The effect of sulfur treatments on growth and phytoextraction of cobalt and nickel by *Berkheya coddii*

By

Thendo Peterson Nethengwe (300850)
Research report presented in partial fulfilment of the degree of Master of Science (by Coursework and Research Report) in the School of Animal, Plant and Environmental Sciences,

University of the Witwatersrand, Johannesburg

DECLARATION

The work described in this research report was carried out in the School of Animal, Plant and Environmental Sciences and the School of Chemistry, University of Witwatersrand, Johannesburg from August 2008 to November 2011 under the supervision of Ms. I.M. Weiersbye and Prof. L. Chimuka. This study represents original work by the author and has not been submitted in any form to any other University. Where use was made of the work of others, it has been duly acknowledged in the text.

Mr. Thendo Peterson Nethengwe
Date: 14th May 2012

This research report is submitted for examination after approval by the Research Supervisors consisting of the following:
I. Weiersbye, L. Chimuka.

ABSTRACT

One of the environmental concerns associated with mining waste is the contamination of soil. This study addresses the decontamination of soil, particularly of Co and Ni using *Berkheya coddii* (*B. coddii*). *B. coddii* is a hyperaccumulater plant that is able to decontaminate Co and Ni from the contaminated land. The use of *B. coddii* to decontaminate soil or waste must be based on a cognizance of the complicated, integrated effects of pollutant sources and soil-plant variables.

Phytoextraction pot trials using *B. coddii* were carried out under green house condition, with controlled watering. A contaminated metallurgical waste residue known as Rustenburg Base Mine Refineries waste (RBMR waste soil) collected from Rustenburg while a serpentine (native) soil (N soil) where *B. coddii* grows naturally was collected from Mpumalanga. The experiment involved the addition of sulfur doses to both soils in order to test whether acidification and higher sulfur availability could enhance the uptake of both Co and Ni by *B. coddii*. The results indicate that the addition of sulfur from 2.0 to 8.0 g per kilogram decreased pH in both substrates. RBMR waste soil pH was found to have decreased from 7.8 to 7.4 while the N soil pH was found to have decreased from 6.4 to 4.7. The reduction oxidation potential (redox potential) in both substrates was observed to have decreased along with the increase in sulfur dosage. The mean redox potential for RBMR waste soil was found to be 350 mV and 506 mV for the N soil after the addition of sulfur. Conductivity increased along with the increase in sulfur dosage in both substrates. The mean conductivity for the N soil was found to be 961 µS/cm while that of the RBMR waste soil was found to be 1453 µS/cm after the addition of sulfur.

The decrease in soil pH was significant ($p = 0.00115$) in the N soil than RBMR waste soil. Despite the increase in sulfur dosage and decrease in soil pH in both substrates, *B. coddii* observed growing. Although it was evident that *B. coddii* is able to grow in the RBMR waste soil, it was observed that the RBMR waste soil limits the root depth of the *B. coddii*, reducing chances for the roots to penetrate into the ground especially when dry. The RBMR waste soil becomes more compacted than the N soil when dry. It is therefore

crucial to ensure that there is enough moisture to allow for the *B. coddii* being able to survive effectively in the RBMR waste soil. *B. coddii* plant height in the RBMR waste soil after four months was observed to be in the range of 190 to 200 mm tall. This was found to be less than the height observed for the *B. coddii* planted in the N soil, which was in the range of 350 to 400 mm.

Nonetheless, plants grown in both substrates were able to absorb Ni and Co into their tissues. More Co and Ni were found to have accumulated into the leaf tissues than in other parts of the plant. This could be an advantage since one would harvest only the leaf part or the canopy (shoots) and allow *B. coddii* to resprout in order to continue taking up more Co and Ni from the same waste substrate to remediation levels that could be stipulated by Government as desirable for the ecosystem and the protection of human health. Although the accumulated Ni and Co can be recovered from biomass, this alone might not provide sufficient economic justification for phytoextraction due to the low concentrations that could be recovered.

B. coddii was found to absorb higher concentrations of Co and Ni from the N soil than from the RBMR waste soil. However, the results found in this study may not be conclusive. This could be due to many variables that could control metal uptake which were not investigated. These include mycorrhizal fungi and metal forms in the soil. Moreover, this study was performed in a green house and not in the outdoor environment. Ni is generally toxic to most plants, hyperaccumulators (i.e. *B.coddii*) contain elements that nullify the toxic effect of nickel, and in this case the accumulated nickel is bound to malate to form a harmless nickel complex which could be absorbed by the plants as nutrients.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my supervisors Ms. I. Weiersbye and Prof L. Chimuka for their guidance, support and valuable contribution to this research report.

Many thanks go to Dr H. Tutu, Dr S. Costly, Prof. J. Okonkwo, Dr. Sashnee Raja, Mark Cooper, David Furniss, T.N. Mutshatshi, Z. Ndlovu and T. Mabaso and laboratory staff in the Department of Chemistry, Environmental Analytical Chemistry Research Group (University of Witwatersrand) for their cooperation and assistance in the analyses of the samples.

My special thanks go to my daughter Adaho Nduvho Nethengwe, my Wife Vhutshilo Nethengwe and to my Parents Mr. Thambatshira Samuel Nethengwe and Mrs Gladys Humbulani Nethengwe, as well as to my brothers and sister Paul, Thusanani and Anzadakalo Nethengwe. Thank you so much for your loyal support, encouragement, patience, understanding and believing in me.

I further greatly appreciate the encouragement received from friends at the Department of Environmental Affairs. May the glory of the lord be with you.

Above all, I would like to submit to the almighty God who gave me energy, power and wisdom to be able to finish this research and say a prayer of gratitude for all the blessings that he bestowed upon me during the entire time of my studies.

TABLE OF CONTENTS

Contents **Pages**

DECLARATION .... **ii**

ABSTRACT .... **iii**

ACKNOWLEDGMENTS .... **iii**

Chapter One: Introduction, aims and objectives **1**

1.1. Background to the research 1
1.2. Statement of the research problem 5
1.3. Research aims and objectives 7
1.3.1. Aims 7
1.3.2. Key questions 8
1.4. Hypotheses of the Research 8
1.5. Research Assumptions 9
1.6. Justification of the Research 9

Chapter Two: Literature review **10**

2.1. Introduction 10
2.2. Basic Plant Physiology 14
2.3.Total and bio-available fraction of heavy metals in the soil 18
2.4. Plant response to heavy metals 19
2.4.1. Metal excluders 19
2.4.2. Metal indicators 19
2.4.3. Metal accumulators 20
2.5. Toxicity of heavy metals in the environment 20
2.5.1 Nickel (Ni) 21
2.5.2. Cobalt (Co) 22
2.6. Major sources of heavy metals 23

2.6.1. Weathering process as natural sources of heavy metals 23
2.6.2. Anthropogenic sources of heavy metals in the environment 25
2.7. Environmental remediation technologies 28
2.7.1. Isolation and containment 28
2.7.2. In situ treatment (soil flushing) 29
2.7.3. Soil washing (chemical leaching) 30
2.7.4. Mechanical separation 31
2.7.5. Pyrometallurgical separation 32
2.7.6. Chemical treatment 33
2.7.7. Electrokinetics 33
2.7.8. Bioremediation 35
2.7.9. Phytoremediation 36
2.8. Vegetation indices (VIs) used for the determination of any plant stress 47
2.9. Methodology for extracting metals in soil and plants 48
2.9.1. Microwave digestion 48
2.9.2. Acid digestion 49
2.9.3. Optimized European Community Bureau of Reference Sequential extraction procedure (BCR-SE) 49
2.10. Analytical methods for the determination of metal concentrations 52
2.11. Ion Chromatography 55

Chapter Three: Materials and methods 57

3.1. Description of the study area 57
3.2. Materials and methods used prior to *B. coddii* growth (part one) 60
3.2.1. Soil and seed sample collection and preparation prior to growth experiment 60
3.2.2. Grinding of soil samples 61
3.2.3. Physical and chemical properties of the soil and waste samples 62
3.3. Plant growth pot trials 67
3.3.1. Seed germination 67
3.3.2. Soil preparation before transplanting 67

3.3.3. Transplanting of the seedlings 69
3.3.4. Determination of plant growth rate and plant stress 69
3.3.5. Treatment of soil types with sulfur 72
3.4. After plant growth experiment (part two) 72
3.4.1. Physical and soil chemistry procedures 73
3.4.2. Plant sample collection and preparation procedures 73
3.4.3. Plant digestion procedure 75
3.5. Quality assurance 76
3.5.1. Calibration curve and certified reference materials for Carbon, Nitrogen and Sulfur (CNS) 76
3.5.2. Statistical analysis 77

Chapter Four: Results 84

4.1. Determination of the soil solution pH, reduction oxidation potential and conductivity 78
4.1.1. Determination of soil pH 78
4.1.2. Determination of soil conductivity 79
4.1.3. Soil reduction oxidation potential (redox potential) 80
4.2. Soil total concentration of carbon, nitrogen, sulfur and nitrate, sulphate 81
4.3. Sequential extractions for sulfur treated and untreated substrates 84
4.3.1. Extraction of Co and Ni concentration in soil solution (aqueous) 85
4.3.2. Weak acid extractable of Co and Ni 87
4.3.3. Extraction of Co and Ni bound to oxides 88
4.3.4. Extraction of Co and Ni bound to organic matter 90
4.4. Aqua regia (total digestion) extraction of Co and Ni 92
4.5. Growth of *B. coddii* in different soils treated and untreated with sulfur 94
4.6. *B. coddii* stress determination 97
4.7. Dry matter production (yield) and Root: shoot ratios of *B. coddii* plant 99
4.8. Ni and Co concentrations in *B. coddii* organs 103
4.9. Allocation of Ni and Co concentration between *B. coddii* organs. 110
4.10. Efficiency of *B. coddii* metals uptake 113

Chapter Five: Discussion... 118

5.1. Soil physical and chemical properties effects on plant growth and sulfur treatment 118

5.2. Effect of sulfur treatment on soil metal bioavailability 120

5.2.1. Extraction of Ni and Co concentration in soil solution (aqueous)..................... 120

5.2.2. BCR-sequential extraction for metal in various constituents of the soil............ 121

5.3. Plant height increment and leaf production 125

5.4. Plant stress determination 127

5.5. Potential of sulfur treatment for enhancing metal uptake...................................... 128

5.6. Efficiency of *B. coddii* in metal uptake 131

Chapter Six: Conclusion and recommendation 132

References.. 135

Appendices... 146

Appendix 1: Operational and ecological definition 146

Appendix 2: Soil solution conductivity, pH and redox potential obtained prior to the growth 150

Appendix 3: Soil conductivity, pH and redox potential obtained after the growth 151

Appendix 4: Concentration of Co and Ni (mg/kg) in soil, prior growth experiment 154

Appendix 5: Concentrations of Co and Ni in the soil.. 157

Appendix 6: Concentrations of Co and Ni in *B. coddii* organs. 159

Appendix 7: Dry matter production (g) of *B. coddii* 163

Appendix 8: Number of leaves on *B. coddii* plants over 266 days growing period 165

Appendix 9: Height of *B. coddii* plants over 266 days growing period 165

Appendix 10: Determination of stress indicators obtained before sulfur and after sulfur has been applied.. 171

Appendix 11: Soil Carbon, Nitrogen and Sulfur (CNS) before plant growth 175

Appendix 12: Soil Carbon, Nitrogen and Sulfur (CNS) after the growth experiment . 175

Appendix 13: Results for nitrate and sulphate obtained before the growth experiment. 177

Appendix 14: Plant CHNS results obtained after plant growth experiment 178
Appendix 15: Concentration factors in plant organs 184
Appendix 16: Mass ratios - proportion of total mass allocated to each *B. coddii* organs and root: shoot ratios 190
Appendix 17: Total shoots concentration of Co and root: shoot ratio of Co concentration 191
Appendix 18: Total shoots concentration of Ni and root: shoot ratio of Ni concentration 193
Appendix 19: Statistical analysis output 196

List of figures

Figure 2.1: Plant physiological processes 14

Figure 2.2: Phytoextraction mechanism 40

Figure 2.3: Schematic representation of an ICP – OES 53

Figure 3.1: Distrubution of the two study areas (i.e. RBMR waste soil and N soil) 59

Figure 3.2: Photograph demonstrating how RBMR waste soil samples were collected .. 61

Figure 3.3: Photograph showing of *B. coddii* seed collection 61

Figure 3.4: Showing how *B. coddii* seeds were geminated in the tray using the N soil... 67

Figure 3.5: Demonstrating how soil was prepared before transplanting. 68

Figure 3.6: Illustration of how plant height was measured.. 70

Figure 3.7: Illustration of how reflectance was measured 72

Figure 3.8: Illustration on how a plant samples were collected and grounded 75

Figure 3.9: Calibration curve for ICP-OES instrument obtained before analysis 76

Figure 4.1: Soil pH after plant growth experiment. 79

Figure 4.2: Soil conductivity after plant growth experiments 80

Figure 4.3: Soil reduction oxidation potential 81

Figure 4.4: Carbon percentage after plant growth 83

Figure 4.5: Nitrogen percentage after plant growth 83

Figure 4.6: Sulfur percentage after plant growth 84

Figure 4.7: Deionised water extractable Co and Ni concentration in soil 86

Figure 4.8: Acetic acid extractable Co and Ni concentration soil. 88

Figure 4.9: Hydroxylamine extractable Co and Ni concentration in the two soils........... 90

Figure 4.10: Hydrogen peroxide and ammonium acetate extractable Co and Ni concentrations in the two soils.. 91

Figure 4.11: Aqua regia (total digestion) extractable Co and Ni concentrations from the two soils 93

Figure 4.12: Measured plant height measurement over the experiment period................ 95

Figure 4.13: Number of plant leaves over the experiment period 96

Figure 4.14: Measured mNDVI705 and PSRI... 98

Figure 4.15: Leaf dry mass of *B. coddii* after sulfur amendments of both soils............ 100

Figure 4.16: Stem dry mass of *B. coddii* after sulfur amendments of both soils. 100

Figure 4.17: Root dry mass of *B. coddii* after sulfur amendments of both soils. 101

Figure 4.18: Comparison of *B. coddii* total biomass in different soils with various sulfur treatments. 101

Figure 4.19: Roots: shoot ratio 103

Figure 4.20: Ni and Co concetrations in *B. coddii* leaves from different soils and with various sulfur dosage. 105

Figure 4.21: Ni and Co concetrations in roots of *B. coddii* grown in two soils with sulfur dosage. 107

Figure 4.22: Ni concentrations in stem of *B. coddii* grown in two soils with sulfur dosage 108

Figure 4.23: Total shoot concentration (stem and leaf) after *B. coddii* growth experiment 109

Figure 4.24: Total metal concentrations (Ni and Co) in *B. coddii* after plant growth experiments in the two soils. 110

Figure 4.25: Allocation of Ni concentration on *B. coddii* organs (a) *B. coddii* grown in Native soil; (b) *B. coddii* grown in RBMR waste soil with sulfur dosages. . 111

Figure 4.26: Allocation of Co concentration on *B. coddii* organs (a) B. coddii grown in Native soil; (b) *B. coddii* grown in RBMR waste soil with sulfur dosages. . 113

List of tables

Table 2.1: Examples of plant metal indicators 20

Table 2.2: Examples of plants that have been used in phytoremediation 46

Table 2.3: The modified four-stage BCR sequential extraction scheme 51

Table 4.1: Nitrate and sulphate concentration in soil 82

Table 4.2: Mass proportion and root: shoot ratios of *B. coddii* 102

Table 4.3: Root: shoot ratios of Co and Ni in *B. coddii* 113

Table 4.4: Average concentration factors for nickel and cobalt in 266 days 115

Table 5.1: The pH and ammonium-acetate-extractable Ni and Co concentrations 124

GLOSSARY

AAS	Atomic Absorption Spectrometry
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
ASD	Analytical Spectral Devices
BCR-SE	European Community's Bureau of References -Sequential Extraction
BCR	European Community Bureau of Reference,
CCME	Canadian Council of Ministers of the Environment
EDTA	Ethylenediamine tetra-acetic acid
EPA	Environmental Protection Agency (U.S.A)
FAAS	Flame Atomic Absorption Spectrometry
FRTR	Federal Remediation Technologies Roundtable (U.S.A)
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-AES	Inductively Coupled Plasma – Atomic Emission Spectroscopy
KCl	Potassium chloride
$\mathbf{mNDVI_{705}}$	Modified red edge Normalised Difference Vegetation Index
N	Native soil for *B. coddii* (substrate 1)
PGE	Platinum group element
PGM	Platinum group metal
PSRI	Plant Senescent Reflectance Index
RBMR	Rustenburg base metal refinery waste (substrate 2)
VI	Vegetation Index

Chapter One: Introduction, aim and objectives

1.1. Background to the research

One of the environmental concerns associated with mining waste is the contamination of soil with heavy metals. Controlled and uncontrolled disposal of tailings and metallurgical waste contaminated with heavy metals, as a result of mining and smelting of metalliferous ores, are responsible for the migration of many contaminants into the environment such as in soil and water (Ghosh *et al*., 2005). Heavy metals and organic contaminants are becoming increasingly common in soil and water across many regions of the world. These contaminants often cause adverse effects in many organisms, including humans.

Heavy metal is a collective term generally applied to the group of metals and metalloids with an atomic density greater than 3.6 g/cm^3 (Alloway, 1990). Many of these metals are micronutrients for plants and other biota, but most of them are also toxic in high concentrations. Heavy metals are toxic because they have the potential to disrupt functions of the animal body and plant tissues. There are various ways that heavy metals may enter the body such as through food, water, dust/air, or absorption through the skin when they come into contact with humans in activities such as mining (Alloway, 1990).

Heavy metals like Pb are associated with low intelligence in children. Cr^{6+} is said to be carcinogenic while Mn is linked to causing mental disorders (Singh *et al*., 2003). The world-wide release of heavy metal, in the year 2002 was estimated at 22000, 93900, 783000, 234000, 156000 and 1350 000 tons for Cd, Cu, Pb, Co, Ni and Zn respectively (Singh *et al*., 2003). Once released, metals are naturally persistent in the environment due to their limited water solubility and resistance to most conventional treatment techniques.

Governments throughout the world are spending huge amounts of money trying to develop mechanisms that could be used to remediate contaminated soil, particularly contamination by heavy metals (Singh *et al*., 2003). In this regard, mining tailings and metal processing plants (refineries and smelters) are considered a major anthropogenic source of heavy metals found in the environment to date. Many of these sites leach heavy metals to the non-contaminated soils and groundwater due to improper storage of waste materials (Singh *et al*., 2003). These sites, therefore, need immediate attention.

Traditionally, heavy metal contaminated sites have been cleaned by techniques that are destructive such as excavation of the contaminated soils. Apart from being destructive, some remediation techniques are costly to implement (Weiersbye, 2007). They often employ the use of high energy, a by-product of which in the end results in further contamination. Interestingly, certain types of plants can not only tolerate soil contaminated with metals and other potentially harmful compounds, but also seem to thrive in these harsh conditions. In fact, some plant species can accumulate high concentrations of heavy metals in their tissues which are otherwise fatal to other organisms. The use of green plants and their associated micro biota to remediate or decontaminate contaminated soil is called phytoremediation (ITRC, 2009).

Phytoremediation is an environmentally friendly green technology that is cost effective and incredibly inexpensive. In some cases, metal hyper-accumulator plants used to remove metal from terrestrial as well as aquatic environments. This technique makes use of the intrinsic capacity of these plants to selectively accumulate metals and transport them to shoots (Shah *et al*., 2006). The U.S.EPA (1997) defines phytoremediation as “the direct use of plants for in situ, risk reduction for contaminated soil, sediments, and groundwater through contaminant removal, degradation, or containment.” Similarly, Chaney (1995) defines phytoremediation as “the use of plants and plant processes to remove, degrade or render harmless hazardous materials present in the soil or groundwater.”

The idea of using metal accumulating plants to remove heavy metals and other contaminants was introduced in 1983 (Brooks, 1987) and the concept has been implemented in some ways for the past 300 years (Brooks, 1987). Plants that are able to tolerate and accumulate specific elements in their canopy to order of magnitude above specified levels are called hyperaccumulators (Brooks, 1987).

Phytoremediation has been classified as a biological technique (Phytotechnologies) since plants are directly involved in extraction of pollutants from (or stabilizing within) the contaminated soil. Sometimes the element can be concentrated within the above biomass; this reduces the amount of pollutants in the soil. For example, several sequential crops of selected plant species can be cultivated and harvested to reduce the concentrations of heavy metals in contaminated soils to environmentally acceptable concentrations. The plants used in the contaminated soil may take up or stabilize the concentration of heavy metals from those sites (ITRC, 2009).

Phytoextraction is considered one of the best approaches in removing heavy metals from contaminated soil without destroying the soil structure and its fertility. Plants can absorb, concentrate and precipitate toxic metals and radionuclides from contaminated soils and wastes into the biomass from diffusely polluted areas, especially where pollutants occur only at relatively low concentration. Several approaches have been used, but the two basic strategies of phytoextraction are: (i) Chelate assisted phytoextraction or induced phytoextraction, in which artificial chelates such as fertilizers are added to increase the mobility and uptake of a metal contaminants, (ii) Continuous phytoextraction where the removal of metal depends on the natural ability of the plant to take up or remediate the contaminated sites and the number of plant growth repetitions are controlled (ITRC, 2009).

Phytoextraction refers to the ability of plants to take up contaminants into the root structures and translocate those pollutants (for example Ni and Co) to the aboveground shoots (i.e. leaves and stems). In order for this technique to be effective, contaminants to be extracted by plants must be bioavailable and bioaccessible (come into contact with the plant roots). Alternatively, the uptake may occur through vapor absorption into the organic root membrane in the vadose zone (ITRC, 2009). Once absorbed, the contaminant may dissolve into the transpiration water or be actively taken up through plant transport mechanisms (ITRC, 2009). Ideally the plant should also produce a large quantity of biomass. The heavy metals stored in the plant biomass or tissues can be extracted, and recovered for reuse.

Based on its effectiveness, phytoextraction can also be applied in the recovery of metals from mining waste and polluted areas, particularly those associated with sub-economic mineralization and contamination by industries. Many sites across the world polluted with heavy metals, such as Co and Ni from mining emissions, can now be potentially phytoremediated (Brooks et al., 2001). There are several factors that influence the survival of plants in a particular environment. These include plant growth rate, metal selectivity, and resistance to disease as well as methods of harvesting. Slow growth rates, shallow root systems, and low biomass production can limit the total yield of metals taken from the soil per hectare and this can render phytoextraction inefficient for commercial use (Baker *et al*., 1994).

However, the root/shoot biomass ratio of plants can be changed by adding or with-holding nutrients to the soil, or by mycorrhizal fungi (Marschner, 1986). Also, the addition of chemicals (e.g. sulfur) that lower the pH of the soil can increase the solubility of some metals for uptake (Robinson *et al*., 1999). For example, sulphate (or acid mine drainage), this can make the soil more acidic, and result in increased bioavailability of Co and Ni (Alloway, 1990).

Remediation of polluted environments in South Africa, especially by using green technology such as plants, under controlled conditions, can help to reduce the problem of soil pollution without having to move people away and excavate the soil. In South Africa there are a number of indigenous plants that are being studied as potential phytoextractors of Ni and Co (Nindi, 2005). One of those species is *B. coddii* Roessler (Asteraceas). This plant grows naturally in serpentine soil of the Badplaas and Barberton mountain area in the Mpumalanga Province (Nindi, 2005). *B. coddii* has also been introduced to the polluted soils around Rustenburg Base Metal Refinery waste (RBMR waste soil) in Rustenburg (Slatter, 2007). This species has an unusually fast growth rate and high leaf biomass for a metal hyperaccumulator. *B. coddii* has been recognised world-wide as effective in cleaning-up soil contaminated by Co and Ni (Nindi, 2005). Co and Ni are two metals which are trace nutrients for most of the plants, but can be toxic in high concentrations. Both are economically valuable metals, but are also pollutants in base metal smelter waste and mine tailings.

This study focused on the effect of sulfur treatment on *B. coddii* growth and phytoextraction of Co and Ni from contaminated RBMR waste soil and a natural serpentine soil. These will be referred to as the serpentine or native (N) soil and the Rustenburg Base Mine Refineries waste (RBMR waste soil). A study of the same nature was conducted by Robinson *et al*., (1999) but on the native soil only. The study found that sulfur could be used as a low cost soil amendment to enhance the metal uptake of crops grown on ultramafic soils. The current study explored the possibility of using sulfur to enhance the bioavailability of metals in both substrates. This is the first time such a study has been conducted on this particular RBMR waste soil, where sulfur is added.

1.2. Statement of the research problem

Mining in South Africa provides a vast contribution to the economy, both in terms of the actual materials that are mined and in the creation of literally hundreds of thousands of jobs, with benefits to many aspects of society. However, mining activities inherently hold extensive adverse effects for the biophysical, social, and economic environment and results in severe disturbance of large land areas. Tailings material is processed at a rate of millions of tons per year and massive tailings dumps originating from the coal, gold, and base metal mining have gradually changed the South African landscape and the quality of water (Ashton et al., 2001).

In 1996, the mining industry was responsible for the production of 377 million tons of tailings, accounting for 81% of the solid waste stream in South Africa (Nindi, 2005). Currently, mining waste is still the principal contributor to the solid waste stream, followed by pulverized fuel ash (6.7%), agricultural waste (6.1%), urban waste (4.5%) and sewage sludge (3.6%). Other impacts of mining include destruction of land and vegetation as well as pollution and changes in surface drainage. As a result, environments are prone to increased soil erosion, compaction, subsidence, changes in topsoil characteristics, and a reduced capacity to support crops, livestock and biodiversity (Nindi, 2005).

Although mines are expected to provide for and apply rehabilitative measures before closure is granted (Weiersbye *et al*., 2006), it is much more complicated than simply restoring the disturbed area. The consequence of mining activities can led to soil contamination and also poor physical characteristics, such as poor textural material properties, combined with the effect of the slopes of the discard sites; low concentrations of plant nutrients and organic matter; salts; pH extremes; and the presence of heavy metals. The processing of mine tailings and discard material usually results in an elevated topography which means that these discard sites are particularly exposed to the adverse effects of wind and water erosion. These aspects, often accompanied by difficult climatic conditions characteristic to arid and semi-arid areas of Southern Africa, deter the establishment of permanent self-sustaining vegetation cover on mine stockpiles and tailings (Weiersbye *et al*., 2006). A number of case studies of an array of mining materials have been studied that illustrate the line of argumentation as indicated above (Weiersbye *et al*., 2006).

This research tested the use of sulfur to enhance the phytoextraction of Co and Ni in native soil and base metal smelter waste soil. This was done by growing *B. coddii* under semi-controlled conditions in a greenhouse at University of Witwatersrand (Wits), Johannesburg, South Africa. The findings support recommendations on how to remediate platinum and base-metal mine wastes in South Africa whilst recovering Co and Ni, using plants as green technology.

1.3. Research aims and objectives

Rationale – *B. coddii* is a hyperaccumulator of Ni, and also accumulates Co to some extent (Robinson *et al*., 1999). Co is more valuable than Ni, and therefore it is of interest to determine whether it is possible to increase the uptake and yield of Co by *B. coddii*. However, according to Robinson *et al*. (1999), uptake of Co by *B. coddii* is competitively inhibited at high Ni concentrations. It is desirable to maintain high concentrations of Ni as well as Co in *B. coddii* in order to remove both contaminants simultaneously from polluted soil.

Therefore any soil or plant treatment to boost Co concentrations in *B. coddii* would need to alter competitive interactions between these metals in such a way that will significantly benefit Co bio concentration, without significantly impairing Ni bio concentration. The solubility of both metals in the substrate is influenced by pH, conductivity, redox potential and interaction of other ions and chemicals as well as plant root exudates, and interactions with micro-organisms such as Arbuscular mycorrhizal fungi (AMF). Robinson *et al*., 1999 have suggested simply acidifying the growth substrate in order to increase the availability of Co, and other metals. However, an experiment using sulfur treatments on *B. coddii* were inconclusive, possibly due to the short term duration of the experiment (Robinson *et al*., 1999).

1.3.1. Aim

- To determine whether the addition of sulfur to both the N soil and RBMR waste soil increases the solubility of Ni and or Co in the selected soils, and whether this results in increased metal uptake by *B. coddii* plants in a sulfur, pH or soluble metal dose-dependent fashion.

1.3.2. Objectives and or key questions

- Is sulfur (S) dose associated with changes in the height increment or biomass of *B. coddii* if grown in a metallurgical waste (RBMR waste soil) or N soil?
- Is sulfur (S) dose associated with increasing concentrations of Ni or Co in B. coddii plants or plant organs?
- To determine the influence of sulfur on metal speciation and potential bioavailability in both substrates (i.e. what is in solution, weak acid dissoluble, bound to oxide, and organics).
- To determine the elemental composition of both substrates before and after planting or harvesting, using a sequential extraction method in order to estimate the reactions of Ni and Co that are potentially bioavailable.
- To determine concentration of Ni and Co on different parts (i.e. root, stem and leaf) of *B.coddii*, before and after addition of S to both substrates.

1.4. Hypotheses of the Research

- There is no difference between *B. coddii* survival or dry matter production in both substrates.
- The addition of sulfur dosage increases the concentrations of Co and Ni in *B. coddii* biomass in both substrates.
- Adding S will acidify both substrates and increase the bioavailability of Co and Ni and its uptake by *B.coddii*.
- Adding S will result in increased Co and Ni bio-concentration at the cost of plant stress and reduced plant dry matter production.

1.5. Research Assumptions

This research assumes that *B. coddii* cannot only be used to extract nickel, but also to extract cobalt from soil or waste.

1.6. Justification of the Research

Anglo Platinum has the challenge of remediating mining waste and recovering valuable metals such as nickel and cobalt from wastes and contaminated soil. Therefore, this research tried to develop optimum conditions at which *B. coddii* can easily be used to decontaminate a platinum and base metal refinery waste.

Chapter Two: Literature review

2.1. Introduction

Soil pollution is a growing environmental problem which has been attracting considerable public attention over the last decades. Increasingly widespread pollution has caused vast areas of land to become non-arable and hazardous for both wildlife and human populations living in those areas. Unfortunately, the cost associated with the removal of pollutants from the soil by means of traditional physicochemical methods has led to many companies ignoring the problem. An example of this is the commonly used technology for the remediation of metal polluted soils which is the excavation and burial of the soil at a hazardous waste disposal site and is estimated to an average cost of $1 000 per acre (Garbisu *et al*., 2000 sited Raskin *et al*., 1997).

Other common approaches used to treat metal-polluted soils are fixation (which is known as chemical processing of the soil to immobilize the metals) and leaching (using acid solutions or other solutions to desorb and leach metals from soil followed by the return of clean soil residue to the site) (Salt *et al*., 1995). Apart from minimizing the impact of future incidents by means of controlling pollution input, it is imperative to deploy innovative technologies which could economically remediate polluted soils with minimum cost (Garbisu *et al*., 2000).

In contrast to many organic pollutants, which are results of anthropogenic factors which often degrade into the soil, metals occur naturally and are not biodegraded (Wade *et al*., 1993). The danger of heavy metals is therefore aggravated by their persistence in the environment. Although some metals are essential for life (i.e. they provide essential nutrients and cofactors for metalloproteinase and enzymes), at high concentrations they can block essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules (Collins *et al.,* 1989).

Above specified concentration, heavy metals are toxic for both higher organisms and micro organisms. Many heavy metals directly affect various physiological and biochemical processes causing reduction in growth, inhibition of photosynthesis and respiration (Garbisu *et al*., 2000). Some of the heavy metals can be accumulated in the roots, and some are then transported to the aerial parts of the plants. Metals cannot be destroyed biologically or degraded; they can just be transformed from one oxidation state to the other. As a result of the oxidation state and binding properties, metals may become either: (i) more water or fat soluble and subject to removal by leaching, (ii) less soluble, and thus (iii) less water soluble so that it precipitates and then becomes less bio-available or removed from the contaminated site (Garbisu *et al*., 2000).

South Africa has the largest deposits of platinum group elements (PGEs) in the world followed by Russia, USA and Canada (Johnson, 2001). The mining of PGEs has steadily increased since the 1970s reflecting their growing worldwide use especially as auto catalysts and in jewellery (Johnson, 2001). Other metals which are mined alongside PGE's in South Africa include nickel, cobalt, copper and iron as well as other base metals, which occur naturally in sulphide seams (Bradford, 1988). Despite large economic benefits from the mining of these PGEs in South Africa, there are environmental concerns about the large amounts of mine tailings and other waste produced.

In the past, PGEs were considered to have little effect on the biosphere but there is concern at present that they may be toxic as they bio-accumulate in organisms including humans (Cicchella *et al*., 2003). Concentrations of palladium of 1-3 mg/kg have been observed damaging the plants cells (Smith *et al*., 1978). Some complexes of platinum are highly toxic to plants and probable biomethylation of platinum in the aquatic environments may be damaging to plant life similar to methyl mercury (Farago *et al*., 1985).

Traditionally, contaminated sites have been cleaned or remediated by destructive methods such as excavation. In recent years however, approaches to clean-up contaminated areas have begun to focus on the utilization of plants, referred to as phytoremediation (Salt *et al*., 1995, Cunningham *et al*., 1996; Brooks *et al*., 2001; Angle *et al*., 2001). In their studies (Brooks *et al*., 2001), they found the threshold concentration of 1000 mg/kg (0.1%) for nickel in dry material of plant biomass and this was taken to represent a plant's capability to accumulate Ni. In this case the plants concentrate metals in their shoots or canopy (Brooks *et al*., 1983). The plants have the ability to transport and concentrate heavy metals in their aerial parts. Use is being made of these plants for the purpose of remediating polluted soils (Robinson *et al.*, 1997).

Phytoremediation, in particular phytoextraction, has been conducted in various areas for metal uptake and soil stabilization, particularly those metals that result in toxic effects when they enter or come into contact with organisms in the environment (Robinson *et al*., 1997). Phytoremediation is therefore, regarded as a tool for clean-up of most of the heavy metals in the soil. However, the focus of this research is on phytoextraction which is one of the examples of phytoremediation. This technology could potentially be used to phytoremediate the contaminated sites around the world if the plant can survive under different climatic conditions. The idea of phytoextraction was firstly proposed by Chaney (1995) and later by McGrath *et al*., 1993, who further demonstrated that the plants that are tolerant to the soil can be used to recover soil contaminants. Phytoextraction requires cultivating hyperaccumulators selected based on their ability to take up the target contaminants from the contaminated soil (Keeling *et al*. 2003).

Plants can accumulate metals and metalloids essential for growth and development and such metals include: Fe, Mn, Zn, Cu, Mg, Mo, Se, Ni and Co. In addition, some of the plants have the capacity to accumulate metals and metalloids such as Ti, Cd, Cr, As, Pb, Ag and Hg which have no known biological functions to support the growth of such plants (Baker *et al*, 1989; Raskin *et al*., 1994).

The metabolism of metals by plants require a balance between the uptake of essential metal ions to maintain growth and development and the ability to protect sensitive cellular activity and structures from excessive levels of essential and non-essential metals. Resistance of plants to heavy metal ions can be achieved by an avoidance mechanism, which includes keeping the metals in the roots, and mainly the immobilization of metal in root and in cell walls (Raskin *et al*., 1994).

Tolerance to heavy metals is based on the sequestration of heavy metal ions in vacuoles, on binding them by appropriate ligands like organic acids, proteins and peptides and on the presence of enzymes that can function at high levels of metallic ions (Robinson *et al*., 1996). The phytoextraction method of extracting heavy metals from the contaminated soil represents one of the largest economic opportunities for phytoremediation because of the size and scope of environmental problems associated with metal-contaminated soils and the competitive advantage offered by a plant-based remediation technology (Garbisu *et al*., 2001)

The ideal plant to be used in phytoextraction should have the following characteristics or factors (McGrath *et al*, 1993):

- be tolerant to high concentrations of the metal;
- accumulate high concentrations of the metal in its harvestable parts;
- have a rapid growth rate;
- have the potential to produce a high biomass in the field;
- have a profuse root system to be able to absorb metal from a large volume of soil.

The idea of using plants to remove metals from soils came from the discovery of various endemic plants grow naturally in mineralized soils and have the ability to can hyperaccumulate high concentrations of metals in their foliage (Brooks *et al*., 1987; Baker *et al*, 1989; Raskin *et al*., 1997). As pointed out by Garbisu *et al*., (2001) there are two strategies of phytoextraction: (1) chelate-assisted or induced phytoextraction and (2) continuous phytoextraction (Garbisu *et al*., 2001).

2.2. Basic Plant Physiology

Most plants grow by sending their roots into the soil and producing leaf and stem material. To accomplish these basic growth habits (ITRC, 2009), plants use carbon dioxide to harvest light energy from the sunlight, convert it into chemical energy, and produce carbon biomass through the processes of photosynthesis in the leaves and cellular respiration (ITRC, 2009). Plants also take up liquid water and dissolved inorganic nutrients that assist on growth development through the root system, transport them throughout the plant in the xylem, and transpire the water through the leaves as vapor. While carbon dioxide and water vapor are being exchanged, oxygen is also being released to the environment (ITRC, 2009).

Likewise, photosynthetic chemicals (photosynthates or phytochemicals) are transported throughout the plant in the phloem, even into the root to be exuded into the surrounding soil. The upward transport in the xylem and downward transport in the phloem, collectively termed "translocation," depend on the continuous water column that exists throughout the plant. Each biological process contributes to the remediation of contaminants from contaminated soil (ITRC, 2009). Figure 2.1 shows the plant physiological process, which might be required during the phytoextraction process.

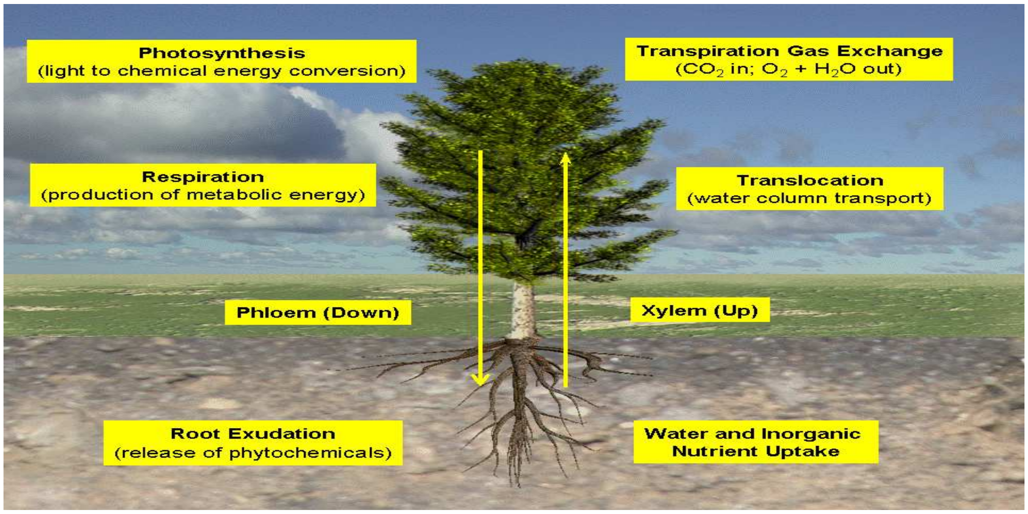


Figure 2.1: Plant physiological processes (ITRC, 2009)

2.2.1 Plant nutrients

Plant nutrients are present in the soil. These nutrients are essential for plant growth and the deficiency can be corrected by the addition only of those specific elements causing the deficiency. The soil elements are often classified into two broad categories, micro-nutrients (Fe, Mn, Cl, Cu, Zn, Na, Co and Ni) and macro-nutrients (N, P, K, Ca, Mg, and S). Micronutrients are defined as organic or inorganic elements or compounds that are needed in only relatively small amounts by living organisms for survival, whereas macronutrients are defined as organic or inorganic elements or compounds which are needed in relatively large amounts by the plants. Organic constituents which include some of these elements include amino acids, carbohydrates and fats, and compounds essential to metabolism such as enzymes. Animals can be exposed to nutrient and non-nutrient metals through consuming plants, fungi, algae and other animals that live on them (i.e. food chain). Even though the chemicals are important to organisms, high exposure can result in a negative impact (Yaron sited Allison, 1995). Below are the examples of plant macronutrients that might be essential for the growth of *B. coddii* in a particular environment.

- **Nitrogen (N)**

Nitrogen as nutrient occurs both in inorganic and organic forms. Inorganic forms of nitrogen that cause pollution in the soil include nitrates, nitrous oxide and nitrogen trioxide. These elements are considered to be the most important inorganic nutrient compounds. Under natural condition their concentrations range from 2 mg/l to 10 mg/l (Yaron, 1995 cited in Allison, 1995). The concentration of nitrogen ions or compounds usually fluctuates throughout the year. The research conducted by Allison, (1973) indicated that nutrients including nitrogen formed during winter in soil are 1 ppm and sometime 50 ppm during spring. Stevenson (1982) stated that 90 % of the nitrogen formed in the surface layer of most soils are organically combined. In the paper by Yaron, (1995) it was highlighted that soil forming factors can influence the concentration of nitrogen in the soil, and the balance of nitrogen in the soil is controlled by fixation of molecular compounds. Nitrates occur naturally as part of the constant cycling of nitrogen in the environment. Over utilization of fertilizers in most areas can, however, generate an additional amount of nitrogen compounds (Roger, 1999).

- **Phosphorus (P)**

This includes organic and inorganic fractions of P consisting of compounds characterized by different solubility and availability. The concentration of P-compounds varies greatly in the soil ranging between 0.1 and 0.8 mg/kg in dry soil. The inorganic phosphorus ions are bound to calcium as mono-, di-or tricalcium phosphates. Under certain soil conditions, the inorganic phosphorus ions are adsorbed to Fe or Au as compounds, and are called fixed phosphates because they are not readily available to plants (Kuun et al, 1987). The pH of soil has an important influence on the availability of phosphorus for plants within a particular environment.

- **Calcium (Ca)**

Calcium is one of the macro-nutritional metals found in the soil. Calcium abundance by weight on the earth is about 2, 1% of calcium. Surface crust has been found to have 3.6% of calcium. Calcium is regarded as a member of the alkaline earth group of metals and a major component of the earth crust. In most cases calcium is found where igneous rock is dominant hence it is the cation found in the basic igneous rock (Neill, 1995).

- **Potassium (K)**

Potassium is one of the macro-nutritional elements found in soil like calcium. The total potassium concentration of the soil is relatively high and only small concentrations of that potassium are available for plants. Potassium is a cation that can be absorbed to a negative charge on clay particles, where it may be exchanged or replaced by other cations and made available for plants again. The introduction of potassium to most soils is through natural processes such as weathering processes. Larger concentrations of potassium in the soil can result in negative impact to some of the organisms that do not need high concentrations of potassium (Neill, 1995).

- **Sulfur (S)**

Sulfur is an essential element for growth and the physiological functioning of plants. In most cases the total sulfur in most of the plants is found to be 0.1 to 2 % of dry weight (0.03 to 0.6 mol S kg^{-1} dry weight) (Robinson *et al*., 1999). The assimilation and uptake of sulfur by plants is strongly interrelated and dependent to that of nitrogen. At adequate concentrations of sulfur supply the organic N/S ratio is around 20:1 on a molar basis. In most plant species a major proportion of sulfur is present in reduced form in the cysteine and methionine residues of proteins. In reality most plants have been found containing a large variety of other organic sulfur compounds such as thiols and or sulpholipids

Some species contain the so called secondary sulfur compounds such as glucosinolates. S compounds are of greater importance in plant functioning, but they are also important for food quality and production of phyto-pharmaceuticals. Plants use sulphate taken up by the roots as a sulfur source for growth. Sulphate is actively taken up across the plasma membrane of the root cells, subsequently loaded into the xylem vessels and transported to the shoot by the transpiration stream. In the chloroplast of the shoot cells, sulphate is reduced to sulphide prior to its assimilation into organic S compound. Plants are able to utilise foliage to absorb sulfur from gases, the chronic atmospheric SO_2 and hydrogen sulphide at a concentration of 0.05 mIand higher. Most of these are found in polluted areas and contribute substantially to the plant's sulfur nutrition.

The sulfur requirement varies strongly between species and it may fluctuate during the plant's growth. The study by Robinson *et al*., (1999), indicate that too much sulfur nutrient could decreases the soil pH to a level not suitable for plants to grow and eventually kills the plants. However, the increase in sulfur dose may increase the availability of heavy metals such as Ni and Co for plant to uptake from the contaminated soil (Robinson *et al*., 1999).

2.3. Total and bio-available fraction of heavy metals in the soil

In soil, metals are associated with several fractions: i) in soil solution as free metal ions and soluble metal complexes; ii) adsorbed to inorganic soil constituents at ion exchange sites; iii) bound to soil organic matter; iv) precipitated such as oxides, hydroxides, and carbonates; v) embedded in the structure of the silicate mineral. In the determination of the different soil fractions, soil sequential extraction is often employed to isolate and quantify the different metal fractions (Lasat, 2000). For phytoextraction to occur, contaminants must be bioavailable i.e. the contaminants must be readily available to be absorbed by the roots. Bioavailability of heavy metals or contaminants depends on metal solubility in soil solution. Metals have high affinity for humic acids, organo clays, and oxides coated with organic matter which makes them less bio available (Berti *et al*., 1996).

The soluble metal forms generally exist as ions or complexed to other liquids. The solubility of metals in soil and groundwater is predominantly controlled by the pH, concentration of metal, cation exchange capacity, organic carbon concentration (Berti *et al.,* 1996), the oxidation state of the mineral components, and the redox potential of the system (Connell., 1984). Soil pH seems to have the greatest effect of any single factor on the solubility or retention of most metals in natural aerobic soils.

Under neutral environmental or basic conditions (i.e. pH of 7 and greater), most soil metals are strongly adsorbed in the clay fractions and hydrous oxides of Fe, or Mn present in soil minerals. However, elevated salt (sulphate or chloride) concentration creates increased competition between cations and anions metals for binding sites (Baste *et al*., 1993). Also competitive adsorption between various metals has been observed in experiments involving various solids with oxide surfaces.

Plants have sulfur rich proteins (metallothioneins) that are involved in the metabolism and binding of metals. Sulfur availability is important in metabolism, as well as changing the pH of the soil and plant metal availability (Marschner, 1995). Robinson *et al*., (1999) found that adding sulfur or acid mine tailings to small pots of serpentine soil or contaminated waste soil resulted in significant increase in Co and Ni uptake by *B. coddii* plants.

2.4. Plant response to heavy metals

There are generally three responses of plants to metals in the soil (Baker *et al*., 1989). These are summarized below.

2.4.1. Metal excluders

These are plants that prevent metal from entering their aerial parts thereby maintaining low and constant metal concentration over a broad range of metal concentration in soil. They mainly restrict metal uptake to their roots. These plants do this by altering their membrane permeability, changing metal binding capacity of cell walls or exude more chelating substances (Cunningham, 1995).

2.4.2. Metal indicators

This refers to plant species which take-up metal in their aerial tissues to similar concentrations as the metal in the soil. It also refers to metallophyte plants that, by their presence, abundance, lack of abundance, or chemical composition, demonstrate some distinctive aspect of the character or quality of a mineral-rich environment (Cunningham, 1995). They tolerate the existing concentration level of metals by producing intracellular metal binding compounds (chelators), or alter metal compartmentalisation patterns by storing metals in non-sensitive parts. Some species of plants can act as sentinels of particular metals in natural (unpolluted) serpentine environments. Some of the plant metal indictors are shown in Table 2.1.

Table 2.1: Examples of plant metal indicators

Indicator plants	**Metal**
Alyssum bertolanii and Alyssum murale	Ni
Becium homblei	Cu
Streptanthus batrachopus, and Streptanthus brachiatus	K and P
Barkheya. coddii (B. coddii)	Ni and Co

Sources: (Cunningham *et al*, 1995 and Kramer *et al*., 1996).)

2.4.3. Metal accumulators

These are plant species that can concentrate metal in their aerial parts to concentration exceeding that in the soil. Hyperaccumulators are plants that can absorb even higher concentration of contaminants either in their roots, shoots and/or leaves (Raskin *et al*., 1994). Baker et al., 1989 defined metal hyperaccumulators as plants that contain more than 0.1 % (1000 mg/g) of Cu, Cr, Pb, Co and Ni or 1 % (>10,000 mg/g) of Zn or Mn in the dry matter. For Cd and rare metals, it is > 0.01 % by dry weight (Baker *et al*., 1989).

Most of the researchers across the world have identified numerous types of hyperaccumulator species by collecting plants from the areas where soil contains greater than usual concentrations of metals, as in the case of geographically metal rich soil and very old polluted areas (contaminated sites). Since 1989 approximately 400 hyperaccumulator species from 22 families have been identified including, *B. coddii* in South Africa. The *Brassicaceae* (cabbage) family contains a large number of hyperaccumulating species with the widest range of metals; these include 87 species from 11 genera (Baker *et al*., 1989).

2.5. Toxicity of heavy metals in the environment

Different types of soil and plants accumulate metals differently depending on their properties. The organic and inorganic soil constituents may have a strong influence on progressive heavy metal accumulation (Alloway, 1995). The characterization of soil heavy metal contamination is commonly based on the determination of total concentrations. However, elements are present in soil in various forms and these can strongly affect their behaviour in terms of solubility and biological availability

Metals and metalloids, including Al, Mg, P, Mn, Pb, Cr, As, Zn, Cd, Ni, Pt, Co and Hg and others can cause significant damage to the environment and human health as a result of their mobilities and solubilities. For the purpose of this research only Ni and Co are discussed below.

2.5.1 Nickel (Ni)

Nickel is a hard, silvery-white metal, which has properties that make it very desirable for combining with other metals to form alloys. Some of the metals that nickel can be alloyed with are Fe, Cu, Cr, and Zn. Mostly Ni is used to make stainless steel. In the environment, Ni can combine with many other elements, including chlorine, sulfur and oxygen (www.atsdr.cdc.gov/toxprofiles). Ni combined with other elements occurs naturally in the earth's crust. It is found in all soil, and is also emitted from volcanoes. Ni is the 24th most abundant element in the environment. It is found primarily combined with oxygen or sulfur as oxides or sulphides. The earth's core contains large concentrations of Ni.

Many of these Ni compounds dissolve fairly easily in water and have a characteristic green colour. Ni and its compounds have no characteristic odour or taste. Ni compounds are used for Ni-plating, to colour ceramics, to make some batteries, and as catalysts that increase the rate of chemical reactions.

Ni is released into the atmosphere during Ni mining and by industries that make alloys or nickel compounds or industries that use Ni and its compounds. These industries may also discharge nickel in wastewater. Nickel is also released into the atmosphere by oil-burning power plants, coal-burning power plants, and trash incinerators. Much of South Africa's nickel comes from recycling nickel-containing alloys.

2.5.2. Cobalt (Co)

Cobalt is present in trace concentrations in soil and plants. Co has potential to occur naturally in many different chemical forms throughout the environment i.e. Co (ii) and Co (iii). This metal normally occurs in association with other metals such as copper, nickel, manganese and arsenic. Small concentrations are found in most rock, soil surface and underground water, plants and animals. The natural sources of Co in the environment are soil, dust, seawater, volcanic eruptions and forest fires. This metal can also be released into the environment through burning of coal, oil, and from car, truck and airplane exhausts and also from industrial processes that use the metal or its compound (www.atsdr.cdc.gov/toxprofiles).

The average mean concentration of Co in soil throughout the world is about 8 mg/kg. Toxic effects of Co on plants are unlikely to occur below soil Co concentrations of 40 mg/kg. However, the concentration of Co in soil is not the only factor determining the toxicity on the plant. The resistance of plants to Co varied in their tolerance and soil type and the soil chemistry. One of the most important soil properties that determine the availability of Co in the soil environment is the soil acidity and oxidation reduction potential (redox potential). Therefore, the more acidic and aerobic the soil the greater the potential for Co mobility and toxicity, at any concentration. In some cases soils with high Co concentrations also have high arsenic (As) and nickel concentrations, and As is generally more toxic to plants and humans than Co. The toxicity of Co is quite low compared to many other metals in soil (www.atsdr.cdc.gov/toxprofiles).

2.6. Major sources of heavy metals

There are various sources and pathways through which metals are introduced into the soil. This includes the natural sources (weathering) and anthropogenic sources such as mining, agriculture and industrial activities. It is important for the source of heavy metal and or chemical to be known when one is conducting remediation process such phytoremediation (Roberts, 1990).

2.6.1. Weathering process as natural sources of heavy metals

Naturally, metals are released to the environment during the time of heavy rainfall or weathering of rock forming minerals. Garburino *et al.,* (2000) indicated that the introduction of a metal in the soil can result from weathering and soil erosion, which also includes various movements of the earth and geochemical reaction. This process may lead to the leaching of some of the metals from the agricultural areas. Other trace metals could be deposited in the soil through blowing of wind and degassing from soil. Metals can also be emitted from volcanoes.

The surface of the continental crust is exposed to the atmosphere, making it vulnerable to physical and chemical processes. Physical weathering is a mechanical process, which fragments rock into smaller particles without substantial change in chemical and or metal composition. This normally happen when a confined pressure of the crust is removed by uplifting of the earth and erosion of the soil from the upland areas, as well as internal stress within the underlying rocks removed, allowing expansion and cracks to open which allows removal and absorption of metal to and from the soil to take place (Andrews *et al*., 1996).

All soil naturally contains levels of metals. The presence of metals in soil is therefore not indicative of contamination. The concentration of metals in uncontaminated soil is primarily related to the geology of the parent material from which the soil was formed. Depending on the local geology of the area, the concentration of metals in soil may exceed the threshold limit set by government in order to regulate the effectiveness of remediation methods introduced at given area. The range of concentration of many metal contaminants in the arable soil of some of the regions is broad e.g. (in mg/kg, dry wt): Cd; 1-143.0, Pb; 4 - 200, and Zn; 13 – 250. The geometric means of the concentration of the elements in the soil are as follow (in mg/kg, dry wt) Cd; 3.2, Pb 102.3 and Zn; 270.2, mostly from the natural process (Andrews *et al*., 1996).

The metals associated with the aqueous phase of soils are subject to movement with soil water, and may be transported through to ground water. The extent of the vertical contamination is intimately related to the soil solution and surface chemistry of the soil matrix. Change in environmental conditions over time, such as degradation of the organic waste from parent material, change in pH of the soil due to various processes as natural weathering may influence vertical contamination.

Soil is a dynamic system, subjected to short-term fluctuation such as variations in moisture status and pH and conductivity of that soil. And soil is also subjected to change due to the environmental management factors hence the change in soil property will eventually affect the form and the bioavailability of those metals in the soil (Alloway, 1995). The trace metals have long lasting effects on the soils because of the relatively strong adsorption.

Only 10% of the arable land has a natural concentration of the trace elements (metals) that has concentrations of up to 0.6, 25, 100 mg/kg for Cd, Pb, Zn, respectively. About 60% of the soils have concentration of higher than the acceptable limit (critical value) of 2 mg/kg for Cd and 200 mg/kg for Zn (Alloway, 1995).

2.6.2. Anthropogenic sources of heavy metals in the environment

Major pollution of the soil with heavy metals is due to anthropogenic sources. Examples of these anthropogenic sources are discussed below

- **Mining activities**

A major environmental problem relating to mining in many parts of the world is uncontrolled discharge of contaminated wastewater (or decant) from abandoned mines (Pulles *et al*., 2005). This is commonly known as acid mine drainage (AMD), there is wide acceptance that this phenomenon is responsible for significant impact on both environmental and socio-economy. Therefore, mining activity is the major source of most of the metals found in the soil currently. This is because methods used to extract mineral often release some metals in soluble form into the environment (Davies, 1990). Although mining industries are playing fundamental role in shaping the current societies in most countries, however, the impact on the environment are more severe. Heavy metals and toxic chemicals removed from the mining areas often affect the health of people living to the proximity of the mine. Ndinya, (1998) indicated that an increase in the environmental awareness has led to an increasing concern over the impacts of mining industries on the environment. The leaching of mine waste and mine drainage are the main culprit behind the problem of soil contamination by mining activities. The leachates can still come from old spoil heaps and tailing at derelict mines (Ashton *et al.*, 2001).

South Africa has made significant progress in shifting policy frameworks to address mine closure and mine water management. Most of the mining industries has changed its practices in order to conform to new legislations and regulations. However, vulnerabilities in the current system remain. AMD is characterized by low pH (high acidity), high salinity levels, elevated concentrations of sulphate, iron, aluminum and manganese. High acidity condition as a result of the AMD is likely to have raised levels of toxic heavy metals such as Cd, Co, Cu, Ni, Zn, and possibly even radionuclide's (Adler *et al*., 2007).

The acidic water dissolves salts and mobilizes metals from mine workings and residue deposits. Dark, reddish-brown water and pH values as low as 2.5 persist at the site (Adler *et al*., 2007). AMD is not only associated with surface and groundwater pollution, but is also responsible for the degradation of soil quality, aquatic habitats and for allowing heavy metals to seep into the environment leading to the contamination of the soil (Adler *et al*., 2007). An exacerbating characteristic of AMD is its persistence and the fact that it is extremely difficult to rectify. Therefore, problems associated with mining activities require joint collaboration among stakeholder to resolve.

The study by the U.S. Environmental Protection Agency conducted in 1987 concluded, "Problems related to mining waste may be rated second as compared to global warming and stratospheric ozone depletion in terms of ecological risk". Therefore, the release of mining waste to the environment can result in profound, generally irreversible destruction of ecosystems. In many cases, the polluted sites may never be fully restored due to lack of available remedial measures or technology (Adler *et al*., 2007).

