



University of Venda

Quantification of the bioaccumulation potential of various chemical elements from coal fly ash using *Brassica juncea* and *Spinacia Oleracea L* and its implication for phytoremediation of coal fly ash dumps.

A Masters Research Thesis submitted to the University of Venda, School of Environmental Sciences,
Department of Ecology and Resource Management.

By

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DEDICATION

I am dedicating this research to my late father, Mashau M.V. who believed in me so much and wanted me to achieve more in life. I am also dedicating it to my mother Madzhie T.G. who supported me all the way and also encouraged me a lot and motivates me to become a better person through my studies. Through the courage of these people and many others I finally managed to finish my thesis, so I am saying that I appreciate all that you have done for me. I am also dedicating this research to my son, Ngobeni Omphile Junior who was born soon before submission of this thesis and my very supportive and loving husband, Ngobeni Basimane.

DECLARATION

I Mashau Aluwani Shiridor declare that *Quantification of the bioaccumulation potential of various chemical elements from coal fly ash using Brassica juncea and Spinacia oleracea L and its implication for phytoremediation of coal fly ash dumps* is my original work and has not been submitted for any degree at any other university or institution. The thesis does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signed (Student):Date:
Mashau A.S.

ABSTRACT

South Africa is highly dependent on coal for production of electricity. The combustion of coal for electricity generation produces waste by-products which include fine ash (fly ash) and coarse ash. Fly ash (FA) is produced in large amounts while its utilization is low due to its classification as a hazardous material. Sasol produces about 7 million tons of FA, while Eskom produces about 28 million tons of coal FA annually. FA is a fine by-product from pulverized coal with detrimental effects on plants, soil or land, animals and human beings, and pollutes the air. The present investigation focuses on the examination of the chemical elements bioaccumulation and phytoremediation potential of Indian mustard (*Brassica juncea*) and Spinach (*Spinacia Oleracea L.*).

X-ray fluorescence (XRF) was used to analyze the elemental composition of FA and soil, Scanning Electron Microscope (SEM) to examine the morphology, while X-ray diffraction (XRD) analysis was used to evaluate the mineralogy of FA. The concentrations of metal and non-metal species that are released from FA on contact with water at different conditions were quantified using ICP-MS. Pot culture experiment was conducted to grow *Brassica juncea* and *Spinacia Oleracea L.* using FA and soil as growth medium. Leachates from the pots were collected and analysed using ICP-MS. Plant parts from harvested *Brassica juncea* and *Spinacia Oleracea L.* were cut separately and analysed using ICP-MS for the concentrations of different metal and non-metal species in plant parts. Plant parts were also used to estimate biomass and chlorophyll content (leaves). To prepare these plant samples for analysis, the powdered plant sample (0.5 g) was digested through aqua regia (HCL:HNO₃ = 3:1 (v/v)) to near dryness using hotplate and filled to 100 mL of MilliQ water. The samples were filtered and directly used to determine the chemical elements concentrations. Blanks and internal standards were used for quality assurance during analysis. Chemical elements that are present in FA and sometimes in higher concentrations are associated with detrimental effects in plants, animals and human beings, hence phytoremediation is vital. Bioconcentration factor (BCF) was used to estimate the metal species accumulation ability of the plants from the FA, FA/soil mixtures, while translocation factor (TF) was used to assess the plant species potential for phytoremediation of coal fly ash dumps. Analysis of Variance (ANOVA) was used to statistically test data using Graphpad software package. Relationship between chemical elements in soil, FA and FA+soil growth media and also different plant parts (root, stem and leaf) of *B. juncea* and *S. Oleracea L.* were calculated using the t - test, ANOVA-Bartlett test, Mann-Whitney Test and Kruskal-Wallis Statistic (KW) depending on each data set.

The physicochemical characterization of coal fly ash showed that FA from Grootvlei power station can be classified as class F with an alkaline pH level of 10.62. It showed that particle morphology of this FA had a lower degree of sphericity with irregular agglomerations of many particles while there were dominant spherical particles and smaller sharp needle like particles. It is also an alumino-silicate material as confirmed by the high SiO₂ and Al₂O₃ content, while soil leachates had an average/neutral pH of 7.22 with very high amounts of Si. In both soil and FA, elements that were expected to be readily available to plants included Ca, Si, K, Ba, Mo, Na, Al, Mg, Sr and non-essential elements (Si, Ba, Na, Al, Sr), which, if uptaken by plants' roots can have negative impacts in plants. Physicochemical analysis of soil, FA and FA+soil leachates showed that the alkalinity of the FA changed over time and there was also a decrease in the EC due to dissolution

of soluble major oxides, which was promoted by continuous water irrigation. The soil and FA+soil growth media showed similar results. Chemical elements like B, Mn, Fe and Ba were occurring at higher concentrations in leachates for most weeks in the pot culture experiments. However, it was observed that in the eleventh week of leachate collection all these chemical elements decreased to very low concentrations. This suggest that these chemical elements can be reduced over time as plants are being irrigated which is either due to uptake by plants or washed off with water. Statistically, there was a significant difference for different chemical elements of leachates from different growth media for each plant species (*B. juncea* and *S. Olearcea L.*).

The overall growth rate shows that *S. Oleracea L* was better than that of *B. juncea* especially in the FA media over time, while the biomass of the two plant species showed similar results. After all, even though *S. Oleracea L* had carotenoid content below detection limits, it had higher chlorophyll b than *B. juncea* for all growth media in general. But, statistically there was no significant difference between the two plant species in terms of growth rate and biomass; even between the plant parts denoting similar growth performance for the two plant species under study. The bioaccumulation potential of the two plant species showed that chemical elements such as Fe, Mn, Ba, Zn and B were highly accumulated by the different parts of the plants. However, the chemical elements such as of Mo, Ni, Cu, and Cr showed the least concentrations. This trend was similar for all growth media and both plant species but this changes over time for different growth media and plant species as increasing and decreasing trends can be noticed. This led to no significant difference between plant species and also growth media, statistically.

High BCF values of Fe, Mn, Ba, Zn and B were observed in the different parts of the plants for both plant species. However, Mo, Ni, Cu, and Cr had BCF values less than 1 for most growth media over time. BCF values in plant parts differed with time, growth media and plant species. Translocation of chemical elements shows that the *B. juncea* plant proved to be an effective phytoremediation plant species since it is effective in translocation of many chemical elements for different growth media to shoots while *S. Oleracea L* failed to translocate most chemical elements from stem to leaves although it translocated some from root to shoots. Hence, it can be concluded from the study that both species can be used in phytoremediation of coal fly ash dumps but with *B. juncea* being the most effective accumulator and translocator of many chemical elements. However, it can be recommended that chelating agents like ethylene diamine tetra acetic acid (EDTA) be introduced to solubilize chemical elements from growth media matrix into growth media solution to facilitate the quick transport of chemical elements into xylem, and increase translocation of chemical elements from the roots to shoots of *B. juncea* and *S. Oleracea L*.

Keywords: *fly ash, phytoremediation, chemical elements, translocation factor, pot culture experiments, bioconcentration factor.*

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TABLE OF CONTENTS

Contents

DEDICATION	i
DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
CHAPTER 1	1
INTRODUCTION	1
1.1 Background information	1
1.2 Problem statement	2
1.3 Research rationale	2
1.4 Objectives	3
1.4.1 Main objective	3
1.4.2 Specific objectives	3
1.5 Research questions	3
1.6 Hypothesis	4
CHAPTER 2	5
LITERATURE REVIEW	5
2.1 Introduction	5
2.2 What is fly ash and how is it generated?	5
2.3 Transport and storage of fly ash	5
2.4 Properties of fly ash	6
2.4.1 Morphology	6
2.4.2 Chemistry	7
2.5 Disposal and Management of ash storage facilities	9
2.6 Stabilization of ash facilities	10
2.7 Uses of fly ash	11
2.7.1 Beneficial utilization of fly ash	11
2.7.2 Application of coal FA in environmental remediation	12
2.8 Impacts of fly ash	12
2.8.1 Environmental impacts of FA and FA ash storage dump	13

2.8.2	Health impacts of fly ash toxicants.....	14
2.9	Revegetation of ash dumps	15
2.10	Bioremediation and the science involved.....	16
2.10.1	Phytostabilization.....	17
2.10.2	Phytoextraction	17
2.10.3	Characteristics of plants suitable for phytoremediation (phytoextraction).....	18
CHAPTER 3		19
MATERIALS AND METHODS.....		19
3.1	Introduction	19
3.2	Plant species description	19
3.3	Experimental Protocol.....	21
3.4	Evaluation of the morphology, chemical and mineralogical properties of fly ash	22
3.4.1	Chemical parameters of fly ash.....	22
3.4.2	Scanning Electron Microscope (SEM)	22
3.4.3	X-ray fluorescence (XRF) analysis.....	22
3.4.4	X-ray diffraction (XRD) analysis	23
3.5	Pot culture experiments.....	23
3.5.1	Sowing of seeds and transplantation.....	25
3.5.2	Harvesting procedure	26
3.6	Preparation of plants samples for analysis and biomass estimation.....	28
3.7	Analysis of chlorophyll-a, chlorophyll-b and carotenoids content	28
3.8	Bioconcentration factor (BCF).....	29
3.9	Translocation factor (TF)	29
3.10	Statistical analysis and data presentation	29
CHAPTER 4		31
RESULTS AND DISCUSSION.....		31
Part 1: The chemical, morphological and mineralogical characterization of FA and soil used in pot culture experiments.....		31
4.1	Introduction	31
4.2	Characterization of fly ash and soil for pot culture experiments	31
4.2.1	Mineralogy of Grootvlei Power Station FA.....	31

4.2.2	Morphology of the Grootvlei Power Station FA	32
4.2.3	Chemical composition of FA and soil.	34
4.3	Physicochemical properties of leachates from coal fly ash and soil	35
4.3.1	Conclusion	39
Part 2: The temporal evolution of the physicochemical characteristics of the leachates as plants were irrigated in the pot culture experiment		40
4.4	Introduction	40
4.4.1	Temporal variation of pH in various growth media.....	40
4.4.2	Temporal variation of EC in various growth media	43
4.4.3	Statistical analysis of EC and pH trends data	46
4.5	Temporal variation of the chemical properties of leachates from pots with different growth media.....	48
4.5.1.	Temporal variation of the chemical elements of leachates from pots of soil growth media for both plants	49
4.5.2	Temporal variation of the chemical elements of leachates from pots of FA growth media for both plants	53
4.5.3	Temporal variation of the chemical elements of leachates from pots of FA+soil growth media for both plants.....	57
4.5.4	Temporal variation of the chemical elements of leachates from pots of FA growth media with no plants grown.....	61
4.5.5	Comparison of soil and FA media leachates for both species	63
4.5.6	Statistical evaluation of the chemical elements trends of leachates for the different growth media	64
4.5.7	Conclusion	69
Part 3: Assessment of the growth of <i>Brassica juncea</i> and <i>Spinacia leracea L.</i> in pot culture experiments grown in FA and soil		71
4.6	Introduction	71
4.6.1	Biomass estimation of <i>B. juncea</i> and <i>S. Oleracea L.</i> in different growth media....	71
4.6.2	Growth performance of <i>B. juncea</i> and <i>S. Oleracea L.</i> in different growth media..	73
4.6.3	Statistical evaluation of the biomass and growth rate trends of <i>B. juncea</i> and <i>S. Oleracea L</i> for the different growth media.....	75
4.6.4	Chlorophyll and carotenoid content of <i>B. juncea</i> and <i>S. Oleracea L.</i> in different growth media	77

4.6.5	Conclusion	81
Part 4:	Evaluation of the concentrations of chemical elements accumulated by roots, stems, and leaves of selected plants.....	82
4.7	Introduction	82
4.7.1	Evaluation of the chemical elements accumulated by <i>B. juncea</i> plant parts (leaf, stem and root) over a period of 115 days for soil media.....	82
4.7.2	Evaluation of the chemical elements accumulated by <i>B. juncea</i> plant parts (leaf, stem and root) over a period of 115 days for FA media	84
4.7.3	Evaluation of the chemical elements accumulated by <i>B. juncea</i> plant parts (leaf, stem and roots) over a period of 115 days for FA+soil media.....	85
4.7.4	Evaluation of the chemical elements accumulated by <i>S. Oleracea L</i> plant parts (leaf, stem and root) over a period of 115 days for soil media	86
4.7.5	Evaluation of the chemical elements accumulated by <i>S. Oleracea L</i> plant parts (leaf, stem and root) over a period of 115 days for FA media.....	88
4.7.6	Evaluation of the chemical elements accumulated by <i>S. Oleracea L</i> plant parts (leaf, stem and root) over a period of 115 days for FA+soil media.....	89
4.7.7	Comparison of the accumulation potential of <i>B. juncea</i> and <i>S. Oleracea L</i>	90
4.7.8	Statistical evaluation of the bioaccumulation of chemical elements in <i>B. juncea</i> and <i>S. Oleracea L</i> for the different growth media.....	90
4.7.9	Conclusion	92
Part 5:	Evaluation of the phytoremediation potential (bioaccumulation capability) of the selected plants using the bioconcentration factor (BCF) and translocation factor (TF).....	94
4.8	Introduction	94
4.8.1	BCF for soil media in both plants	94
4.8.2	BCF for FA media in both plants.....	96
4.8.3	BCF for FA+soil media in both plants.....	98
4.8.4	Statistical evaluation of the BCF of <i>B. juncea</i> and <i>S. Oleracea L</i> for the different growth media with time.....	100
4.9	Assessment of the phytoremediation potential of <i>B. juncea</i> and <i>S. Oleracea L</i> using translocation factor (TF).....	101
4.10	Conclusion.....	104
CHAPTER 5	105
CONCLUSIONS AND RECOMMENDATIONS	105
5.1	Introduction	105

5.2	Conclusions	105
5.3	Recommendations	107
	REFERENCES	108
	APPENDIX.....	122

List of figures

- Figure 2.1: Ash pumped as slurry into ash dams (Chemin, 2014).
- Figure 2.2: SEM micrographs of fly ash adopted from Mahlaba, et al. (2011a).
- Figure 3.1: *Spinacia Oleracea L.*
- Figure 3.2: *Brassica juncea.*
- Figure 3.3: Flow diagram showing steps of the experimental protocol
- Figure 3.4: Illustration of set up of pots (what the pots were filled with and which species were grown in each pot).
- Figure 3.5: Plants ready for transplantation.
- Figure 3.6: Seedlings showing nutrient deficiency after four weeks of germination.
- Figure 3.7: Symptoms of chlorosis, necrosis, nutrient deficiency and death of *Brassica juncea* and *Spinacea Oleracea L.* being stunted.
- Figure 4.1: XRD spectrum of the FA.
- Figure 4.2: SEM images of Grootvlei Power Station FA at (a) x10 000 (b) x20 000 (c) x30 000 and (d) x70 000 magnifications.
- Figure 4.3: Variation of pH in leachates for *B. juncea* grown in soil media with time.
- Figure 4.4: Variation of pH in leachates for *B. juncea* grown in FA media with time.
- Figure 4.5: Variation of pH in leachates for *B. juncea* grown in FA+soil media with time
- Figure 4.6: Variation of pH in leachates for *S. Oleracea L* grown in soil media with time.
- Figure 4.7: Variation of pH in leachates for *S. Oleracea L* grown in FA media with time.
- Figure 4.8: Variation of pH in leachates for *S. Oleracea L* grown in FA+soil media with time.
- Figure 4.9: Variation of pH in leachates where no plants grown in FA media with time.
- Figure 4.10: Variation of EC in leachates for *B. juncea* grown in soil media with time.
- Figure 4.11: Variation of EC in leachates for *B. juncea* grown in FA media with time
- Figure 4.12: Variation of EC in leachates for *B. juncea* grown in FA+soil media with time.
- Figure 4.13: Variation of EC in leachates for *S. Oleracea L* grown in soil media with time.
- Figure 4.14: Variation of EC in leachates for *S. Oleracea L* grown in FA media with time.
- Figure 4.15: Variation of EC in leachates for *S. Oleracea L* grown in FA+soil media with time.
- Figure 4.16: Variation of EC in leachates where no plants grown in FA media with time.
- Figure 4.17: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea* for essential elements B, Zn, Mn, Fe and Cu.
- Figure 4.18: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea* for non-essential elements Cr, Ni and Ba and Mo as an essential element.
- Figure 4.19: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L.* for essential elements B, Zn, Mn, Fe, Mo and Cu.
- Figure 4.20: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L.* for non-essential elements Cr, Ni and Ba.
- Figure 4.21: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea* for essential elements B, Zn, Mn, Fe, Mo and Cu.

Figure 4.22: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea* for non-essential elements Cr, Ni and Ba.

Figure 4.23: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.* for essential elements B, Zn, Mn, Fe, Mo and Cu.

Figure 4.24: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.* for non-essential elements Cr, Ni and Ba.

Figure 4.25: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *B. juncea* for essential elements B, Zn, Mn, Fe, Mo and Cu.

Figure 4.26: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *B. juncea* for non-essential elements Cr, Ni and Ba.

Figure 4.27: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *S. Oleracea L.* for essential elements B, Zn, Mn, Fe, Mo and Cu.

Figure 4.28: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *S. Oleracea L.* for non-essential elements Cr, Ni and Ba.

Figure 4.29: Chemical elements concentrations obtained from FA pots where no plants were grown; for essential elements B, Zn, Mn, Fe, Mo and Cu.

Figure 4.30: Chemical elements concentrations in leachates obtained from FA pots where no plants were grown; for non-essential elements Cr, Ni and Ba.

Figure 4.31: Above and below ground biomass for *B. juncea* in different growth media.

Figure 4.32: Above and below ground biomass for *S. Oleracea L* in different growth media.

Figure 4.33: Growth rate of *B. juncea* for different growth media with time.

Figure 4.34: Growth rate of *S. Oleracea L* for different growth media with time.

Figure 4.35: Chlorophyll a content in *B. juncea* for different growth media with time.

Figure 4.36: Chlorophyll b content in *B. juncea* for different growth media with time.

Figure 4.37: Carotenoid content in *B. juncea* for different growth media with time

Figure 4.38: Chlorophyll a content in *S. Oleracea L* for different growth media with time

Figure 4.39: Chlorophyll b content in *S. Oleracea L* for different growth media with time.

Figure 4.40: Dead plant of *B. juncea* in pot culture experiment.

Figure 4.41: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for soil media pots

Figure 4.42: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for soil media pots.

Figure 4.43: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for FA media pots.

Figure 4.44: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for FA media pots

Figure 4.45: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for the FA+soil media pots.

Figure 4.46: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for FA+soil media pot.

List of tables

Table A1: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L.*

Table A2: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.*

Table A3: Chemical elements concentrations in leachates obtained from FA+soil pots growing *S. Oleracea L.*

Table A4: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea.*

Table A5: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea.*

Table A6: Chemical elements concentrations in leachates obtained from FA+soil pots growing *B. juncea.*

Table A7: Chemical elements concentrations in leachates obtained from FA pots where no plants were grown.

Table A8: Department of Environmental Affairs guidelines for the national norms and standards for the remediation of contaminated land and soil quality (soil screening values for metals).

Table A9: Department of Environmental Affairs guidelines for the national norms and standards for the remediation of contaminated land and soil quality (soil screening values for anions).

Table B1: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* and *S. Oleracea L.* seedlings grown in the agromix media on a seed tray, i.e. raw data from IC-MS (1st harvest – 23 days of pot culture experiments).

Table B2: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the soil media in pot A6 and A10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B3: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the soil media in pot A1 to A5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B4: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA media in pot B1 to B5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B5: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA media in pot B6 to B10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B6: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA+soil media in pot C1 to C5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B7: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media in pot C6 to C10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

Table B8: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the soil media in pot A1 to A5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table B9: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the soil media in pot A6 and A10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table B10: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA media in pot B1 to B5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table B11: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA media in pot B6 to B10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table B12: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA+soil media in pot C1 to C5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table B13: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media in pot C6 to C10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

Table 2.1: ASTM standards classification of fly ash (ASTM C 618, 1993).

Table 2.2: Naturally growing plant species capable of capturing fly ash dust (Chaphekar and Madav, 1999; Pandey, et al., 2009).

Table 2.3: Impacts on the animals and human body that can be caused by exposure to some of the most common toxic contaminants in fly ash (adopted from Ali et al., 2013).

Table 2.4: Summary of the different techniques of phytoremediation (adopted from Ali et al., 2013).

Table 3.1: Plant descriptions for *Spinacia Oleracea L.* and *Brassica juncea* (Learn to grow, 2014; Masley, 2014).

Table 4.1: XRD quantitative results for Grootvlei Power Station FA.

Table 4.2: Comparison of the chemical composition of FA and soil samples.

Table 4.3: Chemical characteristics of FA and soil leachates

Table 4.4: pH and EC leachates' statistical comparisons between *B. juncea* and *S. Oleracea L.* grown in different media.

Table 4.5: Statistical comparisons between four sets of growth media (soil, FA, soil+FA with plants and FA only - without plants) for each plant species.

Table 4.6: Comparison of chemical elements between *B. juncea* and *S. Olearacea L.* grown in soil media.

Table 4.7: Comparison of chemical elements between *B. juncea* and *S. Oleracea L* grown in FA media.

Table 4.8: Comparison of chemical elements between *B. juncea* and *S. Oleracea L* grown in soil+FA media.

Table 4.9: Chemical elements comparisons between four sets of growth media (soil, FA, soil+FA with *B. juncea* and FA only - without plants).

Table 4.10: Chemical elements comparisons between four sets of growth media (soil, FA, soil+FA with *S. Oleracea L* and FA only - without plants).

Table 4.11: Comparison of *B. juncea* and *S. Oleracea L* with regards to their biomass for different growth media.

Table 4.12: Comparison of the different sets of growth media (soil, FA, soil+FA) with regards to their biomass for *B. juncea* and *S. Oleracea L*.

Table 4.13: Comparison of *B. juncea* and *S. Oleracea L* with regards to their growth rate for different growth media.

Table 4.14: Comparison of the different sets of growth media (soil, FA, soil+FA) with regards to their growth rate for *B. juncea* and *S. Oleracea L*.

Table 4.15: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the soil media with time.

Table 4.16: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the FA media with time.

Table 4.17: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media with time.

Table 4.18: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the soil media with time.

Table 4.19: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the FA media with time.

Table 4.20: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the FA+soil media with time.

Table 4.21: Comparison of *B. juncea* and *S. Oleracea L* for the different sets of growth media with regards to the mean concentrations obtained.

Table 4.22: Comparison of *B. juncea* and *S. Oleracea L* plant parts for each set of growth media (soil, FA and soil+FA) with regards to the mean concentrations obtained.

Table 4.23: Comparison of the BCF for *B. juncea* and *S. Oleracea L* plant for the whole plant grown on different sets of growth media.

Table 4.24: Comparison of the BCF for *B. juncea* and *S. Oleracea L* plant parts of same plant species from same growth media with time.

Table 4.25: Translocation factor (TF) of various chemical elements over time in different species of plants grown in different growth media. $TF = BCF_b / BCF_c$ i.e. stem/root.

Table 4.26: Translocation factor (TF) of various chemical elements over time in different species of plants grown in different growth media. $TF = BCF_a / BCF_b$ i.e. leaf/stem.

CHAPTER 1

INTRODUCTION

1.1 Background information

The recent energy crisis has led to the construction of new coal power plants in South Africa which was largely driven by factors of economic growth and social integration. Despite the mounting interest in the renewable sector, coal-fired power stations still account for the largest percentage of South Africa's electricity generating capacity. Specifically, South Africa generates 93 percent of its electricity from coal combustion (Babajide, et al., 2010). By the year 2010, Eskom's coal-fired power stations would be projected to produce about 25 million tons of ash (Eskom fact sheet, 2009). On the other hand, Sasol was producing about 7 million tons of ash, as reported by Matjie et al. (2005).

Ciccu et al. (1999) reported that around 300 billion tons per year of fly ash is produced worldwide and probably this has increased due to the pressing need for generation of sufficient energy for the rapidly increasing population. Fly ash (FA) is defined by Eze et al. (2013) as a byproduct of burning pulverized coal in electric power-generating plants. About 80 % of the solid residue released from combustion of coal is released as FA. Pandey et al. (2009) mentioned that chemically, FA contains oxides, hydroxides, carbonates, silicates, and sulfates of calcium, iron, aluminum, and other chemical elements in trace amounts (Adriano, et al., 1980).

Phytoremediation is the way to go as it is anticipated as a cost-efficient and ecologically kind process, since conventional remediation methods such as acid leaching, land-filling, and excavation process are very expensive and not eco-friendly (Tiwari, et al., 2013). Phytoremediation actually refers to a diverse collection of plant-based technologies that use either naturally-occurring or genetically-engineered plants for cleaning contaminated environments (Flathman and Lanza, 1998). As phytoremediation involves techniques such as rhizofiltration, phytostabilization, phytovolatilization and phytoextraction, the current study will mainly focus on phytoextraction. Phytoextraction (also known as phytoaccumulation, phytoabsorption or phytosequestration) is the uptake of contaminants from the soil or water by plant roots and their translocation to and accumulation in above ground biomass i.e., shoots (Sekara et al., 2005; Yoon et al., 2006; Rafati et al., 2011, Ali et al., 2013). Phytoextraction is relevant in this study because it is the main and most useful phytoremediation technique for removal of chemical elements from polluted soils, sediments or water (Cluis, 2004; Cherian and Oliveira, 2005; Milic et al., 2012, Ali et al., 2013). In addition, phytoextraction is the most promising for commercial application (Sun et al., 2011, Ali et al., 2013).

There are few studies on phytoremediation in South Africa from different sites that have been investigated. These include a study by Mellem et al. (2008) which was undertaken to evaluate the potential of *Amaranthus dubius* for the phytoremediation of selected metal species from three environmental sites which include regular cultivated area, a landfill site, and a wastewater treatment site. *A. dubius* was considered to be able to translocate other chemical elements in certain concentrations, while others were less translocated, and hence it was mentioned that its effectiveness in phytoremediation must be viewed with caution. Another study is by Dye and Weiersbye (2010), which was about the phytoremediation of gold and uranium mine tailing storage facilities (TSFs), which were also unlined under the Mine Woodland Project. Here, the effectiveness of planted woodlands was evaluated to find out if they are capable of reducing or preventing the spread of mine drainage water from TSFs, and providing a sustainable, low-cost solution to the pollution threat. Most studies on phytoremediation in South Africa include the use of plants for the rehabilitation of mine tailings. These include studies by Knoll (1997), where vetiver grass was found to be highly effective in the rehabilitation of mining waste, particularly contaminated tailings. There is therefore a need to further investigate other plants that can be used for the phytoremediation of ash dumps in South Africa, as this approach has not yet been undertaken.

1.2 Problem statement

FA is utilized for many purposes, including in agriculture, for soil amendment. This utilization, together with FA dumping, will continue to cause groundwater contamination, surface water contamination during flooding events, and air pollution relative to dust emission via wind erosion, if preventive measures are not implemented. This is a challenge, as FA contains poisonous, toxic and persistent chemical elements that might accumulate over time and end up being translocated in food webs. The excessive blowing FA through minimal winds or rains can result in deposits on the ground and plant species which may be used in the lives of many organisms, including human beings.

1.3 Research rationale

The literature survey has revealed that chemical elements (B, Mo, Ba, As, Al, Mn, Fe, Ni, Cu, Pb, Zn, Cr and Cd) are present in high concentrations in FA and these have detrimental effects on plants, animals and human beings, as observed by the studies by Davison et al. (1974), Gitari et al. (2005, 2006, 2008 and 2010), Pandey et al. (2009), Wankar and Wadhai (2012) and Tiwari et al. (2013). These chemical elements may be present in the FA in the current study. There are several environmental issues involved with the improper disposal of FA. These include leaching of chemical elements FA from land application; land and water contamination; risk to plants and animals; health hazards; and FA dump slides accidents (Centre for Environment Education, 2007). Because of this, it is significant to conduct a study on phytoremediation of FA to clean, reduce or

stabilize such chemical elements in FA deposits. This study will focus more on phytoextraction than on other phytoremediation techniques based on the aim of the study, which is to investigate the potential of *Spinacia Oleracea L.* and *Brassica juncea* to absorb or extract chemical elements and translocate them from roots to stem, and stem to leaves. Thus the study is important, as it will focus more on cleaning the polluted site rather than reducing or stabilizing such chemical elements.

1.4 Objectives

1.4.1 Main objective

- To determine the potential of *Spinacia Oleracea L.* and *Brassica juncea* in the phytoremediation of chemical elements from coal fly ash.

1.4.2 Specific objectives

- To evaluate the morphology, chemical, mineralogy of coal fly ash and the physicochemical properties of leachates from coal fly ash on contact with water at different conditions.
- To assess the growth of *Spinacia Oleracea L.* and *Brassica juncea* in pot culture experiments using FA and evaluating temporal evolution of chemical elements in leachates from the pots.
- To evaluate the concentrations of chemical elements accumulated by roots, stems, and leaves of selected plants.
- To evaluate the phytoremediation potential of the selected plants using the bioconcentration factor (BCF) and translocation factor (TF).

1.5 Research questions

- What is the chemistry, mineralogy of coal fly ash and physicochemical properties of leachates from coal fly ash on contact with water at different conditions?
- How do plants in pot culture experiments grow in FA, and what is the temporal evolution of chemical elements in leachates from pots?
- What is the concentration of chemical elements accumulated by roots, stem and leaves of selected plants?

- Do the selected plants have the potential for phytoremediation with regards to their bioconcentration factor (BCF) and translocation factor (TF)?

1.6 Hypothesis

The plant species *Spinacia Oleracea L.* and *Brassica juncea* have the potential of phytoremediating FA by removing and translocating the chemical elements in FA.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

South Africa is highly dependent on coal for electricity production. This necessitates coal power stations to burn more coal so that enough electricity can be produced for the people of the country whose population is increasing rapidly. Eskom and Sasol are the major companies which use coal for the production of electricity and in the production of chemicals and liquid fuels in South Africa. Sasol produces about 7 million tons of FA while Eskom produces about 28 million tons (Matjie et al. 2005; Eskom fact sheet, 2009).

2.2 What is fly ash and how is it generated?

In the process of electricity generation, fly ash (FA) is produced. FA is a byproduct of burning pulverized coal in electric power generating plants (Eze et al., 2013). During combustion, mineral impurities in the coal fuse in suspension, and float out of the combustion chamber with the exhaust gases. As the fused material rises, it cools and solidifies into spherical glassy particles called FA. FA is usually collected from the flue gas by means of electrostatic precipitators, baghouses, or mechanical collection devices such as cyclones. In general, there are three types of coal-fired boiler furnaces used in the electric utility industry. They are commonly referred to as dry-bottom boilers, wet-bottom boilers, and cyclone furnaces and the most common type of coal burning furnace is the dry-bottom furnace (Office of Research, Development, and Technology, Office of Infrastructure (RDT), 2012).

FA consists of inorganic and organic components (Liu et al., 2005; Vassilev and Vassileva, 2005). The mineral components in FA have different origins, which include natural and anthropogenic, which can be classified as primary, secondary and tertiary. The primary mineral phases originate from the parent coal and do not undergo phase transformation during coal combustion; the secondary mineral phases are formed during coal combustion, while the tertiary mineral phases are formed during the handling and storage of the FA (Vassilev and Vassileva, 2005; Eze et al., 2013). All FA includes substantial amounts of silicon dioxide (SiO_2) (both amorphous and crystalline) and calcium oxide (CaO), both endemic ingredients in many coal-bearing rock strata (My planet biomass, 2013).

2.3 Transport and storage of fly ash

Many of South Africa's thermal power plants use wet process ashing to dispose ash. This includes the Sasol Synfuels and Kriel Power Station, which is a coal-fired power station owned by Eskom.

At Sasol Synfuels plant, ash from the gasifiers and steam plants are combined and treated in ash handling plants. The waste ash stream is passed over a set of screens that separates the coarse ash particles from the slurry. The coarse ash is deposited on coarse ash heaps. While the finer ash particles that has passed the screens are concentrated in a gravity thickener. The bulk of the water is removed as a clear overflow and is re-used as a carrier for ash. The bottom fraction of the thickener is the slurry of fine ash, as depicted in Figure 2.1. The slurry is pumped out into a fine ash dam (FAD) of which there are currently five at the Sasol complex (three of which have already been decommissioned) with a proposed FAD 6, for which the application is being made (Caddick et al., 2012).



Figure 2.1: Ash pumped as slurry into ash dams (Chemin, 2014).

Usually FA is transported from the power plant either damp or as slurry to a series of holding ponds where the solids are allowed to settle out of suspension. FA is usually then stockpiled and used as landfill. The Matimba Power Station produces approximately 4.8 million tons of ash annually, which is currently being disposed by means of ‘dry ashing’ approximately 3 km (three kilometres) south of the power station. From the boilers FA is transported by overland conveyors to ash disposal facilities and since the ash contains 12 % moisture, dust production is minimal (Kalele et al., 2013). Another similar power station to Matimba in South Africa is Majuba which uses dry ashing system (Eskom, 2013).

2.4 Properties of fly ash

2.4.1 Morphology

A morphological study of FA is of importance as morphology has a bearing on the leachability of heavy metals, as mentioned by Ramesh and Kozinski (2001). The two researchers found that the

presence of non-porous continuous outer surface and a dense particle interior may prevent heavy metal leachability from the FA. Most fly ashes are rich in SiO_2 , Al_2O_3 , and Fe_2O_3 , and contain significant amounts of CaO , MgO , MnO , TiO_2 , N_2O , K_3O , SO_3 , etc (ASTM C 618, 1993; Mbugua, 2012). Other researchers have reported various but similar morphologies of fresh FA (Koukouzas et al., 2009; Muriithi et al., 2009). SEM analysis shows that FA is predominantly made up of spherical particles as shown in Figure 2.2. They concluded that the spherical shape is an indication that the particles were formed under un-crowded freefall conditions and a relatively sudden cooling, which helps to maintain the spherical shape while the agglomerated nature of some particles is an indication that the particles were produced due to high temperature sintering reactions. Researchers have also noted different types of spherical particles, such as cenospheres, plerospheres and spheroids (Vassilev and Vassiliva, 1996; Chancey et al., 2010)

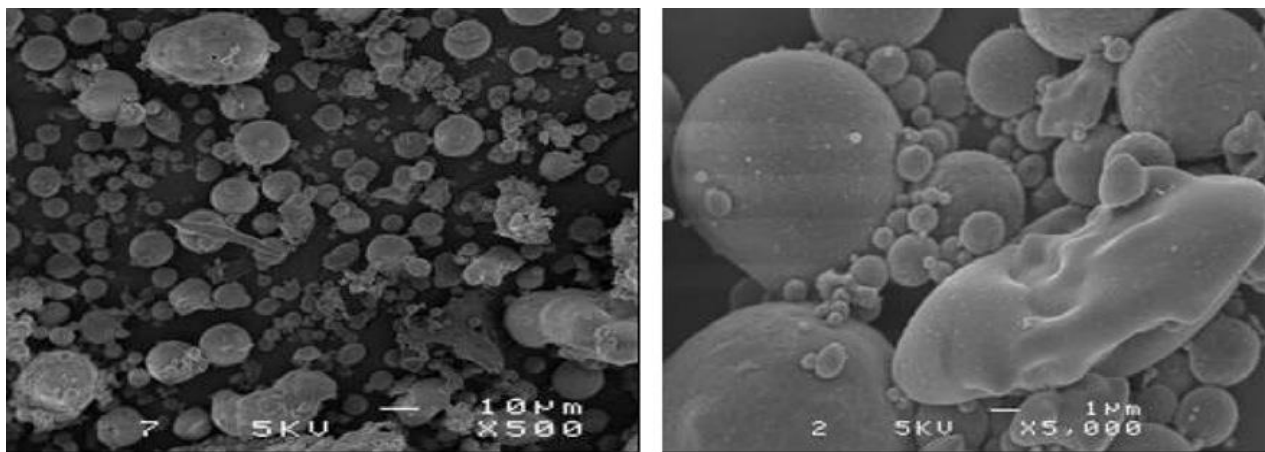


Figure 2.2: SEM micrographs of fly ash adopted from Mahlaba et al. (2011a).

2.4.2 Chemistry

FA chemistry is usually determined by the type of coal from where it is generated (Ojo, 2007; EURELECTRIC, 2000; Mueller et al., 2004). Coal fly ash is a fine-grained material which is made up of mostly spherical, glassy particles. Elemental analysis shows that the main components are silicon, aluminium and calcium. Coal fly ash is very poorly soluble in water. Heavy metals, tightly bonded to ash particles, typically make up less than 1 % of the total mass. Due to its composition and genesis, coal fly ash exhibits pozzolanic properties; it reacts with dissolved calcium hydroxide and water at normal temperature to form strength-developing minerals in a similar manner to cement (Mahlaba et al., 2011). From elemental analysis, FA is observed to be riched in silica, alumina and ferric oxides. FA is also rich in traces of Zn, Pb, Rb, Zr, Sr, Sc, S, Th, Cu, Ni, Mn, Cr, V, Rb, U, Y and Ba (Muriithi et al., 2009). The presence of these trace elements in FA makes it an environmental hazard, as the traces can leach into the ground water and the environment as a whole (Mbugua, 2012). Particle size greatly influences the chemical composition of FA and how it may affect the physical properties of the soil. The pH of FA can vary from 4.5 to 12.0, depending largely on the S content of the parent coal (Plank and Martens, 1974; Pandey et al., 2009).

According to Matjie et al. (2005), the pH values of all the leachates from gasification and steam station ashes (from Sasol) were high with $\text{pH} > 10$, while FA leachates had slightly higher pH values (average pH of 12.7), compared to the gasification ash leachates, which had an average pH value of 11.7. These results are similar to those of Jambhulkar and Juwarkar (2009), who found that FA can be extremely acidic (pH 3–4) but usually it is extremely alkaline (pH 10–12).

FA can be classified into two classes, F and C, which are based on the chemical composition of the FA. According to ASTM C 618 (1993), the chemical requirements for classifying any FA are shown in Table 2.1.

Table 2.1: ASTM standards classification of fly ash (ASTM C 618, 1993)

Properties	Fly Ash Class	
	Class F	Class C
Silicon dioxide (SiO_2) plus aluminum oxide (Al_2O_3) plus iron oxide (Fe_2O_3), min %	70.0	50.0
Sulfur trioxide (SO_3), max %	5.0	5.0
Moisture Content, max %	3.0	3.0
Loss on ignition (LOI), max %	6.0	6.0
Available alkalis, as Na_2O , max %	1.5	1.5

Class F fly ash is produced from burning higher-rank anthracite and bituminous coals, that are pozzolanic in nature (hardening when it reacts with $\text{Ca}(\text{OH})_2$ and water. FA has a low lime content, silica, alumina and iron content (Iyer, 2002). Ahmaruzzaman (2010) reported that the total calcium typically of class F ranges from 1 to 12 %, mostly in the form of calcium hydroxide, calcium sulphate, and glassy components, in combination with silica and alumina. Class F coal fly ash is generated from the combustion of higher rank coals (coal which have a low calcium content). FA (class F) possesses little or no cementitious value but in finely-divided form and in the presence of moisture, will chemically react with calcium hydroxide at ordinary temperature to form cementitious compounds. The bulk chemical composition of class F ash dictates the mineralogical constituents of ash. The most abundant phases in a class F fly ash are the glass that results from the melting of the clays and subsequent exsolution of mullite from the melt (Scheetz and Earl, 1998; Fatoba, 2010).

Class C fly ash is produced normally from lignite and sub-bituminous coals and usually contains significant amounts of calcium oxide (CaO) or lime as high as 30 to 40 % (Ahmaruzzaman, 2010). Type C ashes are generally produced from the combustion of lower rank coals and contain more calcium in their bulk chemical compositions. Due to the high calcium content in FA, the assemblage of the resulting mineralogical phase is quite different (Scheetz and Earl, 1998). This

class of FA, in addition to having pozzolanic properties, also has some cementitious properties, thus, self-hardening when reacted with water (ASTM C 618, 1993).

