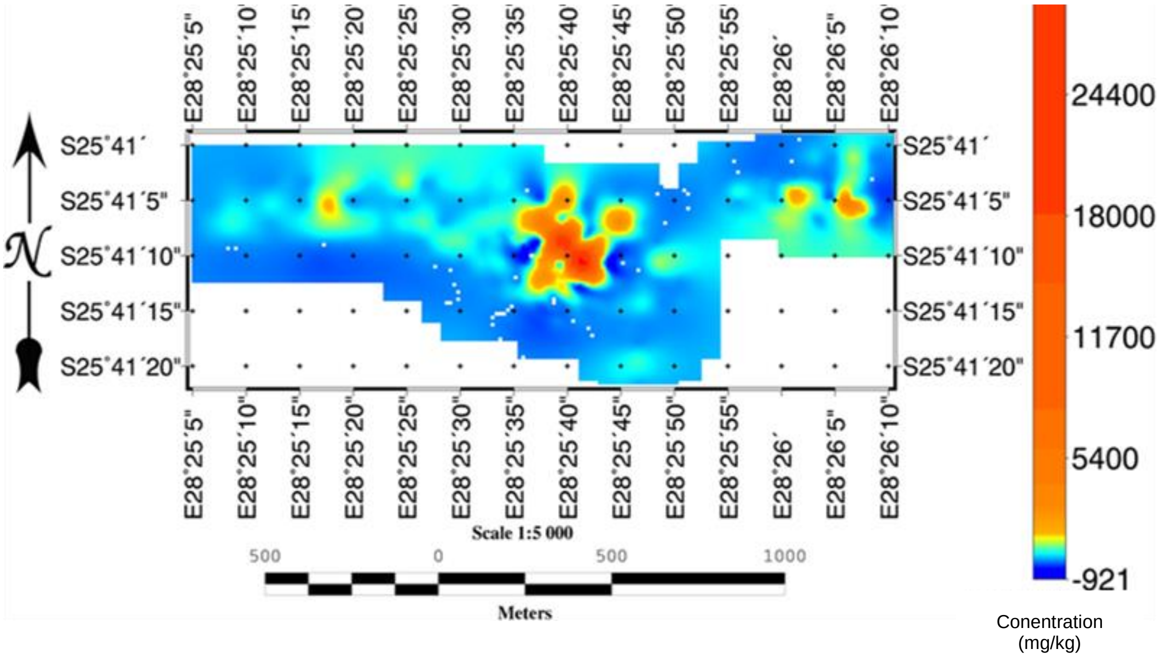
However, comparable and in some cases, higher levels of the metals have been reported in sediments from other countries. The maximum zinc concentration in sediments from the Xiawan Port (Hunan, China) was 5100 mg/kg, whereas the highest concentration of lead was 1040 mg/kg (Zhu *et al*., 2012). Exceptionally high levels of metal contamination were reported for sediments and soils from the floodplain, which had been enriched throughout many centuries of coal and metal mining activities in England and Wales (Hudson-Edwards *et al*., 2008). Maximum concentrations of zinc and lead of 12 203 and 20 310 mg/kg, were found in the River Swale in western Wales (Grove & Sedgwick, 1998), and 38 000 and 15 800 mg/kg in the River Nent in England (Dennis *et al.*, 2003). These elevated concentrations of metals in sediments reported throughout the world mostly resulted from extended periods of mining activity in the vicinity of the rivers.

The values reported in literature were in most cases measured in laboratories using traditional techniques. In contrast, the concentrations of zinc and lead in the current study were determined *in situ* using the pXRF. It was concluded in Chapter 3 that a high water content in sediment results in absorption of the incident XRF beam, thus reducing its excitation capacity and yielding low readings. This implies that the actual contamination by zinc and lead may be more serious than deduced from field measurements.

4.3.3 Zinc and lead distribution maps of the Edendale Mine area

The distribution maps highlight zinc and lead pollution profiles of the area (Figure 4.9). Three centres of contamination for both elements are indicated by red concentric spots. The first is located at Shaft1, north of the R513 (S 25.68403°, E 28.43544°) with maximum concentrations of 1700 mg/kg (S1-19) for zinc and 13000 mg/kg (S1-1) for lead. The second area of high contamination is situated at Shaft 2, south of the road (S 25.68470°, E 28.42 690) with the highest zinc concentration of 4400 mg/kg (S2-16) and the highest lead concentration of 20000 mg/kg (S2-3). The additional contamination centre is evident to the east of the site (S 25.68 420°, E 28.42 190°) with maximum concentrations of 7300 mg/kg for zinc and 21000 mg/kg for lead found in soil sample S2-52. The contamination map integrated to the Google map of the area provides a clear visual representation of the locations of high levels of zinc and lead pollution at the Edendale Mine (Figure 4.10).

Zinc



Lead

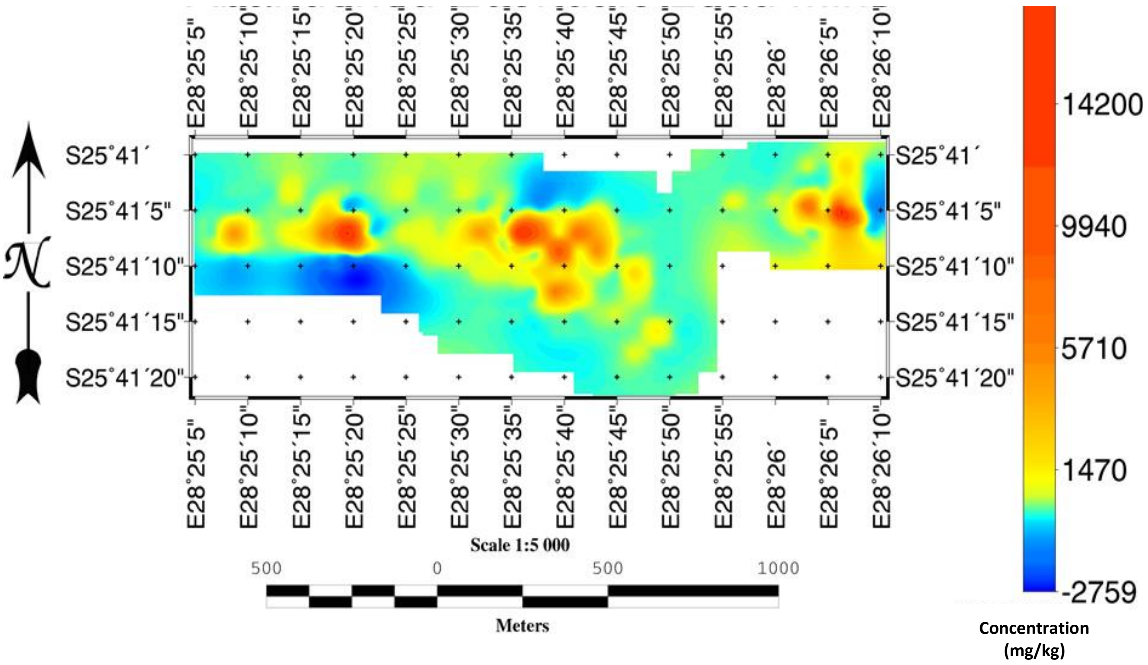
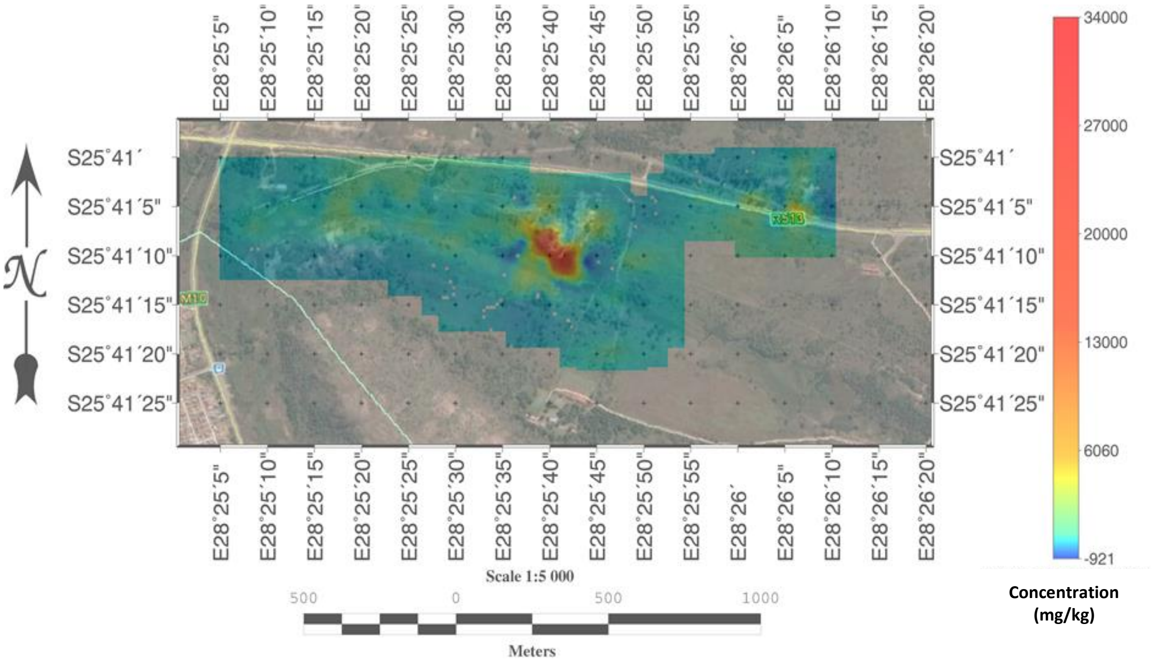


Figure 4.9: Contamination maps for zinc and lead at the Edendale Mine site

Zinc



Lead

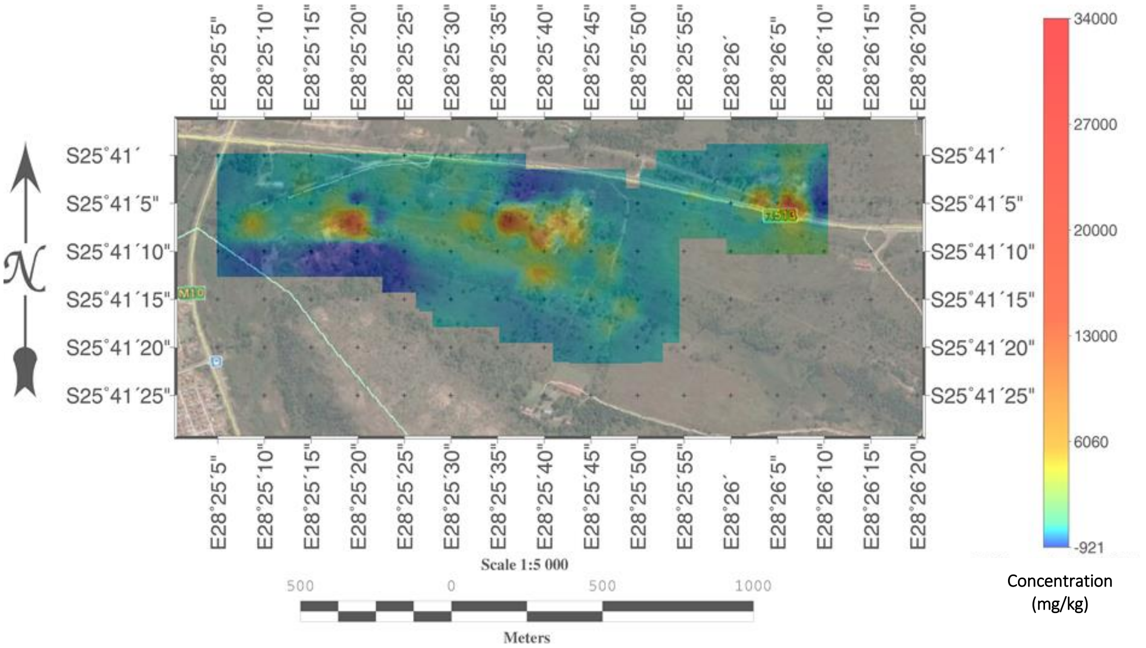


Figure 4.10: Contamination maps for zinc and lead overlaid on the Google map of the region

4.3.4 Assessment of zinc and lead contamination in soils and sediments using a geo-accumulation index

The geo-accumulation indices (I_{geo}) for zinc and lead calculated for the contaminated soils collected from the mine site are presented in Table 4.4. The I_{geo} for zinc fluctuated from - 4.4 (S1-9) to 4.7 (S2-52), with an average of - 0.74, indicating that the area is not contaminated by zinc. Inspection of the samples indicated that 60% of the soil samples were not polluted by zinc ($I_{geo} < 0$), while 14% were moderately polluted ($1 \leq I_{geo} \leq 2$), a further 4.2% of the soil samples were highly polluted ($I_{geo} > 3$) and only 1.8% of soils were extremely polluted ($I_{geo} > 4$). Strong pollution by zinc of soils S2-108, S2-14, S2-8 and S2-40 and extreme pollution of soils S2-97, S2-4 and S2-16 was determined. The calculated I_{geo} confirmed moderate contamination of soils from the Edendale Mine for zinc and severe contamination of soils with lead, as indicated by the concentrations obtained from the *in situ* analysis using the pXRF spectrometer.

Table 4.4: Soil pollution levels at the Edendale Mine

Class	I_{geo}	Pollution level	Percentage of soil polluted with zinc	Percentage of soil polluted with lead
1	< 0	unpolluted	60	32
2	0 - 1	unpolluted to moderately polluted	16	11
3	1 - 2	moderately polluted	14	14
4	2 - 3	moderately to strongly polluted	5.4	13
5	> 3	strongly polluted	4.2	29
6	3 - 4	strongly to extremely polluted	2.4	14
7	> 4	extremely polluted	1.8	15

I_{geo} = geo-accumulation factor

The I_{geo} for lead ranged from –2.8 (S2-79) to 8.0 (S2-52), with an average value of 1.5, indicating an overall moderate contamination of the soils with lead. However, results showed that 67.8% of the soils analysed were polluted with lead compared to only 32.2% that were unpolluted. Lead pollution ranged from moderate (27.1%) to strong (14.5%) and extreme pollution (15.0%). Soils that were extremely polluted with lead as reflected by $I_{geo} > 4$ were: S2-37, S2-136, S2-22, S1-14, S2-19, S2-28, S2-18, S2-95, S2-106, S2-30, S2-100, S2-7, S1-26, S1-19, S2-1, S2-34, S2-14, S2-107, S2-29, S1-3, S2-4, S2-16, S1-1, S2-3 and S2-52.

The I_{geo} was calculated to evaluate zinc and lead contamination of the sediments from the Edendale Spruit and the results are presented in Table 4.5. For zinc, the I_{geo} varied from –1.8 to 3.1, with an average value of 0.75. More than a quarter (27%) of the sediments was free of zinc contamination as reflected by I_{geo} values below zero. The remainder of the sediment samples (73%) were moderately to strongly contaminated with the element. Only sediment Sed8 was characterised by strong zinc pollution (I_{geo} > 4).

Table 4.5: Sediment pollution levels at the Edendale Mine as determined by pXRF

Class	I_{geo}	Pollution level	Percentage of soil polluted with zinc	Percentage of soil polluted with lead
1	< 0	unpolluted	27	0
2	0 - 1	unpolluted to moderately polluted	23	9.4
3	1 - 2	moderately polluted	27	36
4	2 - 3	moderately to strongly polluted	18	9.4
5	> 3	strongly polluted	4.5	45
6	3 - 4	strongly to extremely polluted	4.5	14
7	> 4	extremely polluted	0	31

Minimum and maximum I_{geo} values for lead of 0.34 and 6.0 indicated that all the sediments were moderately to extremely contaminated with lead. No and moderate contamination ($0 < I_{geo} < 1$) were established in sediments upstream (Sed4 and Sed3) from the mine. A total of 46% of the sediment samples, mostly located downstream (Sed17, Sed18, Sed19, Sed20, Sed21 and Sed22) were moderately to strongly polluted with lead. Extreme contamination by lead (I_{geo} > 4) was present in sediments that were located between the two boundaries of the mine (Sed11, Sed14, Sed7, Sed12, Sed10, Sed6 and Sed8).

Adie and Osibanjo (2009) reported high I_{geo} values (5.1 – 13), which correspond to very severe lead pollution, for soil collected in the vicinity of the metropolitan battery company of Ota, Ogun State, Nigeria. The source of the pollution of the soil was attributed to battery manufacturing activities. Agricultural soils collected in the proximity of the abandoned Agios Philipos Mine, Greece, were found to have varying levels of zinc and lead contamination, corresponding to I_{Igeo} values from -0.9 to 7.5 for zinc and -1 to 8.7 for lead (Nikolaidis *et al.*, 2010).

The most contaminated sediments from the stream were found between longitudes E28.42590 (Sed6) and E28.42790 (Sed12). This section of the stream was opposite to the most polluted soils of Site 2, which were located at a higher altitude, and situated between longitudes E28.42540 (S2-21) and E28.42890 (S2-7). From these results, it can be deduced that soils, remaining ore and mine waste, containing the toxic metals, have been eroded and that the metals migrated from Site 2 to the stream. Younger *et al.* (1997) described the role of water drained from an abandoned mine in the contamination of a freshwater system in England. Sediments of Lake Victoria collected to the north of Mwanza municipality (an industrial area) in Tanzania were found to be contaminated when compared to the southern and central areas of the town (Kishe & Machiwa, 2003). The latter areas were the least affected by anthropological activities. The authors attributed metal contamination of the sediments to the discharge of industrial and sewage waste from the industrial zone into the lake.

4.3.5 Analysis of soil samples at different depths

To investigate changes in concentration with depth, boreholes were drilled at the three centres of contamination identified from the maps. Due to the rocky nature of the soil, the holes were only drilled to a depth of 1 m, although the drill bit could be extended to reach a depth of 2 m. The soil at Centre 1 was composed of a mixture of dry soil and hard rock. Compared to Centre 1, soils at Centres 2 and 3 were moist, but the composition was a mixture of clay and gravel that caused the extension bit to block and made further drilling impossible. The concentrations and the GPS coordinates of the drilling sites are presented in Table 4.6.

Concentrations of the contaminants measured at the surface (0- 0.15 m) were generally higher. The decrease in concentration with depth was greater with lead than in the case of zinc. At Centre 1, for example, the zinc concentration decreased from 5200 to 2200 mg/kg from the surface to a depth of 0.5 m, whereas the lead concentration decreased from 45 000 to 6300 mg/kg over the same distance. Similar results were obtained for the other centres (Table 4.6).

Table 4.6: Concentrations (mg/kg) of zinc and lead in soil from different depths (m) at Edendale Mine

	C1 (S 25.68 416°; E 28.43 537°)		C2 (S 25.69 694°; E 28.428 72°)		C3 (S 25.68 572°; E 28.42 838°)	
Depth	Zn	Pb	Zn	Pb	Zn	Pb
0 – 0.1	5200	45000	5600	24000	2000	2000
0.5 – 0.6	2200	6300	3400	560	860	240
0.9 – 10	-	-	2200	12000	880	53

C1 = centre 1, C2 = centre 2, C3 = Centre 3

The results of this investigation are comparable to those of a study undertaken in Congo-Brazzaville to assess metal contamination of soils at an abandoned lead mine. The study revealed a decrease in the concentration of zinc from 2400 to 1200 mg/kg and lead from 11800 to 2000 mg/kg in soils when samples were analysed at depths of 15 and 150 cm, respectively (Matini *et al*., 2011). The rapid decrease in the concentrations of the contaminants observed in the soil profile was attributed to deposition of waste material on the soil surface and limited leaching of contaminants into the soil. This may also be the reason for the soil profile obtained at the Edendale Mine. The difference in the mobilities of the zinc and lead ions may explain the smaller reduction in the concentration of zinc (42%) compared to that of lead (14%). In contrast, a different soil contamination profile was obtained by Ololade (2014), when investigating heavy metal contamination of soils located near an auto-mechanic workshop in Ikare, Nigeria. Zinc and lead concentrations were found to increase with depth. The researcher attributed the profile to the mobility of the elements originating from vehicle lubricants.

4.3.6 Distribution of contaminants according to soil particle sizes

Six soil samples (S1-1, S1-7, S1-14, S2-1, S2-3 and S2-15) from the most contaminated area of the Edendale Mine were selected to study the distribution of zinc and lead in soils according to particle size. Eight soil fractions were separated and the concentrations of zinc and lead were determined in each fraction using the pXRF spectrometer. Results of the soil fractionation are presented in Figure 4.11.

Zinc and lead were most abundant in the finest fractions (F7 and F8) (Figure 4.11). The second set of sample fractions containing a high concentration of contaminants were the coarsest fractions (F1, F2 and F3). This trend was true for soils S1-7, S1-14, S2-1 and S2-3, whereas soils S1-1 and S2-15 contained relatively low concentrations in the first three fractions. The most contaminated soil fractions (F7 and F8) corresponded to the lowest mass percentage of the soils.