In South Africa, for example, AMD generation occurred in 2002 on the West Rand in Gauteng Province because of the decanting of AMD from a closed mine. The decanting AMD flooded underground mine workings on the West Rand. "Decant has subsequently been manifested at various mine shafts and diffuse surface seeps in the area. Up until early-2005, and despite completion of storage and pumping facilities to contain and manage on average 15 Mega-Liters per day (ML/d) of decant, the AMD found its way into an adjoining natural water course and flowed northward through a game reserve, and towards the Cradle of Humankind World Heritage Site" (Oelofse *et al*., 2007).

The legacies of the historic mine sites in South Africa will remain problematic for many years to come due to the vast magnitude of the associated impacts. There are no easy solutions to the problem, but concerted efforts could lead to vast improvements and reductions in the environmental impacts associated with the historic mine sites. The primary management issues therefore include long term decant risk, acid mine drainage, water pumping and treatment and allocation of responsibility especially in light of the interconnectedness of the mines (Pulles *et al*., 2005).

- **Industrial activities**

The industrial sources of metals include the use of items containing metals such as paints, batteries and tanning. Other sources are from metal processing industries that release metal through furnaces or effluents. The effluents from industries are released to the environment often without any removal measures. Ombrotrophic peat's in northern England and Scotland for example, close to industrial areas, have substantial contents of toxic metals (Cd, Cu, Zn, Ni, Co and Pb) as well as sulfur. All these metals were driven from atmospheric deposition (Anderson *et al*., 1997).The peat's sulfur may be converted to sulfuric acid under drought conditions, due to the entry of oxygen into the peat. The consequent lowering of soil solution pH is predicted to cause the release of metals held on legend sites of the peat organic matter (Nindi., 2005). A number of investigations have been carried out on assessment of metals in soil and movement of metal in industrially contaminated land in former industrial sites especially in Europe (Anderson *et al*., 1997).

- **Agricultural activities**

Agricultural activities have been found as the main source of metals in some areas. An example is soil watered at a number of volcanic agricultural sites from the Solofrana River valley (Southwest of Italy), irrigated for a long time with contaminated river water and subjected to overflowing. These was found to contain high amount of heavy metals i.e. Fe, Cr, Cu, Mn, Ni, Pb, and Zn (Imperato *et al*., 2003)

Micro-morphological properties, the effect of main soil characteristics on the distribution of metals in the various forms, and metal uptake or effects on vegetables were also investigated (Imperato *et al*., 2003).

- **Fossil fuel combustion**

This is the burning of coal, oil (including gasoline), or natural gas. This burning, usually is for the generation of energy. Fossil fuel combustion results in the release of carbon dioxide, as well as combustion of by products that can include unburned hydrocarbons, methane, and carbon monoxide. Carbon monoxide, methane, and many of the unburned hydrocarbons slowly oxidize into carbon dioxide in the atmosphere. Common sources of fossil fuel combustion include cars and electric utilities. A wide range of metals is found in fossil fuels. These can be emitted into the environment as particles through combustion ash, which may be transported in air and contaminate soil (Alloway *et al*., 1997). In general, fossil fuel combustion results in a wider range of heavy metals which include: Pb, Cd, Cr, Zn, Ag, Cu, and Ni, etc that end in the environment.

2.7. Environmental remediation technologies

Human evolution has led to immense scientific and technological progress even in the field of environmental protection and conservation. Technological ingenuity has enhanced the potential for improving industrial development and rapid progress is being made not only in the field of electronics but also in technologies to be used to remediate polluted environment. These technologies are discussed below.

2.7.1. Isolation and containment

Contaminants can be isolated and contained, to prevent further movement, to reduce the permeability of the waste to less than 1×10^{-7} m/s (as required by the U.S.EPA) and to increase the strength or bearing capacity of the waste (U.S.EPA, 1983). In this case physical barriers made of steel, cement, bentonite and grout walls can be used for capping, vertical and horizontal containment. Capping is a site-specific water infiltration which will involve the use of synthetic membranes.

The vertical barriers reduce the movement of contaminated ground water or uncontaminated ground water through a contaminated area. To prevent the transportation of contaminants past the barrier, the barrier should extend to the bedrock layer of low permeability. If this cannot be done, a groundwater extraction system would be required to avoid the passage of contaminants below the barrier (U.S.EPA, 1983). In this case the slurry walls, grout or geo-membrane curtains, and sheet pile walls are often employed.

Slurry walls are the least expensive and are thus the most common. Horizontal barriers within the soils (cut off trenches or wells) are under development and have not been demonstrated as effective but are potentially useful in restricting downward movement of metal contaminants by acting as underlying liners without the requirement for excavation.

2.7.2. In situ treatment (soil flushing)

Extracting solutions are infiltrated into the soil using surface flooding, sprinklers, leach fields, horizontal drains, basin infiltration systems, surface trenches, or vertical drains. Water with or without additives is employed to solubilise contaminants. The efficiency of the extraction depends on the hydraulic conductivity of the soil. High permeability gives better results (greater than 1x 10^{-3} cm/s). Other factors that influence the method include the solubility of pollutants in water. Prior mechanical mixing of the soil can disturb the infiltration of the extract. Understanding the chemistry of the binding of the contaminant and the hydrogeology of the site are very important (U.S.EPA, 1983).

Since water solubility is the controlling removing mechanism, additives are used to enhance efficiencies. In an analysis of a test site, it was determined that 400 years would be required to treat a site with water alone compared to 4 years with chemical enhanced flushing. This technique is still quite limited, particularly where metal removal is a major concern (U.S.EPA, 1983). Chemical enhanced flushing has potential for a wide variety of metals, and little handling of soil is required.

To enhance the extraction, chemicals such as organic and inorganic acids, sodium hydroxide which can dissolve organic soil matter, can be added. The soil pH, soil type, cations exchange capacity (CEC), particle size, permeability and types of contaminants all have the ability to influence the removal efficiencies. High clay and organic matter contents are particularly detrimental. Once the water is pumped from the soil, it must be extracted and then treated to remove metals in the wastewater treatment facilities or reused in the flushing processes.

Based on this idea, full-scale treatment was performed at a chromium plant for removal of Cr (vi) (U.S.EPA, 1997). Water was used as the flushing solution with three methods of infiltration; that is, infiltration basins, injection wells and an infiltration trench. Due to this method, the concentration of chromium was reduced from 2000 mg/l to 18 mg/l. At another site, about 30 000 m^3 volume of soil was successfully treated in the Netherlands to decrease the cadmium concentration in 90% of the soil from 10 mg/kg to less than 1 mg/kg with dilute hydrochloric acid (pH 3). More demonstrations of the technique are still needed, in addition to developing more understanding into the mechanisms for solution, to be used for metal recovery (U.S.EPA, 1997).

2.7.3. Soil washing (chemical leaching)

In this method, chemicals are used to remove target pollutants from the soil. This method can be performed in a reactor or as heap leaching. The chemicals to be used in this method are as follow: (i) inorganic acids such as sulfuric and hydrochloric acids with pH less than 2, (ii) organic acids including acetic and citric acids (pH not less than 4), (iii) chelating agents such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetate (NTA), and various combination of the above (ITRC, 2009). Once the soil has been treated it is then returned back to the original site. In other words, the cleaned soil is returned back to the original site where it can be used for the rehabilitation of the damaged site.

Soil with less than 10 – 20 % clay and organic concentration (i.e. sandy soils) are most effectively remediated with these extractants. After treatment, both the heavy metals and organic chemicals are removed from the soil. In general, soils with low concentrations of cyanide, fluoride and sulphide, CEC of 50-100 mg/kg and particle sizes of 0.25-2 mm, with contaminant solubility in water of greater than 1000 mg/l, can be effectively cleaned by soil washing.

The use of biodegradable bio-surfactants to remove heavy metals from oil contaminated soils was recently demonstrated in a laboratory using scale batch washes with surfactin, rhamnolipid and sophorolipid (Mulligan *et al*., 2001). The first two agents are produced by bacteria while the last is produced by yeast. The results indicate the feasibility of removing metals with the anionic bio surfactants. These bio-surfactants were also able to remove metals from the sediments. Since these agents are biodegradable, they can enhance hydrocarbon removal and can potentially be produced in situ, since they have a great potential for soil washing and soil flushing applications.

2.7.4. Mechanical separation

This is a physical separation involving size selection of different particles. The main aim of size selection processes is to remove the larger, cleaner particles from the smaller, more polluted ones. The characterization in terms of particle size and contaminant level in each fraction is the most important parameter that determines the suitability of this process (Mulligan *et al*., 2001).

The technique involves hydro-cyclones which separate particles greater than 10–20 mm from small particles by centrifugal force and fluidized bed separation which removes smaller particles at the top in the counter current overflow in a vertical column, by gravimetric settling and flotation which is based on the different surface characteristics of the contaminated particles. This technique has been used in mineral ore processing with the addition of special chemicals such as frothers or flotation agents and aeration causing contaminated particles to float. Magnetic separation, which is based on the magnetic properties of metals, can also be used to separate these ferrous materials from the contaminated soils (Mulligan *et al*., 2001).

The application of physical separation techniques is becoming more common and will continue to increase either as standalone techniques or in combination with other techniques. These can be used prior to another technique thus reducing the volume of soil to be treated.

2.7.5. Pyrometallurgical separation

Pyrometallurgical separation process involves the use of high temperature furnaces to volatilize metals in contaminated soil. In this case about 200–700^{o} C is used to evaporate the contaminants. After volatilization, metals are then recovered or immobilized. This method is mostly applicable to Hg since it is easily converted to its metallic form at high temperatures. Other metals such as Pb, As, Cd and Cr may require pre-treatment with reducing or fluxing agents to assist melting and provide a uniform feed. This type of treatment is usually performed off site due to a lack of mobile units and is mostly applicable to highly contaminated soils (5-20 %) where metal recovery is profitable. The soil must be concentrated with metals of interest by physical or soil washing processes prior to pyrometallurgical processes. Mercury, however, can easily be recovered at lower concentrations (Mulligan *et al*., 2001). Other valuable metals such as Au and Pt can also be recovered from low soil concentrations. Rotary kilns, arc furnaces or rotary health furnaces are the main type of equipment used in this process. Often a sludge with a high concentration of heavy metals is produced which can then be recovered (U.S.EPA. 1997).

This type of technology also includes the use of fluidized bed thermal desorption and high –vacuum retort. In this type of technology it has been found that about 228 mg/kg of mercury were obtained after treatment of soil contaminated with 1300 mg/kg to 34, 000 mg/kg of mercury at a U.S.EPA site. About 20 000 to 100 000 tons of contaminated soil was remediated, however not all contaminates are removed by this method, some of the metals remains in the residuals to be disposed of. Therefore pre-treatment could be important since it will reduce the volume of soil to be treated and further increase the recovery of metals to be extracted from this contaminated soil.

2.7.6. Chemical treatment

Chemical treatment by reductive as well as oxidative mechanisms may be used to detoxify or decrease the mobility of metal contaminants (Mulligan et al, 2001). This method is commonly used for wastewater treatment. Oxidization reactions which detoxify precipitate or solubilize metals, involve addition of potassium permanganate and hydrogen peroxide. As metals cannot be destroyed, chemical treatment helps to change the oxidation state to that of less toxic metals. Neutralization reactions are performed to adjust the pH of acidic or basic soils. Reduction reactions are induced through the addition of alkali metals such as sodium, sulfur dioxide, sulphite salts and ferrous sulphate.

Sometimes chemical treatment is used to pre-treat the soil for solidification or other treatments. These chemical treatments can be performed in situ by injection into the groundwater but have the potential to introduce further contamination (Mulligan *et al*., 2001). A good example is the use of As. In this case a co-precipitation of high concentration of As (v) and Fe (iii) forms $FeAsO_4$ while low concentrations of As (v) co-precipitate with $FeHO_2$ with high concentration of Fe(iii) to form arsenic ferrihydride, a product that is resistant to acid and neutral leaching (Mulligan *et al*., 2001). However other than the As, Hg, Pb, Se. Si can also be used in the reduction process.

2.7.7. Electrokinetics

This technique can also be referred to as electrokinetic soil processing, electro migration, electrochemical decontamination, or electro reclamation. It can be used to extract radio nuclides, metals and other types of organic wastes from saturated and unsaturated soils and sediments. It is also regarded as an in situ soil processing technology.

The principle of electrokinetic remediation relies upon application of a low intensity direct current through the soil between two or more electrodes. Most soils contain water in the pores between the soil particles and have an inherent electrical conductivity that results from salts present in the soils. Based on this type of technology, the current mobilizes charged particles and ions in the soils by the following processes: (i) Electro migration which involve the transportation of charged chemical species under an electric gradient, (ii) Electro-osmosis which involve the transport of pore fluid under an electric gradient, (iii) Electrolysis which is the chemical reactions occurring at the electrode under influence of electric fields. This type of technology can be efficient in extracting contaminants from the fine grained and high permeability soils (Mulligan *et al*., 2001).

There are a number of factors that determine the direction and the extent of the migration of the contaminants. Such factors include the type and concentration of the contaminants, the type and structure of the soil and the interfacial chemistry of the system. Unlike soil washing, this process is effective with clay soils of low permeability. Water and other suitable salt solutions may be added to the system to enhance the mobility of the contaminants and increase the effectiveness of the technology.

The contaminants at the electrodes may be removed by any several methods including electroplating at the electrode, precipitation or co- precipitation at the electrode, pumping of water near the electrode or complexing with ion exchange resins. The electrochemistry associated with this process involves an acid front that is generated at the anode if water is the primary pore fluid present. Demonstrations of this technology have been performed, such as using the Lasagne$^{\text{TM}}$ technology, but are limited (Mulligan *et al*., 2001). In Europe, this technology is used for remediation of pollutants such Cu, Zn, Pb, As, Cd, Cr, and Ni (Mulligan *et al*., 2001).

2.7.8. Bioremediation

These techniques include bioleaching and oxidation/reduction reactions. Bioleaching involves the use of *thiobacillus* bacteria under aerobic and acidic conditions (pH 4) at temperatures between 15 and 55°C, depending on the strain. Leaching can be performed by direct means, oxidation of metal sulphides to produce sulfuric acid, which then desorbs metals on the soil by substitution of protons. Indirect leaching involves conversion of Fe^{2+} to Fe^{3+} which in turn oxidizes sulfur minerals to Fe^{2+} producing acidity (Mulligan *et al*., 2001).

Several options are available for bioleaching including heap leaching, bio slurry reactors and in situ processes. Anoxic sediments are more suitable for treatment since the bacteria can solubilise the metal which has already been exposed to oxidising conditions. For both heap leaching and reactors, the bacteria and sulfur compounds are added. In the reactor, mixing is used and pH can be controlled more easily. Leaching is recycled during heap leaching (Mulligan *et al*., 2001).

Another leaching technique that has potential for remediation of metal-contaminated soil is through the production of citric and gluconic acids by the fungus *aspergillus niger* which can produce citric and gluconic acid. These can act as acids (pH 3.5) and chelating agents for the removal of metals such as copper from oxide mining resides (Mulligan *et al*., 2001).

Biosorption is a biological treatment method which involves the absorption of metals into biomass such as algal or bacterial cells that can be dead or alive. Micro-organisms are also known to oxidize and reduce metal contaminants. Hg and Cd can be oxidized while As and Fe can be reduced by micro-organisms. Cr (vi) can be reduced to Cr (iii) that is less mobile and less toxic. Bacteria such as *bacillus subtilis* and sulphate reducing bacteria in the presence of sulfur can perform this reaction (Mulligan *et al*., 2001).

2.7.9. Phytoremediation

The word “phytoremediation” is from the Greek prefix: phyto- meaning “plant” and the Latin root word: remidium- meaning “to correct or remove an evil”. In soil, the “evil” could be anthropogenic (man-made) contaminants such as organic pollutants and heavy metals.

Phytoremediation takes the advantage of natural processes of plants. These processes include water and chemical uptake, metabolism within the plants, exudates released into the soil that leads to contaminated loss from the soil and the physical and biochemical impacts of the plant roots. The growth of the plants depends on photosynthesis, in which water and carbon dioxide are converted into carbohydrates and oxygen, using heat energy from the sunlight. Roots are effective on extracting water from the soil and then transported through the xylem. The transportation occurs primarily at the stomata, with additional transportation at the lenticels. Carbon dioxide uptake from the atmosphere occurs through the stomata, along with release of oxygen. Breakdown of the carbohydrates produced during photosynthesis are necessary for the active transport of nutrients by roots. Diffusion and advection of oxygen into the soil are necessary for the survival of the plants and the high and saturated soil water content will greatly slow oxygen transport. Few woody plants species can transport oxygen to the root zone except for flood tolerance trees such as poplar, due to coping mechanisms other than transportation of oxygen (ITRC, 2009).

Plants require macronutrients (N, P, Ca, K, Mg, and S) and micronutrients (B, Ci, Cu, Fe, Mn, Zn and possibly Co, Ni, Se, Si, and V). Lack of chlorophyll due to stresses on the plant, such as lack of nutrients, can results in chlorosis (the yellowing of normally green plants leaves). Nutrients uptake pathways can take up contaminants that are similar in chemical form or behaviour to the nutrients. Cd can be subjected to plant uptake due to its similarity to plant nutrients Ca and Fe although poplar leaves in the field study did not accumulate a significant concentration of cadmium (Mulligan *et al*., 2001).

As (as arsenate) might be taken up by plants due to the similarity to plant nutrients phosphate. For the uptake into plants the chemical or contaminates should be in soluble form to be absorbed from the soil solution to the outer tissue of the roots. The contaminant in the water can move through the epidermis to and through the casparin strip and then through the endodermis, where they can be adsorbed, bound or metabolized. Chemicals passing through the endodermis and reaching the xylem are then transported in the transpiration stream or sap.

The compound might react with the partition into plant tissue, be metabolized or be released to the atmosphere through stomata pores (Paterson *et al*, 1994). The uptake of organic chemicals can depend on the type of plants and the age of the contaminants and other physical and chemical properties of the soil.

The breaking up of soil aggregate is a physical effect of root tips pushing through the soil as the root tip grows. This helps to reach the pollutants in the soil. Roots can form large openings in the soil, especially as roots decay, which can contribute to water, gas and contaminant transport through the soil and change the aeration and water status of the soil. The increased workability of soil due to the incorporation of organic matter by plants might make soil conditions more amenable to various types of soil treatment. Plants material and roots can have a chemical and biological impact on the soil. Just like in other environmental remediation technologies, a phytoremediation technique has advantages and disadvantages; these are highlighted below (ITRC, 2009).

Advantages of phytoremediation

- Early cost of phytoremediation indicated substantial savings over cost of traditional technologies. As actual cost data are developed during pilot-scale studies, it reflects that phytoremediation can be a lower-cost technology.
- Phytoremediation has been perceived to be a more environmental – friendly "green" and low – tech alternative to more active and intrusive methods. As such

the need for phytoremediation is of greater demand by the public and is widely accepted by the communities.

- Phytoremediation can be applied in situ to remediate shallow soil and ground water, and it can also be used in surface water bodies.
- Phytoremediation does not have destructive impact on soil fertility and structure that some more vigorous conventional technologies may have on the environment such as acid extraction and soil washing. Since the method involves the use of plants this tends to improve the overall condition of the soil, regardless of the degree of contaminants reduced.
- Vegetation also provides vegetation cover which can eventually reduce air pollution through fugitive dust emission and prevent erosion.

Disadvantages of phytoremediation

- The depth limitation due to shallow distribution of plant roots. To avoid this disadvantage the selection of deep rooted plants is essential for pollutants that are far below the surface.
- This process requires a longer period of time. This is because the technology is dependent on plant growth rates for establishment of an extensive roots system or significance above ground biomass. Based on the biomass estimate, a heavy metal contaminated site would require 13 to 14 years to be remediated (Salt *et al*., 1995).
- Plant matter that is contaminated will require either proper disposal or an analysis of risk pathways. Harvesting and proper disposal is required for plant biomass that accumulates heavy metals or radionuclide in phytoextraction and rhizofiltration. It may be necessary for the other forms of phytoremediation where contaminants accumulate within the plants. The biomass may be subject to regulatory requirements for handling and disposal.
- During winter season phytoremediation tends to loss its effectiveness (when plant growth slows or stops). Therefore a back–up remedial technology might be required.

- As with all remedial technologies, in some cases, there may be uncertainty about attainment of remedial goals, such as meeting concentration goals in soil or ground water, or in achieving hydraulic containment. The bench scale or pilot–scale tests to assess attainment might not be possible in some cases if rapid remediation is desired, due to the potential relatively long periods of time for some forms of phytoremediation.
- High initial contaminant concentrations can be phytotoxic and prevent plant growth. Preliminary phytotoxicity studies are likely to be necessary to screen candidate plants.
- Since phytoremediation requires planting of plants, this requires great care due to stresses of climate and pest; under the adverse conditions of contaminated soil or ground water, successful cultivation can be much more difficult.
- Amendments and cultivation practices might have unintended consequences on contaminant mobility. For example, application of many fertilizers can result in the lowering of soil pH, which might result in increased metal mobility and leaching of metals to groundwater. This will require the person who needs to conduct phytoremediation to understand the soil amendments process.

2.7.9.1 Types of phytoremediation

There are a number of different types of phytoremediation methods which need to be discussed. Defining these types of phytoremediation is crucial since it can help in understanding the phytoremediation process. Different phytoremediation processes may be applied to a specific type of contaminant within the given environmental media, and this may require different types of plants such as grass, herbs, shrubs and trees. Different plants absorb and tolerate different concentrations of metals from the soils but sometimes plants can absorb common metals depending on the contaminated media. There are six major processes of phytoremediation, which are discussed below.

- **Phytoextraction**

Phytoextraction is a green technology which uses plants and their associated micro-organisms to diminish the concentration of inorganic and organic chemical elements in contaminated soils to such a level that the treated soil can be re-used for agriculture, horticulture, forestry or amenity (Figure 2.2). This is done by extracting the contaminants from the soil into its biomass (ITRC, 2009). The plant has to be cropped each year and the biomass has to be removed from the area. Phytoextraction therefore takes up contaminants by roots with subsequent accumulation in the above ground portion of a plant, generally to be followed by harvest and ultimate disposal of the plants biomass. This is regarded as a contaminants removal process from both soil and water. This process applies to metals such as Ag, Cd, Co, Hg, Mn, Ni, Pb, and Zn), of metalloids (e.g. As, and Se), radionuclides (e.g. Sr, Cs, U) and non metals (e.g. Persistent organic pollutants)

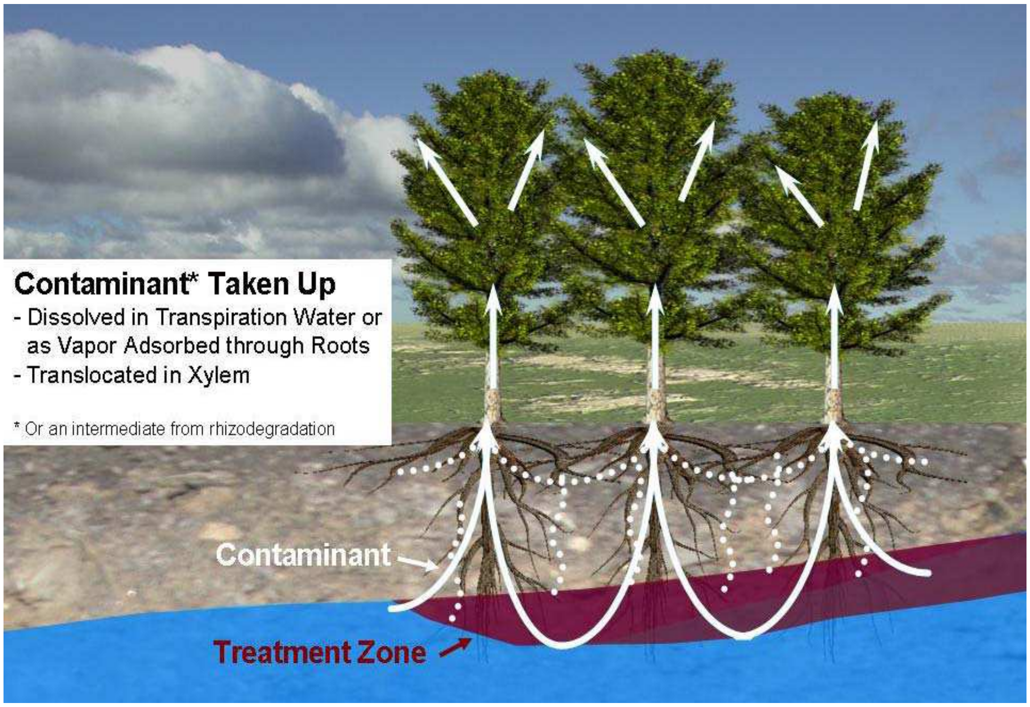


Figure 2.2: Phytoextraction mechanism (ITRC, 2009)

The success of phytoextraction depends on two components, the contaminated soil and the plant species (ITRC, 2009). The decisive soil factors are the degree of contamination, the metal availability for uptake into roots (bioavailability), the nutrient status and the water-holding capacity of the soil (Ernst, 1972). The plant characteristics are the ability to explore the soil by the roots and their associated micro-organisms, the genetically steered uptake efficiency by the roots via general and/or metal-specific transporters, the translocation of the metals from the root to the shoot, the accumulation in shoots without hampering the basic plant metabolism, the growth form, and the metal-resistance of the plant species or ecotype (Ernst, 1972; Lasat, 2000).

Phytoextraction is generally less considered for organic or nutrient contaminants taken up by plants as these can be metabolized, changed, or volatilized by the plants, thus preventing accumulation. However, some studies have shown accumulation of unaltered organic contaminants within the above ground portion of the plants. The target medium is generally soil, although contaminants in sediments or sludge can also undergo phytoextraction. Phytoextraction has also been referred to as phytomining or biomining. A narrower definition of the so called phytomining is the use of plants to obtain an economic return from metals extracted by a plant, whether from contaminated soils or from soil having naturally high concentrations of metals. Phytoextraction occurs in the root zone of plants. The root zone may typically be relatively shallow, with the bulk of roots at shallower rather than deeper depths. This can be a limitation of method. In one of the green house studies, phytoextraction coefficients (the ratio of the different metals taken up by *Indian mustard* (*Brassica juncea*) were 58 for Cr (vi), 52 for Cd (ii) and 31 for Ni (ii), 1.7 for Pb (ii), 17 for Zn (ii), 7 for Cu (ii) and 0.1 for Cr (iii). Some of these coefficients are high, thus indicate greater uptake of metals by plant (Mulligan *et al*., 2001).

The effectiveness of the method can be limited by the adsorption of metals to soil particles and low solubility of the metals. However, the metals can be solubilised by addition of chelating agents to allow the uptake of metals by the plants. The phytoextraction of organic pollutants is not straight forward as for the metals since metabolism of the organic pollutants is likely to occur as already mentioned. This technology has already been applied earlier in nature conservation for diminishing the concentration of nitrogen and phosphorus in eutrophicated ecosystems (Ernst, 1972) and more recently for desulfurization of soils (Ernst, 1972).

- **Phytostabilization**

Phytostabilization is the use of vegetation to stabilize soil contaminants *in situ*. This can happen through absorption and accumulation by roots; adsorption onto roots; precipitation, complexation, or metal valence reduction in soil through the process of humification. Further, vegetation can reduce wind and water erosion of the soil, thus preventing dispersal of the contaminant in runoff of fugitive dust emissions, and may reduce leachate generation of the contaminants in the soil (U.S. EPA, 1997).

Phytostabilization is also known as in-place inactivation or phytoimmobilization. Phytostabilization research to date has generally focused on metal contamination, with lead, chromium, and mercury being identified as top potential candidates for phytostabilization (U.S. EPA, 1997). However, organic pollutants may have potential for phytostabilization too, since some organic contaminants or metabolic by-products of these contaminants can be attached to or incorporated into plant components such as lignin (this process is called Phytoliginification). The only difference between the two is that phytostabilization of the metals occurs in the soil, whereas phytostabilization of the organic contaminants occurs above ground (Salt *et al*., 1995).

The metals within the root zone can be stabilized by changing from a soluble to an insoluble oxidation state, through root-mediated precipitation. For example, roots can mediate the precipitation of lead as insoluble lead phosphate (Salt *et al*., 1995). The stabilization of metals also includes the non- biological process of surface adsorption, due to chelation, ion exchange and specific adsorption (U.S. EPA, 1997). Lead is generally toxic to plants and for that reason it does not accumulate in plants under natural conditions. The effective phytostabilization requires understanding of the chemistry of the root zone, root exudates, contaminants, and fertilizers or soil amendment.

- **Rhizofilitration**

Rhizofilitration is similar in concept to phytoextraction but is concerned with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either adsorbed onto the root surface from the water or are absorbed by the plant roots from the soil - water. Plants used for rhizofilitration are not planted directly in situ but are acclimatised to the pollutant first with the idea of familiarising them with the conditions of the metals.

Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place the water supply is substituted for a polluted water supply to acclimatise the plant. After the plants become acclimatised, they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated they are harvested and disposed of safely.

Repeated treatments of the site can reduce pollution to suitable levels as was demonstrated in Chernobyl, where sunflowers were grown in radioactively contaminated pools (U.S. EPA, 1997). This method is mostly used for the decontamination of the heavy metals from the water.

- **Phytodegradation (Phytotransformation)**

Phytodegradation is defined as the degradation or breakdown of organic contaminants by internal and external metabolic processes driven by the plant. Contaminants can be absorbed by the plant and then broken down by plant enzymes. These smaller pollutant molecules may then be used as metabolites by the plant as it grows, thus becoming incorporated into the plant tissues. In other words, those pollutants may then be used as food as the plant grows. Plant enzymes are able to breakdown ammunition wastes, chlorinated solvents such as TCE (Trichloroethane), and others organic herbicides to make food for survival (http://arabidopsis.info/students/dom/mainpage.html).

- **Rhizodegradation**

Rhizodegradation (also called enhanced rhizosphere biodegradation, phytostimulation, and plant assisted bioremediation) is the breakdown of organic contaminants in the soil by soil dwelling microbes which is enhanced by the rhizosphere's presence. Certain soil dwelling microbes digest organic pollutants such as fuels and solvents, producing harmless products through a process known as bioremediation. Plant root exudates such as sugars, alcohols, and organic acids act as carbohydrate sources for the soil microflora and enhance microbial growth and activity. Some of these compounds may also act as chemotactic signals for certain microbes. The plant roots also loosen the soil and transport water to the rhizosphere thus additionally enhancing microbial activity. (http://arabidopsis.info/students/dom/mainpage.html)

- **Phytovolatilization**

Phytovolatilization is the process where plants uptake contaminants which are water soluble and release them into the atmosphere as they transpire the water. The contaminant may become modified along the way, as the water travels along the plant's vascular system from the roots to the leaves, where the contaminants evaporate or volatilize into the air surrounding the plant. There are varying degrees of success with plants as phytovolatilizers with one study showing poplar trees to volatilize up to 90 % of the trichloroethylene (TCE) they absorb (ITRC, 2009).

2.7.9.2. Status of phytoremediation in South Africa

A lot of progress has been made in South Africa on phytoremediation of contaminated soil, particularly with solving the problem of mining waste. The two leading companies in this field are Anglo Platinum and AngloGold Ashanti Ltd. The paper by Slatter (1998) showed that *B. coddii* was chosen as the tool in nickel phytoremediation at Rustenburg Base Metal Refineries as such it was found to accumulate up to 2.5% nickel in the dry biomass. This plant grow rapidly and has a large above-ground biomass with a well developed root system, and it is perennial and so does not need to be planted each season.

Earlier work as cited by Slatter (1998) showed that the nickel concentrations in the roots were comparatively low (up to 0.3% nickel in the dry material) and thus, for ease of harvesting and to ensure the continued vegetative growth of the plant on the planted sites. In addition *B. coddii* was found by Slatter (1998) to accumulate low concentrations (0.006 – 0.3 %) of precious metals, including platinum, palladium and rhodium, within its above ground biomass, making it attractive for the remediation of certain soils that contain low concentrations of these metals.

2.7.9.3. The role of fungi in the phytoremediation process

Fungus plays a major role in phytoremediation technology. Their morphological, physiological and reproductive strategies make them especially suited for their terrestrial habitats. Fungi has the ability of helping the plants to absorb more contaminates from the contaminated soils or sites and allows them to accumulate these in the leaves making the phytoremediation more productive.

Fungi have been shown to transform a wide variety of persistence organic pollutants (POPs) from the contaminated site and make them suitable for use in phytoremediation (Gadd, 2001). Though the use of fungus on phytoremediation has proved to be more promising the mechanisms involved in pollutant transformation by fungi are complex, which might make the process difficult to undertake. Given that soil is not the natural habitat for many of the fungi useful for phytoremediation process, this might lead to the soil being altered, to encourage the growth of the fungus. The effect of soil condition should not be overlooked and could be responsible for the variations in phytoremediation success. This also affects fungal growth which has to compete with indigenous soil micro organisms.

2.7.9.4. Example of plants to be used for phytoremediation process

Data gained from literature clearly indicate that there are numerous plants that have been reported to be metal hyperaccumulators and tolerant to heavy metals. These would be useful for phytoremediation of metal contaminated ecosystems and for further experimentation. Below is an example of the plants that can be used on phytoremediation**.**

Table 2.2: Examples of plants that have been used in phytoremediation of metal contaminated soil

Plants species	**Element**	**Concentration (µg g^{-1})**
Thlaspi caerulescens	Cadmium	3000
Haumaniastrum robertii	Cobalt	10 200
Haumaniastrum katanganse	Copper	8356
Macadamia neurophylla	Manganese	55000
Alyssum bertolonii	Nickel	13400
Astragalus pattersoni	Selenium	6000
Iberis intermedia	Thallium	3070
Atriplex confertifolia	Uranium	100
Thlaspi calaminare	Zinc	10 000
B.coddii	Nickel	17 000

(Howes, 1991)

2.7.9.5. General limitation of environmental remediation technologies

It is currently believed that science and technology can provide effective solutions to most pollution problems, if not all, in today's industrial societies. The validity of this optimistic assumption is highly questionable for at least three reasons: Firstly, current mechanistic, reductionist science is inherently incapable of providing the complete and valid information or accurate information which is required to successfully address environmental problems (Gadd, 2001). Secondly, both the conservation of mass principle and the second law of thermodynamics dictate that most remediation technologies, although successful in solving some pollution problems, tend to cause unavoidable negative environmental impacts elsewhere or in future (Gadd, 2001).

Thirdly, it is intrinsically impossible to design remediation technology that has no negative environmental impacts because of the use of land (biosphere) (ITRC, 2009). This means that although this work focuses on phytoremediation as a possible solution to soil contaminants, the technique cannot solve all pollution problems. Therefore phytoremediation should be seen as a complimentary offer to the existing techniques.

2.8. Vegetation indices (VIs) used for the determination of any plant stress

Vegetation indices (VIs) are derived from the combination of surface reflectance at two or more wavelengths which can reveal particular property of vegetation. They are derived from the reflectance properties of plant foliage. The vegetation index which is much more widely used and solves the problem of the calculated index possibly diverging to infinity is the Normalised Difference Vegetation Index (NDVI). The NDVI was first described by Rouse *et al.,* (1973), cited Tucker, 1979). The combination of its normalised difference formulation and use of the highest absorption and reflectance regions of chlorophyll make it useful over a wide range of conditions. The NDVI was later modified by the Red Edge Normalised Difference Vegetation Index ($NDVI_{705}$), which is intended for use with very high spectral resolution reflectance data, such as from hyperspectral sensors. $NDVI_{705}$ differs to the NDVI because it utilises the bands along the red edge, instead of the main absorption and reflectance peaks of chlorophyll. The $NDVI_{705}$ is mostly used for vegetation stress detection (Sims *et al*., 2002).

The $mNDVI_{705}$ is a modification of the Red Edge NDVI. This VIs Differs from the Red Edge NDVI by incorporating a correction for leaf specular reflection. $mNDVI_{705}$ capitalizes on the sensitivity of the vegetation red edge to small changes in canopy foliage content, gap fraction, and senescence. Just like the $NDVI_{705}$, its' applications include forest monitoring and detection of vegetation stress. The $mNDVI_{705}$ is defined by the following equation: $mNDVI_{705} = P_{750} – P_{705} / P_{750} – 2p_{445}$. One advantage of this index is that all possible values will lie between +1 and –1 (Sims *et al*., 2002).

The $mNDVI_{705}$ value ranges from -1 to 1 with the common range for green vegetation of 0.2 to 0.7. The increased positive $mNDVI_{705}$ values, shows an increasing shades of green on the images, which indicate an increase in the amounts of chlorophyll in green plant

(Karen *et al*, 2007). The $mNDVI_{705}$ values near zero and decreasing negative values indicate stressed vegetation or non-vegetated features such as barren surfaces (rock and soil) and water, snow, ice, and clouds. The unstressed vegetation absorbs most of the visible light that intercepts, and reflects larger portions of the near-infrared light, whereas unhealthy or sparse vegetation reflects more visible light and less near-infrared light (www. earthobservatory.nasa.gov/features/measuring vegetation).

Apart from the $mNDVI_{705}$ Plant Senescence Reflectance Index (PSRI) can also be to determine if the plants are stressed. The PSRI uses the range from -1 to 1 with the common range for green vegetation -0.1 to 2.0 in order to determine if the plant is healthy or not (Sims *et al*., 2002). The PSIR mostly is designed to maximise the sensitivity of the index to the ratio of bulk carotenoids to chlorophyll. Therefore the increase in the PSIR indicates increased canopy stress (carotenoid pigment), and the onset of the canopy senescence. Just like the mNDVI 705, the PSIR also used in vegetation health monitoring, plant physiological stress detection, crop production and yield analysis. The PSIR is defined by the following equation: $PSIR = P_{680} - P_{500}/P_{750}$. Both the PSIR and $NDVI_{705}$ have been used in this study to determine if *B. coddii* on both the substrates is stressed.

2.9. Methodology for extracting metals in soil and plants

2.9.1. Microwave digestion

The closed vessel microwave system is often used for soil digestion in order to extract metals from the soil (maximum pressure 1450 psi, maximum temperature 300°C). The reason why a closed vessel microwave system is often used is to avoid loss of sample or solution which might occur during the digestion process. Based on this, a soil sample of about 0.25 g tends to be placed in a Teflon vessel (100 ml capacity).

2.9.2. Acid digestion/ Aqua regia

Aqua regia is the digestion step used to determine the total concentration of metals available in the soil sample. Several mineral acids (HCI, HNO_3, $HCIO_4$, H_2SO_4) and their mixtures have been used for the dissolution and digestion of metals from soils, but they do not dissolve silicates or silica completely but are vigorous enough to dissolve the heavy metals not bound to silicate phases (Nindi, 2005).

Hydrochloric acid (6M HCI, Nitric acid (16M NHO_3) and aqua regia (HCI +NHO_3, 3+1) have been used to extract Cd, Cr, Cu, Mn, Ni, Pb, as well as Zn in soils. The aqua regia digestion extract between 70-90 % of the total concentrations of these seven trace elements, 30-40 % of the total AI, 30-60 % of Ca, 10-20 % of K as well as 60 – 70 % of Mg but is only able to extract about 2-5 % of Na. In ensuring correctness of the results, care should be taken when extracting with H_2SO_4 as losses of Pb as insoluble $PbSO_4$ may occur and other elements may be lost by occlusion in precipitated $CaSO_4$ in soils with high concentration of Ca. The digestion by $HCIO_4$ with the combination of HNO_3 acid has found consideration application in soil analysis despite the danger of explosion with samples high in organic matter. The H_2SO_4 digestion is not well suited for extracting metals for flame atomic absorption spectrometry (FAAS) analysis because of its complex interference effects (Nindi, 2005).

One of the advantages of aqua regia digestion is that BCR reference soils have indicative values for the aqua regia contents of several elements and this soil can therefore be used to validate the results and extraction procedure required. The extract obtained can be either determined using FAAS or by inductively coupled plasma atomic emission spectrometry (ICP-AES) as it is able to determine most of the heavy metals at the concentration occurring in digestions of normal unpolluted soil (Alloway, 1995).

2.9.3. Optimized European Community Bureau of Reference Sequential extraction procedure (BCR-SE)

BCR – SE is a three step procedure used for the determination of different pools of trace metal contents in the soil. The results obtained through this step can be compared with other similar steps. This enables the scientists to estimate the concentration that is likely to cause significant effects to the receptor, nutritional deficiency and the environmental accessible trace metals upon disposal into the soil (Quevauviller *et al*., 1996). In most cases single extraction tests are commonly used to study the eco-toxicity and mobility of metals in soils. As a result of that dilemma, the BCR Sequential Extraction Procedure was proposed in 1993 by the European Community's Bureau of References, which is now known as the Standards, Measurements, and Testing Programme of the European Commission. This is a recent development towards international standardised methods for geochemical analysis of the soil. This method enables comparison of the results from different laboratories (Smeda *et al*., 2002 as cited by Nindi, 2005).

BCR-SE gives an indication of the concentrations of metals in various pools or fraction in the soil, which can be mobilized by changes in soil chemistry. However, it cannot be used to determine specific geo-chemical associations. BCR-SE results are classified on the basis of their total metal concentration and on the basis of their nominal target phases (i.e. acid soluble metals, metals bound to oxides and those bound to organic matter and sulphides). A typical BCR standard 3 step extraction procedure is indicated in the Table 2.3 below: in terms of Table 2.4 there is acetic acid extraction (known as step 1), which extracts exchangeable and acid soluble metal fraction, hydroxylamine hydrochloric extraction (step 2), this step extracts reducible metals fraction and hydrogen peroxide/ammonium acetate is used for step 3, which extracts oxidisable metal fraction (Margui *et al*., 2004). In recent studies by Rauret *et al*., (2000), the partitioning of metals has been investigated using a sequential extraction procedure. That led to the BCR extraction protocol to be modified by the introduction of other extracting agents such as deionised water, which is mostly used as a first step to extract water-soluble metals and ions in the soil solution or pore-water (Margui *et al*., 2004).

Table 2.3: The modified four-stage Optimized European Community Bureau of Reference (BCR) sequential extraction scheme (Smeda *et al*., 2002)

Extraction step	Reagent(s)	Nominal target phases
1. Water	Distilled and de-ionised water, 16 hours.	Water-soluble metals and other ions in the soil solution
2. Acetic Acid	CH_3COOH (0.11mol l^{-1}) at pH – 2.6, 16 hours	**Weak Acid soluble metals**, soil solution, carbonates, exchangeable metals
3. Hydroxylamine	$NH_2OH.HCl$ (0.1mol l^{-1}) at pH of 2, 16 hours.	**Bound to oxide** Iron/manganese hydroxides
4.Hydrogen peroxide, ammonium acetate	H2O2 (8.8 mol l-1) then CH3COONH4 (1.0 mol l-1) at pH 2, 16 hours	**Bound to organic** matter – organic and sulphides.

The ability of other different extractant to release metal ions associated with specific fractions in soil has been examined (Margui *et al*., 2004). Reagents such as neutral electrolytes, weak acids and complexing agents can leach metals from co-ordination sites, while strong acids and other redox reagents may release more metals as a result of the decomposition of the soil matrix. Procedural parameters, such as extraction time, volume and temperature influence the results of the BCR procedure (Stalika *et al*., 1999 as sited by Nindi, 2005).

2.10. Analytical methods for the determination of metal concentrations

There are various techniques employed for measuring the concentration of elements in a liquid sample. This includes the use of flame atomic absorption spectrometer (FAAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). I will only focus on the techniques relevant to this study, i.e. ICP-OES and the Leco CHN628 for carbon, hydrogen, nitrogen and sulfur (CHNS).

2.10.1. Inductively coupled plasma – optical emission spectroscopy (ICP-OES)

Inductively coupled plasma-optical emission spectroscopy is one of the commonly used instruments. The principle of optical emission spectrometry (OES) is the generation of radiation by a multitude transition (Merian, 1991). An ICP-OES instrument consist of a sample delivery system, IC plasma to generate the signal, optical spectrometer to measure the signal, and computer to control the analysis. The samples are introduced into the sample delivery system manually by inserting the delivery tubes into the liquid samples in the sample container, one at a time (an auto-sampler may be used). The schematic representation of the ICP-OES is shown in Figure 2.3. The components of the ICP-OES are generally similar to the ICP-MS except the determination. In ICP-OES, emission lines are measured and the intensity of these lines represents the concentration in the sample. Both have similar nebulizers and plasma. These different components are briefly discussed.

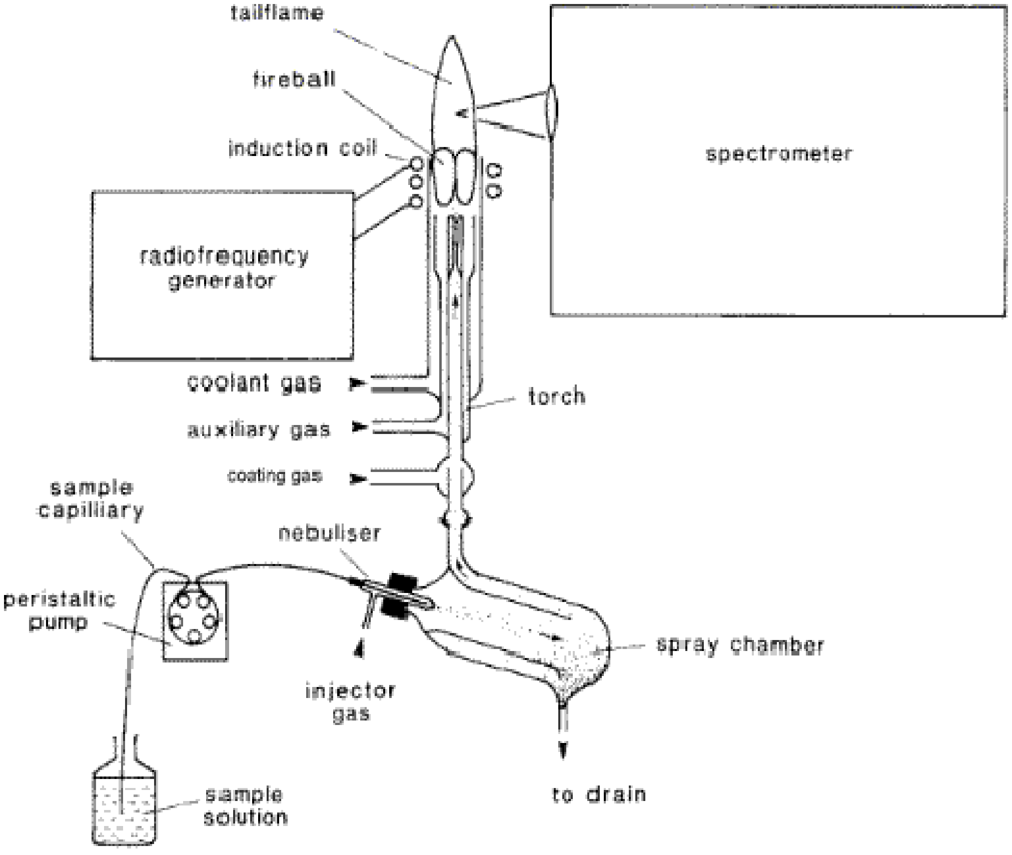


Figure 2.3: Schematic representation of an ICP – OES (Nolte, 2002)

The sample delivery system consists of a peristaltic pump and capillary tubes to deliver a constant flow of analyte into the nebulizer. The nebulizer turns the analyte liquid into droplets. Less than 5 mSg droplets size aerosols are formed from the liquid samples. The largest droplets fall out into a drain in the bottom of spry chamber and the finest droplets are carried by gas into the IC plasma.

Nebulizer

When a liquid is fed into IC plasma it must be in the form of fine droplets otherwise it will not fully atomize. The first stage in forming droplets is the nebulizers. The nebulizer often is the pneumatic which relies on the venture effect. Cross-flow nebulizers are chosen because they are ideal for analyses of heavy metals in aqueous solutions (Nolte, 2002).

Spray chamber

The droplets coming from nebulizers vary in size, from less than 1 um to more than 10 um. Since droplets going into the IC plasma should be kept below 5 um in size, it is necessary to remove the large droplets, and this is done in the spray chamber. The liquid spray from the nebulizer enters the spray chamber. By sheer size, the larger droplets fall to the bottom of the chamber and exit through the drain. The finer droplets in the vapour are transported to the plasma (Nolte, 2002).

ICP torch

The ICP torch produces the IC plasma. Nebulizer gas (inner argon flow), at about 1 L /min, carries the analyte aerosol. Plasma gas, at about 12-16 L/min, sets the plasma condition, for examples, the excitation temperature. The argon and the analyte flow into a toroidal radio frequency (RF) field, usually at 40.68 MHz. The plasma is ignited by a tesla spark (Nolte, 2002).

Principle of vaporization atomization and excitation

As the aerosol vapour enters the plasma, the vapour desolvates and atomization occurs within the plasma. Atoms get exited to atomic and ionic state. Rich spectra are produced because of the presence of both atomic and ionic lines. Different emission lines have different vertical positions in the plasma because of their different excitation energies (Nolte, 2002). These are the ones measured by the detector. Since each metal has got its own emission lines, it allows measuring many elements at once.

Viewing position

The plasma generated in an ICP can be viewed by the spectrometer, side – on or end – on. These viewing positions are also called radial and axial viewing, respectively. Each has advantages and disadvantages, so each tends to be used for different applications e.g. radial viewing for low detection limits in simpler materials (Nolte, 2002).

Advantages and disadvantages of the inductively coupled plasma – optical emission spectroscopy (ICP-OES)

Advantages

- The rapid entry of analyte material in to ICP source does not consume time to dissolve sample.
- It has no problems with dissolution chemistry.
- It is environmentally friendly as there are no problems of chemical waste disposal.
- It has reduced problems of contamination. The ICP-OES is able to analyze non – metallic elements.

Disadvantages

- Spectral interferences can occur as some metals have very close emission lines
- The sensitivity is not as low as ICP-MS
- It is generally an expensive technique compared to FAAS.

2.11. Ion Chromatography

Ion Chromatography is a form of liquid chromatography that uses ion-exchange resins to separate atomic or molecular ions based on their interaction with the resin. This is mostly used for the analysis of anions for which there are no other rapid analytical methods (Weiss *et al*., 2005). This method commonly used for cations and biochemical species such amino acids and proteins. Most of the ion exchange separations are done with pumps and metal columns. The solution to be injected is usually called a *sample*, and the individually separated components are called analyt*e*. In general ion chromatography is one of the most difficult types of liquid chromatography to exploit and most often used for analysis of anions for which there are no other rapid analytical methods to be used (Weiss *et al*., 2005).

Principle of Ion Chromatography

Ion chromatography retains analyte molecules on the column based ionic interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charges. This type of chromatography is further subdivided into cations exchange chromatography and anion exchange chromatography (Weiss *et al*., 2005). The ionic compound consisting of the cationic species M+ and the anionic species B- can be retained by the stationary phase. Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group, where as the anion exchange chromatography retains anions using positively charged functional group. It is important to note that the ion strength of either C+ or A- in the mobile phase can be adjusted to shift the equilibrium position and thus retention time (Weiss *et al*., 2005).

Samples are introduced, either manually or with an auto sampler, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of cellulose beads with covalently bonded charged functional groups. The target analyte (i.e. anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase (Weiss *et al*., 2005). For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions.

The analyte of interest must then be detected by some means, typically by conductivity or UV/Visible light absorbance. This method can be used in water samples and solid samples, however for the solid samples they will need to be extracted with water or acid (cations) to remove ions from sample surface. The liquid samples must be filtered and stored cold until analysis can be performed (Weiss *et al*., 2005).

Chapter Three: Materials and methods

This chapter outlines the materials and methods used in the research project. The methods include sample collection, sample preparation, sample analysis and measurement of *B. coddii* growth. Plant growth trials were performed in the greenhouse situated at the University of the Witwatersrand (Wits). Due to the nature of the research, the methods and materials used is divided into two categories. The first part deals with materials and methods prior to the plant growth experiment. The second part gives materials and methods used after growth experiments have been completed.

3.1. Description of the study area

Two study areas were chosen, for obtaining soil and waste samples and *B. coddii* seeds. One is the native soil also known as serpentine soil. This is the soil where *B. coddii* plants grow naturally and the second study comprised of the contaminated waste found at Rustenburg Base Metal Refinery. The Badplaas soil was described as Native (N) soil where as the Rustenburg Base Metal Refinery wastes were described as RBMR waste soil. Figure 3.1 shows the locations of the two study areas. The Rustenburg Base Metals Refinery (RBMR) waste varies from light grey to dark. The soil texture is difficult to identify simply because the particles are inter-mixed, forming lumps. However, the soil is granular and the grain sizes are considerably greater than the diameter of the sand. The grain shapes are round to sub-angular. The soil is hard when dry, making it difficult for water to penetrate through. This soil has higher moisture retention than serpentine soil. The parent rock is Andesite.

Badplaas is situated in the Lowveld area of Mpumalanga Province in South Africa. The natural soil and seed sampling area encompassed two natural populations of *B. coddii* found at Nelshoogte and SAPPI plantations at Badplaas. The soil contains high Ni and Co concentration.

The Badplaas soil is characterised by red and yellow massive or weak structured soil, with a proportion of 20-30 % sand. The soil consists of a high percentage of silica which implies that the soil has been derived from the acidic rock. The soil has high spore spaces that allow water to infiltrate to the ground rapidly as compared to the RBMR waste soil.

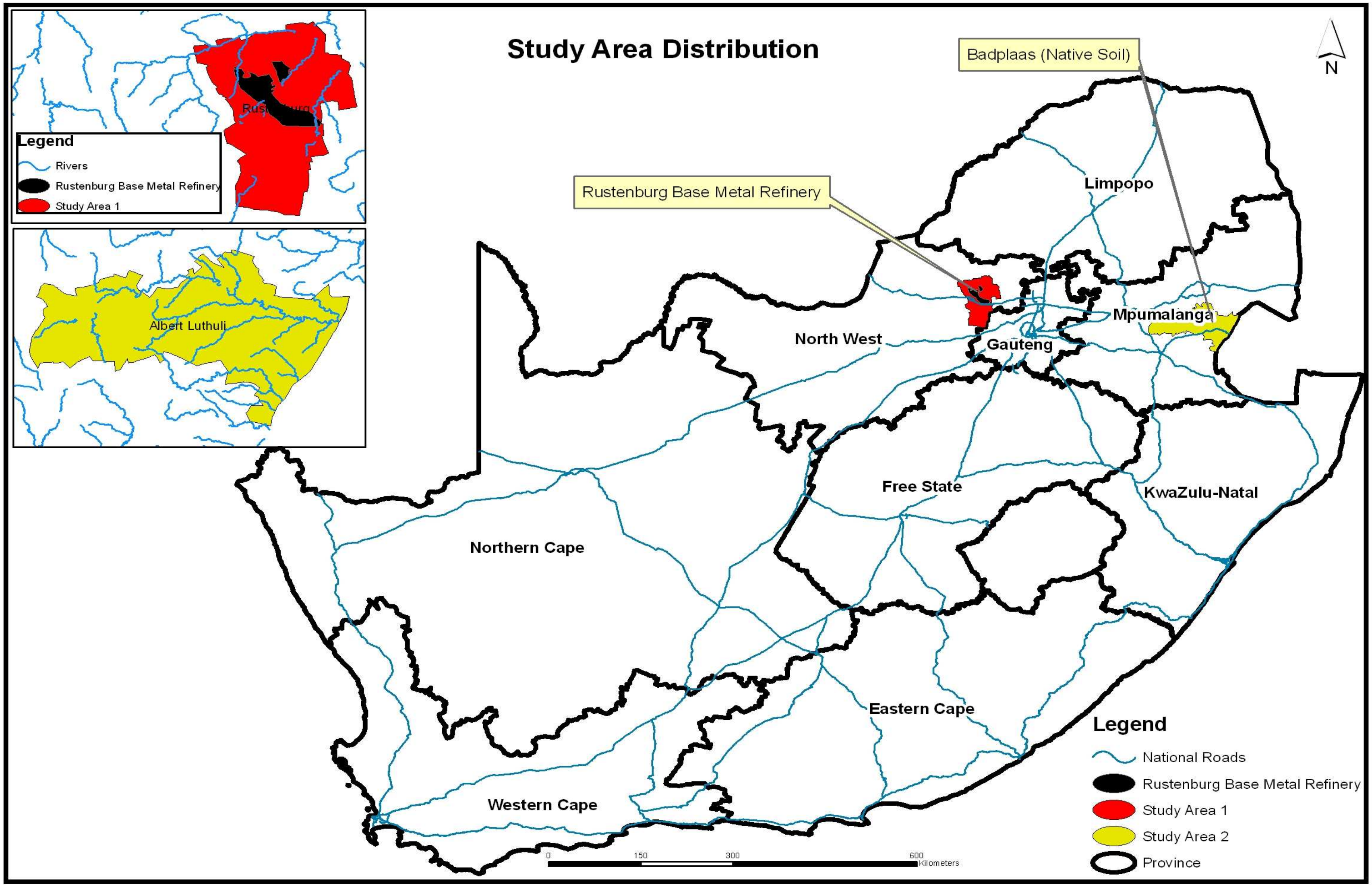


Figure 3.1: Distribution of the two study areas (i.e. RBMR waste soil and N soil)

3.2. Materials and methods used prior to *B. coddii* growth (part one)

3.2.1. Soil and seed sample collection and preparation prior to growth experiment

Prior to the setting up of the *B. coddii* growth experiment, about 6 refuse bins of the native soil (N soil) samples and 6 refuse bins of the Rustenburg Base Metal Refinery (RBMR) waste samples were collected. The Rustenburg Base Metal Refineries were described as RBMR waste soil, whereas native serpentine soil collected from near Badplaas where *B. coddii* grows was described as the N soil. During the collection of the N soil, seed of *B. coddii* plant were also collected from the same area were N soil was collected.

The seeds were used to propagate the seedlings used in the growth experiments. Samples of both soils were collected using a pick and digging spade. The soil samples were stored in a refuse bin and transported to the tunnel situated at City Deep, South of Johannesburg, where growth experiment preparations were carried out. Once all the soil had been prepared, the soil was then taken to the greenhouse situated at Wits University. The seeds were collected and kept in the lab and later transferred to the tray containing N soil for the germination. Figure 3.2 and 3.3(b) show how the soil samples and seeds were collected.

