Field-Scale Phytoremediation of Mixed Contaminated Site in Chicago, USA

BY

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THESIS

Submitted as partial fulfillment of the requirements
For the degree of Master of Science in Civil Engineering
In the Graduate College of the
University of Illinois at Chicago, 2016.

Chicago, Illinois

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This thesis is dedicated to my parents, Emilio Amaya and Encarnación Santos, because they are the cornerstone of my life, and without whom it would never have been accomplished.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor, Prof. Krishna R. Reddy, for his support with my M. S. studies. He was the reason behind making my dream come true. I am very thankful to Prof. Reddy for everything I have learned and the experience I have gained working with him.

I would like also to express my gratitude to my thesis committee members Prof. Benjamin O'Connor and Prof. Amid Khodadoust for taking their time to serve in my committee. This thesis would have never been accomplished without the valuable help of Erin Yargicoglu, Cristina Negri and Daniel Cooper, because they have been the promoters of this project and who helped me to take the first steps towards the realization of this thesis. I would also like to thank my laboratory colleagues and students who helped me with the field and experimental work, especially Yuling Yang and Öznur Karaca, who enriched my research and my personal experience during the time I was fortunate to share with them. My gratitude also to the Professors Sean Vitousek, Kouros Mohammadian and Li An, for his assistance and support and to help me widen my research from various perspectives.

I also would like to thank my previous advisor Prof. J. M. Rodriguez – Maroto, for believe in me and for being a source of inspiration and support. Special gratitude to my "Mandarinas team" at the University of Málaga, for their warmth, support and friendship. I would especially like to thank my mentor, my friend and my previous co-worker Dr. María Villén – Guzmán, for believing in me and for being an inspiration. Thank you "Mimery".

I am thankful to the people that have helped me during my stay at UIC: the OIS team for their tremendous work, Dr. Chandan for guiding my steps in the difficult moments and helped me

to overcome personal difficulties, and Karoline Qasem, whose determination and courage have been an inspiration, besides being a very important personal and academic support. Also, I cannot forget to mention the staff of the Civil and Materials Engineering Department that have helped me throughout all of these months, especially Douglas, Kirk, Kassem, Sara Arevalo and Pam, thanks to all of you for the wonderful job you do.

And last but not least, I would like to express my great gratitude to the most important people in my life, who make it worthwhile, my guide star: My family, especially my parents and aunt Salvadora Santos, my soul sister Karina Arancibia and her family, my friends, for their love and spiritual support and my guardian angel Amjed Al – Taweel, for all the help, support and patience, and making the difficult moments less hard and more enjoyable. Thanks to all of you for being part of my life.

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SUMMARY

Big Marsh is one of the largest expanses of wetland within the Calumet region. The site, which is representative of many other unrestored wetland sites in this region that have been significantly altered by the steel industry and decades of legal and illegal dumping, has been massively altered from original conditions by industrial filling. These fill materials, as well as the soil and surface water have been found to be contaminated with both organic (polycyclic hydrocarbons) and inorganic contaminants (heavy metals). Therefore, the wetlands at Big Marsh are greatly in need of restoration efforts. The large size of the site as well as the shallow contamination make the implementation of phytoremediation technique as the most feasible and sustainable restoration technique to the remediation of the site with mixed contamination. The use of plants to restore areas impacted by industrial activities enhances soil structure and microbial activity, stimulating the biodegradation processes in the soil. The objective of the present work is to study the feasibility of the field – scale implementation of phytoremediation technology in a mixed contaminated site at Big Marsh, and study the final fate of the contaminants (heavy metals and polycyclic aromatic hydrocarbons) in the soil of the three different Areas of Concern found to be representative of the different ecotypes present at Big Marsh: slag disposal area, wet meadow and upland area. In addition, the enhancement of the phytoremediation technique by amending the soil with compost is also evaluated. The study duration extended to three growing seasons. During the first season, replicate test plots are prepared by tilling and homogenizing the soil, sediments and fill material. Soil is also amended with compost only at the slag disposal area. A total of 9 native and restoration plant species specific for each area are planted, and their survival and growth is monitored during two growing seasons. At the end of the second and third growing seasons, sampling of soils and plants is performed, and polycyclic aromatic hydrocarbons and heavy metals are analyzed.

Additionally, sequential extraction is performed in all soil samples to determine the fractionation and mobilization of heavy metals in the soil throughout the experiment. The results showed that only 4 out of the 9 species planted at the slag disposal area, and 1 out of the 9 species planted at the wet meadow and upland area survived at the end of the experiment. All of the surviving plants except the one at the wet meadow area were herbaceous species and prairie grasses. A decrease in PAHs concentrations in the soil of the slag disposal area, and non-detectable levels of PAHs in the above and below ground plant samples was found. While no changes were observable in the soil concentration of PAHs at the wet meadow area and upland area, concentration of those contaminants in the roots of their surviving species was detected. However, the concentration of PAHs in shoots and leaves was also undetectable. Overall, no changes in the concentration of heavy metals in the soil of any of the three areas of concern are observed, except for Manganese, which decreases in the soil of the upland area. Concentrations of heavy metals were detected in the roots of all the surviving species analyzed but not in stems and leaves, except for Manganese, that was uptaken by the plant. Results from sequential extraction showed that the exchangeable fraction of the metals in the soil at each experimental area was very small, indicating that these metals have very low mobility. In the case of Mn, results from sequential extraction showed that it was mainly retained in the Fe and Mn oxides – bound fraction, what makes this element more bioavailable. Overall, the native grasses showed the best survival rates, and in combination with compost amendment at the slag disposal area showed the best performance. Additionally, the addition of compost amendment seemed to enhance the process of biodegradation of PAHs in the soil, and buffered the negative impact of high concentration of toxic metals in the soil that could potentially cause phytotoxicity.

CHAPTER 1

INTRODUCTION

1.1.Problem Statement

The ecosystemic importance of wetlands is well known all over the world, since they are the habitat for numerous threatened and endangered species, as well as being an important sink of carbon. Furthermore, healthy wetlands perform crucial functions for the environment, such as flood mitigation and water cleaning and detoxifying. However, despite all the focused efforts, these ecosystems are severely disappearing. In northwestern Indiana and Southeast Illinois, the Lake Calumet region contains some of the richest remaining wetlands. Due to the heavy presence of the industrial activity that exists in the region, a great fraction of those wetlands has been degraded. Part of the sediments have been contaminated and some of the uplands are barren due to phytotoxicity.

Big Marsh is one of the wetland extensions that exist within the Calumet region. This site covers approximately 121 hectares within the Great Lakes Basin, and is representative of many other wetlands in the area negatively impacted by steel industry and decades of spills and both, legal and illegal dumping. The natural conditions of this wetland have been massively altered, with a great amount of fill material within its sediments. Big Marsh contains upland habitat areas, which were created largely with foundry slag. 9 hectares in the southeast corner of Big Marsh are composed of innocuous fill that contains a high percentage of iron and is suspected to be blast furnace slag or similar material. Sixteen hectares of the southern filled section contain impenetrable slag and has been devoid of vegetation for 35 years. Only a few eastern cottonwood trees (*Populus deltoides*) and low herbaceous vegetation have managed to establish growth during this time. Surface waters in Big Marsh are less than two feet deep in most areas. Fill materials across the site range from 2 to 3 m thick and consists of steel-mill

slag, with some construction/demolition debris and dredge spoils from Lake Calumet and the Calumet River. Water quality is impacted by high pH levels; in some areas the pH reaches 12.6. Bottom sediments in the marsh are natural muck soil that has not been dredged. The southeastern part of the site is covered with white calcite that leaches out of the slag from adjacent upland fill. Although there is no known contamination source at the site, the fill materials as well as the soil and sediments have been found to be contaminated with both heavy metals and organic contaminants such as polycyclic hydrocarbons (PAHs). Therefore, wetlands at Big Marsh are severely in need of restoration efforts. Several Phase II Environmental Site Assessments that have been completed at Big Marsh show that there are three different Areas of Concern in terms of soil and fill conditions that are representative of three different ecotypes. The specific areas identified for the investigation are a slag disposal area located at the East side, a degraded wet meadow area located at the South East side, and an upland area near emergent wetland at the North West.

The great areal extent of the site as well as the shallow contamination make the implementation of conventional remediation techniques not feasible due to their applicability is often limited to a single sort of contamination or site conditions (Cameselle et al., 2013). A previous study, Reddy and Chirakkara (2013) identified that the phytoremediation technique has potential to be the most feasible and sustainable restoration technique to the remediation of a site with similar characteristics as Big Marsh. Plants utilize several mechanisms to remove, degrade, or contain soil and groundwater contaminants. Some mechanisms target certain types of contaminants over others (e.g. only volatile compounds will be capable of being evapo-transpired (phytovolatilization) through the leaves and shoots of the plants). Several organic compounds (e.g. TCE, PCE) can be completely degraded by the plant, while inorganic contaminants tend to be sequestered or accumulated within the plant. One of the main advantages of phytoremediation implementation is the restoration of an area affected by the

impact of industrial activity without altering the soil structure or disturbing its biological activity. Moreover, the establishment of plants in an eroded site stabilizes and holds the soil, curbing such erosion and minimizing the risk of contaminant exposure by windblown dust. In addition to that, the soil structure is enhanced and the organic content increases, ameliorating microbial activity (Marmiroli et al., 2006; Ovurard et al., 2011).

Most of the reported studies on phytoremediation are focused on a single type of contamination and on laboratory testing which study do not reflect all of the factors that really affect the growth and survivorship of the plants. With the aim of a better understanding of the effect of mixed contamination on phytoremediation, a previous study has been performed in our laboratory (Chirakkara, 2014; Chirakkara and Reddy 2014, 2015) with the objective of investigating the effects of the mixed contamination on the growth and survivorship of the plants and their contaminant uptake. This study demonstrated that phytoremediation has a great potential as a green, sustainable and effective remediation technology for the treatment of soils polluted with organic contaminants and heavy metals, and also, highlighted the beneficial use of biomass soil amendment to enhance the plants survival and growth. Nonetheless, despite the satisfactory results, the field–scale implementation of this technique still represents a challenge, due to site specific variable ground conditions and contaminants that should be taken into account in the design of phytoremediation application (Chirakkara et al., 2016). Therefore, further investigation on field–scale phytoremediation of mixed contaminated soils is needed. The present work is developed with the aim of studying in detail the challenges posed by the field–scale implementation of phytoremediation technique at Big Marsh, a site with mixed contamination.

1.2. Research Objectives

The combined effects of mixed contamination on plant uptake and their subsequent effect of growth as well as the enhancement of the phytoremediation in mixed contaminated soil has been previously studied in detail (Chirakkara, 2014). However, very little is known about the field–scale implementation of this technique. In order to identify the main limitations and to optimize the strategy for mixed contaminated sites sustainable restoration, the field – scale phytoremediation of mixed contaminated soil needs to be further investigated.

The main goal of the present work is to investigate the feasibility of the field–scale phytoremediation technology implementation at a site contaminated with heavy metals and organic contaminants (PAHs), specifically at Big Marsh site at each of the three different Areas of Concern identified at the site site. Additionally, the addition of soil amendment to ensure successful growth of new vegetation is also evaluated. The specific objectives of the research are to:

1. Study the survival and growth of 18 native and restoration species at three Big Marsh's Areas of Concern.
2. Assess the feasibility of the selected plants to be used in phytoremediation and reduce soil contamination exposure at Big Marsh.
3. Study how the phytoremediation technique affects the soil characteristics.
4. Study the final fate of organic contaminants (PAHs) and heavy metals in the soil and uptake or degradation of the contaminants in the roots and stems.
5. Study the effect of using compost amendment in the soil with mixed contamination and assess its potential to enhance the field – scale implementation of the phytoremediation technique.

1.3. Thesis Organization

This thesis is organized in six chapters as follows:

- Chapter 2 describes the phytoremediation of the slag disposal area at Big Marsh and evaluates the potential benefits of compost amendment addition to the mixed contaminated soil.
- Chapter 3 explains the phytoremediation of the wet meadow area.
- Chapter 4 describes the phytoremediation of the upland area.
- Chapter 5 makes a comparison of the important results obtained from the three areas and studies the effect of varying site conditions on phytoremediation of mixed contaminants.
- Chapter 6 provides the overall conclusions and recommendations.

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CHAPTER 2

PHYTOREMEDIATION OF MIXED CONTAMINATION IN SLAG DISPOSAL AREA: FIELD – SCALE INVESTIGATION AT BIG MARSH WETLAND SITE, CHICAGO, USA.

2.1. Introduction

Throughout the United States and internationally, wetlands are important resources that, despite focused efforts, have been steadily disappearing. Wetlands serve as habitats for threatened and endangered species and are enormous sinks for carbon, and provide crucial environmental functions including cleaning and detoxifying water and mitigating floods. In northwestern Indiana and northeastern Illinois, the Lake Calumet region contains some of the richest of the remaining wetlands. Because of the heavy industrial presence in the region, a high fraction of these wetlands have been degraded. Many of the wetland sediments are contaminated, some of the upland areas are barren due to plant toxicity, and concern exists that surface and ground water in the area are being negatively impacted by residual contaminants.

Big Marsh is one of the largest expanses of wetland within the Calumet region. This site is representative of many other unrestored wetland sites in this region which have been significantly altered by the steel industry and decades of legal and illegal dumping. The wetland has been massively altered from original conditions by industrial filling and these fill materials as well as the groundwater and surface water have been found to be contaminated with polyaromatic hydrocarbons; benzene, toluene, ethylbenzene, and xylenes; organic solvents; polychlorinated biphenyls, and heavy metals. Therefore, the wetlands at Big Marsh are greatly in need of restoration efforts.

Sites with mixed contamination pose technical challenges associated with the present of various classes of contaminants with different physico – chemical properties, because they

will respond in a different way to the remediation technologies. Several technologies for the remediation of contaminated soils have been developed over the past three decades. Their applicability is often limited to a particular kind of contaminant. In the case of contaminated sites with mixed contamination, few technologies have proven to be efficient, but they also have important limitations, plus their application at field scale results very expensive. In this context, phytoremediation arises as a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al., 2013).

A previous study showed that the mixed contamination in the soil had a significant effect of the plant growth (Chirakkara and Reddy, 2014). The ability of the plants to survive in high impacted areas and the low bioavailability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency. Phytoremediation can be enhanced either by increasing the capability of contaminant uptake by the plant or amending the soil to increase the bioavailability of the contaminants. The addition of organic matter to the soil can improve the soil and increase the plant biomass in phytoremediation (Masciandaro et al., 2013). The addition of compost to the soil can improve plant growth as well as increase soil microbial activity (Ghanem et al., 2013). Compost is expected to immobilize the metal contaminants in the soil, thus plant germination and growth are expected to improve in composted soil (Alvarenga et al., 2014).

The present work investigates the use of phytoremediation in a slag disposal upland area at Big Marsh, a wetland in southeast Chicago (Illinois, USA), contaminated with PAHs and Heavy Metals. This study, conducted over three growing seasons, includes planting, monitoring, subsequent analysis and all the data was used to analyze and study the plant survival and growth and contaminant uptake, with the aim of evaluating the plants species and phytoremediation feasibility at the site.

2.2. Site Background

The contaminated site, with 121 hectares of open space classified as wooded/marshland without any on – site structures, and 35 hectares of wetland, is one of the largest expanses of wetlands within the Calumet region. It falls within the Great Lakes Basin and is hydrologically connected to Lake Michigan through Lake Calumet and the Calumet River. Big Marsh is relatively level and undeveloped with large areas of open water, degraded wetlands, and upland fill areas covered with invasive species of vegetation.

Big Marsh has historically been used for storage and disposal of potentially hazardous substances, being the site representative of many other unrestored wetland sites in this region which have been significantly altered by the steel industry and decades of legal and illegal dumping. The fill material at the site is largely composed of steel mill slag, foundry sand, construction and demolition debris, and dredge spoils from Lake Calumet. Much of the soil and sediment at the site is contaminated and some of the upland areas are barren due to slag fill.

Phase I Environmental Site Assessment (ESA) was used with the purpose of identify all the Recognized Environmental Conditions (RECs) that are present on the Big Marsh (ASTM, 2013). More specifically, the ESA aimed to determine the nature, concentration, direction and rate of movement, and extent of the contaminants of concern. All significant physical features of the remediation site and vicinity were evaluated to predict their effects on contaminant fate and transport, as well as human health, safety, and the environment.

The investigations revealed that site geology can be classified into three types: Fill material, stratified drifts, and silt and clay. Fills material surfaces are generally underlain by an undisturbed layer of sand which is underlain by an unconsolidated layer of till consisting of sand, silt, and clays. Stratified drifts are usually ice channel deposits, consisting of coarser grained materials that are found at the base of the till layer. There are two aquifers in this

region separated by dense glacial till confining soil. Regional shallow aquifers are found in fill material and Carmi lacustrine sediments. Based on regional topography, shallow groundwater flows to the west towards Lake Calumet. Deeper groundwater flows towards Lake Michigan. Site hydrogeology enters from the northeast, southeast, and northwest. Areas of unexcavated sand and fill flow southwest to Lake Calumet. Deeper groundwater follows the bedrock surface contours to the south and southeast to Lake Michigan.

Soil, surface water, sediment and groundwater samples at various locations throughout the site were analyzed for the presence of Polynuclear Aromatic Hydrocarbons (PAHs), Semi – volatile Organic Compounds (SVOCs), pesticides, metals and pH. Soil contamination was predominantly metals and SVOCs, which exceeded the limits of IEPA Taco Tier 1 residential ingestion and/or inhalation SROs (IEPA 2001). Soil mounds and localized debris piles were also in excess for metals and SVOCs. Site sediments at many of the site surface water had excessive metal concentration. However, groundwater and surface water samples determined contamination was not an issue.

The risk assessment is performed to quantify the threat posed to human health and environmental health of plants and animals. Human risk assessment is performed according to the Illinois Environmental Protection Agency (IEPA) methodology (Illinois Administrative Code, Part 742: Tiered Approach to Corrective Action Objectives) (Sharma and Reddy, 2004). Table 2.1 compares the maximum concentrations at the site to the IEPA TACO Tier 1 standards for the critical exposure pathway. The highlighted numbers show the critical exposure pathway for that specific contaminant. The highlighted cells indicate that the maximum detection by media on the site exceeds the IEPA TACO Tier 1 standard. From the table, it is noticeable that a high number of contaminants that could possibly be a harm to human health. The ecological risk assessment was completed by taking the maximum concentrations at the site and comparing them to the soil and sediment benchmarks given in the Calumet Area Ecotoxicology

Table 2.1. Human risk assessment as per IEPA TACO Tier 1

Contaminant	Exposure Rout Specific Values for Soil		Soil Component of Groundwater Ingestion	Max Detection by media (mg/Kg)				
	Residential Ingestion	Residential Inhalation	Class 1 Groundwater	Surface Soil	Sediment	Shallow Subsurface Soil	Deep Subsurface Soil	Surface water
pH								12.02
2,4 - Dimethylpheol	NA	NA	0.14			0.11		
2-Butanone	47000	25000	17					0.17
2-Methylnaphthalene	NA	NA	NA			0		
3,4-Methylphenol	NA	NA	NA	1.94		23.1		
4,4'-DDD	3	NA	16			1.3		0.017
4,4'-DDE	2	NA	54		0.02	0.1		
4,4'-DDT	2	1500	32		0.13	0		
Acenaphthene	4700	NA	570		0.04			
Acenaphthylene	NA	NA	NA	2.03	0.11	2.1		
Acetone	70000	100000	25	0.67	0.08	0.4		
Anthracene	23000	NA	12000	6.71	0.42	4.8		
Araclor 1248 (PCB 1248)	NA	NA	NA	1.27				
Arsenic	13	750	0.05	70	26.9		168	
Barium	5500	690000	2	342	672		555	0.02
Benzene	12	0.8	0.03	0.01		1		0.24
Benzo(a)pyrene	0.1	NA	8	11.4	1.18	7.7		
Benzo(a)anthracene	0.9	NA	2	10.9	1.09	8.2		
Benzo(b)fluoranthene	0.9	NA	5	12.7	1.15	6.6		
Benzo(g,hi)perylene	NA	NA	NA	7.06	0.51	3.8		
Benzo(k)fluoranthene	9	NA	49	6.6	1.07	5.7		
bis(2-ethylhexyl)phthalate	46	31000	3600			1.3		
Cadmium	78	1800	0.005	24	43.6		26.7	
Carbazole	32	NA	0.6	11.2		1.5		
Carbon disulfide	7800	720	32			0.02		
Cholorofm	100	0.3	0.6	0.013		0.01		
Chromium	230	270	0.1	1620	202		2010	0.011
Chrysene	88	NA	160	8.55	1.09	8.3		
Copper	2900	NA	0.65	158	233		775	0.013
Dibenz(a,h)anthracene	0.2	NA	2	2.38	0.18	1.2		
Dienzofuran	310	NA	NA	3.04		1.3		
Diethylphtyalate	0.1	2000	2000			1.3		
Ethylbenzene	7800	400	13			2.8		
Fluoranthene	3100	NA	4300	23.7	1.94	16.8		
Fluorene	3100	NA	560	5.41	0.16	2.4		
Indeno(1,2,3,-cd)pyrene	0.9	NA	14	6.75	0.67	4.6		
Lead	400	NA	0.01	1440	2720		1860	0.03
Mercury	23	10	0.002	0.51	1.93		0.57	
Naphtyalene	1600	170	12	2.75	0.278	9.2		
Nickel	1600	13000	0.1	54.1	87.2		462	0.01
Phenanthrene	NA	NA	NA	33.2	1.79	15.3		
Pyrene	2300	NA	4200	17.5	2.39	14.9		
Selenium	390	NA	0.05	7.1	7.4		9.5	
Silver	390	NA	0.05	6.3	9.7		10	
Toluene	16000	650	12	0.03		0		
TotalXylenes	16000	320	150	0.04		13.7		
Zinc	23000	NA	5	9100	3860		22000	0.012

NA: Not available.

Report (CDM 2010). The threshold and benchmark values were acquired by a technical team by researching many different sources. The definitions for benchmark and threshold from the Calumet Area Eco-toxicology Report are presented below:

- Calumet Open Space Reserve (COSR) Threshold values: Chemical concentrations derived from toxicity studies that identified no-observable-adverse-effect levels (NOAEL) for a variety of plants and animals.
- COSR Benchmarks values: Chemical concentrations derived from toxicity studies that identified lowest-observable-adverse effect levels (LOAEL). (Calumet Eco-toxicology Roundtable Technical Team).

Table 2.2 compares the on-site concentrations with the benchmark and threshold. The columns highlighted are the benchmark and threshold columns. Cells that are highlighted mean that the maximum concentration exceeded the benchmark value for that specific contaminant.

2.3. Research Methodology

2.3.1. *Initial Soil Characterization*

A delineation survey was conducted to determine the extent and boundary of the slag disposal area at Big Marsh. The initial baseline sampling was conducted on the site in order to identify the existing heavy metal and organic contaminants present in the soil. Five composite samples were taken along transects representing roughly equivalent conditions at the Slag Disposal Area. Sampling locations were recorded using a GPS.

Soil samples were collected to perform soil characterization and contaminant concentration analysis.

Table 2.2. Ecological risk assessment.

Contaminant	Ecological Assessment: Calumen Open Space Reserve (mg/Kg)				Max Detection by media (mg/Kg)					
	Soil Threshold	Soil Benchmark	Sediment Threshold	Sediment Benchmark	Surface Soil	Sediment	Shallow Subsurface Soil	Deep Subsurface Soil	Surface water	Ground Water
pH									12.08	
2,4 - Dimethylpheol							0.1		0.17	0.017
2-Butanone										
2-Methylnaphthalene							0.0			
3,4-Methylphenol					1.94		23.1			0.11
4,4'-DDD	0.004	0.04	0.005	0.06			1.3		0.017	
4,4'-DDE	0.004	0.04	0.003	0.03		0.02	0.1			
4,4'-DDT	0.004	0.04	0.004	0.03		0.13	0.0			
Acenaphthene	4	20	1.3	1.3		0.04				
Acenaphthylene	NA	NA	0.01	0.13	2.03	0.11	2.1			
Acetone					0.67	0.08	0.4			
Anthracene	11400	51000	0.06	0.85	6.71	0.42	4.8			
Araclor 1248 (PCB 1248)					1.27					
Arsenic	18	31	9.79	33	70	26.9		168		
Barium	330	585	NA	NA	342	672		555	0.02	0.003
Benzene					0.01		1.0		0.24	0.005
Benzo(a)pyrene	11.3	113	0.15	1.45	11.4	1.18	7.7			
Benzo(a)anthracene	NA	NA	0.11	1.05	10.9	1.09	8.2			
Benzo(b)fluoranthene	1	10	10	NA	12.7	1.15	6.6			
Benzo(g,hi)perylene	NA	NA	0.17	3.2	7.06	0.51	3.8			
Benzo(b)fluoranthene	1	10	0.24	13.4	6.6	1.07	5.7			
Benzo(g,hi)perylene	NA	NA	0.17	3.2	7.06	0.51	3.8			
Benzo(k)fluoranthene	1	10	0.24	13.4	6.6	1.07	5.7			
bis(2-ethylhexyl)phthalate	NA	NA					1.3			
Cadmium	0.4	3.37	0.99	4.98	24	43.6		26.7		0.01
Carbazole					11.2		1.5			
Carbon disulfide							0.02			
Cholorofm					0.013		0.01			
Chromium	26	131	43.4	111	1620	202		2010	0.011	
Chrysene					8.55	1.09	8.3			
Copper	54	910	31.6	149	158	233		775	0.013	
Dibenz(a,h)anthracene	NA	NA	0.03	0.14	2.38	0.18	1.2			
Dienzofuran					3.04		1.3			
Diethylphtyalate							1.3			
Ethylbenzene							2.8			
Fluoranthene	1380	2750	6.2	6.2	23.7	1.94	16.8			
Fluorene	6	30	0.54	0.54	5.41	0.16	2.4			
Indeno(1,2,3,-cd)pyrene	1	10	0.2	2	6.75	0.67	4.6			
Lead	16	430	35.8	128	1440	2720		1860	0.03	
Mercury	0.07	1.3	0.18	1.06	0.51	1.93		0.57		
Naphtyalene	852	17000	0.47	0.56	2.75	0.278	9.2			
Nickel	44	210	22.7	48.6	54.1	87.2		462	0.01	0.02
Phenanthrene	5	50	1.8	1.8	33.2	1.79	15.3			
Pyrene	83	1350	0.2	1.52	17.5	2.39	14.9			
Selenium	0.8	1	4	4	7.1	7.4		9.5		
Silver	0.4	2	1	3.7	6.3	9.7		10		
Toluene					0.03		0.0			
TotalXylenes					0.04		13.7			
Zinc	113	250	121	459	9100	3860		22000	0.012	

NA: Not available.

2.3.2. *Test Section Preparation*

The experimental area was identified based on preliminary soil initial baseline sampling. An experimental and adjacent plot of size 16m x 16m each, were demarcated in the slag disposal area, representative of one of the three different ecotypes present at Big Marsh. Ground was prepared by adding a thin layer of compost on the surface and then tilling and homogenizing the fill material to approximate depth of 1m.

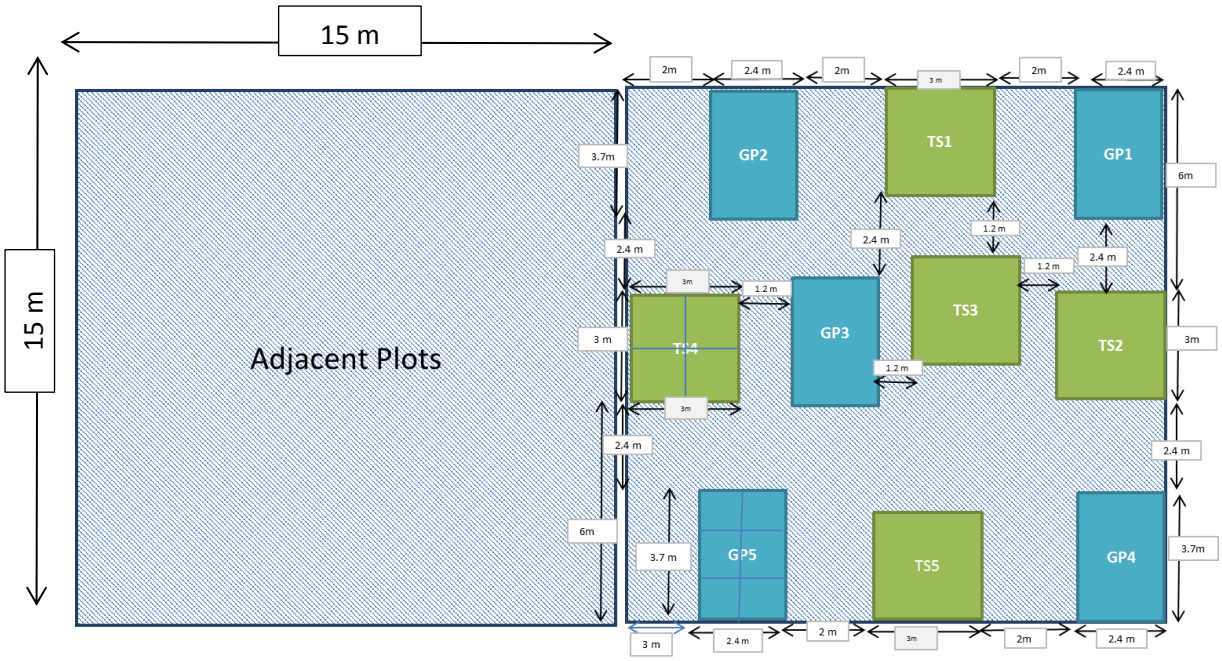
At the experimental plot (15m x 15m), two different types of subplots were designed in order to establish herbaceous and woody plants. Those parcels intended for planting herbaceous plants were called GP (Grasses and Plugs) plots, and those plots used for planting trees and shrubs were called TS (Trees and Shrubs) plots (Figure 2.1a). A total number of 5 subplots, each 3m x 4m were selected as GP plots. Each subplot was divided into 6 groups of size 1.3m x 1.3m, and each group was divided into 16 cells of size 0.3m x 0.3m (Figure 2.1b). Another 5 subplots of size 3m x 3m each, were selected as TS plots, and each subplot was divided into 4 groups, each 1.5m x 1.5m (Figure 2.1c).

The adjacent plot (15m x 15m) was delineated next to the experimental plot with the purpose of monitoring plant survival and grow characteristics of the grass species. Figure 2.1 shows the experimental and adjacent plots layout, and Figure 2.2 shows the images of the area of study during the first stage of the experiment.

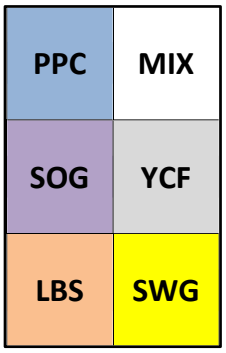
One composite soil sample from each group at each subplot of the experimental plot was collected for baseline contaminant concentration analysis and soil characterization.

2.3.3. *Plant Selection and Planting*

The selection of plants was based on the potential phytoremedial properties and the soil characteristics. For the slag disposal area, a total of 9 native and restoration species that included 5 species of grass and plugs and 4 species of trees and shrubs were chosen.



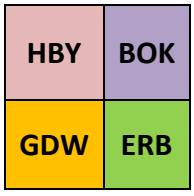
a. Overview of Plot Layout



1	2	3	4	1	2	3
4	5	7	8	4	5	6
9	10	11	12	7	8	9
13	14	15	16	10	11	12
				13	14	15

1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16

b. Grass and plugs (GS) subplots planting layout



1	2	1	2
3	4	3	4
1	2	1	2
3	4	3	4

c. Trees and shrubs (TS) subplots planting layout.

Figure 2.1. Plots and subplots delineation layout.

Figure 2.2: Planting at Slag Disposal Experimental Area. (a) Before any treatment. (b) Plot delineation after tilling and homogenization. (c) Trees and Shrubs planting. (d) Experimental Area after planting.

According to the delineation, the GS subplots were divided into 6 subgroups, 5 of which were designed with the aim of planting the grass samples grouped by species, and the remaining group with the aim of planting all the species together. A total of 16 samples of the same species were planted at each subgroup, and 3 species of each sample were planted at the remaining subgroup. A total of 96 grass samples were planted within the experimental plot, and 20 samples were planted in the adjacent plot.

Within the plot intended for planting trees and shrubs (TS plots), a subdivision into groups for the different species was also performed. In this case, no subgroup was intended for planting mixed species. At each subgroup, only one woody species was planted, and a total of 20 woody species (trees and shrubs) were planted within the experimental plot. No woody samples were planted in the adjacent plot. Table 2.3 shows the species selected for this experimental area.

2.3.4. *Watering and Monitoring*

Once soil preparation and planting was completed, the test plot was watered twice a week throughout summer months (June to August) and monitored weekly for survival, leaves, pests and infection, and height of the woody plants during the first growing season. At the adjacent plot, only survival monitoring was performed. Table 2.4 shows the rating system used to assess plant health.

During the second growing season, the test plots were monitored bi-weekly during the summer. No additional water or pest control was performed at the experimental area, in order to let the plants grow under normal conditions and assess the suitability of the native plants to cope with the natural site conditions and compete against the invasive species. Figures 2.3 and 2.4 show the monitoring plant survival and growth of a representative species of grasses (Switchgrass) and a representative woody species (Gray Dogwood), respectively.

Table 2.3. Species selected for restoration of slag disposal area

Type	Scientific Name	Common Name	Sample ID	Number of samples	
				Experimental Plot	Adjacent Plot
Grasses and Plugs	*Andropogon scoparius*	Little Bluestem	LBS	96	50
	Bouteloua curtipendula	Side Oats Grama	SOG	96	50
	Dalea purpurea	Purple Prairie Clover	PPC	96	50
	Panicum virgatum	Switch Grass	SWG	96	50
	Ratibida pinnata	Yellow Coneflower	YCF	96	50
Trees	*Celtis occidentalis*	Hackberry	HBY	20	0
	Quercus velutina	Black Oak	BOK	20	0
Shrubs	*Cornus racemose*	Gray Dogwood	GDW	20	0
	Circis canadensis	Eastern Redbud	ERB	20	0

a. After planting **b. 1 month** **c. 2 months**

d. End of the 1st season **e. End of the 2nd season** **f. End of the 3rd season**

Figure 2.3: Growth monitoring pictures of Switchgrass at the adjacent plot

a. After planting **b. 1 month** **c. 2 months**

d. End of the 1st season **e. End of the 2nd season**

Figure 2.4. Growth monitoring pictures of Gray Dogwood at the experimental plot TS1

Table 2.4. Monitoring rating system

Parameter	Measurement
Height (H)	Height in cm[1]
Survival (S)	Scale 1-4 (1 =dead; 2 =dying; 3 =no change in growth; 4 =evidence of new growth)
Leaves (L)	Scale 1-4 (1 = >50% leaves are dead; 2 = >25% leaves are dead, discoloration and/or wilting is present; 3 = <25% of leaves are discolored and/or wilting with no dead or dying leaves present; 4 = No discoloration, wilting or dead/dying leaves.)

1For woody species only.

2.3.5. *Termination Sampling*

At the end of the second growing season, a first set of soil and plants sampling was performed at the Slag Disposal Area. A total of 30 soil samples were taken from each GP subplot (6 soil samples/plot for 5 plots) from 2-3 representative samples randomly selected from surviving plants within the plot. All soil samples were kept on ice during the day. Vegetative biomass from 2-3 representative plants were taken from each GP plot, divided into above ground (leaves and shoots) and belowground (roots) biomass.

At the end of the third growing season, a terminal sampling was performed. A total of 30 soil samples were collected from all GP subplots. Vegetative samples consisting of roots, leaves and shoots were also collected from each surviving species. Additionally, two grab samples from invasive vegetation (Sweet Clover) were also taken. Target contaminants (BaP, As, Cr, Pb and Mn) were analyzed in all soil and vegetative tissues sampled. Also, complete analysis of metals and PAHs was performed on selected soil and vegetative samples. Soil characterization tests were also performed on soil samples in the lab.

2.3.6. *Soil and Plant Sample Tests*

The soil characterization tests performed in the lab consisted of physicochemical properties that mainly included measurements of the pH, electrical conductivity (EC), Organic Carbon (OC), and Oxidation – Reduction Potential (ORP), Water Holding Capacity (WHC), Grain size distribution (GSD),– and Exchangeable Nutrients Content.

The soil pH and ORP were determined according to the ASTM D4972 – 01 Standard Test Method for pH of Soils (ASTM 2007). The values were measured in the laboratory using an Orion Model 720-A pH/ISE meter. Water content values were measured in the laboratory according to ASTM D 2216 Standard Test method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass (ASTM 2005). Organic Carbon was determined

using ASTM D 2974 Standard Test Method for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils (ASTM 2000). The Electrical Conductivity of the soil was measured in a 1:5 soil: water suspension, using a Fischer Scientific model TRACEABLE^{TM} conductivity meter. Grain Size Distribution was determined according to ASTM D 422-63 Standard Test Method for Particle Analysis of Soils (ASTM 2002). To analyze exchangeable nitrogen, 1 g soil was shaken with 10 mL of 2M KCl solution for 1 h (Xu et al., 2013). The filtered extractant was analyzed using Spectronic Genesys Spectrophotometer, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of potassium and phosphorus, 1 g soil was shaken with 1M ammonium acetate for 1 h. The solution was filtered, and the extractant was analyzed for phosphorus with Spectronic Genesys spectrophotometer, as per the procedure given by Sattayatewa et al. (2011). The Water Holding Capacity (WHC) of the soil was determined following the ASTM D2980 – 04 Standard Test Method for Volume Mass, Moisture – Holding Capacity and Porosity of Saturated Peat Materials (ASTM 2010).

Soil and vegetative samples were sent to STAT Analysis Corporation (Chicago, IL, USA) for sample acid digestion and analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS). Polynuclear Aromatic Hydrocarbons (EPA method SW8270C) were also tested by Gas Chromatography Mass Spectrometry (GC/MS).

Sequential Extraction analyses were performed using the procedure summarized in Table 2.5 in order to determine the speciation of the contaminants in the soils both before and after the phytoremediation technique is implemented. This procedure was originally developed by Tessier et al. (1979) and has been extensively used for the speciation of trace metals in natural soils and sediments into five fractions: (1) Exchangeable, (2) Bound to carbonates, (3) Bound to Fe-Mn oxides, (4) Bound to Organic Matter, and (5) Residual (which consists of the prior four fractions summed – up and subtracted from the total concentration). However, the

Table 2.5. Sequential extraction procedure for speciation of heavy metals

Fraction	Designation	Extraction Procedure (per 1g dry soil sample)
1	Exchangeable	An amount of 8 mL of 1 M sodium acetate solution (pH = 8.2) was added and mixed continuously for 1h.
2	Carbonates – bound	To the residue from above, 8 mL of 1M sodium acetate (pH adjusted to 5 with acetic acid) was added and mixed continuously for 5h.
3	Fe – Mn oxides – bound	To the residue from above, 20 mL of 0.04 M hydroxylamine hydrochloride (NH2OH.HCL) was added in 25% (v/v) acetic acid, and heat to 96ºC with occasional stirring for 6h.
4	Organic – bound	To the residue from above, 3 mL of 0.02M nitric acid (HNO3) and 5mL of 30% Hydrogen Peroxide (H2O2) (pH adjusted to 2.0 with HNO3) was added and mixed continuously for 3h. Cool the mixture and add 5mL of 3.2 M ammonium acetate (NH4OAc) in 20% (v/v) HNO3. Finally, dilute to 20 mL and mixed continuously for 30 min.
5	Residual	Acid digestion EPA 3050 method.

residual fraction of the soil in this work was obtained by acid digestion, following the EPA 3050 method. Samples from sequential extraction and blank were sent to STAT Analysis Corporation (Chicago, IL, USA) for analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

For the test results, mean and standard deviation were calculated using Microsoft Office Excel 2013. To check whether a significant difference exists between the result sets, the t-test was performed with Microsoft Office Excel 2013. The alpha value was taken as 0.05 for the t-test.

2.4. Results and Discussion

In this section, the results of soil characterization and contaminant concentrations in soil and vegetative tissue during the three growing seasons are presented and discussed.

2.4.1. *Initial Soil Characterization.*

Soil physical properties were tested in the lab for every soil composite sample. The results for the initial soil characterization are shown in Table 2.6. The average pH value of the surface soil at the beginning of this study was 7.48. The slag dumped throughout the region is mainly iron slag generally characterized by a high alkalinity. The results for pH found in the surface, were lower than expected, possibly due to that soil was sampled mainly from the surface, where there was a thin top soil layer that covered the high pH slag layer underneath. The Oxidation – Reduction Potential (ORP) is an index of the exchange activity of electrons among elements in solution. The results show a negative potential, which indicates reducing conditions in the initial soil. The organic matter content found initially in the soil was also very low, evidencing the need to amend the soil to increase the survival probabilities of the plants. Grain size distribution shows that the soil has a high fraction of coarse grained particles, mainly due to

Table 2.6. Soil characterization before, after tilling and at the end of the third growing season.

Soil Parameter	Initial Values	After Tilling Values	Season 3
pH	7.48	9.26	8.16
ORP (mV)	-44.36	-156.79	-91.76
OC (%)	4.2	8.02	7.68
EC (mS/cm)	0.18	0.01	0.06
mc (%)	16.47	17.62	11.82
WHC (%)	27.08	37.06	30.91
Exc. Phosphate (mg/L)	0.08	0.067	0.06
Exc. Nitrate (mg/L)	1.51	2.16	2.39
% Gravel	51.8	56.4	29.5
% Sand	26.8	31.5	32.9
% Fines	21.4	12.1	37.6

the predominance of debris and fill material in the experimental area. The distribution of the grain size in the soil at the beginning of the experiment can be found in Figure 2.6(a).

Table 2.7 shows the concentration (mg/Kg – dry soil) for different Polynuclear Aromatic Hydrocarbon (PAHs) compounds and metals that were found initially in the soil at the experimental area. Benzo(a)pyrene, with the highest concentration in the initial soil (0.43 mg/Kg –dry soil) is the target contaminant representative of the presence of PAHs in the experimental area, due its known carcinogenic and mutagenic potential. Numerous heavy metal species were found in the initial soil. Some of them such as Chromium (300 mg/kg – dry soil) and Lead (400 mg/Kg- dry soil) were found in concentrations above the maximum allowable risk – based levels.