2.5 Disposal and Management of ash storage facilities

Basically, for disposing FA a large area is required. The cumulative accumulation of FA in the coming years will pose a serious and hazardous waste disposal problem. Thus, FA disposal creates environmental health hazards. The most widely-accepted disposal practices for FA are holding it in settling ponds, stockpiling, land filling and land disposal. Sluicing to holding ponds, where most solids settle and accumulate until they are removed for land filling or stockpiling is by far the most common method in South Africa. High-rate land application of FA is also a potential method of disposal. On disposal much of surface enriched sublimates are rapidly released into surrounding aquatic environment, including trace elements (Pandey et al., 2009).

At Sasol Synfuel, with regards to the deposition system, the fine ash is deposited to form a day wall in a series of paddocks constructed by raising low walls of previously deposited fine ash. The paddock walls are raised mechanically by means of excavators. Between 100 and 150 mm of FA, FA is deposited in the paddock, and after settling, the supernatant water is drained off towards the pool of clear ash effluent (CAE) dam. After a period of drying, the paddock walls are raised again, and the cycle repeated. A chain of paddocks is constructed around the dam perimeter to form the day wall. Each paddock is used in turn for deposition, and the balance of the material will be deposited in the body of the impoundment. The deposition cycle decides the rate of rise of the dam and is kept as long as possible to allow the previous layer of deposition to dry out by draining and evaporation before the next layer is deposited. The day wall is built during daylight hours and provides freeboard to the dam. When the fine ash delivery is not used for wall building, it is directed into the floor of the dam. The fine ash then beaches from the delivery points towards the pool (Caddick et al., 2012).

Majuba disposes approximately 3256 kilotons of ash on the ash dump per year (Eskom, 2013). Trucks and bulldozers spread the ash over the dump, which would eventually be covered with a 300 mm layer of topsoil (as and when required, depending on the amount of dust) and vegetation for management purposes. Most widely-used means of preventing the spread of metals in groundwater in many disposal facilities is the liner system, as it is less expensive. The liner or barrier design is governed by the waste classification of the ash material, as per the National Department of Environmental Affairs (DEA) waste classification regulations for the disposal of waste and regulations governing the design of waste disposal facilities (Sewmohan, 2014). The ash and waste water classified as Type 3 wastes and a Class C single composite liner is needed over the total footprint of the facility. A clay liner with drainage is usually provided, in addition to the Class C liner over the backfilled area, to separate pit backfill material from ash that will be used to fill up the remaining void in the pit to a free draining surface level. A single composite

liner consists of a clay and geomembrane layer as mentioned by Sewmohan (2014). The other control measures for managing FA facilities in South Africa include the use of dust suppress sprayers around the ash facility, which are opened every day to eliminate dust from the ash facility (Eskom, 2013).

2.6 Stabilization of ash facilities

South Africa generally stabilizes FA facilities through spraying on a daily basis with water and revegetation of these ash facilities. Stabilization technologies are designed to suppress the movement of contaminants in soils, FA, sludges, and liquids by reducing their solubility or by changing the permeability of the matrix (Erakhrumen, 2007). Plant species can be used as stabilizers, and this includes a few of them with high dust capturing efficiency, self-regenerating and can be found growing in local vegetation. Examples of such plant species are given in Table 2.2 (Chaphekar and Madav, 1999).

Table 2.2: Naturally growing plant species capable of capturing fly ash dust (Chaphekar and Madav, 1999; Pandey et al., 2009).

English Name	Botanical name	Family	Habit
Opposite-leaved fig	<i>Ficus hispida</i>	Moraceae	Tree
Gigantic swallow wort	<i>Calotropis gigantean</i>	Asclepiaceae	Shrub
Sebastian plum	<i>Cordia dichotoma</i>	Boraginaceae	Tree
Banyan tree	<i>Ficus benghalensis</i>	Moraceae	Tree
Jackfruit	<i>Artocarpus heterophyllus</i>	Moraceae	Tree
Wild sage	<i>Lantana camara</i>	Verbenaceae	Shrub
Country Mallow	<i>Abutilon indicum</i>	Malvaceae	Herb
Sweet basil	<i>Ocimum basilicum</i>	Labiatae	Herb
Jujube tree	<i>Ziziphus mauritiana</i>	Rhamnaceae	Tree
African Tulip	<i>Spathodea campanulata</i>	Bignoniaceae	Tree
Coral Jasmine	<i>Nyctanthes arbor-tristis</i>	Oleaceae	Shrub
Fig	<i>Ficus glomerata</i>	Moraceae	Tree
Vasan vel	<i>Cocculus hirsutus</i>	Menispermaceae	Shrub
French mulberry of Ghats	<i>Callicarpa tomentosa</i>	Verbenaceae	Shrub
Indian tulip tree	<i>Thespesia populnea</i>	Malvaceae	Tree
Bastard cedar	<i>Guazuma ulmifolia</i>	Sterculiaceae	Tree
Monkey face tree	<i>Mallotus philippinensis</i>	Euphorbiaceae	Tree
Teak	<i>Tectona grandis</i>	Verbenaceae	Tree
Gunpowder tree	<i>Trema orientalis</i>	Ulmaceae	Tree
-	<i>Heterophragma quadriloculare</i>	Bignoniaceae	Tree

2.7 Uses of fly ash

2.7.1 Beneficial utilization of fly ash

a) Soil amendment

Besides the use of FA for construction purposes, soil amendments are the most extensively studied reutilization option. FA will change some soil properties. Mixed soil generally experiences a decreased bulk density, decreased moduli of rupture (index of the general strength), increased water holding capacities, and an increased hydraulic conductivity initially, which is later followed by a lower hydraulic conductivity (El-Mogazi et al., 1988). Chang et al. (1977) observed that the increased water holding capacity did not necessarily increase the water available to vegetation. FA has a cementing effect on the soil which contributes to the reduced hydraulic conductivity.

Different types of FA can change the pH of the soil either up or down. The pH influences the mobility of toxic elements (Boron) and many necessary nutrients. Therefore, the soil and FA pH could determine the available concentration of these elements (Cheremisinoff, 1988). Depending on the initial state of the soil, the results could be quite desirable. The extent of physical and chemical changes is dependent on the amount of FA added. Relevant studies have normally added between 5 and 50 % FA. It has generally been found that more positive results are realized at lower (5 %) FA additions (Chang et al. 1977; El-Mogazi et al., 1988; Wong and Wong, 1989). Unfortunately, this small volume will contribute, but will not significantly alleviate, the disposal problem for FA (Openshaw, 1992). The amendment with FA in various concentrations (w/w) adds synergistic action for uptake of micro- and macro- nutrients (Wankar and Wadhai, 2012).

b) Improvement of degraded lands by FA

Pandey and Singh (2010) found that sometimes soil loses fertility and quality due to environmental causes and unmanaged exploitation by human. For increasing soil productivity, waste FA could be used as an exploitable resource for the management of degraded soils, because FA possesses several similarities, like soil and contains essential micro-nutrients (Fe, Mn, Zn, Cu, Co, B and Mo) and macro-nutrients (P, K, Ca, Mg and S). FA also can be used in the reclamation of wastelands (sodic soil, acidic soil and mine spoil) as FA possesses many of the functional properties of lime and gypsum (Shainberg et al., 1989; Kumar and Singh, 2003). Pandey et al. (2009) also suggested the use of FA in the reclamation of wastelands.

c) Other uses

FA may be used as an effective soil stabilizer and in positive correlation, with soil microbial flora, it enhances soil fertility and plant health. FA also might be used as a plant growth media additive in horticulture, floriculture and forestry. Beneficial utilization of FA from Thermal Power Plants (TPPs) shows marked effect in agricultural field and it proves to be a sustainable amendment agent for agriculture (Wankar and Wadhai, 2012). Other uses include the manufacturing asbestos-fly ash based sheets; use as a structural fill material; for manufacture of light weight aggregates; use as a filter for low-grade refractory bricks; for recovery of cenospheres and rare metals; back-filling of abandoned mines; lining of irrigation canals; and used as a filter in paints, plastics and rubber (Centre for Environment Education, 2007).

2.7.2 Application of coal FA in environmental remediation

Several studies including Gitari et al. (2008), Madzivire et al. (2010), Doucet et al. (2014) and Muriithi et al. (2009) have been undertaken in South Africa with regards to the application of FA in environmental remediation. Studies by Gitari et al. (2008) reported that FA was used in the treatment of Acid Mine Drainage (AMD) by using FA as a liming agent. Here, the treatment of AMD with FA was observed to be site-specific, and thus, the effectiveness of the treatment process will depend on the quality of the FA and AMD. In another study by Madzivire et al. (2010) FA was applied to circumneutral mine waters for the removal of sulphates as gypsum and ettringite. It was concluded that sulphate can be removed from circumneutral mine water by treating the soil with FA to a pH greater than 11, followed by seeding with gypsum and adding amorphous $\text{Al}(\text{OH})_3$ with subsequently precipitation of sulphate as ettringite. In the study by Doucet et al. (2014), FA slurry was injected in deep saline formations for improved CO_2 confinement and since this was a theoretical concept, it was mentioned that it may be conceivable, while numerous challenges need to be overcome before the proposed scenario can become technologically feasible for the safe, long-term geo-sequestration of CO_2 in such environments. Another study was conducted by Muriithi et al. (2009) on brine remediation using FA and accelerated carbonation and it was concluded that most of the brine species, with the exception of B, V, Mo and Cl are stabilized within the ash matrix and that enhanced carbonation leads to co-precipitation of some of the traces present in brine, hence producing cleaner effluents.

2.8 Impacts of fly ash

FA contains a range of toxic constituents that are known to leach, leak, or spill out of coal ash disposal sites and adversely affect human and environmental health.

2.8.1 Environmental impacts of FA and FA ash storage dump

a) Leaching from land application of FA

Coal ash particles are essentially insoluble aluminosilicate glasses. However, trace substances on the ash surface may be soluble. The dissolved elements or ash particles could potentially end up in a drinking water source, such as groundwater, a river or a lake. Studies by the Centre for Environment Education (2007) have shown that even where some leaching of trace elements from FA has occurred, its effects did not pose any public health risk. Only groundwater wells located directly beneath the ash deposit showed elevated concentration of some elements. The quantity of elements that would be available for leaching in an aqueous media will depend on the fixation of these elements on the ash particles, pH of the ash-aqueous medium, ash source and leaching time. In general, under acidic conditions, the rate and quantity of leaching is higher (Pandey et al. 2009).

b) Water contamination

The improperly disposed FA slurry can mix with the open water bodies and change its colour from greenish to silvery grey. The FA can overflow from the ponds and may find its way into drains, which in turn can result into the clogging of drains. During the rainy season, FA laden with water may leave behind a blanket of FA on a large tract of agricultural land. FA slurry makes the soil less porous and impedes aeration as a result of which land turns fallow. The ash also finds its way to agricultural land through the use of ash laden water for irrigation. The disposal of FA in improperly lined FA ponds may cause leaching of chemical elements to the ground water source. Hence FA ponds should be lined with an impermeable barrier, i.e. clay layer or synthetic liner to prevent the leaching (Centre for Environment Education, 2007).

Pandey et al. (2009) observed the effect of FA on groundwater as a function of physicochemical characteristics of ash and hydrogeologic and climatic conditions of the disposal site. Erosion and leaching of heavy metals such as Zn, Cu and Pb were found in high concentration in tube well water located in the vicinity of an ash pond in Orissa, while Cu, Mn, Pb and Zn were the major contaminants in groundwater (Praharaj et al., 2002). Weathered FA deposits cause more groundwater contamination due to the presence of higher levels of soluble salts. Groundwater quality due to application of FA remained unaffected with respect to radionuclide contamination (IIT Kharagpur, 1999).

c) Risk to soil, plants and animals

Overall, FA poses a minimal risk to plants and animals. As noted previously, it consists chiefly of common compounds found in the earth. For accepted practices of disposal, transportation, and use, effects from trace metals leaching and inhalation of ash are both localized and minimal, as shown

in human health studies. FA can adversely affect aquatic life if it is present in sufficient quantities, mostly because of increased turbidity of the water (which inhibits plant growth), gill clogging and increased alkalinity. FA has been extensively studied as soil amendments; researchers have found that bottom ash can improve soil drainage and limited dose of FA can be used to provide nutrients for plant growth. Used alone, however, FA can have drawbacks; aggregate instability, a tendency to erode, nitrogen deficiencies, boron phytotoxicity and low cation nutrient retention capacity. Studies have also found that trace metals in FA can be absorbed by vegetation. Since plants grown on FA can absorb trace elements, care is needed in determining appropriate application rate when ash is used as a soil amendment. A proper soil/ash mixture has been shown to be beneficial for crops (Centre for Environment Education, 2007).

In soil, heavy metals cause toxicological effects on soil microbes, which may lead to a decrease in their numbers and activities. High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Khan et al., 2010).

d) Air pollution

FA is a serious source of air pollution since it remains air-borne for a long period and causes health hazards for the local masses. It causes irritation to eyes, skin, nose, throat, and respiratory tract. Repeated inhalation of FA dust containing crystalline silica can cause bronchitis, silicosis (scarring of the lung), and lung cancer. Inhalation of high levels of FA dust may result in severe inflammation of the small airways of the lung and asthma-like symptoms. Besides being a health hazard, FA corrodes exposed metallic structure in its vicinity and degrades environment before it may also increase the risk of scleroderma (Davison et al., 1974; Finkelman et al., 2000). The dust of FA can end up contaminating surface water and land caused by wind. Hence, these are risks and need to be attended soon enough and some strategies should be adopted to check air pollution around coal-fired thermal power plants, including plantations of tree species with high dust capturing efficiency (Pandey et al. 2009).

2.8.2 Health impacts of fly ash toxicants

As with many substances, the recycling and disposal of FA raises concerns about its human health and ecological effects. Most health-related questions about FA revolve around the inhalation of its particles, ingestion of its particles or dissolved trace elements or direct skin contact due to wind erosion of dry FA. FA may cause severe health problems like asthmatic disorders, eye and skin problems. It is a serious source of air pollution since it remains air-borne for a long period and causes health hazards. Fugitive dust and heavy metal contamination in the ground water are the

major problems (Centre for Environment Education, 2007). Table 2.3 summarizes the impacts of some metal species on human beings and animals.

Table 2.3: Impacts on the animals and human body that can be caused by exposure to some of the most common toxic contaminants in fly ash (adopted from Ali et al., 2013).

Heavy metal	Harmful effects	References
As	As (as arsenate) is an analogue of phosphate and thus interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis	Tripathi et al. (2007)
Cd	Carcinogenic, mutagenic, and teratogenic; endocrine disruptor; interferes with calcium regulation in biological systems; causes renal failure and chronic anemia	Degraeve (1981), Salem et al. (2000), and Awofolu (2005)
Cr	Causes hair loss	Salem et al. (2000)
Cu	Elevated levels have been found to cause brain and kidney damage, liver cirrhosis and chronic anemia, stomach and intestinal irritation	Salem et al. (2000), Wuana and Okieimen (2011)
Ni	Allergic dermatitis known as nickel itch; inhalation can cause cancer of the lungs, nose, and sinuses; cancers of the throat and stomach have also been attributed to its inhalation; hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, and hepatotoxic; causes hair loss	Salem et al. (2000), Khan et al. (2007), Das et al. (2008), Duda-Chodak and Baszczyk (2008), and Mishra et al. (2010)
Pb	Its poisoning causes problems in children such as impaired development, reduced intelligence, loss of shortterm memory, learning disabilities and coordination problems; causes renal failure; increased risk for development of cardiovascular disease	Salem et al. (2000), Padmavathamma and Li (2007), Wuana and Okieimen (2011) and Iqbal (2012)
Zn	Over dosage can cause dizziness and fatigue	Hess and Schmid (2002)
B	Inhalation of boron can lead to over the short-term to eye, nose, and throat irritation. Ingestion of large amounts, however can cause damage to the testes, intestines, liver, kidneys and brain, and eventually lead to death	Gottlieb et al. (2010)
Mo	In animals it causes slowed growth, low birth weight and infertility	Gottlieb et al. (2010)

2.9 Revegetation of ash dumps

FA management is becoming more challenging due to strict environmental regulations of its utilization and disposal, which may affect soil fertility, soil quality and soil biota and, eventually,

groundwater quality. To overcome this environmental problem, the revegetation of FA facilities with tolerant plants is one of the cheapest alternatives. This may reduce atmospheric pollution, leaching of toxic metals and develop a bioaesthetic pleasant environment for local inhabitants (Rai et al., 2004). Various categories of plants can be grown on FA. However, members of Leguminosae seem to be advantageous for revegetating FA cover. This is because Leguminosae has a *Rhizobium* symbiont for nitrogen input, which exerts a metal detoxification mechanism such as phytochelatin (PC) synthesis. Leguminosae also demonstrate the possibility of being suitable for use in FA-contaminated environments. With non-leguminous plant species, biofertilisers comprising free-living nitrogen-fixing organisms may be helpful. Plants that yield edible oils, because metals are lipid-insoluble, may be suitable species to revegetate FA. In metal-contaminated soil from FA contamination, bioremediation processes using plants that are metal-tolerant can help the subsequent ecosystem and enable biological equilibrium, which can then help the successive phytocolonisation of economically important plants (Pandey et al., 2009). Pandey et al. (2009) mentioned that the successful revegetation of alkaline FA is limited by phytotoxicity due to boron and aluminum; restriction of root growth due to the fine particle size of the ash; nutrient deficiency; and reduction in the number of free-living and symbiotic N₂-fixing microorganisms and the physico-chemical conditions of the ash (Adriano et al., 1980).

For developing effective revegetation on FA landfills/dykes/lagoons, some FA-tolerant species are recommended by researchers. These include species such as *Calotropis procera*, *Cassia tora*, *Chenopodium album*, *Sida cardifolia*, *Blumea lacera* (Gupta and Sinha, 2008); *Prosopis juliflora* (Rai et al., 2004); *Acacia auriculiformis*, *L. leucocephala* (Cheung et al., 2000); *Cicer arietinum* (Gupta et al., 2004), *C. surattensis* (Vajpayee et al., 2000); *Sesbania cannabina* (Sinha and Gupta, 2005); *Cassia siamea* (Tripathi et al., 2004); *Agropyron elongatum*, *Festuca arundinacea*, *Melilotus officinalis* (Mulhern et al., 1989); *Liquidambar styraciflua*, *Platanus occidentalis* (Carlson and Adriano, 1991); *Mesembryanthemum nodiflorum*, *Enchylaena tomentosa*, *Halosarcia halocnemoides*, *Halosarcia pergranulata* (Jusaitis and Pillman, 1997); *M. officinalis*, *Melilotus alba* (Gutenmann et al., 1976).

2.10 Bioremediation and the science involved

Bioremediation is the use of biological interventions of biodiversity for mitigation (and wherever possible, complete elimination) of the noxious effects caused by environmental pollutants in a given site. Bioremediation has been successfully applied for cleaning up of soil, surface water, groundwater, sediments and ecosystem restoration. It has been unequivocally demonstrated that a number of xenobiotics, including nitro-glycerine (explosive), can be cleaned up through bioremediation. Bioremediation is generally considered to include natural attenuation (little or no human action), bio-stimulation or bio-augmentation, the deliberate addition of natural or engineered micro-organisms to accelerate the desired catalytic capabilities. Thus bioremediation, phytoremediation and rhizoremediation contribute significantly to the fate of hazardous waste and

can be used to remove these unwanted compounds from the biosphere (Ma et al., 2011; Schroeder and Schwitzguebel, 2004). Phytoremediation is a technique under bioremediation and actually refers to a diverse collection of plant-based technologies that use either naturally-occurring or genetically-engineered plants for cleaning contaminated environments (Prasad, 2011). Techniques of phytoremediation include phytoextraction (or phytoaccumulation), phytofiltration, phytostabilization, phytovolatilization, and phytodegradation (Alkorta et al., 2004).

Phytoremediation groundcovers have been widely applied to soils impacted by recalcitrant compounds such as PAHs, PCBs, and other persistent organic pollutants that are typically less mobile, soluble, biodegradable, and bioavailable. Furthermore, these groundcover systems can also be used as certain types of landfill covers that also promote the degradation of the underlying waste. These have been referred to as bioreactor landfills. Finally, phytoremediation groundcovers have been used to extract specific inorganic contaminants such as metals, salts, and radionuclides in concentrations higher than what existed in the soil (Prasad, 2011).

2.10.1 Phytostabilization

Phytostabilization refers to the holding of contaminated soils and sediments in place by vegetation, and immobilizing toxic contaminants in soils. The establishment of rooted vegetation prevents windblown dust, an important pathway for human exposure at hazardous waste sites. Hydraulic control is possible, in some cases, due to the large volume of water that transpires through plants, which prevents migration of leachate towards groundwater or receiving waters. Phytostabilization is especially applicable for metal contaminants at waste sites where the best alternative is often to hold contaminants in place. Metals do not ultimately degrade, so capturing them *in situ* is the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas, where a large-scale removal action or other *in situ* remediation is not feasible (Prasad, 2011).

2.10.2 Phytoextraction

Phytoextraction refers to the ability of plants to take up contaminants into the roots and translocate them to the above ground shoots or leaves (Prasad, 2011). For contaminants (heavy metals) to be extracted by plants, the constituent must be dissolved in soil water and come into contact with the plant roots through transpiration stream. Alternatively, the uptake may occur through vapour adsorption onto the organic root membrane in the vadose zone. Once adsorbed, the contaminant may dissolve into the transpiration water or be actively taken up through plant transport mechanisms.

Metal translocation to shoots is a crucial biochemical process and is desirable in effective phytoextraction because the harvest of root biomass is generally not feasible (Zacchini et al., 2009; Tangahu et al., 2011).

Table 2.4: Summary of the different techniques of phytoremediation (adopted from Ali et al., 2013)

Technique	Description
Phytoextraction	Accumulation of pollutants in harvestable biomass i.e., shoots
Phytofiltration	Sequestration of pollutants from contaminated waters by plants
Phytostabilization	Limiting the mobility and bioavailability of pollutants in soil by plant roots
Phytovolatilization	Conversion of pollutants to volatile form and their subsequent release to the atmosphere
Phytodegradation	Degradation of organic xenobiotics by plant enzymes within plant tissues
Rhizodegradation	Degradation of organic xenobiotics in the rhizosphere by rhizospheric microorganisms
Phytodesalination	Removal of excess salts from saline soils by halophytes

2.10.3 Characteristics of plants suitable for phytoremediation (phytoextraction)

As phytoremediation (especially phytoextraction) is a plant-based technology, the success of phytoextraction is essentially dependent upon proper plant selection (Ali et al., 2013). Plants suitable for phytoextraction should ideally have the following characteristics, as mentioned in the study by Mejare and Bulow, 2001; Tong et al., 2004; Adesodun et al., 2010; Sakakibara et al., 2011; Shabani and Sayadi, 2012; Ali et al., 2013.

- High growth rate.
- Production of more above-ground biomass.
- Widely-distributed and highly branched root system.
- More accumulation of the target heavy metals from soil.
- Translocation of the accumulated heavy metals from roots to shoots.
- Tolerance to the toxic effects of the target heavy metals.
- Good adaptation to prevailing environmental and climatic conditions.
- Resistance to pathogens and pests.
- Easy cultivation and harvest.
- Repulsion to herbivores to avoid food chain contamination

The metal translocation to shoots is an important biochemical process and is advantageous in effective phytoextraction because the harvest of root biomass is generally not feasible in many instances (Zacchini et al., 2009; Tangahu et al., 2011; Ali et al., 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This chapter focuses on materials and methods which were used in the generation of the data. It also includes a brief description of plants chosen for the evaluation of chemical elements bioaccumulation and phytoremediation potential.

3.2 Plant species description

Plants tend to be different or similar in many or few characteristics. It is important to know the plant species before using it for phytoremediation. This is important as one should have information about where, when and how those plants grow, their maximum height and biomass production, its scientific name and also the family it comes from. The descriptions for *Brassica juncea* and *Spinacia Oleracea L* are shown in Table 3.1 while Figure 3.1 and 3.2 show how they look like.



Figure 3.1: Spinach (*Spinacia Oleracea L*)

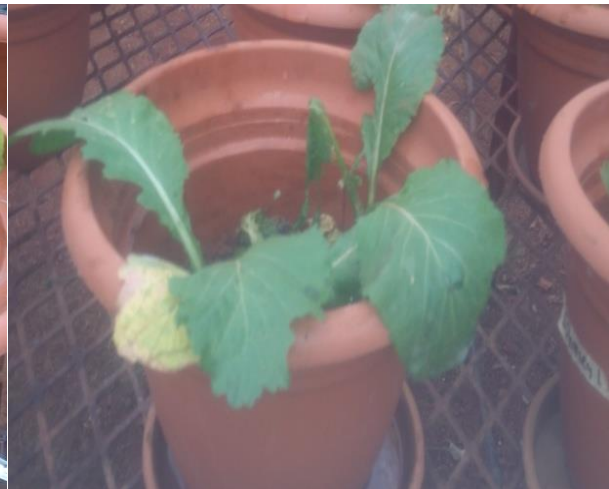


Figure 3.2: Indian mustard (*Brassica juncea*)

Table 3.1: Plant descriptions for *Spinacia Oleracea L.* and *Brassica juncea* (Learn to grow, 2014; Masley, 2014).

	<i>Spinacia Oleracea L.</i>	<i>Brassica juncea</i>
Scientific name	<i>Spinacia Oleracea L.</i>	<i>Brassica juncea</i>
Common name	Spinach	Indian mustard
Family	Amaranthaceae	Brassicaceae
Native to	South west Asia	Asia
Height	8-10 inches (20-25 cm)	Up to 3 feet tall (91 cm)
Harvest time	30-46 days after sowing	45 days after sowing
Environment	Cool-season crop that should be planted in early spring and tolerates partial shade	Does best in full sun, and does fine in partial shade
Soil	Does best in loamy well-drained garden soil rich in organic matter	Sandy to rich and well-drained soil
Germination	6-25 days	6-20 days
Growth habit	Fast growing herb/forb	Fast growing herb/forb
Duration	Annual	Annual, perennial

3.3 Experimental Protocol

Figure 3.3 shows the flow diagram of the methods followed for the current study. This starts on the collection of coal FA, then characterization of FA, pot culture experiments using FA and soil mixtures, analysis of leachates and plant parts, then calculation of BCF and TF and discussion of all the results, recommendations and conclusions.

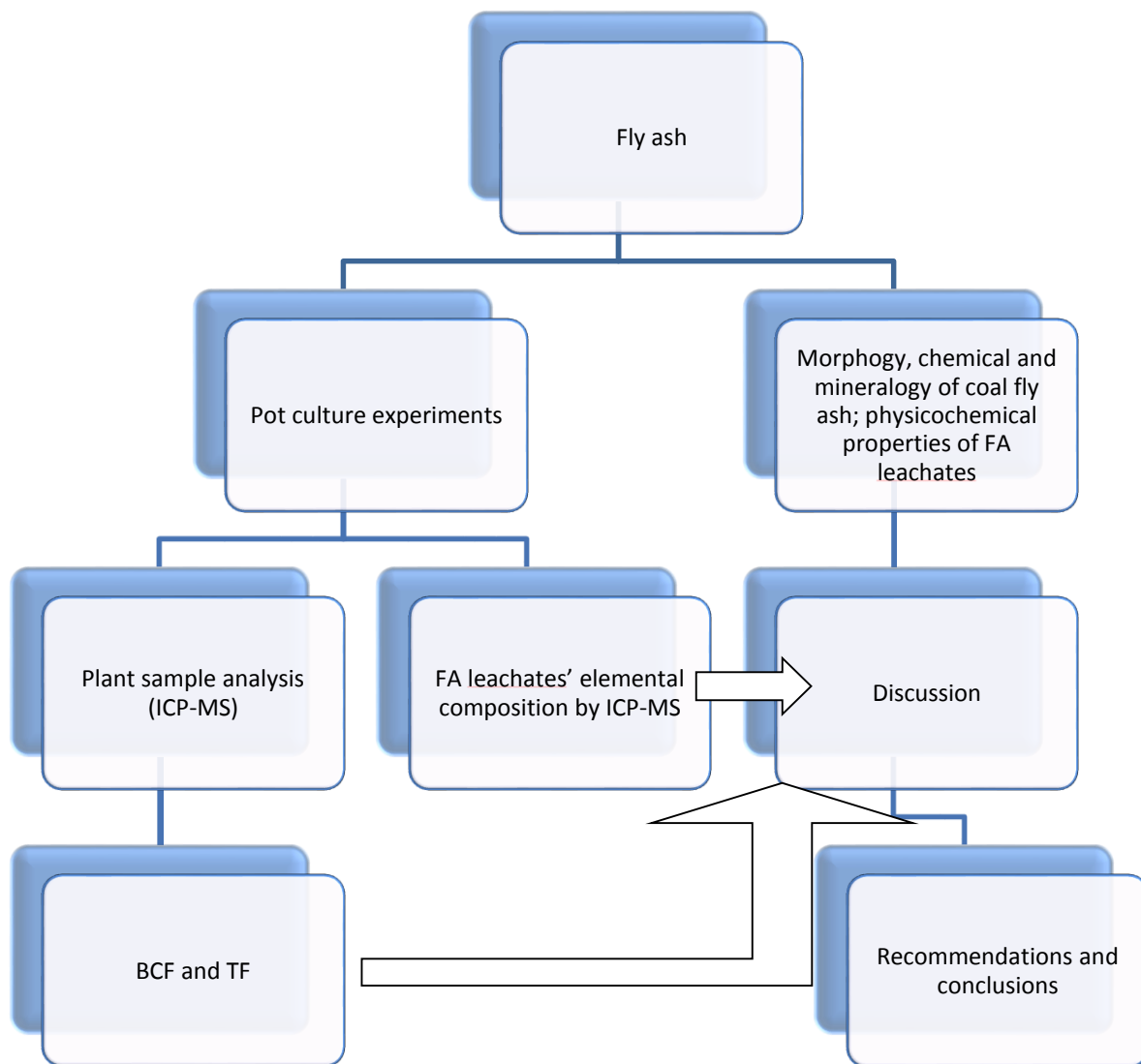


Figure 3.3: Flow diagram showing steps of the experimental protocol.

3.4 Evaluation of the morphology, chemical and mineralogical properties of fly ash

3.4.1 Chemical parameters of fly ash

Coal FA samples were collected from Grootvlei Power Station. The leaching test was done using the European pre-standard leaching test (European Committee for Standardization, 1998); which is a one-step batch test at an L/S ratio. FA was mixed sufficiently on a clean paper and about 500g of the homogenized samples were oven-dried at 60 °C for 2 hours. Samples were then ground to pass through a 2 µm stainless steel sieve. For the determination of pH and EC, a ratio of 1:4 (1 L, 250 g) deionized water to FA was used. The samples were shaken for 24 hours at room temperature (27 °C) in an end-over-end mechanical shaker operating at 90 rpm. The samples were then filtered and then pH and EC was measured by a CRISON multimeter port MM40 probe and recorded.

3.4.2 Scanning Electron Microscope (SEM)

The morphology of the FA was determined by scanning the FA powder with Scanning Electron Microscope (SEM) to examine the pore size and shape at the surface. Practically, the samples of FA were prepared by coating an aluminium SEM stub with carbon glue. A small amount of the sample was sprinkled onto the glue and the excess gently blown away with compressed air. The stubs were then sputter coated with gold palladium alloy to make them conductive. The SEM that was used for analysis was a Zeiss 1450 fully analytical scanning electron microscope. The images were taken at 5 kV, probe current of 400 pA and working distance of 14 mm, using the secondary detector.

3.4.3 X-ray fluorescence (XRF) analysis

The FA and soil samples were milled in a tungsten-carbide milling pot to achieve particle sizes <75 micron. The samples were dried at 100 °C and roasted at 1000 °C to determine Loss On Ignition (LOI) values. Approximately 1.00 g sample was mixed with 6.00 g Lithiumtetraborate flux and fused at 1050 °C to make a stable fused glass bead. The Thermo Fisher ARL Perform'X Sequential XRF with OXSAS software was used for analysis. Blanks and certified reference materials were analysed with each batch of sample. The elemental analysis was reported in terms of the weight % of the oxides of the preferred elements such as SiO₂, Al₂O₃, Fe₂O₃, CaO, MgO and TiO₂. Both coal FA and soil were analysed by XRF Spectroscopy and Van der Merve et al. (2011) also used similar method.

3.4.4 X-ray diffraction (XRD) analysis

XRD is used to determine the mineralogical composition of raw material components as well as for qualitative and quantitative phase analysis of multiphase mixtures (Nayak and Singh, 2007). The powdered FA was used for XRD analysis to investigate the mineralogy of FA. The samples were prepared according to the standardized Panalytical backloading system, which provides nearly random distribution of the particles. They were analysed using a PANalytical X'Pert Pro powder diffractometer in θ - θ configuration with an X'Celerator detector and variable divergence- and fixed receiving slits with Fe filtered Co-K α radiation ($\lambda=1.789 \text{ \AA}$). The phases were identified using X'Pert Highscore plus software. The relative phase amounts (weight %) were estimated using the Rietveld method (Autoquan Program). Errors are on the 3 sigma level; and the amorphous phases, if present were not taken into consideration in the quantification. Only coal FA samples were analysed by XRD.

3.5 Pot culture experiments

Pot culture experiments were conducted for four successive months (from April to July 2015). The dry coal FA used was collected from Grootvlei Power Station which is one of ESKOM's power stations. Good and viable seeds of *Spinacia Oleracea L* and *Brassica juncea* were purchased for the experiments. A total of 35 plastic pots of 16 cm long and 9 cm diameter, which were used in the experiment, were thoroughly cleaned and filled up with different compositions of soil and coal FA. Figure 3.4 shows how the pots were set-up in pot culture experiments.

There are few studies which had similar pot culture experiments but not exactly the same as the current study. This includes the study by Lai et al. (2008) and Subhashini et al. (2013) where only soil was used as a growth media for uptake of chemical elements by plant species. Also the study by Jamil et al. (2009) where FA and garden soil were introduced as growth media but with an addition of EDTA chelation agent in different compositions into different amounts of FA. All of these studies are similar but does not have similar aims as the current study; for instance, they do not investigate the leachates which comes from the pot culture experiments with time while the current study is covering that aspect. So the current study is a bit unique with respect to the pot culture experiments and bringing about new knowledge.

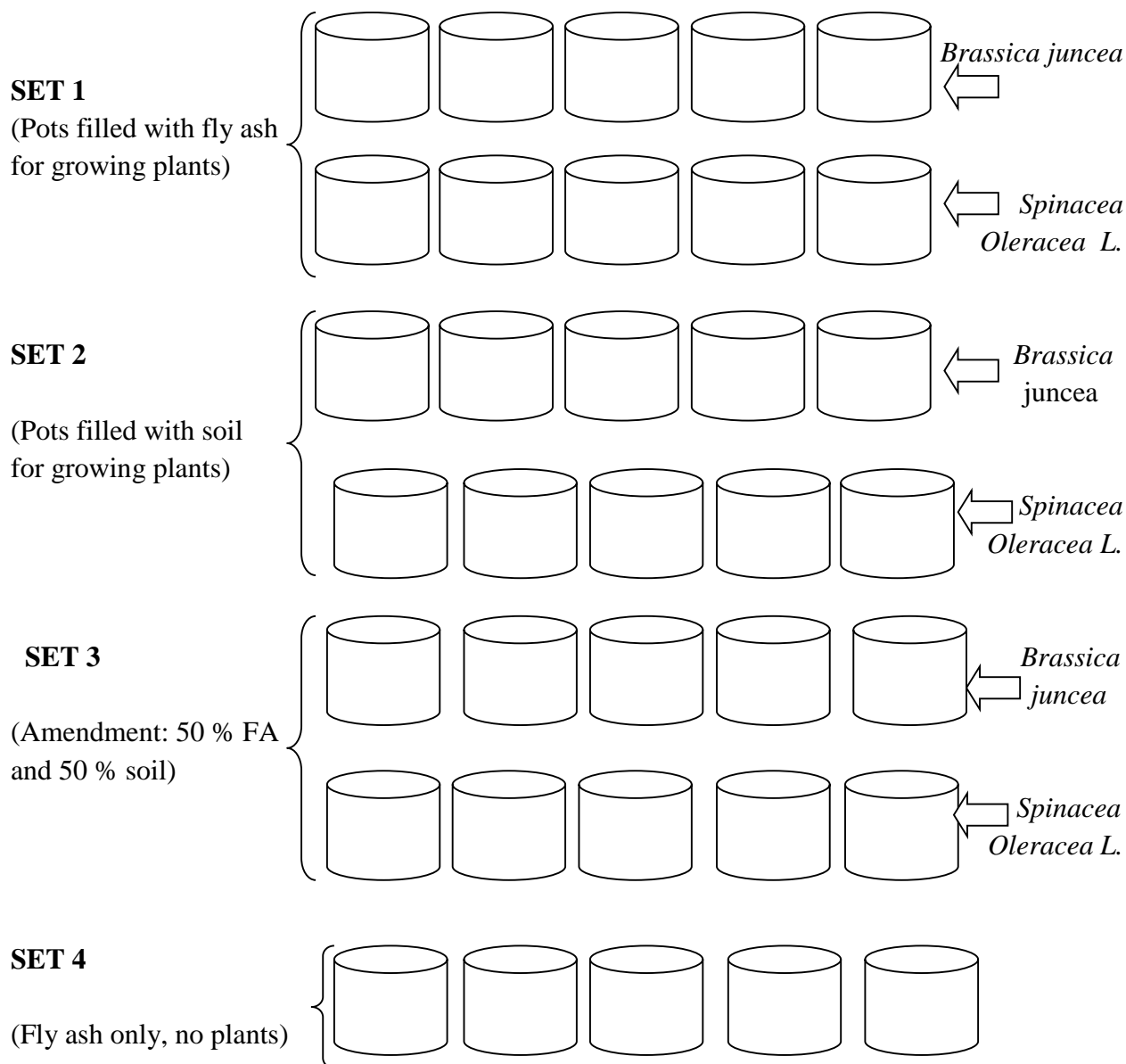


Figure 3.4: Illustration of set up of pots (what the pots were filled with and which species were grown in each pot).

Thus, there were 35 pots with four sets of compositions taken; first with 10 replica pots filled with FA only, where 5 pots were for growing *Spinacia Oleracea L* and the other 5 pots for *Brassica juncea*; for set two , 10 replica pots were filled with soil (5 for growing *Spinacia Oleracea L* and 5 were used for growing *Brassica juncea*); for set three, 10 replica pots were filled with a mix (amendment) of 50 % FA and 50 % of soil with similar growth of plant species as set two; the last five pots for set four were filled with coal fly ash only, without any plants grown as a control for leachate analysis, as they were irrigated as well. All this is illustrated in Figure 3.4

Other materials which were used include an auger, garden gloves and a watering can for the irrigation of plants. The physicochemical parameters for the soil used in the experiments as a control were assessed before set-up of the experiments. Before the soil was used for growing plants, it was assessed for pH, EC and temperature, as it might affect the growth of plants. These parameters were measured using a CRISON multimeter port MM40 probe.

3.5.1 Sowing of seeds and transplantation

Seeds of *Spinacia Oleracea L.* and *Brassica juncea* were sown 2.54-10.16 cm apart and about 1.27 cm deep in a seed tray filled with agro-mix and the planting date was noted. This was done in May 2015 for the purpose of winter harvest. The soil and FA were mulched to reduce or eliminate weeds and firmed gently and watered with enough tap water using a watering can to moisten the soil. This was done every morning until the plants were harvested or as per requirements, even after germination. Seeds germinated within 5 days after sowing and the seedlings were ready for transplantation to pots soon after having more than two larger leaves and tall enough, this can be seen in Figure 3.5. It took about 3 weeks and two days for seedlings to become ready for transplantation to the pots.



Figure 3.5: Plants ready for transplantation.

Seedlings in Figure 3.5 were transplanted with some of the agro-mix from the seed tray for better adaptation to the new environment after transplantation. Four seedlings were transplanted to each pot and leaves were thinned when they touched for better space of growth in each pot. Each pot was labeled in terms of FA or soil and plant species (*Spinacia Oleracea L.* or *Brassica juncea*). All pots were put on pot saucers for collecting leachates from the pots on drainage from pots. This

was done to determine the chemical elements available in the leachates and consequently available to the roots of the plants.