In order to avoid cross contamination of the samples, cleaned equipment was used when collecting those bulk samples from both areas.

Figure 3.2: Photograph demonstrating how RBMR waste soil samples were collected.

Figure 3.3: Photograph showing: a) how *B. coddii* seed were collected and b) *B. coddii* population growing naturally at Badplaas site.

3.2.2. Grinding of soil samples

The soil samples were labelled. The bulk of the soil samples collected from each site were well mixed and broken down at the tunnel situated at City Deep South of Johannesburg to ensure good representation of the sub-samples and also to ensure that soil size are small and are able to support the seedling before final transplanting. Any vegetation (roots and leaves) were removed from the soil to avoid interference with the experiment once started.

Approximately 5000 g of soil were re-sampled from each bulk sample collected and used for soil chemistry analysis. From the 5000 g soil sample, about 400 g of each soil type was subdivided into four samples comprising of 100 g each. This was used for BCR sequential extraction. Another 800 g of each soil type was subdivided into four samples comprising of 200 g was used for the determination of the soil pH, conductivity and redox potential. About 2 mg from each soil type were used for CHNS analysis to determine carbon, hydrogen, nitrogen and sulfur in the soil. 100 g of each soil was weighed and used for the determination of moisture content. The remaining samples from the N soil were used during the germination of seedlings.

3.2.3. Physical and chemical properties of the soil and waste samples

Soil moisture-holding capacity, and solution pH, conductivity (EC) and reduction oxidation potential (ORP or Eh) were measured. These are described below:

- **Determination of moisture content**

Approximately 100 g of substrate sub-samples were precisely weighed. Four samples were taken from both the N soil and RBMR waste soil, and transferred to the paper sampling bag. The sampling bags of the substrata were kept at 40^{o}C for three days in the oven. The mass of the sub-samples were measured on each of the three days until a constant mass was obtained (i.e. all water had been evaporated). This was done in order to determine the moisture capacity of both soils (i.e. in black bags). The results obtained were then used in determining the volumes of water to be provided to the plants so that no drainage of water and thus leaching of ions from the bags could occur during the experiment.

- **Determination of the substrate pH, conductivity and Eh**

A Cyberscan PC 10 instrument was used to measure pH, conductivity and reduction oxidation potential (Eh) (although it was recognised that the reduction oxidation potential would not be representative of *in situ* conditions in undisturbed soil) in a 1:2 v/v soil solution. Four samples each consisting of 100 g of soil from each soil sample were measured from 800 g of each sub-sample. This was then transferred into 250 ml glass beaker. 200 ml of distilled and de-ionised water were added to each sample beaker (four for N soil and four for RBMR waste soil) to make a slurry solution. The remaining 400 g from 800 g of each soil were sub-divided into four samples of each soil type and were again transferred to 250 ml beakers. 200 ml of potassium chloride (0.1. M KCL) were added to these samples. From this preparation the pH of both substrates were measured. For pH measurements the instrument was calibrated with pH buffer of 4, 7 and 10 before use.

The same procedure was used to measure the soil pH, conductivity and reduction oxidation potential in soil samples after the plant growth experiment. However, after pot trial about 32 samples consisting of 50 g of soil samples (16 from the N soil and 16 from the RBMR waste soil) and 100 ml of deionised water were mixed together in a 250 ml beaker for measurements of these parameters.

- **Soil carbon, hydrogen, nitrogen and sulfur analysis procedure**

2 mg of each substrate (RBMR and N soils) was weighed into foil capsules for the determination of total carbon, hydrogen, nitrogen and sulfur (CHNS) using LECO CHN628 instrument supplied by LECO Corporation; Saint Joseph, Michigan USA. The weighed samples from each substrate were run for 150 seconds in order to determine the available CHNS within the soil and waste. The CHNS analyses were carried out before and after *B. coddii* growth experiments. The same procedure was used for the analyses of CHNS in freeze-dried plants parts (i.e. the roots, stems and leaves).

- **Extraction of soil aqueous solution**

4 sets of 100 g samples of each soil type were weighed and transferred into 250 ml beakers, labelled as per the soil type. 200 ml of de-ionised water was added to each soil. The samples were shaken for 16 hours (overnight) at ambient temperature (+/- 20 degrees centigrade) on a mechanical shaker, operating at approximately 80 rpm. The extracts were separated from the solid residue by centrifugation and decanted into a polyethylene container using a filter paper and stored at 4 degrees centigrade for analysis with ICP - OES for metals and ion chromatography for anions. The same procedures were used after plant growth experiment. However, only 4 g of each soil were weighed together with 40 ml of de-ionised water.

- **Optimized European Community Bureau of Reference Sequential extraction (BCR-SE)**

BCR sequential extraction (BCR-SE) provides an indication of the concentrations of metals in various reservoirs or `pools' in the soil, which can be mobilized by changes in soil chemistry. However, it cannot be used to determine specific geochemical associations of each soil in this study.

The solutions for BCR sequential extraction were prepared as follows: doubly de-ionized water (milliQ) was used in the preparation of all solutions where necessary. Acetic acid, 0.11mol L^{-1} solution was prepared by taking 25 ± 0.2 ml glacial acetic acid and mixed with 0.5 L of distilled water in a 1 L graduated polypropylene bottle and made up to 1 L with de-ionized water in a fume cupboard. 500 ml of this solution (acetic acid, 0.43 mol L^{-1}) was taken and diluted to 2 L with de-ionized water to obtain an acetic acid solution of 0.11mol L^{-1}.

Hydroxyl ammonium chloride (hydroxylamine hydrochloride), 0.5 mol L^{-1} was prepared by weighing 69.5 g of hydroxyl ammonium chloride and dissolving in 800 ml de-ionized water. The solution was transferred to a 2 L volumetric flask. 50 ml of 2 mol L^{-1} HNO_3 was added by means of a volumetric pipette in a 2 L volumetric flask. The solution was made up to the mark using de-ionised water. Hydrogen peroxide, 300 mg g^{-1} (8.8 mol L^{-1}) was used as supplied by the manufacturer but was acid stabilized to pH 2-3. A solution of ammonium acetate (0.1mol L^{-1}) was prepared by dissolving 154.16 g of ammonium acetate in 1800 ml of de-ionized water. The pH was adjusted to 2.0 +/- 0.1 with concentrated HNO_3 and made up to 2 L with de-ionized water.

- **Weak acid soluble extraction**

The BCR procedure was performed as follow: 200 ml volume of acetic acid (0.11mol L^{-1}) was added to residue of each 100 g of each sample in a 250 ml conical flask. The samples were shaken for 16 hours (overnight) at ambient temperature (± 20 degrees centigrade) on a mechanical shaker operating at approximately 80 rpm. The extracts were separated from the solid residue by centrifugation and decanted into a polyethylene container using a filter paper and stored at 4 degrees centigrade for analysis. The residues were washed with 60 ml of de-ionized water by shaking for 20 minutes, centrifuged and the washings were discarded.

- **Bound to oxide extraction**

The residue from the step above was used for step above extractions with hydroxyl ammonium chloride solution. A 170 ml of hydroxyl ammonium chloride (0.1mol L^{-1}, adjusted to pH 2 with nitric acid) was added to the residues from step one and transferred quantitatively back to the Conical flask. Extra 30 ml reagent was added to make it 200 ml after being used for rinsing. The samples were shaken for 16 hours (overnight) at ambient temperature (+/-20 degrees centigrade) on a mechanical shaker, operating at approximately 80 rpm as before. The extracts were separated and stored as described above. The residues were washed with 60 ml of de-ionized water by shaking for 20 minutes, centrifuged and the washings were discarded. These residues were used for step three extractions with H_2O_2.

- **Bound to organic extraction**

In this case 50 ml of H_2O_2 (8.8 mol L^{-1}) was added in small aliquots to avoid losses due to possible violent reaction, to each residue from step above. The containers were then covered loosely with their caps and digested at room temperature for 1 hour with occasional manual shaking. The digestion was then continued for 1 hour at 85 ± 2^oC in a water bath, where after the volume was then reduced to about 30 ml through further heating of the covered containers. Another 20 ml of H_2O_2 (8.8 mol L^{-1}) was added to each container which was then heated at 85 +/- 2^oC continued for 1 hour. After that, the covers were removed and volume reduced to 10 ml through heating of the uncovered containers. Thereafter 130 ml of ammonium acetate was added to the cool moist residue and then shaken for about 16 hours at 22 ± 5^oC (overnight) in order to complete the extraction process. After 16 hours the extracts were separated and stored as described above using ICP – OES.

The same BCR sequential extraction procedure was used to determine the availability of heavy metals found in the soil after growth experiments were completed. However, the amount of soil and solution was different. In this case only 4 g of soil was measured from each soil type. The equivalent extraction solution used was calculated accordingly.

- **Aqua Regia extraction**

0.1 g of the residue left from BCR steps above (i.e. 1+ 2 + 3) was weighed from the 8 selected samples out of the 32 samples digested through BCR-SE; in this case, 4 samples were taken from the RBMR waste soil and another four from the N soil. In addition to these samples, two samples analysed before the experiment through BCR sequential extraction were also digested to determine the total available metals. 2 ml of HNO_3, 6 ml of HCL and 1 ml of HF was added into the digestion tube containing 0.1 g of soil sample and run for 30 minutes using the Anton Paar microwave assisted extraction system. After digestion, samples were allowed to cool down for 30 minutes and then transferred into a 100 ml volumetric flask. 6 ml of boric acid was added into that volumetric flask in order to neutralise the HF concentration, thereafter de-ionised water was added up to the mark. After the total digestion the samples were stored in the fridge at 5 ^{o}C for metal analyses using ICP- OES.

3.3. Plant growth pot trials

3.3.1. Seed germination

The plants were germinated from seeds collected from *B. coddii* in Badplaas. Germination was conducted in a black plastic tray containing the same soil where the plant seeds were collected. This was to ensure the same AMF-inoculation in all the experimented plants. The seedlings were allowed to grow for a period of 6 weeks to allow them to be established. At this stage seedlings had 3 to 5 leaves before transplanting into containers of two soils. These are the native (N) soil and RBMR waste soil. Seed germination was done as from mid July to the end of August 2008. Figure 3.4 shows seed germination in the tray.

Figure 3.4: Showing how B. coddii seeds were geminated in the tray using the N soil.

3.3.2. Soil preparation before transplanting

Prior to the transplanting, stones and other rubble were removed from the soils (Figure 3.5). 2.4 kg of each soil was packaged into about 2.5 L planting bags. These were weighed to ensure same amount of soil to avoid the variation in plant growth rate. All the bags were blocked at the bottom to ensure that soil does not come out of the planting bag. Transplanting was performed in August 2008. Approximately 100 to 200 ml tap water was added to the plants three times a week.

Figure 3.5: Demonstrating how soil was prepared before transplanting.

The bags were put on dishes to prevent any contamination of the roots by contaminates from the ground, or possible loss of water and metals in solution during watering. The plants were kept in the green house for the rest of the experiment in order to allow the plants to grow under controlled conditions to avoid hail or rain interference with the experiment.

3.3.3. Transplanting of the seedlings

96 of 2.5 L planting bags of each containing about 2.4 kg of the soil from each soil type were transplanted with one seedling. All together there were 192 bags since experiments were carried out with four replicates. *B. coddii* seedlings were transplanted on the 31st August 2008. The removal of seedlings from the tray was done by means of adding more water in the tray that contains the seedlings to allow for the seedlings to float on the water so that they can be easily removed from the soil within the tray. The reason why this has been done was to try to minimize damage which might happen to the roots of the seedlings, and to avoid transferring the N soil into RBMR waste soil bags.

Just after transplanting, each plant was given about 400 ml of tap water. Thereafter, all plants were watered with approximately 100 to 200 ml of water every two days. It was important that water availability did not differ between the treatments and limit plant growth, but also that water did not drip out of the bags and result in loss of leachable elements. The bags were each placed on a plastic dish, and the amount of water was determined to be slightly less than the soil moisture holding capacity in order to reduce leaching. Pot plants were randomized and rotated at weekly intervals to ensure that there was no influence of light or temperature gradients to the growth of the plants from each soil.

3.3.4. Determination of plant growth rate and plant stress

Soil acidity is known to limit root development, growth rate and biomass production by *B. coddii* (Nindi, 2005). In addition, it would not be practical to add sulfur to very young plants as once the sulfur commenced oxidation to sulphate (a fairly rapid process in the presence of moisture, air and soil micro-organisms), the metals would leach before the plants had developed adequate roots to explore the soil volume for metal uptake. In the field the addition of metal solubilising or chelating agents has been shown to result in an increased risk of metal leaching to groundwater if plants are unable to extract the available metals. Therefore the *B. coddii* plants in this experiment were allowed to grow until the mid exponential growth phase before adding the sulfur treatments.

The growth curve was identified by regular measurements of plant height, number of leaves and leaf chlorophyll, and sulfur added at the mid exponential phase of growth. Plant height measurements and the number of leaves were taken every two weeks from planting, and every week from the date at which sulfur was added into the pots. Both the measurement and number of leaves was recorded in an experimental log book for presentation at the end of the experiment. A measuring tape was used to determine the height of each plant to an accuracy of 1 mm. The numbers of leaves from each plant was determined by means of counting leaves from the tip of the plant to the bottom (base) of the plant. In this regard, the live leaves and dead leaves were counted. Figure 3.6 shows how the height measurements were taken from each plant.

Figure 3.6: Illustration of how plant height was measured

During the growth experiments, a spectral radiometer (made by Analytical Spectral Devices or ASD) was used to determine leaf reflectance at wavelengths from 400nm to 2500nm, and derive vegetation indices (VI's) (i.e. $mNDVI_{705}$ and PSIR) that are an indication of plant leaf chlorophyll and caerotenoid pigments that change in concentrationsdetectable when plants are under some form of growth stress. The pigments were not directly quantified using biochemical methods, as it was not necessary to do this in order to simply determine relative plant productivity or stress over time between treatments, plus there is extensive literature to confirm that the VI's used are reliable indicators of these pigments. In this case, ASD measurements were taken 14 days before sulfur treatment and again 14 days after the sulfur treatment and 1 day before *B. coddii* harvesting. The sensor of the instrument with an internal light source was placed directly on the first most fully expanded leaves in order to capture the reflectance. Black paper was placed under the selected leaves in order to avoid the measurement of different coloured reflective backgrounds underneath or around the leaf which might interfere with the results.

Two points from each leaf were measured for reflectance, namely the apex and the base of the leaf, in all cases avoiding the mid-rib. The sensor generates heat, which results in a small, circular oval burn mark on the leaf and thus an applied stress [Figure 3.7 (e)]. However, this stress was consistent throughout the experiment with all plants being measured, and no differences in the number of leaf measured and scarred between plants. The reference codes for each plant were recorded to enable plant identification by reference code. Three leaves were selected from each plant. Where a very young or small or stressed plant was not having enough leaves to choose from, all the leaf selected and measured. Figure 3.7 show how the ASD measurements have been taken from each plant.

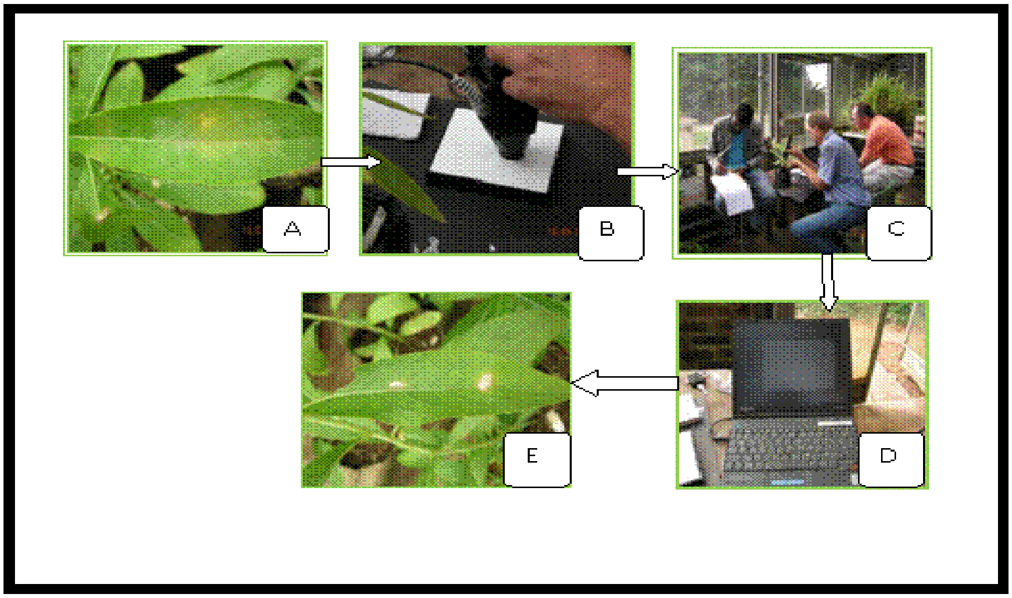


Figure 3.7: Illustration of how reflectance was measured from the *B. coddii* leaf surface using a spectral radiometer. (A= *B. coddii* leaf, B= Sensor, C =Data capturing, D=Computer used for data capturing connected to the sensor, E= leaves with a burn from the internal light source used after measurement. Each leaf was measured once).

3.3.5. Treatment of soil types with sulfur

After the plants had grown to about 200 mm height, about 16 out of 96 plants were randomly selected from each substrate amounting to 32 plants. Twenty-six (24) of these plants were treated with sulfur and the remaining 8 plants were used as control. Various amounts of sulfur of 2.0, 4.0 and 8.0 g per kg of soil were added in powder form in each pot selected for sulfur treatment in March 2009. Sulfur was allowed to dissolve into the soil until the date of harvesting that happened in May 2009. The experiment was done in 4 replications of each of the two types of soil containing the plants of different height.

3.4. After plant growth experiment (part two)

This section provides information related to the materials and methods followed when collecting samples after the plant growth experiments.

3.4.1. Physical and substrates chemistry procedures

After the completion of the plant growth trial, 100 g of each substrates sample was collected from the 32 pots used for sulfur treatment. These were transferred to the plastic sampling bags. All the samples were properly labelled and within a day stored in the refrigerator for physical and soil chemistry analysis.

Out of the 32 substrate samples collected, four samples from each soil type served as control, the remaining 24 of the sample were those treated with sulfur of which 12 was for the N soil and another 12 was for RBMR waste soil. These were stored in the cold room at 5^{o}C prior to the determination of the pH, conductivity and reduction oxidation potential (redox). Only 50 g of fresh substrate samples were then used for the determination of pH, conductivity, redox in de-ionised water as described earlier. The remaining 50 g of each substrate sample were dried in the oven at 40^{o}C until constant weight was obtained and later grounded using pestle and mortar in the fume cardboard.

The samples were then ground using a high purity agate pestle and mortar in a fume cabinet. The grinding of the soil samples was undertaken in the fume cabinet because the soil contained heavy metals that might contaminate the air and other samples within the laboratory. All the stone and grass and other material not suitable for analysis were removed before grinding as before. 4 g of the ground samples was weighed from each sample and used for the 4 steps BCR sequential extraction followed by microwave digestion of the remaining residues. Another 2 mg of the same substrate re-sampled from the pots was weighed and used for CHNS analysis.

3.4.2. Plant sample collection and preparation procedures

All the plants from the bags that were treated with sulfur, together with the four controls from each substrate, were harvested by means of cutting the stem from the surface of the soil using a plastic knife. A plastic knife was used in this regard as small metal particles or oxides (rust) can come off a steel knife and contaminate the samples, and this would affect the results. Before cutting another plant with the same knife, the knife was firstly rinsed with tap water and then followed with de-ionised water. This was done in order to avoid contamination from one sample to the other since *B. coddii* is a Ni and Co hyperaccumulator.

The plants harvested were then separated into stems and leaves, hence all plant parts (roots, stems, leaves) were washed using the normal tap water once followed by de-ionised water three times. All the plants were thoroughly washed to avoid the soil influence on the results of the plants parts. All the plants sample parts namely the stem, roots and leaves were then kept in the plastic sampling bags and stored in the refrigerator at 5^{o}C (it is important to note that this was done on the same day and in winter season) waiting to be freeze dried.

The samples where freeze dried at the Department of Chemistry Laboratory at Wits University using a freeze drier called Labcolco instrument. The samples were freeze dried at a temperature of about -23^{o} for about 2 to 3 days. Once the samples were freeze dried to a constant weight, they were ground to a coarse powder in an agate pestle and mortar (refer to appendix 7). Ground plant samples were then transferred to the plastic specimen jar which was labelled accordingly and continued milling with a glass ball using the shaker until a fine powder was obtained. This was done in order to ensure complete mixing and representation of each plant sample. Figure 3.8 shows how the plants were harvested, dried, grounded and milled.

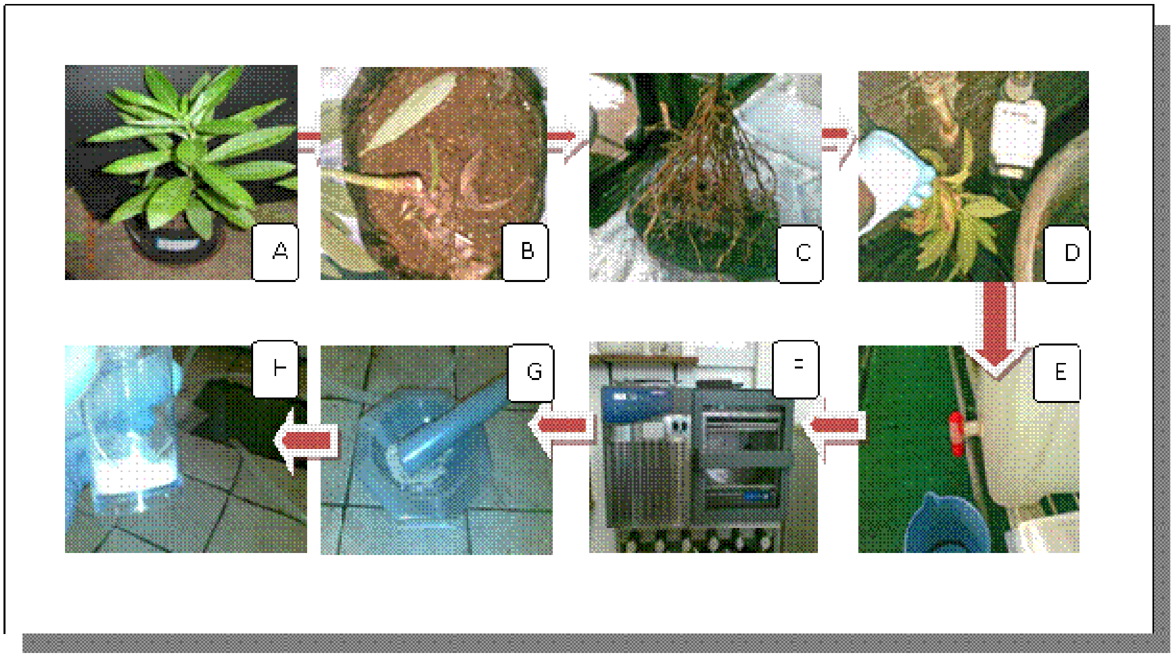


Figure 3.8: Illustration on how a plant sample was collected, washed, grounded and milled

(A= *B. coddii* plant, B= cut plant, C= plant root system, D= plant shoots and tap water, E= container with de-ionised water, F= freeze dryer, G= agate pestel and mortar with ground sample, H= specimen jar with milled sample)

3.4.3. Plant digestion procedure

±0.1g of each plant samples was weighed into Teflon digester tubes together with 4 ml of nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2). The samples were digested for 15 minutes using the Anton Paar Sealed Microwave digestion system. After digestion the samples were allowed to cool down for 15 minutes and later transferred to 100 ml volumetric flasks diluted with de-ionised water up to 100 ml, and stored in the refrigerator for elemental analyses using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) as described in chapter two (2) of this research.

3.5. Quality assurance

3.5.1. Calibration curve and certified reference materials for Carbon, Nitrogen and Sulfur (CNS) and ICP- OES

CHNS and ICP-OES analysis was carried out on Orchard Leaf Certified Reference Material (CRM) at the same time as *B.coddii* samples. Validations of the results of the *B.coddii* analyses were done by comparing the Orchard Leaf CRM results with the CRM data sheet. Instruments used for analysis of soil and waste Ni and Co and plant metals concentrations were calibrated prior to use. The lowest detection limit set for ICP – OES instrument was 0.05 ppm where as the highest detection limit set was 1.2 ppm, therefore anything below 0.05 was considered not detected hence it could not be reported. However, it should also be noted that detection limit varies as per the element and the wavelength for a given element. Below are the calibration curves for Ni and Co obtained through ICP- OES of the standard solution.

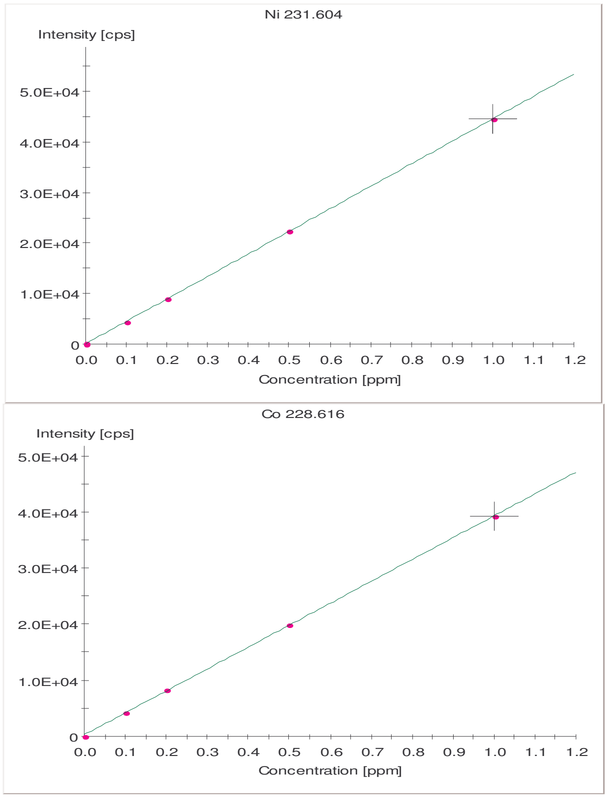


Figure 3.9: Calibration curve for ICP - OES instrument obtained before analysis

3.5.2. Statistical analysis

Data was entered on Microsoft Excel 2007 spread sheets in order to be analyzed using statistical software (Statistical version 10). The data were analysed using 1-way ANOVA, 2-way ANOVA and liner regression. The t-test was performed to determine if there is any significance between the two soils. In this case p value = <0.05 were considered significant. In addition, in order to identify the differences between treatment, data were further analysed using tukey HSD test. The results for this analysis are included in the results sections were necessary as well as on the appendices.

Chapter Four: Results

4.1. Determination of the soil solution pH, reduction oxidation potential and conductivity

A number of parameters such as pH, conductivity and reduction oxidation potential influences heavy metals availability to plants in the soil. To ascertain the effect of the plant growth experiments on these three determinants in both N soil and RBMR waste soil soil, determinants analyses was conducted prior to and post the experiments and the results are presented in Figures 4.1 to 4.3.

4.1.1. Determination of soil pH

Using two different solvents in the analyses, de-ionised water and 0.1M potassium chloride (KCl), RBMR waste soil was found to have a pH of 8.0 and 7.9, respectively, before the plant growth experiments. This compares favourably with the value of 7.7 reported by Nandi (2005) for RBMR waste soil. In contrast, the N soil, using the same two solvents, recorded a pH of 6.4 and 6.0, respectively, before the plant growth experiments.

The soil pH results recorded after the plant growth experiments (Figure 4.1) revealed that the pH of both soils decreased along with the increase in sulfur dosage. However, the decrease of the RBMR soil was not very pronounced and was within margin of error. The soil pH for the N soil decreased from 6.4 to 4.7. The addition of sulfur had a more dramatic effect on the soil pH of the N soil compared to the RBMR waste soil. Furthermore, there was a significant difference in soil pH between both treatments (ANOVA, p= 0.004 for both the RBMR and N soil) although both were found to be within the range of soil pH suitable for plant growth (Donahue *et al*., 1983).

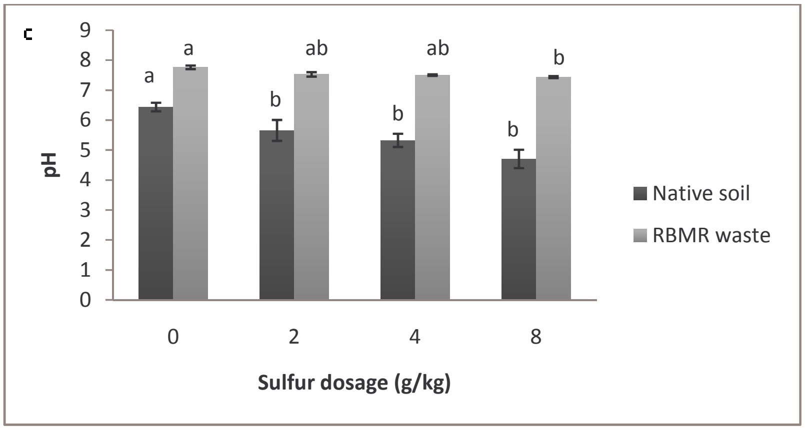


Figure 4.1: Soil pH in both substrates after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p= 0.004 (1-way ANOVA.

4.1.2. Determination of soil conductivity

As for the pH, soil conductivity was also measured before and after the plant growth experiment, using de-ionised water. The soil conductivity recorded for RBMR and N soil were 1494 and 65 µS/cm, respectively. The soil conductivity results obtained before plant growth experiment indicated that RBMR waste soil had higher soil conductivity than the N soil. To determine the effect of sulfur dosage in both soils, soil conductivity was studied after plant growth experiments. The results are shown in Figure 4.2.

The soil conductivity results showed that RBMR waste soil conductivity increases from 1344 to 1593 µS/cm (liner regression, p= 0.31). The soil conductivity for the N soil increases from 428 to 1323 µS/cm along with the increase in sulfur dosage (liner regression, p= <0.001). The results suggest that sulfur did not have significant effect on the conductivity of the soil from RBMR waste soil. However, the results suggest that sulfur had significant effect on soil conductivity for the N soil. In both substrates there was a positive correlation between sulfur dose and soil conductivity (liner regression, r^2 = 0.65 for N soil and 0.07 for RBMR waste soil). Furthermore the ANOVA results suggest that there was a significant difference in soil conductivity between S treatment recorded for the N soil (ANOVA, p= 0.001) but no significant difference between S treatment for soil conductivity of the RBMR waste soil (ANOVA, p= 0.6).

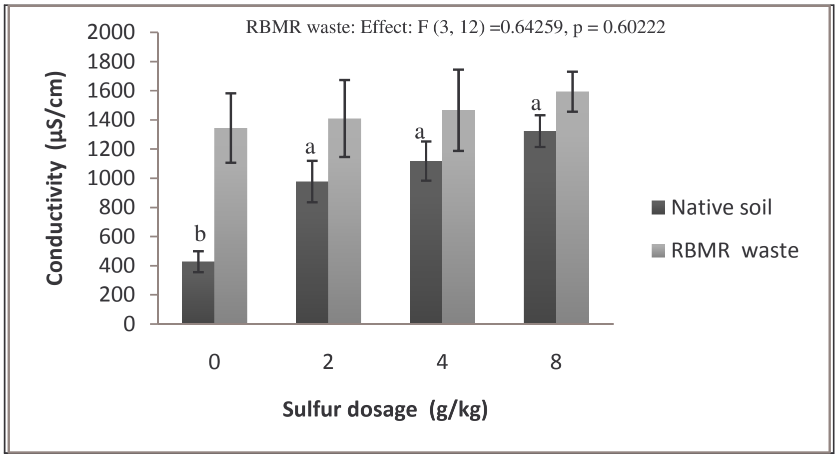


Figure 4.2: Soil solution conductivity in both substrates after plant growth experiments. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p = 0.001 (ANOVA).

4.1.3. Soil reduction oxidation potential (redox potential)

The soil redox potential obtained before the plant growth experiments (appendix 2 and 19) revealed that N soil had higher redox potential than the RBMR waste soil soil. The N soil and RBMR waste soil redox potential was found to be 358 and 234mV respectively.

The soil reduction oxidation potential found after the experiment was completed revealed that there was no significant difference in reduction oxidation potential between the two substrates. The p values obtained using one-way ANOVA for both substrates (RBMR waste soil soil and N soil) were found to be 0.88 and 0.05 respectively. The addition of sulfur did not have significant effect on soil reduction oxidation potential (Figure 4.3), the p-values obtained through liner regression for both the RBMR and N soil was reported to be 0.77 and 0.31 respectively.

The results obtained for the RBMR waste soil further suggest that there was a no strong positive correlation between the additional of sulfur and redox potential (liner regression, $r^2 = 0.006$). However, the results obtained for the N soil suggest that there was negative correlation between the addition of sulfur and the redox potential (liner regression, $r^2 = 0.07$). Although, the result further suggests a slight increase in redox potential from sulfur dosage of 2.0 g/kg soil and then decreased thereafter for the N soil. It is difficult to link that with the increase in sulfur. Sulfur did not significantly affect the RBMR waste soil redox potential.

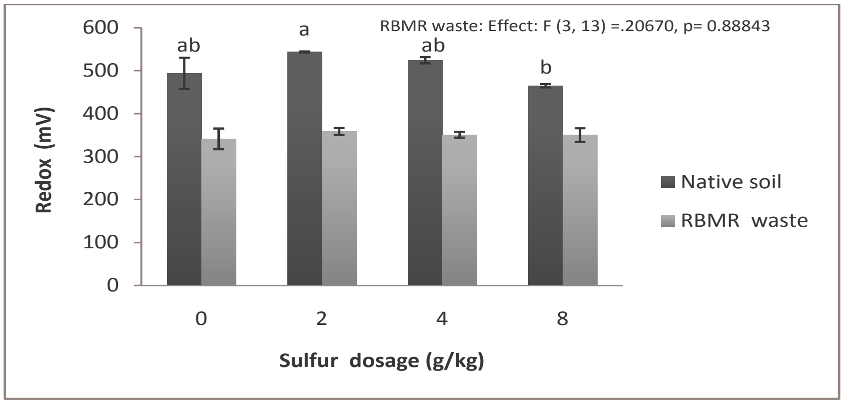


Figure 4.3: Reduction oxidation potential in both substrates after the plant growth experiment (measurements performed with soil dissolved in deionised water). Values represent mean ± SE, different letters on the graph indicate significant difference among means at p=0.05.

4.2. The total concentration of carbon, nitrogen and sulfur, and of nitrate and sulphate in soil and waste.

The RBMR waste soil has a higher concentration of both nitrate and sulphate as compared to the N soil (Table 4.1). The nitrate concentration was recorded to be 1.76 mg/kg for the RBMR waste soil and 1.13 mg/kg for the N soil. The concentration of sulphate was found to be 34.88 mg/kg for the RBMR waste soil and 0.29 mg/kg for the N soil. The standard deviation especially for sulphate is very high and reflects heterogeneity distribution in the soil.

Table 4.1: Nitrate and sulphate concentration before plant growth experiments in soils.

	RBMR waste soil (mgkg)	**N soil (mg/kg)**
Nitrate Mean	**1.76**	**1.13**
STDEV	1.37	0.08
Standard error	0.69	0.04
Sulphate Mean	**34.88**	**0.29**
STDEV	19.68	0.18
Standard error	9.84	0.09

The analysis done before the plant growth experiment on both substrates revealed a low percentage of carbon (i.e. 2.5% and 2.4% for RBMR waste soil and the N soil respectively) but higher than the percentage of nitrogen and sulfur. No detectable percentage of nitrogen was identified before the growth experiment in both soils. The N soil was observed to have higher sulfur concentration as compared to the RBMR waste soil soil. However, the result showed that there is no significant difference in carbon percentage between the two substrates (ANOVA, $p = 0.02$), despite slightly higher percentage of carbon in the RBMR waste soil.

The results as shown in Figure 4.4, seem to suggest that the increase in sulfur dosage to 2.0 g increased the concentration of carbon in the N soil and marginally in the RBMR waste soil and that continuous increase in sulfur dosage (i.e. 4.0 to 8.0 g) eventually decreased the concentration of carbon in both the substrates. However, such a trend is unlikely because no external carbon source was added to the two soils during experiments. The observation is perhaps as a result of variations in the data which had high standard deviations.

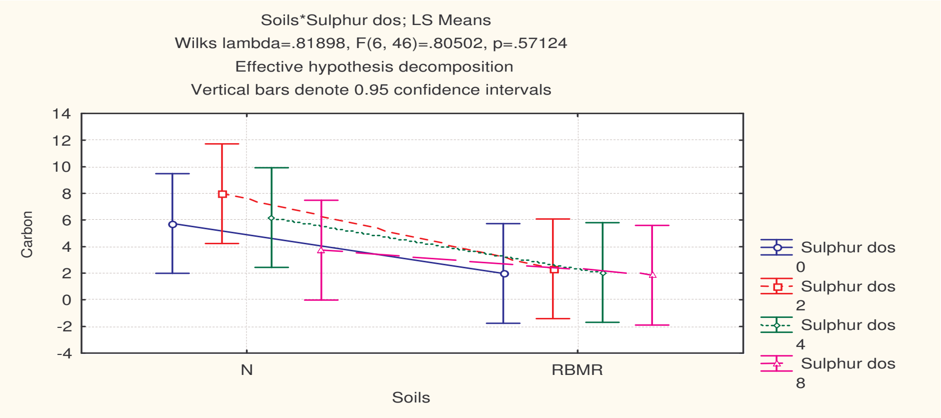


Figure 4.4: Carbon percentage after plant growth experiment.

The results in Figure 4.5 suggest that there is no significant difference between the percentages of nitrogen and sulfur (ANOVA, p = 0.84).There is also no significant difference between the nitrogen percentage in both soils (ANOVA, p = 0.02). The percentage of nitrogen obtained in the control (i.e. 0 g of sulfur) in both soils was higher than the nitrogen one in the soil treated with sulfur. The percentage of nitrogen in the soil seemed to increase along with the increase in sulfur dosage in the N soil. The RBMR waste soil nitrogen percentage also suggested increase along with the addition of sulfur, but reached a plateau at 4 g/kg of sulfur.

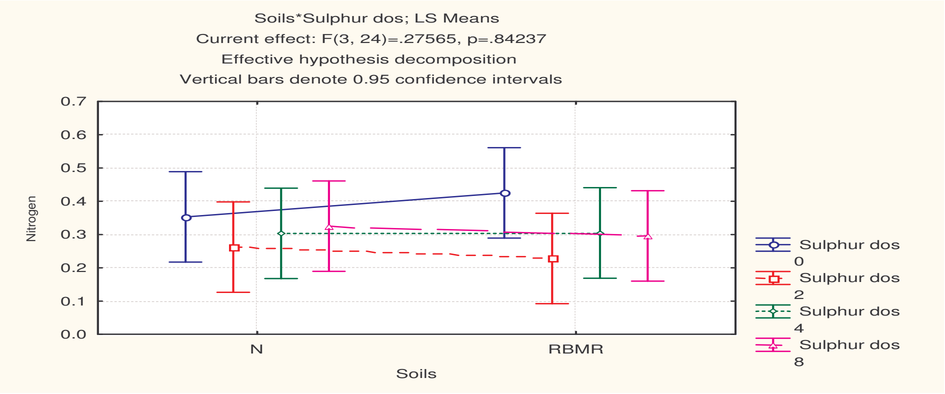


Figure 4.5: Nitrogen percentage after plant growth experiments.

The results shown in Figure 4.6 suggest that there is no significant difference in sulfur percentage in both soils (P = 0.77), although there was a positive correlation between the sulfur concentration recorded for both soils with the increase in the amount of sulfur dosage in both soils. This positive correlation was more pronounced in the RBMR waste soil than in the native soil (N soil).

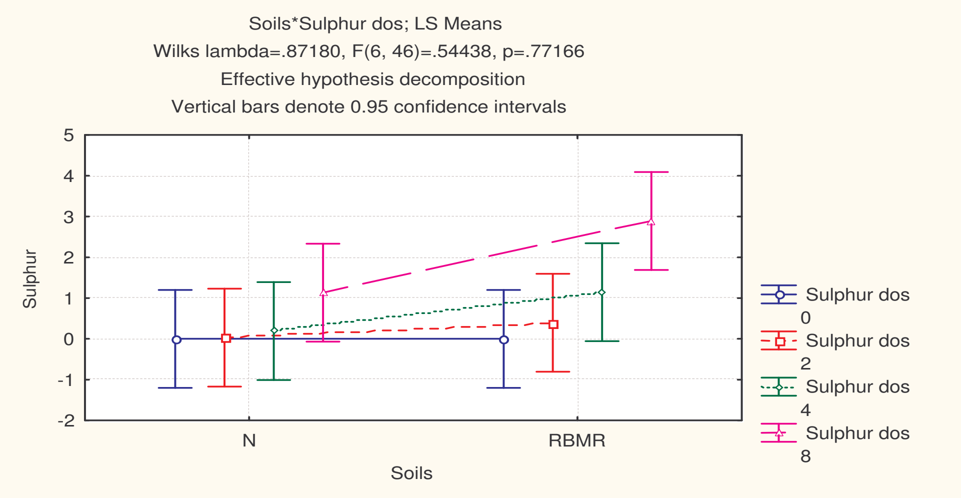


Figure 4.6: Sulfur percentage after plant growth experiments.

4.3. Sequential extractions for sulfur treated and untreated soil

The main objective of this research was to determine if the addition of sulfur would increase the availability of Co and Ni in the soil and further enhance the chances for uptake of both metals by *B. coddii*. In attempt to respond to the objective and the focus of the study, sequential extraction method was used to determine the concentration of Co and Ni in both soils, before and after plant growth experiment. The results for Co and Ni obtained through sequential extraction method are showed in Figures 4.7 to 4.10 below.

4.3.1. Extraction of Co and Ni concentration in soil solution (aqueous)

Water extraction was used to determine concentration of Co and Ni freely available for the plant to uptake (Figure 4.7). The extraction of N soil and RBMR waste soil with deionised water, before plant growth experiment showed that the concentration of Co and Ni available in the N soil (C1) is 0.3 and 2.9 mg/kg, respectively while the results for Co and Ni in the RBMR waste soil (C1) was found to be 0.03 and 0.3 mg/kg, respectively. These results showed that the N soil had higher concentration of Co and Ni compared to the RBMR waste soil. The results of water extraction obtained for the control group after the plant growth experiments showed no detectable concentration of Co and Ni in both soils. In addition to the results reported, further analysis found no detectable concentration of Co and Ni in both substrates (i.e. soil and waste) despite the addition of sulfur dosage in both the substrates with the exception of the N soil where Co concentration was found to be 0.6 mg/kg in soil treated with 8.0 g/kg of sulfur.

The water extractable fraction of Ni in the RBMR waste soil seemed to have decreased along with the addition of sulfur dosage except in soil where sulfur was added up to 2.0 g/kg; in this case, no Ni fraction was detected. Furthermore no extractable fraction of Ni was detected in the N soil (Figure 4.7). Generally one would say that the addition of sulfur decrease the concentration of Ni and Co extractable with water in both the substrates. However, due to measurement errors related to sampling of the soil, one would be cautious in evaluating the above results.

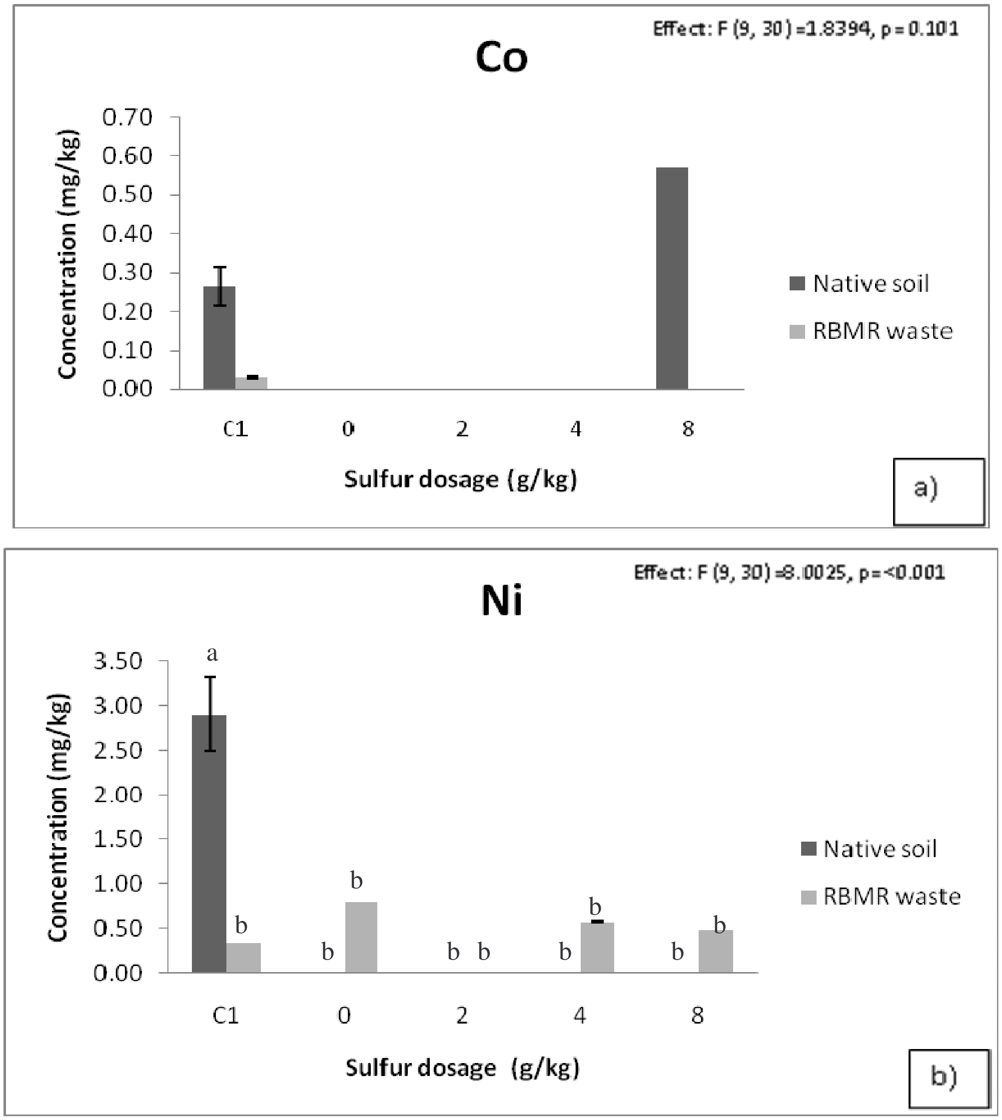


Figure 4.7: De-ionized water extractable Co and Ni concentration in both substrates i.e. a) RBMR waste soil and b) N soil before and after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at P=<0.001 (one-way ANOVA).

Note: C 1 = control N soil/ RBMR waste soil before experiment, (0 g of S/kg) **=** control N soil/ RBMR waste soil during experiment, S 2.0 g of S/kg = N soil and RBMR waste **both** treated with 2.0 g of S/kg of soil , 4.0 g of S/kg = N soil and RBMR waste both treated with 4.0 g of S/kg of soil, 8.0 g of S/kg = N soil and RBMR waste soil treated with 8.0 g of S/kg of soil. N soil = Native soil, RBMR waste soil= Rustenburg Base Metal Refinery waste.

4.3.2. Weak acid extractable Co and Ni

After the water extraction, the same soil was again extracted using acetic acid. The results are showed in Figure 4.8. Acetic acid extractable fractions of Co and Ni in the RBMR waste soil without S addition (control group (C1)) were found to be 1.5 and 16 mg/kg, respectively while the result for Co and Ni in the N soil control group (C1) was found to be 0.7 and 2.6 mg/kg, respectively. The acetic acid extractable fractions of Co and Ni recorded for the RBMR waste soil was found to be higher compared to the N soil. The results found for Co after the plant growth experiments showed that the N soil with no sulfur added had about 1.9 mg/kg higher than the concentration of Co reported before the experiment.

In addition, the N soil with no sulfur added was found to have higher Co concentrations than the RBMR waste soil where sulfur was not added. Subsequently Ni concentration extractable with acetic acid in the N soil with zero sulfur dosage was found to be 6.8 mg/kg. This result was found to be higher than those reported for the N soil control group (C1) before the growth experiment, which was found to be 2.6 mg/kg. However, there was no detectable Ni extractable with acetic acid in the RBMR waste soil with zero sulfur dosage after plant growth experiments.

The concentration of Co extractable with acetic acid was found to increase along with the addition of sulfur in both the substrates. Concentration of Co in the N soil increased from 2.2 to 2.8 mg/kg with a sulfur dose of 0 to 8 g/kg, whereas in the RBMR waste soil, it was observed to increase from 1.9 to 3.8 mg/kg. The influence of sulfur dosage on the acetic acid extractable Ni concentration did not give a clear pattern as compared to the Co. Although in the RBMR waste soil, the addition of sulfur had an influence in the concentration of Ni extracted with acetic acid. In the N soil, the same observation was seen as for the RBMR waste soil. However, the concentration of Ni extracted from the N soil was less than in the RBMR waste soil. The increase in the concentration of Ni extracted from the N soil with an increase in sulfur dosage was not very pronounced.

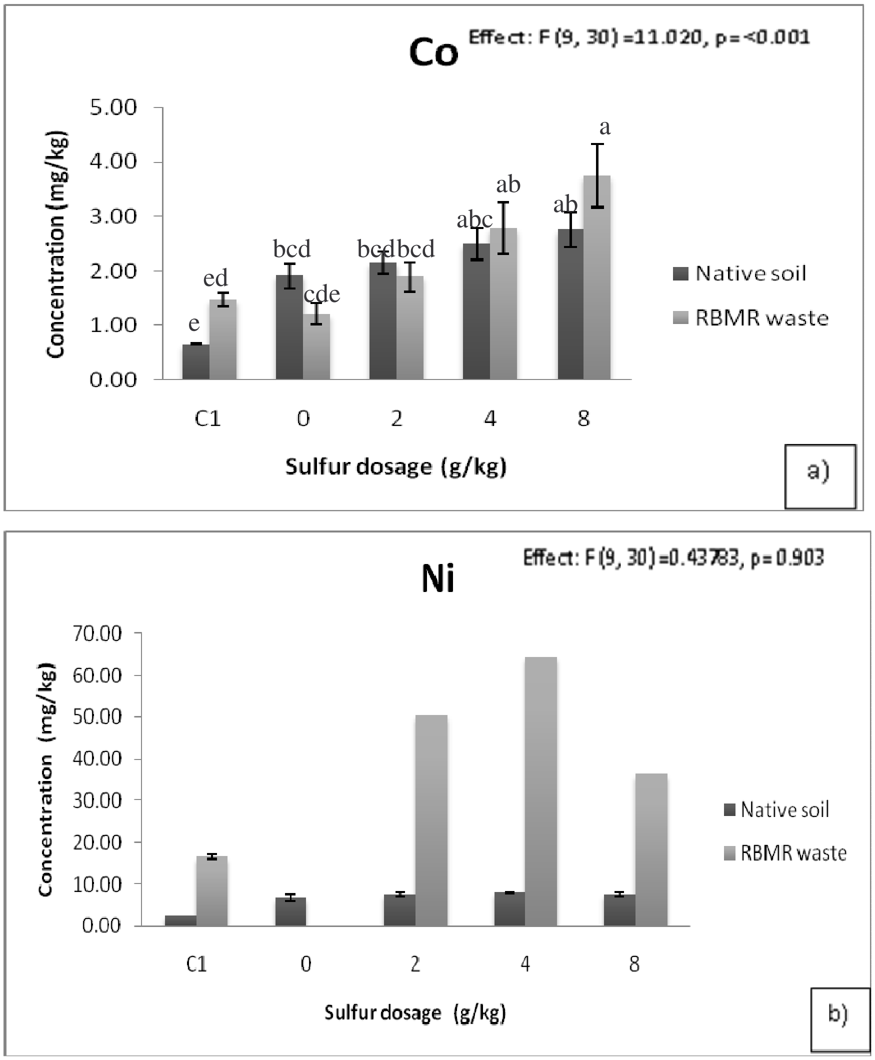


Figure 4.8: Acetic acid extractable Co and Ni concentration both substrates i.e. a) RBMR waste soil and b) N soil before and after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p=<0.001 (one- way ANOVA).

4.3.3. Extraction of Co and Ni bound to oxides

The soil residue remaining after extraction with acetic acid was again extracted using hydroxylamine for the determination of Co and Ni bound to oxides. The hydroxylamine extractable fractions of Co and Ni are shown in Figure 4.9. The results found for Co before the plant growth experiments (i.e. N soil control group (C1)) was found to be 31.4 mg/kg and similar to the concentration of Co obtained for the RBMR waste soil control group (C1). After the plant growth experiments have been completed, there was no detectable concentration of Co in the N soil treated with different sulfur dosages. The Co concentration found in the control group after the plant growth experiment (i.e. RBMR (0 g of S/kg)) was higher than the Co concentration obtained for the RBMR waste soil control group (C1) before plant growth experiments.

Hydroxylamine extractable fraction of Ni before experiment was found to be more in the N soil (i.e. N soil (C1)) than in RBMR waste soil (i.e. RBMR waste soil (C1)). After plant growth experiments, Ni concentration extractable with hydroxylamine was found to be more than Ni concentration obtained before the experiment in both soils. Ni concentration was higher in the RBMR waste soil treated with sulfur than in the N soil. The same was seen in the RBMR waste soil with zero addition of sulfur.

Figure 4.9b clearly shows that an addition of sulfur in both soils might have contributed to the increases Ni extractable with hydroxylamine compared to the control group. It should also be noted that increase in Ni extractable with hydroxylamine for both N soil and RBMR waste soil not treated with sulfur after the plant growth could have been attributed by other factors other than the addition of sulfur. The results obtained for the RBMR waste soil suggest that Ni concentration extracted with hydroxylamine increased with sulfur dosage; however, continuous addition of sulfur to 8.0 g/kg decreased Ni concentration in the RBMR waste soil. For the N soil, added sulfur increased slightly the concentration of Ni extracted; continuous addition of sulfur i.e. 4 g/kg to 8 g/kg decreased the concentration of Ni extractable with hydroxylamine. Again this observation needs to be treated with caution because of errors which could be coming from sampling.

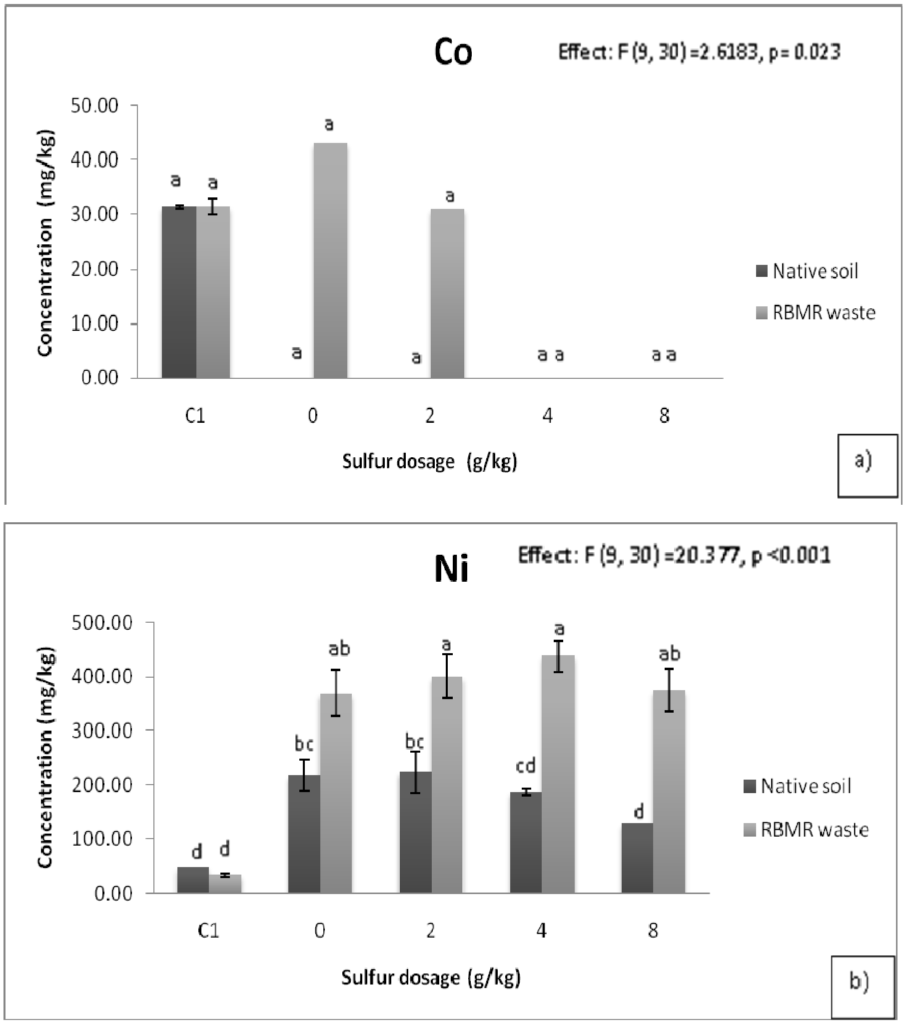


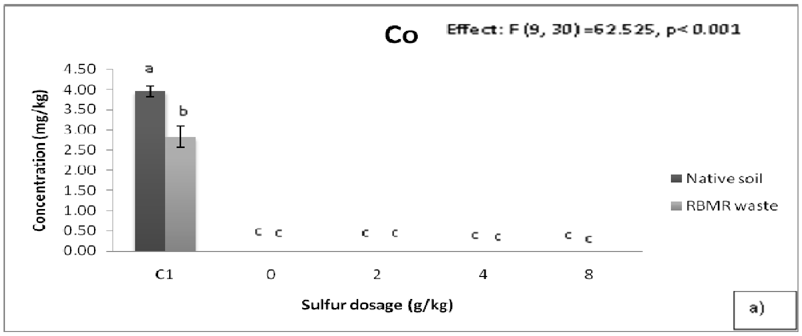
Figure 4.9: Hydroxylamine extractable Co and Ni concentration in both substrates i.e. a) RBMR waste soil and b) N soil before and after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p=<0.001 and p= 0.023 (one- way ANOVA).