2.4.2. *Soil Characterization after Compost Amendment and Tilling.*

As expected, tilling and homogenization affected the soil physical properties. The soil pH after the treatment increased up to 9 as compared with the values obtained for the initial soil sampling (Table 2.6). The mixing of the alkaline slag layer underneath the top soil during homogenization could have induced this increase, masking the possible effect of the addition of compost on the soil pH. However, the organic matter incorporation, had a direct immediate impact on organic carbon content which increased from 4 to 8%, as well as in the magnitude of the reduction potential, which also increased. The exchangeable Nitrate concentration increased up to 46% after the addition of soil amendment. The water holding capacity increased in the soil after adding compost, resulting in more water available for the plants. The grain size distribution showed a decrease of coarse fraction and an increase of fines after tilling, indicating the degradation of compost.

The total PAHs concentrations of the soil after adding compost are shown in Table 2.7. No significant differences in the concentration values were found before and after the soil

Table 2.7. Contaminant Concentrations in Soil

Contaminant	Concentration (mg/Kg – dry soil)			
	Initial Soil	After Tilling	Season 2	Season 3
PAHs				
Acenaphthene	<DL (0.03)	<DL (0.03)	<DL (0.03)	<DL (0.03)
Acenaphthylene	0.03	<DL (0.03)	<DL (0.03)	<DL (0.03)
Anthracene	0.05	0.05	0.05	0.06
Benz(a)anthracene	0.2	0.29	0.13	0.12
Benzo(a)pyrene	0.43	0.41	0.48	0.25
Benzo(b)fluoranthene	0.36	0.36	0.23	0.31
Benzo(g,h,i)perylene	0.35	0.27	0.18	0.2
Benzo(k)fluoranthene	0.16	0.31	0.17	0.21
Chrysene	0.29	0.36	0.19	0.2
Dibenz(a,h)anthracene	<DL (0.03)	0.12	0.07	<DL (0.03)
Fluoranthene	0.26	0.45	0.2	0.17
Fluorene	<DL (0.03)	<DL (0.03)	<DL (0.03)	<DL (0.03)
Indeno(1,2,3-cd)pyrene	0.21	0.24	0.15	0.18
Naphthalene	0.08	<DL (0.03)	<DL (0.03)	<DL (0.03)
Phenanthrene	0.18	0.14	0.06	0.09
Pyrene	0.26	0.36	0.17	0.14
Metals				
Aluminum	5200	7925	6325	6675
Antimony	3.7	4	5	4.3
Arsenic	6.8	9.6	10	12.1
Barium	95	138	140.0	124
Beryllium	0.7	0.9	1	1
Cadmium	4.4	14	13.5	13.6
Calcium	130000	150000	155000	123750
Chromium	300	275	241	248
Cobalt	9.2	8.6	7.6	8.4
Copper	85	79.5	79	94
Iron	360000	257500	180000	192500
Lead	745	938	966	1011
Magnesium	23000	20000	21250	20500
Manganese	19000	20750	17195	18308
Mercury	0.03	0.03	0.05	0.04
Nickel	64	128	51	54.4
Potassium	320.0	2175	1333	1538
Selenium	0.9	1.1	1.4	1.8
Silver	0.9	2.5	2.1	2.1
Sodium	200	625	533	485
Thallium	0.9	1	1.3	1.7
Vanadium	150	173	210	181
Zinc	3900	6075	7675	7275

DL= Detection Limit.

preparation. However, concentrations of metals such as As and Pb increased in soil after tilling. The reason why the presence of heavy metals in the soil is greater after tilling, could be the same as that for the pH increase. When digging the soil, the underlying slag layer that is rich in metals, is mixed with the top soil cover, thus increasing these values in the mixed soil.

The results of the percentages of the different fractions of metals in the soil before and after tilling are shown in Table 2.8, and the results for heavy metals such as As, Cr, Pb and Mn, the target contaminants of the present study, are plotted in Figure 2.7a. As it can be observed, the exchangeable fraction, of metals in the soil is very low (Table 2.8), and it remains constant after tilling and compost addition. Overall, the amended soil showed an increase in the organic bound fraction of the metals, which was likely due to the effect of compost addition. The residual fraction of As and Cr decreased slightly while Pb and Mn increased after adding compost (Figure 2.7a).

2.4.3. *Plant Monitoring*

Figure 2.5 shows the surviving percentages at the slag disposal area based on monitoring results for the first and second growing season. During the first growing season, the native grass species had stronger growth and survivorship rates than the woody species. Such is the case of Switchgrass (SWG), Purple Prairie Clover (PPC) and Little Bluestem (LBS), which performed the best across the test plot area with 100% of survival at the end of the first growing season. The survival rates found in the adjacent plots were similar to those at the experimental plots. The increasingly presence of invasive species in the study area, made it very difficult record the growth monitoring in detail the second year. Trees and shrubs showed a poor performance compared to grass species at the end of the second season. The plants survival and leaf quality assessment (Figure 2.5b) and the height (cm) of the trees and shrubs (Figure 2.5c), were performed only during the first growing season, with the aim of carrying out a detailed monito-

Table 2.8. Percent fractionation of metals in the soil before planting.

Metal	Initial soil					Soil after tilling				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Antimony	8	8	41	17	26	6	11	28	20	35
Arsenic	2	2	7	3	87	2	5	12	9	72
Barium	1	20	57	4	19	1	10	57	13	19
Beryllium	9	9	43	18	22	7	14	36	26	18
Cadmium	3	5	19	5	68	1	6	19	5	69
Chromium	0	0	51	3	46	0	1	52	7	40
Cobalt	2	2	18	5	73	4	7	26	13	50
Copper	1	1	3	17	78	1	3	6	28	62
Lead	0	1	38	2	59	0	1	31	4	65
Manganese	0	1	69	5	24	0	4	59	7	30
Nickel	0	2	29	3	65	1	2	47	9	42
Selenium	9	10	42	18	21	6	11	28	20	35
Thallium	9	9	43	18	22	7	14	36	26	18
Vanadium	0	0	66	2	31	0	1	64	7	29
Zinc	0	0	15	0	84	0	2	13	1	84

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual.

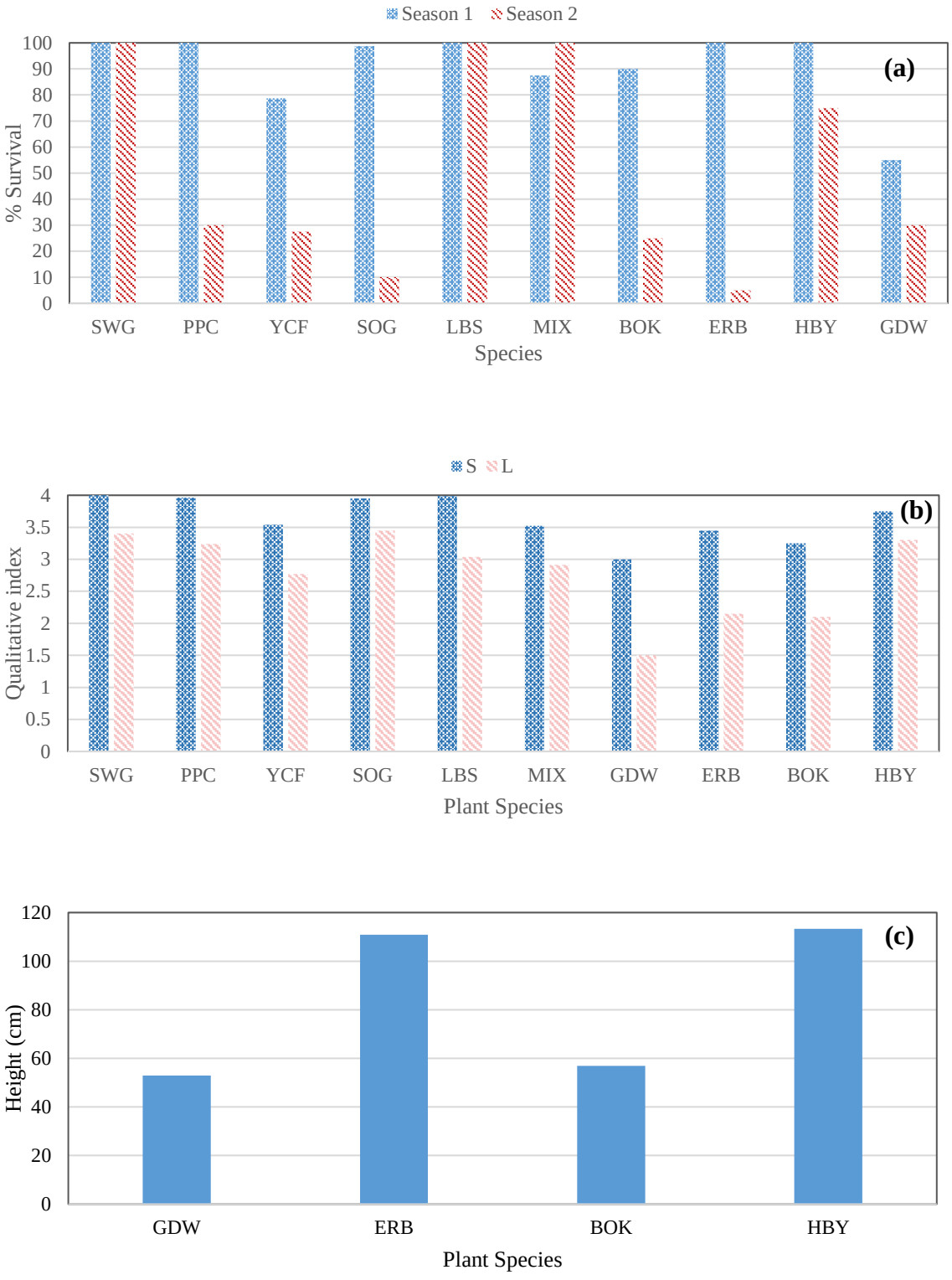


Figure 2.5. Monitoring rating results. (a) Plant survival in the experimental plots at the end of the first and second growing season. (b) Plant survival (S) and leaf quality (L) in grasses and trees at the end of the first growing season. (c) Height of the trees (cm) at the end of the first season

ring of the development and growth of plants and their adaptation to the ground. Due to the presence of invasive species and the poor survival rates of trees species, height values were not recorded during the second season.

No monitoring was performed on the 3rd growing season. However, field observations made when the terminal sampling took place, revealed lower survival rates than in the prior two seasons in the test area. At the end of the experiment, only 4 out of the 9 species initially planted, survived in the contaminated site, all of them grass species. The soil pH and high contaminant concentration could be the main reason of the poor performance of the woody species. A special investigation during the experimental period took place on site, in order to measure the changes of soil pH with depth. These results showed that the soil at a depth 22 cm below the surface within the experimental plot area had an average pH of 9.9 (results not shown). According to the USDA plants database, all of the woody species used in our study, had an optimum pH range from slightly acid to neutral (USDA), being the pH in the experimental area above the optimal growth conditions for all the species. Additionally, the trees and shrubs used in this experiment were also visibly affected by the presence of invasive species and pests in the field.

The use of trees is a promising, sustainable and ecologically solution to remediation of heavy metal – contamination (Dickinson, 2000) and promotes the stabilization of the soil or waste. However, the trees must become established on a site, and this establishment can be challenging, because their growth is inhibited by high concentrations of heavy metals (Pulford et al., 2003). Other factors may limit trees growth, such as macronutrients deficiencies (Pulford, 1991) and physical conditions. The harsh conditions at the experimental area, the presence of invasive species, and the proximity of the slag layer underneath the composted top soil, combined with the high concentration of contaminants could have been the main cause of the poor survival of trees within the study area.

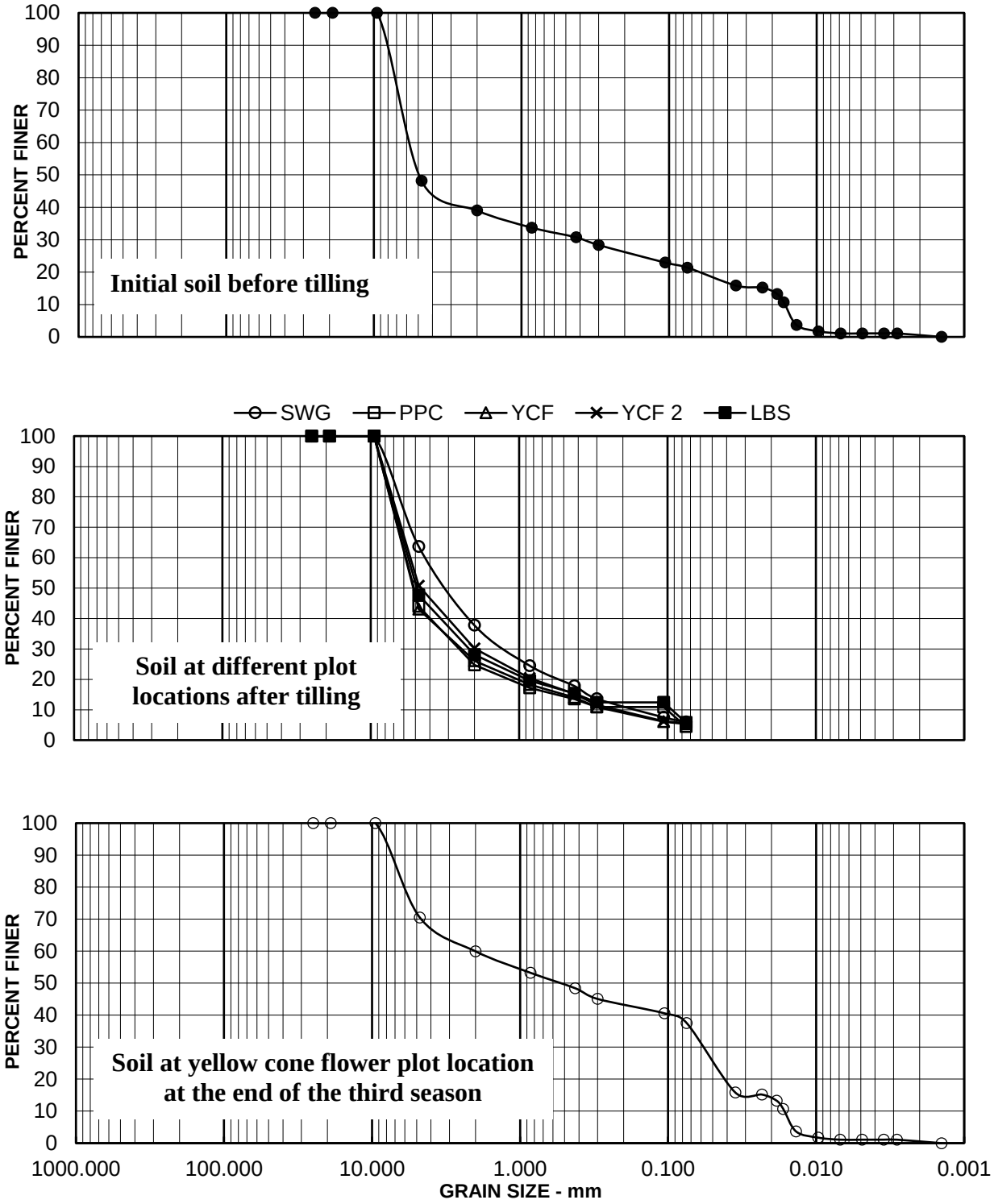


Figure 2.6. Grain Size Distribution of soil before tilling, after tilling and at the end of the third growing season

Previous results obtained in lab scale pointed phytotoxicity as the cause of the poor performance of the plants in the contaminated area, showing that the growth characteristics, as well as the survival rates are greatly influenced by the combined contaminated conditions (Chirakkara and Reddy, 2015). Therefore, the addition of compost may have been the biggest contributing factor for the better performance of grasses and plugs. The experimental area is an upland, barren slag field that was expected to produce the poor results for growth and survivorship due to the lack of topsoil and high pH. However, the presence of native grasses was noticeable in all the subplots at the end of the experiment. The effect of the slag layer did not seem to cause an important impact in grasses. On the other hand, compost did not countered negative effects of the slag layer in the woody species, possibly due to that the soil amendment was not applied at a sufficient soil depth required for the development and establishment of the root system of these trees.

Contrary to what occurs with native species, the presence of invasive species Sweet Clover was predominant in the experimental area, and it seemed to thrive towards the end of the experiment. The ability of this species to fix atmospheric Nitrogen is attributed to rhizobium symbiosis, which could be the key to resist metal toxicity (Chaudri et al., 2000). Other studies showed that legumes are the dominant portion of wild species that survive in long – term metal contaminated environments (Del Rio et al., 2002).

2.4.4. *Fate of PAHs*

The overall results for PAHs concentrations throughout the experiment can be found in Table 2.7. No significant differences ($p>0.05$) were found in the PAHs concentrations of the unplanted initial soil and after tilling and amendment addition. On the contrast, the results in the soil at the end of the third season, in presence of plants, show an overall decrease in the PAHs concentrations. Results show that concentration of BaP in the planted soil decreased at

the end of the third growing season ($p<0.05$). Table 2.9 shows in detail the results for contaminant concentrations in the soil for the surviving species at different plot locations at the end of the second and third season. As compared with the values obtained in the soil samples before planting (Table 2.7), the concentration of PAHs decreases in all the surviving species plots, reaching in some cases undetectable levels. The Benzo(a)pyrene concentrations in the soil decreased 28% at the Switchgrass plot, 38% at Little Bluestem, 45% at Purple Prairie Clover and 47% at Yellow Coneflower plot. Similar tendency is observed for the rest of the PAHs analyzed (Tables 2.7 and 2.9). Table 2.10 shows the results for the PAHs contaminant concentration in the vegetative aerial tissue (stems and leaves). As it can be seen, at the end of the second growing season, no significant presence of PAHs was found either in leaves or stems, since all concentrations were below detection limits. At the end of the third season, only Benzo(a)pyrene concentration was analyzed, and the results show that the concentration of this organic contaminant was below detection limits for all the surviving species. Similar response was observed in the invasive species Sweet Clover, where PAHs content in leaves and stems was insignificant (results not shown). The PAH concentrations in the roots of the surviving species are shown in Table 2.11. As it can be observed, the majority of PAH concentrations were found below detection limits or in a very low concentration. Overall, these results show that the initial concentration of PAHs is dissipated in the soil at the end of the experiments.

Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds with multiple aromatic rings, with low volatility and their persistence in the environment. In soils, these compounds can be dissipated by either one or a combination of mechanisms such as microbial degradation and volatilization (Gabet, 2004; Park et al., 1990; Saison, 2001). Although the dissipation of low molecular weight and volatile PAHs can be achieved by natural dissipation

Table 2.9. Soil contaminant concentrations at different plot locations

	Concentration (mg/kg – dry soil)							
	Season 2				Season 3			
Contaminant	**SWG**	**LBS**	**PPC**	**YCF**	**SWG**	**LBS**	**PPC**	**YCF**
PAHs								
Acenaphthene	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)
Acenaphthylene	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)
Anthracene	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		0.06
Benz(a)anthracene	0.08	0.13	0.16	0.15	0.11	0.11		0.13
Benzo(a)pyrene	0.18	0.28	0.20	0.28	0.3	0.25	0.23	0.22
Benzo(b)fluoranthene	0.16	0.27	0.26	0.24	0.27	0.13		0.34
Benzo(g,h,i)perylene	0.12	0.20	0.17	0.22	0.16	0.14		0.29
Benzo(k)fluoranthene	0.1	0.17	0.19	0.22	0.1	0.1		0.32
Chrysene	0.11	0.19	0.22	0.22	0.14	0.17		0.25
Dibenz(a,h)anthracene	0.05	0.08	0.08	0.09	<DL (0.03)	0.08		<DL (0.04)
Fluoranthene	0.11	0.20	0.26	0.24	0.13	0.15		0.21
Fluorene	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)
Indeno(1,2,3-cd)pyrene	0.10	0.17	0.15	0.18	0.12	0.12		0.23
Naphthalene	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)
Phenanthrene	0.04	0.06	0.09	0.08	0.06	0.07		0.12
Pyrene	0.10	0.17	0.22	0.2	0.11	0.11		0.17
Metals								
Aluminum	5900	6700	5900	6800	7850	6200	6600	6050
Antimony	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)
Arsenic	10	10	10	9	12	12	12	12
Barium	120	150	160	130	135	110	130	120
Beryllium	0.9	1	1	1.1	1.2	1	1.1	0.9
Cadmium	14	10	18	12	11	12	19	12.5
Calcium	140000	170000	140000	170000	130000	110000	140000	115000
Chromium	256	298	302	284	237	240	260	253
Cobalt	7.4	7.1	8.4	7.3	8	8	8.8	9.2
Copper	87	63	94	73	82	87	110	99
Iron	200000	140000	210000	170000	200000	200000	170000	200000
Lead	1213	1006	1066	1018	995	1070	1006	973
Magnesium	18000	23000	23000	21000	18000	16500	32000	15500
Manganese	19400	20000	21400	21400	18000	18333	18400	18500
Mercury	0.03	0.05	0.06	0.06	0.04	0.04	0.05	0.04
Nickel	64	40	48	50	53	48	49	68
Potassium	930	1400	1400	1600	1400	1700	1300	1750
Selenium	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)
Silver	2.1	1.7	2.8	1.9	1.65	1.95	3.1	1.75
Sodium	540	440	680	470	460	410	660	410
Thallium	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)
Vanadium	170	240	220	210	190	155	190	190
Zinc	8800	6100	8800	7000	6800	7950	7300	7050

SWG= Switch Grass;
LBS= Little Bluestem;
YCF = Yellow Cone Flower;
PPC = Purple Prairie Clover.
DL = Detection Limit.

Table 2.10 Contaminant concentration per surviving species in stems and leaves

Contaminant	Concentration (mg/Kg)							
	Season 2				Season 3			
	SWG	LBS	YCF	PPC	SWG	LBS	YCF	PPC
PAHs								
Acenaphthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Acenaphthylene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Benz(a)anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Benzo(a)pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)
Benzo(b)fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Benzo(g,h,i)perylene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Benzo(k)fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Chrysene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Dibenz(a,h)anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Fluorene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Indeno(1,2,3-cd)pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Naphthalene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Phenanthrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)				
Metals								
Aluminum	54	48	56	<DL(38)				
Antimony	<DL(4)	<DL(4)	<DL(4)	<DL(4)				
Arsenic	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(13)	<DL(15)	<DL(10)	<DL(19)
Barium	4	7.4	5.2	5.5				
Beryllium	<DL(1)	<DL(1)	<DL(1)	<DL(1)				
Cadmium	<DL(1)	<DL(1)	<DL(1)	<DL(1)				
Calcium	8200	5100	26000	16000				
Chromium	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(13)	<DL(15)	<DL(10)	<DL(19)
Cobalt	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Copper	<DL(5)	<DL(5)	12	6.3				
Iron	330	570	850	570				
Lead	6.37	3.85	12	4.22	<DL(6)	<DL(8)	<DL(6)	<DL(9)
Magnesium	1300	1600	4800	1400				
Manganese	100	87	153	70	63	117	99	72
Mercury	<DL(0.02)	<DL(0.02)	<DL(0.02)	<DL(0.02)				
Nickel	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Potassium	5200	9300	38000	14000				
Selenium	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Silver	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Sodium	<DL(110)	<DL(110)	<DL(110)	<DL(110)				
Thallium	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Vanadium	<DL(2)	<DL(2)	<DL(2)	<DL(2)				
Zinc	24	75	97	72				

SWG= Switch Grass;
LBS= Little Bluestem;
YCF = Yellow Cone Flower;
PPC = Purple Prairie Clover.
DL = Detection Limit.

through soil indigenous microbes (Huang et al., 2004, Zhang et al., 2012), the compost has been shown to enhance PAH degradation in a number of studies by improving soil texture for oxygen transfer, and providing energy to the microbial population (Haritash and Kaushik, 2009). The presence of plants can also be the cause of the dissipation of PAHs in the soil, since they can break down or degrade the contaminants by metabolic processes (Kang 2014, Balasubramaniyam 2015, Wang et al., 2012). However, there are no congruent evidences that there are synergistic effects between plants and compost in terms of PAHs dissipation. While there are studies that confirm that the presence of organic contaminants are lower in presence of plants and amended soils (Vouillamoz and Milke., 2001; Chirakkara and Reddy., 2015), there are also studies in which degradation of PAHs did not show such synergistic effect (Ghanem et al., 2013, Wang et al., 2012). Although the results of this study show that there is a degradation of the organic contaminants initially present in the soil, these results do not show how this degradation occurs. The dissipation of the organic contaminants can occur either by one or a combination of the following mechanisms: Rhizodegradation, degradation in the roots of the plants as a result of microorganisms activity and root enzymes and exhudates (Myresiotis et al., 2012, Huesemann et al., 2009, Schnoor et al., 1995), and phytodegradation direct uptake of contaminants and metabolization in the plant tissues (Al-Baldawi et al., 2015).

Regardless of the degradation mechanism, numerous factors can contribute to the degradation rate of the PAHs, such as the number of rings (Park et al., 1990, Huang et al., 2004). In the present study, the soil samples at Yellow Coneflower plot show a higher concentration of PAHs of 5 and 6 rings such as Benzo(b)fluoranthene (Table 2.9), and in some cases, such as Benzo(g,h,i)perylene and Benzo(k)fluoranthene, the dissipation is null. A high pH media such as the soil of this study, can also affect PAHs dissipation. The results obtained by Moretto et al. (2005) showed that PAHs degradation decreased in a high pH when organic matter is added.

Table 2.11. Contaminant concentration in roots of surviving plant species

Contaminant	Concentration (mg/Kg)							
	Season 2				Season 3			
	SWG	LBS	PPC	YCF	SWG	LBS	PPC	YCF
PAHs								
Acenaphthene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Acenaphthylene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Anthracene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Benz(a)anthracene	<DL(0.05)		<DL(0.04)	0.08	<DL(0.03)	<DL(0.03)		<DL(0.03)
Benzo(a)pyrene	0.05	<DL(0.04)	<DL(0.04)	<DL (0.05)	<DL(0.03)	<DL(0.03)	<DL(0.03)	<DL(0.03)
Benzo(b)fluoranthene	<DL(0.05)		<DL(0.04)	0.18	<DL(0.03)	<DL(0.03)		<DL(0.03)
Benzo(g,h,i)perylene	<DL(0.05)		<DL(0.04)	0.14	<DL(0.03)	<DL(0.03)		<DL(0.03)
Benzo(k)fluoranthene	<DL(0.05)		<DL(0.04)	0.1	<DL(0.03)	<DL(0.03)		<DL(0.03)
Chrysene	<DL(0.05)		<DL(0.04)	0.1	<DL(0.03)	<DL(0.03)		<DL(0.03)
Dibenz(a,h)anthracene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Fluoranthene	<DL(0.05)		<DL(0.04)	<DL (0.01)	0.05	<DL(0.03)		<DL(0.03)
Fluorene	<DL(0.05)		<DL(0.04)	<DL (0.01)	0.07	<DL(0.03)		<DL(0.03)
Indeno(1,2,3-cd)pyrene	<DL(0.05)		<DL(0.04)	0.11	<DL(0.03)	<DL(0.03)		<DL(0.03)
Naphthalene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Phenanthrene	<DL(0.05)		0.51	<DL (0.01)	0.05	0.04		0.04
Pyrene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)
Metals								
Aluminum	1600		240	600	200	290		330
Antimony	14		17	15	<DL(5)	<DL(5)		<DL(5)
Arsenic	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)
Barium	52		11	17	4.95	7.4		12
Beryllium	<DL(2)		<DL(2)	<DL(2)	<DL(1)	<DL(1)		<DL(1)
Cadmium	<DL(2)		<DL(2)	<DL(2)	<DL(1)	<DL(1)		<DL(1)
Calcium	100000		16000	23000	6400	9400		18000
Chromium	76	13	6	9	4.1	7.83	<DL(3)	12
Cobalt	<DL(3)		<DL(3)	<DL(3)	<DL(3)	<DL(3)		<DL(3)
Copper	11		10	34	23	19		22
Iron	44000		6400	8000	6200	7100		7300
Lead	52	110	39	78	24	53	6	88
Magnesium	8100		2500	2600	2450	860		3000
Manganese	5523	697	238	468	221	285	69	650
Mercury	<DL(0.02)		<DL(0.02)	<DL(0.02)	<DL(0.02)	<DL(0.02)		<DL(0.02)
Nickel	<DL(3)		<DL(3)	<DL(3)	<DL(3)	<DL(3)		<DL(3)
Potassium	4800		14000	19000	5250	890		9800
Selenium	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)
Silver	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)
Sodium	<DL(180)		240	<DL(190)	<DL(170)	<DL(130)		<DL(110)
Thallium	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)
Vanadium	<DL(160)		13	19	4	8		21
Zinc	430		430	470	275	400		390

SWG= Switch Grass;
LBS= Little Bluestem;
YCF = Yellow Cone Flower;
PPC = Purple Prairie Clover.
DL = Detection Limit.

The results of the present work are consistent with numerous studies that have evidenced the degradation of PAHs in presence of Switchgrass (Pradhan et al. 1998, Murphy et al. 2011, Meggo et al, 2013), and Little Bluestem (Pradhan et al, 1998). However, little is known about the effect of Yellow Coneflower and Purple Prairie Clover in the degradation of those organic compounds.

Despite some studies observed that the addition of organic matter to the soil improves the degradation of organic contaminants, the effect of the plants in absence of soil amendment in the degradation of PAHs has not been object of the present study. Therefore, the synergistic effect of compost amendment and the surviving species in PAHs degradation is not conclusive. However, results in the present study confirm that the presence of compost seems to enhance the plant growth under hard surviving conditions and reduce the toxicity produced by soil pollution. Therefore, compost amendments provide a promising approach for enhancing phytoremediation of mixed contaminated soils (Chirakkara and Reddy, 2015).

2.4.5. *Fate of Heavy Metals*

The overall results of heavy metal concentrations in soil are shown in Table 2.7. As compared to the unplanted soil after tilling and compost addition, no significant differences can be found at the end of the third growing season, except for As, which concentration in soil increased slightly ($p<0.05$) throughout the experiment. The results for the total metals concentration in soil at the plots of the surviving species are presented in Table 2.9. These results show that the metals in the soil tend to remain constant in the soil after compost addition (Table 2.7) and throughout the experiment. This tendency in heavy metals present in the soil such as Cr, Pb and Mn is repeated in all surviving species plots, which suggests that the presence of plants in the experimental area did not affect the concentration of heavy metals in the soil. Arsenic, on the other hand, has a different behavior, and its concentration tends to increase when compared

to the unplanted soil (Table 2.7). However, it does not show significant differences between the different plot locations ($p>0.05$).

The total metals concentrations in stems and leaves of the surviving species can be found in Table 2.10. At the end of the third growing season, only the results for the target contaminants (As, Cr, Pb and Mn) are shown. As it can be observed, the concentrations of heavy metals in the plants were below detection limits in all cases, except for Mn, which was detected in the aerial vegetative tissue at the end of the third growing season, although there are no significant differences in the average values at the end of the second and third growing seasons ($p>0.05$). The total metal concentrations in roots was also measured and results can be found in Table 2.11. The concentration of heavy metals such as Cd and As was not detectable in roots throughout the experiment. However, it did not occur the same way for Cr, Pb, Mn and Zn, which concentrations in the roots of the surviving plants were detected. Although the concentration of Cr and Mn did not show significant difference in the roots of the surviving species throughout the experiment ($p>0.05$), their average values in SWG roots when they were collected at the end of the second growing season showed high variability (SD ±108 and ±8223, respectively). The concentration of Pb only showed differences in SWG roots, were slightly decreased at the end of the experiment ($p<0.05$).

The percentages of the metal fractionation in the soil at the plots of the different surviving species at the end of the experiment are shown in Table 2.12, and the results for the target heavy metals are plotted in Figure 2.7b. As it can be observed, at the end of the third growing season, the exchangeable fraction of the metals present in soil remains very low, without significant changes in the soil for the different species. Generally no changes are observed in the distribution of the fractions in which metals appear retained in the soil along the experiment. As explained above, the percentage of metal retained on the organic fraction

Table 2.12. Soil Sequential Extraction of different plots at Season 3

	SWG					LBS					YCF				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Aluminum	0	0	32	12	56	0	0	31	9	59	0	0	28	8	63
Antimony	6	13	16	24	40	6	11	28	18	37	7	14	36	24	18
Arsenic	2	5	12	9	72	3	6	14	9	68	3	7	17	11	62
Barium	1	9	54	17	19	1	10	59	15	15	2	18	51	11	18
Beryllium	9	17	21	32	21	7	14	36	24	18	7	14	36	24	18
Cadmium	1	5	19	4	72	2	7	26	6	60	2	10	16	8	64
Calcium	3	32	54	6	5	2	29	59	5	5	5	54	33	4	5
Chromium	0	0	52	10	37	0	0	58	12	29	0	1	44	6	48
Cobalt	4	7	21	14	55	4	8	30	14	44	4	8	21	14	53
Copper	1	1	43	23	32	2	3	7	37	52	1	2	5	46	47
Iron	0	0	62	6	31	0	0	73	4	23	0	0	16	2	82
Lead	0	0	27	5	67	0	1	41	3	55	0	1	29	6	64
Magnesium	2	10	54	12	22	2	7	67	11	13	4	14	45	13	24
Manganese	0	3	63	7	28	0	3	76	8	14	0	8	58	3	31
Nickel	1	1	33	21	43	1	1	42	20	36	1	2	40	14	43
Potassium	30	11	13	2	44	26	18	13	2	41	44	18	7	2	30
Selenium	7	14	35	26	18	7	14	36	24	18	7	14	36	24	18
Silver	6	12	15	22	45	6	11	28	19	36	7	14	35	23	20
Sodium	53	42	4	0	0	43	52	5	0	0	50	47	2	0	0
Thallium	9	17	21	32	21	7	14	36	24	18	9	18	22	29	22
Vanadium	0	0	59	12	28	0	0	67	10	23	0	1	53	14	31
Zinc	0	1	10	1	87	0	2	21	2	75	0	3	12	1	85

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual.

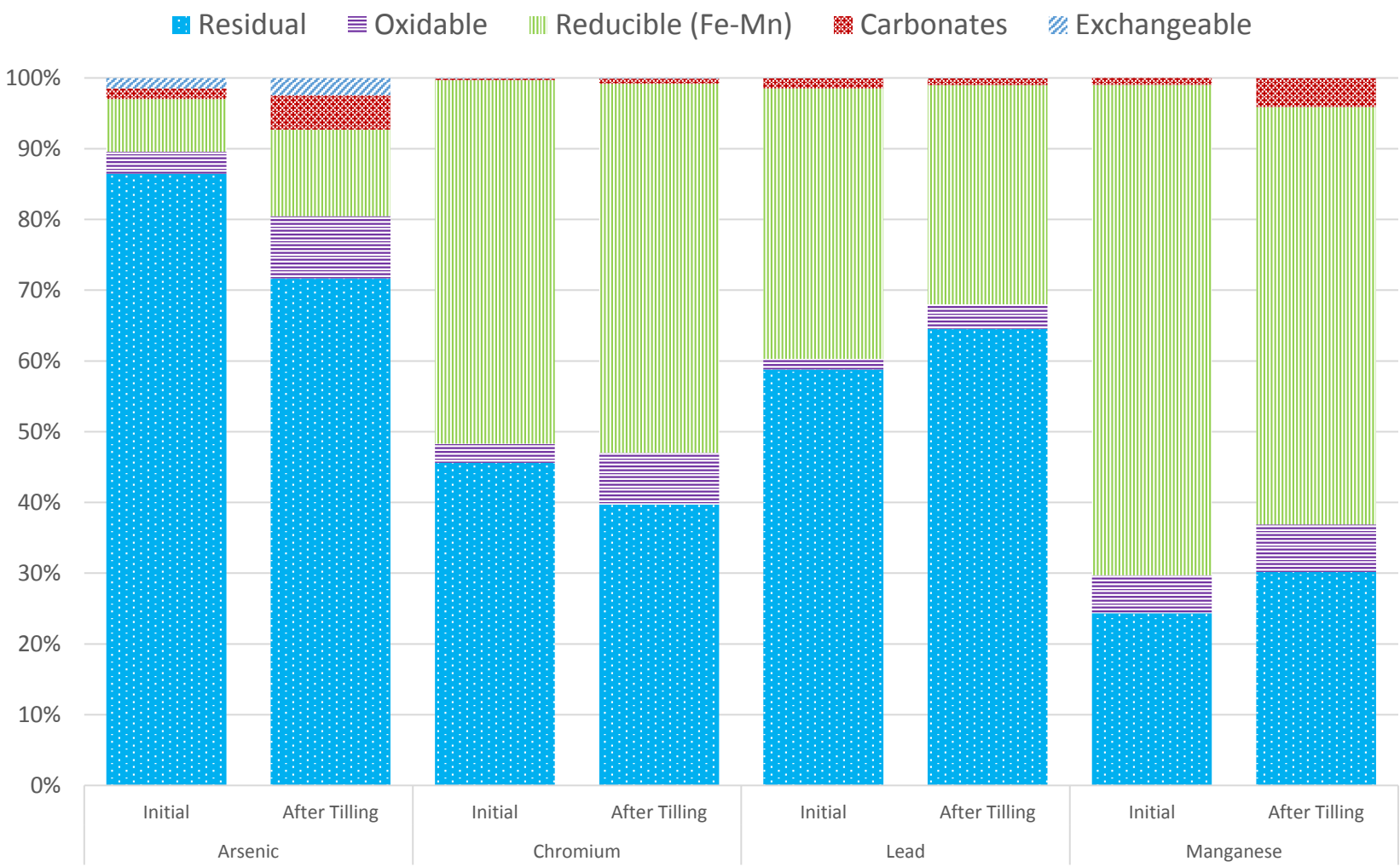


Figure 2.7a. Metal distribution comparison between soil before and after tilling

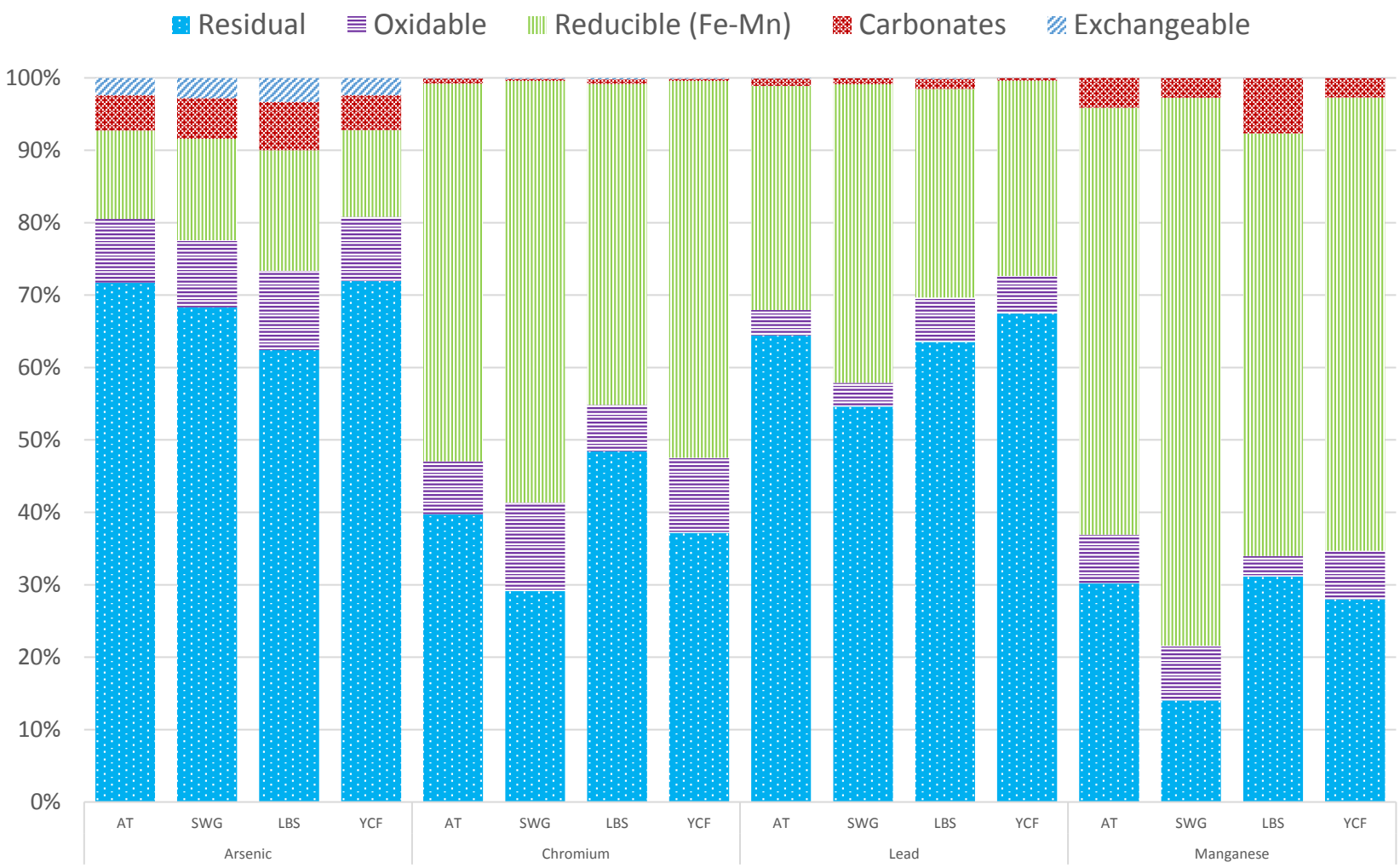


Figure 2.7b. Metal distribution comparison between soil after tilling (AT) and soils at surviving plant plots at the end of the third season, Switchgrass (SWG), Little Bluestem (LBS) and Yellow Cone Flower (YCF)

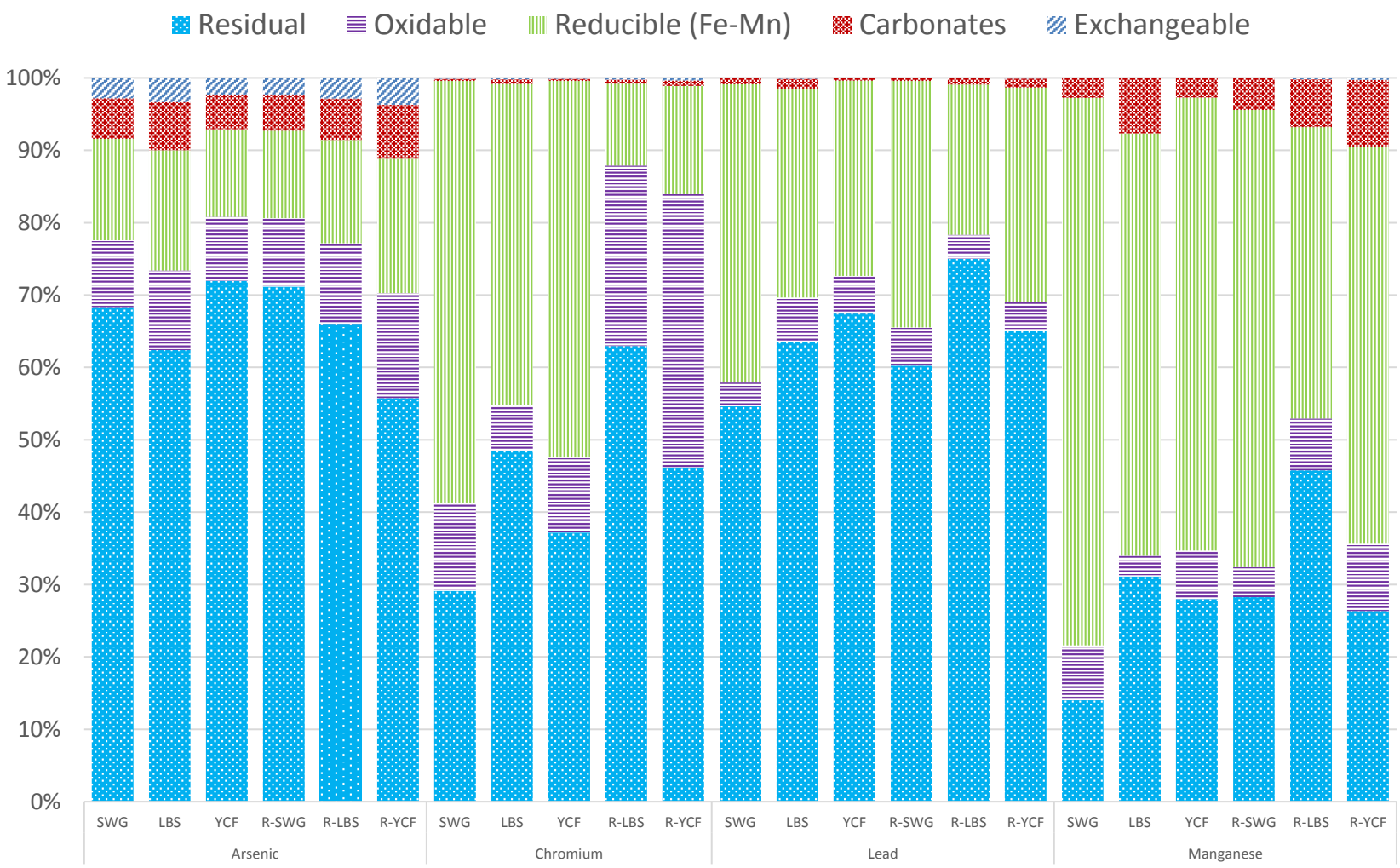


Figure 2.7c. Metal distribution comparison between soils of surviving plant plots, Switchgrass (SWG), Little Bluestem (LBS) and Yellow Cone Flower (YCF) and root soil (R-SWG, R-LBS, R-YCF) at the end of the third season

increased after the addition of compost amendment during soil preparation. However no significant changes were observed in the distribution of metals from then.