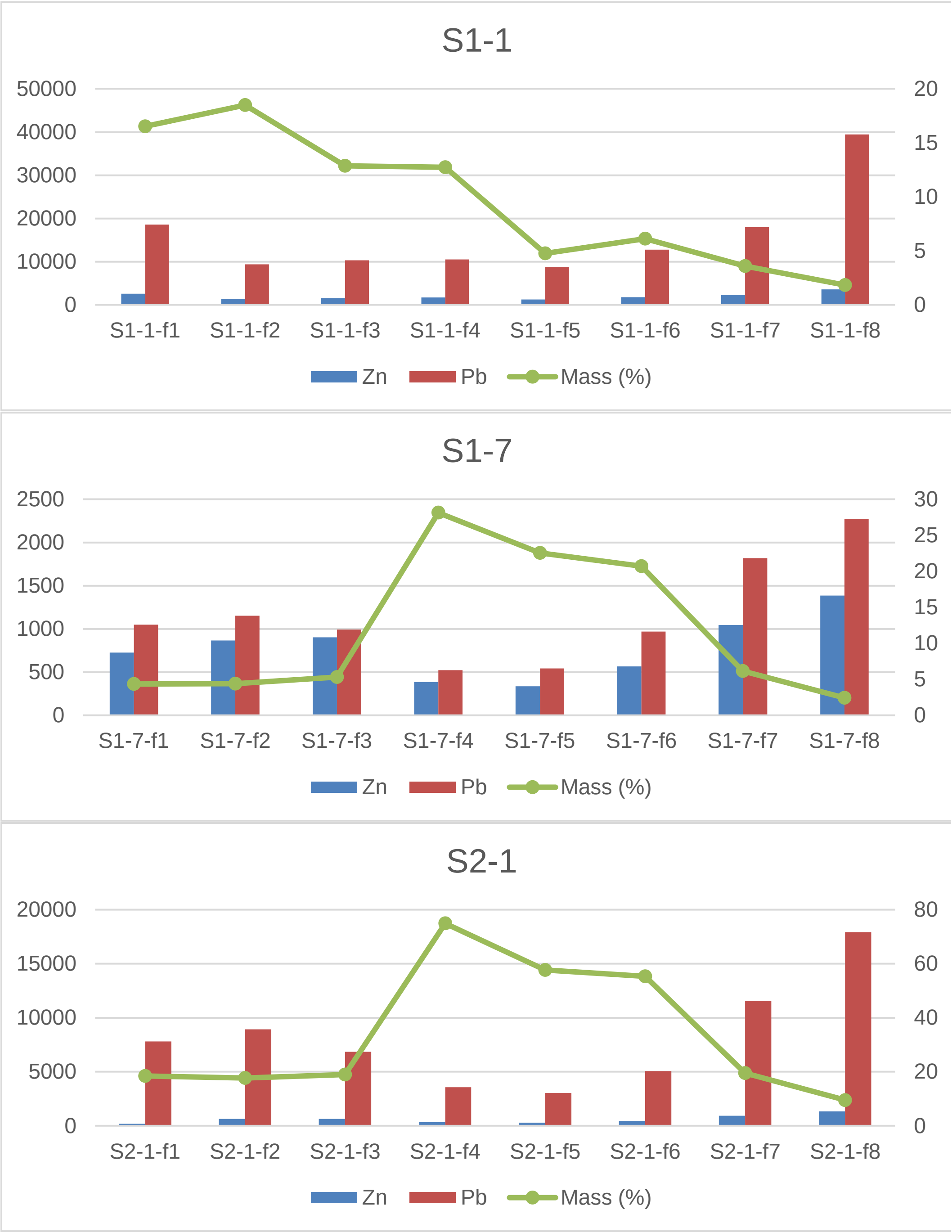


Figure 4.11: Distribution patterns for zinc and lead for individual soil particle size fractions for S1-1, S1-7, and S2-1

Figure 4.11 cont: Distribution patterns for zinc and lead for individual soil particle size fractions S1-14, S2-3 and S2-15

The occurrence of the highest levels of metal contaminants in the finest soil fraction correspond to the results of Wei *et al.* (2015). The high concentrations of metal and metalloid contaminants were attributed to the large surface area offered by these fine soil particles to adsorb the pollutants.

Soil fractions composed of fine particles may be converted to dust, which easily enters the atmosphere and consequently may cause remote contamination. Polluted dust constitutes a threat as it can be taken in orally, by inhalation or even through contact with the skin (Khan *et al*., 2016). Tthe risk of this occurring at the Edendale Mine is negligible, since the finest fractions comprised the smallest percentage (less than 10%) of the soil mass.

4.3.7 Determination of the pH and electrical conductivity of soil samples

Thirty-six soil samples of low, medium and high lead and zinc concentrations were selected to determine the pH and EC trends in soils at the Edendale Mine. The soil properties were correlated to elemental concentrations in the soils to determine if they influence the availability and mobility and finally, the uptake of the contaminants by plants (Figure 4.12). The logarithmic concentrations of zinc and lead were plotted as a function of pH and EC.

The average soil pH for the site was found to be almost neutral (pH=6.9), with the exception of soils S2-1 and S2-7, which were acidic (both pH=5.6), and soil S1-4 (pH=8.2), which was basic. The average EC for the site was 138 µS/cm and it ranged from 25.0 µS/cm (S2-123) to 502 µS/cm (S2-95).

Soil pH controls the mechanism by which soils adsorb and desorb metals (Houben *et al*., 2013). In acidic soils, the metals are usually more freely available, whereas in a basic soil, precipitation of the metals may occur, rendering them immobile (Zheng *et al*., 2012; Salazar & Pignata; 2014; Khan *et al.*, 2016). High EC of soil reflects elevated amounts of ionised elements, which include potentially toxic metals, in the soil solution. However, low pH and high EC failed to correlate with high total concentrations of zinc and lead in the soils. Also, high pH and low EC did not correspond to low total concentrations of the elements (Figure 4.12). Metal adsorption and mobility, which also depend on soil properties including organic matter, salinity, moisture, soil texture and structure, as well as temperature, may govern the fluctuations of pH and EC observed at the Edendale Mine (ATSDR, 2007; Sherene, 2010; Fernandes & Nayak, 2014).

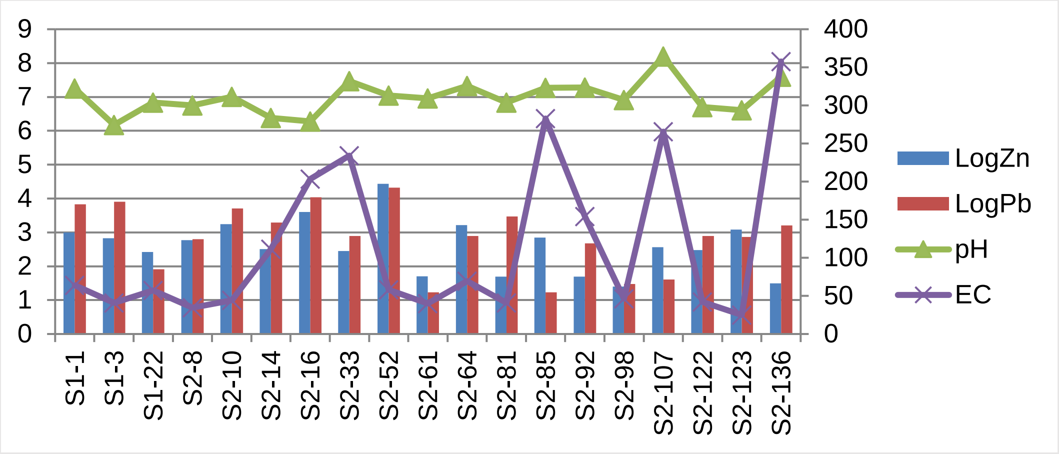


Figure 4.12: Total zinc and lead concentrations (expressed in logarithmic form), as well as the pH and EC of soil samples from the Edendale Mine. The scale on the left applies to the log of the concentrations and the pH, while the scale on the right applies to the EC values

4.3.8 Mobility of soil contaminants at the Edendale Mine

The mobility and bioavailability of iron, zinc and lead in soils were evaluated by performing the BCR sequential analysis. This method entails the sequential use of solvents of increasing extracting strengths and is used to assess the nature of association of elements with the soil solid phases, and consequently determines the degree of mobility of the elements in the soil. The weaker these elements are bound to the soil particles, the easier they are mobilised by the extracting solvents. Conversely, the stronger the element is bound, the lesser the degree of mobility. Four fractions, the acid soluble (F1), reducible (F2), oxidisable (F3) and residual (F4), are obtained using acetic acid, hydroxylammonium chloride, ammonium acetate and acid digestion with a mixture of hydrofluoric and nitric acids, respectively. Fractions F1 to F4 contain elements of decreasing mobility. The acid-soluble fraction contains elements that are weakly adsorbed to soil particles and are associated with carbonates. Toxic elements contained in this fraction are easily released into the environment under low pH conditions (Passos *et al*., 2011). The reducible fraction consists of elements associated with manganese and iron oxides. These elements are released from amorphous manganese and iron, as well as from manganese oxyhydroxides and oxides (Schramel *et al*., 2000). In the environment, the release of these elements may be influenced by the redox potential of the soil solution (Silveira *et al*., 2006). The oxidisable fraction, extracted following the application of hydrogen peroxide and ammonium acetate, contains elements bound to organic matter or metal sulfides. Metals in this fraction can be released into the environment following dredging or flooding that results in the sediment coming into contact with oxygen-enriched water (Kumar *et al*., 2013). Elements from the residual fraction are present in the crystalline structure of the soil, and cannot be released into the environment under normal conditions. The mobilities of the contaminants, zinc and lead, identified in soils from the Edendale Mine, were assessed using the BCR sequential extraction method, together with the reference element, iron.

An average of only 1% of the total iron was found in the exchangeable and carbonate fractions, but the amount ranged from 0% (S2-3 and S2-31) to 5% (S1-13) of the total. The reducible and oxidisable fractions contained 22% and 10%, of total iron, respectively. Iron was predominant in the residual fractions of the samples (average of 67% of the total iron) (Figure 4.13), but varied from 45% (S2-29) to 89% (S2-3). These results indicate that the iron was mostly bound to the crystalline components of the surface soils at the Edendale Mine and cannot be mobilised under natural conditions. This is evidence that the iron found on the surface is of lithogenic origin (Passos *et al.*, 2011). The finding was expected, since the total iron concentrations measured using the pXRF spectrometer were consistently high. Moreover, previous mineralogical analysis of soil samples from the area using XRD established the presence of $(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2\cdot(Mg,Fe)_3(OH)_6)$, the chlorite mineral, in which iron is one of the main elements integrated in the structure (Glass, 2006).

Similarly, zinc was found predominately in the residual fraction with an average percentage of 61% of the total zinc content. The minimum and maximum percentages of zinc in this fraction were 48% (S2-31) and 85% (S1-13) (Figure 4.13). However, the exchangeable and carbonate fraction contained the second largest average percentage (18%), ranging from 7% (S2-13) to 21% (S1-1, S2-3 and S2-55). The concentrations of zinc in the reducible and oxidisable fractions were low (14% and 7% of the total, respectively). However, the high levels of zinc in the residual fraction indicate that the element is also mainly bound to the crystalline structure of the soil. The degree of bonding suggests that zinc would not be leached under natural environmental conditions. However, some of the metal was loosely bound (18% of the total in the exchangeable fraction), suggesting that it could be released readily into the environment, whereas 7% of the metal may be leached under reducing conditions and 14% in an oxidising environment.

The average percentages of total lead in the soil fractions displayed a different trend: residual (8.0%) < oxidisable (15%) < exchangeable (35%) < reducible (42%) (Figure 4.14). These percentages ranged from 4.0 - 55%, 36 - 69%, 7.0 - 39% and 0 -1.0% for the exchangeable and carbonate, the reducible, the oxidisable and the residual fractions, respectively (Figure 4.13).

Figure 4.13: Results of the BCR sequential extraction procedure indicating the distribution of metals (%) amongst soil fractions taken from highly contaminated areas at the Edendale Mine

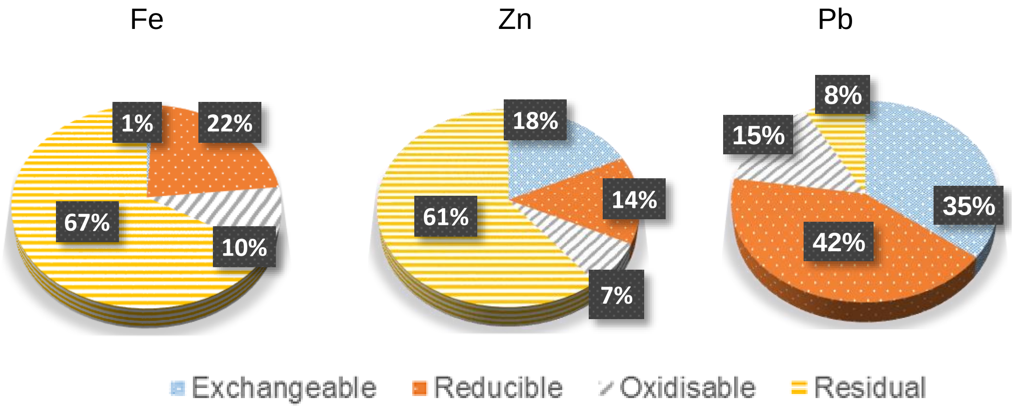


Figure 4.14: Average percentages of metal fractions in soils from the Edendale Mine as determined by BCR sequential extraction

Exchangeable fraction: iron (1%) < zinc (18%) < lead (35%)

Reducible fraction: zinc (14%) < iron (22%) < lead (42%)

Oxidisable fraction: zinc (7%) < iron (10%) < lead (14%)

Residual fraction: lead (8%) < zinc (61%) < iron (67%)

The first fraction, representing the soil fraction containing the most mobile forms of lead, constituted 35% of the total lead, which was a higher percentage than for zinc or iron. This finding indicates that lead is the most mobile of the three elements in the soils from the Edendale Mine. Moreover, the reducible fraction combined with the oxidisable fraction accounted for 57% of the total. The lead present in these two fractions represents lead that is bound to soil with the potential to be mobilised under favourable conditions. The residual fraction, containing the least mobile forms of lead, represented only 8% of the lead.

The comparison of the average percentages of iron, zinc and lead in the fractions followed the trends presented in Figure 4.14. A high variability in the total concentrations of lead and zinc was observed in the soil fractions, which were attributed to different mobility patterns of the elements in the soils at the Edendale Mine. For example, soils S1-1 and S2-3 from the contamination centres C1 and C2, respectively, were the most contaminated. These soils yielded exchangeable fractions containing high concentrations of the metals, hence higher mobility compared to soils S1-13, and S2-43 (from C1 and C2, respectively), which were characterised by exchangeable fractions containing low concentrations of the metals with low mobility. It could be concluded that lead and zinc are probably from the same origin in soils (S1-1 and S2-3) and (S1-13 and S2-43), but they are of different origin between the two groups of soils. The variability of the soil fractions was reduced for iron, particularly for the exchangeable and residual fractions, which suggests a common origin of the metal in the soils.

These findings confirm that lead is the most mobile element of the three in soils from the mine, followed by zinc and iron. Lead is bound weakly to the soil solid phases and may be associated with carbonate, suggesting that the lead is of anthropogenic origin.

The mining activities at the Edendale area that extended over 40 years (1898 -1938) may explain the high proportion of lead present in the exchangeable and the reducible fractions. Anthropogenic activities may have mobilised lead previously confined to the ore comprising minerals such as anglesite ($PbSO_4$), cerussite ($PbCO_3$) and galena (PbS), which were identified from XRD analysis of soils collected from the Edendale Mine (Glass, 2006).

Sequential extraction (BCR) of agricultural soils collected from the vicinity of a mining area in Zamopa Province, Spain, was performed by Álvarez-Ayuso *et al.* (2012). The reported results were comparable to those of the Edendale Mine. Lead was found to be the most mobile element compared to antimony and arsenic, with the exchangeable and reducible fractions containing 15.8 to 75.5% of the total lead. Half of the total lead was found in the easily soluble fraction (exchangeable fraction), following BCR extraction of contaminated soils collected adjacent to a mining area in the Mezica Valley, Slovenia (Pustisek *et al*., 2001). These soils were considered hazardous to the environment.

A more appropriate method to evaluate the mobility of an element in soil is to determine the associated bioavailability index (BI), which is calculated according the formula:

$$BI = (F1 + F2 + F3) \times 100 / C_{tota} \qquad \text{Equation 4.3}$$

where C_{total} represents the total concentration of the element in the soil sample (Hanzhou *et al*., 2009).

The BI values calculated for the three elements investigated were as follows: iron (5%) < zinc (37%) < lead (42%). The high mobility of lead and zinc, and the low mobility of iron in the soils from the Edendale Mine agreed with the speciation of these elements, as well as their different origins. Regarding the type of association of these elements in the soils from the Edendale Mine, lead is considered of anthropogenic origin. The high bioavailability of the metal poses a serious threat to human health and indicates that it is an environmental risk. Although zinc was associated with fractions that indicate a potentially high mobility, a large proportion of the element was found to be bound to the crystalline structure of the soils. Iron was the most strongly bound to soil particles and consequently, was the least mobile. This indicates that the element is from lithogenic origin and therefore does not present a threat to the environment (Passo *et al*., 2011).

Average percentages of iron, zinc and lead obtained in each of the fractions after the BCR sequential extraction of soils from each of the three centres of contamination (C1, C2 and C3) were calculated and compared to determine any similarities between the contamination centres (Figure 4.15).

For iron:
Exchangeable fraction: C3 (0%) < C2 (2%) < C1 (5%)
Reducible fraction: C3 (9%) < C1 (13%) < C2 (35%)
Oxidisable fraction: C3 (2%) < C1 = C2 (15%)
Residual fraction: C2 (48%) < C1 (67%) < C3 (89%)
For zinc:
Exchangeable fraction: C1 (7%) < C2 (10%) < C3 (21%)
Reducible fraction: C1 (3%) < C2 (4%) < C2 = C3 (4%)
Oxidisable fraction: C2 (2%) < C1 = C3 (5%)
Residual fraction: C3 (70%) < C2 (84%) < C1 (85%)
For lead:
Exchangeable fraction: C1 (55%) < C2 (28%) < C3 (16%)
Reducible fraction: C1 (36%) < C2 (42%) < C3 (59%)
Oxidisable fraction: C1 (7%) < C2 (17%) = C3 (21%)
Residual fraction: C1 (2%) < C3 (4%) < C2 (13%)

The mobility and bioavailability of iron is poor, irrespective of the centre of contamination, since the concentration proportions representing the exchangeable fractions were all below 6%, despite small variations in proportions between the fractions observed for samples within the same centre. Moreover, a large percentage of the total concentration of the element was found in the residual fractions, confirming the strong binding of the metal to the crystalline structure of the soils. This result is in agreement with the general mobility trend found for iron within the entire site.

The mobility and bioavailability of zinc is therefore relatively low. Lead was found to be highly mobile atCentre 1 > Centre 2 > Centre 3. Metals that could potentially be mobilised occur in the exchangeable, reducible and oxidisable fractions in extracted soils from the three centres of contamination. These fractions represented more than 70% of the total lead. The metals in these composite fractions could be easily mobilised as a result of a change of environment to acidic, reducing or oxidising conditions (Filgueiras *et al*., 2002). Lead contamination of the soils at the Edendale Mine is therefore serious and poses a real risk to the environment and people living in the vicinity.

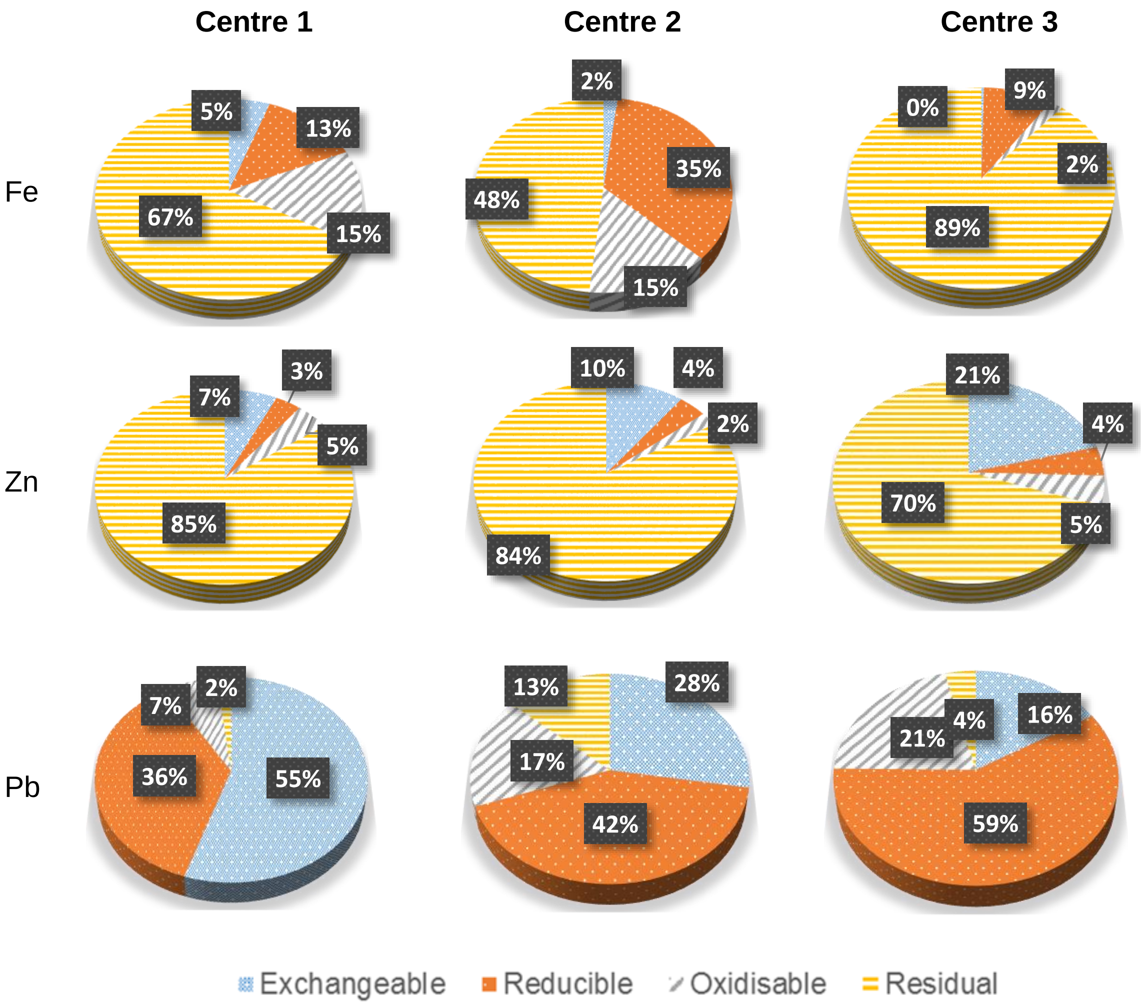


Figure 4.15: Pie charts indicating the average percentages of metals in the four fractions obtained through BCR sequential extraction of soils from the three centres of contamination at the Edendale Mine

4.3.9 Mobility of metal contaminants in sediments at the Edendale Mine

The proportions of iron, zinc and lead in the sediment fractions, obtained after applying the BCR sequential extraction, were determined using the ICP-OES. The average percentages of total iron in the sediment fractions were as follows: exchangeable (2%; range 0 - 5.0%), reducible (15%; range 10 - 26%), oxidisable (5%; range 2 - 10%) and residual (80%; 70 - 90%) (Figures 4.16 and 4.17). It can be deduced from these results that iron is strongly bound to the sediment particles and has poor mobility, since a large percentage of the element remained in the residual fraction, while only a small portion was recovered from the exchangeable fraction.

