



University of Venda

SCHOOL OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF HYDROLOGY AND WATER RESOURCES

**Analysis of Heavy Metals and Persistent Organic Pollutants in Sewage Sludge
from Thohoyandou Wastewater Treatment Plant and transfer to Vegetables**

BY

AKINSANYA, NURUDEEN AKINWALE

Student No: 11629723

Supervisor: Prof. J.O. Odiyo

Co-Supervisor: Dr. E.O. Popoola

Co-Supervisor: Prof. T.A.M. Msagati

**A Master's dissertation submitted to the Department of Hydrology and Water
Resources in fulfilment of the requirements of Masters of Environmental
Sciences**

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DECLARATION

I, Akinsanya Nurudeen Akinwale, hereby declare that this dissertation for the Master in Environmental Sciences degree at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this or any other institution. This is my work in design and execution, and all reference materials contained herein have been duly acknowledged.

Signature _____

Date: _____

I hereby certify that the above statement is correct.

Supervisor: Prof. J.O. Odiyo _____

Date: _____

Co-Supervisor: Dr. E.O. Popoola _____

Date: _____

Co-Supervisor: Prof. T.A.M. Msagati _____

Date: _____

DEDICATION

This research project is dedicated to God Almighty, the giver of life, grace, knowledge and wisdom to accomplish all tasks.

To my wife Modupe, my children Oluwadamilola, Oreoluwa and Oluwatumininu for their patience, support, encouragement and understanding.

To my late father, Pa Lamidi Ishola Akinsanya, who always encouraged me to further my studies.

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Table of Contents

DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	vi
LIST OF FIGURES	viii
LIST OF TABLES	x
CHAPTER 1: INTRODUCTION	1
1.1: Background of the research	1
1.2: Statement of the Problem	3
1.3: Justification	4
1.4: Research Questions	5
1.5: Objectives	5
1.5.1: Main objective	5
1.5.2: Specific Objectives	5
1.6: The Study Area	6
CHAPTER 2: LITERATURE REVIEW	8
2.1: Status of waste generation	8
2.2: Wastewater treatment processes	8
2.3: Sewage sludge composition, treatment and disposal	10
2.4: POPs in soil and sludge	11
2.5: Conventions on Toxic Contaminants	12
2.6: POP Regulations	14
2.7: POP Methods of Analysis	15
2.8: Heavy metals in Sludge and Soil	16
2.9: Heavy metals in plants	19
2.10: Heavy Metal Analysis	19
2.11: Spinach Vegetable	21
CHAPTER 3: METHODOLOGY	22
3.1: Research Design	22
3.2: Sampling	25
3.2.1: Sludge Sample	25
3.2.2: Soil Sample	26
3.2.3: Plant Sample	26
3.3: Sample Pre-treatment	27
3.3.1: Sludge Sample	27
3.3.2: Soil Samples	28
3.3.3: Plant Samples	28
3.4: Analytical procedures and sample preparation	28
3.4.1: pH determination	28

3.4.2: Alkalinity determination	30
3.4.3: Organic carbon determination.....	31
3.4.4: Cation exchange capacity (CEC) determination.....	32
3.4.5: Conductivity determination	33
3.4.6: POPs Analysis.....	33
3.4.7: Heavy Metal Analysis.....	35
3.5: Instrumental analysis	37
3.5.1: pH determination	37
3.5.2: Conductivity determination	37
3.5.3: POPs determination	37
3.5.4: Metal determination.....	39
3.6: Cultivation of <i>spinacia oleracea</i>	39
3.6: Ethical Issues	39
3.7: Data Analysis.....	40
CHAPTER 4: RESULTS AND DISCUSSION.....	41
4.1: Physicochemical properties	41
4.2: Heavy metal content in soil.....	42
4.3: Speciation of metals in <i>spinacia oleracea</i>	59
4.4: Mobility factor (%) of the metals in the <i>spinacia oleracea</i> leaf.....	67
4.5: Heavy Metal Concentration in the Vegetables Grown using sewage sludge.....	71
4.5.1 Transfer Factors (TF) of the Heavy Metals from Soils to <i>Spinacia oleracea</i> Leaf.....	71
4.6: Polycyclic aromatic hydrocarbons (PAHs).....	74
4.7: Statistical analysis of variance (ANOVA) of total metal determination.....	76
4.8 Hierarchical cluster analysis (HCA) of trace metals in sewage sludge samples	77
4.9: Intermetal and Sampling Points Association	79
CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS	80
5.1: CONCLUSION.....	80
5.2: RECOMMENDATIONS.....	81
REFERENCES	83

ABSTRACT

Sewage sludge (biosolids) from wastewater treatment plants (WWTPs) has been widely used as a soil improver in Europe, United States of America and some developing countries including South Africa. It has its benefits for farmers as a good source of organic matter and minerals, however, sludge after treatment still contains pathogenic organisms, heavy metals and persistent organic pollutants (POPs). The POP and heavy metal contaminants that accumulate in sludge may transfer through the food chain and cause adverse effects on human beings.

In this study, a field experiment was carried out on farmland fertilized with sewage sludge from a wastewater treatment plant (WWTP) that vasically receives domestic wastewater and storm water. Vegetable spinach (*Spinacia oleracea*) was used for this study and was planted on a farmland under controlled conditions. Ten ridges each of dimensions 20 m × 0.3 m was made and dry sludge weights of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 kg were applied as manure on each of the ridges, respectively.

Representative samples of sludge and soil were taken for analysis of heavy metals and POPs. At maturity, in twelve weeks, the root and leave samples of the vegetable were taken from all the ridges including the control. The soil, sludge, and vegetable samples were analyzed for total heavy metal content (Cd, Cr, Cu, Ni, Pb, Co, Zn, Al, Fe, Mn), speciated heavy metal content and POP (PAH, PCB). Soil and sludge samples were also analyzed for total organic content, pH, cation exchange capacity (CEC), conductivity and alkalinity. The analysis for total heavy metals and speciated heavy metal content was carried out using inductively coupled plasma optical emission spectrophotometer (ICP-OES), and CEC analysis was carried out using atomic absorption spectrophotometer (AAS). A two-dimensional gas chromatograph with time of flight mass spectrometry detector (GC X GC TOFMS) was used for POP measurements. pH measurement was made using a pH meter and conductivity measurement using a conductivity meter. Alkalinity and total organic content analysis was performed using titrimetric apparatus. The highest total heavy metal concentration of 378.9 mg / kg was recorded in Fe metal in soil and Leaf sample while the lowest total metal concentration of 0.0003 mg / kg was in Cu metal in root sample. The highest heavy metal concentration of 1002 mg / kg in speciated forms was in Mn metal in F1 fraction and the lowest of 0.0004 mg / kg was in Cd metal in F5 fraction. PAHs were only found in soil samples and their concentrations ranged from 2.53 mg / kg to 146.5 mg / kg. There were no

PCB detected in all the samples analysed. The results indicated that the trace metals concentrations found in the exchangeable fraction were higher than those observed in any of the preceding extractions except in the case of Cd, Cr, Fe and Pb where Fe-Mn oxide and organic matter fractions predominated and were closely followed by exchangeable fraction.

LIST OF FIGURES

Fig 1.1: Map of Thulamela Municipality showing the study sites of Thohoyandou WWTP and School of Agriculture, University of Venda.....	7
Fig 3.1: Spinacea oleracea seed germinated at five days old	23
Fig 3.2: Spinacia olerecea seedling at fourteen days old	23
Fig 3.3: Spinacia oleracea seedling transferred to the field at twenty eight days old.....	24
Fig 3.4: Spinacia oleracea on the field at fifty six days old	24
Fig 3.5: Spinacea oleracea on the field at seventy four days old ready for harvest.....	25
Fig 3.6: Sludge sample been collected on a sludge drying bed.....	26
Fig 3.7: Sludge sample for POP analysis preserved in a cooler box of ice.....	27
Fig 3.8: Pulverizer used for grinding samples.....	29
Fig 3.9: Vacuum filtration pump with 0.45 µm pore size filter paper	29
Fig 3.10: Sand and sludge sample slurry in deionize water	30
Fig 3.11: Titration of soil and sludge samples for total organic carbon analysis.....	32
Fig 3.12: Soxhlet extraction of POPs from samples.....	34
Fig 3.13: Vacuum evaporator for concentration of sample extracts.....	35
Fig 3.14: Sample digestion for total heavy metals on a hot plate.....	36
Fig 3.15: GC × GC TOFMS instrument used for POP determination.....	38
Fig 4.1: Bar chat showing the concentrations of Aluminium metal in soil samples	45
Fig 4.2: Bar chat showing the concentrations of Chromium metal in soil samples	46
Fig 4.3: Bar chat showing the concentrations of Copper metal in soil samples.....	46
Fig 4.4: Bar chat showing the concentrations of Iron metal in soil samples.....	47
Fig 4.5: Bar chat showing the concentrations of Manganese metal in soil samples.....	47
Fig 4.6: Bar chat showing the concentrations of Zinc metal in soil samples	48
Fig 4.7: Bar chat showing the concentrations of Cobalt metal in soil samples.....	48
Fig 4.8: Bar chat showing the concentrations of Lead metal in soil samples	49
Fig 4.9: Bar chart showing the concentration of Aluminium metal in Root samples.....	49
Fig 4.10: Bar chart showing the concentration of Cobalt metal in root samples.....	50
Fig 4.11: Bar chart showing the concentration of Chromium metal in Root samples.....	50
Fig 4.12: Bar chart showing the concentration of Copper metal in root sample.....	51
Fig 4.13: Bar chart showing the concentration of Iron metal in root samples	51
Fig 4.14: Bar chart showing the concentration of Manganese metal in root samples	52

Fig 4.15: Bar chart showing the concentration of Nickel metal in root samples.....	52
Fig 4.16: Bar chart showing the concentration of Lead metal in root samples	53
Fig 4.17: Bar chart showing the concentration of Zinc metal in root samples.....	53
Fig 4.18: Bar chart showing the concentration of Aluminium metal in leaf samples	54
Fig 4.19: Bar chart showing the concentration of Chromium metal in leaf samples.....	55
Fig 4.20: Bar chart showing the concentration of Copper metal leaf samples.....	55
Fig 4.21: Bar chart showing the concentration of Iron metal in leaf samples.....	56
Fig 4.22: Bar chart showing the concentration of Manganese metal in leaf samples.....	56
Fig 4.23: Bar chart showing the concentration of Zinc metal in leaf samples	57
Fig 4.24: Bar chart showing the concentration of Cadmium metal in leaf samples	57
Fig 4.25: Bar chart showing the concentration of Cobalt metal in leaf samples.....	58
Fig 4.26: Bar chart showing the concentration of Nickel metal in leaf samples.....	58
Fig 4.27: Bar chart showing the concentration of Lead metal in leaf samples	59

LIST OF TABLES

Table 4.1: Physicochemical properties of soil and sludge samples	42
Table 4.2: Total metal concentration in soil samples (mg/Kg) and the summary statistics....	43
Table 4.3: Total metal concentration in root samples (mg/kg).....	44
Table 4.4: Total metal concentration in leaf samples (mg/Kg) and the summary statistics ...	54
Table 4.5: Percentage Metals in various Geochemical Fractions of Spinacia oleracea leaf...	60
Table 4.6: Aluminium levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf.....	61
Table 4.7: Cadmium levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf.....	61
Table 4.8: Cobalt levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf	62
Table 4.9: Chromium levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf.....	62
Table 4.10: Copper levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf	63
Table 4.11: Iron levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf .	64
Table 4.12: Manganese levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf.....	64
Table 4.13: Nickel levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf	65
Table 4.14: Lead levels (mg/kg) in different geochemical fraction in Spinacia oleracea leaf	65
Table 4.15: Zinc levels (mg/kg) in different geochemical fraction in Spinaciaoleracea leaf .	66
Table 4.16: Mobility factor (%MF) of metals in the Spinacia oleracea leaf samples	68
Table 4.17: Transfer factor of the heavy metals from sludge to Spinacia oleracea leaf.....	74
Table 4.18: PAH concentration in soil samples.....	76
Table 4.19: Analysis of variance of total metal determination.....	77
Table 4.20: Intermetal and sampling points association	79

LIST OF UNITS, SYMBOLS, ACRONYMS AND ABBREVIATIONS

$^{\circ}\text{C}$degree celcius
cm.....centimeter
g.....grams
 g^{-1}per gram
 g/cm^3grams per centimeter cube
kg.....kilogram
kg/d....kilogram per day
L...litre
 L^{-1}per litre
meq....milliequivalent
mg/g....milligram per gram
min...minute
ML....mega litre
M....molar
m....metre
mL....millilitre
mL/min.....millilitre per minute
mm...millimetre
mg/kg....milligram per kilogram
N....normal
ng/g.....nanogram per gram
pg....picogram
ppm.....parts per million
rpm....revolutions per minute
 $\mu\text{g}/\text{kg}$microgram per kilogram
 μLmicrolitre
 μmmicrometer
 $\mu\text{s}/\text{cm}$microsemens per centimeter
v/v....volume per volume
A....alkalinity
Al....aluminium
 Ca^+calcium ion

CaCO₃...calcium carbonate

Cd....cadmium

Co....cobalt

Cr...chromium

Cu....copper

Fe....iron

FeSO₄....iron (ii) sulphate

HNO₃....nitric acid

HOAc....acetic acid

H₂O₂....hydrogen peroxide

H₃PO₄....phosphoric acid

H₂SO₄....sulphuric acid

K⁺....potassium ion

K₂Cr₂O₇....potassium dichromate

LS C....leave sample of vegetable grown on ridge with 0 kg sludge (control)

LS 5.... leave sample of vegetable grown on ridge with 5 kg sludge

LS 10.... leave sample of vegetable grown on ridge with 10 kg sludge

LS 15.... leave sample of vegetable grown on ridge with 15 kg sludge

LS 20.... leave sample of vegetable grown on ridge with 20 kg sludge

LS 25.... leave sample of vegetable grown on ridge with 25 kg sludge

LS 30.... leave sample of vegetable grown on ridge with 30 kg sludge

LS 35.... leave sample of vegetable grown on ridge with 35 kg sludge

LS 40.... leave sample of vegetable grown on ridge with 40 kg sludge

LS 45.... leave sample of vegetable grown on ridge with 45 kg sludge

Mg²⁺....magnesium ion

MgCl₂....magnesium chloride

Mn...manganese

Na⁺....sodium ion

NaOAc....sodium acetate

NH₄OAc....ammonium acetate

NH₂OH.HCl....hydroxylamine chloride

Ni....nickel

Pb...lead

RS C....root sample of vegetable grown on ridge with 0 kg sludge (control)

RS 5... root sample of vegetable grown on ridge with 5 kg sludge
RS 10... root sample of vegetable grown on ridge with 10 kg sludge
RS 15.... root sample of vegetable grown on ridge with 15 kg sludge
RS 20.... root sample of vegetable grown on ridge with 20 kg sludge
RS 25.... root sample of vegetable grown on ridge with 25 kg sludge
RS 30.... root sample of vegetable grown on ridge with 30 kg sludge
RS 35.... root sample of vegetable grown on ridge with 35 kg sludge
RS 40.... root sample of vegetable grown on ridge with 40 kg sludge
RS 45.... root sample of vegetable grown on ridge with 45 kg sludge
SS C...soil sample on ridge with 0 kg sludge
SS 5... soil sample on ridge with 5 kg sludge
SS 10.... soil sample on ridge with 10 kg sludge
SS 15.... soil sample on ridge with 15 kg sludge
SS 20.... soil sample on ridge with 20 kg sludge
SS 25.... soil sample on ridge with 25 kg sludge
SS 30.... soil sample on ridge with 30 kg sludge
SS 35.... soil sample on ridge with 35 kg sludge
SS 40.... soil sample on ridge with 40 kg sludge
SS 45.... soil sample on ridge with 45 kg sludge
v...volume
Zn....zinc
°....degree
'....minute
"....second
%....percent
≤....less than and equal to
>....greater than
<....less than
±....plus or minus
AAS...Atomic Absorption Spectrophotometer
Aoac....Association of Analytical Chemist
Ase...Accelerated Solvent Extraction
Astm.....American Society for Testing and Materials
Bcr....Community Bureau of Reference

CEC....cation exchange capacity
CR...consumption rate
CRM...certified reference material
CSF....cancer slope factor
DDT...Dichlorodiphenyltrichloroethane
DTIE....division of technology, industry and economics
DWA....Department of Water Affair
ECD...electron capture detector
EDC...endocrine destruction chemical
US EPA...United States Environmental Protection Agency
EU...European Union
FAO... Food and Agriculture Organization
GC....gas chromatograph
HCB....hexachloro benzene
HPLC...high performance liquid chromatography
HRs....hazard ratios
HRMS...high resolution mass spectrometry
ICP/OES....inductive couple plasma optical emission spectrophotometer
LDCs....less developed countries
MAE....microwave assisted extraction
OCPs....octa calcium phosphate
PAH...polyaromatic hydrocarbon
PCB...polychlorinated biphenyl
PCDD....polychlorinated dibenzo dioxine
PCDF...polychlorinated dibenzo furan
POPs...persistent organic pollutants
RfD...reference dose
SFE....supercritical fluid extraction
SMAT....standards measurement and testing programme
SPE....solid phase extraction
TDI...Tolerable Daily Intake
Teq...Toxic Equivalent
UNEP....United Nation Environmental Protection
USA....United State of America

USEPA...united state environmental protection agency

WHO...world health organisation

WWT...wastewater treatment

WWTP....wastewater treatment plant

XRFS....x-ray fluorescence spectroscopy

bw/d...body weight per day

SVs.....screening values

RL...maximum acceptable risk level

CHAPTER 1: INTRODUCTION

This chapter introduces the topic of study by narrating the background, objectives, aims, problem statement, research questions as well as justification. It also gives the breakdown and structure of the dissertation.

1.1: Background of the research

The sediments that result from the sedimentation of suspended solids during the wastewater treatment process in wastewater treatment plants (WWTP) are called sewage sludge. Various sources of release of wastewater include runoffs, homes, industries, medical facilities, and businesses. Sewage sludge contains nitrogen, phosphorus and organic matter that can provide soil with benefits and this explains why it is widely used for soil amendment (During and Gath, 2002). Sewage sludge also contains contaminants, including metals, pathogens and organic pollutants (Harrison et al., 2006).

Generally waste water treatment processes inevitably generate sludge. The production of sludge is estimated at about 40-50g dry weight generated from waste water use per person per day (Sanchez et al., 2007). Sludge may be disposed of by applying to agricultural land, disturbed land or dedicated disposal sites, forest, however, any form of disposal needs to be under control in order to protect human and animal health and the environment and considering that sewage sludge potentially contains chemical pollutants (Jones and Alcock, 1996; Jiayin et al., 2006).

The organic pollutants that are of particular interest to this study are the ones that persist in the environment, which are called persistent organic pollutants (POPs). POPs are organic compounds of natural or anthropogenic origin and can resist photolytic, chemical and biological degradation, bio accumulate through the food web and can affect human health and the environment adversely (Letcher et al., 2010). There are evidences of POP long range transport to parts of the world where they have never been used or produced, thus resulting in exposure of most human population to POPs (Letcher et al., 2010; Linda et al., 2012).

Of the inorganic pollutants that are of concern, heavy metals which are defined as metals that have density/specific gravity that is greater than 5 g/cm^3 (During and Gath, 2002) have been reported as immensely forming part of the sludge composition. They also include transition metals and higher atomic weight metals of group (III) to (V) of the periodic table. Heavy metals can persist in the environment for a very long time and can be found in different environments. They are highly toxic such that minute quantities can affect life adversely (Ademoroti, 1996). The distribution, mobility and toxicity of metals are strongly related to the different forms in which they exist (Ure and Davidson, 1995). Heavy metals contamination due to natural and anthropogenic sources is now a global environmental concern and accumulation of such metals in living beings often result in several disease conditions (USEPA, 2004).

As advancements in technology takes place in manufacturing industries, this also creates changes in the compounds that are produced and discharged and in the resulting waste water characteristics. It is difficult and costly to treat numerous by products generated from industrial processes by conventional wastewater treatment processes. To enforce industrial pretreatment program is an intimidating task, and some of the regulated pollutants still find their way into the municipal waste water collection system and therefore must be treated (Tchobanoglous et al., 2004).

Amongst numerous contaminants in sludge, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxin and dibenzofurans (PCDD/Fs) and heavy metals persist in the environment and thus of particular interest. They have lower metabolism and degradability by most living organisms and thus have longer half-life making them persistent in the environment. Heavy metals are also caught up easily and tend to gather onto sludge particles, so that all these compounds are present in sludge. (Baveye et al., 1999a; Songa et al., 2006). Moreover, these pollutants are powerful poisons, for instance, some of the PAHs are a known carcinogen (Song et al., 1995; Songa et al., 2006), PCBs can also give rise to various toxicities, such as hepatotoxicity, dermal lesions, endocrine disruption, body weight loss, immunosuppression, reproductive and developmental toxicity, neurotoxicity and carcinogenicity, despite the cessation of PCB exposure (Negoita et al., 2003; Songa et al., 2006). The biological and physicochemical reactions of organic and inorganic pollutants with naturally occurring inorganic compounds in soils may also cause the formation of by-products which are mutagenic or carcinogenic. Interaction between metal

ions and other compounds around them results in the formation of many different metal species (e.g simple hydrated ions, ion pairs hydroxyl polymers, complex ions involving inorganic and organic ligands, sorbed in colloidal material or fine particulate matter) (Negoita et al., 2003; Songa et al., 2006).

1.2: Statement of the Problem

Research on the composition of sludge generated after wastewater treatment processes in countries in Europe, USA and Asia have shown that they contain heavy metals and persistent organic pollutants (Latumus et al., 2007; Soler – Rovira et al., 1996; Harrison et al., 1999). These constituents of sludge can be toxic, carcinogenic, mutagenic, teratogenic, endocrine disruptors and also cause developmental, behavioural and reproduction problems in a variety of species (Roefler et al., 2000; Tchobanoglous et al., 2004).

Sewage sludge composition in South Africa probably follows the same trend as the case observed in the western developed countries as well as in the developing countries of Asia. The WWTP in the study area is the Thohoyandou WWTP in Thulamela Municipality. It has catchment area covering Sibasa, Shayandima, Thohoyandou, Manini, sand Singamari settlements. Shayandima is also home to some industries and a hospital. Thus, wastewater is released from homes, industries, medical facility and businesses to the sewers for treatment.

Visits to WWTPs around Thulamela Municipality revealed that the treatment processes are the same in all WWT plants. It also revealed that the treatment processes are generalised perhaps with the assumption that effluents from industries producing specific chemical pollutants would have been treated before being discharged into the sewer.

Sewage sludge produced after WWT processes has to be disposed. The cheapest and economical means of disposal is to use it as soil improver or manure. Investigation has revealed that Thohoyandou WWTP disposes its sludge by selling it to farmers and receipt of purchase is issued by the Municipality.

Farmers around the municipality have been using sewage sludge as manure to grow their crops and have attested to the fact that their crops do very well with the manure. Farmers are also aware that there are health implications to the consumers of crops grown with this

manure, thus, most farmers do not own up to using sewage sludge as manure for their crops for fear of rejection of their produce.

1.3: Justification

Sludge may be applied to agricultural land, disturbed land, forest or dedicated disposal sites. However, in order to defend human health and the environment from harm, any form of disposal needs to be controlled in view of the fact that sewage sludge potentially carries chemical pollutants (Jones and Alcock, 1996; Jiayin et al., 2006).

In order to restrict human food chain contamination by heavy metals, limits are imposed on the quantity of sewage sludge that can be applied to agricultural land. Heavy metals are often highly lasting in soil and they stay there for as long as thousands of years (Alloway, 1990; Toribioa and Romanyab, 2006). Enhancement of total metals in soil has been ascribed to long term sludge application (During and Gath, 2002). Metals applied with sewage sludge may be maintained in the soil as a result of their fastening on hydroxides, clays and organic matter, the forming of insoluble salts, or the presence of residual sewage sludge particle (Alloway and Jackson, 1991; Toribioa and Romanyab, 2006).

The guideline value of 1-4 pg WHO-TEQ/Kg bw/day PCDD/Fs is the WHO tolerable daily intake and this could be surpassed if a person's food was obtained solely from places originating from the products growing on sewage sludge containing high concentrations of POPs (Wild et al., 1994; Jiayin et al., 2006).

Investigations have been carried out to determine the existence of heavy metals and persistent organic pollutants in water bodies. Scanty studies have also been done on the POPs concentration in sewage sludge and for the heavy metal accumulation in sewage sludge independently. However, few, if any, have combined the determination of toxicity concentrations of both heavy metals and POPs. Such a study will give a holistic picture of all or most of the individual or combined levels of contamination, so that comprehensive remediation decisions can be pursued. It is therefore important to investigate the PAH, PCB, PCDD/Fs and heavy metal levels in sludge amendment soil.

It is obvious that the application of sewage sludge as a soil improver by farmers will not stop because of the perceived advantage. Thus, the cause, effect and trend of sludge usage as manure will therefore be beneficial and informative. These findings will be compared to the United States Environmental Protection Agency (US EPA) intake limits on these toxic pollutants.

1.4: Research Questions

- To what extent is the sewage sludge from Thohoyandou wastewater treatment plant contaminated with heavy metals and POPs?
- How effective is the uptake of heavy metals and POPs from sewage sludge amended soil by *Spinacia oleracea* vegetables?
- What is the extent of the relationship between the quantity of sludge applied and the uptake by *Spinacia oleracea* vegetables?

1.5: Objectives

1.5.1: Main objective

The main objective of this study is to determine the concentration of some heavy metals (Cd, Cr, Cu, Co, Pb, Ni, Zn, Al, Fe, Mn) and POPs (PAH, PCB) in sludge sample from Thohoyandou WWTP, and to assess the extent of of plant uptake of the POPs and heavy metals.