4.3.4. Extraction of Co and Ni bound to organic matter

The residue remaining after extraction with hydroxylamine was again extracted using hydrogen peroxide and ammonium acetate for determination of Co and Ni bound to organic matter. The results of this extraction are shown in Figure 4.10. Figure 4.10a shows that Co concentration extracted with this solution was more in the N soil than the

RBMR waste soil. However, no detectable Co concentration was found in both substrates treated with different sulfur dosage after plant growth.

Hydrogen peroxide and ammonium extractable fraction of Ni in both substrates [i.e. N soil and RBMR waste soil control group (C1)] was found to be lower (10 and 46.6 mg/kg, respectively) compared to those results treated with different sulfur dosages. Ni concentration obtained for both the N (0 g of S/kg) and RBMR (0 g of S/kg) soils were higher than that of the N soil and RBMR waste soil control group (C1). Hence sulfur treatment had no influence on Ni fraction extractable with ammonium acetate. The overall extraction of Ni with hydrogen peroxide and ammonium acetate as indicated in Figure 4.10b revealed that RBMR waste soil had higher Ni concentration as compared to the N soil



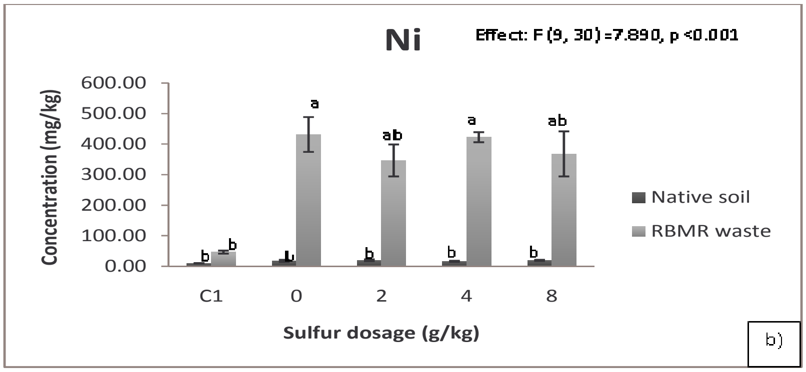


Figure 4.10: Hydrogen peroxide and ammonium acetate extractable Co and Ni concentrations in both substrates i.e. a) RBMR waste soil and b) N soil before and after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p= <0.001 (one-way ANOVA).

4.4. Aqua regia extraction of Co and Ni

Aqua regia was used in this research to extract Co and Ni that strongly bound to soil matrices such as silicates. The results of this extraction are shown in Figure 4.11 (a) and (b). The results shown indicate that the RBMR waste soil [i.e. RBMR waste soil control group (C1) (4000 mg/kg)] had higher concentration of Ni compared to the N soil [i.e. N soil control group (C1) (2900 mg/kg)]. However, the results obtained after the experiment indicated that Ni strongly bound to soil was lower than the concentration of Ni obtained before the experiment in both the soils. The concentration of Co and Ni recorded for the control group sample i.e. the RBMR waste soil (0 g of S/kg) and N soil (0 g of S/kg) were 661 mg/kg, 1160 mg/kg and 7 mg/kg and 140 mg/kg, respectively. However, concentration of Co on the control group i.e. RBMR waste soil (0 g of S/kg) and N soil (0 g of S/kg) was found to be lower than those reported before the experiment. Both the substrates treated with sulfur recorded to be more than the Co obtained before the experiments. It is important to note that this could be an outlier.

The results for Ni obtained for both substrates (i.e. RBMR waste soil and N soil) treated with 2.0 g of S/kg of sulfur was found to be 1181.5 mg/kg, and 1852.5 mg/kg respectively, higher than the result for Ni concentration obtained for control group during the experiment (i.e. RBMR (0 g of S/kg) and N (0 g of S/kg). However, the concentration of Co for soil treated with 2.0 g of S/kg of sulfur (i.e. N soil) was found to be 157 mg/kg and no trace of Co extractable through Aqua regia on RBMR waste soil treated with 2.0 g of S/kg was identified after the experiment.

The results for Ni concentrations obtained for the N soil treated with 4.0 g of S/kg of sulfur was lower than the results obtained for N soil treated with 2.0 g of S/kg of sulfur. RBMR waste soil treated with 4.0 g of S/kg of sulfur showed an increase in Ni concentration (1334 mg/kg) as compared to RBMR waste soil treated with 2.0 g of S/kg of sulfur; however, there was a decline in Ni concentration extractable through aqua regia on the RBMR waste soil treated with 8.0 g of S/kg of sulfur. The N soil treated with 8.0 g of S/kg of sulfur showed an increase in Ni concentration as compared to N soil treated with 4.0 g of S/kg of sulfur.

Although there were some changes in Co and Ni concentrations extractable through aqua regia it is difficult to tell at this point if that has something to do with sulfur added in both the soils. From what is expected, concentration of this fraction should remain the same and should not increase since no external addition of these metals to the soils was done. Variations in the measurements in Figure 4.11 are due to heterogeneity of the metals in the two soils and more samples were needed.

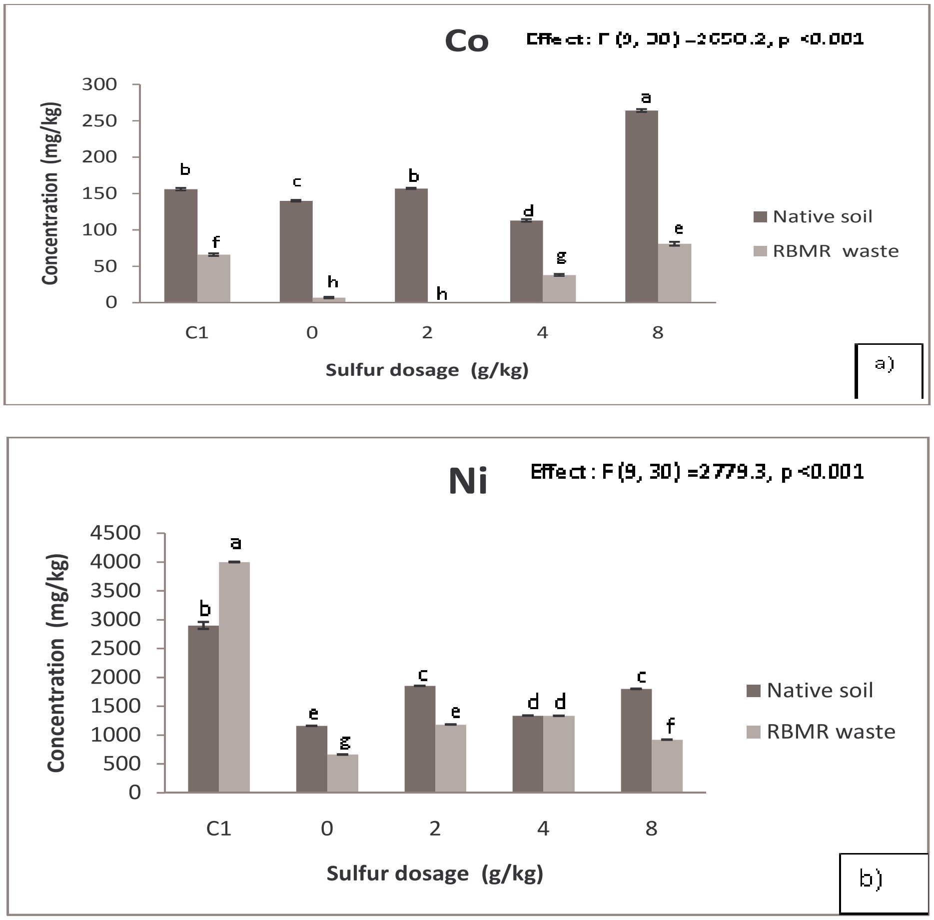


Figure 4.11: Aqua regia extractable Co and Ni concentrations in both substrates i.e. a) RBMR waste soil and b) N soil before and after plant growth experiment. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p=<0.001(one- way ANOVA).

4.5. Growth of *B. coddii* in different soils treated and untreated with sulfur

The results for the mean plant height measurements and the number of leaf counted during the plant growth experiments are shown in Figure 4.12 and 4.13. The results obtained from day 1 to 205 represent measurements taken before sulfur was added in both soils. Results obtained from day 219 to 266 represent measurements taken after the addition of sulfur in both soils. The plant height results (Figure 4.12) indicate that plants from both the substrates were growing at the same rate until day 94 (i.e. ±3 months). The measurements taken on day 108 indicate a significant increase in the B. coddii planted in the N soil as compared to the B. coddii planted in the RBMR waste soil. From day 150 to day 266 plants from both the substrates were observed slightly stable in plant height growth with minimum changes to the B. coddii planted in the N soil. Although the height measurement was found to be stable from day 150 to 226, the number of leaves however, increased from days 150 to 266 (Figure 4.13) despite the plant heights reaching a plateau. This was more pronounced in the plants grown in the N soils.

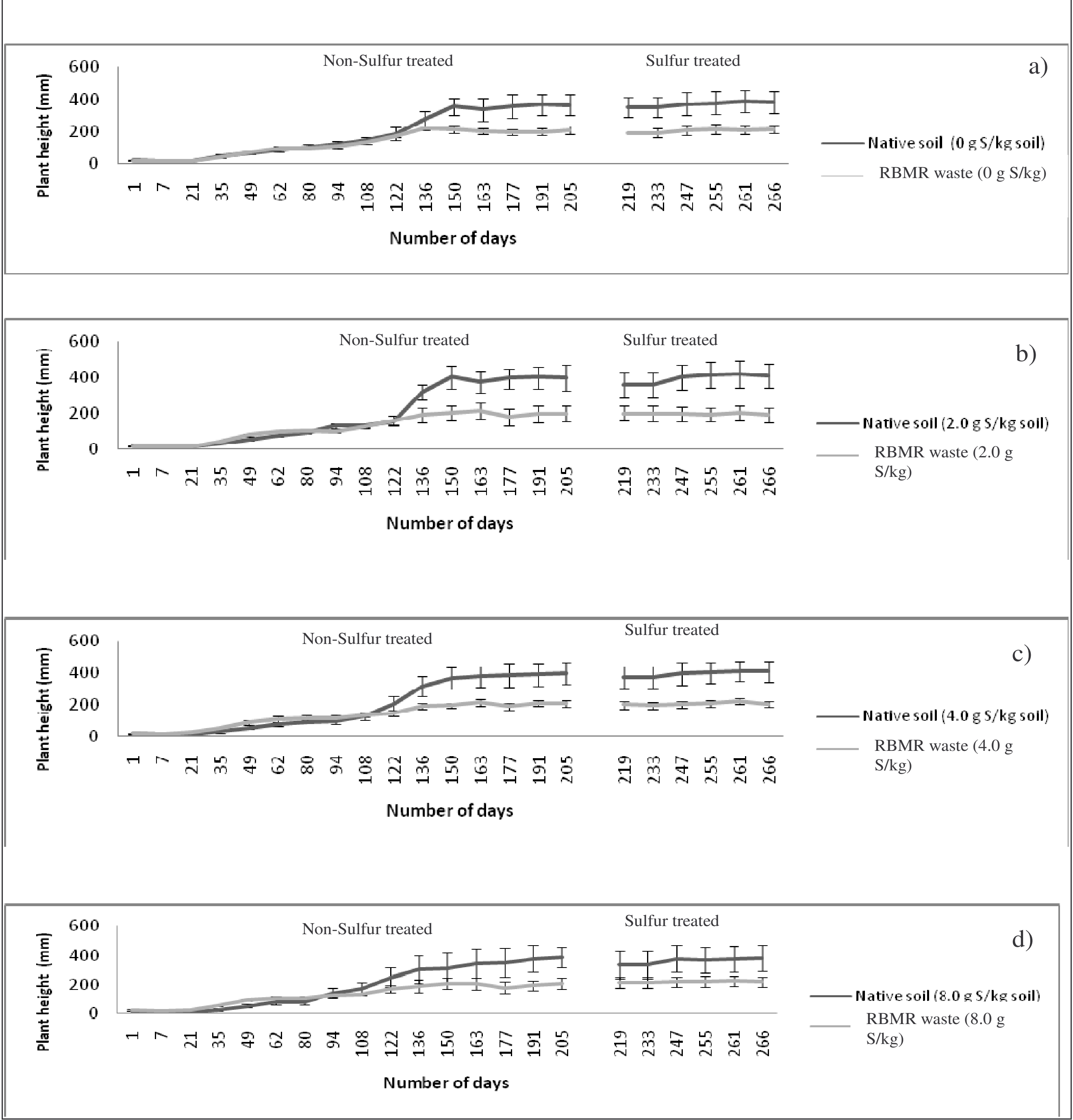


Figure 4.12: Measured plant height measurement over the experiment period. Values represent mean ± SE.

Note:

RBMRp = plants grown in the RBMR waste soil and Np = plants grown in the N soil; Np (0 g of S/kg) control for native soil during the experiment, RBMRp (0 g of S/kg) control for RBMR waste soil during experiment, Np (2.0g of S/kg) = native soil treated with 2.0 g of sulfur, RBMRp (2.0 g of S/kg) = RBMR waste soil treated with 2.0 g of sulfur, Np (4.0 g of S/kg) = native soil treated with 4.0 g of sulfur, and RBMRp (4.0 g of S/kg) = RBMR waste soil treated with 4.0 g of sulfur, Np (8.0 g of S/kg) = native soil treated with 8.0 g of sulfur, RBMRp (8.0 g of S/kg) = RBMR waste treated with 8.0 g of sulfur, Results reported here are mean values for each treatments. Full results are reported in Appendix 8.

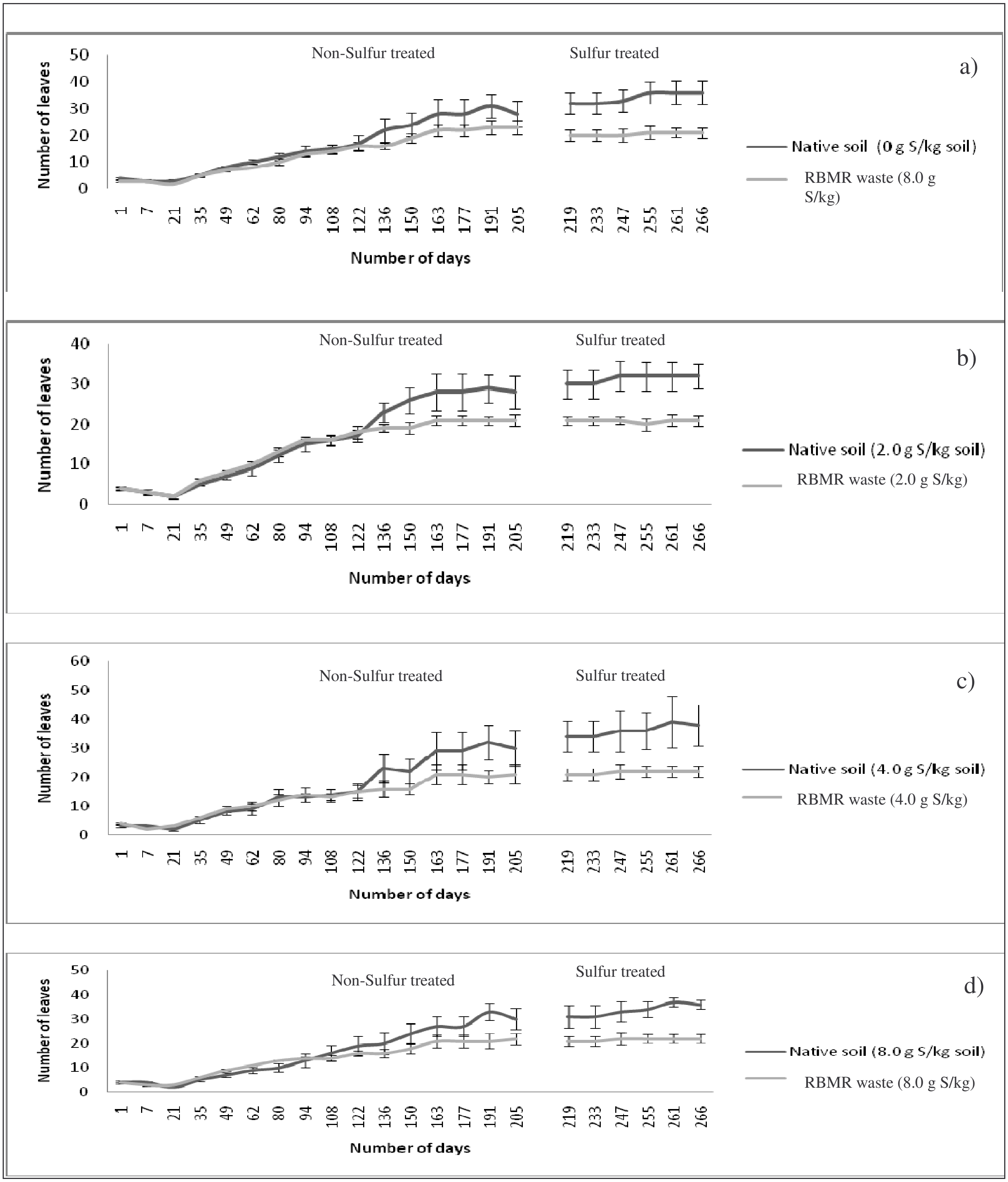


Figure 4.13: Number of plant leaves over the experiment period (for more details see Figure 4.12). Values represent mean ± SE).

4.6. *B. coddii* stress determination

During the growth experiment 32 plants were assessed for their vegetation indices (VIs) i.e. the red edge normalized difference vegetation index ($mNDVI_{705}$) and plant senescence reflectance index (PSRI). Indices were derived from spectral radiometry of leaf surfaces. This was done in order to determine plant stress both the substrates (i.e. N soil and RMBR waste) after sulfur were added into both substrates. The results are reported in Figure 4.14.

The results reported for the $mNDVI_{705\ (1)}$ for both the substrates were within the range of 0.2 to 0.5. Therefore, both plants were observed to absorb most of the visible light and reflect larger portions of the near infrared. However, *B. coddii* grown in the N soil had higher $mNDVI_{705\ (1)}$ in comparison to the *B. coddii* grown in the RBMR waste soil. *B. coddii* in the RBMR waste soil (Figure 4.14 a & b), there was no change in the $mNDVI_{705}$ between sulfur dosages. However, the plants treated 4.0 g of sulfur in the N soil decreased in $mNDVI_{705}$. The results obtained 1 day before harvesting (i.e. $mNDVI_{705\ (3)}$) also reported no difference $mNDVI_{705}$ in *B. coddii* except *B. coddii* planted in the soil treated with 2.0 g of sulfur that showed a decline in $mNDVI_{705}$ value.

The PSIR results obtained (Figure 4.14 c & d) for the plants planted in the RBMR and N soils revealed that their canopy values were within the normal range value of -0.1 to 0.2, regarded as the range of healthy plants. PSIR results obtained before sulfur treatment for the plants in the RBMR waste soil were found to be within the normal range for healthy plants. The results obtained for the PSIR in the plants after sulfur was added in the soil showed an increase in the PSIR value. However, the value was still found to be within the common range of the healthy plant. The *B. coddii* planted on the RBMR waste soil treated with 8.0 g of sulfur was observed unchanged. This was similar to before sulfur was added and even 1 day before plant harvest, despite yellowish colour observed. The results obtained for PSIR before harvest suggests that *B. coddii* planted in the RBMR waste soil treated with 4.0 and 8.0 g of sulfur) were still within the normal range with no significant increase on the PSIR value. In general, the addition of sulfur was observed to have decreased the PSIR values in both the substrates. The decrease in PSIR value was not significant

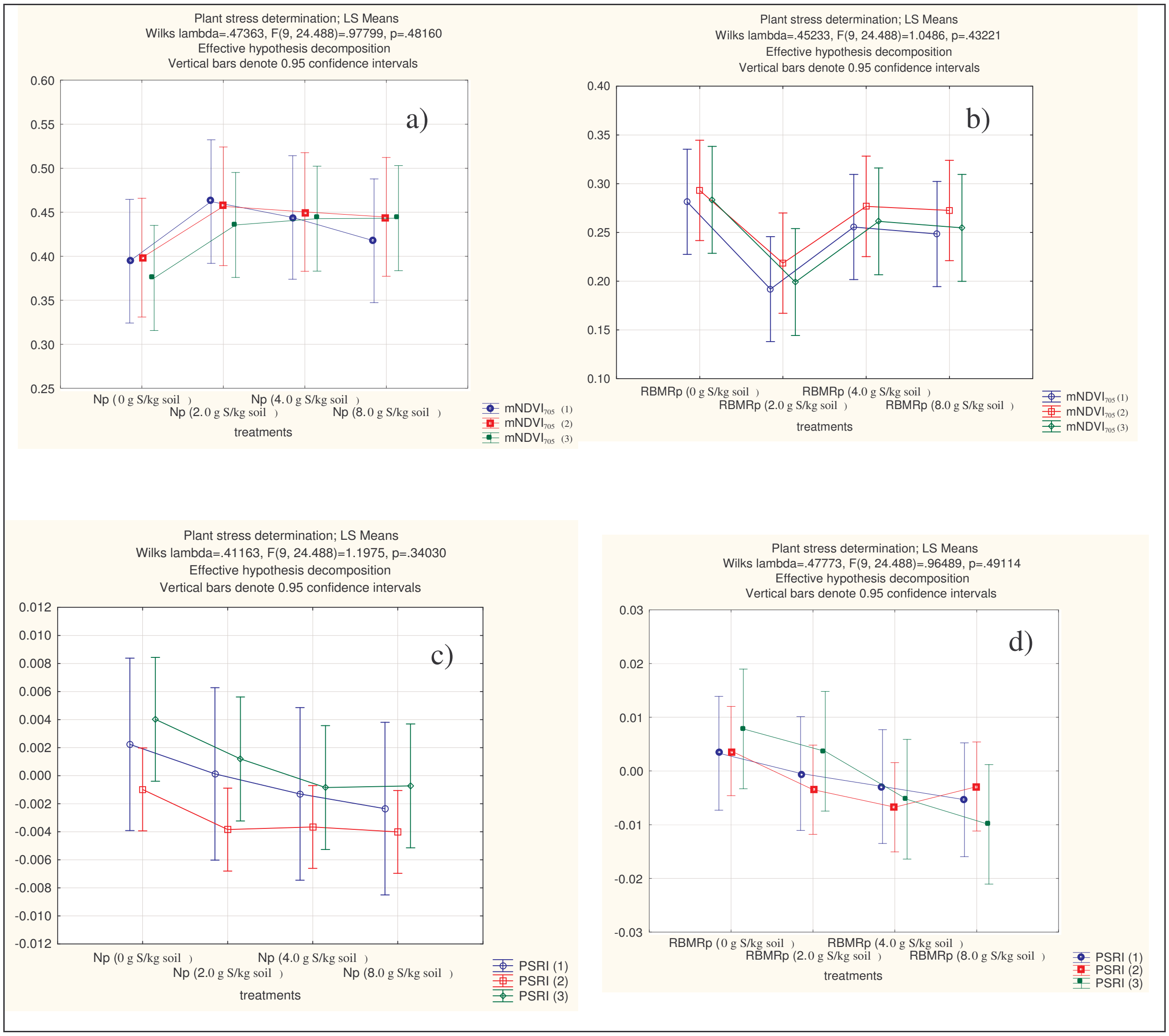


Figure 4.14: Measured mNDVI$_{705}$ and PSRI: (a) mNDVI$_{705}$ measurements for Native (N) soil, **(b)** mNDVI$_{705}$ measurements for RBMR waste soil, **(c)** PSIR measurements for N soil and **(d)** PSIR measurements for the RBMR waste soil.

Notes:

mNDVI$_{705\ (1)}$ and PSIR $_{(1)}$ = mNDVI$_{705}$ and PSIR measured before sulfur treatment respectively, mNDVI$_{705\ (2)}$ and PSIR $_{(2)}$ = mNDVI$_{705}$ and PSIR measured 14 days after sulfur treatment respectively, mNDVI$_{705\ (3)}$ and PSIR $_{(3)}$ = mNDVI$_{705}$ and PSIR measured 1 day before harvesting respectively. RBMRp = plants growth in the RBMR waste soil and Np = plants growth in the N soil.

4.7. Dry matter production (yield) and Root: Shoot ratios of *B. coddii* plant

To estimate plant productivity (dry matter production), and the total concentration of Ni and Co accumulated by the entire *B. coddii* organs (root, stem and leaves), the dry mass of the plant parts was determined. The results for the comparison of dry matter production within the different part of the plants and with various sulfur dosages are shown in Figures 4.15, 4.16 and 4.17. The comparison with total biomass is shown in Figure 4.21.

There is significant difference in leaf and stem dry mass in two substrates. The leaf structure of the *B. coddii* planted on the RBMR waste soil treated with 2.0 and 4.0 g of sulfur was found increased with the increase in sulfur dosage and further decrease as more sulfur was added, however, the stem structure of the B.coddii in the RBMR waste soil was observed decreased with the increase in sulfur dosage i.e. 2.0 to 4.0 g /kg. The results further suggest that the increase in sulfur has the potential to increase stem dry mass in the RBMR waste soil (Figure 4.15 and 4.16). For the N soil the results obtained for the leaf dry mass was observed stable along with the increase in sulfur dosage from 2 to 8.0 g/kg, however, the stem dry mass was observed increased with the increase in sulfur dosage (i.e. 2.0 to 4.0 g/kg), this results further suggests that further increase in sulfur dosage have the potential to decrease the stem dry mass.

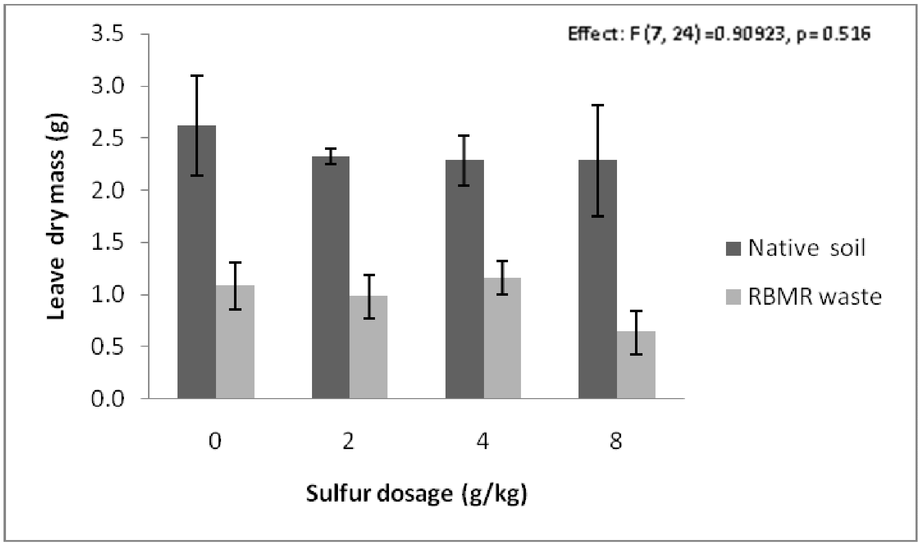


Figure 4.15: leaf dry mass of *B. coddii* after sulfur addition in both substrates. Values represent mean ± SE.

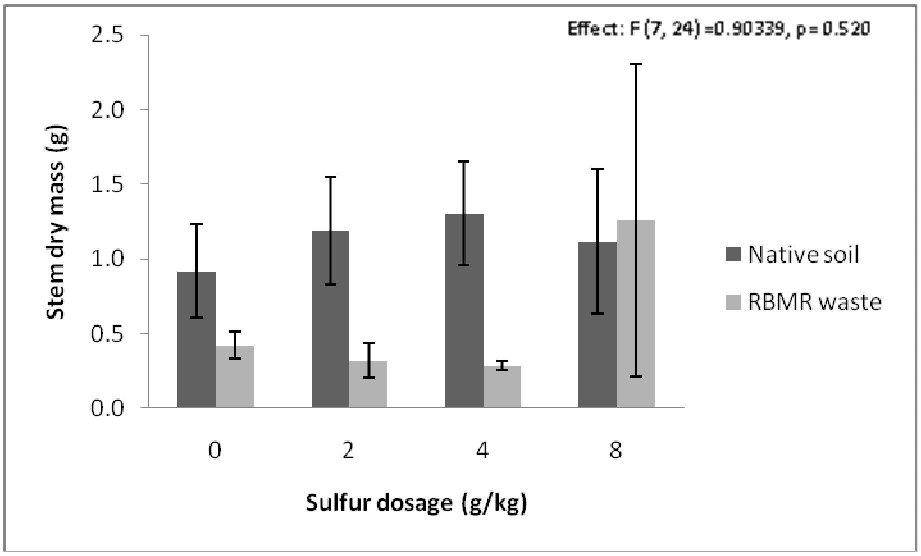


Figure 4.16: Stem dry mass of *B. coddii* after sulfur addition in both substrates. Values represent mean ± SE.

There was a significant difference between the root mass of the plants grown on the N soil (one-way ANOVA, p= 0.004). The result reported for the dry root mass for the plants grown in N soil showed that root structure decreased with increase in sulfur dosage from 2.0 to 8.0 g of sulfur (Figure 4.17), where as the root structure for the plant grown in the RBMR waste soil showed that root structure increased with the increase in sulfur dosage and further decrease as more sulfur is added (Figure 4.17). Though roots structure of the

B.coddii planted on the RBMR waste soil was observed increased, there was no significant difference in root mass.

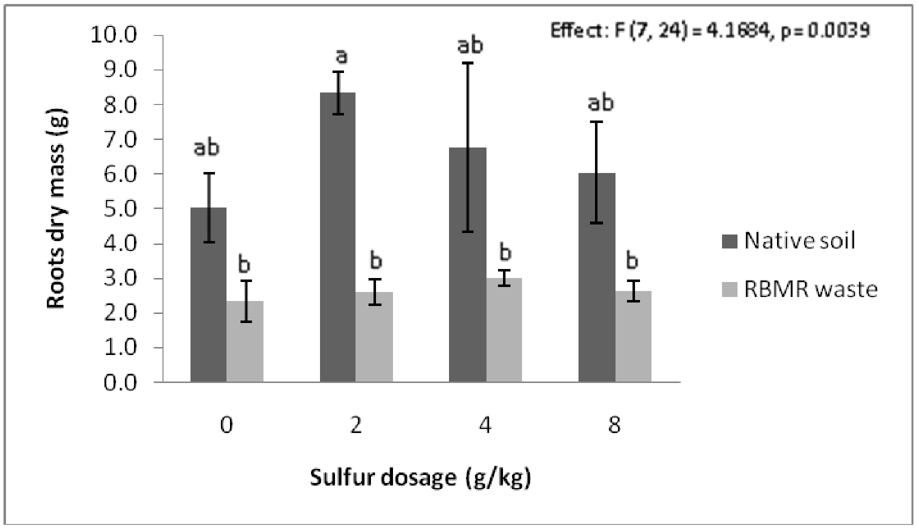


Figure 4.17: Root dry mass of *B. coddii* after sulfur addition in both substrates. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p= 0.003.

The total dry matter comparison shown in Figure 4.18 reveals that the *B. coddii* planted on the N soil had higher biomass than to the *B. coddii* planted on the RBMR waste soil. The *B. coddii* biomass increased relative to the addition of sulfur in the N soil up to of 2.0 g/kg. The total biomass in RBMR waste soil was constant up to 4.0 g S/kg soil although increased slightly. There was no significant different in total dry mass in both the soil (one-way ANOVA, p= 0.516).

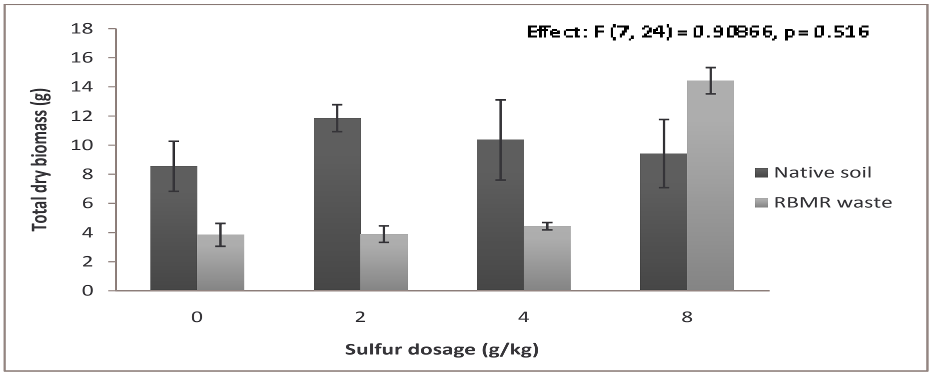


Figure 4.18: Comparison of B. coddii total biomass in different substrates with various sulfur treatments. Values represent mean ± SE.

Table 4.2: Mass proportion and root: shoot ratios of *B. coddii*.

	Root mass proportion (SE)	Shoot mass proportion (SE)	Root/ shoot ratio (SE)
N (0 g S /kg soil)	0.59 (0.03)	0.41 (0.03)	1.46 (0.16)
N (2.0 g S /kg soil)	0.71 (0.01)	0.29 (0.01)	2.39 (0.16)
N (4.0 g S /kg soil)	0.62 (0.06)	0.38 (0.06)	1.84 (0.52)
N (8.0 g S /kg soil)	0.65 (0.02)	0.35 (0.02)	1.85 (0.17)
RBMR (0 g S /kg soil)	0.59 (0.05)	0.41(0.05)	1.56 (0.35)
RBMR (2.0 g S /kg soil)	0.68 (0.05)	0.32 (0.05)	2.26 (0.38)
RBMR (4.0 g S /kg soil)	0.67 (0.03)	0.33 (0.03)	2.16 (0.31)
RBMR (8.0 g S /kg soil)	0.62 (0.08)	0.38 (0.08)	2.01(0.53)

The relative growth rates of roots and shoots are important parameters of dry matter production. This parameter reflects, for instance, the proportion of total dry matter gain that is reinvested in photosynthetic tissue. Roots and shoots are functionally interdependent and these two systems maintain a dynamic balance of *B. coddii* biomass which reflects relative abundance of above ground resources compared with the root-zone resources. Root: shoot ratios are thus indicative of *B. coddii* growth response to condition. Table 4.2., indicate that the root mass proportion of *B. coddii* grown in the N soil increased with the increased in sulfur compared to the untreated *B. coddii* and further observed decrease as more sulfur was added to 4.0 g S/kg soil. In addition further increase in sulfur dosage i.e. 8.0 g S/kg soil, root mass proportion was observed increased from 0.62 to 0.65. On the other hand the root mass proportion of *B. coddii* grown in the RBMR waste soil was observed decreased from 0.68 to 0.62 with the increase in sulfur dosage i.e. 2.0 to 8.0 g S/kg soil. However, the root mass proportion observed in both the soil treated with sulfur dosage was found to be more than those which were not treated with sulfur.

The shoot mass proportion of *B. coddii* grown in the RBMR waste soil was observed increased along with the increase in sulfur dosage, hence the shoot mass proportion of *B. coddii* grown in the N soil was observed decreased along with the increase in sulfur dosage i.e. 2.0 g S/kg soil. However, an increase in sulfur dosage (i.e. 4.0 to 8.0 g S/kg soil) did not results to the change in the shoot mass proportion as this was observed to be slightly stable. From the mass proportion as indicated in Table 4.2., it is clear that *B.*

coddii had heavier roots mass proportion than the shoots mass proportion. As indicated in Figure 4.19., and Table 4.2., the root: shoot ratio of *B. coddii* in the N soil was observed increased with the increase in sulfur. Whereas the root: shoot ratios of the *B. coddii* grown on the RBMR waste soil was observed decreased with the increase in sulfur addition.

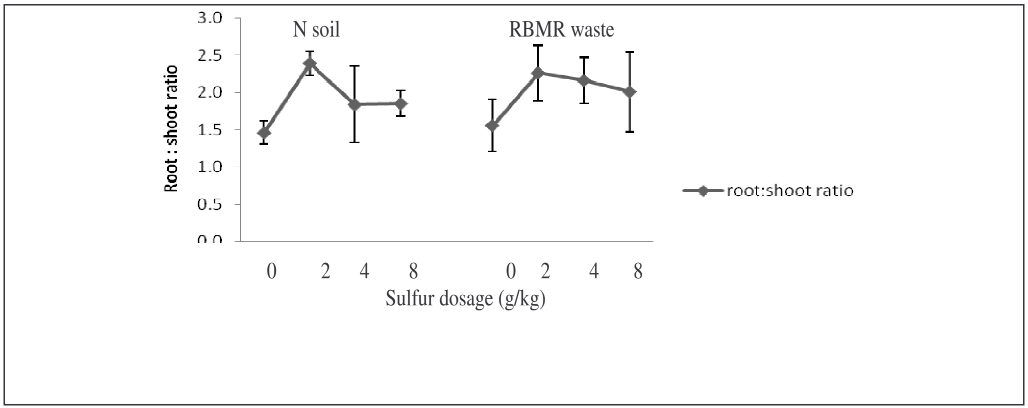


Figure 4.19: Roots: shoot ratio. Values represent mean ± SE.

4.8. Ni and Co concentrations in *B. coddii* organs (i.e. roots, stem and leaf) after addition of sulfur

Comparison of Ni and Co in different organs of *B. coddii* is shown in Figures 4.20, 4.21 and 4.22.

B. coddii accumulates detectable concentration of Ni and Co in the leaves despite the treatment in both the soils (Figure 4.20). The results revealed that significant concentration of Ni and Co are accumulated in leaf organs as compared to other organs of the *B. coddii*. Notably more Ni concentration is accumulated in the leaves of the *B. coddii* from the N soil than in RBMR waste soil (one –way ANOVA, p = 0.03). There was significantly different in Ni concentration in *B.coddii* untreated with sulfur with those treated with sulfur, however, *B.coddii* treated with 2 and 4 g S/kg soil were not significantly different. The concentration of Ni in the leaf organs from the N soil ranged from 3392 to 5025 mg/kg while from the RBMR waste soil was 780 to 1512 mg/kg. The same trend was observed for the Co concentration wherein plants grown in the N soil had generally higher concentration of Co in the leaves compared to those in the RBMR waste soil.

The accumulated concentration of Co from the plants grown in RBMR waste soil ranged from 14 -432.3 mg/kg. The concentration of Co in the N soil was found ranging from 28 - 114 mg/kg. When comparing the influence of sulfur on metal accumulation, results obtained for the plant grown in N soil treated with 2.0 g of sulfur was found to be 5025 mg/kg, showing an increase in Ni concentration in the leaves followed by a decrease in Ni concentration as more sulfur was added into the soil (Figure 4.20a). These results suggest that there is an optimum dosage of sulfur required for Ni uptake by *B. coddii* (i.e. 2.0 g of sulfur). Beyond this dosage it does suggest that sulfur reduces the Ni uptake by the plant.

Looking at the accumulation of Ni in leaves from plants grown in RBMR waste soil; results suggest that addition of sulfur in the RBMR plants reduces accumulation of Ni in the leaf (linear regression, $r^2 = 0.0986$, $p = 0.254$). These results suggest that sulfur did not have significant effect on Ni availability in the leaves. The results obtained for Co indicate a negative correlation in the concentration of Co concentration detected in leaves relative to the amount of sulfur added into the N soil (linear regression, $r^2 = 0.1172$, $p = 0.194$). However, looking at the trend without considering statistic results it is not clear from the results if the decrease was sulfur dependent. The addition of 2.0 and 4.0 g of sulfur in the RBMR waste soil showed an increase in Co concentration in the leaf (linear regression, $r^2 = 0.0103$, $p = 0.718$).

The concentration of Co recorded to be 432 mg/kg suggest being an outlier and therefore does not give clear picture. Most of the plants that were planted in the RBMR waste soil and treated with sulfur were observed to be unhealthy at the time of harvest compared to those that were planted in the N soil (Figure 4.14 b and d).

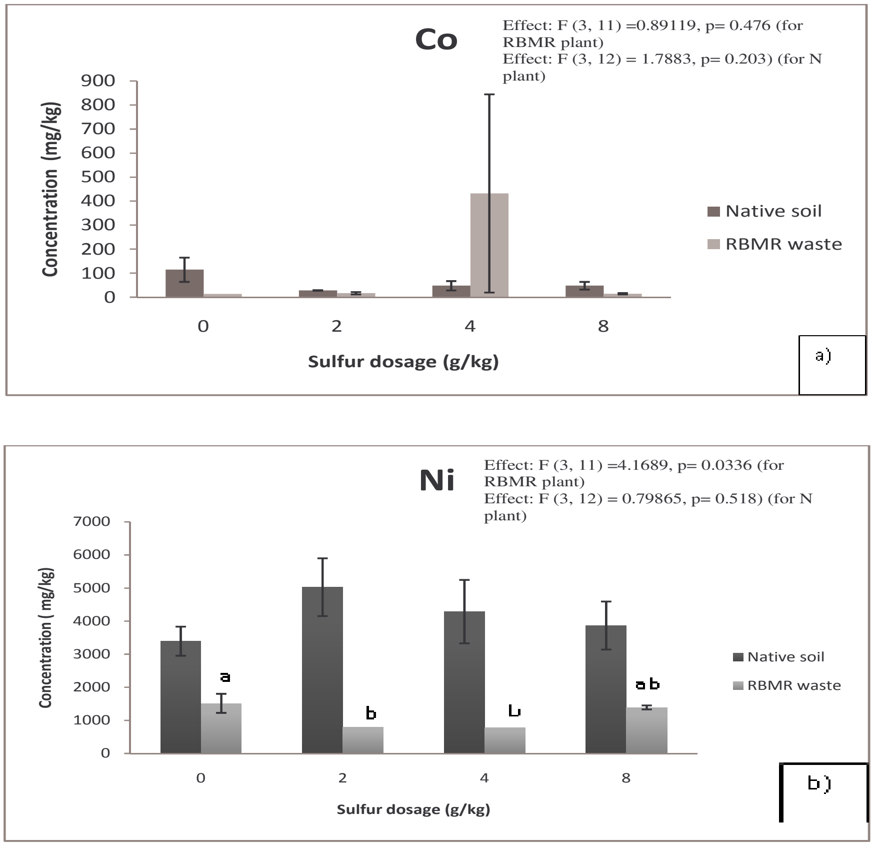


Figure 4.20: Co and Ni concetrations in *B. coddii* leaves from different substrates and with various sulfur dosage. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p<0.03.

The roots were analysed for Ni and Co concentration and used to determine which organs of the *B. coddii* is able to accumulate higher concentrations of Ni and Co (Figure 4.21). Results reflect that in all the *B. coddii* that was planted in the RBMR waste soil, Co was not detectable in their root system. However, *B. coddii* planted in the N soil with various sulfur dosages accumulated a small fraction of Co concentration in their root system. In this soil the concentration of Co accumulated decreased with increase in sulfur dosage (Figure 4.21b) (liner regression, $r^2 = 0.0125$, $p = 0.680$).

The concentration of Ni accumulated in the root system of *B. coddii* grown in the N soil show that the concentration increased with increase in sulfur dosage (Figure 4.21b) (liner regression, $r^2 = 0.5209$, $p = 0.002$). However, the increase was much observed from sulfur dosage of 0 to 4.0 g/kg soil. Thereafter the increase seemed to have levelled. This suggests that perhaps optimum sulfur dosage of 4.0 g/kg soil for Ni accumulation in the root system of *B. coddii* grown in the N soil. For the RBMR waste soil there was also an observed increase in the concentration accumulated Ni with increase in sulfur dosage (liner regression, $r^2 = 0.1009$, $p = 0.231$) but this increase was not very pronounced as compared to the N soil.

Although inconclusive, the addition of sulfur in both soils had an influence on the availability of Ni which can be absorbed by the *B. coddii* root system in particular in the N soil (one – way ANOVA, $p = 0.014$). More Ni was accumulated in the plants grown in the N soil than those grown in the RBMR waste soil; this different was more pronounced in soils added with sulfur.

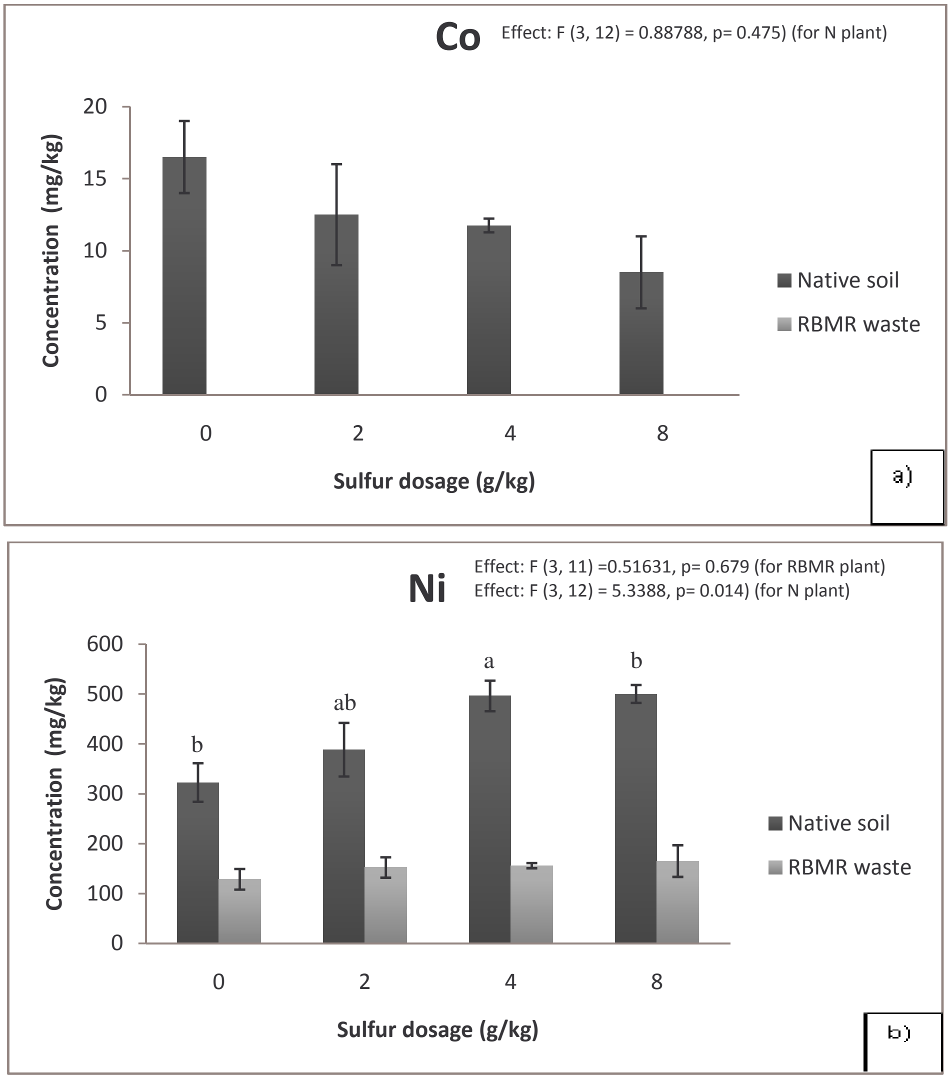


Figure 4.21: Co andNi concetrations in roots of *B. coddii* grown in two substrates with sulfur dosage.Values represent mean ± SE, different letters on the graph indicate significant difference among means at p= 0.014.

The stem results revealed that as the rate of sulfur increased, the concentration of Ni available in the stem also increased (Figure 4.22) (liner regression, $r^2 = 0.5723$, $p = 0.004$, for *B. coddii* planted in the N soil and $r^2 = 0.0560$, $p= 0.377$, for *B. coddii* planted in the RBMR waste soil). This suggests that there was a positive correlation between the additions of sulfur with the availability of Ni accumulated in the *B. coddii* stem. However, the influence of sulfur dosage on Ni accumulation was more pronounced in plants grown in the N soil than the RBMR waste soil (Figure 4.22). The concentration of

Ni accumulated from plants grown in the N soil was much higher than that from RBMR waste soil (one-way ANOVA, p = 0.05). Ni concentration obtained for the plants grown in RBMR waste soil and N soils with no sulfur added (i.e. control treatment) was found to be 208 mg/kg and 962 mg/kg, respectively, which suggest that though sulfur could not be added *B. coddii* could still accumulate certain concentration of Ni from both substrates.

The result obtained for Co on stems from plant grown in both substrates revealed that no Co concentration was detected even with sulfur treatment. The results obtained for Co concentration suggests that large fraction of the Co were transported from the root and stem to the leaf with little Co concentration able to be detected in the stem samples.

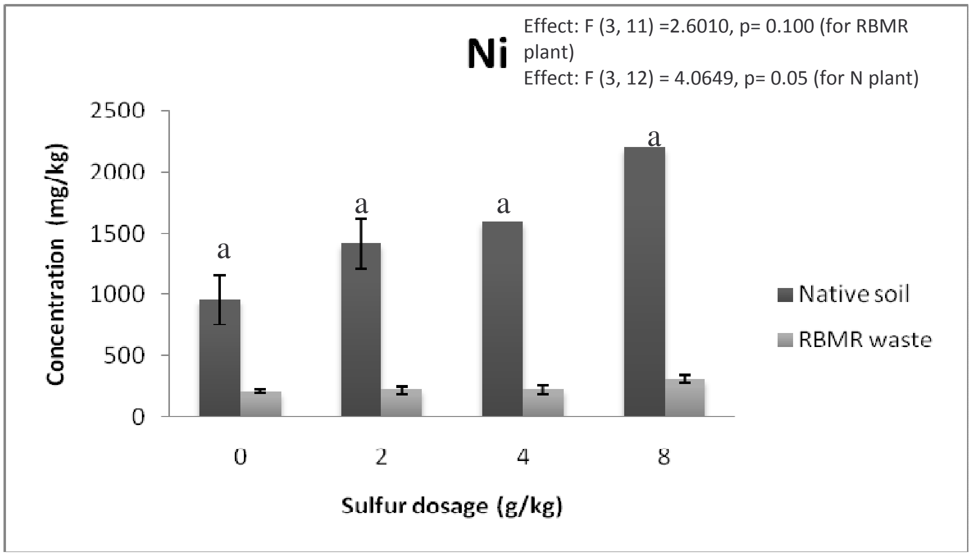


Figure 4.22: Ni concentrations in stem of *B. coddii* grown in both substrates with sulfur dosage. Values represent mean ± SE, different letters on the graph indicate significant difference among means at p= 0.05.

Figure 4.23a and 4.24a showed that concentration of Co in shoot of the *B. coddii* grown in the N soil increased along with the increase in sulfur dosage (i.e. 2.0 g S/kg soil to 4.0 g S/kg soil) and later showed no change as more sulfur was added (i.e. 8.0 g S/kg). The concentration of Co in the shoot of *B. coddii* showed no significant change as the amount of sulfur was added in the RBMR waste soil. The high concentration of Co reported in the shoot of *B. coddii* treated with 4.0 g S/kg soil could be an outlier therefore it does not give a clear picture if sulfur addition had an influence.

Figure 4.23b revealed that concentration of Ni in shoot of *B. coddii* decreased along with the increase in sulfur dosage in the RBMR waste soil, however, the total shoot concentration of Ni in the N soil was observed decreased along with the increase in sulfur (i.e. 2.0 to 4.0 g S/kg soil) and further increased as more sulfur was added (i.e. 8.0 g S/kg soil).

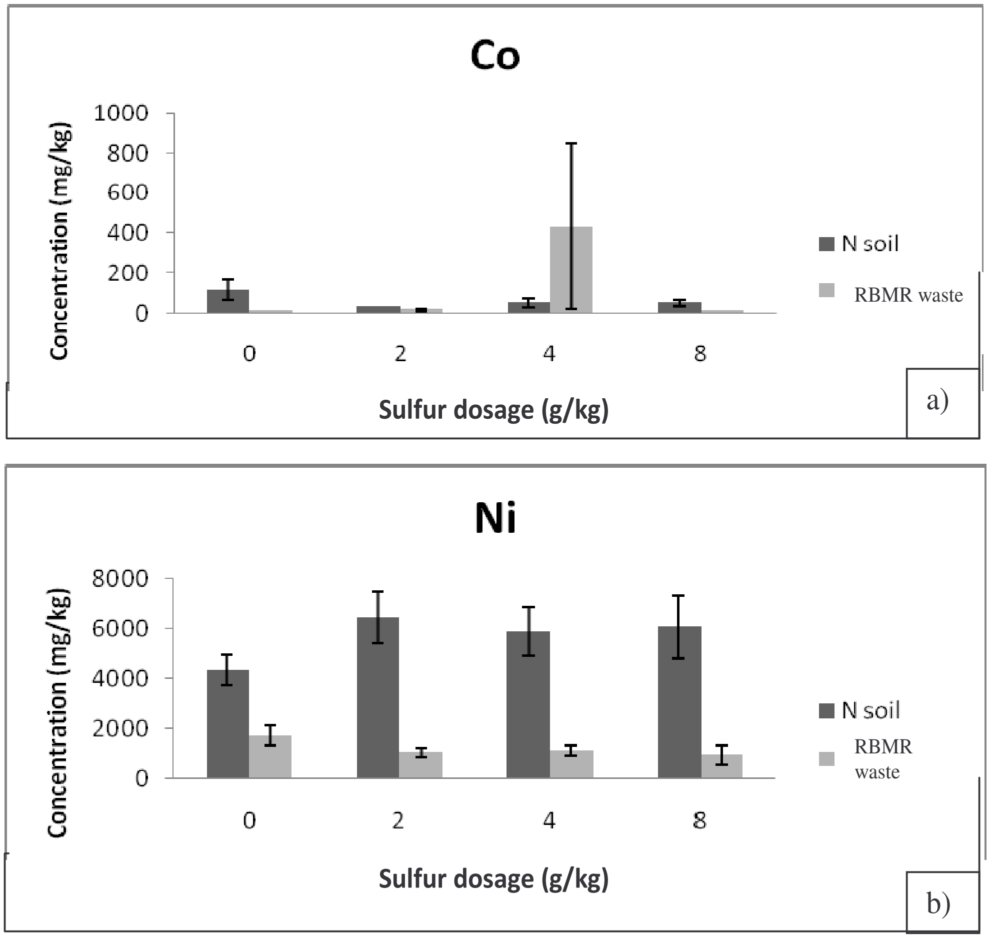


Figure 4.23: Total shoot concentration (stem and leaf) after *B. coddii* growth experiment. Values represent mean ± SE.

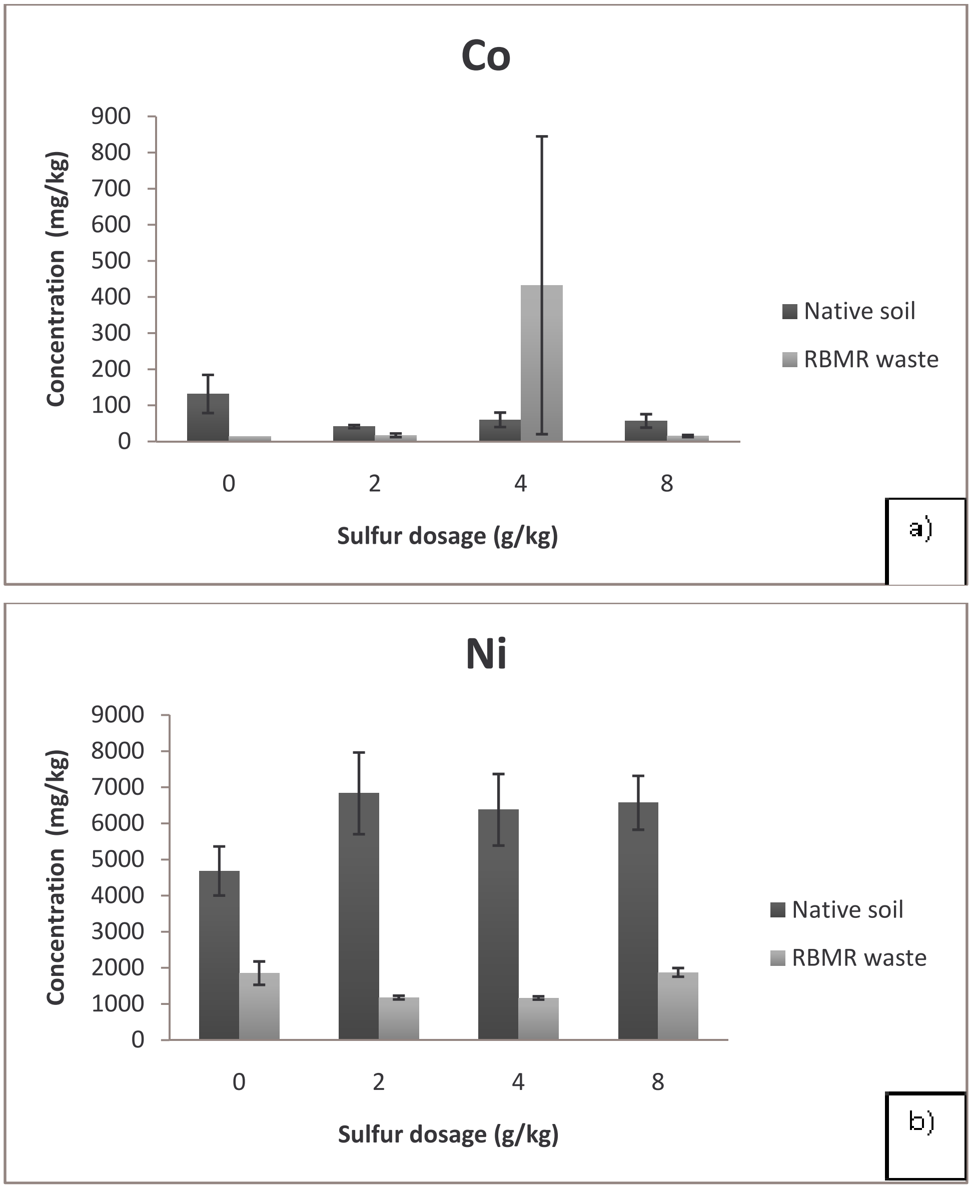


Figure 4.24: Total metal concentrations (Co and Ni) in *B. coddii* after plant growth experiments in both substrates. Values represent mean ± SE.