Among the target contaminants studied in the present work (Figure 2.7b), As is the metal (metalloid) that presents certain percentage retained in the exchangeable fraction. However, the concentration of this element in the vegetative tissue of the surviving species was undetectable (Tables 2.10 and 2.11). The low mobility of As could be due to the fact that a major percentage was retained in the residual fraction. Although Pb also presents a high percentage retained in the residual fraction, concentrations of this metal in the roots of the surviving species were detected. The distribution of Pb in other fractions more assimilable by the plant, such as Fe – Mn oxides bound, could be a determinant factor to its uptake. Cr and Mn, however, present a higher percentage of retention in the Fe – Mn oxides bound fraction, being Mn almost completely retained in this fraction. The presence of these metals in the plant suggest the existence of some mechanism to uptake Mn and Fe oxides by the plant, which make other metals retained in this fraction available for the plant as well.

The results obtained in the present study show a low mobility of heavy metals in the soil during the experiment, possibly due to its retention in the solid phase. Welp and Brümmer. (1999) showed that the partitioning of metals between the solid and liquid phase is mainly controlled by pH, becoming stronger with high values of pH. In general, sorption increases when increasing pH, which means that the more acidity, the higher metal solubilization and thus the more mobilization. The adsorption mechanisms tend to be higher when the values of pH are also high (Sherene, 2010). However, the mobilization in alkaline soils can also be subjected to kinetic limitations (Villén – Guzmán et al., 2015). With the addition of organic amendments, such as compost, and in presence of plants, heavy metals are expected to form soluble complexes with organic ligands (McLean and Bledsoe, 1992; Karami et al., 2011). Nevertheless, at the conclusion of this study, no significant variation in the metal concentration

values ($P>0.05$) were found in the soil. These results are consistent with those obtained by Alvarenga et al. (2009), in which pseudo total concentration of soil metals did not change significantly when organic residues were applied, pointing that, besides of pH, the addition of organic amendments with a high proportion of humified organic matter is an important factor to control metal bioavailability.

Although the of As in soil is detected at the end of the experiment, no presence of this metalloid was found either in the plant nor roots, suggesting that the As was not chemically mobilized, and therefore was not available. The chemical speciation of As is a major concern when remediation techniques are applied. Studies suggest that the presence of soil amendment with high content of organic matter, such as compost, reduces the content of As (III) (Maňáková et al., 2014) or promotes the oxidation of Arsenic (III) to Arsenic (V) (Hartley et al., 2009). The latter study, suggests that the microbial mediated activity, favored by the presence of compost, plays an important role in changing the speciation of this compound. Similarly, the toxicity and mobility of Cr in soil depends on its oxidation state. Thus, Rendina et al. (2011) reported that the addition of compost to the contaminated soil increased Cr (III) concentration, less toxic, compared to the unamended soil that presented higher concentration of Cr (VI). The results obtained by Banks et al. (2006) suggested that the organic matter played a significant role in mobility of Chromium in soil due to the reduction of the mobile Cr (VI) to the relatively immobile Cr (III). In the present study, oxidation state of metals was not studied, but the low concentration of Cr and As in the vegetative tissue, suggest that the solubility of these compounds is very low, making them not available for plant uptake.

According to Shahid et al. (2012) and Hashimoto et al. (2009), organic ligands are capable to modify Pb speciation by forming organo – metallic complexes and hydroxyl complexes that can increase solubility, bioavailability and toxicity of this element. Although this could explain the presence of Pb in roots, the concentration of this element decreases in

the plant at the end of the experiment, being negligible in the leaves and stems (Table 2.10), indicating that there is any effect on the mobilization of this element, inside the plants.

Juárez-Santillán et al. (2010) showed that alkaline and reducing substrate conditions favor the presence of Mn^{2+}, which is the most soluble form of Mn, as well as the most assimilable form by plants. This could explain the higher concentration of Mn found in the present study in stems and leaves (Table 2.10) and in roots (Table 2.11), as compared to the rest of heavy metals. However, the results for the fractionation of Mn in the present study, are not consistent with those obtained by Juárez - Santillán (2010), where the residual soil fraction was the highest, followed by Mn – Fe oxides. In the present work, the highest retention of Mn was found in the fraction bounded to Mn – Fe oxides followed by the residual fraction.

The ability of Switchgrass and Little Bluestem to uptake or immobilize heavy metals in soil has been found in the literature. Results obtained by Levy et al. (1999) showed that metal concentration in alkaline soils amended with organic matter were found under detection limits in Switchgrass shoots, consistently to those results obtained in the present study. Gudichuttu (2014) showed that the Pb concentrations in Switchgrass and Little Bluestem shoots are lower at high compost treated soil, as compared to the unamended soils. The same tendency is shown in the results obtained by Chen et al. (2012), where metal concentrations in the plant were directly proportional to the increase of heavy metal in the solution. However, the conditions of the latter study are slightly different, while hydroponic cultures with contaminated solutions instead of soils are used. No evidences in the use of Yellow Coneflower or Purple Prairie Clover have been found in the literature.

Although unfortunately the plant growth in unamended contaminated soil was not studied in the present work, literature suggests that the presence of compost is key in the mobilization of the contaminants. As far as it has been surveyed in the present study, the addition of compost amendment was the main contributor to plants survivorship and reduced

the toxicity caused by the presence of heavy metals. However, the results in the present work show that the effect of compost amendment in the stabilization of heavy metals in the soil are not conclusive.

2.4.6. *Fate of Contaminants in Root Soil*

In order to evaluate the differences in the bulk soil versus in the root zone soil (rhizosphere), soil from the surviving species roots were collected and analyzed. The rhizosphere mainly consists of the millimeters of soil surrounding the plant root, where take place complex biological and ecological processes. Table 2.13 compares the bulk soil characterization results to those obtained from the root zone soil characterization, at each surviving species subplots. As it can be observed, the pH values in the root zone soil is slightly lower for LBS and YCF, compared to the pH in the bulk soil in the same plots. As it was expected, higher values of organic content are found in the root zone soil, likely due to the presence of humic acids, roots exudates and living organisms in the root system. On the other hand, the moisture content of the soil at the root zone was very low as compared to the bulk soil, due to this soil was collected from the roots once the samples were oven dried. The results of the root zone soil sequential extraction are shown in Table 2.14, and the comparison of the fractionation percentages in the soil inside and outside the root zone can be found in Figure 2.7c. As it can be observed, the distribution of As in the root zone soil does not present significant changes in the studied species, with the exception of YCF, that exhibits a mobilization of the residual fraction towards the organic and residual phases. In the case of Cr, there are observable certain changes in the distribution of this metal. For SWG, the fraction retained in the organic phase increases to the detriment of the fractions retained in the Fe and Mn oxides and in the organic – bound fraction. Moreover, in LBS the fraction of Cr retained in the residual fraction is also higher in the root

Table 2.13. Comparison of bulk soil vs. root soil characterization results

	Bulk Soil			Root Zone Soil		
Parameter	SWG	LBS	YCF	SWG	LBS	YCF
pH	8.25	8.05	8.18	8.08	7.87	7.78
MC (%)	10.88	13.37	11.22	6.07	5.96	9.01
OC (%)	8.34	7.52	8.66	31.58	38.42	49.79
EC (mS/cm)	0.047	0.06	0.06	0.05	0.05	0.04
ORP (mV)	-97.08	-85.65	-85.65	-69.46	-55.85	-50.87
Exc. Nitrate (mg/L)	1.45	3.2	2.5	3.2	4	
Exc. Phosphate (mg/L)	0.05	0.06	0.07	0.5	0.2	

Table 2.14. Root Soil Sequential Extraction.

	R-SWG					R-LBS					R-YCF				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Antimony	6	11	28	21	34	20	9	23	18	29	13	9	51	17	11
Arsenic	2	5	12	9	71	3	6	14	11	66	4	7	19	14	56
Barium	2	10	61	10	18	3	15	38	12	31	2	15	44	15	24
Beryllium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17
Cadmium	2	5	25	6	62	1	5	17	5	71	2	7	26	8	57
Chromium	0	0	46	8	46	0	1	11	25	63	0	1	15	38	46
Cobalt	3	7	27	13	50	4	7	20	14	55	3	7	34	17	39
Copper	1	2	9	40	48	1	1	7	64	26	1	1	7	72	19
Lead	0	0	34	5	60	0	1	21	3	75	0	1	30	4	65
Manganese	0	4	63	4	28	0	7	40	7	46	0	9	55	9	26
Nickel	1	1	43	11	44	1	1	18	24	56	1	1	27	37	34
Selenium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17
Thallium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17
Vanadium	0	1	60	14	24	0	1	31	40	28	0	1	31	45	22
Zinc	0	1	20	1	77	0	1	12	3	84	0	2	19	6	73

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual.

zone soil, where also increases the fraction bounded to the organic matter. This tendency is also observed for YCF. The distribution of Pb in the root soil is very similar to that in the bulk soil, with the difference of a higher retention in the residual fraction observed in the three species analyzed. Mn, on the other hand, is the metal that presents major changes. While it remains predominantly retained in the Fe – Mn oxides bound fraction, the percentage decreases slightly in the root zone soil, increasing the percentage retained in the residual fraction. However, an increase of retention in the carbonates – bound fraction is also observed, which could explain a higher mobility of this element.

Rhizodegradation is plant-assisted biodegradation or bioremediation in the rhizosphere (the soil around the roots of a plant). The root exudates of plants can improve the living environment of indigenous microorganisms indirectly by reducing the toxicity of soil contaminants, improving the spatial heterogeneity of the rhizosphere environment, and promoting the growth of rhizosphere microorganisms, thereby resulting in the enhancement of biological activity of microorganism, and ultimately improving the degradation ability of the rhizosphere microorganism to PAHs (Chaudhry et al., 2005 and Parrish et al., 2005).

2.4.7. *Practical Implications*

The harsh conditions of the experimental area were created when the ground was tilled. The mixing of underlying slag with the soil could have jeopardized the success of the plants. It would be advisable, accordingly with the results obtained from this study, to homogenize and mix the top soil layer, without mixing with the underlying slag as much as possible.

In phytoremediation, plants are ideally chosen such that they can cover a significantly large root surface area and are capable of adapting to the conditions of the soil. From an economic viewpoint, plants that require less maintenance such as fertilizing or frequent trimming are preferable. As such, feasibility studies have focused on the *Graminaeae* family

or commonly known as grass since these species have very fibrous root systems which extend over a large surface area and penetrate deeper into the soil. The use of deep rooted prairie grasses to stimulate the degradation and detoxification of toxic and recalcitrant organic chemicals at low soil concentrations represents a potential low-cost, effective, and low-maintenance remedial option. Due to the low survival rate of trees and shrubs, they are not recommended to use for remediation in the study area. However, due to the beneficial effects of their use in phytoremediation, it would be appropriate to establish the necessary conditions for their development by planting and growing herbaceous plants in the first instance. Herbs, due to their high rate of renewal and their proven efficiency in reducing soil toxicity, would help prepare the ground for the subsequent implementation of woody species, thus enhancing the effect of this sustainable technique.

The phytoremediation of contaminated soils with the two types of contaminants (organic and inorganic), can be enhanced with several strategies. The addition of compost amendment to the slag disposal area enhanced the survival rates by providing nutrients and organic matter, promotes the stabilization of the heavy metals in the soil and expedites the biodegradation of organic contaminants by reducing the stress of the plants.

Although the technique developed showed highly effectiveness in removing organic contaminants from the soil, proving that phytoremediation is highly suitable for this purpose, it would be highly recommendable the study of the presence of byproducts and or metabolites derived from PAHs degradation, in order to study the final fate of these compounds and assess the effectiveness of this technique in reducing the toxicity produced by this organic contaminants.

The toxic inorganic contaminants, such as Cr or Pb, although they did not manage to be removed from the soil, neither were uptaken by plants, which represents a decrease in the risk of exposure from living organisms. The results from sequential extraction of metals

showed that the exchangeable fraction of the metals studied had the lowest concentration. However, since the heavy metals total concentrations remain above the established limits, it is highly recommended to track the remediated area, as it is a dynamic system, it is important to check the bioavailability of inorganic contaminants would not be modified over the time.

Big Marsh is representative of many other unrestored wetland sites in the region which have been significantly altered by the steel industry. Many other sites in the Calumet area and the Grand Calumet Area of Concern have similar conditions to Big Marsh and this project results are immensely valuable in evaluating the potential for using native plants to remediate other wetland sites.

This study identified several native plant species suitable for re-vegetation and restoration of heavily impacted, urban/industrial sites with historic soil contamination. The results suggest that native plant species may promote organic contaminant degradation and soil neutralization once established in slag-impacted zone.

2.5. Conclusions

The present study confirms that the compost amendment significantly improves long term growth and survival of plants at high impacted slag areas with very thin top soil. Although results showed that addition of soil amendment together with the presence of plants promotes the biodegradation of organic pollutants (PAHs), the synergistic effect of compost amendment and the surviving species in PAHs degradation is not conclusive. On the other hand, the presence of plants did not affect the mobility of heavy metals in soil, which either were assimilated by the plants, with exemption of Mn. Data here show that native grass species have larger phytoremedial potential than woody species, which show higher vulnerability to soil contamination, invasive species, and pests. Overall, compost amendment provides a promising approach for enhancing phytoremediation of mixed contaminated soils using native species

such as Switchgrass and Little Bluestem, which are shown to survive under the harsh site slag conditions and reduce the organic contaminants while not affecting heavy metals mobilization, lowing the risk of the contaminants to public and the environment.

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CHAPTER 3

FIELD INVESTIGATION OF PHYTOREMEDIATION OF MIXED CONTAMINANTS IN WET MEADOW AREA AT BIG MARSH SITE, CHICAGO, USA

3.1. Introduction

Throughout the United States and internationally, wetlands are important resources that, despite focused efforts, have been steadily disappearing. Wetlands serve as habitats for threatened and endangered species and are enormous sinks for carbon, and provide crucial environmental functions including cleaning and detoxifying water and mitigating floods. In northwestern Indiana and northeastern Illinois, the Lake Calumet region contains some of the richest of the remaining wetlands. Because of the heavy industrial presence in the region, a high fraction of these wetlands have been degraded. Many of the wetland sediments are contaminated, some of the upland areas are barren due to plant toxicity, and concern exists that surface and ground water in the area are being negatively impacted by residual contaminants.

Big Marsh is one of the largest expanses of wetland within the Calumet region. The site, with 121 hectares of open space classified as wooded/marshland without any on–site structures, and 35 hectares of wetland, is one of the largest expanses of wetlands within the Calumet region. It falls within the Great Lakes Basin and is hydrologically connected to Lake Michigan through Lake Calumet and the Calumet River. Big Marsh is relatively level and undeveloped with large areas of open water, degraded wetlands, and upland fill areas covered with invasive species of vegetation.

The area object of the present study is located at the Southern part of Big Marsh and comprises approximately 9 hectares of innocuous fill material that contains a high percentage of iron presumably blast furnace slag. Furthermore, approximately 16 hectares of the southern

filled section contain impenetrable slag and has been devoid of vegetation for 35 years. Fill materials across the site range from 2 to 3 meters thick and consists of steel-mill slag, with some construction and demolition debris and dredge spoils from Lake Calumet and the Calumet River. Water quality is impacted by high pH levels; in some areas the pH reaches 12.6. Bottom sediments in the marsh are natural muck soil that has not been dredged. The southeastern part of the site is covered with white calcite that leaches out of the slag from adjacent upland fill.

Big Marsh is representative of many other unrestored wetland sites in this region which have been significantly altered by the steel industry and decades of legal and illegal dumping. The wetland has been massively altered from original conditions by industrial filling, and these fill materials as well as the groundwater and surface water have been found to be contaminated with polyaromatic hydrocarbons; benzene, toluene, ethylbenzene, and xylenes; organic solvents; polychlorinated biphenyls, and heavy metals. Therefore, the wetlands at Big Marsh are greatly in need of restoration efforts.

Sites with mixed contamination pose technical challenges associated with the present of various classes of contaminants with different physico–chemical properties, because they will respond in a different way to the remediation technologies. Several technologies for the remediation of contaminated soils have been developed over the past three decades. Their applicability is often limited to a particular kind of contaminant. In the case of contaminated sites with mixed contamination, few technologies have proven to be efficient, but they also have important limitations, plus their application at field scale results very expensive. In this context, phytoremediation arises as a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al., 2013).

A previous study showed that the mixed contamination in the soil had a significant effect on the plant growth (Chirakkara and Reddy, 2014). The ability of the plants to survive

in high impacted areas and the low bioavailability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency.

The present work investigates the use of phytoremediation in a wet meadow area at Big Marsh, a wetland in southeast Chicago (Illinois, USA), contaminated with PAHs and Heavy Metals. This study includes planting, monitoring, subsequent analysis and all the data was used to analyze and study the plant survival and growth and contaminant uptake, with the aim of evaluating the plants species and phytoremediation feasibility of the site.

3.2. Research Methodology

3.2.1. *Initial Soil Characterization*

A delineation survey was conducted to determine the extent and boundary of the experimental area at Big Marsh. The initial baseline sampling was conducted on the site in order to identify the existing heavy metal and organic contaminant present in the soil. Three composite samples were taken in clusters in the flooded wetland area. Sampling locations were recorded using a GPS. Soil samples were oven dried and soil characterization and contaminant concentration analysis were performed. Additional pH tests were performed *in situ* at Season 2, at different locations and depths in order to get a better understanding of pH distribution in the soil of the experimental area.

3.2.2. *Test Section Preparation*

The experimental area was identified based on preliminary soil initial baseline sampling. An experimental and adjacent plot of size 15m x 15m each, were demarcated. Ground was prepared tilling and homogenizing the fill material to approximate depth of 1m.

At the experimental plot (15m x 15m), two different types of subplots were designed in order to establish herbaceous and woody plants. Those parcels intended for planting herbaceous

plants were called GP (Grasses and Plugs) plots, and those plots used for planting trees and shrubs were called TS (Trees and Shrubs) plots (Figure 3.1a). A total number of 5 subplots, each 2.4m x 3.7m were selected as GP plots. Each subplot was divided into 6 groups of size 1.2m x 1.2m, and each group was divided into 16 cells of size 0.3m x 0.3m (Figure 3.1b). Another 5 subplots of size 3m x 3m each, were selected as TS plots, and each subplot was divided into 4 groups, each 1.5m x 1.5m (Figure 3.1c).

The adjacent plot (15m x 15m) was delineated next to the experimental plot with the purpose of monitoring plant survival and grow characteristics of the grass species. One composite soil sample from each group at each subplot of the experimental plot was collected for baseline contaminant concentration analysis and soil characterization.

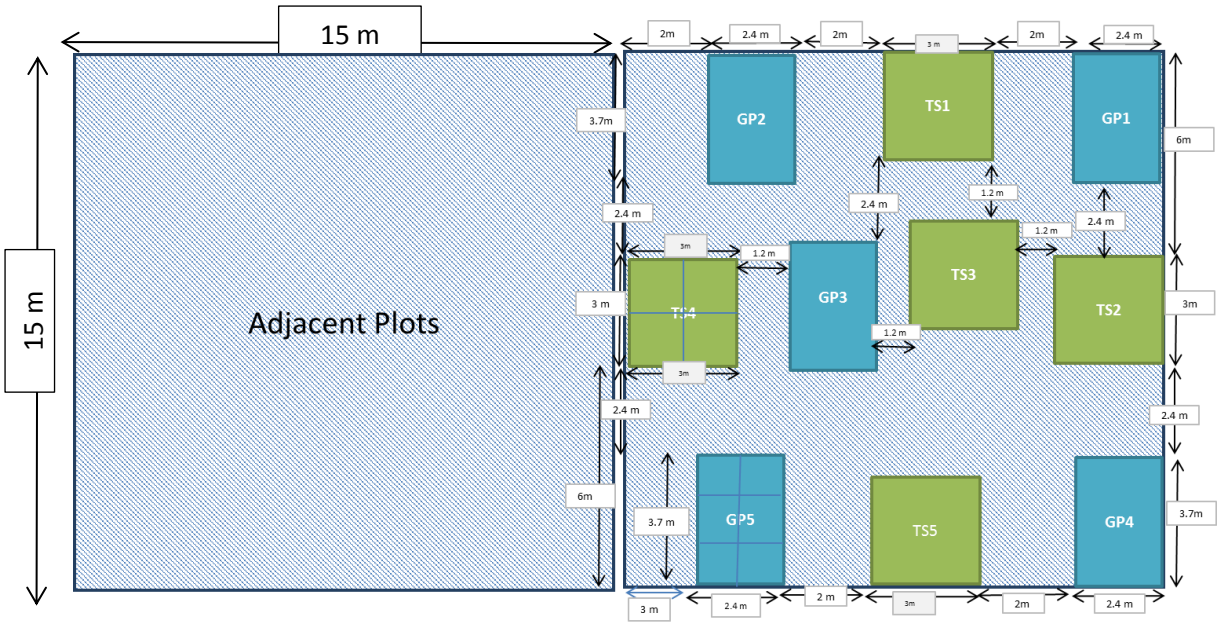
3.2.3. *Plant Selection and Planting*

The selection of plants was based on the potential phytoremedial properties and the soil characteristics existing at the wetland test location. A total of 9 native and restoration species, that included 5 species of grass and plugs and 4 species of trees and shrubs were chosen.

According to the delineation, the GS subplots were divided into 6 subgroups, 5 of which were designed with the aim of planting the grass samples grouped by species, and the remaining group with the aim of planting all the species together. A total of 16 samples of the same species were planted at each subgroup, and 3 species of each sample were planted at the remaining subgroup. A total of 96 grass samples were planted within the experimental plot, and 20 samples were planted in the adjacent plot. Within the plot intended for planting trees and shrubs (TS plots), a subdivision into groups for the different species was also performed. In this case, no subgroup was intended for planting mixed species. At each subgroup, only one woody specie was planted, resulting of a total of 20 woody species (trees and shrubs) planted within the experimental plot (Table 3.1). No woody samples were planted in the adjacent plot. The

Table 3.1. Species selected for restoration of wetland area

Type	Scientific Name	Common Name	Sample ID	Number of Samples	
				Experimental Plot	Adjacent Plot
Grasses and Plugs	*Asclepias incarnata*	Swamp milkweed	SMW	96	50
	Cassia hebecarpa	Wild Senna	WSA	96	50
	Deschampsia caespitosa	Tufted hair grass	THG	96	50
	Solidago graminifolia	Common grass-leaved goldenrod	CGG	96	50
	Spartina pectinata	Prairie cord grass	PCG	96	50
Trees	*Acer saccharinum*	Silver maple	SMP	125	0
	Quercus bicolor	Swamp white oak	SWO	125	0
Shrubs	*Amorpha fruticosa*	False indigo bush	FIB	125	0
	Cornus stolonifera	Red-osier dogwood	ROD	125	0



a. Overview of Plot Layout

THG	MIX
WSA	PCG
SMW	CGG

1	2	3	4	1	2	3	
4	5	7	8	4	5	6	
9	10	11	12	7	8	9	
13	14	15	16	10	11	12	
				13	14	15	
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16

b. Grass and plugs (GS) subplots planting layout

SMP	SWO
ROD	FIB

1	2	1	2
3	4	3	4
1	2	1	2
3	4	3	4

c. Trees and Shrubs (TS) subplots planting layout.

Figure 3.1. Plots and subplots delineation layout.

pictures of the experimental area before, during and after soil preparation and planting can be found in Figure 3.2.

3.2.4. *Watering and Monitoring*

Once completed soil preparation and planting, the test plot was watered twice a week throughout summer months (June to August) and monitored weekly for survival, leaves quality and pests infection during the first growing season. At the adjacent plot, only survival monitoring was performed. Table 3.2 shows the rating system used to assess plant health.

At the second growing season, the test plots were monitored bi-weekly during the summer. No additional water or pest control was performed at the experimental area, in order to let the plants grow under normal conditions and assess the suitability of the native plants to cope with the natural site conditions and compete against the invasive species. Figures 3.3 and 3.4 show the monitoring plant survival and growth of a grass and woody species, respectively.

3.2.5. *Termination Sampling*

At the end of the second growing season, a first sampling was conducted at the experimental area. Composite soil samples were taken from the surviving species FIB plot. All soil samples were kept on ice during the day. Vegetative biomass, divided into above ground (leaves, shoots and fruiting bodies) and belowground (roots), was taken from one representative sample at each TS-FIB plot. All the samples were transported back to the lab, weighed and oven – dried. Contaminant concentration analysis was performed to soil and vegetative samples.

At the end of the third growing season, a terminal sampling was performed. Soil was sampled from all TS-FIB subplots. Vegetative samples consisting of roots, leaves and shoots were also collected from the FIB surviving species. Additionally, two grab samples from invasive vegetation (Phragmites) were also collected.

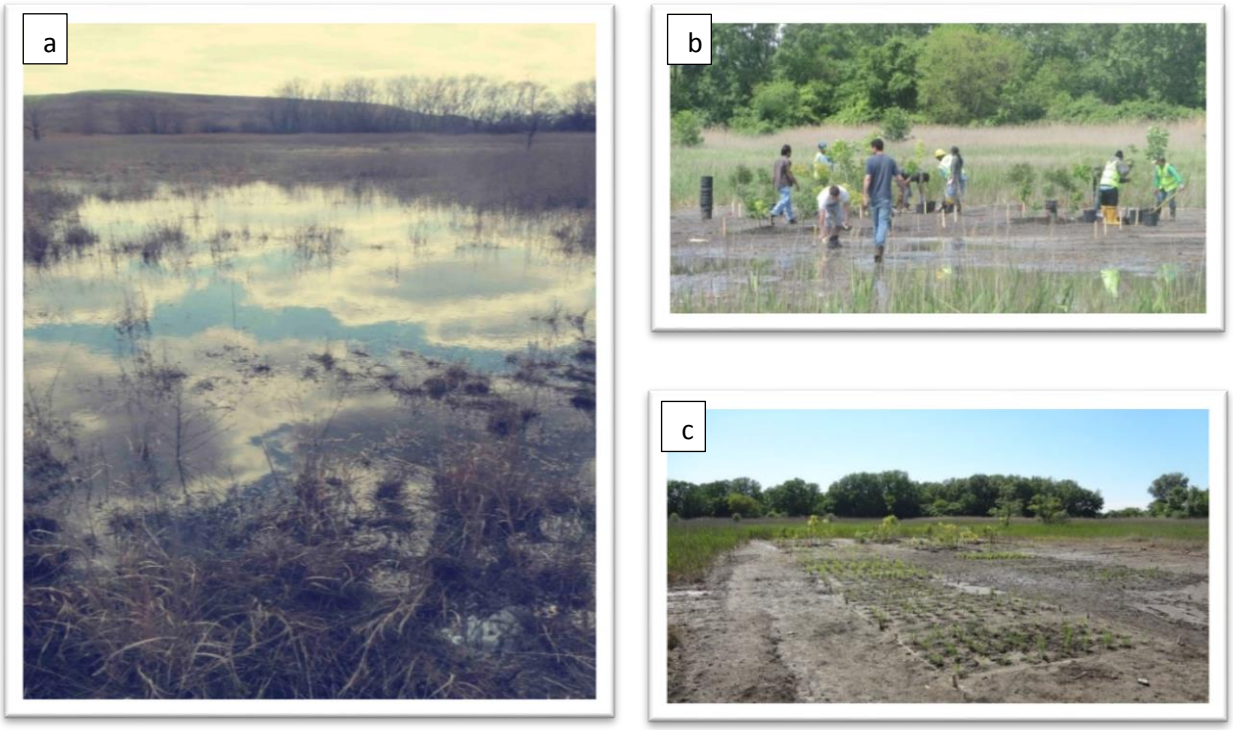


Figure 3.2. Experimental area. (a) Before any treatment. (b) Planting after tilling and homogenization. (c) After planting.

Table 3.2. Monitoring rating system

Parameter	Measurement
Survival (S)	Scale 1-4 (1 =dead; 2 =dying; 3 =no change in growth; 4 =evidence of new growth)
Leaves (L)	Scale 1-4 (1 = >50% leaves are dead; 2 = >25% leaves are dead, discoloration and/or wilting is present; 3 = <25% of leaves are discolored and/or wilting with no dead or dying leaves present; 4 = No discoloration, wilting or dead/dying leaves.)

a. After planting **b. 1 week** **c. 2 months**

d. End of the 1st season **e. End of the 2nd season**

Figure 3.3: Monitoring of Grass-leaved Goldenrod (CGG) at the adjacent plot

a. After planting **b. 1 week** **c. 1 month**

d. 2 months **e. End of the 2nd Season** **f. End of the 3rd season**

Figure 3.4. Monitoring of False Indigo Bush (FIB) at the experimental plot TS1

Soil physical properties were tested in the lab and total contaminant concentration (PAHs and metals) was also analyzed for soil and vegetative biomass samples collected.

3.2.6. *Soil and Plant Sample Tests*

The soil characterization tests performed in the lab consisted of physicochemical properties that mainly included measurements of the pH, electrical conductivity (EC), Organic Carbon (OC), and Oxidation – Reduction Potential (ORP), Water Holding Capacity (WHC), Grain size distribution (GSD), and Exchangeable Nutrients Content.

The soil pH and ORP were determined according to the ASTM D4972 – 01 Standard Test Method for pH of Soils (ASTM 2007). The values were measured in the laboratory using an Orion Model 720-A pH/ISE meter. Water content values were measured in the laboratory according to ASTM D 2216 Standard Test method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass (ASTM 2005). Organic Carbon was determined using ASTM D 2974 Standard Test Method for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils (ASTM 2000). The Electrical Conductivity of the soil was measured in a 1:5 soil: water suspension, using a Fischer Scientific model TRACEABLE^{TM} conductivity meter. Grain Size Distribution was determined according to ASTM D 422-63 Standard Test Method for Particle Analysis of Soils (ASTM 2002). To analyze exchangeable nitrogen, 1 g soil was shaken with 10 mL of 2M KCl solution for 1 h (Xu et al., 2013). The filtered extractant was analyzed using Spectronic Genesys Spectrophotometer, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of phosphorus, 1 g soil was shaken with 1 M ammonium acetate for 1 h. The solution was filtered, and the extractant was analyzed with Spectronic Genesys spectrophotometer, as per the procedure given by Sattayatewa et al. (2011). The Water Holding Capacity (WHC) of the soil was determined

following the ASTM D2980 – 04 Standard Test Method for Volume Mass, Moisture – Holding Capacity and Porosity of Saturated Peat Materials (ASTM 2010).

Soil and vegetative samples were sent to STAT Analysis Corporation (Chicago, IL, USA) for sample acid digestion and analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS). Polynuclear Aromatic Hydrocarbons (EPA method SW8270C) were also tested by Gas Chromatography Mass Spectrometry (GC/MS).

Sequential Extraction was performed using the Tessier procedure (Tessier et al., 1979) with slight modifications in order to determine the speciation of the contaminants in the soils both before and after the phytoremediation technique was implemented. Further information regarding the sequential extraction procedure can be found in Chapter 2. Samples from sequential extraction and blank were sent to STAT Analysis Corporation (Chicago, IL, USA) for analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

In order to assess the amount of contaminant uptaken by the plant, the percentage of contaminant in the plant respect to the soil is calculated as follows:

$$\%contaminant\ plant = \frac{mg/Kg\ contaminat\ in\ the\ plant}{\frac{mg}{Kg}contaminant\ in\ the\ soil\ initially} \cdot 100$$

For the test results, mean and standard deviation were calculated using Microsoft Office Excel 2013. To check whether a significant difference exists between the result sets, a one – way analysis of variance (ANOVA) test, followed by the t-test were performed with Microsoft Office Excel 2013. The alpha value was taken as 0.05 for the t-test.

3.3. Results and Discussion

In this section, the results for soil characterization and contaminant concentrations in soil and vegetative tissue during the three growing seasons are presented and discussed.

3.3.1. *Initial Soil Characterization.*

Soil physical properties were tested in the lab for every soil composite sample. In this section results of initial soil survey at the wet meadow area are presented. The physical properties tested to characterize the soil were pH, Oxidation - Reduction Potential (mV), Organic Content (%), Electrical Conductivity (mS/mL), Moisture Content (%), Grain Size Distribution (%), Water Holding Capacity (%), and Exchangeable Nutrients Concentration (mg/L).

The results of the initial soil characterization are shown in Table 3.3. The average pH value of the surface soil at the beginning of this study was 7.29. The results for pH found in the surface, were lower than expected, possibly due to washing effect of weatherization. The Oxidation – Reduction Potential (ORP) is an index of the exchange activity of electrons among elements in solution. The results show a negative potential, which indicates reducing conditions in the initial soil. The soil was predominantly sandy soil, with low organic matter content. The results for additional pH test taken *in situ* at different locations and depths within the experimental area show an increase of pH with depth. According to the results, the soil pH at 36 cm depth is 11, while the pH at the surface ranged from 9 to 10. The pH of the untilled soil measured outside the experimental area was 7.4, whereas the pH inside the experimental area was about 2.5 units higher (10). This increase of pH is likely due to the incorporation of high pH slag material into the upper soil subsurface.

A survey of the contaminants (organics and inorganics) present in the soil was carried out by acid digestion and ICP analysis of soil samples (inorganics) or GC/MS analysis (organics). Table 3.4 shows the concentrations (mg/Kg – dry soil) of different Polynuclear

Table 3.3. Soil characterization before, after and at the end of the third growing season

Soil Parameter	Initial Soil	After Tilling	Season 3	Root soil
pH	7.29	10.68	7.54	7.5
ORP (mV)	-31.13	-244.54		-39.7
OC (%)	2.56	5.45	6.33	15.19
EC (mS/cm)	0.3		0.06	0.23
mc (%)	26.96	40.21	34.68	1.88
WHC (% total mass)	44.73	43.73		
Phosphate (mg/L)	0.06	0.01	0.03	0.07
Nitrate (mg/L)	1.34	0.64	1.79	6.56
%Gravel	17	15	13	
%Sand	51	73	58	
%Fines	31	12	29	

Table 3.4. Contaminant concentrations in soil

Contaminant	Concentration (mg/Kg – dry soil)			
	Initial Soil	After Tilling	Season 2	Season 3
PAHs				
Acenaphthene	0.4	<DL(0.04)	<DL (0.04)	<DL (0.04)
Acenaphthylene	0.07	<DL(0.04)	<DL (0.04)	<DL (0.04)
Anthracene	0.5	<DL(0.04)	<DL (0.04)	<DL (0.04)
Benz(a)anthracene	1.5	0.05	<DL (0.04)	0.06
Benzo(a)pyrene[a]	0.4	0.1	0.1	0.1
Benzo(b)fluoranthene	1.6	0.04	<DL (0.04)	0.05
Benzo(g,h,i)perylene	1	0.07	<DL (0.04)	0.09
Benzo(k)fluoranthene	1	0.07	<DL (0.04)	0.09
Chrysene	2	0.07	<DL (0.04)	0.09
Dibenz(a,h)anthracene	0.5	<DL(0.04)	<DL (0.04)	<DL (0.04)
Fluoranthene	4	0.07	<DL (0.04)	0.09
Fluorene	0.4	<DL(0.04)	<DL (0.04)	<DL (0.04)
Indeno(1,2,3-cd)pyrene	0.8	<DL(0.04)	<DL (0.04)	0.07
Naphthalene	0.4	<DL(0.04)	<DL (0.04)	<DL (0.04)
Phenanthrene	5	0.05	<DL (0.04)	0.05
Pyrene	4	0.07	<DL (0.04)	0.09
Metals				
Aluminum	9900	47500	52000	47167
Antimony	<DL(5)	<DL(5)	<DL(5)	<DL(5)
Arsenic[b]	7	<DL(3)	4.6	4
Barium	63	560	480	580
Beryllium	1	9	7	6
Cadmium	1	<DL(1)	<DL(1)	<DL(1)
Calcium	52000	220000	230000	225000
Chromium[c]	36	60	62	68
Cobalt	8	<DL(2)	<DL(2)	<DL(2)
Copper	27	7.2	6.7	9
Iron	28000	17500	31000	16667
Lead[d]	111	59	51	53
Magnesium	24000	13000	15000	13333
Manganese[e]	1400	8150	8650	6767
Mercury	0.04	<DL(0.02)	0.03	<DL(0.02)
Nickel	22	7	5.5	5.8
Potassium	1400	2950	2100	2900
Selenium	<DL(1)	6.6	6.4	10.6
Silver	<DL(1)	<DL(1)	<DL(1)	<DL(2)
Sodium	110	1250	950	1183
Thallium	<DL(1)	<DL(1)	<DL(1)	<DL(2)
Vanadium	41	24	23	26
Zinc	470	200	130	188

Target contaminant concentrations Average±SD (number of samples) Before tilling – Season 3, Respectively:

a. BaP: 0.4±0.7 (5); 0.1±0.04 (4); 0.1±0.02 (7); 0.1±0.02 (6)
b. As: 7±0.69 (5); <DL(3) ±0.7 (4); 4.6±2.75 (7); 4±0.99 (6)
c. Cr: 36 (1); 60±18 (4); 62±16 (7); 68±14 (6).
d. Pb: 111±24 (5); 59±13 (7); 51±15 (7); 53±19 (6).
e. Mn: 1400 (1); 8150±353 (2); 8457±1827 (7); 6767±1015 (6).

Aromatic Hydrocarbon (PAHs) compounds and metals that could be found initially in the soil at the experimental area. Benzo(a)pyrene was one of the target PAH contaminants of this study due its known carcinogenic and mutagenic potential. Among the heavy metal species found in the initial soil, Arsenic, Chromium, Lead and Manganese were target contaminants of the present study. The target contaminants BaP, As and Pb were analyzed from 5 composite soil samples, whereas for the rest of metals and PAHs were analyzed in a representative sample.

3.3.2. *Soil Characterization After Tilling*

According to what was expected, tilling and homogenization (Table 3.3) affected the soil physical properties. The soil pH after the treatment increased up to 10.7 as compared to the value obtained for the initial soil sampling. The mixture of the alkaline slag layer underneath with the top soil coverage during homogenization could have induced this increase on the soil pH. The organic carbon content in the unplanted soil increased from 2.5 to 5.5% after tilling. The organic content after the soil preparation was found higher than expected, taking into account that no additional organic matter was added to the soil while tilling, and it was likely due to the high spatial heterogeneity that affected the soil characteristics from one location to other. The magnitude of the reduction potential increased one order of magnitude, as a result of soil tilling, resulting in highly reductive conditions. The exchangeable nutrients concentration decreased for both, Phosphate and Nitrate up to 83% and 52%, respectively, probably due to dilution effects after mixing the soil. The water holding capacity did not change significantly in the soil after tilling, likely due to the predominant presence of the sand fraction, which increased after tilling. The grain size distribution of the soil at the experimental area is represented in Figure 3.6.