1.5.2: Specific Objectives

The specific objectives of the study are:

- To determine the concentrations of heavy metals, and POPs in sludge sample and soil samples before and after sludge applications.
- To determine the physico-chemical properties such as pH, total organic content, conductivity, cation exchange capacity (CEC) and alkalinity of the sludge sample and soil samples before and after sludge application.
- To determine the concentrations of heavy metals and POPs in *Spinacia oleracea* grown on amended soil with different quantities of sludge applications.
- To determine the concentrations of the chemical/speciation forms of heavy metals in the sludge sample, soil, root and leave samples after sludge applications.

1.6: The Study Area

This project involved two study areas, both located in the Vhembe district in Thulamela Municipality of Limpopo Province, South Africa. The specific study areas are; Thohoyandou wastewater treatment plant (WWTP) and the experimental farm in the School of Agriculture at University of Venda. Sludge sample was collected from the sludge drying beds at Thohoyandou WWTP. Soil and plant samples were collected at the experimental farm of the School of Agriculture at the University of Venda.

Thohoyandou WWTP is located in Muledani, in the Vhembe District of the Limpopo Province in South Africa and lies between longitude $30^{\circ} 28' 28''$ E and latitude $23^{\circ} 0' 13''$ S, as shown in Figure 1.1. It has a catchment area covering Sibasa, Thohoyandou, Shayandima, Manini, and others. These areas are populated with industries, hospitals, various urban and semi urban settlements as well as numerous scattered rural villages. The WWTP has an installed capacity of 6 Megalitres per day and receives effluent wastewater at a rate of 45 litres per second. The treatment processes include physical, biological and chemical processes. These treatment processes include the abstraction of solid substances by physical screening or sedimentation, the abstraction of soluble and fine suspended organic pollutants by biological oxidation and adsorption processes and the adjustment of pH and bacteria load by chemical addition of lime and chlorine.

The School of Agriculture's demonstration farm is located between longitude $30^{\circ} 31' 40''$ E and latitude $22^{\circ} 47' 39''$ S. The climate around the study area is arid and semi-arid, the annual rainfall is ± 500 mm per annum and the temperature range between a minimum of 10°C during winter and a maximum of 40°C during summer. The site has a characteristics of a deep well drained clay soil (Odhambo et al., 2010). Odhambo, et al. (2010) also revealed some pre-sowing analysis of physical and chemical properties of the site as follows; 62% clay, 27% silt, 11% sand, organic carbon 2.09%, total Nitrogen 0.052%, Phosphorus 3.49 mg/kg and pH of 5.75.

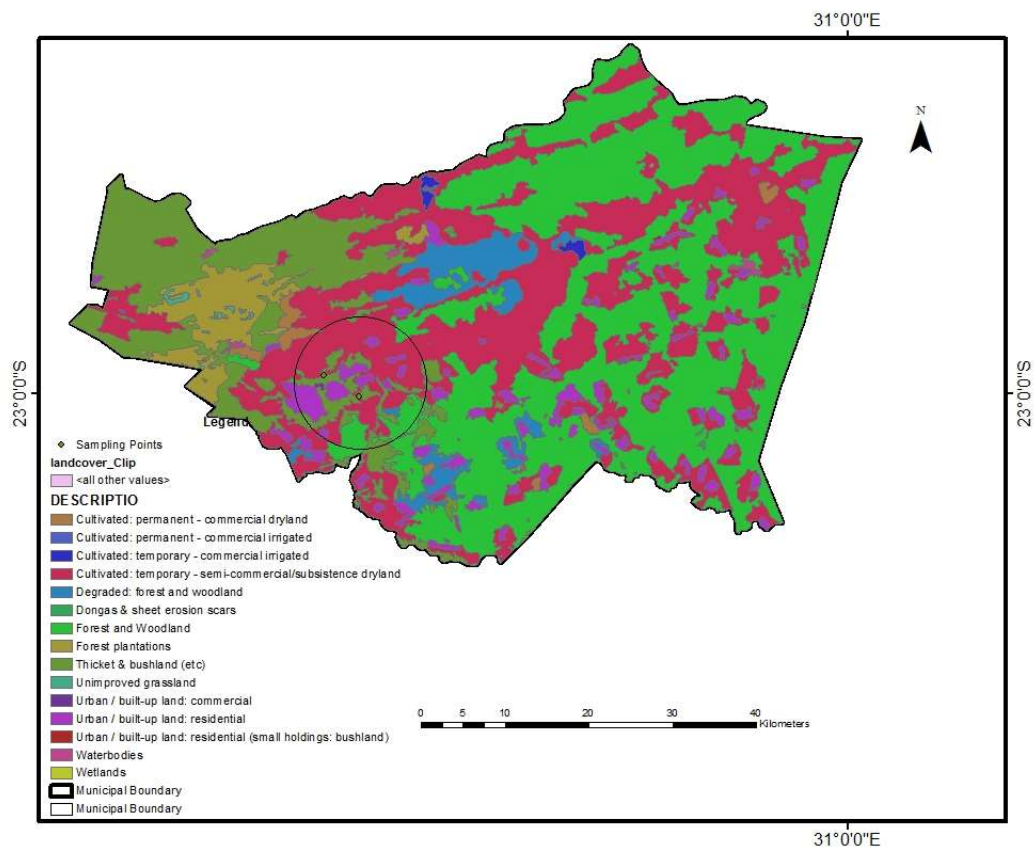


Fig 1.1: Map of Thulamela Municipality showing the study sites of Thohoyandou WWTP and School of Agriculture, University of Venda

CHAPTER 2: LITERATURE REVIEW

This chapter critically reviews the existing literature and work done previously in the field that is within the research questions and research objectives of this project. This is in a bid to acknowledge the previous work done in the field of study and to highlight the place of this research project in the overall context.

2.1: Status of waste generation.

The domestic and industrial activities generate waste materials that end up being disposed to the environment. These activities include; agriculture and livestock production, processing and aquaculture, oil and gas, mining, food, manufacturing, and forestry (Ikehata and Liu, 2011). Depending on the sources, they can be referred to as domestic, municipal, agricultural, or industrial wastes. Patterns of production and consumption of consumer products has evolved over the years leading to the presence of new end-of-life products, including new chemical and microbial substances in waste that may endanger sanitary, cause environmental risks have evolved and these act differently from previously known substances (Marcoux et al., 2013). Municipal effluents are recognized as a major source of many environmental contaminations due to the presence of contaminants, such as polyaromatic hydrocarbons, pesticides, surfactants, steroids, and metals (Gagne et al., 2006).

2.2: Wastewater treatment processes

Municipal wastewater is a combination of liquid or water-carried wastes originating from various places such as sanitary conveniences of dwellings, commercial or industrial facilities and institutions, in addition to any groundwater, surface water and storm water that may as well be present (United Nation, 2003). Contaminants are normally removed from wastewater using physical operation, chemical and biological processes (Tchobanoglous et al., 2004). Individual wastewater treatment procedures are put together into a variety of systems, classified as preliminary, primary, secondary, and tertiary wastewater treatment. This is in order to achieve different levels of contaminant removal (USEPA, 2004).

The preliminary treatment, a physical operation, involves the use of bar screens and grit chambers. The bar screen removes waste such as large trash, sticks, leaves, plastic material,

rags, and cans. In the grit chamber, sand, grit, cinders, and small stones settle to the bottom (USEPA, 2004). The primary treatment phase involves the use of sedimentation method to take out the floating and settleable materials present in wastewater. To improve the removal of suspended solids and dissolved solids chemicals are at times added (Tchobanoglous et al., 2004). A secondary treatment, which uses biological processes, involves two methods mainly, the attached and suspended growth processes. In both processes, microorganisms are used to remove biodegradable organic materials in an aerobic condition converting it to carbon dioxide, water and biomass and in anaerobic conditions converting it into carbon dioxide methane gas and biomass (USEPA, 2004). Piero (1993) described tertiary treatment of wastewater as involving the use of ion exchange columns, activated carbon, ozone, sand filtration and reverse osmosis to further purify the water.

Currently, there are new directions and concerns in the field of wastewater treatment and according to Tchobanoglous et al. (2004) these concerns are apparent in the following specific areas of wastewater treatment; (1) The changing quality in the composition of wastewater (2) Emerging human health and environmental concerns, (3) The problems in industrial waste, (4) Upgrading of wastewater treatment plants, (5) Treatment plant performance and reliability, (6) Process analysis and control.

Information from the website of the Department of Water Affairs (DWA) of the Republic of South Africa (June 2009), revealed that there are 986 WWTPs in South Africa and among these 50% are relatively small systems. The challenges relating to these small-scale operations include having to establish new skills and competence, infrastructural, mechanical and electrical maintenance of the plants.

Wastewater treatment processes removes dissolved metals and biodegradable organic compounds. Depending on the catchment area a WWTP covers, its sewage may contain plenty of heavy metals which cannot be entirely removed during treatment. It also contains persistent organic pollutants mostly in the form of chlorinated hydrocarbon which are not biodegradable and thus cannot be removed during the course of treatment.

2.3: Sewage sludge composition, treatment and disposal

Sewage sludge is the solid byproduct of wastewater treatment (Lowman et al., 2013). Potentially toxic elements, hydrophobic organic contaminants, and pathogens transfer to the sewage sludge during wastewater treatment with potential implications for the use of sludge (Werther and Ogada, 1997). These undesired constituents may have an impact on the environment and also affect soils, plants, animals and human health (Langenkamp and Part, 2001).

Treated sewage sludge is called biosolids and can be grouped into two main classes, A and B. In Class A biosolids pathogens are reduced below current detectable levels, while in Class B biosolids, pathogens are reduced to levels that are not likely to create a hazard to public health and the environment under regulated use. Class B biosolids cannot be sold, given away in bags or other containers, nor applied on lawns or home gardens (Tchobanoglous et al., 2004).

Biosolids are stabilized prior to utilisation or throwing away, to control odours and reduce the number of pathogens. They are usually thickened and may be dewatered to reduce the volume to be transported for final processing, disposal or beneficial use (USEPA, 2004). The methods of stabilization of biosolids practiced in wastewater treatment plants include digestion, lime stabilization, heat-treatment, and composting (Werther and Ogada, 1997). After stabilization, the biosolids can be safely spread on land. Some scientists have suggested that the rules regulating sludge treatment and land application are insufficient to protect the environment and public health because they are based on outdated science (Lowman et al., 2013).

There are various methods of disposal of biosolids and they include; landfilling, incineration, sea dumping, and agricultural use. Biosolids are a good source of organic matter if properly treated and applied, for improving soil structure and for the supply of nitrogen, phosphorus and micronutrients that are needed by plants (USEPA, 2004). Some of the limitations of monitoring land application, enforcing regulatory rules, and systematic tracking and investigation of public concerns are staff shortages and budget constraints at federal, state, and local Government levels (Lowman et al., 2013).

2.4: POPs in soil and sludge

Airborne POPs, transported from the production and usage sites, condense when in soils and sediments at favourable temperatures. Due to their hydrophobic nature, POPs in sewage adsorb to sediments during treatment processes and thus form part of the constituent of sludge. Soils and sediments contain the majority of the load of POPs in the environment where they primarily partition into organic matter (Jones and deVoogt, 1999).

The fact that POPs can bio-accumulate and magnify in the food chain raises concerns around their effect on top predator species including humans (Jones and deVoogt, 1999). The transfer of POPs through some food chains and this tendency has been comprehensively studied. The balance of POPs research has been on aquatic and terrestrial ecosystems: algae – plankton – fish – bird pathway (Skoglund et al., 1996) and air – grass – grazing animals – milk/meat – human pathway (Welch-Paush and McLachlan, 1998). The above mentioned terrestrial pathway is of importance for human exposure and there are other suspected terrestrial pathways for human exposure.

Purported POPs effects include harm to the immune system of predator species at the top of the group (Ross et al., 1995), they are also implicated in enhancing susceptibility to other diseases and effects on the patterns of behavior (Leonards, 1997). Clearly the anxiety over adverse health effects in human and wildlife provide the momentum for research on their sources, environmental faith and food chain transfer (Jones and deVoogt, 1999).

A major issue of concern for the degradation and ecotoxicity of organic pollutants which are hydrophobic in soil environments is mainly due to pollutant bioavailable fraction (Heitzer et al., 1992). Reid et al. (2000) in their study reported that the bioavailability of organic compounds can be studied from two different perspectives; chemical and biological. For biodegradation to occur in biological assessment of bioavailability, two requirements as a prior condition must be satisfied; the compound must be bioavailable that is, easy access to the target organism and the compound must be inherently biodegradable.

Many different factors influence the fate and behavior of organic pollutants in the soil, these factors include soil characteristics, compound properties and environmental factors, such as temperature (Baveye et al., 1999b). Pollutants entering the soil environment can be leached

into ground water, biodegrade, volatilize, or bind to soil solid phase and transfer to organisms (Jones et al., 1996; Reid et al., 2000). Soil – compound interactions have been shown to be affected by some factors such as soil inorganic constituents with particular reference to pore size and structure; soil processing by microorganisms; soil organic matter, both in amount and nature; and pollutant concentration (Reid et al., 2000). Many researchers have proposed that the influence of soil organic matter is the most important factor dominating the interaction of organic compound with soil (Cornelissen et al., 1997).

It has been noticed that pollutants bioavailability and extractability decrease as soil – pollutant contact time increases, a phenomenon termed as “ageing”. These intra soil processes have also been reported to limit the bioavailability of compounds and the formation of non – bioavailable residues increase with time (Reid et al., 2000). Generally, there are two soils – associated compound pools recognized to exist after compound ageing: a fraction of the compound which can be quickly desorbed, and a fraction of the compound which is more slowly desorbed (Pignatello and Xing, 1996). Ageing is widely accepted to be controlled by sorption while the ageing process has been shown to be enhanced by wetting and drying of soil (Reid et al., 2000).

Due to the presence of toxic molecules in the sludge that can be used to enhance the soil productivity, international and national health regulatory bodies have imposed guidelines to regulate their presence in agricultural food products.

1-4 pg WHO – TEQ/Kg bw/day PCDD/Fs is the WHO tolerable daily intake (TDI) guide line value. This could be exceeded if a person’s food is obtained solely from sources originating from the products grown on land that was enhanced with the application of sewage sludge containing high concentration of PCDD/Fs (Dai et al., 2007).

2.5: Conventions on Toxic Contaminants

There are various Conventions, formulated under the auspices of UNEP, this provide an international platform to govern the environmental management of hazardous chemicals throughout their life cycles. They include; The Basel Convention of 1989, The Rotterdam Convention of 1998, and The Stockholm Convention of 2001(UNEP, 2013).

The Basel Convention was to control the movement of hazardous Wastes and their disposal across international boundaries. It is an international agreement that was designed to reduce the transport of hazardous waste between countries, and especially to stop the transfer of hazardous waste from developed to least developed countries (LDCs). The Convention also aims to reduce the quantity and hazardous nature of wastes generated, to ensure they are given sound environmental management as closely as possible to the source of generation, and to assist LDCs in environmentally sound management of the hazardous and other wastes they produce (Short, 2004). The Convention was opened for signature on 22 March 1989, and the enforcement started on 5 May 1992. As at October 2013, there were 53 signatories and 180 states that have indicated to be parties to the Convention, though South Africa is not yet a signatory.

The Rotterdam Convention had to do with the prior informed consent procedure for particular hazardous chemicals and pesticides in international trade. It was adopted in September 1998. As of May 2013, the Convention had 72 signatories and 153 parties. The objective of this Convention was to promote among parties shared responsibility and cooperative efforts in the trans-boundary trade of certain hazardous and toxic chemicals in order to prevent harm to human health and the environment and promote the safe use of those hazardous chemicals. This could be done by encouraging information exchange about their characteristics, providing for a national decision-making process on their import and export, and disseminating these decisions to parties. However, South Africa is not a signatory (Short, 2004).

The Stockholm Convention was specifically on persistent organic pollutants (POPs) and was adopted in 2001. It was in response to protect human health and environment urgently from POPs. The Convention aimed to eliminate or restrict the production and use of intentionally produced POPs and reduce the unintentionally produced POPs. As of May 2013, the Convention had 152 signatories and 179 parties. South Africa ratified the Convention in September 2002 (Short, 2004).

2.6: POP Regulations

The Stockholm Convention presently focuses on eliminating or reducing the release of 12 POPs, the so-called ‘Dirty Dozen’. They are either present as single substances or a mixture of congeners. These twelve chemicals include aldrin, chlordane, dichloro diphenyl trichloroethane (DDT), dieldrin, endrin, heptachlor, mirex, and toxaphene. These are used mostly for the production of pesticides and there are also two industrial chemicals PCBs and hexachlorobenzene (HCB) that are used in industry but also produced unintentionally together with dioxins and furans.

Different international organizations have established guidelines to assess POPs intake risk for human health (Huan-yun et al., 2010). A tolerable daily intake for dioxins plus dioxin-like PCBs of 1–4 pg World Health Organization toxic equivalent (WHO-TEQ)/kg bw/d was specified by the World Health Organization (WHO) (Van den Berg et al., 2000); and a tolerable monthly intake of 70 pg WHO TEQ/kg bw/month was specified by the Joint FAO/WHO Expert Committee on Food Additives (2.33 pg WHO-TEQ/kg bw/d) (Moon et al., 2009). The U.S. Food and Drug Administration developed the action levels of 2,000 ng/g wet weight for PCBs for residues in edible commodities, (US EPA., 2000); [www.epa.gov/ost/fishadvice/volume2/index.htm]). The FAO/WHO has recommended acceptable daily intakes and provisional tolerable daily intakes of 1.0×10^4 ng/kg bw/d and 5,000 ng/kg bw/d for DDTs and hexachlorocyclohexanes (HCHs), respectively (IPCS, 2009; http://www.who.int/ipcs/publications/jmpr/jmpr_pesticide/en/index.html).

Screening values (SVs) has been established by the U.S. Environmental Protection Agency for contaminants such as PCBs, octacalcium phosphates (OCPs), and PBDEs. These are described as concentrations of analytes aimed at in fish and shellfish tissue which can be used as minimum values to evaluate possible risk associated with consumption of poisoned fish or seafood and to suggest that more thorough investigation should be carried out (US EPA, 2000; Cheung et al., 2007). The SVs are calculated using the following equations:

To calculate non-carcinogens: $SV_n = (RfD.BW) / CR \dots\dots\dots(1)$

To calculate carcinogens: $SV_c = ([RL/CSF].BW)/CR \dots\dots\dots(2)$

Screening value (SV) is in mg/kg; body weight (BW) is in kg; consumption rate (CR) is in kg/d; and maximum acceptable risk level (RL) is in 10^{-5} (US EPA, 2000; <http://www.epa.gov/earth1r6/6pd/qa/qadevtools/mod4references/supplemental/volume1.pf>).

The values for the oral reference dose (RfD) is in mg/kg/d while the oral cancer slope factor (CSF) is in mg/kg/d⁻¹. These values are available online at <http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris>.

HR is the hazard ratios and is calculated by dividing the average daily exposure (EDI) by the screening values. A ratio greater than unity, suggest that the average daily exposure level is more than the SVs (Jiang et al., 2005).

There is no particular legislation as regards the production, use, transportation and disposal of endocrine disruptive chemicals (EDCs) and POPs in the countries of the Orange-Senqu River basin. In addition, South Africa with its sophisticated water law of 1998, still have to deal with the problems presented by EDC and POPs (Water Wheel, 2008). No limits have been set for EDCs in South Africa, and water quality guidelines make no reference to these substances.

2.7: POP Methods of Analysis

According to the “Guidance for Analysis of Persistent Organic Pollutants”, published by UNEP Chemicals Branch, Division of Technology, Industry and Economics (DTIE), POP analysis involves; extraction of the POP, purification/clean up and separation and quantification. In doing these, Laboratories may adopt European Union (EU), United States Environmental Protection Agency (USEPA), Association of Analytical Chemist (AOAC), and American Society for testing and Materials (ASTM) methods of analyses.

There are various methods of extraction. These include Soxhlet extraction, sonication extraction, supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), liquid-liquid extraction, microwave assisted extraction (MAE), and solid phase extraction (SPE). The traditional classical methods are the sonication, Soxhlet and liquid-liquid, while MAE, ASE and SFE are recent methods.

The classical methods have the advantages of low cost equipment and easy handling and use. Their disadvantages however include; long extraction times, requiring large solvent volumes, giving a diluted extract and not being automated (Camel, 2000). The recent extraction methods have advantages of fast extraction, low solvent volume required and extracts are concentrated. The disadvantages include; high cost of equipment, care owing to high pressures and elevated temperatures that may degrade thermolabile analytes (Camel, 2000).

For the purpose of this project, Soxhlet extraction was employed because it is easily available, easy to operate and it normally results into high enrichment and recoveries. After extraction, the extract will be concentrated by evaporation under vacuum or with nitrogen.

Purification is usually performed to remove substances/materials that will interfere with the analyte in order not to obtain results that will be understood wrongly. Purification can be performed with different types of adsorbents and with different solvents depending on selectivity, conditioning and column flow.

Separation, identification and quantification of POPs can be carried out using high performance liquid chromatography (HPLC) or gas chromatography (GC). GC however have the following advantages; high sample resolution, high speed and accuracy, high sensitivity and easy to use. The analysis of POPs was conducted through Gas chromatography with mass spectrometric detector (MS detector).

2.8: Heavy metals in Sludge and Soil

Researches done on the chemical constituents of sewage sludge have revealed the presence of heavy metals e.g Zn, Cd, Cr, Ni, Cu etc, in stabilized sludges. This may be due to a number of reasons which include; the residue of the chemicals used during the process of treatment and stabilization, metals contained in the influent sewage that did not get removed by the treatment process or according to Panov et al. (2008), the accumulation of heavy metals by microorganism in active sludge treatment from biological treatment plants.

Blackeslec (1973) sampled sludge from 57 treatment plants in Michigan U.S.A and Berrow and Webber (1972), sampled sludge from 42 plants in England and found that metal concentrations in sludge exhibit wide variations from one city to another reflecting the proportion of domestic and industrial input into the waste water system.

Traditionally, it was of the view that the migration of heavy metals in sewage sludge amended soils is insignificant and that metals remain at the site of input that is, in the topsoil (Dowdy et al., 1991; Ashworth and Alloway, 2004). However, several field-based experiments have confirmed the apparent migration of metals to depth parts of the soil (McBride et al., 1997a; Ashworth and Alloway, 2004).

It has widely been reported that the amendment of soils with sewage sludge often leads to higher concentrations of metals in the food chain (Wei and Liu, 2005). The increase might be from the fact that repeated application of sewage sludge has reduced the capacity of the soil to retain heavy metals and thus heavy metals leach into the ground water or soil solution and will be available for plant uptake. This can strongly influence the speciation and bioavailability of metals derived from anthropogenic sources (Chary et al., 2007).

Results from other studies have shown that when contaminated soils are used for crop production heavy metals are potentially toxic to crops, animals and humans (Xian, 1989). Heavy metals are non-biodegradable, become toxic at some concentrations and they also tend to accumulate along the food chain where man is the last link (Amir et al., 2005).

The determination of total heavy metal content does not provide useful information about the risks of bioavailability, the capacity for remobilization and the behaviour of the metal in the environment (Hsu and Lo, 2001). The estimation of heavy metal bioavailability is from its chemical forms or speciation as this is related to the different natures of the metals, their bonding strength either in free ionic form or complexed by organic matter or incorporated in the mineral fraction of a sample (Amir et al., 2005).

The different forms heavy metals may be found in soils are;

- Dissolved in soil solution
- Exchangeable, in organic and inorganic compounds

- As structural components of the lattices of soil minerals
- As insoluble precipitates with other soil components

It is the first two forms that are available to plants, the other two are potentially available in the longer term (Amir et al., 2005). In general, the equilibrium between the soil solution and solid phase determines the concentration of an element in the soil solution with pH playing the decisive role (Aydinalp and Marinova, 2003). The soil's ability to immobilize heavy metals increases with rising pH and peaks under mildly alkaline conditions (Aydinalp and Marinova, 2003). Investigation of speciation of metals in sludge, soil or sludge amended soil, may help in the assessment of the bioavailable metal fractions which is considered important for predicting the release of metals into soil and subsequently absorption by plants.

Stover et al. (1976) in their study of the forms of metals in wastewater sludge reported that sludge contains a wide variety of sites capable of metal retention. Retention mechanisms include ion exchange, sorption, chelation and precipitation. Retention depends on the chemical properties of sludge and the nature of the metal being investigated. Different forms of metal exist in a given wastewater sludge and the predominant form of metal present varies for the different sludge. Thus, each metal will respond differently after incorporation into the soil system and the quantity available for the sorbed and exchangeable fractions may indicate which metals can be most readily used by plants growing in soils treated with wastewater sludge. The concentrations of Zn, Cd, Cu, and Ni increased significantly with the application of sludge on forages (Lopez – Mosquera et al., 2005).

The adsorption capacity of soils is influenced by soil properties such as pH, content of clay, organic matter, exchangeable bases and hydrolytic acidity (Sprynskyy et al., 2011). Fractionation studies have shown that distribution of metal ions in the different chemical fractions depended not only on the solid matrix composition and its physico-chemical properties but mainly on the nature of the metal itself (Paulauskas et al., 2011).

Heavy metal limits for the sludge and the receiving soils are always presented as total metal concentrations and rarely consider soil type and land use practices (Ross, 1994). Of much greater importance than total metal is metal solubility, which is a function of properties such

as chemical form of the metal, soil pH, texture, organic matter content, etc (McBride et al., 1997b).

2.9: Heavy metals in plants

The different ways heavy metals can enter into the human body include (1) through inhalation of dust, (2) consumption of contaminated drinking water, (3) direct ingestion of soil and (4) consumption of food plants grown in metal contaminated soil (Cambra et al., 1999). One important pathway through dietary uptake of metals could be through crops planted in soil enhanced with sewage sludge (Wei and Liu, 2005).

Analyses of heavy metal contents in the roots, stems and leaves of plants grown in sludge fields showed the presence of heavy metals (Panov et al., 2008). Vegetables contain carbohydrates, proteins, antioxidants as well as vitamins, minerals and trace elements and thus constitute an important part of the human diet. It is known that serious systematic health problems can develop as a result of excessive accumulation of heavy metals such as Cd, Cr, and Pb in the human body (Oliver, 1997). Dospatliev et al. (2012) also pointed out that vegetables poisoned with heavy metals can cause serious human health risks, such as renal failure, symptoms of chronic toxicity and liver damage. Vegetable products require strict regulatory control regarding heavy metals contents.