4.9. Allocation of Ni and Co concentration between *B. coddii* organs.

The results obtained, as indicated in Figures 4.25 and 26, revealed that about 70-80% of Ni concentration absorbed by *B. coddii* plant was accumulated in the leaf structure. With only 5-10% of the Ni accumulated in the stem and the remaining 5 % of Ni was found to be accumulated in the root system. This means that 80% of Ni is likely to be recovered from the soil without killing the plant. This can be done by using the same plant repeatedly until all the available Ni from the soil is absorbed. With regard to Co accumulation in various parts of the plant, 80-90% of the Co was found to be accumulated in the leaf parts with 10 % accumulated in the root system of the N soil. The results obtained revealed that no Co was noticed in the stem in both the soils which means Co was transported to the leaves with no accumulation of Co in the stem (Figure 4.24).

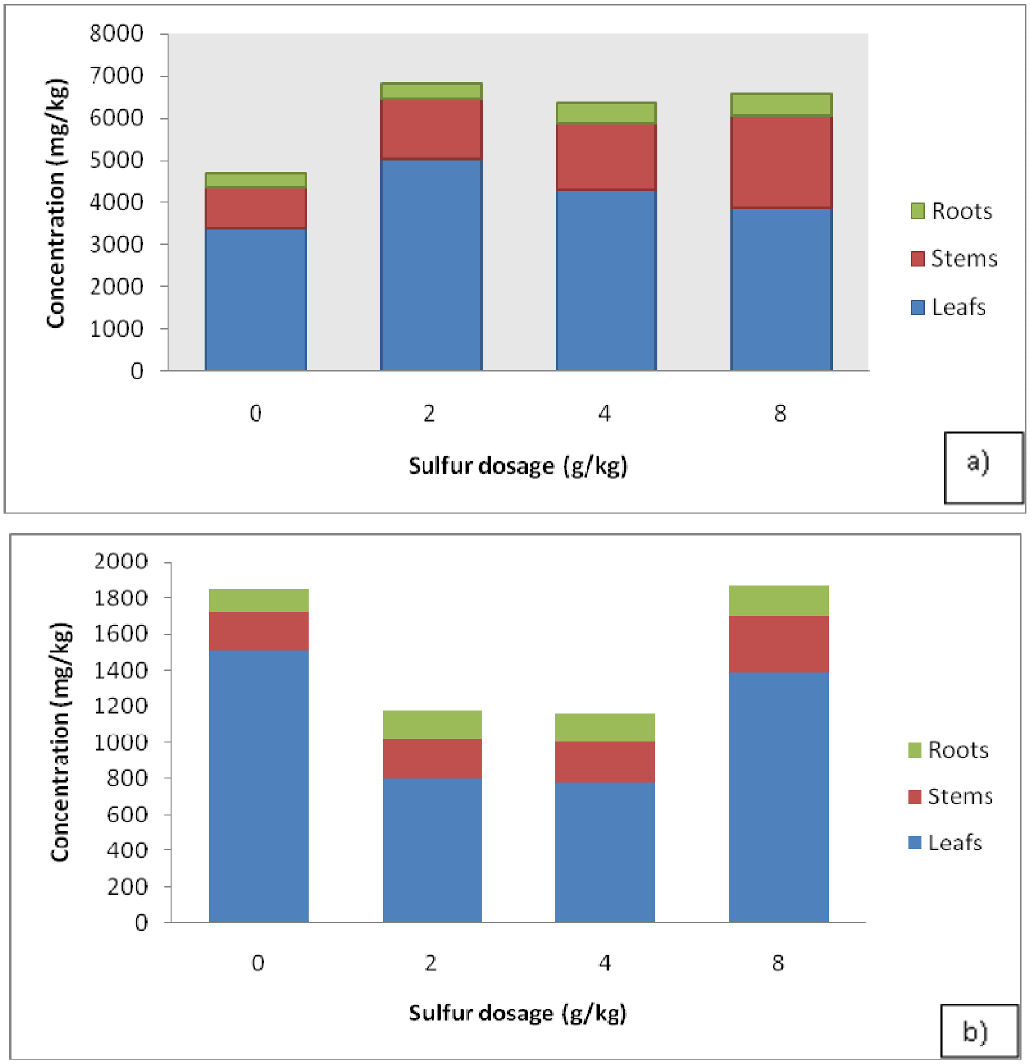


Figure 4.25: Allocation of Ni concentration on *B. coddii* organs (a) *B. coddii* grown in Native soil; (b) *B. coddii* grown in RBMR waste soil with sulfur dosages.

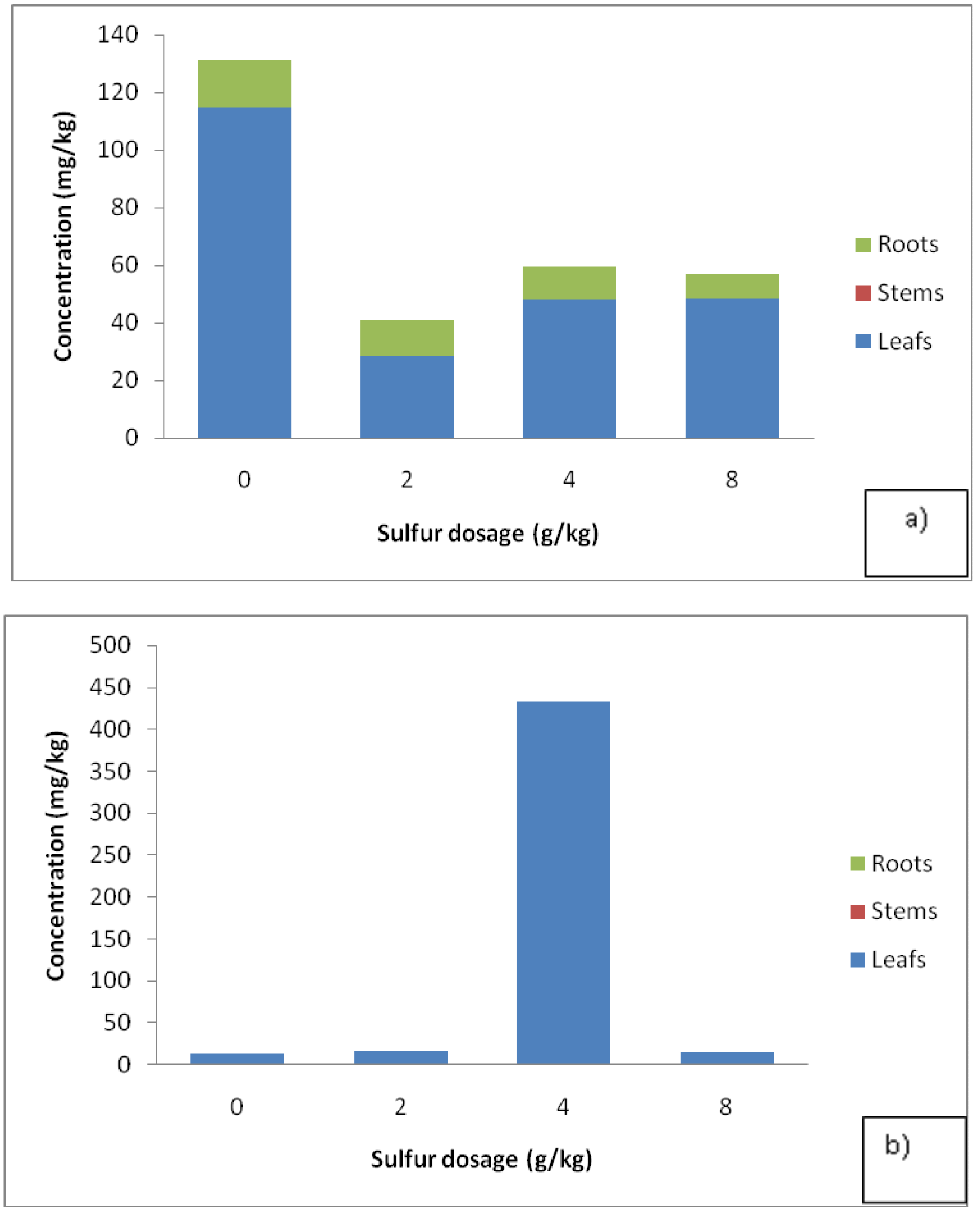


Figure 4.26: **Allocation of Co concentration on *B. coddii* organs (a) *B. coddii* grown in Native soil; (b) *B. coddii* grown in RBMR waste soil with sulfur dosages.**

Table 4.3: Root: shoot ratios of Co and Ni in *B. coddii* (mean ± SE).

Samples	**Co concentration shoot ratio (mean (SE))**	**Co concentration root ratio (mean (SE))**	**Ni concentration shoot ratio (mean (SE))**	**Ni concentration root ratio (mean (SE)**
N soil (0 g S/kg)	1.17 (0.12)	0.67	2.79 (0.02)	0.21 (0.02)
N soil (2.0 g S/kg)	1.20 (0.13)	0.59	2.82 (0.03)	0.18 (0.03)
N soil (4.0 g S/kg)	1.47 (0.17)	0.53 (0.17)	2.50 (0.22)	0.25 (0.05)
N soil(8.0 g S/kg)	1.28 (0.17)	0.44	2.00 (0.28)	0.23 (0.04)
RBMR waste soil (0 g S /kg)	NC	NC	2.44 (0.32)	0.31 (0.08)
RBMR waste soil (2.0 g S /kg)	NC	NC	1.57 (0.67)	0.68 (0.14)
RBMR waste soil (4.0 g S /kg)	NC	NC	1.63 (0.35)	0.62 (0.11)
RBMR waste soil (8.0 g S /kg)	NC	NC	1.98 (0.44)	0.52 (0.16)

Note: NC = not calculated and SE = standard error

Ratios results obtained, as indicated in Table 4.3, revealed that the shoot ratios concentration of Ni decreases with the increase in sulfur in the N soil, however, the shoot ratios Ni concentration in *B. coddii* grown in the RBMR waste soil showed that shoot ratio increased with the increase in sulfur dosage (i.e. 2.82 to 2.00 and 1.57 to 1.98, respectively). The shoot ratio obtained for treated substrates in both cases where lower than the shoot ratio obtained for the untreated soil except for the substrates that was treated with 2.0 g of S. In addition all the root ratios for Ni concentration reported was found to be below 1 with an evidence of decrease as more sulfur was added in both the substrates. Both shoot and root ratio for Co concentration decreased as more sulfur was added in the substrates.

4.10. Efficiency of *B. coddii* in transferring Co and Ni from soil to various parts of the plant

To determine if the addition of sulfur to soil increased the bio-concentration of both Co and Ni in *B. coddii*, the ratio of concentration of these metals in plants to the soil was calculated. The bio-concentrations of Ni and Co in the *B. coddii* were obtained using the following equations:

- Concentration factor 1 (LCF, SCF, and RCF 1) = (plant tissues concentration (i.e. leaf, stem or roots) / water extractable concentration)
- Concentration factor 2 (LCF, SCF, and RCF 2) = (plant tissues concentration (i.e. leaf, stem or roots) / acetic acid extractable concentration)
- Concentration factor 3 (LCF, SCF, and RCF 3) = (plant tissue concentration (i.e. leaf, stem or roots) / aqua regia extractable concentration)

Where: CF = concentration factor; LCF 1, 2 and 3 = leaf concentration factors; SCF1, 2 and 3 = stem concentration factors and RCF 1, 2 and 3 = roots concentration factors.

The concentration factor results obtained are summarised in Table 4.5. below.

Table 4.4: Average concentration factors for nickel and cobalt in 266 day old *B. coddii* plants grown in RBMR waste soil and N soil

Soil treatments	*B. coddii* tissues	**Concentration factor 1** (water extractable)		**Concentration factor 2** (acetic acid extractable)		**Concentration factor 3** (Aqua regia extractable)	
		Co	Ni	Co	Ni	Co	Ni
N soil (0 g of S /kg)	***Leaf (LCF)***	NC	NC	73	506	1	3
	Stem (SCF)	NC	NC	NC	140	NC	0.8
	Root (RCF)	NC	NC	8	48	0.1	0.3
N soil (2.0 g of S /kg)	***Leaf (LCF)***			14	641	0.2	3
	Stem (SCF)	NC	NC	NC	184	NC	1
	Root (RCF)	NC	NC	7	51	0.1	0.2
N soil (4.0 g of S /kg)	***Leaf (LCF)***	NC	NC	18	215	0.3	1
	Stem (SCF)	NC	NC	NC	195	NC	1
	Root (RCF)	NC	NC	5	62	0.1	0.4
N soil (8.0 g of S /kg)	***Leaf (LCF)***	NC	NC	11	155	0.1	1
	Stem (SCF)	NC	NC	NC	238	NC	1
	Root (RCF)	27	NC	3	68	0.03	0.3
RBMR waste soil (0 g of S /kg)	***Leaf (LCF)***	NC	1434	8	NC	3	2
	Stem (SCF)	NC	237	NC	NC	NC	0.3
	Root (RCF)	NC	144	NC	NC	NC	0.2

RBMR waste soil (2.0 g of S /kg)	***Leaf (LCF)***	NC	NC	10	NC	NC	1
	Stem (SCF)	NC	NC	NC	6	NC	0.2
	Root (RCF)	NC	NC	NC	4	NC	0.13
RBMR waste soil (4.0 g of S /kg)	***Leaf (LCF)***	NC	NC	NC	NC	NC	NC
	Stem (SCF)	NC	594	NC	6	NC	0.2
	Root (RCF)	NC	297	NC	2	NC	0.1
RBMR waste soil (8.0 g of S /kg)	***Leaf (LCF)***	NC	NC	NC	NC	NC	NC
	Stem (SCF)	NC	629	NC	6	NC	0.2
	Root (RCF)	NC	506	NC	3	NC	0.2

Note: NC: Not calculated because of missing values in the plant parts or in the soil. CF1, CF2 and CF3, see details in the equations used to calculate the concentration factors.

From Table 4.4, it can be observed that no clear trend in the concentration factors is seen from the water extractable fraction. Little calculations were also performed because of the missing data. It is only in the RBMR waste soil with 0, 4 and 8 g/kg of sulfur added that concentration factors were calculated for Ni. For 0 g/kg of sulfur added, the concentration factors decreased from leaf to roots. This is expected because Ni is known to accumulate more in the leaves than the stems and roots. The acetic acid extractabke fraction was the only one where concentration factor for both Co and Ni was more available.

The concentration factors decreased from the leaf to roots for both metals. This means that both metals prefer to accumulate more in the leaves compared to the stems or roots. For Ni, the concentration factors in the leaves tend to increase with increase in sulfur added. This may suggest that perhaps there is an optimum sulfur dosage beyond which, poor Ni uptake results. This is contrary to Ni concentration factors observed in the stem where this ratio increased with amount of sulfur added. The same trend was also observed for Ni in the roots.

The concentration factors in the aqua regia fraction were mostly around 1, show little accumulation for both metals in the plant. This may mean that this fraction is not bioavailable for plant uptake. The results may also suggest that plant uptake of metals in the diffirent fraction is highest in water extractable where little Co and Ni was detected followed by the acetic acid extractable fraction.

Chapter Five: Discussion

5.1. Substrates physical and chemical properties effects on plant growth and sulfur treatment

5.1.1. Soil pH

The pH controls the solubility of many metals in the soils. Acidification increases the solubility of Ni and Co, therefore facilitating their movement in the soil, and thus increasing the availability of Ni and Co for uptake by the *B. coddii* as nutrients. The soil pH conditions can be regarded as major limitation to soil productivity.

The soil pH results reported in this research suggest being suitable for the survival of most of the plants including *B. coddii* plants. Donahue *et al*., (1983) indicated that *B. coddii* grows in soils with pH that ranges from pH 5 to pH 8.5. Therefore, if all other factors are kept constant *B. coddii* should be able to grow in both soils despite the RBMR waste soil having a higher pH than the N soil.

The results further revealed that although sulfur addition decreased soil pH in the two soils especially the N soil, the soil pH decreases did not negatively affect *B. coddii* dry matter production. The biomass was observed to slightly increase with the decrease in soil pH. The *B. coddii* biomass production in the N soil increased and then decreased along with the decrease in soil pH due to sulfur addition. As suggested by Robinson *et al.,* (1999), the decrease in soil pH enhances the availability of Ni and Co in both the soils which allows more metals (i.e. Ni and Co) to be taken-up by *B. coddii* to its tissues.

The small change in RBMR waste soil pH suggests that the rate at which the sulfur was dissolving was very slow. The soil type, soil porosity and duration of exposure are the most important factors which could influence soil pH behaviour (Alexandra, 2005). The slow change on soil pH in the RBMR waste soil could be linked to the low infiltration rate of sulfur solution. The RBMR waste soil was found to have a small pore space that can allow for the infiltration of more sulfur solution into the soil. These results concur with the finding by Robinson *et al*., (1997), which suggest that there were significantly decreased in soil pH along with the increase in sulfur dosage. However, it should be noted that the soil which Robinson *et al.,* (1997) experimented was different from the RBMR waste soil and N soils. It appears from literature that no study has been done before on the effect of sulfur addition to the soil pH in particular for the RBMR waste soil and the N soil.

5.1.2. Soil conductivity

This indicates the total Co and Ni concentration available in the soil. The Aqua regia fraction after sequential extraction indicate the concentration of Co and Ni not available for plant uptake while the total include fractions of these metals available for plant uptake but bound to different substances. In the study by Nindi (2005), it was noted that the availability of Co and Ni, is dependent on what type of soil matrices these metals are bound to. The fraction of metals strongly bound to the soil matrices is not easily extracted from the soil by the plant. An increase in the total Ni determined after the addition of sulfur, suggests that the addition of sulfur had an effect on the aqua regia extraction of Ni. This could also be the case in Co extracted on both soils.

However, this observation cannot be correct because no external sources of these metals were added. This can be attributed to measurement errors especially due to sampling which gave results with high variations. Nemutandani *et al*., (2006) used similar digesting procedures when extracting Ni and Co from the N soil found concentration ranging from 237 to 1057 and 15 to 81 mg/kg^{-1}, respectively. These results were lower than the results of both Ni and Co recorded in this study on the same soil. In the study by Nindi, (2005) Ni and Co concentration of 297 mg/kg^{-1} and 30 mg/kg^{-1}, respectively was reported for the RBMR waste soil and these results are lower than the results reported here for similar soil. These variations are again attributed to heterogeneity distribution of the metals in the soils.

The fraction of metals left and bound to silicates is not bioavailble to plants and this should not be affected by addition of sulfur in the soil. One expects the pH changes especially the lowering of pH like in this study not to affect this fraction. Variations of Co and Ni concentrations in this fraction should be as indicated due to heterogeneity distribution of the metals in the soils. Large number of samples is therefore needed than taken here.

5.1.3. Soil reduction oxidation potential (Eh) (redox potential)

The redox potential for both soils was observed to decrease along with the increase in sulfur dosage. This suggests that there is a positive relationship between soil pH and the redox potential, since both decreased as the sulfur dosage increased. The soil redox potential results revealed that the N soil has higher redox potential than the RBMR waste soil, however, the soil pH for the N soil was lower than the RBMR waste soil. The decrease in redox potential influenced the solubility of Ni and Co in both the soils, at that time precipitation of Co and Ni is believed to have occurred. However, the influence of redox potential on the status of Ni and Co will vary with soil type.

5.2. Effect of sulfur treatment on soil metal bioavailability

5.2.1. Extraction of Ni and Co concentration in soil solution (aqueous)

The results for soil pH reported in chapter four pointed out that the addition of sulfur in both soils decreases the soil pH although this was more evident in the N soil. It was thus assumed that the decrease in soil pH would increase the bioavailability and uptake of Co and Ni by *B. coddii.* The concentrations of both Co and Ni were higher in the control group obtained after plant growth experiments. This might suggest that most of these metals previously identified in an aqueous solution are taken up by the plant during plant growth experiments. Therefore, to get a complete picture one has to look at the concentration of these metals in the actual plants.

It was expected that the soil type added with highest dosage of sulfur will have the highest metal accumulation, assuming that the pH decrease resulted in more bioavailability of these metals in aqueous solution. This is however, not what was observed, except for Co in the N soil and Ni in the RBMR waste soil with highest sulfur dosage. This could mean that uptake of these metals depend on other factors and not solely on the bioavailability of the metals (i.e. plant tolerance and thresholds).

5.2.2. BCR-sequential extraction for metal bonds to various constituents of the substrates.

5.2.2.1. Effects of sulfur on weak acid extractable fraction of Co and Ni in the soil

Acetic acid was used to extract Co and Ni weakly bound to weak organic acids in both the soils. The results for Co and Ni obtained before the plant growth experiments were compared with those reported after the plant growth experiments with different amounts of sulfur. The results obtained demonstrate that sulfur had an influence on the availability of both Co and Ni fraction. The concentration of these metals extracted by weak acid increased with sulfur dosage. This increase was exceptionally pronounced for Ni in the N soil. The ability of weak acids in the soil to bind metals is pH dependent (Siebielec *et al*., 2005). Depending on the pKa of the acids, generally as the pH decreases, these acids become less ionised. This process favours the release of the metals, making them bioavailable. Plants generally release weak acids from their roots into the soil. This helps to release the metals in the soil making them available for uptake. This explains why the addition of sulfur resulted in more metals being extracted by weak acid. However, this result may also suggest that most of these metals were not taken up by *B. Coddii.*

Robinson *et al*. (1999) conduct a similar study, where he investigated the effect of sulfur addition on metal availability and metal uptake by the plant, but on different types of soils. The study showed that as the amounts of sulfur increased, the pH decreased, resulting in more Co and Ni availability in the soil for plant uptake. In this case, ammonium acetate was used as extraction solution. An increase in Ni and Co concentration was also observed in the *B. coddii*. The results obtained in this study revealed that decrease in soil pH was observed resulting in more Co and Ni which could be extracted with ammonium acetate to become available for *B.coddii* to uptake.

5.2.2.2. Effects of sulfur on hydroxylamine extractable fraction of Co and Ni in the soil

Although inconclusive, the addition of sulfur had increased hydroxylamine extractable fraction of Ni in both soils to a certain extent. However, continuous increase of sulfur showed a decrease in hydroxylamine extractable fraction of Ni. This was more pronounced in the N soil. The reason for this decrease is not easy to explain; perhaps pH played a role since pH decrease was more pronounced in the native soil with increase in sulfur. However, there are no clear relationships between hydroxylamine extractable fractions of Ni from the soil with those accumulated in *B. coddii* plant parts except those in the leaves. The concentration of Ni accumulation in the leaf shows a similar pattern.

The Co concentrations extractable with hydroxylamine before the growth experiment were reported to be the same for both the soils. After the experiment, the results for the N soil with different sulfur dosage showed no detectable fraction of Co when extracted with hydroxylamine. The same was observed for the RBMR waste soil treated with 8.0 g of sulfur. The decrease in Co in this case could have been attributed to the increase in sulfur, which might have resulted in Co being more available for *B. coddii* uptake. There was no clear indication that Co that was not detected in the various soil treatments after the addition of sulfur was taken up the plants. Only plants grown in the N soil showed an increase in Co uptake along with increase in sulfur dosage but the pattern is not the same as that in the soil. This suggests that other reasons may be the cause for less Co detected in the N soils after the addition of sulfur. However, the homogeneity of the soil during sampling after addition of sulfur could also explain what is observed.

5.2.2.3. Effects of sulfur on hydrogen peroxide and ammonium acetate extractable fraction of Co and Ni in the soil

Robinson *et al*., (1999) conduct similar studies, but on different soils and environmental conditions. In this study, sulfur was added up to 5 g /kg of soil. The results indicate that the addition of sulfur caused a decrease in soil pH, accompanied by an increase in the ammonium acetate extractable fraction of Co and Ni in the soil. The results obtained in the study by Robinson *et al*., (1999), though he was focusing on different soil to this study, are shown in Table 5.1 together with results obtained in this study. The behaviour of Ni in the N soil (i.e. native soil) after addition of sulfur is similar to that observed by Robinson *et al*., (1999). In both studies, concentration of Ni extracted from N soil with ammonium acetate and hydrogen peroxide combustion tend to increase with the increase in sulfur. This suggests that concentration of Ni extracted with hydrogen peroxide and ammonium acetate was not available for *B.coddii* uptake. However, for the RBMR waste soil, the opposite trend was observed in this study.

The concentration of Ni extracted by hydrogen peroxide and ammonium acetate decreased along with an increase in sulfur dosage. This means that the N soil (i.e. native soil) composition could have been similar to the soil used by Robinson *et al*., (1999). The behaviour of Co with the addition of sulfur in the soil in a study by Robinson et al., (1999) showed a decrease in the concentration extracted by hydrogen peroxide and ammonium acetate. This is also in line with what has been observed in this study where no Co concentration was detected after sulfur was added to the two soils. However, in this study, the picture is not clear because in the control group (sulfur not treated with sulfur), no Co was detected in the two soils. A more detailed study is therefore still needed.

Table 5.1: The pH and ammonium-acetate-extractable Ni and Co concentrations (mg/kg) for sulfur treated and untreated soils

	Sulfur treatment (g/kg)				
Sulfur dosage (g/kg) (Robinson *et al*., (1999))	**0**	**0.63**	**1.25**	**2.5**	**5**
Sulfur dosage (g/kg) (this study)	**0**	**-**	**2**	**4**	**8**
pH (Robinson *et al)*	6.9	6.3	6.1	5.7	5.5
pH (Native soil)	6.4	-	5.7	5.3	4.7
pH (RBMR waste soil)	7.8	-	7.5	7.5	7.4
Ni (Robinson *et al)*	4.1	4.6	4.2	7.4	12.6
Ni (Native soil)	17.9	-	19.9	16.4	19.4
Ni (RBMR waste soil)	431.3	-	346.3	422.5	367.7
Co (Robinson *et al)*	2	1.2	1.6	1	2.1
Co (Native soil)	Nd	-	nd	Nd	nd
Co (RBMR waste soil)	Nd	-	nd	Nd	nd

Notes: nd= not detected.

5.2.3. Aqua regia extraction of total Co and Ni in the soil

The results reported earlier in chapter 4 indicate that Co and Ni fraction which could not necessarily be available for *B. coddii* to uptake. This fraction is also influenced by soil constituents. In the study by Nindi (2005), it was noted that the availability of Co and Ni, is dependent on what type of soil matrices these metals are bound to. The fraction of metals strongly bound to the soil matrices is not easily extracted from the soil by the plant. An increase in the total Ni determined after the addition of sulfur, suggests that the addition of sulfur had an effect on the aqua regia extraction of Ni ($p= <0.001$). This could also be the case in Co extracted on both soils. Nemutandani *et al*., (2006) used similar digesting procedures when extracting Ni and Co from the N soil found concentration ranging from 237 to 1057 and 15 to 81 mg/kg^{-1}, respectively. These results were lower than the results of both Ni and Co recorded in this study on the same soil. In the study by Nindi, (2005) Ni and Co concentration of 297 mg/kg^{-1} and 30 mg/kg^{-1}, respectively was reported for the RBMR waste soil, this result are lower than the results reported on this study on similar soil.

This suggests that additional of sulfur had an influence in making more and more Ni and Co extractable through aqua regia to be available for plant to uptake, it is clear from the results that the additional of sulfur had an influence in the breaking down of the silicates that could have inhabit both Ni and Co. However, it should be noted that, though sulfur had an influence on breaking down silicate on the soil matrix, silicate was not broken down completely. This could be the reason why Ni and Co as still been detected even after sulfur was added in both substrates. The high amount of Co obtained in both substrates at 8.0 g of S/kg could be seen as an outlier, this is because the results obtained after the experiment was supposed to be lower than those obtained before the experiment. It is clear from this results that sulfur addition on both substrates influence the availability of Ni taken up by the *B.coddii*, however the situation was different on N soil treated with 8.0 g of S/kg that show higher amount of Co.

5.3. Plant height increment and leaf production

The results obtained revealed that *B. coddii* planted in the N soils has a higher growth compared to the *B. coddii* planted in the RBMR waste soil. The higher growth rate results recorded for the *B. coddii* in the N soil could be linked to the fact that this plant grows naturally in this soil (Nindi, 2005, Robinson *et al*, 1999). This also could be because plant growth rates depend on many factors such as soil types, soil nutrient, non nutrients metals and soil pH. None of these factors was investigated in detail. The soil conductivity was also much higher on the RBMR waste soil compared to the native soil. However, the reduction oxidation potential was highest in the native soil. All these differences could have accounted for the higher growth rate of the plants in the native soil. Other factors such as the role of fungi in the two soils were not investigated which could also influence plant growth.

The obtained results indicate that *B. coddii* growth does not depend only on the concentration of Ni and Co since these metals were highest in the RBMR waste soil. The native soil also had a coarser texture compared to the RBMR waste soil. This was seen as a positive property as it enhanced the solubilising of the added sulfur. The RBMR waste soil had a fine texture and added sulfur was seen on the surface for a much longer period compared to the N soil.

During the planning stage of the research it was assumed that once sulfur is added into the soil, it might result in *B .coddii* plants dying due to an increased acidity condition. In other words, the pH was lower than what the *B. coddii* plants could tolerate, as seen growing naturally on neutral soil pH. However, despite the fact that pH decreased from 6.4 to 4.7 in the N soil, and from 7.7 to 7.4 in the RBMR waste soil, no plant died although some of the plants become yellowish after sulfur was added in the soil, particularly in the RBMR waste soil. All the plants where sulfur was added were observed growing until the day they were harvested.

Although the plant grown in both the RBMR and N soils were found to increase overtime, correlation results obtained indicated that there was a significant difference between the growth rates in the two soils at $p = 0.77$. The results obtained showed that there was a positive correlation between the height and the number of leaf. In other words, plant height increase was accompanied by an increase in the number of leaves. In addition in first 35 - 49 days all plants were observed to be growing at more or less the same rate. This could have resulted from the physical properties of the soil where they were propagated. At this time they were still utilising nutrients stored in their tissues during their seeding period. The same trend was also observed in the study by Euliss, (2004) who conducted a study on the growth of *Brassica napus* in selenium contaminated soil.

5.4. Plant stress determination

The mNDVI_{705} value ranged from -1 to 1 with the normal range for green vegetation of 0.2 to 0.8 (Rouse *et al*., 1973). Increasingly positive mNDVI_{705} values are associated with increasing green-ness i.e. it indicates increasing amounts of chlorophyll (Karen *et al*, 2007). NDVI values near zero and decreasing negative values indicate low to negligible chlorophyll concentrations, and indicate plant stress or death. The mNDVI_{705} results are also summarised in chapter 4. Majority of *B. coddii* planted in the RBMR waste soil were found to be between 0.2 and 0.3 whereas the mNDVI_{705} value obtained for the *B. coddii* planted in the N soil were found to be between 0.4 and 0.5 which is within 0.2 and 0.8 mNDVI_{705} for the healthy vegetation (Rouse *et al*., 1973). Despite the sulfur addition, the majority of *B. coddii* plants were observed as healthy, in particular those *B. coddii* that are planted in the N soil.

Although the mNDVI_{705} and the PSRI results obtained were within the normal range of healthy vegetation, mNDVI_{705} results obtained for the RBMR plants were reported to be lower than those reported for the N plants. These results suggest that *B. coddii* planted in the RBMR waste soil were slightly stressed compared to those planted in the N soil. However, the mNDVI_{705} and PSRI results obtained in both the soils were closer to each other, making it difficult to link the results reported with the addition of sulfur as a cause of stress. During the harvesting period, the majority of the *B. coddii* plants planted in the RBMR waste soil were observed to be yellowish when compared to the *B. coddii* plants planted in the N soil, this could indicates plant stress.

The PSIR results obtained for the *B. coddii* planted in the RBMR waste soil showed that the PSIR value obtained was within the normal range value of -0.1 to 0.2. However, it is not clear if sulfur addition had an influence on the change of the chlorophyll and carotenoid pigment of the *B. coddii* in both substrates. PSIR results obtained in both cases were found to have increased over time, more so for plants planted in the RBMR waste soil. The increase in the PSIR value indicates that *B. coddii* planted in the RBMR waste soil was stressed. Despite the increase in the PSIR value for the *B. coddii* planted in the

RBMR waste soil, *B. coddii* were observed to tolerate the harsh condition of the RBMR waste soil and was able to grow until the date of harvest. A larger portion of the dry matter produced was found in the root structure followed by the leaf structure and stem structure being the least

B. coddii in both soils where observed to have more root mass proportion than the shoots. This suggests that *B. coddii* roots in both the soils were able to grow in length in order to explore the availability of nutrients (i.e. Ni and Co). The roots were able to supply the shoot with water and nutrients required by *B. coddii* to grow in both the soils. The root: shoot ratios revealed that *B. coddii* grown on the RBMR waste soil was growing under favourable conditions, as this was identified to adapt and tolerate harsh condition.

5.5. Potential of sulfur treatment for enhancing the uptake of Co and Ni in harvestable *B. coddii*

In the study by Robinson *et al*., (1999) it was noted that there were highly significant ($p = < 0.01$) positive correlations between the concentrations of sulfur added, and the Co and Ni concentrations in *B. coddii*. The highest rate of the addition of sulfur in the soil in the study by Robinson *et al*., (1999), was (5 g/kg of soil) and the mean Ni and Co concentrations recorded for the *B. coddii* were 1331 and 290 mg/kg, representing a three and five-fold increase relative to the controls (400 and 56 mg/kg), respectively. However, the plants were 180 cm taller than in this study which means that plants were allowed to grow for much longer periods. In this study sulfur was added up from 0, 2, 4 and 8.0 g of sulfur in both soils, higher than what was added in Robinson *et al*., (1999). The reason that more sulfur was added was to determine if more Co and Ni would be taken up by the plants with increased sulfur dosage.

The results reported in this research showed that increase in sulfur, increased in the concentration of Ni in the leaf organ of *B. coddii* planted in the RBMR waste soil. However, concentration of Ni in the N soil was observed to decrease. Although inconclusive, sulfur addition in both the soils might have had an influence on the availability of Co. It is however; clear, as shown in the results that a further increase in sulfur could have reduced the availability of Co, in particular for those plants in the RBMR waste soil.

A positive relationship between the addition of sulfur and the concentration of Ni reported in the *B. coddii* root and stem structures was observed. Sulfur, plays an important role in reducing the toxicity of Ni in the *B. coddii* planted in the RBMR waste soil by being involved in metal-binding complexes (Nemutandani *et al*., 2006). The study by Robinson *et al*., (1999) found that the addition of sulfur caused a highly significant increase in Ni and Co uptake by *B. coddii*. The results of Ni in this study for leaves and roots (i.e. N soil) are slightly lower than that reported on Nemutandani *et al* (2006)'s paper, where the concentration of Ni on leaves and roots were found to be 13,980 ± 10,780 and 2046 ± 789 mg/kg^{-1} dry mass in the same soil (N soil), respectively. This is not surprising becuase Nemutandani et al., never grow the plants but got them from their natural forest.

The study by Brooks *et al*., (2001) reported a concentration of 14,000 mg/kg^{-1} in the same soil (i.e. N soil). However, it should be noted that this research did not add sulfur in their experiment and they only looked at N soil not RBMR waste soil. The decrease in Ni concentration reported in this study in the leaves from the N soil is not very clear to know especially because of limited number of samples. Although Ni concentration was observed to increase in the leaves of *B. coddii* planted on the RBMR waste soil soil treated with sulfur, this did not exceed the concentration Ni, reported for the leaves of *B. coddii* planted in the N soil. As reported in chapter 4 the concentrations of Co was observed to have decreased in the *B. coddii* root structures for the plants planted in the N soil with an increase in sulfur relative to the control group (N soil not treated with sulfur).

The findings of this study revealed that much of the Co and Ni accumulated in the leaf's structure in both soils. The high accumulation of those metals in the leaf than in roots organs could render these plants suitable for phytoextraction of Co and Ni. Therefore only the leaf and stem structure of the *B. coddii* can be harvested, leaving the plant base and the roots to re-generate in the same area. The findings of this study concurred with the finding by Slatter (1998), Nemutandani *et al*., (2006) and Brooks *et al*., (2001) which suggest that Ni and Co appeared to be transported from the roots, stem to the leaf structure via xylem. As found in Slatter (1998)'s research the Ni concentration on the roots were comparatively low thus, for ease of harvesting and ensuring continuous vegetative growth of *B. coddii* on site. However, in the studies the factors that influence transportation of Co to the leaves were not determined. It should also be noted that sulfur was not added in this study in order to influence bioavailability and enhance uptake of Ni by *B. coddii*.

Having said that, it is clear from the results obtained that sulfur addition in RBMR waste soil increases the Ni uptake by *B. Coddii*. Therefore, continuous addition of sulfur could increase the concentration of Ni in dry mass of *B. coddii* planted in the RBMR waste soil. The results obtained in this research concur to some extent with the findings by Robinson *et al., (*1997 and 1999) which suggested that an increase in sulfur increased concentration of Co and Ni uptake by *B. coddii*. Although inconclusively, it is clear that there is a number of parameters that regulate Ni and Co fate in the soil. Therefore the uptake of Ni and Co by *B. coddii* is largely dependent on a number of these factors and not just the availability of sulfur.

As observed previously by Paterson *et al*., (1994), there are about three types of factors that affect the uptake and distribution of chemical compounds within plants. First and foremost are physicochemical properties of the compound such as water solubility, vapour pressure, molecular weight, and water partition coefficient. Second are environmental characteristics including temperature of the soil as well as organic and mineral matter concentration and water content of the soil. Third are plant characteristics such as the type of root system, shape and composition of the leaves, and lipid (oil) content.

5.6. Efficiency of *B. coddii* in transferring Co and Ni from soil to various organs of the plant (soil - plant pathways)

The concentration factors of less than 1.00 means that no bio-accumulation of Ni and Co by *B. coddii* will occur. The high Ni and Co concentration factors value of greater than 1 were observed for the CF 1 and CF 2 than in the CF 3, this suggest that Ni and Co were highly bio-accumulated and bio-magnified in the tissues of the B. coddii planted in the N soils. Little Ni and Co bioaccumulated in the plants grown in the RBMR waste soil. Though inconclusively, the high accumulation of Ni and Co in the plant grown in the N soil as opposed to those grown in the RBMR waste soil could not be linked with the increase in the amount of sulfur rather it might have been atributed by other factors (i.e moresture, temperature and soil type). This is because no clear trend or relationship can be observed between the additional of sulfur and the increase in concentration factors.

The average concentration factors reported in this study were found to be higher than those reported in the study by Nemutandani *et al*., (2006) and Robinson *et al.*, (1999). The paper by Robinson *et al*., (1999) demonstrated that Co was readily taken up by the plant with and without the presence of Ni. Ni uptake was however, inhibited by the presence of an equal concentration of Co. This could indicate competition for the binding sites in the root zone and suggested a significant limitation to phytoextraction in case both metals are present like in the RBMR waste soil. Ni average concentration factors (i.e. CF 2) was found to be higher than the Co average concentration factors in both the soils. This result suggests that there was a competition between those metals by *B. coddii*.

This was also confirmed by the root: shoot ratios for the Co concentration that showed that Co concentration root: shoot ration decreased with the increase in sulfur in the N soil. Though all the shoot ratios for Ni concentration were observed to decrease with the increase in sulfur, they were found to be greater than 1 which suggests that *B. coddii* could be best suited for the remediation of Ni contaminants in both the soils. However, the RBMR shoot was found to have a lower ratio of Ni than the N soil shoot. These results suggest that *B. coddii* can be regarded as a hyperaccumulator for Ni and Co.

Chapter Six: Conclusion and recommendation

The results reported in chapter four of this research suggests that *B. coddii* can be a potential candidate for phytoextraction regardless of whether sulfur was added or not. The results in growth rate measurement as well as leaf count suggest that *B. coddii* is well adapted to the poor soil conditions of the RBMR waste soil and is able to absorb both Co and Ni. The Ni and Co being absorbed in this way means that it can be extracted in the mining industry for sale. The addition of sulfur in the RBMR waste soil did not result in a significant increase in growth rate of the plants planted or a decrease in soil pH.

The results pointed out that the growth of *B. coddii* in the RBMR waste soil was within the range of 190 to 250 mm, which was lower than what was achieved in the N soil (350 – 400 mm). Despite the addition of sulfur, growth results revealed that *B. coddii* was found growing healthily and able to uptake larger quantities of both Co and Ni to their structural part. Therefore sulfur addition did not have a significant negative impact on the growth rate of *B. coddii* in both the soils even though some of the plants on the RBMR waste soil were observed to be turning yellow.

The addition of sulfur in the RBMR waste soil could increase both the growth rate and uptake of target metals (Ni and Co). The results further showed that sulfur addition increased the total biomass of the *B. coddii* planted in the RBMR waste soil and decreased the total biomass of the *B. coddii* in the N soil. Total biomass results revealed that production yield was higher in the N soil than in the RBMR waste soil. However, the good thing is that the total biomass of the plants in the RBMR waste soil increased with the increase in sulfur. This implies that this process will be useful for mining waste remediation. The success of phytoextraction is largely dependent on the biomass production especially the root structure. The bigger the root structure the more *B. coddii* will be able to survive and accumulate Co and Ni from the soil. From the results obtained and explained in chapter 4 it is clear that *B. coddii* plants are able to grow in both substrates regardless of how much sulfur was added. However, it should be noted that continuous increases in sulfur might decrease the root structure impacting on the growth of *B. coddii*. The soil pH condition of both soil types did not interfere with the growth of *B. coddii* plant planted in both the soils. The soil pH results were found to be within the soil pH required for the survival of any plant in a given environment. *B. coddii* was observed growing in both soil types and able to absorb larger concentrations of Co and Ni from the contaminated soil despite their sizes. However, it could increase the availability of targeted metals (Co and Ni).

B. coddii could be the best candidate for phytoextraction in particular for RBMR waste soil. The results showed that *B. coddii* was able to grow in both soil types despite larger quantities of Ni that were observed in the extraction with hydroxylamine and hydrogen peroxide and ammonium acetate than in water and acetic acid extraction. The concentration of Ni and Co found to be available in both soil types after sulfur treatment in this experiment did not limit the growth of *B. coddii*. Although it is inconclusive, phytoremediation of the RBMR waste soil through phytoextraction with the use of sulfur could be a low cost operation compared with the addition of other chemicals. Further research is required to determine other factors that might affect the growth rate and metal uptake by *B. coddii* in the RBMR waste soil.

The findings of this research further suggests that larger concentrations of Co and Ni are located in the leaves or canopy parts of the *B. coddii,* followed by the stem parts, which means only the upper part (shoots) of the plants can be harvested for Co and Ni extraction. The remaining parts of *B. coddii* inclusive of the root structure and the plant bases can be allowed to regenerate in the same area to continue up-taking more Co and Ni from the same soil to remediation levels that could be stipulated by Government as desirable concentration for ecosystem and human health's protection.

The regenerated parts of the *B. coddii* can again be harvested to extract Co and Ni likely to have accumulated from the same contaminated land. *B. coddii* can be a potential candidate for use in phytoremediation not only despite sulfur addition but also because of its resistance to the harsh condition of the RBMR waste soil and its ability to absorb a larger fraction of Ni from the RBMR waste soil. Although sulfur to some extent enhanced Co and Ni uptake by *B. coddii,* an excessive amount of sulfur can be toxic resulting in more plants being threatened and eventually dying.

Therefore, sulfur addition that ranges between 5-10 g /kg of soil can be recommended specifically for RBMR waste soil and other soils that have similar characteristics as RBMR waste soil. The duration of exposure to sulfur in the RBMR waste soil could have an impact on the availability of Co and Ni taken up by the plant. Future experiments should explore possibilities for adding sulfur at the initial stages of the plant growth experiment. This could result in more metal uptake by *B.coddii* plants.

The N soil results reported, revealed that even during short periods of exposure, the presence of sulfur resulted in increased concentrations of Co and Ni being absorbed by *B.coddii* leaving less and less concentration of Co and Ni in the soil. However, this was not the case in the RBMR waste soil. Further studies are required to expand on the findings and results of this research specifically looking at the RBMR waste soil. However, it should also be noted that *B. coddii* plants do not grow naturally in the RBMR waste soil. Although there was more Co and Ni available in the soil as a result of sulfur addition, not all of the Co and Ni were taken up by the plants. Some residual fractions remained in the soil. These results suggest that *B. coddii* plants have adaptive mechanisms that allow for the selection of Co and Ni of their choice.

Although the findings of this research have shown the potential of *B. coddii* to absorb Ni and Co, field trials now need to be conducted particularly in the area where Ni and Co is a concern. The results of this study are from a semi-controlled environment and therefore cannot be used to measure the field performance and yield of metals using *B. coddii*. However, the results of this study can be used to demonstrate that sulfur fertilisation has potential for use in developing a phytoextraction technology for the RBMR waste soil. Furthermore, the results of this study can be used to guide the design of the field trials such as the amounts of sulfur which appear to be optimal, and provide evidence with regard to macronutrient fertilization required.

References

Adler, R. and Rascher, J. 2007. A strategy for the management of acid mine drainage from gold mines in Gauteng. Report No. CSIR/NRE/PW/ER/2007/0053/C. CSIR, Pretoria.

Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for cobalt. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Alexandra, B. 2005, the importance of soil organic matter: key to drought resistance soil and sustained food production, Food and Agriculture Organisation of United Nation, Rome, Italy.

Alloway, B.J, and Ayres, D.C., (1997) Chemical Principles of environmental Pollution 2nd edition, Chapman and hall, London, UK.

Alloway, B.J. 1990. Soil processes and behaviour of metals. In Alloway, B.J. (Ed.) heavy metals in soils. Halsted Press, New York, NY, p. 320-345.

Alloway, B.J.1995. Heavy Metals in Soils, Second Edition. Chapman and Hall, London, UK.

Anderson, T.R., Howes, A.W., Slatter, KA and Dutton, MF.1997. “Studies on the Nickel Hyperaccumulator, Berkheya coddii”. The Ecology of Ultramafic and Metalliferous Areas. Proceedings of the Second International Conference on Serpentine Ecology (Eds: Jaffré, T; Reeves, RD and Becquer, T) pp 261-266. Orstom, Nouvelle-Calédonie.

Angle J.S., Chaney R.L., Baker A.J.M., Li Y., Reeves R., Volk V., Roseburg R., Brewer E., Burke S., and Nelkin J. 2001. Developing commercial phytoremediation technologies: practical considerations. South African Journal of Sciences: 97, p619-623.

Ashton, P.J., Love, D., Mahachi, H., and Dirks, P.H.G.M. 2001. An Overview of the Impact of Mining and Mineral Processing Operations on Water Resources and Water Quality in the Zambezi, Limpopo and Olifants Catchments in Southern Africa. Contract Report to the Mining, Minerals and Sustainable Development (SOUTHERN AFRICA) Project, by CSIR Environmentek, Pretoria, South

Africa and Geology Department, University of Zimbabwe, Harare, Zimbabwe. Report No. ENV-P-C 2001-042: p. xvi + 336

Baker A. J. M. and Brooks R. R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. – Biology Recovery 1: p. 81-126.

Baker, A.J.M. 1981. Accumulators and Excluders – Strategies in the Response of Plants to Heavy Metals. Journal of Plant Nutrition 3: p. 643-654.

Baker, A.J.M. 1987. Metal Tolerance. New Phytol. 106: 93-111.

Baker, A.J.M., Reeves, R.D., and Hajar, S.M. 1994. Heavy Metal Accumulation and Tolerance in British Populations of the Metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). New Phytol. 127: p. 61 - 68.

Baker, A.J.M., and Brooks, RR. 1989. Terrestrial Higher Plants which Hyperaccumulate Metallic Elements - A Review of their Distribution, Ecology, and Phytochemistry. Biorecovery 1: p. 81 - 126.

Baker, A.J.M., McGrath, SP., Sidoli, C.M.D., and Reeves, R.D. 1995. The Potential for Heavy Metal Decontamination. Mining Environmental Management 3 (3): p. 12-14.

Baker, A; Brooks, R and Reeves, R. 1988. Growing for Gold and Copper and Zinc. New Scientist, 10 March 1988: p. 44 - 48.

Basta N.T., Pantone, D.J., and Tabatabai, M.A. 1993. Path analysis of heavy metal adsorption by soil, p. 1054-1057.

Benjamin, M.M., and Leckie, J.D. 1980. Analysis, Chemistry, Biology Volume 2. In Baker R.A., (Ed)– MI: Ann Arbor Science Publishers, Inc.

Berti, W.R., and Jacobs L.W. 1996. Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge applications. Journal of Environmental Quality: 25, p. 44.

Bradford C.W. 1988. Handbook of toxicity of inorganic compounds. New York –Basel: Marcel Dekker, p. 533-539.

Brooks R.R. 1987. Copper and cobalt uptake by Humaniastrum species. Plant and Soil, 48: p. 541-544.

Brooks R.R., Robinson B.H., Howes A.W., and Chiarucci A. 2001. An evaluation of *Berkheya coddii* Roessler and Alyssum bertolonii Desv. for phytoremediation and phytomining of nickel. South African Journal of Science. 97:p. 558-560.

Canadian Council of Ministers of the Environment (CCME). 1996. A protocol for the derivation of environmental and human health soil quality guidelines. Report No. CCME -EPS-101E. Environment Protection Publications. Winnipeg, MA, Canada.

Chaney, R., Brown, S., Li, Y-M., Angle, J.S., Homer, F., and Green, C. 1995. Potential Use of Metal Hyperaccumulators. Mining Environmental Management September: p 9-11.

Cicchella D., De Vivo B., and Lima A. 2003. Palladium and platinum concentrations in soils from Napoli metropolitan area, Italy: Possible effects of catalytic exhausts. Science of the Total Environment. 308: p. 121 – 131.

Collis, Y.E., and Stotzky, G. 1989. Factors affecting the toxicity of heavy metals to microbes. In: Beveridge, T.J., Doyle, R.J. (Eds), Metal Ions and Bacteria. Wiley, Toronto, Canada: p. 31-90

Connell, D.W., and Miller, G.J. 1984. Chemistry and ecotoxicology of pollution. Journal of Environmental Quality 26: p. 259 – 264.

Cunningham S.D., Betti W.R., and Huang J.W. 1995. Phytoremediation of contaminated soils. Trends Biotechnology 13: p. 393 – 397.

Davies, B.E. 1990. Heavy metals in soils. Halsted Press. New York, NY, p. 14-21.

Elliot, H. A., Liberali, M.R. and Huang C.P. 1986. Competitive adsorption of heavy metals by soils. – Journal of Environmental Quality 15:p 214 – 219.

Environmental Protection Agency (EPA). 1998. A citizen's guide to phytoremediation. Technology Innovation Office. EPA 542-F-98-011. Online: http://www.clu-in.

Ernst, W. (1972). "Ecophysiological Studies on Heavy Metal Plants in South Central Africa". Kirkia 8: p. 125 - 145.

Euliss, K.W. 2005. The effect of selenium accumulation in hydroponically grown Canola (*Brassica napus*), Purdue University.

European Environmental Bureau. 2000. The environmental performance of the mining industry and the action necessary to strengthen European legislation in the wake of the Tisza-Danube pollution. European Environmental Bureau. Document No. 2000/016. 32, p. 33

Farago M.E., and Parsons P.J. 1985. Effects of platinum metals on plants. In: Hemphill D.D., (Ed). Trace Substance. Environmental health, Vol 19. Columbia, MO: University of Misouri, p. 397.

Federal Remediation Technologies Roundtable (FRTR). 2003. Remediation technologies screening matrix and reference guide. Version 4. Online: http://www.frtr.gov.

Gadd G.M. 2001. Fungi in Bioremediation, United Kingdom, University Press, Cambrigde.

Garbisu, C., and Alkorta, I. 2000. Phytoextraction: a cost-effective plant-based technology for the removal of metals from environment. Journal of Bioresource technology 77 (2001): p. 229 - 236

Gatliff, E. G. 1994. Vegetative Remediation Process Offers Advantages over Traditional Pump and-Treat Technologies, Remediation 4 (3): p. 343–52.

Ghosh, M, and Singh, S.P. 2005. A review on phytoremediation of heavy metals and utilization of its by-products, Biomass and Waste Management Laboratory, School of Energy and Environmental Studies, Faculty of Engineering Sciences, Devi Ahilya University, Indore – 452017, India.

Heaton, C. P., Rugh, C. L., Wang, N.-J., and Meagher, R. B. 1998. Phytoremediation of Mercury- and Methylmercury-Polluted Soils Using Genetically Engineered Plants, Journal of Soil Contamination 7: p. 497–509.

Howes A.W. 1991. Investigations into nickel hyper-accumulation in the plant *B. coddii*. M.Sc. Thesis, University of Natal, Pietermaritzburg – South Africa.

Imperato, M., Adamo, P., Naimo, D., Arienzo, M., Stanzione, D., Violante, P. 2003. Spatial distribution of heavy metals in urban soils of Naples city (Italy). Environmental Pollution 124: p 247-256

Interstate Technology & Regulatory Council (ITRC). 2009. Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised. PHYTO-3. Washington, D.C.: Interstate Technology & Regulatory Council, Phytotechnologies Team. www.itrcweb.org.

Johnson M. 2001. Precious Metal Division, Johnson Matthey Publishing Company, London, UK (Web page: http//www.matthey.com/divisions/precious).

Karen,L and G. Arturo Sanchez-Azofeifa. 2007. Changes in spectral properties, chlorophyll content and internal mesophyll structure of senescing *Populus balsamifera* and *Populus tremuloides* leaves, Center for Earth Observation Sciences (CEOS) & Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada.

Keeling et al. 2003. Nickel and Cobalt phytoextraction by the hyperaccumulator *B. coddii*: Implications for polymetallic phytomining and phytoremediation. Soil and Earth Sciences, Institute of Natural Resources, Palmerston North, New Zealand.

Kennish, M.J. 1992. Ecology of estuaries: anthropogenic effects. – CRC Press, Inc., Boca Raton, FL

Krämer,U., Charnack, J.M., and Baker, A.J.M. 1996. Free histidine as a metal chelator in

Lasat M.M. 2000. Phytoextraction of metals from contaminated soil: a review of plant/soil/ metals interaction and assessment of pertinent agronomic issue, publisher, Washington, D.C

Margui, E., Salvoda, V., Queralt, I. and Hildalgo, M. 2004. Comparison of three-stage sequential extraction and toxicity characteristic leaching tests to evaluate metal mobility in mining wastes. Analytica Chimica Acta 524: p. 151 - 159

Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, Harcourt Brace Jovanovich, Publishers, London.

Martinez, C.E., and Motto, H.L. 2000. Solubility of lead, zinc, and copper added to mineral soils. Environmental Pollution 12: p. 56

McGrath, S.P., Sidoli, C.M., Baker, A.J.M., and Reeves, RD. 1993. Plants Clean Up Soils. Paper presented at the Baas “Science for Life” Meeting.

McKone T.E, and Maddalena R.L. 2007. Plant uptake of organic pollutants from soil: critical review of bioconcentration estimates based on models and experiments. Environmrntal Energy Technologies Division, Berkeley, California, USA.

McNair, M.R., Tilstone, G.H., and Smith, S.S. 2000. The genetics of metal tolerance and accumulation in higher plants. - In: Terry, N., and Bañuelos, G. (eds.): Phytoremediation of Contaminated Soil and Water, Lewis Publishers, Boca Raton.

Merian, H., (1991) Metals and their compounds in the Environment, Cambridge, New York.

Mulligan C.N., Yong, R.N., and Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering Geology volume 60: p. 193 – 207

Naicker, K., Cukrowska, E.M., and McCarthy, T.S. 2003. Acid mine drainage from gold mining activities in Johannesburg, South Africa. Environmental Pollution 122: p. 29-40.

Ndinya, E.B., (1998) Voltammetric study of stability constants and speciation of lead in a tropical freshwater environment (lake Baringo). Master's Thesis. Kenyatta University, Nairobi.

Neill, P.O. 1993. Environmental Chemistry Second edition, by Chapman and hall, London , UK.

Nemutandani, T. 2004. Potential of *Berkheya coddii* for the phytoremediation of nickel contaminated land around Barberton, Mpumalanga Province. BSc Honours dissertation, University of Venda for Science and Technology, South Africa.

Nindi, M. 2005, Comparison of soil chemistry of two metal contaminated soils where *Berkheya coddii* grows to the platinum mining tailings, MSc Thesis, University of Venda, School of Environmental Sciences, South Africa. p. 44-88

Nolte, J. 2002. ICP Emission spectrometry: A Practical Guide. Wiley – VCH, Germany.

Ochieng L. 2002. Mobility studies of heavy metals in mining polluted soil. Rustenburg Section of Anglo Platinum. MSc Thesis, University of Witwatersrand, Johannesburg.

Oelofse, S.H.H., Hobbs, P.J., Rascher, J. and Cobbing, J.E. 2007. The pollution and destruction threat of gold mining waste on the Witwatersrand - A West Rand case study. Symposium on environmental issues and waste management in energy and mineral production (SWEMP 2007), 11-13 December 2007. Bangkok.

Paterson S, Mackay D, McFarlane C. 1994. A model of organic chemicals uptake by plants from soil and the atmosphere. Environmental Science Technology 28: p. 2259 – 2266.