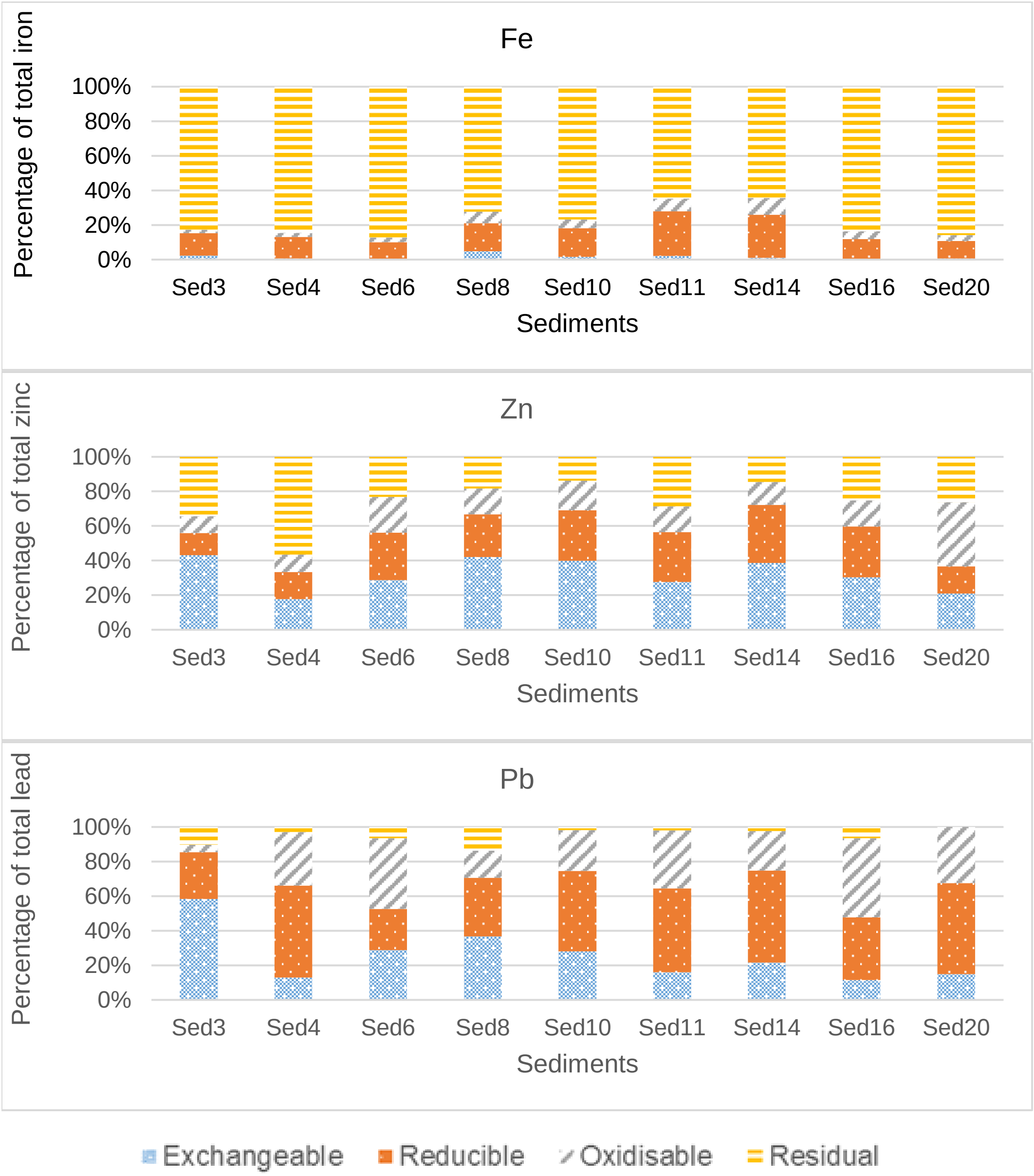


Figure 4.16: Results of the BCR sequential extraction procedure indicating the percentage proportion of iron (Fe), zinc (Zn) and lead (Pb) in sediment fractions taken from a tributary of the Edendale Spruit

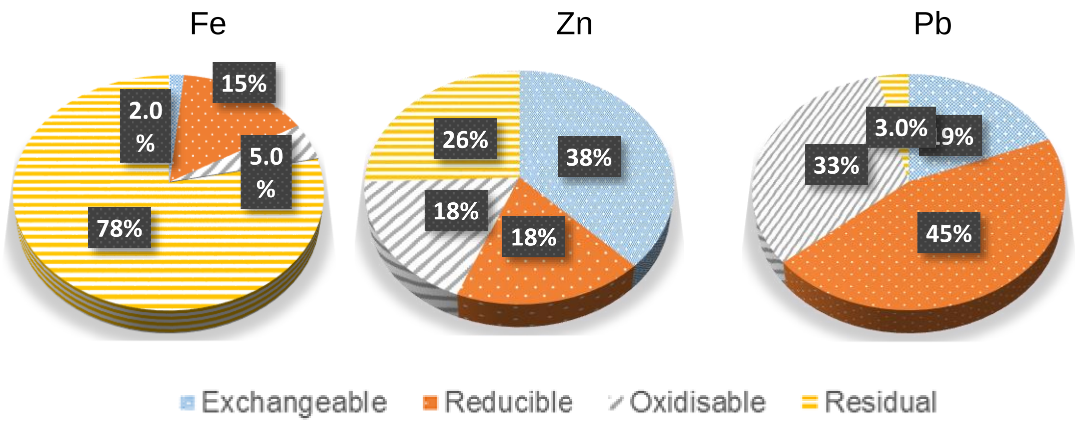


Figure 4.17: Average percentages of metals in sediment fractions from the tributary of the Edendale Spruit as determined by the BCR sequential extraction

Large variations in the percentage of total lead was observed within the sediment fractions. Sediments Sed3, Sed6 and Sed8 displayed a common trend that was different to that of the other sediment samples. For these sediments, the percentage of total lead was high in the exchangeable, but lower in the reducible fraction, due a large enrichment with lead from the contamination source. This was particularly true for Sed6 and Sed8, which were the samples most highly polluted with lead.

However, no apparent reason could be found to explain the high percentage of total lead in the exchangeable fraction obtained from Sed3, a sample which contained a relatively low lead concentration and was only considered to be moderately contaminated.

By considering the concentrations of the three metals in the exchangeable fractions, which followed the order of zinc > lead > iron, it was established that zinc is the most mobile element in the sediments, followed by lead and iron (Figure 4.17). However, total lead in the exchangeable, reducible and oxidisable fractions (87%) represented the greater mobilisable fraction when compared to zinc (74%), and therefore posed the greatest potential threat to the environment. This trend continued with the residual fraction (3.0%) containing the smallest proportion of lead, followed by zinc (26%). Iron was the most retained element by the sediment particles, since only 2.0% was found in the exchangeable fraction, whereas 78% of the total was recovered from the residual fraction.

4.3.10 Risk assessment for zinc and lead in soil and sediment from Edendale Mine

The mobility of metals in soils and sediments depend on their association with the geochemical fractions. The threat posed by zinc and lead in soils from the Edendale Mine to the environment was evaluated based on the risk assessment code (Muriithi *et al.,* 2011). The proportions of zinc and lead represented in the exchangeable fractions relative to the total concentration of those elements in soils, randomly selected from the contamination centres C1, C2 and C3, were calculated from the results obtained from the BCR sequential extraction. The degree of environmental risk associated with the RAC, ICF and GCF for iron, zinc and lead present in the individual soils were determined.

According to the RAC, iron and zinc are of low risk at the site, since the exchangeable ratios were below 10% for all the soils (Table 4.7). However, RAC values for lead ranged from 4 to 55% and corresponded to low, medium or high degrees of risk. The highest risk of lead to the environment was determined for Soil S1-1 from C1. Similarly, using the ICF, it was determined that iron and zinc are of a low risk, with the exception of Samples S2-19 and S2-29 for iron and S2-31 for zinc, which reflected a medium risk (Table 4.8). For lead, the ICF-values corresponded to medium, considerable and high risk. Samples S1-1, S2-31 and S2-29 reflected a high risk of lead to the environment, while the GCF-values calculated for Samples S1-31, S2-3, S2-31 and S1-1 and S2-29 revealed low, medium, considerable to high environmental risk, respectively, at the Edendale Mine site.

Table 4.7: Risk assessment for contaminated soils using RAC

Centre of contamination	Samples	Fe	Risk level	Zn	Risk level	Pb	Risk level
C1	S1-13A	5.4	L	7.2	L	9.4	L
	S1-14C	0.80	L	14	M	4.0	L
	S1-1C	0.60	L	21	M	55	H
C2	S2-43	2.9	L	10	M	9.2	L
	S2-19	1.9	L	19	M	22	M
	S2-3	0.30	L	21	M	28	M
C3	S2-55	2.2	L	21	M	18	M
	S2-31	0.50	L	9.3	L	4.3	L
	S2-29	0.60	L	12	M	16	M
Site		0.90	L	18	M	35	H

L = Low, M = Moderate and H = High

Results of the risk assessment calculations using RAC, ICF and GCF were similar for the individual samples. Iron and zinc were consistently classified as low to medium risk, whereas the classification for lead ranged from low to high risk in soils from the abandoned Edendale Mine. These results were consistent with the pollution assessment using the I_{geo}, which determined that soils from the Edendale Mine were unpolluted to strongly polluted for zinc and moderately polluted to severely polluted for lead.

Table 4.8: Risk assessment for contaminated soils using ICF and GCF

Centre	Sample	GCF	Risk Level	ICF Fe	Risk level	ICF Zn	Risk Level	ICF Pb	Risk Level
C1	S1-13	-	-	0.49	L	0.18	L	-	-
	S1-14	4.6	L	0.58	L	0.34	L	3.7	C
	S1-1	55	H	0.38	L	0.88	L	53	H
C2	S2-43	-	-	0.51	L	0.20	L	-	-
	S2-19	-	-	1.1	M	0.70	L	-	-
	S2-3	7.6	M	0.13	L	1.0	L	6.5	M
C3	S2-55	-	-	0.48	L	0.43	L	-	-
	S2-31	23	C	0.90	L	1.1	M	21	H
	S2-29	27	H	1.2	M	0.57	L	25	H
Site		13	C	0.50	L	0.65	L	12	C

L = Low, M = Moderate, C = Considerable and H = High

The ecological risk associated with the mobility of iron, zinc and lead in sediments from the tributary of the Edendale Spruit was assessed using the modified degree of contamination scales of RAC, ICF and GCF (Zhao *et al*., 2012). Low, medium to considerable, and medium to high ecological risk was found for iron, zinc and lead, respectively, using the RAC (Table 4.9). For lead, the highest risk was reflected by Sed3, while results from Sed8 indicated considerable risk. The remaining sediments revealed a medium environmental risk for that element. When applying the ICF, a low risk for iron and high risk for lead were indicated by the sediments, while for zinc the ecological risk varied from medium to considerable (Table 4.10). Using the GCF, a high risk was reflected by Sed4, Sed10, Sed11 and Sed14, a considerable risk for Sed6 and Sed16, and a medium risk for Sed3 and Sed8. The risk assessment results were consistent within the three assessment indices (RAC, ICF and GCF) for the three metals. A low ecological risk was posed by iron, but a medium to considerable risk was indicated for zinc by the RAC and ICF. The GCF, which results from the contribution of individual risk from iron, zinc and lead, indicated medium, considerable to high ecological risk depending on the sample. It can be concluded that iron poses no risk, but that zinc and lead may pose a considerable to high ecological risk to the tributary of the Edendale Spruit located at the Edendale Mine. This result is in agreement with the I_{geo} contamination index, which indicated that the sediments were highly contaminated with zinc and extremely contaminated with lead.

Table 4.9: Risk assessment for contaminated sediments using RAC

Sample	RAC Fe	Risk level	RAC Zn	Risk level	RAC Pb	Risk level
Sed3	2.4	L	21	M	58	H
Sed4	0.71	L	29	C	13	M
Sed6	0.54	L	39	C	29	M
Sed8	4.8	L	28	M	37	C
Sed10	1.5	L	39	C	28	M
Sed11	2.0	L	42	C	16	M
Sed14	1.0	L	29	M	22	M
Sed16	0.24	L	18	M	11	M
Sed20	0.68	L	43	C	15	M

L = Low, M = Moderate, C = Considerable and H = High

Table 4.10: Risk assessment for contaminated sediments using ICF and GCF

Sample	GCF	Risk level	ICF Fe	Risk level	ICF Zn	Risk level	ICF Pb	Risk level
Sed3	12	M	0.21	L	2.8	M	8.7	H
Sed4	37	H	0.18	L	2.9	M	34	H
Sed6	19	C	0.15	L	5.9	C	14	H
Sed8	9.1	M	0.39	L	2.5	M	6.3	H
Sed10	59	H	0.30	L	6.2	H	53	H
Sed11	54	H	0.55	L	4.4	C	49	H
Sed14	44	H	0.55	L	3.3	C	40	H
Sed16	15	C	0.19	L	0.77	L	14	H
Sed20	-	-	0.16	L	1.9	M	-	-

L = Low, M = Moderate, C = Considerable and H = High

4.3.11 Contamination of vegetation at the Edendale Mine site

Zinc and lead concentrations present in leaves and roots of *Calamagrostis acutifolia* (grass), *Tagetes minuta* (kakiebos), *Lantana camara*, *Lippia javanica*, a *Solanum* species and *Hyparrhenia filipendula* (thatching grass) were determined using ICP-OES. Concentrations of zinc and lead in the plant parts from the contaminated sites (S2-1, S2-3 and S2-7) were higher than that of their counterparts growing in areas with low levels of the metals, as determined by pXRF (control). Zinc and lead concentrations in the roots of the plant species from the contaminated sites were higher than the concentrations in the leaves. Among the plants species investigated, *C. acutifolia* was the most capable of accumulating zinc and lead, both in leaves and roots, whereas the *Solanum* species, thatching grass and *L. camara* to a lesser extent, indicated accumulation of the metals mostly in the roots compared to other parts (Table4.11).

Table 4.11: Mean concentrations and standard deviations (SD) for zinc and lead (mg/kg) (n=3) in plants from the Edendale Mine measured in the digests using ICP-OES

Plant species and part		Control				S1				S3				S7			
		Zn	SD	Pb	SD	Zn	SD	Pb	SD	Zn	SD	Pb	SD	Zn	SD	Pb	SD
Calamagrostis	L	13.1	0.960	<LOD	NA	132	24.6	115	20.3	95.4	3.26	95.2	5.85	122	6.96	28.9	2.15
acutifolia	R	50.5	26.1	2.90	0.170	301	14.7	879	35.7	177	24.1	399	16.5	247	49.8	63.8	31.5
Tagetes	L	34.2	25.9	<LOD	NA	62.	2.99	<LOD	-	67.9	3.30	<LOD		59.9	13.7	<LOD	-
minuta	R	8.68	0.580	<LOD	NA	85.3	13.8	22.6	7.15	63.6	31.4	471	39.0	36.9	30.7	158	128
Lantana	L	NA	NA	NA	NA	11.0	1.45	<LOD	-	71.8	16.3	31.9	4.65	155	45.3	33.9	10.5
camara	R	NA	NA	NA	NA	11.0	2.58	10.3	3.58	22.6	9.51	68.7	24.1	97.8	24.7	347	98.2
Lippia	L	12.2	0.120	<LOD	NA	37.9	1.61	5.08	0.720	43.1	12.1	21.5	5.16	25.4	0.410	5.24	0.730
javanica	R	3.28	0.840	<LOD	NA	31.0	1.96	<LOD	-	45.3	5.18	182	20.0	42.5	8.70	75.3	15.3
Solanum	L	5.16	1.91	8.39	0.230	189	11.0	<LOD	-	NA	-	NA	-	534	76.2	2.51	0.580
species	R	16.8	1.81	<LOD	NA	217	70.3	116	1.73	NA	-	NA	-	325	321	116	69.9
Thatching	L	22.6	3.21	<LOD	NA	83.1	8.19	<LOD	-	NA	-	NA	-	44.8	4.05	24.6	4.52
grass	R	22.9	2.63	<LOD	NA	185	14.3	62.6	6.60	NA	-	NA	-	375	46.2	132	12.7

L = Leaves, R = Roots NA = Not available and SD = Standard deviation

The ability of plants to take up and accumulate metals, such as zinc and lead, from polluted soils has been proven by other researchers. Salazar and Pignata (2014) investigated the potential of plants growing on contaminated residential and agricultural soils located next to a former lead smelter plant in Cordoba, Argentina, to take up heavy metals from the soils. Results obtained from the investigation were comparable to those of this present study. Substantially higher concentrations of lead were found in roots of plants such as *Sorghum halepense* (1047 mg/kg), whereas *Tagetes minuta* was found to accumulatte 859 mg/kg in roots and 380 mg/kg in the leaves (Salazar & Pignata, 2014). Accumulation of zinc and lead in leaves and roots of plants growing on a former landfill containing waste from an iron and steel foundry in Firminy, France, was reported by Remon *et al.* (2005). The levels of the metals were significantly lower than those found in the current study, possibly because the landfill soils were formerly excavated (Remon *et al*., 2005). Metal uptake and accumulation by commercial plants has been widely reported. Plants grazed by sheep and pigs in Norway were found to contain up to 500 mg/kg of zinc, originating from soils polluted by atmospheric deposits (Brekken & Steinnes, 2004).

The presence of lead and zinc in the various plant parts is of significant importance regarding the ecological risk posed by these elements at the Edendale Mine. The abandoned mine site is used as pasture for cattle, which have probably accumulated lead and zinc in their tissues from grazing contaminated grass. Evidence of growing vegetables and maize was found at the abandoned mine site. These plants probably absorb the mobile toxic elements in their aboveground parts. The health of the people consuming the contaminated beef and crops is at risk.

4.4 CONCLUSIONS

- The screening of soils at the old Edendale Mine using the pXRF revealed an average concentration of 480 mg/kg for zinc and 1100 mg/kg for lead. However, three centres that are highly polluted with zinc and lead were identified and superimposed on a Google map of the area. The maximum concentrations for zinc and lead were (1700 and 13 000 mg/kg), (4400 and 20 000 mg/kg) and (7300 and 21000 mg/kg), for the contamination centres C1, C2 and C3, respectively. Although the concentrations of zinc and lead decreased with increasing depth, high concentrations of the toxic elements are present at a depth of 1 m. From the total number of sampling points visited (n=167), 46% of these contained more zinc and 77% contained more lead than the baseline concentrations set for South

African agricultural soils (Herselman *et al*., 2005). The threshold set for waste destined for landfill (Bomo *et al*., 2013) was exceeded by 3% of the soils by a factor of two. This is an important consideration, as one method of reclaiming the land would be to remove the polluted soil and place it in a landfill.