2.10: Heavy Metal Analysis

Techniques of heavy metals analysis can be broadly categorized into;

- Single-element methods, such as atomic absorption spectrometry (AAS) or
- Simultaneous multi-element methods, such as inductively coupled plasma optical emission spectrometry (ICP-OES) or X-ray fluorescence spectrometry (XRFS).

They can further be categorized into methods such as AAS, which carry out the analysis in solution i.e with dissolved samples, or methods such as XRFS, which analyse solid samples more or less directly.

The choice of method for a particular application will take account of those factors as well as their sensitivity, precision and accuracy. The choice of method may well, however, be

dominated by the relative cost of the (mainly instrumental) techniques currently in use. Atomic spectrometric methods are by far the most commonly employed. For total metal analysis, several mineral acids (HCl, HNO₃, HClO₄, H₂SO₄) and their mixtures have been used for the dissolution and extraction of elements from soils.

For the analysis of the different metal forms, a number of different sequential extraction procedures (SEP) for heavy metal fractionation have been presented by researchers from time to time, such as by McLaren and Crawford (1973), Tessier *et al.* (1979), Forstner (1985), Salomons and Forstner (1984), Emmerich *et al.* (1982), Shuman (1985), Sims and Kline (1991), Ure *et al.* (1993), Ma and Rao (1997), Maiz *et al.* (1997), Luo and Christie (1998), Basta and Sloan (1999), Nemati *et al.* (2011) and the community bureau of reference (BCR) method, currently known as SMAT (The standards measurement and testing programme). These sequential extraction procedures could be very helpful when seeking to isolate the fractions of heavy metals more specifically.

In SEP, the most mobile metals are removed first with the first fractionation procedure and continue in order of decreasing mobility. All SEPs facilitate fractionation. These fractions are named according to Tessier *et al.* (1979) as exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound, and residual. These fractions can also be referred to as exchangeable, weakly absorbed, hydrous-oxide bound, organic bound, and lattice material components, respectively (Maiz *et al.*, 2000). Metals of anthropogenic sources tend to reside in the first four fractions and metals of natural sources in parent rock are found in the residual fraction (Ratuzny *et al.*, 2009).

The sequential extraction procedure for removing the various metal fractions is as follows; the ionic composition of water is changed to allow metals sorbed to the exposed surfaces of sediment to be removed easily as the exchangeable fraction, this is commonly done using a salt solution. The carbonate-bound fraction is removed using an acidic solution because this fraction is susceptible to changes in pH. Metals bound to Fe and Mn oxides are particularly susceptible to anoxic (reducing) conditions thus a third solution capable of dissolving insoluble sulfide salts is used. The fourth fraction of metals bound in the organic phase is removed by oxidizing the organic material. The residual fraction consists of metals incorporated into the crystal structures of primary and secondary minerals. This fraction is the

hardest to remove and requires the use of strong acids to break down silicate structures (Tessier et al., 1979).

2.11: Spinach Vegetable

Spinach (*Spinacia oleracea*) belongs to the order Caryophyllales and family Amaranthaceae. It originated from Iran and is now widely grown in the temperate regions of the world (Department of Agricultural Forestry and Fisheries, 2011). It is a quick maturing, cool-season vegetable crop which germinates at 2°C to 30°C but 7°C to 24°C is optimal. Spinach can withstand low temperatures of -9°C to -6°C without great injury (Koike et al., 2011).

A constant and uniform supply of water is required by the plant in order to obtain a good crop of high quality. The soil should always be moist and never allowed to dry out (Department of Agricultural Forestry and Fisheries, 2011).

A variety of soils are used for spinach production but in most regions sandy loam soils with high organic matter content are preferred. Spinach is particularly sensitive to saturated soil conditions and to acidity. The optimum soil pH is 6.2 to 6.9 (Koike et al., 2011). Spinach can be eaten as raw salads and cooked as a pot-herb. It is a source of vitamin A (retinol), B₁ (thiamine), B₂ (riboflavin), B₃ (niacin) and calcium. The seeds can be used as a laxative and for treating breathing difficulties and liver inflammation (Department of Agricultural Forestry and Fisheries, 2011).

CHAPTER 3: METHODOLOGY

This chapter highlights the procedures followed in carrying out the project. It explains the research designs, the sampling and all laboratory activities carried out to achieve the research objectives and get answers to research questions.

3.1: Research Design

This project employed experimental research strategies relevant for the exploration and finding of explanations for the cause and effect relationship between the quantities of sewage sludge applied on soil and the concentrations of some heavy metals and POPs that eventually get into vegetables grown on this amended soil (Saunders et al., 2012).

A preliminary survey of farms suspected to be using sludge as a soil improver was conducted around Thulamela municipality in Limpopo Province in South Africa. When interviewed, farmers did not admit to be using sludge on their farmlands for fear of rejection of their produce by prospective buyers. Therefore, this project was simulated by planting spinach vegetables on sludge amended soil at the demonstration farm of School of Agriculture, University of Venda.

Eleven ridges of dimensions 0.3 m by 20 m were made on a plot of land 20 m by 30 m and sludge quantities of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Kg were measured on each of ten ridges respectively using a top loading balance, the eleventh ridge contained no sludge to serve as the control. The sludge was pulverized and mixed with the top soil of 1 – 10 cm depth

Vegetable spinach seeds were purchased from a reputable agricultural product outlet and planted on trays using agro-mix as the substrate. The trays were placed in a greenhouse for four weeks for the seeds to germinate and grow into seedlings (Figures 3.1 and 3.2).



Fig 3.1: *Spinacea oleracea* seed germinated at five days old



Fig 3.2: *Spinacia oleracea* seedling at fourteen days old

The seedlings were then transferred at four weeks old to the already prepared and labeled ridges on the farmland (Figures 3.3 - 3.5) and allowed to grow for another eight weeks before harvesting. Irrigation was done twice daily using sprinklers.



Fig 3.3: *Spinacia oleracea* seedling transferred to the field at twenty eight days old



Fig 3.4: *Spinacia oleracea* on the field at fifty six days old

At maturity, the vegetable parts, namely the root and the leaves, were sampled on each of the ridge on the farm and the soil before sludge addition and after harvesting.



Fig 3.5: *Spinacea oleracea* on the field at seventy-four days old ready for harvest

Sludge was sampled from the drying beds at the Thohoyandou WWTP. Total heavy metals, speciated heavy metals and POPs concentrations were analyzed for in the sludge, soil and plant samples. Moreover, pH, total organic carbon and alkalinity were measured in sludge and soil samples. Controls were incorporated to ensure the validity of data and the research was a cross – sectional study in that it involved a field study at one point in time or over a short period.

3.2: Sampling

3.2.1: Sludge Sample

Sludge was sampled from the sludge drying beds at Thohoyandou wastewater treatment plant in Muledane and the representative sample was achieved by stratified random sampling by taking a number of sample units with a stainless-steel shovel from the field and combining them to form a bulk sample. The sampler was cleaned with deionized water and dried between samples to avoid cross contamination (Harvey, 2000). For POP analysis, the sludge sample was collected in a Borosilicate glass wrapped with dark tape to keep sunlight out, 40-mL, with a teflon-lined screw cap capable of forming an air tight seal and kept at $\leq 6^{\circ}\text{C}$ when transporting to the laboratory (Figure 3.6). Containers used to collect samples for the determination of POP was washed with soap and water followed by methanol (or isopropanol) rinsing prior to use (USEPA, 2007). For heavy metals analysis, the sludge sample was kept in a polypropylene bag.



Fig 3.6: Sludge sample been collected on a sludge drying bed

3.2.2: Soil Sample

Soil sample was sampled from the ridges of the simulated farm located at the demonstration farmland of University of Venda. Soil sampling was done before sludge application and after harvesting on the ridges. A control soil sample was also taken from a ridge with plants but no sewage sludge. Representative samples were taken as above from top soil of depth 0-10 cm. For POPs and heavy metals analyses, the soil samples were collected and put in containers as was done for the sludge sample in section 3.2.1.

3.2.3: Plant Sample

Plant samples were taken from the simulated farm located at the demonstration farmland of the University of Venda. Control plant samples were also taken from a ridge that had no sewage sludge applied to it. The roots and leaves of the plant were handpicked and representative samples were achieved by randomly taking a number of sample units and combining them to form a bulk sample (Howman et al., 2001). For POPs analysis, the plant samples were collected in a borosilicate covered glass, 40-mL, with a Teflon-lined screw cap capable of forming an air tight seal and kept at $\leq 6^{\circ}\text{C}$ when transporting to the laboratory. Containers that were used to collect samples for the determination of POP were washed with soap and water followed by methanol (USEPA, 2007). The integrity of the samples was preserved during the process of transportation from the study area to the laboratory by

transporting the samples in coolers and away from direct sunlight (Figure 3.7). For heavy metals analysis, the plant samples were kept in polypropylene bags when transporting to the laboratory.



Fig 3.7: Sludge sample for POP analysis preserved in a cooler box of ice

3.3: Sample Pre-treatment

3.3.1: Sludge Sample

Sludge samples for heavy metal analysis were prepared for drying by removing all dirt, breaking down aggregates, using a pulverizer (FRITSCH analysette 3 SPARTAN pulverisette 0). Samples were mixed by coning and quartering, it was poured in the form of a cone and then flattened to form a circular layer of material. The circular materials were divided into four even parts and two opposite quarters were discarded. The two remaining quarters were combined and the coning and quartering was repeated until a manageable sample size was obtained (Radojevic and Bashkin, 2006). The samples were then dried at 70°C in an electric oven (Scientific series 200) overnight. The dried sludge was passed through a sieve with a 2 mm mesh size. The < 2 mm sludge was then stored in polyethylene bags until required for analysis. This sample was also used to determine the pH, alkalinity, total organic carbon, cation exchange capacity and conductivity measurements of the sludge.

For POPs analysis, matrix molecules were removed from the samples, crushed and stored in a wrapped borosilicate glass which was then kept in the refrigerator at $\leq 6^{\circ}\text{C}$ until the time for sample preparation.

3.3.2: Soil Samples

Soil samples for heavy metal analysis were prepared for drying by breaking down aggregates, using a pulverizer, and were mixed by coning and quartering as in section 3.3.1 above. It was then dried at 70°C in an electric oven overnight the dried soil was passed through a sieve with a 2 mm mesh size. The < 2 mm soil was stored in polyethylene bags until required. This sample was also used to determine pH, Alkalinity, total organic carbon, cation exchange capacity and conductivity of the soil. Sample pretreatment for POPs analysis was done following the same procedure described for sample pretreatment for POPs analysis in section 3.3.1.

3.3.3: Plant Samples

Plant sample (leave and root) for heavy metals analysis were washed with deionize water, oven dried using an electric oven at 70°C overnight and then crushed using a pulverizer (Figure 3.8). The sample was then stored in polyethylene bags until required.

For POPs analysis, the root and leave samples were washed with deionize water to remove the attached soil material. They were then stored in a wrapped borosilicate bottle in a refrigerator at $\leq 6^{\circ}\text{C}$ until required.

3.4: Analytical procedures and sample preparation

3.4.1: pH determination

The pH measurements of soil and sludge samples were carried out using a method described by Blackeslec (1973). 20 g of dried and sieved soil was measured and placed into a 50-mL beaker and 100 mL of deionized water added, covered and suspension continuously stirred for 5 minutes. The soil suspension was allowed to stand for about 1 hour and for the sludge for about 15 minutes to allow most of the suspended clay and waste to settle out from the

suspension. The aqueous phase was filtered using a 0.45 μm pore size disc filter (Figure 3.9). The filtrate was then measured for pH using a pH meter (Ohaus Starter 2100).



Fig 3.8: Pulverizer used for grinding samples



Fig 3.9: Vacuum filtration pump with 0.45 μm pore size filter paper

3.4.2: Alkalinity determination

Twenty (20) g of soil or sludge sample was measured using an analytical balance into a conical flask (Figure 3.10). 100 mL of deionized water was added and stirred. It was allowed to stand for 30 minutes and filtered through a 0.45 μm pore size filter paper. The initial pH of the filtrate was measured. To the sample solution of pH below 8.3, three drops of bromo cresol green indicator were added and titrated with 0.2 M hydrochloric acid solutions. The colour change was from blue to yellow. To the sample solution of pH above 8.3, three drops of phenolphthalein indicator were added and titrated against 0.2 M hydrochloric acid solutions. The colour change was from pink to colourless. This was followed by the bromo cresol green titration to a yellow end point (USEPA, 1975). Alkalinity of the filtrate was calculated in $\text{mg CaCO}_3 \text{ L}^{-1}$ while the alkalinity of the soil was calculated using the following formula

$$\text{Alkalinity (mg CaCO}_3 \text{ g}^{-1}) = A \times V/M$$

A = alkalinity of the extract ($\text{mg CaCO}_3 \text{ L}^{-1}$)

V = volume of water (L)

M = weight of the soil sample (g)



Fig 3.10: Sand and sludge sample slurry in deionize water

3.4.3: Organic carbon determination

Sludge and soil samples extraction involved rapid dichromate oxidation of organic matter, using the Walkley-Black procedure which has been the reference method. 1 g of the soil or sludge sample was measured on an analytical balance. 10 mL of 0.16 M potassium dichromate ($K_2Cr_2O_2$) solution and 20 mL of concentrated H_2SO_4 was added. The mixture was heated to $135\ ^\circ C$ on a hot plate for 30 minutes and allowed to cool. It was then made up to 125 mL with distilled water and 1 mL of H_3PO_4 was added to the digestive mix to eliminate interferences from the ferric (Fe^{3+}) iron that may be present in the sample (Tiessen and Moir, 1993).

The digestive mix was titrated with 1 M $FeSO_4$ solution using five drops of a solution of ortho-phenanthroline ferrous complex as the indicator (Figure 3.11). Colour change associated with this indicator was green to reddish brown for the orthophenanthroline ferrous complex. Organic carbon was then calculated according to equation 3.1;

$$\text{Organic carbon (mg/g)} = \frac{18 \times C \times V}{M} \times (1 - V_1/V_2) \dots\dots\dots 3.1$$

M

C = concentration of dichromate solution (mol/L)

V = volume of dichromate solution used (mL)

V_1 = volume of titrant used in sample determination (mL)

V_2 = volume of titrant used in blank determination (mL)

M = weight of sample used (g)

Organic carbon (%) = organic carbon (mg/g)/10



Fig 3.11: Titration of soil and sludge samples for total organic carbon analysis

3.4.4: Cation exchange capacity (CEC) determination

5 g of soil was weighed into a 100 mL bottle and 25 mL of 1 M ammonium acetate solution adjusted to pH 7 with acetic acid was added and was shaken for 1 hour on a flask shaker. The supernatant was filtered directly through a 0.45 μm filter paper into a 100 mL volumetric flask. The soil was left in the bottle; 20 mL of 95 % ethanol was added, shaken and allowed to settle. The supernatant was again filtered into the same 100 mL flask. The washing, shaking and filtering procedure was repeated twice more, each time letting the soil remain in the bottle. The extract was made up to 100mL mark with deionized water and the concentrations of the exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ and K^+) were determined using ICP – OES (Radojevic and Bashkin, 2006). CEC is reported in meq per 100 g of soil. The conversion is done as follows;

$$\text{mg Ca g}^{-1} \text{ soil} = 0.1 \times C_{\text{Ca}}/5$$

C_{Ca} = concentration of Ca in soil extract in mg/L

$$\text{mg Ca (100)}^{-1} \text{ soil} = 100 \times 0.1 \times C_{\text{Ca}}/5 = 20 \times 0.1 \times C_{\text{Ca}}$$

$$\text{meq Ca (100)}^{-1} \text{ soil} = 2 \times C_{\text{Ca}}/20$$

3.4.5: Conductivity determination

2 g was weighed into a conical flask using an analytical balance, and 40 mL of deionized water was added. This was stirred vigorously and was allowed to stand for 30 minutes. The conductivity was determined with a conductivity meter (Mettler Toledo), and was ensured that the electrode cell was in the supernatant above the settled particles.

3.4.6: POPs Analysis

Samples for POPs determination was taken through the process of extraction, using Soxhlet extraction method, purified to remove interfering substances from the analyte using an adsorbent packed in a column and dried using a drying agent also packed in a column. Samples were then filtered using a disc filter of 0.45 μm pore size. Samples, prior to or after pretreatment were kept in the refrigerator at $\leq 6^{\circ}\text{C}$.

The Soxhlet extraction process described by USEPA sw-846 method 3540C was adopted (Figure 3.12). 10 g of the solid sample was homogenized with 10 g of anhydrous sodium sulfate and placed in an extraction thimble. The extraction thimble drained freely for the duration of the extraction period. 300 mL of the extraction solvent (Acetone/Hexane (1:1) (v/v), $\text{CH}_3\text{COCH}_3 / \text{C}_6\text{H}_{14}$) was placed into a 500-mL round bottom flask containing one or two clean boiling chips. The flask was attached to the extractor and condenser. Samples were extracted for 24 hours at 4 - 6 cycles/hour.

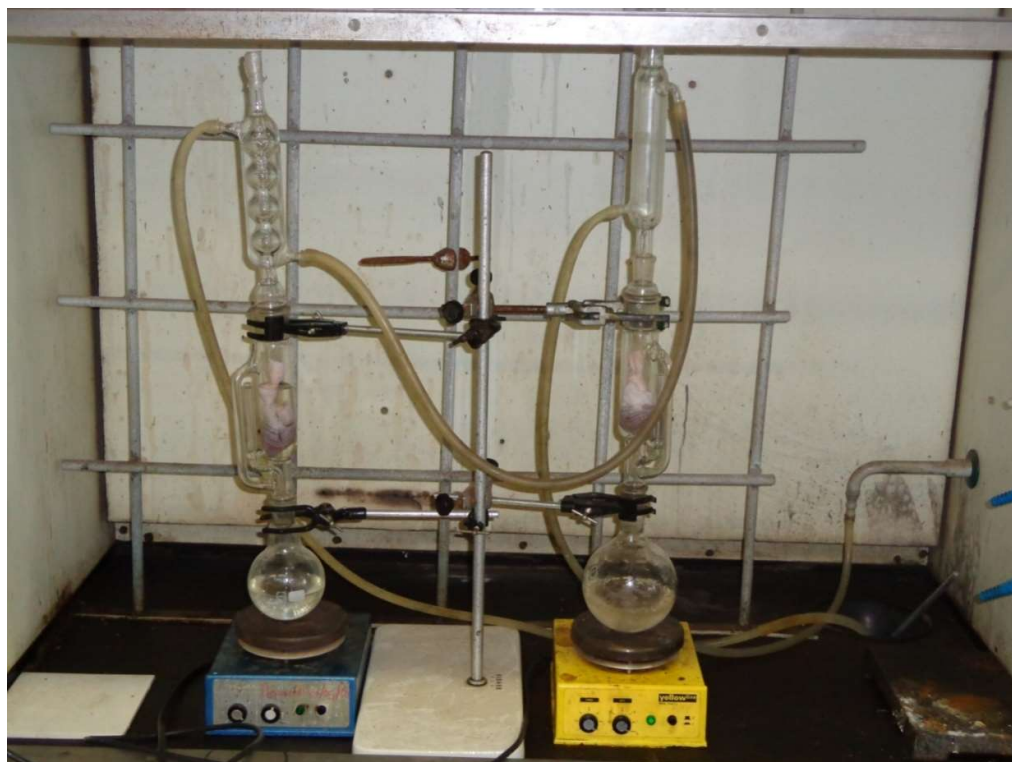


Fig 3.12: Soxhlet extraction of POPs from samples

For the cleanup process described by USEPA SW-846 method 3630C, slurry of 10 g of activated silica gel was prepared in methylene chloride and placed into a 10 mm ID chromatographic column. The column was tapped to settle the silica gel and the excess methylene chloride was eluted and 2 cm of anhydrous sodium sulfate was added to the top of the silica gel. The column was pre-eluted with 40 mL of pentane. The rate for all elutions was 2 mL/min. The eluate was discarded and, just prior to exposure of the sodium sulfate layer to the air, 2 mL cyclohexane sample extract was transferred onto the column using an additional 2 mL cyclohexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, 25 mL of pentane was added and the elution of the column was continued. This pentane eluate was discarded. Next, the column was eluted with 25 mL of methylene chloride/pentane (2:3) (v/v) into a flask and was concentrated, using a vacuum evaporator (BuchI Rotavapor R- 210) and water bath (Heating Bath B- 491), to 5 mL volume (Figure 3.13). The extract was then analysed using a GC × GC TOFMS (Pegasus 4D LECO Corporation).



Fig 3.13: Vacuum evaporator for concentration of sample extracts

3.4.7: Heavy Metal Analysis

(a) Total heavy metal

The USEPA method 3050B was used with minor modifications. One (1) g dry weight of sample was weighed using an analytical balance (RADWAG WAS 220/C/2) and transferred into a digestion vessel (Figure 3.14). 10 mL of 1:1 nitric acid was added and the slurry mixed. The vessel was covered with a watch glass and sample was heated to $95^{\circ}\text{C} \pm 5^{\circ}\text{C}$ on a hot plate (Labcon HPE 3118u). It was refluxed for 15 minutes without boiling. The sample was allowed to cool and 5 mL of concentrated nitric acid was added, the cover replaced and refluxed for 30 minutes. This step was repeated until no brown fumes were given off by the sample. The sample was then heated at $95^{\circ}\text{C} \pm 5^{\circ}\text{C}$ without boiling for 2 hours. The vessel was covered at all times. The sample was allowed to cool and 2 mL of water and 3 mL of 30% hydrogen peroxide was added. The vessel was again covered and heated until effervescence subsides and the volume reduced to approximately 5 mL then the vessel cooled. This step was repeated until the effervescence is minimal. After cooling, the digestate was diluted to 100 mL with distilled water in a standard flask. Particulates were removed by

filtration with 0.45 μm pore size disc filter and the concentration of metal was done using the ICP – OES (Thermo Fischer scientific).



Fig 3.14: Sample digestion for total heavy metals on a hot plate

(b) Heavy metal speciation

The modified form of the extraction procedure by Tessier et al. (1979) for heavy metals analysis was used to fractionate the soil, sludge and plant samples. The method was aimed at differentiating metal fractions into five stages. The procedure was as follows: For the first fraction referred to as exchangeable fraction, 1 g of the sample was placed in a 50 mL tube and was shaken at room temperature with 8 mL of 1.0 M MgCl_2 solution using a flask shaker for one hour. For the second fraction referred to as carbonate bound or weakly absorbed fraction, 8 mL of 1.0 M NaOAc adjusted to pH 5 with acetic acid was added to the residue from the first fraction and then shaken for 5 hours at room temperature and centrifuged. The third fraction, also called the hydroxide bound or Fe and Mn oxide bound fraction, was achieved by adding 20 mL 0.04 M hydroxylamine chloride ($\text{NH}_2\text{OH} \cdot \text{HCl}$) in 25% (v/v) HOAc , to the residue from second fraction and was shaken for 6 hours at 96 ± 3 $^\circ\text{C}$ in a thermostated water bath shaker. The organic matter bound fraction which is the fourth, was extracted by adding to the residue from fraction three, 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3 with the aid of a pH meter and the mixture was heated to 85 ± 2 $^\circ\text{C}$ in a water bath for 2 hours with occasional agitation. A second 3 mL aliquot of 30% H_2O_2 (pH 2 with HNO_3) was added and the sample was again heated to 85 ± 2 $^\circ\text{C}$ for 3 hours with intermittent agitation. After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3

was added and the sample diluted to 20 mL and agitated continuously for 30 minutes. Each fraction was separated from the supernatant by centrifugation at 10,000 rpm, for 30min in a centrifuge. The supernatants were filtered using a 0.45 μm pore size disc filter ready for instrumental analysis. The sediments were rinsed with 8 mL of deionized water and centrifuged again. For the fifth fraction, the USEPA method 3050B for the determination of total metal content was used, as described in section 3.4.2. Concentration of metals was determined using the ICP –OES.

3.5: Instrumental analysis

3.5.1: pH determination

A pH meter (OHAUS STARTER 2100), was used for all pH determination. Prior to use, the instrument was calibrated using standard buffer solutions. Buffer solutions of pH 4 and 7 were used for calibration when measuring acidic samples while buffer solutions of pH 9 and 7 were used when measuring samples with pH in the alkaline range. After this was done, the instrument was then used for pH determination of the analytes.

3.5.2: Conductivity determination

Conductivity measurement was done using a conductivity meter (Mettler Toledo). Prior to the measurement, the meter was calibrated using two potassium chloride solution calibration standards of value 1413 $\mu\text{S}/\text{cm}$ and 12.88 mS/cm after which the samples conductivity was measured.

3.5.3: POPs determination

Quantitative and qualitative determination of PAHs and PCBs standards and samples were performed with the GC \times GC TOFMS (Pegasus 4D LECO Corporation, St Joseph, MI, USA) (Figure 3.15). Calibration standards were prepared using certified reference materials (CRM) supplied by Sigma Aldrich (Johannesburg, South Africa). The PCB CRMs included PCB 3, PCB 77, PCB 209 and the PAH CRMs included Benzo(k) fluoranthene, Acenaphthene, Naphthalene and Benzo (e) pyrene. Other PAH standards used include; Anthracene, Pyrene, Ferrocene, Phenanthrene, and Flouranthene. 1000 mg/L stock solutions of PCB standards

were prepared in hexane and 1000 mg/L stock solutions of PAH were made in acetonitrile. Mixed standards of 10, 20, 30, 40 and 50 mg/L of PCB and PAH were made from the stock solutions, and this were analyzed in the GC × GC-TOF-MS to produce the calibration standards.