The pots were kept in a nursery rather than a growth chamber/temperature controlled Green House to mimic what really happens in the environment. The pots were monitored, including the heights of plants. These were noted every 10 days after germination and during harvesting. Heights were also observed in the study by Jamil et al. (2009) to investigate growth rate of plants.

3.5.2 Harvesting procedure

After 46 days, plant species were expected to have reached maturity and ready to be harvested as was expected in the study by Masley (2014). First harvest was that of seedlings ready for transplantation, second harvest was done after 46 days of transplantation, while the final harvest was done after another 46 days. Thus, the plants were harvested 3 times. Two random plant samples were collected from each pot (in every harvest), uprooted or dug carefully with their roots intact, to ensure adequate and representative material for analytical testing. Under good conditions plants grow rapidly and leaves are harvestable after 3 weeks, when plants have developed 6–8 fully expanded leaves. With the current study, it was going to take longer than 3 weeks so that plants can have more than 4 leaves since after week 3; seedlings were having only 4 leaves. Seedlings ended up being harvested (first harvest) having at least 4 small leaves. With the second and third harvest, 46 days' period was allowed to pass then harvest was done for each harvest. This is similar with the study by Lai et al. (2008)

3.5.3 Challenges experienced in the growth of the plants and how they were solved

There were challenges with regards to the germination of the seedlings as it had to be repeated twice. Seedlings were growing very slow, being stunted and having symptoms of lacking essential nutrients (nutrient deficiency). The leaves were becoming chlorotic and necrotic, thus yellowing of plant tissues and dying with brown tissues, the images of these is shown in Figure 3.6.

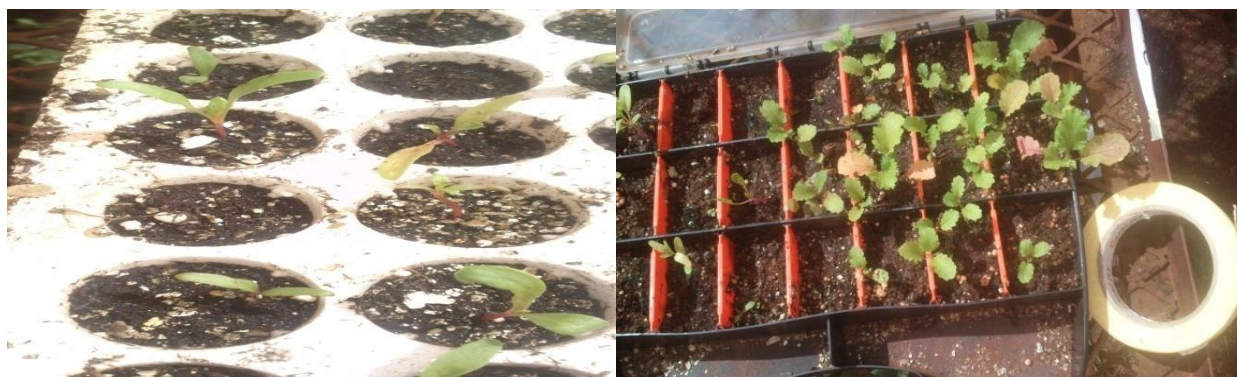


Figure 3.6: Seedlings showing nutrient deficiency after four weeks of germination.

The nutrient deficiency problem was solved by sowing new seeds to start the growth afresh. The new seedlings survived better.

In the current study there were plant growth problems in the pot culture experiments, especially with *Brassica juncea*. This led to plants being harvested having at least 4 small leaves as 46 days was over. The *S. Oleracea L* species were growing very slow being stunted but survived better than *Brassica juncea* species; these challenges in plants can be seen at Figure 3.7. These plants were harvested the way they were and analysed.



Figure 3.7: Symptoms of chlorosis, necrosis, nutrient deficiency and death of *Brassica juncea* and *Spinacea Oleracea L*. being stunted in FA and soil growth media.

The chemical properties of soils and FA used for pot culture experiments was assessed and analysed with XRF Spectroscopy for the concentrations of the remaining chemical elements in the substrates. This was done after pot culture experiments were finished, when all plants have been harvested.

3.6 Preparation of plants samples for analysis and biomass estimation

Plant samples were repeatedly washed with deionised water to remove the adhering FA, soil and other contaminants. Plants were then made moisture free by wiping them with a paper towel. As samples of the whole plant was collected, the plants were cut to separate leaves and roots from each other in order to determine chemical elements' concentrations of each part of the plant and for the estimation of biomass. Samples were weighed quickly to obtain fresh weight (FW) in grams. The same plant parts were air-dried in the open at room temperature and weighed to obtain the dry weight (DW) in grams and results used to estimate biomass. The dried samples were ground into a powder fine enough to pass through a 2 μm stainless steel sieve using mortar and pestle ready for analysis. For the estimation of selected chemical elements and bioaccumulation potential, well-mixed powdered plant samples of 0.5 g were weighed into a beaker for digestion through aqua regia ($\text{HCL}:\text{HNO}_3 = 3:1$ (v/v)) to near dryness or until a white-colored solution was formed. The acid digestion was carried out on a hotplate. After complete digestion of samples, MilliQ water (18.2 $\text{M}\Omega/\text{cm}$) was added and left to cool down. The samples were filtered, the volume increased to 100 ml with MilliQ water. These filtered solutions were used to determine the chemical elements concentrations. Blanks and internal standards were included for quality assurance during chemical elements analysis of plant parts samples. The concentration of different chemical elements in plant parts was analysed using ICP-MS.

3.7 Analysis of chlorophyll-a, chlorophyll-b and carotenoids content

Leaves of *Brassica juncea* and *Spinacea Oleracea L.* were accurately weighted and 0.5 g of each fresh plant leaf sample was taken. This was sufficiently homogenized in a blender with 10 ml of the extracting solvent (90 % ethanol). The homogenized sample mixture was centrifuged at 10,000 rpm for 15min at 40 $^{\circ}\text{C}$. The supernatant was separated and 0.5 ml of it was mixed with 4.5 ml of ethanol. The solution was transferred into a corresponding cell and put in cell compartment and analysed for Chlorophyll-a, Chlorophyll-b and carotenoids content using spectrophotometer (SQ Pharo 100). The chlorophyll contents were then determined using the following equations (Lichtenthaler, 1987):

$$\text{Ch-a} = 13.36A_{664} - 5.19 A_{649} \quad (1)$$

$$\text{Ch-b} = 27.43A_{649} - 8.12 A_{664} \quad (2)$$

$$C_{x+c} = (1000A_{470} - 2.13\text{Ch-a} - 97.63\text{Ch-b})/209 \quad (3)$$

Where A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C_{x+c} = Carotenoids

3.8 Bioconcentration factor (BCF)

Bioconcentration factor (BCF) is a tool used to estimate chemical elements accumulation ability of the plants from the substrate (Zayed et al., 1998; Pandey, 2012a). BCF was calculated for each plant part (root, stem, leaf) using the following equations for the FA and soil:

$$BCF^a = \text{Metal in stem} / \text{Metal in FA or soil.} \quad (4)$$

$$BCF^b = \text{Metal in roots} / \text{Metal in FA or soil.} \quad (5)$$

$$BCF^c = \text{Metal in leaves} / \text{Metal in FA or soil.} \quad (6)$$

3.9 Translocation factor (TF)

TF is an asset to assess a plant's potential for phytoremediation purpose. TF is based on the ratio of metal concentration in plant stem as compared to that of the plant root (Pandey et al., 2012).

Thus:

$$TF = BCF^a / BCF^b \quad (7)$$

3.10 Statistical analysis and data presentation

Data was subjected for one-way analysis of variance (ANOVA) using Graphpad software package. Relationship between chemical elements in soil, FA and FA+soil growth media and also different plant parts (root, stem and leaf) of *B. juncea* and *S. Oleracea L* were calculated using the t - test, ANOVA-Bartlett test, Mann-Whitney Test and Kruskal-Wallis Statistic (KW) depending on each data set. This was done to determine how well the sets of data are related to each other with regards to the concentrations of chemical elements in each plant part and also in different growth media.

The dry weight concentrations of the chemical elements in the plant parts were calculated using the following formula for both *B. juncea* and *S. Oleracea L*:

$$PPM = \frac{C \times V}{W}$$

Where C = concentration value from ICP-MS

V = volume of the solution used in the analysis

W = weight of the plant part used

For example, Fe in leaves sample of *B. juncea* for FA as a growth media after 69 days of pot culture experiments had a concentration value from ICP-MS of 13.76 mg L^{-1} , with volume of MilliQ water used being 100 mL, while the mass of powdered plant part samples used was 0.5 g.

$$13.76 \text{ mg L}^{-1} = 13.76 \text{ mg kg}^{-1}$$

13.76 mg of Fe \equiv 1000 mL of solution

$$X \equiv 100 \text{ mL}$$

$$X/100 = 13.76 / 1000$$

$$X = 100 \times 13.76 / 1000$$

$$X = 1.376 \text{ mg of Fe}$$

i.e. 1.376 mg of Fe \equiv 0.5 g of leaves sample

$$\text{Level of Fe in the leaves} = 1.376 \text{ mg} / 0.5 \text{ g}$$

$$= 2.752 \text{ mg/g}$$

$$2.752 \times 1000 \text{ mg} / 1000 \text{ g} = 2752 \text{ mg kg}^{-1}$$

$$= 2752 \text{ mg kg}^{-1}$$

Therefore, there is 2752 mg/ kg of Fe in the leaves of *B. juncea*.

$$\begin{aligned} \text{This is the same as } PPM &= \frac{C \times V}{W} \\ &= 13.76 \times 100 / 0.5 \\ &= 2752 \text{ mg kg}^{-1} \\ &= 2752 \text{ mg kg}^{-1} \end{aligned}$$

The concentrations of each chemical element was calculated for B, Cr, Mn, Ni, Cu, Zn, Mo, Ba and Fe in different growth media for *B. juncea* and *S. Oleracea L*; data was presented in tables. The tables for results obtained straight from ICP-MS are presented in the appendix as Table B1 to B13 while the calculated concentrations into mg kg^{-1} are presented in Part 4 of Chapter 4 as Table 4.15 to 4.20 in mean \pm standard deviation.

CHAPTER 4

RESULTS AND DISCUSSION

Part 1: The chemical, morphological and mineralogical characterization of FA and soil used in pot culture experiments

4.1 Introduction

This chapter focuses on the presentation and discussion of the physicochemical, morphological and mineralogical characterization of FA and soils which were used in pot culture experiments. It will also dwell on the chemical composition of the plants biomass for *Spinacea Oleracea L* and *Brassica juncea* grown on coal FA, coal FA: soil mixture and soil media only.

4.2 Characterization of fly ash and soil for pot culture experiments

4.2.1 Mineralogy of Grootvlei Power Station FA

Qualitative results for the FA are shown in Figure 4.1. The representative diffraction peaks for each phase are labeled accordingly. Mullite was observed as a major phase in coal FA followed by quartz.

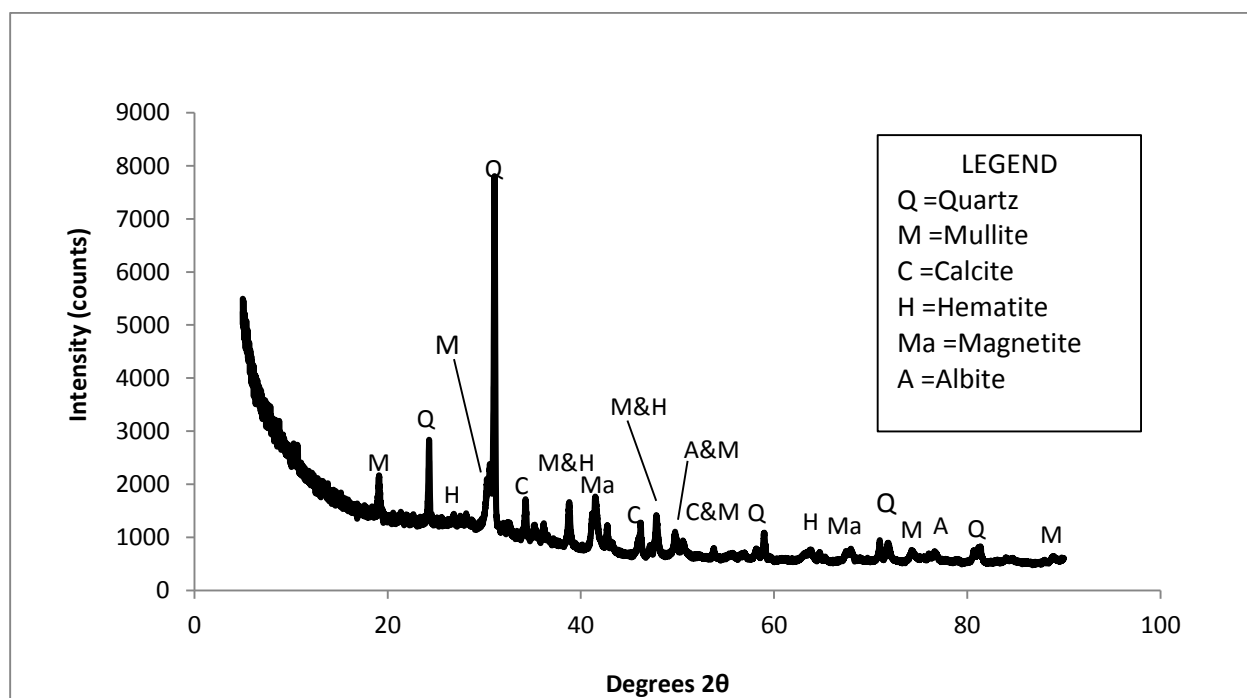


Figure 4.1: XRD spectrum of the FA.

In the study by Mainganye et al. (2012) similar results were obtained for Arnot, Tukuta, Matla, Lethabo and Hendrina power station FAs. In all the XRD peaks obtained by this researcher, the highest peak was that of quartz phase which was the same as observed in the current study. Major peaks observed were that of mullites, followed by quartz, then magnetite, calcites, hematite and albite in minor amounts or low intensity peaks; similar peaks were also obtained by Mainganye et al. (2012) in South African coal FAs although albite and calcites were not reported.

Table 4.1 shows the quantitative results for FA and it is observed that mullite (48.14 %) and quartz (28.51 %) are present as major constituents having higher weight percentage. Minor amounts of magnetite, calcite, hematite and plagioclase (albite) are also present in the FA.

Table 4.1: XRD quantitative results for Grootvlei Power Station FA.

Content	Weight %	3 σ error
Calcite; CaCO ₃	6.66	0.66
Hematite; Fe ₂ O ₃	5.21	0.36
Magnetite; Fe ₃ O ₄	7.88	0.42
Mullite; Al ₆ Si ₂ O ₃	48.14	1.17
Plagioclase; Na (Al Si ₃ O ₈)	3.61	0.63
Quartz; SiO ₂	28.51	0.75

Studies by Gitari et al. (2006 and 2010), Madzivire et al. (2010), van der Merwe (2011) and Mainganye et al. (2012) also observed presence of similar mineral phases. van der Merve et al. (2011) mentioned that the presence of mullites and quartz as major phases can make such FA to be used as particulate reinforcements in composites for wear-resistant applications as well.

4.2.2 Morphology of the Grootvlei Power Station FA

The morphology of the FA was evaluated using Scanning Electron Microscopy (SEM). The FA sample was placed on sample holder and the images were captured under various magnifications. SEM micrographs of FA are shown in Figure 4.2.

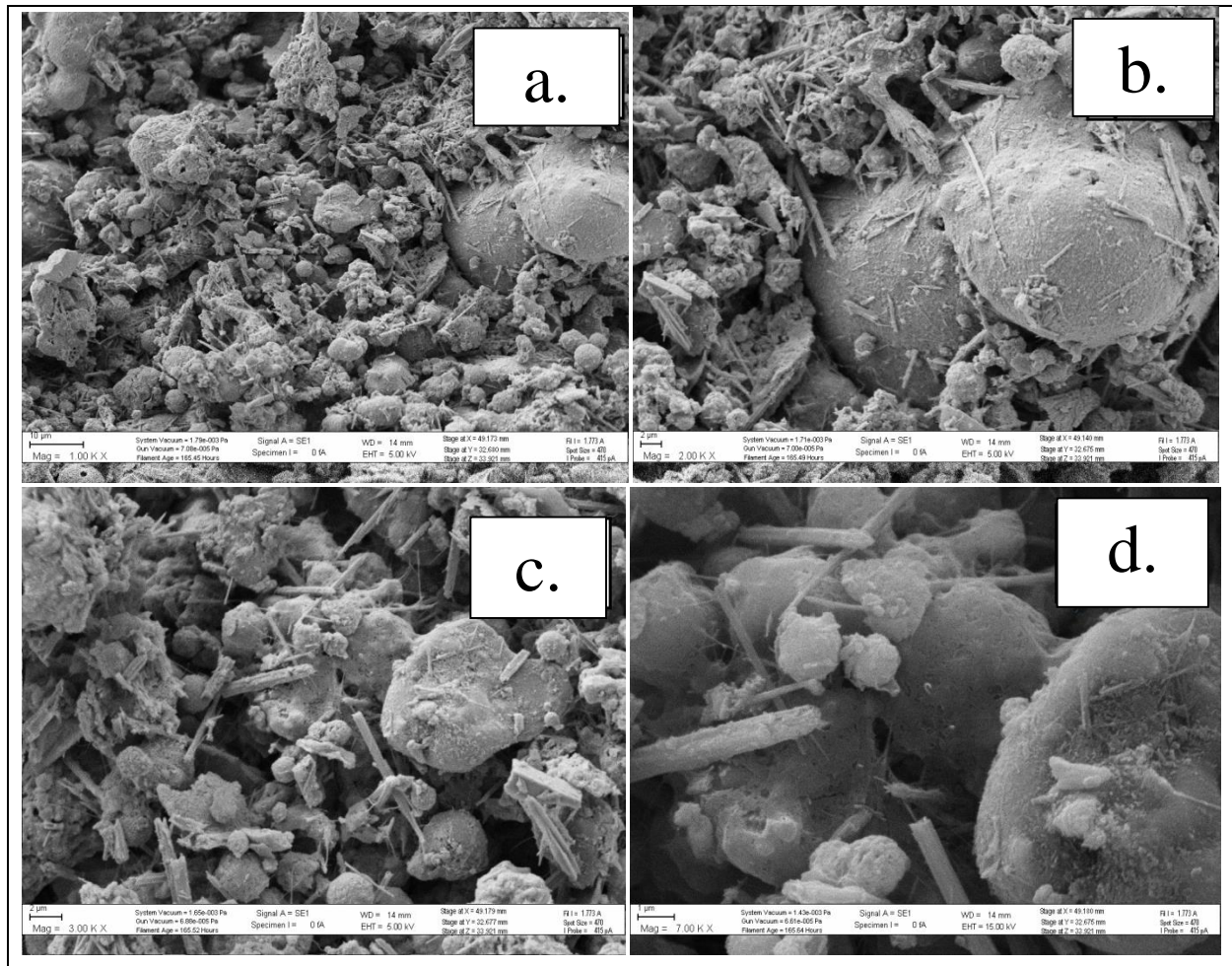


Figure 4.2: SEM images of Grootvlei Power Station FA at (a) x10 000 (b) x20 000 (c) x30 000 and (d) x70 000 magnifications.

Morphological study of fly ashes is of importance as morphology has a bearing on the leachability of heavy metals (Muriithi et al., 2009). Particles morphology of this FA has a lower degree of sphericity with irregular agglomerations of many particles. The FA particles are of various sizes and apart from the dominant spherical particles (Figure 4.2. d.) smaller sharp needle like particles are also observed.

In a study by Mahlaba et al. (2011b), of two South African fly ashes, one had majority of particles being spherical while the second had rather irregular morphology. Another study by Singh and Singh (2011) also observed FA particles that were irregular in shape, size and solid in cross section similar to the FA of the current study. Li et al. (2003); Saikia et al. (2006) and Kutchko and Kim (2006) concluded that the spherical shape in FA is an indication that the particles were formed under un-crowded freefall conditions and a relatively sudden cooling, which helps to maintain the

spherical shape while the agglomerated nature of some particles is an indication that the particles were produced due to high temperature sintering reactions.

4.2.3 Chemical composition of FA and soil.

The chemical composition of the FA and soil was evaluated using X-ray fluorescence (XRF) and the results are presented in Table 4.2. The results show concentrations of different major and trace elements in FA and soil.

Table 4.2: Comparison of the chemical composition of FA and soil samples.

Fly ash sample				Soil sample			
Major elements (as oxides)	(w/w) %	Trace elements	mg kg ⁻¹	Major elements (as oxides)	(w/w) %	Trace elements	mg kg ⁻¹
SiO ₂	43.65	As	22	SiO ₂	82.90	As	24
TiO ₂	1.23	Cu	57	TiO ₂	0.54	Cu	17
Al ₂ O ₃	22.68	Ga	35	Al ₂ O ₃	7.33	Ga	9
Fe ₂ O ₃	10.89	Mo	9	Fe ₂ O ₃	3.31	Mo	8
MnO	0.06	Nb	31	MnO	0.03	Nb	9
MgO	1.88	Ni	63	MgO	0.25	Ni	20
CaO	7.04	Pb	29	CaO	0.36	Pb	6
Na ₂ O	0.19	Rb	39	Na ₂ O	0.71	Rb	51
K ₂ O	0.81	Sr	2001	K ₂ O	1.09	Sr	46
P ₂ O ₅	0.54	Th	41	P ₂ O ₅	0.05	Th	13
Cr ₂ O ₃	0.18	U	28	Cr ₂ O ₃	0.01	U	5
NiO	0.01	W*	48	NiO	0.00	W*	235
V ₂ O ₅	0.02	Y	65	V ₂ O ₅	0.01	Y	10
ZrO ₂	0.07	Zn	40	ZrO ₂	0.07	Zn	21
CuO	<0.01	Zr	507	CuO	0.00	Zr	405
SO ₃	0.36			LOI	3.02		
LOI	9.50			TOTAL	99.69		
TOTAL	99.11						

The results show that this FA consists of three main components which are Fe₂O₃, SiO₂ and Al₂O₃. The American Society for Testing and Materials (ASTM, 1993) uses these three major phases to classify fly ashes based on coal source. From the results SiO₂ + Al₂O₃ + Fe₂O₃ > 70 %. This South African fly ash can be categorized as class F (SiO₂-rich), which is either derived from anthracitic

or bituminous coals (Mattigod et al., 1990). High concentration of Fe_2O_3 , SiO_2 and Al_2O_3 confirms that this fly ash is an alumino-silicate material as reported by Gitari et al., (2010). In other coal FA studies, the high concentrations of MgO and CaO indicate presence of soluble alkaline oxides that react in alkaline pH. Among the minor elements, Sr (2001 mg kg^{-1}), Zr (507 mg kg^{-1}), Y (65 mg kg^{-1}), Cu (57 mg kg^{-1}) and Ni (63 mg kg^{-1}) occur in high concentration with traces of Mo at 9 mg kg^{-1} . This is similar to the results of FA reported by Gitari et al. (2008), Mahlaba et al. (2011a and 2011b).

Koukouzas et al. (2009) describes the loss on ignition (LOI) as a measure of the amount of the unburned carbon remaining in the fly ash. He also mentioned that it may also represent losses in mass from some of the minerals in the ash, such as portlandite (Ca(OH)_2), calcite (CaCO_3) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). In the present study, FA had LOI of 9.5 % which is high and above ASTM standards classification of class F fly ash with maximum 6 % (ASTM, 1993). The soil had SiO_2 (82.9 %) as the main component of the soil, while Fe_2O_3 and Al_2O_3 were available in small amounts. There was also trace amounts of Zr, W, Sr, Ni, As and Rb in the soil.

There is similarity in terms of the elemental composition of FA and soil since SiO_2 is available in both mediums as a major element. Except for SO_3 which is not present in the soil, all the other major elements are present in both soil and FA and just differing slightly in their concentration. This is also the case with trace elements; all trace elements that are there in FA are there in the soil sample but just differing in concentrations. These results can help us conclude that the growth of the plants in both mediums can be similar as their elemental characteristics are very similar. But because the soil has very high SiO_2 concentration, which can imply a sandy type of soil, the growth of *Brassica juncea* and *Spinacea Oleracea L* can be expected to be slow as they tolerate loam soil which is not very rich in SiO_2 .

4.3 Physicochemical properties of leachates from coal fly ash and soil

Table 4.3 shows the physicochemical parameters (pH and EC) of the FA and soil leachates used as media for growth of the phytoremediation plants, the cationic species and the anions. The results were obtained from samples prepared from the European pre-standard batch leaching test (European Committee for Standardization, 1998).

Table 4.3: Chemical characteristics of FA and soil leachates

	Values in mg L⁻¹	
	FA sample	Soil sample
Al	5.46±0.01	4.09±5.09
B	0.64±0.01	ND
Ca	37.89±0.50	3.18±0.04
K	10.53±0.40	7.10±0.31
Mg	0.24±0.00	2.66±0.07
Na	10.67±0.04	2.73±0.05
P	0.01±0.00	0.10±0.00
Si	2.01±0.04	16.49±1.85
Fe	ND	6.38±0.74
Sr	1.32±0.02	ND
	Values in µg L⁻¹	
Li	173.84±5.39	2.59±0.24
Be	0.00±0.00	0.13±0.04
B	ND	17.17±0.23
V	22.98±1.23	14.50±0.25
Cr	17.48±0.64	9.88±0.42
Mn	0.84±0.04	126.74±12.90
Fe	3.39±1.24	ND
Co	0.19±0.06	1.52±0.02
Ni	0.42±0.06	11.35±0.27
Cu	0.57±0.08	9.15±0.28
Zn	<0.00±0.00	10.53±0.25
As	1.55±0.20	0.64±0.21
Se	3.28±0.10	<0.582±0.00
Sr	ND	26.37±1.35
Mo	30.37±1.50	0.17±0.07
Cd	0.07±0.08	0.01±0.00
Sb	1.34±0.04	0.07±0.01
Ba	110.07±2.94	74.61±3.99
Hg	0.00±0.00	0.11±0.01
Pb	0.06±0.05	0.97±0.01
F⁻	1.12±0.15	3.15±0.9
Cl⁻	2.42±0.26	3.99±0.67
NO₃⁻	46.71±0.33	3.27±0.93
SO₄²⁻	3.35±0.91	87.57±2.78
PO₄³⁻	11.74±0	ND
pH	10.62±0.08	7.22±0.03
EC	364±2	75.91±0.95

Results in Mean±SD where n=3
***ND- not detected**

The average pH of the FA leachates was measured to be 10.62, with an average electrical conductivity (EC) of $364 \mu\text{S cm}^{-1}$. This shows that fly ash under study is alkaline. The soil leachate revealed an average pH level of 7.22 with $75.9 \mu\text{S cm}^{-1}$ average EC. Jamil et al. (2009) mentioned that the pH value of FA depends on the S content of parent coal; hence, coal with high S produces acidic ashes and with low S content produces alkaline ashes. FA under study possesses low content of S leading to the Grootvlei FA being alkaline. This can be confirmed further by the presence of sulphates in low concentration as shown in Table 4.3. For soil, sulphates are seen in Table 4.3 being the highest in all anions recorded, and this is why soil leachates tend to have a neutral pH level. The EC estimates the amount of total dissolved salts in soil solution, so the EC of FA was generally higher than that of normal soil because of the presence of more soluble salts in FA (Bilski et al., 1995).

The XRF results for the FA are further confirmed by the ICP-MS results for the FA leachates in Table 4.3. It is shown that there are high amounts of Ca, Na, K, Al, Sr, Si, and B, however, there are also other elements available in small amounts such as Ba, Li, Fe, V, Cr, Se, and Mo. The Fe results in Table 4.3 are inconsistent with results shown in tables 4.1 and 4.2. Subsequently it was higher in Table 4.1 and 4.2 were FA was analysed in a solid form but low in Table 4.3 were FA leachates were analysed. This phenomenon might have been caused by Fe slowly solubilizing in FA leachate when FA gets in contact with water. Results in Table 4.3 also shows that there are high amounts of Si in the soil leachates. This is followed by elements such as K, Fe, Al, Ca, Na, Mg and P. There are also elements available as minor elements such as Mn, Sr, V, Ni, Zn, Cr, Cu, Li, Co, Pb, As, Se, Mo, Be, Hg, Sb and Cd occurring in lowest concentration. The contact of water and soil (even FA) make other chemical elements to be available, for example, elements like Ba and B were also observed as minor elements in the leachates but in higher concentrations. Chemical elements such as Si, Mn, Ba, K, Fe and Al will be readily available to plant roots as they occur in high concentrations in solutions of these materials. Prasad and Mondal (2008) reported that the percentage of leached amounts found to follow the trend $\text{Zn} > \text{Fe} > \text{Mn} > \text{Cr} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$ for the Chandrapura FA and $\text{Fe} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Cd}$ for Ramagundan FA which is not very similar to what was observed in the current study. They also mentioned that the effect of pH on chemical elements released from ash surface in aqueous solution followed a predictable pattern of decreasing release with increasing pH.

The presence of these elements in the leachates can lead to translocation of them from roots to shoots of the plants especially those that are soluble in water as they will be absorbed by roots. An example of these chemical elements from FA leachates can include those that are available as major elements including Ca, Na, K, Al, Si, Sr and B. Other minor elements (including Fe, Cu, Li, Ba, Cr, Mo and V) can accumulate over time and be absorbed by plants and become translocated to the stems and shoots.

A comparison of the chemical characteristics of the soil and FA leachates shows that Ca, Na, K, Al, Si, Mg, and P are available as major elements in both media except for Sr and B which occur as minor elements in the soil leachates. Fe is also occurring as a major element in soil while in FA occurs as a minor element. For minor elements, Li and Ba occur in high concentrations than other minor elements in FA while in soil Mn is the highest followed by Ba. This indicates that these growth media are likely to supply the same type of nutrients to the plants, what will differ between the two is the amount available in solution which in turn is available to plants.

According to Silva and Uchida (2000), plants require 16 essential elements such as C, H, O, N, P, K, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo, and Cl for their growth and development. In the FA under study Ca, Na, K, Al, Sr, Si, and B occur in high amounts, while Na, Al, Sr, Si are regarded as non-essential nutrients, this can lead to direct toxicity to plants if they are absorbed by plant roots (Silva and Uchida, 2000). The same goes for elements like Ba, Li, V, Cr and Se that are available in the leachate solutions (even in lower concentration) which, if taken up by plants' roots can cause toxicity to plants.

The anions content in FA leachates was also analysed and results are shown in Table 4.3, where nitrates (NO_3^-) are available in higher concentrations while phosphates (PO_4^{3-}), sulphates (SO_4^{2-}), chloride (Cl^-) and fluoride (F^-) follows in lower concentrations. Soil leachates are rich in sulphates, followed by chloride, nitrates and then fluoride. The occurrence of these sulphates in high amounts accounts for soil being neutral unlike in FA leachates where sulphates are very low in concentration. All these anions are essential to plants. N is available to plants as nitrate (NO_3^-) and important for plants as well. Each type of plant is unique and has an optimum nutrient range as well as a minimum requirement level. Below this minimum level, plants start to show nutrient deficiency symptoms, while the excessive nutrient uptake can also cause poor growth because of toxicity (Silva and Uchida, 2000). N is biologically combined with C, H, O, and S to create amino acids, which are the building blocks of proteins. Amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development. Since all plant enzymes are made of proteins, N is needed for all of the enzymatic reactions in a plant. N is a major part of the chlorophyll molecule and is therefore necessary for photosynthesis. N is a necessary component of several vitamins and it improves the quality and quantity of dry matter in leafy vegetables and protein in grain crops (Silva and Uchida, 2000).

4.3.1 Conclusion

From the results and discussion of this chapter it can be concluded that FA from Grootvlei power station is a class F coal FA with an average alkaline pH level of 10.62. Particles morphology of this FA shows a lower degree of sphericity with irregular agglomerations of many particles. The FA particles are of various sizes and apart from the dominant spherical particles, smaller sharp needle like particles are also observed. It is also an alumino-silicate material as confirmed by the high SiO_2 and Al_2O_3 content. In the FA under study Ca, Na, K, Al, Sr, Si, and B will be readily available to plants from FA solution with water. Since Na, Al, Sr, Si are regarded as non-essential nutrients and are available in FA leachates in high concentrations, their uptake can lead to direct toxicity of plants. This also applies to elements like Ba, Li, V, Cr and Se that are available in the FA leachate solution. Soil leachates had a neutral pH of 7.22 with very high amounts of Si. In both soil and FA, elements that can be readily available to plants includes Ca, Si, K, Ba, Mo, Na, Al, Mg, Sr and here there are non-essential nutrients (Si, Ba, Na, Al, Sr), which, if uptaken by plants' roots can have negative impacts in plants.

Part 2: The temporal evolution of the physicochemical characteristics of the leachates as plants were irrigated in the pot culture experiment

4.4 Introduction

This part of Chapter 4 focuses on the physicochemical characteristics of the leachates from different pots used in pot culture experiments. This includes pots with soil, FA, and a mixture/amendment of FA and soil (50/50) where *B. juncea* and *S. Oleracea L* are grown, and also those pots with FA only where no plants were grown for control purposes. The measured pH and EC parameters and analysed chemical elements available in those leachates are discussed.

4.4.1 Temporal variation of pH in various growth media

Figures 4.3 to 4.9 show the variation in pH for leachates of various growth media over 11 weeks of the monitoring period.

Figure 4.3 shows the pH levels of leachates from soil media pot where *B. juncea* was grown. The trend shows that within first week of the leachates collection, pH levels were relatively high and then plummeted to lower levels in the second week. Similar pH values trend was observed in the soil media where *S. Oleracea L* was grown (Figure 4.6) within the first week but the average pH (i.e. 8.52) was relatively higher than that of *B. juncea* (i.e. 7.56), then it started decreasing until the eleventh week. This observed trend could have been due to the different adaptation mechanisms of the two plant species during early stages of the pot culture experiments. Therefore, it can be concluded that *S. Oleracea L* adapted faster to soil as growth media than *B. juncea* as it could not continue moderating pH levels at the same rate.

Nonetheless, similar trend was observed in all other leachates from different growth media (FA only, FA with plants and FA+soil media). The average pH of leachates from FA pot media show initial high value (i.e. 10.62) but with continuous irrigation the pH decreases until it reaches an equilibrium for both plant species (Figure 4.4, 4.7 and 4.9 where no plants were planted). This was similar to what was observed for FA+soil media for both plant species (Figure 4.5 and 4.8), wherein pH starts high but decreases over time and reaches equilibrium. This could be attributed to dissolution of soluble major oxides in the FA which correspondingly lower the pH values over time.

In summary, the raw FA from Grootvlei power station starts with an alkaline pH of 10.62 (same as in batch experiments - Part 1) but this change over time as FA is irrigated with water and it ends up losing its alkalinity with leachate pH decreasing to neutral pH levels.

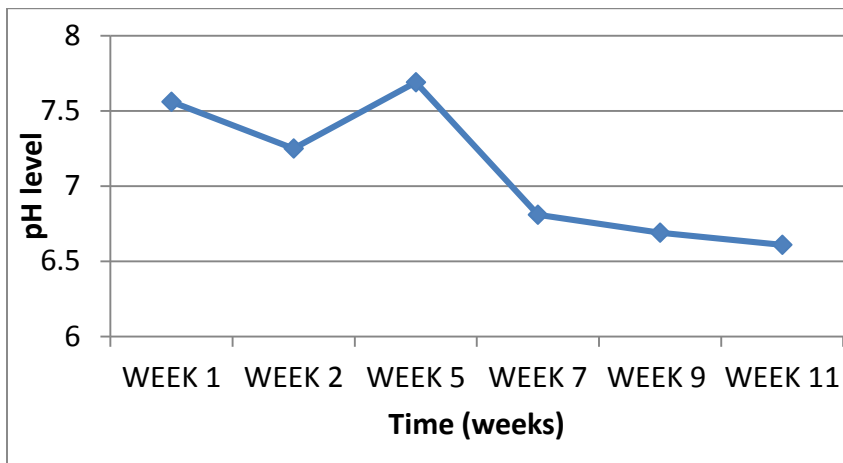


Figure 4.3: Variation of pH in leachates for *B. juncea* grown in soil media with time.

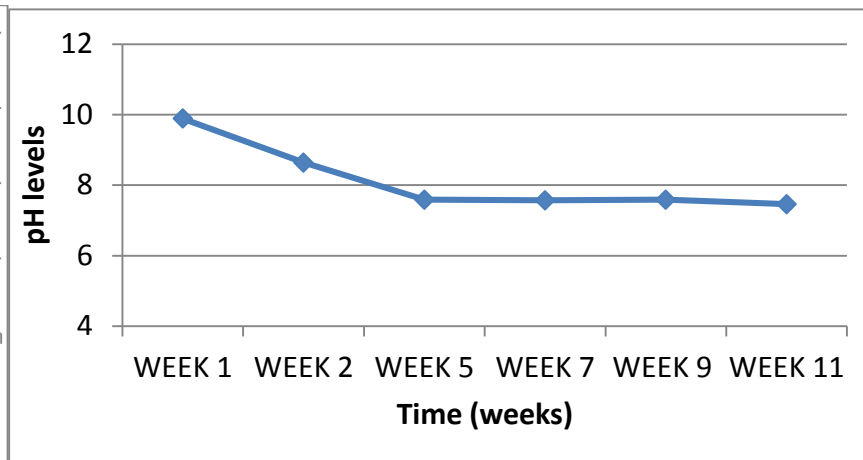


Figure 4.4 Variation of pH in leachates for *B. juncea* grown in FA media with time.

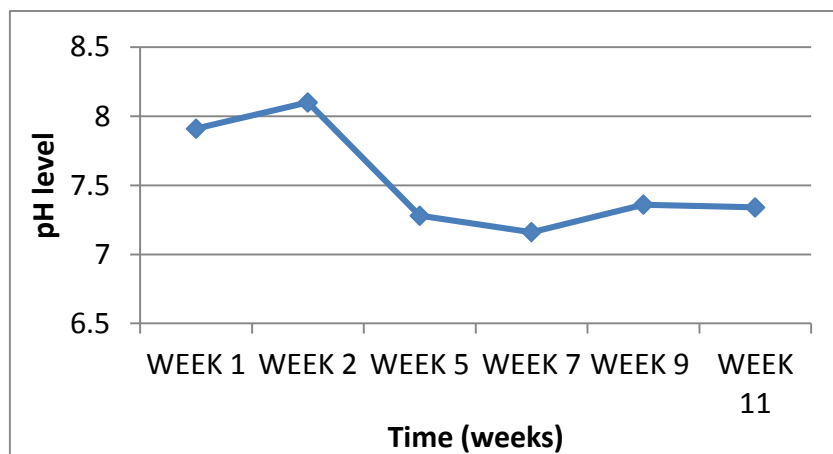


Figure 4.5: Variation of pH in leachates for *B. juncea* grown in FA+soil media with time.

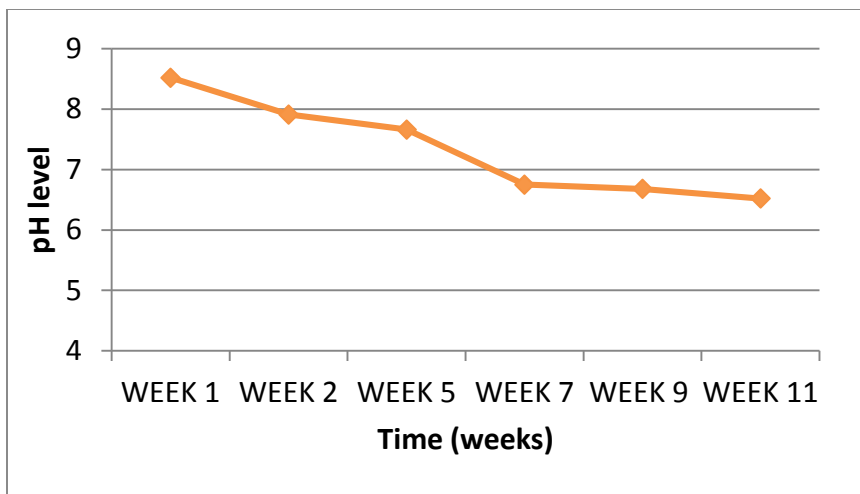


Figure 4.6: Variation of pH in leachates for *S. Oleracea L* grown in soil media with time.