The total PAHs concentrations of the soil are shown in Table 3.4. The target contaminant BaP was analyzed from 4 composite soil samples, whereas for the rest of PAHs

were analyzed in only one sample. No significant differences were found in BaP concentration values before and after the soil treatment ($p<0.05$). Unfortunately, the number of samples was not enough to compare the statistical significance of the rest of PAHs concentrations in the soil before and after the tillage.

Heavy metal concentrations can be found in Table 3.4. As it can be observed, As and Pb concentrations in the soil decrease after tilling ($p<0.05$). It was not possible to compare statistically the concentrations of Cr and Mn, due to the low number of samples available. The differences found in As and Pb concentrations after tilling the soil might be due to spatial variability. At the initial soil, only the target contaminants As and Pb were measured in enough replicates, and the results obtained were 7±0.69 and 111±24.30, respectively. Concentrations of Cr and Mn were 36 and 1400 mg/Kg – dry soil, respectively. After tilling, concentration values changed with respect to the initials. In the case of Pb and As, contaminant concentrations decrease after homogenizing the soil. These results suggests that there is a dilution effect of these contaminants when mixing the soil. On the other hand, concentrations of Cr and Mn increase dramatically after tilling the soil. Unfortunately, the number of samples did not allow to perform statistical analysis to the results obtained. As it has been explained above, the generalized flooding in the study area prevented from taking sufficient representative samples, allowing only samples at the edges of the area to be taken. Thus, this lack of representation, followed by the flooding cycles and the variable moisture conditions, could explain the enormous spatial variability of the contaminant concentrations in the study area.

The results of the percentages of the different fractions of metals in the soil are shown in Table 3.6. The results for heavy metals such as As, Cr, Pb and Mn, the target metal contaminants of the present study, are plotted in Figure 3.7. As it can be observed, the predominant fraction before tilling is the Residual Fraction for As and Cr, while more than 50% of the Pb and Mn is retained in the Fe and Mn oxides bound fraction. On the other hand

the exchangeable fraction, which is the most bioavailable fraction of metals in the soil is very low for all the metals. The tilled soil shows an increase in the Fe – Mn oxides for As and Cr while Pb and Mn kept approximately the same proportion. Organic fraction increased for all metals after tilling, while Carbonates – bound fraction only increased in As, remaining the same in Cr and decreased for Pb and Mn.

3.3.3. *Plant Monitoring*

Figures 3.3 and 3.4 show the results for monitoring of Grass – leaved Goldenrod (CGG) and False Indigo Bush (FIB) respectively, throughout the experiment. As it can be observed from the pictures, FIB thrived at the end of the third season, while CGG, as well as the rest of the grass species did not survive. None of the species except FIB survived by the end of the experiment. However, some signs of stress were found in the plant during the terminal sampling. The roots of the tree appeared restricted to the original potting soil, seemed to grow laterally over straight down into the slag material, and showed a high density of nodules.

Figure 3.5 shows the surviving results for the first and second growing season at the wet meadow area based on the monitoring parameters (Table 3.2). During the first growing season, all the species showed high survival rates. However, a sharp decrease is found at the second season for all the species, with exemption of FIB. The survival rates found in the adjacent plots and in the mix subplot were similar to those at the experimental plots. The presence of invasive species in the study area was also very low, highlighting the harsh conditions for the establishment of any plants on the site. The plants survival and leaf quality assessment (Figure 3.5b) was performed on trees, only during the first growing season, with the aim of carrying out a detailed monitoring of their development and growth and their adaptation to the ground. As it can be observed, FIB reached the best performance out of all the selected species. An intensive plant growth monitoring was performed during the first

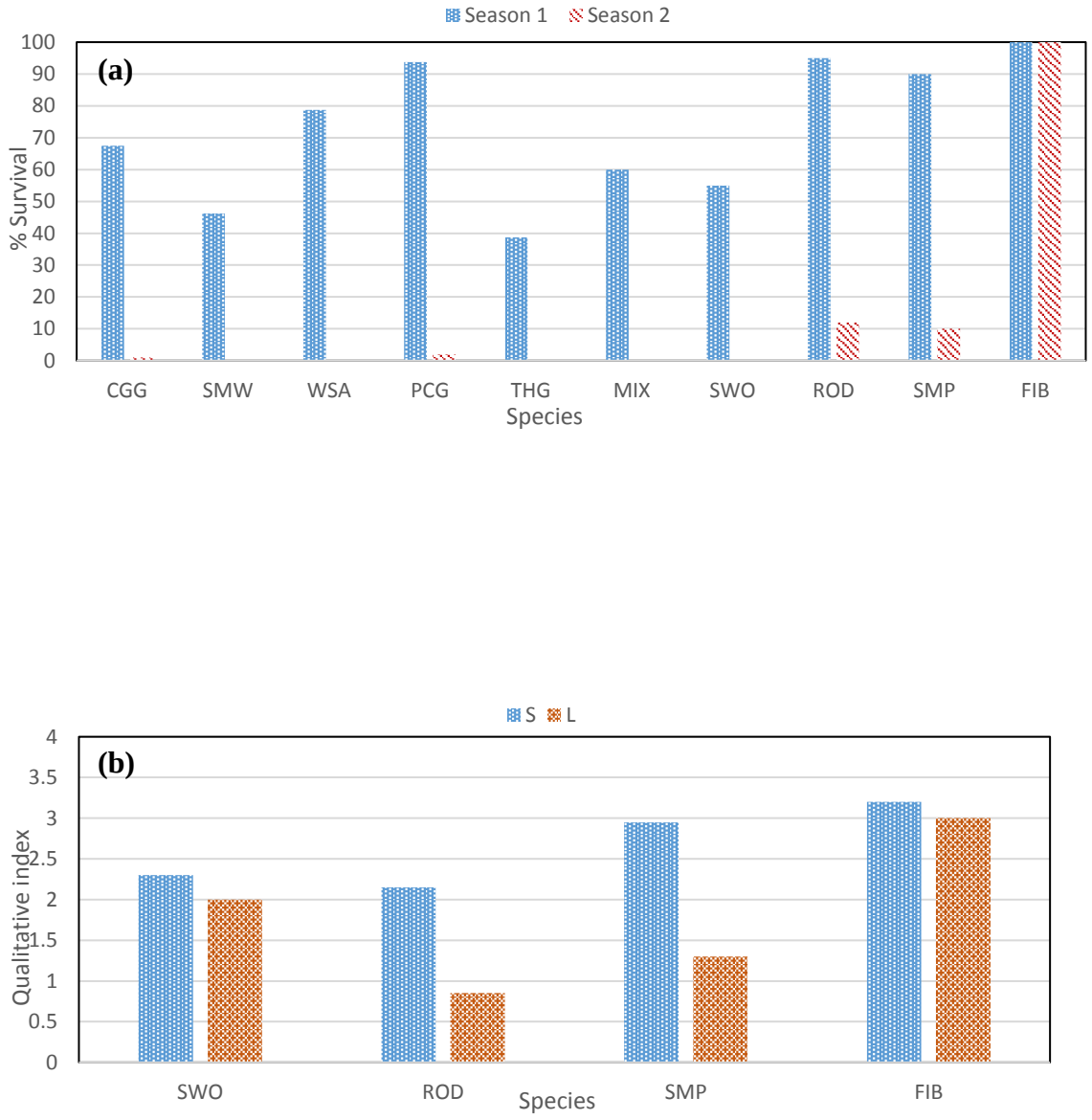


Figure 3.5. Monitoring rating results. (a) Plant survival in the experimental plots at the end of the first and second growing season. (b) Plant survival and leaf quality in grass and trees at the end of the first growing season.

growing season, and the plants were watered weekly during the summer, allowing the soil moisture to remain constant. This, together with the greater availability of nutrients present in the potting soil which seedlings and shrubs were planted with, may have helped the species survive the first year. During the second season, on the contrast, no watering or pest control was performed, and the potting soil nutrients progressive consumption could affect the plant survival rates. No monitoring was performed during the 3rd growing season. However, field observations made during the terminal sampling, showed that all species planted, except FIB, survived at the end of the experiment.

The results for pH distribution in the soil of the experimental area showed that the soil at a depth 36 cm below the surface within the experimental plot area had an average pH of 11, and in the surface remained around 10. This high value of pH is due to the existence of an extremely basic slag layer below the soil surface, which increased the overall pH of the soil during tilling. The high pH of the soil was likely one of the main reasons of the poor performance of the experimental species. According to the USDA plants database, all of the species used in our study, had an optimum pH range from slightly acid to neutral (USDA). Additionally, the drought tolerance of the experimental species was null or low, with the exception of FIB, which also had the wider pH range.

The presence of cycles of soil drought and flooding in the experimental area could have also been a determining factor in ensuring the survival of plants (USDA). Wetland species have been observed to have less biomass accumulation when exposed to extended flooding-drought periods (Kercher and Zedler, 2004; Dylewski et al., 2011; Ewing, 1995). Other factors may also had limited plants growth, such as macronutrients deficiencies (Pulford 1991). The harsh conditions at the experimental area, the presence of invasive species, and the proximity of the slag layer underneath the composted top soil, combined with the high concentration of contaminants could have been the main cause of the poor survival of the native species planted

in the experimental area. FIB, on the other hand, showed extraordinary resistance to the severe conditions of the experimental area, and thrived by the end of the experiment. As a result, only this species was considered for further detailed evaluation.

Previous results obtained in lab scale pointed phytotoxicity as the cause of the poor performance of the plants in the contaminated area, showing that the growth characteristics, as well as the survival rates are greatly influenced by the combined contaminated conditions, (Chirakkara and Reddy, 2015). On the other hand, FIB, like other species of the family *Fabaceae,* has properties that make it resistant to heavy metal pollution. The advantages of this group lies in their self – sufficiency in terms of nitrogen supply, and their favorable level of tolerance to drought (Gawronski and Gawronska, 2007). The ability of this species to fix Nitrogen due to rhizobium symbiosis, could be the key to face metal toxicity (Chaudri et al., 2000). In addition, FIB can tolerate a wide range of soil moisture, being able to survive in saturated or very wet soil and also survive under prolonged periods of drought (Cornell University). This adaptability, together with the capacity of fixing Nitrogen, can explain the suitability of FIB to survive the conditions of the study area.

3.3.4. *Fate of PAHs*

The PAH concentrations in the soil in FIB plot can be found in Table 3.4, where the EPA 16 priority PAHs are shown. However, only the target PAH contaminant, Benzo(a)Pyrene (BaP) was analyzed with enough replicates to perform statistical analysis (the average and standard deviation results are shown below the table). Results from the initial soil (Table 4) reveal a high concentration of PAHs, as compared to the samples taken the subsequent two growing seasons. The experimental area was flooded when the initial survey took place, and only a few samples could be taken at the edge of the site. The results of BaP in the initial soil show a high spatial variability that is tempered after tilling the soil, suggesting the presence of a hot spot in

the location where the initial samples were taken. In general, concentration of PAH in soil after tilling are very low, in some cases undetectable. As it can be observed, no significant differences were found in BaP concentrations throughout the experiment ($p>0.05$). Table 3.5 shows that all PAHs concentrations in leaves and stems of FIB are below detection limits at the end of the second growing season, including BaP, which was the only organic contaminant measured at the end of the third growing season. Concentration of BaP measured in the roots of FIB was also very low (0.03 mg/Kg) close to the detection limit value. These results suggest that the organic compound object of this study is not sorbed, or degraded by the plant. The results in the present study are consistent with Chekol et al. (2002) and Yan (2012) in which the presence of plants does not affect the dissipation of organic contaminants in the soil such as Pyrene or TNT.

No detailed studies have been found to investigate the fate of PAHs when FIB is used for phytoremediation. However, literature shows similar results when the phytoremediation technique is implemented using legume species. The results obtained by Fu et al (2012) using alfalfa as a candidate for phytoremediation of BaP from a PAH – contaminated soil show that planting alfalfa inhibited BaP removal from the contaminated soil. These researches point the competition between plants and microorganisms for nitrogen as the main reason that could have impeded BaP removal from the rhizosphere of alfalfa. The presence of nitrogen – fixing plants could increase the likelihood of removal inhibition of the pollutant due to the competition for nutrients between plants and microorganisms. Smith et al. (2008) reported that total N removal by plants was negatively correlated with loss percentage of phenantrene, chrysene, fluoranthene and pyrene in a 3–year field study. Thus, under low availability of nutrients, the result of mycorrhiza scavenging of N could lead to a depletion of the soil critical nutrients needed for microbial degradation of the contaminants, resulting in less efficient phytoremediation of PAHs.

Table 3.5. Contaminant concentration in leaves and stems of False Indigo Bush

Contaminant	Concentration (mg/kg) Season 2[a]	Season 3
PAHs		
Acenaphthene	<DL (0.3)	
Acenaphthylene	<DL (0.3)	
Anthracene	<DL (0.3)	
Benz(a)anthracene	<DL (0.3)	
Benzo(a)pyrene[b]	<DL (0.3)	<DL (0.03)
Benzo(b)fluoranthene	<DL (0.3)	
Benzo(g,h,i)perylene	<DL (0.3)	
Benzo(k)fluoranthene	<DL (0.3)	
Chrysene	<DL (0.3)	
Dibenz(a,h)anthracene	<DL (0.3)	
Fluoranthene	<DL (0.3)	
Fluorene	<DL (0.3)	
Indeno(1,2,3-cd)pyrene	<DL (0.3)	
Naphthalene	<DL (0.3)	
Phenanthrene	<DL (0.3)	
Pyrene	<DL (0.3)	
Metals		
Antimony	<DL (40)	
Arsenic[c]	<DL(4)	<DL(2)
Barium	<DL(3)	
Beryllium	<DL(1)	
Cadmium	<DL(1)	
Calcium	6100	
Chromium[d]	<DL(3)	<DL(2)
Cobalt	<DL(2)	
Copper	<DL(5)	
Iron	125	
Lead[e]	<DL(2)	<DL(1)
Magnesium	1350	
Manganese[f]	66	72
Mercury	<DL0.02	
Nickel	<DL(2)	
Potassium	8350	
Selenium	<DL(2)	
Silver	<DL(2)	
Sodium	<DL(130)	
Thallium	<DL(2)	
Vanadium	<DL(2)	
Zinc	23	

aContaminant concentration in stems, leaves and fruit body.
Target contaminant concentrations Average±SD (number of samples) Season 2; Season 3. Respectively:

b. BaP: <DL(0.3)±0.06 (10); <DL(0.03)±0.003 (4).
c. As: <DL(4)±0.0.75 (10); <DL(2) ±0.44 (4).
d. Cr: <DL (3)±0.7 (10); <DL(2) ±0.44 (4).
e. Pb: <DL(2) ±0.6 (10); <DL(1) ±0.2 (4).
Mn: 66.3±25.7 (10); 72±15.2 (4).

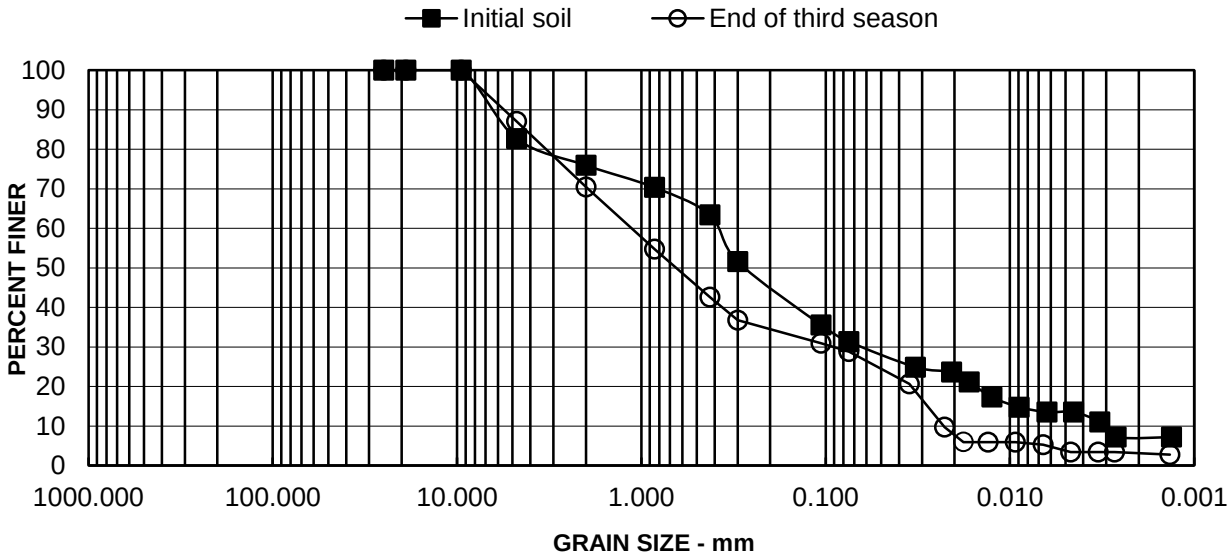


Figure 3.6. Grain Size Distribution of soil before tilling and at the end of the third growing season.

3.3.5. *Fate of Heavy Metals*

The average results for heavy metal concentrations in the bulk soil at the FIB subplot can be found in Table 3.5. Only the target contaminants, Arsenic (As), Chromium (Cr), Lead (Pb) and Manganese (Mn) were analyzed with enough replicates to perform statistical analysis (The average and standard deviation results are shown below the table). The results show a high spatial variability in terms of heavy metal concentrations in the soil.

As compared to the unplanted soil after tilling, no significant differences can be found at the end of the third growing season, except for Mn, which concentration in soil decreased slightly ($p<0.05$) throughout the experiment. These results show that very little mobilization of the metals in the soil occurs, tending to remain constant, despite the presence of plants. This tendency in the target contaminants suggests that the presence of plants in the experimental area did not affect the concentration of heavy metals in the soil. Manganese, on the other hand, has a different behavior, and its concentration tends to decrease when compared to the unplanted tilled soil.

The total metals concentrations in stems and leaves of the surviving species can be found in Table 3.5. At the end of the third growing season, only the results for As, Cr, Pb and Mn are shown. As it can be seen, the concentrations of heavy metal in the plants were below detection limits in all cases, except Mn for which concentration in the aerial vegetative tissue was detected. The targeted metals concentrations were analyzed in the roots at the end of the third growing season. The concentration of As was not detectable in roots. However, it did not occur the same way for Cr, Pb and Mn, which concentrations in the roots of the surviving plants were detected (5 mg/Kg, 10 mg/Kg and 480 mg/Kg, respectively). Although significant concentrations of Pb and Mn were found in the root biomass, the proportion of contaminant uptake from the soil is very reduced (Table 3.7).

Table 3.6. Percentage of metal fractionation from sequential extraction at False Indigo Bush plot soil

	Initial Soil					After Tilling					Season 3					Root Soil				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Antimony	7	7	33	13	41	15	7	45	11	22	26	17	17	24	17	6	12	32	21	29
Arsenic	3	3	16	6	72	5	11	27	16	41	6	12	18	21	44	5	10	25	18	43
Barium	9	29	33	2	26	1	6	44	15	34	2	10	39	20	30	3	9	43	20	25
Beryllium	9	9	43	17	22	2	3	61	9	25	2	8	51	11	28	2	4	64	12	18
Cadmium	6	12	29	11	42	4	8	51	12	25	9	17	22	31	22	6	12	30	22	30
Chromium	1	2	30	3	64	0	1	44	13	42	0	2	32	19	47	1	1	40	23	35
Cobalt	3	3	38	6	49	4	8	51	12	25	7	14	35	26	18	6	12	30	22	30
Copper	3	3	14	13	67	4	8	47	15	27	4	9	21	31	35	2	3	3	71	20
Lead	0	7	56	2	34	1	1	58	8	32	0	5	59	8	27	0	3	62	11	25
Manganese	0	8	64	2	26	0	4	58	9	29	0	6	55	12	27	1	9	60	12	19
Nickel	1	6	27	3	64	3	9	46	10	32	4	22	29	14	32	2	6	31	22	39
Selenium	7	8	34	13	38	3	7	41	11	38	3	10	33	19	35	2	7	35	40	16
Thallium	9	9	43	17	22	7	15	37	22	19	9	17	22	31	22	6	12	30	22	30
Vanadium	1	1	42	4	53	1	2	53	12	32	1	2	54	14	30	1	2	47	17	32
Zinc	0	3	19	1	77	1	1	27	5	67	1	5	35	7	52	0	5	38	6	50

F1. Exchangeable fraction. F2. Carbonates - bound fraction. F3. Fe – Mn oxides – bound fraction. F4. Organic fraction. F5. Residual fraction.

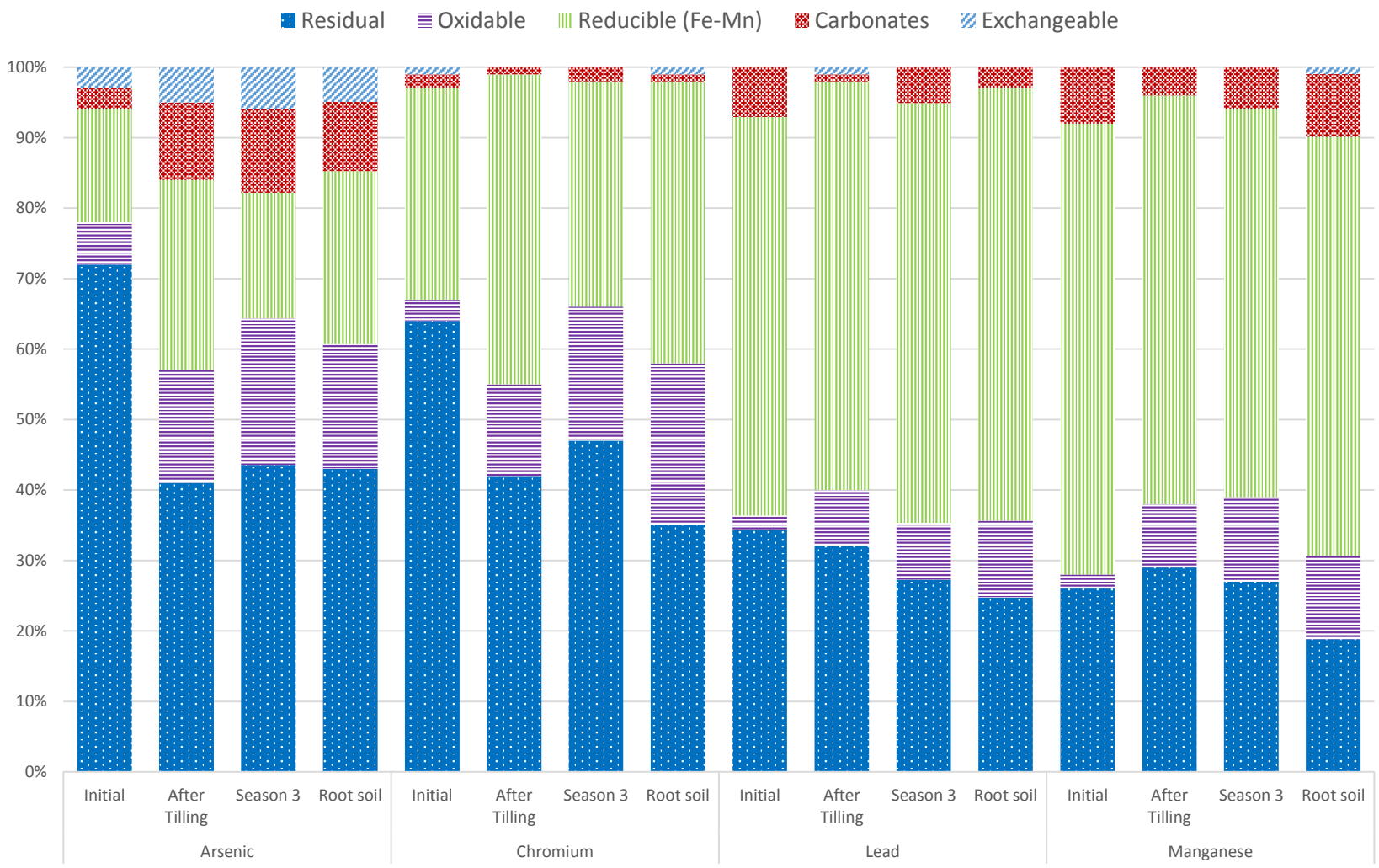


Figure 3.7. Metal distribution comparison between soil before and after tilling, at the end of the third season and root soil at FIB plot

The percentages of the metal fractionation in the soil at the plot of FIB are shown in Table 3.6, and the fractionation of the target heavy metals are plotted in Figure 3.7. Two different tendencies are observed in the fractionation of the target heavy metals. On one hand, As and Cr, reduce dramatically their residual fraction after tilling the soil, and the metals tend to be retained in the organic and reducible fraction. The more bioavailable fractions (exchangeable and carbonates) also tend to increase, although the proportion of contaminant uptaken by the plant was very small (Table 3.7). The highest percentage of Pb and Mn, on the other hand, tends to remain retained in the fraction bounded to Fe and Mn oxides (reducible fraction). The significant presence of these metals in the plants suggests the existence of some sort of Fe and Mn oxides assimilation, causing that heavy metals also retained in this fraction become part of the vegetative tissue. However, despite the presence of Pb and Mn, no signs of toxicity were shown in FIB.

The results obtained in the present study show a low mobility of heavy metals in the soil during the experiment, possibly due to the high soil pH, its retention in the solid phase and the reduced presence of the bioavailable fraction. However, the presence of Mn and Pb in the roots indicates that there is contaminant uptake by FIB.

The chemical form of a metal is determined by the biological availability and chemical reactivity in the soil–water environment, such as sorption-desorption and precipitation-dissolution and so on. The mobility of heavy metals in the soil can be affected by the pH on the aqueous phase present in the soil, as well as local equilibriums but also by kinetic limitations (Tack and Verloo, 1995; Villén – Guzmán et al., 2015). Among the variables that control the solubility and therefore the mobility of these inorganic elements in the soil, pH is the most important one, as it controls the solubility of metal hydroxides, carbonates and phosphates (Clemente et al., 2003; Carrillo – González et al., 2006). Other variables can also affect the transformation rate of heavy metals, such as the soil moisture regime

Table 3.7. Contaminant uptake

contaminant	mass of contaminant (mg)		% contaminant uptake	
	roots	leaves	roots	leaves
BaP	0	0	13	0
As	0	0	0	0
Cr	1.8	0	3	0
Pb	3.5	0	7	0
Mn	167	25	2	15

(Zheng and Hang 2011; Li et al., 2015). The latter study found that when both factors, high pH and wetting – drying cycles are combined, the available fraction of metals decrease. According to that, the moisture cycles along with the high pH of the soil in the study area, could be the main reason to explain the low mobilization of metals.

The results of this study show that Pb and Mn, with greater presence in the root of the plant (10 mg/Kg and 480 mg/Kg, respectively) are those with a larger fraction retained in the iron and manganese oxides (Figure 3.7). Both, Fe and Mn are two essential micronutrient for the development of the plant, but its bioavailability is subjected to the chemical conditions in the environment. Thus, an oxidizing atmosphere and alkaline pH, maintain these elements forming insoluble oxides, difficult to assimilate by the plant. In soils prone to flooding as in the present study, the reduction of these oxides with subsequent solubility of Fe and Mn is favored (Hong et al., 2010). Microbial activity, is another key factor to the transformation of these oxides, because when there is oxygen deficiency in the environment, changes in the redox potential occur, and NO_3^-, Mn and Fe serve as alternative electron acceptors for microbial respiration, being transformed to their reduced ionic species. Therefore, this process can also increase the solubility and availability of Mn and Fe (Rengel, 2000).

Although some amount of Mn, followed by Pb are uptaken by the plant, no signs of toxicity were found in FIB at the end of the experiment. Symptoms associated with toxicity caused by excess of Mn in the plant include chlorosis and necrotic lesions in older leaves, dark - brown or red necrotic spots, dry tips on the leaves and stunted roots (Kabata – Pendias and Pendias, 2001). However, terminal samples of FIB did not show any of these symptoms, indicating low presence of toxicity. Plants have homeostatic mechanisms to avoid getting intoxicated with an excess of nutrients. The limited presence of heavy metals in the above-ground plant tissue, could indicate the existence of some mechanism whereby the plant assimilates the metal but it remains retained in the roots, without allowing it to affect the rest

of the growing tissue. Numerous mechanisms can protect plants from toxicity caused by the presence of high concentrations of heavy metals, such as vacuolar sequestration (Maestri et al., 2010), detoxification in the aerial parts (Rascio and Navari – Izzo, 2011) or the presence of metal – binding ligands in the plant cells, known as metallothioneins and phytochelatins (Rea, 2012). While the presence of heavy metals does not affect plant development in the present study, it could affect the development and activity of the Nitrogen–fixation–bacteria. Microbial activity associated to Nitrogen–fixation is a parameter frequently used to monitor heavy metal pollution (Giller et al., 1989, Lorenz et al., 1992, Brookes, 1995). In the present study, nonetheless, microbial activity associated to Nitrogen–fixation was not monitored. However, the high density of nodulation in the sampled roots, and the exchangeable nitrogen levels in the rootzone soil (Table 3.3) indicate no evidence of inhibition of activity caused by the presence of heavy metals.

The plants of the family Fabaceae, have been documented as accumulators of heavy metals (Piechalak et al., 2002). Many Fabaceae species are good for phytoremediants of heavy metal pollution. The advantage of this group is their self – sufficiency in terms of nitrogen supply, and their favorable level of tolerance to drought. *A. fruticosa,* the specie subject of this study, is an ornamental tree widely cultivated in urban areas and well known by their ability to absorb Pb (Gawronsky and Gawronska, 2007). However, the results in the present study do not agree to that, since Pb concentrations found in FIB were very small as compared to the concentration of this metal in soil (Table 3.9).

The results of the present study are consistent with those obtained in the work of Shi et al. (2011), in which a total of 6 species were planted in alkaline mine tailings with high content of Pb, Cu and Zn. *A. fruticosa* was the only that thrived without being affected by heavy metal toxicity, and the concentration of Pb in the root (4.11 mg/Kg) was much lower than in the above

ground tissue (1.23 mg/Kg) of the plant sampled in the Pb contaminated soil, showing a low translocation index and bioaccumulation of this element.

Other studies (Seo et al., 2008, Zhao et al., 2014) also concluded that the heavy metals uptake in the root is much higher than in the aerial vegetative tissue. However, these results show Pb uptake concentration values much higher than in the present study, probably due to a higher presence of contaminants in the soil.

3.3.6. *Root Soil Characterization*

Results of the soil characterization outside and inside the root – zone can be found in Table 3.3 (Season 3 and Root Soil columns, respectively). As it can be observed, after 3 growing seasons, the pH of the experimental subplot decreased in both, the bulk soil and the root zone soil, with no significant differences between them. This decrease of pH in the soil could be mainly due to the weatherization of the surface, caused by the moisture – drying cycles the area of study was exposed. As it was expected, organic content in the root – zone soil at the surviving FIB subplots is 100% higher than the percentage of organic carbon in the bulk soil. This is due to the presence of humic acids, roots exudates and biological activity that takes place in the root system. The extraordinary low value of moisture content of the root – soil compared to the bulk soil is due to the soil from the root zone was collected after the samples were oven – dried. As expected, the Nitrogen content at the bulk soil at the end of the experiment was higher than what had been found before and after tilling, but lower that the Nitrogen content in the root – zone soil. According to what was expected, the higher presence of organic matter, along with the presence of Nitrogen fixing symbiont mycorrhiza in the root system, are likely the main reason of this increase of exchangeable nitrogen in the soil. The presence of Phosphate, however, did not increase significantly throughout the experiment, indicating a low availability of this nutrient in the soil subject of study.

3.3.7. *Practical Implications*

The harsh conditions of the experimental area were brought to light when the ground was tilled. The effect of the high pH slag layer could have jeopardized the success of the plants. It would be advisable, accordingly with the results obtained from this study, either to homogenize and mix the top soil layer, without mixing the deeper soil and or fill materials, or neutralize soil pH before planting. Furthermore, in order to improve the success of the phytoremediation technique applied in situ, it would be important to study and evaluate the soil conditions and contaminant concentrations in the soil after tilling and homogenizing, to complement the first survey of the site characteristics, since important changes that could affect plants performance can be detected.

Due to the low survival rate performed by all the species except FIB, it would be recommendable to establish the necessary conditions for a better development and growth of the selected species, by amending the soil in order to buffer the toxicity of the heavy metals and provide nutrients to the plants. Therefore, the addition of compost amendment in the experimental area is highly recommended.

On the other hand, the high tolerance to metal concentration of FIB, makes it ideal to use for phytoremediation of wetland sites with similar characteristics. In addition to that, the use of legumes in the area of concern is highly recommended, due to their capacity to survive and their resistance to heavy metal toxicity.

The presence of Pb and Cr in roots, and the presence of Mn in roots and stems/leaves of FIB could be of concern. The correlation between the heavy metal concentration in the soil and the capacity of FIB to accumulate heavy metals should be studied. Furthermore, a better investigation of the fate of these contaminants would be recommended.

Big Marsh is representative of many other unrestored wetland sites in the region which have been significantly altered by the steel industry. Many other sites in the Calumet area and

the Grand Calumet Area of Concern have similar conditions to Big Marsh and this project will be immensely valuable in evaluating the potential for using native plants to remediate other wetland sites.

3.4. Conclusions

Field investigation revealed only 1 of the 9 selected native plant species survived in the area of study during the three growing seasons. FIB (*A. fruticosa*) is the only species that showed higher tolerance to the harsh conditions of the site. The ability of this species to survive is attributed to its ability to fix atmospheric nitrogen.

The degradation of Benzo(a)Pyrene by the surviving species was not observed, nor was assimilated by the plant.

The presence of FIB did not affect the mobility and speciation of heavy metals in the soil. Only significant decrease of initial Mn occurred. Mn was also detected in roots and shoots of FIB, indicating that there exist assimilation of this element by the plant. On the other hand, the presence of Pb and Cr in the roots and its absence in the aerial tissues of FIB, indicated that these elements were uptaken by the plant, but were not translocated to the rest of the plant. The adaptability and survival of FIB and its high tolerance to toxicity demonstrated the potential of this species for its use in the remediation of the study area.

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CHAPTER 4

FIELD-SCALE PHYTOREMEDIATION OF MIXED CONTAMINANTS IN UPLAND AREA AT BIG MARSH SITE, CHICAGO, USA

4.1. Introduction

Throughout the United States and internationally, wetlands are important resources that, despite focused efforts, have been steadily disappearing. Wetlands serve as habitats for threatened and endangered species and are enormous sinks for carbon, and provide crucial environmental functions including cleaning and detoxifying water and mitigating floods. In northwestern Indiana and northeastern Illinois, the Lake Calumet region contains some of the richest of the remaining wetlands. Because of the heavy industrial presence in the region, a high fraction of these wetlands have been degraded. Many of the wetland sediments are contaminated, some of the upland areas are barren due to plant toxicity, and concern exists that surface and groundwater in the area are being negatively impacted by residual contaminants.

Big Marsh is one of the largest expanses of wetland within the Calumet region. The site covers approximately 121 hectares within the Great Lakes Basin. The northern part of the site contains upland habitat areas which were created largely with foundry slag. The area of study is located in the North West of Big Marsh, and its soil and sediments are mixed with slag and construction and demolition debris. Bottom sediments are natural muck soil that has not been dredged. Some low vegetation is also found in the upland area, together with some eastern cottonwood trees (*Populus deltoids*).

This site is representative of many other unrestored wetland sites in this region which have been significantly altered by the steel industry and decades of legal and illegal dumping. The wetland has been massively altered from original conditions by industrial filling and these fill materials as well as the groundwater and surface water have been found to be contaminated

with polyaromatic hydrocarbons, benzene, toluene, ethylbenzene, and xylenes, organic solvents, polychlorinated biphenyls, and heavy metals. Therefore, the wetlands at Big Marsh are greatly in need of restoration efforts.

Sites with mixed contamination pose technical challenges associated with the presence of various classes of contaminants with different physicochemical properties, because they will respond in a different way to the remediation technologies. Several technologies for the remediation of contaminated soils have been developed over the past three decades. Their applicability is often limited to a particular kind of contaminant. In the case of contaminated sites with mixed contamination, few technologies have proven to be efficient, but they also have important limitations, plus their application at field scale can be expensive. In this context, phytoremediation has potential to be a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al., 2013).

A previous study showed that the mixed contamination in the soil had a significant effect on the plant growth (Chirakkara and Reddy, 2014). The ability of the plants to survive in high impacted areas and the low bioavailability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency. The present work investigates the use of phytoremediation in an upland area at Big Marsh, a wetland in southeast Chicago (Illinois, USA), contaminated with PAHs and Heavy Metals. This study includes planting, monitoring, subsequent analysis to evaluate the plant survival and growth and contaminant uptake, with the aim of evaluating the plants species for phytoremediation feasibility of the site.

4.2. Research Methodology

4.2.1. *Initial Soil Characterization*

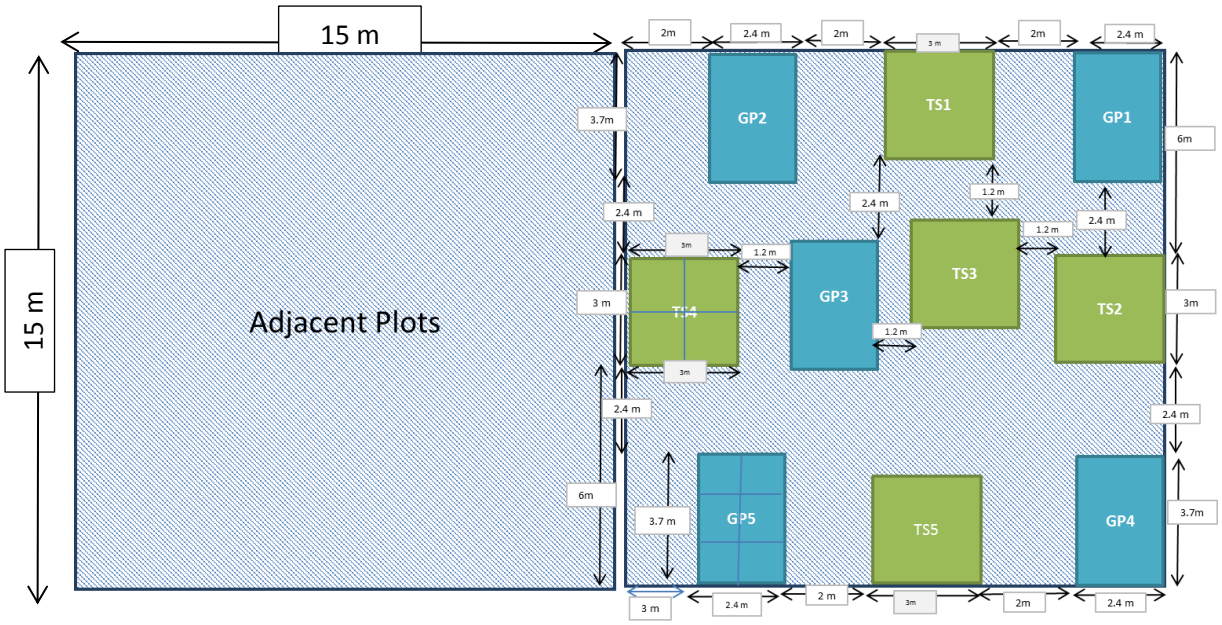
A delineation survey was conducted to determine the extent and boundary of the area of concern at Big Marsh. An initial baseline sampling was conducted to identify the existing heavy metal and organic contaminants present in the soil. Five composite samples were taken along transects representing roughly equivalent conditions at the area of study. Sampling locations were recorded using a GPS.

In order to get a better understanding of pH distribution in the soil, additional soil samples at different locations and depths were collected during on the second growing season at the experimental area.

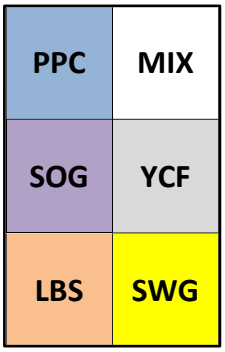
4.2.2. *Test Section Preparation*

An experimental and adjacent plots of size 15m x 15m each, were demarcated in the area of study, based on the preliminary initial baseline sampling (Figure 4.1a). The soil was tilled and homogenized to approximate 1m depth.

Two different types of subplots were designed at the experimental plot, GP and TS, to name the parcels intended for planting herbaceous plants (grasses and plugs) and woody plants (trees and shrubs), respectively. A total of 5 GP subplots and 5 TS subplots were selected. Each GP subplot, 2.4m x 3.7m, was divided into 6 groups of parcels 4ft x 4ft, and each parcel was divided into 16 cells 0.3m x 0.3m (Figure 4.1b). Analogously, each TS plot, 3m x 3m, was divided into 4 groups, 1.5m x 1.5m each (Figure 4.1c). Next to the experimental area, the adjacent plot was delineated in order to monitor the survival and growth of the herbaceous species. Pictures of the experimental area before, during and after soil preparation and planting are shown in Figure 4.2.