- Considering the average geo-accumulation index of soils at the Edendale Mine, it indicated that the soils were not polluted by zinc, and only moderately polluted by lead. However, individual I_{geo} for the soils indicated that 2% of the soils for zinc, and 15% for lead, were extremely polluted. Applying the BCR sequential extraction scheme, lead was found predominantly in the exchangeable and mobilisable (exchangeable, reducible, and oxidisable) fractions of extracted soils, implying potential mobility of the lead under reducing and oxidising conditions. Zinc has moderate mobility, since the element was mostly found in the residual fraction (61%), whereas the exchangeable fraction represented less than 15% of the total zinc. Iron, which was used as a reference element, was found mainly in the residual fraction. This metal was considered to be of lithogenic origin, whereas lead was considered to be of anthropogenic origin. The origin of the zinc remains uncertain.
- The evaluation of the soils using the RAC, the ICF and the GCF, consistently confirmed that zinc constitutes a low to moderate ecological risk, while lead was found to be of moderate to high ecological risk. This finding correlates with results obtained from the investigation of the mobility of those elements in the soil. The levels of contamination, mobility and ecological risk of zinc and lead in the soils were further confirmed by the uptake of these elements by plants growing on the Edendale Mine site. Although zinc and lead were mainly confined to the roots of the plants investigated, a significant amount accumulated in the leaves. The best zinc and lead accumulator, either in the roots or in the leaves, was *C. acutifolia* followed by the *Solanum* species, thatching grass and*Tagetes minuta.*
- Average concentrations of 750 mg/kg for zinc and 1200 mg/kg for lead were determined in sediments of the Edendale Spruit. The highest zinc (2400 mg/kg) and lead (5400 mg/kg) concentrations found were possibly underestimated, since the pXRF analyses were performed on wet sediments. Nevertheless, according to the I_{geo}, calculated from sediments collected over a distance of 1000 m along the stream, 73% of samples for zinc and 100% for lead, were moderately to strongly contaminated. Following the fractionation of the sediments using the BCR sequential extraction, zinc and lead were found to be highly mobile or potentially mobile since (34%, 73% and 27%) for zinc and (25%, 95% and 5%) for lead were

found in the exchangeable, oxidisable and reducible, and residual fractions, respectively. A moderate to high ecological risk for zinc and lead was indicated when applying the ICF and GCF, which confirms the high mobility of the toxic elements in soils from the site.

Soils and sediments from the Edendale Mine are highly contaminated with zinc and lead. These elements are mobile and pose a severe ecological risk. The health of livestock and of people exposed to the site are at risk. This investigation provides the local authority with a database, maps and GPS coordinates to identify the sites of contamination. This study has provided sufficient data to justify the implementation of a remediation programme.

CHAPTER 5
INVESTIGATION OF COPPER CONTAMINATION OF ORCHARD SOILS IN HOEDSPRUIT USING pXRF SPECTROMETRY

5.1 BACKGROUND AND MOTIVATION

South Africa is renowned for fruit production and export. The diversity of the climate of the country allows for the production of various fruits of economic importance, including grapes, apples, peaches, oranges and mangoes. Vineyards of grapes and orchards of apples and pears, for example, occupy 15 835, 22 925 and 12 211 ha, with an annual production of 46 051, 792 549 and 413 614 tons, valued at R0.7, R4.8 and R2.5 billion, respectively[7]. Globally, South Africa is one of the top producers and the largest exporter of fresh Valencia oranges (*Citrus sinensis*)[8]. In 2015, 1 783 666 tons of Valencia oranges were produced, representing about 65% of citrus fruit harvested in the country. In 2013, Limpopo was the leading province with 56% of 25 710 ha planted with Valencia oranges, followed by the Eastern Cape (15%), Mpumalanga (14%), Western Cape (8%) and KwaZulu Natal (3%)[9]. Mango (*Mangifera indica*) is among the export fruits of South Africa, but contributes less to the national and international market than Valencia oranges. Only 48 372 tons were produced in 2013, representing a 25% decline compared to 2012, due to adverse weather conditions.

Fruit sales generate finances for the South African economy, however, the degree to which this takes place is often jeopardised by losses due to crop and plant diseases. These are usually caused by parasites including microbes, insects, viruses, yeasts and fungi. Many of the pests that plague fruit trees and cause a decline in the commercial value of the fruit are well characterised. These pests attack all plant parts including fruit, leaves, seeds, trunk, branches and flowers. For mango, insects such as *Othreis maternal, Gonodonta pyrgo, G. clotilda* and *Leptoglossus stigmai* are known to feed from the ripening fruits (Pefia *et al*., 1998). *Procontarinia matteiana,* the most prevalent of gall fly in South Africa, parasites mango leaves, which may result in a reduction in production (Augustyn *et al*., 2010). Mango pests such as thrips, midges, mites, scales, whiteflies,

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[7] www.hortgro.co.za/wp-content/uploads/2016/02/Industry-Perspectives-2014.pdf. [Accessed]: 26/07/2016.

[8] www.namc.co.za/upload/South-African-Fruit-Trade-Flow-May-2014-Issue. [Accessed]: 26/07/2016.

[9] www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20 Profiles/. [Accessed]: 26/07/2016.

mealybugs, weevils, ants, locusts and caterpillars, feed from the foliage and consequently reduce the photosynthetic activity of the trees (Pefia *et al*., 1998). Parasites including *Hemicriconemoides mangiferae* and *Xylosandrus compactus* attack mango trees, causing chlorosis and necrosis of seeds, trunks, twigs and roots (McSorley & Campbell, 1980). Lee (2015) listed a number of common viruses and graft transmissible diseases, including the citrus tristeza virus, citrus psorosis virus, impietratura, cristacortis disease, citrus vein enation, citrus blight disease, citrus viroids, citrus tatterleaf, viral citrus leaf blotch, measles disease, yellow vein citrus leprosis and citrus yellow mosaic. He proposed methods to control these diseases. In Africa, 17 fruit fly species of economic importance, belonging to four genera, *Ceratitis, Dacus, Trirhitrum* and *Bactrocera*, have been identified (Badii *et al*., 2015; De Meyer *et al*., 2016). *Ceratiti capitata* and *Ceratitis rosa* are reported as the flies causing the most damage to mangoes in South Africa. They are an indigenous species, specific to African, and causes losses of between 20 and 30% on average (Badii *et al.*, 2015). Fungal infestation of orchards is also a major cause of crops losses. Fungi such as *Colletotrichum gloeosporioides* and *Gloeosporium mangiferae* cause anthracnose of mango fruits. *Erwinia mangiferae, Oidium mangiferae, Cephaleuros virescens* and *Elsinoe mangiferae* are responsible for mango bacterial black spot, powdery mildew, red rust and scrab, respectively (Rusjan, 2012).

The large number of pests affecting fruit result in major financial losses for farmers globally. In the Maghreb (Algeria, Libya, Morocco and Tunisia, one of the major regions of fruit production), 214.4 tons of stone, pome and citrus fruits were lost due the Mediterranean fly over the period 1980 to 1989, which corresponded to annual losses of \$60.11 million (IAEA, 1995). The average annual loss of oranges due to pest infestations of oranges in Brazil (2012 – 2013) was estimated at 2 million tons, corresponding to \$525 million (Oliveira *et al*., 2014). In South Africa, a survey (1991) that involved approximately 61 000 avocado trees was conducted in Nelspruit and Hazyview regions of Mpumalanga, revealed that 84% of fruit damage was due to flies, resulting in losses estimated at R2.93 million per annum to the farmers (Erichsen & Schoeman, 1992).

To minimise financial losses attributed to flies, fungal and microbial infestations of fruit orchards, growers implement various pest control methods. Pesticide sprays are the most commonly used technique for fruit pest control in developing countries. Chemical formulations used for pest control are often based on potentially toxic elements such as copper, mercury, manganese, lead, chromium, arsenic and zinc (Wuana & Okieimen, 2011). Copper-based chemical formulations, such as the Bordeaux mixture ($CuSO_4•5H_2O + Ca(OH)_2$), which was accidentally discovered in France in 1880, and the Burgundy mixture ($CuSO_4 \cdot 5H_2O + Na_2CO_3$), have been sprayed in orchards for centuries across the world (van-Zwieten *et al*., 2004; Rusjan, 2012). The copper in fungicides destroys cell proteins and therefore targets the enzyme functions of parasites (Mackie *et al*., 2012). Copper-based pesticides used in Australia include copper sulfate, copper dihydrazine sulfate, copper oxychloride, copper carbonate and copper hydroxide (van-Zwieten *et al*., 2004). Copper salt-based formulations are also used in South Africa for pest control in granadilla, grape, guava, mango, olive, peach, pear and plum orchards (Quinn *et al*., 2011). Copper sulfate was previously used in horticulture, but is considered too toxic and has been replaced largely by copper hydroxide (Mackie *et al*., 2012). The use of copper formulations to combat fungal infestations is currently the only organic alternative in horticulture.

Application of copper-based pesticides in orchards may effectively improve fruit production and marketability, however, the accumulation of copper in soils, resulting from intensive and long-term application of these pesticides, is irrefutable. Li *et al.* (2005) investigated copper contamination in Chinese apple orchard soils, which had been sprayed with the Bordeaux mixture for up to 30 years. Compared to the soil of an adjacent forest, the researchers found the orchard soils to be contaminated with copper. Copper concentrations in the orchard soils increased with the age of the orchards (Li *et al*., 2005). Accumulation of copper in orchard surface soil was also reported by Silva *et al.* (2012). These researchers observed an increased copper concentration in surface soils of orchards aged from 10 to 26 years in Brazil. The accumulation was ascribed to the long-term use of copper-based fungicides (Silva *et al*., 2012). Copper accumulation in agricultural soils has been reported globally. Drouineau and Mazoyer (1962) reported a copper concentration as high as 500 mg/kg in surface agricultural soil in France, while Flores-Veles *et al.* (1996) reported levels of up to 1500 mg/kg in vineyard soils in the same country.

The accumulation of copper in agricultural soils raises concerns related to soil fertility, food safety, water contamination, human health and environmental risk (Hans, 2012). High concentrations of copper in soil impacts negatively on biota and activities of soil organisms and micro-organisms, since these organisms reduce plant infestation by digesting pathogen spores present in soils (van-Zwieten *et al*., 2004). Living organisms in soils, such as microbes and earthworms, fertilise soils by improving its structure. Elevated copper concentrations in soils may result in the destruction of the microflora and fauna. Wang *et al.* (2009) found a significant reduction in soil microbial biomass with an increase in the total copper concentration in soil.

Rural communities rely only on boreholes, rivers and streams for their supply of drinking water. Toxic elements may leach into groundwater from polluted soils. Fresh water may be enriched with toxic elements leaching from soils after storms. Contaminated groundwater and fresh water become unfit for human consumption and pose a threat to public health.

Copper is an essential element for the growth of plants and animals. The micronutrient contributes to the protection of seeds, resistance to plant infestations and the regulation of water in plants (Wuana & Okieimen, 2011). In humans, copper contributes to the synthesis of haemoglobin. However, a toxic dose and chronic exposure to even low concentrations of the element may lead to various conditions including diarrhoea, vomiting, liver damage, skin and hair decolouration[10], and male sterility (Vigeh *et al.*, 2011). In plants, toxic levels of copper may prevent growth of shoots and roots, and cause chlorosis of leaves (Rusjan, 2012).

The implementation of remediation programmes are necessary to minimise the risks associated with metal contamination of orchard soils. Several remediation techniques are available to restore contaminated soils. These aim at reducing the total concentration or the bioavailable fraction of metals to levels below those set by national and international regulations (Wuana & Okieimen, 2011). Phytoremediation is one of the most appropriate remediation techniques (Wuana & Okieimen, 2011; Mahar *et al.*, 2016). It is based on the use of plants to remove organic and inorganic contaminants from polluted lands and water. Phytoremediation offers the advantage of low cost, minimum disruption of the environment, wide acceptance, absence of problems relating to the disposal of contaminants irrespective of form, avoidance of spreading contaminants, multi-element removal capability and large scale applicability, compared to other methods of remediation (Wuana & Okieimen, 2011; Etim, 2012). Phytostabilisation is a related technique used to reduce the mobility of soil and groundwater contaminants by metal-tolerant perennial plants (Abdullahi, 2015). When planted in contaminated soils, metal contaminants are absorbed by the roots or adsorbed onto the roots to restrict their bioavailability and prevent biomagnification in the food chain. During phytoextraction, soils are cleaned as a result of absorption by the roots and translocation of the contaminants to the aboveground portions of the plants (Lasat, 2000; Abdullahi, 2015). In the case of highly toxic elements,

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[10] www.copperinfo.com/health/index.html. [Accessed]: 28/07/2016.

plants are harvested after an appropriate period and incinerated. The resulting ashes are disposed in hazardous waste landfills.

Although the use of plants for the benefit of cleaning soil, water and air pollution dates back hundreds of years, research into the technique and applications in the field are relatively recent (Etim, 2012). Plants capable of translocating large amounts of metals from the roots to the aboveground portions of the plants, are known as hyperaccumulators, and are selected for phytoextraction. The term ‘hyperaccumulator’ was first coined by Jaffre *et al.* (1976) to characterise plants containing unusually high nickel concentrations in New Caledonia. Plant species, such as *Sebertia acuminata* and *Psychotria douarrei*, were found to accumulate nickel (1.2, 2.5 and 1.1% dry weight) and (3.4, 2.3 and 5.5% dry weight) in leaves, trunk and twig barks, respectively. Brummer and Woodward (1999) also reported copper concentrations of 2500 mg/kg in leaves and 4500 mg/kg in roots of the “copper flower” *Becium centraliafricanum (*previously referred to as *Becium homblei*) and *Ocimum homblei* growing on copper deposits in Zambia. However, a more recent definition for a hyperaccumulator states that the metal concentration must exceed the threshold value of 1000 mg/kg in shoots and that the biological factors must be greater than 1. The biological factors determine the shoot to soil ratio of the element (bioaccumulation factor; BAF) and shoot to root ratio (translocation factor; TF) of the metal concentrations (van der Ent *et al*., 2013).

The family *Lamiaceae* is one of 45, comprising approximately 400 species. Many of these species are hyperaccumulators of various elements (Brummer & Woodward, 1999; Lio, 2000; Mahar *et al.*, 2016). Plant species from the *Lamiaceae* family, including *Haumaniastrum katangense, Haumaniastrum robertii* and *Becium homblei are* reputed to tolerate extremely high copper concentrations in soils and accumulate unusually large amounts of the copper in their areal parts (8000, 15000 and 10000 mg/kg, respectively) (Brummer & Woodward, 1999; Lasat, 2002; Sheoran *et al*., 2011). *Rosmarinus officinalis* and *Ocimum labiatum* (formerly *Orthosiphon labiatus*) are perennial shrubs, belonging to this family. Although *R. officinalis* (rosemary) is cultivated globally for its multi-functionality, it originates from the Mediterranean region. The plant is used for food and beverage flavouring, in cosmetics, for essential oil production and for therapeutic treatments (Madejón *et al*., 2009; Habtemariam, 2016). Only a few reports are available in literature concerning the exploitation of *R, officinalis* for phytoremediation (Madejon *et al*., 2009; Testiati *et al*., 2013; Ziarati *et al*., 2014), whereas no report was found for *O. labiatum*. Some pot trials, undertaken to determine the degree of copper uptake by *O. labiatum*, have indicated its tolerance towards copper, but field trials were not included in the study (Maphakela, 2015).

Public awareness of health risks associated with agricultural products led countries to set regulations and standards for fruit imports. The European Community, which is a major importer of fresh fruit from South Africa (DAFF, 2014), established numerous stringent legal requirements. Fruit and vegetable exporters to the European Community are required to comply with sanitary and phytosanitary measures, product traceability, hygiene rules, microbiological criteria for foodstuffs, safe and effective application of pesticides and must provide a list of potential contaminants (Graham, 2006). Fruit such as orange, are also required to satisfy the “market standard grade” for which a certificate of conformity must be issued prior to export. Moreover, private organisations (European Retailers’ Protocol for Good Agricultural Practice (EUREPGAP) and the British Retail Consortiums (BRC) have set standards for “good agriculture practices”, which allow the verification of the implementation of necessary management and control systems by suppliers (Graham, 2006). The determination of the levels and risk assessment of toxic element contamination in soils are part of good agricultural practice, which farmers need to comply with. Concerns were raised regarding the environmental and health risks associated with the contamination of topsoils with copper in orchards, which led the IFOAM (International Federation of Organic Agriculture Movement) to restrict total copper input on organic farms to a maximum of 8 kg/ha/year (van-Zwieten *et al.,* 2004).

South Africa has a long history of the application of copper-based fungicides in mango, avocado and, to lesser extent, in citrus orchards. Mr C. Redelinghuys (2016), a farmer from Hoedspruit, revealed in a personal communication that copper oxychloride (2.5 g/L) is used as fungicide and is sprayed once every month in mango orchards, while it is applied in citrus orchards only once a year on young trees. However, no investigations to determine the levels of copper contamination of orchard soils have been undertaken.

The applicability of the pXRF technique for *in situ* screening of agricultural soils for the determination of copper levels has been proven (Chapter 3). In contrast to pollution at the Edendale site, copper(II) is applied in the form of a solution in orchards. In addition orchard pollution is ongoing. Differences in copper concentrations found between *in situ* screening and results obtained after the soil samples had been processed were not significant at the 95% confidence level. The field results correlated well with data obtained from the ICP-OES and WDXRF laboratory techniques. In the current chapter, the determination of the spatial and depth distribution of copper in soils of mango and citrus orchards from Hoedspruit (Limpopo) is described. Copper concentrations were measured using the pXRF spectrometer and the enrichment factor (EF) was calculated to determine the contamination level. Sequential extraction (BCR) was applied to representative soil samples to assess the mobility and bioavailability of copper in the contaminated soils. The ecological risk associated with the copper contamination was determined using the RAC. A pot trial was conducted to investigate copper uptake by mango trees. Finally, a phytoextraction field experiment was carried out in a contaminated mango orchard using *R. officinalis* and *O. labiatum* as phytoremediators.

5.2 MATERIALS AND METHODS

5.2.1 Description of the study area

Hoedspruit (Limpopo) is located in the north-east region of South Africa and has a sub-tropical climate. Many mango and citrus orchards, located in the surroundings of the R527 to the south-west of the town, have been developed. Data collected from 2000 to 2012 indicated that average maximum monthly temperatures fluctuate between 26 °C (June and July) and 33 °C[11] (December, January and February). The minimum monthly temperature varies from 9 °C (July) to 21 °C (January and February). The region received from 1999 to 2004 an average annual rainfall of 900 mm with a maximum of 1463 mm in 2000 and a minimum of 629 mm in 2002 (Barrett *et al.*, 2006). The Hoedspruit area is composed of arid and semi-arid land, mostly rocky and sandy or clay loam and the top soil is shallow (Barrett *et al.*, 2006). The natural savannah biome found in the area is dispersed between and around a large number of farms. The mango and citrus orchards from three farms (J, K and T), which formed part of the study, are indicated in Figure 5.1. The names of these farms will not be revealed.

The samples were coded according to the following method: the farm is coded J, K or T, the type of orchard (M = mango and C = citrus), followed by the number of the plot (example C3). Thereafter follows the specific sample number i.e. C3-R1-3 where R1 = number of row (row 1) and 3 = number of the tree. The code JMC3-R1-3 therefore indicates that the sample originates from a mango orchard (specifically Orchard C3) on Farm J and was selected from the third tree in the first row.

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[11] www.worldweatheronline.com/hoedspruit-weather-averages/limpopo/za.aspx. [accessed]: 30/07/2016.

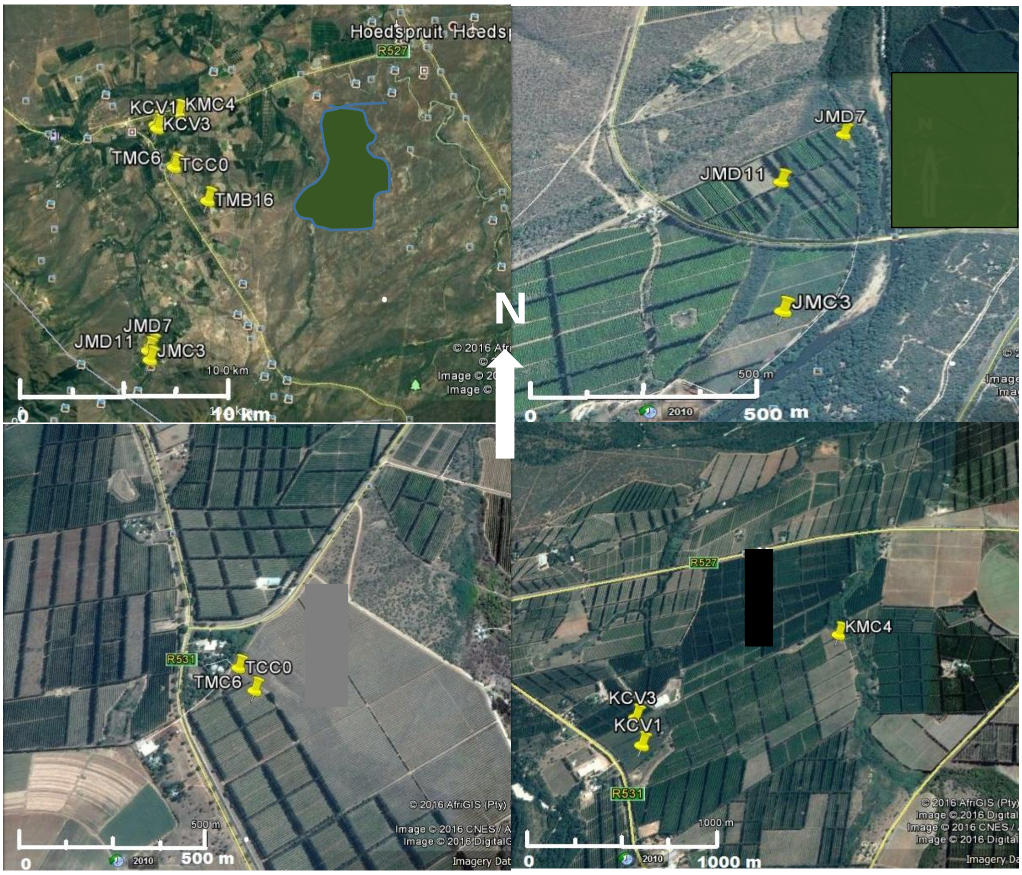

Figure 5.1: Location of farms (J, K and T) and orchards (JM, KM, KC, TM and TC) in Hoedspruit (Limpopo, South Africa), that were sampled to determine the levels of copper contamination in the soils. Scale bars indicate distances on the images. Direction North is indicated for all images.