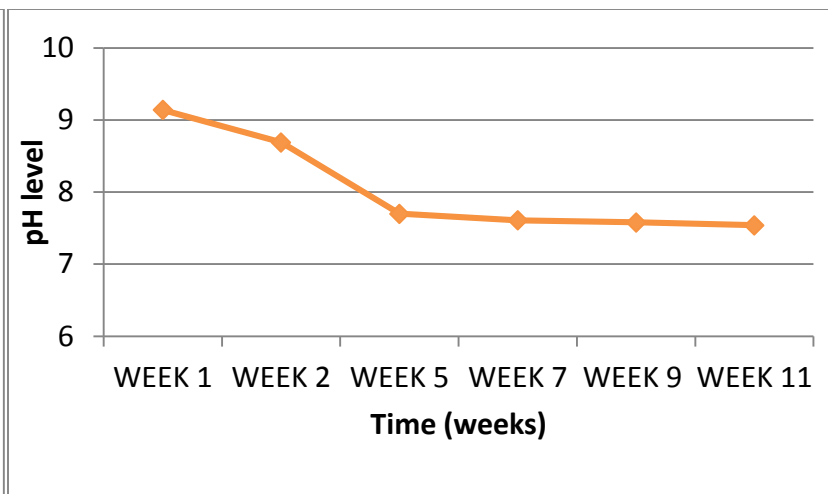


Figure 4.7: Variation of pH in leachates for *S. Oleracea L* grown in FA media with time.

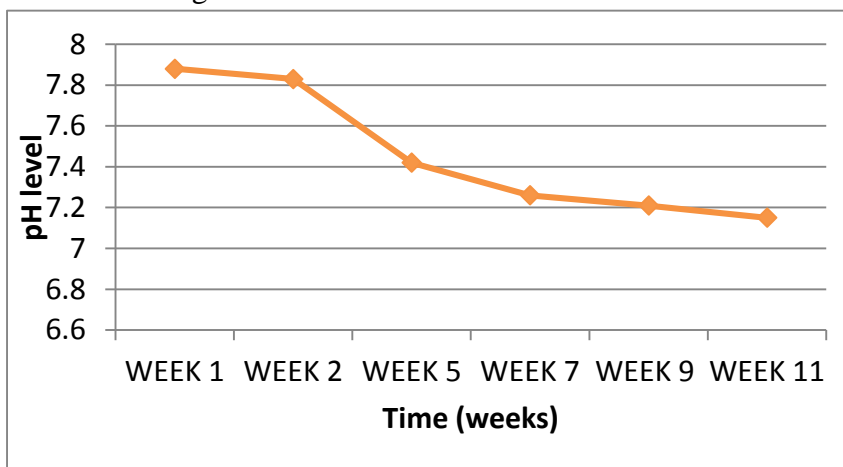


Figure 4.8: Variation of pH in leachates for *S. Oleracea L* grown in FA+soil media with time.

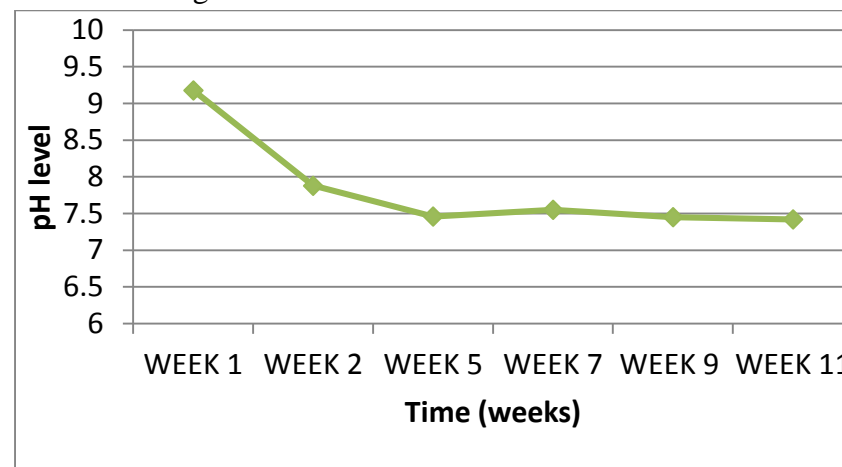


Figure 4.9: Variation of pH in leachates where no plants grown in FA media with time.

4.4.2 Temporal variation of EC in various growth media

Figures 4.10 to 4.16 show the variation in the Electrical Conductivity (EC) for leachates of various growth media over 11 weeks of the monitoring period.

The trends of Figures 4.10 and 4.11 show relatively low EC within the first week and increased in the second week of the leachate collection. This might have been due to the slow adaptation of *B. juncea* to soil and FA as a growth media. Then in week 5, that started to change and EC was lowering again showing that the plants were using up chemical elements responsible for high conductivity. Similar thing happened in week 5 of Figure 4.12 where a mixture of soil+FA was a growth media.

Figure 4.10 to Figure 4.16 shows decreasing trends where EC was high in week 1 of leachates collection while as weeks go by; it was continuing to decrease for all growth media and plant species. This shows that *S. Oleracea L* and *B. juncea* growth could have influenced lower EC values as they used most of the chemical elements over time which can increase electrical conductivity of the leachates. This includes those metals that are semiconductors and conductors of electrical current such as CO_3^{2-} , OH^- , SO_4^{2-} , Cl^- , NO_3^- and PO_4^{3-} . This can be explained in conjunction with chemical elements trends found in leachates of different growth media in Part 3 of this chapter 4; some chemical elements started very high in the first week and decreased with time until the eleventh week of the pot culture experiments which is a similar trend for the overall EC. Only nine chemical elements were chosen for discussions which includes B, Cr, Mn, Ni, Cu, Zn, Mo, Ba and Fe and they behaved in a decreasing trend for different growth media and for both plant species over time honoring to the trend of EC behaving in a similar manner. The salts from the nine chemical elements and some which were not discussed could have caused the high EC at the first week (as they are soluble in water) and with continuous irrigation EC decreased over time until the eleventh week with the concentration of those chemical elements decreased as well.

In comparison of EC values obtained from the leachates where FA was a growth media of *S. Oleracea L* and *B. juncea* (Figure 4.11 and Figure 4.14), and those pots without plants (Figure 4.16); it can be observed that EC was relatively above $300 \mu\text{S cm}^{-1}$ for all three graphs and it plummeted to below $250 \mu\text{S cm}^{-1}$ at eleventh week for all three graphs. Therefore, decrease in the EC of the FA is due to dissolution of soluble salts which is promoted by continuous irrigation in the FA rather than plant growth. This can further influence the issue of phytoremediating FA dumps since such chemical elements can end up in our groundwater and surface waters (like rivers) when it rains. The EC estimates the amount of total dissolved salts in soil solution, so the EC of FA was generally higher than that of normal soil in most instances because of the presence of more soluble salts present in FA (Bilski et al., 1995).

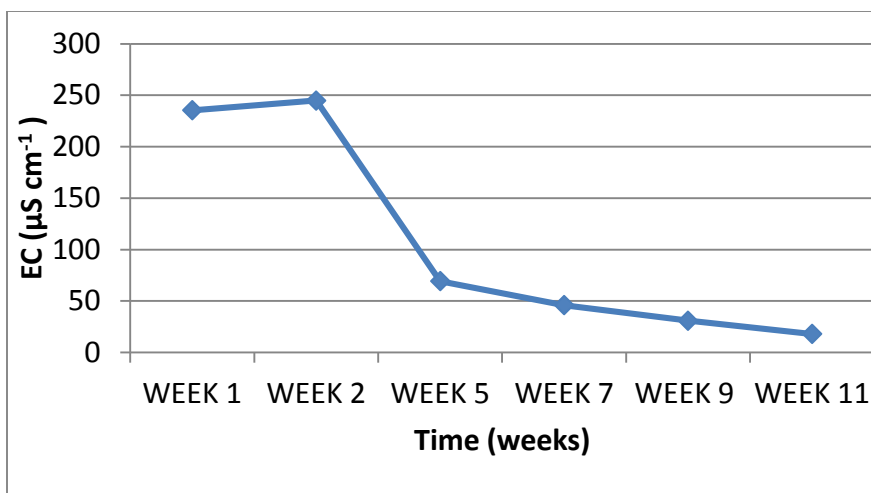


Figure 4.10: Variation of EC in leachates for *B. juncea* grown in soil media with time.

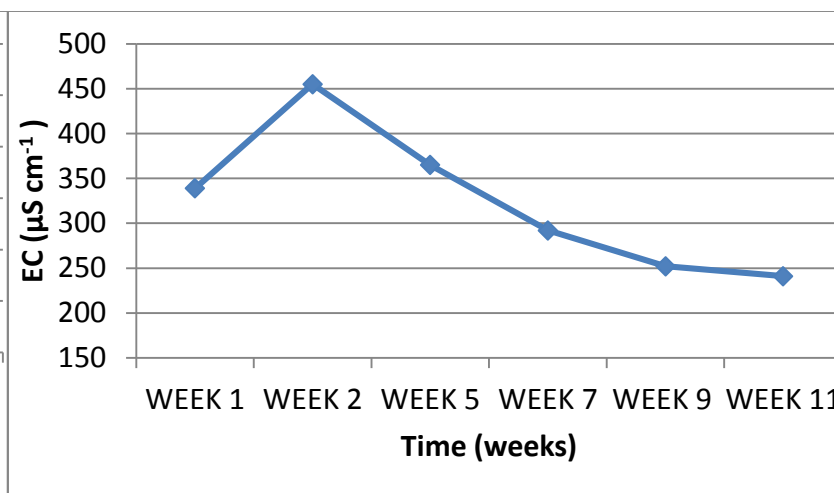


Figure 4.11: Variation of EC in leachates for *B. juncea* grown in FA media with time

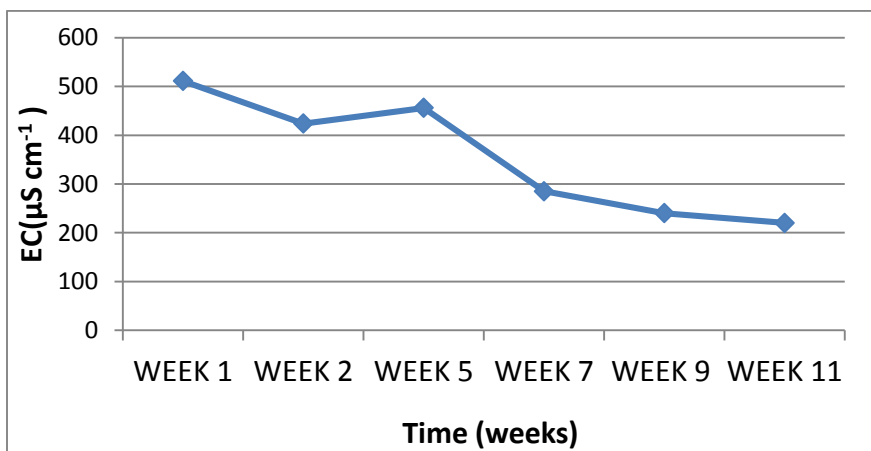


Figure 4.12: Variation of EC in leachates for *B. juncea* grown in FA+soil media with time

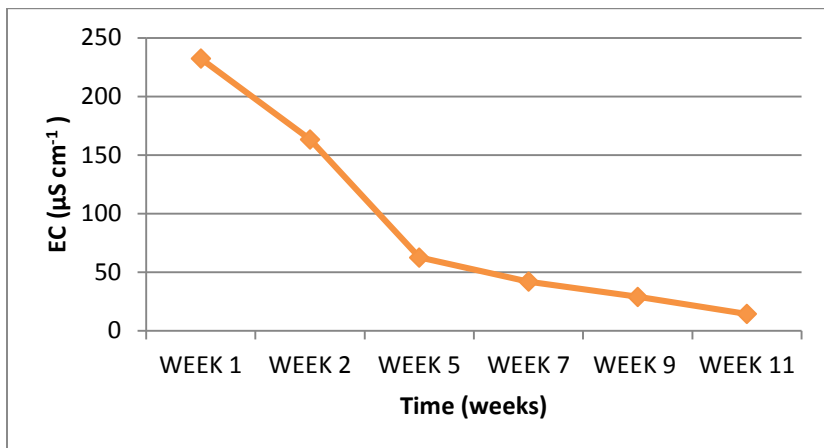


Figure 4.13: Variation of EC in leachates for *S. Oleracea L* grown in soil media with time.

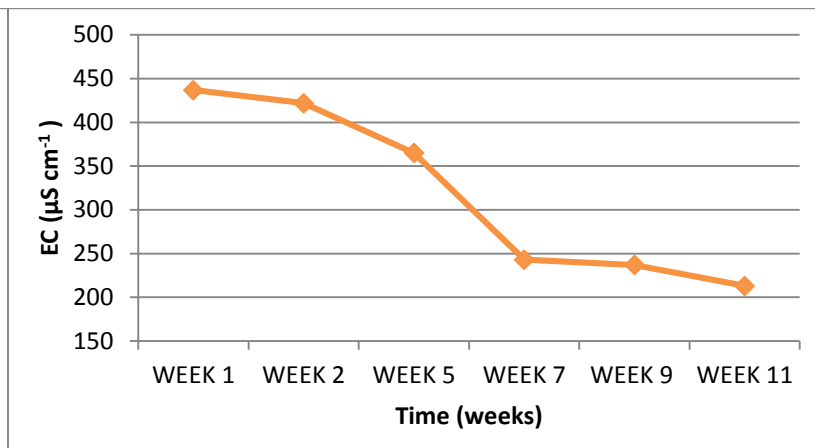


Figure 4.14: Variation of EC in leachates for *S. Oleracea L* grown in FA media with time.

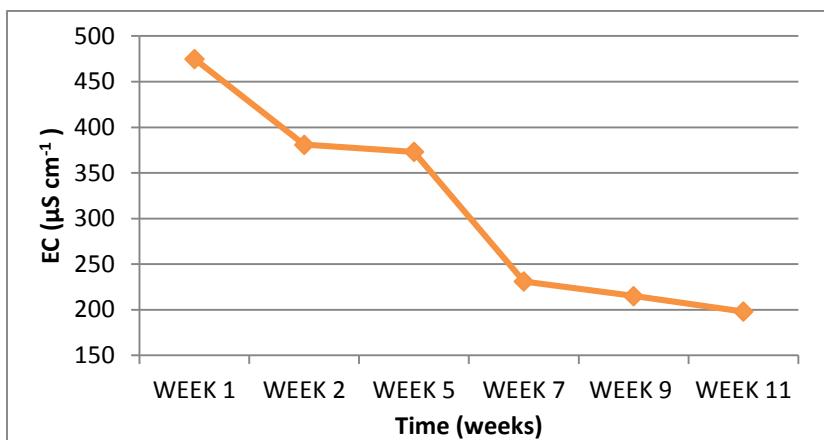


Figure 4.15: Variation of EC in leachates for *S. Oleracea L* grown in FA+soil media with time.

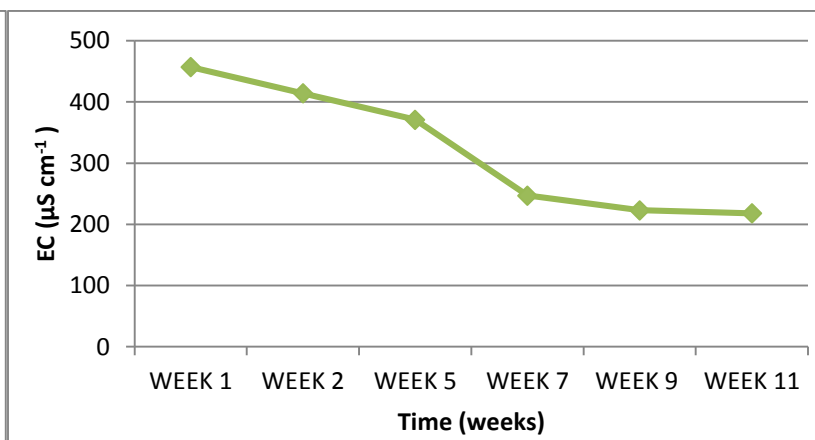


Figure 4.16: Variation of EC in leachates where no plants grown in FA media with time.

4.4.3 Statistical analysis of EC and pH trends data

A one-way analysis of variance (ANOVA) was carried out using Graphpad software for the EC and pH levels of the three growth media under study. Results were considered significant at a P level <0.05. For the other data, t-test was considered not to be effective and accurate so it was advised by the Graphpad software that we consider Wilcoxon matched-pairs signed-ranks test as a measure of variation. The results of analysis are presented in Table 4.4.

Table 4.4: pH and EC leachates statistical comparisons between *B. juncea* and *S. Oleracea L* grown in different media.

pH					
Growth media	P value	Degrees of freedom (DF)	t - test	Wilcoxon matched-pairs signed-ranks test (Sum of positive ranks)	Significance
Soil	>0.999			10.000	X
FA	0.5625			7.000	X
Soil+FA	0.3665	5	0.9926		X
EC					
Soil	0.0313			21.000	✓
FA	0.8431	5	0.2084		X
Soil+FA	0.0050	5	4.772		✓ **

Note:

- X - not significant
- ✓ - significant
- ✓ * - very significant
- ✓ ** - extremely significant

This statistical test results depicted in Table 4.4 show that there was no significant difference in the EC and pH levels of leachates over eleventh week period for many growth media where *B. juncea* and *S. Oleracea L* were grown, except for the EC of the leachates of soil and soil+FA media for both species. These results imply that there was no significant difference between leachates from pots where soil/FA/soil+FA were growth media for both species on the basis of pH levels. In case of EC for soil media, there was a significant difference between leachates from pots where *B. juncea* and *S. Oleracea L* were grown. With respect to the EC of the soil+FA media the P value

was 0.005 which is way less than 0.05, implying extremely significant difference between *B juncea* and *S. Oleracea L* leachates with respect to uptake of chemical elements responsible for high EC. This indicates that these plants species have different nutrient requirements derived from both soil and FA media.

Table 4.5 shows the statistical comparison of leachates from four sets of growth media which includes soil, FA, soil+FA with *B juncea* and *S. Oleracea L* and FA only (without plants grown on it).

Table 4.5: Statistical comparisons between four sets of growth media (soil, FA, soil+FA with plants and FA only - without plants) for each plant species.

pH				
Growth media (soil, FA, soil+FA with plants and FA only - without plants).	P value	Kruskal-Wallis Statistic (KW)	ANOVA (Bartlett statistic)	Significance
<i>B. juncea</i> and FA only	0.0046		1.205	✓ *
<i>S. Olearacea L</i> and FA only	0.0054		0.8067	✓ *
EC				
<i>B. juncea</i> and FA only	0.0670	7.158		X
<i>S. Olearacea L</i> and FA only	0.3118	3.570		X

Note:

- X - not significant
- ✓ - significant
- ✓ * - very significant

From Table 4.5, it can be seen that for the pH levels of leachates where *B juncea* was grown for all three growth media (soil, FA and soil+FA) and for FA only; there was a strong significance in the pH levels of the three sets of growth media were *B juncea* plant species were planted as compared to that media without plants grown in it. This implies that there is a difference between all the four sets of growth media (including the one without any plants grown in it). This might have been the reason why pH levels tend to differ for different media for similar plant species, although they exhibited a similar decreasing trend as discussed in section 4.4.1 in this part 2 of Chapter 4. Similar observation with pH levels of *S. Oleracea L* leachates for all the growth media

since the P value was 0.0054, which is very significant, hence indicating a difference between all the four sets of growth media for the same plant species.

With regards to the EC of the leachates for *B juncea* and FA media with no plants, the two-tailed P value was 0.0670 which is above 0.05 and it was considered not significant. This analysis confirmed that there was no significant difference in the EC of leachates over the 11-week period for the two plant species between the four sets of growth media unlike with pH levels.

4.5 Temporal variation of the chemical properties of leachates from pots with different growth media

Figures 4.17 – 4.30 show the temporal variation of the chemical elements in the leachates from different growth media. They are based on the results for the leachates collected from first week to the eleventh week of the pot culture experiments for different growth media. The full analysis results are presented in the appendix in Tables A.1 to A.7. The chemical elements chosen for the current study includes B, Cr, Mn, Ni, Cu, Zn, Mo, Ba and Fe. They were chosen after a complete ICP-MS analysis was done and it was noted that these nine chemical elements occurred in the leachates at higher concentrations than others. Studies carried out indicated that these chemical elements were present in FA and were in high concentrations (Davison et al., 1974; Gitari et al., 2006; Pandey et al., 2009; Pandey, 2012a; Wankar and Wadhai, 2012 and Tiwari et al., 2013).

A summary of the statistical test results for these leachates are shown in Table 4.6 to 4.10. Statistics is the science of learning from data and it offers essential insight in determining which data and conclusions are trustworthy (The World of Statistics, 2013). When statistical principles are correctly applied, statistical analysis tend to produce accurate results which is why statistics is also important for the current study to be able to draw reliable conclusions. In different studies of phytoremediation and others, statistics is used to quantify results such as Lai et al. (2008), Jamil et al. (2009), Pandey (2012a) and Subhashini et al. (2013). In the current study, the two plant species are compared to see if their presence in the similar growth media had effect on the availability of different chemical elements under study in the leachates. Also the three different growth media (soil, FA and FA+soil) where similar plant species were grown were compared here to see how variable was the availability of chemical elements against the one growth media (FA) where no plant species were grown. Significant difference obtained from statistical data helps in concluding if there was a difference or not between growth media and also between plant species.

4.5.1. Temporal variation of the chemical elements of leachates from pots of soil growth media for both plants

The Figures 4.17 and 4.18 shows the chemical elements concentrations obtained from soil pots growing *B. juncea* for essential elements (B, Zn, Mn, Fe, Mo and Cu) and non-essential elements (Cr, Ni and Ba) while Figures 4.19 and 4.20 shows similar concentrations but plant species being *S. Oleracea L.*

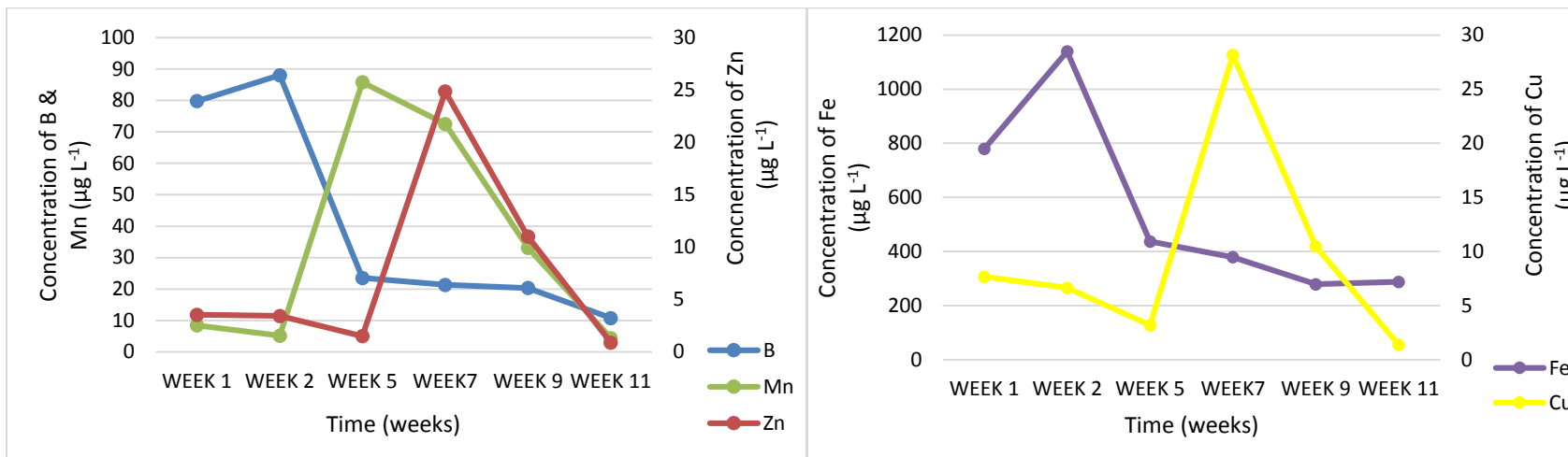


Figure 4.17: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea* for essential elements B, Zn, Mn, Fe and Cu.

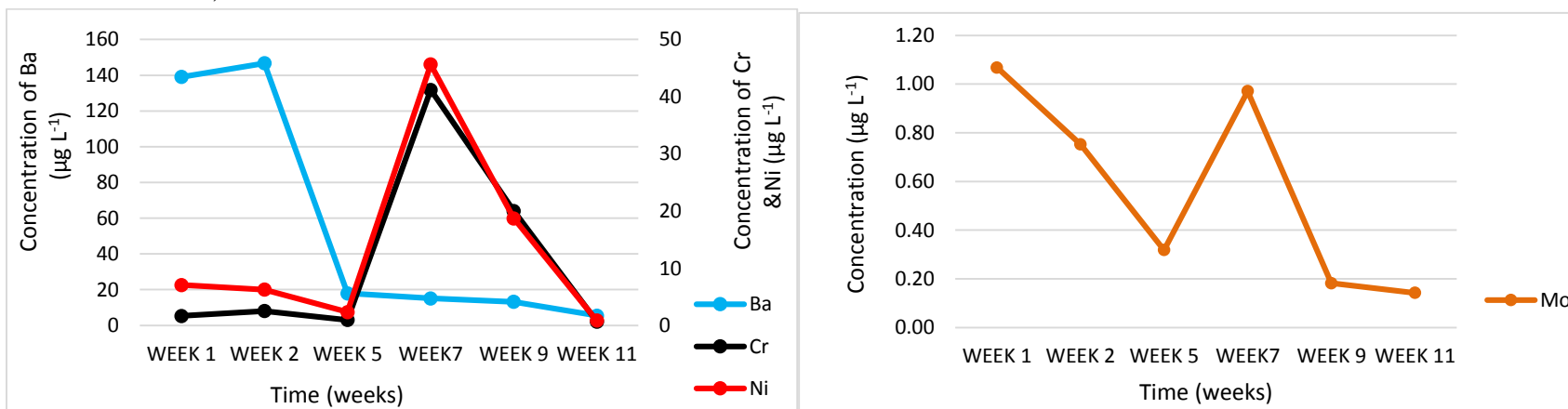


Figure 4.18: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea* for non-essential elements Cr, Ni and Ba and Mo as an essential element.

There is a common trend for Zn, Cu, Cr, Mo and Ni which at high pH they all form soluble hydroxides, hence the high concentration at week 7 corresponding to high pH for *B. juncea* species in Figure 4.17 and 4.18. Similar trend can be observed for *S. Oleracea L* species in Figure 4.19 and 4.20 for Mn, Zn, B, Ba, Cr, Ni, Fe and Cu. This can imply that the chemical elements behaved similarly in leachates where both plant species were grown.

Trace metals such as B, Mn, Fe and Ba recorded higher concentrations in the first week to fifth week of soil media (Figures 4.17 and 4.18). Fe shows the highest concentration of about 779.13 $\mu\text{g L}^{-1}$ while Mn, Ba and B concentrations were 8.38 $\mu\text{g L}^{-1}$, 139.06 $\mu\text{g L}^{-1}$ and 79.81 $\mu\text{g L}^{-1}$ during the first week. It continued to rise in two weeks, while it was still higher at five weeks than many chemical elements presented in the figures even though it decreased drastically in five weeks for B, Fe, and Ba. This can further support the claim that Fe, Mn, Ba and B can be potentially uptaken by *B. juncea* plant since they are found in high concentration in leachate solution. Other chemical elements that were available even though in lower concentrations in the leachate solution includes Zn, Mo, Cu, Ni and Cr for soil media with *B. juncea* (Figures 4.17 and 4.18). Similar results were observed for soil media where *S. Oleracea L* was grown as shown in Figures 4.19 and 4.20.

However, some chemical elements like B, Mn, Fe and Ba were occurring at higher concentrations for most weeks in the pot culture experiments. It was observed from Figures 4.17 to 4.20 that in the eleventh week of leachate collection all these chemical elements plummeted to very low concentrations (Figures 4.17 to 4.20). This indicates that these chemical elements can be reduced over time as plants are being irrigated which is either due to uptake by plants or wash out by water. It can also be concluded that for the two species (*B. juncea* and *S. Oleracea L.*) both in soil media, show no remarkable difference in the trends of these investigated chemical elements in the leachates from the first week to the eleventh week.

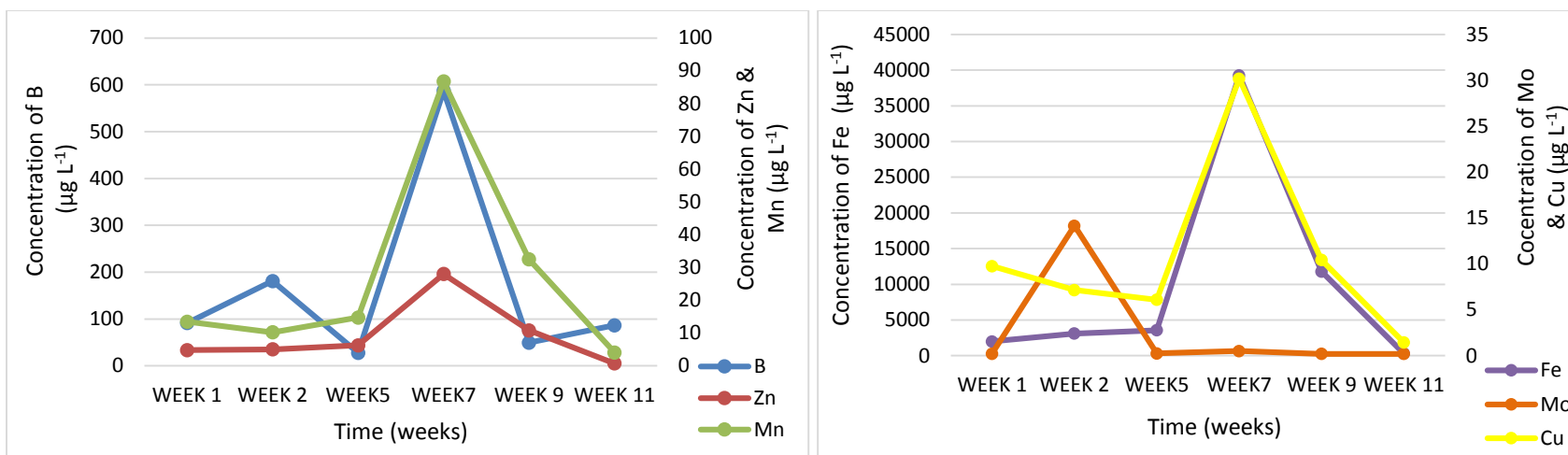


Figure 4.19: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L* for essential elements B, Zn, Mn, Fe, Mo and Cu.

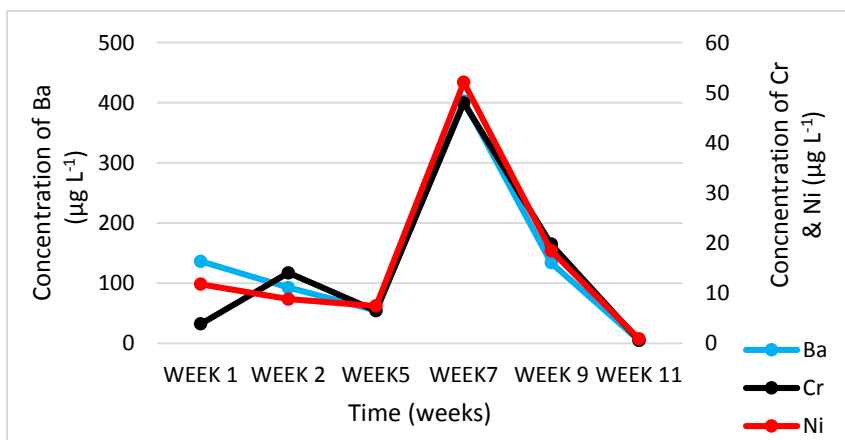


Figure 4.20: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L* for non-essential elements Cr, Ni and Ba.

4.5.2 Temporal variation of the chemical elements of leachates from pots of FA growth media for both plants

The Figures 4.21 and 4.22 shows the chemical elements concentrations obtained from FA pots growing *B. juncea* for essential elements (B, Zn, Mn, Fe, Mo and Cu) and non-essential elements (Cr, Ni and Ba) while Figures 4.23 and 4.24 shows similar concentrations but plant species being *S. Oleracea L.*

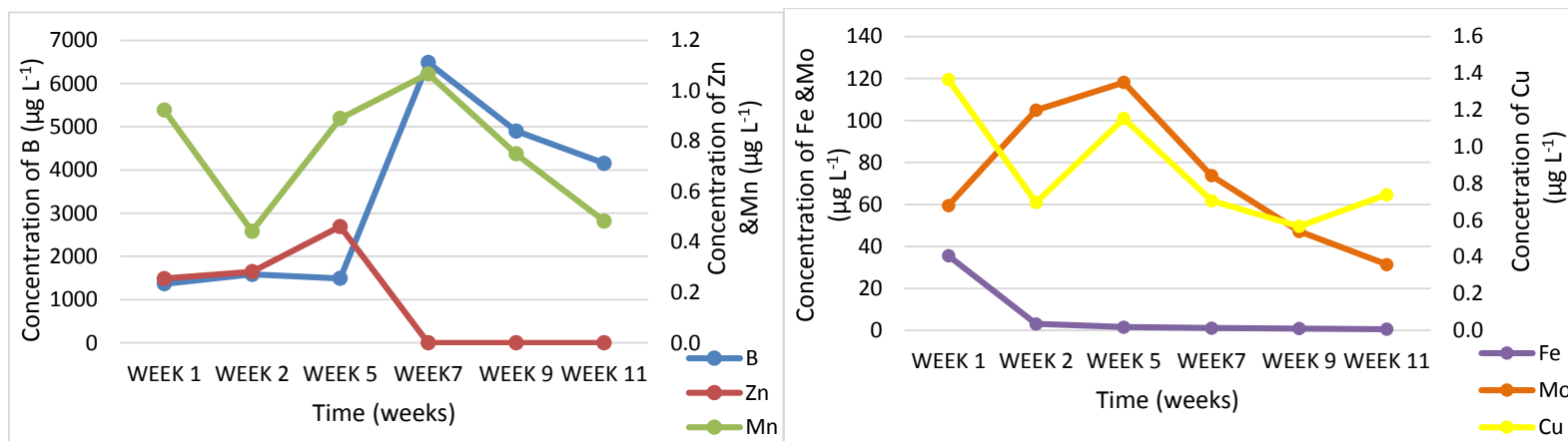


Figure 4.21: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea* for essential elements B, Zn, Mn, Fe, Mo and Cu.

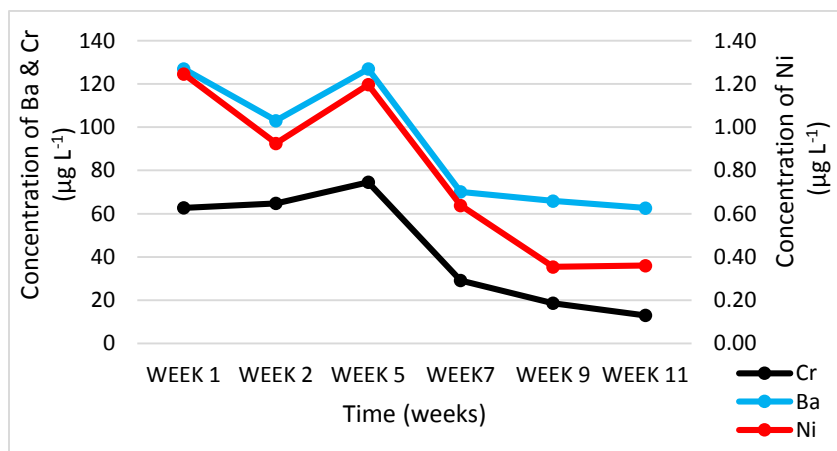


Figure 4.22: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea* for non-essential elements Cr, Ni and Ba.

In general, for FA media where both species were grown, B, Mo, Cr and Ba occurred in higher concentrations for most weeks (Figures 4.21 to 4.24). Trace metals such as Zn, Mn, Fe, Cu and Ni recorded lower concentrations for most weeks. There is a marked difference in Mn, Mo, Fe, and Cr trends between the two plant species in the FA media. However, the Cu trend was different only in the first week for the two plant species, but similar throughout until the eleventh week. While Mo started high for *S. Oleracea L* (Figure 4.23) and continued to decline for all the weeks, *B. juncea* (Figure 4.21) started low, increased in two weeks and five weeks then it started decreasing at the seventh week and continued to decrease until the eleventh week. This marked difference might be associated with pH levels which showed a little difference in leachates for the two plant species in different weeks even though trends were similar. Also the ability of plant uptake of chemical elements from FA media solution might have been different resulting in the difference of such chemical elements' availability in the leachates.

Opposite was the case with B, Zn, Ni and Ba trends which showed to be decreasing over time for the two plant species with no big difference in terms of their concentrations. These chemical elements followed a similar trend and pH levels might have influenced the decreasing trend over time for the chemical elements. Where there were high pH levels there was high concentrations of many chemical elements because it allows for a faster dissolution of chemical elements. Even though some trends of FA leachates differed for the two plant species in-between the weeks, in the eleventh week all of them ended up declining to lower concentrations than when they started off in the first week.

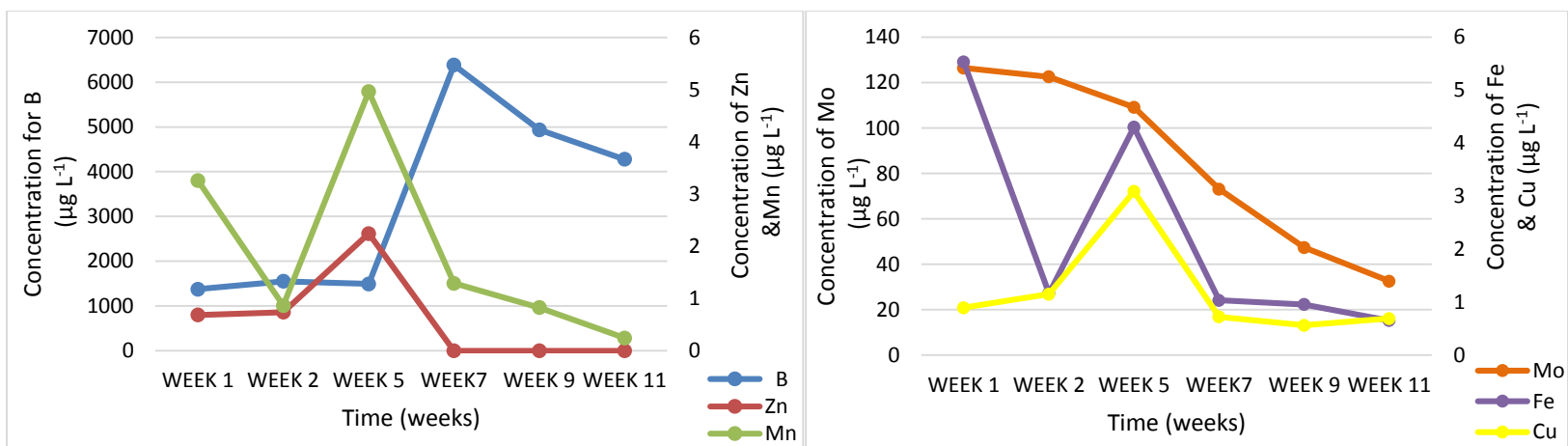


Figure 4.23: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.* for essential elements B, Zn, Mn, Fe, Mo and Cu.