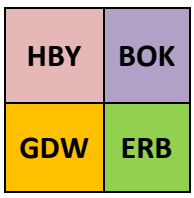


a. Overview of Plot Layout



1	2	3	4	1	2	3	
4	5	7	8	4	5	6	
9	10	11	12	7	8	9	
13	14	15	16	10	11	12	
				13	14	15	
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16

b. Grass and plugs (GS) subplots planting layout



1	2	1	2
3	4	3	4
1	2	1	2
3	4	3	4

c. Trees and Shrubs (TS) subplots planting layout

Figure 4.1. Plots and subplots delineation layout

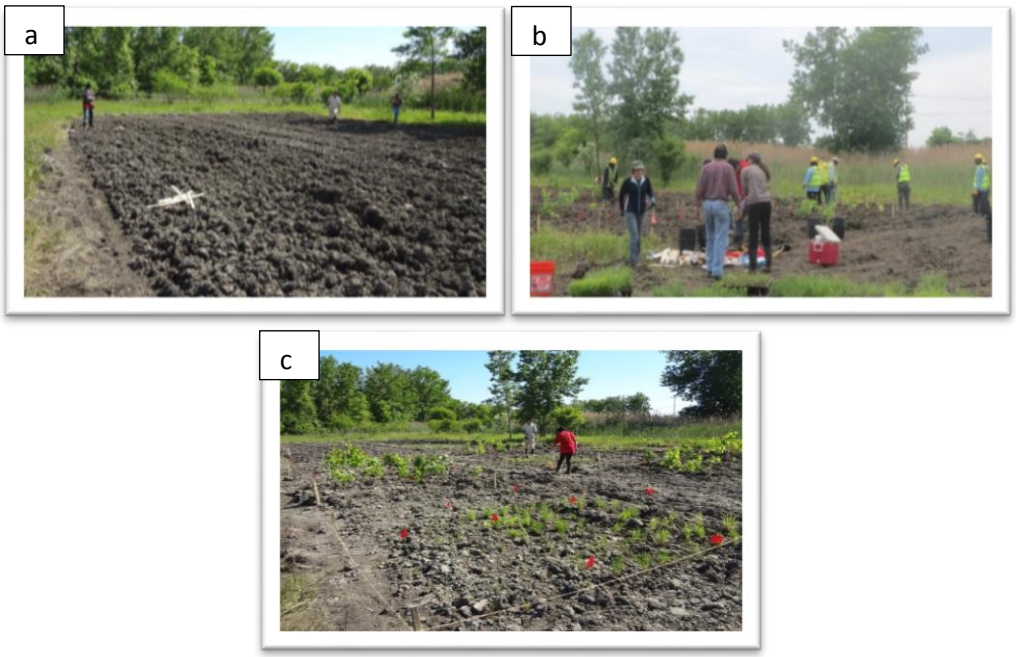


Figure 4.2: Planting at Experimental Area. (a) After tilling. (b) Trees and shrubs planting (c). After planting

4.2.3. *Plant Selection and Planting*

Based on the potential phytoremedial properties and the soil characteristics, a total of 5 species of grass and plugs and 4 species of trees and shrubs were selected.

The plants were planted by species at each subgroup of the GS and TS subplot. One of the remaining groups at each GS subplots was intended to plant 3 samples of each species up to a total of 15 samples. A total of 116 GS samples were planted, 20 of them were established in the adjacent plot. At the TS experimental plot, a total of 20 woody plants were planted. No woody samples were planted in the adjacent plot. The species selected are summarized in Table 4.1.

4.2.4. *Watering and Monitoring*

The experimental area was watered twice a week throughout summer months (June to August) and monitored weekly for survival, leaves, pests and infection, and height of the woody plants during the first growing season. At the adjacent plot, only survival monitoring was performed. The rating system used to assess plant health is shown in Table 4.2.

During the second growing season, the test plots were monitored bi-weekly during the summer. No additional water or pest control was performed at the experimental area, in order to let the plants grow under normal conditions and assess the suitability of the plants to cope with the natural site conditions. Monitoring and survival of two representative species of the study, Little Bluestem (GS) and Eastem Red Bud (TS), throughout the experiment are shown in Figures 4.3 and 4.4, respectively.

Table 4.1. Species selected for restoration of the upland area

Type	Scientific Name	Common Name	Sample ID	Number of samples	
				Experimental Plot	Adjacent Plot
Grasses and Plugs	*Andropogon scoparius*	Little Bluestem	LBS	96	50
	Bouteloua curtipendula	Side Oats Grama	SOG	96	50
	Dalea purpurea	Purple Prairie Clover	PPC	96	50
	Panicum virgatum	Switch Grass	SWG	96	50
	Ratibida pinnata	Yellow Coneflower	YCF	96	50
Trees	*Celtis occidentalis*	Hackberry	HBY	20	0
	Quercus velutina	Black Oak	BOK	20	0
Shrubs	*Cornus racemose*	Gray Dogwood	GDW	20	0
	Circis canadensis	Eastern Redbud	ERB	20	0

Table 4.2. Monitoring rating system

Parameter	Measurement
Survival (S)	Scale 1-4 (1 =dead; 2 =dying; 3 =no change in growth; 4 =evidence of new growth)
Leaves (L)	Scale 1-4 (1 = >50% leaves are dead; 2 = >25% leaves are dead, discoloration and/or wilting is present; 3 = <25% of leaves are discolored and/or wilting with no dead or dying leaves present; 4 = No discoloration, wilting or dead/dying leaves.)

a. After planting **b. 1 week** **c. 1 month**

d. End of the 1st season **e. End of the 2nd season** **f. End of the 3rd season**

Figure 4.3. Growing monitoring pictures of Little Bluestem at the adjacent plot

a. 1 week **b. 1 month** **c. 2 months**

d. End of the 1st season **e. End of the 2nd season**

Figure 4.4. Growing monitoring pictures of Eastem Red Bud at the experimental plot TS1

4.2.5. *Termination Sampling*

Two sets of termination sampling were performed at the end of the second and third growing seasons. Composite soil samples and vegetative biomass, divided into above ground (leaves and shoots) and belowground (roots), were collected.

At the end of the second growing season, 2 soil samples and 4 vegetative biomass (above and below ground) were sampled at the GP subplots of the surviving species.

At the end of the third growing season, a terminal sampling was performed. Only Little Bluestem (LBS) was sampled as it was the only plant that survived. A total of 5 composite soils samples and 5 above and below ground biomass were collected at LBS subplots at the experimental area. No other soil or plant samples were collected at GP or TS plots due to heavy invasive weed growth and low survival of other plants (with the exception of LBS). Additionally, two grab samples from indigenous vegetation (*Asclepias sp.*) were also collected to assess any uptake by existing vegetation. Soil and vegetative samples were analyzed for target contaminants. Also, complete analysis of metals and PAHs was performed on selected soil and vegetative samples. Soil characterization tests were also performed on all soil samples in the lab.

4.2.6. *Soil and Plant Sample Tests*

The soil physicochemical characterization tests performed in the lab included measurements of pH, Electrical Conductivity (EC), Organic Carbon (OC), Oxidation – Reduction Potential (ORP), Water Holding Capacity (WHC), Grain Size Distribution (GSD), and Exchangeable Nutrients Content. Soil and vegetative samples were sent to STAT Analysis Corporation (Chicago, IL, USA) for sample acid digestion and analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS). Polynuclear Aromatic Hydrocarbons (EPA method SW8270C) were also tested by Gas Chromatography

Mass Spectrometry (GC/MS). The soil pH and ORP were determined according to the ASTM D4972 – 01 Standard Test Method for pH of Soils (ASTM 2007). The values were measured in the laboratory using an Orion Model 720-A pH/ISE meter. Water content values were measured in the laboratory according to ASTM D 2216 Standard Test method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass (ASTM 2005). Organic Carbon was determined using ASTM D 2974 Standard Test Method for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils (ASTM 2000). The Electrical Conductivity of the soil was measured in a 1:5 soil: water suspension, using a Fischer Scientific model TRACEABLETM conductivity meter. Grain Size Distribution was determined according to ASTM D 422-63 Standard Test Method for Particle Analysis of Soils (ASTM 2002). To analyze exchangeable nitrogen, 1 g soil was shaken with 10 mL of 2M KCl solution for 1 h (Xu et al., 2013). The filtered extractant was analyzed using Spectronic Genesys Spectrophotometer, following the procedure given by Sattayatewa et al. (2011). To determine the exchangeable fractions of phosphorus, 1 g soil was shaken with 1 M ammonium acetate for 1 h. The solution was filtered, and the extractant was analyzed with Spectronic Genesys spectrophotometer, as per the procedure given by Sattayatewa et al. (2011). The Water Holding Capacity (WHC) of the soil was determined following the ASTM D2980 – 04 Standard Test Method for Volume Mass, Moisture – Holding Capacity and Porosity of Saturated Peat Materials (ASTM 2010).

Soil and vegetative samples were sent to STAT Analysis Corporation (Chicago, IL, USA) for sample acid digestion and analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS). Polynuclear Aromatic Hydrocarbons (EPA method SW8270C) were also tested by Gas Chromatography Mass Spectrometry (GC/MS).

Sequential Extraction analyses were performed using the procedure developed by Tessier et al. (1979) with slight modifications, to determine the speciation of the contaminants in the soils both before and after the phytoremediation technique was implemented. The information regarding the sequential extraction procedure can be found in Chapter 2. Samples from sequential extraction were sent to STAT Analysis Corporation (Chicago, IL, USA) for analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

The contaminant uptake by the plant was calculated as the percentage of contaminant in the plant with respect to the soil amount that was initially present in soil as follows:

$$\%contaminant\ plant = \frac{mg\ contaminant\ in\ the\ plant}{mg\ contaminant\ in\ the\ soil\ initially} \cdot 100$$

Mean and standard deviation of results were calculated using Microsoft Office Excel 2013. The t-test was performed with Microsoft Office Excel 2013 to check whether a significant difference exists between the result sets, the. The alpha value was taken as 0.05 for the t-test.

4.3. Results and Discussion

In this section, the results for soil characterization and contaminant concentrations in soil and vegetative tissue during the three growing seasons are presented and discussed.

4.3.1. *Initial Soil Characterization*

The results for the initial soil characterization are shown in Table 4.3. The physical properties tested to characterize the soil were pH, Oxidation - Reduction Potential (mV), Organic Content (%), Electrical Conductivity (mS/mL), Moisture Content (%), Grain Size Distribution (%), Water Holding Capacity (%), and Exchangeable Nutrients Concentration (mg/L).

Table 4.3. Soil characterization before, after and at the end of the third growing season

Soil Parameter	Initial Soil	After Tilling	Season 2	Season 3	Root Soil
pH	7.1	7.3	7.7	7.6	7.6
ORP (mV)	-22.69	-36.62	-54.4		-41.46
OC (%)	7.75	5.55	9.24		22
EC (mS/cm)	0.43	0.25	0.04		0.09
mc (%)	47.4	25.3	22.6	18	8.2
WHC (% total mass)	52	45	46		
Phosphate (mg/L)	0.16	0.16	0.08		0.14
Nitrate (mg/L)	9.41	4.83	3.04		3.67
%Gravel	3	3			
%Sand	67	78			
%Fines	30	19			

The average pH value of the surface soil at the beginning of this study was 7.1. The Oxidation – Reduction Potential (ORP) is an index of the exchange activity of electrons among elements in solution. The results show a negative potential, which indicates reducing conditions in the initial soil. The organic matter content found initially in the soil was 7.75%, the water holding capacity was 52%, the moisture content was 47%, and the nutrients concentration were 0.16 mg/L for Phosphate and 9.41 mg/L for Nitrate. The grain size distribution analysis results show that the soil is predominantly sandy to silty clay, with a low percentage of coarse grained fraction. Figure 4.6 shows the grain size distribution of the soil before, after tilling and at the end of the third growing season. As it can be observed, the distribution of the grain size in the soil was affected by the tilling and compost addition and the planting.

Table 4.4 shows the concentration (mg/Kg – dry soil) of different Polynuclear Aromatic Hydrocarbon (PAHs) compounds and metals that could be found initially in the soil at the experimental area. Benzo(a)pyrene, with a concentration in the initial soil of 1.8 mg/Kg –dry soil, was the target organic contaminant of this study due its known carcinogenic and mutagenic potential. The target contaminant BaP was analyzed from 7 composite soil samples, whereas the rest of PAHs were analyzed only in one sample.

Numerous heavy metal species were found in the initial soil. Among them, Arsenic (10 mg/kg – dry soil), Chromium (32 mg/Kg- dry soil), Lead (187 mg/Kg – dry soil) and Manganese (800 mg/kg – dry soil) were the target heavy metals analyzed. Concentrations of As and Pb were initially analyzed from 6 composite samples, whereas the rest of metals, included Cr and Mn were measured only in one sample.

4.3.2. *Soil Characterization after Tilling*

The results of the soil characterization after tilling are shown in Table 4.3. The soil pH after the treatment did not show significant difference as compared with the value obtained for the

Table 4.4. Contaminant concentrations in soil

Contaminant concentration (mg/Kg – dry soil)				
Contaminant	**Initial Soil**	**After Tilling**	**Season 2**	**Season 3**
PAHs				
Acenaphthene	0.1	0.5	0.1	
Acenaphthylene	<DL(0.04)	0.17	<DL(0.04)	
Anthracene	0.4	1.3	0.4	
Benz(a)anthracene	1	3	1	
Benzo(a)pyrene[a]	1.8	2.7	1.3	5.3
Benzo(b)fluoranthene	1	3	1	
Benzo(g,h,i)perylene	0.6	1.7	0.5	
Benzo(k)fluoranthene	0.7	2.0	0.7	
Chrysene	1	3	1	
Dibenz(a,h)anthracene	0.3	0.8	0.3	
Fluoranthene	2.4	7.9	2.3	
Fluorene	0.1	0.6	0.1	
Indeno(1,2,3-cd)pyrene	0.5	1.6	0.5	
Naphthalene	<DL(0.04)	0.2	<DL(0.04)	
Phenanthrene	1.6	6.8	1.4	
Pyrene	2	7	2	
Metals				
Aluminum	13000	11900	12000	11400
Antimony	5	<DL(4)	3	<DL(2)
Arsenic[b]	10	9	10	10
Barium	110	106	97	120
Beryllium	<DL(1)	<DL(1)	<DL(1)	<DL(1)
Cadmium	<DL(1)	<DL(1)	<DL(1)	<DL(1)
Calcium	47000	51500	50000	49500
Chromium[c]	32	31	26	27
Cobalt	16	18	12	12
Copper	86	48	55	63
Iron	60000	28500	26000	19800
Lead[d]	187	143	163	158
Magnesium	25000	24500	26000	25500
Manganese[e]	800	700	513	402
Mercury	0.2	0.1	0.2	0.2
Nickel	44	42	33	35
Potassium	2900	3200	2300	2560
Selenium	<DL(2)	<DL(2)	<DL(2)	<DL(2)
Silver	<DL(2)	<DL(2)	<DL(2)	<DL(2)
Sodium	120	120	160	268
Thallium	<DL(2)	<DL(2)	<DL(2)	<DL(2)
Vanadium	34	31	24	28
Zinc	490	140	180	198

Target contaminant concentrations Average±SD (number of samples) initially, after tilling, at season 2 and season 3, respectively. :

a. BaP: 1.8±0.74 (7); 2.7±2.8 (7); 1.35±0.7 (4); 5.3±4.2 (5).
b. As: 10±3 (6); 9±0.6 (6); 10±0.9 (4); 10±1.4 (5).
c. Cr: 32 (1); 31±0.7 (2); 26±2.4 (4); 27±3 (11).
d. Pb: 187±64 (6); 142±49 (6); 163±15 (4); 158±23 (5).
e. Mn: 800 (1); 700±226 (2); 513±103 (4); 402±103 (5).

initial soil sampling. The organic carbon content of the soil after tilling was 5.55%, and the magnitude of the oxidation – reduction potential increased. The exchangeable Nitrate concentration decreased up to 51% after the tillage of the soil, while the exchangeable Phosphate remained the same. The results for water holding capacity were very similar before and after tillage. However, the grain size distribution results show an increase in the sand fraction, while gravel remained the same and the percentage of fines decreased after tilling.

The results for the special investigation of pH in soil performed *in situ* showed that the pH is uniform in the soil, being 7.3 in surface, inside and outside the experimental area, and 7.4 in depth.

The total PAHs concentrations of the soil after tilling are shown in Table 4.4. In general, there exist a high variability of contaminants in the study area. Concentration of Benzo(a)Pyrene (BaP) increases in the soil after tilling. This could be likely due to the existence of high concentrations of the organic contaminant underneath the top soil, or high concentration in certain areas, so that the concentration after tilling increases due to the mixing effect.

The concentration of heavy metals can also be found at Table 4. As it can be observed, no significant differences were found in the concentrations of the target heavy metals (As, Cr, Pb, Mn) in the soil after tilling ($p>0.05$). These results indicate that the concentrations remain constant at the surface and at depth, and the mixing effect has no significant impact on the concentrations of metals in the soil.

The fractionation of heavy metals in the soil before and after tilling are shown in Table 4.7, and the target heavy metals fractionation is compared in Figure 4.7. As it can be observed, no significant changes occur in As fractionation before and after tilling, with the exception of the exchangeable fraction, which was higher than the rest of the target contaminants, and which slightly increases in the soil after tilling. Overall, the exchangeable fraction of metals in the soil is very low (Table 7), and it remains the same after tilling. The oxidable fraction increases

in Cr, Pb and Mn after tilling, while the Fe – Mn oxides – bounded fraction tends to decrease. On the other hand, Carbonates – bound fraction increases only in Pb and Mn after tilling, while the residual fraction remains similar in all of them before and after the treatment.

4.3.3. *Plant Monitoring Results*

Figures 4.3 and 4.4 show the results of monitoring of plant species representative of the herbaceous and woody plants used in the present study, Little Bluestem (LBS) and Eastem Red Bud (ERB) respectively, throughout the experiment. At the end of the experiment (after the three growing seasons), only LBS out of the total 9 selected species survived.

The surviving percentages at the upland area based on monitoring results for the first and second growing season (Table 4.2) are shown in Figure 4.5. The plants survival and leaf quality assessment (Figure 4.5b) were performed only on woody species, during the first growing season, with the aim of carrying out a detailed monitoring of the development and growth of plants and their adaptation to the ground. All the species showed high survival rates during the first growing season, probably due to the comprehensive monitoring of the plants and continued irrigation. However, all the species experienced a sharp decrease by the end of the second growing season, with exception of SWG and LBS. The survival rates found in the adjacent plots were similar to those at the experimental plots. The presence of invasive species in the study area was also very low, as compared to the surroundings.

No monitoring was performed during the 3rd growing season. However, field observations made when the terminal sampling took place, revealed that, at the end of the experiment, invasive species as well as the indigenous Milkweed took over the experimental area, and only LBS out of the 9 species initially planted, survived in the contaminated study area. According to the USDA plants database, all of the species used in our study, had an optimum pH range from slightly acid to neutral (USDA), and the pH of the planted soil both

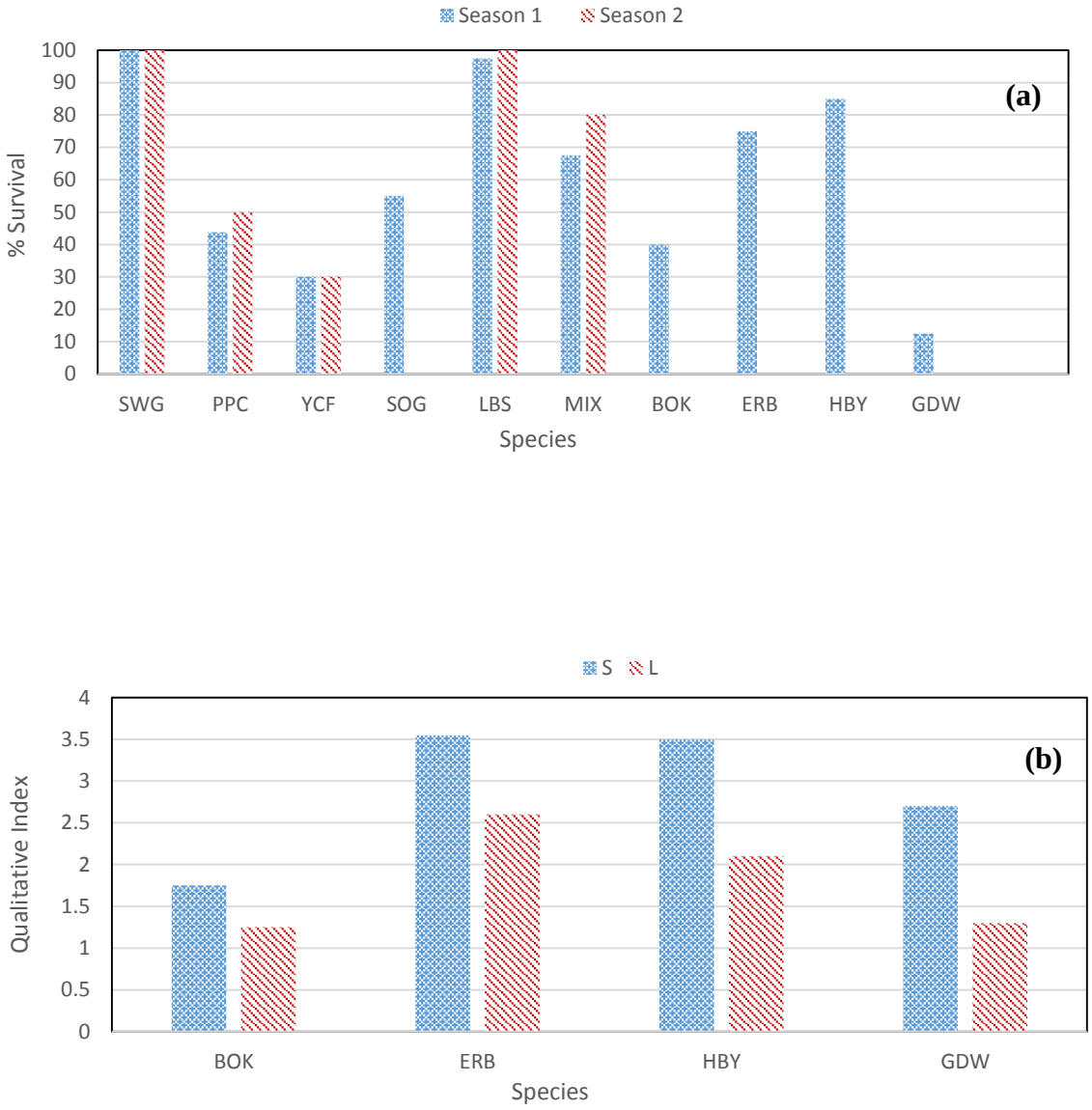


Figure 4.5. Monitoring Rating results. (a) Plant survival in the experimental plots at the end of the first and second growing season. (b) Plant survival and leaf quality in trees at the end of the first growing season

in the surface and at 64cm depth, was within the tolerance range. Therefore, the competition with other existing species might be the main reason for the poor performance of the native species. On the other hand, LBS showed high tolerance to the adverse conditions without showing any symptoms of toxicity, with healthy roots and abundance of living organisms in the rhizosphere. Therefore, in the present work, only LBS and its plot soil were studied in detail.

4.3.4. *Fate of PAHs*

Table 4.4 shows the results of PAHs contaminant concentrations in the soil at the end of second growing season. At the end of the third growing season, only BaP was measured in the planted soil. As it can be observed, the concentration levels of BaP in the experimental area do not decrease during the experiment. The high variability existing in the study area could explain the extraordinary high concentration of BaP found in soil at the end of the third growing season. Due to the lack of replicates analyzed, it was not possible to determine the existence of significant changes in the concentration of the rest of PAHs in the soil throughout the experiment. Unfortunately, only results of BaP concentration in soil are available at the end of the third growing season.

Table 4.5 shows the results for the PAHs contaminant concentration in the vegetative aerial tissue (stems and leaves). Results show that all PAHs concentrations were below the detection limit or very close, indicating the poor presence of these contaminants in the plant. Similar response occurs with the indigenous species Milkweed, with PAHs content in leaves and stems insignificant (results not shown). However, the results for PAH concentrations in the roots of LBS, found in Table 4.6, show a high concentration of these contaminants at the end of the third growing season. As it can be observed, the high spatial variability also affected the yield of BaP uptake by the roots. Overall, detectable concentrations of PAHs were found

Table 4.5. Contaminant concentration in Leaves and Stems of Little Bluestem

Contaminant	Concentration (mg/Kg) Season 2	Season 3
PAHs		
Acenaphthene	<DL(0.3)	<DL(0.03)
Acenaphthylene	<DL(0.3)	<DL(0.03)
Anthracene	<DL(0.3)	<DL(0.03)
Benz(a)anthracene	<DL(0.3)	<DL(0.03)
Benzo(a)pyrene	<DL(0.3)	<DL(0.03)
Benzo(b)fluoranthene	<DL(0.3)	<DL(0.03)
Benzo(g,h,i)perylene	<DL(0.3)	<DL(0.03)
Benzo(k)fluoranthene	<DL(0.3)	<DL(0.03)
Chrysene	<DL(0.3)	<DL(0.03)
Dibenz(a,h)anthracene	<DL(0.3)	<DL(0.03)
Fluoranthene	<DL(0.3)	<DL(0.03)
Fluorene	<DL(0.3)	0.03
Indeno(1,2,3-cd)pyrene	<DL(0.3)	<DL(0.03)
Naphthalene	<DL(0.3)	0.03
Phenanthrene	<DL(0.3)	0.04
Pyrene	<DL(0.3)	<DL(0.03)
Metals		
Aluminum	<DL(370)	<DL(370)
Antimony	<DL(3)	<DL(4)
Arsenic	<DL(2)	<DL(2)
Barium	10	22
Beryllium	<DL(1)	<DL(1)
Cadmium	<DL(1)	<DL(1)
Calcium	9500	8800
Chromium	<DL(2)	<DL(2)
Cobalt	<DL(2)	<DL(2)
Copper	<DL(5)	<DL(45)
Iron	190	156
Lead	<DL(1)	<DL(21)
Magnesium	1800	1940
Manganese	14	23
Mercury	<DL(0.02)	<DL(0.02)
Nickel	<DL(2)	<DL(2)
Potassium	4300	9500
Selenium	<DL(2)	<DL(2)
Silver	<DL(2)	<DL(2)
Sodium	<DL(110)	116
Thallium	<DL(2)	<DL(2)
Vanadium	<DL(2)	<DL(2)
Zinc	10	30

Table 4.6. Contaminant concentration in roots of Little Bluestem

Contaminant	Concentration (mg/Kg) Season 2	Season 3
PAHs		
Acenaphthene		0.6
Acenaphthylene		2.2
Anthracene		7
Benz(a)anthracene		10.3
Benzo(a)pyrene[a]	2.7	7
Benzo(b)fluoranthene		6
Benzo(g,h,i)perylene		11
Benzo(k)fluoranthene		8
Chrysene		10
Dibenz(a,h)anthracene		5
Fluoranthene		17
Fluorene		2
Indeno(1,2,3-cd)pyrene		7
Naphthalene		0.1
Phenanthrene		11
Pyrene		21
Metals		
Aluminum	6500	1722
Antimony	17	<DL(4)
Arsenic[b]	<DL(4)	<DL(2)
Barium	57	18
Beryllium	<DL(4)	<DL(1)
Cadmium	<DL(4)	<DL(1)
Calcium	21000	6340
Chromium[c]	10	4
Cobalt	<DL(8)	3
Copper	72	36
Iron	11000	3240
Lead[d]	51	21
Magnesium	8500	2252
Manganese[e]	173	93
Mercury	0	<DL(0.02)
Nickel	19	6
Potassium	4700	2630
Selenium	<DL(8)	<DL(2)
Silver	<DL(8)	<DL(2)
Sodium	500	158
Thallium	<DL(8)	<DL(2)
Vanadium	13	6
Zinc	100	38

Target contaminant concentrations Average±SD (number of samples) throughout the experiment:

a. BaP: 2.7±2 (3); 7±16 (5).
b. As: <DL(4)±2.6 (4); <DL(2)±0.2 (5).
c. Cr: 10±6 (4); 4±3 (5).
d. Pb: 51±14 (4) 21±12 (5).
e. Mn: 173±75 (4); 93±30 (5).

in the roots of LBS at the end of the experiment, indicating that the organic contaminants could be potentially accumulated in the roots of the surviving species. These results suggest that the organic contaminants are bioavailable in the soil and that there exists contaminant uptake in the plant. On the other hand, according to the PAHs concentrations in soil throughout the experiment (Table 4.4), there was no indication of dissipation nor degradation of these contaminants in the soil. However, the practically nonexistent presence of contaminants in the stems and leaves of LBS (Table 4.5) indicate the existence of some mechanism of metabolizing these pollutants performed by the plant itself.

Phytodegradation, also known as phytotransformation, consists of the uptake of organic contaminants from soil, sediments and water through the roots, and its transformation inside the plant. Once incorporated, these contaminants are transformed through various internal mechanisms and metabolic processes involving the breakage of these compounds for further storage in the vegetative tissue or elimination through transpiration (phytovolatilization) (Aisien et al., 2013). The potential of LBS in phytoremediation of PAHs contaminated soils was demonstrated by Aprill and Sims (1990). This study revealed that the presence of plants increased the percentage of dissipation of soil contaminants. However, these researchers did not study the individual phytoremediation capacity of different species used, and neither the amount of pollutant that could have been assimilated by the plants was measured. Pradhan et al. (1998) conducted a laboratory–scale experiment for 6 months, using soil from a gas manufacturing plant. Two types of PAHs contaminated soils were used, one of them was performed an initial pretreatment to reduce the initial concentration of PAHs. The results show that there is a reduction in both soils in the presence of LBS, finding no evidence of the presence of such contaminant in the aerial parts of the plants. The results of the present study are in agreement with those findings obtained by Pradhan et al. (1998) in terms of pollutant uptake.

However, the decrease of contaminant in the soil resulting from the treatment is not noticed in the present study due to, as already explained above, the high spatial variability.

4.3.5. *Fate of Heavy Metals*

Table 4.4 shows the heavy metal concentrations in the bulk soil at the LBS subplot. Only the analysis of the target contaminants, As, Cr, Pb and Mn was replicated enough to perform statistical analysis. Contrary to what is observed in PAHs concentrations, low spatial variability in terms of metal concentrations is observed. While there is some variability in the initial soil, this is decreased after the site preparation. As it can be observed in Table 4.4, while the concentrations of As, Cr and Pb remain constant throughout the three growing seasons, it does not happen the same with the concentration of Mn, which significantly decreases ($p<0.05$) towards the end of the experiment.

The metal concentrations in the aerial vegetative tissue of LBS (stems and leaves) is shown in Table 4.5. As it can be observed, from the target contaminants, only Mn concentration is detected in the plant, increasing up to 82% at the end of the third growing season, whereas the rest of the target contaminants remain below detection limits. In addition, it is also noticeable the concentration of Zn in the plant, its concentration seems to increase towards the end of the third growing season. Unfortunately, not enough samples were analyzed to perform statistical analysis of this metal.

Table 4.6 shows the concentration of metals in the roots of LBS. The presence of the target contaminants in the roots is noticeable, except for As, with concentration remaining below detection limits throughout the experiment. Different concentrations of heavy metals in the roots of the surviving LBS are detected in varying degrees according to the assimilated element: Mn (93±30 mg/Kg), Zn (38±13 mg/Kg), Pb (21±12.5 mg/Kg), Cr 4±0.76 mg/Kg). It is observed that the concentration of metals uptaken by the roots is directly related to the

concentration of the element in the soil (Table 4.4). In all cases, the presence of contaminant in the roots of LBS significantly decreases during the third growing season ($p<0.05$) as compared to the second growing season. This tendency is noticed in other metals than the target contaminants, with presence in the root of the plant also detected. On the other hand, the concentration of Mn and Zn in the aerial tissue of LBS tends to increase with time, indicating the existence of some mechanism of translocation. Results show that, while the absorption of Cr, Pb, Mn and Zn by the roots of LBS takes place, only Mn and Zn are assimilated. However, despite the presence of these contaminants in the plants, the percentage of contaminant uptaken is very low as compared to the concentration initially found in soil (Table 4.8).

Results of percentages of metal fractionation from sequential extraction are shown in Table 4.7, and the fractionation of the targeted heavy metals are plotted in Figure 4.7. In general, results in Table 4.7 suggest a very little mobilization of the contaminants in the soil, with a tendency to remain retained in the less available fractions of the soil. As it can be observed, the main changes are observed in the soil after tillage, while in the root zone soil the distribution of the metal retentions tends to be similar to what it was in the soil initially. Observing carefully the fractionations of the heavy metals (Figure 4.7), two different behaviors are noticed in terms of the retention distribution. The major percentage of retention of As and Cr is found in the residual fraction throughout the experiment, followed by the fraction bounded to Fe and Mn – oxides, carbonates, and exchangeable fraction. While a noticeable percentage of those metals is retained in the exchangeable fraction, the more easily extractable, the presence of these contaminants in the plant is very small in the case of Cr, and non-detectable in the case of As. Pb and Mn, on the other hand, show the higher percentage of retention in the fraction bounded to Fe – Mn – oxides, and even though the percentage of retention tends to slightly decrease, this fraction is predominant, followed by carbonates and residual, being the oxidable and exchangeable fractions the less representative. This tendency is also followed by

Table 4.7. Percentage of metal fractionation from sequential extraction at Little Bluestem plot soil

	Initial Soil					After Tilling					Root – Zone Soil				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Antimony	6	12	30	15	37	19	15	19	27	19	23	10	26	9	32
Arsenic	4	7	19	10	60	7	5	16	10	62	4	7	20	6	63
Barium	15	30	28	7	20	5	23	37	9	26	6	18	33	5	38
Beryllium	7	14	35	18	25	11	9	53	15	13	9	15	38	13	25
Cadmium	5	19	27	14	34	17	22	19	24	17	7	20	28	10	35
Calcium	5	29	57	6	2	3	31	58	6	3	11	25	57	4	3
Chromium	1	2	18	7	72	2	2	13	12	70	1	2	10	9	79
Cobalt	2	4	47	8	39	5	5	39	10	40	3	12	33	7	46
Copper	2	4	16	47	31	3	3	12	52	30	2	10	8	57	24
Lead	0	10	68	8	14	0	19	59	11	11	1	22	53	3	21
Manganese	0	9	67	6	17	0	16	50	14	19	1	25	48	3	23
Nickel	1	5	36	14	44	2	5	31	17	45	1	6	28	11	54
Selenium	6	12	31	16	34	14	11	28	20	27	8	13	32	11	37
Thallium	8	15	38	20	19	19	15	19	27	19	10	16	40	14	20
Vanadium	1	2	27	9	61	3	2	25	13	58	1	2	20	8	68
Zinc	1	9	54	6	31	2	7	53	7	30	1	13	54	4	28

F1. Exchangeable fraction. F2. Carbonates - bound fraction. F3. Fe – Mn oxides – bound fraction. F4. Organic fraction. F5. Residual fraction.

Table 4.8. Mass of contaminant uptake

contaminant	mass of contaminant (mg)		% contaminant uptake	
	roots	leaves	roots	leaves
BaP	0.8	0	15	0
As	0	0	0	0
Cr	0.4	0	1.5	0
Pb	2.4	0	1.5	0
Mn	10	0.1	2.6	1

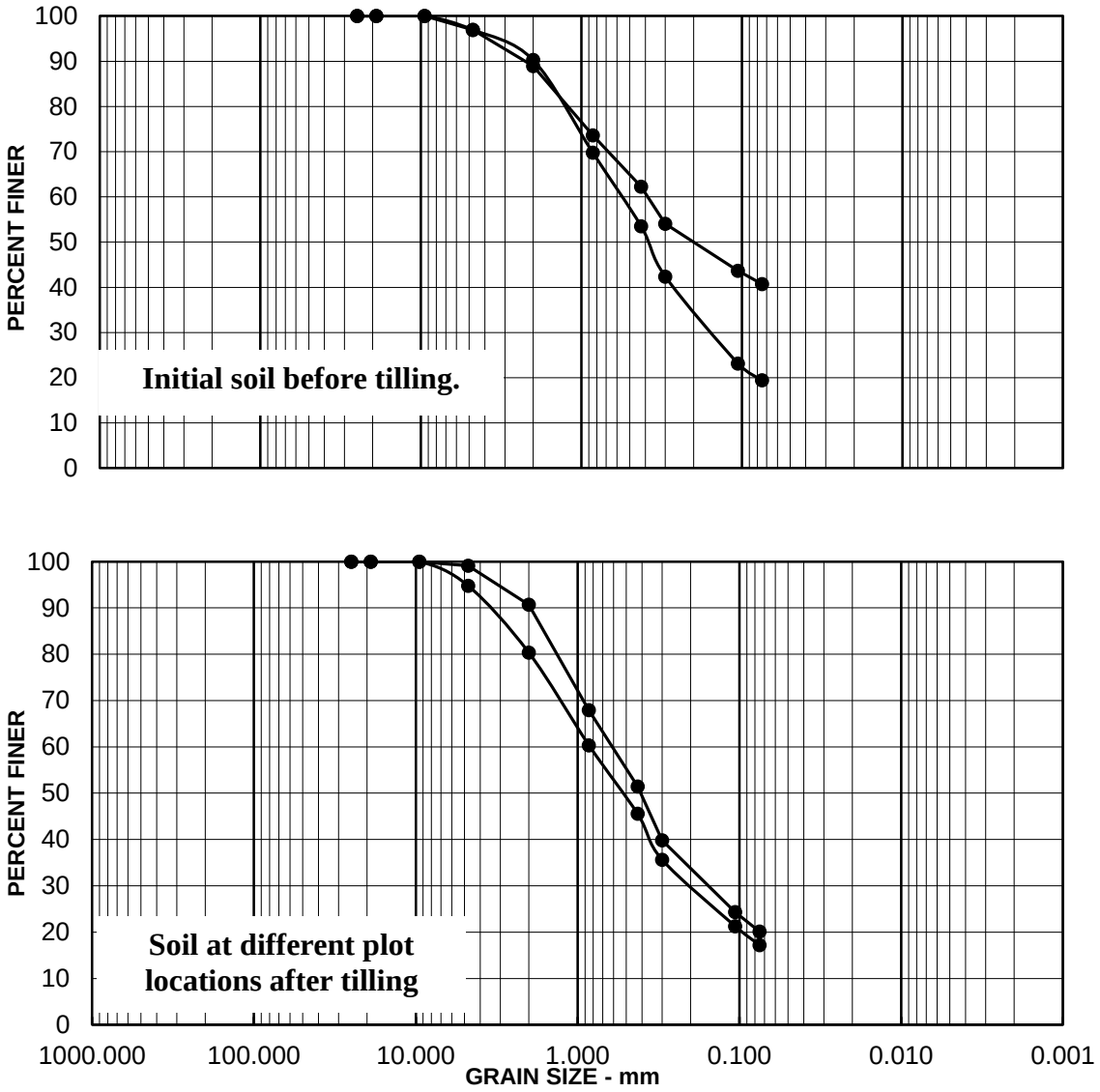


Figure 4.6. Grain Size Distribution of soil before and after tilling

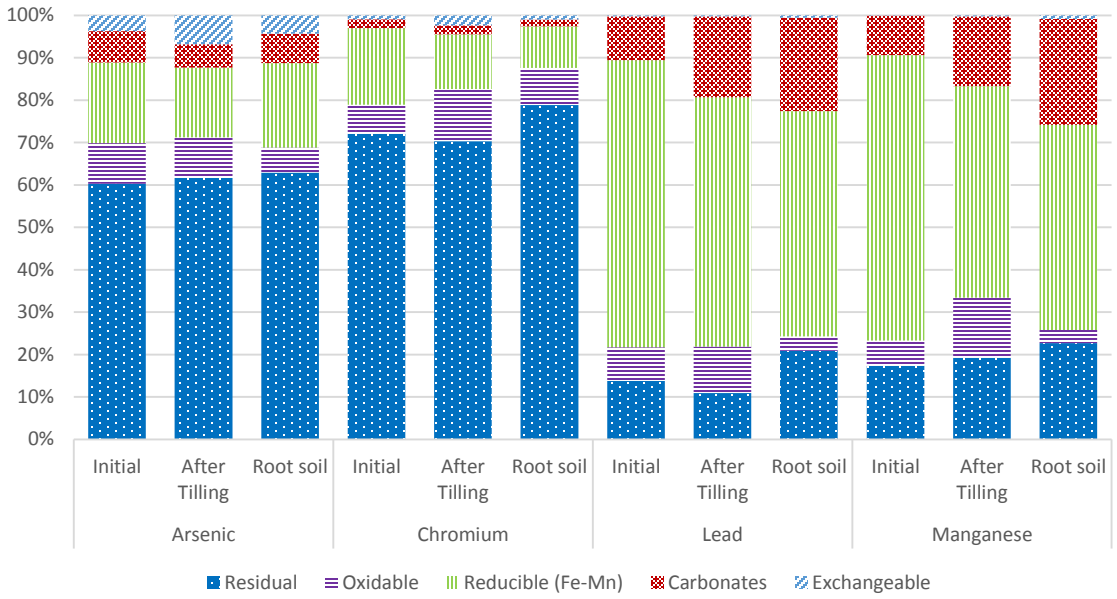


Figure 4.7. Heavy metals fractionation in soil before, after tilling and in root soil of LBS

Zn (Table 4.7), with presence in the plant also noticeable. The presence of these elements in the aerial tissue of LBS suggest the existence of some sort of Fe–Mn oxides assimilation mechanism in the plant, which could cause that the heavy metals retained in this fraction can also be uptaken. Despite the little change of the fractionation of heavy metals in the soil, results indicate that the uptake of some of these contaminants takes place due to the establishment of the plant.

The uptake of trace elements by the roots can occur in two ways: passive, which does not involve metabolic processes, or active, that do implies. While assimilation is due to the diffusion of ions from the external solution to the endodermis of the root, metabolic energy is required for the active absorption, which occurs against the chemical gradient. Although both mechanisms may occur, when the concentration in soil exceeds the physiological barrier, all elements are passively absorbed (Kabata-Pendias and Pendias, 2001). Results in the present work show undetectable concentration of As, Pb and Cr in the aerial vegetative tissue despite their presence in the roots, indicating that these contaminants are being immobilized in the root system. This could be due to the existence of certain defense mechanism performed by the plant to protect itself against toxicity produced by an excess of such contaminants. These defense mechanisms are usually related to root exudates and production of phyto–chelating molecules capable of immobilize metals, storing them in vacuoles or cell membranes, being the roots the most common storage for excess of metals (Tyler et al. 1989).

Tolerance of LBS to As has been studied by other researchers (Rocovich and West, 1975). In order to be absorbed, As needs to be bioavailable (Mirza et al., 2014). The chemical form commonly used of As by the plants is Arsenate, since Arsenite is unstable and tends to oxidize to Arsenate through biochemical processes in the soil (Mcnair and Cumbes, 1987). Arsenate is chemically analogous to Phosphate, and competes with it for absorption into the plant (Mehrag

and Macnair 1990). Therefore, since the sensitivity to the Arsenate is closely linked to the assimilation of phosphate, a greater presence of phosphate in the soil will result in lower absorption of Arsenate, due to the suppression of the phosphate-arsenate assimilation system (Meharg and Macnair, 1991, 1992). Meharg and Macnair (1990), observed that in the plants with more tolerance to the presence of As, the competence for Phosphate was lower. In their study, tolerant plants exposed to a high affinity phosphate range (between 0 and 0.01 mol m^{-3}), showed a higher affinity to the Phosphate uptake, being less affected by the presence of arsenate. The concentration of Phosphate at the study site is within the high affinity range (2 mmol $m^{-3)}$, which suggests that this mechanism likely takes also place in LBS. Nonetheless, the affinity of the plant for As in the present study should be further investigated.