Fig 3.15: GC × GC TOFMS instrument used for POP determination

Helium was used as the carrier gas whereas nitrogen, compressed air and liquid nitrogen were for the operation of the jet thermal modulator. The sample injector temperature was set at 250⁰C and samples were injected at a volume of 1 μL with splitless mode. The flow of carrier gas was set at a rate of 1.5 mL/min. the GC × GC column set comprised of a 29.59 m, Rxi-5Sil MS (0.25 mm internal diameter, 0.25 μm stationary film thickness) for the first while the second column was 1.180 μm Rxi-17Sil MS (0.15 mm internal diameter, 0.15 μm stationary film thickness). Temperature programme on the first column oven was 80⁰C held for 0.5 min, and increased to 220⁰C at a ramping rate of 20⁰C then to 290⁰C at a ramping rate of 10⁰C held for 1.5 min.

3.5.4: Metal determination

Both total and speciated metal analysis was done using ICP-OES (Thermo Fisher scientific). The instrument was allowed to equilibrate for about 15 minutes and all the instrument settings were optimized. It was then calibrated using a multi element standard containing 100 parts per million (ppm) concentrations of each metal. This stock standard solution was then used to prepare dilute standard solutions of 5, 10, 15, 20, 25 ppm, 30, 35 and 40 mg/L. These were introduced into the instrument to generate an instrument response which was then used to plot a calibration curve, after which the sample analytes were then introduced into the instrument to determine the metal concentrations in all the samples.

3.6: Cultivation of *spinacia oleracea*

Spinach was propagated by seed. The soil was ploughed to a depth of at least 20 cm and then harrowed. The seed was planted 1.2 to 1.9 cm deep (Koike et al., 2011). The seeds were sown in rows on wide beds and spacing between rows was 60 cm and between plants in a row 20 cm (Department of Agriculture, Forestry and Fisheries, 2011). Three irrigations were done between seeding and emergence and then throughout the entire crop growth (Koike et al., 2011). The fields were sprinkler irrigated to ensure the germination of seed; the first irrigation was performed immediately after planting and the second one followed just before emergence and continued (Department of Agriculture, Forestry and Fisheries, 2011). Fertilization was done using broadcasting before planting and a management strategy was employed for the control of diseases and pest. This included spraying with registered insecticide and fungicide (Department of Agriculture, Forestry and Fisheries, 2011). The crop was hand harvested when it was 90 days old. This ensured a good uptake of nutrients (Koike et al., 2011). Higher yield harvest was delayed until plants have 10 to 12 leaves (Seaman, 2012).

3.6: Ethical Issues

This research project involved the collection of sludge samples from a Government owned waste water treatment plants. Thus, access to the sampling points was processed in writings to the section of water affairs under the Thulamela municipality. The research also involved a simulation process for cultivation of spinach vegetables on sludge amended soil at the

University of Venda demonstration farm. Therefore, permission for the use of the farm was asked from the Dean of School of Agricultural sciences.

This research project maintained high level of integrity and objectivity and promoted accuracy throughout the period and stages of the research.

Harm of physical health or embarrassment to those who participated in or are affected by this research project was avoided by making sure that the appropriate personal protective equipment was worn and that confidentiality and anonymity was guaranteed respectively.

3.7: Data Analysis

To answer the project's research questions and to meet the set research objectives, numerical data were generated (Saunders et al., 2012). The data were analyzed using PAST 3.1 statistical tool.

The data generated included the independent data variables; the different weights of sludge, and the dependent data variables; concentrations of metal uptake by leaves, roots and in soil, concentration of POPs uptake in leaves, roots and in soil. Also determined were the initial concentrations of heavy metals and POPs in sludge and soil, total organic carbon, pH and alkalinity.

The data presentation for individual variables was done using tables and bar charts, to summarize data, show highest and lowest values, show trends and compare trends for two or more numerical values respectively. The relationships, differences and trends between data variables was done statistically using correlation analysis, and one way ANOVA to quantify the strength of the linear relationship between two variables and the level of significance between them.

CHAPTER 4: RESULTS AND DISCUSSION

4.1: Physicochemical properties

Table 4.1 summarizes the results of the physicochemical characteristics for soil and sludge samples determined in this study. Physicochemical characteristics of soil such as pH, organic matter (OC), cation exchange capacity (CEC) are among the factors that influence the interactions and dynamics of metals within the soil matrix (Pam et al., 2013) and the quantity of trace elements available for mobilisation and release or sorption in a soil.

The mean values of the pH of soil samples ranged from 6.50 to 6.81. pH value for sludge sample is 5.65. This suggests that the soils and sludge are weakly acidic, the most acidic being the sludge sample. pH is one of the important parameters that have profound effect on soil properties as well as control the availability of heavy metals in the soil. At low pH, metals are easily bioavailable because there is an increase in solubility of metals and the movement of metal ions from the surface to the underlying soil layer will be definite (Pam et al., 2013). Movement of metal ions from the sludge into the profile of soil investigated in this study was very slow because of the very low acidity.

The soil conductivity gave a range of 286.4 $\mu\text{s}/\text{cm}$ to 525.0 $\mu\text{s}/\text{cm}$. The cation exchange capacity (CEC) for the soil samples ranged from 1.9034 to 2.9897 meq/100g while the organic matter ranged between 19.9 to 30.5 mg/g for soil samples and 50.8 mg/g for sludge sample. The CEC of the soil can regulate the mobility of metals in soils since as it increases as pH increases. Organic matter of soils affects the availability of metals due to metal-organic complexation which immobilizes heavy metals at low pH; and mobilizes metals at weakly acidic to alkaline pH (Gupta and Sinha, 2006).

Table 4.1: Physicochemical properties of soil and sludge samples

	Alkalinity (mg/g)	pH	OC (mg/g)	Conductivity (μ s/cm)	CEC (meq/100g soil)
SSC	0.19	6.68	19.9	286.4	2.7022
SS 5	0.23	6.52	20.7	452.0	2.9504
SS 10	0.25	6.54	19.9	512.0	2.8997
SS 15	0.31	6.66	20.9	499.0	2.9589
SS 20	0.39	6.75	23.6	393.0	2.9897
SS 30	0.43	6.81	27.7	525.0	2.7174
SS 35	0.26	6.70	29.1	485.0	2.7551
SS 40	0.36	6.50	30.5	479.0	1.9034
SS	2.08	5.65	50.8	-----	-----

*SS 5= Soil sample with 5 Kg of sludge added etc. **C= control ***SS= Sludge sample

4.2: Heavy metal content in soil

Table 4.2 gives the values of the total heavy metal concentrations in soil samples and the summary statistics. In the soil samples, the concentrations range of the metals were; Al (9.21 - 260.8 mg/Kg), Cr (0.05 - 4.006 mg/Kg), Cu (0.0009 - 3.05 mg/Kg), Fe (3.52 - 378.9 mg/Kg), Mn (1.74 - 45.75 mg/Kg), Zn (0.01 - 2.647 mg/Kg), Co (0.02 - 0.8638 mg/Kg), and Pb (0.1864 - 0.2238 mg/Kg), respectively. Cadmium and Ni metals had concentrations that were not detectable by the instrument. Summary statistics analysis using PAST 3 software gave the lowest mean concentration of 62.2454 mg/Kg ($s = 122.9504$), which was in sample SS15 and the highest mean concentration of 67.8060 mg/Kg ($s = 132.6974$), which was in sample SSC. The results of correlation analysis showed that there was weak positive correlation between all the sample pairs suggesting that concentrations increase as amount of sludge increases in all samples. One-way ANOVA analysis gave the difference between sample groups occurring by chance alone as high ($F = 2.031$, $p = 0.0667$) and thus, not statistically significant at $p < 0.05$ (Table 4.19).

Bar charts in Figures 4.1 to 4.9 show the effect of increasing quantities of sludge on heavy metal concentrations in the different soil samples. All metal had the same concentration pattern in all samples with no significantly visible increase through all samples. This may be due to the soil natural phenomenon of maintaining balance within the environment since all soil samples were collected after ten weeks of sludge application. This trend corresponds with similar research carried out by Wuana et al. (2012). The relative concentration pattern of

metals in soil is as follows; Fe > Al > Mn > Cr > Cu > Zn > Co > Pb while Cd and Ni were not detected in sludge.

Table 4.2: Total metal concentration in soil samples (mg/Kg) and the summary statistics

	SSC	SS 5	SS 10	SS 15	SS 20	SS 30	SS 35	SS 40	SS
Al	260.8	238.7	220.5	221.8	241.1	226.3	9.21	226.9	11.9
Cr	4.006	3.839	3.7	3.586	3.815	3.471	0.05	3.634	ND
Cu	2.915	2.53	2.353	2.476	2.605	2.424	0.0009	2.446	0.03
Fe	367.1	372.5	360.3	352.1	378.9	361.8	3.52	376.9	0.07
Mn	40.58	44.04	40.58	40.04	43.98	45.73	1.74	45.75	ND
Zn	1.68	1.65	1.495	1.858	2.177	2.178	0.01	2.256	4.38
Cd	ND	ND	ND	ND	ND	ND	ND	ND	2.16
Co	0.8558	0.835	0.7788	0.7719	0.817	0.8638	0.02	0.8337	0.02
Ni	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pb	0.1864	0.2049	0.1979	0.2068	0.2204	0.2206	ND	0.2238	0.03
Min	0	0	0	0	0	0	0	0	0
Max	367.1	372.5	360.3	352.1	378.9	361.8	9.21	376.9	11.9
Mean	67.80597	66.42874	62.94647	62.2454	67.32587	64.2597	2.0787	65.86105	2.32
Std. error	41.96261	41.30783	39.50848	38.88033	41.9428	39.85149	1.29	41.13374	4.181
Variance	17608.6	17063.4	15609.2	15116.8	17591.98	15881.41	11.66	16919.85	17.47
Stand. dev	132.6974	130.6268	124.9368	122.9504	132.6348	126.0215	3.41	130.0763	4.18

*SS 5= Soil sample with 5 Kg of sludge added etc **C= Control ***ND= Not detectable

Tables 4.3 shows the total heavy metals concentrations in root samples. The range of concentration of the individual metals were as follows: Co (0.02 – 1.13 mg/Kg), Cr (0.05 – 1.47 mg/Kg), Cu (0.17 – 0.97 mg/Kg), Fe (3.19 – 186.40 mg/Kg), Mn (1.62 – 71.28 mg/Kg), Ni (0.02 – 0.05 mg/Kg), Pb (0.02 – 0.06 mg/Kg), Zn (0.01 – 3.31 mg/Kg) and Al (6.76 – 211.40 mg/Kg), respectively. Chromium, Fe, Al and Cu metals were all found to have concentration values above the WHO maximum limits (Appendix B1) of 0.05, 2.5, 75 and 0.4 mg/kg, respectively. The maximum and minimum mean concentrations are 43.3201 mg/kg ($s = 74.5977$) and 1.1658 mg/Kg ($s = 2.2328$) in samples RS 40 and RS 10, respectively. There was a weak positive correlation among sample groups and the difference in the groups is not statistically significant ($F = 1.986$, $p = 0.07$) at the statistical level of significance of 95 % thus occurring by chance alone. Figures 4.10 to 4.18 are the bar charts showing the trends of the total heavy metal concentrations in root samples. There was a general trend of sharp increase from control sample and then gradual increase until the sample with the highest quantity of sludge.

Table 4.3: Total metal concentration in root samples (mg/kg)

Metal	RSC	RS 5	RS 10	RS 15	RS 20	RS 30	RS 35	RS 40
Al	13.38	211.40	6.76	191.50	99.52	8.37	130.10	171.40
Cd	ND	ND	ND	ND	ND	ND	ND	ND
Co	0.03	0.34	0.02	0.29	0.64	0.02	1.02	1.13
Cr	0.07	0.65	0.05	0.55	1.27	0.05	1.15	1.47
Cu	ND	0.21	ND	0.17	0.72	ND	0.66	0.97
Fe	4.35	86.08	3.19	68.21	134.20	3.21	120.80	186.40
Mn	2.18	29.14	1.62	23.46	44.18	1.64	71.28	68.64
Ni	0.02	0.05	0.02	0.04	ND	0.02	ND	ND
Pb	ND	0.02	ND	0.02	0.05	ND	0.04	0.06
Zn	0.03	1.14	ND	0.97	1.78	0.01	2.52	3.31
min	0	0	0	0	0	0	0	0
max	13.38	211.40	6.76	191.50	134.20	8.37	130.10	186.40
mean	2.01	32.90	1.17	28.52	28.22	1.33	32.74	43.32
std error	1.34	21.63	0.71	19.36	15.60	0.85	16.96	23.59
variance	18.05	4680.61	4.99	3749.0	2434.0	7.26	2876.3	5564.8
std dev.	4.25	68.41	2.23	61.23	49.34	2.69	53.63	74.60

RS 5= Root sample with 5 Kg of sludge added etc **C= Control ***ND= Not detectable

Table 4.4 gives the total heavy metals concentrations in leaf samples and the summary statistics. The concentration range of the individual metals were: Cd (0.0015- 0.0017 mg/kg), Cr (0.0494- 1.092 mg/kg), Co (0.0112- 0.0239 mg/kg), Cu (0.0012 – 0.2787 mg/kg), Fe (3.23 – 25.47 mg/kg), Mn (1.611 – 5.417 mg/kg), Ni (0.0211 – 0.1587 mg/kg), Pb (0.0005 – 0.0055 mg/kg), Zn (0.012 – 2.858 mg/kg) and Al (8.634 – 61.93), respectively. All the metals have concentrations below FAO/WHO maximum limits (Appendix B1) except for Cr, Fe, Zn and Mn which had some of the samples having values higher than 0.05 mg/kg, 2.5 mg/Kg, 2 mg/Kg and 0.6 mg/Kg, respectively, which are the FAO/WHO maximum limits for Cr, Fe, Zn and Mn in vegetables. Cobalt and Ni metals have no permissible guidelines/limits as yet because there are no evidences of high levels in human diet (Javed and Usmani, 2013). Sample LS 30 had the highest mean concentration value of 9.3689 mg/kg ($s = 20.0657$) and sample LS 5 had the lowest mean concentration of 1.3579 mg/kg ($s = 2.7716$). Correlation analysis was carried out and showed that all the leave samples had weak positive correlations between all pairs of values, that is, all the samples generally increased in concentration of heavy metals with increase in the amount of sludge added. One-way ANOVA analysis carried out showed that the difference between sample groups occurring by chance alone is high ($F = 0.6662$, $p = 0.7165$). Based on the F value, it can be deduced that the difference

between sample groups is not statistically significant. Figures 4.19 to 4.28 are bar charts comparing the effect of increasing quantities of sludge on concentration of heavy metals in leaf samples. All metals follow the same pattern in all samples with an overall upward increase in concentration from sample LS C to LS 40. This suggests that there is uptake of metals by the vegetable which is different with quantities of sludge added. These results substantiated a similar finding by Wang *et al.* (2008). The comparison of metal concentrations in all the samples is as follows: Al > Fe > Mn > Zn > Cr > Cu > Ni > Co > Pb > Cd.