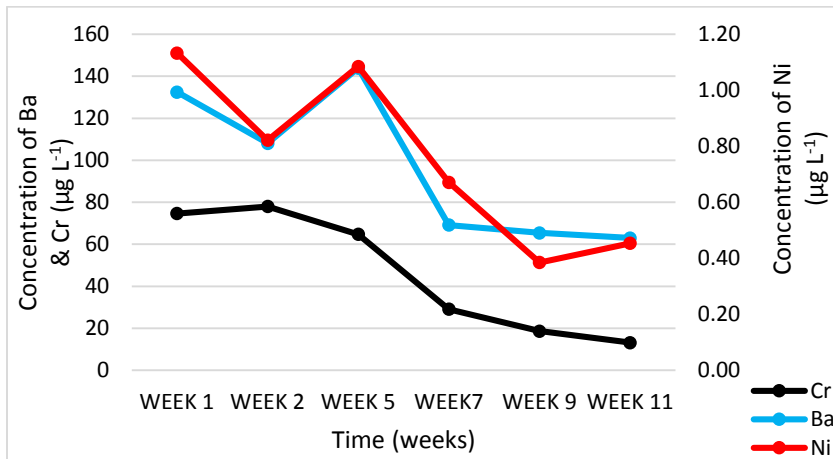


Figure 4.24: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.* for non-essential elements Cr, Ni and Ba

4.5.3 Temporal variation of the chemical elements of leachates from pots of FA+soil growth media for both plants

The Figures 4.25 and 4.26 shows the chemical elements concentrations obtained from FA+soil pots growing *B. juncea* for essential elements (B, Zn, Mn, Fe, Mo and Cu) and non-essential elements (Cr, Ni and Ba) while Figures 4.27 and 4.28 shows similar concentrations but plant species being *S. Oleracea L.*

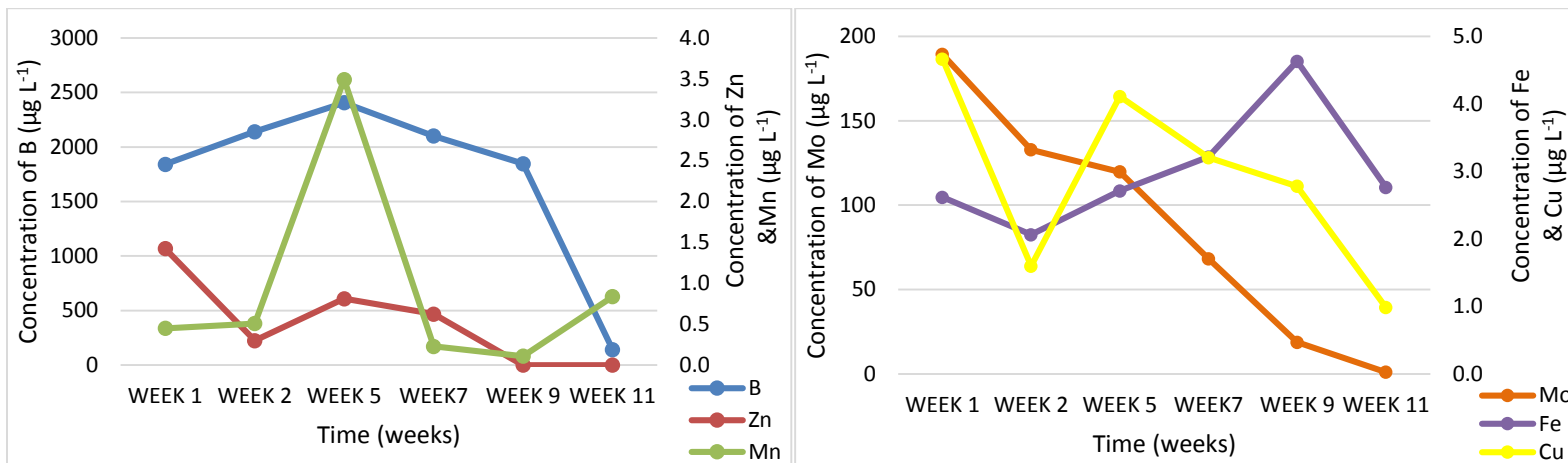


Figure 4.25: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *B. juncea* for essential elements B, Zn, Mn, Fe, Mo and Cu.

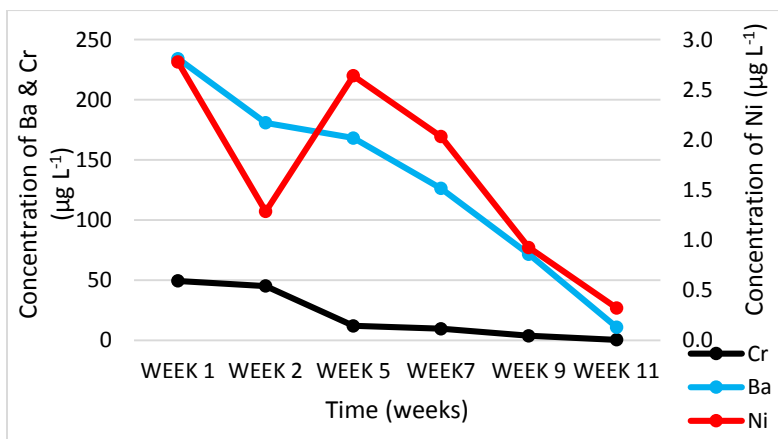


Figure 4.26: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *B. juncea* for non-essential elements Cr, Ni and Ba.

In the FA+soil media, B, Mo and Ba occurred in high concentrations for most weeks for both *B. juncea* and *S. Oleracea L* (Figures 4.25 to 4.28). Figure 4.27 shows Mn occurring at the fifth week in high concentrations but decreased towards the end for *S. Oleracea L*, while similar trend was observed for *B. juncea* (Figure 4.25) with a bit of increase in the eleventh week. Other chemical elements like Zn, Fe, Cu and Ni recorded low concentrations for most weeks for both *B. juncea* and *S. Oleracea L* in FA+soil leachates.

There is a difference in the trends of B, Fe, Cu and Ni for both species, while there is a common trend for Mo and Cr for both species. Ba on the other hand shows similar decreasing trend starting at the fifth week until the eleventh week for both species. However, the only difference can be noticed at the second week of leachate collection in the pot culture experiments as for *B. juncea* the second week shows decrease (Figure 4.26) while there is increase for *S. Oleracea L* in the second week (Figure 4.28). After all, there is a common decrease in the eleventh week for all the chemical elements under study for both *B. juncea* and *S. Oleracea L*.

High concentrations of non-essential elements like Cr and Ba could cause growth problem for both investigated plant species. On the other hand, essential elements such as B, Mn and Mo also occurred in high concentrations which can be good for plants but can cause toxicity problems if they exceed normal amounts that plants need. It is envisaged that the investigated plant species would tolerate such concentrations of chemical elements in their system. There is no marked difference in the concentrations of chemical elements in leachates where *B. juncea* and *S. Oleracea L* was grown. The implication is that these species will grow taking up similar chemical elements because of their availability in leachates when growing even though there will be a slight difference in the concentrations of such chemical elements.

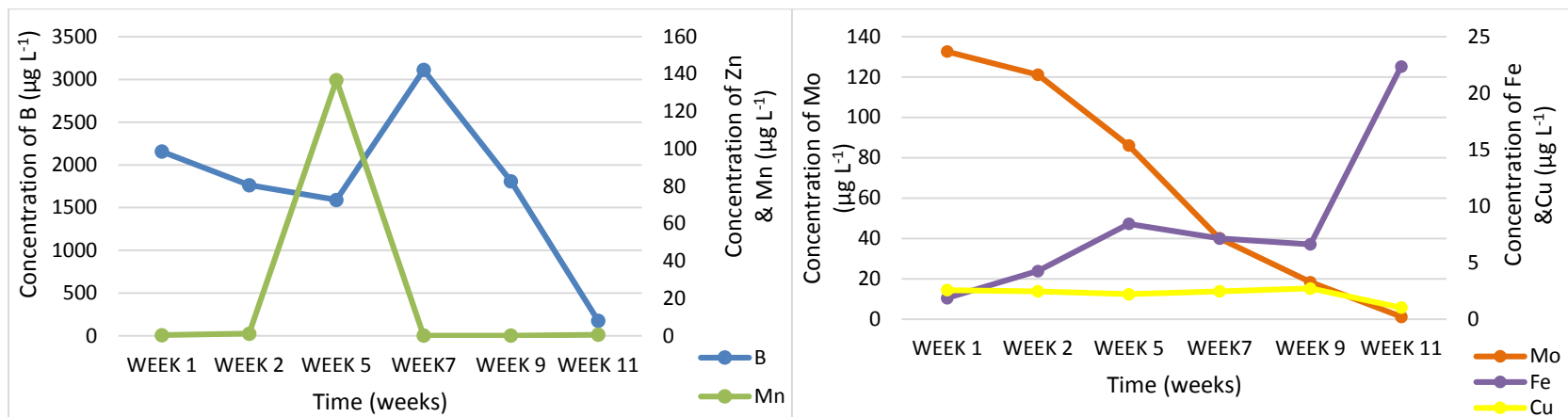


Figure 4.27: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *S. Oleracea L* for essential elements B, Zn, Mn, Fe, Mo and Cu.

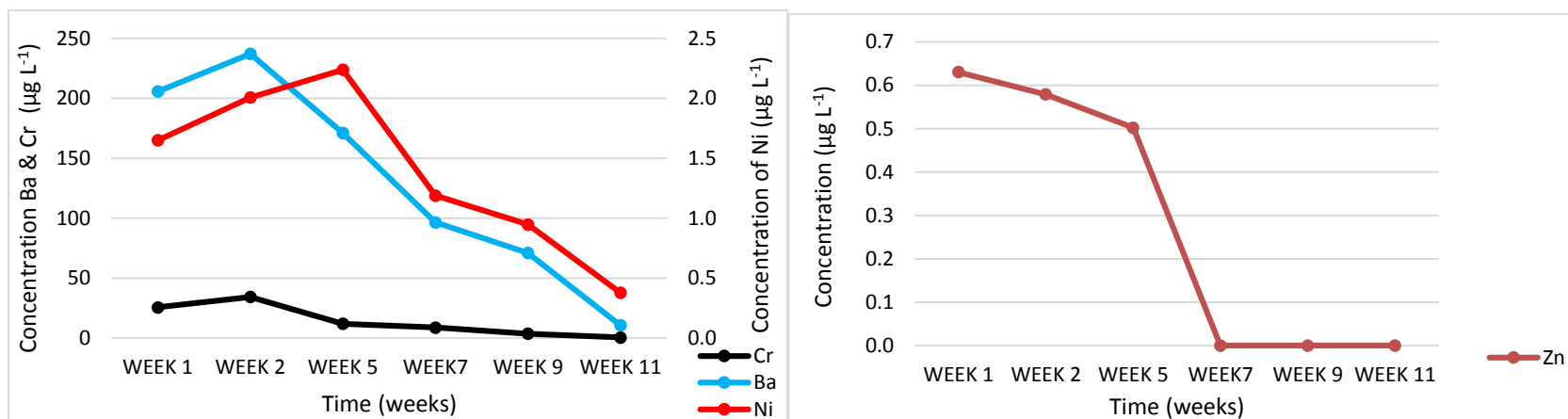


Figure 4.28: Chemical elements concentrations in leachates obtained from FA+soil mixture pots growing *S. Oleracea L* for non-essential elements Cr, Ni and Ba.

4.5.4 Temporal variation of the chemical elements of leachates from pots of FA growth media with no plants grown

The Figures 4.29 and 4.30 shows the chemical elements concentrations obtained from FA pots growing no plant species for essential elements (B, Zn, Mn, Fe, Mo and Cu) and non-essential elements (Cr, Ni and Ba).

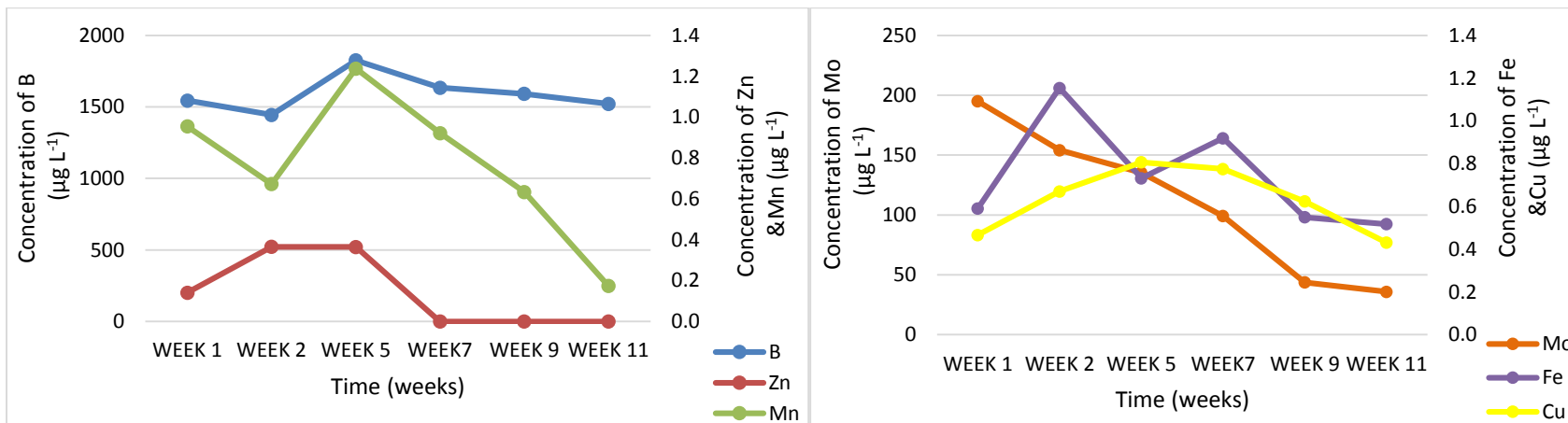


Figure 4.29: Chemical elements concentrations obtained from FA pots where no plants were grown; for essential elements B, Zn, Mn, Fe, Mo and Cu.

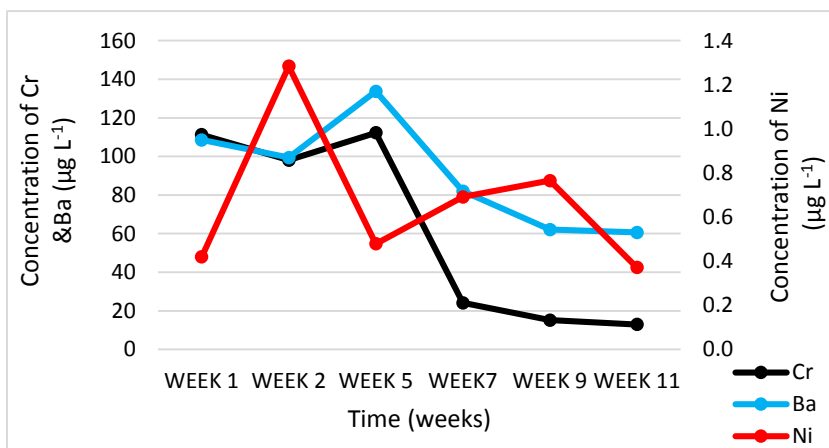


Figure 4.30: Chemical elements concentrations in leachates obtained from FA pots where no plants were grown; for non-essential elements Cr, Ni and Ba.

The Figures 4.21 to 4.24 where two plant species were grown in FA media; and Figures 4.29 and 4.30 where no plants were grown shows that B, Ba, Cr and Mo had the highest concentrations for all the figures while Cu, Zn, Ni, Mn and Fe were the lowest over time. When comparing FA media without plants and the one with *B. juncea* as a plant species, the trends of chemical elements are similar for Zn, Cr and Ba, while they are different for Fe, Mo, Cu, B, Mn and Ni. Ba trends were similar for both growth media (no plants and where there were plants) as it elevated on the fifth week and decreased in the seventh week of leachate collection. However, where *S. Oleracea L* was the plant species, the Ba, Mo, Mn, Cu and Zn trends were similar to that of FA leachates where no plants were grown but different for B, Fe, Ni and Cr. This shows that the growth of plant species in FA did have some effect on the availability of chemical elements in leachates of both growth media over time with respect to their trends. Hence there was a remarkable difference between leachates from FA media where no plants were grown and FA media where plants were grown for specific chemical elements with respect to the two plant species under study. The only common trend was that of Ba which was the same for *B. juncea* and *S. Oleracea L* when comparing it to leachates of FA media where no plants were grown.

After all, for all the chemical elements in both FA media (one with plants and the other without plants), the trends showed to have declined and ending up very low in the eleventh week. This shows that chemical elements were being washed away during irrigation (as not all leachates coming out of the pots were analysed) and others uptaken by plants where there was growth.

4.5.5 Comparison of soil and FA media leachates for both species

The results for the leachates of FA and soil media turned out to be considerably different since other chemical elements which occurred in high concentrations at soil media occurred in lower concentrations in FA media and vice versa. However, in both soil and FA media, essential and non-essential elements were present but in different concentrations. The non-essential chemical element Ba occurred at higher concentrations in leachates of both growth media and it might not be good for plants since they do not need it when growing. This suggests that plants which will be growing in FA and this type of soil media will have to deal with issues like toxification from these non-essential chemical elements. This is worse for soil media since also Cr and Ni which are non-essential chemical elements occurred in higher concentrations than in the FA media (other non-essential elements were not reported but appear in the appendix). This can lead to low growth rate, effects on the normal functioning of the plants' metabolism, or even death of plants if these plants take up much of these non-essential elements.

According to the Department of Environmental Affairs (DEA, 2012) guidelines with respect to the national norms and standards for the remediation of contaminated land and soil quality, it can be noted that this type of soil will not have much negative impacts on the environment since the chemical elements possessed by its leachates is below or within the recommended limits by DEA

especially for Cu, Mn, Cr, Ni and Zn as presented in the appendix as Table A8 (compared to Figure 4.17 to 4.20). The limits for other chemical elements like Fe, Ba, B and Mo were not reported by DEA. With respect to the results of anions obtained from batch experiments for the soil reported in Part 1 of Chapter 4, sub-section 4.3 in Table 4.3, it can be compared with the DEA standards in Table A9 in the appendix to see if this type of soil possesses recommended limits for the anions. It is clear that this type of soil has F^- , Cl^- , NO_3^- and SO_4^{2-} anions that are very low and within the recommended limits by DEA. Hence, the availability of such anions makes the soil not to be an environmental hazard or pollutant.

In terms of amending the two types growth media, this type of soil can be suitable and environmental friendly. But the concern may be on concentrations of different chemical elements coming with the soil since it will increase the non-essential chemical elements in the amendment with FA. This is because FA media already has those chemical elements which can make them to exceed the recommended limits when amendment is done. Hence soils with lesser concentrations of non-essential chemical elements can be recommended for amendment with this type of FA to be environmental friendly and good for plants.

4.5.6 Statistical evaluation of the chemical elements trends of leachates for the different growth media

Tables 4.6 to 4.10 depict statistical summary of the evaluation of different chemical elements trends of leachates for the different growth media. In Tables 4.6 to 4.8 is where *B. juncea* and *S. Olearacea L* are compared in terms of the chemical elements which were available in their leachates for the similar growth media to find out if the difference was significant or not (whether the difference was due to chance or not); i.e. difference between plants. This was done to be able to determine if the difference in plant species had effect on the occurrence or bioavailability of chemical elements in leachates from each growth medium. The t - test was used here but where data was not suitable for t – test, Mann-Whitney test was used in the analysis to obtain accurate results.

While Tables 4.9 and 4.10 presents the difference between the four growth media (soil, FA, soil+FA with a certain plant species and FA only - without plants) with respect to the occurrence of different chemical elements in leachates. This was done to compare these four growth media and find out if they have effect on the occurrence of such chemical elements in the leachate and also to find out how significant the difference was for a similar plant species. The Kruskal-Wallis Statistic (KW) was used here but where data was not suitable for it, ANOVA-Bartlett test was used in the analysis to obtain accurate results.

Table 4.6 shows the occurrence of different chemical elements in leachates from pots where *B. juncea* and *S. Oleracea L* was grown in the soil media.

Table 4.6: Comparison of chemical elements between *B. juncea* and *S. Oleracea L* grown in soil media.

SOIL MEDIA	P value	Degrees of freedom	t - test	Mann-Whitney test	Significance
B	0.0411			5.000	✓
Cr	0.5887			14.000	X
Mn	0.6908	10	0.4095		X
Ni	0.7655	10	0.3065		X
Cu	0.8182			16.000	X
Zn	0.5887			14.000	X
Mo	0.9361			17.000	X
Ba	0.4848			13.000	X
Fe	0.0260			4.000	✓

Note:

X - not significant

✓ - significant

As shown in Table 4.6, for all the other chemical elements (Cr, Mn, Ni, Cu, Zn, Mo and Ba) there was no significant difference in their occurrence from soil media leachates between *B. juncea* and *S. Oleracea L* with exception of B and Fe. Thus, there was a significant difference between the leachate chemistry from soil media where both plants were grown with respect to B and Fe only. In all the investigated chemical elements, there was a slight difference which occurred in the same growth media (i.e. soil). In case of B and Fe, the difference might have been caused by the difference in the speed and ability of the plants to uptake such chemical elements. Also the fact that these plants are not the same could have caused such difference as they behave differently.

Table 4.7 shows the occurrence of different chemical elements in leachates from pots where *B. juncea* and *S. Oleracea L* was grown in the FA media.

Table 4.7: Comparison of chemical elements between *B. juncea* and *S. Oleracea L* grown in FA media.

FA MEDIA	P value	Degrees of freedom	t - test	Mann-Whitney test	Significance
B	0.9965	10	0.004472		X
Cr	0.8764	10	0.1595		X
Mn	0.3939			12.000	X
Ni	0.4530	5	0.8133		X
Cu	>0.999			17.500	X
Zn	0.4143			12.500	X
Mo	0.5647	10	0.5955		X
Ba	0.8237	10	0.2287		X
Fe	>0.999			18.000	X

Note:

X - not significant

✓ - significant

Table 4.7 shows that absolutely no significant difference was found. For each investigated chemical elements, there was no P value which was less than 0.05 and therefore the slight difference observed from previous graphs between leachates of FA media where *B. juncea* and *S. Oleracea L* were grown can be due to difference in plant species. However, this suggest that the investigated plant species have similar capacity in terms of the uptake of chemical elements as the difference is not significant enough.

Table 4.8 shows the occurrence of different chemical elements in leachates from pots where *B. juncea* and *S. Oleracea L* was grown in the FA+soil media.

Table 4.8: Comparison of chemical elements between *B. juncea* and *S. Oleracea L* grown in soil+FA media.

SOIL+FA MEDIA	P value	Degrees of freedom	t - test	Mann-Whitney test	Significance
B	0.6991			15.000	X
Cr	0.5781	10	0.5748		X
Mn	0.9372			17.000	X
Ni	0.3836	5	0.9546		X
Cu	0.3303	10	1.023		X
Zn	0.3530	10	0.9740		X
Mo	0.5693	10	0.5885		X
Ba	0.9994	10	0.0007925		X
Fe	0.0931			7.000	X

Note:

X - not significant

✓ - significant

There was some similarity in Table 4.7 for FA and Table 4.8 for FA+soil media since absolutely no significant difference was found for both of them for all chemical elements. This might have been a result of mixing FA and soil here as a medium of growth since in Table 4.6 for soil media, Fe showed a significant difference between leachates from media where *B. juncea* and *S. Oleracea L* were grown. But in this case, FA media seems to be the one that suppressed that difference since Fe in Table 4.7 showed no significant difference. It can be concluded that the little difference that may be found is not significant enough since statistical results confirms that there was no significant difference between the leachates where these two species were grown in terms chemical elements availability in leachates.

Table 4.9 shows comparison of leachates from all sets of growth media for the same kind of specie which is *B. juncea* in this case.

Table 4.9: Chemical elements comparisons between four sets of growth media (soil, FA, soil+FA with *B. juncea* and FA only - without plants).

<i>B. juncea</i>	P value	Kruskal-Wallis Statistic (KW)	ANOVA-Bartlett test	Significance
B	0.0022	14.599		✓ *
Cr	0.0333	8.720		✓
Mn	0.0019	14.926		✓ *
Ni	0.0444		71.536	✓
Cu	0.0008	16.642		✓ **
Zn	0.0012	14.688		✓ *
Mo	0.0076		43.038	✓ *
Ba	0.3523	3.267		X
Fe	0.0003	18.500		✓ **

Note:

- X - not significant
- ✓ - significant
- ✓ * - very significant
- ✓ ** - extremely significant

Table 4.9 can clearly show that there was considerable difference in the availability of different chemical elements in leachates from different growth media. This is attributed to the different chemical characteristics of each set of growth media before continuous irrigation. The significant difference was extremely high for Fe and Cu, while it was very significant for Mo, Mn and B; Cr and Ni had significance difference but not a strong one for same plant species (*B. juncea*) in different growth media. Ba did not show significant difference for all the growth media sets since it had a P value of 0.3523 which is very high. The difference which can be seen between leachates from all these four sets can be further confirmed by these statistical results that indeed there is a difference between the growth media sets as would be expected. Hence, each growth media coming into play with its own physicochemical characteristics.

Table 4.10 shows comparison of leachates from all sets of growth media for the same kind of species which is *S. Oleracea L.* in this case.

Table 4.10: Chemical elements comparisons between four sets of growth media (soil, FA, soil+FA with *S. Oleracea L.* and FA only - without plants).

<i>S. Oleracea L.</i>	P value	Kruskal-Wallis Statistic (KW)	ANOVA-Bartlett test	Significance
B	0.0019		29.99	✓ *
Cr	0.0448	8.060		✓
Mn	0.0147	10.514		✓
Ni	0.0158		79.080	✓
Cu	0.0013	15.647		✓ *
Zn	0.0024	14.392		✓ *
Mo	0.0032	13.799		✓ *
Ba	0.3896	3.013		X
Fe	0.0002	19.580		✓ **

Note:

- X - not significant
- ✓ - significant
- ✓ * - very significant
- ✓ ** - extremely significant

Table 4.10 for *S. Oleracea L.* is similar to the one for *B. juncea* at Table 4.9 since there was a significant difference for all chemical elements for different sets, except for Ba which showed no significant difference between the leachates from the four sets. This similarity might have emerged from statistical results in Table 4.6 to 4.8 since generally there was no big difference in chemical elements for the leachates of the two species of plants. The only difference is that Cu showed extremely high significance in Table 4.9 while here in Table 4.10 the significance was no longer extreme but just significant; also Mn was very significant in Table 4.9 while in Table 4.10 Mn was just significant.

4.5.7 Conclusion

In summary, the raw FA from Grootvlei power station started with an alkaline pH of 10.62 (same as in batch experiments - Part 1) but this changed over time as FA was irrigated with water and it ends up losing its alkalinity to neutral pH levels. There was also a decrease in the EC of the FA due to dissolution of soluble major oxides, which was promoted by continuous water irrigation in the FA rather than plant growth. Even though some chemical elements like B, Mn, Fe and Ba were occurring at higher concentrations in leachates for most weeks in the pot culture experiments; it was observed that in the eleventh week of leachate collection all these chemical elements plummet to very low concentrations. This suggest that these chemical elements can be reduced over time as

plants are being irrigated which is either due to uptake by plants or washed off with water. The similar trend was observed for soil and FA+soil growth media. There was a significant difference for different chemical elements of leachates from different growth media for each plant species (*B. juncea* and *S. Olearcea L*). In conclusion, the chemical elements present in leachate solution are readily available for plants uptake and these results are important for making concluding remarks in the phytoremediation of FA.

Part 3: Assessment of the growth of *Brassica juncea* and *Spinacia leracea L.* in pot culture experiments grown in FA and soil

4.6 Introduction

This Part 3 of Chapter 4 focuses on the biomass estimation, growth rate and the chlorophyll content of the plants during the pot culture experiments. The results obtained from the growth assessment experiments are shown in Figures 4.31 to 4.39.

4.6.1 Biomass estimation of *B. juncea* and *S. Oleracea L.* in different growth media

The above and below ground biomass of *B. juncea* and *S. Oleracea L.* are presented in Figures 4.31 and 4.32 for the period of 115 days. The results were obtained by weighing plant parts after each harvest in pot culture experiments.

Figure 4.31 shows the above and below ground biomass for *B. juncea* in different growth media in terms of weight with respect to different plant parts.

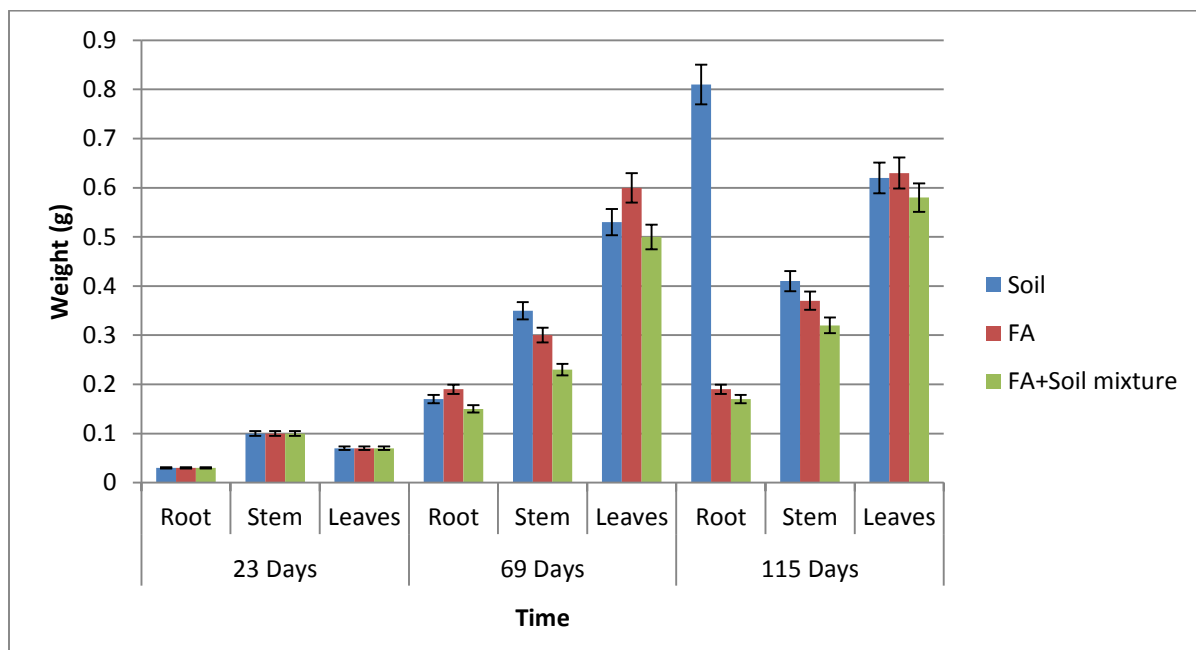


Figure 4.31: Above and below ground biomass for *B. juncea* in different growth media.

The overall biomass of *B. juncea* for all the considered media showed increase from 23 to 115 days (Figure 4.31). The roots of *B. juncea* in soil media recorded the highest biomass than other parts of plants for all growth medium during the experiments especially in 115 days with an

increase from 0.17 g to 0.81 g for soil media. This indicates good survival skills of the *B. juncea* species in soil media as well as the FA and FA+soil media.

However, the leaves of *B. juncea* recorded a higher biomass in the FA media than in soil media and the FA+soil media but was equal in 23 days of pot culture experiments. In the previous study reported by Jamil et al. (2009), different case was observed since biomass content of *Jatropha curcas* was much lower in 100 % FA when compared with control/soil. Generally, there is an increasing trend in the biomass of plant parts for the three growth media over a period of 115 days. This trend is expected or common for plants that are growing.

Figure 4.32 shows the above and below ground biomass for *S. Oleracea L* in different growth media in terms of weight with respect to different plant parts.

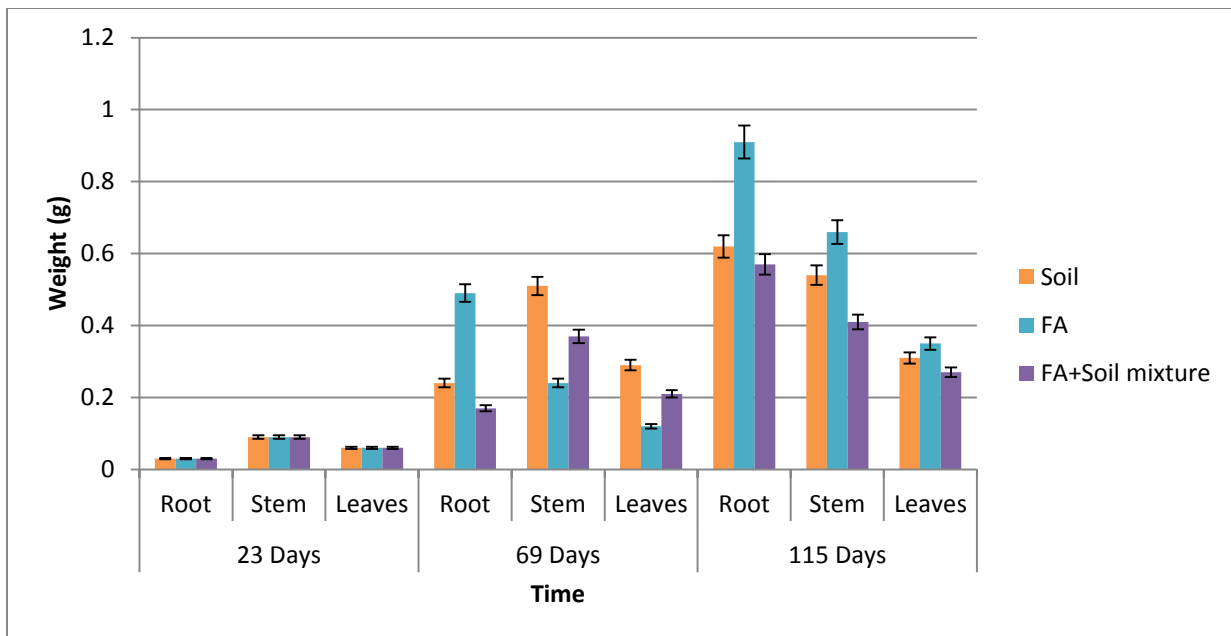


Figure 4.32: Above and below ground biomass for *S. Oleracea L* in different growth media.

Figure 4.32 shows that *S. Oleracea L* recorded the highest root biomass and its constant increase over time in FA medium than in the soil or FA+soil medium except for 23 days were they were equal for all growth media. The results further show that *S. Oleracea L* has an increase in the average biomass of roots, stems and leaves for all growth media over time even though increase was not that great for the leaves. This is like the pattern observed in *B. juncea* plant species (Figure 4.31). This result trend suggests that *S. Oleracea L* can grow very well in FA media especially after some time as in 115 days the biomass showed to be higher than that of soil and FA+soil media for the three plant parts. Jamil et al. (2009) reported that the performance of their plants improved in FA which was amended with garden soil, and this was due to improved physicochemical

properties of the substrate and increased availability of nutrients to the plants resulting in increased biomass. Hence in the current study, the soil which was amended with FA could have reduced the availability of nutrients instead of adding them up since its biomass was lower in 115 days than that of the FA media, i.e. soil weakened the physicochemical properties of the FA.

4.6.2 Growth performance of *B. juncea* and *S. Oleracea L.* in different growth media

The growth rate of *B. juncea* and *S. Oleracea L.* in terms of height or length of plant parts are presented in Figures 4.33 and 4.34 for the period of 115 days for different growth media. The results were obtained by measuring plant parts after each harvest in pot culture experiments.

Figure 4.33 shows the growth rate for *B. juncea* in different growth media in terms of height with respect to different plant parts over time.

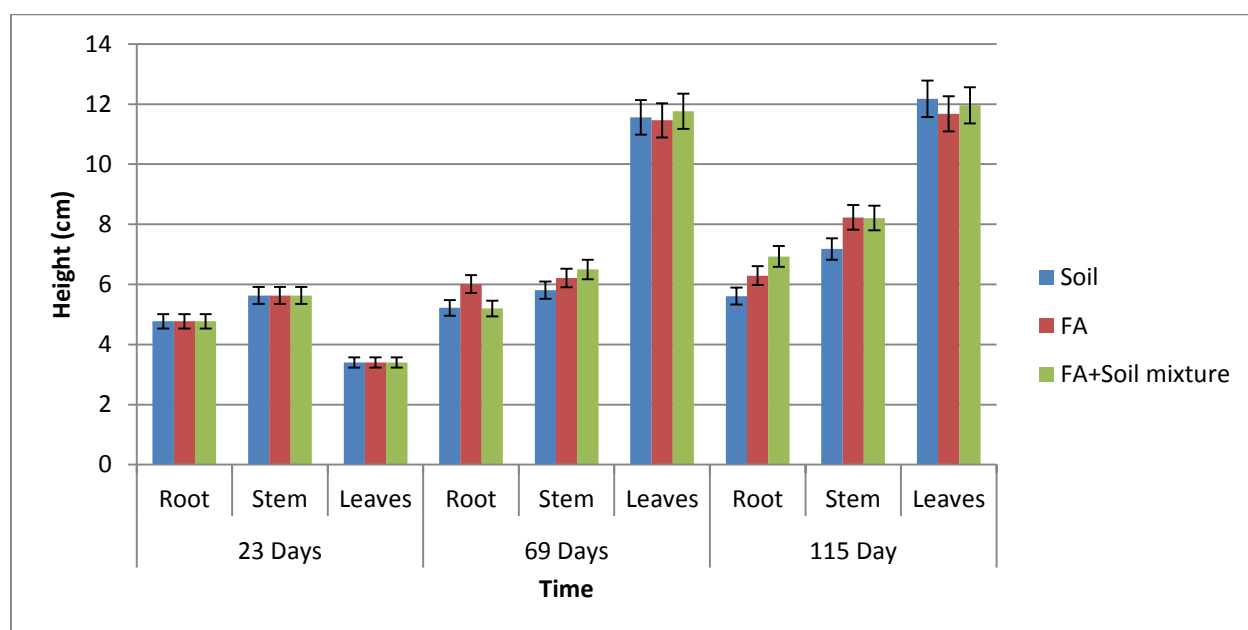


Figure 4.33: Growth rate of *B. juncea* for different growth media with time.

In this study, *B. juncea* shows similar survival patterns in the three growth media with respect to various plant parts as depicted in Figure 4.33. Although, leaf parts of the plants showed healthy growth over time for all the considered growth media. Their heights started very low at about 3.4 cm in 23 days and grew to above 10 cm in 69 days. The leaf growth was almost constant for FA media from 69 days until 115 days of pot culture experiments. However, there was an overall increase in growth rate of the *B. juncea* plants even though the increase was minimal over time for all the growth media. Different case was observed by Goswami and Das (2015) as they reported that in their study, plant height and biomass decreased with Cd treatments and when compared to control, there was a significant decline in dry biomass of root, shoot and leaves at all the Cd treated

sets of *Brassica* after 21 days. Gisbert et al. (2006) mentioned that growth of *Brassica* species had been reported to be greatly retarded by the accumulation of Cd which is not discussed in the current study.

When comparing the overall growth rate (Figure 4.33) and biomass (Figure 4.31) of *B. juncea* for all the three growth media, it is clear that leaves were doing very well as they had the highest biomass and lengths while stems followed and roots being the least. When biomass increased so does the growth rate or heights of plants; in the study by Goswami and Das (2015), decrease for both biomass and height was observed instead because of Cd presence.

Figure 4.34 shows the growth rate for *S. Oleracea L* in different growth media in terms of height with respect to different plant parts over time.