Results of the present study indicate that only a small percentage of Chromium is uptaken by LBS, remaining in the roots of the plant, while the concentration in the aerial vegetative tissue of the plant is not detectable. Higher concentration of Cr in roots compared to shoots has been reported (Shahandeh and Hossner 2000). The toxicity of Cr depends on its oxidation state. The most bioavailable and soluble form of Cr is also the most toxic one (Cr VI). Smith et al. (1989) observed that once Cr is uptaken by the plant, it is generally retained in the roots, due to the existence of many linkage sites in the cell wall. The low assimilation rate of Cr by plants is related to the uptake mechanism existing in the roots. Apparently, radical tissues are not able to stimulating the reduction of Cr^{3+} to Cr^{2+}, soluble, which is the key process in Fe absorption and uptake by plants (Cary et al., 1977; Tiffin 1972).

The concentration of Pb found in the roots of LBS, was 21 mg/Kg at the end of the third growing season, being undetectable in stems and leaves. Results in the present study are in agreement with those obtained by Levy et al. (1999), in which concentration of Pb in a species

from the gender *Andropogon* was very low compared to the concentration of this element in the soil.

It has been observed that for the range of pH in between 7.2 and 7.8, the uptake of Zn is linked to the concentration of this element in the soil (Kabata–Pendias and Pendias, 1999), being often found in higher concentrations in the roots as compared to shoots, especially when this element is abundant in the soil (Kabata–Pendias and Pendias, 2001). When there is Zn in excess in the soil, this element can be translocated from the roots to be accumulated in the aerial vegetative tissues of the plant. The tolerance of *A. scoparius* to Zn has been studied by Ehinger and Parker (1979) where it was observed that in hydroponic cultures, the plants that had been collected in an urban site with high Zn concentration in the soil, were able to survive under higher concentrations of Zn in solution as compared to those that had been collected in an uncontaminated rural site. Vesicular arbuscular mycorrhizae, symbiont fungi known as a plant – growth stimulator commonly found in the root system of Big and Little Bluestem (Anderson et al., 1994) have been found to be involved in plant survival in Zn contaminated sites and in the uptake and retention of this metal in the roots (Shetty et al., 1994). On the other hand, concentration of Zn uptake by Big Bluestem observed at Levy et al. (1999), was found higher than in the present study, probably due to a higher concentration range of Zn in the soil.

Similarly to Zn, the presence of mycorrhiza increases the uptake yield of Mn, being the plants interconnected by the mycorrhiza network the ones which obtain higher concentration of this element, compared to those which grow in absence of the symbiont fungi (Weremijewicz and Janos 2013). Mn is essential to the plant growth, and it is also involved in the Nitrogen assimilation and participates in the chlorophyll synthesis together with Fe (Labanauskas, 1966). Paschke et al. (2005) observed that the toxicity levels produced by Mn depended on the type of plant. Thus, the

plants destined to human consumption were usually less tolerant to the presence of an excess of Mn than those species destined to land reclamation such as those from the genus *Andropogon* (Big Bluestem) tested in the study. Results of the present study are in agreement with those obtained by these researchers showing that, for Big Bluestem, the amount of Mn retained in the root was higher than that translocated to the shoots. Zn also plays an important role in the plant, since it is the essential component of many enzymes. The phytotoxicity produced by an excess of Zn is frequently reported, and its toxic effects are usually similar to those observed in other heavy metals. According to the literature (Kabata–Pendias and Pendias 2001), the normal levels of Zn and Mn in leaves are in between 27 – 150 and 30 – 300, respectively; the concentrations of these elements found in stems and leaves were within this range. The low toxicity of the metal concentration in the soil – root – plant system is also confirmed by the presence of numerous organisms found in leaves and stem as well as the root – zone soil of LBS when it was collected.

4.3.6. *Root soil characterization*

The results of Season 2, Season 3 and Root Soil in Table 4.3 correspond soil characterization results outside and inside the root – zone respectively. As it can be observed, no significant changes are found in the pH of the soil in any stage. Organic content, however, is nearly doubled in the soil during Season 2, likely due to the establishment of plants in the experimental area. As it was expected, the percentage of organic carbon in the root – zone soil increases nearly 150% as compared to the soil at Season 2. The abundant presence of living organisms in the root system of the surviving plants along with the presence of humic acids, microbial activity and root exudates, might be the main reason of this increase. The lower value of moisture content found in the root soil is due to that the soil from the root – system was collected after oven drying the samples. As

it can be observed in the Table 4.3, no significant changes are found in the Water Holding Capacity of the soil at Season 2, compared to the soil after tilling. Values of nutrient concentrations in the soil at Season 2 and in the root – soil do not present significant differences, and are lower than at the beginning of the experiment.

4.3.7. *Practical Implications*

The low survival of plants in the area of study highlights the importance of the selection of plants that can survive the harsh conditions of a contaminated site. Since the lack of nutrients could have been one of the main reasons that could have led the plants to perish, it would be highly recommended to provide the soil with the sufficient nutrients supply in form of soil amendments, so that the nutrients can be slowly released and be readily available to be uptaken by the plants.

The presence of other indigenous plants such as Milkweed, which took over the area of study at the end of the third growing season, could have affected the survival of the planted species due to a high competition for the nutrients available. Some of the species from the genus *Asclepias* such as Swamp Milkweed (*A. incarnata*) is a native wildflower widely distributed across the US and Canada that can be used as a wetland restoration. Some species of this gender are also the food plants of some butterflies such as monarch butterfly (*Danaus plexippus)*, playing an important role in the conservation of these species, as well as countless pollinating insects and birds that feed on its nectar (Kirk and Belt 2011). Due to the high rate of survival observed in Milkweed, it is recommended to study the species of the genus *Asclepia* present in the area of study and consider, if applicable, its potential use in the phytoremediation of areas with similar conditions.

The low survival of trees makes them appropriate to use once the necessary conditions for their development have been established by planting and growing herbaceous plants are achieved.

The high spatial variability of the organic contaminant concentrations in the soil, jeopardized the representability of the site conditions, masking valuable conclusions potentially obtained by the use of this technique. It would be highly recommended, therefore, to investigate the organic contaminant concentrations distribution in the soil, and to perform a sampling design according to this distribution, to account for the spatial variability effect in the results.

The results of the present study show high concentrations of BaP in the vegetative tissue of the surviving plant, Little Bluestem, revealing the existence of a high rate of contaminant uptake and accumulation of this contaminant in the roots. The low presence of contaminant in stems and leaves of BaP indicates the existence of degradation mechanisms in the plant. Therefore, it would be recommended to further investigate, to study the presence of byproducts and metabolites derived from BaP degradation that could be potentially toxic. Similarly the fate of other contaminants present in the study area should be further explored.

4.4. Conclusions

The following conclusions can be drawn from this study:

- Little Bluestem (*Andropogon scoparius*) was the only species that survived the three growing seasons, showing higher tolerance to the presence of contaminants.
- Despite the high spatial variability found in the organic contaminants distribution in the soil, results of contaminant concentrations in the roots of LBS show that a high rate of BaP uptake and accumulation/degradation takes place in the plant, with the presence of organic contaminants undetectable in stems and leaves.
- Heavy metals, contrary to the response of PAHs did not display a high spatial variability in the soil. However, the presence of plants did not affect the mobility and speciation of

these elements in the soil. While the presence of Cr, Pb, Mn and Zn was detectable in the roots of LBS, indicating contaminant uptake, the concentration of Cr and Pb was not detected in stems and leaves. Mn and Zn on the other hand, were detected in the aerial tissues of the plants, indicating the existence of uptake and assimilation.

- The survival of LBS and its high tolerance to the site conditions, make it a suitable species for the phytoremediation of upland areas in the Calumet region under the same characteristics. However, in order to increase the viability of the other species, the areas should be provided with available nutrients to achieve a higher yield of plants survival and suitability.

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CHAPTER 5

PHYTOREMEDIATION OF MIXED CONTAMINANTS UNDER VARIABLE SITE CONDITIONS: FIELD-SCALE INVESTIGATION AT IMPACTED BIG MARSH SITE

5.1. Introduction

Due to increased industrialization and overpopulation, numerous sites exist where soil and groundwater are contaminated, presenting health risks to humans and the environment. Remediation of these toxins is essential to protect public health and sustainable development (Chirakkara et al., 2016). Big Marsh is one of such sites, being the largest expanses of wetland within the Calumet region. The site, which is representative of many other unrestored wetland sites in this region that has been significantly altered by the steel industry and decades of legal and illegal dumping that has massively altered from original conditions by industrial filling.

Big Marsh site is located in the South East of Chicago, and covers approximately 121 hectares within the Great Lakes Basin. Approximately 35 hectares of the central portion of it is a wetland. The study site contains upland habitat areas, which were created largely with foundry slag. Nine hectares in the southeast corner of Big Marsh are composed of innocuous fill that contains a high percentage of iron, mainly blast furnace slag. Sixteen hectares of the southern filled section contain impenetrable slag and is devoid of vegetation. Only a few eastern cottonwood trees (*Populus deltoides*) and low herbaceous vegetation have managed to establish growth on the site. Surface waters in Big Marsh are less than one meter deep in most areas. Fill materials across the site range from 2 to 3 meters thick and consists of steel-mill slag, with some construction and demolition debris and dredge spoils from Lake Calumet and the Calumet River. Water quality is impacted by high pH levels; in some areas the pH reaches 12.6. Bottom sediments in the marsh

are natural muck soil that has not been dredged. The southeastern part of the site is covered with white calcite that leaches out of the slag from adjacent upland fill. These fill materials, as well as the soil and surface water have been found to be contaminated with both organic (polycyclic hydrocarbons) and inorganic contaminants (heavy metals). Therefore, the wetlands at Big Marsh are greatly in need of restoration efforts.

Sites with mixed contamination pose technical challenges associated with the presence of various classes of contaminants with different physicochemical properties, because they will respond in a different way to the remediation technologies. Several technologies for the remediation of contaminated soils have been developed over the past three decades. Their applicability is often limited to a particular kind of contaminant. In the case of contaminated sites with mixed contamination, few technologies have proven to be efficient, but they also have important limitations, plus their application for large field sites can be very expensive. In this context, phytoremediation has potential to be a benign, cost effective alternative for the treatment of contaminated sites with mixed contamination (Cameselle et al. 2013). The large area and the variable distribution of contamination throughout the shallow subsurface make Big Marsh uniquely suited for phytoremediation. This green and sustainable remedial option can be adopted to remediate soils with a mixture of organic and inorganic contaminants that can be removed by the plants through different mechanisms. Some mechanisms target certain types of contaminants over others, e.g. volatile compounds can be evapotranspired through the leaves and shoots of the plants (phytovolatilization). Several organic compounds can be completely degraded by the plant, while inorganic contaminants tend to be sequestered or accumulated within the plant. Many other sites in the Calumet area and the Grand Calumet Area of Concern have similar conditions as Big

Marsh; therefore, this project results will be immensely valuable in evaluating the potential for using native plants to remediate other wetland sites.

As it has been reported in a previous study (Chirakkara and Reddy, 2014), the mixed contamination in the soil has a significant effect on the plant growth. Therefore, the design of a field–scale phytoremediation application presents limitations related not only to the nature of the contaminants present in the soil, but also to the extension of this contamination, because it can inhibit plant growth and survival (Chirakkara and Reddy, 2015). Phytoremediation can be enhanced either by increasing the capability of contaminant uptake by the plant or amending the soil to increase the bioavailability of the contaminants.

The present work investigates the use of phytoremediation in a mixed contaminated site at Big Marsh, a wetland in southeast Chicago (Illinois, USA), contaminated with PAHs and Heavy Metals. This study, with an extension of three completed growing seasons, takes place in three different areas within the contaminated site (a slag disposal area, a wet meadow area, and an upland area). The present work includes the amending of one of the experimental areas with compost to enhance plant growth, planting, monitoring, subsequent analysis and assessment of the plant survival and growth, and contaminant uptake, with the aim of evaluating the performance of the plants species and phytoremediation feasibility of the site.

5.2. Research Methodology

5.2.1. *Initial Soil Characterization*

A delineation survey was conducted at Big Marsh in order to determine the extent and boundary of the three areas representative of different ecotypes present in the wetland. The specific areas identified for the investigation are: (1) Slag disposal area: slag – filled upland located at the East

side; (2) wet meadow: degraded wet meadow located at the Southeastern side; and (3) upland area: upland area near emergent wetland at the Northwestern side. Figure 5.1 shows the location of the study area at the experimental site.

The initial baseline sampling was conducted on the site in order to identify the existing heavy metal and organic contaminant present in the soil. Five composite samples were taken along transects representing roughly equivalent conditions at the upland and slag disposal area, and three composite samples were taken in clusters at the wet meadow. Sampling locations were recorded using a GPS. Additionally, soil pH was tested at different locations and depths in the three experimental areas, in order to study the pH distribution in the soil. Soil samples were collected to perform soil characterization and contaminant concentration analysis.

5.2.2. *Preparation of Test Section at Each Area*

The experimental areas were identified based on preliminary soil initial baseline sampling. An experimental and adjacent plot of size 15m x 15m each, were demarcated at each of the three areas representative of the three different ecotypes present at Big Marsh. Ground was prepared by tilling and homogenizing the soil. A thin layer of compost was incorporated to the soil only in the slag disposal area. The soil and fill material at the surface was tilled and homogenized to approximate depth of 1 meter.

At the experimental plot (15m x 15m), two different types of subplots were designed in order to establish herbaceous and woody plants. Those parcels intended for planting herbaceous plants were called GP (Grasses and Plugs) plots, and those plots used for planting trees and shrubs were called TS (Trees and Shrubs) plots (Figure 5.2a). A total number of 5 subplots, each 2.4m x 3.7m. were selected as GP plots. Each subplot was divided into 6 groups of size 1.2m x 1.2m, and

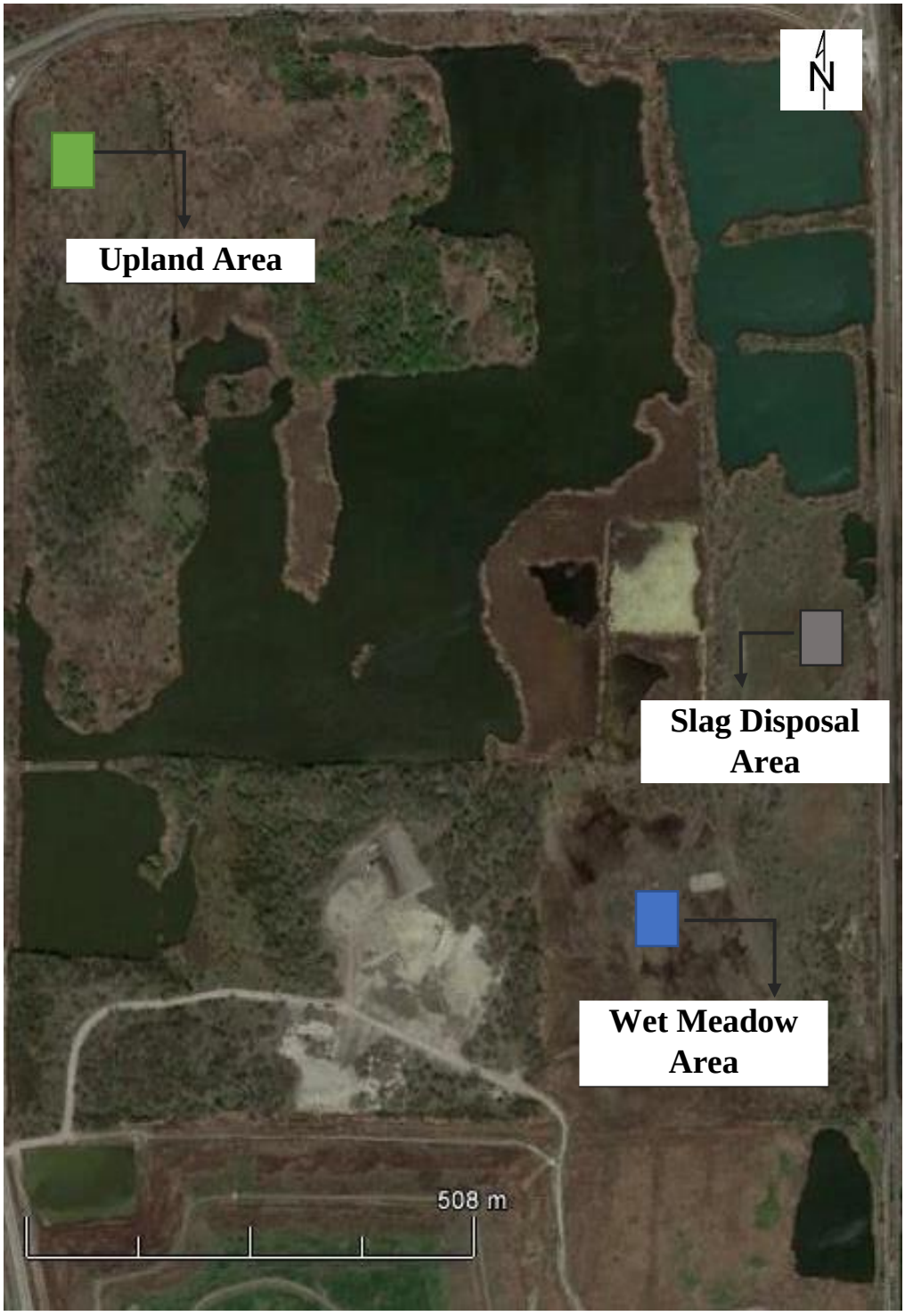


Figure 5.1. Study site map

each group was divided into 16 cells of size 0.3m x 0.3m (Figure 5.2b). Another 5 subplots of size 3m x 3m each, were selected as TS plots, and each subplot was divided into 4 groups, each 1.5m x 1.5m (Figure 5.2c).

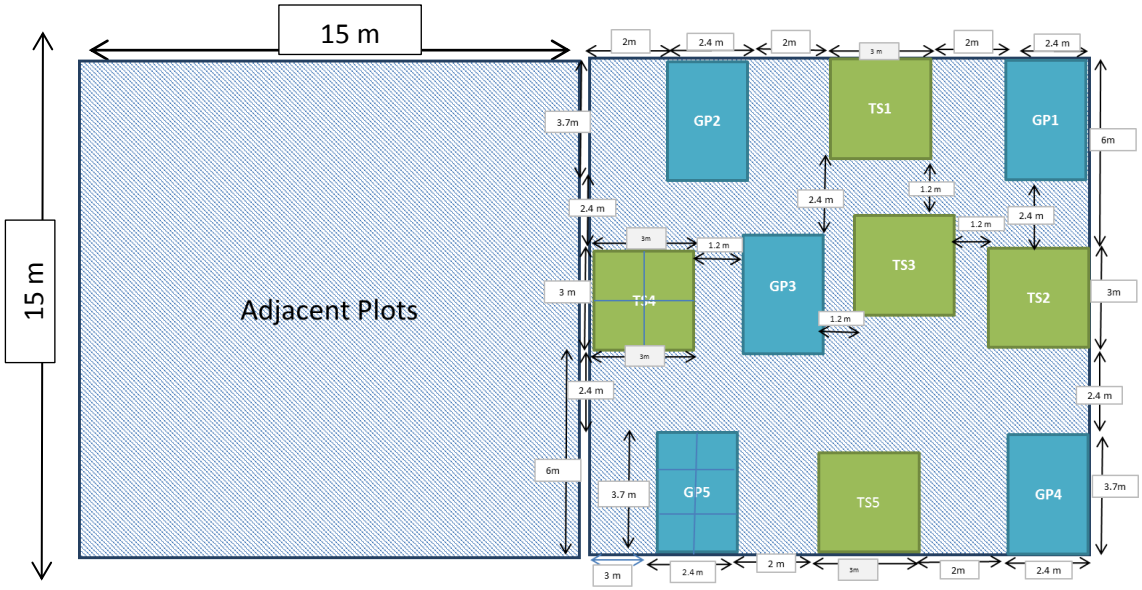
The adjacent plot (15m x 15m) was delineated next to the experimental plot with the purpose of monitoring plat survival and grow characteristics of the grass species. One composite soil sample from each group at each subplot of the experimental plot was collected for baseline contaminant concentration analysis and soil characterization.

5.2.3. *Plants Selection and Planting*

The selection of plants was based on the potential phytoremedial properties and the soil characteristics at each area. Five different species of grasses and plugs (herbaceous), 2 species of trees and 2 species of shrubs based on the area conditions were selected. Thus, the slag disposal and the upland area were planted with the same group of restoration plant species (Figure 5.3), whereas at the wet meadow area, a different group of 9 native and restoration species was chosen (Figure 5.4). The species selected for each experimental area are listed in Table 5.1.

According to the delineation, the GS subplots were divided into 6 subgroups, 5 of which were designed with the aim of planting the grass samples grouped by species, and the remaining group with the aim of planting all the species together. A total of 16 plants of the same species were planted in each subgroup, and 3 plants of each species were planted in the remaining subgroup. A total of 96 grass plants were planted within the experimental plot, and 20 samples were planted in the adjacent plot.

Within the plot intended for planting trees and shrubs (TS plots), a subdivision into groups for the different species was also performed. In this case, no subgroup was intended for planting



a. Overview of Plot Layout

PPC	MIX
SOG	YCF
LBS	SWG

1	2	3	4	1	2	3	
4	5	7	8	4	5	6	
9	10	11	12	7	8	9	
13	14	15	16	10	11	12	
				13	14	15	
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16
1	2	3	4	1	2	3	4
5	6	7	8	5	6	7	8
9	10	11	12	9	10	11	12
13	14	15	16	13	14	15	16

b. Grass and plugs (GS) subplots planting layout

HBY	BOK
GDW	ERB

1	2	1	2
3	4	3	4
1	2	1	2
3	4	3	4

c. Trees and Shrubs (TS) subplots planting layout

Figure 5.2. Plots and subplots delineation layout

Table 5.1. Plant species selected for field-scale phytoremediation experiments

Area	Type	Scientific Name	Common Name	Plant ID	Number of Plants	
					Exp. Plot	Adj. Plot
Slag Disposal Area and Upland Area	Grasses and Plugs	*Asclepias incarnata*	Swamp milkweed	SMW	96	50
		Cassia hebecarpa	Wild Senna	WSA	96	50
		Deschampsia caespitosa	Tufted hair grass	THG	96	50
		Solidago graminifolia	Common grass-leaved goldenrod	CGG	96	50
		Spartina pectinata	Prairie cord grass	PCG	96	50
	Trees	*Acer saccharinum*	Silver maple	SMP	125	0
		Quercus bicolor	Swamp white oak	SWO	125	0
	Shrubs	*Amorpha fruticosa*	False indigo bush	FIB	125	0
		Cornus stolonifera	Red-osier dogwood	ROD	125	0
Wet Meadow Area	Grasses and Plugs	*Andropogon scoparius*	Little Bluestem	LBS	96	50
		Bouteloua curtipendula	Side Oats Grama	SOG	96	50
		Dalea purpurea	Purple Prairie Clover	PPC	96	50
		Panicum virgatum	Switch Grass	SWG	96	50
		Ratibida pinnata	Yellow Coneflower	YCF	96	50
	Trees	*Celtis occidentalis*	Hackberry	HBY	20	0
		Quercus velutina	Black Oak	BOK	20	0
	Shrubs	*Cornus racemose*	Gray Dogwood	GDW	20	0
		Circis canadensis	Eastern Redbud	ERB	20	0

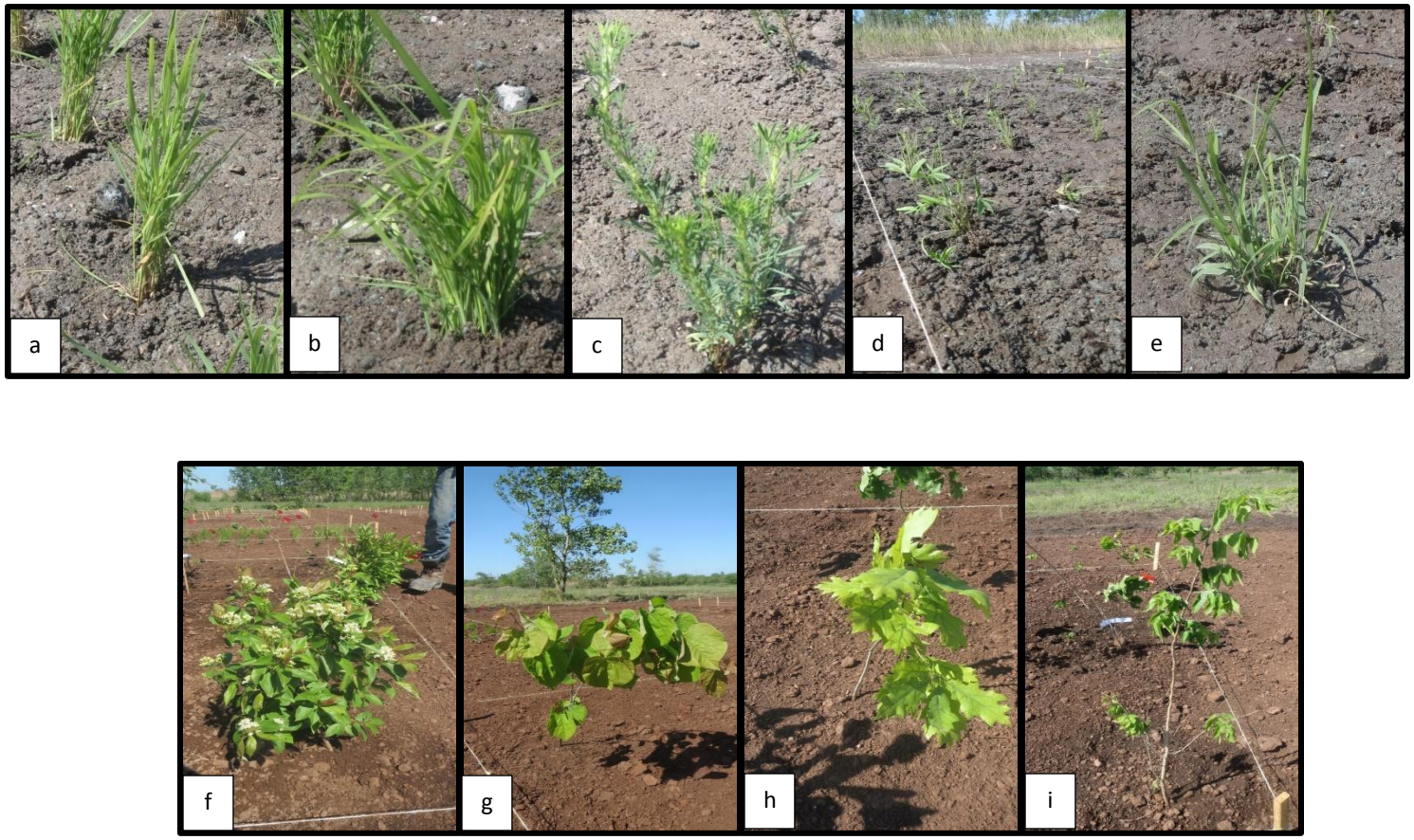


Figure 5.3. Species selected for restoration of slag disposal and upland areas. (a)Switchgrass (*Panicum virgatum*). (b)Little Bluestem (*Andropogon scoparius*). (c)Purple Prairie Clover (*Dalea purpurea*). (d)Yellow Coneflower (*Ratibida pinnata*). (e)Side Oats Gramma (*Bouteloua curtipendula*). (f)Gray Dogwood (*Cornus racemose*). (g)Eastern Redbud (*Circis canadiensis*). (h)Black Oak (*Quercus velutina*). (i) Hackberry (*Celtis occidentalis*)

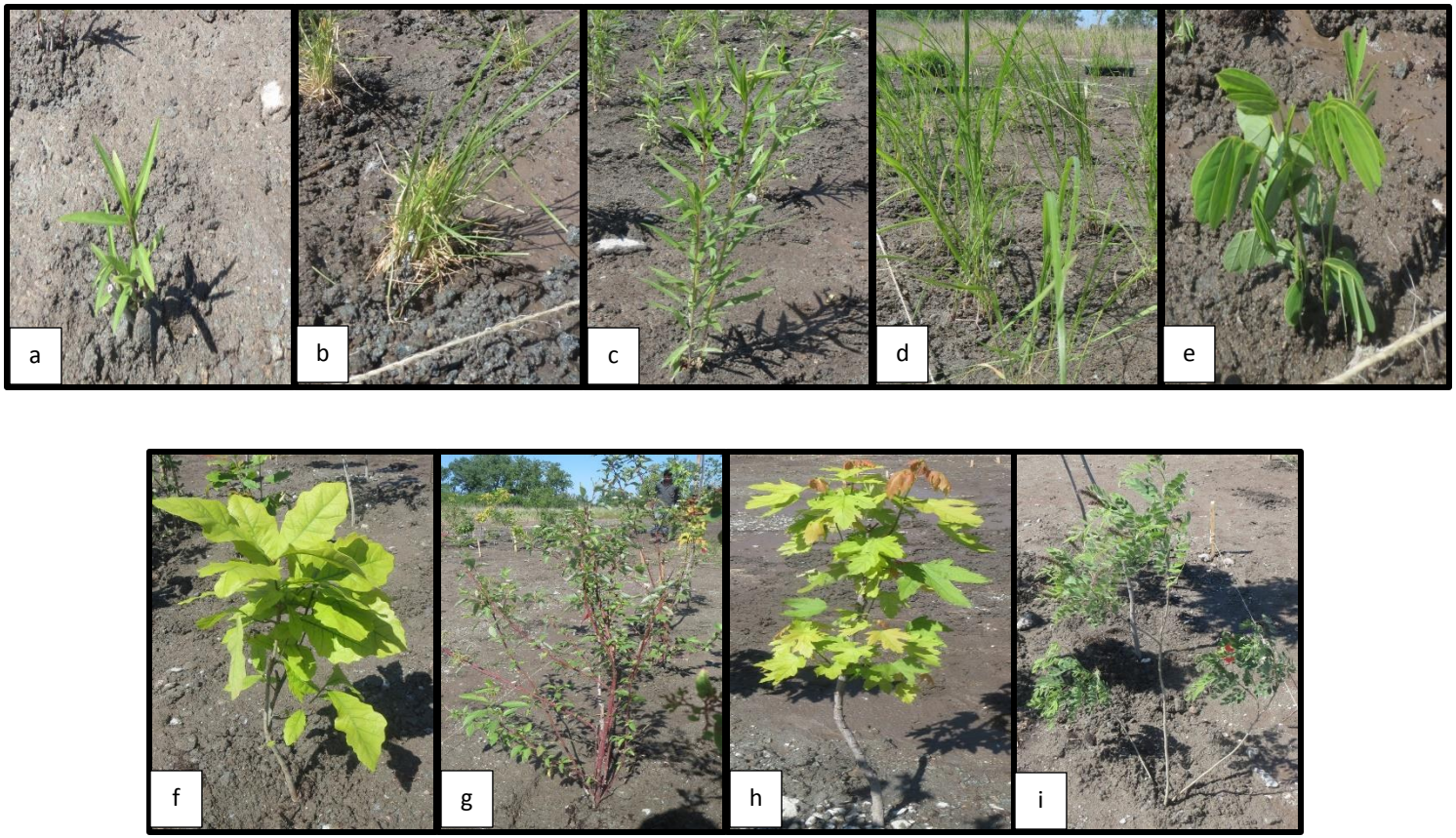


Figure 5.4. Species selected for restoration of the wet meadow area. (a) Swamp Milkweed (*Asclepias incarnata*). (b)Tufted Hairgrass (*Deschampsia caespitosa*). (c)Common Grass – leaved Goldenrod (*Solidago gramnifolia*). (d)Prairie Cord Grass (*Spartina pectinata*). (e)Wild Senna (*Cassia hebecarpa*). (f)Swamp White Oak (*Quercus bicolor*). (g)Red – osier Dogwood (*Cornus stolonifera*). (h)Silver Maple (*Acer saccharinum*). (i)False Indigo Bush (*Amorpha fruticosa*)

mixed species. At each subgroup, only one woody species was planted, resulting of a total of 20 woody species (trees and shrubs) planted within the experimental plot. No woody samples were planted in the adjacent plot.

5.2.4. *Watering and Monitoring*

Once soil preparation and planting completed, the test plots were watered twice a week throughout summer months (June to August) and monitored weekly for survival, leaves, pests and infection, and height of the woody plants during the first growing season. At the adjacent plots, only survival of plants was monitored.

During the second growing season, the test plots were monitored bi-weekly during the summer. No additional water or pest control was performed at any of the experimental areas, in order to let the plants grow under normal conditions and assess the suitability of the plants to cope with the natural site conditions and compete against the invasive species. Figure 5.5 shows the evolution of the three experimental areas throughout the three growing seasons.

5.2.5. *Termination Sampling*

At the end of the second growing season, a total of 30 soil samples were taken from GP subplots (6 soil samples per plot in a total of 5 plots) from 2-3 representative samples randomly selected from surviving plants within the plot. All soil samples were kept on ice during the day. Vegetative biomass from 2-3 representative plants were taken from each GP plot, divided into above ground (leaves and shoots) and belowground (roots) biomass.

At the end of the third growing season, a terminal sampling was performed, and soil and vegetative samples (consisting on roots, leaves and shoots) of each surviving species were

a. Slag disposal area

b. Wet meadow area

c. Upland area

Figure 5.5. Overall monitoring pictures of the three experimental areas. From left to right: Season 1 before tilling, season 1 after tilling, season 2 and season 3, respectively

collected. Additionally, two grab samples of vegetation from outside each experimental area (Sweet Clover at slag disposal area, Phragmites at wet meadow and Milkweed at the upland area) were also taken. Target contaminants (BaP, As, Cr, Pb and Mn) were analyzed in all soil and vegetative tissues sampled. Also, in order to have a complete view of the changes in soil and phytoremediation potential of native plants on the ground an additional complete analysis of metals and PAHs was performed on selected soil and vegetative samples. One soil sample from the initial soil at each area, and one soil sample of each surviving species (SWG, PPC, YCF and LBS at the slag disposal area, FIB at the wet meadow and LBS at the upland area) were selected from the soil after tilling (and compost addition), and from the soil at the end of the third growing season, with exception of LBS samples from the upland experimental plot, due to the lack of soil samples from that area. Soil characterization tests were also performed to soil samples in the lab.

5.2.6. *Soil and Plant Sample Tests*

The soil characterization tests performed in the lab consisted of physicochemical properties that mainly included measurements of the pH, electrical conductivity (EC), Organic Carbon (OC), and Oxidation – Reduction Potential (ORP), Water Holding Capacity (WHC) Grain size distribution (GSD) and Exchangeable Nutrients Content. A detailed description of soil physicochemical characterization tests can be found in Chapter 2. Soil and vegetative samples were sent to STAT Analysis Corporation (Chicago, IL, USA) for sample acid digestion and analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS). Polynuclear Aromatic Hydrocarbons (EPA method SW8270C) were also tested in these samples by Gas Chromatography Mass Spectrometry (GC/MS).

Speciation of contaminants in the soil before and after the phytoremediation technique implementation was determined by sequential extraction. Those analyses were performed using the Tessier procedure (Tessier et al., 1979), with slight modifications. The information regarding the sequential extraction procedure followed in the present work is provided in Chapter 2. Samples from sequential extraction and blank were sent to STAT Analysis Corporation (Chicago, IL, USA) for analysis of Total Metal Concentrations (EPA method SW6020) with Inductively Coupled Plasma Mass Spectrometry (ICP/MS).

For the test results, mean and standard deviation were calculated using Microsoft Office Excel 2013. To check whether a significant difference exists between the result sets, the t-test was performed with Microsoft Office Excel 2013. The alpha value was taken as 0.05 for the t-test.

5.3. Results and Discussion

In this section, the results for soil characterization and contaminant concentrations in soil and vegetative tissue during the three growing seasons are presented and discussed.

5.3.1. *Initial Soil Characterization*

Soil physical properties were tested in the lab for every soil composite sample. The results for the initial soil characterization are shown in Table 5.2. The soil pH values obtained during the initial sampling at the slag disposal area, wet meadow area, and upland area are 7.5±0.11, 7.3±0.04 and 7.1±0.11, respectively. Thus, according to these results, the initial soil pH at the three different ecotypes present in the site of study were close to neutral, being the soil pH at the upland area slightly more acidic than the other two. The results of the soil pH found in the surface of the three areas were lower than expected, likely due to weatherization.

Table 5.2. Soil characterization before, after and at the end of the third growing season

	Slag Disposal Area								Wet Meadow Area				Upland Area				
							Root Soil Season 3					Root Soil Season 3					Root Soil Season 3
Soil	Season 1		Season 3						Season 1		Season 3		Season 1		Season 2	Season 3	
Parameter	IS	AT	SWG	LBS	YCF	SWG	LBS	YCF	IT	AT	FIB	FIB	IS	AT	LBS	LBS	LBS
pH	7.5	9.3	8.3	8.1	8.2	8.1	7.9	7.8	7.3	10.7	7.5	7.5	7.1	7.3	7.7	7.6	7.6
ORP (mV)	-44.4	-156.8	-97.1	-85.7	-85.7	-69.5	-56	-51	-31.1	-244.5		-39.7	-22.7	-36.6	-54.4		-41.5
OC (%)	4.2	8	8.3	7.5	8.7	31.6	38.4	49.8	2.6	5.5	6.3	15.2	7.8	5.6	9.2		22
EC (mS/cm)	0.18	0.01	0.05	0.06	0.06	0.05	0.05	0.04	0.3		0.06	0.23	0.43	0.25	0.04		0.09
mc (%)	16.5	17.6	10.9	13.4	11.2	6.1	6	9	27	40.2	34.7	1.9	47.4	25.3	22.6	18	8.2
WHC (%)	27	37		31					45	44			52	45	46		
P(mg/L)	0.08	0.07		0.06		0.47	0.2		0.06	0.01	0.03	0.07	0.16	0.16	0.08		0.14
N (mg/L)	1.5	2.2		2.4		3.21	3.98		1.3	0.6	1.8	6.6	9.4	4.8	3		3.7
% Gravel	52	56		30					17	15	13		3	3			
% Sand	27	32		33					51	73	58		67	78			
% Fines	21	12		38					31	12	29		30	19			

SWG= Switch Grass
LBS= Little Bluestem
YCF = Yellow Cone Flower
PPC = Purple Prairie Clover
<DL = Below Detection Limit

The initial organic content (OC) found in the soil was 4.2%±2.4, 2.6%±0.1 and 7.8%±2.32 at the slag disposal area, wet meadow area, and upland area, respectively. The slag disposal area and the wet meadow area had lower OC values as expected due to the non-existence of plants or living organisms found in these sites during the initial surveys (Figure 5.5).

The area which presents initially higher water holding capacity (WHC) is the upland area (52%), followed by the wet meadow (44.7%) and the slag disposal area (27%). The results of the WHC of these three experimental areas are related to their grain size distribution. Thus, the lowest WHC was expected to be obtained in the slag disposal area, due to the higher percentage of coarse-grained particles (52%). The wet meadow and the upland areas present similar grain size distribution, but the upland area had a higher WHC, because of the lowest coarse-grained fraction (3%). Figure 5.6 compares the initial grain size distribution of site at the three experimental areas.

The presence of exchangeable Nitrogen and Phosphate is also very low in the slag disposal area (1.5 mg/L and 0.08 mg/L, respectively), followed by the wet meadow area (1.3 mg/L and 0.06mg/L). on the other hand, the upland area was the one with higher exchangeable Nitrogen (9.4 mg/L) and exchangeable Phosphate (0.16 mg/L) found initially in the soil.

The low organic matter content found initially in the soil at the slag disposal area, together with the low WHC and exchangeable nutrients scarcity, evidenced the need to amend the soil to increase the success of the plants performance at this experimental plot.

Table 5.4 shows the concentration (mg/Kg – dry soil) of the different Polynuclear Hydrocarbons (PAHs) that were found initially in the soil. The concentration of Benzo(a)pyrene (BaP), which is the target PAHs of the present study, is initially 0.43±0.3 mg/Kg – dry soil at the slag disposal area, 0.43±0.7 mg/Kg – dry soil at the wet meadow area and 1.8±0.7 (mg/Kg – dry soil) at the upland area, being these two latter the areas with higher overall PAH concentrations

Table 5.3. Variation of soil pH at study areas measured during growing season 2

Area	Location	Depth	pH
Slag Disposal Area	Inside experimental plot area	surface	7.9
	Inside experimental plot area	22 cm	9.9
	TS experimental plots	surface	8
	GP experimental plots	surface	8
Wet Meadow Area	Inside experimental plot area	36 cm	11
	Outside experimental plot	10 cm	7.4
	TS experimental plots	surface	9.6
	GP experimental plots	surface	10
Upland Area	Outside experimental plot	64 cm	7.4
	TS experimental plots	surface	7.3
	GP experimental plots	surface	7.4

Table 5.4. PAHs concentrations in soil

Concentration	Slag Disposal Area										Wet Meadow Area				Upland Area			
	Season 1		Season 2				Season 3				Season 1		S2	S3	Season 1		S2	S3
mg/Kg –dry soil	IS	AT	SWG	LBS	PPC	YCF	SWG	LBS	PPC	YCF	IS	AT	FIB	FIB	IS	AT	LBS	LBS
Acenaphthene	<DL (0.03)	<DL (0.03)	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)	0.4	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.1	0.5	0.1	
Acenaphthylene	0.03	<DL (0.03)	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)	0.07	<DL (0.04)	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.17	<DL (0.04)	
Anthracene	0.05	0.05	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		0.06	0.5	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.4	1.3	0.4	
Benz(a) anthracene	0.2	0.29	0.08	0.13	0.16	0.15	0.11	0.11		0.13	1.5	0.05	<DL (0.04)	0.06	1	3	1	
Benzo(a)pyrene	0.43	0.41	0.18	0.28	0.2	0.28	0.3	0.25	0.23	0.22	0.4	0.1	0.1	0.1	1.8	2.7	1.3	5.3
Benzo(b) fluoranthene	0.36	0.36	0.16	0.27	0.26	0.24	0.27	0.13		0.34	1.6	0.04	<DL (0.04)	0.05	1	3	1	
Benzo(g,h,i) perylene	0.35	0.27	0.12	0.2	0.17	0.22	0.16	0.14		0.29	1	0.07	<DL (0.04)	0.09	0.6	1.7	0.5	
Benzo(k) fluoranthene	0.16	0.31	0.1	0.17	0.19	0.22	0.1	0.1		0.32	1	0.07	<DL (0.04)	0.09	0.7	2	0.7	
Chrysene	0.29	0.36	0.11	0.19	0.22	0.22	0.14	0.17		0.25	2	0.07	<DL (0.04)	0.09	1	3	1	
Dibenz(a,h) anthracene	<DL (0.03)	0.12	0.05	0.08	0.08	0.09	<DL (0.03)	0.08		<DL (0.04)	0.5	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.3	0.8	0.3	
Fluoranthene	0.26	0.45	0.11	0.2	0.26	0.24	0.13	0.15		0.21	4	0.07	<DL (0.04)	0.09	2.4	7.9	2.3	
Fluorene	<DL (0.03)	<DL (0.03)	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)	0.4	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.1	0.6	0.1	
Indeno(1,2,3-cd) pyrene	0.21	0.24	0.1	0.17	0.15	0.18	0.12	0.12		0.23	0.8	<DL (0.04)	<DL (0.04)	0.07	0.5	1.6	0.5	
Naphthalene	0.08	<DL (0.03)	<DL (0.04)	<DL (0.04)	<DL (0.05)	<DL (0.04)	<DL (0.03)	<DL (0.04)		<DL (0.04)	0.4	<DL (0.04)	<DL (0.04)	<DL (0.04)	<DL (0.04)	0.2	<DL (0.04)	
Phenanthrene	0.18	0.14	0.04	0.06	0.09	0.08	0.06	0.07		0.12	5	0.05	<DL (0.04)	0.05	1.6	6.8	1.4	
Pyrene	0.26	0.36	0.1	0.17	0.22	0.2	0.11	0.11		0.17	4	0.07	<DL (0.04)	0.09	2	7	2	

IS – Initial soil (before tilling); AT – After tilling

S2 – Season 2; S3 – Season 3.

initially found in the soil. This contaminant was analyzed in a minimum of 5 composite samples, whereas the rest of PAHs were analyzed only in one selected soil composite sample from each area.