5.2.2 Field measurements and soil sample collection

Copper concentrations in mango and citrus orchards soils were screened *in situ* using the pXRF spectrometer (Niton XL 3t GOLDD^{TM})+ as described in Chapter 4. The device was calibrated in the mining mode, since high concentrations of copper were expected in the soils and a measurement time of 3 min was applied. Copper concentrations were measured in soils under the trees, as well as in the corridors between the tree lines. Measurements were made of surface soil (0 - 20 cm) at intervals of three, five, eight, ten or fifteen trees, depending on the size of the orchard. At the measurement points, the soil was exposed by removing plants and vegetation debris. The soil was loosened and levelled before the reading was taken (Figure 5.2). The GPS coordinates of the plots were recorded. A total of 170 measurements were made in six mango orchards, including in JMC3, JMD7 and JMD11, TMB16 and TMC6, and KMC4 belonging to farms J, T and K, respectively. A total of 109 measurements were made of soils of citrus orchards (KCV0, KCV1 and KCV3) on Farm K and in Orchard TC belonging to Farm T.

Figure 5.2: *In situ* analysis of soil between the rows of trees in a citrus orchard using the pXRF spectrometer

5.2.3 Calculation of the enrichment factor for assessment of orchard soil contamination

Copper concentrations determined by field measurement using the pXRF spectrometer were used to calculate an environmental index to determine the extent of copper pollution of the orchard soils. The enrichment factor (EF), as proposed by Buat-Menerd and Chesselt (1979), was selected for the assessment. The index is expressed as follows:

$$EF= \frac{C_n(\text{sample}) / C_{ref}(\text{sample})}{B_n(\text{background}) / B_{ref}(\text{background})} \qquad \text{Equation 5.1}$$

where C_n is the concentration of the contaminant in the sample, C_{ref} is the concentration of the reference element in the sample, B_n is the background concentration of the contaminant and B_{ref} is the background concentration of the reference element.

The EF value is used to assign the level of contamination according to the degree of enrichment into five categories: an EF < 2 corresponds to deficiency to minimal enrichment, 2 < EF ≤ 5 corresponds to moderate enrichment, 5 < EF ≤ 20 corresponds to significant enrichment, 20 < EF ≤ 40 corresponds to very high enrichment and EF > 40 is an indication of extremely high enrichment.

In this study, high concentrations of aluminium were consistently found in the soil, and the metal was therefore selected as a reference element for the calculations of the EFs (Saleem *et al*., 2015). Aluminum is one of the elements commonly used as reference, in addition to iron, manganese, scandium and titanium. The average concentrations of copper and aluminum in the control samples were used as the background values for these elements in the study area. This same approach was followed in a study conducted in Lagos (Nigeria) to profile the contamination of soil by heavy metals at an old power generation station (Adeyi & Torto, 2014).

5.2.4 Analysis of orchard soils at different depths

Drill soil samples were collected from Orchard JMC3, which was identified as the most severely contaminated with copper. A Stihl drilling device (Germany) was used to collect the drill samples from four sites (D1, D2, D3 and D4) in Orchard JMC3. Concentrations of copper in the drill soil samples were determined on-site using the pXRF spectrometer. Drill soil samples were removed and measurements were taken at the soil surface as well as at intervals of 0.25, 0.50, 0.75, 1.0, 1.25 and 1.5 m. Measurement was discontinued when the copper concentration was near the background level or no longer detected by the pXRF spectrometer.

5.2.5 Determination of pH and electrical conductivity of soils from the orchards

Soil samples were collected from sites where the copper concentration was relatively high compared to the background. Fifteen soils samples were randomly selected from the mango orchards (JMC3, JMD7, JMD11 and KMC4) for the determination of pH and EC. A combined meter (Martini Instruments, MI 805, Romania) was used for the determination of the pH and EC of the orchard soil samples. Three buffer solutions (pH 4.00, 7.00 and 10.0) were used to calibrate the instrument for pH measurements, while a solution of 1415 µS/cm provided by the supplier was used to calibrate the device for the EC measurements. The soil samples were suspended in water at a ratio of 1:5 (v/v) and shaken for 30 min before the measurements were taken (Blakemore & Daly, 1987). Triplicate measurements were made by introducing the electrode into the supernatant after the suspension had settled.

5.2.6 Mobility and bioavailability of copper in orchard soils

The sequential extraction scheme proposed by the European Union (BCR) was applied to evaluate the mobility and bioavailability of copper in the orchard soils. The sequential extraction scheme includes four steps, of which the details have been provided in Section 4.2.8. The first three steps exploit the increasing extraction power of three solvents (acetic acid, hydroxylammonium chloride, and hydrogen peroxide combined with ammonium acetate) to selectively isolate the exchangeable, reducible and oxidisable copper fractions from soils. The last step consisted of total digestion of the residue obtained from step 3 using a hydrofluoric (2 mL) and nitric acid (3 mL) mixture to determine the residual fraction. Percentages of total copper in each of the fractions were calculated to evaluate the degree of mobility and bioavailability of copper in the orchard soils. Iron, which was found in high concentration and with little variation, was selected as a reference element for comparison in this mobility study.

5.2.7 Risk assessment of copper pollution of the orchard soils

The ecological risk of copper in the mango and citrus orchards was assessed from the results of the fractionation of the metal in soils. Ecological risk levels associated with copper contamination of the orchard soils were obtained from the application of the RAC. The degree of contamination related to the RAC value is presented in Table 4.2 (Chapter 4). The exchangeable fractions resulting from the BCR sequential extraction of the most contaminated soils from three mango orchards, JMC3, KMC4 and TMB16, were selected for the evaluation of the risk caused to the environment by the copper contamination.

5.2.8 Investigation of copper uptake by mango trees

A pot trial was conducted to determine the degree of absorption and accumulation of copper in roots, twigs and leaves of mango trees. Solutions of copper(II) sulfate pentahydrate (AR) ($CuSO_4 \cdot 5H_2O$) (Merck, Johannesburg, South Africa) (250, 500 and 1000 mg/L) were prepared in distilled water, corresponding to copper(II) concentrations of 65, 130 and 260 mg/L, respectively. Twenty healthy six month-old mango trees grown in potted soils (approximately 4 L) were purchased from Malelane Nursery (Mpumalanga, South Africa) for the trial in a greenhouse. The plants were divided into four groups of five plants, and were randomly arranged in the greenhouse. The first group received distilled water (control), the second 65 mg/L of the Cu(II), the third 130 and the fourth 260 mg/L. Each individual plant was watered with 150 mL of the corresponding solution daily, for sixty days. This volume of distilled water and $CuSO_4 \cdot 5H_2O$ corresponded to the maximum quantity that could be held by the potted soils without drainage. The plants were harvested after sixty days.

Leaves, twigs and roots were separated, washed under running water for 5 min and rinsed three times using deionised water (Ataabadi *et al.*, 2012). The fresh plant parts were dried in an oven at 70 °C for 48 h. The dried plant parts were then pulverised. A mass of 0.250 g of the plant material was microwave digested with 5.0 mL of nitric acid (65% w/w). The resulting digests were transferred to 50 mL volumetric flasks and diluted with deionised water. The copper concentrations of the plant parts were determined using ICP-OES as described in Section 3.2.4.

5.2.9 Phytoremediation trial of copper-contaminated soils from the orchards

A phytoremediation trial was conducted in the mango orchard JMC3 using two plant species. *Rosmarinus officinalis* and *Ocimum labiatum.* Plants (25 each) approximately 20 and 10 cm tall, respectively were obtained from Random Harvest Nursery (Honeydew, Johannesburg, South Africa). The plants were planted between mango trees in the orchard most contaminated with copper on 5 April 2015. Control plants were also grown on uncontaminated soils. Five plants of each species were randomly selected and harvested on 13 June 2016. Soil samples were taken from each of the growing sites sampled. Roots, twigs and leaves of the plants were separated, washed for 5 min under running tap water and rinsed three times using deionized water. The fresh roots, twigs and leaves of the plants were dried in an oven at 70 °C for 48 h and milled. Copper concentrations of the soils and plant parts were determined using ICP-OES after acid microwave digestion as described in Section 3.2.4.

Accumulation of copper, taken up from the soils, in roots, twigs and leaves was evaluated using the calculated bioaccumulation factor (BAF). The translocation factor (TF) for copper in twigs and leaves of each plant was calculated and used in combination with the BAF to determine the phytoextraction capacity of plants for copper. These factors were obtained as follows:

$$\mathrm{BAF} = \frac{C_{\mathrm{part}}}{C_{\mathrm{soil}}} \qquad \text{Equation 5.2}$$

$$\mathrm{TF} = \frac{C_{\mathrm{part}}}{C_{\mathrm{root}}} \qquad \text{Equation 5.3}$$

where C_{part} is the concentration of copper in the plant part, C_{soil} is the copper concentration in soil and C_{root} is the concentration of copper in the roots of the plants.

5.3 RESULTS AND DISCUSSION

5.3.1 Copper levels in mango and citrus orchard soils from Hoedspruit

Soils from mango and citrus orchards (JMC3, JMD7 and JMD11), (KMC4, KCV0, KCV1 and KCV3) and (TC, TMC6 and TMB16) from Farms J, K and T, respectively, were screened for copper using the pXRF spectrometer. The analyses were carried out under the trees and in the corridors between the rows. The concentrations of copper obtained in soils of the mango orchards are presented in Figure 5.3.

The average concentrations of copper measured in soils of the mango orchards from Farm J were 230 mg/kg (Orchard JMD7), 240 mg/kg at JMD11and 580 mg/kg at JMC3. For Farm T, the average concentration of copper in soils was 450 mg/kg in Orchard TMB16 and 320 mg/kg in Orchard TMC6. The only mango orchard (KMC4) investigated at Farm K revealed an average concentration of 350 mg/kg (Table 5.1). Maximum copper concentrations of 670, 970, 980, 1200, 2200 and 2600 mg/kg were measured in soils from orchards JMD7, KMC4, JMD11, TMC6, JMC3 and TMB16, respectively. The copper concentrations within and between orchards, rows and in corridors between the rows varied extremely as indicated by the standard deviations (Table 5.1). The average copper concentrations measured in soils between the rows of the mango trees were always lower than those under the trees.

Four citrus orchards (TC, KCV0, KCV1 and KCV3) from Farms T and K were investigated. The concentrations of copper obtained in soils of the citrus orchards are presented in Figure 5.4. The average concentrations of copper obtained in the soils were 80, 120 and 140 mg/kg for orchards KCV3, KCV1 and KCV0, respectively (Table 5.1). For Orchard TC, the average copper concentration was only 39 mg/kg. The highest levels of copper in the citrus soils were found for Orchards KCV1 (450 mg/kg) and KCV0 (310 mg/kg). Citrus orchard KCV0 was found to be the most contaminated, and TC the least contaminated. For these orchards, the copper concentrations measured under the citrus trees were lower than those measured in the corridors between the rows (Table 5.1). Concentrations of copper in soils from the citrus orchards were lower than those from the mango orchards. Large differences in copper concentrations were observed between the mango and citrus orchards, and also within each orchard.

Figure 5.3: Copper concentrations in soils of mango orchards from Hoedspruit measured using the pXRF spectrometer. Name of orchards (i.e. JMC3) J = name of the farm, M= type of farm (mango) and number of the plot (C3). Name of the soils (i.e. C3-R1-3): C3 = plot number, R1 = number of row (row 1) and 3 = number of the tree

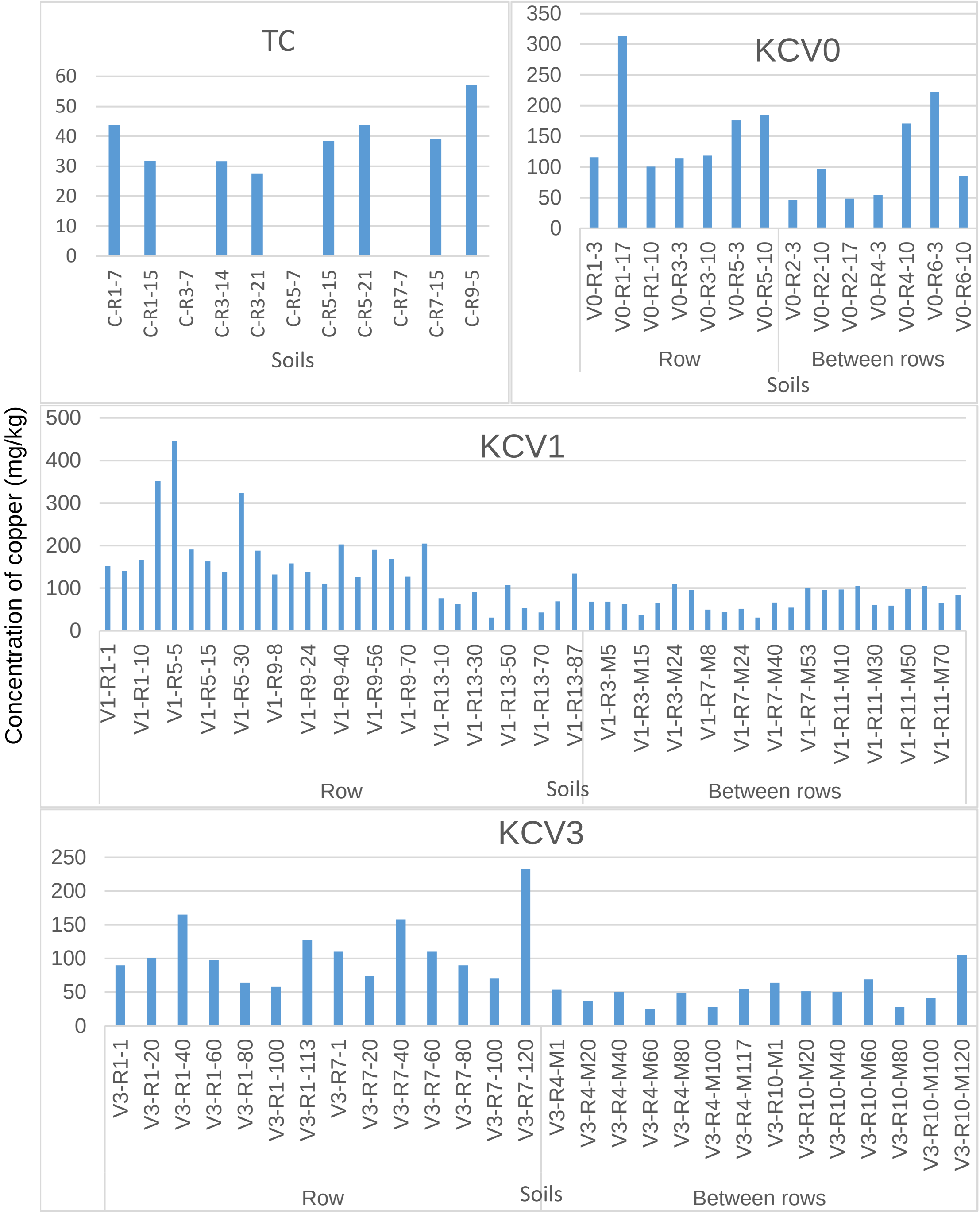

Figure 5.4: Concentrations of copper in soils from citrus orchards TC, KCV0, KCV1 and KVC3 measured using the pXRF spectrometer

Table 5.1: Mean copper concentrations (n=3), standard deviations (SD), and concentration ranges (mg/kg) in mango and citrus orchard soils in Hoedspruit

	Orchard	Rows			Between rows			Rows + Between rows		
		Average	SD	Range	Average	SD	Range	Average	SD	Range
Mango	JMC3	730	480	119 - 2170	410	310	130 - 1050	580	430	120 - 2200
	JMD7	270	170	52 - 670	140	72	28 - 240	230	160	28 - 670
	JMD11	290	270	80 - 980	230	190	71- 490	240	240	71 - 980
	TMB16	510	560	28 - 2600	140	120	26 - 360	450	530	26 - 2600
	TMC6	380	270	120 - 1200	230	210	54 - 660	320	250	54 - 1200
	KMC4	450	300	26 - 970	230	150	55 - 550	350	260	26 - 970
Citrus	TC	NA	NA	NA	NA	NA	NA	39	9.0	28- 57
	KCV0	140	74	46 - 310	100	100	46 - 220	140	74	46 - 310
	KCV1	160	91	31 - 450	73	24	31 - 110	120	80	31- 450
	KCV3	110	48	58 - 230	50	21	25 - 110	81	47	25 - 230

NA = Not analysed

The large differences in copper concentration observed in the orchards are probably due the properties of individual soils, spraying technique used, frequency of spraying and the age of the orchards. Wightwick *et al.* (2008) attributed differences in copper concentrations observed in soils within an orchard, which was sprayed using the same copper concentration of the fungicide and frequency, could be attributed to local variation of soil properties and topography. High concentration of copper observed in rows of mango and citrus trees, compared to concentrations in the corridors between rows, may be related to the spray technique used. Farmers in Hoedspruit commonly use copper oxychloride solutions for the treatment of fungal diseases. The solution is applied onto the trees using a sprayer pulled by a tractor (Figure 5.5).

The sprayer, assisted by a fan delivering rapid air movement, vaporises enough pesticide solution to reach the target pest (Endalew *et al*., 2010). Deposition of droplets of the sprayed solution mainly occurs on the leaves, but also takes place on the ground below the trees. Endalew *et al.* (2010) reported percentages of deposition of 55.8% and 54.2% for leaves, and 9.5% and 12.2% for the ground, when using a normal orchard sprayer arrangement with ISO TXA 80 08 hollow cone nozzles and a boom sprayer nozzle arrangement with ISO TT 110 03 flat fan nozzle, respectively. Significant amounts of the pesticide drips to the ground in the immediate surroundings of the tree, onto the leaf litter covering the soil. This may explain the relatively higher levels of copper under the trees compared to soils between rows found in the mango orchards. Soils under the plant canopy are left largely undisturbed, while soils between rows are frequently ploughed to remove weeds that restrict the movement of the tractor (Mackie *et al*., 2012).

Figure 5.5: Spray technique used by farmers in Hoedspruit to apply copper oxychloride to trees

Correlation between copper accumulation in orchard soils and the age of the orchards has been reported elsewhere. Park and Cho (2011) investigated copper accumulation in apple orchard soils that had been sprayed repeatedly using Bordeaux mixture for 15 years. Copper concentrations of approximately 9, 10.5, 13 and 16.5 mg/kg were reported in soils for apple orchards aged 1-3, 4-6, 7-9 and 10-15 years, respectively (Park & Cho, 2011). However these concentrations were very low compared to those found in this study. The large differences in copper levels in soils within the mango and citrus orchards could be explained by the difference in age and spray frequency. According to Mr C. Redelinghuys (2016), mango and citrus farmers use copper oxychloride ($3Cu(OH)_2•CaCl_2$) at a concentration of 2.5 g/L. Mango orchards are sprayed once every four weeks, while only young citrus plants are sprayed once a year. The highest copper level was found in the soils of mango orchard JMC3, the oldest mango orchard studied (more than 30 years), followed by KMC4

(18-years old) and the younger orchard, TMB16. The citrus orchards studied were younger than the mango orchards.

The copper concentrations measured in the orchards were higher than the average (34 mg/kg) from uncultivated soils found in the vicinity of the orchards, and used as a control. Moreover, 86% of the mango orchard soils and 42% of the citrus orchard soils had concentrations above 100 mg/kg, which is the maximum copper concentration permissible in South African agricultural soils (DNHPD, 1991; Herselman *et al*., 2005). It has been found that 67% of the mango orchard soils and 28% of the citrus orchard soils analysed had copper concentrations higher than 120 mg/kg, which is the recommended maximum threshold level for copper for South African soils (Herselman *et al*., 2005). These high concentrations of copper in soils from the orchards could be ascribed to the application of copper-based fungicides used to combat fungal infestation of orchards. Copper accumulation in soils following long-term usage of copper-based fungicides has been reported elsewhere. Conradie (2004) analysed nursery soils from vineyards in the Western Cape Province (South Africa) that had been sprayed with copper-based fungicides over a period of 5 to 30 years. A maximum average copper concentration of 20 mg/kg was reported, which is substantially lower than the average concentrations found in the mango and citrus orchard soils in Hoedspruit. The values determined in the current study are comparable to those reported by other researchers for agricultural soils where copper-containing fungicide formulations were applied. Long-term usage of a metal-based pesticide in apple orchards in Japan resulted in copper accumulation ranging from 21 to 931 mg/kg in the soil (Aoyama & Tanaka, 2013). Similarly, copper concentrations ranging from 21.8 to 141 mg/kg were reported for apple orchards of 5 to 45-years old in China (Wang *et al*., 2009). High levels of copper (200 – 500 mg/kg) were present in a large tract of land in France (Brun *et al*., 1998), 250 mg/kg in 20 to 30-year old apple orchards in Australia (Pietrzak & McPhail, 2004) and 280 – 340 mg/kg in avocado orchards in New South Wales (Australia) (Merrington *et al*., 2002). Concentrations of copper exceeding 1300 mg/kg have been found in vineyard soils from Brazil, which had been repeatedly sprayed with copper-containing fungicides for more than 15 years (Nachtigall *et al*., 2007). Higher copper levels (1508 - 3215 mg/kg) than those obtained in this study were reported for soils from vineyards in Brazil that had been treated with copper-based fungicides for over 50 years (Mirlean *et al*., 2007).