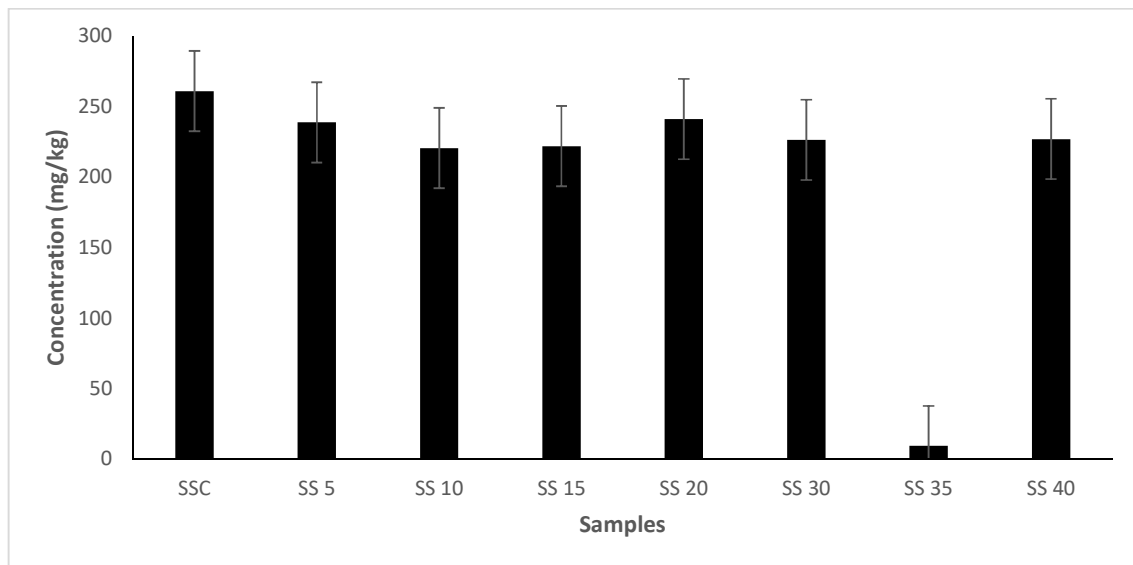


Fig 4.1: Bar chat showing the concentrations of Aluminium metal in soil samples

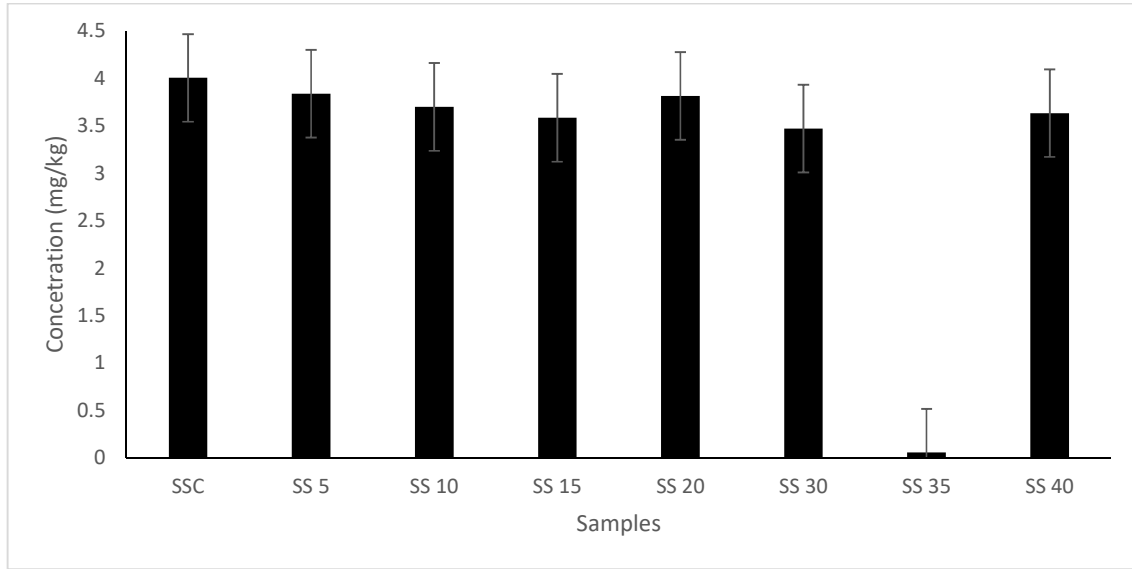


Fig 4.2: Bar chat showing the concentrations of Chromium metal in soil samples

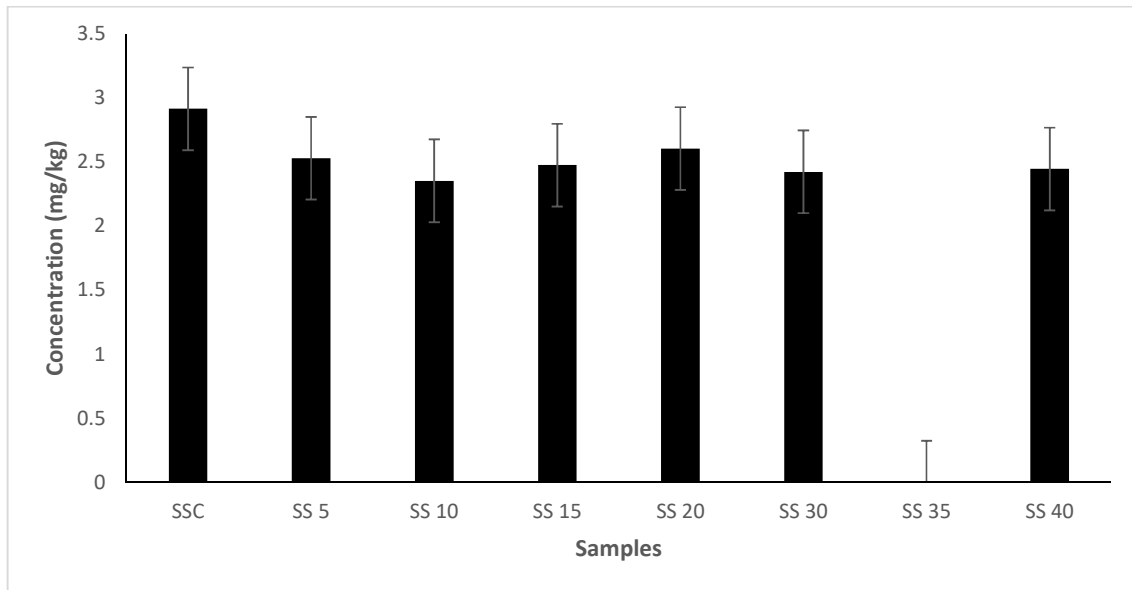


Fig 4.3: Bar chat showing the concentrations of Copper metal in soil samples

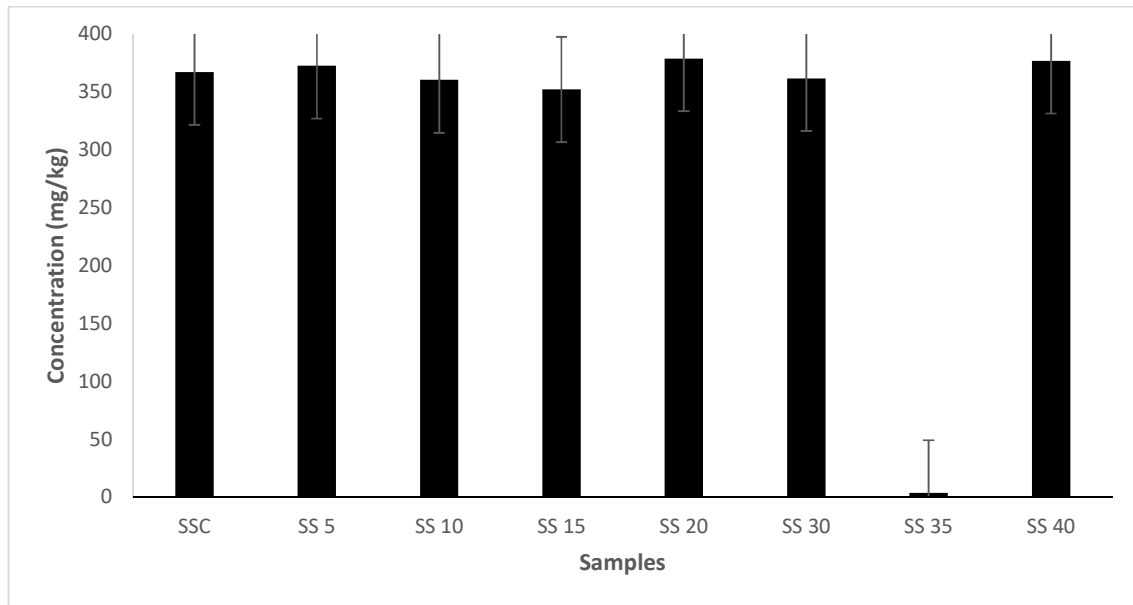


Fig 4.4: Bar chat showing the concentrations of Iron metal in soil samples

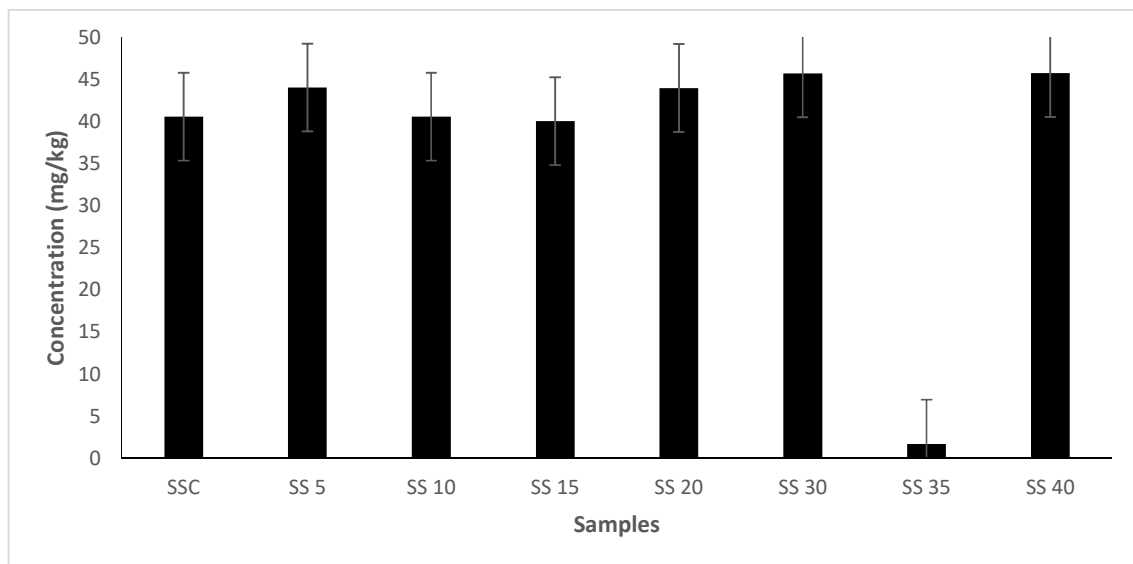


Fig 4.5: Bar chat showing the concentrations of Manganese metal in soil samples

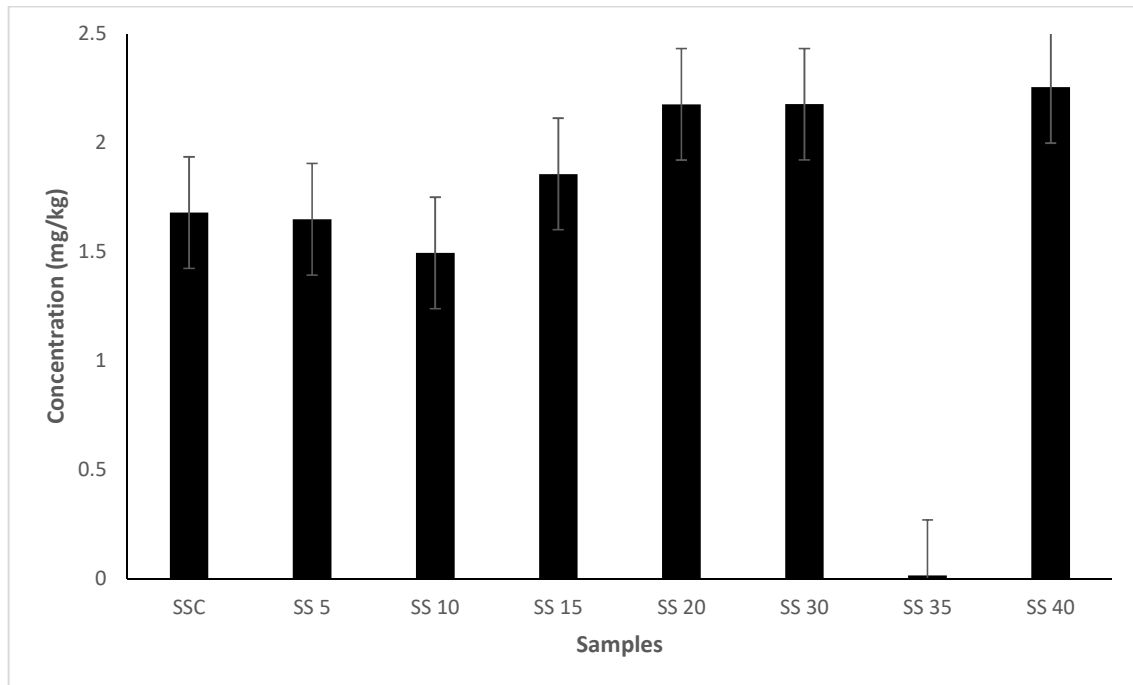


Fig 4.6: Bar chat showing the concentrations of Zinc metal in soil samples

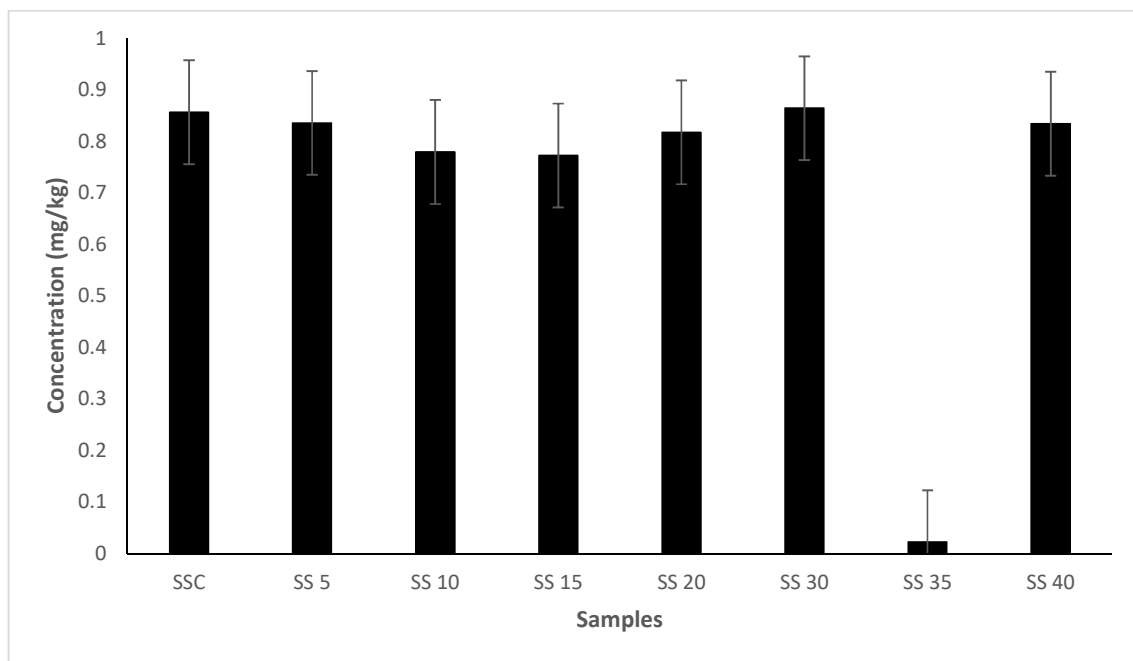


Fig 4.7: Bar chat showing the concentrations of Cobalt metal in soil samples

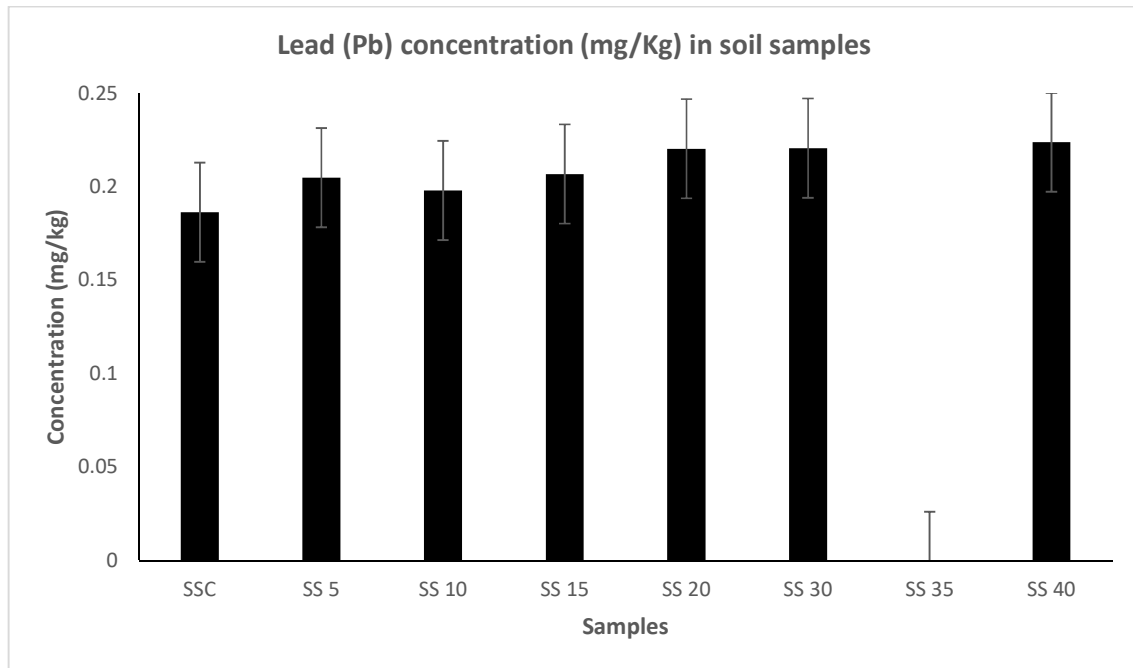


Fig 4.8: Bar chat showing the concentrations of Lead metal in soil samples

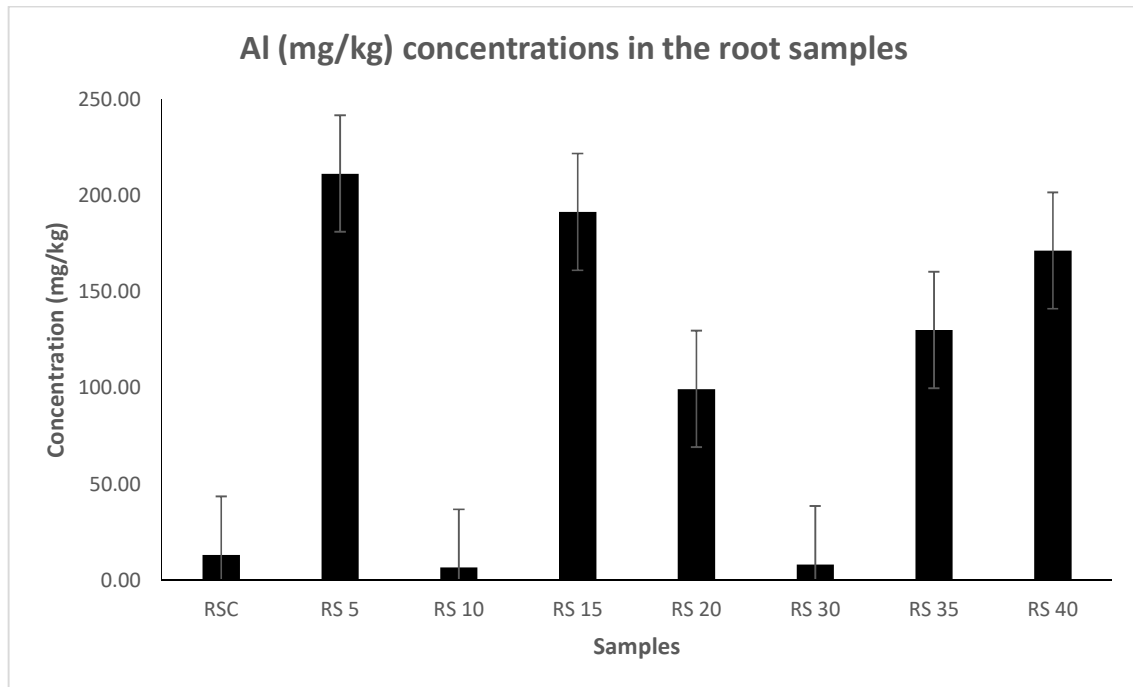


Fig 4.9: Bar chart showing the concentration of Aluminium metal in Root samples

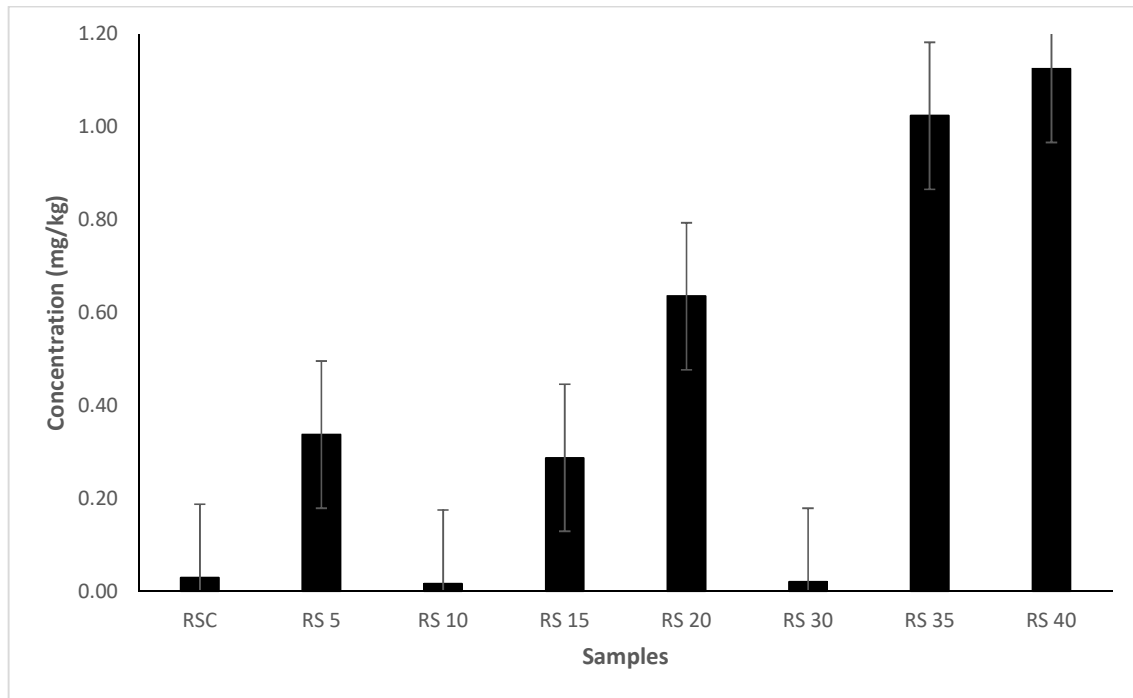


Fig 4.10: Bar chart showing the concentration of Cobalt metal in root samples

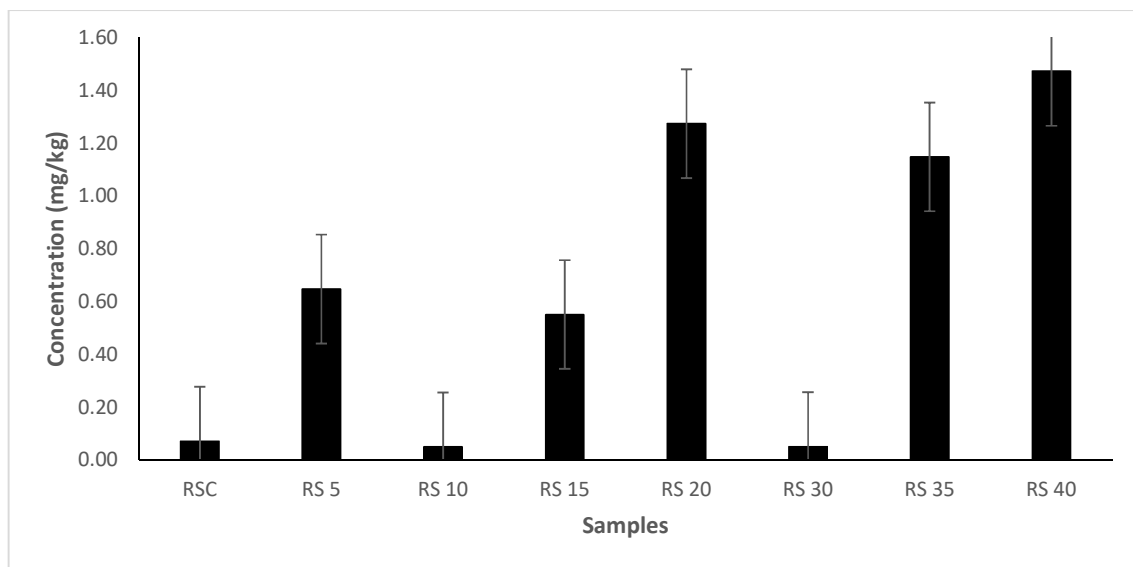


Fig 4.11: Bar chart showing the concentration of Chromium metal in Root samples

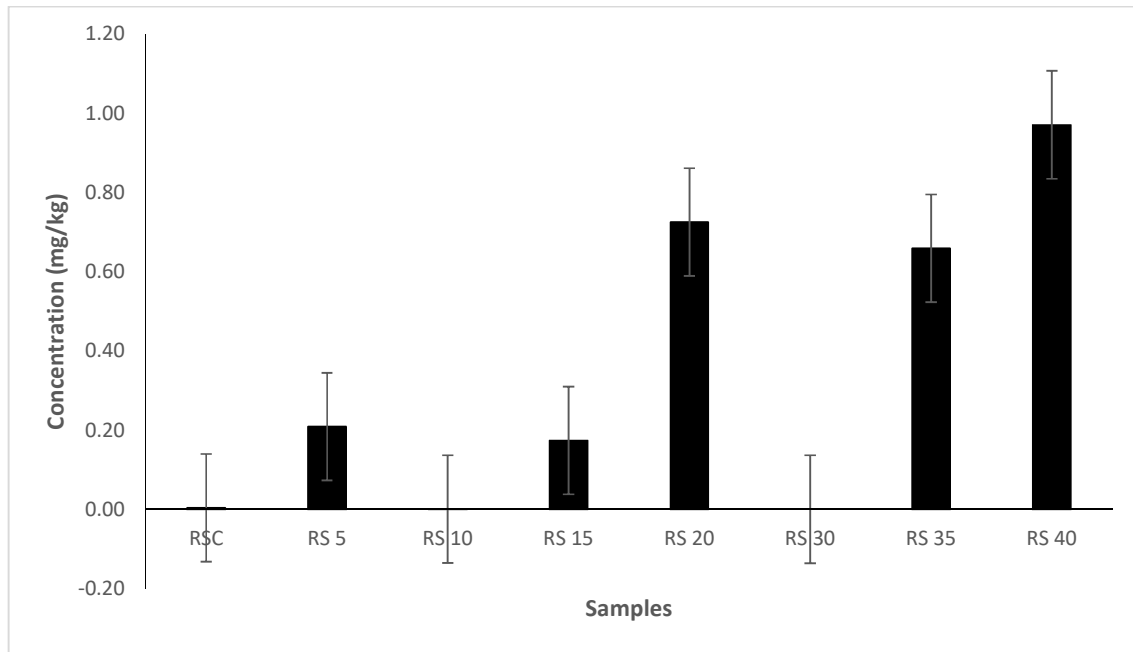


Fig 4.12: Bar chart showing the concentration of Copper metal in root sample

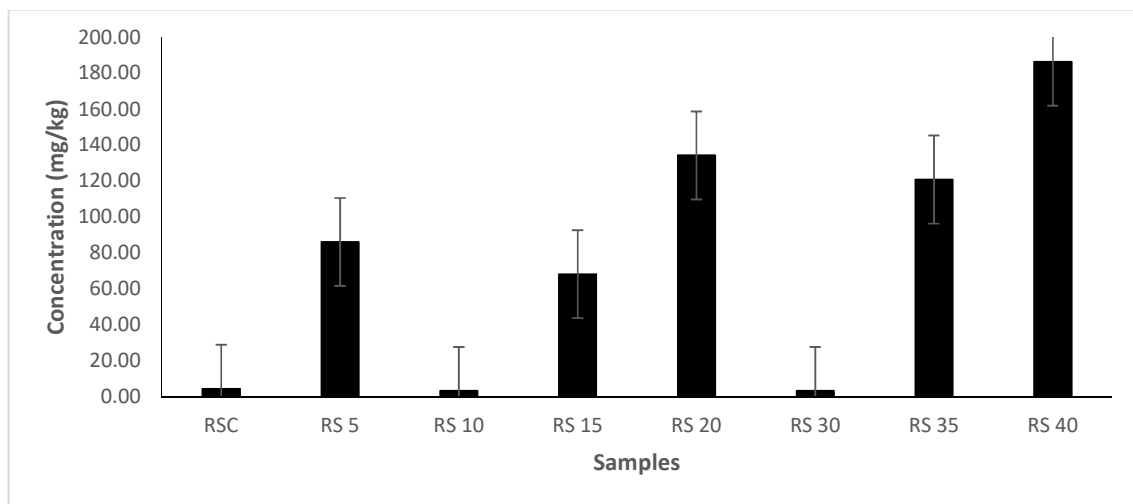


Fig 4.13: Bar chart showing the concentration of Iron metal in root samples

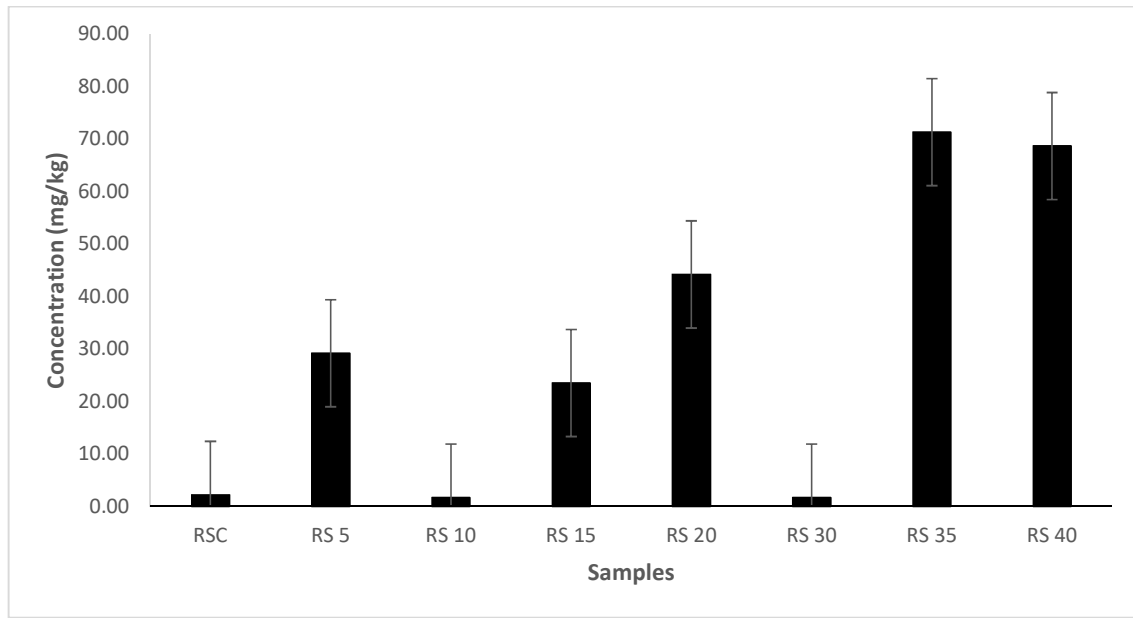


Fig 4.14: Bar chart showing the concentration of Manganese metal in root samples

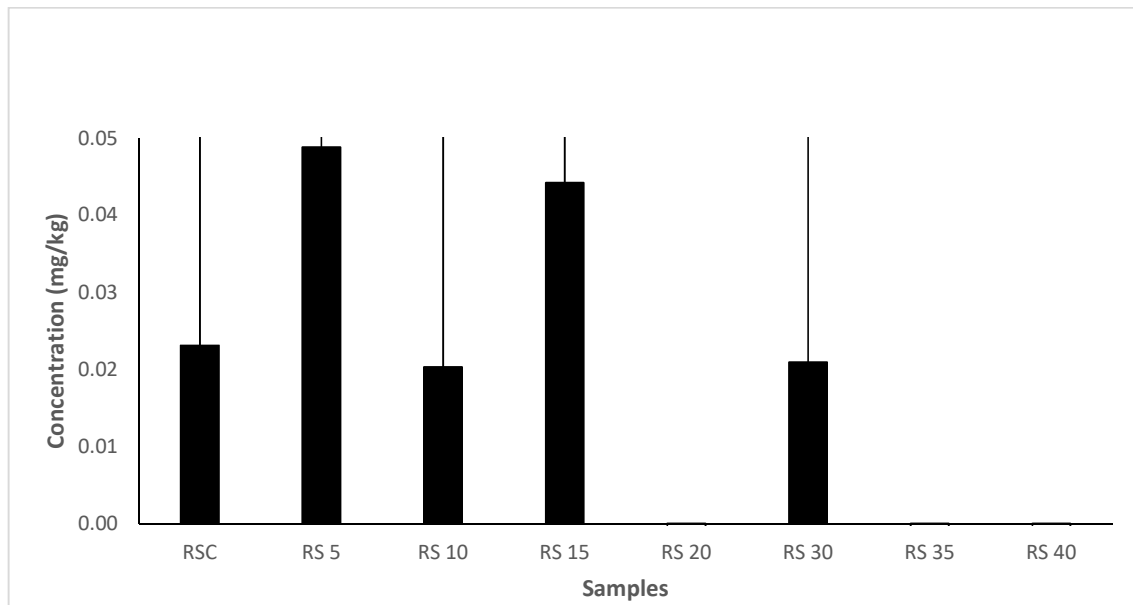


Fig 4.15: Bar chart showing the concentration of Nickel metal in root samples

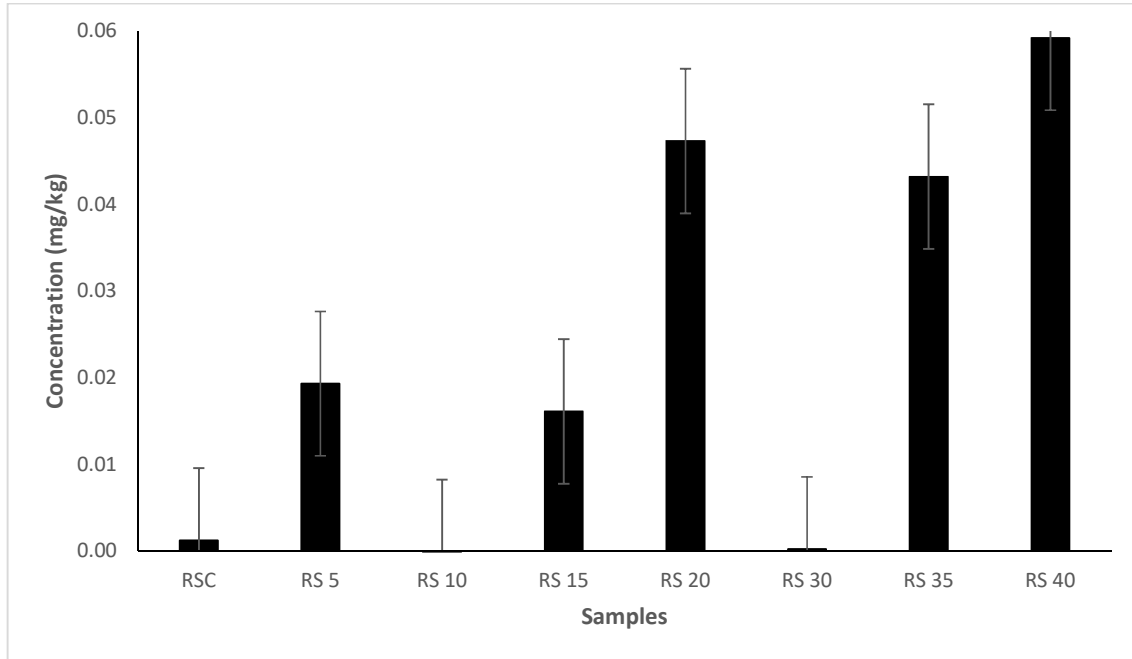


Fig 4.16: Bar chart showing the concentration of Lead metal in root samples

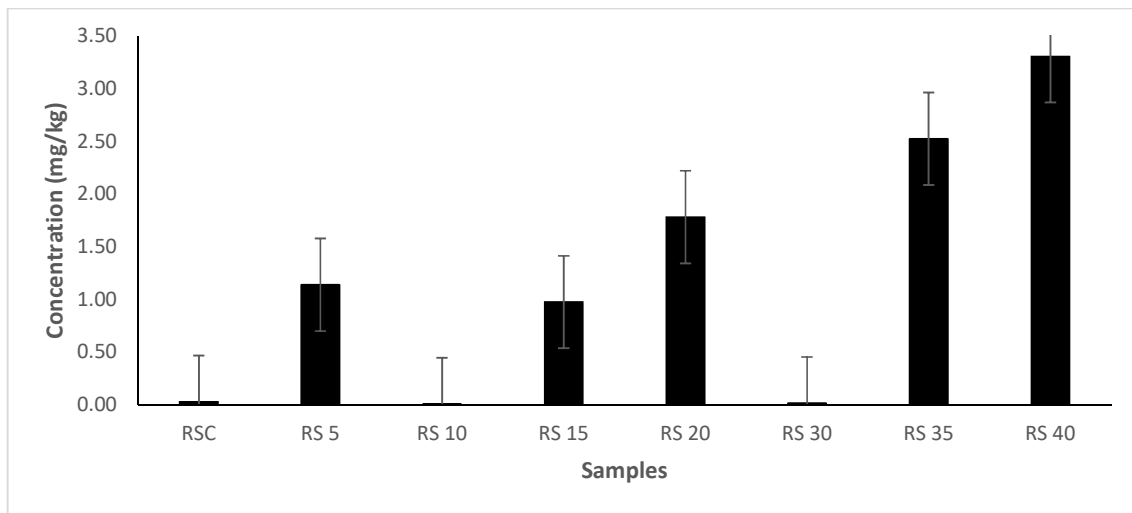


Fig 4.17: Bar chart showing the concentration of Zinc metal in root samples

Table 4.4: Total metal concentration in leaf samples (mg/Kg) and the summary statistics