Pepper, I.L., Bengson, S. A., Rao, P. R. and Josephson, K. L. 2003. Revegetation of Mine tailings through the use of biosolid amendment. A paper presented at the 2003 National Meeting of the American Society of Mining and Reclamation and 9th Billings Land Reclamation Symposium, Billings MT, June 3-6, 2003.
plants that accumulate nickel. *Nature,* 379: 635-638.

Przybylowicz, J. M., Przbylowicz, W.J. and Pineda, C. A. 2001. Nuclear Microprobe studies of elemental distribution in apical leaves of the Ni hyperaccumulator *B. coddii*. South African, Journal of Science 97: p. 591-599.

Pulles, W., Banister, S. and Van Biljon, M. 2005. The development of appropriate procedures towards and after closure of underground gold mines from a water management perspective. Report No. 1215/1/05. Water Research Commission, Pretoria.

Quevauviller, P.H., Lachicha, M., Barahona, E., Rauret, G., Ure, A., Gome, A. and Muntau, H. 1996. Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. The Sciences of Total Environmrnt 178: p. 127 – 132

Quevauviller, Ph., Lachicha, M., Barahona, E., Rauret, G., Ure, A., Gome, A. and Muntau, H. 1996. Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. The Science of the Total Environment 178: p. 127-132.

Raskin, I, Kumar, P.B.A.N., Dushenkov, S. and Salt, D. 1994. Bio concentration of heavy metals by plants. – Current Opinion in Biotechnology 12: p. 44.

Raskin, I. 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13: p. 468-473.

Raskin, I., Smith, R.D., Salt, D.E. 1997. Phytoremediation of metals: using plant to remove pollutants from environment. Biotechnology 8: p. 221 – 226

Reeve, R.N. 1999. Environmental Analysis. Analytical chemistry by open learning. John Willey & Sons, New York.

Reeves, R. D. and Brooks, R. R. 1983. Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. Environmental Pollution 201: p. 277-285.

Richardson, M.L. 1990 Risk Assessment of Chemicals in the Environment, Royal Society of Chemistry, England.

Robinson B.H., Brooks R.R., and Clothier B.E. 1999. Soil amendments affecting nickel and cobalt uptake by *B. coddii*: potential for use in phytomining and phytoremediation. Annals of Botany 84: p 689-694.

Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H., Gregg, P.E.H., and De Dominicis V. 1997. The potential of the high-biomass nickel hyperaccumulator Berkheya coddii for phytoremediation and phytomining . Journal of Geochemical Exploration 60 (2) : p. 115 – 126.

Robinson, B.H., Brooks, R.R., Kirkman, J.H., Gregg, P.E.H., and Gremigni. P. 1996. Plant-available elements in soils and their influence on the vegetation over ultramafic (serpentine) rocks in New Zealand. J.R. Sot. N.Z. 26: p. 455-466.

Robinson, B.H., Lombi, E., Zhao, F.J., McGrath, S.P. 2003. Uptake and Distribution of nickel and other metals in the hyperaccumulator *B. coddii*. New Phytoremediation 158: p. 279- 285.

Rouse, J.W., Haas, R.H., Schell, J.A., and Deering, D.W. 1973. Monitoring vegetation systems in the Great Plains with ERTS. Third ERTS Symposium, NASA SP-3511: p. 309 – 317.

Salt D.E., Baylock, M., Kumar, Dushenkov, N.P.B.A., Ensely B.D., Chet. I. and Raskin I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from environment using plants. Biotechnology 13: p. 468-478.

Schwartz, C., Guimont, S., Saison, C., Perronnet, C. and Morel J.L. 2001. Phytoextraction of Cd and Zn by the hyperaccumulator plant Thlaspi caerulescens as affected by plant size and origin. South Africa Journal of Science 5: p 23 - 34

Shah, K., and Nongkynrih, J.M. 2006. Metal hyperaccumulation and bioremediation. Biological Plantarum 51: p 618 - 634.

Siebielec, G., Stuczyński, T., and Korzeniowska-Pucułek, R. 2005 Metal Bioavailability in Long-Term Contaminated Tarnowskie Gory Soils, Department of Soil Science Erosion and Land Conservation, Institute of Soil Science and Plant Cultivation,Czartoryskich 8, 24-100 Puławy, Poland

Sims, D.A. and Gamon, J.A. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species leave structures and development stages. Remote Sensing of the Environment 81: p. 337 – 354.

Singh, O.V., Labana, S., Pendey, G. and Budhirahja, R. 2003. Phytoremediation: an overview of metallic ion decontamination from soil. Applied Microbiology and Biotechnology 61: p. 405 - 412.

Singh, S. P., Ma, L. Q. and Harris, W. G. 2001. Heavy metal interactions with phosphoric clay: sorption and desorption behaviour. Journal of Environmental Quality 30: p. 61-68.

Smeda, A., and Zyrinicki, W. 2002. Application of sequential extraction and the ICP-AES Method for study of the partitioning of metals in fly ashes. Journal of Microchemical 72: p. 9-16.

Smith I.C., Carson B.L. and Hoffmeister F. 1978. Trace metals in the environment, Vol.2. Ann Arbor scientific Publications, p.405

Stalika, C.D., Pilidis, S. M. and Tzouwara-Karayami, S. 1999. Use of sequential extraction scheme with data normalisation to assess the metal distribution in agricultural soils irrigated by lake water. The Science of the Total Environment 236: p. 7-18.

U.S.EPA. 1983. Hazardous waste land treatment. USEPA SW- 874. Municipal Environmental Research Laboratory,Cincinnati, OH.

U.S.EPA. 1996. A Citizen's Guide to Phytoremediation.

U.S.EPA. 1997. Status of in situ phytoremediation technology. pp. 31-42. In Recent developments for in situ treatment of metal contaminated soils. March. EPA-542-R-97-004.

U.S.EPA. 1999. Screening Level Ecological Risk Assessment Protocol, Appendix C, Media-to-Receptor Bioconcentration Factors (BCFs) and Appendix D, Bioconcentration Factors (BCFs) for Wildlife Measurement Receptors. www.epa.gov/osw/hazard/tsd/td/combust/ecorisk/volume3/appx-c.pdf. www.epa.gov/osw/hazard/tsd/td/combust/eco-risk/volume3/appx-d.pdf.

U.S.EPA. 2000. Introduction to Phytoremediation. EPA/600/ R-99/107. Office of Research

U.S.EPA. 2000. Introduction to Phytoremediation. EPA/600/R-99/107. Cincinnati: Office of Research and Development. www.cluin.org/download/remed/introphyto.pdf.

U.S.EPA. 2008. Assessing Relative Bioavailability in Soil at Superfund Sites. www.epa.gov/superfund/health/contaminants/bioavailability/index.htm.

Vandegrift, G. F., Reed, D. T. and Tasker, I. R. 1992. Environmental remediation, removing organic and metal ion pollution. American Chemical Society, USA

Viloen, M. J. and Reimold, W. U. 1999. An introduction to South Africa's geological and mining heritage. South African Journal of Geology 102: p. 14-25.

Wade, M.J., Davis, B.K., Carlisle, J.S., Klein, A.K. and Valoppi, L.M. 1993. Environmental transformation of toxic metals. Occupational medicine 8: p. 576 - 601

Weiersbye I.M. 2007. Review and cost comparison of conventional and phyto-technologies for mine closure. Cite the publication i.e. Foune, A.B, Tibbeh, M., and Wiertz, J., (eds). Mine closure 2007: proceeding of the 2nd international seminer on mine closure.

Weiersbye, I.M. and Witkowski, E.T.F. 2003. Acid rock drainage from gold tailings dams on the Witwatersrand basin impacts on tree seed fate and seedling morphology. In: D. Armstrong, A.B. de Villiers, R.L.P. Kleinmann, T.S. McCarthy and P.J. Norton (eds). Mine Water and the Environment VIII. Proceedings of the 8th International Congress on Mine Water and the Environment, International Mine Water Association, p. 311-328.

Weiersbye, I.M. and Witkowski, E.T.F. 2007. Impacts of acid mine drainage (AMD) on the regeneration potential of highveld phreatophyte plants. In J.J. Bester, A.H.W. Seydack, Vorster, T., Van der Merwe, I.J. and Dzivhani, S. Multiple Use Management of Natural Forests and Woodlands: Policy Refinements and Scientific Progress IV. Department of Water Affairs and Forestry of South Africa. www.dwaf.gov.za/forestry, p. 224 – 237.

Weiersbye, I.M. Straker, C.J., and Przybylowicz, W.J. 1999 Micro-PIXE mapping of elemental distribution in arbuscular mycorrhizal roots of the grass, *Cynodon dactylon*, from gold and uranium mine tailings. Nuclear Instruments and Methods in Physics Research 158: p. 335-343.

Weiersbye, I.M., Witkowski, E.T.F. and Reichardt, M. 2006. Floristic composition of gold and uranium tailings dams, and adjacent polluted areas, on South Africa's deep-level mines. Bothalia 36 : p. 101-122

Weiss, J. and Weiss, T. 2005. Handbook of Ion Chromatography, Third, Completely Revised and Enlarged Edition. John Wiley and Sons, Inc. ISBN: 3-527-28701-9: p. 931

Wild, A. 1996. Soils and the Environment. Cambridge University Press, UK.

Yaron, B. 1996. Soil Pollution Processes and Dynamics, library of congress, Germany.

Appendices

Appendix 1: Operational and ecological definition

- **Phytoremediation:**

  Is defined as the direct use of living green plants to clean up polluted and contaminated soils with both organic and inorganic contaminants through removal, degradation, or containment
- **Phytoextraction:**

  Is defined as a process that has applications for recovery of metals from substrates such as soil, particularly those associated with sub-economic mineralization and contamination by industries

- **Arbuscular mycorrhizal / fungi:**

  Is defined as a symbiosis between a roots fungus of a higher plants in which the fungus penetrates the cortical cells of the roots of a vascular plant.

- **Above ground biomass:**

  For the purpose of this research this refers to the plant shoot (stem and leaves).

- **Below ground biomass:**

  This refers to the roots.
- **Heavy metals and metalloids:**

  These are transition elements with high density, malleability, electrical and thermal conductivity that have high specific gravity and high atomic mass, such as lead, cadmium, zinc, copper, silver, and mercury. However some heavy metals are essential plant micronutrients, but all are toxic at high concentrations because they are very reactive in the organisms body.

- **Metals and metalloids:**

Are defined as any element that has a silvery lustre and is a good conductor of heat and electricity

- ***Berkheya coddii Roesler*:**

  Is an asteraceous perennial plant that typically grows to a height of about 1.5 meters. This species has been found naturally only on serpentine soils in South Africa Mpumalanga province. The plant belongs to a group of very unusual plants termed hyperaccumulators (Brooks *et al*., 1977). *B. coddii* is defined as a fast-growing, high biomass nickel (Ni) and cobalt (Co) hyperaccumulator plant that has attracted attention for the last decades for its possible use in phytoextraction and phytoremediation of Ni and Co contaminated soil (Robinson *et al*., 2003).

- **Hyperaccumulator plants:**

  This refers to plants that tend to accumulate heavy metals from the soil into the aerial parts to concentrations in excess of threshold concentrations set by Brooks *et al*. (1977) in dry biomass. The root:shoot quotient must be greater than 1 (i.e. more metal in shoot than in root).

- **Environmental standard:**

  This is regarded as a level or limit of contaminants (i.e. heavy metals) that should not be exceeded, and this will normally be specified as the concentration of pollutant in a particular environmental media (Harrison, 1999).

- **Serpentine soil:**

  This refers to a type of ultramafic soil that is derived from mineral-rich rocks and contains high concentrations of base metals.

- **Pollution:**

  Is defined as the introduction into the environment of natural and man-made substances liable to cause hazards to human health, harm to living resources and ecological systems damage to structures or amenity or interference with legitimate uses of environment

- **Tailing dumps:**

  Refers to those mine waste materials that are characterized of fine waste residue deposits.

- **Seed germination:**

- This is defined as the process that leads to the growth of an embryonic plant contained within a seed which results in the formation of the seedling

- **Seedling establishment:**

  For the purpose of this project the seed establishment is basically referring to the seedlings that manage to grow after having been transplanted in both substrates, following the seed germination process

- **Plant percentage survival**

  This is defined as the percentage of the original plants which are still living at each observation period while carrying out the experiment.

- **Bio-concentration Factor**

  This is used to describe the accumulation of chemicals in organisms (plants and animals), from contaminated environments (soil and water).

- **Bio-concentration**

  This is defined as the increase in concentration of a chemical in an organism (plants and animals) resulting from tissue absorption levels exceeding the rate of metabolism and excretion.

- **Enrichment factor**

  The amount by which an element must be increased above its normal crustal abundance in an ore to make it commercially extractable

- **Metallophyte**

  This is a plant that can tolerate high constitute of metals in the growth substrate such as Ni, Co and Pb. Such plants range from "obligate metallophytes" (which are the plants that can only survive in the presence of these metals), "facultative metallophytes" which can tolerate such conditions but are not confined to them. An example of this type of plant is *B. coddii*

- **Tolerance**

  This is defined as the ability of plants to survive in a soil that is toxic to other plants and is manifested by an interaction between a genotype and its environment (McNair *et al.* 2000)

Appendix 2: Soil solution conductivity, pH and redox potential obtained prior to the growth experiment

Soil pH, EC, and Eh obtained prior to the commencement of the growth experiment			
Water extract:			
Sample no	pH	Sample	pH
N 1	6.4	RBMR 1	8.02
N 2	6.3	RBMR 2	8.02
N 3	6.5	RBMR 3	8.01
N 4	6.3	RBMR 4	8.01
Mean	6.375	Mean	8.015
STDEV	0.095743	STDEV	0.005773503
Standard Error	0.047871	Standard Error	0.002886751
Sample no	Conductivity (µS/cm)	Sample no	Conductivity (µS/cm)
N 1	65	RBMR 1	1494
N 2	64	RBMR 2	1496
N 3	66	RBMR 3	1493
N 4	65	RBMR 4	1494
Mean	65	Mean	1494.25
STDEV	0.816497	STDEV	1.258305739
Standard Error	0.408248	Standard Error	0.62915287
Sample no	Reduction oxidation potential (mV)	Sample no	Reduction oxidation potential (mV)
N 1	360.6	RBMR 1	233.4
N 2	357.6	RBMR 2	233.4
N 3	357.8	RBMR 3	234.4
N 4	357.7	RBMR 4	233.8
Mean	358.425	Mean	233.75
STDEV	1.452297	STDEV	0.472581563
Standard Error	0.726149	Standard Error	0.236290781
0.1 M KCl extract:			
Sample no	pH	Sample no	pH
N 1	6.02	RBMR 1	7.91
N 2	6.03	RBMR 2	8.01
N 3	6.02	RBMR 3	7.99
N 4	6.02	RBMR 4	7.95
Mean	6.0225	Mean	7.965
STDEV	0.005	STDEV	
Standard Error	0.0025	Standard Error	0.022173558

Appendix 3: Soil conductivity, pH and redox potential obtained after the growth experiment

Soil pH, EC, Eh results obtained after the growth experiment: Water Extracts)			
NATIVE SOIL(N)			
untreated sample (control) (Native soil) (0 g s/kg soil)	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
N 1	6.6	418	551.3
N 2	6.7	230	546.3
N 3	6.2	502	479.6
N 4	6.2	561	396.3
Mean	6.4	427.5	493.4
STDEV	0.3	144.3	72.5
RSD	0.04	0.3	0.1
Standard Error	0.14	72.2	36.3
Sample treated with 2.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
N 1	6.3	753	544.6
N 2	6.1	789	539.8
N 3	5.4	996	543.4
N 4	4.8	1371	546.1
Mean	5.7	977.25	543.5
STDEV	0.7	283.5	2.7
RSD	0.1	0.29	0.005
Standard Error	0.35	141.8	1.3
Sample treated with 4.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
N 1	5.7	1109	541.3
N 2	5.6	926	529.5
N 3	5.2	933	517.6
N 4	4.7	1501	508.1
Mean	5.3	1117.3	524.1
STDEV	0.4	269.5	14.4
RSD	0.08	0.2	0.02
Standard Error	0.2	134.7	7.2
Sample treated with 8.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
N 1	5.33	1008	472.6
N 2	4.91	1468	456.9
N 3	4.69	1345	458.7

N 4	3.87	1470	470.3
Mean	4.7	1322.75	464.625
STDEV	0.613731755	217.8246619	7.970518595
RSD	0.130581224	0.164675609	0.017154735
Standard Error	0.306865877	108.912331	3.985259297
RBMR waste soil			
untreated sample (control) 0.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
RBMR 1	7.59	1397	370.8
RBMR 2	7.82	685	356.6
RBMR 3	7.76	1816	367.3
RBMR 4	7.86	1477	269.4
Mean	7.7575	1343.75	341.025
STDEV	0.118988795	475.2412545	48.13054297
RSD	0.015338549	0.35366791	0.14113494
Standard Error	0.059494397	237.6206273	24.06527149
Sample treated with 2.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
RBMR 1	7.36	769	376.4
RBMR 2	7.44	667	342.3
RBMR 3	7.6	1787	347.1
RBMR 4	7.69	1373	366.8
Mean	7.5225	1149	358.15
STDEV	0.149749791	527.2393511	16.13701749
RSD	0.019906918	0.458868017	0.045056589
Standard Error	0.074874896	263.6196755	8.068508743
Sample treated with 4.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)
RBMR 1	7.53	730	348.2
RBMR 2	7.52	1818	362.7
RBMR 3	7.51	1349	332.4
RBMR 4	7.42	1965	359.5
Mean	7.495	1465.5	350.7
STDEV	0.050662281	556.264026	13.69403763
RSD	0.006759477	0.37957286	0.039047726
Standard Error	0.02533114	278.132013	6.847018816
Sample treated with 8.0 g S/kg soil	pH	Conductivity (µS/cm)	Reduction oxidation potential (mV)

RBMR 1	7.39	1852	374.6
RBMR 2	7.51	1777	374.1
RBMR 3	7.36	1483	343.3
RBMR 4	7.46	1259	307.8
Mean	7.43	1592.75	349.95
STDEV	0.0678233	273.613322	31.68432841
RSD	0.009128304	0.171786735	0.090539587
Standard Error	0.03391165	136.806661	15.8421642

Appendix 4: Concentration of Co and Ni (mg/kg) in soil, prior growth experiment

BCR sequential extraction results obtained before the commencement of the growth experiment		
C1 = before growth experiment	Co	Ni
Water extraction (soil 100 g soil = 200 ml solution)		
N1 (C1)	0.26	2.5
N1 (C1)	0.158	1.7
N1 (C1)	0.068	0.971
N1 (C1)	0.044	0.643
MEAN (mg/L)	0.1325	1.4535
STDEV	0.098147848	0.82576
RSD	0.740738473	0.568119
Final conc. (mg/kg)	0.265	2.907
Standard Error	0.049073924	0.41288
RBMR (C1)	0.012	0.183
RBMR (C1)	0.007	0.172
RBMR (C1)	0.021	0.165
RBMR (C1)	0.019	0.155
MEAN (mg/L)	0.01475	0.16875
STDEV	0.006448514	0.011786
RSD	0.437187393	0.069845
Final conc. (mg/kg)	0.0295	0.3375
Standard Error	0.003224257	0.005893
Acetic acid extraction (soil 100 g soil = 200 ml solution)		
C1 = before growth experiment		
N1 (C1)	0.353	1.5
N1 (C1)	0.328	1.4
N1 (C1)	0.326	1.1
N1 (C1)	0.34	1.2
MEAN (mg/L)	0.33675	1.3
STDEV	0.012473305	0.182574
RSD	0.037040252	0.140442
Final conc. (mg/kg)	0.6735	2.6
Standard Error	0.006236652	0.091287
RBMR (C1)	0.549	9.5
RBMR (C1)	1.113	8.2
RBMR (C1)	0.707	6.8
RBMR (C1)	0.586	8.8
MEAN (mg/L)	0.73875	8.325
STDEV	0.258462602	1.147098
RSD	0.349864774	0.13779
Final conc. (mg/kg)	1.4775	16.65
Standard Error	0.129231301	0.573549

Hydroxylamine extraction (soil 100 g soil = 200 ml solution)		
C1 = before growth experiment		
N1 (C1)	15	24.1
N1 (C1)	16.1	23.3
N1 (C1)	16.3	24.6
N1 (C1)	15.4	22.5
MEAN (mg/L)	15.7	23.625
STDEV	0.605530071	0.921502
RSD	0.038568794	0.039005
Final conc. (mg/kg)	31.4	47.25
Standard Error	0.302765035	0.460751
RBMR (C1)	20	29
RBMR (C1)	13.4	11
RBMR (C1)	15.8	13
RBMR (C1)	13.6	15
MEAN (mg/L)	15.7	17
STDEV	3.065941943	8.164966
RSD	0.195282926	0.480292
Final conc. (mg/kg)	31.4	34
Standard Error	1.532970972	4.082483
Hydrogen peroxide and ammonium acetate extraction (soil 100 g soil = 200 ml solution)		
C1 = before growth experiment		
N1 (C1)	1.6	5.3
N1 (C1)	2.2	6
N1 (C1)	2.1	4.2
N1 (C1)	2	4.4
MEAN (mg/L)	1.975	4.975
STDEV	0.262995564	0.834166
RSD	0.133162311	0.167672
Final conc. (mg/kg)	3.95	9.95
Standard Error	0.131497782	0.417083
RBMR (C1)	0.906	19.3
RBMR (C1)	1.15	14.7
RBMR (C1)	1.5	22.1
RBMR (C1)	2.1	37
MEAN (mg/L)	1.414	23.275
STDEV	0.518250904	9.645163
RSD	0.366514077	0.4144
Final conc. (mg/kg)	2.828	46.55
Standard Error	0.259125452	4.822581
Aqua regia results obtained before the growth experiment (soil 100 g soil = 200 ml solution)		
C1 = before growth experiment	Co	Ni
N (C1)	156	2970

N (C1)	152	2730
N (C1)	160	2900
N (C1)	156	3000
Mean	156	2900
STDEV	3.265986	120.8305
RSD	0.020936	0.041666
Standard Error	1.632993	60.41523
RBMR (C1)	62	4010
RBMR (C1)	64	3999
RBMR (C1)	68	4006
RBMR (C1)	70	3985
Mean	66	4000
STDEV	3.651484	10.98484
RSD	0.055326	0.002746
Standard Error	1.825742	5.492419

Appendix 5: Concentrations of Co and Ni (mg/kg dry mass) in the soil substrate after sulfur treatment and growth of *B. coddii* for 266 days. (BDL: indicate concentrations below detection limit) (mg/kg)

BCR sequential extraction results obtained after the growth experiment									Aqua regia obtained after the growth experiment	
Samples	Water extraction (Ni)	Water extraction (Co)	Acetic acid extraction (Ni)	Acetic acid extraction (Co)	Hydroxylamine extraction (Ni)	Hydroxylamine extraction (Co)	Hydrogen peroxide and ammonium acetate extraction (Ni)	Hydrogen peroxide and ammonium acetate extraction (Co)	Aqua regia (Ni)	Aqua regia (Co)
N (0 g S /kg soil)	BDL	BDL	5.48	1.31	220	BDL	27	BDL	1157	140
N (0 g S /kg soil)	BDL	BDL	5.27	2.03	290	BDL	BDL	BDL	1162	143
N (0 g S /kg soil)	BDL	BDL	9.06	1.99	150	BDL	15.7	BDL	1161	139
N (0 g S /kg soil)	BDL	BDL	7.32	2.35	210	BDL	20	BDL	1160	138
Mean	BDL	BDL	6.7825	1.92	217.5	BDL	17.85	BDL	1160	140
STDEV	#DIV/0!	#DIV/0!	1.77577	0.437417	57.37305	#DIV/0!	5.703508	#DIV/0!	2.160246899	2.160246899
Standard Error	#DIV/0!	#DIV/0!	0.887885	0.218708	28.68652	#DIV/0!	3.292922	#DIV/0!	1.08012345	1.08012345
N (2.0 g S /kg soil)	BDL	BDL	6.37	1.645	240	BDL	22.3	BDL	1851	158
N (2.0 g S /kg soil)	BDL	BDL	7.215	2.665	280	BDL	27.2	BDL	1852	156
N (2.0 g S /kg soil)	BDL	BDL	8.815	2.25	150	BDL	15	BDL	1850	159
N (2.0 g S /kg soil)	BDL	BDL	8.3	2.06		BDL	15.2	BDL	1855	155
Mean		BDL	7.675	2.155	223.3333	BDL	19.925	BDL	1852	157
STDEV	#DIV/0!	#DIV/0!	1.096183	0.423576	66.58328	#DIV/0!	5.920234	#DIV/0!	2.160246899	1.825741858
Standard Error	#DIV/0!	#DIV/0!	0.548091	0.211788	38.44188	#DIV/0!	2.960117	#DIV/0!	1.08012345	0.912870929
N (4.0 g S /kg soil)	BDL	BDL	8.476667	1.72	BDL	BDL	20	BDL	1336.5	118
N (4.0 g S /kg soil)	BDL	BDL	7.725	3.075	180	BDL	14.4	BDL	1340	111
N (4.0 g S /kg soil)	BDL	BDL	7.875	2.64	180	BDL	19.2	BDL	1335.5	112
N (4.0 g S /kg soil)	BDL	BDL	8.15	2.55	200	BDL	12.1	BDL	1334	111
Mean	BDL	BDL	8.056667	2.49625	186.6667	BDL	16.425	BDL	1336.5	113
STDEV	#DIV/0!	#DIV/0!	0.330715	0.566	11.54701	#DIV/0!	3.798574	#DIV/0!	2.549509757	3.366501646
Standard Error	#DIV/0!	#DIV/0!	0.165357	0.283	6.666667	#DIV/0!	1.899287	#DIV/0!	1.274754878	1.683250823

N (8.0 g S /kg soil)	BDL	BDL	7.075	2.03	BDL	BDL	15.6	BDL	1810	270
N (8.0 g S /kg soil)	BDL	BDL	9.246667	2.726667	BDL	BDL	20.5	BDL	1797	262
N (8.0 g S /kg soil)	BDL	0.91	6.526667	2.686667	130	BDL		BDL	1798	261
N (8.0 g S /kg soil)	BDL	0.233	7.51	3.585		BDL	22.1	BDL	1795	263
Mean	BDL	0.5715	7.589583	2.757083	130	BDL	19.4	BDL	1800	264
STDEV	#DIV/0!	0.478711	1.175705	0.637699	#DIV/0!	#DIV/0!	3.386739	#DIV/0!	6.782329983	4.082482905
Standard Error	#DIV/0!	0.3385	0.587852	0.318849	#DIV/0!	#DIV/0!	1.955335	#DIV/0!	3.391164992	2.041241452
RBMR (0 g S /kg soil)	BDL	BDL	BDL	0.9	350	BDL	321	BDL	661	9
RBMR (0 g S /kg soil)	BDL	BDL	BDL	1.78	349.5	43	511	BDL	663	5
RBMR (0 g S /kg soil)	BDL	BDL	BDL	1	290	BDL	BDL	BDL	659	8
RBMR (0 g S /kg soil)	0.8	BDL	BDL	1.22	490	BDL	462	BDL	661	6
Mean	0.8	BDL	BDL	1.225	369.875	43	431.3333	BDL	661	7
STDEV	#DIV/0!	#DIV/0!	#DIV/0!	0.393404	84.89246	#DIV/0!	98.64245	#DIV/0!	1.632993162	1.825741858
Standard Error	#DIV/0!	#DIV/0!	#DIV/0!	0.196702	42.44623	#DIV/0!	56.95125	#DIV/0!	0.816496581	0.912870929
RBMR (2.0 g S /kg soil)	BDL	BDL	BDL	1.54	362	31	242	BDL	1186	BDL
RBMR (2.0 g S /kg soil)	BDL	BDL	50.35	2.39	420	BDL	396	BDL	1178.5	BDL
RBMR (2.0 g S /kg soil)	BDL	BDL	BDL	2.34	500	BDL	BDL	BDL	1179	BDL
RBMR (2.0 g S /kg soil)	BDL	BDL	BDL	1.34	320	BDL	401	BDL	1182.5	BDL
Mean	BDL	BDL	50.35	1.9025	400.5	31	346.3333	BDL	1181.5	BDL
STDEV	#VALUE!	#DIV/0!	#DIV/0!	0.54064	77.98077	#DIV/0!	90.3899	#DIV/0!	3.488074923	#DIV/0!
Standard Error	#VALUE!	#DIV/0!	#DIV/0!	0.27032	38.99038	#DIV/0!	52.18663	#DIV/0!	1.744037461	#DIV/0!
RBMR (4.0 g S /kg soil)	BDL	BDL	64.2	3.03	500	BDL	381	BDL	1340	34
RBMR (4.0 g S /kg soil)	0.45	BDL	BDL	3.38	380	BDL	445	BDL	1332	40
RBMR (4.0 g S /kg soil)	0.47	BDL	BDL	3.37	470	BDL	453	BDL	1333	39
RBMR (4.0 g S /kg soil)	0.81	BDL	BDL	1.38	400	BDL	411	BDL	1331	39
Mean	0.576667	BDL	64.2	2.79	437.5	BDL	422.5	BDL	1334	38
STDEV	0.20232	#DIV/0!	#DIV/0!	0.953974	56.78908	#DIV/0!	33.12099	#DIV/0!	4.082482905	2.708012802

Standard Error	0.116809	#DIV/0!	#DIV/0!	0.476987	28.39454	#DIV/0!	16.5605	#DIV/0!	2.041241452	1.354006401
RBMR (8.0 g S /kg soil)	BDL	BDL	BDL	3.76	290	BDL	BDL	BDL	926	88
RBMR (8.0 g S /kg soil)	BDL	BDL	BDL	3.3	350	BDL	515	BDL	916	76
RBMR (8.0 g S /kg soil)	0.48	BDL	BDL	5.33	380	BDL	300	BDL	920	78
RBMR (8.0 g S /kg soil)	BDL	BDL	36.3	2.63	480	BDL	288	BDL	918	82
Mean	0.48	BDL	36.3	3.755	375	BDL	367.6667	BDL	920	81
STDEV	#DIV/0!	#DIV/0!	#DIV/0!	1.14794	79.37254	#DIV/0!	127.7354	#DIV/0!	4.320493799	5.291502622
Standard error	#DIV/0!	#DIV/0!	#DIV/0!	0.57397	39.68627	#DIV/0!	73.74807	#DIV/0!	2.160246899	2.645751311

Appendix 6: Concentrations of Co and Ni in *B. coddii* organs (i.e. roots, stems and leaves) after sulfur treatments and growth of *B. coddii* for 266 days.

Samples/Plant parts	Roots (Co)	Roots (Ni)	Stems (Co)	Stems (Ni)	Leaves (Co)	Leaves (Ni)	Total Ni Concentration in dry matter	Total Co Concentration in dry matter
Np (0 g S/kg soil)	BDL	357.5	BDL	957	244	2800	1371.5	244
Np (0 g S/kg soil)	BDL	208.5	BDL	531	145	3070	1269.8	145
Np (0 g S/kg soil)	14	379	BDL	1500	45	4700	2193	29.5
Np (0 g S/kg soil)	19	344.5	BDL	861	25	3000	1402	22
Mean	16.5	322.375		962.25	114.75	3393	1559	66
STDEV	3.535533906	77.23813285	#DIV/0!	402.2564481	100.8972249	879.1425747	453	106
Standard Error	2.5	38.61906643	#DIV/0!	201.128224	50.44861247	439.5712874	226	52.7614028
Np (2.0 g S/kg soil)	16	259	BDL	1300	28	3300	1620	22
Np (2.0 g S/kg soil)	9	516	BDL	1300	30	3900	1905	20

Np (2.0 g S/kg soil)	BDL	417	BDL	2000	26	7100	3172.333333	26
Np (2.0 g S/kg soil)	BDL	360.5	BDL	1050	30	5800	2403.5	30
Mean	12.5	388.125		1412.5	28.5	5025	2275.208333	20.5
STDEV	4.949747468	107.4277858	#DIV/0!	409.0130397	1.914854216	1746.186321	754.2090488	4.607511982
Standard Error	3.5	53.71389291	#DIV/0!	204.5065199	0.957427108	873.0931604	377.1045244	2.303755991
Np (4.0 g S/kg soil)	11	519	BDL	1587	104	6600	2902	57.5
Np (4.0 g S/kg soil)	12	412	BDL	BDL	40	4745	2578.5	26
Np (4.0 g S/kg soil)	11	557	BDL	1590	35	3800	1982.333333	23
Np (4.0 g S/kg soil)	13	496	BDL	1590	13	2000	1362	13
Mean	11.75	496		1589	48	4286.25	2123.75	29.875
STDEV	0.957427108	61.38946707	#DIV/0!	1.732050808	39.13225439	1917.226186	660.1159014	19.2370086
Standard Error	0.478713554	30.69473353	#DIV/0!	1	19.56612719	958.6130932	330.1026089	9.618504302
Np (8.0 g S/kg soil)	BDL	503	BDL	BDL	96	3300	1901.5	96
Np (8.0 g S/kg soil)	BDL	450	BDL	2200	37	5900	2850	37
Np (8.0 g S/kg soil)	6	534.1	BDL	BDL	31	3760	2147.05	18.5
Np (8.0 g S/kg soil)	11	514	BDL	BDL	29	2500	1507	20
Mean	8.5	500.275		2200	48.25	3865	2188.425	28.375
STDEV	3.535533906	35.90500197	#DIV/0!	#DIV/0!	32.01431971	1453.123073	564.4340091	36.39682907
Standard Error	2.5	17.95250099	#DIV/0!	#DIV/0!	16.00715986	726.5615367	282.2170045	18.19841454

RBMRp (0 g S /kg soil)	BDL	79	BDL	225	BDL	BDL	152	BDL
RBMRp (0 g S /kg soil)	BDL	177	BDL	177	14	1310	554.6666667	14
RBMRp (0 g S /kg soil)	BDL	142	BDL	239	BDL	2080	820.3333333	BDL
RBMRp (0 g S /kg soil)	BDL	115	BDL	189.5	BDL	1147.5	484	BDL
Mean	BDL	128.25		207.625	14	1512.5	616.125	14
STDEV	#DIV/0!	41.5	#DIV/0!	29.17011884	#DIV/0!	498.1402915	189.6034701	#DIV/0!
Standard Error	#DIV/0!	20.75	#DIV/0!	14.58505942	#DIV/0!	287.6014314	107.6454969	#DIV/0!
RBMRp (2.0 g S /kg soil)	BDL	196	BDL	206.5	22	BDL	201.25	22
RBMRp (2.0 g S /kg soil)	BDL	177	BDL	301	BDL	BDL	239	BDL
RBMRp (2.0 g S /kg soil)	BDL	111	BDL	144	12	798	351	12
RBMRp (2.0 g S /kg soil)	BDL	124	BDL	236	BDL	BDL	180	BDL
Mean	BDL	152		221.875	17	798	390.625	17
STDEV	#DIV/0!	40.93083597	#DIV/0!	65.22061918	7.071067812	#DIV/0!	76.14005051	7.071067812
Standard Error	#DIV/0!	20.46541799	#DIV/0!	32.61030959	5	#DIV/0!	38.07002526	5
RBMRp (4.0 g S /kg soil)	BDL	145.5	BDL	353	BDL	780	426.1666667	BDL
RBMRp (4.0 g S /kg soil)	BDL	152	BDL	351	844.5	BDL	251.5	844.5
RBMRp (4.0 g S /kg soil)	BDL	170	BDL	357	20	BDL	263.5	20
RBMRp (4.0 g S /kg soil)	BDL	155	BDL	196	BDL	BDL	175.5	BDL
Mean	BDL	155.625		314.25	432.25	780	416.625	432.25
STDEV	#DIV/0!	10.37123426	#DIV/0!	78.87278787	583.0095411	#DIV/0!	105.4619468	583.0095411

Standard Error	#DIV/0!	5.185617128	#DIV/0!	39.43639394	412.25	#DIV/0!	52.7309734	412.25
RBMRp (8.0 g S /kg soil)	BDL	190	BDL	214	18	1330	578	18
RBMRp (8.0 g S /kg soil)	BDL	117.5	BDL	164	BDL	1450	577.1666667	BDL
RBMRp (8.0 g S /kg soil)	BDL	243	BDL	302	12	BDL	272.5	12
RBMRp (8.0 g S /kg soil)	BDL	109	BDL	217	BDL	BDL	163	BDL
Mean	BDL	164.875		224.25	15	1390	593.0416667	15
STDEV	#DIV/0!	63.51164591	#DIV/0!	57.25018195	4.242640687	84.85281374	68.53821387	4.242640687
Standard error	#DIV/0!	31.75582296	#DIV/0!	28.62509098	3	60	40.12697131	3

Appendix 7: Dry matter production (g) of *B. coddii* in response to sulfur (g/kg) treatment of Native soil (Np) and RBMR waste soil soil (RBMRp) = *B. coddii* planted on the RBMR waste soil, Np = *B. coddii* planted on the N soil) treated with various amount of sulfur.

N plants with 0 g of S/kg soil	Root dry mass	stem /shoot dry mass	Leaves dry mass	Total dry mass
N plants				
Np (0 g S /kg soil)	2.83	0.28	1.53	4.64
Np (0 g S /kg soil)	3.88	0.69	2.55	7.12
Np (0 g S /kg soil)	6.4	0.93	2.51	9.84
Np (0 g S /kg soil)	6.96	1.77	3.88	12.61
Mean	5.0175	0.9175	2.6175	8.5525
STDEV	1.980208322	0.628510143	0.964827273	3.573545738
RSD	0.394660353	0.685024679	0.368606408	1.44829144
Standard Error	0.990104161	0.314255072	0.482413636	1.786772869
N plants treated with 2.0 g S/kg soil				
Np (2.0 g S /kg soil)	6.59	0.23	2.26	9.08
Np (2.0 g S /kg soil)	9.23	1.24	2.19	12.66
Np (2.0 g S /kg soil)	9.04	1.29	2.55	12.88
Np (2.0 g S /kg soil)	8.5	1.99	2.3	12.79
Mean	8.34	1.1875	2.325	11.8525
STDEV	1.206952084	0.724356036	0.156737573	2.088045693
RSD	0.144718475	0.60998403	0.06741401	0.822116515
Standard Error	0.603476042	0.362178018	0.078368786	1.044022846
N plants treated with 4.0 g S/kg soil				
Np (4.0 g S /kg soil)	2.83	0.36	1.62	4.81
Np (4.0 g S /kg soil)	13.83	1.41	2.73	17.97
Np (4.0 g S /kg soil)	4.56	2.04	2.49	9.09
Np (4.0 g S /kg soil)	5.85	1.41	2.3	9.56
Mean	6.7675	1.305	2.285	10.3575
STDEV	4.86818498	0.696491206	0.476969601	6.041645787
RSD	0.719347614	0.533709736	0.208739431	1.461796782
Standard Error	2.43409249	0.348245603	0.2384848	3.020822893
N plants treated with 8.0 g S/kg soil				
Np (8.0 g S /kg soil)	1.75	0.18	0.72	2.65
Np (8.0 g S /kg soil)	6.64	0.79	2.98	10.41
Np (8.0 g S /kg soil)	7.84	1.03	2.44	11.31
Np (8.0 g S /kg soil)	7.88	2.46	2.99	13.33
Mean	6.0275	1.115	2.2825	9.425
STDEV	2.909127876	0.965418735	1.072889401	4.947436012
RSD	0.482642534	0.865846399	0.470050121	1.818539054
Standard Error	1.454563938	0.482709367	0.5364447	2.473718006
RBMR plants				
RBMR plants with 0.0 g S/kg soil	Roots dry mass	Stem dry mass	Leaves dry mass	Total dry mass
RBMRp (0 g S/kg soil)	2.67	0.16	0.94	3.77
RBMRp (0 g S/kg soil)	1.94	0.42	1.28	3.64
RBMRp (0 g S/kg soil)	0.96	0.58	0.53	2.07
RBMRp (0 g S/kg soil)	3.8	0.53	1.57	5.9
Mean	2.3425	0.4225	1.08	3.845

STDEV	1.197897464	0.187327699	0.448032737	1.833257899
RSD	0.511375651	0.443379168	0.414845127	1.369599947
Standard Error	0.598948732	0.093663849	0.224016368	0.91662895
RBMR plants treated with 2.0 g S/kg soil sulfur				
RBMRp (2. 0 g S/kg soil)	2.54	0.26	0.68	3.48
RBMRp (2. 0 g S/kg soil)	1.85	0.03	0.63	2.51
RBMRp (2. 0 g S/kg soil)	3.56	0.42	1.08	5.06
RBMRp (2. 0 g S/kg soil)	2.44	0.56	1.53	4.53
Mean	2.5975	0.3175	0.98	3.895
STDEV	0.710228836	0.227504579	0.418330013	1.356063428
RSD	0.273427848	0.716549854	0.42686736	1.416845063
Standard Error	0.355114418	0.113752289	0.209165007	0.678031714
RBMR plants treated with 4.0 g S/kg soil				
RBMRp (4. 0 g S/kg soil)	2.78	0.31	0.9	3.99
RBMRp (4. 0 g S/kg soil)	2.64	0.22	1.14	4
RBMRp (4. 0 g S/kg soil)	3.64	0.26	0.98	4.88
RBMRp (4. 0 g S/kg soil)	2.91	0.35	1.62	4.88
Mean	2.9925	0.285	1.16	4.4375
STDEV	0.445524036	0.056862407	0.32249031	0.824876753
RSD	0.148880213	0.199517218	0.278008888	0.626406318
Standard Error	0.222762018	0.028431204	0.161245155	0.412438376
RBMR plants treated with 8.0 g of S/kg soil				
RBMRp (8. 0 g S/kg soil)	3.4	0.13	0.93	4.46
RBMRp (8. 0 g S/kg soil)	2.13	0.23	0.8	3.16
RBMRp (8. 0 g S/kg soil)	2.31	0.28	0,79	2.59
RBMRp (8. 0 g S/kg soil)	2.68	44	0.02	46.7
Mean	2.63	11.16	0.583333333	14.37333333
STDEV	0.562079472	21.89342215	0.492172057	22.94767368
RSD	0.213718431	1.961776178	0.843723526	3.019218134
Standard Error	0.281039736	10.94671107	0.284155669	11.51190648

Appendix 8: Number of leaves on *B. coddii* plants over 266 days growing period

Leaf count before sulfur treatment																Leaf count after sulfur treatment					
Samples/days	1	7	21	35	49	62	80	94	108	122	136	150	163	177	191	205	219	233	247	255	261	266
Np (0 g S/kg soil)	3	2	1	4	6	8	8	9	12	12	12	14	17	17	23	17	25	25	26	27	29	28
Np (0 g S/kg soil)	5	4	4	5	7	10	13	13	13	12	20	23	25	25	29	28	31	31	32	33	35	37
Np (0 g S/kg soil)	4	3	3	6	9	12	12	15	14	21	21	24	26	26	29	26	29	29	30	35	30	31
Np (0 g S/kg soil)	4	2	3	6	8	11	14	19	19	24	33	36	43	43	43	41	44	44	45	47	49	49
Mean Leaf	4	3	3	5	8	10	12	14	15	17	22	24	28	28	31	28	32	32	33	36	36	36
STDEV	1	1	1	1	1	2	3	4	3	6	9	9	11	11	8	10	8	8	8	8	9	9
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	1	1	1	1	1	1	2	2	3	4	5	6	6	4	5	4	4	4	4	5	5
Np (2.0 g S/kg soil)	4	4	3	5	6	7	9	11	15	13	16	16	15	15	19	17	20	20	22	22	22	23
Np (2.0 g S/kg soil)	3	4	2	5	9	14	16	19	19	17	23	29	35	35	33	36	35	35	36	36	35	34
Np (2.0 g S/kg soil)	3	1	1	4	5	7	10	13	13	18	26	28	31	31	33	30	35	35	39	38	39	38
Np (2.0 g S/kg soil)	4	3	2	4	7	8	13	17	17	20	26	29	32	32	32	30	30	30	32	32	31	31
Mean Leaf	4	3	2	5	7	9	12	15	16	17	23	26	28	28	29	28	30	30	32	32	32	32
STDEV	1	1	1	1	2	3	3	4	3	3	5	6	9	9	7	8	7	7	7	7	7	6
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	1	0	0	1	2	2	2	1	2	2	3	5	5	3	4	4	4	4	4	4	3

Np (4.0 g S/kg soil)	3	2	2	4	6	4	6	9	9	9	11	11	13	13	19	15	22	22	24	26	25	27
Np (4.0 g S/kg soil)	3	3	1	3	7	11	19	14	14	13	35	32	44	44	48	45	47	47	56	54	64	58
Np (4.0 g S/kg soil)	2	2	1	5	8	10	9	16	18	23	23	21	30	30	32	29	35	35	36	35	35	36
Np (4.0 g S/kg soil)	4	4	3	7	10	12	16	12	13	16	21	22	27	27	30	30	30	30	29	29	30	29
Mean Leaf	3	3	2	5	8	9	13	13	14	15	23	22	29	29	32	30	34	34	36	36	39	38
STDEV	1	1	1	2	2	4	6	3	4	6	10	9	13	13	12	12	10	10	14	13	17	14
RSD	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	1	1	1	1	2	3	2	2	3	5	4	6	6	6	6	5	5	7	6	9	7
Np (8.0 g S/kg soil)	3	3	1	7	4	6	5	6	7	8	7	13	16	16	23	18	18	18	22	25	35	33
Np (8.0 g S/kg soil)	3	4	2	5	8	10	10	12	19	22	24	32	34	34	40	38	38	38	40	39	40	38
Np (8.0 g S/kg soil)	5	4	2	5	7	10	12	14	15	25	22	23	27	27	31	28	31	31	32	32	32	33
Np (8.0 g S/kg soil)	3	3	2	4	8	11	13	19	21	22	27	29	31	31	36	34	37	37	39	39	39	40
Mean Leaf	4	4	2	5	7	9	10	13	16	19	20	24	27	27	33	30	31	31	33	34	37	36
STDEV	1	1	1	1	2	2	4	5	6	8	9	8	8	8	7	9	9	9	8	7	4	4
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	1	0	0	1	1	1	2	3	3	4	5	4	4	4	4	4	5	5	4	3	2	2
RBMRp (0 g S/kg soil)	4	3	2	5	8	7	9	12	13	15	14	16	17	17	18	19	14	14	15	16	17	16
RBMRp (0 g S/kg soil)	3	2	2	3	7	8	13	15	14	14	15	15	19	19	21	19	19	19	19	19	20	21
RBMRp (0 g S/kg soil)	3	2	2	6	7	8	11	13	17	20	17	22	25	25	30	22	24	24	27	27	25	24
RBMRp (0 g S/kg soil)	2	3	2	4	5	8	7	12	13	14	19	21	26	26	24	30	22	22	19	22	23	24

Mean Leaf	3	3	2	5	7	8	10	13	14	16	16	19	22	22	23	23	20	20	20	21	21	21
STDEV	1	1	0	1	1	1	3	1	2	3	2	4	4	4	5	5	4	4	5	5	4	4
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	0	0	1	1	0	1	1	1	1	1	2	2	2	3	3	2	2	3	2	2	2
RBMRp (2.0 g S/kg soil)	3	2	1	5	9	11	14	16	18	17	19	18	21	21	20	21	20	20	20	19	19	19
RBMRp (2.0 g S/kg soil)	5	3	2	5	7	9	10	15	14	16	16	16	18	18	18	17	18	18	21	18	20	20
RBMRp (2.0 g S/kg soil)	5	4	4	7	9	11	14	17	17	22	21	19	22	22	22	21	23	23	24	25	25	25
RBMRp (2.0 g S/kg soil)	3	3	2	5	8	8	15	16	14	15	19	23	24	24	23	24	22	22	19	19	19	20
Mean Leaf	4	3	2	6	8	10	13	16	16	18	19	19	21	21	21	21	21	21	21	20	21	21
STDEV	1	1	1	1	1	2	2	1	2	3	2	3	3	3	2	3	2	2	2	3	3	3
RSD	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	1	0	1	1	1	1	1	0	1	2	1	2	1	1	1	1	1	1	1	2	1	1
RBMRp (4.0 g S/kg soil)	4	2	2	5	6	6	7	7	10	11	13	15	17	17	17	16	19	19	16	22	22	22
RBMRp (4.0 g S/kg soil)	4	2	3	5	8	10	11	14	12	14	12	11	15	15	17	16	18	18	21	17	18	18
RBMRp (4.0 g S/kg soil)	3	2	3	7	11	11	12	15	13	12	15	18	20	20	21	20	21	21	22	21	22	21
RBMRp (4.0 g S/kg soil)	4	2	4	6	11	13	16	19	17	21	25	20	30	30	26	30	27	27	28	26	27	27
Mean Leaf	4	2	3	6	9	10	12	14	13	15	16	16	21	21	20	21	21	21	22	22	22	22
STDEV	1	0	1	1	2	3	4	5	3	5	6	4	7	7	4	7	4	4	5	4	4	4
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	0	0	1	1	2	2	3	2	2	3	2	3	3	2	3	2	2	3	2	2	2

RBMRp (8.0 g S/kg soil)	4	3	2	6	9	11	13	13	13	14	14	15	16	16	13	18	19	19	16	22	22	22
RBMRp (8.0 g S/kg soil)	4	3	2	7	10	10	13	16	17	15	12	13	17	17	19	17	18	18	21	17	18	18
RBMRp (8.0 g S/kg soil)	4	3	3	6	10	11	12	13	15	19	18	20	24	24	25	25	21	21	22	21	22	21
RBMRp (8.0 g S/kg soil)	4	1	4	4	8	11	13	14	11	15	20	22	27	27	27	26	27	27	28	26	27	27
Mean Leaf	4	3	3	6	9	11	13	14	14	16	16	18	21	21	21	22	21	21	22	22	22	22
STDEV	0	1	1	1	1	1	1	1	3	2	4	4	5	5	6	5	4	4	5	4	4	4
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	0	1	1	1	1	0	0	1	1	1	2	2	3	3	3	2	2	2	3	2	2	2

Appendix 9: Height of *B. coddii* plants over 266 days growing period

	Height measurements taken before sulfur treatment																Height measurements taken after sulfur treatment					
Samples/^{days}	1	7	21	35	49	62	80	94	108	122	136	150	163	177	191	205	219	233	247	255	261	266
Np (0 g S/kg soil)	19	12	12	31	60	60	60	80	110	140	130	230	150	160	200	200	190	190	190	200	205	210
Np (0 g S/kg soil)	18	16	15	40	60	80	90	120	120	125	280	305	305	340	340	330	320	320	340	340	360	350
Np (0 g S/kg soil)	25	20	21	58	70	85	120	150	200	300	350	470	450	460	440	440	420	420	450	460	470	460
Np (0 g S/kg soil)	25	20	20	70	80	109	140	150	150	175	340	420	445	470	490	490	470	470	510	515	520	520
Mean height	22	17	17	50	68	84	103	125	145	185	275	356	338	358	368	365	350	350	373	379	389	385
STDEV	4	4	4	18	10	20	35	33	40	79	101	109	142	144	128	129	124	124	141	140	140	136
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	2	2	2	9	5	10	18	17	20	40	51	54	71	72	64	64	62	62	70	70	70	68
Np (2. 0 g S/kg soil)	19	20	15	35	50	55	70	100	100	130	200	210	210	250	230	200	170	170	205	220	210	220

Np (2. 0 g S/kg soil)	24	24	20	45	70	80	100	110	110	125	330	440	375	400	410	390	330	330	410	415	420	420
Np (2. 0 g S/kg soil)	10	14	10	20	40	80	95	150	150	170	360	505	430	410	440	470	420	420	470	500	505	470
Np (2. 0 g S/kg soil)	21	14	20	40	55	90	110	160	160	185	390	455	490	520	525	530	510	510	525	530	540	530
Mean height	19	18	16	35	54	76	94	130	130	153	320	403	376	395	401	398	358	358	403	416	419	410
STDEV	6	5	5	11	13	15	17	29	29	30	84	131	120	111	124	144	145	145	140	140	148	134
RSD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	3	2	2	5	6	7	9	15	15	15	42	66	60	55	62	72	73	73	70	70	74	67
Np (4. 0 g S/kg soil)	21	17	15	10	35	50	60	65	70	130	160	195	195	180	200	220	190	190	195	230	240	220
Np (4. 0 g S/kg soil)	15	15	10	20	45	80	90	110	110	110	280	320	350	370	370	380	350	350	390	395	400	400
Np (4. 0 g S/kg soil)	19	14	17	30	55	80	100	150	180	300	402	470	450	460	455	460	460	460	475	450	460	480
Np (4. 0 g S/kg soil)	21	20	18	65	70	100	120	70	140	275	420	480	510	530	540	530	500	500	520	530	540	530
Mean height	19	17	15	31	51	78	93	99	125	204	316	366	376	385	391	398	375	375	395	401	410	408
STDEV	3	3	4	24	15	21	25	40	47	98	121	136	138	152	145	133	139	139	144	127	127	136
RSD	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	1	1	2	12	7	10	13	20	23	49	60	68	69	76	73	67	69	69	72	63	64	68
Np (8. 0 g S/kg soil)	15	12	5	8	17	20	30	50	70	80	70	100	100	110	170	260	120	120	170	175	180	180
Np (8. 0 g S/kg soil)	15	17	16	30	45	80	70	130	150	210	270	194	325	330	330	330	280	280	310	315	320	312
Np (8. 0 g S/kg soil)	16	20	13	22	70	110	110	150	235	330	330	370	375	370	400	370	370	370	430	390	400	435
Np (8. 0 g S/kg soil)	19	25	15	39	75	119	130	230	240	380	540	580	585	600	610	595	580	580	600	600	610	605
Mean height	16	19	12	25	52	82	85	140	174	250	303	311	346	353	378	389	338	338	378	370	378	383
STDEV	2	5	5	13	27	45	44	74	81	134	193	211	199	201	182	145	192	192	182	177	180	181
RSD	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0
Standard Error	1	3	2	7	13	22	22	37	40	67	97	106	100	100	91	72	96	96	91	89	90	90

RBMRp (0 g S/kg soil)	14	20	18	65	95	120	110	130	150	155	160	170	155	150	150	140	138	139	150	157	159	160
RBMRp (0 g S/kg soil)	19	10	10	20	65	90	100	105	137	180	240	245	220	200	190	200	160	160	180	190	200	190
RBMRp (0 g S/kg soil)	15	15	16	40	85	110	120	140	150	200	270	205	195	180	190	220	200	200	220	225	210	225
RBMRp (0 g S/kg soil)	26	20	24	25	45	64	70	60	115	150	220	235	240	260	270	275	275	278	285	289	280	280
Mean height	19	16	17	38	73	96	100	109	138	171	223	214	203	198	200	209	193	194	209	215	212	214
STDEV	5	5	6	20	22	25	22	36	17	23	46	34	37	46	50	56	60	61	58	56	50	52
RSD	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	3	2	3	10	11	12	11	18	8	12	23	17	18	23	25	28	30	31	29	28	25	26
RBMRp (2. 0 g S/kg soil)	14	15	15	49	70	85	100	80	110	95	100	110	145	120	135	133	129	125	110	100	110	95
RBMRp (2. 0 g S/kg soil)	15	19	18	53	80	100	110	100	120	150	175	155	150	100	115	130	150	150	165	170	180	170
RBMRp (2. 0 g S/kg soil)	30	25	25	45	70	90	100	110	150	170	190	230	230	190	200	200	200	200	190	200	210	200
RBMRp (2. 0 g S/kg soil)	9	3	10	35	105	110	99	100	130	220	300	310	330	310	330	320	320	323	316	300	305	300
Mean height	17	16	17	46	81	96	102	98	128	159	191	201	214	180	195	196	200	200	195	193	201	191
STDEV	9	9	6	8	17	11	5	13	17	52	83	88	87	95	97	89	86	88	87	83	81	85
RSD	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Standard Error	5	5	3	4	8	6	3	6	9	26	41	44	43	47	49	44	43	44	44	42	40	42
RBMRp (4. 0 g S/kg soil)	16	7	11	37	55	60	70	60	100	90	140	150	150	120	170	170	140	140	150	160	180	155
RBMRp (4. 0 g S/kg soil)	4	2	30	40	70	90	100	120	135	170	190	185	220	200	180	170	175	176	175	180	200	177
RBMRp (4. 0 g S/kg soil)	19	15	16	62	115	140	130	140	150	160	230	230	240	220	245	245	230	230	235	235	240	240
RBMRp (4. 0 g S/kg soil)	24	25	41	55	110	140	160	145	145	160	200	210	235	210	240	250	250	235	240	250	260	240
Mean height	16	12	25	49	88	108	115	116	133	145	190	194	211	188	209	209	199	195	200	206	220	203
STDEV	9	10	14	12	30	39	39	39	23	37	37	34	42	46	39	45	50	46	45	43	37	44

RSD	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	4	5	7	6	15	20	19	20	11	18	19	17	21	23	20	22	25	23	22	22	18	22
RBMRp (8. 0 g S/kg soil)	22	25	19	60	85	100	100	115	120	120	120	160	150	120	120	120	130	130	135	140	145	135
RBMRp (8. 0 g S/kg soil)	41	30	31	75	95	110	100	140	145	165	190	190	175	160	190	220	210	210	220	225	230	225
RBMRp (8. 0 g S/kg soil)	19	10	15	50	76	90	89	90	125	145	130	155	155	130	180	190	200	200	205	205	210	205
RBMRp (8. 0 g S/kg soil)	14	4	26	40	107	130	135	150	140	245	310	320	335	300	290	300	310	310	310	315	320	310
Mean height	24	17	23	56	91	108	106	124	133	169	188	206	204	178	195	208	213	213	218	221	226	219
STDEV	12	12	7	15	13	17	20	27	12	54	87	77	88	83	70	75	74	74	72	72	72	72
RSD	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Standard Error	6	6	4	7	7	9	10	13	6	27	44	39	44	42	35	37	37	37	36	36	36	36