5.3.2 Assessment of copper contamination of the mango and citrus orchards

Enrichment factor (EF) is a common index used to measure levels of contamination of soil (Zhao *et al*., 2012; Ololade, 2014; Saleem *et al*., 2015; Matong *et al*., 2016). Adeyeye *et al.* (2006) also used the calculated EFs to evaluate the degree of soil contamination in a 24-year old cocoa farm that had been regularly fumigated using copper-based fungicides. However, relatively little contamination was found by the researchers. The EF calculated for the mango and citrus orchards soils for copper established the magnitude of contamination of soils for each orchard. Soils from the mango orchards were more contaminated by copper than the citrus. Fifty percent of the 290 soil samples taken from the mango orchards had EFs of between 5 and 165. These values correspond to significant to extremely high enrichment. For the citrus orchards, only 8% of the 106 soil samples analysed displayed significant enrichment, while 92% were deficient in copper or scored values indicating minimal, or moderate enrichment. The mango orchard, JMC3, of which more than 40% of soils scored values reflecting extremely high enrichment (EF > 40), 32% very high enrichment (20 < EF < 40) and 28% significant enrichment (5 < EF < 20). This orchard was considered the most contaminated of all the orchards investigated (Figure 5.6). Citrus orchard TC was the least contaminated, since all of the soil samples yielded an EF below 5, indicating copper deficiency, to minimal or moderate enrichment with copper (Figure 5.6). Moderate enrichment was the highest level of contamination reached for the citrus orchards. According to the percentage of soils with an EF above 5, corresponding to significant, very high and extremely high enrichment, soils from mango orchards corresponded to the following contamination order: JMC3 (100%) > TMC6 (81%) > JMD7 (77%) > JMD11 (75%) > TMB16 (67%) > KMC4 (51%) (Figure 5.6). For citrus orchards, the contamination order was as follows: KCV0 (14%) > KCV1 (12%) > KCV3 (3%) > TC (0%).

The percentages of soils from the mango orchards having EFs above 5 were calculated and used to compare soil contamination levels from Farms J, T and K. Mango orchard soils from Farm J were the most contaminated (88%), followed by Farm T (74%) and Farm K (51%) (Figure 5.7).

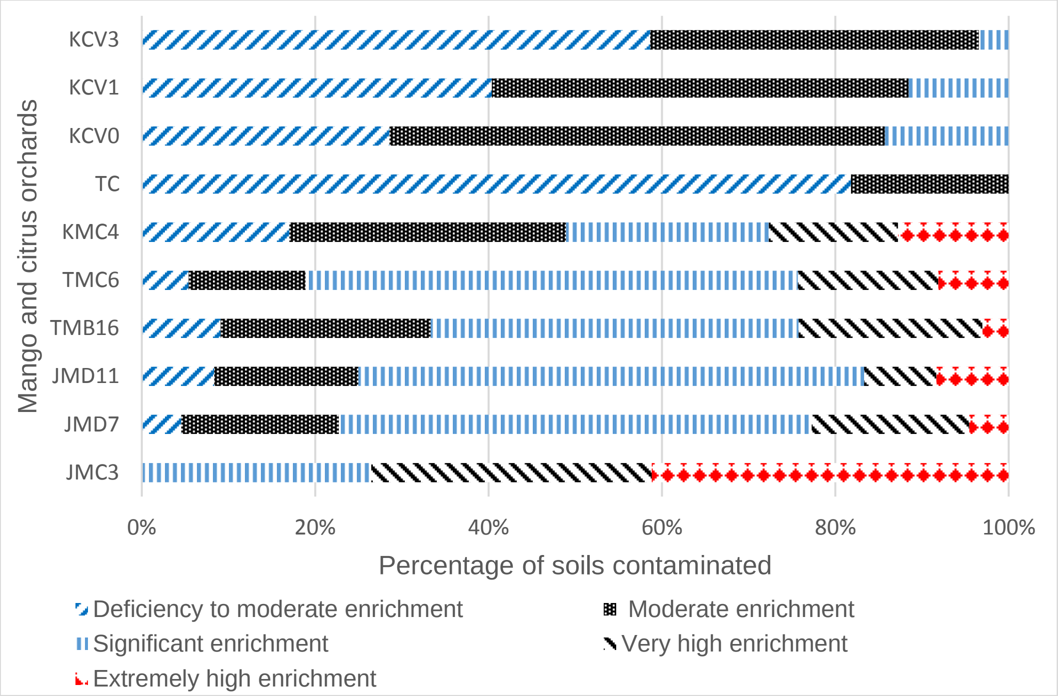

Figure 5.6: Levels of copper contamination according to values obtained from the calculation of the EFs, and percentages of contaminated mango and citrus orchard soils with EFs above 5

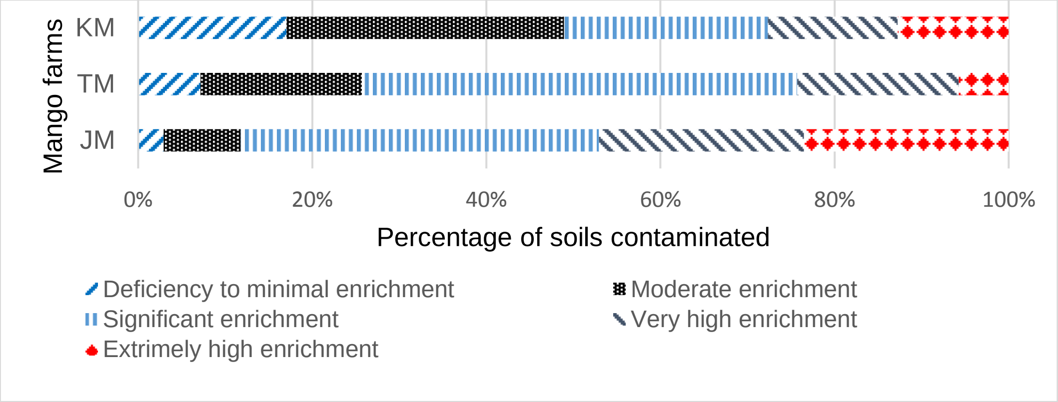

Figure 5.7: Comparison of levels of copper contamination of soils found in farms J, K and T, in Hoedspruit

Total concentrations of copper determined in the mango and citrus orchard soils using the pXRF agreed with the calculated EFs. High levels of copper in the soils corresponded to extreme enrichment of soils with copper, while low concentrations corresponded to deficiency or minimal contamination. The enrichment of soils with copper substantiates the anthropogenic intrusion of the element. The copper contamination of the soils in Hoedspruit is essentially due to repeated application of copper salts over many years. The variation in the levels of contamination observed in the soils from the orchards are ascribed to local variation of soil properties and topography, the age of orchards and the spray technique used.

5.3.3 Changes in copper accumulation with the depth of the orchard soils

Changes in the soil copper concentration with depth was investigated by drilling four auger holes in the mango orchard, JMC3. The results obtained are presented in Table 5.2. A decrease in the copper concentration from the soil surface to a depth of 0.75 m was observed for auger holes D1, D2 and D3. For these holes the copper concentrations were near the background value or lower than the LOD in the case of the sample from 0.75 m. A decrease in copper concentration was also observed for auger hole D4, from 766 mg/kg at the soil surface to 165 mg/kg at 0.75 m, but the concentration was found to increase to 700 mg/kg at a depth of 1.0 m. It then decreased again to 198 mg/kg at 1.50 m. Although a relatively high concentration was found (198 mg/kg) at this depth, drilling could not continue due to the presence of wet clay, which caused the drill bit to become encased in the soil.

The accumulation of copper on the surface of the soil is probably due to the high content of organic matter comprising orchard litter (Nascimento & Fontes, 2004). The distribution pattern of copper in the mango orchard soils from Hoedspruit agreed with results obtained by other researchers who conducted similar studies. Silva *et al.* (2012) investigated soil metal contamination of mango orchards in Petrolina (Brazil), which were sprayed with copper-based fungicide for 6 to 26 years. They discovered an accumulation of copper on the surface of the soils, which decreased with an increase in depth. The author ascribed the accumulation to a strong interaction of copper with the organic matter, due to selective adsorption of copper to the surfaces of colloidal particles (Nascimento & Fontes, 2004). The accumulation of copper at 1.00 m depth for auger hole D4 may be due to the presence of clay, which stopped the movement of the drill bit. The high concentration observed at this depth suggested that copper probably leached slowly from the surface to build up in the clay layer. Soil clay is also a major factor in metal adsorption. A high clay content in soil provides more adsorption sites to dissolved metals (Reichman, 2002).

Table 5.2: Concentrations of copper (mg/kg) in soil at different depths in Orchard JMC3

Depth (m)	D1	D2	D3	D4
0	432	725	250	766
0.25	126	350	198	623
0.50	49.0	50.0	< LOD	264
0.75	45.0	32.0	< LOD	165
1.00	NA	NA	NA	700
1.25	NA	NA	NA	360
1.50	NA	NA	NA	198

NA = Not analysed

5.3.4 Mobility of copper in the orchard soils

The BCR sequential extraction scheme was applied to nine soils of low, medium and high copper contamination from the most polluted mango orchards (JMC3, TMB16 and KMC4) to yield four soil fractions F1 (exchangeable) F2 (reducible), F3 (oxidisable) and F4 (residual). The concentrations of iron (reference element) and copper were determined in each of the fractions using ICP-OES as described in Section 4.2.8.

Iron was found predominantly in the residual fraction. The element could not be detected in the exchangeable fraction and only small portions were present in the reducible and oxidisable fractions. The same trend was observed for all of the individual soils (Figure 5.8). The distribution pattern of iron in the soil fractions suggests that the element is strongly bound to components of the surface soil in the orchards, consequently it could not be mobilised. Such strong attachment of the element to the soil particles demonstrates a lithogenic origin (Yusuf, 2006; Sundaray *et al*., 2011). This conclusion is supported by the low bioavailability (ranging from 2.5 to 13%) calculated for the element in the mango orchard soils.

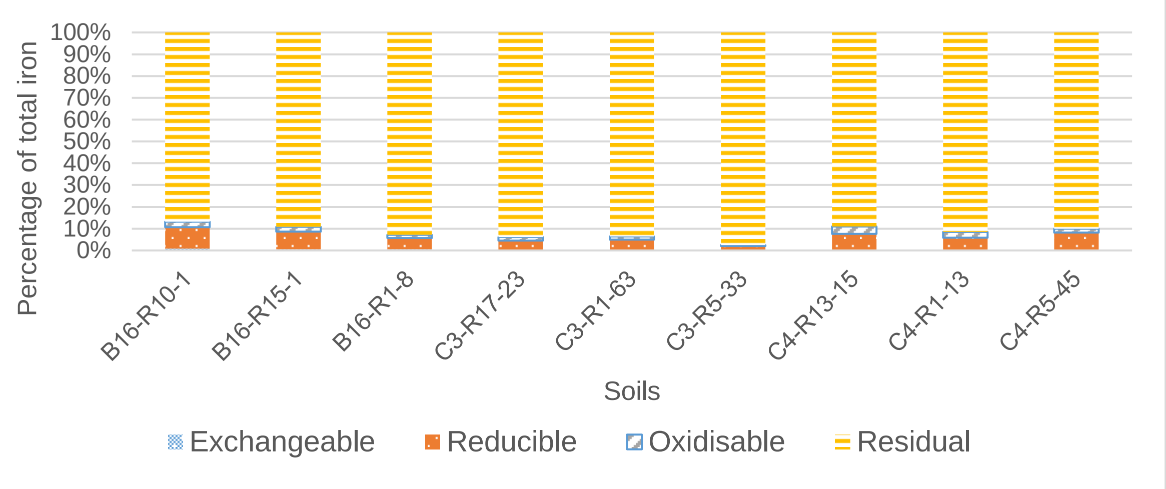

Figure 5.8: Distribution of iron (%) amongst fractions obtained following BCR sequential extraction of soils from the contaminated orchards

Copper was found mostly in the oxidisable fraction (32%), followed by the residual fraction (27%), the reducible fraction (26%) and the exchangeable fraction (15%). The average BI calculated was 65% and ranged from 28 to 89%. The relatively low percentage in the residual fraction, combined with the high BI, indicate that copper is mobilisable in the mango and citrus orchard soils. However, most of the mobilisable copper portion is confined to the reducible and oxidisable fractions. Differences were observed within the fractionation results of individual soils. A high copper content in the exchangeable fraction coincided with low proportion of copper in the residual fraction. This variation was particularly pronounced with soils B16R10T1, B16R15T1, C3R5T53, KR13T15 and KR1T13 (Figure 5.9). Soil B16R15T1 yielded the most mobile fraction for copper, while the least mobile was determined to be fraction from soil samples KR13T15 and KR1T13.

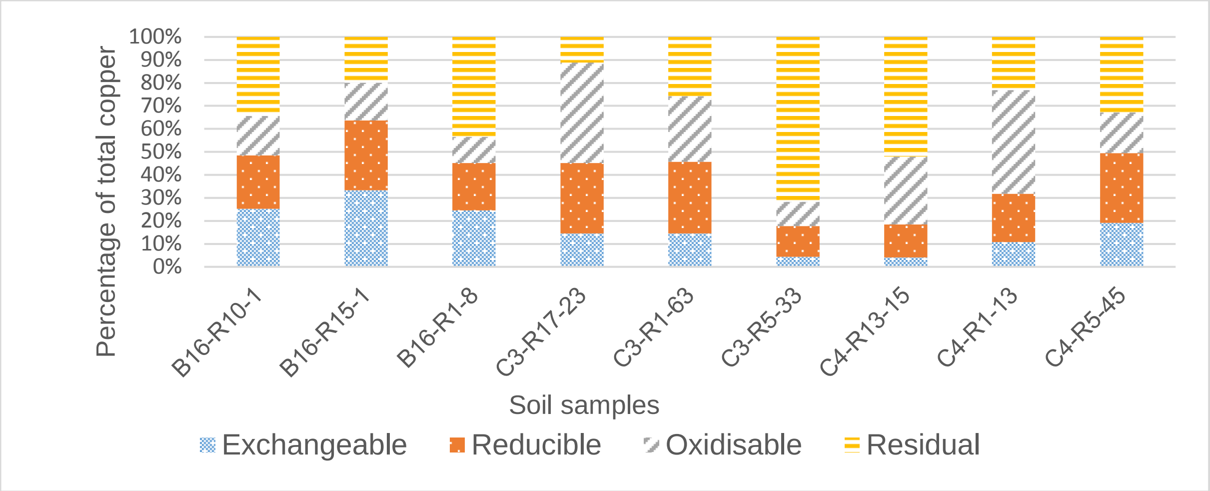

Figure 5.9: Distribution of copper (%) amongst fractions obtained after BCR sequential extraction of soils from the contaminated orchards

Comparing the mobility of copper in soils from the mango orchards JMC3, KMC4 and TMB16, it was found that the percentage copper in the exchangeable fraction followed the order KMC4 < JMC3 < TMB16 (Figure 5.10). This implies that copper is more mobile in soil from Orchard TMB16, than in JMC3 and KMC4. The potential mobility of copper was obtained by summing the copper concentrations in F1, F2 and F3. The following order was found, KMC4 < TMB16 < JMC3, whereas the residual fractions were in the reverse order, JMC3 < TMB16 < KMC3.

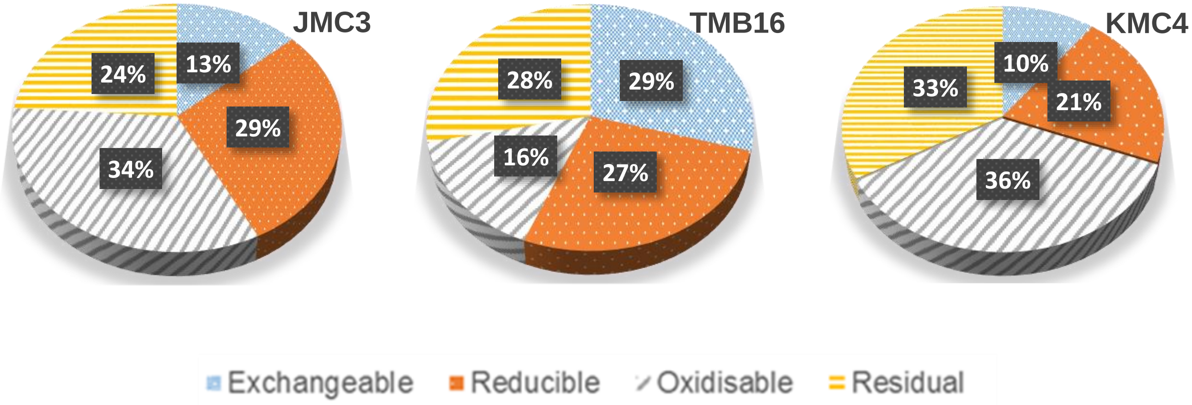

Figure 5.10: Average percentages of copper in fractions prepared through sequential extraction of soils from mango orchards JMC3, TMB16 and KMC4

The relatively high levels of copper in the oxidisable fraction, followed by the reducible fraction, in fractions prepared from soils from mango orchards in Hoedspruit corresponded to the trend generally found in soils from contaminated vineyards with long histories of copper-based fungicide application (Komárek *et al*., 2010). This also confirmed that copper is immobilised readily in soils by iron and manganese hydroxides and oxides, as well as by soil organic matter (Komárek *et al.*, 2010).

Copper is known to readily bind in the oxidisable fraction by forming stable sulfides and copper humic acid complexes (Schramel *et al.,* 2000). The release of copper from the oxidisable fraction depends on changes in the environment that lead to oxidising conditions (Sarkar *et al*., 2014). The high percentage of copper in the oxidisable fraction is probably due to the high content of organic matter in the soil. Although the organic matter content of the soils was not determined, a high content was assumed since the soils are continuously enriched with dead leaves, branches and fruit, which are incorporated into the soil over several years. The high levels of copper in the exchangeable fractions and the low concentrations in the oxidisable fractions in soil from Orchard TMB16 were the reverse of results obtained for fractions prepared from Orchards JMC3 and KMC4. The low copper concentrations in exchangeable and high concentrations in oxidisable fractions are thought to result from differences in organic matter content in the mango orchards soils, since solid and dissolved organic matter in soils substantially affect the mobility of copper (Janoš *et al*., 2010). Copper is strongly bound to soil oxides and organic matter in soils from Orchards JMC3 and KMC4, which consequently limits the mobility of the element. For these orchards, the risk of migration of copper into the groundwater is low. However, the risk of migration of copper into the groundwater is relatively elevated in soils from Orchard TB16, because high copper concentrations were found in the exchangeable fraction and relatively low levels in the oxidisable fraction prepared from that soil.

The anthropogenic origin of copper in soil from mango orchards in Hoedspruit was confirmed by the high concentrations of the element in the exchangeable, reducible and oxidisable fractions compared to low concentrations in the residual fractions. However, the mobility of copper was found to be limited, because the element was mainly bound to soil manganese and iron oxides, humic acid and sulfide complexes present in the organic matter.