Arsenic, Chromium, Lead and Manganese were the target inorganic contaminants selected in this study among the heavy metal species found initially in the soil at the three experimental areas (Table 5.5). The concentration of As initially found in the surface was 6.8±0.7 mg/Kg – dry soil at the slag disposal area, 7±0.6 mg/Kg – dry soil at the wet meadow and 10±3 mg/Kg – dry soil at the upland area. Concentrations of Cr were higher at the slag disposal area (300 mg/Kg – dry soil), whereas in the wet meadow and the upland area remained below 40 mg/Kg – dry soil. Similarly, the concentration of Pb found initially in the soil at the slag disposal area (745±200 mg/Kg – dry soil) was higher as compared to the wet meadow area (111±24 mg/Kg – dry soil) and the upland area (187±64 mg/Kg-dry soil). Initial Mn concentration at the slag disposal area (19000 mg/Kg–dry soil) was also the highest as compared to the other two areas, where initial Mn concentration remained around 1000 mg/Kg – dry soil). Concentrations of As and Pb were initially analyzed in a minimum of 5 composites samples, whereas the rest of metals included Cr and Mn, were only analyzed in one selected sample. Among the heavy metals that are not included in the group of target contaminants, Zn was found in a high concentration at the slag disposal area, followed by the upland and the wet meadow areas (3900, 490 and 470 mg/Kg – dry soil, respectively). Noticeable presence of Ni with initial concentration at the slag disposal area was also the highest (64 mg/Kg – dry soil), followed by the upland area (44 mg/Kg – dry soil) and the wet meadow (22 mg/Kg-dry soil). Some presence of Cd (4.4 mg/Kg-dry soil) was also found initially at the slag disposal area.

Table 5.5. Metal concentrations in soil

Conc. mg/Kg – dry soil	Slag Disposal Area										Wet Meadow Area				Upland Area			
	Season 1		Season 2				Season 3				Season 1		S2	S3	Season 1		S2	S3
	IS	AT	SWG	LBS	PPC	YCF	SWG	LBS	PPC	YCF	IS	AT	FIB	FIB	IS	AT	LBS	LBS
Al*1000	5	8	6	7	6	7	8	6	7	6	10	48	52	47	13	12	12	11
Sb	3.7	4	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	<DL (5)	5	<DL (4)	3	<DL (2)
As	6.8	9.6	10	10	10	9	12	12	12	12	7	<DL (3)	4.6	4	10	9	10	10
Ba	95	138	120	150	160	130	135	110	130	120	63	560	480	580	110	106	97	120
Be	0.7	0.9	0.9	1	1	1.1	1.2	1	1.1	0.9	1	9	7	6	<DL (1)	<DL (1)	<DL (1)	<DL (1)
Cd	4.4	14	14	10	18	12	11	12	19	12.5	1	<DL (1)	<DL (1)	<DL (1)	<DL (1)	<DL (1)	<DL (1)	<DL (1)
Ca*1000	130	150	140	170	140	170	130	110	140	115	52	220	230	225	47	52	50	50
Cr	300	275	256	298	302	284	237	240	260	253	36	60	62	68	32	31	26	27
Co	9.2	8.6	7.4	7.1	8.4	7.3	8	8	8.8	9.2	8	<DL (2)	<DL (2)	<DL (2)	16	18	12	12
Cu	85	79.5	87	63	94	73	82	87	110	99	27	7.2	6.7	9	86	48	55	63
Fe*1000	360	258	200	140	210	170	200	200	170	200	28	18	31	17	60	29	26	20
Pb	745	938	1213	1006	1066	1018	995	1070	1006	973	111	59	51	53	187	143	163	158
Mg*1000	23	20	18	23	23	21	18	17	32	16	24	13	15	13	25	25	26	26
Mn*1000	19	21	19	20	21	21	18	18	18	19	1	8	9	7	1	1	0.5	0.4
Hg	0.03	0.03	0.03	0.05	0.06	0.06	0.04	0.04	0.05	0.04	0.04	<DL (0.02)	0.03	<DL (0.02)	0.2	0.1	0.2	0.2
Ni	64	128	64	40	48	50	53	48	49	68	22	7	5.5	5.8	44	42	33	35
K	320	2175	930	1400	1400	1600	1400	1700	1300	1750	1400	2950	2100	2900	2900	3200	2300	2560
Se	0.9	1.1	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (1)	6.6	6.4	10.6	<DL (2)	<DL (2)	<DL (2)	<DL (2)
Ag	0.9	2.5	2.1	1.7	2.8	1.9	1.65	1.95	3.1	1.75	<DL (1)	<DL (1)	<DL (1)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)
Na	200	625	540	440	680	470	460	410	660	410	110	1250	950	1183	120	120	160	268
Tl	0.9	1	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (1)	<DL (1)	<DL (1)	<DL (2)	<DL (2)	<DL (2)	<DL (2)	<DL (2)
V	150	173	170	240	220	210	190	155	190	190	41	24	23	26	34	31	24	28
Zn	3900	6075	8800	6100	8800	7000	6800	7950	7300	7050	470	200	130	188	490	140	180	198

IS – Initial soil (before tilling)
AT – After tilling
S2 – Season 2; S3 – Season 3

5.3.2. *Soil Characterization after Compost Amendment and Tilling*

Only the slag disposal area was amended with compost and tilled while the other two areas were tilled without any amendment. The soil physico-chemical properties after tilling are found in Table 5.2. Tilling and ground homogenization affected the soil physical properties. As it can be observed, soil pH increases sharply at the slag disposal area as well as in the wet meadow (9.3±0.36 and 10.7±0.16, respectively), whereas no significant differences were found in the soil pH at the upland area (7.3±0.06). Results of soil pH distribution (Table 5.3) show that the soil pH increases with depth at the slag disposal and wet meadow areas, while in the upland area remains approximately uniform. The high pH found in those areas could be due to the presence of a highly alkaline slag layer underneath the top soil coverage. The mixture of this slag layer with the soil when tilling could have induced this increase in the soil pH, masking the possible effect of the addition of compost in the soil pH at the slag disposal area.

As expected, the organic content of the soil at the slag disposal area increased after tilling and compost addition about 100% of the initial value, as well as the exchangeable Nitrate concentration, which increased up to 46%, and WHC, that increased 10%. However, no significant changes in soil exchangeable Phosphate were found after tilling and soil amendment addition ($p>0.05$). The increase of the values of these soil properties results on more available organic matter, nutrients and water for the plants that could potentially lead to a higher success of its survivorship and growth. Although OC also increased at the wet meadow area after tilling, lower WHC and exchangeable nutrients were found in the surface. In the upland area these soil properties (OC, WHC, exc. Nitrogen and Phosphate) decreased after tilling.

The grain size distribution of soils also changed after the tillage of the three experimental areas. At the slag disposal area, the percentage of the sand fraction increased after tilling, similar

to other experimental areas. The abundant presence of coarse-grained fill material and debris at the slag disposal area resulted in a higher percentage of the coarse fraction after tilling (56%). The addition of compost could have also led to increase in the sand-size fraction at this area. The grain distribution of the soils after tilling at the three experimental areas is represented in Figure 5.6.

The concentration of BaP after tilling (Table 5.4) remains constant after tilling at the slag disposal area (0.41±0.08), as well as in the wet meadow area (0.1±0.03), where slightl decrease of the target contaminant was observed, but not statistically significant ($p>0.05$). However, the concentration of BaP in the soil after tilling at the upland area (2.7±3.2) reveals a high spatial variability. The existence of high concentrations of organic contaminants in hot – spots could have likely increased the concentration after the soil tillage due to mixing effect.

Metal concentrations in soil after tilling are shown in Table 5.5. As it can be observed, the tillage had different effects on the soil metal distribution. Thus, while the concentrations of As (9.6±1.23 mg/Kg – dry soil) and Pb (938±100 mg/Kg – dry soil) increase at the slag disposal area ($p<0.05$), it remain without significant changes at the upland area (9±0.57 mg/Kg – dry soil of As and 143±48.7 mg/Kg – dry soil of Pb), and decrease at the wet meadow area (below detection limits). Due to the lack of samples analyzed, it was not possible to compare statistically the concentrations of Cr and Mn, although the same tendency as for As and Pb at each area is observed on their average values.

The percentage of each metal fraction in the soil before planting is shown in Table 5.10 and the target heavy metal fractionation is compared in Figure 5.7. As it can be observed, As is mainly retained in the residual fraction, before and after tilling; however, the soil tillage seems to have certain effect on the mobilization of the metal(loid) towards the Mn and Fe oxides bound and Organic – bound fractions, with percentages increase slightly. Cr and Mn are mainly retained in

the Fe and Mn oxides – bound fraction, and their distribution remains constant after the soil tillage. Pb is mainly retained in the residual fraction, which percentage increases after tilling, as well as the one bound to organic fraction. In the wet meadow area, As, which initially is also found mainly retained in the residual fraction, shows certain mobilization after tilling the soil, increasing its proportion in the rest of the soil fractions. Cr is mainly retained in the residual fraction and after tilling is mobilized towards the Fe and Mn oxides – bound and organic – bound fraction. Mn and Pb on the other hand, are mainly retained in the Fe and Mn oxides – bound fraction, and their distribution remains without noticeable changes after soil tillage. This tendency is also observed at the upland area, except for the residual fraction of Pb and Mn is much lower than in the wet meadow area. A higher presence of Pb and Mn in mobile soil fractions could mean a higher bioavailability of those elements to be uptaken by the plants. Less noticeable are the changes of As and Cr mobilization at the upland area, with distribution between the different soil fractions remains unchanged, with predominant fraction as the residual.

5.3.3. *Plant Monitoring Results*

Figure 5.5 shows the evolution of the plants performance at the three experimental areas throughout the experiment. As it can be observed, the presence of other species in the study areas makes it difficult the visual recognition of the experimental plots at the end of the experiment in the slag disposal and the upland areas. Especially at the slag disposal area, where during the third growing season, the presence of other species than those selected for the study took over the experimental site, hindering the identification of surviving species. However, it does not occur the same in the wet meadow area, where the overall growth of plants is very limited.

During the first growing season, all the species showed high survival rates and good performance, probably due to the comprehensive monitoring and continued irrigation. At the end of the second growing season, the native grass species had stronger growth and higher survivorship rates than the woody species at both the slag disposal area and the upland area. These results are consistent with those obtained by Palmroth et al. (2006) in which the soil amendment did not enhance trees survivorship, which was very low as compared to grasses. Survivorship rates of all species at the wet meadow area sharply decreased with the exception of False Indigo Bush (FIB). The survival rates found in the adjacent plots and in the mix subgroup of the grasses and plugs subplot were similar to those found in the experimental plots at the three areas.

Although no monitoring was performed during the third growing season, field observations made during the terminal sampling revealed that the highest number of surviving species was found at the slag disposal area, where 4 out of the total 9 species survived at the end of the experiment: Switchgrass (SWG), Little Bluestem (LBS), Yellow Coneflower (YCF), and Purple Prairie Clover (PPC). On the other hand, only one species out of all the species initially planted survived at the wet meadow area (FIB) and at the upland area (LBS). In the upland area, other invasive as well as indigenous species such as Milkweed dominated the experimental site.

The soil pH at both the slag disposal and the wet meadow areas was highly alkaline. According to the USDA plants database (USDA), the soil pH tolerated by all the plant species selected for the present study is ranged between slightly acidic (5.5 – 6) and slightly alkaline (7.5 – 8). The soil pH found in these experimental areas is above the ideal values at both soil surface and with depth (Tables 5.2 and 5.3). A previous study carried out by Chirakkara (2014) revealed that toxicity associated with the soil contamination, especially when the contamination is mixed, the plant growth and survival are affected. Therefore, the high pH as well as the contaminant

presence in the soil could be the main causes of low survivorship of plants at the wet meadow area. However, although the slag disposal area has the highest heavy metal concentrations, it is also the area with higher number of surviving species. The grain size distribution could have also affected the performance of the plants. Thus, those species that grow in soils with higher percentage of medium-sized grain tend to develop higher biomass than those that grow in coarse grained soil (Huang et al., 2013). However, contrary to what was expected, neither the grain distribution, nor the effect of the slag layer and the high contaminant concentrations seemed to negatively impact the grasses and plugs at the slag disposal area. The addition of compost might have been the most important factor for the good performance of these species. However, the negative effects of the soil characteristics in the woody species were not countered by the addition of soil amendment, likely due to compost not extended to deeper for a good development and successful establishment of the root system of trees and shrubs. On the other hand, pH did not seem to be the main cause of the poor survivorship of the native species planted at the upland area, since the soil pH remained below 7.5 throughout the three growing seasons. The toxicity may not be caused by the presence of contaminants as the presence of living organisms above and below ground was noticeable during the terminal sampling at the end of the third growing season. In this case, the competition with other existing species could have been the main reason for the poor performance of the species planted at this experimental area.

The beneficial effects of using trees and shrubs in phytoremediation has been reported (Dickinson, 2000). However, the establishment of woody species in the soil can be inhibited by high concentrations of heavy metals (Pulford and Watson, 2003), as well as deficiency of macronutrients (Pulford, 1991). The harsh conditions at the wet meadow area, the competition for nutrients uptake due to the presence of invasive and indigenous species at the upland area, as well

as the negative effect of the slag layer underneath the top soil coverage at both the slag disposal and the wet meadow areas could have been the main causes of the poor survival of trees. The woody species FIB, on the other hand, was the one that reached the best performance out of all the selected species at the wet meadow area. The soil in this area experienced long periods of flooding and drought, which could also be a cause of the deterioration of the species initially planted. Nevertheless, FIB not only resisted the severe soil conditions, but also thrived by the end of the experiment, being the only surviving species in this experimental area.

Only the surviving species SWG, LBS, YCF and PPC at the slag disposal area, FIB at the wet meadow and LBS at the upland area, were considered for further detailed evaluation.

5.3.4. *Fate of PAHs*

Table 5.4 shows the soil concentrations of the EPA 16 priority PAHs at the end of the growing seasons 2 and 3 for each surviving species plot at each experimental area. Due to the high carcinogenic potential of BaP, this contaminant was used as an indicator for the risk due to total PAHs (Harms et al., 2003). As it can be observed, the concentration of BaP at the slag disposal area decreases 28% at SWG plot, 38% at the LBS plot, 45% at the PPC plot, and 47% at the YCF plot. The same tendency is also observed for the rest of the PAHs detected in the initial soil. In general, concentrations of PAHs in soil after tilling at the wet meadow area were very low, and undetectable for certain compounds. No significant differences in the concentration of BaP were found in the soil at FIB plot at the end of the second and third growing seasons ($p<0.05$). A high spatial variability exists at the upland area, and concentrations of BaP in the soil at the end of the third growing season are significantly higher than at the beginning of the experiment. The rest of the PAHs seem to decrease at the end of the second growing season in the soil, compared to the

unplanted soil in this area. Unfortunately, due to the lack of enough replicates, it was not possible to assess statistical significance of changes in concentrations. Furthermore, only results of BaP concentrations were analyzed at the end of the third growing season. According to these results, the highest concentration of BaP in the soil after planting is found at the upland area (5.3±4.2 mg/Kg – dry soil), and only decrease of soil BaP concentrations have been observed at the slag disposal area. At the wet meadow area, however, the soil concentrations of BaP do not show significant differences at the end of the experiment.

Results of PAH concentrations in stems and leaves of the surviving plants are shown in Table 5.6. As it can be observed, results show that all PAHs concentrations were below detection limits (or very close to) in all the aerial vegetative tissue analyzed from the surviving species at the three experimental areas. The levels of the target contaminant BaP, is undetectable in all stems and leaves samples. Similar results found for the invasive and indigenous species collected outside of the three experimental areas with PAH concentrations in their aerial vegetative tissues insignificant (results not shown).

PAHs concentrations in the roots are found in Table 8. According to those results, BaP concentrations in the roots of SWG, LBS, YCF, and PPC at the slag disposal area were found below detection limits at the end of the third growing season. Similar results are found for the rest of the PAHs analyzed. Results of BaP concentrations in the roots of FIB were also very low, close to detection limits. However, results of PAHs concentrations in the roots of LBS at the upland area show that the concentration of these organic contaminants were in all cases detectable. BaP concentrations in the roots of LBS seem to be affected by the high spatial variability (7±16 mg/Kg) of the organic contaminants distribution at this area. Besides, the detectable concentrations of the rest of PAHs in the roots of LBS indicate that the organic contaminants can potentially be sorbed

and accumulated in the roots of the surviving plants. In accordance with these results, the dissipation of PAHs at the slag disposal area are observed in the soil, suggesting that these compounds are degraded before they are uptaken by the plants. At the upland area, the only surviving species LBS, is also found among the surviving species at the slag disposal area. However, results obtained for the same species at these two areas are very different. While the concentration of BaP in the roots of LBS at the slag disposal area is negligible, there exist accumulation of this compound in the roots of LBS at the upland area. Among the differences between one area and the other is the addition of compost at the slag disposal area, while the upland area is planted in absence of soil treatment other than tilling. Additionally, pH at the LBS subplot of the upland area is slightly more acidic (7.6±0.05) than at the slag disposal area (8.05±0.03). Unfortunately, the rest of the soil characteristics at the end of the third season cannot be compared due to the lack of samples of soil from the upland area for analysis. Therefore, since the more noticeable difference between both areas is the presence of compost, it is possible that the addition of soil amendment helped these compounds to be degraded in the soil at the slag disposal area. On the other hand, the absence of BaP in stems and leaves of LBS at the upland area indicates that this compound is not translocated to the rest of the plant.

The addition of organic matter to the soil is expected to produce a positive effect in the organic contaminants degradation, due to the compost amendment in general, improves soil texture, oxygen transfer and increase the nutrients availability, stimulating and enhancing the growth of the populations of microorganisms capable of degrading those compounds (Haritash and Kaushik, 2009). Unplanted soil amendment with compost reduce significantly the concentrations of aromatic organic compounds, compared to unplanted and unamended soils (Wischmann and Steinhart, 1997).

Table 5.6. PAH concentrations in stems and leaves

Concetration (mg/Kg)	Slag Disposal Area								Wet Meadow Area		Upland Area	
	Season 2				Season 3				Season 2	Season 3	Season 2	Season 3
	SWG	LBS	YCF	PPC	SWG	LBS	YCF	PPC	FIB		LBS	
Acenaphthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Acenaphthylene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Benz(a)anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Benzo(a)pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL (0.3)	<DL(0.03)	<DL(0.3)	<DL(0.03)
Benzo(b)fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Benzo(g,h,i)perylene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Benzo(k)fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Chrysene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Dibenz(a,h)anthracene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Fluoranthene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Fluorene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	0.03
Indeno(1,2,3-cd) pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)
Naphthalene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	0.03
Phenanthrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	0.04
Pyrene	<DL(0.3)	<DL(0.3)	<DL(0.3)	<DL(0.3)					<DL (0.3)		<DL(0.3)	<DL(0.03)

Aprill and Sims (1990) studied the degradation of 4 PAHs, one of them was BaP, using prairie grasses, including Little Bluestem and Switchgrass. While the degradation of contaminant by each species is not shown, the results of their study shows that there exists degradation of PAHs in the soil in presence of plants and soil amendment (cow manure). The researchers suggest that the humification processes are stimulated by the roots in the planted soils, which is likely reason for the higher degradation of PAHs than in the unplanted soil, due to plants stimulating the microbial metabolic processes. The degradation of PAHs using Little Bluestem and Switchgrass was also studied by Pradhan et al. (1998). It was observed that the reduction of these contaminants in a fertilized soil and in presence of plants was significantly higher compared to the unplanted soils. Palmroth et al. (2006) studied the degradation of hydrocarbons in a field–scale study using trees and herbaceous plants and with and without soil amendment. It was observed that at the end of the 39 months study, the degradation of hydrocarbons in the soils amended with compost and NKP fertilizer was higher than that obtained in the planted soil without amendment.

On the other hand, in a previous study carried by Chirakkara (2014), the lab scale assay of soil spiked with heavy metals and PAHs, the unplanted soil amended with compost presented a lower concentration of Phenanthrene than in the unplanted unamended soil. Furthermore, a higher reduction is observed in planted amended soil than in planted soils without any kind of amendment. However, in another assay carried out with real contaminated soil from Big Marsh (Chicago, IL), it was observed that there exists a reduction of PAHs in the planted soil amended with compost; this reduction was not significant as compared to the unplanted soil. The presence of plants could also degrade these contaminants through rhizodegradation. Although evidences in the literature of the enhanced degradation by the combined effects of plants and compost are not congruent, results in the present study show that the fate of PAHs in soil in presence of LBS is different depending

on whether the soil is amended or not. Thus, in the amended soil at the slag disposal area, the degradation of PAHs in the soil is observed, while in the unamended soil at the upland area, the degradation of PAHs, and more specifically of BaP, in the soil is not observed. Furthermore, this compound is uptaken and accumulated in the roots of the plant. The results of the present study suggest, therefore, that the biodegradation of PAHs in the soil of the slag disposal area is stimulated by the addition of compost. The primary factor that influences phytoremediation of PAHs in the soil is the microbial activity (Hutchinson et al., 2003). The microbial populations and their activity are associated with the water content and the nutrients availability in the soil, as well as the pH. Tate (1995) determined that the optimum pH range in which the maximum microbial activity is achieved in the soil is comprised in between 7 and 9. The pH of the soil at the slag disposal area is found within the range in the LBS subplot (8.05±0.03). The increase of nutrients availability as well as the oxygenation of the soil after tilling and compost amendment in this area can, therefore, account for these observations.

PAHs are hydrophobic and non-polar molecules persistent in the environment that can be uptaken by the plants in different ways, including the absorption through their roots, since it has been observed that there exists a correlation between the lipophilicity and the uptake of non–ionic compounds by the roots (Briggs et al., 1982, 1983; Ryan et al., 1988). While many of these molecules can be adsorbed by the roots but not absorbed, Fismes et al. (2002) observed that plants which grow in industrial soils uptake PAHs from soil through their roots. However, results in terms of PAHs translocation to the aerial part of the plants obtained by the researchers are not consistent with those obtained in the present work. According to Fismes et al. (2002) there exists translocation from the roots to the aboveground part of the plants. Although concentration of the contaminants is very small compared to that in the roots it is detectable in all cases and it is correlated to the

concentration of contaminant in the soil. On the other hand, the results obtained in the present work show that the concentration of PAHs analyzed in the plants that survived in Big Marsh is negligible in all cases. Therefore, results in the present study suggest the existence of a mechanism of metabolization of those contaminant that prevents its translocation to the aerial parts of the plant. Metabolism of BaP was studied by Harms et al. (1977), and it was observed that plants are able to metabolize BaP and convert it into oxygenated derivates due to the activity of quinones and other enzymes type cytochrome P – 450.

In the rhizosphere of LBS collected from the upland area there was a noticeable presence of soil invertebrates, especially earthworms. These animals are used as a bio-indicator to measure the toxicity of different contaminants (OECD 2000). The bioaccumulation of PAHs in earthworms have been object of numerous studies (Parrish et al., 2006; Contreras–Ramos et al., 2006; Ma et al., 1998), having been observed that they are able to survive under high concentrations of BaP in soil (Shaub and Achazi, 1996). Those animals are in direct contact with the PAHs, which can be adsorbed in the detritus in which earthworms feed on (Johnsen et al., 2005). Although PAHs tend to become less bioavailable when aging (Chung and Alexander, 1998), the presence of these living organisms, that act mixing the soil and increasing aeration, could have certain influence in the mobilization of these contaminants increasing their availability to be uptaken by the plants.

On the other hand, results of the unamended soil at the wet meadow area suggest that there is no degradation of BaP in the soil, and uptake by the roots or leaves and stems is negligible. Some differences can be pointed out between the two unamended areas. The contaminant concentrations, the availability of nutrients and the organic content in the wet meadow area was significantly lower than in the upland area. Furthermore, the presence of symbiont organisms in the rhizosphere of FIB responsible of atmospheric Nitrogen fixation can be the main factor that

governs the fate of this contaminant. Studies performed in alfalfa, a Nitrogen fixing plant such as FIB (Fu et al., 2012) revealed that the removal of BaP from the rhizosphere was not affected by the presence of the plant. It was suggested that, when the availability of nutrients is reduced, the presence of Nitrogen – fixing organisms can inhibit the degradation of organic contaminants by autochthonous microorganisms, due to competition for the nutrients.

The degradation rate of the organic pollutants depends on the soil properties and microflora. In addition to the biodegradation that can take place in soils, leaching and volatilization should also be taken into account to quantify the degradation rate. PAHs are usually found highly retained in the solid phase, and this, together with their high molecular weight, such is the case of the target contaminant Benzo(a)Pyrene, are the reason for the natural degradation rates in soil of these compounds results very slow (Haritash and Kaushik, 2009). According to Grosser et al. (1991), the degradation of Benzo(a)pyrene in the soil, presents a first – order rate constant that ranges from 0.00024 day-1 to 0.0009 days-1, in a study site located in southern Illinois. The addition of organic matter to the soil can accelerate this degradation rate, due to the improvement of the soil conditions to the establishment and growth of microbial populations, increases the availability of nutrients and enhances the oxygen transfer (Wischmann and Steinhart, 1997). In presence of compost amendment, Wischmann and Steinhart (1997), observed that the degradation of all Benzo(a)pyrene was reduced 46% in the amended soil after 15 weeks, while this compound was not degraded in the unamended soil. On the other hand, assuming that the phytodegradation of this compound follows a pseudo-first order kinetic rate, (Medina and McCutcheon, 1996; Pavlostathis et al., 1998), the degradation of Benzo(a)pyrene observed by Aprill and Sims (1990) and Prahdan et al. (1998) presents a degradation rate constant that ranges from 0.004 days-1 to 0.009 days-1. Therefore, under specific circumstances, the degradation rate observed in

phytoremediation can be higher than the observed by the biodegradation in unamended soils, but is very slow as compared to the observed the soil amended with compost. However, the phytoremediation rates are specific to each study site conditions.

5.3.5. *Fate of Heavy Metals*

Table 5.5 shows the metal concentration average values in the soil at each surviving species subplot. Only the analysis of the target contaminants (As, Cr, Pb and Mn) was replicated enough to perform statistical analysis. As it can be observed, the concentration of As, Cr and Pb in the soil tends to remain constant after tilling (with compost addition only at the slag disposal area) in all areas. The concentration of Mn at the upland area, on the other hand, decreases slightly at the end of the third growing season. The concentration of heavy metals in stems and leaves is shown in Table 5.7. According to these results, only the concentration of Mn is detected in the aboveground tissue of the surviving species. The concentration of this element decreases in stems and leaves of the surviving species of FIB and LBS at the two unamended areas (8% and 39%, respectively), while no significant changes are observed in stems and leaves of the surviving species at the slag disposal areas ($p>0.05$). Table 5.9 shows the concentration of metals in roots of the surviving species. Results from the analysis of heavy metals in roots show that, except for As, all the target contaminants were detected in the belowground tissue of the surviving species. Additionally, a significant decrease in the concentrations of Cr, Pb and Mn is observed in the roots of all species with exception of YCF, with concentration of those target contaminants decreases slightly at the end of the third growing season ($p<0.05$).

Table 5.11 shows the results of the percentages of metal fractionation from the sequential extraction performed on the soil at the slag disposal and the wet meadow area, and the fractionation of the targeted heavy metals are plotted in Figure 5.8. Due to the lack of soil samples, sequential

Table 5.7. Metal concentrations in stems and leaves

Concetration (mg/Kg)	Slag Disposal Area								Wet Meadow Area		Upland Area	
	Season 2				Season 3				Season 2	Season 3	Season 2	Season 3
	SWG	LBS	YCF	PPC	SWG	LBS	YCF	PPC	FIB		LBS	
Aluminum	54	48	56	<DL(38)							<DL(370)	<DL(370)
Antimony	<DL(4)	<DL(4)	<DL(4)	<DL(4)					<DL (40)		<DL(3)	<DL(4)
Arsenic	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(13)	<DL(15)	<DL(10)	<DL(19)	<DL(4)	<DL(2)	<DL(2)	<DL(2)
Barium	4	7.4	5.2	5.5					<DL(3)		10	22
Beryllium	<DL(1)	<DL(1)	<DL(1)	<DL(1)					<DL(1)		<DL(1)	<DL(1)
Cadmium	<DL(1)	<DL(1)	<DL(1)	<DL(1)					<DL(1)		<DL(1)	<DL(1)
Calcium	8200	5100	26000	16000					6100		9500	8800
Chromium	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(13)	<DL(15)	<DL(10)	<DL(19)	<DL(3)	<DL(2)	<DL(2)	<DL(2)
Cobalt	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Copper	<DL(5)	<DL(5)	12	6.3					<DL(5)		<DL(5)	<DL(45)
Iron	330	570	850	570					125		190	156
Lead	6.37	3.85	12	4.22	<DL(6)	<DL(8)	<DL(6)	<DL(9)	<DL(2)	<DL(1)	<DL(1)	<DL(21)
Magnesium	1300	1600	4800	1400					1350		1800	1940
Manganese	100	87	153	70	63	117	99	72	66	72	14	23
Mercury	<DL(0.02)	<DL(0.02)	<DL(0.02)	<DL(0.02)					<DL0.02		<DL(0.02)	<DL(0.02)
Nickel	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Potassium	5200	9300	38000	14000					8350		4300	9500
Selenium	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Silver	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Sodium	<DL(110)	<DL(110)	<DL(110)	<DL(110)					<DL(130)		<DL(110)	116
Thallium	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Vanadium	<DL(2)	<DL(2)	<DL(2)	<DL(2)					<DL(2)		<DL(2)	<DL(2)
Zinc	24	75	97	72					23		10	30

Table 5.8. PAH concentrations in roots

	Slag Disposal Area								WM	Upland Area	
	Season 2				Season 3				S3	S2	S3
Concetration (mg/Kg)	**SWG**	**LBS**	**PPC**	**YCF**	**SWG**	**LBS**	**PPC**	**YCF**	**FIB**	**LBS**	**LBS**
Acenaphthene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			0.6
Acenaphthylene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			2.2
Anthracene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			7
Benz(a)anthracene	<DL(0.05)		<DL(0.04)	0.08	<DL(0.03)	<DL(0.03)		<DL(0.03)			10.3
Benzo(a)pyrene	0.05	<DL(0.04)	<DL(0.04)	<DL (0.05)	<DL(0.03)	<DL(0.03)	<DL(0.03)	<DL(0.03)	0.03	2.7	7
Benzo(b)fluoranthene	<DL(0.05)		<DL(0.04)	0.18	<DL(0.03)	<DL(0.03)		<DL(0.03)			6
Benzo(g,h,i)perylene	<DL(0.05)		<DL(0.04)	0.14	<DL(0.03)	<DL(0.03)		<DL(0.03)			11
Benzo(k)fluoranthene	<DL(0.05)		<DL(0.04)	0.1	<DL(0.03)	<DL(0.03)		<DL(0.03)			8
Chrysene	<DL(0.05)		<DL(0.04)	0.1	<DL(0.03)	<DL(0.03)		<DL(0.03)			10
Dibenz(a,h)anthracene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			5
Fluoranthene	<DL(0.05)		<DL(0.04)	<DL (0.01)	0.05	<DL(0.03)		<DL(0.03)			17
Fluorene	<DL(0.05)		<DL(0.04)	<DL (0.01)	0.07	<DL(0.03)		<DL(0.03)			2
Indeno(1,2,3-cd)pyrene	<DL(0.05)		<DL(0.04)	0.11	<DL(0.03)	<DL(0.03)		<DL(0.03)			7
Naphthalene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			0.1
Phenanthrene	<DL(0.05)		0.51	<DL (0.01)	0.05	0.04		0.04			11
Pyrene	<DL(0.05)		<DL(0.04)	<DL (0.01)	<DL(0.03)	<DL(0.03)		<DL(0.03)			21

WM – Wet Meadow Area
S2 – Season 2
S3 – Season 3

Table 5.9. Metal concentrations in roots

Concentration (mg/Kg)	Slag Disposal Area								Wet Meadow Area	Upland Area	
	Season 2				Season 3				S3	S2	S3
	SWG	LBS	PPC	YCF	SWG	LBS	PPC	YCF	FIB	LBS	LBS
Aluminum	1600		240	600	200	290		330		6500	1722
Antimony	14		17	15	<DL(5)	<DL(5)		<DL(5)		17	<DL(4)
Arsenic	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(3)	<DL(2)	<DL(4)	<DL(2)
Barium	52		11	17	4.95	7.4		12		57	18
Beryllium	<DL(2)		<DL(2)	<DL(2)	<DL(1)	<DL(1)		<DL(1)		<DL(4)	<DL(1)
Cadmium	<DL(2)		<DL(2)	<DL(2)	<DL(1)	<DL(1)		<DL(1)		<DL(4)	<DL(1)
Calcium	100000		16000	23000	6400	9400		18000		21000	6340
Chromium	76	13	6	9	4.1	7.83	<DL(3)	12	5	10	4
Cobalt	<DL(3)		<DL(3)	<DL(3)	<DL(3)	<DL(3)		<DL(3)		<DL(8)	3
Copper	11		10	34	23	19		22		72	36
Iron	44000		6400	8000	6200	7100		7300		11000	3240
Lead	52	110	39	78	24	53	6	88	10	51	21
Magnesium	8100		2500	2600	2450	860		3000		8500	2252
Manganese	5523	697	238	468	221	285	69	650	480	173	93
Mercury	<DL(0.02)		<DL(0.02)	<DL(0.02)	<DL(0.02)	<DL(0.02)		<DL(0.02)		0	<DL(0.02)
Nickel	<DL(3)		<DL(3)	<DL(3)	<DL(3)	<DL(3)		<DL(3)		19	6
Potassium	4800		14000	19000	5250	890		9800		4700	2630
Selenium	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)		<DL(8)	<DL(2)
Silver	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)		<DL(8)	<DL(2)
Sodium	<DL(180)		240	<DL(190)	<DL(170)	<DL(130)		<DL(110)		500	158
Thallium	<DL(3)		3.1	<DL(3)	<DL(3)	<DL(3)		<DL(3)		<DL(8)	<DL(2)
Vanadium	<DL(160)		13	19	4	8		21		13	6
Zinc	430		430	470	275	400		390		100	38

S2 – Season 2
S3 – Season 3

Table 5.10. Percent fractionation of metals in the soil before planting

	Slag Disposal Area										Wet Meadow Area										Upland Area									
	Before Tilling					After Tilling					Before Tilling					After Tilling					Before Tilling					AfterTilling				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Sb	8	8	41	17	26	6	11	28	20	35	7	7	33	13	41	15	7	45	11	22	6	12	30	15	37	19	15	19	27	19
As	2	2	7	3	87	2	5	12	9	72	3	3	16	6	72	5	11	27	16	41	4	7	19	10	60	7	5	16	10	62
Ba	1	20	57	4	19	1	10	57	13	19	9	29	33	2	26	1	6	44	15	34	15	30	28	7	20	5	23	37	9	26
Be	9	9	43	18	22	7	14	36	26	18	9	9	43	17	22	2	3	61	9	25	7	14	35	18	25	11	9	53	15	13
Cd	3	5	19	5	68	1	6	19	5	69	6	12	29	11	42	4	8	51	12	25	5	19	27	14	34	17	22	19	24	17
Cr	0	0	51	3	46	0	1	52	7	40	1	2	30	3	64	0	1	44	13	42	1	2	18	7	72	2	2	13	12	70
Co	2	2	18	5	73	4	7	26	13	50	3	3	38	6	49	4	8	51	12	25	2	4	47	8	39	5	5	39	10	40
Cu	1	1	3	17	78	1	3	6	28	62	3	3	14	13	67	4	8	47	15	27	2	4	16	47	31	3	3	12	52	30
Pb	0	1	38	2	59	0	1	31	4	65	0	7	56	2	34	1	1	58	8	32	0	10	68	8	14	0	19	59	11	11
Mn	0	1	69	5	24	0	4	59	7	30	0	8	64	2	26	0	4	58	9	29	0	9	67	6	17	0	16	50	14	19
Ni	0	2	29	3	65	1	2	47	9	42	1	6	27	3	64	3	9	46	10	32	1	5	36	14	44	2	5	31	17	45
Se	9	10	42	18	21	6	11	28	20	35	7	8	34	13	38	3	7	41	11	38	6	12	31	16	34	14	11	28	20	27
Tl	9	9	43	18	22	7	14	36	26	18	9	9	43	17	22	7	15	37	22	19	8	15	38	20	19	19	15	19	27	19
V	0	0	66	2	31	0	1	64	7	29	1	1	42	4	53	1	2	53	12	32	1	2	27	9	61	3	2	25	13	58
Zn	0	0	15	0	84	0	2	13	1	84	0	3	19	1	77	1	1	27	5	67	1	9	54	6	31	2	7	53	7	30

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual

Table 5.11.Percent fractionation of metals in soil at different plots after season 3

	Slag Disposal Area															Wet Meadow Area				
	SWG					LBS					YCF					FIB				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Antimony	6	13	16	24	40	6	11	28	18	37	7	14	36	24	18	26	17	17	24	17
Arsenic	2	5	12	9	72	3	6	14	9	68	3	7	17	11	62	6	12	18	21	44
Barium	1	9	54	17	19	1	10	59	15	15	2	18	51	11	18	2	10	39	20	30
Beryllium	9	17	21	32	21	7	14	36	24	18	7	14	36	24	18	2	8	51	11	28
Cadmium	1	5	19	4	72	2	7	26	6	60	2	10	16	8	64	9	17	22	31	22
Chromium	0	0	52	10	37	0	0	58	12	29	0	1	44	6	48	0	2	32	19	47
Cobalt	4	7	21	14	55	4	8	30	14	44	4	8	21	14	53	7	14	35	26	18
Copper	1	1	43	23	32	2	3	7	37	52	1	2	5	46	47	4	9	21	31	35
Lead	0	0	27	5	67	0	1	41	3	55	0	1	29	6	64	0	5	59	8	27
Manganese	0	3	63	7	28	0	3	76	8	14	0	8	58	3	31	0	6	55	12	27
Nickel	1	1	33	21	43	1	1	42	20	36	1	2	40	14	43	4	22	29	14	32
Selenium	7	14	35	26	18	7	14	36	24	18	7	14	36	24	18	3	10	33	19	35
Thallium	9	17	21	32	21	7	14	36	24	18	9	18	22	29	22	9	17	22	31	22
Vanadium	0	0	59	12	28	0	0	67	10	23	0	1	53	14	31	1	2	54	14	30
Zinc	0	1	10	1	87	0	2	21	2	75	0	3	12	1	85	1	5	35	7	52

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual

extraction was not performed to the soil from the upland area at the end of the third growing season. From results of Table 5.11, very little mobilization of the metals in the soil is observed as compared to those obtained in the soil after tilling (Table 5.10). According to what is observed in Figure 5.8, a very small percentage of the target contaminants is retained in the mobile fractions (Exchangeable and Carbonates).

According to these results, the presence of plants does not affect the mobilization of the target contaminants in the soil, as the concentration in the soil did not change significantly throughout the experiment, except for Mn at the upland area.

Despite being the contaminant with higher percentage retained in the exchangeable fraction, the concentration of As is undetectable in all vegetative tissues analyzed.