Appendix 10: Determination of stress indicators obtained before sulfur and after sulfur has been applied

	Modified Red Edge Normalized Difference Vegetation Index (mNDVI$_{705}$)			Plant Senescence Reflectance Index (PSRI)		
Sample name	mNDVI$_{705}$ (1)	mNDVI$_{705}$ (2)	mNDVI$_{705}$ (3)	PSRI (1)	PSRI (2)	PSRI (3)
Np (0 g S/kg soil)	0.389106298	0.406298975	0.430848201	0.005132309	0.001228063	-0.000876191
Np (0 g S/kg soil)	0.361329252	0.369515589	0.371466857	0.002203489	0.000356104	0.003826855
Np (0 g S/kg soil)	0.417253813	0.466910737	0.36420097	-0.008112255	-0.002863855	0.006830082
Np (0 g S/kg soil)	0.409899703	0.35056437	0.334676158	0.009729247	-0.002644442	0.006301872
Mean	0.394397266	0.398322418	0.375298046	0.002238198	-0.000981033	0.004020655
STDEV	0.025061492	0.051246618	0.040306247	0.007563614	0.002080063	0.003517266
RSD	0.063543778	0.128656123	0.107397965	3.379332627	-2.120279059	0.874799406
Standard Error (SE)	0.012530746	0.025623309	0.020153123	0.003781807	0.001040032	0.001758633

Np (2.0 g S/kg soil)	0.486239962	0.358047754	0.486465292	-0.004943021	-0.004690798	0.002917232
Np (2.0 g S/kg soil)	0.479070553	0.494962365	0.451299288	-0.0018216	-0.003051464	-0.000291406
Np (2.0 g S/kg soil)	0.518761475	0.489123129	0.455726832	0.00540594	-0.001299788	0.001185981
Np (2.0 g S/kg soil)	0.364406365	0.484610504	0.348515459	0.001877363	-0.006304108	0.000965648
Mean	0.462119589	0.456685938	0.435501718	0.00012967	-0.003836539	0.001194364
STDEV	0.067392643	0.065895192	0.060062505	0.004488248	0.002150202	0.001320122
RSD	0.145833772	0.144289952	0.137915657	34.61275468	-0.560453519	1.105293367
Standard Error (SE)	0.033696321	0.032947596	0.030031253	0.002244124	0.001075101	0.000660061
Np (4.0 g S/kg soil)	0.496326726	0.462533211	0.486901743	-0.005861164	-0.006551656	0.004289897
Np (4.0 g S/kg soil)	0.340317484	0.532918574	0.356134174	0.006420335	-0.000125624	0.001167169
Np (4.0 g S/kg soil)	0.48131211	0.362196177	0.496629004	-0.000754307	-0.00258091	-0.005347701
Np (4.0 g S/kg soil)	0.458556707	0.443646674	0.43114377	-0.005008372	-0.005365992	-0.003506961
Mean	0.444128257	0.450323659	0.442702173	-0.001300877	-0.003656045	-0.000849399
STDEV	0.070927606	0.070195034	0.064522141	0.005611227	0.002882607	0.00438848
RSD	0.159700728	0.15587685	0.145746159	-4.313418635	-0.788449415	-5.166571201
Standard Error (SE)	0.035463803	0.035097517	0.032261071	0.002805613	0.001441304	0.00219424
Np (8.0 g S/kg soil)	0.465531343	0.466460574	0.379344058	0.004111553	0.00014953	-0.008405136
Np (8.0 g S/kg soil)	0.42429726	0.37186709	0.434088504	-0.004966257	-0.008016045	0.003647187
Np (8.0 g S/kg soil)	0.477751458	0.510009765	0.45750356	-0.004299635	-0.002836329	0.003523056
Np (8.0 g S/kg soil)	0.302506463	0.430484087	0.502015866	-0.004238907	-0.005342007	-0.001695674
Mean	0.417521631	0.444705379	0.443237997	-0.002348311	-0.004011213	-0.000732642
STDEV	0.080014504	0.058439749	0.05107019	0.004319163	0.003488162	0.005688833
RSD	0.191641577	0.131412282	0.115220694	-1.839263324	-0.869602847	-7.764821541
Standard Error (SE)	0.040007252	0.029219875	0.025535095	0.002159581	0.001744081	0.002844416

RBMRp (0 g S/kg soil)	0.285357086	0.328163174	0.314280208	0.000499174	0.000650458	0.014433633
RBMRp (0 g S/kg soil)	0.307522141	0.25510046	0.324526489	0.002541293	0.005051817	-0.001372378
RBMRp (0 g S/kg soil)	0.207829811	0.258088994	0.223003127	0.008811425	0.004130087	0.014191309
RBMRp (0 g S/kg soil)	0.324700208	0.331238147	0.271774206	0.001334098	0.005053366	0.004030983
Mean	0.281352311	0.293147694	0.283396008	0.003296497	0.003721432	0.007820886
STDEV	0.051592947	0.042244017	0.046288647	0.003770976	0.002092992	0.007814307
RSD	0.183374882	0.144104894	0.163335565	1.14393431	0.56241588	0.999158718
Standard Error (SE)	0.025796473	0.021122008	0.023144324	0.001885488	0.001046496	0.003907153
RBMRp (2.0 g S/kg soil)	0.189025444	0.204296604	0.210181213	0.00540594	-0.013957737	-0.000349521
RBMRp (2.0 g S/kg soil)	0.193655819	0.212818445	0.170868685	-0.019876157	-0.016128208	-0.007411646
RBMRp (2.0 g S/kg soil)	0.180122588	0.209307433	0.159359989	0.005909317	0.006153382	0.018538187
RBMRp (2.0 g S/kg soil)	0.204424347	0.247462357	0.25604742	0.006663042	0.009984093	0.003878602
Mean	0.191807049	0.21847121	0.199114327	-0.000474465	-0.003487118	0.003663906
STDEV	0.010113986	0.019641237	0.043749578	0.012944774	0.013464073	0.010955452
RSD	0.05273	0.089903092	0.219720895	-27.28291308	-3.861089415	2.990102102
Standard Error (SE)	0.005056993	0.009820619	0.021874789	0.006472387	0.006732036	0.005477726
RBMRp (4.0 g S/kg soil)	0.247889374	0.293134839	0.202526124	0.001283111	-0.003770052	-0.006031307
RBMRp (4.0 g S/kg soil)	0.261401495	0.317142611	0.227749904	-0.009527831	-0.003461705	-0.011978942
RBMRp (4.0 g S/kg soil)	0.22201792	0.234241352	0.300515456	-0.006097133	-0.013122419	-0.000356617
RBMRp (4.0 g S/kg soil)	0.291009612	0.262247637	0.314755542	0.002781964	-0.006673873	-0.002751157
Mean	0.2555796	0.27669161	0.261386756	-0.002889972	-0.006757012	-0.005279506
STDEV	0.028721108	0.036135467	0.054697027	0.005885931	0.004483536	0.005035707
RSD	0.112376371	0.130598348	0.209257071	-2.036673689	-0.663538283	-0.95382166

Standard Error (SE)	0.014360554	0.018067734	0.027348514	0.002942965	0.002241768	0.002517853
RBMRp (8.0 g S/kg soil)	0.155236992	0.264341954	0.252942424	-0.023884549	-0.000559867	-0.003681677
RBMRp (8.0 g S/kg soil)	0.212488378	0.33057596	0.185608303	0.00449962	0.000576529	-0.030084803
RBMRp (8.0 g S/kg soil)	0.325396191	0.324021495	0.321134093	0.000920609	-0.001085713	0.003796918
RBMRp (8.0 g S/kg soil)	0.300268567	0.171285063	0.259128017	-0.002983926	-0.010517619	-0.009827367
Mean	0.248347532	0.272556118	0.254703209	-0.005362061	-0.002896668	-0.009949232
STDEV	0.078714127	0.073797665	0.055407123	0.012720886	0.005127774	0.014533811
RSD	0.316951516	0.270761359	0.217536021	-2.372387139	-1.770231981	-1.460797233
Standard Error (SE)	0.039357063	0.036898833	0.027703561	0.006360443	0.002563887	0.007266905

Appendix 11: Soil Carbon, Nitrogen and Sulfur (CNS) results obtained before the growth experiment commenced (in % dry mass)

	Carbon	**Nitrogen**	**Sulfur**
RBMR waste soil			
RBMR 1	2.363	-0.185	0.067
RBMR 2	2.723	-0.174	0.16
Mean	**2.543**	**-0.1795**	**0.1135**
STDEV	0.254558	0.007778	0.065761
RSD	0.100102	-0.04333	0.579391
Standard Error	0.18	0.0055	0.0465
Native soil			
	Carbon	**Nitrogen**	**Sulfur**
N 1	1.754	0	0.548
N 2	2.495	0	0.242
N 3	3.189	0	0.279
Mean	2.479333	0	0.356333
STDEV	0.717628	0	0.167016
RSD	0.289444	#DIV/0!	0.468707
Standard Error	0.414323	0	0.096427

Appendix 12: Soil Carbon, Nitrogen and Sulfur (CNS) results obtained after the growth experiment (in % dry mass)

N soil			
	Carbon	**Nitrogen**	**Sulfur**
Control native plants sample			
N (0 g S/kg soil)	2.191	0.25	BDL
N (0 g S/kg soil)	11.5	0.406	BDL
N (0 g S/kg soil)	3.836	0.375	BDL
N (0 g S/kg soil)	5.454	0.381	BDL
Average value	5.74525	0.353	BDL
STDEV	4.061194518	0.069967	BDL
RSD	0.706878642	0.198206	#DIV/0!
Standard Error	2.030597259	0.034983	BDL
Sample treated with 2.0 g sulfur per kg of soil			
N (2.0 g S/kg soil)	10.87	BDL	0.023
N (2.0 g S/kg soil)	14.6	0.354	0.032
N (2.0 g S/kg soil)	3.44	0.407	0.029
N (2.0 g S/kg soil)	3	0.287	0.033
Average value	7.9775	0.262	0.02925
STDEV	5.703463129	0.181437	0.0045
RSD	0.71494367	0.692507	0.153846154
Standard Error	2.851731565	0.090718	0.00225
Sample treated with 4.0 g sulfur per kg of soil			

N (4.0 g S/kg soil)	16.63	0.36	0.123
N (4.0 g S/kg soil)	3.068	0.301	0.324
N (4.0 g S/kg soil)	2.416	0.279	0.101
N (4.0 g S/kg soil)	2.616	0.273	0.206
Average value	6.1825	0.30325	0.1885
STDEV	6.970337414	0.039702	0.1010165
RSD	1.127430233	0.130922	0.535896553
Standard Error	3.485168707	0.019851	0.05050825
Sample treated with 8.0 g sulfur per kg of soil			
N (8.0 g S/kg soil)	1.81	0.272	1.195
N (8.0 g S/kg soil)	7.707	0.409	0.36
N (8.0 g S/kg soil)	3.472	0.378	1.038
N (8.0 g S/kg soil)	1.959	0.242	1.938
Average value	3.737	0.32525	1.13275
STDEV	2.751105111	0.080752	0.647668318
RSD	0.736180121	0.248277	0.571766337
Standard Error	1.375552556	0.040376	0.323834159
RBMR waste soil			
	Carbon	**Nitrogen**	**Sulfur**
Control sample			
RBMR (0 g S/kg soil)	2.011	0.447	BDL
RBMR (0 g S/kg soil)	1.905	0.512	BDL
RBMR (0 g S/kg soil)	2.002	0.505	BDL
RBMR (0 g S/kg soil)	2.007	0.236	BDL
Average value	1.98125	0.425	BDL
STDEV	0.050966492	0.129324	BDL
RSD	0.025724412	0.304291	#DIV/0!
Standard Error	0.025483246	0.064662	BDL
Sample treated with 2.0 g sulfur per kg			
RBMR (2.0 g S/kg soil)	2.686	0.161	0.354
RBMR (2.0 g S/kg soil)	2.264	0.164	0.522
RBMR (2.0 g S/kg soil)	2.374	0.163	0.334
RBMR (2.0 g S/kg soil)	2.009	0.424	0.366
Average value	2.33325	0.228	0.394
STDEV	0.280491681	0.130673	0.086348133
RSD	0.120215014	0.573126	0.219157697
Standard Error	0.140245841	0.065336	0.043174066
Sample treated with 4.0 g sulfur per kg			
RBMR (4.0 g S/kg soil)	2.233	0.448	0.343
RBMR (4.0 g S/kg soil)	1.925	0.162	3.346
RBMR (4.0 g S/kg soil)	2.182	0.453	0.638
RBMR (4.0 g S/kg soil)	1.886	0.156	0.255
Average value	2.0565	0.30475	1.1455
STDEV	0.17631884	0.168328	1.476115736
RSD	0.08573734	0.552347	1.288621332
Standard Error	0.08815942	0.084164	0.738057868

Sample treated with 8.0 g sulfur per kg			
RBMR (8.0 g S/kg soil)	1.441	0.143	5.48
RBMR (8.0 g S/kg soil)	1.97	0.474	0.755
RBMR (8.0 g S/kg soil)	2.169	0.148	5.241
RBMR (8.0 g S/kg soil)	1.816	0.419	0.083
Average value	1.849	0.296	2.88975
STDEV	0.308001082	0.175239	2.867796759
RSD	0.166577113	0.592023	0.992403066
Standard Error	0.154000541	0.087619	1.433898379

Appendix 13: Results for Nitrate and sulphate obtained before the growth experiment

Nitrate and Sulphate concentration obtained before growth experiment		
	RBMR waste soil (Mg/kg)	**N soil (Mg/kg)**
Nitrate	1.084	1.028
Nitrate	1.018	1.112
Nitrate	1.12	1.172
Nitrate	3.822	1.21
Mean	1.761	1.1305
STDEV	1.374649046	0.079354
SE	0.687324523	0.039677
Sulphate	25.698	0.552
Sulphate	21.086	0.17
Sulphate	64.016	0.208
Sulphate	28.726	0.21
Mean	34.8815	0.285
STDEV	19.67538024	0.178949
SE	9.837690121	0.089474

Appendix 14: Plant CHNS results obtained after the growth experiment (i.e. roots, stems and leafs) (in % dry mass)

Roots parts	Carbon	Nitrogen	Sulfur
Control native plants sample			
Native soil			
Np (0 g S/kg soil)	41.75	0.906	0.278
Np (0 g S/kg soil)	37.7	0.929	0.234
Np (0 g S/kg soil)	41.83	0.927	0.198
Np (0 g S/kg soil)	35.03	1.833	0.243
Mean	39.0775	1.14875	0.23825
STDEV	3.3165381	0.4562853	0.0328672
RSD	0.0848708	0.3972015	0.1379524
Standard Error (SE)	1.6582691	0.2281426	0.0164336
Sample treated with 2.0 g sulfur per kg			
Np (2.0 g S/kg soil)	32.21	25.97	0.034
Np (2.0 g S/kg soil)	37.95	1.045	0.045
Np (2.0 g S/kg soil)	36.97	0.764	0.038
Np (2.0 g S/kg soil)	34.03	36.49	0.023
Mean	35.29	16.06725	0.035
STDEV	2.6439869	18.027855	0.0092014
RSD	0.0749217	1.1220249	0.2628985
Standard Error (SE)	1.3219934	9.0139273	0.0046007
Sample treated with 4.0 g sulfur per kg			
Np (4.0 g S/kg soil)	36.89	32.32	0.025
Np (4.0 g S/kg soil)	39.83	0.833	0.043
Np (4.0 g S/kg soil)	39.18	2.366	0.042
Np (4.0 g S/kg soil)	30.59	0.755	0.031
Mean	36.6225	9.0685	0.03525
STDEV	4.2147153	15.518736	0.0087321
RSD	0.1150854	1.7112793	0.2477199
Standard Error (SE)	2.1073576	7.759368	0.0043661
Sample treated with 8.0 g sulfur per kg			
Np (8.0 g S/kg soil)	41.1	1.586	0.234
Np (8.0 g S/kg soil)	35.62	0.678	0.243
Np (8.0 g S/kg soil)	36.25	0.826	0.134

Np (8.0 g S/kg soil)	37.61	27.39	0.123
Mean	37.645	7.62	0.1835
STDEV	2.4484621	13.186001	0.063773
RSD	0.0650408	1.7304463	0.347537
Standard Error (SE)	1.2242311	6.5930004	0.0318865
RBMR waste soil soil			
Control sample			
RBMRp (0 g S/kg soil)	42.45	0.772	0.031
RBMRp (0 g S/kg soil)	0.058	0.24	BDL
RBMRp (0 g S/kg soil)	40.64	1.202	0.003
RBMRp (0 g S/kg soil)	45.24	0.606	BDL
Mean	32.097	0.705	0.0085
STDEV	21.442974	0.398967	0.0150665
RSD	0.6680679	0.5659106	1.7725316
Standard Error (SE)	10.721487	0.1994835	0.0075333
Sample treated with 2.0 g sulfur per kg			
RBMRp (2.0 g S/kg soil)	36.85	0.735	0.246
RBMRp (2.0 g S/kg soil)	42.55	0.716	0.04
RBMRp (2.0 g S/kg soil)	43.47	0.762	0.069
RBMRp (2.0 g S/kg soil)	41.31	0.981	0.092
Mean	41.045	0.7985	0.11175
STDEV	2.9333655	0.1231219	0.0919941
RSD	0.0714671	0.1541915	0.8232135
Standard Error (SE)	1.4666828	0.0615609	0.0459971
Sample treated with 4.0 g sulfur per kg			
RBMRp (4.0 g S/kg soil)	41.1	0.855	0.123
RBMRp (2.0 g S/kg soil)	37.24	0.636	0.292
RBMRp (2.0 g S/kg soil)	40.43	44.45	0.056
RBMRp (2.0 g S/kg soil)	42.44	0.867	BDL
Mean	40.3025	11.702	0.11775
STDEV	2.2060579	21.832258	0.1265817
RSD	0.0547375	1.8656861	1.0750035
Standard Error (SE)	1.103029	10.916129	0.0632908
Sample treated with 8.0 g sulfur per kg			
RBMRp (8.0 g S/kg soil)	42.65	0.672	0.719

RBMRp (2.0 g S/kg soil)	42.82	41.26	0.129
RBMRp (2.0 g S/kg soil)	43.31	59.46	0.197
RBMRp (2.0 g S/kg soil)	41.25	0.822	0.107
Mean	42.5075	25.5535	0.288
STDEV	0.8837939	29.592122	0.2898758
RSD	0.0207915	1.1580457	1.0065133
Standard Error (SE)	0.441897	14.796061	0.1449379

Stems parts	Carbon	Nitrogen	Sulfur
Control native plants sample			
Np (0 g S/kg soil)	38.86	37.32	0.043
Np (0 g S/kg soil)	36.66	0.665	0.085
Np (0 g S/kg soil)	40.33	1.666	0.067
Np (0 g S/kg soil)	39.55	1.501	0.071
Mean	38.85	10.288	0.0665
STDEV	1.57867	18.02666	0.0174643
RSD	0.040635	1.7522026	0.2626203
Standard Error (SE)	0.78934	9.01333	0.0087321
Sample treated with 2.0 g sulfur per kg			
Np (2.0 g S/kg soil)	38.47	0.884	0.131
Np (2.0 g S/kg soil)	42.26	4.61	0.057
Np (2.0 g S/kg soil)	41.5	0.828	0.171
Np (2.0 g S/kg soil)	40.39	34.92	0.089
Mean	40.655	10.3105	0.112
STDEV	1.64666	16.501514	0.0496521
RSD	0.0405033	1.6004572	0.4433225
Standard Error (SE)	0.82333	8.250757	0.0248261
Sample treated with 4.0 g sulfur per kg			
Np (4.0 g S/kg soil)	42.86	1.082	0.185
Np (4.0 g S/kg soil)	39.41	45.98	0.054
Np (4.0 g S/kg soil)	38.87	7.724	0.03
Np (4.0 g S/kg soil)	35.7	10.54	0.213
Mean	39.21	16.3315	0.1205
STDEV	2.93247	20.159459	0.0918858

RSD	0.0747888	1.2343911	0.7625378
Standard Error (SE)	1.46624	10.079729	0.0459429
Sample treated with 8.0 g sulfur per kg			
Np (8.0 g S/kg soil)	36.43	6.987	0.19
Np (8.0 g S/kg soil)	38.31	6.236	0.188
Np (8.0 g S/kg soil)	37.57	0.757	0.174
Np (8.0 g S/kg soil)	38.77	16.91	0.178
Mean	37.77	7.7225	0.1825
STDEV	1.02098	6.7250524	0.0077244
RSD	0.0270315	0.8708388	0.0423256
Standard Error (SE)	0.51049	3.3625262	0.0038622
RBMR waste soil treated with Sulfur			
Control sample			
RBMRp (0 g of S/kg)	36.74	0.928	2.763
RBMRp (0 g S/kg soil)	34.51	32.48	0.112
RBMRp (0 g of S/kg)	36.39	1.397	0.289
RBMRp (0 g of S/kg)	35.48	0.84	0.123
Mean	35.78	8.91125	0.82175
STDEV	0.99943	15.714402	1.2966972
RSD	0.0279326	1.7634341	1.5779704
Standard Error (SE)	0.49972	7.857201	0.6483486
Sample treated with 2.0 g sulfur per kg			
RBMRp (2.0 g S/kg soil)	35.92	0.867	1.098
RBMRp (2.0 g S/kg soil)	37.31	2.682	0.345
RBMRp (2.0 g S/kg soil)	38.49	36.93	0.395
RBMRp (2.0 g S/kg soil)	36.87	0.652	0.309
Mean	37.1475	10.28275	0.53675
STDEV	1.06653	17.788152	0.3758248
RSD	0.0287107	1.7299022	0.7001859
Standard Error (SE)	0.53327	8.8940759	0.1879124

Sample treated with 4.0 g sulfur per kg			
RBMRp (4.0 g S/kg soil)	35.29	27.3	0.473
RBMRp (4.0 g S/kg soil)	38.29	1.947	0.441
RBMRp (4.0 g S/kg soil)	36.03	10.48	0.399
RBMRp (4.0 g S/kg soil)	39.69	0.651	0.211
Mean	37.325	10.0945	0.381
STDEV	2.02836	12.271095	0.1173144
RSD	0.0543432	1.2156218	0.3079118
Standard Error (SE)	1.01418	6.1355473	0.0586572
Sample treated with 8.0 g sulfur per kg			
RBMRp (8.0 g S/kg soil)	35.99	38.75	0.479
RBMRp (8.0 g S/kg soil)	36.65	0.911	1.031
RBMRp (8.0 g S/kg soil)	36.12	0.827	1.546
RBMRp (8.0 g S/kg soil)	37.24	BDL	0.222
Mean	36.5	10.122	0.8195
STDEV	0.56997	19.08976	0.5903335
RSD	0.0156156	1.8859672	0.7203582
Standard Error (SE)	0.28499	9.54488	0.2951668

Leaf parts			
Control native plants sample	Carbon	Nitrogen	Sulfur
Np (0 g S/kg soil)	27.6	1.904	0.042
Np (0 g S/kg soil)	37.2	2.117	0.11
Np (0 g S/kg soil)	43.06	2.539	0.055
Np (0 g S/kg soil)	43.86	2.399	0.086
Average value	37.93	2.23975	0.07325
STDEV	7.499413	0.284433	0.030674
RSD	0.1977172	0.1269932	0.4187577
Standard Error (SE)	3.749707	0.142217	0.015337
Sample treated with 2.0 g sulfur per kg			
Np (2.0 g S/kg soil)	38.1	1.908	0.125
Np (2.0 g S/kg soil)	38.53	2.388	0.069
Np (2.0 g S/kg soil)	39.02	2.414	0.003
Np (2.0 g S/kg soil)	37.94	2.864	0.144

Mean	38.3975	2.3935	0.08525
STDEV	0.484037	0.390526	0.063405
RSD	0.012606	0.1631611	0.7437537
Standard Error (SE)	0.242018	0.195263	0.031703
Sample treated with 4.0 sulfur per kg			
Np (4.0 g S/kg soil)	34.63	2.689	0.123
Np (4.0 g S/kg soil)	38.35	2.354	0.171
Np (4.0 g S/kg soil)	39.85	2.949	0.092
Np (4.0 g S/kg soil)	42.68	2.236	0.314
Mean	38.8775	2.557	0.175
STDEV	3.352823	0.324211	0.0982
RSD	0.0862407	0.1267935	0.5611429
Standard Error (SE)	1.676412	0.162105	0.0491
Sample treated with 8.0 sulfur per kg			
Np (8.0 g S/kg soil)	31.64	2.805	0.219
Np (8.0 g S/kg soil)	36.97	2.077	0.162
Np (8.0 g S/kg soil)	43.26	2.488	0.217
Np (8.0 g S/kg soil)	43.93	1.885	0.059
Mean	38.95	2.31375	0.16425
STDEV	5.794624	0.412943	0.074973
RSD	0.1487708	0.1784735	0.4564566
Standard Error (SE)	2.897312	0.206472	0.037486
RBMR waste soil			
Control sample			
RBMRp (0 g S/kg soil)	36.87	1.271	0.183
RBMRp (0 g S/kg soil)	30.91	1.462	0.317
RBMRp (0 g S/kg soil)	37.61	1.958	0.091
RBMRp (0 g S/kg soil)	37.18	1.603	0.043
Mean	35.6425	1.5735	0.1585
STDEV	3.169557	0.290201	0.120581
RSD	0.0889263	0.1844303	0.7607634
Standard Error (SE)	1.584779	0.1451	0.06029
Sample treated with 2.0 sulfur per kg			
RBMRp (2.0 g S/kg soil)	35.11	1.27	0.158
RBMRp (2.0 g S/kg soil)	2.21	0.179	0.096
RBMRp (2.0 g S/kg soil)	31.73	1.314	0.169
RBMRp (2.0 g S/kg soil)	33.62	1.313	0.297

Mean	25.6675	1.019	0.18
STDEV	15.69938	0.560375	0.08436
RSD	0.6116443	0.5499264	0.4686667
Standard Error (SE)	7.849688	0.280188	0.04218
Sample treated with 4.0 sulfur per kg			
RBMRp (4.0 g S/kg soil)	37.48	1.522	0.189
RBMRp (4.0 g S/kg soil)	33.05	1.159	0.319
RBMRp (4.0 g S/kg soil)	37.06	1.416	0.248
RBMRp (4.0 g S/kg soil)	42.97	1.586	0.192
Mean	37.64	1.42075	0.237
STDEV	4.075905	0.188056	0.06103
RSD	0.1082865	0.1323639	0.2575105
Standard Error (SE)	2.037952	0.094028	0.030515
Sample treated with 8.0 sulfur per kg			
RBMRp (8.0 g S/kg soil)	31.93	1.378	1.055
RBMRp (8.0 g S/kg soil)	35.89	1.86	0.294
RBMRp (8.0 g S/kg soil)	32.29	1.776	0.231
RBMRp (8.0 g S/kg soil)	34.3	1.506	0.442
Mean	33.6025	1.63	0.5055
STDEV	1.847437	0.225903	0.376858
RSD	0.0549792	0.1385908	0.7455153
Standard Error (SE)	0.923719	0.112951	0.188429

Appendix 15: Concentration factors in plant organs for soil solution extracts and total soil concentrations.

Calculation used for the determination of concentration factors

Concentration Factor = [plant] / [substrate]

	Concentration factors (Leaves)					
	LCF 1 (water)		LCF 2 (Acetic Acid)		LCF 3 (Acid digestion)	
N soil with 0 g/kg of sulfur	Co	Ni	Co	Ni	Co	Ni
N (0 g S/kg soil)	NC	NC	186.2595	510.9489	1.742857	2.420052
N (0 g S/kg soil)	NC	NC	71.42857	582.5427	1.013986	2.641997
N (0 g S/kg soil)	NC	NC	22.61307	518.7638	0.323741	4.048234
N (0 g S/kg soil)	NC	NC	10.6383	409.8361	0.181159	2.586207
Mean	NC	NC	72.73487	505.5229	0.815436	2.924122
STDEV	#DIV/0!	#DIV/0!	69.38612	61.83199	0.621209	0.654121
Standard Error	#DIV/0!	#DIV/0!	40.0601	35.69872	0.358655	0.377657
N soil treated with 2.0 g/kg of sulfur						
N (2.0 g S/kg soil)	NC	NC	17.02128	518.0534	0.177215	1.78282
N (2.0 g S/kg soil)	NC	NC	11.25704	540.5405	0.192308	2.105832

N (2.0 g S/kg soil)	NC	NC	11.55556	805.4453	0.163522	3.837838
N (2.0 g S/kg soil)	NC	NC	14.56311	698.7952	0.193548	3.126685
Mean			13.59924	640.7086	0.181648	2.713294
STDEV	#DIV/0!	#DIV/0!	2.361247	117.8878	0.012283	0.81705
Standard Error	#DIV/0!	#DIV/0!	1.363266	68.06257	0.007091	0.471724
N soil treated with 4.0 g/kg of sulfur						
N (4.0 g S/kg soil)	NC	NC	60.46512	778.6079	0.881356	4.938272
N (4.0 g S/kg soil)	NC	NC	13.00813	614.2395	0.36036	3.541045
N (4.0 g S/kg soil)	NC	NC	13.25758	482.5397	0.3125	2.845376
N (4.0 g S/kg soil)	NC	NC	5.098039	245.3988	0.117117	1.49925
Mean	#DIV/0!	#DIV/0!	18.07515	214.761	0.253922	1.319154
STDEV	#DIV/0!	#DIV/0!	25.29071	225.2054	0.326436	1.432488
Standard Error	#DIV/0!	#DIV/0!	12.64536	112.6027	0.163218	0.716244
N soil treated with 8.0 g/kg of sulfur						
N (8.0 g S/kg soil)	NC	NC	47.29064	466.4311	0.355556	1.823204
N (8.0 g S/kg soil)	NC	NC	13.56968	638.0678	0.141221	3.28325
N (8.0 g S/kg soil)	34.06593	NC	11.53846	576.0981	0.118774	2.091212
N (8.0 g S/kg soil)	124.4635	NC	8.089261	332.8895	0.110266	1.392758
Mean	#DIV/0!	#DIV/0!	11.3975	154.6981	0.068075	0.743364
STDEV	63.92075	#DIV/0!	18.25314	133.9912	0.1168	0.809918
Standard Error						
	LCF 1 (water)		LCF 2 (Acetic Acid)		LCF 3 (Acid digestion)	
RBMR waste soil with 0 g/kg of sulfur	Co	Ni	Co	Ni	Co	Ni
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	NC
RBMR (0 g S/kg soil)	NC	NC	7.865169	NC	2.8	1.975867
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	3.156297
RBMR (0 g S/kg soil)	NC	1434.375	NC	NC	NC	1.736006
Mean	NC	1434.375	7.865169	NC	2.8	2.28939
STDEV	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.760282
Standard Error	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.438949
RBMR waste soil treated with 2.0 g/kg of sulfur						
RBMR (2.0 g S/kg soil)	NC	NC	14.28571	NC	NC	NC
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
RBMR (2.0 g S/kg soil)	NC	NC	5.128205	NC	NC	0.676845
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
Mean	NC	NC	9.70696	NC	NC	0.676845
STDEV	#DIV/0!	#DIV/0!	6.475337	#DIV/0!	#DIV/0!	#DIV/0!
Standard Error	#DIV/0!	#DIV/0!	4.578755	#DIV/0!	#DIV/0!	#DIV/0!
RBMR waste soil treated with 4.0 g/kg of sulfur						
RBMR (4.0 g S/kg soil)	NC	NC	NC	12.14953	NC	0.58209
RBMR (4.0 g S/kg soil)	NC	NC	249.8521	NC	21.1125	NC
RBMR (4.0 g S/kg soil)	NC	NC	5.934718	NC	0.512821	NC
RBMR (4.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
Mean	NC	NC	NC	NC	NC	NC
STDEV	NC	NC	127.8934	12.14953	10.81266	0.58209
Standard Error	#DIV/0!	#DIV/0!	121.9587	#DIV/0!	10.29984	#DIV/0!
RBMR waste soil treated with 8.0 g/kg of sulfur						
RBMR (8.0 g S/kg soil)	NC	NC	4.787234	NC	0.204545	1.436285
RBMR (8.0 g S/kg	NC	NC	NC	NC	NC	1.582969

soil)						
RBMR (8.0 g S/kg soil)	NC	NC	2.251407	NC	0.153846	NC
RBMR (8.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
Mean	NC	NC	NC	NC	NC	NC
STDEV	NC	NC	3.519321	NC	0.179196	1.509627
Standard Error	#DIV/0!	#DIV/0!	1.267913	#DIV/0!	0.02535	0.073342

	Concentration factors (stems)					
	<u>SCF 1 (water)</u>	<u>SCF 2 (Acetic Acid)</u>			<u>SCF 3 (Acid digestion)</u>	
N soil with 0 g/kg of sulfur	Co	Ni	Co	Ni	Co	Ni
N (0 g S/kg soil)	NC	NC	NC	174.635	NC	0.827139
N (0 g S/kg soil)	NC	NC	NC	100.759	NC	0.456971
N (0 g S/kg soil)	NC	NC	NC	165.5629	NC	1.29199
N (0 g S/kg soil)	NC	NC	NC	117.623	NC	0.742241
Mean	NC	NC	NC	139.645	NC	0.829585
STDEV	NC	NC	NC	36.02375	NC	0.346552
Standard Error	NC	NC	NC	18.01187	NC	0.173276
N soil treated with 2.0 g/kg of sulfur						
N (2.0 g S/kg soil)	NC	NC	NC	204.0816	NC	0.702323
N (2.0 g S/kg soil)	NC	NC	NC	180.1802	NC	0.701944
N (2.0 g S/kg soil)	NC	NC	NC	226.886	NC	1.081081
N (2.0 g S/kg soil)	NC	NC	NC	126.506	NC	0.566038
Mean	NC	NC	NC	184.4135	NC	0.762846
STDEV	NC	NC	NC	43.05789	NC	0.221645
Standard Error	NC	NC	NC	21.52894	NC	0.110822
N soil treated with 4.0 g/kg of sulfur						
N (4.0 g S/kg soil)	NC	NC	NC	187.2198	NC	1.18743
N (4.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
N (4.0 g S/kg soil)	NC	NC	NC	201.9048	NC	1.190565
N (4.0 g S/kg soil)	NC	NC	NC	195.092	NC	1.191904
Mean	NC	NC	NC	194.7389	NC	1.189966
STDEV	NC	NC	NC	7.348838	NC	0.002296
Standard Error	NC	NC	NC	4.242854	NC	0.001326
N soil treated with 8.0 g/kg of sulfur						
N (8.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
N (8.0 g S/kg soil)	NC	NC	NC	237.9236	NC	1.224263
N (8.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
N (8.0 g S/kg soil)	NC	NC	NC	NC	NC	NC
Mean	NC	NC	NC	237.9236	NC	1.224263
STDEV	NC	NC	NC	#DIV/0!	NC	#DIV/0!
Standard Error	NC	NC	NC	#DIV/0!	NC	#DIV/0!

	SCF 1 (water)	SCF 2 (Acetic Acid)			SCF 3 (Acid digestion)	
RBMR waste soil with 0 g/kg of sulfur	Co	Ni	Co	Ni	Co	Ni
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.340393
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.266968
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.362671
RBMR (0 g S/kg soil)	NC	236.875	NC	NC	NC	0.286687
Mean	NC	236.875	NC	NC	NC	0.31418
STDEV	NC	#DIV/0!	NC	NC	NC	0.044808
Standard Error	NC	#DIV/0!	NC	NC	NC	0.022404
RBMR waste soil treated with 2.0 g/kg of sulfur						
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.174115
RBMR (2.0 g S/kg soil)	NC	NC	NC	5.978153	NC	0.255409
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.122137
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.199577
Mean	NC	NC	NC	5.978153	NC	0.18781
STDEV	NC	NC	NC	#DIV/0!	NC	0.055403
Standard Error	NC	NC	NC	#DIV/0!	NC	0.027702
RBMR waste soil treated with 4.0 g/kg of sulfur						
RBMR (4.0 g S/kg soil)	NC	NC	NC	5.498442	NC	0.263433
RBMR (4.0 g S/kg soil)	NC	780	NC	NC	NC	0.263514
RBMR (4.0 g S/kg soil)	NC	759.5745	NC	NC	NC	0.267817
RBMR (4.0 g S/kg soil)	NC	241.9753	NC	NC	NC	0.147258
Mean	NC	593.8499	NC	5.498442	NC	0.235505
STDEV	NC	304.9034	NC	#DIV/0!	NC	0.058867
Standard Error	NC	176.0361	NC	#DIV/0!	NC	0.029434
RBMR waste soil treated with 8.0 g/kg of sulfur						
RBMR (8.0 g S/kg soil)	NC	NC	NC	NC	NC	0.231102
RBMR (8.0 g S/kg soil)	NC	NC	NC	NC	NC	0.179039
RBMR (8.0 g S/kg soil)	NC	629.1667	NC	NC	NC	0.328261
RBMR (8.0 g S/kg soil)	NC	NC	NC	5.977961	NC	0.236383
Mean	NC	629.1667	NC	5.977961	NC	0.243696
STDEV	NC	#DIV/0!	NC	#DIV/0!	NC	0.062032
Standard Error	NC	#DIV/0!	NC	#DIV/0!	NC	0.031016

	Concentration factors (Roots)					
	RCF 1 (water)		RCF 2 (Acetic Acid)		RCF 3 (Acid digestion)	
N soil with 0	Co	Ni	Co	Ni	Co	Ni

g/kg of sulfur						
N (0 g S/kg soil)	NC	NC	NC	65.23723	NC	0.308989
N (0 g S/kg soil)	NC	NC	NC	39.56357	NC	0.179432
N (0 g S/kg soil)	NC	NC	7.035176	41.83223	0.100719	0.326443
N (0 g S/kg soil)	NC	NC	8.085106	47.06284	0.137681	0.296983
Mean	NC	NC	7.560141	48.42397	0.1192	0.277962
STDEV	NC	NC	0.742413	11.64039	0.026136	0.066791
Standard Error	NC	NC	0.524965	5.820195	0.018481	0.033395
N soil treated with 2.0 g/kg of sulfur						
N (2.0 g S/kg soil)	NC	NC	9.726444	40.65934	0.101266	0.139924
N (2.0 g S/kg soil)	NC	NC	3.377111	71.51767	0.057692	0.278618
N (2.0 g S/kg soil)	NC	NC	NC	47.30573	NC	0.225405
N (2.0 g S/kg soil)	NC	NC	NC	43.43373	NC	0.19434
Mean	NC	NC	6.551777	50.72912	0.079479	0.209572
STDEV	NC	NC	4.489656	14.12452	0.030811	0.058025
Standard Error	NC	NC	3.174667	7.062262	0.021787	0.029013
N soil treated with 4.0 g/kg of sulfur						
N (4.0 g S/kg soil)	NC	NC	6.395349	61.2269	0.09322	0.388328
N (4.0 g S/kg soil)	NC	NC	3.902439	53.33333	0.108108	0.307463
N (4.0 g S/kg soil)	NC	NC	4.166667	70.73016	0.098214	0.417072
N (4.0 g S/kg soil)	NC	NC	5.098039	60.8589	0.117117	0.371814
Mean	NC	NC	4.890623	61.53732	0.104165	0.371169
STDEV	NC	NC	1.126624	7.126717	0.010622	0.046406
Standard Error	NC	NC	0.563312	3.563359	0.005311	0.023203
N soil treated with 8.0 g/kg of sulfur						
N (8.0 g S/kg soil)	NC	NC	NC	71.09541	NC	0.277901
N (8.0 g S/kg soil)	NC	NC	NC	48.66619	NC	0.250417
N (8.0 g S/kg soil)	6.593407	NC	2.233251	81.8335	0.022989	0.297052
N (8.0 g S/kg soil)	47.2103	NC	3.06834	68.44208	0.041825	0.286351
Mean	26.90185	NC	2.650795	67.50929	0.032407	0.27793
STDEV	28.72048	NC	0.590498	13.83204	0.013319	0.019946
Standard Error	20.30845	NC	0.417545	6.91602	0.009418	0.009973
	<u>RCF 1 (water)</u>		<u>RCF 2 (Acetic Acid)</u>		<u>RCF 3 (Acid digestion)</u>	
RBMR waste soil with 0 g/kg	Co	Ni	Co	Ni	Co	Ni

of sulfur						
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.119516
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.266968
RBMR (0 g S/kg soil)	NC	NC	NC	NC	NC	0.215478
RBMR (0 g S/kg soil)	NC	143.75	NC	NC	NC	0.173979
Mean	NC	143.75	NC	NC	NC	0.193985
STDEV	NC	#DIV/0!	NC	NC	NC	0.062542
Standard Error	NC	#DIV/0!	NC	NC	NC	0.031271
RBMR waste soil treated with 2.0 g/kg of sulfur						
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.165261
RBMR (2.0 g S/kg soil)	NC	NC	NC	3.515392	NC	0.150191
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.094148
RBMR (2.0 g S/kg soil)	NC	NC	NC	NC	NC	0.104863
Mean	NC	NC	NC	3.515392	NC	0.128616
STDEV	NC	NC	NC	#DIV/0!	NC	0.034451
Standard Error	NC	NC	NC	#DIV/0!	NC	0.017226
RBMR waste soil treated with 4.0 g/kg of sulfur						
RBMR (4.0 g S/kg soil)	NC	NC	NC	2.266355	NC	0.108582
RBMR (4.0 g S/kg soil)	NC	337.7778	NC	NC	NC	0.114114
RBMR (4.0 g S/kg soil)	NC	361.7021	NC	NC	NC	0.127532
RBMR (4.0 g S/kg soil)	NC	191.358	NC	NC	NC	0.116454
Mean	NC	296.946	NC	2.266355	NC	0.11667
STDEV	NC	92.22096	NC	#DIV/0!	NC	0.007958
Standard Error	NC	53.2438	NC	#DIV/0!	NC	0.003979
RBMR waste soil treated with 8.0 g/kg of sulfur						
RBMR (8.0 g S/kg soil)	NC	NC	NC	NC	NC	0.205184
RBMR (8.0 g S/kg soil)	NC	NC	NC	NC	NC	0.128275
RBMR (8.0 g S/kg soil)	NC	506.25	NC	NC	NC	0.26413
RBMR (8.0 g S/kg soil)	NC	NC	NC	3.002755	NC	0.118736
Mean	NC	506.25	NC	3.002755	NC	0.179081
STDEV	NC	#DIV/0!	NC	#DIV/0!	NC	0.068648
Standard Error	NC	#DIV/0!	NC	#DIV/0!	NC	0.034324

Appendix 16: Mass ratios - proportion of total mass allocated to each *B. coddii* organs and root: shoot ratios

Mass ratios - proportion of total mass allocated to each *B. coddii* organs					
	Root	Stem	Leaf	Shoot	Root: shoot ratio
N (0 g S /kg soil)	0.609913793	0.060344828	0.329741379	0.390086207	1.563536
N (0 g S /kg soil)	0.54494382	0.096910112	0.358146067	0.45505618	1.197531
N (0 g S /kg soil)	0.650406504	0.094512195	0.255081301	0.349593496	1.860465
N (0 g S /kg soil)	0.551942902	0.14036479	0.307692308	0.448057098	1.231858
Mean	0.589301755	0.098032981	0.312665264	0.410698245	1.463348
STDEV	0.050073136	0.032792457	0.04359182	0.043364607	0.311975
Standard Error	0.025036568	0.016396229	0.02179591	0.025036568	0.155988
N soil treated with 2.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
N (2.0 g S /kg soil)	0.725770925	0.025330396	0.248898678	0.274229075	2.646586
N (2.0 g S /kg soil)	0.72906793	0.097946288	0.172985782	0.27093207	2.690962
N (2.0 g S /kg soil)	0.701863354	0.10015528	0.197981366	0.298136646	2.354167
N (2.0 g S /kg soil)	0.664581704	0.155590305	0.179827991	0.335418296	1.981352
Mean	0.705320979	1.639615359	0.923010132	0.294679021	2.418267
STDEV	0.029742011	1.222613039	0.525048766	0.025757337	0.327361
Standard Error	0.014871006	0.611306519	0.262524383	0.014871006	0.163681
N soil treated with 4.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
N (4.0 g S /kg soil)	0.588357588	0.074844075	0.336798337	0.411642412	1.429293
N (4.0 g S /kg soil)	0.769616027	0.078464107	0.151919866	0.230383973	3.34058
N (4.0 g S /kg soil)	0.501650165	0.224422442	0.273927393	0.498349835	1.006623
N (4.0 g S /kg soil)	0.611924686	0.14748954	0.240585774	0.388075314	1.576819
Mean	0.617887117	0.131305041	0.250807843	0.382112883	1.838329
STDEV	0.11171427	0.070504831	0.077054529	0.096747396	1.030241
Standard Error	0.055857135	0.035252415	0.038527264	0.055857135	0.515121
N soil treated with 8.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
N (8.0 g S /kg soil)	0.660377358	0.067924528	0.271698113	0.339622642	1.944444
N (8.0 g S /kg soil)	0.637848223	0.075888569	0.286263208	0.362151777	1.761273
N (8.0 g S /kg soil)	0.693191866	0.09106985	0.215738285	0.306808134	2.259366
N (8.0 g S /kg soil)	0.591147787	0.184546137	0.224306077	0.408852213	1.445872
Mean	0.645641308	0.104857271	0.249501421	0.354358692	1.852739
STDEV	0.042850448	0.053986484	0.034731719	0.037109577	0.340423
Standard Error	0.021425224	0.026993242	0.01736586	0.021425224	0.170211
RBMR waste soil with 0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
RBMR (0 g S /kg soil)	0.708222812	0.042440318	0.24933687	0.291777188	2.427273
RBMR (0 g S /kg soil)	0.532967033	0.115384615	0.351648352	0.467032967	1.141176
RBMR (0 g S /kg soil)	0.463768116	0.280193237	0.256038647	0.536231884	0.864865
RBMR (0 g S /kg soil)	0.644067797	0.089830508	0.266101695	0.355932203	1.809524
Mean	0.587256439	0.13196217	0.280781391	0.412743561	1.560709
STDEV	0.109631351	0.103338446	0.047744392	0.094943535	0.700725

Standard Error	0.054815675	0.051669223	0.023872196	0.054815675	0.350363
RBMR waste soil treated with 2.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
RBMR (2.0 g S /kg soil)	0.729885057	0.074712644	0.195402299	0.270114943	2.702128
RBMR (2.0 g S /kg soil)	0.737051793	0.011952191	0.250996016	0.262948207	2.80303
RBMR (2.0 g S /kg soil)	0.703557312	0.083003953	0.213438735	0.296442688	2.373333
RBMR (2.0 g S /kg soil)	0.538631347	0.123620309	0.337748344	0.461368653	1.167464
Mean	0.677281377	0.073322274	0.249396349	0.322718623	2.261489
STDEV	0.093548393	0.046158594	0.063290182	0.081015285	0.651312
Standard Error	0.046774197	0.023079297	0.031645091	0.046774197	0.376035
RBMR waste soil treated with 4.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
RBMR (4.0 g S /kg soil)	0.696741855	0.077694236	0.22556391	0.303258145	2.297521
RBMR (4.0 g S /kg soil)	0.66	0.055	0.285	0.34	1.941176
RBMR (4.0 g S /kg soil)	0.745901639	0.053278689	0.200819672	0.254098361	2.935484
RBMR (4.0 g S /kg soil)	0.596311475	0.071721311	0.331967213	0.403688525	1.477157
Mean	0.674738742	0.064423559	0.260837699	0.325261258	2.162835
STDEV	0.063024761	0.012143306	0.059131572	0.054581044	0.532544
Standard Error	0.031512381	0.006071653	0.029565786	0.031512381	0.307464
RBMR waste soil treated with 8.0 g/kg of sulfur	Root	Stem	Leaf	Shoot	
RBMR (8.0 g S /kg soil)	0.762331839	0.029147982	0.208520179	0.237668161	3.207547
RBMR (8.0 g S /kg soil)	0.674050633	0.07278481	0.253164557	0.325949367	2.067961
RBMR (8.0 g S /kg soil)	0.683431953	0.082840237	0.233727811	0.316568047	2.158879
RBMR (8.0 g S /kg soil)	0.377464789	0.61971831	0.002816901	0.622535211	0.606335
Mean	0.624319803	0.201122835	0.174557362	0.375680197	2.01018
STDEV	0.169265198	0.280035083	0.115943222	0.146587961	0.926002
Standard Error	0.084632599	0.140017542	0.057971611	0.084632599	0.534628

Appendix 17: Total shoots concentration of Co and root: shoot ratio of Co concentration

Samples/Plant parts	Total concentration of Co in shoots dry mass	Total Co Concentration in dry matter	(Co) shoot ratio	(Co) root ratio
Np (0 g S/kg soil)	244	244	1	#VALUE!

Np (0 g S/kg soil)	145	145	1	#VALUE!
Np (0 g S/kg soil)	45	29.5	1.5254237	0.4745763
Np (0 g S/kg soil)	25	22	1.1363636	0.8636364
Mean	114.75	110.125	1.1654468	0.6691063
STDEV	100.89722	105.52281	0.2151595	#VALUE!
Standard Error	50.448612	52.761403	0.1242224	#VALUE!
Np (2.0 g S/kg soil)	28	22	1.2727273	0.7272727
Np (2.0 g S/kg soil)	30	19.5	1.5384615	0.4615385
Np (2.0 g S/kg soil)	26	26	1	#VALUE!
Np (2.0 g S/kg soil)	30	30	1	#VALUE!
Mean	28.5	20.5	1.2027972	0.5944056
STDEV	1.9148542	4.607512	0.2580789	#VALUE!
Standard Error	0.9574271	2.303756	0.1290395	#VALUE!
Np (4.0 g S/kg soil)	104	57.5	1.8086957	0.1913043
Np (4.0 g S/kg soil)	40	26	1.5384615	0.4615385
Np (4.0 g S/kg soil)	35	23	1.5217391	0.4782609
Np (4.0 g S/kg soil)	13	13	1	1
Mean	48	29.875	1.4672241	0.5327759
STDEV	39.132254	19.237009	0.3381064	0.3381064
Standard Error	19.566127	9.6185043	0.1690532	0.1690532
Np (8.0 g S/kg soil)	96	96	1	#VALUE!
Np (8.0 g S/kg soil)	37	37	1	#VALUE!
Np (8.0 g S/kg soil)	31	18.5	1.6756757	0.3243243
Np (8.0 g S/kg soil)	29	20	1.45	0.55
Mean	48.25	28.375	1.2814189	0.4371622
STDEV	32.01432	36.396829	0.3377628	#VALUE!
Standard Error	16.00716	18.198415	0.1688814	#VALUE!
RBMRp (0 g S /kg soil)		BDL	#VALUE!	#VALUE!
RBMRp (0 g S /kg soil)	14	14	1	#VALUE!
RBMRp (0 g S /kg soil)		BDL	#VALUE!	#VALUE!
RBMRp (0 g S /kg soil)		BDL	#VALUE!	#VALUE!
Mean	14	14	#VALUE!	#VALUE!
STDEV	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Standard Error	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

RBMRp (2.0 g S /kg soil)	22	22	1	#VALUE!
RBMRp (2.0 g S /kg soil)	BDL	BDL	#VALUE!	#VALUE!
RBMRp (2.0 g S /kg soil)	12	12	1	#VALUE!
RBMRp (2.0 g S /kg soil)	BDL	BDL	#VALUE!	#VALUE!
Mean	17	17	#VALUE!	#VALUE!
STDEV	7.0710678	7.0710678	#VALUE!	#VALUE!
Standard Error	5	5	#VALUE!	#VALUE!
RBMRp (4.0 g S /kg soil)	BDL	BDL	#VALUE!	#VALUE!
RBMRp (4.0 g S /kg soil)	844.5	844.5	1	#VALUE!
RBMRp (4.0 g S /kg soil)	20	20	1	#VALUE!
RBMRp (4.0 g S /kg soil)		BDL	#VALUE!	#VALUE!
Mean	432.25	432.25	#VALUE!	#VALUE!
STDEV	583.00954	583.00954	#VALUE!	#VALUE!
Standard Error	412.25	412.25	#VALUE!	#VALUE!
RBMRp (8.0 g S /kg soil)	18	18	1	#VALUE!
RBMRp (8.0 g S /kg soil)		BDL	#VALUE!	#VALUE!
RBMRp (8.0 g S /kg soil)	12	12	1	#VALUE!
RBMRp (8.0 g S /kg soil)	15	BDL	#VALUE!	#VALUE!
Mean	15	15	#VALUE!	#VALUE!
STDEV	3	4.2426407	#VALUE!	#VALUE!
Standard error	1.7320508	3	#VALUE!	#VALUE!