5.3.5 The pH and elecrical conductivity of the orchard soils

The pH and EC of the mango orchard soil samples were determined and correlated to the percentage of copper in the exchangeable fractions, obtained using the BCR sequential extraction scheme. The correlation was done to determine the influence of pH and EC on the mobility of copper in the mango orchard soils (Figure 5.11)

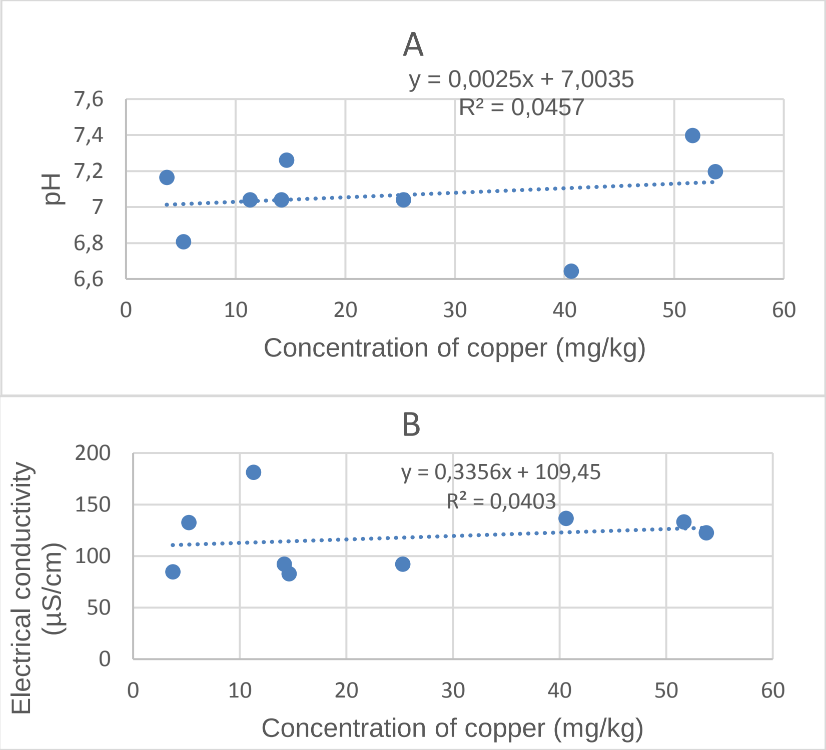

Figure 5.11: Values for pH (A) and EC (B) plotted as a function of copper concentration (mg/kg) in the exchangeable fraction, following sequential extraction of soil samples to determine if these parameters influence copper mobility in the soil

The mean pH obtained for the mango orchard soils was 7.1, with fluctuations between 6.6 (B16-R15-1) and 7.4 (C3-R1-63). The mean EC for the mango orchard soils was 118 µS/cm, and varied from 83 to 181 µS/cm. The EC values for the mango orchard soils were low compared to those for natural soils, which varied between 200 and 800 µS/cm (Sadhu *et al.*, 2012). The coefficient of correlation (r^2) of soil pH with copper concentration was found to be 0.046, while that of EC with copper concentration was 0.04. These correlation coefficients obtained demonstrate a poor relationship between the mobile fraction and both the pH and EC of the mango orchard soils. Therefore pH and EC do not influence the mobility of copper in the mongo orchard soils from Hoedspruit and *vice versa*.

Several soil properties, including pH, organic matter and clay content, have been proven to greatly influence metal availability and mobility in soils. In tropical soils, probably because of intensive weathering, pH has been identified as the major factor regulating metal availability and mobility in soils (Rieuwerts, 2007). It is generally accepted that metal mobility and availability in soils increase with a decrease in soil pH, which is represented by a high negative correlation coefficient (Rieuwerts *et al*., 1998; Zheng *et al*., 2012; Li *et al*., 2014). However, in many cases, an absence or poor correlation between soil pH and copper mobility have been reported. Katyal and Sharma (1991) found no correlation between the copper mobile fraction extracted by DTPA and soil pH for 57 soils in India. Instead, the mobility of copper correlated with the soil clay content. Similarly, Gomes *et al.* (2001) found no correlation between soil pH and the availability of copper in seven soils of different chemical and mineralogical characteristics in Brazil. The availability of copper was found to depend on the organic carbon, clay and gibbsite content of the soils (Gomes *et al*., 2001).

Electrical conductivity is an important soil property that reflects the number of ionic species in the soil solution (Mahapatra *et al*., 2013). A high EC implies the presence of a large number of dissolved ions in the soil. Ionic species in the soil solution represent dissolved macro- and micronutrients of plants, as well as soluble toxic contaminants. The EC values measured in the contaminated mango orchard soils in Hoedspruit, which were below the levels of uncontaminated soils, indicate a low content of dissolved copper. This means that copper is mostly adsorbed by soil solid fractions, therefore a large portion of the copper in the soil is not readily available or mobile. Copper mobility in the mango orchard soils in Hoedspruit, which was not found to be influenced by the pH or correlated to the EC of the soils, probably depends on soil properties, including organic matter and clay contents (Yusuf, 2006; Sundaray *et al*., 2011).

5.3.6 Risk assessment of copper contaminated orchard soils

The high concentrations of copper found in the soils of the mango orchards indicated a need to evaluate the ecological risk related to the contamination, as recommended by Schramel *et al.* (2000*)*. The ecological risk of the iron and copper contamination was estimated using the RAC, which refers to the exchangeable fraction obtained following the fractionation of the mango orchard soils.

The RAC values for iron ranged from 0.1 to 0.8, with an average value of 0.4. Iron therefore poses a low overall ecological risk (Table 5.3). This result was expected, despite the high concentration of the element in the soils, because of its low mobility.

Table 5.3: Risk assessment using RAC for sampled mango orchard soils

Soil	Fe	Risk level	Cu	Risk level
B16-R10-1	0.8	L	25	M
B16-R15-1	0.6	L	33	C
B16-R1-8	0.6	L	25	M
C3-R17-23	0.3	L	15	M
C3-R1-63	0.2	L	15	M
C3-R5-33	0.1	L	4	L
C4-R13-15	0.1	L	4	L
C4-R1-13	0.2	L	11	M
C4-R5-45	0.4	L	19	M
Average	0.4	L	17	M

L= Low, M = Moderate and C = Considerable

For copper, RAC values ranged from 4 to 35, with an average value of 17. This average corresponds to a moderate ecological risk for copper, but RAC values ranged from low to considerable for individual soils sampled. The highest ecological risk posed by copper was found for soil B16-R15, which corresponded to considerable risk, while low risk was indicated for samples C3-R1-63 and C3-R17-23. Despite high total copper concentrations and extensive pollution found in the mango orchards in Hoedspruit, the environmental risk associated with copper contamination can be described as moderate. This risk assignment can be ascribed mainly to the retention of copper by the iron and manganese oxides within the reducible fraction, as well as the humic and fulvic complexes in the oxidisable fraction.

5.3.7 Investigation of copper uptake by mango trees

A pot trial was conducted to assess copper uptake by young mango trees growing in soils, which had been artificially contaminated by using copper(II) solution over 60 days. No literature could be found relating to copper accumulation in leaves, twigs and roots of young mango trees. Concentrations of copper in the mango tree parts obtained for the different concentrations applied are presented in Figure 5.12.

Copper concentrations in the plant parts increased with an increasing application concentration of copper(II) and were higher than the concentrations in the control. However, only a slight increase in the copper concentration in the leaves and twigs was found when the application concentration was increased from 130 mg/L to 260 mg/L. In the leaves, the copper concentrations were 1.3, 1.7 and 1.6 times higher when 65, 130 and 260 mg/L of Cu(II) solution was applied. The concentration in the control was 9.03 ± 0.15 mg/kg of copper. For the twigs, the copper concentrations were 10.0 ± 0.3 mg/kg for 65 mg/L, 13.1 ± 0.8 mg/kg at 130 mg/L and 13.5 ± 1.7 mg/kg at 260 mg/L of copper(II) solution. Copper concentrations obtained in the roots were 19.5 ± 0.109, 21.2 ± 7.76 and 33.6 ± 10.1 mg/kg for 65, 130 and 260 mg/L of dosed copper(II) solution, which corresponded to a 5, 6 and 9 times increase in concentration obtained relative to the control (3.78 ± 0.20 mg/kg). The copper concentrations obtained in parts of the mango plants grown in contaminated soils were statistically different ($p < 0.05$) to concentrations in the control plants. Copper concentrations in leaves, twigs and roots were also found to be statically different from each other, regardless of the contamination level of the growing media. However, the average copper concentration in leaves was only 1.1 higher than that of the twigs, whereas the average copper concentration in the roots was approximately twice that in the leaves and twigs. This means that young mango trees predominantly accumulate copper in their roots, rather than in their leaves and twigs. The standard deviations for copper concentrations after triplicate analysis of mango tree parts were generally low, which implies that the method of analysis had good precision.

In the control plants, copper was distributed in the order: roots < twigs < leaves. However, this order changed to twigs < leaves < roots when plants were treated with increasing concentrations of copper(II). Copper concentrations in the mango leaves and twigs for the contaminated soils were lower than the toxicity level (20 - 30 mg/kg), while the concentrations in roots, after applying 65 and 260 mg/L, were within the toxicity range (Vangrosveldt & Clijster, 1994).

Roots did not translocate much copper to the leaves and twigs, since little variation in copper concentration was observed in the plant parts between the control and plants grown in contaminated substrates. However, the mango trees absorbed and accumulated relatively large amount of copper in the roots, since increasingly high levels of the metal were found with increased concentration in the soils when compared to the control.

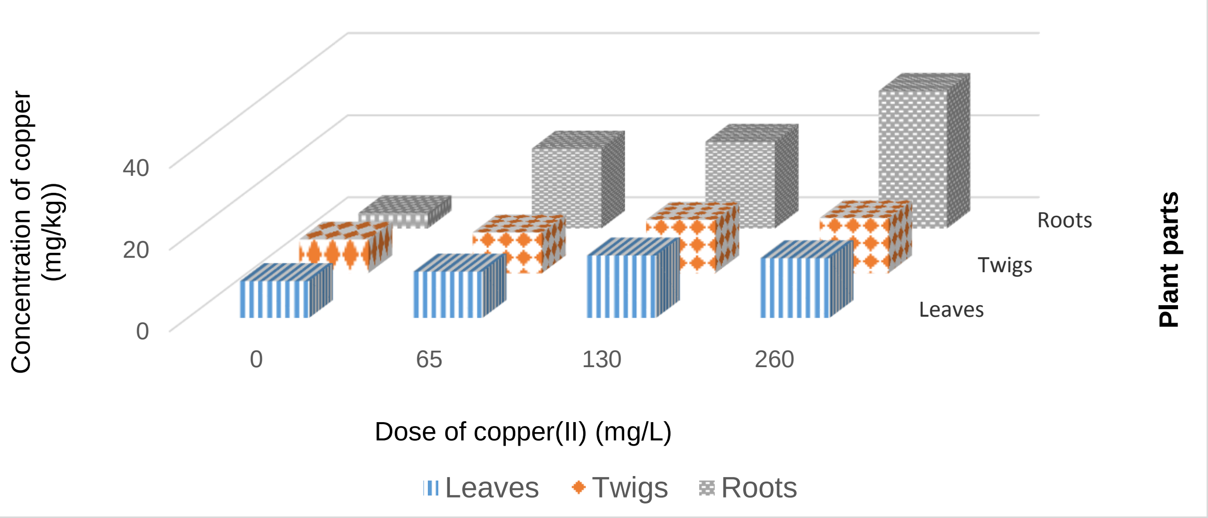

Figure 5.12: Concentrations of copper in mango leaves, twigs and roots when different concentrations of copper(II) solution were applied in a pot trial

5.3.8 Phytoremediation of the contaminated orchard soils

Rosmarinus officinalis and *O. labiaum*, planted in the most copper-contaminated mango orchard (JMC3), and control plants were harvested after 15 months. Samples of soils on which the plants grew were also collected. Soils, roots, twigs and leaves of the plants were analysed to determine their copper content. Results of the analysis are presented in Table 5.4.

In areas where *R. officinalis* was planted, the copper concentrations in the contaminated soils (122 - 243 mg/kg) were 6 to 11 times higher than the concentration in the control soil (21.9 mg/kg). Levels of copper in the roots (7.52 - 23.8 mg/kg) and twigs (16.0 - 37.9 mg/kg) of *R. officinalis* grown on the contaminated soils were lower than their counterparts in the control soil (roots = 26.5 mg/kg and twigs = 8.54 mg/g). However, in leaves of the plants, copper concentrations varied from 73.6 mg/kg to 236 mg/kg, representing 9 to 30 times more than in the leaves of the control plant (Table 5.4). Copper concentrations in the leaves increased with an increase in copper concentration in the soil. A correlation coefficient (r^2) of 0.82 was found between the total copper concentration in the soil and in the leaves, while no correlation was obtained between the plant roots and twigs when compared to the copper concentrations in the soil. *Rosmarinus officinalis* did not accumulate significant amounts of copper in roots and twigs of plants grown in the contaminated orchard soils, since the BAF obtained for each plant part was below 1. However, slight accumulation was observed in the leaves of the plant, because the BAF values equalled (Table 5.5). The TFs obtained for the migration of copper from the roots of the plant to the twigs ranged from 1 to 3 for plants collected from the contaminated soils, which were higher than for the control (0.3) (Table 5.5). In addition, the TFs varied from 6 to 10 for the leaves, and these were also higher than the value determined for the control.

For *O. labiatum*, copper concentrations in the contaminated soils on which the plants grew for 15 months were 6 to 10 times higher than the copper concentration in the control soil (17.2 mg/kg). Copper concentrations in the roots of the plants varied from 6.14 to 21.5 mg/kg comparable to that of the control roots (17.7 mg/kg) (Table 5.4). In twigs, the copper concentrations were 1 to 3 times higher than the concentration obtained in the control (12.4 mg/kg). Copper concentrations in the leaves of plants from the contaminated sites varied from 333 to 675 mg/kg and were 31 to 62 times higher than the concentration found in the leaves of the control plant. Copper concentrations in the leaves were correlated to the concentrations in the soils ($r^2 = 0.71$), since the concentration in the leaves increased with an increase of copper concentration in the soils. No correlation was found between the copper in roots and twigs and soil copper concentrations. The BAFs calculated for copper accumulation in *O. labiatum* roots and twigs were below 1 and were lower than was calculated for the control plant. This means that the plant did not accumulate significant amounts of copper in these plant parts from the soil. Conversely, the BAFs obtained for the leaves of the plants were higher than values found for the control and ranged from 3 to 5, whereas the TFs varied from 22 to 110 (Table 5.6).

Copper concentrations measured in leaves of plants, which were grown on contaminated soils for 15 months were 3 to 9 times higher for *R. officinalis* and 13 to 27 times higher for *O. labiatum*, than the maximum natural concentration of copper in leaves of plants, estimated to be between 5 and 25 mg/kg (Ziarati *et al*., 2014).

Generally, exposure of plants to toxic levels of copper leads to interveinal foliar chlorosis, which may be characterised by the presence of cream/white spots or lesions on the leaves, purpling of leaves and the development of necrotic tips and margins of leaves (Reichman, 2002; Yruela, 2005). Toxic levels may also hinder plant growth and development due to disruption of cellular and molecular processes (Ziarati *et al*., 2014). Despite high leaf copper accumulation, both *R. officinalis* and *O. labiatum* did not display any signs of growth disruption or chlorosis (Figure 5.13). These plants are therefore tolerant to high concentrations of copper and may be potential candidates for phytoextraction of copper from the contaminated mango orchard soils. This assumption is supported by higher copper transfer (TF > 1) from the roots to the aboveground portions of the plants compared to the control. Yoon *et al.* (2006) evaluated the phytoremediation potential of 36 plants, representing 17 species, growing in a site contaminated by metals through prior industrial activities in Jacksonville North (Florida, USA). They concluded that *Phyla nodiflora*, for which the leaf copper concentration was 460 mg/kg, and TF (shoot/root) = 12, was a potential copper phytoremediator. Reports on the performance of *R. offinalis* for phytoremediation of metal contaminated soils are limited in the literature and non-existent for *O. labiatum*. Among the few reports, a high lead concentration of 935 mg/kg and biological factors (BAF and TF) > 1 were obtained for shoots of *R. officinalis*, when the plants were grown hydroponically (Ardalan *et al*., 2014), which were comparable to values obtained in this study for copper. A higher concentration of lead (1507 mg/kg) accumulated in roots of *R. officinalis* than was found for copper in this study (maximum concentration = 23.8 ± 0.4 mg/kg). However, low copper concentrations in shoots (0.1 - 17 mg/kg) and biological factors < 1 calculated for *R. officinalis*, grown on soils highly contaminated with copper (up to 7900 mg/kg) in the Mediterranean areas of France were lower than values determined in this study. Consequently, the authors did not consider *R. officinalis* as a potential candidate for phytoremediation.

Table 5.4: Copper concentrations (mg/kg) in roots, twigs and leaves of *Rosmarinus officinalis* and *Ocimum labiatum,* and in soils on which the plants grew for 15 months in the mango orchards. The standard deviations are provided in brackets

		Control	Site1	Site 2	Site 3	Site 4	Site 5
R. officinalis	Soil	21.9 (1.4)	122 (5)	156 (9)	139 (5)	243 (8.03)	122 (13)
	Roots	26.5 (0.8)	7.52 (0.78)	15.2 (2.9)	11.5 (1.2)	13.8 (1.30)	23.8 (0.4)
	Twigs	8.54 (0.94)	16.6 (1.0)	35.7 (2.4)	16.0 (1.1)	36.2 (2.90)	37.9 (2.4)
	Leaves	7.80 (0.32)	73.6 (1.6)	194 (7)	99.8 (5.5)	236 (14.9)	154 (14)
O. labiatum	Soil	17.2 (1.3)	137 (9)	114 (10)	109 (5)	170 (8.66)	97.6 (11.9)
	Roots	17.7 (0.2)	6.14 (1.02)	18.0 (1.2)	21.5 (3.7)	14.9 (0.944)	14.2 (0.7)
	Twigs	12.4 (0.1)	23.4 (2.3)	14.5 (0.6)	33.0 (3.3)	13.8 (1.64)	15.5 (1.7)
	Leaves	10.9 (0.3)	675 (54)	333 (17)	564 (52)	504.5 (43.5)	333 (23)

Table 5.5: Accumulation and translocation of copper in roots (R), twigs (T) and leaves (L) of *Rosmarinus officinalis* planted in mango orchards in Hoedspruit

	BAF-R	TF-T	BAF-T	FT-L	BAF-L
Control	1	0.3	0.4	0.3	0.4
Site 1	0.1	2	0.1	10	1
Site 2	0.1	2	0.2	13	1
Site 3	0.1	1	0.1	9	1
Site 4	0.1	3	0.1	17	1
Site 5	0.2	2	0.3	6	1

Table 5.6: Accumulation and translocation of copper in roots (R), twigs (T) and leaves (L) of *Ocimum labiatum* planted in mango orchards in Hoedspruit

	BAF-R	TF-T	BAF-T	TF-L	BAF-L
Control	1	0.7	0.7	0.6	0.6
Site 1	0.04	4	0.2	110	5
Site 2	0.1	1	0.1	22	3
Site 3	0.2	2	0.3	26	5
Site 4	0.09	2	0.08	34	3
Site 5	0.1	1	0.2	24	3

To be selected for phytoextraction of metal contaminants from soils, plant species must possess hyperaccumulation capacity. Numerous criteria have been used to characterise hyperaccumulator plant species. However, three criteria have been consistently used: a threshold concentration value of 1000 mg/kg must be reached, the BAF for leaves and TF roots to leaves translocation must be > 1 (Wei *et al*., 2005; van der Ent *et al.*, 2013; Ardalan *et al*., 2014). When applying these criteria to results obtained for *R. officinalis* and *O. labiatum* in this study, the plants failed to qualify as phytoremediators, because the concentrations of copper found in the leaves were all below the threshold value of 1000 mg/kg. However, Krämer (2010), followed by van der Ent *et al*. (2013), suggested that for copper the threshold value indicating phytoremediation potential be reduced to 300 mg/g, because of the low average copper concentrations observed in leaves of plants from natural environments.

Considering this approach, *O. labiatum* qualifies as a phytoremediator for copper in orchard soil, since the leaf concentration of copper in all the plant specimens harvested from the orchard exceeded 300 mg/kg, and the BAF and TF for the leaves were > 1. However, *R. officinalis* did not qualify, since leaf concentrations of copper were lower than the suggested threshold value.

Considering that the accumulation in the leaves in the current study resulted from copper absorption from contaminated soil only, bioavailable copper cations must have been translocated through the cellular membrane of plant roots, from the soil solution to the root intracellular medium with the aid of protein-based membrane transporters (Lasat, 2000). Copper cations bind to specific sites of the membrane transporters from the extracellular medium and diffuse through the membrane structure to the intracellular medium of the roots. From the roots, copper is translocated to aboveground portions *via* vascular tissues (xylem and phloem).

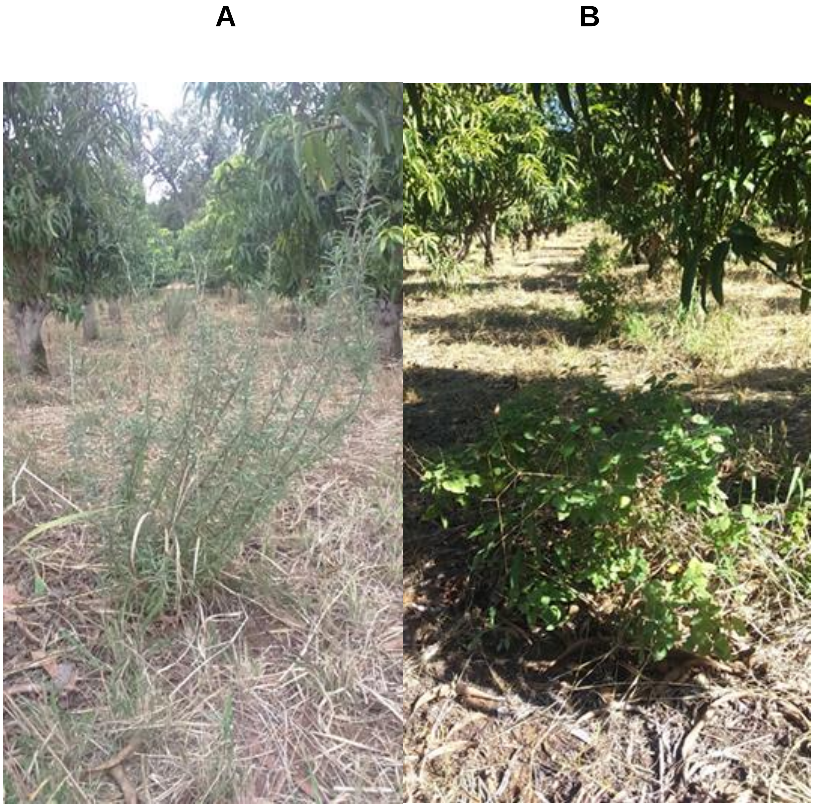

Figure 5.13: *Rosmarinus officinalis* (A) and *Ocimum labiatum* (B) in the mango orchard fifteen months after being planted

However, copper accumulation in the plant leaves may not only have resulted from copper uptake from the soil, but possibly also through foliar deposition of copper, followed by subsequent incorporation into the leaf body (Hughes *et al*., 1980; Hogan & Wotton, 1984).