	LSC	LS 5	LS 10	LS 15	LS 20	LS 30	LS 35	LS 40
Al	10.01	8.634	17.03	48.17	25.51	61.93	28.01	29.84
Cr	0.058	0.0494	0.8817	1.092	0.8164	0.9347	1.045	1.022
Cu	0.0012	ND	0.2787	0.265	0.2093	0.2698	0.2517	0.2515
Fe	3.684	3.23	7.697	17.91	11.88	25.47	14.3	11.81
Mn	1.834	1.611	1.738	3.034	4.831	3.93	2.638	5.417
Zn	0.0173	0.012	0.7332	0.9333	2.858	1.01	1.573	2.169
Cd	ND	ND	0.0015	0.0015	0.0017	0.0015	0.0017	0.0017
Co	0.0239	0.021	0.0112	0.0161	0.0163	0.0236	0.0202	0.0237
Ni	0.0212	0.0211	0.1383	0.0925	0.1038	0.1167	0.1148	0.1587
Pb	0.0005	0.0009	0.0007	0.0042	0.0015	0.0029	0.0055	0.0046
Min	ND	ND	0.0007	0.0015	0.0015	0.0015	0.0017	0.0017
Max	10.01	8.634	17.03	48.17	25.51	61.93	28.01	29.84
Mean	1.56498	1.3579	2.85103	7.15186	4.6228	9.36892	4.79599	5.06982
Std. error	1.014608	0.876475	1.739775	4.877356	2.604312	6.345344	2.924299	2.996392
Variance	10.29429	7.68208	30.26817	237.886	67.82442	402.6339	85.51525	89.78366
Stand. Dev	3.208471	2.771657	5.501652	15.42355	8.235558	20.06574	9.247446	9.475424

*LS 5= Leaf sample with 5 Kg of sludge added etc. **C= control ***ND= Not detected

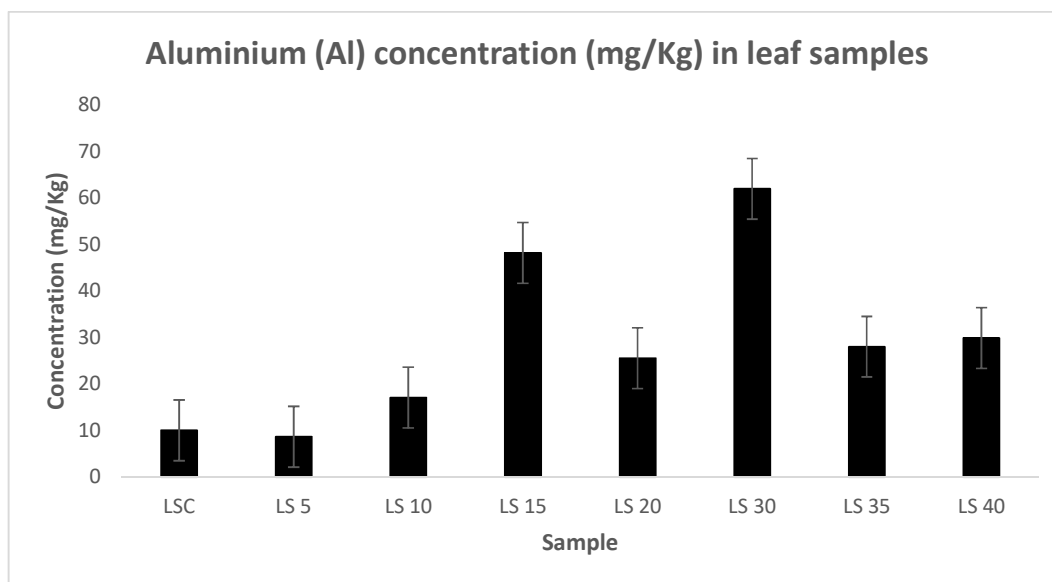


Fig 4.18: Bar chart showing the concentration of Aluminium metal in leaf samples

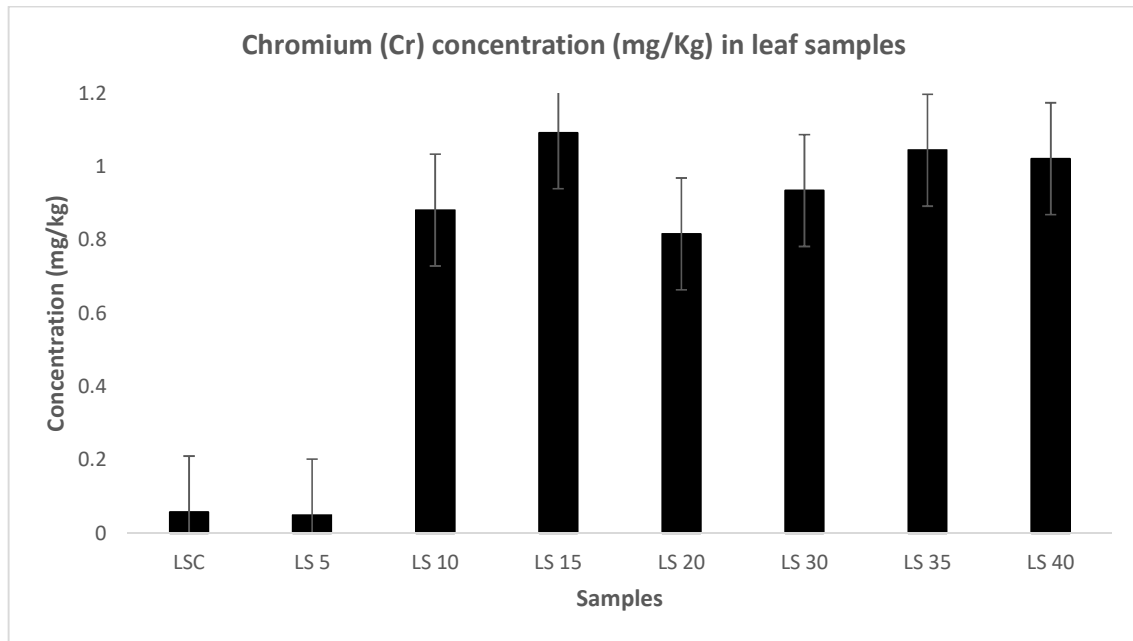


Fig 4.19: Bar chart showing the concentration of Chromium metal in leaf samples

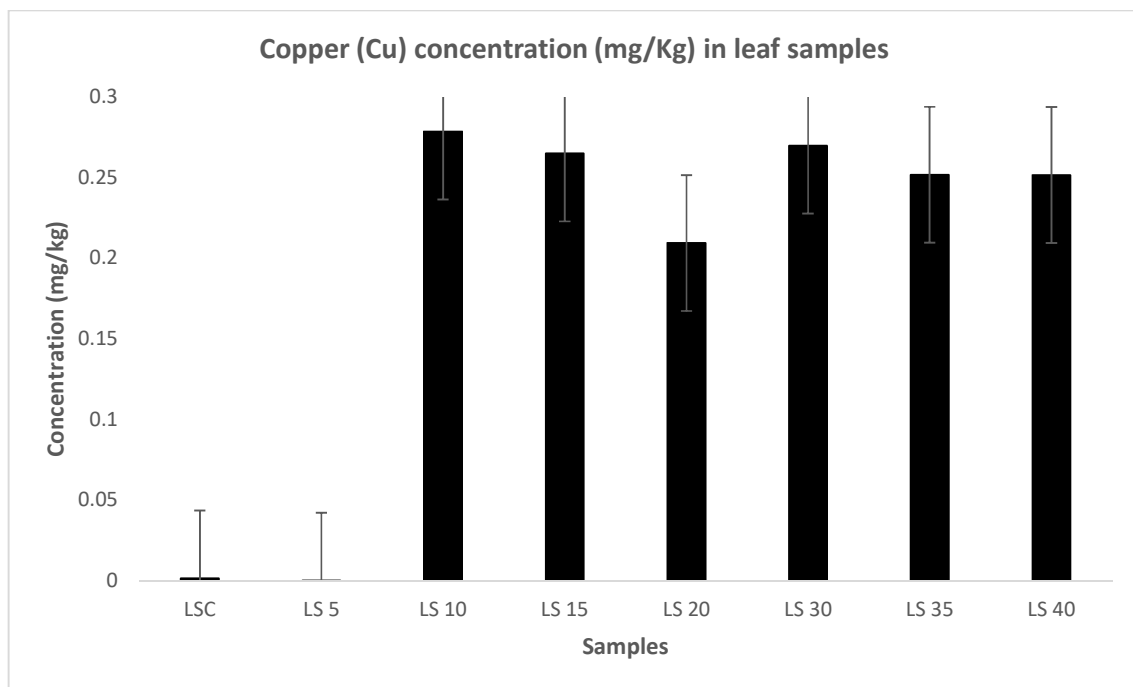


Fig 4.20: Bar chart showing the concentration of Copper metal leaf samples

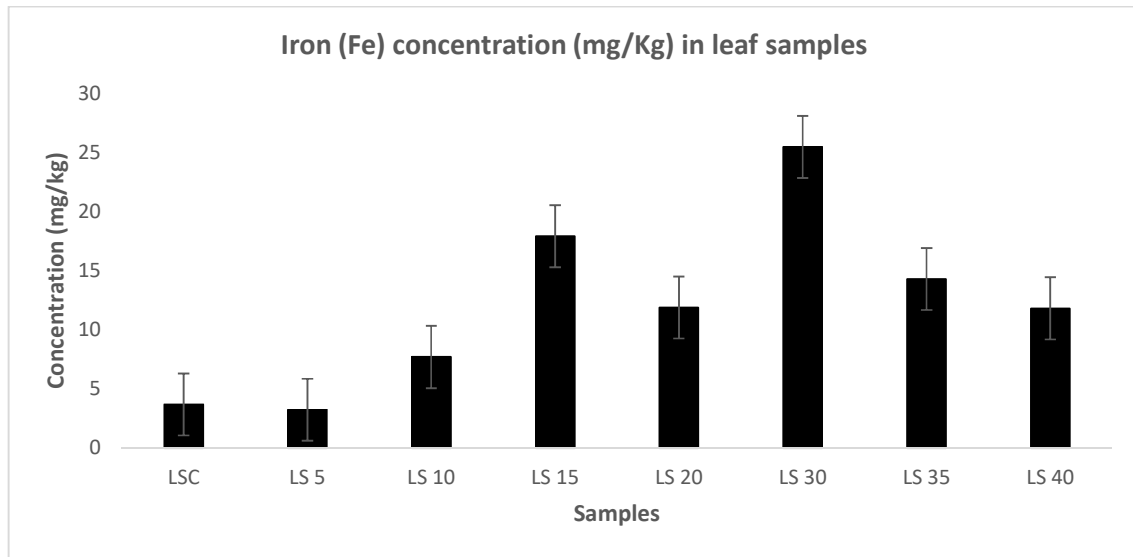


Fig 4.21: Bar chart showing the concentration of Iron metal in leaf samples

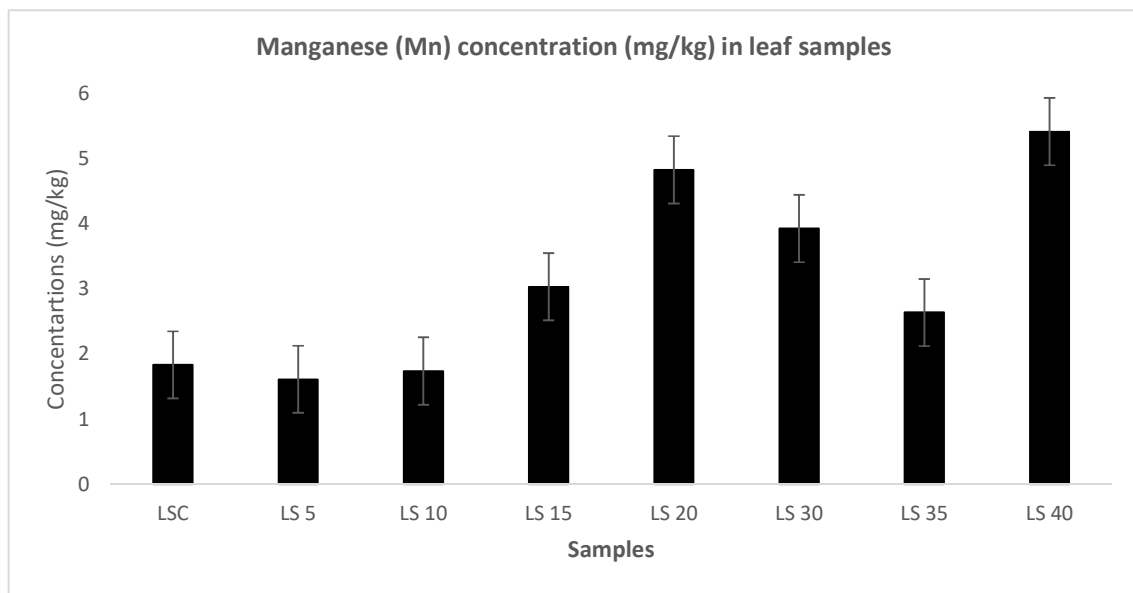


Fig 4.22: Bar chart showing the concentration of Manganese metal in leaf samples

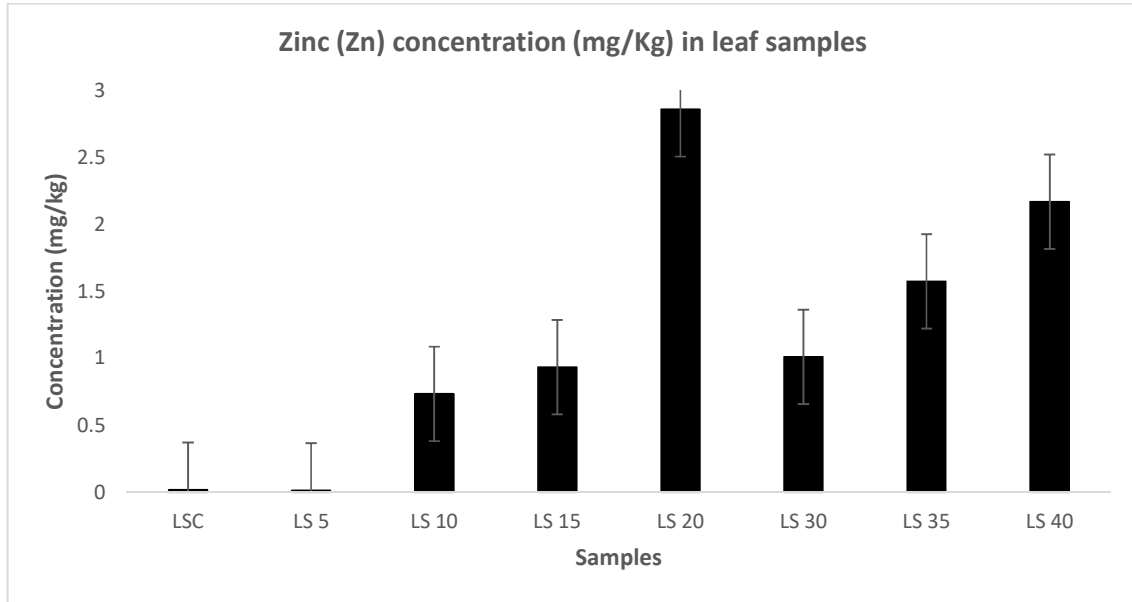


Fig 4.23: Bar chart showing the concentration of Zinc metal in leaf samples

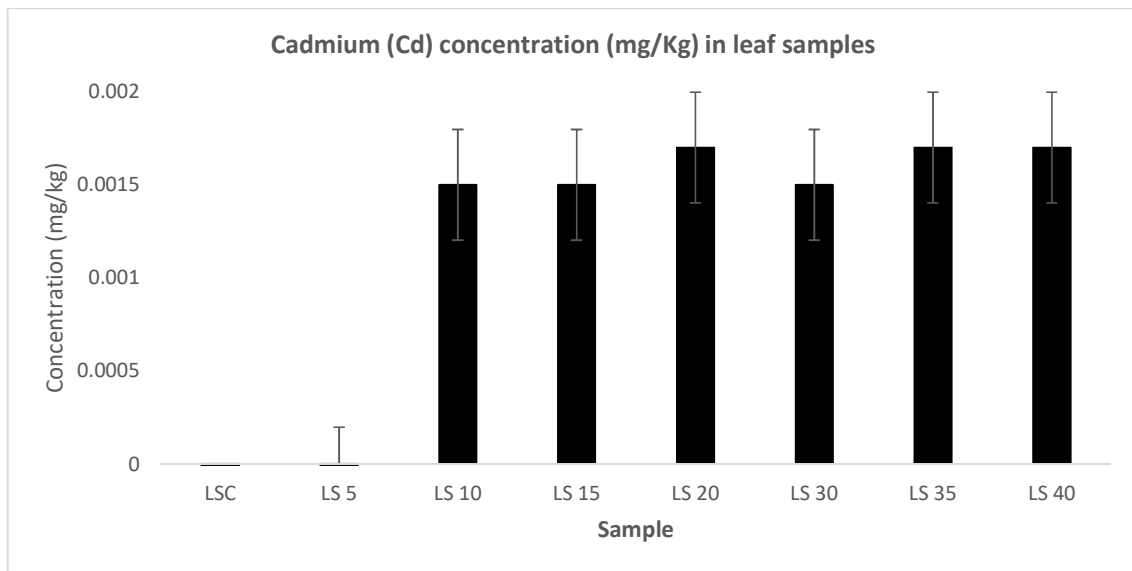


Fig 4.24: Bar chart showing the concentration of Cadmium metal in leaf samples

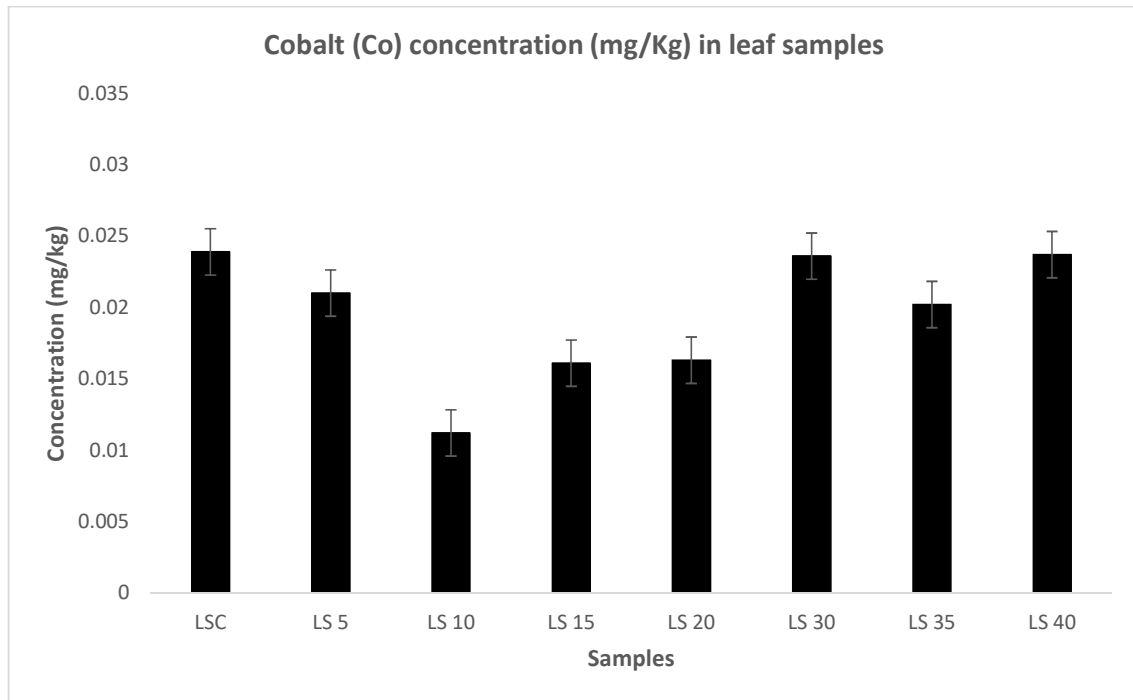


Fig 4.25: Bar chart showing the concentration of Cobalt metal in leaf samples

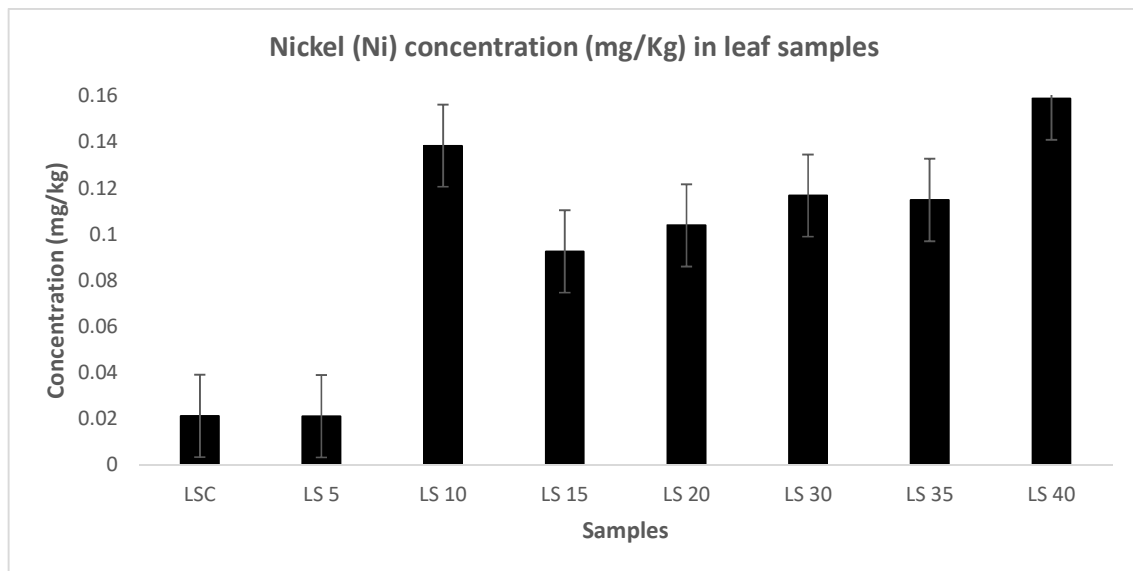


Fig 4.26: Bar chart showing the concentration of Nickel metal in leaf samples

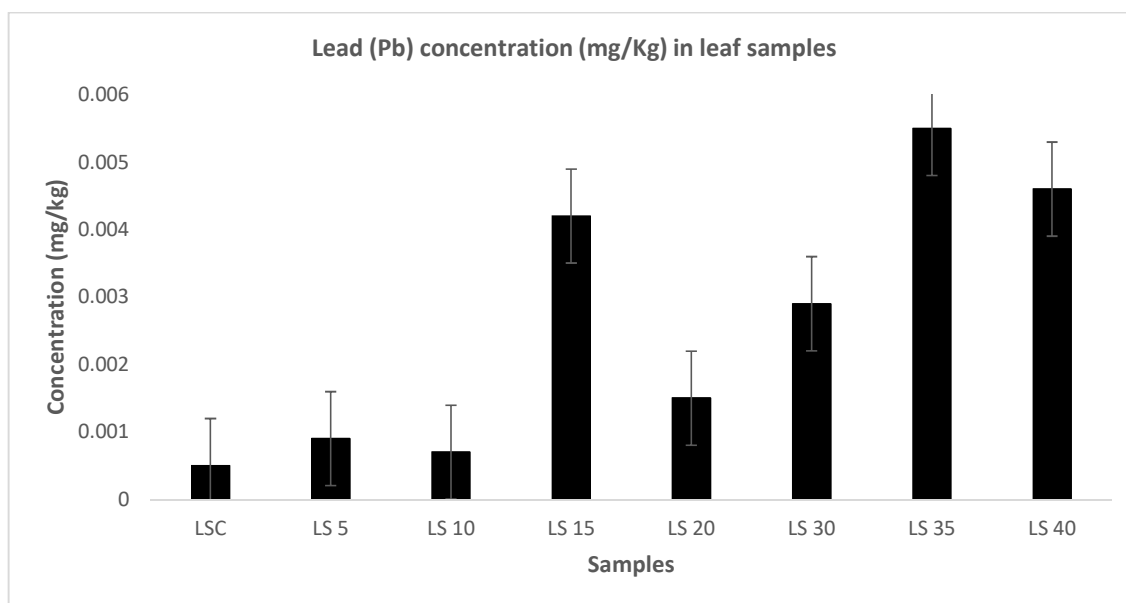


Fig 4.27: Bar chart showing the concentration of Lead metal in leaf samples

4.3: Speciation of metals in *spinacia oleracea*

Metal speciation has helped in assessing the mobility, bioavailability and toxicity of metals and hence gives a better insight into the ultimate fate of metals which are introduced into the *spinacia oleracea* leaf. The results of the heavy metals speciation on eight leaf samples are presented in Tables 4.6 to 4.15. Metal chemical speciation carried out by sequential extraction of the metals is essential to the metal mobility (Tessier et al., 1979). The order of abundance of metals in the fractions in this study was: Exchangeable > Organic matter > Bound to Fe and Mn oxides > Carbonate bound > Residual.