Appendix 18: Total shoots concentration of Ni and root: shoot ratio of Ni concentration

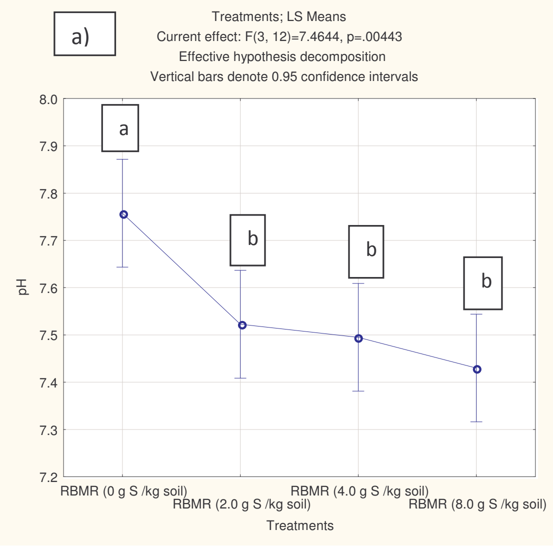
Samples/Plant parts	Total concentration of Ni in shoots dry mass	Total Ni Concentration in dry matter	(Ni) shoot ratio	(Ni) root ratio
Np (0 g S/kg soil)	3757	1371.5	2.739336493	0.260664
Np (0 g S/kg soil)	3601	1269.8333	2.835805225	0.164195
Np (0 g S/kg soil)	6200	2193	2.827177383	0.172823
Np (0 g S/kg soil)	3861	1401.8333	2.754250387	0.24575

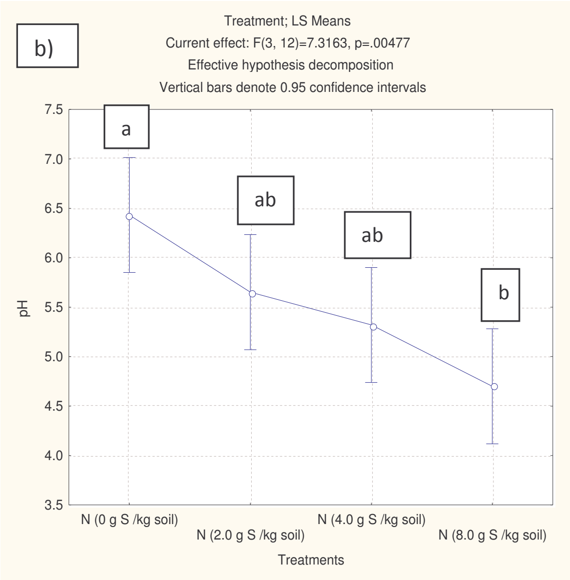
Mean	4354.75	1559.0417	2.789142372	0.210858
STDEV	1234.79833	426.3922	0.049403644	0.049404
Standard Error	617.3991652	213.1961	0.024701822	0.024702
Np (2.0 g S/kg soil)	4600	1619.6667	2.840090553	0.159909
Np (2.0 g S/kg soil)	5200	1905.3333	2.729181246	0.270819
Np (2.0 g S/kg soil)	9100	3172.3333	2.868551014	0.131449
Np (2.0 g S/kg soil)	6850	2403.5	2.850010401	0.14999
Mean	6437.5	2275.2083	2.821958304	0.178042
STDEV	2013.858237	680.1555	0.062966027	0.062966
Standard Error	1006.929119	340.0777	0.031483014	0.031483
Np (4.0 g S/kg soil)	8187	2902	2.821157822	0.178842
Np (4.0 g S/kg soil)	4745	2578.5	1.840217181	0.159783
Np (4.0 g S/kg soil)	5390	1982.3333	2.719017993	0.280982
Np (4.0 g S/kg soil)	3590	1362	2.635829662	0.36417
Mean	5875.25	2123.75	2.504055664	0.245944
STDEV	1953.480484	679.5918	0.449002042	0.095099
Standard Error	976.7402418	339.7959	0.224501021	0.04755
Np (8.0 g S/kg soil)	3300	1901.5	1.735471996	0.264528
Np (8.0 g S/kg soil)	8100	2850	2.842105263	0.157895
Np (8.0 g S/kg soil)	3760	2147.05	1.751240074	0.24876
Np (8.0 g S/kg soil)	2500	1507	1.658925017	0.341075
Mean	6065	2188.425	1.996935587	0.2286
STDEV	2511.221482	564.43401	4.449096691	0.063612
Standard Error	1255.610741	282.217	0.282443567	0.037587
RBMRp (0 g S /kg soil)	225	152	1.480263158	0.519737
RBMRp (0 g S /kg soil)	1487	554.66667	2.680889423	0.319111
RBMRp (0 g S /kg soil)	2319	820.33333	2.826899634	0.1731
RBMRp (0 g S /kg soil)	1337	484	2.762396694	0.237603
Mean	1720.125	616.125	2.437612227	0.312388
STDEV	860.865456	275.0343	0.641022795	0.150591
Standard Error	430.432728	137.5172	0.320511397	0.075295
RBMRp (2.0 g S /kg soil)	206.5	201.25	1.026086957	0.973913
RBMRp (2.0 g S /kg soil)	301	239	1.259414226	0.740586

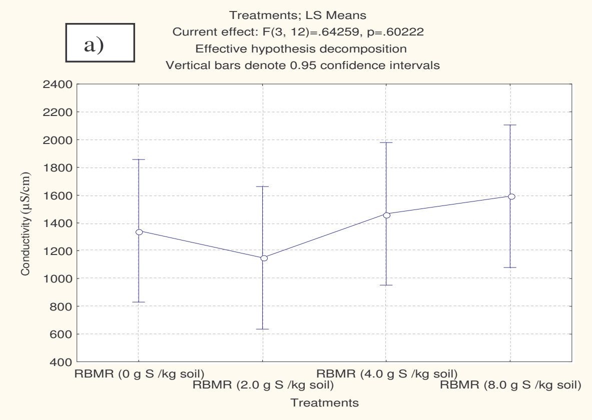
RBMRp (2.0 g S /kg soil)	942	351	2.683760684	0.316239
RBMRp (2.0 g S /kg soil)	236	180	1.311111111	0.688889
Mean	1019.875	1817.875	1.570093244	0.679907
STDEV	349.3210975	76.14005	0.752726423	0.272309
Standard Error	174.6605488	38.07003	0.665798431	0.136154
RBMRp (4.0 g S /kg soil)	1133	426.16667	2.658584278	0.341416
RBMRp (4.0 g S /kg soil)	351	251.5	1.395626243	0.604374
RBMRp (4.0 g S /kg soil)	357	263.5	1.35483871	0.645161
RBMRp (4.0 g S /kg soil)	196	175.5	1.116809117	0.883191
Mean	1094.25	416.625	1.631464587	0.618535
STDEV	422.4581833	105.4619	0.695697902	0.221921
Standard Error	211.2290917	52.73097	0.347848951	0.110961
RBMRp (8.0 g S /kg soil)	1544	578	2.671280277	0.32872
RBMRp (8.0 g S /kg soil)	1614	577.16667	2.796419289	0.203581
RBMRp (8.0 g S /kg soil)	302	272.5	1.108256881	0.891743
RBMRp (8.0 g S /kg soil)	217	163	1.331288344	0.668712
Mean	919.25	397.6667	1.976811198	0.523189
STDEV	660.8976377	212.5053	0.880365659	0.31463
Standard error	381.569429	106.2526	0.44018283	0.157315

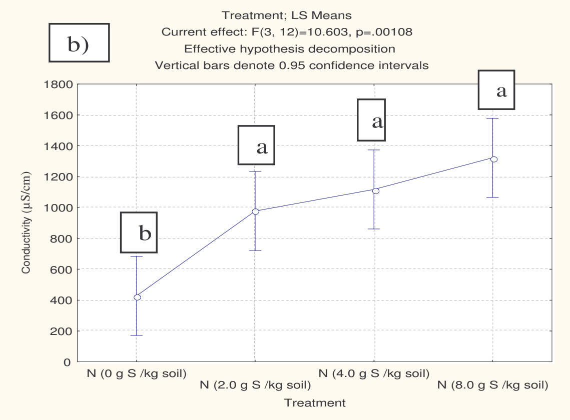
Appendix 19: Statistical analysis output

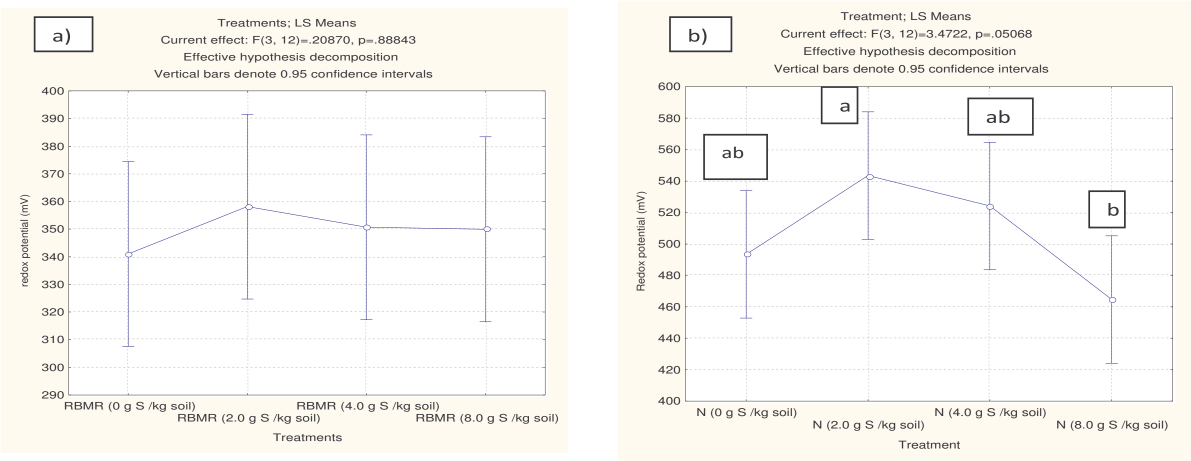
Analysis of soil pH, conductivity and redox potential for both soils





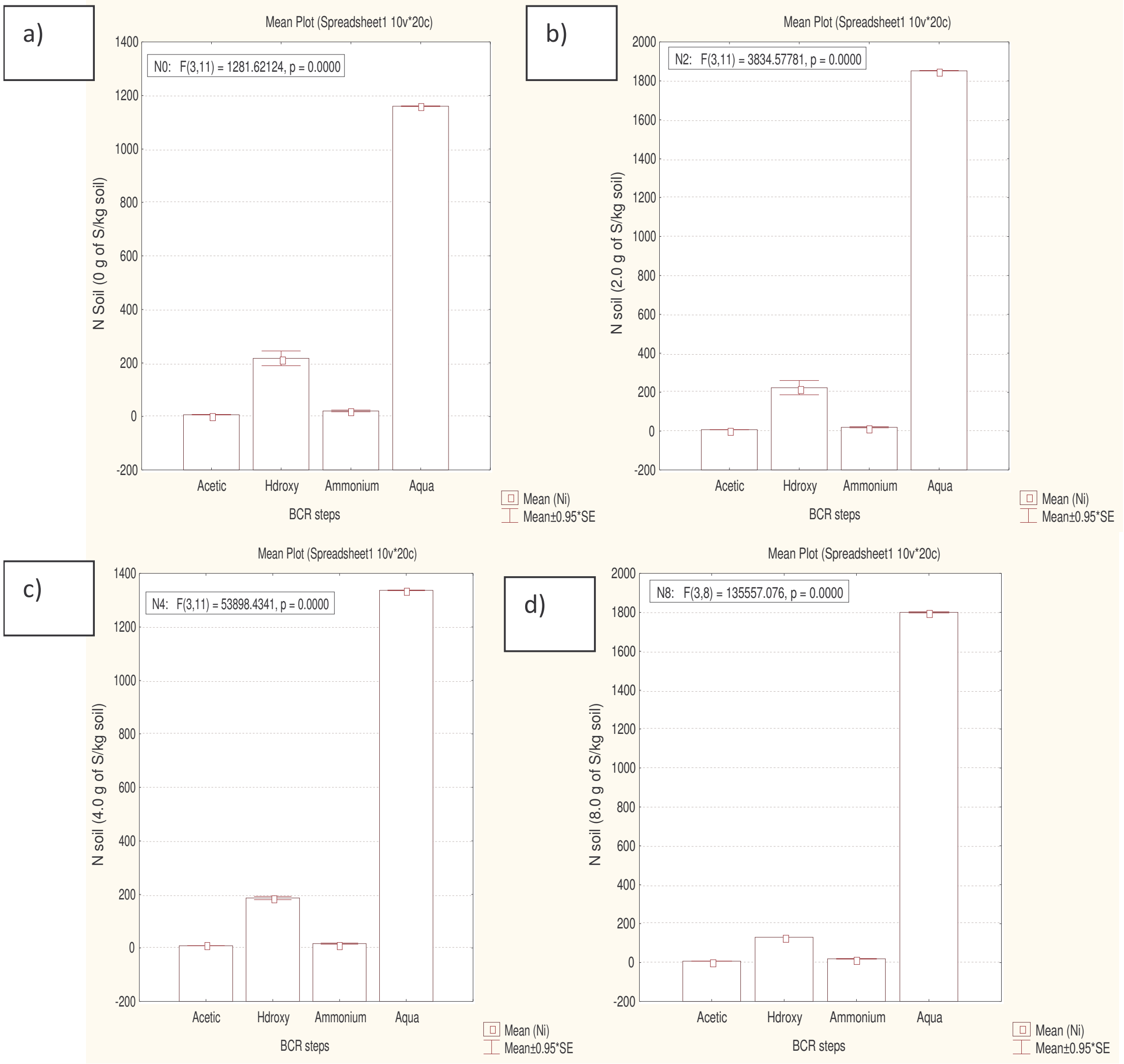





a)
Treatments; LS Means
Current effect: F(3, 12)=.20870, p=.88843
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
redox potential (mV)
400
390
380
370
360
350
340
330
320
310
300
290
RBMR (0 g S /kg soil)
RBMR (2.0 g S /kg soil)
RBMR (4.0 g S /kg soil)
RBMR (8.0 g S /kg soil)
Treatments
b)
Treatment; LS Means
Current effect: F(3, 12)=3.4722, p=.05068
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
Redox potential (mV)
600
580
560
540
520
500
480
460
440
420
400
ab
a
ab
b
N (0 g S /kg soil)
N (2.0 g S /kg soil)
N (4.0 g S /kg soil)
N (8.0 g S /kg soil)
Treatment

One way ANOVA analysis demonstrating the behaviour of Ni on various BCR steps and treatment

N soil



Tukey HSD test; N (0 g of S/kg soil)
Homogenous Groups, alpha = .05000
Error: Between MS = 686.08, df = 15.000

Cell No.	treatments	N0 Mean	c	b	a
1	Water	0.000	****		
2	Acetic	6.783	****		
4	Ammonium	15.675	****		
3	Hdroxy	217.500		****	
5	Aqua	1160.000			****

Tukey HSD test; variable N2
Homogenous Groups, alpha = .05000
Error: Between MS = 3093.2, df = 15.000

Cell No.	treatments	N2 Mean	c	b	a
1	Water	0.000	****		
2	Acetic	7.675	****		
4	Ammonium	19.925	****		
3	Hdroxy	167.500		****	
5	Aqua	1852.000			****

Tukey HSD test; variable N4
Homogenous Groups, alpha = .05000
Error: Between MS = 1764.2, df = 15.000

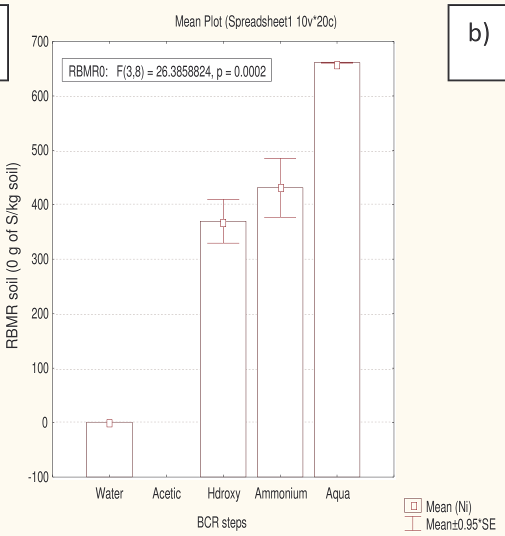
Cell No.	treatments	N4 Mean	c	b	a
1	Water	0.000	****		
2	Acetic	8.057	****		
4	Ammonium	16.425	****		
3	Hdroxy	140.000		****	
5	Aqua	1336.500			****

Tukey HSD test; variable N8
Homogenous Groups, alpha = .05000
Error: Between MS = 874.82, df = 15.000

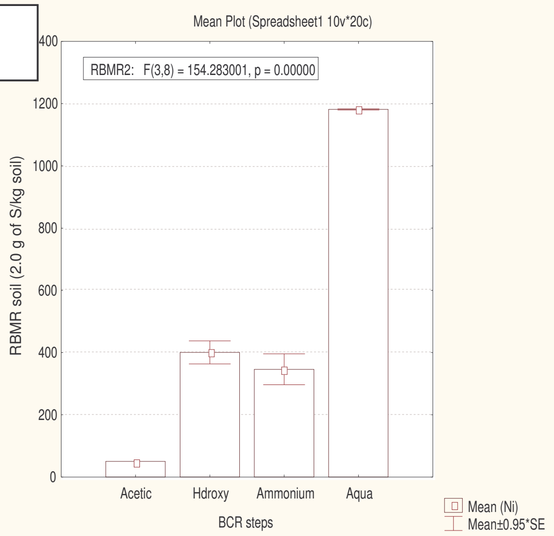
Cell No.	treatments	N8 Mean	b	a
1	Water	0.000	****	
2	Acetic	7.590	****	
4	Ammonium	14.550	****	
3	Hdroxy	32.500	****	
5	Aqua	1800.000		****

RBMR waste soil

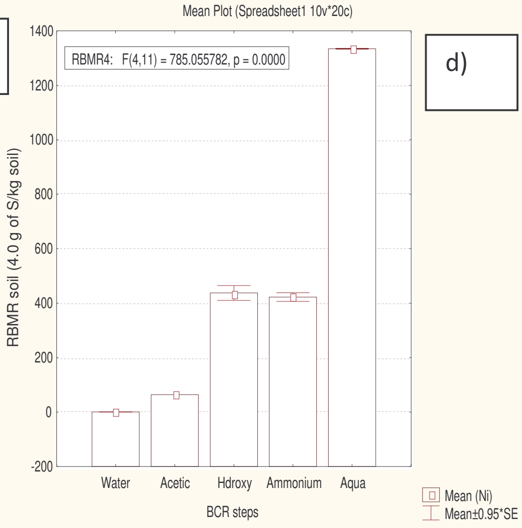
a)



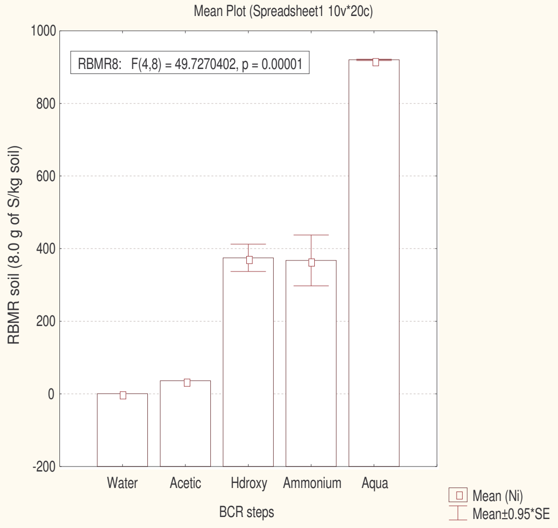
b)



c)



d)



Tukey HSD test; variable RBMR0
Homogenous Groups, alpha = .05000
Error: Between MS = 12042., df = 15.000

Cell No.	treatments	RBMR0 Mean	c	b	a
2	Acetic	0.0000	****		
1	Water	0.2000	****		
4	Ammonium	323.5000		****	
3	Hdroxy	369.8750		****	
5	Aqua	661.0000			****

Tukey HSD test; variable RBMR2 (Spreadsheet1)
Homogenous Groups, alpha = .05000
Error: Between MS = 8432.1, df = 15.000

Cell No.	treatments	RBMR2 Mean	c	b	a
1	Water	0.000	****		
2	Acetic	12.587	****		
4	Ammonium	259.750		****	
3	Hdroxy	400.500		****	
5	Aqua	1181.500			****

Tukey HSD test; variable RBMR (4.0 g of S/kg)
Homogenous Groups, alpha = .05000
Error: Between MS = 1073.8, df = 15.000

Cell No.	treatments	RBMR4 Mean	c	b	a
1	Water	0.433	****		
2	Acetic	16.050	****		
4	Ammonium	422.500		****	
3	Hdroxy	437.500		****	
5	Aqua	1334.000			****

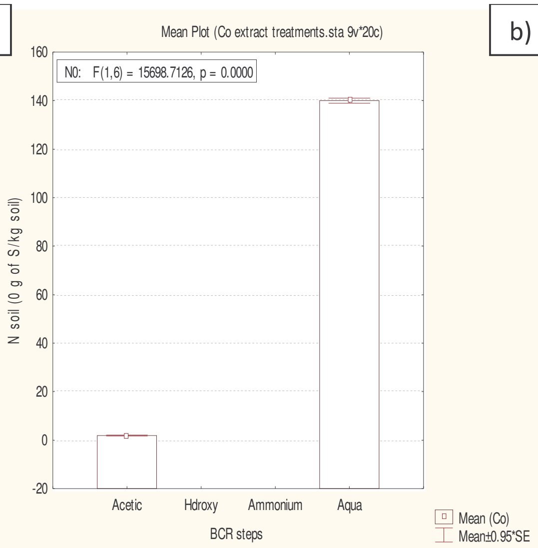
Tukey HSD test; variable RBMR (8.0 g of S/kg soil)ror: Between MS = 10264.,

Cell No.	treatments	RBMR 8 Mean	c	b	a
1	Water	0.1200	****		
2	Acetic	9.0750	****		
4	Ammonium	275.7500		****	
3	Hdroxy	375.0000		****	
5	Aqua	920.0000			****

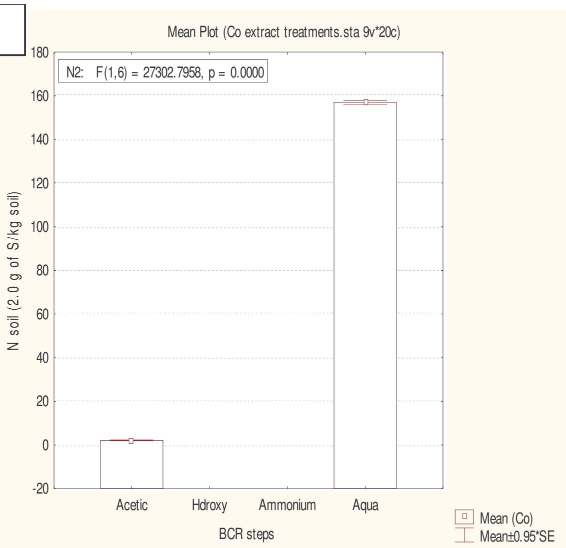
One way ANOVA analysis demonstrating the behaviour of Co on various BCR steps and treatment

N soil

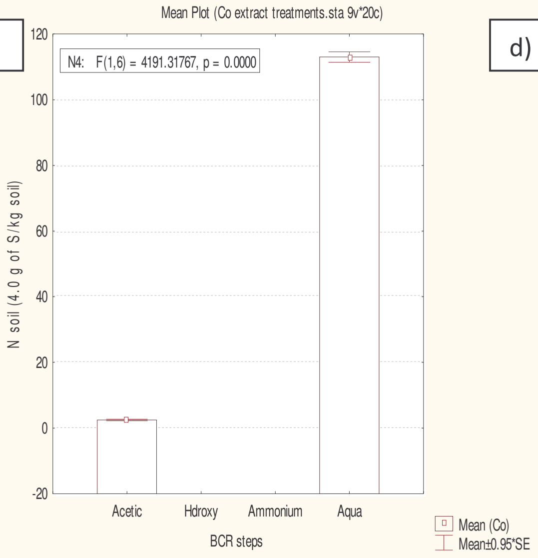
a)



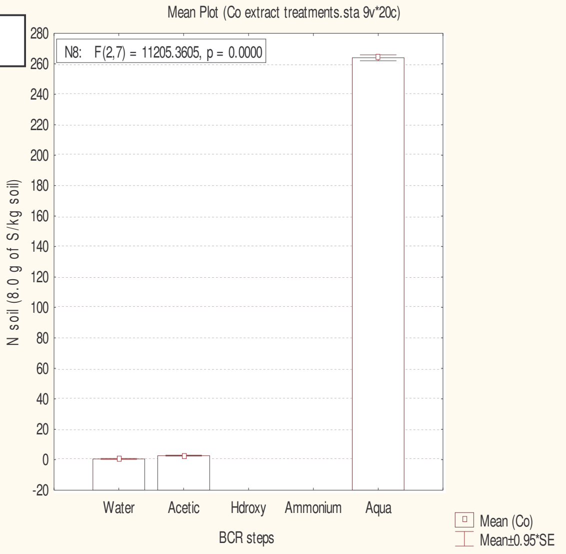
b)



c)



d)



Tukey HSD test; variable N0 (Co extract treatments.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = .97160, df = 15.000

Cell No.	treatments	N0 Mean	b	a
4	Ammonium	0.0000	****	
3	Hdroxy	0.0000	****	
1	Water	0.0000	****	
2	Acetic	1.9200	****	
5	Aqua	140.0000		****

Tukey HSD test; variable N2 (Co extract treatments.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = .70255, df = 15.000

Cell No.	treatments	N2 Mean	c	b	a
4	Ammonium	0.0000	****		
3	Hdroxy	0.0000	****		
1	Water	0.0000	****		
2	Acetic	2.1550		****	
5	Aqua	157.0000			****

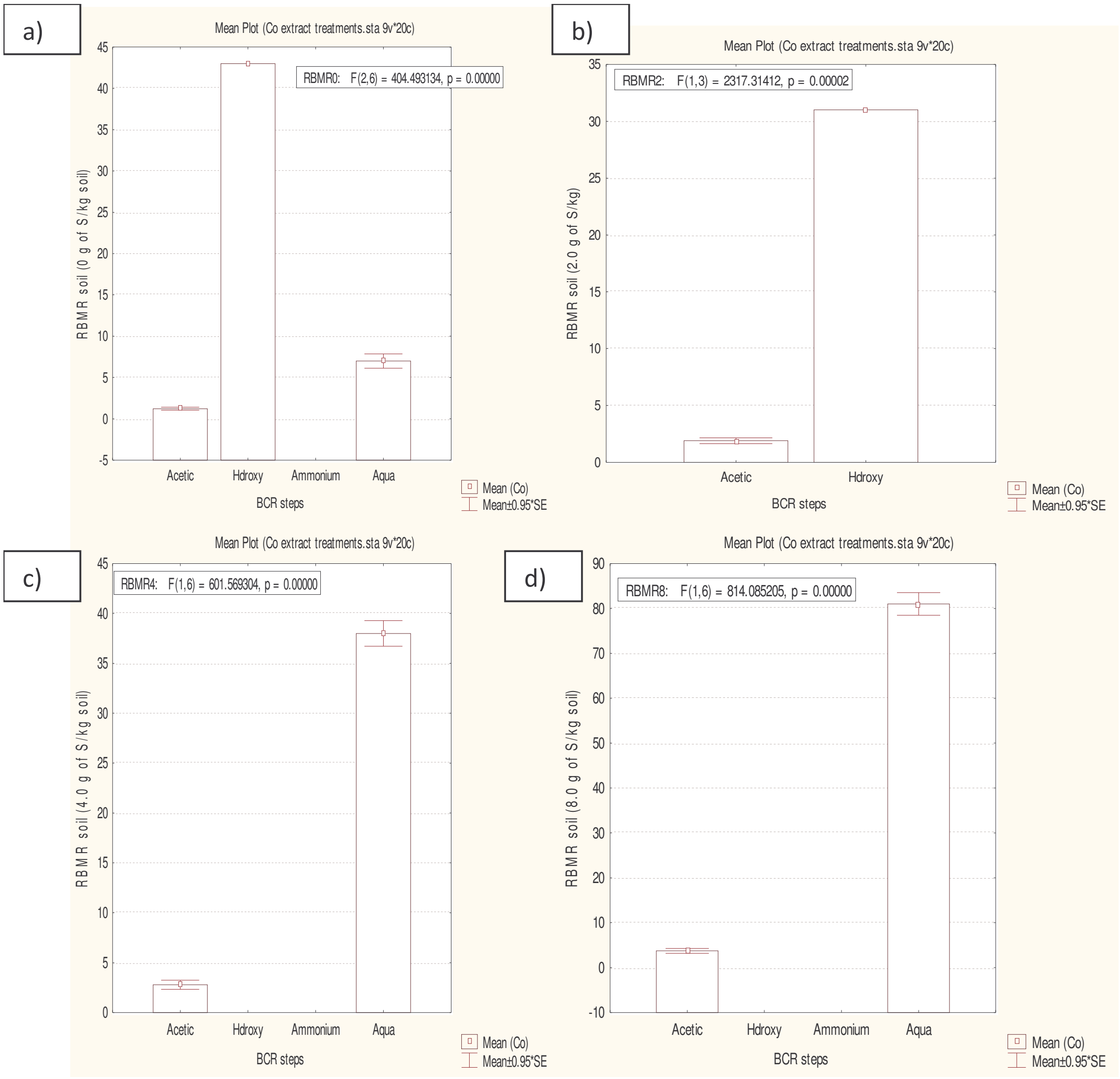
Tukey HSD test; variable N4 (Co extract treatments.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = 2.3307, df = 15.000

Cell No.	treatments	N4 Mean	b	a
4	Ammonium	0.0000	****	
3	Hdroxy	0.0000	****	
1	Water	0.0000	****	
2	Acetic	2.4962	****	
5	Aqua	113.0000		****

Tukey HSD test; variable N8 (Co extract treatments.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = 3.4517, df = 15.000

Cell No.	treatments	N8 Mean	b	a
4	Ammonium	0.0000	****	
3	Hdroxy	0.0000	****	
1	Water	0.2858	****	
2	Acetic	2.7571	****	
5	Aqua	264.0000		****

RBMR waste soil



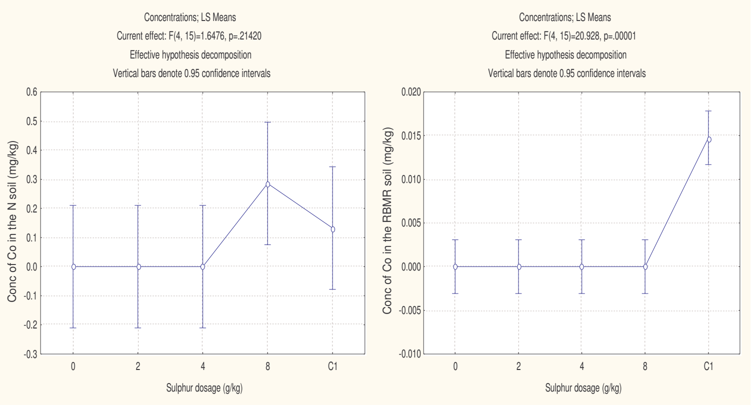
Cell No.	Tukey HSD test; variable RBMR0 (Co extract treatments.sta) Homogenous Groups, alpha = .05000 Error: Between MS = 93.148, df = 15.000 treatments	RBMR0 Mean	a
4	Ammonium	0.00000	****
1	Water	0.00000	****
2	Acetic	1.22500	****
5	Aqua	7.00000	****
3	Hdroxy	10.75000	****

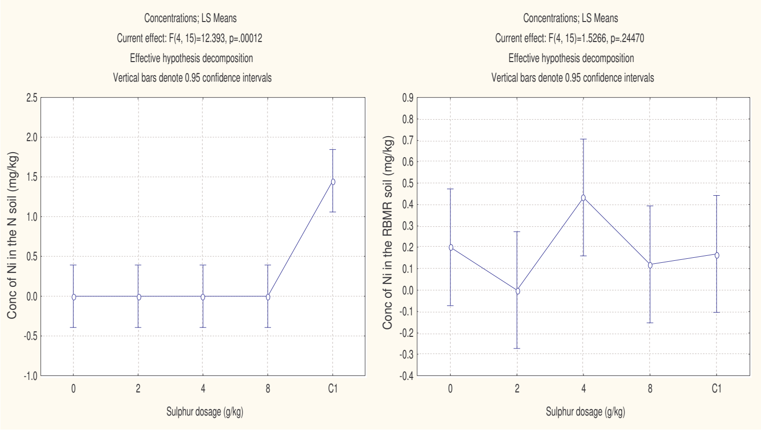
Cell No.	Tukey HSD test; variable RBMR2 (Co extract treatments.sta) Homogenous Groups, alpha = .05000 Error: Between MS = 48.108, df = 15.000 treatments	RBMR2 Mean	a
4	Ammonium	0.000000	****
5	Aqua	0.000000	****
1	Water	0.000000	****
2	Acetic	1.902500	****
3	Hdroxy	7.750000	****

Cell No.	Tukey HSD test; variable RBMR4 (Co extract treatments.sta) Homogenous Groups, alpha = .05000 Error: Between MS = 1.6487, df = 15.000 treatments	RBMR4 Mean	b	a
4	Ammonium	0.00000	****	
3	Hdroxy	0.00000	****	
1	Water	0.00000	****	
2	Acetic	2.79000	****	
5	Aqua	38.00000		****

Cell No.	Tukey HSD test; variable RBMR8 (Co extract treatments.sta) Homogenous Groups, alpha = .05000 Error: Between MS = 5.8636, df = 15.000 treatments	RBMR8 Mean	b	a
4	Ammonium	0.00000	****	
3	Hdroxy	0.00000	****	
1	Water	0.00000	****	
2	Acetic	3.75500	****	
5	Aqua	81.00000		****

One way- ANOVA analysis for Water extraction of Co and Ni concentration in the RBMR and N soil





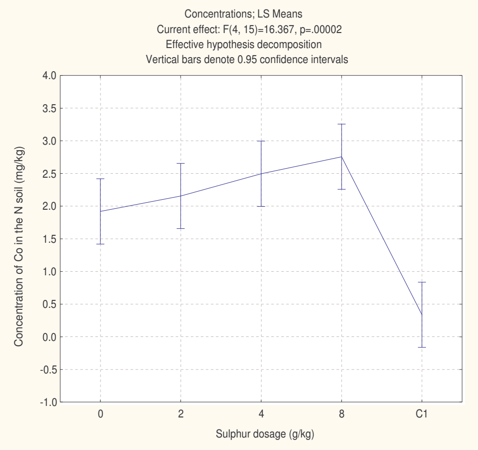
Tukey HSD test; variable native Ni (effect of sulphur on water extracts of Ni.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = .13638, df = 15.000

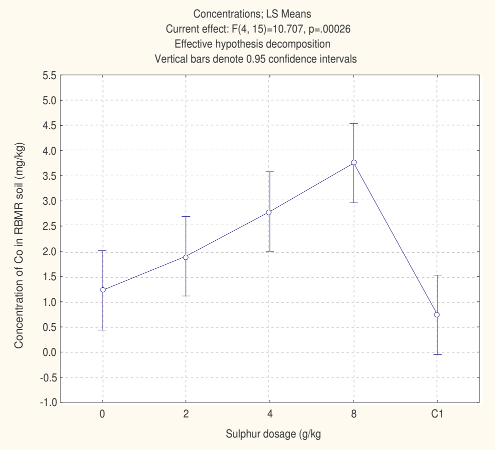
Cell No.	Sulphur dosage	Native soil (Ni)	b	a
2	2	0.000000	****	
3	4	0.000000	****	
4	8	0.000000	****	
1	0	0.000000	****	
5	C1	1.453500		****

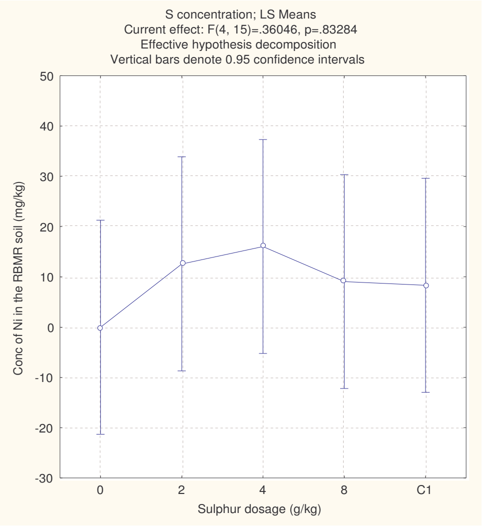
Tukey HSD test; variable RBMR soil (Co) (effect of sulphur on water extracts of Co)
Homogenous Groups, alpha = .05000
Error: Between MS = .00001, df = 15.000

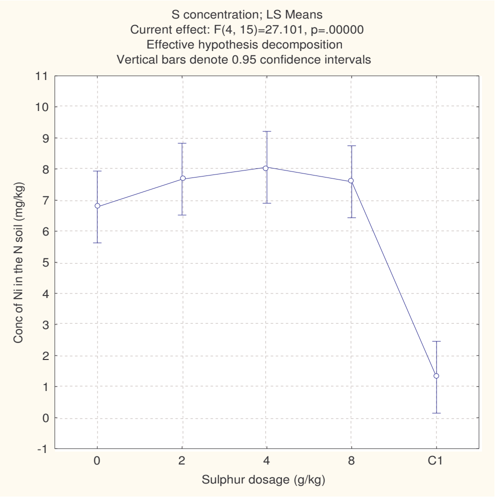
Cell No.	Sulphur dosage	RBMR soil (Co)	b	a
2	2	0.000000	****	
3	4	0.000000	****	
4	8	0.000000	****	
1	0	0.000000	****	
5	C1	0.014750		****

One way- ANOVA analysis for acetic acid extraction of Co and Ni concentration in the RBMR and N soil









Tukey HSD test; variable Ni (Native soil) (Acetic acid extractable)
Homogenous Groups, alpha = .05000
Error: Between MS = 1.1760, df = 15.000

Cell No.	S concentration	Ni (native) Mean	b	a
5	C1	1.300000	****	
1	0	6.782500		****
4	8	7.589583		****
2	2	7.675000		****
3	4	8.056667		****

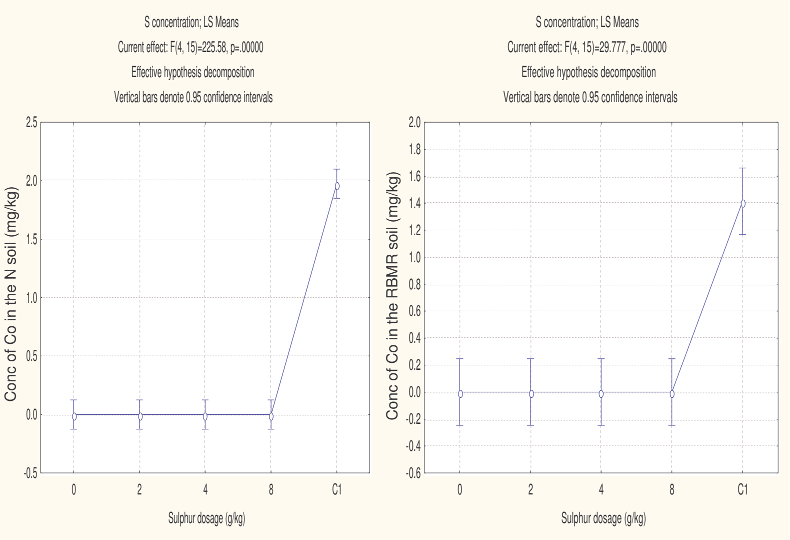
Tukey HSD test; variable Native soil Co (Acetic acid extraction)
Homogenous Groups, alpha = .05000
Error: Between MS = .21958, df = 15.000

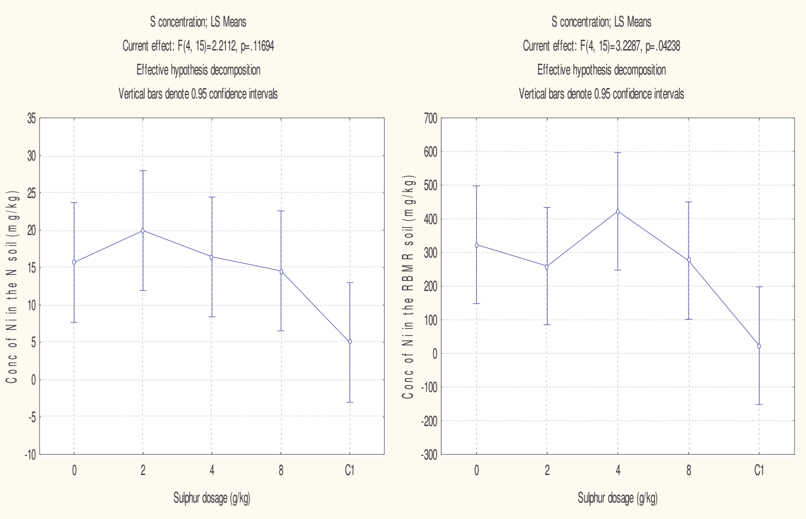
Cell No.	Sulphur dosage	Native soil (Co)	b	a
5	C1	0.336750	****	
1	0	1.920000		****
2	2	2.155000		****
3	4	2.496250		****
4	8	2.757083		****

Tukey HSD test; variable RBMR soil (Co) (Acetic acid extractior
Homogenous Groups, alpha = .05000
Error: Between MS = .54834, df = 15.000

Cell No.	Sulphur dosage	RBMR soil (Co)	c	b	a
5	C1	0.738750	****		
1	0	1.225000	****	****	
2	2	1.902500	****	****	
3	4	2.790000		****	****
4	8	3.755000			****

One way- ANOVA analysis for hydrogen peroxide and ammonium acetate extraction of Co and Ni concentration in the RBMR and N soil





Tukey HSD test; variable Ni (RBMR) (effect of sulphur on ammonium extract of Ni)
Homogenous Groups, alpha = .05000
Error: Between MS = 26859., df = 15.000

Cell No.	Sulphur dosage	RBMR soil (Ni)	b	a
5	C1	23.2750	****	
2	2	259.7500	****	****
4	8	275.7500	****	****
1	0	323.5000	****	****
3	4	422.5000		****

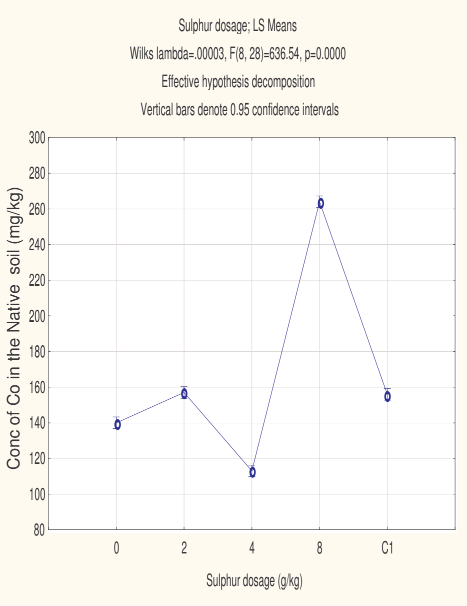
Tukey HSD test; variable Co (Native) (effect of sulphur on ammonium ext
Homogenous Groups, alpha = .05000
Error: Between MS = .01383, df = 15.000

Cell No.	Sulphur dosage	Native soil (Co)	b	a
2	2	0.000000	****	
3	4	0.000000	****	
4	8	0.000000	****	
1	0	0.000000	****	
5	C1	1.975000		****

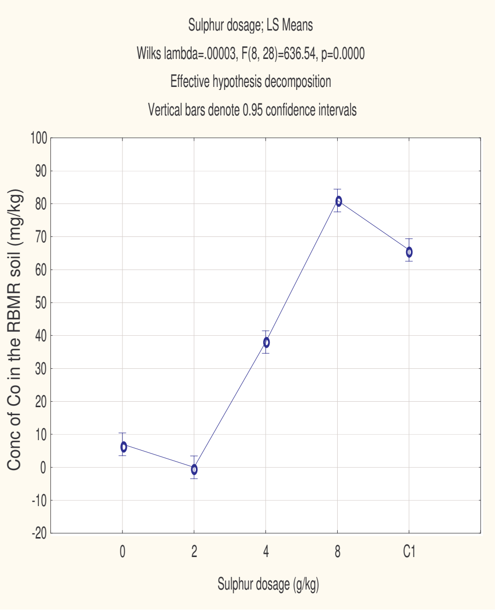
Tukey HSD test; variable Co (RBMR) (effect of sulphur ammonium extrac
Homogenous Groups, alpha = .05000
Error: Between MS = .05372, df = 15.000

Cell No.	S concentration	Co (RBMR) Mean	b	a
2	2	0.000000	****	
3	4	0.000000	****	
4	8	0.000000	****	
1	0	0.000000	****	
5	C1	1.414000		****

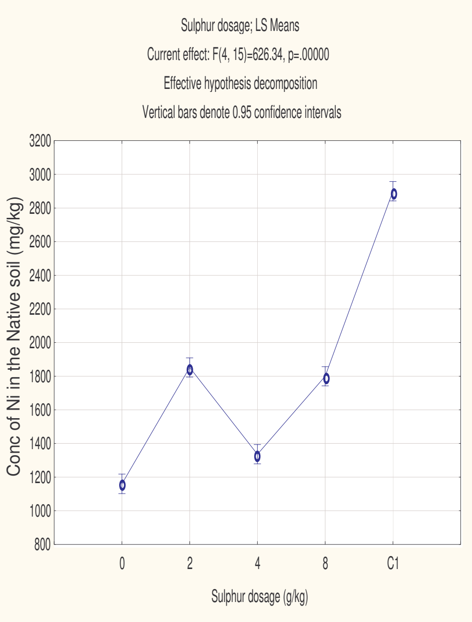
One way- ANOVA analysis for aqua regia extraction of Co and Ni concentration in the RBMR and N soil



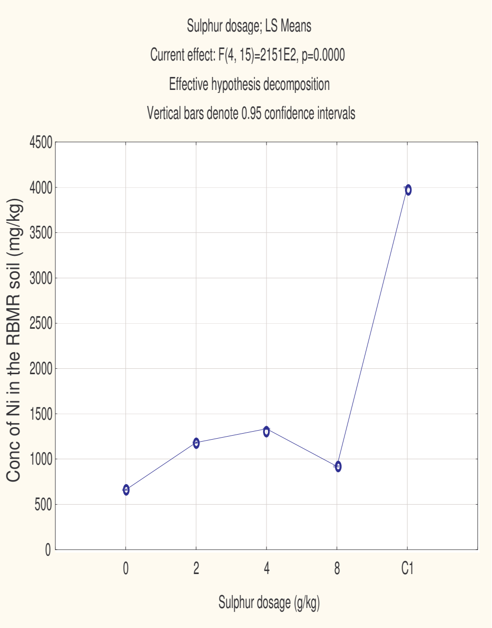
Sulphur dosage; LS Means
Wilks lambda=.00003, F(8, 28)=636.54, p=0.0000
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals



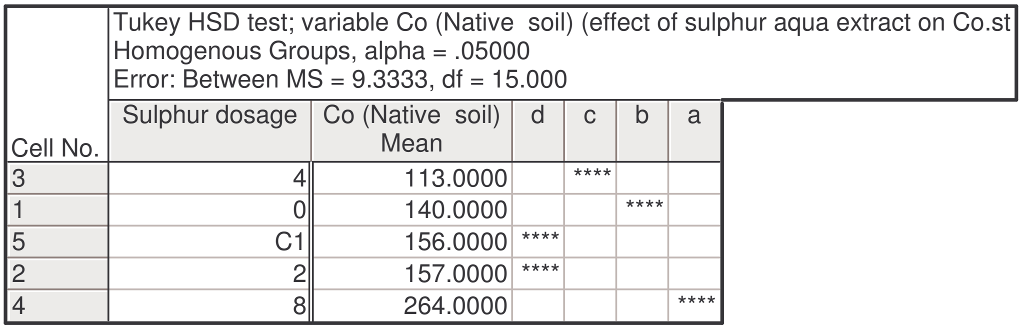
Sulphur dosage; LS Means
Wilks lambda=.00003, F(8, 28)=636.54, p=0.0000
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals



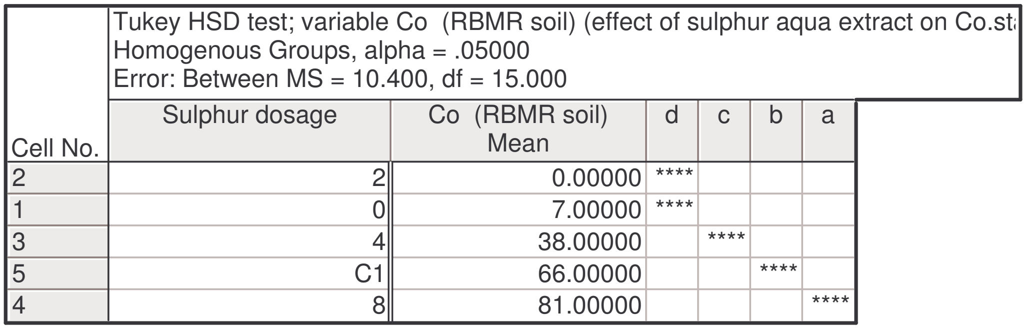
Sulphur dosage; LS Means
Current effect: F(4, 15)=626.34, p=.00000
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals



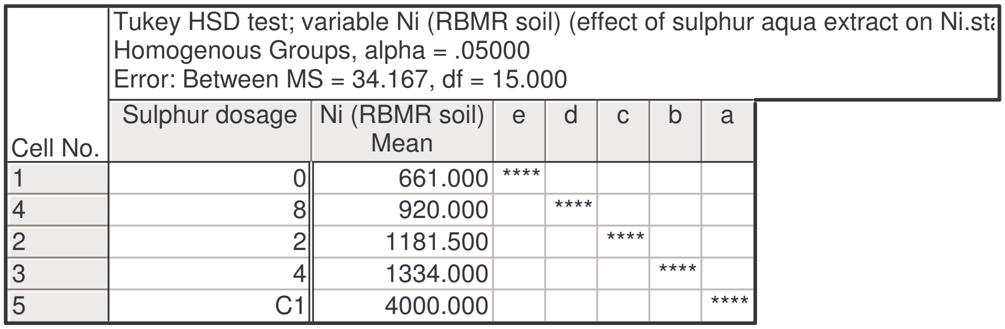
Sulphur dosage; LS Means
Current effect: F(4, 15)=2151E2, p=0.0000
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals


Tukey HSD test; variable Co (Native soil) (effect of sulphur aqua extract on Co.st
Homogenous Groups, alpha = .05000
Error: Between MS = 9.3333, df = 15.000

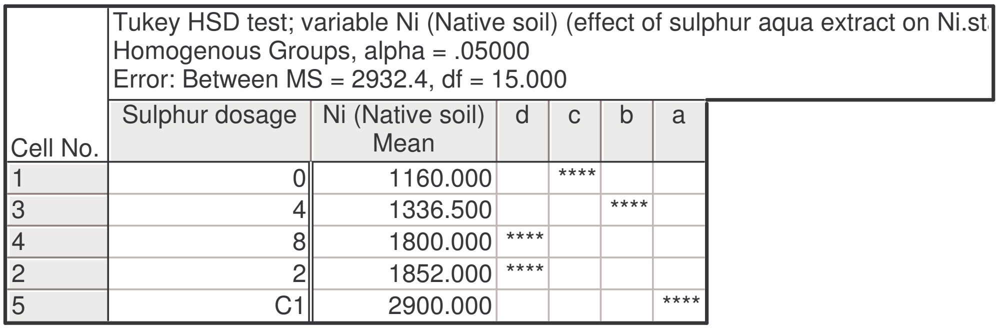
Cell No.	Sulphur dosage	Co (Native soil) Mean	d	c	b	a
3	4	113.0000		****		
1	0	140.0000			****	
5	C1	156.0000	****			
2	2	157.0000	****			
4	8	264.0000				****


Tukey HSD test; variable Co (RBMR soil) (effect of sulphur aqua extract on Co.sta
Homogenous Groups, alpha = .05000
Error: Between MS = 10.400, df = 15.000

Cell No.	Sulphur dosage	Co (RBMR soil) Mean	d	c	b	a
2	2	0.00000	****			
1	0	7.00000	****			
3	4	38.00000		****		
5	C1	66.00000			****	
4	8	81.00000				****

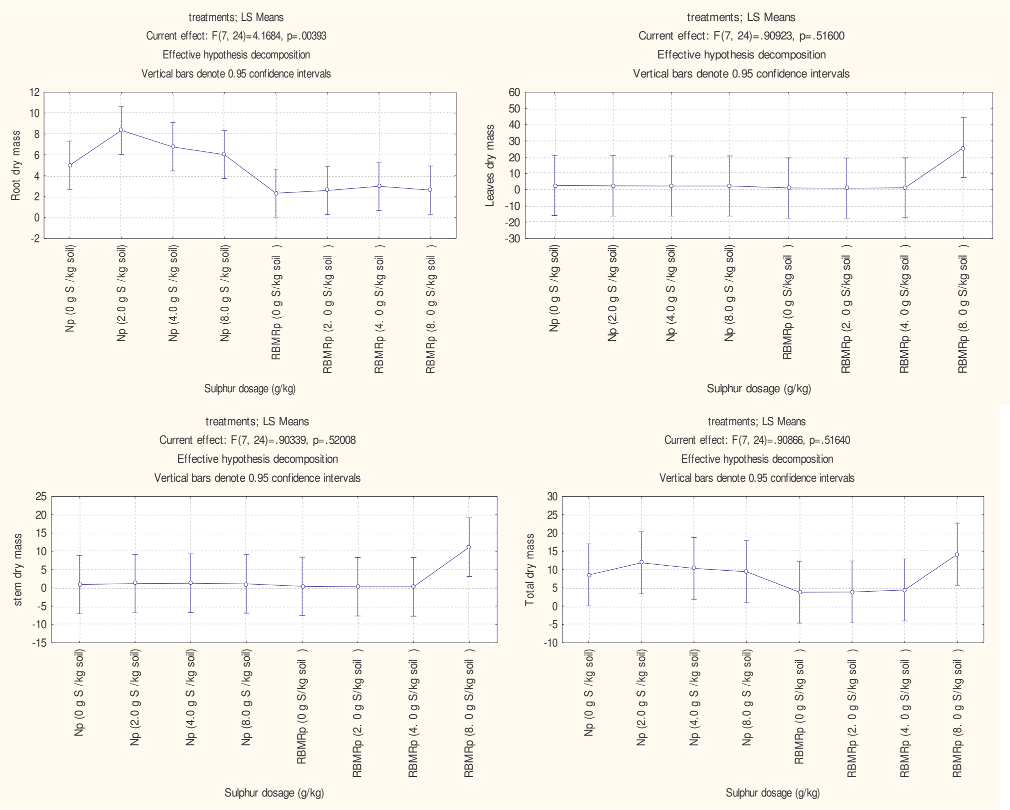

Tukey HSD test; variable Ni (RBMR soil) (effect of sulphur aqua extract on Ni.sta
Homogenous Groups, alpha = .05000
Error: Between MS = 34.167, df = 15.000

Cell No.	Sulphur dosage	Ni (RBMR soil) Mean	e	d	c	b	a
1	0	661.000	****				
4	8	920.000		****			
2	2	1181.500			****		
3	4	1334.000				****	
5	C1	4000.000					****


Tukey HSD test; variable Ni (Native soil) (effect of sulphur aqua extract on Ni.st
Homogenous Groups, alpha = .05000
Error: Between MS = 2932.4, df = 15.000

Cell No.	Sulphur dosage	Ni (Native soil) Mean	d	c	b	a
1	0	1160.000		****		
3	4	1336.500			****	
4	8	1800.000	****			
2	2	1852.000	****			
5	C1	2900.000				****

One way- ANOVA analysis: Dry mass production

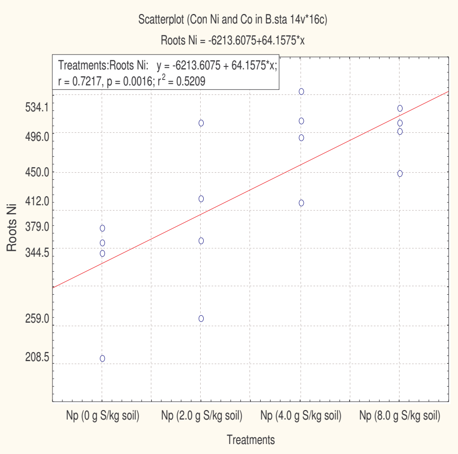


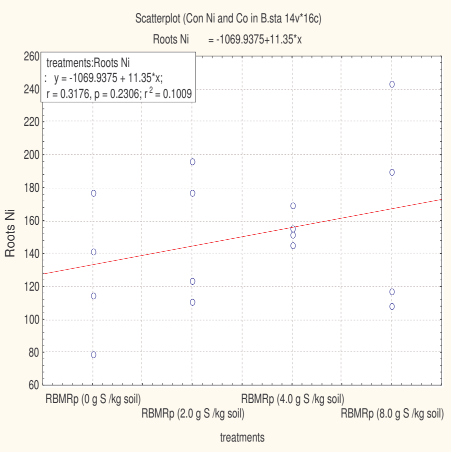
Tukey HSD test; variable Root dry mass (dry mass production.sta)
Homogenous Groups, alpha = .05000
Error: Between MS = 4.9993, df = 24.000

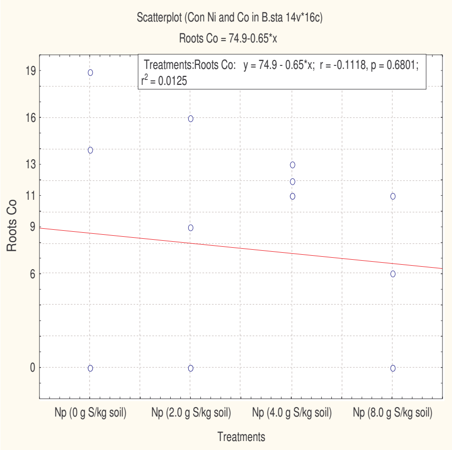
Cell No.	treatments	Root dry mass Mean	b	a
5	RBMRp (0 g S/kg soil)	2.342500	****	
6	RBMRp (2. 0 g S/kg soil)	2.597500	****	
8	RBMRp (8. 0 g S/kg soil)	2.630000	****	
7	RBMRp (4. 0 g S/kg soil)	2.992500	****	
1	Np (0 g S /kg soil)	5.017500	****	****
4	Np (8.0 g S /kg soil)	6.027500	****	****
3	Np (4.0 g S /kg soil)	6.767500	****	****
2	Np (2.0 g S /kg soil)	8.340000		****

Linear regression analysis: concentration of Co and Ni on various parts of *B.coddii*

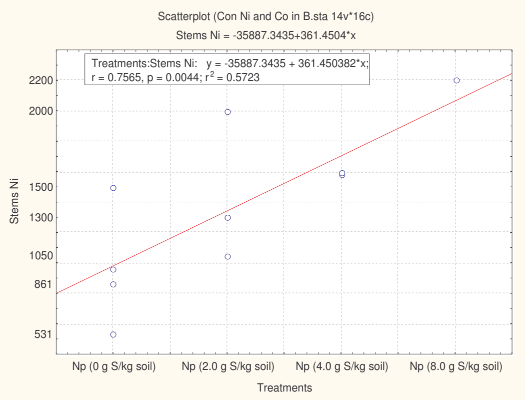
Roots

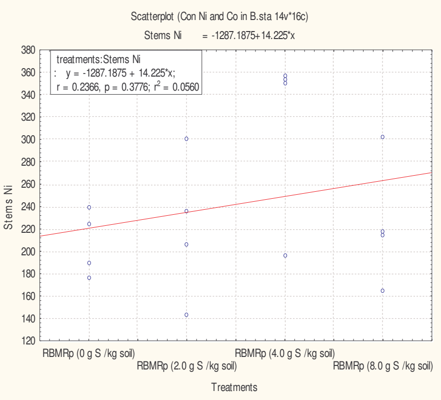




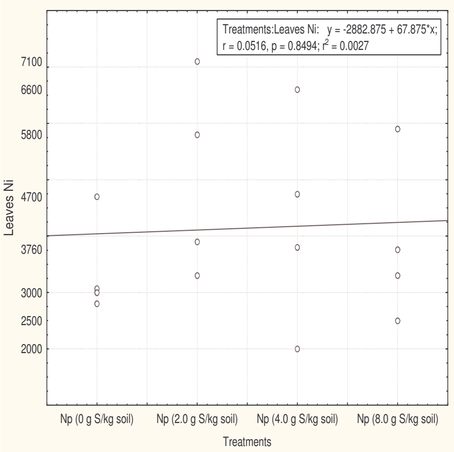


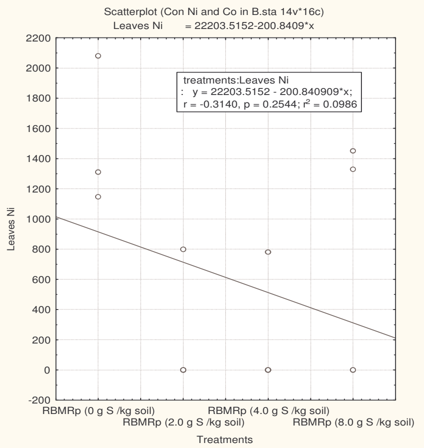
Stems

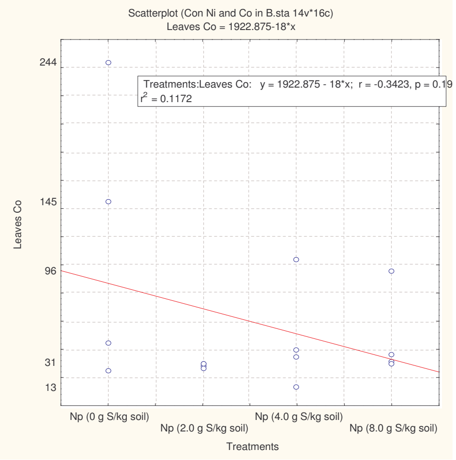


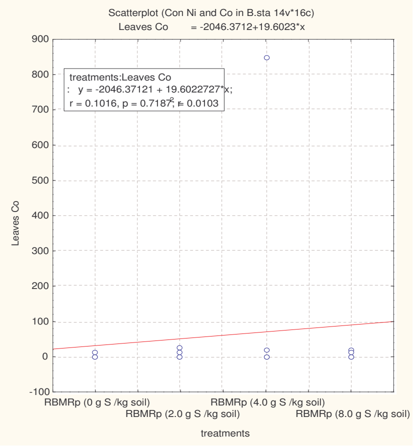


Leaves









One way- ANOVA: allocation of Co and Ni on different parts of *B.coddii*