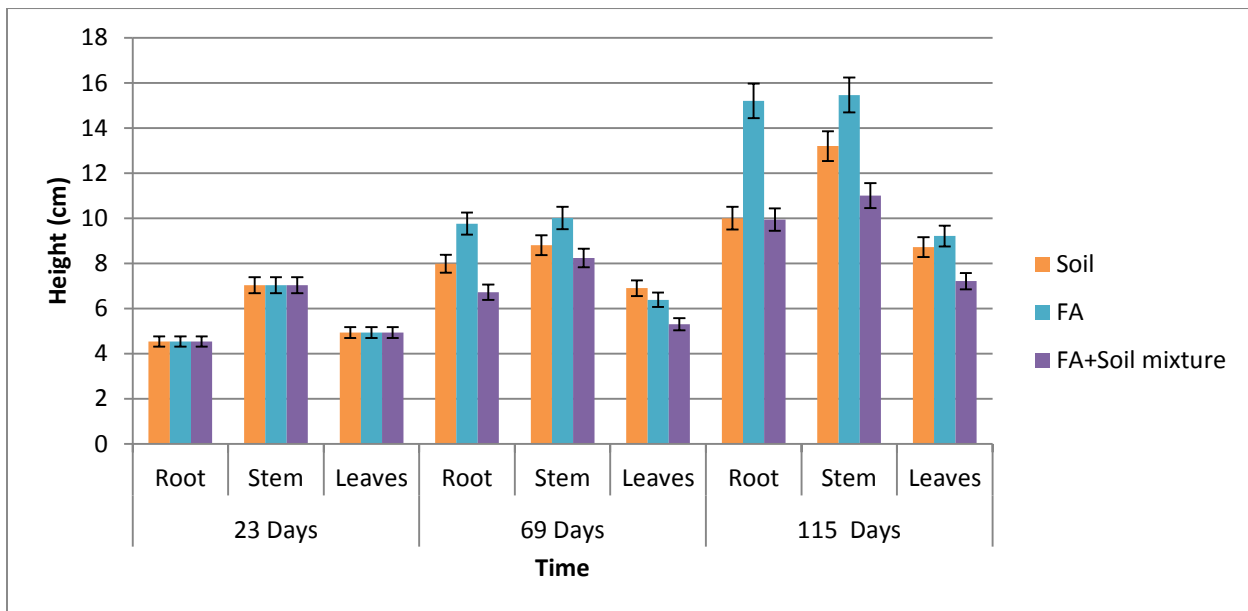


Figure 4.34: Growth rate of *S. Oleracea L* for different growth media with time.

Figure 4.34 shows the growth rate of roots, stems and leaves for *S. Oleracea L* being the highest and continuing to increase over time in the FA media than in the soil or FA+soil media, except for 23 days where growth rate was similar for all growth media. The results further show that *S. Oleracea L* exhibited an increase in the average growth rate of all plant parts for soil as well as the FA+soil media over time. The stems show growth increase after 69 days for all growth media similar to the roots. Jamil et al. (2009) stated that plants with extensive root system are capable of trapping more heavy metals due to better exploration in soil or growth media (Keller et al., 2003), hence such increase in the biomass and growth rate of roots is an advantage for the current study in phytoremediation.

Biomass of *S. Oleracea L* (Figure 4.32) was highest for roots, stems and leaves in the FA media in 115 days while also the growth rate (Figure 4.34) shows similar increasing trend. Therefore, it shows that the growth rate and biomass are directly proportional to each other, nonetheless the species grew very well in FA media than the other two growth media under study. Singh et al. (2011) observed a different trend since they discovered that the plant parts of vegetable crop were significantly increased at all amended in FA combinations as compared to the inoculated control set. In the current study, FA amendment had the least overall biomass and growth rate in the last day of pot culture experiments for all plant parts of *S. Oleracea L*.

4.6.3 Statistical evaluation of the biomass and growth rate trends of *B. juncea* and *S. Oleracea L* for the different growth media

Table 4.11 to 4.14 shows the statistical summary of the evaluation of biomass and growth rate of *B. juncea* and *S. Oleracea L* for the three growth media under study.

Table 4.11 shows comparison of *B. juncea* and *S. Oleracea L* in terms of their biomass for each growth media. These statistical test were done to find out if the species of plants has effects on the biomass of the plants grown on each growth media and t-test was used to test such difference in biomass of the two plant species.

Table 4.11: Comparison of *B. juncea* and *S. Oleracea L* with regards to their biomass for different growth media.

GROWTH MEDIA	P value	Degrees of freedom	t - test	Significance
Soil	0.7080	16	0.3813	X
FA	0.6826	16	0.4104	X
Soil+FA	0.9392	16	0.0775	X

Note:

- X - not significant
- ✓ - significant

There was no significant difference in biomass ($P \leq 0.05$) for all the growth media were *B. juncea* and *S. Oleracea L* where grown (Table 4.11). This suggests that there is no obvious effect of plant species grown in a specific media. Figures 4.31 and 4.32 shows some variation in growth rates of *B. juncea* and *S. Oleracea L* plant parts but statistically the difference was not significant. Jamil et al. (2009) reported similar results where there was no significant difference in biomass under the treatments they used.

Table 4.12 shows comparison of the different sets of growth media (soil, FA, soil+FA) with regards to the biomass for each plant species (either *B. juncea* or *S. Oleracea L.*). These statistical test were done to find out if the type of growth media has effects on the biomass of the plants grown on them and ANOVA-Bartlett test was used to test such difference in biomass.

Table 4.12: Comparison of the different sets of growth media (soil, FA, soil+FA) with regards to their biomass for *B. juncea* and *S. Oleracea L.*

PLANT SPECIES	P value	ANOVA-Bartlett test	Significance
<i>B. juncea</i>	0.6282	0.962	X
<i>S. Oleracea L.</i>	0.6877	2.501	X

Note:

- X - not significant
- ✓ - significant

In the Table 4.12, even with respect to all three sets of growth media where similar plant was grown; there was no significant difference for both *B. juncea* and *S. Oleracea L.* These statistical results confirm that the little difference we saw in Figures 4.33 and 4.34 was just not significant enough to have effect on the biomass of the plants.

Table 4.13 shows comparison of *B. juncea* and *S. Olearacea L* in terms of their growth rate for each growth media. These statistical test were done to find out if the species of plants has effects on the heights of the plants grown on each growth media and t-test was used to test such difference in biomass of the two plant species. Mann-Whitney test was used when data was not suitable for t-test to obtain accurate results.

Table 4.13: Comparison of *B. juncea* and *S. Oleracea L.* with regards to their growth rate for different growth media.

GROWTH MEDIA	P value	Degrees of freedom	t - test	Mann-Whitney test	Significance
Soil	0.1359			23.000	X
FA	0.1903			25.000	X
Soil+FA	0.7196	16	0.3654		X

Note:

- X - not significant
- ✓ - significant

Table 4.13 shows that the growth rate of *B. juncea* and *S. Oleracea L.* in each specific growth media (in soil, FA, FA+soil); does not indicate significant difference. Hence, statistically showing that the difference discussed earlier for Figures 4.33 and 4.34 was not significant enough to have effect on the growth rate of these plant species in each set of growth media.

Table 4.14 shows comparison of the different sets of growth media (soil, FA, soil+FA) with regards to the growth rate for each plant species (either *B. juncea* or *S. Oleracea L.*). These statistical test were done to find out if the type of growth media has effects on the heights of the plants grown on them and ANOVA-Bartlett test was used to test such difference in growth rate. Kruskal-Wallis Statistic (KW) was used when data was not suitable for ANOVA-Bartlett test to obtain accurate results.

Table 4.14: Comparison of the different sets of growth media (soil, FA, soil+FA) with regards to their growth rate for *B. juncea* and *S. Oleracea L.*

PLANT SPECIES	P value	Kruskal-Wallis Statistic (KW)	ANOVA-Bartlett test	Significance
<i>B. juncea</i>	0.8260	0.3824		X
<i>S. Oleracea L.</i>	0.7065		4.000	X

Note:

- X - Not significant
- ✓ - significant

Table 4.14 shows that for *B. juncea* plants grown on the three sets of growth media there was also no significant difference found over time ($P \leq 0.05$), similar case was observed with *S. Oleracea L.* grown on the three sets of growth media. After all, for both growth rate and biomass of the plant species under study, there was no significant difference. This has the implication that the type of growth media and the species of the plant did not have significant effect on the biomass and growth rate of plants in the current study.

4.6.4 Chlorophyll and carotenoid content of *B. juncea* and *S. Oleracea L.* in different growth media

Total leaf pigment includes chlorophyll-a, chlorophyll-b and carotenoids that are necessary for photosynthesis process as mentioned by Sumanta et al. (2014). They also stated that the content of foliar pigments varies depending on species, nonetheless variation in leaf pigments (chlorophylls and carotenoids) and its relation can be due to internal factors and environmental conditions. Chlorophyll a is the principal photosynthetic pigment while chlorophyll b is the accessory photosynthetic pigment. Sumanta et al. (2014) indicated that in excess of light carotenoids play an important role in photoprotection and also have ecological significance. Strzalka et al. (2003)

reported that they protect plants against over excitation in strong light and dissipate the excess of absorbed energy, they scavenge reactive oxygen species formed during photooxidative stress and moderate the effect of extreme temperatures. One of the important factors involved in the protective action of carotenoids is their influence on the molecular dynamics of membranes, making flowers and fruits colored, they play an important role in ecosystems, attracting pollen-dispersing insects and fruit-eating animals (Sumanta et al., 2014).

Figure 4.35 and 4.36 depicts chlorophyll a and b content in *B. juncea* for different growth media with time.

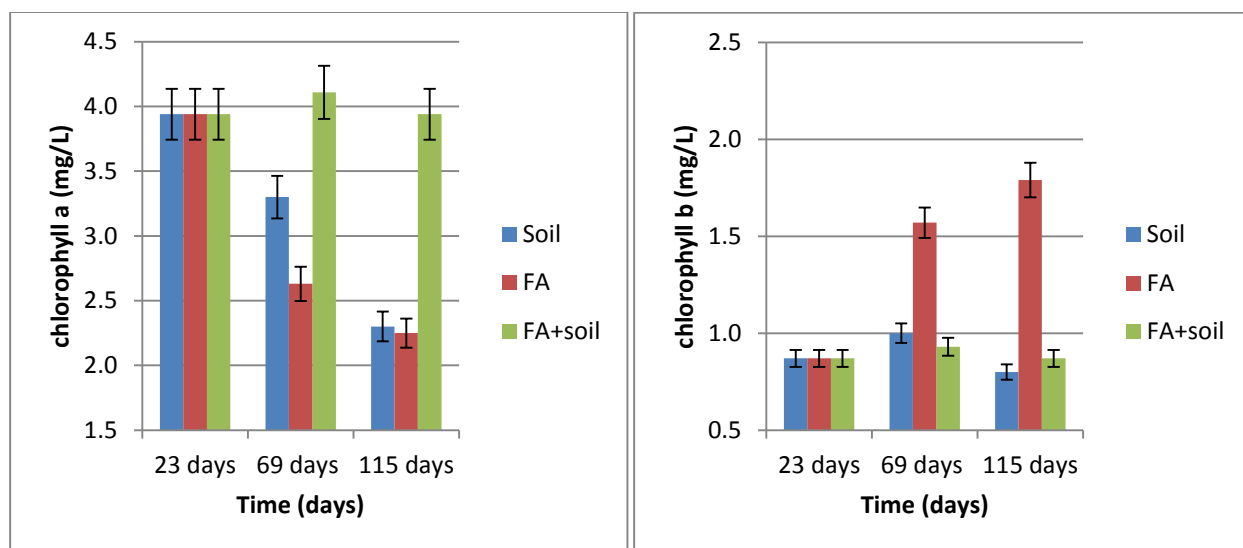


Figure 4.35: Chlorophyll a content in *B. juncea* for different growth media with time.

Figure 4.36: Chlorophyll b content in *B. juncea* for different growth media with time.

In the study, there is no common trend for chlorophyll a and b in the *B. juncea* species since there was increase overtime for other growth media while there was decrease for other growth media with time as shown in Figures 4.35 and 4.36. Chlorophyll a recorded in the FA+soil media increased to 4.11 mg L^{-1} over 69 days but in 115 days it decreased to about 3.94 mg L^{-1} which is the same as in 23 days (Figure 4.35). However, chlorophyll a for *B. juncea* growing in soil and in FA decreased over time.

As shown in Figure 4.36, the chlorophyll b showed a steady increase over time for *B. juncea* grown in FA medium; but for soil and FA+soil growth media, increase was observed at 69 days but decreased at 115 days. With these results, one cannot conclude which media is the best for the *B. juncea* plants, but FA only and FA+soil seems to be good in terms of chlorophyll a and b. These results can imply that the FA under study cannot be amended with this type of soil since it will not

promote higher levels of pigments, another type of soil with better physicochemical properties will have to be introduced.

Jamil et al. (2009) reported a reduction in chlorophyll pigment (a + b) in all the treatments when compared with control. However, chlorophyll pigment (a + b) content had been found to be more in EDTA treated plants than in non EDTA treated plants.

Figure 4.37 shows the carotenoid content which was found in *B. juncea* for different growth media from 23 days to 115 days.

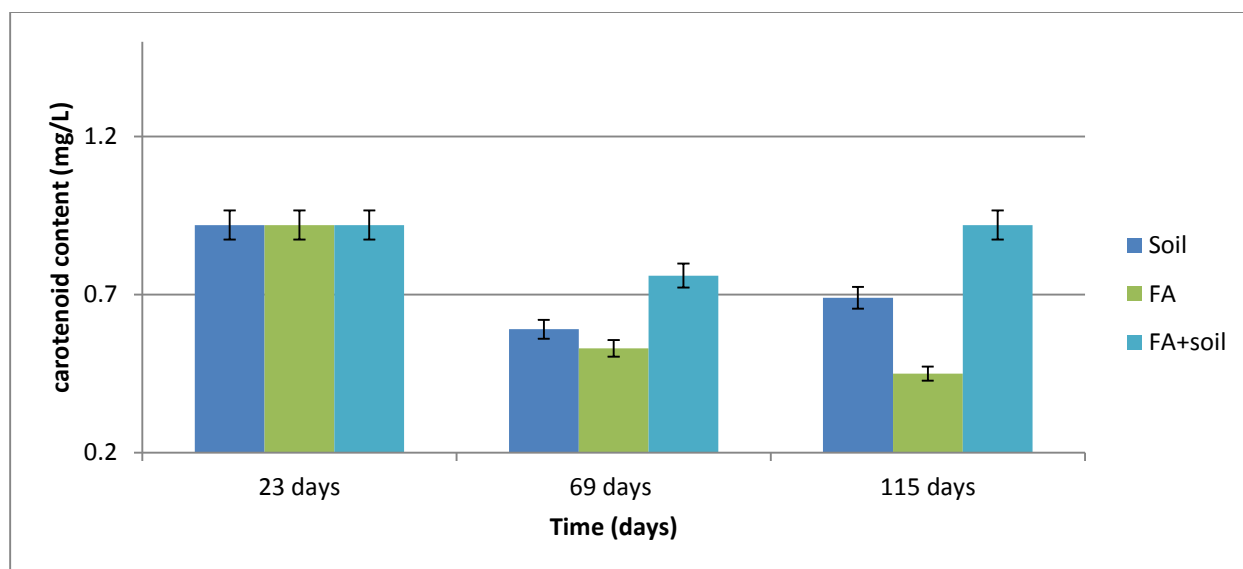


Figure 4.37: Carotenoid content in *B. juncea* for different growth media with time.

Figure 4.37 shows that the carotenoid content over the 11 weeks' period was high to the level of 0.92 mg L^{-1} for FA+soil media than for FA and soil media. This decrease of pigments experienced over time might have been due to the concentrations of different non-essential chemical elements which were found in the substrates or growth media as discussed in the previous section. The present findings can be supported by Nouairi et al. (2006), who found a significant reduction in the total chlorophyll content of the young leaves of *B. juncea* and *B. napus* on exposure to 10 mM Cd for 15 days (Goswami and Das, 2015). This decline in plant pigments (chlorophyll and carotenoid) might probably justify the steady decline in plant biomass with time. This is related to what Goswami and Das (2015) reported even though there was involvement of the exposure to cadmium in the study.

This decrease in pigments is not good for plants since they are very important in the growth of plants with respect to photosynthesis as mentioned at the start of this sub-section about pigments,

hence, FA+soil media is the best medium for growth of the *B. juncea* plants as it had increased carotenoid content over time.

Figure 4.38 and 4.39 depicts chlorophyll a and b content in *S. Oleracea L* for different growth media with time.

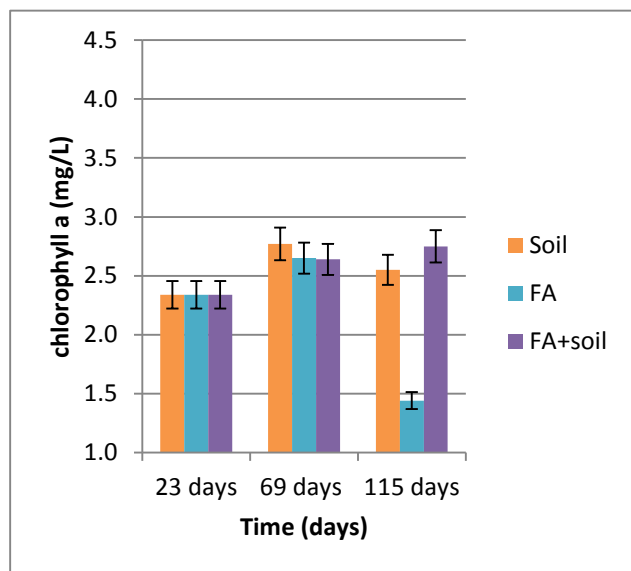


Figure 4.38: Chlorophyll a content in *S. Oleracea L* for different growth media with time.

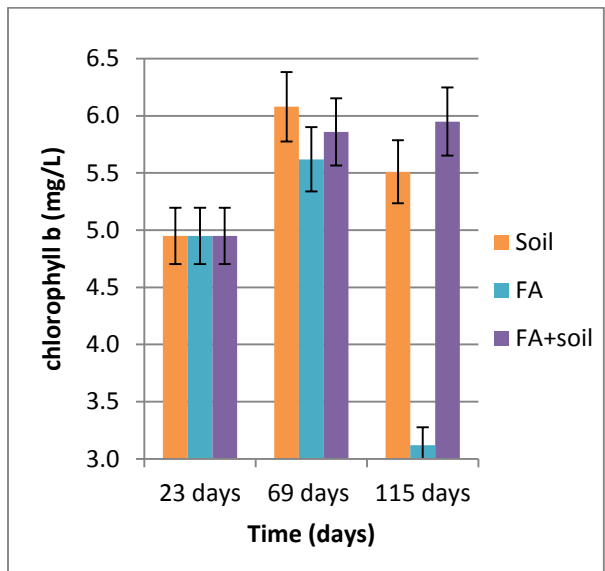


Figure 4.39: Chlorophyll b content in *S. Oleracea L* for different growth media with time.

There was no carotenoid content found in *S. Oleracea L* when calculations for chlorophyll content were done, hence it was below the detection limits of the spectrophotometer (SQ Pharo 100).

Figures 4.38 and 4.39 shows increase in the chlorophyll a content of *S. Oleracea L* over 69 days of pot culture experiments for all three sets of growth media. However, in the 115 days a decrease in chlorophyll a and b for *S. Oleracea L* grown in FA and soil only media was observed. Nevertheless, in the FA+soil medium, the increase continued even in 115 days. From these results, one can conclude that FA+soil media is good for growth of *S. Oleracea L* even though the carotenoid content was not detected.

The impact of decrease in plant pigments might have been due to the high concentrations of non-essential chemical elements which were found in the growth media used and hence causing failure in chlorophyll biosynthesis (Stobart et al., 1985).

4.6.5 Conclusion

In conclusion of this Part 3 of Chapter 4, the biomass, growth rate, chlorophyll and carotenoid content exist in a direct proportional relationship. In other words, increase in the biomass will lead to proportional increase in the growth rate, chlorophyll and carotenoid content. Therefore, in this present study, it cannot be easily concluded which media was most effective in growing a productive plant with high growth rate, biomass and pigments since there was an increasing and decreasing trends with time. The overall growth rate shows that *S. Oleracea L* was better than that of *B. juncea* especially in the FA media over time, while similar was the case with biomass of the two plant species. After all, even though *S. Oleracea L* had carotenoid content below detection limits, it had higher chlorophyll b than *B. juncea* for all growth media in general.

Part 4: Evaluation of the concentrations of chemical elements accumulated by roots, stems, and leaves of selected plants

4.7 Introduction

This part focuses on the concentrations of nine selected chemical elements which were found in different plant parts of *B. juncea* and *S. Oleracea L* in each pot during pot culture experiments. The mean concentrations of chemical elements and their standard deviations shown in Tables 4.15 to 4.20 are from different plant parts (i.e. leaf, stem and roots) of the two species under study which were grown in different sets of growth media (soil, FA and FA+soil) with respect to time. The concentrations for the seedlings were the same for all the pots to different growth media as this was the start of pot culture experiments leading to a standard deviation of zero for 5 pots. Plants were harvested three times, i.e. seedlings as the first harvest, then second and third harvest from pots. The raw data for Tables 4.15 to 4.20 are presented in Tables B1 to B13 in the appendix before any calculations were done, these are the results obtained straight from ICP-MS.

4.7.1 Evaluation of the chemical elements accumulated by *B. juncea* plant parts (leaf, stem and root) over a period of 115 days for soil media

In this subsection mean concentrations of chemical elements under study for different plant parts of *B. juncea* are discussed with respect to time in days. Plants were harvested from 5 pots for each harvest and average of them was taken. This was different for the second harvest (69 days) in Table 4.15 since plants were collected from only two pots because plants from other three pots had already died, hence average of two pots was recorded. Such deaths and challenges experienced by plants when growing were discussed in Chapter 3, Figure 4.40 shows one of the pot were plants died.



Figure 4.40: Dead plant of *B. juncea* in pot culture experiment.

Table 4.15 shows the mean \pm standard deviation of the nine chemical elements under study for the three harvests that were done for the soil media and results were reported in mg kg^{-1} .

Table 4.15: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the soil media with time.

mg kg^{-1}	1 st Harvest (23 days)			2 nd Harvest (69 days) n=2			3 rd Harvest (115 days) n=5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	0.60 \pm 0	0.51 \pm 0	5.76 \pm 0	15.43 \pm 12.74	1.11 \pm 1.13	4.33 \pm 2.6	21.73 \pm 15.81	8.24 \pm 2.68	19.50 \pm 21.32
Cr	2.07 \pm 0	2.43 \pm 0	4.59 \pm 0	15.25 \pm 7.59	3.49 \pm 0.72	3.15 \pm 0.21	12.64 \pm 0.76	9.76 \pm 1.20	4.61 \pm 1.68
Mn	11.17 \pm 0	13.74 \pm 0	64.22 \pm 0	170.00 \pm 84.85	83.00 \pm 15.56	44.00 \pm 5.66	167.00 \pm 18.38	110 \pm 14.14	54 \pm 31.11
Ni	1.46 \pm 0	1.53 \pm 0	2.61 \pm 0	4.92 \pm 2.91	1.80 \pm 0	1.22 \pm 0.21	3.24 \pm 0.06	3.55 \pm 0.69	1.60 \pm 0.47
Cu	1.82 \pm 0	2.09 \pm 0	6.33 \pm 0	17.68 \pm 11.63	5.45 \pm 1.02	4.06 \pm 0.19	12.52 \pm 0.36	11.41 \pm 0.99	4.72 \pm 1.55
Zn	4.24 \pm 0	6.07 \pm 0	24.10 \pm 0	62.50 \pm 61.47	12.26 \pm 4.50	14.79 \pm 7.41	24.78 \pm 13.92	43.06 \pm 8.75	17.95 \pm 2.53
Mo	0.05 \pm 0	0.06 \pm 0	0.20 \pm 0	0.75 \pm 0.23	0.02 \pm 0.02	0.08 \pm 0.07	0.83 \pm 0.70	0.13 \pm 0.01	0.37 \pm 0.36
Ba	1.57 \pm 0	2.60 \pm 0	18.28 \pm 0	41.05 \pm 32.54	9.69 \pm 2.25	15.55 \pm 9.39	30.62 \pm 0.46	26.56 \pm 0	20.95 \pm 9.43
Fe	190 \pm 0	254 \pm 0	1194 \pm 0	8523 \pm 4707.92	2075 \pm 456.79	1583 \pm 35.36	7517 \pm 60.81	5559 \pm 315.37	1969 \pm 810.34

In Table 4.15 for the period of 115 days, the chemical elements which were greatly accumulated by plant parts than others were Fe and Mn which were reported in mg kg^{-1} for both pots. Zn, Ba, Cr and Cu were also mainly accumulated by different plant parts; Ni, Cr and B followed with lower concentrations in plant parts, while Mo was accumulated in very low mean concentrations in plant parts. There is a common increasing trend of mean concentrations of most chemical elements in different plant parts with time generally. Subhashini et al. (2013) reported an increasing trend of Zn concentration over time accumulated by different plant species in their plant parts.

If we can compare the mean concentrations of this *B. juncea* plant species in 69 days and the ones in 23 days while they were still seedlings, it can clearly be observed that for Fe stems were having the highest concentration (Table 4.15), then roots followed while leaves had the lowest concentration. This changed in 69 days since leaves accumulated higher concentration of Fe, roots followed and then stems now had the lowest concentration of this Fe. In 115 days, similar trend continued but with increased values. This was similar for Cr, Mn and Cu since leaves had the

highest concentrations in 69 and 115 days while they were very low before in 23 days. *Jatropha curcas* studied by Jamil et al. (2009) showed to have the potential of accumulating chemical elements such as in the present study like Fe, Al, Cr, Cu and Mn.

This can further give us some sort of insight as to the fact that translocation could have been happening, but to say how effective it was will be emphasized by translocation factor in the next sections. The results in Table 4.15 show that *B. juncea* can accumulate chemical elements at different concentration even when planted on the soil media.

4.7.2 Evaluation of the chemical elements accumulated by *B. juncea* plant parts (leaf, stem and root) over a period of 115 days for FA media

Table 4.16 shows accumulation of chemical elements under study by *B. juncea* which was grown in the FA media only. These results were reported as mean \pm standard deviation at n=5 since all plants were available during harvesting time (no death this time).

Table 4.16: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the FA media with time.

mg kg ⁻¹	1 st Harvest (23 days)			2 nd Harvest (69 days) n=5			3 rd Harvest (115 days) n=5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	0.60 \pm 0	0.51 \pm 0	5.76 \pm 0	88.46 \pm 38.43	14.86 \pm 15.16	17.18 \pm 7.71	81.88 \pm 76.39	50.85 \pm 92.36	37.51 \pm 18.8
Cr	2.07 \pm 0	2.43 \pm 0	4.59 \pm 0	11.64 \pm 7.87	7.48 \pm 11.46	3.51 \pm 1.75	8.74 \pm 5.99	9.46 \pm 7.66	4.83 \pm 3.48
Mn	11.17 \pm 0	13.74 \pm 0	64.22 \pm 0	136.80 \pm 122.39	18 \pm 5.29	30.80 \pm 20.18	47.20 \pm 18.03	30.40 \pm 13.52	40 \pm 28.84
Ni	1.46 \pm 0	1.53 \pm 0	2.61 \pm 0	4.36 \pm 2.21	3.03 \pm 4.50	1.50 \pm 0.55	3.88 \pm 2.93	3.84 \pm 3.49	1.86 \pm 1.12
Cu	1.82 \pm 0	2.09 \pm 0	6.33 \pm 0	12.89 \pm 7.03	9.20 \pm 15.39	3.86 \pm 1.90	4.80 \pm 1.87	3.47 \pm 2.48	4.40 \pm 2.24
Zn	4.24 \pm 0	6.07 \pm 0	24.10 \pm 0	30.09 \pm 5.58	23.51 \pm 35.59	9.23 \pm 3.38	12.26 \pm 6.72	11.96 \pm 8.94	12.98 \pm 4.46
Mo	0.05 \pm 0	0.06 \pm 0	0.20 \pm 0	3.10 \pm 2.60	0.56 \pm 0.57	0.57 \pm 0.16	4.08 \pm 4.33	2.22 \pm 2.86	2.16 \pm 2.23
Ba	1.57 \pm 0	2.60 \pm 0	18.28 \pm 0	62.18 \pm 29.64	41.01 \pm 64.73	20.03 \pm 12.55	22.59 \pm 5.36	20.32 \pm 13.02	25.56 \pm 20.97
Fe	190 \pm 0	254 \pm 0	1194 \pm 0	5645.2 \pm 5117.17	826 \pm 380.23	1536 \pm 1044.7	1400 \pm 481.49	990.4 \pm 859.6	1570.4 \pm 1599.81

Fe, Mn, B, Ba and Zn were highly accumulated by the *B. juncea* plant parts than other chemical elements. It is clear that Fe had the highest mean concentrations recorded for leaves as plant parts

than others in 69 days but decreased in 115 days and this is so for many chemical elements as shown in Table 4.16 such as B, Ba, Cr, Mn, Ni, Cu and Zn for the leaves. Pandey (2012a) reported a similar scenario that all chemical elements accumulation in different plant parts was significantly higher in pre-monsoon than that of post-monsoon, except Cr in stem of post-monsoon. He also mentioned that this might be due to the run off and leaching effect that are capable of removing heavy metals from the FA, hence in the current study irrigation might be the cause. Even though there is decrease in the mean concentrations of chemical elements in plant parts, there is still accumulation taking place which can allow for translocation to happen.

4.7.3 Evaluation of the chemical elements accumulated by *B. juncea* plant parts (leaf, stem and roots) over a period of 115 days for FA+soil media

In this subsection mean concentrations of chemical elements under study for different plant parts of *B. juncea* are discussed with respect to time in days. Plants were harvested from 5 pots for each harvest and average of them was taken. This was different for the third harvest (115 days) in Table 4.17 since plants were collected from only three pots because plants from other two pots had already died, hence average of three pots was recorded.

Table 4.17 shows the mean \pm standard deviation of the nine chemical elements under study for the three harvests that were done for the FA+soil media and results were reported in mg kg^{-1} .

Table 4.17: Chemical elements' mean concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media with time.

mg kg^{-1}	1 st Harvest (23 days)			2 nd Harvest (69 days) n=5			3 rd Harvest (115 days) n=3		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	0.60 \pm 0	0.51 \pm 0	5.76 \pm 0	19.98 \pm 10.35	4.92 \pm 2.62	11.17 \pm 7.28	25.25 \pm 10.51	6.73 \pm 2.44	8.95 \pm 5.21
Cr	2.07 \pm 0	2.43 \pm 0	4.59 \pm 0	3.39 \pm 1.29	2.19 \pm 0.66	3.29 \pm 1.55	8.52 \pm 5.30	2.31 \pm 1.10	2.61 \pm 1.04
Mn	11.17 \pm 0	13.74 \pm 0	64.22 \pm 0	28.40 \pm 13.74	11.2 \pm 4.60	26.40 \pm 19.36	58.67 \pm 18.15	22 \pm 13.11	20 \pm 12.17
Ni	1.46 \pm 0	1.530	2.61 \pm 0	1.55 \pm 0.44	1.15 \pm 0.43	1.54 \pm 0.72	3.54 \pm 2.37	1.15 \pm 0.49	1.09 \pm 0.42
Cu	1.82 \pm 0	2.09 \pm 0	6.33 \pm 0	4.07 \pm 1.12	2.22 \pm 0.87	3.39 \pm 1.61	5.38 \pm 3.30	2.57 \pm 0.79	2.18 \pm 0.72
Zn	4.24 \pm 0	6.07 \pm 0	24.10 \pm 0	10.78 \pm 2.40	7.11 \pm 3.36	8.71 \pm 3.10	13.48 \pm 7.54	7.00 \pm 0.96	5.66 \pm 2.95
Mo	0.05 \pm 0	0.06 \pm 0	0.20 \pm 0	1.77 \pm 2.05	0.68 \pm 1.04	0.53 \pm 0.35	0.52 \pm 0.30	0.27 \pm 0.31	0.47 \pm 0.55
Ba	1.57 \pm 0	2.60 \pm 0	18.28 \pm 0	23.32 \pm 7.93	12.45 \pm 7.05	19.06 \pm 15.63	34.85 \pm 22.60	13.08 \pm 6.66	13.69 \pm 7.13
Fe	190 \pm 0	254 \pm 0	1194 \pm 0	1124 \pm 845.23	410.8 \pm 165.19	1240 \pm 1199.19	2428 \pm 1728.09	676.67 \pm 557.71	628.67 \pm 287.73

The accumulation of chemical elements in plant parts over time is not showing a common trend since other chemical elements increase with time in specific plant parts while others decrease. But generally, in Table 4.17, Fe is the chemical elements that is abundantly accumulated by plant parts and this is similar to the study by Pandey (2012b) where Fe was highly accumulated in roots and frond of the water fern. It was also mentioned by Maiti and Jaiswal (2008) that metal accumulation trend depends on some factors such as age and type of plant species, metal concentrations in FA, pH, and season of sampling. Mn, Ba, B and Zn followed Fe on their accumulation while Mo was the lowest.

4.7.4 Evaluation of the chemical elements accumulated by *S. Oleracea L* plant parts (leaf, stem and root) over a period of 115 days for soil media

In this subsection mean concentrations of chemical elements under study for different plant parts of *S. Oleracea L* are discussed with respect to time in days. Plants were harvested from 5 pots for each harvest and average of them was taken.

Table 4.18 shows the mean \pm standard deviation of the nine chemical elements under study for the three harvests that were done for the soil media and results were reported in mg kg^{-1} .

Table 4.18: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the soil media with time.

mg kg^{-1}	1 st Harvest (23 days)			2 nd Harvest (69 days) n=5			3 rd Harvest (115 days) n=5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	11.90 \pm 0	2.24 \pm 0	2.10 \pm 0	1.19 \pm 0.80	2.11 \pm 0.75	8.48 \pm 2.67	26.86 \pm 4.95	21.09 \pm 5.14	25.46 \pm 8.49
Cr	2.94 \pm 0	3.34 \pm 0	3.79 \pm 0	2.72 \pm 1.04	6.03 \pm 6.43	2.53 \pm 0.82	31.67 \pm 57.64	16.17 \pm 14.58	12.43 \pm 10.5
Mn	101.59 \pm 0	40.57 \pm 0	13.44 \pm 0	57.63 \pm 28.42	27.25 \pm 7.76	97.55 \pm 25.81	840.4 \pm 592.54	2991.6 \pm 1039.12	1341.6 \pm 181.8
Ni	1.58 \pm 0	2.04 \pm 0	1.53 \pm 0	2.39 \pm 1.05	2.36 \pm 2.19	1.82 \pm 0.22	3.22 \pm 0.78	7.06 \pm 1.54	3.28 \pm 0.54
Cu	7.29 \pm 0	5.74 \pm 0	2.13 \pm 0	2.32 \pm 1.09	2.34 \pm 1.36	3.29 \pm 1.37	9.64 \pm 1.90	13.48 \pm 4.39	8.23 \pm 2.02
Zn	27.12 \pm 0	10.540	4.89 \pm 0	7.40 \pm 2.36	6.92 \pm 1.95	17.02 \pm 3.04	25.69 \pm 9.65	58.04 \pm 19.35	32.17 \pm 9.98
Mo	0.28 \pm 0	0.09 \pm 0	0.10 \pm 0	0.08 \pm 0.03	0.39 \pm 0.61	0.13 \pm 0.03	0.22 \pm 0.06	0.17 \pm 0.08	0.13 \pm 0.01
Ba	23.82 \pm 0	8.84 \pm 0	4.79 \pm 0	8.53 \pm 3.79	11.62 \pm 5.06	19.75 \pm 13.24	58.00 \pm 24.95	148.95 \pm 46	96.91 \pm 53.17
Fe	328 \pm 0	916 \pm 0	150 \pm 0	336.4 \pm 218.42	466.4 \pm 398.18	183.2 \pm 65.26	452.8 \pm 181.5	315.2 \pm 140.86	189.6 \pm 46.74

Accumulation for all plant parts was recorded to be highest for Fe which was reported in mg kg^{-1} . The mean concentration of Fe was highest in root plant part than in leaves and stems, that is when we look at 69 and 23 days (seedlings in Table 4.18). This changed in 115 days although concentrations were still high but leaves accumulated the highest mean concentration. Even in the study by Morariu et al. (2013), similar scenario was observed where Fe was highly accumulated than other chemical elements. Although there are some plant parts where there is decrease over time in mean concentration, accumulation seems to be high for Mn, Ba, and Cu with Zn and Cr which has a drastic increase in mean concentration at 115 days than the days before.

4.7.5 Evaluation of the chemical elements accumulated by *S. Oleracea L* plant parts (leaf, stem and root) over a period of 115 days for FA media

In this subsection mean concentrations of chemical elements under study for different plant parts of *S. Oleracea L* are discussed with respect to time in days. Plants were harvested from 5 pots for each harvest and average of them was taken.

Table 4.19 shows the mean \pm standard deviation of the nine chemical elements under study for the three harvests that were done for the FA media and results were reported in mg kg^{-1} .

Table 4.19: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the FA media with time.

mg kg^{-1}	1 st Harvest (23 days)			2 nd Harvest (69 days) n=5			3 rd Harvest (115 days) n=5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	11.9 \pm 0	2.24 \pm 0	2.10 \pm 0	8.55 \pm 3.02	11.44 \pm 7.83	27.64 \pm 10.62	42.11 \pm 8.59	33.23 \pm 15.5	32.62 \pm 6.13
Cr	2.94 \pm 0	3.34 \pm 0	3.79 \pm 0	7.89 \pm 2.99	2.55 \pm 0.93	17.04 \pm 30.41	3.73 \pm 1.14	10.88 \pm 6.33	4.48 \pm 1.81
Mn	101.59 \pm 0	40.57 \pm 0	13.44 \pm 0	48.63 \pm 21.20	26.11 \pm 18.84	77.28 \pm 34.87	85.26 \pm 17.56	98.29 \pm 52.33	55.28 \pm 21.26
Ni	1.58 \pm 0	2.04 \pm 0	1.53 \pm 0	5.07 \pm 2.40	1.98 \pm 1.03	11.37 \pm 20.43	0.36 \pm 0.12	1.43 \pm 0.92	0.42 \pm 0.13
Cu	7.29 \pm 0	5.74 \pm 0	2.13 \pm 0	5.99 \pm 3.27	4.32 \pm 3.51	4.3 \pm 0.96	1.47 \pm 0.34	3.58 \pm 2.19	1.59 \pm 0.41
Zn	27.12 \pm 0	10.54 \pm 0	4.89 \pm 0	12.70 \pm 4.13	11.14 \pm 4.40	18.99 \pm 12.05	4.71 \pm 1.57	8.62 \pm 4.94	5.05 \pm 2.86
Mo	0.28 \pm 0	0.09 \pm 0	0.10 \pm 0	0.61 \pm 0.24	0.34 \pm 0.24	2.48 \pm 3.99	1.95 \pm 0.95	1.34 \pm 0.67	1.17 \pm 0.70
Ba	23.820 \pm	8.84 \pm 0	4.79 \pm 0	37.39 \pm 23.69	19.93 \pm 8.01	22.76 \pm 10.71	63.12 \pm 12.17	111.84 \pm 80.47	57.19 \pm 16.26
Fe	328 \pm 0	916 \pm 0	150 \pm 0	1277.2 \pm 1295.73	294.8 \pm 363.25	211.6 \pm 168.75	625.2 \pm 176.68	1334 \pm 992.13	831.2 \pm 243.11

Table 4.19 shows that accumulation is there for all plant parts but those chemical elements that are accumulated highly includes Fe, Mn, Ba, B and Zn for *S. Oleracea L*. Chemical elements like Cu, Ni, Cr and Mo were accumulated the least. The study by Jambhulkar and Juwarkar (2009) shows that the results of the metal analysis of the species under study indicated that Fe was accumulated to the greatest extent in vegetation followed by Mn, Ni, Zn, Cu, Cr and Pb. This is similar to what was observed in the current study. Zn accumulation in plant parts decreased drastically in 115 days and this might have been caused by dying of leaves as dead leaves were not analysed for the results and they have some concentrations when they fall off.

4.7.6 Evaluation of the chemical elements accumulated by *S. Oleracea L* plant parts (leaf, stem and root) over a period of 115 days for FA+soil media

In this subsection mean concentrations of chemical elements under study for different plant parts of *S. Oleracea L* are discussed with respect to time in days. Plants were harvested from 5 pots for each harvest and average of them was taken.

Table 4.20 shows the mean \pm standard deviation of the nine chemical elements under study for the three harvests that were done for the FA+soil media and results were reported in mg kg^{-1} .