Sum of concentrations of metals in different geochemical phases can be used to express the potential mobility of metals. The potential mobility of a metal can be assessed by adding up the results of the exchangeable phase, carbonate phase, Fe and Mn oxide phase and organic phase of that metal (Haung *et al.*, 2007). As shown (Table 4.5), the potential mobility of Al to *spinacia oleracea* leaf was 96.35%. It was 36.96% for the exchangeable phase which represents the mobile and bioavailable metal fraction. In exchangeable phase, the metals have more labile bounds and can be easily released into the environment. Thus, the presence of metals in this phase where they can be readily taken up by plants from the soils represents the most hazardous condition to the ecosystem. In our study, mostly all metals in *spinacia oleracea* leaf samples were present in abundance in this phase. From the results of the

sequential extraction in all the *spinacia oleracea* leaf studied, Mn was mostly abundant in the exchangeable fraction (82.19%). High abundance of a metal in the exchangeable fraction compared with its abundance in other geochemical phases indicates higher mobility of the metal in the environment. Analysed heavy metals such as Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were highly abundant in the exchangeable phase, signifying that they were derived from anthropogenic sources which could be from the sewage sludge. The high percentage of the total extractable fraction in the mobile phase indicates that all these metals in this *spinacia oleracea* leaf were potentially more bioavailable for plants uptake (Haung et al., 2007).

Table 4.5: Percentage Metals in various Geochemical Fractions of *Spinacia oleracea* leaf

Phase	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Total
Exchangeable	36.96	12.50	77.11	26.19	44.03	23.33	82.19	16.57	30.23	50.85	399.96
Carbonate bound	9.36	12.50	12.97	13.81	20.93	4.26	12.30	0.00	14.51	24.74	125.38
Fe-Mn oxide	13.85	62.50	6.88	12.78	10.85	10.77	4.40	0.00	14.87	12.29	149.19
Organic matter	36.18	12.50	2.65	31.96	20.00	47.10	1.02	0.00	37.48	11.29	200.18
Residual	3.66	0.00	0.39	15.26	4.19	14.54	0.08	83.43	2.90	0.84	125.29

The results of speciation analysis of Al in Table 4.6 indicated that the mean values of Al in the various fractions were: Exchangeable (746.08 mg/kg) > Organic matter (730.24 mg/kg) > Fe-Mn oxide (279.49 mg/kg) > Carbonate bound (188.93 mg/kg) > Residual (73.80 mg/kg), respectively. The high level of Al in exchangeable and organic matter fractions might be ascribed to the ease of complexation products formed between the metal and natural organic matter such as humic and fulvic acids. Since Al was more abundant in the exchangeable fraction, it implied that Al was more mobile in this environment than other metals that are mostly abundant in the remaining four geochemical phases.

Total extractable Cd values in the leaf samples was low (Table 4.7) and within the tolerable limit (3.0 mg/kg) set by USEPA (1986) for agricultural lands. The high percentage of Cd in the reducible phase in all the *spinacia oleracea* leaf might be ascribed to the precipitation of amorphous hydrous oxides of Cd during the decomposition of the sewage sludge (Obasi et al., 2013). The high percentage of the total extractable fraction in the mobile phase suggests that Cd in this sludge was potentially more bioavailable for uptake to *spinacia oleracea* leaf (Obasi et al., 2013).

Table 4.6: Aluminium levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	894.40	197.10	76.75	123.00	18.88	1310.13
LS 5	1166.00	133.70	171.10	795.50	83.30	2349.60
LS 10	1020.00	346.40	168.45	415.90	84.08	2034.83
LS 15	1325.00	193.60	259.45	605.00	80.74	2463.79
LS 20	551.90	209.40	205.20	881.50	80.74	2463.79
LS 30	133.10	127.50	435.45	680.50	100.60	1477.15
LS 35	536.10	106.90	690.50	1416.50	63.20	2813.20
LS 40	342.10	196.80	229.00	924.00	78.73	1770.63
Mean	746.08	188.93	279.49	730.24	73.80	2018.54
SD	43.20	22.75	31.66	40.31	13.82	108.80

Table 4.7: Cadmium levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	0.00	0.00	0.02	0.01	0.00	0.03
LS 5	0.00	0.00	0.01	0.00	0.00	0.01
LS 10	0.00	0.00	0.00	0.00	0.00	0.00
LS 15	0.00	0.00	0.00	0.00	0.00	0.00
LS 20	0.00	0.00	0.00	0.00	0.00	0.00
LS 30	0.01	0.00	0.01	0.00	0.00	0.02
LS 35	0.00	0.01	0.00	0.00	0.00	0.01
LS 40	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.001	0.001	0.005	0.001	0.000	0.008
SD	0.000	0.000	0.000	0.000	0.000	0.000

The results of speciation analysis of Co in the sample (Table 4.8) gave the mean of Co in the following decreasing order: Exchangeable (7.85 mg/kg) > Carbonate bound (1.32 mg/kg) > Fe-Mn oxide (0.70 mg/kg) > Organic matter (0.27 mg/kg) > Residual (0.04 mg/kg). The high percentage of the total extractable fraction in the mobile phase indicates that Co in these *spinacia oleracea* leaf was potentially more bioavailable for uptake (Obasiet al., 2013).

Table 4.8: Cobalt levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	3.66	0.55	0.09	0.14	0.01	4.45
LS 5	3.26	0.59	0.14	0.19	0.03	4.21
LS 10	12.63	1.42	0.27	0.28	0.04	14.64
LS 15	5.51	1.21	0.26	0.43	0.02	7.43
LS 20	7.40	1.04	0.26	0.32	0.03	9.05
LS 30	8.91	1.68	0.74	0.31	0.11	11.75
LS 35	11.55	1.73	3.36	0.15	0.01	16.80
LS 40	9.91	2.36	0.46	0.36	0.05	13.14
Mean	7.85	1.32	0.70	0.27	0.04	10.18
SD	2.28	0.50	0.06	0.04	0.02	2.74

Organic fraction contained the predominant species of Cr. The mean values of Cr in the geochemical fractions ranged from 0.62 mg/kg to 1.55 mg/kg with an overall total mean of 4.85 mg/kg. The Cr content was strongly associated with the organic matter and exchangeable fractions which is in agreement with those reported by other researchers (Obasiet al., 2013), but however, differ from those reported by Gupta and Sinha (2006) for tannery sludge. The association of Cr with the organic matter phase implicated that it is strongly bound to organic matter which is an indication that the availability of the metals to the *spinacia oleracea* leaf might be reduced due to organic complexation. Thus, the relative mild mobility of this metal in the *spinacia oleracea* leaf studied may be due to the slightly acidic nature of the sewage sludge (Table 4.1).

Table 4.9: Chromium levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	2.10	0.32	0.58	0.83	0.40	4.23
LS 5	0.71	1.01	0.60	1.52	0.89	4.73
LS 10	1.66	0.22	0.66	1.00	0.99	4.53
LS 15	0.70	1.07	0.17	1.51	0.71	4.16
LS 20	1.86	0.19	0.59	1.34	0.74	4.72
LS 30	1.24	0.21	0.82	1.81	0.88	4.96
LS 35	1.31	1.15	0.85	2.44	0.60	6.35
LS 40	0.58	1.20	0.66	1.96	0.68	5.08
Mean	1.27	0.671	0.620	1.550	0.740	4.850
SD	0.080	0.040	0.050	0.090	0.080	0.370

The result of speciation analysis of Cu in the sample (Table 4.10) gave the mean of Cu in the following decreasing order in *spinacia oleracea* leaf: Exchangeable (2.84 mg/kg) > Carbonate bound (1.35 mg/kg) > Organic matter (1.29 mg/kg) > Fe-Mn oxide (0.70 mg/kg) > Residual (0.27 mg/kg). Copper concentrations in the sewage sludge were all below the toxic limit of 250 mg/kg set by USEPA (1986) for agricultural lands. The results signified that Cu mostly occurred in the exchangeable phase (i.e. most labile phase) which is a deviation from the reports of Uba et al. (2008) and Obasi et al. (2013). The high percentage of the total extractable fraction in the most labile phase implies that Cu in this sewage sludge will be readily bioavailable to the *spinacia oleracea* leaf in this environment.

The results of speciation analysis of Fe in Table 4.11 also showed that the mean levels of Fe in the various fractions were: Organic matter (315.24 mg/kg) > Exchangeable (156.15 mg/kg) > Residual (97.29 mg/kg) > Fe-Mn oxide (72.06) > Carbonate bound (28.49 mg/kg), respectively. The majority of Fe in the *spinacia oleracea* leaf was associated with the organic phase having varied from 50.95 mg/kg to 469.40 mg/kg with a mean of 315.24 mg/kg. High Fe content (315.24 mg/kg) in the organic fraction has been reported (Osakwe, 2010). Organic compounds of heavy metals can be directly or indirectly introduced into sewage sludge through the formation of a complex and can be taken up by *spinacia oleracea* leaves. The high levels of Fe also in the organic and exchangeable phases suggested that the metal might be potentially toxic if not regulated due to their mild mobility (Table 4.16).

Table 4.10: Copper levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	2.55	1.84	0.32	0.96	0.04	5.71
LS 5	3.16	0.45	0.63	1.54	0.27	6.05
LS 10	2.25	2.07	0.56	0.88	0.36	6.12
LS 15	3.16	0.63	1.35	1.81	0.21	7.16
LS 20	3.30	2.35	0.67	1.73	0.24	8.29
LS 30	1.49	1.83	0.97	1.05	0.58	5.92
LS 35	2.15	0.56	0.77	0.76	0.16	4.40
LS 40	4.68	1.03	0.36	1.56	0.27	7.90
Mean	2.84	1.35	0.70	1.29	0.27	6.45
SD	0.90	0.70	0.31	0.39	0.11	0.87

Table 4.11: Iron levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	292.40	32.61	47.76	50.95	10.53	434.25
LS 5	163.30	21.16	41.25	306.95	140.30	672.96
LS 10	216.70	23.24	43.59	298.60	163.50	745.63
LS 15	185.90	41.66	32.71	323.25	77.78	661.30
LS 20	88.78	11.77	45.07	227.95	115.00	488.57
LS 30	71.92	15.89	152.60	391.25	153.90	785.56
LS 35	106.10	13.47	164.70	453.55	42.12	779.94
LS 40	124.10	68.13	48.78	469.40	75.22	785.63
Mean	156.15	28.49	72.06	315.24	97.29	669.23
SD	19.22	28.49	12.30	25.77	14.67	40.65

The results of speciation analysis of Mn in Table (4.12) revealed that the mean levels of Mn in the various fractions were: Exchangeable (650.84 mg/kg) > Carbonate bound (97.43 mg/kg) > Fe-Mn oxide (34.88 mg/kg) > Organic matter (8.05 mg/kg) > Residual (0.66 mg/kg), respectively. Manganese was distributed mainly as exchangeable and carbonate fractions in our study. Total extractable Mn concentrations in the sewage sludge were high and exceeded the tolerable limits (100-300 mg/kg) set by USEPA (1986) for agricultural lands. The high levels of Mn in the exchangeable phase in all *spinacia oleracea* leaves might be ascribed to being mobile in that environment for the plant uptake.

Table 4.12: Manganese levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	298.80	37.97	5.65	2.54	0.08	345.04
LS 5	237.10	35.08	5.66	5.00	0.69	283.53
LS 10	757.20	76.86	14.13	3.07	0.83	852.09
LS 15	480.80	88.26	12.19	10.86	0.42	592.53
LS 20	640.00	81.45	18.81	8.11	0.58	748.95
LS 30	982.50	156.90	26.74	13.92	1.32	1181.38
LS 35	1002.00	136.30	169.90	10.59	0.44	1319.23
LS 40	808.30	166.60	25.99	10.28	0.91	1012.08
Mean	650.84	97.43	34.88	8.05	0.66	791.86
SD	37.38	13.45	6.58	2.84	0.08	48.56

The level of Ni in all the leaf sample (Table 4.13), fell within the permissible limit of 150 mg/kg set by CCME (1991) for residential and agricultural lands. Most of the Ni was found

in the residual and exchangeable phases. The high association of Ni in the residual fraction of the *spinacia oleracea* leaves might be attributed to the weak acidic stabilization process of the sewage sludge (Obasi et al., 2013). Similar results have been reported by Gupta and Sinha (2006) using tannery sludge.

Table 4.13: Nickel levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	0.00	0.00	0.00	0.00	0.17	0.17
LS 5	0.00	0.00	0.00	0.00	0.25	0.25
LS 10	0.00	0.00	0.00	0.00	0.27	0.27
LS 15	0.01	0.00	0.00	0.00	0.29	0.30
LS 20	0.00	0.00	0.00	0.00	0.23	0.23
LS 30	0.00	0.00	0.00	0.00	0.00	0.00
LS 35	0.00	0.00	0.00	0.00	0.00	0.00
LS 40	0.23	0.00	0.00	0.00	0.00	0.23
Mean	0.03	0.00	0.00	0.00	0.151	0.181
SD	0.001	0.00	0.00	0.00	0.02	0.06

The Pb levels in *spinacia oleracea* leaves (Table 4.14) fell below USEPA (1986) allowed limits of 30-300 mg/kg set limit for residential and agricultural lands. Similar results were obtained by Uba et al. (2008). More than 97% of Pb was found in the non-residual fraction while high percentage of the total extractable fraction contributed to the mobile phase (organic and exchangeable phases) and as such implies high risk for Pb contamination.

Table 4.14: Lead levels (mg/kg) in different geochemical fraction in *Spinacia oleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	0.37	0.00	0.11	0.24	0.00	0.72
LS 5	0.04	0.23	0.09	0.27	0.02	0.65
LS 10	0.36	0.03	0.12	0.11	0.03	0.65
LS 15	0.15	0.12	0.03	0.32	0.02	0.64
LS 20	0.36	0.05	0.17	0.39	0.03	1.00
LS 30	0.21	0.09	0.07	0.33	0.05	0.75
LS 35	0.39	0.15	0.34	0.36	0.02	1.26
LS 40	0.11	0.25	0.05	0.44	0.02	0.87
Mean	0.25	0.120	0.123	0.310	0.024	0.827
SD	0.040	0.030	0.040	0.050	0.003	0.080

The results of speciation analysis of Zn in (Table 4.15) indicated that the mean values of Zn in the various fractions were: Exchangeable (18.87 mg/kg) > Carbonate bound (9.18 mg/kg) > Fe-Mn oxide (4.56 mg/kg) > Organic matter (4.19 mg/kg) > Residual (0.31 mg/kg),

respectively. Zinc contents in leaf samples was below the permissible limits of 300 mg/kg set limit for agricultural lands USEPA (1986). The percentage of mobile fractions were high suggesting that Zn will be readily bioavailable to the environment. The association of Zn with labile fraction has been reported earlier by several other researchers (Ramos et al., 1994; Obasi et al., 2013).

Table 4.15: Zinc levels (mg/kg) in different geochemical fraction in *Spinaciaoleracea* leaf

Sampling site	F ₁	F ₂	F ₃	F ₄	F ₅	Total
LS C	12.19	6.28	2.02	0.09	0.19	20.77
LS 5	13.99	6.49	4.97	5.31	0.30	31.06
LS 10	16.96	6.85	5.03	0.59	0.36	29.79
LS 15	14.85	8.72	3.27	6.12	0.27	33.23
LS 20	23.74	8.79	5.72	5.74	0.32	43.31
LS 30	15.34	10.32	8.89	5.35	0.55	40.45
LS 35	28.26	9.75	2.89	4.58	0.13	45.61
LS 40	25.65	16.27	3.69	5.73	0.36	51.70
Mean	18.87	9.18	4.56	4.19	0.31	37.11
SD	5.69	3.03	1.00	0.96	0.12	9.38

Finally, results of the speciation study showed that the highest levels of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the *spinacia oleracea* plant leaves grown using sewage sludge from Thohoyandou wastewater treatment plant as manure, occurred mostly in the exchangeable fraction. Fractionation profile revealed that some major proportion of metals were associated with the exchangeable fraction at most treatments (36.96% Al; 12.50% Cd; 77.11% Co; 26.19% Cr; 44.03% Cu; 23.33% Fe; 82.19% Mn; 16.57% Ni; 30.23% Pb and 50.85% Zn) (Table 4.5). This indicates that in *spinacia oleracea* leaves, these metals principally exist in the mobile phase, which is readily bioavailable; therefore, they provide elevated environmental risk. The high percentage of exchangeable fraction most likely reflect anthropogenic properties of the samples (Ogunwale, 2017).

The results also suggested that accumulation of selected metal varied greatly among sewage sludge treatment concentrations in the *spinacia oleracea* plant leaves. This is in line with the report of Chunilall et al. (2005) that uptake of an element by a plant is solely dependent on the plant species, its inherent controls and the soil quality. It was also observed that the levels of metals in *spinacia oleracea* leaves were dependent upon their concentrations in their habitual soil environment. This observation is similar with the finding of Obasi et al. (2013).

4.4: Mobility factor (%) of the metals in the *spinacia oleracea* leaf

The operationally defined extraction sequence fractionates the heavy metals in the soil or sewage sludge in order of decreasing solubility. As a result, the Exchangeable and Carbonate bound fractions, ($F_1 + F_2$), which are the early fractions, capture the most reactive and presumably the most mobile and bioavailable fractions (Kersten and Forstner, 1989). The relative index of metal mobility was calculated as a mobility factor (%MF) (Salbu et al., 1998; Narwal et al., 1999; Kabala and Singh, 2001; Ogunwale, 2017) on the basis of the following equation:

$$MF = \frac{F_1 + F_2}{F_1 + F_2 + \dots + F_5} \times 100 \quad \dots\dots\dots (4.1)$$

The values of % mobility factor (MF) for Al (see Table 4.16), with a mean of 47.23 ± 5.08 , decreased in the following order: LSC (83.30) > LS 10 (67.15) > LS 15 (61.64) > LS 5 (55.32) > LS 20 (39.47) > LS 40 (30.44) > LS 35 (22.86) > LS 30 (17.64). Aluminum in this study for *spinacia oleracea* leaf reflected the level of exchangeable and organic matter fractions. Exchangeable fraction is a measure of those trace metals which are released most readily to the environment. It is acknowledged that the reactivity and potential bioavailability of Al generally increases with increasing solubility. Thus, high MF values of Al is an indication of liability and bioavailability. LS Control had the highest susceptibility to Al exposure, while LS 30 had the lowest risk.

The mobility factor (MF) for Cd in (Table 4.16) in this study with a mean value of 18.75 ± 2.11 , and which ranged from 0.00 at the LSC, LS 5, LS 10, LS 15, LS 20 and LS 40 to 100 at LS 35, were generally lesser than those for Al, Co, Cr, Cu, Fe, Mn, Pb and Zn but greater than Ni. Hence, Cd is among the least bioavailable in the sampling location for the *spinacia oleracea* plant leaf treated with wastewater treatment plant. This might be due to the properties of the sewage sludge from Thohoyandou wastewater treatment plant, the sources of Cd were minute and low rate of evaporation that might take place in the plant.

Table 4.16: Mobility factor (%MF) of metals in the *Spinacia oleracea* leaf samples

LS C	83.30	0.00	94.61	57.21	76.88	74.84	97.60	0.00	51.39	88.93
LS 5	55.32	0.00	91.45	36.36	59.67	27.41	96.00	40.48	41.54	65.94
LS 10	67.15	0.00	95.97	41.50	70.59	32.18	97.88	0.00	60.00	79.93
LS 15	61.64	0.00	90.44	42.55	52.93	34.41	96.04	3.45	42.19	70.93
LS 20	39.47	0.00	93.26	43.43	68.15	20.58	96.33	0.00	41.00	73.41
LS 30	17.64	50.00	90.13	29.23	56.08	11.19	96.45	0.00	40.00	63.44
LS 35	22.86	100.00	79.05	38.74	61.59	15.33	86.29	0.00	42.86	83.34
LS 40	30.44	0.000	93.38	35.04	72.28	24.47	96.33	0.00	41.38	81.08
Mean	47.23	18.75	91.04	40.51	64.77	30.05	95.37	5.49	45.05	75.88
SD	5.08	2.11	8.53	2.88	4.01	2.09	8.82	0.11	3.83	6.05

The mobility factor (%MF) for Co in (Table 4.16) in this study with a mean value of 91.04 ± 8.53 , which varied from 79.05 at the LS 35 to 95.97 at LS 10, were generally greater than those for Al, Cd, Cr, Cu, Fe, Ni, Pb and Zn. Therefore, Co is one of the most bioavailable in the plant. The values of % mobility factor (MF) for Cr (Table 4.16), with a mean of 40.51 ± 2.88 , decreased in the following order: LSC (57.21) > LS 20 (43.43) > LS 15 (42.55) > LS 10 (41.50) > LS 35 (38.74) > LS 40 (35.04) > LS 30 (29.23).

Chromium revealed the level of organic matter and exchangeable fractions. It occurs naturally in soils, at least in trace quantities. Chromium in sludge can be dissolved in the soil solution or bound to exchange sites. This fraction is seen to be phytoavailable. In general, their availability depends on soil parameters and processes such as the pH value, the organic matter (OM), the cation exchange capacity (CEC), and the clay content. The plant at LS Control was rated as the most susceptibility to Cr exposure, while those plant around LS 30 had the least at risk. The reported low MF value for Cr in the treatment plant wastewater sludge of RS 30 *spinacia oleracea* leaf is therefore an index of the high stability of Cr in the sample.

The mobility factor (%MF) for Cu in (Table 4.16) in this study with a mean value of 64.77 ± 4.01 , ranged from 52.93 (LS 15 *spinacia oleracea* leaf) to 76.88 (LS Control), were generally higher than those for Al, Cd, Cr, Fe, Ni and Pb. Copper was found most in the exchangeable fraction. Although Cu is essential for human diet, too much of it can cause eminent health problem. At elevated concentrations, Cu is also dangerous to plant (Keen and Ziderberg-Cherr, 1993). From this study, the sewage sludge of LS Control had the greatest vulnerability to Cu exposure, while those treatment around LS 15 had the least. The reported low MF value

for Cu in the sewage sludge from Thohoyandou wastewater treatment plant of LS 15 *spinacia oleracea* leaf is therefore a mark of the high stability of Cu in the sample.

The mobility factor (%MF) for Fe in (Table 4.16) in this investigation with a mean value of 30.05 ± 2.09 , which ranged from 11.19 at LS 30 to 74.84 at LS Control, were generally lower than those for Cd and Ni. Hence, Fe is more bioavailable than Cd and Ni. In terms of toxicity, however, levels of Cd and Pb may give cause for concern even at lower concentrations compared to Fe, and their effects may be several-fold higher than those of the Fe equivalents. Although Fe is primarily regarded as an essential element, excessive exposure to it, however, can lead to very serious health problems (Keen and Ziderberg-Cherr, 1993). From the result, the treatment of the LS Control environs in this study had the greatest vulnerability to Fe exposure, while those treatment along LS 30 had the lowest vulnerability.

The mobility factor (%MF) for Mn in (Table 4.16) in this study with a mean value of 95.37 ± 8.82 , which ranged from 86.29 at the LS 35 to 97.88 at LS 10, were generally higher than all those metals examined. Hence, Mn is more bioavailable than other metals in this study. In terms of toxicity, however, levels of Pb and Cd may give cause for concern even at lower concentrations compared to Mn, and their effects may be several-fold higher than those of the Mn equivalents. Although Mn is mainly considered a necessary element, undue exposure to it, however, can also lead to very grave health problems (Keen and Ziderberg-Cherr, 1993).

The values of % mobility factor (MF) for Ni (Table 4.16), with a mean of 5.49 ± 0.11 , decreased in the following order: LS 5 (40.48) > LS 15 (3.45) > ND at LSC, LS 10, LS 20, 30, 35, and LS 40. From the result, the treatment in LS 5 of *spinacia oleracea* leaf environs in this study had the greatest vulnerability to Ni exposure, while those treatment along LSC, LS 10, LS 20, 30, 35, and LS 40 *spinacia oleracea* leaf had the lowest vulnerability.

The values of % mobility factor (MF) for Pb (Table 4.16), with a mean concentration of 45.05 ± 4.83 , decreased in the following order: LS 10 (60.00) >LS Control (51.39) >LS 35 (42.86) >LS 15 (42.19) >LS 5 (41.54) >LS 40 (41.38) >LS 20 (41.00) >LS 30 (40.00). Apart from reflecting the levels of organic and exchangeable phases of Pb in this study and medium MF values, an indication of liability and biological availability of heavy metals (Salbu et al., 1998), showed the extent of the vulnerability of living things generally to heavy metals. Evidently, the treatment of plant at LS 10 of the *spinacia oleracea* leaf environment in this

study had the highest vulnerability to Pb exposure, while the treatment of plant around LS 30 had the least vulnerability. The low MF value reported for Pb in the treatment of plant of LS 30 is therefore a sign of the high stability of this metal in the sample.