Adeyeye *et al.* (2006) considered foliar absorption of copper as one of the routes of copper accumulation in cocoa leaves (190-1435 mg/kg) in orchards frequently treated by copper-rich fungicide in Nigeria. Hogan and Watton (1984) agreed that foliar absorption was a probable route for the copper accumulation observed in leaves of plants that had been exposed to smelter effluents. The mechanism of foliar absorption of elements is still unclear. However, researchers identified the cuticle and stomata as the leaf structures through which nutrients are incorporated into the plant by both passive and active transport (Cheristensen, 2005; Njoloma, 2012). Foliar absorption may have taken place to some extent, contributing to the copper accumulation in leaves of *R. officinalis* and *O. labiatum* in this study. This assumption is supported by the poor mobility of copper, which was found mainly bound to soil organic matter and to manganese and iron oxides, thereby restricting root absorption and translocation to the plant leaves.

5.4 CONCLUSIONS

- Mango and citrus orchard soils from Hoedspruit, which were suspected of copper contamination based on a long history of the application of copper-based fungicides were screened using a pXRF spectrometer. Average copper concentrations in the mango orchard soils varied from 200 to 580 mg/kg, whereas in the citrus orchards, the average concentration fluctuated from 80 to 140 mg/kg. A maximum concentration of 2600 mg/kg was measured in mango orchard soils and 450 mg/kg in citrus orchards. Differences in copper concentrations between the orchards were attributed to the age of the orchards and to the frequency of spraying of the copper-containing fungicides. Copper in the orchards accumulated mainly on the soil surface and decreased with an increase of depth due to the high content of organic matter in the surface soil. However, accumulation of copper was found at a depth of 1.00 m, which was attributed to the presence of a layer of clay at this depth.

- Assessment of copper contamination, using the EF, indicated that 185 of the 290 (representing 64%) mango orchard soils analysed were significantly to extremely contaminated, while only 8% of 106 citrus soils were significantly contaminated. High enrichment of the mango and citrus orchard soils with copper corresponded to the high concentrations found, which confirmed the anthropogenic origin of the contaminant. The origin of the contamination of the soils was attributed to the copper-based fungicide formulations used in the orchards, since no other sources of copper were present in the region.

- The BCR sequential extraction scheme was applied to assess the mobility of copper in the orchard soils. Copper was predominant in the oxidisable (32%) and reducible (28%) fractions, which confirmed the adsorption of copper by organic and clay colloids. Less than 14% of the total copper was present in the easily mobile fraction, represented by the exchangeable fraction. The mobility of copper was consequently limited in the mango orchard soils from Hoedspruit. An overall moderate environmental risk was found from the risk assessment using RAC, which corroborated with the low mobility of copper in the orchard soils.

- An increase in copper concentration was determined in the plants when young mango trees were treated with increasing concentrations of copper sulfate. The increase was more pronounced in the roots than in the twigs and leaves. The results suggest that mango trees take up bioavailable copper from contaminated soils, which predominantly accumulates in the roots, rather than in the twigs and leaves.

- High copper concentrations were present in leaves of *R. officinalis* (73.6 to 236 mg/kg) and *O. labiatum (*333 to 675 mg/kg), which grew in the contaminated mango orchard soils for 15 months. However, copper concentrations in the roots and twigs of the plants were low and similar to concentrations present in the control. The BAFs and TFs calculated for plant leaves were all above 1. However, the plants did not qualify as phytoaccumulators. Nevertheless, *O. labiatum* was found to have good potential as a phytoremediator for copper-contaminated soils from mango orchards in Hoedspruit.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

Many improperly closed mines in the Tshwane Metropolitan date back to early periods of intensive mining activities. Mine wastes dumped on soils often contain high concentrations of toxic elements and serve as a source of soil pollution. Fruit orchards, developed decades ago in the Hoedspruit area, have contributed to securing South Africa's dominant position as an international supplier of fresh fruit. However, the application of copper-based fungicides to control fungal growth in orchards, has led to the accumulation of copper in the soil. The presence of high concentrations of toxic elements in soils and sediments is of environmental concern, because their potential mobility and leachability may result in their migration into groundwater and fresh water, before they ultimately enter the food chain through biomagnification in fish and plants.

The broad aim of this study was to investigate the feasibility of using pXRF spectrometry to screen areas of South Africa for the presence of toxic elements in soil and sediment, by correlating the results with those obtained by more traditional techniques. This is a technique that is easy to apply for the rapid screening of large land tracts for metal contaminants. A further aim was to determine the levels of toxic elements in plant samples from contaminated areas by analysing the digested material using ICP-OES.

6.2 ACHIEVEMENT OF OBJECTIVES

The pXRF spectrometer was successfully calibrated by adjusting the factory fundamental parameters through the use of CRMs, which yielded good precision and accuracy in the measurement of copper (Table 3.9), zinc (Table 3.10) and lead (Table 3.11) in soils and sediments. The RSDs obtained for seven replicate analyses of CRMs, containing low to high concentrations of the toxic elements, were below 5%, whereas the percentage recoveries were between 90 and 110%. The LODs, when using the pXRF, were 23.7, 16.8 and 17.4 mg/kg for copper, zinc and lead, respectively. Results obtained in the field using the field portable XRF were confirmed by ICP-OES and the coefficients of determination (r^2) obtained were 0.8250, 0.9743 and 0.9423 for copper, zinc and lead, respectively (Figure 3.10). These values were comparable to values recommended by the US EPA. Representative samples from the Edendale Mine and orchards were collected, processed (dissolved or dried, sieved, and pelletized) and analysed using ICP-OES and WDXRF. The results obtained correlated well with data obtained from the pXRF analysis, indicating that the technique could be successfully used for screening large numbers of sampling points in the field to identify elemental contamination.

The soil of the abandoned Edendale Mine, which had previously been reported as being contaminated, and orchard soils in Hoedspruit that had a high probability of being polluted with copper, were screened using the pXRF spectrometer. Average concentrations of 480 mg/kg and 1100 mg/kg, with maximum concentrations of 7300 mg/kg and 21000 mg/kg of zinc and lead, respectively, were measured in soils at the old mine (Figure 4.7). Sediments from the tributary of the Edendale Spruit, which flows through the abandoned mine site, were also investigated *in situ* using the pXRF, to determine zinc and lead contamination. Average and maximum concentrations of 750 and 2400 mg/kg for zinc, and 1200 and 5400 mg/kg for lead, were determined in the sediment (Figure 4.8). Average copper concentrations were found to range from 80 to 580 mg/kg in soils of the citrus and mango orchards. The maximum concentration measured was 2600 mg/kg (Table 5.1).

The levels of copper, zinc and lead contamination of soils and sediments were established using I_{geo} as a measure for the Edendale Mine and EF for the orchards. From the analysis of soils at the mine it was determined that 18% of the sampling points were contaminated moderately to extremely with zinc. In the case of lead, 68% of the soils were contaminated. Most of the sediments (73%) were contaminated moderately to strongly, with zinc, while 46% were contaminated with lead. About 63% of the mango orchard soils and 8% of the citrus orchard soils analysed were contaminated significantly to extremely with copper. Metal contamination was generally the highest at the soil surface and decreased progressively with an increase in depth.

Contamination maps were constructed for the abandoned Edendale Mine using the measured concentrations of zinc and lead, in combination with the GPS coordinates acquired for each sampling point. These maps were integrated into the Google map of the area for locating the contaminated zones.

The mobility and bioavailability of the soil contaminants were determined by the application of a BCR fractionation scheme. The percentage of total concentration in the exchangeable fraction was used to determine the mobility of copper, zinc and lead in soils and sediments. Lead was found to be highly mobile, whereas the mobility of zinc was moderate. Zinc (34%)

and lead (25%) were found to be relatively mobile in the sediments. In contrast, the mobility of copper in the orchard soils was found to be limited. This was attributed to the binding of the metal to soil organic matter and clay.

Using ICP-OES, the concentrations of zinc and lead were determined in plants growing on contaminated soils at the Edendale Mine. Accumulation of these elements was observed in roots and leaves. A maximum of 375 mg/kg for zinc was found in roots of thatching grass, while the highest value for lead was 534 mg/kg in leaves of a *Solanum* species.

A phytoremediation study was conducted in the most contaminated mango orchard using two aromatic plants, *O. labiatum* and *R. officinalis*. Analysis using ICP-OES revealed leaf copper concentrations of up to 236 mg/kg for *R. officinalis* and as high as 675 mg/kg for *O. labiatum* (Table 5.4). The determined biological factors were above 1 for these plant species, indicating promising potential for copper phytoextraction.

The origins of metal pollutants in soils and sediments of the Edendale Mine and fruit orchards in Limpopo was confirmed based on their speciation, spatial distribution and distribution in the soil profile. Lead in the soil, and both lead and zinc in sediments from the Edendale Mine, as well as copper in the orchard soils, were proven to be of anthropogenic origin. High concentrations of these elements were confined to the site or at specific zones. These elements were generally found in abundance in the topsoil, and the concentrations decreased with depth. The speciation of copper and lead in soils, and lead and zinc in sediments, indicated that the major proportion of the total concentration was present in the mobilisable (exchangeable, reducible and oxidisable), rather than in the residual fraction. On the other hand, zinc in soil had a comparable spatial and soil profile distribution, but was mainly present in the residual fraction (61%), instead of in the mobilisable fraction (39%). Zinc in soils from the Edendale Mine was probably of mixed (anthropogenic and geogenic) origin.

6.3 CONTRIBUTION OF THE STUDY

This study confirmed the superiority of the Compton normalisation calibration over the use of fundamental parameters for pXRF, when applied for the determination of elements at trace levels in soil matrices dominated by low Z elements. The suitability of pXRF spectrometry for *in situ* analysis of soils that are heavily contaminated with copper, zinc and lead, using the fundamental parameter calibration, was proven by this study.

The concentrations of zinc and lead present in soils and sediments at the abandoned Edendale Mine were determined and used to establish contamination maps for these elements. These maps may be useful to officials of the Tshwane Metro, and to environmentalists and stakeholders involved in a variety of activities in the vicinity of the abandoned mine. The ecological risk associated with zinc and lead contamination of soil and sediment at the mine was determined. Lead in soils, as well as lead and zinc in sediments, were found to be highly mobile and reflect a serious ecological risk that must be addressed urgently. Zinc in soil was found to be moderately mobile and corresponded to a moderate ecological risk. The mobility and bioavailability of these toxic elements were confirmed by their presence, at relatively high concentrations, in plants growing at the contaminated site. This finding indicates that these elements have already infiltrated the lower levels of the food chain, thereby increasing the risk of biomagnification. While we were conducting field measurements, we observed that power lines had been erected, as part of a housing development. We subsequently alerted the local municipal officials to the dangers of lead contamination in the area. Development seems to have halted since, although no communication was received from the local authorities.

The levels of copper contamination in mango and citrus orchard soils in Hoedspruit (and in other regions of South Africa) have not been investigated, despite repeated applications of copper-based fungicides over many years. This study established that high concentrations of copper are present in these soils. The study also revealed that copper contamination was more pronounced in mango orchards than in citrus orchards, due to the higher frequency of spraying. The most contaminated soils were directly under the trees, rather than in the passages between trees (Table 5.1). Fortunately, the mobility of copper in the orchard soils was limited, probably because the element is primarily attached to organic matter, and to iron and manganese oxides of the soil, thereby limiting the ecological risk (Figure 5.10). Although this study has proven that mango trees absorb and accumulate copper in their roots, the contaminant is not translocated to the aboveground portions of the plants (Figure 5.12). This suggests that the fruit will not carry a risk to consumers.

Two aromatic plants, *R. officinalis* and *O. labiatum*, were evaluated for their ability to extract copper from contaminated mango orchard soils. The results obtained are promising, since substantial amounts of copper accumulated in the leaves during the 15 month field trial. These findings indicate that intercropping of orchards with aromatic plants could be a potential solution to mitigate the high concentrations of copper in the soil. Plants used for this purpose could be removed and distilled to yield essential oils, thereby contributing an additional income. It is known that the high boiling points of metals prevent their transfer to the essential oil during steam distillation. The remaining plant material can then be used to prepare compost for distribution in areas deficient in copper. However, the use of aromatic plants, grown on copper-contaminated land, as culinary ingredients and medicinal decoctions, should be avoided due to safety issues.

6.4 SHORTFALLS OF THE STUDY

The most reliable method to interpret the origin of pollutants, particularly when various sources are involved, is to apply geostatistical analysis to combined data involving spatial distribution, soil profile distribution, speciation of the pollutants, and other geological information. Multivariate statistical analysis, including principal component analysis (PCA) and factor analysis (FA), are the most commonly used methods for this purpose. These statistical methods are applied to large datasets collected for individual contaminants. In the present study, only two toxic elemental pollutants (zinc and lead) from the Edendale Mine and one (copper) from the orchards, were present in soils at concentration levels of interest. The limited number of contaminants determined at each site did therefore not permit the application of these methods.

6.5 RECOMMENDATIONS AND FUTURE WORK

It is recommended that widespread use of the pXRF spectrometer should be encouraged for the screening of copper, zinc and lead, as well as for other elemental pollutants, in mining and orchard soils during site assessments.

Lead and zinc contamination at the Edendale Mine must be addressed. We recommend that the mine site should be closed off, as a matter of urgency, to prevent people from coming into contact with the polluted soils. Evidence of maize being cultivated on small patches of land was seen during our field surveys. Currently, it is also an attractive site for squatters, since it is close to the road and provides access to water (the stream). An appropriate remediation programme, including phytoremediation, soil washing or excavation, must be implemented. The stream that flows through the Edendale Mine and areas that the sediments were heavily contaminated with zinc and lead should be dredged.

Copper pollution of mango orchard soils must be addressed by phytoremediation using plants, which could include *R. officinalis* and *O. labiatum,* since these have demonstrated a potential for phytoextraction. It is also recommended that, if the use of copper-based fungicides cannot be avoided altogether, the frequency of spraying should be reduced as far as possible.

Direct exposure of humans to toxic elements in soil may occur from dust. The Mamelodi Township is adjacent to the Edendale Mine site and houses are only a few hundred metres away from the polluted area. Analysis of collected dust at, and in the vicinity of, the polluted mine should be conducted to determine the health risk associated with dust ingestion. A screening of the population (blood and breast milk) living in close proximity to, and interacting with the Edendale Mine, for the presence of zinc and lead should provide an indication of the health impact of the contaminated soils from the abandoned Edendale Mine.

The approximately six thousand inappropriately closed mines, dispersed throughout the country, should be more closely monitored and possibly rehabilitated. A larger number of orchards and vineyards that have a long history of the application of metal-based fungicides should be screened, so that the situation with regard to soil contamination and the potential associated environmental risks can be assessed. This will allow South Africa to be more closely aligned to developed countries with regard to the GAP requirements for fruit exports. It is proposed that the South African government and stakeholders in the fruit industry facilitate the generation of contamination maps so that the ecological risk associated with the contamination can be assessed. The pXRF is an ideal tool for such surveys. Remedial action can then be implemented in areas where it is deemed necessary.

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APPENDICES

Appendix 1: Copper concentrations in soil samples from mango orchards measured by ICP-OES, WDXRF and pXRF in the unprepared, dried and sieved, and pelletized samples

Sample	ICP-OES	WDXRF	Unprepared	Dried and sieved	Pellet
O1	59.9	97.0	28	52	59
O2	249	283	450	310	230
O3	242	243	140	310	190
O4	219	604	210	590	-
O5	452	1060	550	83	760
O6	274	625	310	590	510
O7	118	201	180	150	170
O8	578	629	540	770	510
O9	413	444	450	380	330
O10	132	202	130	180	68
O11	171	272	270	120	230
O12	51.7	39.0	28	24	19
O13	64.0	0.00	37	41	29

Appendix 2: Zinc concentrations in soil samples from the Edendale mine measured by ICP-OES, WDXRF and pXRF in the unprepared, dried and sieved, and pelletized samples

Sample	ICP-OES	WDXRF	Unprepared	Dried and sieved	Pellet
M1	1120	1990	990	1600	1700
M2	208	122	230	78	89
M3	21.0	152	<LOD	99	150
M4	22.4	76.0	37	42	76
M5	405	675	530	470	680
M6	28.0	118	25	84	120
M7	124	174	42	26	71
M8	435	1110	650	760	710
M9	118	224	89	93	120
M10	211	586	31	340	390
M11	311	628	590	570	460
M12	1710	3020	1800	2200	2500
M13	97.8	151	160	120	150
M14	40.7	55.0	36	38	55
M15	3770	7890	4400	6100	6900
M16	53.7	534	480	530	390
M17	671	806	660	990	670
M18	447	359	280	270	270
M19	226	301	410	310	280
M20	109	231	330	180	180
M21	209	178	330	110	120
M22	5430	10200	7300	8800	8300
M23	60.3	86.0	51	72	73
M24	217	484	69	420	170
M25	74.2	467	51	62	22
M26	30.2	49.0	19	19	31
M27	5.22	45.0	51	41	<LOD
M28	7.25	36.0	25	24	310
M29	242	402	370	320	73
M30	174	106	29	73	130
M31	437	739	710	590	520
M32	25.5	40.0	32	21	<LOD
M33	<LOD	<LOD	24	<LOD	<LOD
M34	868	1250	1600	1300	940

Appendix 3: Lead concentrations in soil from the Edendale mine measured by ICP-OES, WDXRF and pXRF in the unprepared, dried and sieved, and pelletized samples

Sample	ICP-OES	WDXRF	Unprepared	Dried and sieved	Pelletized
M1	9130	16600	11000	13000	14000
M2	351	373	770	250	270
M3	38.0	219	44	42	150
M4	114	389	290	250	260
M5	301	1090	1700	860	740
M6	97.8	296	82	72	250
M7	42.4	263	38	27	150
M8	1740	3640	3900	2900	2800
M9	121	168	59	78	110
M10	176	608	210	254	380
M11	3680	7680	5100	6100	5500
M12	18300	31900	19000	23000	24000
M13	107	161	170	160	140
M14	34.7	45.0	36	37	33
M15	9260	11100	11000	6900	8500
M16	104	1250	860	1100	910
M17	2510	4790	2100	7100	4200
M18	1250	1270	890	940	870
M19	25.9	39.0	28	34	34
M20	591,	708	77	530	560
M21	373	331	520	240	230
M22	19800	29100	21000	21000	23000
M23	46.80	78.0	17	59	61
M24	129	321	470	260	260
M25	61.7	398	41	51	17
M26	15.4	37.0	17	<LOD	22
M27	5.34	31.0	28	41	26
M28	13.9	31.0	41	32,82	23
M29	546	961	780	840	780
M30	159	162	730	150	130
M31	867	1110	730	910	860
M32	49,79	44.0	36	34	31
M33	<LOD	<LOD	45	<LOD	<LOD
M34	1195	2260	1600	1700	1700

Appendix 4: Zinc concentrations in sediment samples from the Edendale mine measured by ICP-OES, WDXRF and pXRF in the wet, dried and sieved, and pelletized samples

Sample	ICP-OES	WDXRF	Wet	Dried and sieved	Pelletized
S1	266	611	150	170	340
S2	136	162	79	99	110
S3	186	288	77	130	210
S4	226	486	160	230	310
S5	434	1030	540	530	630
S6	2990	3200	1700	3800	2600
S7	1660	2610	910	2700	2100
S8	2730	5140	2500	5900	3800
S9	821	1250	620	810	850
S10	1470	3460	2100	2600	2600
S11	1130	861	1100	1700	1100
S13	1180	922	350	1700	1100
S14	705	1010	990	640	650
S15	637	1550	660	1300	990
S16	1945	4260	650	3600	2900
S17	878	1410	340	1500	1100
S18	852	1970	360	1600	1400
S20	165	265	130	190	170
S21	283	1080	650	570	680
S22	141	323	120	120	210

Appendix 5: Lead concentrations in sediment samples from the Edendale mine measured by ICP-OES, WDXRF and pXRF in the wet, dried and sieved, and pelletized samples

Sample	ICP-OES	WDXRF	Wet	Dried and sieved	Pelletized
S1	596	1020	320	270	660
S2	307	238	110	140	160
S3	428	411	130	310,	280
S4	443	871	210	350	540
S5	479	888	280	420	550
S6	606	10500	5200	9900	7800
S7	3640	6130	1900	5700	4600
S8	10200	19500	5400	18000	13000
S9	1260	2160	920	1700	1400
S10	211	92.0	3100	3600	3100
S11	1840	2160	1500	2500,	1900
S12	3360	4540	2200	6300	3900
S13	1250	951	2600	1400	1100
S14	1140	2030	1700	1300	1400
S15	959	1920	710	1700	1200
S16	2070	4060	680	3500	2800
S17	1340	1970	410	1900	1500
S18	1130	1890	380	1600	1500
S20	307	279	170	240	180
S21	477	981	310	470	620
S22	426	522	230	230	360