Table 4.20: Chemical elements' mean concentrations in leaves, roots and stems of *S. Oleracea L* grown in the FA+soil media with time.

mg kg^{-1}	1 st Harvest (23 days)			2 nd Harvest (69 days) n=5			3 rd Harvest (115 days) n=5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	11.90 \pm 0	2.24 \pm 0	2.10 \pm 0	3.37 \pm 2.36	22.76 \pm 43.96	12.10 \pm 8.13	59.02 \pm 23.56	27.69 \pm 10.93	21.45 \pm 6.41
Cr	2.94 \pm 0	3.34 \pm 0	3.79 \pm 0	19.26 \pm 29	28.88 \pm 39.83	28.90 \pm 37.34	3.55 \pm 1.26	12.07 \pm 4.88	7.93 \pm 7.44
Mn	101.59 \pm 0	40.57 \pm 0	13.44 \pm 0	16.56 \pm 9.97	65.94 \pm 45.29	46.04 \pm 32.75	225.13 \pm 49.39	102.97 \pm 38.38	60.89 \pm 32.80
Ni	1.58 \pm 0	2.04 \pm 0	1.53 \pm 0	10.98 \pm 20.59	16.09 \pm 26.77	12.51 \pm 23.21	0.31 \pm 0.10	1.37 \pm 1.11	0.54 \pm 0.43
Cu	7.29 \pm 0	5.74 \pm 0	2.13 \pm 0	2.77 \pm 1.66	2.82 \pm 1.64	4.17 \pm 2.20	1.37 \pm 0.19	5.31 \pm 3.47	2.21 \pm 1.21
Zn	27.12 \pm 0	10.54 \pm 0	4.89 \pm 0	3.92 \pm 2.73	7.46 \pm 3.47	9.08 \pm 7.30	7.18 \pm 1.10	7.75 \pm 4.02	5.07 \pm 2.30
Mo	0.28 \pm 0	0.09 \pm 0	0.10 \pm 0	3.17 \pm 4.07	5.19 \pm 6.08	4.54 \pm 5.23	2.01 \pm 0.53	0.84 \pm 0.38	0.56 \pm 0.20
Ba	23.82 \pm 0	8.84 \pm 0	4.79 \pm 0	7.4 \pm 4.74	27.25 \pm 41.60	11.78 \pm 7.16	96.32 \pm 15.66	148.39 \pm 63.67	82.80 \pm 52.49
Fe	328 \pm 0	916 \pm 0	150 \pm 0	196 \pm 234.26	261.2 \pm 227.66	142.4 \pm 201.91	568.8 \pm 289.90	1574 \pm 1041.72	956 \pm 537.85

In Table 4.20, it can be noticed that Fe is still the major chemical elements being accumulated by plant parts and roots are accumulating the highest mean concentrations. Jambhulkar and Juwarkar (2009) mentioned that the rhizospheric microorganisms may interact symbiotically with roots to enhance the potential for metal uptake, because microorganisms may excrete organic compounds, which increase bioavailability, and facilitate the root absorption of essential metals such as Fe (Crowley et al., 1991). Also Jamil et al. (2009) reported similar results that Fe and Mn were retained more in roots while Cu, Al and Cr were translocated more to the shoot. The results show that the accumulation in most plant parts was higher in 69 days than it was in 23 days (when they were still seedlings). However, Ba, Mn, B and Cr followed as being highly accumulated in plant

parts while Zn, Ni, Cu and Mo fell under that category of the least accumulated chemical elements. The increased accumulation over time for some chemical elements might have been due to the amendment of soil with FA used as a media for the growth of *S. Oleracea L* as they were both strengthening the accumulation over time.

4.7.7 Comparison of the accumulation potential of *B. juncea* and *S. Oleracea L*

When comparing *B. juncea* and *S. Oleracea L* in the different growth media it is clear that accumulation is there for all plant parts but those chemical elements that are accumulated highly includes Fe, Mn, Ba, B and Zn for both *B. juncea* and *S. Oleracea L*. But when comparing their mean concentrations all the five chemical elements are highly accumulated by *B. juncea* plant parts than *S. Oleracea L*. Hence, *B. juncea* is expected to have better translocation than *S. Oleracea L* because of having highest accumulation in its plant parts. The study by Jambhulkar and Juwarkar (2009) discovered that *Cassia siamea* was found to accumulate all metals at higher concentrations compared to other species. The same study also shows that the results of the metal analysis of the species under study indicated that Fe was accumulated to the greatest extent in vegetation followed by Mn, Ni, Zn, Cu, Cr and Pb. In the current study, there is no big difference in the accumulation of chemical elements in plant parts of *B. juncea* and *S. Oleracea L*. Hence, even translocation of chemical elements is expected to be similar. In all the studies used in this section, the highest chemical elements that was accumulated was Fe and this is the same as the current study.

4.7.8 Statistical evaluation of the bioaccumulation of chemical elements in *B. juncea* and *S. Oleracea L* for the different growth media

Table 4.21 shows the comparison of the whole plant (not plant parts) of *B. juncea* and *S. Oleracea L* for the different sets of growth media (soil, FA and soil+FA) with regards to the mean concentrations obtained. Plant parts which are leaf, stem and roots' concentrations were summed up to get the total concentration of the whole plant. These statistical test were done to find out if the species of plant has effects on the bioaccumulation of the chemical elements. Mann-Whitney Test was used to test difference in the bioaccumulation of chemical elements.

Table 4.21: Comparison of *B. juncea* and *S. Oleracea L* for the different sets of growth media with regards to the mean concentrations obtained.

Time (in days)	Growth media	P value	Mann-Whitney Test	Significance
23 DAYS	Agromix	0.67	35.000	X
69 DAYS	Soil	0.45	31.500	X
	FA	0.73	36.000	X
	FA+Soil	0.26	27.000	X
115 DAYS	Soil	0.67	35.000	X
	FA	0.73	36.000	X
	FA+Soil	0.80	37.000	X

Note:

- X - Not significant
- ✓ - significant

Table 4.21 shows that in 23 days there was no significant difference in the accumulation of chemical elements by the two plant species under study. The same goes for 69 and 115 days of pot culture experiments for each growth media presented since there was no significant difference found between the two plant species. This might mean that the two plant species did not have effect on the accumulation of chemical elements and they might be having similar characteristics which makes them similar on the uptake of chemical elements.

Table 4.22 shows comparison of the plant parts of *B. juncea* and *S. Oleracea L* for each set of growth media (soil, FA and soil+FA) with regards to the mean concentrations obtained. Plant parts which are leaf, stem and roots' concentrations were tested using Kruskal-Wallis Statistic (KW) to find the difference in the bioaccumulation of chemical elements. These tests were done to find out if there is a significant difference between plant parts of the same species of plant which were grown in the similar type of growth media. This confirms what was discussed earlier in section 4.7.7 where it was mentioned that similar chemical elements are commonly accumulated by both plant species.

Table 4.22: Comparison of *B. juncea* and *S. Oleracea L* plant parts for each set of growth media (soil, FA and soil+FA) with regards to the mean concentrations obtained.

Time (in days)	Plant species	Growth media	P value	Kruskal-Wallis Statistic (KW)	Significance
23 DAYS	<i>B. juncea</i>	Agromix	0.12	4.22	X
	<i>S. Oleracea L.</i>	Agromix	0.48	1.49	X
69 DAYS	<i>B. juncea</i>	Soil	0.23	2.90	X
		FA	0.36	2.04	X
		FA+Soil	0.61	0.99	X
	<i>S. Oleracea L.</i>	Soil	0.81	0.41	X
		FA	0.51	1.34	X
		FA+Soil	0.27	2.65	X
115 DAYS	<i>B. juncea</i>	Soil	0.74	0.60	X
		FA	0.87	0.27	X
		FA+Soil	0.38	1.93	X
	<i>S. Oleracea L.</i>	Soil	0.95	0.11	X
		FA	0.81	0.41	X
		FA+Soil	0.83	0.37	X

Note:

- X - Not significant
- ✓ - significant

No significant difference was found for leaf, stem and roots of each plant species for all the growth media from 23 days to 115 days of pot culture experiment in Table 4.22. This have the implication that plant parts of *B. juncea* and *S. Oleracea L* accumulated chemical elements similarly as there is no significant difference between what leaves, stems and roots accumulated. Although these statistical results say so, the translocation factor is still important to confirm if translocation of chemical elements from root to shoot occurred or not as this is very important for the current study.

4.7.9 Conclusion

In terms of the overall accumulation of chemical elements, in 23, 69 and 115 days of pot culture experiments for all the three growth media, chemical elements Fe, Mn, B, Ba and Zn were the highest for most plant parts and with *B. juncea* experiencing highest overall accumulation than *S. Oleracea L*. When it comes to 115 days, for soil and also FA+soil as a growth media, *S. Oleracea L* accumulated highest overall mean concentration for Fe, Mn, B, Ba and Zn, while for FA as a

growth media, chemical elements Fe, Mn, B, Ba and Zn were accumulated more by the *B. juncea* plant parts. Hence, this can imply that *B. juncea* can be used as an accumulator for these chemical elements which will be coming from FA than *S. Oleracea L*; while for FA+soil and also soil only as a growth media, *S. Oleracea L* can be an effective accumulator for long periods like 115 days than *B. juncea*. For short periods like in 69 days, *B. juncea* can be concluded as the perfect plant species than *S. Oleracea L* for all the three growth media under study. There was no significant difference for *B. juncea* with respect to all the three plant parts which were analysed, similar was the case with *S. Oleracea L*. This means that the accumulation was not very different in stems, roots and leaves of either *B. juncea* or *S. Oleracea L* in 23, 69 and 115 days.

Part 5: Evaluation of the phytoremediation potential (bioaccumulation capability) of the selected plants using the bioconcentration factor (BCF) and translocation factor (TF)

4.8 Introduction

After the assessment of chemical elements (B, Zn, Mn, Fe, Mo, Cu, Cr, Ni, and Ba) in different plant parts, bioconcentration factor (BCF) and translocation factor (TF) were studied to comprehend the possible role of *S. Oleracea L* and *Brassica juncea* in the phytoremediation of abandoned FA deposits. BCF and TF can be used to assess a plant's potential for phytoremediation purpose (Yoon et al., 2006; Pandey, 2012a). The BCF value more than 1 indicates that the plant is a potential accumulator of chemical elements, while the TF value greater than 1 indicates that the plant is a potential translocator of chemical elements. Figure 4.41 to 4.46 shows the BCF of chemical elements that were accumulating in plant parts of both *B. juncea* and *S. Oleracea L* in 69 and 115 days for all the three growth media under study.

4.8.1 BCF for soil media in both plants

The Figure 4.41 depicts BCF values of all the chemical elements in different plant parts in 69 days while Figure 4.42 shows similar variables but in 115 days for soil as a growth media. The calculated BCF values of Fe, Mn, Zn and Cu were very high in all plant parts (especially in the leaves) of *B. juncea* in Figure 4.41 which indicates the phytoremediation potential of it. Although for Ba, B and Ni, the BCF values were less than 1 for other plant parts meaning *B. juncea* was not an effective accumulator of these certain chemical elements at these 69 days of pot culture experiment. For *S. Oleracea L* in the same Figure 4.41, Mn and Zn had the highest BCF values for different plant parts while for B, Cr, Ni, Cu, Fe, Mo and Ba were less than 1 showing lack of accumulation in the plant species.

For Figure 4.42, *B. juncea* continued to accumulate more of Fe, followed by Zn, Mn and Cu for all plant parts, while Ba, Ni and B were the least accumulated with BCF values less than 1. For *S. Oleracea L* in the same Figure 4.42, chemical elements B, Ba, Ni, Mo and Fe were not effectively accumulated while Mn, Zn, Cr and Cu were accumulated effectively by all the plant parts. From these two Figures (4.41 and 4.42) we can conclude that *B. juncea* is more suitable for accumulation of many chemical elements than *S. Oleracea L* which managed to accumulate only few chemical elements in time with soil as a growth media. Jambhulkar and Juwarkar (2009) mentioned that the rhizospheric microorganisms may interact symbiotically with roots to enhance the potential for metal uptake, because microorganisms may excrete organic compounds, which increase bioavailability, and facilitate the root absorption of essential metals such as Fe (Crowley et al., 1991). This might have contributed to the current results because Mn was found to be abundant in roots than other plant parts in 115 days in Figure 4.42.

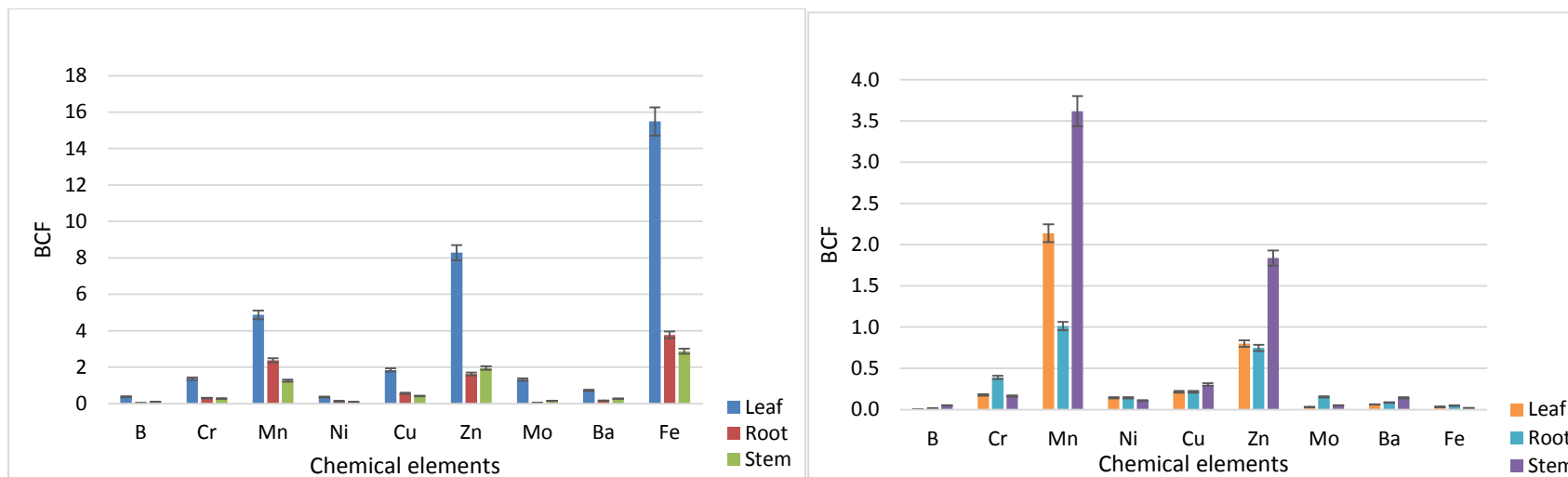


Figure 4.41: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for soil media pots.

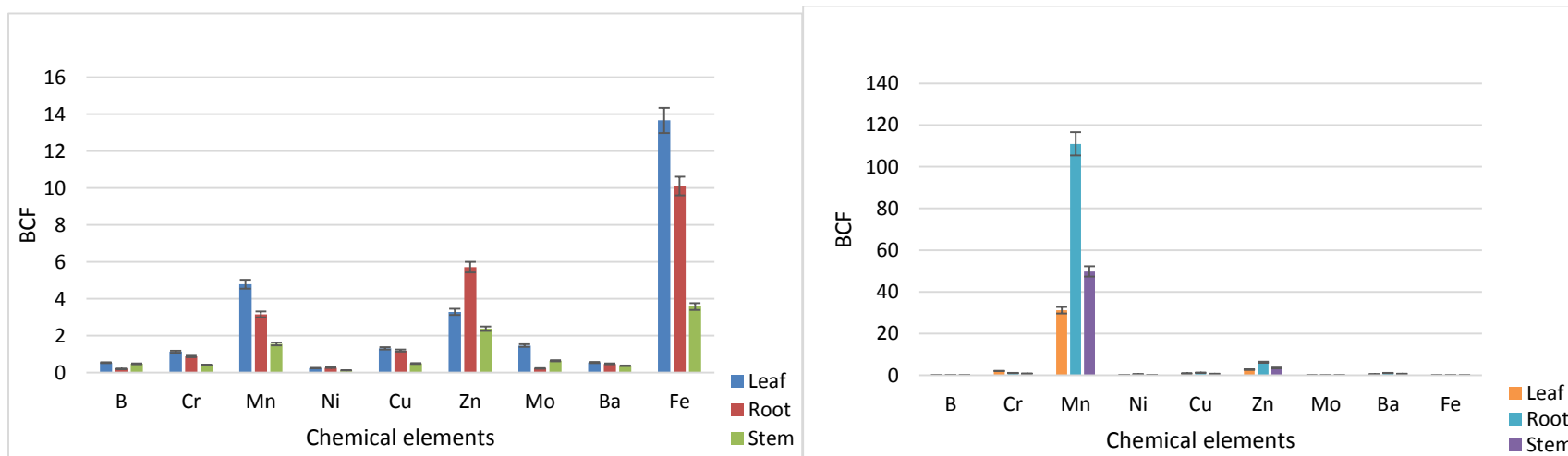


Figure 4.42: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for soil media pots.

4.8.2 BCF for FA media in both plants

Figures 4.43 and 4.44 depicts BCF values of different chemical elements which were accumulated in plant parts of both species grown in the FA media in 69 and 115 days of pot culture experiment. For both Figures, it can be clearly seen that Fe is highly accumulated with BCF values above 700 for *B. juncea* in 69 days which is reduced in 115 days (Figure 4.44) to about 220 with regards to leaf plant parts. This high accumulation might have been due to its high concentration which was noticed in the previous section retained in different plant parts of both plants which were grown in the FA media. Morariu et al. (2013) reported such scenario of having high Fe accumulation in plants.

In 69 days of pot culture experiments (Figure 4.43), *B. juncea* plant parts did not manage to effectively accumulate B, Cr, Mo and Ba for different plant parts with BCF values less than 1, while it managed for Fe, Mn, Zn, Cu and Ni with BCF values greater than 1. Figure 4.43 depicts that *S. Oleracea L* effectively retained Fe, Mn, Zn, Cu and Ni in its plant parts while it did not do so for B, Cr, Mo and Ba with BCF values less than 1. The two plant species accumulated effectively same chemical elements (Fe, Mn, Zn, Cu and Ni) while they did not accumulate similar chemical elements (B, Cr, Mo and Ba) in FA media in 69 days. Hence they had similar accumulating potential in 69 days of pot culture experiments.

When it comes to 115 days, *B. juncea* retained similar chemical elements effectively in Figure 4.44, while for *S. Oleracea L* increase in BCF value of Fe for roots can be noticed. In this FA growth media, *B. juncea* can be a more suitable plant species for accumulation of different chemical elements since it accumulates many different chemical elements than *S. Oleracea L*, and there might be a possibility of accumulating more different chemical elements over time by *B. juncea*.

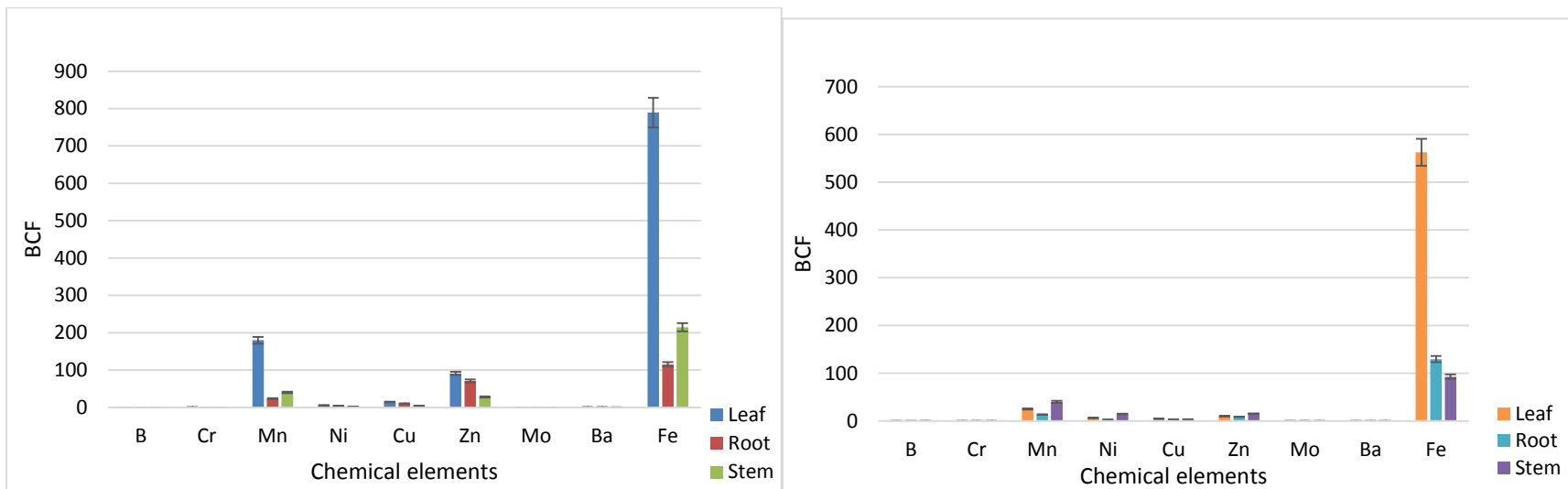


Figure 4.43: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for FA media pots.

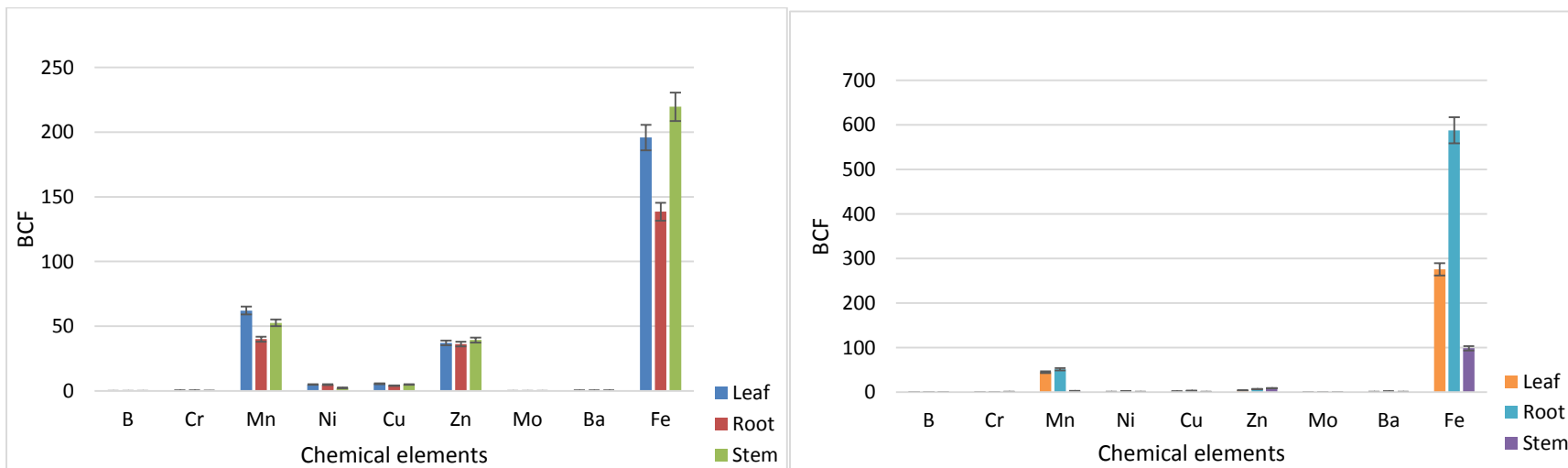


Figure 4.44: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for FA media pots.

4.8.3 BCF for FA+soil media in both plants

Figure 4.45 shows BCF values for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for the FA+soil media pots and Figure 4.46 showing similar variables but in 115 days. It can be clearly seen from the two figures that again Fe is highly accumulated by plant parts of both plant species with BCF values very high and in most cases Zn and Mn followed. In Figure 4.45, *B. juncea* plant parts showed to have retained more of Fe, Zn and Mn with BCF values greater than 1, while for B, Cr, B and Mo were less than 1 which shows lack of accumulation in plant parts in 69 days. But only three chemical elements (i.e. B, Ba and Mo) were not accumulated effectively by plant parts of *S. Oleracea L* with BCF value less than 1, but all the other 6 chemical elements (i.e Fe, Mn, Zn, Cu, Ni and Cr) were accumulated effectively in 69 days.

With Figure 4.46 was not very different from Figure 4.45 since *S. Oleracea L* accumulated the 6 chemical elements (i.e. Fe, Mn, Zn, Cu, Ni and Cr) effectively while the other three (i.e. B, Ba and Mo) were not accumulated effectively by plant parts.

From the results of the two Figures 4.45 and 4.46, it can be concluded that *S. Oleracea L* accumulated many different chemical elements than *B. juncea* in the FA+soil growth media from 69 days to 115 days. Chaney (1983) and Pandey (2012a) reported that several hyper-accumulator species have a high BCF, but due to very low biomass the amount of phytoextraction is less. This might have been one of the causes of one plant being better than the other since *S. Oleracea L* had better biomass than *B. juncea* over time in the previous section (Part 3 in this Chapter 4). In the study by Jamil et al. (2009), it was observed that Fe and Mn were retained more in root while Cu, Al and Cr were translocated more to the shoot, indicating phytoextraction of these three elements from substrate. Even in the current study for the soil, FA and FA+soil media for both plants, there is a common trend of Fe having high BCF values for plant parts, this might have been attributed by Fe being highly accumulated by plant parts as presented in Part 4 of Chapter 4.

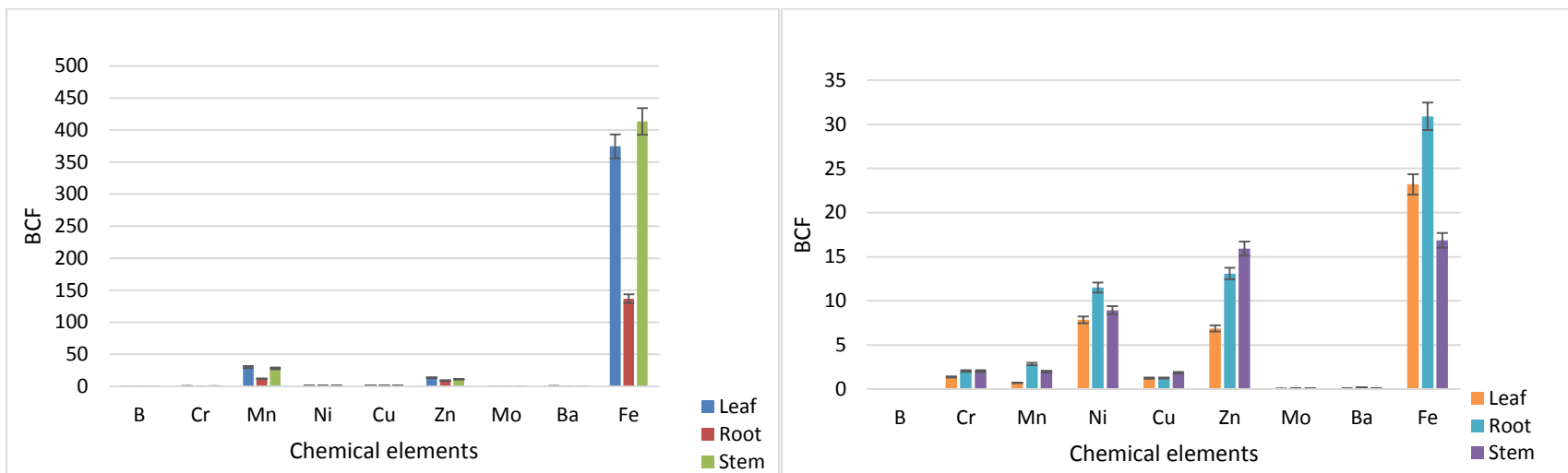


Figure 4.45: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 69 for the FA+soil media pots.

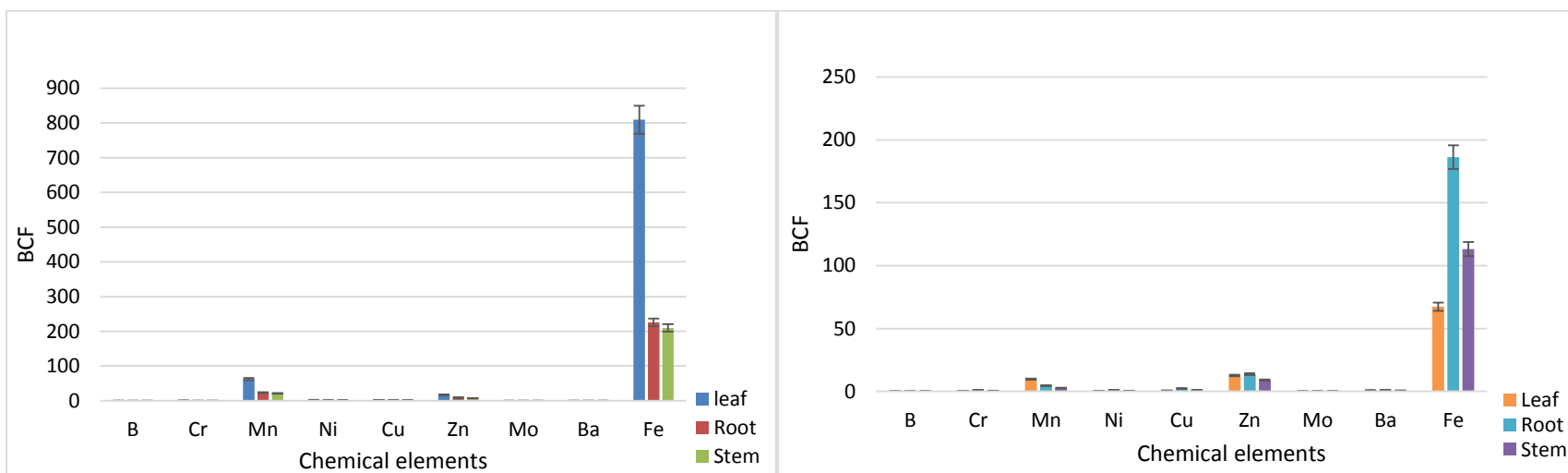


Figure 4.46: BCF for chemical elements accumulating in *B. juncea* and *S. Oleracea L* in day 115 for FA+soil media pot.

4.8.4 Statistical evaluation of the BCF of *B. juncea* and *S. Oleracea L* for the different growth media with time

Table 4.23 shows the comparison of the BCF of the whole plant (not plant parts) of *B. juncea* and *S. Oleracea L* for the different sets of growth media (soil, FA and soil+FA) with regards to the mean concentrations obtained. Plant parts which are leaf, stem and roots' concentrations were summed up to get the total concentration of the whole plant. These statistical test were done to find out if the species of plant has effects on the BCF. Mann-Whitney Test was used to test difference in the BCF values obtained for the plants.

Table 4.23: Comparison of the BCF for *B. juncea* and *S. Oleracea L* plant for the whole plant grown on different sets of growth media.

Time (in days)	Growth media	P value	Mann-Whitney Test	Significance
69 DAYS	Soil	0.03	16.000	✓
	FA	0.76	36.500	X
	FA+Soil	0.83	37.500	X
115 DAYS	Soil	0.49	32.000	X
	FA	0.80	37.000	X
	FA+Soil	0.86	38.000	X

Note:

X - Not significant

✓ - significant

With regards to Table 4.23, it is clear that there was no significant difference in the BCF of chemical elements in the two plant species under study except for soil media were significant difference was found. These only means that the two plant species have a lot in common and they did not have effect on the BCF of chemical elements.

Table 4.24 shows comparison of the BCF for *B. juncea* and *S. Oleracea L* plant parts of same plant species from same growth media with time. Plant parts which are leaf, stem and roots' BCF were tested using Kruskal-Wallis Statistics (KW) to find the difference in the BCF. These tests were done to find out if there is a significant difference between plant parts of the same species of plant which were grown in the similar type of growth media.

Table 4.24: Comparison of the BCF for *B. juncea* and *S. Oleracea L* plant parts of same plant species from same growth media with time.

Time (in days)	Plant species	Growth media	P value	Kruskal-Wallis Statistic (KW)	Significance
69 DAYS	<i>B. juncea</i>	Soil	0.07	5.36	X
		FA	0.71	0.68	X
		FA+Soil	0.72	0.66	X
	<i>S. Oleracea L.</i>	Soil	0.88	0.25	X
		FA	0.74	0.59	X
		FA+Soil	0.67	0.79	X
115 DAYS	<i>B. juncea</i>	Soil	0.44	1.65	X
		FA	0.95	0.10	X
		FA+Soil	0.80	0.45	X
	<i>S. Oleracea L.</i>	Soil	0.87	0.27	X
		FA	0.84	0.34	X
		FA+Soil	0.74	0.60	X

Note:

- X - Not significant
- ✓ - significant

There was no significant difference was found for leaf, stem and roots of each plant species for all the growth media from 23 days up to 115 days of pot culture experiment in Table 4.26. This have the implication that plant parts of *B. juncea* and *S. Oleracea L* accumulated chemical elements similarly as there is no significant difference between what leaves, stems and roots accumulated.

4.9 Assessment of the phytoremediation potential of *B. juncea* and *S. Oleracea L* using translocation factor (TF)

Table 4.25 and 4.26 in this section shows TF of various chemical elements over time in different species of plants grown in different growth media. Particularly Table 4.25 shows how effective translocation from root to stem was for that specific plant species after accumulating different chemical elements. The translocation factor (TF) value greater than 1, indicates the translocation of chemical elements from root to above ground part (Jamil et al., 2009).

Table 4.25: Translocation factor (TF) of various chemical elements over time in different species of plants grown in different growth media. $TF = BCF^b / BCF^c$. i.e. stem/root.

$TF = BCF^b / BCF^c$ i.e. stem/root	Brassica juncea						S. Oleracea L.					
	69 Days			115 Days			69 Days			115 Days		
	Soil	FA	FA+Soil	Soil	FA	FA+Soil	Soil	FA	FA+Soil	Soil	FA	FA+Soil
B	3.90	1.16	2.27	2.37	0.74	1.33	4.03	2.42	0.53	1.21	1.85	0.77
Cr	0.90	0.47	1.50	0.47	0.51	1.13	0.42	6.68	1.00	0.77	1.35	0.66
Mn	0.53	1.71	2.36	0.49	1.32	0.91	3.58	2.96	0.70	0.45	0.05	0.59
Ni	0.67	0.50	1.33	0.45	0.48	0.94	0.77	5.73	0.78	0.46	0.16	0.40
Cu	0.75	0.42	1.53	0.41	1.27	0.85	1.41	0.99	1.48	0.61	0.24	0.42
Zn	1.21	0.39	1.23	0.42	1.09	0.81	2.46	1.70	1.22	0.55	1.25	0.65
Mo	3.82	1.02	0.77	2.88	0.97	1.72	0.33	7.34	0.88	0.76	1.12	0.66
Ba	1.60	0.49	1.53	0.79	1.26	1.05	1.70	1.14	0.43	0.65	0.38	0.56
Fe	0.76	1.86	3.02	0.35	1.59	0.93	0.39	0.72	0.55	0.60	0.17	0.61

Table 4.25 clearly shows that in 69 days B and Mo were translocated well from root to stem of *B. juncea* for all the three growth media, but that was not so in 115 days since B recorded 0.74 for the FA growth media which is less than 1. Mo on the other side was not effective for soil as well as soil+FA in 115 days. For *S. Oleracea L*, Zn was the dominant one on being effective in 69 days but not in 115 days.

Basically for *B. juncea* in 69 days, FA+soil growth media translocated most chemical elements to stems, while in 115 days FA media translocated about 5 chemical elements with FA+soil media translocating 4 chemical elements. Hence FA+soil media is suitable to translocate chemical elements from root to stem than soil and FA media using *B. juncea*. With *S. Oleracea L*, FA growth media translocated most chemical elements to stems in 69 and 115 days. Hence, *S. Oleracea L* can be an effective translocator of many chemical elements in coal fly ash dumps. This might be dependent on the extent of bioaccumulation of these chemical elements under study in plant parts. However, Jamil et al. (2009) reported that Fe and Mn were retained more in roots while Cu, Al and Cr were translocated more to the shoot.

Table 4.26 shows how effective translocation from stem to leaf was for that specific plant species after accumulating different chemical elements.

Table 4.26: Translocation factor (TF) of various chemical elements over time in different species of plants grown in different growth media. $TF = BCF^a / BCF^b$ i.e. leaf/stem.

TF=BCF ^a /BCF ^b i.e.leaf\stem	Brassica juncea						S. Oleracea L.					
	69 Days			115 Days			69 Days			115 Days		
	Soil	FA	FA+Soil	Soil	FA	FA+Soil	Soil	FA	FA+Soil	Soil	FA	FA+Soil
B	3.57	5.15	1.79	1.11	2.18	2.82	0.14	0.31	0.28	1.06	0.68	2.75
Cr	4.84	3.32	1.03	2.74	1.81	3.26	1.07	0.46	0.67	2.55	0.25	0.45
Mn	3.86	4.44	1.08	3.09	1.18	2.93	0.59	0.63	0.36	0.63	18.75	3.70
Ni	4.04	2.90	1.01	2.02	2.09	3.25	1.31	0.45	0.88	0.98	1.58	0.57
Cu	4.35	3.34	1.20	2.65	1.09	2.46	0.71	1.39	0.66	1.17	1.75	0.62
Zn	4.22	3.26	1.24	1.38	0.94	2.38	0.43	0.67	0.43	0.80	0.44	1.42
Mo	8.95	5.46	3.36	2.25	1.89	1.12	0.61	0.25	0.70	1.72	1.30	3.59
Ba	2.64	3.10	1.22	1.46	0.88	2.54	0.43	1.64	0.63	0.60	1.50	1.16
Fe	5.38	3.68	0.91	3.82	0.89	3.86	1.84	6.04	1.38	2.39	2.80	0.59

In the current study, as shown in Table 4.26, *B juncea* recorded that it translocated all chemical elements effectively for all the growth media in 69 days except for Fe in the FA+soil growth media. While in 115 days all chemical elements were translocated from stem to leaf except for Zn, Ba and Fe in the FA media. With *S. Oleracea L.*, in 69 and 115 days very few chemical elements were translocated to the leaves in different growth media but in the soil media translocation was better since five chemical elements were translocated to leaves in 115 days while three were translocated in 69 days. In the study by Subhashini et al. (2013), among the 10 sampled plant species, *Abutilon indicum*, *Cleome viscosa*, *Catharanthus roseus* and *Ruellia tuberosa*, *Perotisindica*, *Echinocloacolona* and *Cyperusrotundus* was discovered to have the potential to be used in phytoextraction of Zn (TF and BCF>1).

In Pandey (2012a), the TF values were found lower than 1 for all the examined metals except Cd and Cr indicating that *Ipomoea carnea* did not transfer effectively the metals from root to stem or stem to leaf. Poor translocation of heavy metals to the above ground parts could be due to metal sequestration in the root cell vacuoles of the plant to render it non-toxic (Shanker et al., 2005). Baker and Walker (1990) suggested that it is expected that metal uptake, translocation and bioaccumulation mechanisms of the plant systems differ for metal to- metal and species-to-species. Normally, the most tolerant plant species take-up the lowest proportion of the total soil-chemical elements and contains the smallest shoot metal contents (Liu et al., 2004).

4.10 Conclusion

From the results of BCF, it can be concluded that *B juncea* is more suitable for the accumulation of many chemical elements than *S. Oleracea L*, which managed to accumulate only few chemical elements in time with soil as a growth media. For the FA+soil growth media, it can be concluded that *S. Oleracea L* accumulated many different chemical elements than *B juncea* from 69 days to 115 days. While again in FA growth media, *B juncea* can be a more suitable plant species for accumulation of different chemical elements since it accumulates many different chemical elements than *S. Oleracea L*, and there might be a possibility of accumulating more different chemical elements over time by *B. juncea*. From the results of translocation of chemical elements from root to stem and stem to leaves, conclusions can be drawn that the *B. juncea* species can be an effective phytoremediation plant species since it is effective in translocation of many chemical elements to shoots while *S. Oleracea L* is failing to translocate most chemical elements from stem to leaves although it translocated some from root to shoots. After all, one cannot really conclude which chemical elements were translocated more for all the growth media since one chemical elements can be translocated well in one growth media and not the other. However, Fe seems to be having many TF values that are greater than 1 showing better translocation for most growth media than other chemical elements, similar phenomenon was reported by Jamil et al. (2009).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter focuses on the concluding remarks of all the chapters including the results and discussions that were done in Chapter 4. This gives conclusions in all the finding for the current study and recommendations on what can be done further to improve the study for future application are made.