The values of % mobility factor (MF) for Zn (Table 4.16), with a mean value of 75.88 ± 6.05 , decreased in the following order: LS Control (88.93) > LS 35 (83.34) > LS 40 (81.08) > LS 10 (79.93) > LS 20 (73.41) > LS 15 (70.93) > LS 5 (65.94) > LS 30 (63.44). From the results, the treatment of plant at LS Control environment in this study had the highest vulnerability to Zn exposure, while the treatment around LS 30 had the least vulnerability. The low MF value reported for Zn in the *spinacia oleracea* leaf of LS 30 is therefore an index of the high stability of Zn in the sample. The amount of Zn in sewage sludge is at least a magnitude bigger than the amount of some other heavy metals. Its concentration in the sewage sludge solution is usually smaller in comparison with total amounts. Zinc easily complexes with organic matter and clay minerals, but its soluble forms are very mobile and readily available for plants, especially under slightly acid conditions (Kabata-Pendias, 2011).

The mobility factor (%MF) values of the metals in all the treated plant samples follow the order: Mn > Co > Zn > Cu > Al > Pb > Cr > Fe > Cd > Ni. These values do not follow any particular trend with respect to sewage sludge treatment as measured in *spinacia oleracea* leaf at different concentrations. Similar observation was reported by Osakwe and Egharevba (2008). The relatively high mobility factor (MF) observed for Mn is quite in agreement with the high percentage of exchangeable fraction recorded from the chemical fractionation results. The low mobility factor (MF) value reported in some treatments at different concentrations is therefore a mark of the high stability of these metals in the samples (Ogunwale, 2017). A medium % mobility factor (MF) value for heavy metals in the sewage sludge as determined from *spinacia oleracea* leaf at different concentrations of the sewage sludge have been interpreted as an evidence of relatively slight liability and biological availability (Kabala and Singh, 2001; Obasi et al., 2013). Generally, the mobility factors in all the studied sewage sludge treatments were less than 100, indicating low pollution due to the metals.

The chemical fractionation of the metals studied revealed the geochemical nature of the ten heavy metals and their probable association with different chemical forms in the *spinacia oleracea* leaf around the sewage sludge from Thohoyandou wastewater treatment plant. The

results showed that the heavy metal concentrations found in the exchangeable fractions were higher than those observed in any of the preceding extractions except in the case of Cr, Fe, Ni and Pb where organic fraction predominated while Cd was abundant in reducible fraction. The heavy metal fractionation results gave an overall picture that high percentage of the metals were found to be strongly bound to *spinacia oleracea* leaf matrix i.e. in a form readily available for introduction into the food chain.

The overwhelming importance of the exchangeable fraction in this study illustrates clearly the difficulty of distinguishing between background (control sample) and anomalous levels of heavy metal contamination when only total metal analyses are performed. The relatively high mobility factor observed in Mn confirms the high liability and biological availability of Mn in some of the *spinacia oleracea* leaf studied. The present study indicates that the metals studied do not pose environmental risks at present since the total metal concentration of Mn which showed high liability and biological availability in this study was very low. Consequently, farmers can be encouraged to reclaim and utilize the sewage sludge for agricultural, residential,

4.5: Heavy Metal Concentration in the Vegetables Grown using sewage sludge

4.5.1 Transfer Factors (TF) of the Heavy Metals from Soils to *Spinacia oleracea* Leaf

The determination of heavy metal concentration in *Spinacia oleracea* leaf was done using atomic absorption spectrophotometer. Heavy metal concentrations values were higher in sludge samples compared to *Spinacia oleracea* leaf samples. Naser *et al.* (2011) reported that the levels of heavy metals in vegetables were generally lower than the soil samples. These results may be attributed to root activity which seems to act as a barrier for translocation of metals (Table 4.17). It also suggests that other soil properties and plant physiological processes play a major role in trace element bioavailability and uptake.

Heavy metals concentration showed variation among *Spinacia oleracea* leaf treated with different sewage sludge levels from SS Control, SS 5, SS 10, SS 15, SS 20, SS 30, SS 35 and SS 40 (Table 4.17). The variations in heavy metals concentrations in *Spinacia oleracea* leaf of the different treatments concentrations may be attributed to the differences in their morphology and physiology for heavy metal uptake, exclusion, accumulation and retention (Kumar *et al.*, 2009). Also, concentration of all the elements analysed varies from one

treatment to the other. *Spinacia oleracea* leaf differed in their ability to accumulate and concentrate metals in their edible parts, differences between them were significant which was well supported from the studies carried out by Sharma *et al.* (2006).

The variations in heavy metals concentrations in *Spinacia oleracea* leaf were due to variations in their absorption and accumulation tendency. The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils and sludges, the nature of sludge and the degree of maturity of the plants at the time of the harvest (Voutsas *et al.*, 1996). The minimum, maximum mean, standard deviation and variance concentration of trace metals (mg/kg) in *Spinacia oleracea* leaf were presented in Table 4.17, respectively.

In treatment (SS Control, SS 5, SS 10, SS 15, SS 20, SS 30, SS 35 and SS 40) the concentrations of trace elements ($\mu\text{g/g}$) ranged between 0.0000-0.9187 for SS C, 0.0000-0.8411 for SS 5, 0.0000-2.0803 for SS 10, 0.0000-4.9889 for SS 15, 0.0000-3.1099 for SS 20, 0.0000-7.3401 for SS 30, 0.0000-286.00 for SS 35 and 0.0000-3.2534 for SS 40 (Table 4.17). The mean concentrations were between (0.16201 and 30.3255 mg/kg) for all the treated samples which were in good agreement with concentrations (11-60 mg/kg) observed in vegetables by Arora *et al.* (2008).

The mean uptake of metal from the sewage sludge was in the order: SS 35 (30.3255 mg/kg) > SS 30 (1.00855 mg/kg) > SS 15 (0.72176 mg/kg) > SS 40 (0.66269 mg/kg) > SS 20 (0.58019 mg/kg) > SS 10 (0.39484 mg/kg) > SSC (0.16984 mg/kg) > SS 5 (0.16201 mg/kg), respectively (Table 4.17).

Transfer factor (TF) is the ratio of the concentration of heavy metal in a plant to the concentration of heavy metal in soil. It indicates the amount of heavy metals in the soil that ended up in the vegetable crop (Njagi, 2013). Transfer factor (TF) was calculated to understand the extent of risk and associated hazard due to ingestion consequent upon heavy metal accumulation in edible portion of vegetables (Njagi, 2013).

The transfer factors for each heavy metal were computed based on the method described by Harrison and Chirgawi (1989). The heavy metal transfer from soil to the vegetables was calculated as follows:

$$\text{Transfer factor} = \frac{\text{Metal content in plant } \mu\text{g/g}}{\text{Metal content in soil } \mu\text{g/g}}$$

Transfer factor of heavy metals depends upon bioavailability of metals, which in turn depends upon its concentration in the soil or sludge, their chemical forms, difference in uptake capability and growth rate of different plant species (FAO/WHO, 2011). Higher values of TF signify poor retention of metals in soil or sludge and/or more translocation into plants. The Table 4.17 shows the transfer factor (TF) of different heavy metals from soil to vegetable calculated as the ratio between the concentrations of heavy metals in *Spinacia oleracea* leaf and its mean concentration in soil. In *Spinacia oleracea* leaf the TF was Fe > Cu > Mn > Al > Ni > Co > Zn > Pb > Cd > Cr. The results revealed that Fe had the highest TF in all the *Spinacia oleracea* leaf in all the treatments. Similar results were reported by Naser *et al.* (2011) where they found that Fe had the highest TF among other metals and the order was Zn, Fe, Cd, Ni, Co and Pb, they also reported that the high mobility of Fe with a natural occurrence in the soil and the low retention of Zn in the soil than other toxic cations may elevate the TF of Fe.

In a study conducted by Opaluwa *et al.* (2012), the highest TF of metals was for Cu and the order was Cu, Co, Fe, As, Zn, Ni and Pb. The food-chain crops might absorb enough amounts of heavy metals to become potential health hazards to human (Abulude, 2005), that means that Fe, Cu, Mn, Ni and Co may pose the greatest threat among metals studied because of the elevated TF. Among the heavy metals, TF values were found to be higher for Fe, Cu and Mn whereas relatively lower TF values were found in Al, Ni, Co, Zn, Pb, Cd and Cr. The food chain (soil-plant-human) is mainly known as one of the major pathways for exposure of human to soil contaminants. Soil-to-plant transfer is one of the key processes of human exposure to toxic heavy metals through the food chain (Zhuang *et al.*, 2009). When TF < 1 or TF = 1, it denotes that the plant only absorbs the heavy metal but does not accumulate and when TF > 1, this indicates that plant accumulates the heavy metals. The mean TF values of Al, Ni, Co, Zn, Pb, Cd and Cr in the sludge were less than one in the vegetables which indicate that plants only absorb these heavy metals while for Fe, Cu and Mn were greater than one which implied that vegetable accumulates them in the sample. Different types of plants can absorb and tolerate metals differently. In general, there is little evidence of crop contamination through sludge operation. The application of sludge manures (biosolid) may, however, increase the metal content of uncontaminated soils. This may pose a risk to animals or children in the area who might ingest the Thohoyandou soil directly. The variations in heavy metals concentrations in

Spinacia oleracea leaf were due to variations in their absorption and accumulation tendency. Soil properties such as pH, organic matter, cation exchange capacity, redox potential, soil texture, and clay content may also affect the heavy metal uptake (Overesch *et al.*, 2007).

Table 4. 17: Transfer factor of the heavy metals from sludge to *Spinacia oleracea* leaf

Site/ Metal	LS C	LS 5	LS 10	LS 15	LS 20	LS 30	LS 35	LS 40	Total
Al	0.0384	0.0362	0.0772	0.2171	0.1058	0.2737	3.0413	0.1315	3.9212
Cr	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cu	0.0014	0.000	0.3573	0.3442	0.2552	0.3137	12.585	0.3030	14.1598
Fe	0.9187	0.8411	2.0803	4.9889	3.1099	7.3401	286.00	3.2534	308.5324
Mn	0.6281	0.6368	0.7396	1.2234	1.8510	1.6240	0.000	2.2110	8.9139
Zn	0.0015	0.00003	0.0020	0.0027	0.0075	0.0028	0.4469	0.0058	0.4678
Cd	0.000	0.000	0.00004	0.00004	0.00004	0.00003	0.0010	0.00004	0.0020
Co	0.000	0.000	0.000	0.000	0.000	0.000	1.0100	0.000	1.0100
Ni	0.1116	0.1055	0.6915	0.4405	0.4718	0.5305	0.0000	0.7214	3.0728
Pb	0.0001	0-0005	0-0005	0.0008	0.0007	0.0007	0.1700	0.0008	0.1741
Min.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Max.	0.9187	0.8411	2.0803	4.9889	3.1099	7.3401	286.00	3.2534	
Mean	0.16984	0.16201	0.39484	0.72176	0.58019	1.00855	30.3255	0.66269	
SD	0.3105	0.29380	0.62669	1.4675	1.00152	2.16327	85.3044	1.0822	
Variance	0.3273	0.3096	0.6606	1.5469	1.0557	2.2803	89.9188	1.14071	

4.6: Polycyclic aromatic hydrocarbons (PAHs)

The content of polycyclic aromatic hydrocarbons in the sewage sludge studied was characterized by clear differences. The two PAH concentration ranged from 4.20 to 146.50 mg/Kg (Table 4.18). It should be emphasized that the value of 146.50 mg/Kg was noted in only one sewage sludge sample from Thohoyandou wastewater treatment plant and a considerable contribution of treated industrial sewage in SS 5 (Table 4.18). In the remaining samples the maximum content of the two PAHs did not exceed 50 mg/Kg (Table 4.18). In more than half of the sewage sludge samples studied, EU standards (2006) limit of 6 mg/Kg were markedly exceeded (for sludge sample SS 5, as much as 8 times), in the case of the remaining samples, the values were also above the critical levels. When evaluating relations between the individual PAH contents studied, the amount of sewage added to each sample, the progressive state of the fermentation process, as well as the character of the sewage treated. To some extent this can point to the fact that with an increase in the contribution of industrial sewage in the sewage treated, the content of the PAHs did not follow any particular trend.

Spinacia oleracea grown on different levels of sludge from Thohoyandou wastewater treatment plant exhibited diverse increase concentrations of PAHs compared to one another.

Additionally, most of the concentrations were well above safety standards established in European Union (2006) revealing that this sludge grown vegetable is unsafe for human consumption (Table 4.18). In most of the sewage sludge samples studied, 2- and 3-ring PAHs had the highest contribution level. The highest content in most of the sewage sludge studied was observed for naphthalene. In one sample there was benzo[e]pyrene. The share of naphthalene in almost all of the studied sludge samples (except for SS 45) was higher than 87% (on average – 87.83%).

Taking into account the data presented above, it seems more plausible to take into consideration some chosen (mutagenic and carcinogenic) compounds from the PAH group when evaluating the possibility of the natural utilization of sewage sludge since the standards implemented in the countries of the European Union determine the maximum concentration of 3 PAH, i.e. naphthalene (6 mg/kg), benzo[a]pyrene (2.5 mg/kg) and benzo[b]fluoranthene (2 mg/kg). Similar standards concerning a higher number of PAHs are also valid in Austria (Fürhacker and Haberl, 1995). According to the Austrian standards, the maximum concentration of the 5 PAHs (fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene and indeno[1,2,3-cd] pyrene) in sewage sludge utilized for agricultural purposes cannot exceed 9.6 mg/kg.

Based on European Union standards, only in the case of naphthalene and benzo[e]pyrene was slightly exceeded levels observed in the sludge with the highest total content of PAH (SS 5; SS 10; SS 15; SS 20; SS 25 and SS 25). This sludge did not fulfil the requirements of the Austrian standard either, only the content of one individual PAH (SS 45) met the standards required.

Some authors attempt to identify the source of PAH in order to determine the origin based on the presence of individual compounds or determining relations between individual PAHs. The above method is used mainly for soils (Baran *et al.*, 2002). and sediments (Baran *et al.*, 2002). In the case of sewage sludge, the most representative seems to be the method proposed by Budzinski *et al.* (1997). Further to this method, it is proposed that the value of the naphthalene/benzo[e]pyrene (Nap/Ben) > 10 indicates that the source of the origin of PAHs are the processes to which crude oil is subjected, whereas the value of thenaphthalene/benzo[e]pyrene coefficient lower than 10, points to the predominance of pyrolytic processes. In the case of the fluoranthene/pyrene coefficient, it is assumed that values higher than 1 are related to pyrolytic processes, mainly coal burning. They showed

that the main share of PAHs in most sewage sludge samples was related to pyrolytic processes.

On the basis of the above data, it can be assumed that discharges from the wastewater treatment plant could have contributed to the pollution of sewage sludge with PAHs. It would also affirm the high contribution of naphthalene when compared to other sludges studied as suggested by Perez *et al.* (2001). Evaluating the content of the PAHs, it was found that in almost all cases there occurs an almost similar value with the exception of benzo[e]pyrene in SS 25 treatment. The contaminants that could have appeared in samples would have come from Thohoyandou wastewater treatment plant. Since one of the major contributors to increase in PAH concentrations in cities is industrial activities in this case observed mostly from Thohoyandou wastewater treatment plant where contaminants accumulate in sludge. The presence of PAH in all the treated samples could be due to the quality of sludges, chemical treatment, disinfectant, soil, air, other agrochemicals, urbanization and industrialization.

Table 4.18: PAH concentration in soil samples

Sample	PAH	Concentration (mg/kg)
SS 5	Naphthalene	146.5
SS 10	Naphthalene	32.49
SS 15	Naphthalene	21.49
SS 20	Naphthalene	43.26
SS 25	Benzo[e]pyrene	2.53
SS 25	Naphthalene	35.86
SS 45	Naphthalene	4.2

4.7: Statistical analysis of variance (ANOVA) of total metal determination

Data collected were subjected to one-way analysis of variance (ANOVA) to assess whether trace metals varied significantly between treatment and samples from the Thohoyandou wastewater treatment plant, with possibilities less than 0.05 ($p < 0.05$) considered statistically significant. From the statistical results obtained, all the trace metals across the treatment stands indicated that there were no significant differences in the metal distribution at $p > 0.05$

which implies that there is insignificant relationship between the means of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the sewage sludge of the study area at 95% probability level $\{F_{\text{calculated}} (2.82) < F_{\text{tabulated}} (2.91)\}$ (Table 4.19). This strongly signifies that the ten analytes might have come from the same source.

The insignificant ANOVA could be related to the texture and origin of the soil parent material as reported by Yassoglou *et al.* (1987). We can also infer that the activities carried out in this plant were similar and the effects of external forces were negligible.

Table 4.19: Analysis of variance of total metal determination

Source of variation	Sum of squares	Degree of freedom	Mean squares	$F_{\text{calculated}}$	P value	$F_{\text{tabulated}}$
Between groups	35936.70	7	5133.81	2.82	0.93	2.91
Within groups	1.04194E06	72	14471.40			
Total	1.07788E06	79				

4.8 Hierarchical cluster analysis (HCA) of trace metals in sewage sludge samples

Agglomeration schedule of cluster analysis (CA) was performed on data using nearest neighbour linkage and Euclidean distance as a measure of proximity between samples. Results of CA are shown in Figure 4.2. The hierarchical cluster analysis using nearest neighbour method produced three clusters, between which the variables were significantly ($P < 0.05$) different. It classified the eight treatments into three statistically significant clusters which resulted into dendrogram (Figure 4.1). According to the ten metals, HCA categorized each treatment into three distinctive clusters based on pollution magnitude as anthropogenic, mixed (anthropogenic and lithogenic), and lithogenic for all stands, respectively. Hierarchical cluster analysis (HCA) was executed to determine the correspondence between the treatments stands in the study area.

For all the treatment plants, three clusters were evident. The first cluster contained Cd and Mn group as emanating from anthropogenic. The second cluster comprised Ni, Cr, Co, Cu,

Mn, Pb and Fe group. These elements were classified as mixed source (anthropogenic and lithogenic). The third cluster discriminated the lithogenic Zn and Al. The association between Cd and Mn were stronger than Zn and Al.

The relationship among the treatments between the sewage sludge were established using cluster analysis as presented in Figure 4.28 below. As the samples were taken from different parts of Thohoyandou wastewater treatment plant, differences in biosolid composition and amount of sludge added all presented differences in the concentrations of trace metals in different treatments studied.

Identical studies by Obasi et al. (2013) found Cd to be strongly associated with sewage sludge and Pb, Fe and Ni to be associated with various industries and wastewater processes. According to Njagi (2013), Cr, Co, Cu, Mn and Zn were associated with wastewater related sources such as primary, secondary and tertiary treatments, etc.

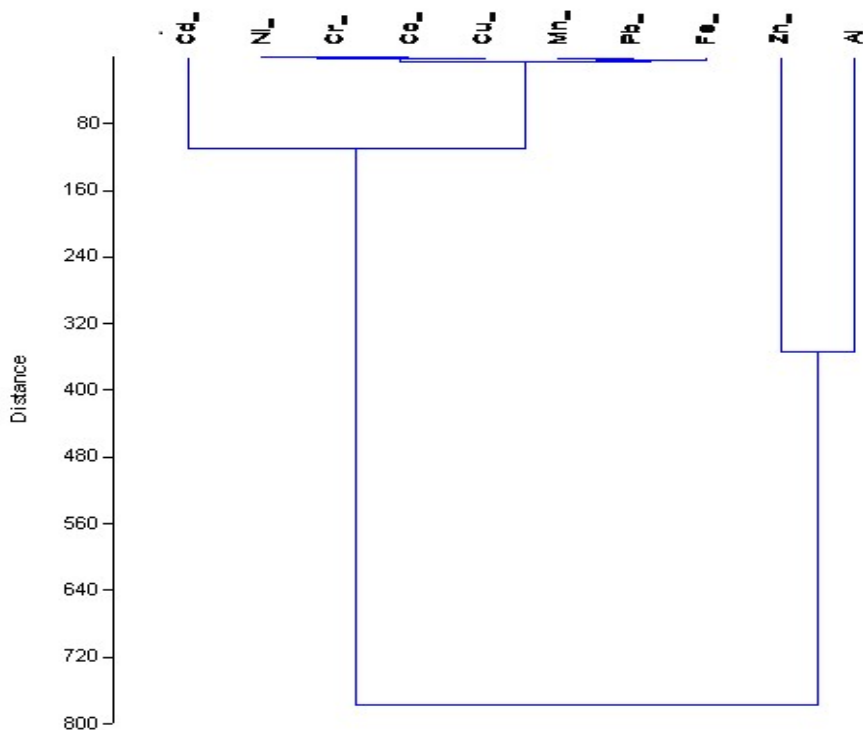


Figure 4.28: Cluster diagram showing relationship among trace metals in the sewage sludge

4.9: Intermetal and Sampling Points Association

Linear correlation coefficient was used to establish the interrelationship between heavy metal levels from soil/ sewage sludge samples (Tables 4.20). This coefficient measures the strength of a linear relationship between two variables on a scale of -1 (perfect inverse relation) through 0 (no relation) to +1 (perfect sympathetic relation).

In this study, the raw total metal determination data was used in calculating the correlation coefficient using the PAST (Pateontological Statistics Software (PAST Version 2.17). The correlation calculation performed on the trace metals in the treatment soil revealed that strong positive correlations were present among all the trace metals. The significant positive correlation coefficients from these metals may imply that these elements, originated from the same source which in this case could be linked to sewage sludge/or agricultural activities. The values obtained for correlation coefficients for these metals are in agreement with the results from Njagi (2013) for some positive correlations for all the metals listed above.

In a nutshell, the descriptive statistics, cluster analysis, correlation analysis and PCA results indicated that the elements had chemical affinity and/or were from similar sources. For instance, Cu, Fe, Mn, Ni and Zn were transition metals having variable oxidation states, while Al, Cd, Fe and Pb were pollutants from agricultural activities. These results followed the same trend in all the treatment stands as that of contaminated soils and supported their principal analysis results, indicating that the soils were contaminated via similar sources.

Table 4.20: Intermetal and sampling points association

	Al	Cu	Fe	Mn	Zn	Cd	Ni	Pb
Al	1.0000							
Cu	0.99169	1.0000						
Fe	0.99649	0.98900	1.0000					
Mn	0.99840	0.98598	0.99346	1.0000				
Zn	0.98883	0.99474	0.99307	0.98265	1.0000			
Cd	0.97112	0.99128	0.97229	0.96110	0.99202	1.000		
Ni	0.96249	0.98137	0.96703	0.95578	0.98879	0.99272	1.0000	
Pb	0.89901	0.92795	0.89028	0.89375	0.93008	0.95492	0.96367	1.000

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1: CONCLUSIONS

Based on the results obtained, it was concluded that there were narrow variations in the physicochemical parameters measured in the sewage sludge and soil samples. The pH of the sewage sludge and soil suggested that most of the metals would exist in free form while other properties like Conductivity, CEC, OC and Alkalinity load support leaching of metals from the sewage sludge from treatment plant. The order of the mean of trace metals levels in the soil and sludge were: Fe > Al > Mn > Cr > Cu > Zn > Co > Pb > Cd > Ni. Mean trace metals levels in all the samples were lower than the maximum allowable limits of the FAO/WHO standards. Although, trace metals levels sludge/soil were within the permissible limits, however, the ongoing agricultural practices could lead to increase in accumulation of trace metals in the soil overtime.

A five-stage sequential extraction procedure was adopted to fractionate trace metals in the *Spinacia oleracea* leaf. The levels of trace metals in the geochemical fractions evaluated for the treatment plant followed the following order: Exchangeable > Organic matter > Fe-Mn oxide > Carbonate bound > Residual fractions, respectively. The chemical fractionation of the metals studied revealed the geochemical nature of the ten trace metals and their probable association with different chemical forms in the *Spinacia oleracea* leaf soils and sludge around the school farms. The results indicated that the trace metals concentrations found in the exchangeable fraction were higher than those observed in any of the preceding extractions except in the case of Cd, Cr, Fe and Pb where Fe-Mn oxide and organic matter fractions predominated and were closely followed by exchangeable fraction. The trace metal fractionation results gave an overall picture that high percentage of the metals were found to be strongly bound to vegetable matrix i.e. in a form readily available for introduction into the food-chain except for Ni.

The relatively medium mean mobility factor observed in trace metals confirms the mild liability and biological availability of trace metals in some of the *Spinacia oleracea* leaf soil and sludge studied. The present study suggests that the metals studied do pose environmental risks with time as the rate of accumulation continues.

The variability of trace element transfer factors (TFs) was shown to be inherently mild. Part of that variability could be explained by the effect of environment on physiological functions responsible for the uptake, translocation and accumulation of trace elements. This study also revealed that the vegetable under study may pose health risk to the people who consume them as they were found to be deficient of essential metals such as Co and Zn. The study also confirmed the suitability of *Spinacia oleracea* as a good bioindicator of environmental pollutants.

Generally, the PAHs identified in the sample SS 5 was higher than the other samples. Among the PAHs detected in sewage sludge samples, naphthalene had the highest value (146.53 mg/kg) while benzo[e]pyrene had the least values in all the treatments. In view of this, wastewater treatment plant of Thohoyandou was not effective in removing the PAHs completely.

Statistical analysis of the data shows that metal pollutants in the sludge from Thohoyandou wastewater treatment plant used in growing vegetables originated from the source, which might be industrial activities and agrochemical inputs. The results obtained from soils, vegetable and sewage sludge analysis for Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn indicate appreciable levels of these metals in all these samples.

In summary, the largest contributors to trace metal concentrations still seem to be of industrial sources along with land use activities. It can be pointed out that wastewater industry should be located far away from human residence, not be used for agricultural purposes and trace metals should be regulated in a stringent manner.

5.2: RECOMMENDATIONS

In order to decrease soil pollution resulting from Thohoyandou wastewater treatment plant practices, the following are therefore recommended:

1. The method of separation at the Thohoyandou wastewater treatment plant with respect to PAHs removal should be improved upon.
2. There is the need for continuous monitoring of South African wastewater plants to ascertain their quality status. This will serve as a guide to the farmer and vegetable consumers on action plans to be taken.

3. Future studies should focus on investigating the levels of xenobiotics such as OCPs, phthalate esters and so on in crops and vegetables cultivated on the soil/sludge using Thohoyandou wastewater treatment plant. This is important to ascertain the consumption safety of such food crops.
4. Further investigations on environmental pollution should be carried out often in order to monitor the seasonal variations in levels of trace metals and compare with the previous speciation studies.

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