5.2 Conclusions

Part 1 of the study was focusing on the chemical, morphological and mineralogical characterization of FA and soil used in pot culture experiments. It showed that particles morphology of FA from Grootvlei power station shows a lower degree of sphericity with irregular agglomerations of many particles while there are dominant spherical particles and smaller sharp needle like particles. It is also an alumino-silicate material as confirmed by the high SiO_2 and Al_2O_3 content. In the FA under study it was expected that Ca, Na, K, Al, Sr, Si, and B will be readily available to plants from FA solution with water since they were in high concentrations. Since Na, Al, Sr, Si are regarded as non-essential nutrients and are available in FA leachates in high concentrations, their uptake can lead to direct toxicity of plants. This also applies to chemical elements like Ba, Li, V, Cr and Se that are available in the FA leachate solution. Soil leachates had a neutral pH of 7.22 with very high amounts of Si. FA from Grootvlei power station can be classified as a class F coal FA with an alkaline pH level of 10.62. In both soil and FA, elements that were expected to be readily available to plants included Ca, Si, K, Ba, Mo, Na, Al, Mg, Sr and non-essential elements (Si, Ba, Na, Al, Sr), which, if uptaken by plants' roots can have negative impacts in plants.

Part 2 of the study focused on the temporal evolutions of the physicochemical characteristics of the leachates (with time) as plants were irrigated in the pot culture experiment. In summary, the raw FA from Grootvlei power station started with an alkaline pH of 10.62 (same as in batch experiments - Part 1) but this changed over time as FA was irrigated with water and it ends up losing its alkalinity to neutral pH levels. There was also a decrease in the EC of the FA due to dissolution of soluble major oxides, which was promoted by continuous water irrigation in the FA rather than plant growth. Even though some chemical elements like B, Mn, Fe and Ba were occurring at higher concentrations in leachates for most weeks in the pot culture experiments; it was observed that in the eleventh week of leachate collection all these chemical elements plummet to very low concentrations. This suggest that these chemical elements can be reduced over time as plants are being irrigated which is either due to uptake by plants or washed off with water. The

similar trend was observed for soil and FA+soil growth media. There was a significant difference for different chemical elements of leachates from different growth media for each plant species (*B. juncea* and *S. Oleracea L.*).

Part 3 focused on the assessment of the growth of *Spinacia oleracea L.* and *Brassica juncea* in pot culture experiments grown in soil, FA and FA+soil media. In this present study, it cannot be easily concluded which media shows to be effective in growing a productive plant with high growth rate, biomass and pigments since there was flexibility over the results. After all, even though *S. Oleracea L* had carotenoid content below detection limits, it had higher chlorophyll b than *B. juncea* for all growth media in general until 115 days especially for the FA growth media. Even in terms of growth rate and biomass, *S. Oleracea L* showed to be better than *B. juncea* especially with the root part of plant in different growth media over time. But, statistically there was no significant difference between the two plant species in terms of growth rate and biomass; even between the plant parts denoting similar growth performance for the two plant species under study.

Part 4 was focusing on the evaluation of the concentrations of chemical elements accumulated by roots, stems, and leaves of selected plants. In terms of the overall accumulation of chemical elements, in 23, 69 and 115 days of pot culture experiments for all the three growth media, chemical elements Fe, Mn, B, Ba and Zn were the highest for most plant parts and with *B. juncea* experiencing highest overall accumulation than *S. Oleracea L.* When it comes to 115 days, for soil and also FA+soil as a growth media, *S. Oleracea L* accumulated highest overall mean concentration for Fe, Mn, B, Ba and Zn, while for FA as a growth media, chemical elements Fe, Mn, B, Ba and Zn were accumulated more by the *B. juncea* plant parts. Hence, this can imply that *B juncea* can be used as an accumulator for these chemical elements which will be coming from FA than *S. Oleracea L*; while for FA+soil and also soil only as a growth media, *S. Oleracea L* can be an effective accumulator for long periods like 115 days than *B. juncea*. For short periods like in 69 days, *B juncea* can be concluded as the perfect plant species than *S. Oleracea L* for all the three growth media under study. There was no significant difference for *B. juncea* with respect to all the three plant parts which were analysed, similar was the case with *S. Oleracea L.* This means that the accumulation was not very different in stems, roots and leaves of either *B. juncea* or *S. Oleracea L* in 23, 69 and 115 days.

Part 5 focused on the evaluation of the phytoremediation potential (bioaccumulation capability) of the selected plants using the bioconcentration factor (BCF) and translocation factor (TF). From the results it can be concluded that *B juncea* is more suitable for the accumulation of many chemical elements than *S. Oleracea L*, which managed to accumulate only few chemical elements in time with soil as a growth media. For the FA+soil growth media, it can be concluded that *S. Oleracea L* accumulated many different chemical elements than *B juncea* from 69 days to 115 days. While again in FA growth media, *B juncea* can be a more suitable plant species for accumulation of different chemical elements since it accumulates many different chemical elements than *S. Oleracea L*, and there might be a possibility of accumulating more different

chemical elements over time by *B. juncea*. TF further proved this because the *B. juncea* species was proved to be an effective phytoremediation plant species since it is effective in translocation of many chemical elements for different growth media to shoots while *S. Oleracea L* failed to translocate most chemical elements from stem to leaves although it translocated some from root to shoots.

5.3 Recommendations

Few studies have revealed that in numerous cases it has been observed that in spite of presence of high concentration of chemical elements in growth media; they have low mobility into the plant system (Alkorta et al., 2004; and Jamil et al., 2009). This was also the case in the current study especially for *B. juncea*, which took long for certain chemical elements to be taken up and be translocated. For these reasons chelate induced phytoextraction is necessary. With the objective of desorbing chemical elements from growth media matrix into growth media solution to facilitate the transport of chemical elements into xylem, and increase translocation of chemical elements from the roots to shoots of *B. juncea* and *S. Oleracea L* (Nowack et al., 2009; and Madrid et al., 2002). This can also help in reducing the amount of chemical elements that are washed off with irrigation leachates and instead, be taken up and accumulated in plant parts. Also combining with biomass material to provide organic growth media can be an advantage and this can be further studied. Future studies are recommended involving several chelating agents like ethylene diamine tetra acetic acid (EDTA), *trans*-1,2-diaminocyclohexane-N', N', N'-tetraacetic acid (CDTA), diethylene triamine pentaacetic acid (DTPA), citric acid, maleic acid, etc. form more soluble complexes with the metal ions, and facilitate their uptake (Wu et al., 2003). Out of these, EDTA was found to be the perfect chelating agent in studies of phytoremediation because of its high efficiency in extracting many chemical elements (Luo et al., 2005).

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APPENDIX

Table A1: Chemical elements concentrations in leachates obtained from soil pots growing *S. Oleracea L.*

<i>S. Oleracea L.</i>						
Values in mg L ⁻¹						
Soil media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	4.26	7.38	8.22	6.22	3.14	1.28
Ca	25.78	23.30	4.74	109.90	3.26	1.05
K	29.44	27.45	9.30	48.88	7.52	0.69
Mg	15.22	8.85	2.39	60.79	2.45	0.33
Na	21.26	17.78	2.16	85.59	2.47	0.29
P	0.01	0.02	0.05	< 0.1	0.08	0.01
Si	14.65	16.13	11.25	167.90	32.96	0.95
Sr	0.14	0.37	0.03	0.75	0.03	0.01
Values in µg L ⁻¹						
Li	<0.680	73.41	1.06	10.75	4.66	1.37
Be	0.01	0.11	0.07	1.34	0.42	<0.039
B	91.45	180.82	27.81	587.25	49.49	86.59
Ti	75.33	99.20	127.72	840.48	422.86	12.64
V	5.72	13.43	9.14	70.40	27.65	1.38
Cr	3.89	14.08	6.53	47.96	19.78	0.62
Mn	13.46	10.23	14.72	86.73	32.47	4.07
Fe	1997.88	3110.97	3564.41	39260.00	11790.00	297.21
Co	1.18	1.58	1.13	7.24	2.57	0.08
Ni	11.81	8.84	7.47	52.09	18.47	0.92
Cu	9.76	7.15	6.10	30.19	10.43	1.44
Zn	4.79	5.02	6.24	27.98	10.79	0.74
As	1.01	1.39	0.72	2.92	1.10	0.15
Se	1.48	1.84	0.66	2.06	<0.456	<0.456
Mo	0.22	14.16	0.24	0.52	0.21	0.21
Cd	0.03	0.03	0.00	0.03	0.02	0.01
Sb	0.12	0.34	0.09	0.22	0.11	0.04
Ba	136.54	92.90	54.80	402.76	133.97	5.50
Hg	0.01	0.01	<0.004	<0.003	0.00	0.00
Pb	0.40	0.49	0.55	6.56	1.70	0.05

Table A2: Chemical elements concentrations in leachates obtained from FA pots growing *S. Oleracea L.*

<i>S. Oleracea L.</i>						
Values in mg L ⁻¹						
FA media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	0.28	2.45	0.91	0.42	0.30	0.27
Ca	116.70	124.40	154.90	147.70	96.80	71.71
K	66.39	64.01	31.97	8.86	7.55	6.24
Mg	4.40	4.96	8.66	46.05	32.10	24.12
Na	60.37	50.95	16.64	9.53	7.95	6.42
P	0.03	< 0.005	< 0.005	0.03	0.03	0.03
Si	1.65	0.76	1.89	1.92	1.87	1.89
Sr	3.33	3.53	4.39	3.06	1.95	1.54
Values in µg L ⁻¹						
Li	711.93	595.96	302.86	346.20	260.15	226.12
Be	0.01	0.00	0.01	<0.039	<0.039	<0.039
B	1377.09	1550.94	1492.71	6382.09	4936.09	4277.50
Ti	0.22	0.08	0.26	0.08	<0.070	<0.070
V	68.47	81.70	64.09	25.84	25.43	24.68
Cr	74.64	78.00	64.76	29.15	18.70	13.16
Mn	3.26	0.87	4.96	1.29	0.83	0.24
Fe	5.53	1.17	4.30	1.04	0.96	0.65
Co	0.51	0.50	0.37	0.31	0.25	0.21
Ni	1.13	0.82	1.08	0.67	0.39	0.45
Cu	0.89	1.15	3.09	0.72	0.56	0.69
Zn	0.69	0.74	2.24	<0.181	<0.181	<0.181
As	9.63	8.17	4.34	5.90	5.95	5.47
Se	5.71	9.84	8.48	13.11	9.70	8.79
Mo	126.45	122.56	109.19	73.12	47.39	32.61
Cd	0.02	0.03	0.03	0.04	0.03	0.04
Sb	3.78	4.53	4.29	9.85	8.94	8.55
Ba	132.43	108.06	143.88	69.18	65.46	63.00
Hg	0.01	0.01	0.02	0.02	0.01	0.01
Pb	0.04	0.02	0.06	0.01	0.01	0.00

Table A3: Chemical elements concentrations in leachates obtained from FA+soil pots growing *S. Oleracea L.*

<i>S. Oleracea L.</i>						
Values in mg L ⁻¹						
FA+Soil media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	0.03	0.03	0.03	0.03	0.02	0.02
Ca	235.30	226.10	135.20	68.88	50.29	6.27
K	42.39	37.46	25.42	3.46	7.04	0.52
Mg	25.77	21.71	18.55	23.72	15.71	1.40
Na	32.15	31.00	8.37	7.99	6.06	0.41
P	0.05	0.04	0.08	0.13	0.37	0.06
Si	4.26	5.05	3.05	4.69	3.60	0.60
Sr	3.66	3.42	2.23	1.70	1.11	0.13
Values in µg L ⁻¹						
Li	25.32	20.34	52.78	35.08	19.00	3.20
Be	0.00	0.01	0.00	<0.039	<0.039	<0.039
B	2155.13	1762.79	1591.22	3110.40	1809.38	177.83
Ti	0.18	0.17	0.48	0.41	0.34	1.26
V	15.36	12.41	13.67	31.27	22.91	4.85
Cr	25.62	34.31	11.95	8.75	3.60	0.41
Mn	0.40	1.18	136.78	0.12	0.17	0.67
Fe	1.87	4.26	8.44	7.14	6.63	22.36
Co	1.54	1.45	1.99	1.25	0.70	0.07
Ni	1.65	2.01	2.24	1.19	0.95	0.38
Cu	2.57	2.46	2.21	2.47	2.72	1.03
Zn	0.63	0.58	0.50	<0.181	<0.181	<0.181
As	10.16	8.18	7.76	9.63	6.95	1.33
Se	5.05	4.17	3.11	1.54	0.75	<0.456
Mo	132.64	121.16	86.08	40.06	18.36	1.16
Cd	0.03	0.04	0.02	0.02	0.01	0.00
Sb	6.00	4.66	4.00	6.72	4.38	0.32
Ba	205.63	237.13	170.99	96.47	70.92	10.63
Hg	0.01	0.02	0.01	0.02	0.01	0.00
Pb	0.03	0.03	0.03	0.00	0.01	0.03

Table A4: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea*.

<i>B. juncea</i>						
Values in mg L ⁻¹						
Soil media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	1.79	2.64	0.89	0.67	0.52	0.32
Ca	29.50	30.88	5.73	110.40	3.32	0.99
K	28.49	25.39	11.45	48.95	7.37	0.72
Mg	16.29	17.70	2.33	60.30	2.44	0.33
Na	20.62	17.65	2.35	81.25	2.47	0.32
P	0.01	0.01	0.02	< 0.1	0.07	0.02
Si	11.58	12.52	1.90	159.80	32.58	0.94
Sr	0.17	0.19	0.04	0.76	0.03	0.01
Values in µg L ⁻¹						
Li	<0.680	1.75	0.89	12.13	4.81	0.92
Be	0.05	0.04	0.06	1.96	0.42	<0.039
B	79.81	88.07	23.49	922.35	29.30	10.74
Ti	29.81	38.39	16.47	722.26	434.26	12.85
V	2.70	4.03	1.83	61.38	27.87	1.33
Cr	1.65	2.52	0.94	41.20	20.02	0.66
Mn	8.38	5.11	85.83	72.55	33.10	4.35
Fe	779.13	1140.13	437.07	36620.00	11640.00	287.61
Co	0.89	1.12	0.62	6.02	2.61	0.08
Ni	7.06	6.26	2.32	45.65	18.72	0.85
Cu	7.66	6.64	3.18	28.16	10.47	1.37
Zn	3.54	3.43	1.49	24.86	11.01	0.88
As	0.64	0.67	0.54	2.45	1.09	0.28
Se	1.10	1.26	0.45	1.05	<0.456	<0.456
Mo	1.07	0.75	0.32	0.97	0.18	0.14
Cd	0.03	0.03	0.00	0.03	0.01	0.01
Sb	0.11	0.09	0.05	0.22	0.11	0.03
Ba	139.06	146.75	17.96	367.56	135.07	5.42
Hg	0.00	0.00	<0.004	<0.003	0.00	<0.003
Pb	0.18	0.24	0.07	6.02	1.73	0.05

Table A5: Chemical elements concentrations in leachates obtained from FA pots growing *B. juncea*.

<i>B. juncea</i>						
Values in mg L ⁻¹						
FA media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	1.68	2.44	0.90	0.18	0.21	0.09
Ca	97.71	127.60	151.20	138.20	97.63	78.12
K	47.07	55.85	39.60	8.62	7.72	6.97
Mg	4.00	6.80	7.76	45.79	32.14	24.59
Na	31.67	40.40	19.22	9.22	8.10	7.20
P	< 0.005	0.01	< 0.005	0.03	0.04	0.03
Si	2.02	0.87	1.74	1.91	1.88	1.89
Sr	2.84	3.10	4.32	3.08	1.94	1.55
Values in µg L ⁻¹						
Li	400.76	476.44	331.10	352.88	258.72	216.49
Be	0.00	0.01	0.00	<0.039	<0.039	<0.039
B	1365.75	1583.59	1491.62	6486.03	4901.60	4154.41
Ti	1.16	0.14	0.25	<0.070	<0.070	<0.070
V	54.04	71.46	66.39	25.92	25.28	25.03
Cr	62.75	64.82	74.56	29.15	18.61	13.04
Mn	0.92	0.44	0.89	1.07	0.75	0.48
Fe	35.56	3.12	1.56	1.13	0.93	0.62
Co	0.43	0.56	0.43	0.31	0.25	0.22
Ni	1.25	0.93	1.20	0.64	0.35	0.36
Cu	1.37	0.70	1.15	0.71	0.57	0.74
Zn	0.25	0.28	0.46	<0.181	<0.181	<0.181
As	3.42	8.56	5.42	6.06	5.86	5.17
Se	6.34	9.14	6.51	13.24	9.56	8.97
Mo	59.55	104.97	118.20	73.77	47.21	31.39
Cd	0.01	0.02	0.02	0.04	0.03	0.03
Sb	3.79	4.58	4.11	9.84	8.98	8.38
Ba	126.98	103.05	126.93	70.14	65.90	62.63
Hg	0.01	0.02	0.02	0.02	0.01	0.01
Pb	0.02	0.01	0.02	0.01	0.00	0.00

Table A6: Chemical elements concentrations in leachates obtained from FA+soil pots growing *B. juncea*.

<i>B.juncea</i>						
Values in mg L ⁻¹						
FA+Soil media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	0.03	0.05	0.07	0.05	0.04	0.01
Ca	280.40	217.00	219.30	102.23	51.01	6.20
K	49.45	35.79	35.83	12.89	6.91	0.54
Mg	30.10	23.62	27.80	21.86	15.82	1.38
Na	49.82	29.15	19.87	15.22	6.13	0.44
P	0.03	0.06	0.04	0.04	0.04	0.04
Si	4.13	2.99	2.43	1.23	0.62	0.59
Sr	4.61	3.45	4.27	2.98	1.12	0.13
Values in µg L ⁻¹						
Li	25.90	27.30	187.29	53.62	19.57	2.54
Be	0.01	0.00	0.01	0.04	<0.039	<0.039
B	1841.70	2140.25	2405.82	2100.15	1846.93	141.76
Ti	0.37	0.22	0.29	0.30	0.29	0.34
V	14.56	18.02	26.87	24.12	23.42	4.84
Cr	49.42	45.07	47.02	9.57	3.69	0.42
Mn	0.45	0.51	15.49	0.23	0.11	0.84
Fe	2.62	2.06	2.71	3.21	4.63	20.77
Co	1.78	1.51	2.90	1.23	0.74	0.06
Ni	2.78	1.29	2.64	2.03	0.93	0.32
Cu	4.67	1.60	4.11	3.20	2.78	0.98
Zn	1.42	0.30	0.81	0.62	<0.181	<0.181
As	11.04	10.36	7.83	8.21	7.20	1.33
Se	6.42	4.30	8.83	1.88	0.83	<0.456
Mo	189.41	132.89	163.92	68.25	18.74	1.07
Cd	0.03	0.02	0.03	0.02	0.01	0.01
Sb	5.24	6.15	6.89	6.32	4.46	0.32
Ba	234.17	180.79	208.23	126.24	71.63	10.94
Hg	0.02	0.02	0.03	0.01	0.01	0.00
Pb	0.04	0.02	0.04	0.02	0.01	0.03

Table A7: Chemical elements concentrations in leachates obtained from FA pots where no plants were grown.

No plants in the growth media						
Values in mg L ⁻¹						
FA media	WEEK 1	WEEK 2	WEEK 5	WEEK 7	WEEK 9	WEEK 11
Al	0.49	2.86	0.46	0.39	0.30	0.21
Ca	122.20	100.60	135.90	155.40	99.37	90.51
K	85.04	60.71	39.80	13.07	8.23	7.65
Mg	6.35	6.20	10.92	59.60	28.23	24.64
Na	74.20	49.17	21.15	11.11	6.36	5.76
P	0.01	0.01	0.00	0.03	0.01	0.03
Si	0.90	0.66	1.60	2.27	2.19	2.23
Sr	3.77	3.18	4.18	3.03	1.95	1.83
Values in µg L ⁻¹						
Li	919.53	727.95	423.05	360.85	224.33	213.18
Be	0.00	0.00	0.00	<0.039	<0.039	<0.039
B	1544.72	1444.04	1826.02	6640.43	4556.76	4355.44
Ti	0.07	0.14	0.15	<0.070	<0.070	<0.070
V	75.56	82.59	75.19	30.13	25.87	26.84
Cr	111.31	98.10	112.31	24.13	15.19	12.91
Mn	0.96	0.67	1.24	7.32	39.83	0.17
Fe	0.59	1.15	0.73	0.92	0.55	0.52
Co	0.67	0.51	0.42	0.40	0.25	0.21
Ni	0.42	1.28	0.48	0.69	0.77	0.37
Cu	0.47	0.67	0.81	0.78	1.21	0.43
Zn	0.14	0.37	0.36	<0.181	<0.181	<0.181
As	8.47	9.39	6.42	10.79	6.63	6.05
Se	9.20	10.08	6.80	20.74	10.50	9.55
Mo	194.97	154.18	135.30	99.08	43.72	35.91
Cd	0.04	0.03	0.02	0.06	0.03	0.02
Sb	3.66	4.54	5.02	11.12	8.77	8.51
Ba	108.52	99.40	133.74	81.99	62.13	60.63
Hg	0.02	0.02	0.03	0.02	0.01	0.01
Pb	0.01	0.02	0.02	0.00	0.00	<0.002

Table A8: Department of Environmental Affairs guidelines for the national norms and standards for the remediation of contaminated land and soil quality (soil screening values for metals).

Parameter	Units	SSV1 All Land- Uses Protection of the Water Resource	SSV2 Informal Residential	SSV2 Standard Residential	SSV2 Commercial Residential	Protection of Ecosystem Health
Metals and metalloids						
Arsenic	mg kg ⁻¹	5.8	23	48	150	580
Cadmium	mg kg ⁻¹	7.5	15	32	260	37
Chromium (III)	mg kg ⁻¹	46 000	46 000	96 000	790 000	n/a
Chromium (VI)	mg kg ⁻¹	6.5	6.5	13	40	260
Cobalt	mg kg ⁻¹	300	300	630	5 000	22 000
Copper	mg kg ⁻¹	16	1 100	2 300	19 000	16
Lead	mg kg ⁻¹	20	110	230	1 900	100
Manganese	mg kg ⁻¹	740	740	1 500	12 000	36 000
Mercury	mg kg ⁻¹	0.93	0.93	1.0	6.5	4.1
Nickel	mg kg ⁻¹	91	620	1 200	10 000	1 400
Vanadium	mg kg ⁻¹	150	150	320	2 600	-
Zinc	mg kg ⁻¹	240	9 200	19 000	150 000	240

Table A9: Department of Environmental Affairs guidelines for the national norms and standards for the remediation of contaminated land and soil quality (soil screening values for anions).

Anions	Soil Screening Level (mg kg ⁻¹)
Chlorides	12 000
Fluorides	30
Nitrates-nitrite	120
Sulphates	4000

Table B1: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* and *S. Oleracea L.* seedlings grown in the agromix media on a seed tray, i.e. raw data from IC-MS (1st harvest – 23 days of pot culture experiments).

	B. juncea			S.Oleracea L.		
	In $\mu\text{g L}^{-1}$					
	Leaf	Root	Stem	Leaf	Root	Stem
B	3.02	2.57	28.79	59.50	11.20	10.52
Cr	10.36	12.14	22.93	14.68	16.71	18.97
Mn	55.85	68.70	321.11	507.97	202.85	67.18
Ni	7.29	7.64	13.03	7.88	10.20	7.65
Cu	9.10	10.46	31.67	36.45	28.69	10.64
Zn	21.20	30.37	120.48	135.58	52.72	24.44
Mo	0.23	0.29	1.02	1.42	0.43	0.49
Ba	7.87	12.98	91.40	119.12	44.21	23.97
	In mg L^{-1}					
Fe	0.95	1.27	5.97	1.64	4.58	0.75

Table B2: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the soil media in pot A6 and A10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

	In $\mu\text{g L}^{-1}$					
	A6			A10		
	Leaf	Root	Stem	Leaf	Root	Stem
B	32.09	1.54	12.45	122.19	9.55	30.80
Cr	49.43	14.91	15.02	103.10	19.97	16.50
Ni	14.29	9.01	5.67	34.88	9.02	6.49
Cu	47.27	23.64	19.64	129.54	30.88	20.98
Zn	95.18	45.38	47.75	529.82	77.20	100.18
Mo	2.94	0.05	0.19	4.58	0.17	0.65
Ba	90.21	40.49	44.58	320.30	56.43	110.96
	In mg L^{-1}					
Fe	25.97	8.76	8.04	59.26	11.99	7.79
Mn	0.55	0.47	0.24	1.15	0.36	0.20

*n=2

Table B3: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the soil media in pot A1 to A5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	A1			A2			A3			A4			A5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	12.21	13.71	55.83	4.60	4.26	27.24	6.85	11.68	51.21	4.86	10.31	48.86	1.32	12.70	28.88
Cr	8.19	87.46	18.21	14.39	14.98	7.07	18.15	18.05	12.74	18.97	18.06	11.17	8.25	12.18	14.14
Mn	525.72	92.37	646.41	211.15	116.91	341.69	274.81	136.84	539.83	275.37	137.99	542.92	153.79	197.24	367.90
Ni	7.86	31.06	8.64	19.20	10.20	7.31	13.13	6.62	9.95	13.61	6.73	9.83	5.89	4.39	9.70
Cu	6.54	6.82	16.01	12.91	7.97	7.66	16.58	18.88	23.34	16.77	19.25	23.07	5.25	5.47	12.08
Zn	28.27	19.63	109.85	44.98	29.84	83.16	44.69	40.59	72.88	46.51	42.08	72.64	20.62	40.96	87.09
Mo	0.26	7.43	0.74	0.36	1.19	0.63	0.54	0.39	0.58	0.56	0.39	0.49	0.26	0.46	0.83
Ba	20.12	51.24	72.67	53.97	29.92	35.91	58.68	84.93	170.68	56.63	84.17	168.81	23.97	40.19	45.64
In mg L^{-1}															
Fe	0.50	0.59	0.57	2.88	0.92	0.55	2.26	4.62	1.13	2.23	4.38	1.13	0.54	1.15	1.20

*n=5

Table B4: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA media in pot B1 to B5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	B1			B2			B3			B4			B5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	51.25	121.45	146.02	16.22	64.36	91.88	47.19	35.10	120.11	45.99	21.31	106.66	53.07	43.76	226.26
Cr	36.77	17.67	21.96	18.57	17.71	13.65	60.39	8.94	356.93	38.36	8.17	9.19	43.13	11.31	24.20
Mn	194.87	292.71	255.47	86.54	109.48	263.23	269.69	48.29	258.62	359.46	89.76	572.72	305.22	112.58	581.92
Ni	22.26	10.48	7.71	11.92	18.09	13.84	44.86	5.83	239.54	25.14	5.13	9.61	22.61	10.04	13.45
Cu	20.38	50.41	18.74	11.47	12.13	18.24	30.35	6.32	24.06	55.05	13.61	17.69	32.41	25.61	28.80
Zn	62.25	81.18	71.92	29.03	53.78	72.20	68.47	26.35	36.22	76.09	44.44	99.49	81.64	72.81	195.00
Mo	2.51	3.05	5.01	1.19	2.90	2.35	3.86	1.08	47.98	3.59	0.61	2.26	4.11	0.79	4.30
Ba	119.98	156.60	91.26	46.85	97.19	87.04	335.88	95.83	135.72	152.07	43.72	58.95	279.87	104.93	196.03
In mg L^{-1}															
Fe	5.41	0.84	0.80	0.66	1.13	0.44	17.45	0.38	2.32	5.26	0.35	0.26	3.15	4.67	1.47

*n=5

Table B5: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA media in pot B6 to B10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	B6			B7			B8			B9			B10		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	488.57	14.01	58.73	277.17	20.67	131.00	703.99	51.81	45.63	511.62	42.66	122.64	230.17	56.31	71.52
Cr	39.15	8.69	9.23	47.19	13.97	26.16	43.65	10.38	8.69	127.99	10.98	26.73	33.12	17.18	16.96
Ni	16.05	3.38	6.30	19.38	5.53	9.03	19.36	4.82	4.08	40.99	5.06	11.34	13.10	7.15	6.83
Cu	50.02	9.43	11.99	57.07	18.36	30.00	45.92	10.58	7.45	126.58	11.06	26.48	42.74	15.42	20.66
Zn	182.05	32.83	43.10	131.97	43.58	66.49	158.99	42.86	22.59	166.73	34.19	57.70	112.57	41.97	40.83
Mo	17.82	0.42	3.33	4.52	0.77	3.00	36.17	1.95	3.19	14.60	1.68	3.22	4.49	2.15	1.46
Ba	178.95	24.21	40.73	389.44	78.27	148.25	213.38	62.71	38.68	533.69	74.98	178.22	239.15	80.77	94.98
In mg L^{-1}															
Fe	13.76	2.37	2.58	23.84	7.12	13.39	15.19	3.16	2.44	73.43	3.15	12.48	14.91	4.85	7.51
Mn	0.35	0.05	0.06	0.52	0.12	0.26	0.45	0.10	0.04	1.77	0.08	0.24	0.33	0.10	0.17

*n=5

Table B6: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA+soil media in pot C1 to C5, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	C1			C2			C3			C4			C5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	31.62	23.84	123.26	3.60	8.03	22.39	26.50	506.76	27.34	13.13	21.65	71.13	9.41	8.67	58.26
Cr	348.59	78.50	419.30	6.88	19.36	4.70	90.41	128.00	259.06	25.53	489.89	26.88	10.19	6.33	12.57
Mn	157.20	482.39	438.22	68.30	621.88	121.69	18.25	322.86	17.84	88.00	125.83	267.35	82.16	95.49	305.88
Ni	238.78	53.55	269.94	4.24	10.64	3.39	9.72	17.05	13.65	17.85	317.41	17.39	3.97	3.72	8.44
Cu	23.86	14.37	30.15	8.15	8.80	8.34	21.89	26.01	34.26	9.05	16.81	13.49	6.38	4.51	18.09
Zn	40.79	49.41	81.36	17.41	32.26	18.07	3.00	60.54	7.09	21.00	25.99	33.95	15.74	18.24	86.47
Mo	43.74	9.96	50.56	0.42	1.28	1.59	31.55	58.47	51.97	2.95	59.53	7.07	0.67	0.45	2.39
Ba	79.04	66.25	109.78	22.17	24.77	25.08	26.50	506.76	27.34	30.05	58.04	54.02	27.16	25.32	78.28
In mg L^{-1}															
Fe	3.07	0.92	2.51	0.37	0.31	0.10	0.53	2.53	0.33	0.55	2.51	0.30	0.38	0.26	0.32

*n=5

Table B7: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media in pot C6 to C10, i.e. raw data from IC-MS (2nd harvest - 69 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	C6			C7			C8			C9			C10		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	93.15	9.50	36.81	167.18	44.20	38.17	61.73	17.92	16.02	135.82	29.41	86.41	41.71	22.08	101.74
Cr	26.95	5.39	14.09	14.04	13.99	13.57	11.81	10.88	10.97	19.78	11.82	13.44	12.20	12.76	30.16
Ni	10.40	2.87	5.67	6.07	5.74	6.32	5.33	5.12	5.91	9.55	6.17	6.34	7.42	8.87	14.14
Cu	29.23	5.71	16.12	17.96	16.72	12.43	15.51	7.94	8.89	22.38	11.83	17.30	16.78	13.19	30.12
Zn	64.68	14.68	39.75	54.70	58.43	32.10	44.33	24.86	26.50	66.46	42.87	58.82	39.44	36.79	60.59
Mo	1.88	0.51	1.56	26.45	12.63	1.87	2.01	0.73	0.74	4.81	2.45	4.17	9.11	0.77	4.82
Ba	172.21	25.54	64.56	127.82	112.92	84.93	63.91	47.70	41.70	99.74	42.09	53.26	119.27	83.05	232.13
In mg L^{-1}															
Fe	12.45	1.10	4.45	2.10	2.17	5.10	4.17	2.17	1.40	6.76	1.54	3.42	2.62	3.29	16.63
Mn	0.24	0.03	0.10	0.08	0.08	0.11	0.13	0.04	0.05	0.18	0.05	0.10	0.08	0.08	0.30

*n=5

Table B8: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the soil media in pot A1 to A5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	A1			A2			A3			A4			A5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	91.88	121.74	139.77	153.39	75.69	93.50	144.81	102.79	87.57	147.73	87.46	192.93	133.64	139.55	122.64
Cr	24.38	210.43	42.21	50.04	61.00	68.80	673.41	50.27	150.38	29.46	38.71	25.72	14.48	43.94	23.68
Ni	12.86	37.02	13.85	17.42	22.77	19.89	21.93	36.48	14.54	12.28	36.25	15.03	15.99	43.92	18.72
Cu	37.72	60.36	32.64	47.23	39.91	36.34	63.76	68.82	35.06	46.34	67.06	57.50	45.97	100.82	44.24
Zn	79.63	359.86	155.71	200.43	285.60	176.46	90.19	167.94	85.83	145.19	229.35	224.56	126.81	408.19	161.78
Mo	1.22	1.33	0.60	0.75	0.53	0.62	1.14	0.64	0.59	0.82	0.48	0.70	1.51	1.16	0.65
Ba	141.08	544.67	327.30	385.49	682.76	376.12	366.45	605.82	341.77	389.93	1129.67	955.68	167.02	760.95	421.92
In mg L^{-1}															
Fe	1.68	1.80	0.87	3.67	1.23	1.09	1.55	0.98	0.65	2.69	1.16	1.26	1.73	2.71	0.87
Mn	2.99	18.80	5.58	3.20	8.15	7.69	9.42	20.18	7.31	2.03	10.91	5.93	3.37	16.75	7.03

*n=5

Table B9: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the soil media in pot A6 and A10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$						
	A6			A10		
	Leaf	Root	Stem	Leaf	Root	Stem
B	164.52	50.67	172.89	52.76	31.75	22.12
Cr	65.88	44.56	28.99	60.52	53.08	17.08
Ni	16.00	15.28	9.66	16.42	20.19	6.36
Cu	61.32	53.54	29.08	63.88	60.55	18.09
Zn	74.70	246.25	98.66	173.13	184.38	80.80
Mo	6.62	0.60	3.12	1.69	0.68	0.57
Ba	151.44	132.81	138.08	154.72	132.82	71.38
In mg L^{-1}						
Fe	37.80	26.68	12.71	37.37	28.91	6.98
Mn	0.90	0.50	0.38	0.77	0.60	0.16

*n=2

Table B10: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA media in pot B1 to B5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	B1			B2			B3			B4			B5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	210.08	281.06	126.94	136.59	105.94	165.86	231.95	194.01	146.45	233.07	163.88	166.72	241.17	85.97	209.47
Cr	15.92	97.28	17.63	14.15	34.90	21.86	27.54	46.25	36.66	14.53	75.07	12.37	21.17	18.44	23.50
Mn	546.89	919.75	169.20	328.97	322.77	421.13	421.56	487.83	183.38	474.64	484.20	268.29	359.40	242.62	340.12
Ni	1.19	11.84	1.59	2.20	4.54	3.06	1.69	5.43	2.38	1.24	12.05	1.43	2.62	1.77	1.95
Cu	6.16	24.67	7.14	5.97	11.66	9.40	9.46	15.62	10.19	6.21	32.77	4.96	9.01	4.89	7.97
Zn	34.33	80.67	50.32	18.39	23.34	21.13	29.47	48.70	20.49	17.71	44.26	14.16	17.77	18.48	20.11
Mo	13.65	11.85	5.43	3.69	3.17	2.73	5.69	6.20	2.62	11.81	7.72	7.65	13.90	4.47	10.90
Ba	298.37	1026.10	175.24	252.98	338.68	343.72	332.30	313.65	255.09	411.63	959.21	384.38	282.72	158.48	271.29
In mg L^{-1}															
Fe	2.31	9.58	3.90	3.04	4.24	5.91	4.32	3.77	4.66	2.28	13.91	2.65	3.68	1.85	3.66

*n=5

Table B11: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA media in pot B6 to B10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	B6			B7			B8			B9			B10		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	421.93	62.37	204.25	95.21	31.40	90.55	487.03	1079.76	148.38	997.62	68.34	339.40	45.14	29.39	155.20
Cr	23.38	113.98	54.51	33.23	18.69	19.33	42.20	41.78	21.10	24.11	26.60	12.68	95.49	35.55	13.06
Ni	9.88	49.41	19.11	14.69	8.15	7.17	16.45	16.33	8.46	10.82	6.51	5.83	45.19	15.65	5.86
Cu	24.99	11.88	40.94	12.07	8.15	13.70	35.27	37.32	21.91	30.10	21.35	19.60	17.54	8.14	13.73
Zn	51.66	58.08	99.33	27.51	21.15	38.63	110.16	134.30	64.70	79.55	56.22	67.22	37.57	29.14	54.63
Mo	17.03	8.21	17.06	1.50	0.94	1.53	30.67	35.98	5.53	52.14	7.74	27.37	0.65	2.53	2.50
Ba	148.72	57.62	313.42	79.34	76.63	75.59	127.68	210.82	100.66	111.83	110.30	60.57	97.16	52.59	88.80
In mg L^{-1}															
Fe	6.59	1.65	21.68	4.59	3.39	3.59	11.05	12.32	7.76	6.23	4.95	2.20	6.54	2.45	4.03
Mn	0.25	0.15	0.45	0.14	0.16	0.15	0.38	0.26	0.19	0.19	0.09	0.11	0.22	0.10	0.10

*n=5

Table B12: Chemical elements' concentrations in leaves, roots and stems of *S. Oleracea L.* grown in the FA+soil media in pot C1 to C5, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$															
	C1			C2			C3			C4			C5		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	335.15	227.77	91.55	171.79	85.83	153.21	212.87	105.86	110.97	473.29	144.27	66.22	282.39	128.51	114.27
Cr	12.14	82.61	14.60	17.96	23.63	35.85	17.12	57.72	16.38	28.10	55.08	27.08	13.39	82.79	104.40
Mn	1094.48	821.59	172.09	773.82	338.79	511.34	1040.13	410.77	312.33	1327.25	427.32	115.26	1392.60	575.68	411.32
Ni	1.09	16.66	1.54	2.43	3.81	6.44	1.52	5.79	1.70	1.28	3.88	1.39	1.41	4.03	2.45
Cu	6.60	44.46	6.31	8.26	9.87	18.02	7.26	19.54	6.57	6.25	13.00	7.06	5.84	45.79	17.31
Zn	34.35	74.16	22.02	32.86	23.96	43.32	40.74	30.98	23.13	29.22	32.21	11.62	42.38	32.51	26.57
Mo	10.44	7.45	2.30	6.28	2.45	3.96	10.26	3.39	2.98	13.68	3.63	1.35	9.55	4.11	3.38
Ba	435.86	1120.61	201.22	482.73	588.05	823.88	430.17	672.72	521.55	616.74	996.82	215.42	442.47	331.43	307.99
In mg L^{-1}															
Fe	1.99	17.13	3.30	5.34	4.84	9.38	2.90	5.95	3.72	2.00	6.27	2.67	1.99	5.16	4.83

*n=5

Table B13: Chemical elements' concentrations in leaves, roots and stems of *B. juncea* grown in the FA+soil media in pot C6 to C10, i.e. raw data from IC-MS (3rd harvest - 115 days of pot culture experiments).

In $\mu\text{g L}^{-1}$									
	C7			C8			C9		
	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
B	186.78	39.04	49.24	91.90	19.73	68.29	100.13	42.25	16.77
Cr	41.93	6.72	13.46	69.37	17.54	18.05	16.43	10.39	7.66
Ni	17.94	3.19	6.04	29.43	8.10	7.18	5.72	6.01	3.10
Cu	45.51	8.87	12.84	21.10	16.76	13.13	14.08	12.87	6.77
Zn	110.58	29.81	42.44	50.62	36.02	29.53	40.94	39.22	12.99
Mo	4.32	3.13	5.48	1.48	0.34	1.16	2.00	0.60	0.35
Ba	301.52	37.47	77.38	135.61	102.22	98.85	85.63	56.53	29.19
In mg L^{-1}									
Fe	22.00	1.39	3.41	8.53	6.57	4.43	5.89	2.19	1.59
Mn	0.39	0.04	0.07	0.28	0.17	0.17	0.21	0.12	0.06

*n=3