The soil at the slag disposal area is the one that shows higher concentrations of Cr, Pb and Mn. It was expected that the addition of organic matter increased the mobility of metals in the soil, due to the formation of organo–metallic complexes (Hashimoto et al., 2009; Shahid et al., 2012). However, as compared to the concentration of these metals uptaken by LBS at the upland area, the concentration of the target contaminants uptaken by LBS at the slag disposal area is smaller. Previous studies observed that the presence of compost promotes reduction of Cr (VI) into Cr (III) less mobile (Rendina et al., 2011; Banks et al., 2006). This could be due to the compost amendment enhances the microbial activity, which plays an important role in changing the speciation of this compound (Hartley et al., 2009). The concentration of Pb found in the roots of LBS in the slag disposal area (53±33 mg/Kg) and in the upland area (21±12.5 mg/Kg) are very low as compared to the concentration of this metal in the soil. This study results are in agreement with those obtained by Levy et al. (1999) in which concentration of Pb in Big Bluestem (genus *Andropogon*) was very low compared to the concentration of this metal in the soil. Results in the present work show that,

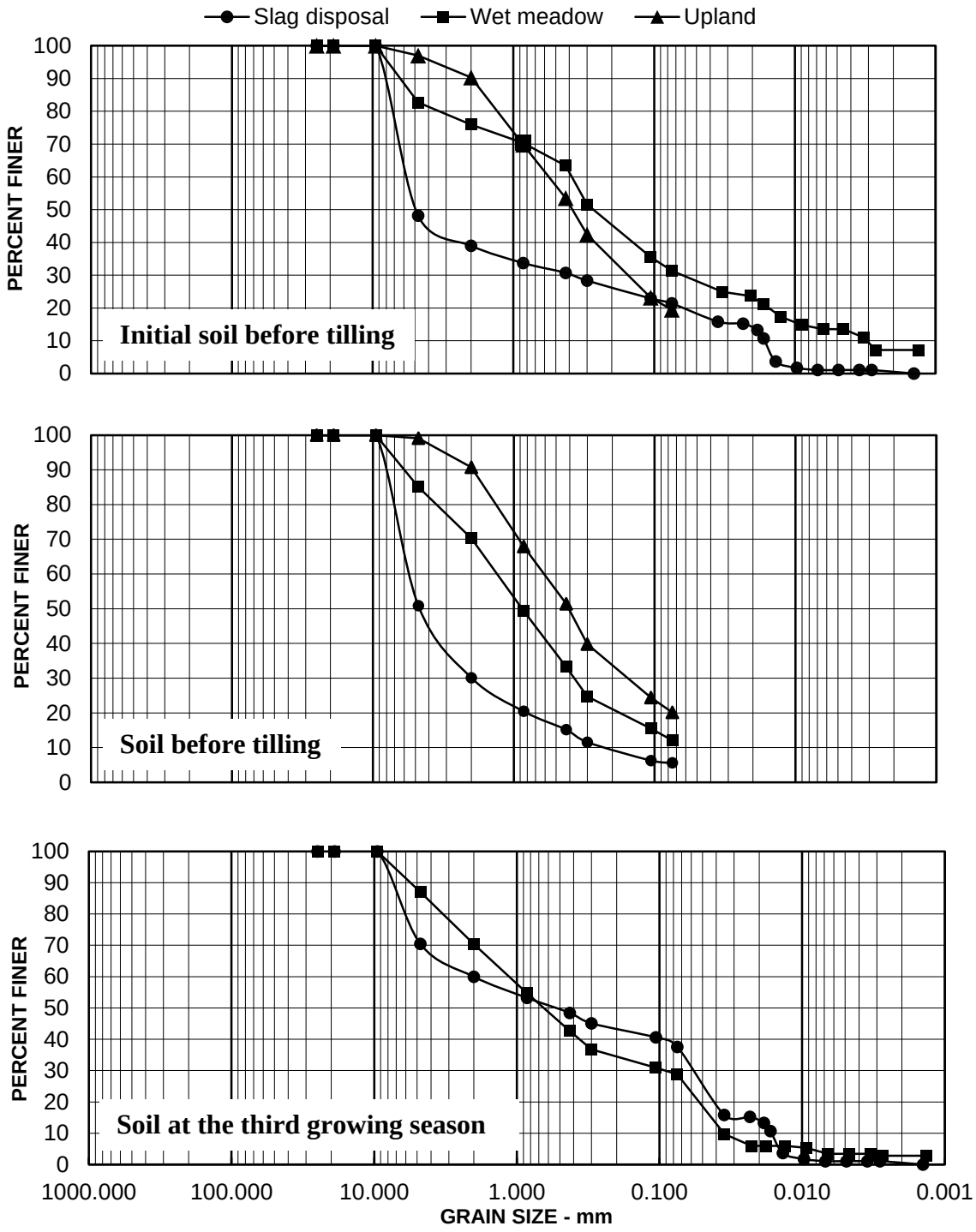


Figure 5.6. Grain size distribution of the soil at the three experimental areas before, after tilling and at the end of the third growing season

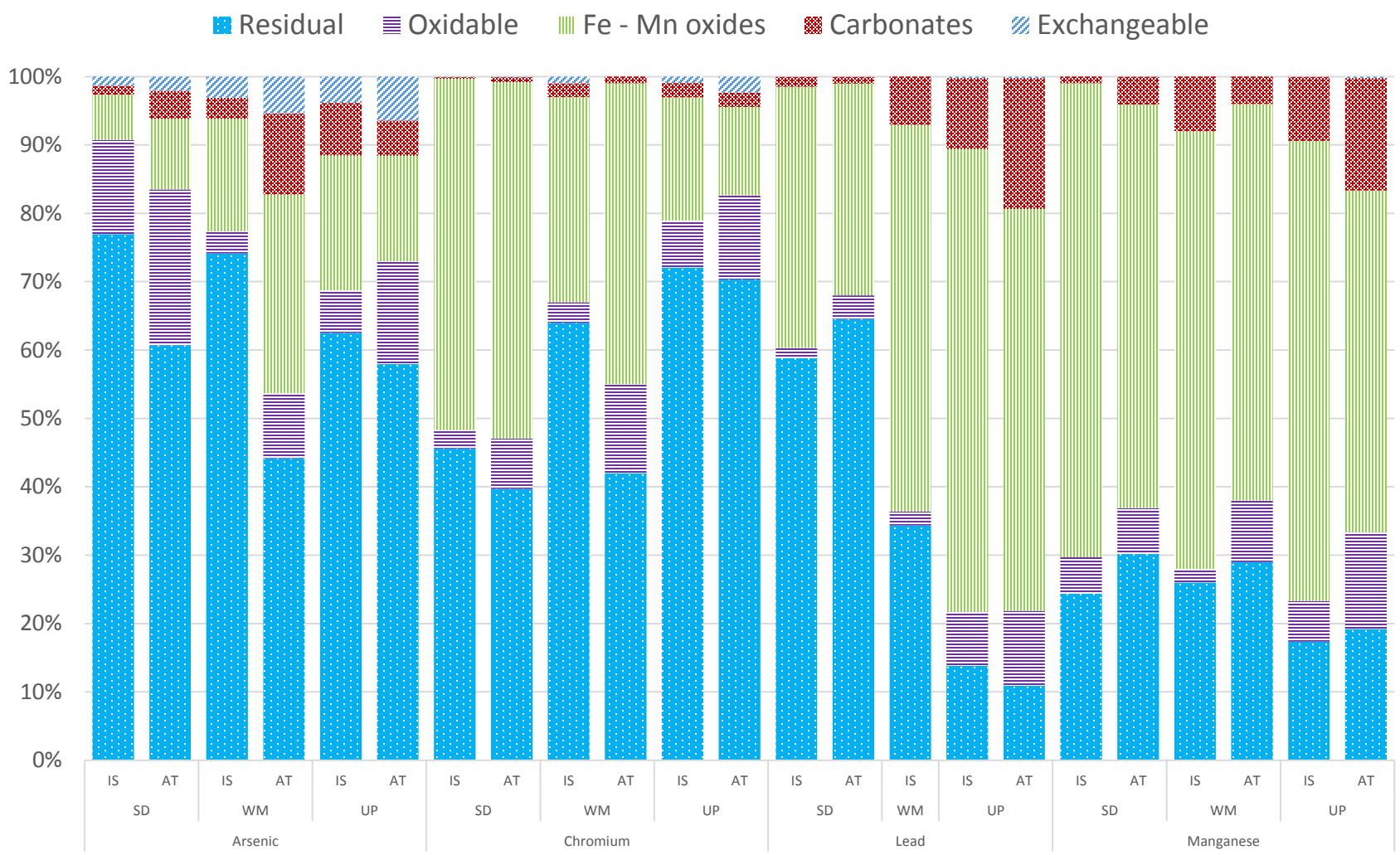


Figure 5.7. Metal distribution comparison between soil before tilling (BT) and after tilling (AT) at the three experimental areas: slag disposal (SD), wet meadow (WM) and upland (UP)

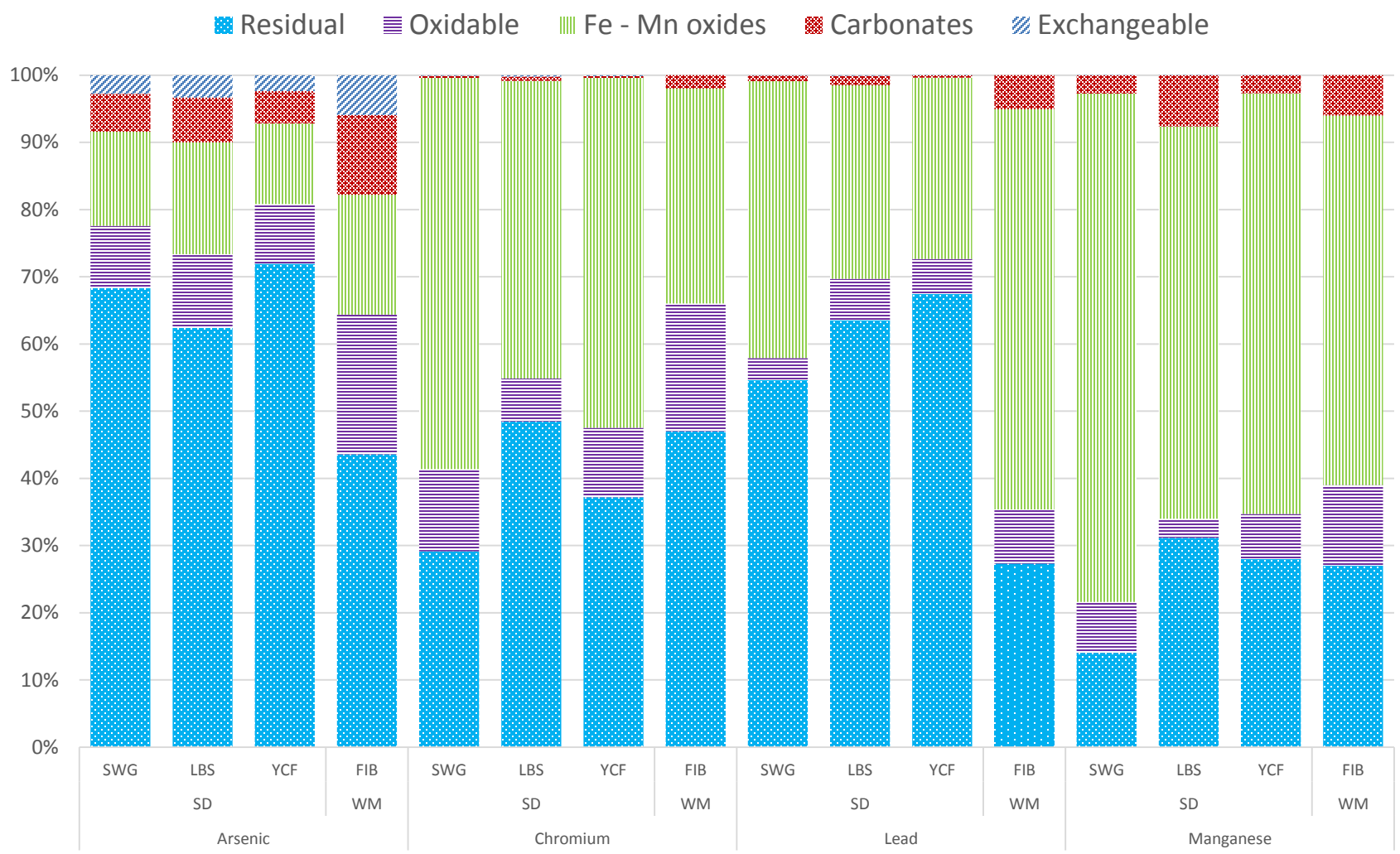


Figure 5.8. Metal distribution comparison of the different surviving plots soil at the experimental areas: slag disposal (SD) and wet meadow (WM) at the end of the third growing season

although there exist Cr and Pb absorption by the roots of the surviving plants, the incorporation of those metals to the aerial tissues are insignificant. This suggests the existence of defense mechanism performed by the plant to protect itself against toxicity produced by an excess of those contaminants. Such defense mechanisms are usually related to root exudates and production of chelating molecules capable to immobilize metals inside the plants, storing them in vacuoles or cell membranes, being the roots the most common storage for excess of metals (Tyler et al., 1989).

Mn is the target contaminant with lowest percentage retained in the residual fraction (Figure 5.8), mainly retained in the Fe–Mn oxides – bound fraction. Its noticeable presence in the plants in both roots and aerial parts suggest the existence of an uptake mechanism of those metallic oxides by the plant, which is able to assimilate and incorporate them to the vegetative tissues. The assimilation of Mn in Big Bluestem has been previously studied. Weremijewicz and Janos (2013) observed that the mycorrhiza naturally present in the roots of LBS increases the uptake yield of Mn.

5.3.6. *Fate of Contaminants in Root Soil*

The soil from the root zone, also called rhizosphere, encompasses the millimeters of soil surrounding the root of the plants, where complex biological and ecological processes occur. In order to study the fate of contaminants in this part of the plant soil system, the soil from the root zone of the survival species was collected and sequential extraction was performed. Results of root–soil characterization can be found in Table 5.2. As it can be observed, no significant changes of the pH at the rhizosphere were observed as compared to the bulk soil of the surviving species subplot. As expected, the organic content found in the rhizosphere of the surviving species at the slag disposal area was the highest (32% - 50%), followed by LBS at the upland area (22%), and

FIB at the wet meadow area (15%). The addition of compost at the slag disposal area, and the presence of invertebrate organism in the rhizosphere of LBS could be the main reason for the higher organic content.

The results of the sequential extraction for the rootzone soil are found in Table 5.12 and the target heavy metals percentage fractionation is represented in Figure 5.9. As it can be observed, the main percentage of As is retained in the residual fraction, followed by the Fe – Mn oxides – bound and organic – bound fractions. This latter is more noticeable in the rhizosphere of FIB at the wet meadow area, followed by the surviving species at the slag disposal area. Similarly, Cr found in the rhizosphere of the surviving species at the slag disposal area and upland area is mainly retained in the residual fraction. The percentage of this metal retained in the organic fraction is higher in the rhizosphere of LBS at the slag disposal area than LBS at the upland area. The main percentage of Pb is found retained in the residual fraction at the surviving species of the slag disposal area. In the rhizospheres of FIB and LBS at the upland area, Pb is mainly retained in the Fe – Mn oxides – bound fraction. This results indicate that this contaminant is more mobile for the unamended areas, and are in agreement with the concentration of this metal found in the vegetative tissues of the surviving species. The distribution of Mn, on the other hand, is very similar in all cases, mainly retained in the Fe–Mn oxides – bound fraction. These results indicate that Mn was the contaminant more easily available.

5.3.7. *Practical Implications*

The harsh conditions at both the slag disposal area and the wet meadow area was exacerbated when the soil was tilled. The pH of the soil became highly alkaline, and in the case of the slag disposal area, the concentration of the inorganic contaminants increased after tilling and homogenize the

Table 5.12.Percent fractionation of metals in the root zone soil after season 3

	Slag Disposal Area															Wet Meadow Area					Upland Area				
	SWG					LBS					YCF					FIB					LBS				
Metal	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
Antimony	6	11	28	21	34	20	9	23	18	29	13	9	51	17	11	6	12	32	21	29	23	10	26	9	32
Arsenic	2	5	12	9	71	3	6	14	11	66	4	7	19	14	56	5	10	25	18	43	4	7	20	6	63
Barium	2	10	61	10	18	3	15	38	12	31	2	15	44	15	24	3	9	43	20	25	6	18	33	5	38
Beryllium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17	2	4	64	12	18	9	15	38	13	25
Cadmium	2	5	25	6	62	1	5	17	5	71	2	7	26	8	57	6	12	30	22	30	7	20	28	10	35
Chromium	0	0	46	8	46	0	1	11	25	63	0	1	15	38	46	1	1	40	23	35	1	2	10	9	79
Cobalt	3	7	27	13	50	4	7	20	14	55	3	7	34	17	39	6	12	30	22	30	3	12	33	7	46
Copper	1	2	9	40	48	1	1	7	64	26	1	1	7	72	19	2	3	3	71	20	2	10	8	57	24
Lead	0	0	34	5	60	0	1	21	3	75	0	1	30	4	65	0	3	62	11	25	1	22	53	3	21
Manganese	0	4	63	4	28	0	7	40	7	46	0	9	55	9	26	1	9	60	12	19	1	25	48	3	23
Nickel	1	1	43	11	44	1	1	18	24	56	1	1	27	37	34	2	6	31	22	39	1	6	28	11	54
Selenium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17	2	7	35	40	16	8	13	32	11	37
Thallium	7	14	35	27	17	7	14	35	27	17	7	14	35	27	17	6	12	30	22	30	10	16	40	14	20
Vanadium	0	1	60	14	24	0	1	31	40	28	0	1	31	45	22	1	2	47	17	32	1	2	20	8	68
Zinc	0	1	20	1	77	0	1	12	3	84	0	2	19	6	73	0	5	38	6	50	1	13	54	4	28

F1. Exchangeable fraction; F2. Carbonates – bound fraction; F3. Fe – Mn oxides bound fraction; F4. Organic – bound; F5. Residual

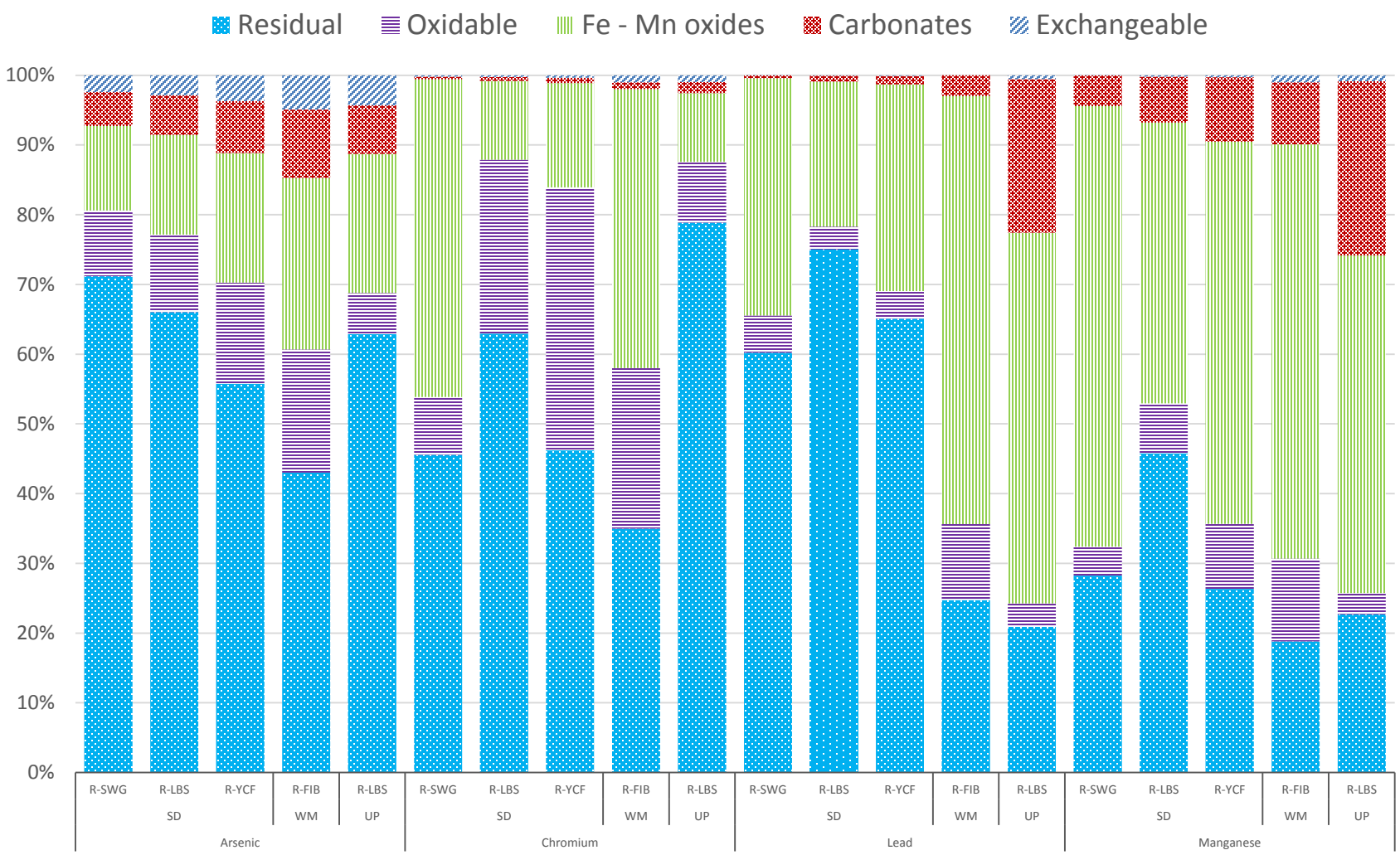


Figure 5.9. Metal distribution comparison of the root zone soil at the different surviving plots at the three experimental areas: slag disposal area (SD), wet meadow area (WM) and upland area (UP) at the end of the third growing season

soil. The high soil pH was one of the main reasons for the low survival of the selected species. Therefore, it would be highly recommended either to perform a shallow tillage of the top soil layer, or neutralize the soil pH after tilling and homogenization. Furthermore, the implementation of the phytoremediation technique should be designed taking into account not only the initial characteristics of the site, but also those results of soil characterization obtained after ground preparation. In addition to that, the soil amendment seemed to be key in the survival of the species in the slag disposal area, leading to buffer the harsh soil conditions and the high concentration of inorganic contaminants, and increasing the growth and survivorship of the selected species. Therefore, the addition of compost is highly recommended. In the upland area, the lack of nutrients and the soil pH were not observed to be the main reason for the low survival of the selected plants. Instead, a high presence of invasive and indigenous plants that took over the area of study was observed to be a plausible cause for the low performance of the selected species, due to competition for the nutrients available. However, the use of indigenous species could be beneficial to the ecosystem, hence recommended to study the features of these plants that grew in the upland area spontaneously, and assess their feasibility as valid species for the restoration of areas under the same conditions.

The use of prairie grasses is highly recommended, due to its higher percentage of survivorship. Furthermore, the suitability of Little Bluestem (*Andropogon scoparius*) has been shown since this species has been able to cope with the harsh conditions of the slag disposal area and the competition against other species for the nutrients available at the upland area. On the other hand, False Indigo Bush (*Amorpha fruticosa*) was the only species that survived at the wet meadow area being able to adapt to the scarcity of organic matter and nutrients, as well as the high soil pH. The property of this leguminous plant to fix atmospheric Nitrogen in its root system was key for

the success of its survivorship. It is highly recommended, therefore, the use of legumes in the remediation of areas under similar conditions, due to their high tolerance to the adverse conditions.

The trees and shrubs were generally more susceptible to the soil conditions. Their tolerance levels to the soil pH, lack of nutrients and high levels of contamination was observed to be lower than in the herbaceous and leguminous plants. It is recommended that the grasses be used in the early stage of the phytoremediation technique, in order to stabilize the ground conditions and increase the organic content in the soil, leading to the optimum conditions to the successful establishment of the woody species.

Non detectable levels of PAHs in stems and leaves of the surviving plants indicates that, regardless the soil conditions, there exists some mechanism of degradation of these contaminants either in the soil in the case of the slag disposal area, or inside the plants in the case of the wet meadow and upland areas. These results suggest that the risk of toxicity due to polycyclic aromatic hydrocarbons contamination is low. In addition, the incorporation of compost has been demonstrated to enhance the degradation of PAHs in the soil. Therefore, it is highly recommended to amend the soil during tilling and homogenization with compost or any organic amendment, in order to not only improve the chances of the selected plants to survive, but also to enhance the phytodegradation of organic contaminants. Furthermore, the addition of compost amendment in the soil of the upland area could help to reduce the concentration of contaminants uptaken by the roots of the plant, where the highest concentration of PAHs were found. However, it is also recommended to analyze the presence of byproducts and metabolites derived from the biodegradation of these organic compounds, in order to determine the final fate of these contaminants in the soil and in the plants.

The results from the sequential extraction showed that the bioavailable fraction of toxic metals in the soil is very low, indicating that the toxic metals present in the three areas of study are immobile. The undetectable concentration of toxic metals in the stems and leaves of the surviving species also confirms that the risk of toxicity related to the heavy metal contamination is very low. Nonetheless, it is recommended to study the mobility of these metals over the time in the soil – plant system, due to its dynamic characteristics, in order to ascertain that their bioavailability does not increase with time.

The results obtained from the present study are valuable to assess the potential for using native plants to remediate other unrestored wetland sites in the Calumet region that have been also significantly impacted by the steel industry and illegal disposal practices.

5.4. Conclusions

The conclusions drawn from the present study are shown below:

- The higher number of surviving species was found at the slag disposal area, which soil was amended with compost. The surviving species were Little Bluestem, Switchgrass, Purple Prairie Clover and Yellow Cone Flower; all of them herbaceous species. On the other hand, only one species survived at each unamended area: False Indigo Bush at the wet meadow area, and Little Blue Stem at the upland area.
- False Indigo Bush (*Amorpha* fruticosa) was the only woody specie that survived among all the trees planted in the three experimental areas, likely due to its capacity to fix atmospheric Nitrogen and its ability to cope with adverse growing conditions. On the other hand, Little Bluestem (*Andropogon scoparius*) was the native prairie grass that survived in both the

amended and the unamended areas, showing a high suitability to its use in the restoration of sites with the same characteristics as Big Marsh.

- Biodegradation of organic pollutants (PAHs) was observed in the compost amended soil at the slag disposal area, while in absence of the soil amendment, the organic contaminant were found uptaken by the roots of the surviving plants. On the other hand, the concentration of PAHs in stems and leaves of all the surviving species was undetectable, which suggests the existence of a metabolic mechanism in the plant that degrades the contaminants before they are translocated to the aerial parts of the vegetative tissue.
- Heavy metals remained immobile in soil with concentrations that remained constant throughout the experiment. No uptake by the aerial tissue of the surviving plants was observed except for Mn with concentrations detected in the stems and leaves of all surviving plants. The presence of heavy metals in the roots was detected in all surviving plants; however, the presence of compost in the soil at the slag disposal area could have helped to the immobilization of the metals, because the concentration found in the roots of the surviving species at this area were very low as compared to the proportion uptaken by the surviving species in the unamended areas, where concentrations of metals in soil was lower.
- Results from the sequential extraction revealed that the bioavailable fraction of heavy metals in soil was very low, being the major part of them retained in the residual fraction. In the case of Mn, the only target contaminant found in the aerial parts of the plants, the main percentage was retained in the Fe – Mn oxides – bound fraction, suggesting the existence of a mechanism in the plants able to uptake these metallic oxides.

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CHAPTER 6

OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1. Overall Conclusions

The feasibility of the field – scale phytoremediation technique application to three different areas contaminated with PAHs and heavy metals at Big Marsh site in Chicago, USA, was studied. The potential benefit of using compost amendment to increase plants survival and growth and organic contaminant degradation was also studied. Three different areas of concern: slag disposal area, wet meadow area and upland area, representative of the three ecotypes existing at Big Marsh site were selected to perform the investigation. The research was carried out systematically, following the same procedure at each experimental area. First, an initial soil survey was performed to identify the contaminant concentrations and soil characteristics at each area. Each of those three areas had different soil characteristics and levels of contamination. Secondly, the experimental areas were delineated and 9 native and restoration species (grasses and trees) specific for each site conditions were selected and planted in the experimental plots. The soil at the slag disposal area, which presented the lowest organic content was amended with compost. The study duration was three complete growing seasons.

The results showed that the compost amendment increased plants survival and growth at the slag disposal area, which displayed the highest survival rates and less phytotoxicity. Additionally, degradation of organic contaminants was observed in the amended soil. Overall the survival of grasses were higher than that observed for trees. False Indigo Bush, at the wet meadow area, was the only woody specie that survived at the end of the experiment of all the woody species selected and planted. Despite soil conditions were the harshest in the wet meadow, the False Indigo

Bush thrived at this experimental area, without showing evidences of phytotoxicity. Only one herbaceous species survived at the upland area, presumably due to the massive presence of indigenous and invasive plant species that took over the experimental area.

Results showed that the PAHs were mainly degraded in the amended soil at the slag disposal area, while no degradation of these contaminants was observed in the unamended soil at the wet meadow area and upland area. The concentrations of these organic contaminants in the roots and shoots of the surviving plants of the slag disposal area were undetectable, whereas in the roots of Little Bluestem at the upland area and False Indigo Bush at the wet meadow area were detected. However, no evidences of the presence of these contaminants in the shoots of those two species were found.

The concentrations of heavy metals in the soil at the three experimental areas did not show significant changes throughout the experiment. The results from the sequential extraction showed that the percentage of exchangeable fraction was very low. However, the analysis of the roots of all the surviving species showed that there existed root uptake of those metals. On the other hand, the concentration of heavy metals in the shoots were undetectable, and Manganese was the only metal found in stems and leaves of all the surviving species at the three experimental areas, although none of them showed evidences of phytotoxicity due to excess of this metal.

The general conclusions from this research are summarized as follows:

- The soil characteristics and contaminant levels changed dramatically after ground preparation. When the soil was tilled, the pH increased in the soil at the slag disposal area and wet meadow area due to the effect of the presence of an alkaline slag layer.

- 6 native and restoration species out of the total 18 species selected and initially planted survived at the end of the experiment. 4 of which survived at the slag disposal area, 1 at the wet meadow and 1 at the upland area.
- The addition of compost amendment to the soil of the slag disposal area increased the probability of success of the surviving plants, increasing their growth and survival rates, and buffering the toxicity due to the presence of contaminants.
- Biodegradation of PAHs was observed uniquely in the soil amended with compost, at the slag disposal area, while in the surviving plants of the wet meadow and upland areas, the degradation of these organic contaminants took place in the roots.
- The establishment of plants in the contaminated soil did not affect the heavy metal mobilization, and concentration of the toxic metals was not dissipated. However, root uptake of those elements is observed in all the surviving species, but there was not translocation of the toxic metals to the aerial parts of the plants.

6.2. Recommendations for Future Research

Based on this study, the following recommendations for future research are presented:

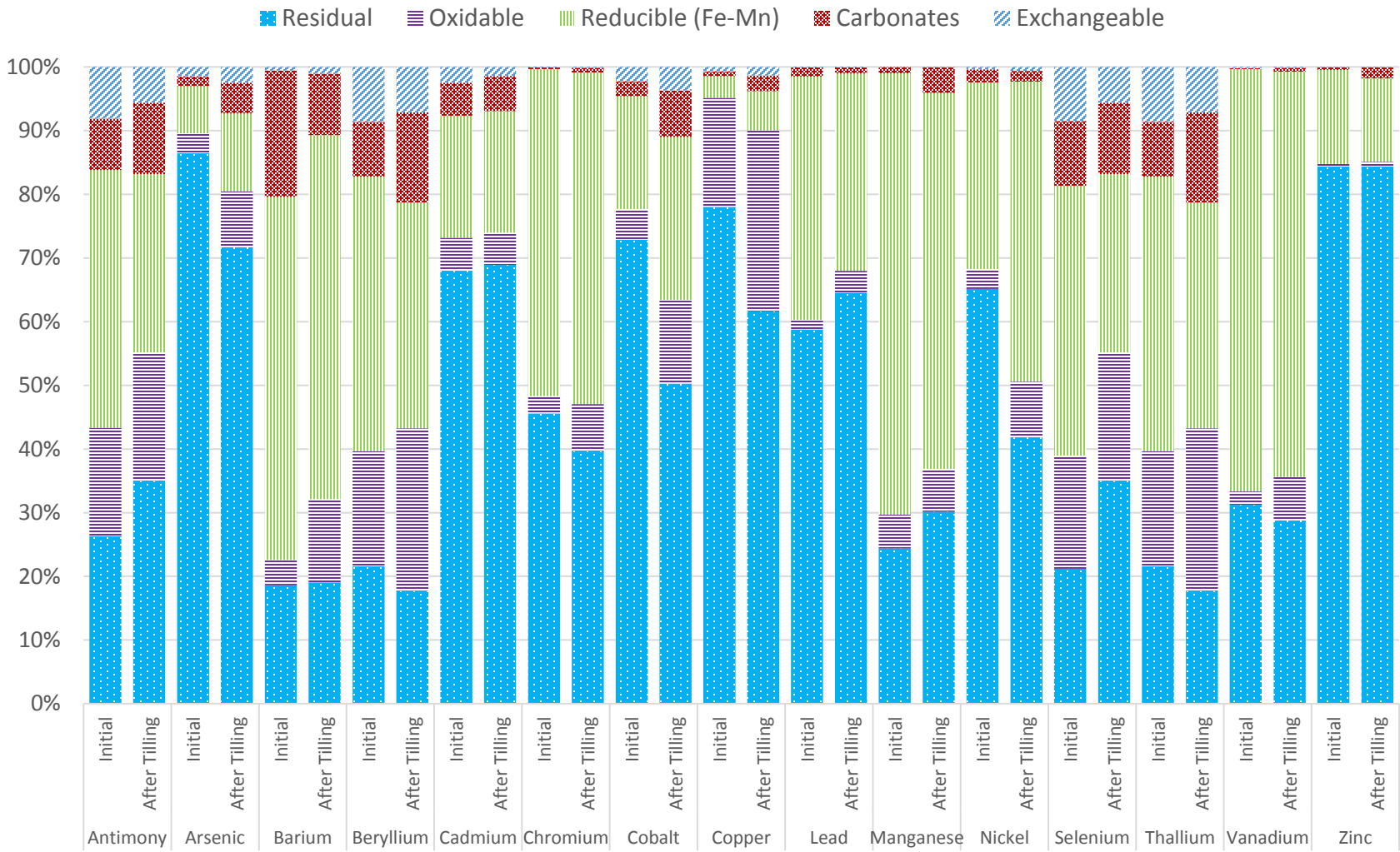
- The soil characteristics and contaminant concentrations after tilling and homogenizing the soil should be taken into account to perform a proper design of the phytoremediation technique.
- Compost amendment or other organic amendments should be added to the soil to increase the successful of plants performance in the contaminated areas.
- In order to have a better knowledge of the influence of compost amendment on the biodegradation of PAHs in the soil, the final fate of those organic contaminants in the

unamended soil should be compared within the same experimental area. Therefore, it is highly recommended to study the fate of PAHs in amended and unamended soils within each experimental area.

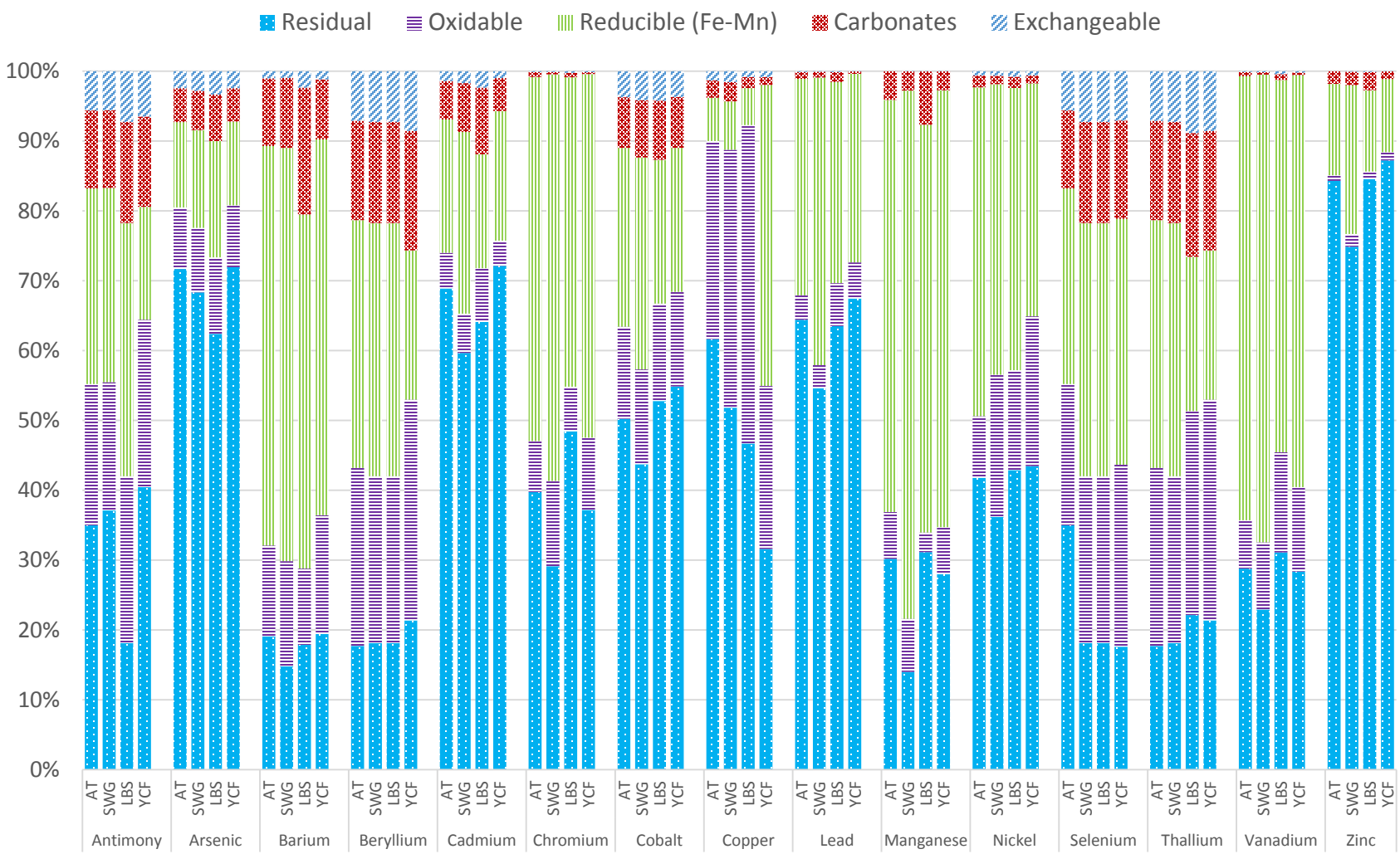
- Results have shown that the prairie grasses are the ones that display better performance in the field–scale implementation of the phytoremediation technology. The species belonging to the families *Graminaceae* and *Fabaceae* are the ones that perform the best, being able to cope with the harsh conditions at each area. Therefore, their use in field – scale phytoremediation is promising.
- It is highly recommended to analyze metabolites and byproducts from the degradation of PAHs in order to assess the human and environmental risk derived from the final fate of those compounds.
- The continuous soil – plant is a dynamic system, and changes in the bioavailability of the heavy metals can take place. It is recommendable to monitor these elements in order to analyze possible changes in their mobility with time.

APPENDIX A

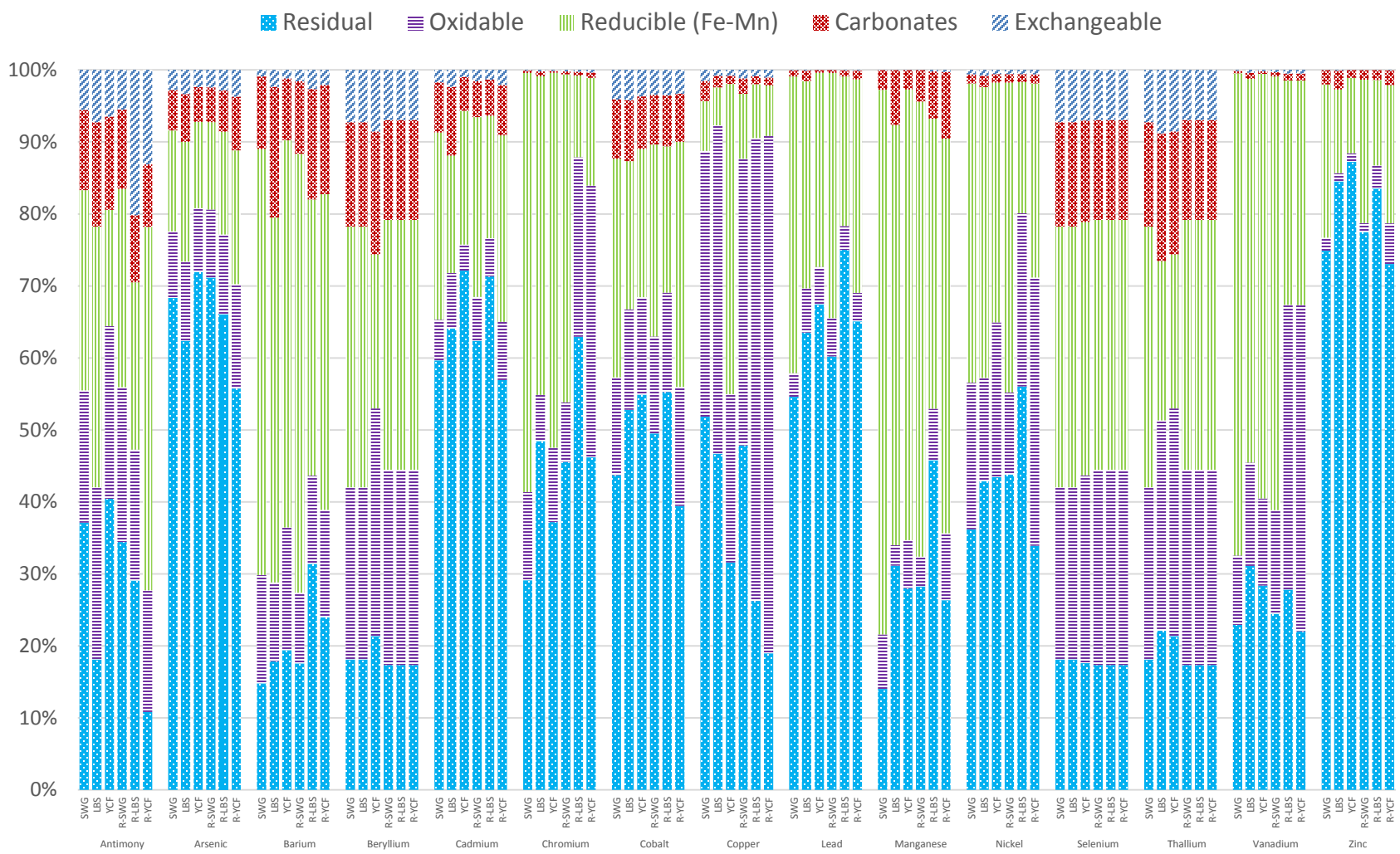
Total metals distribution from sequential extraction



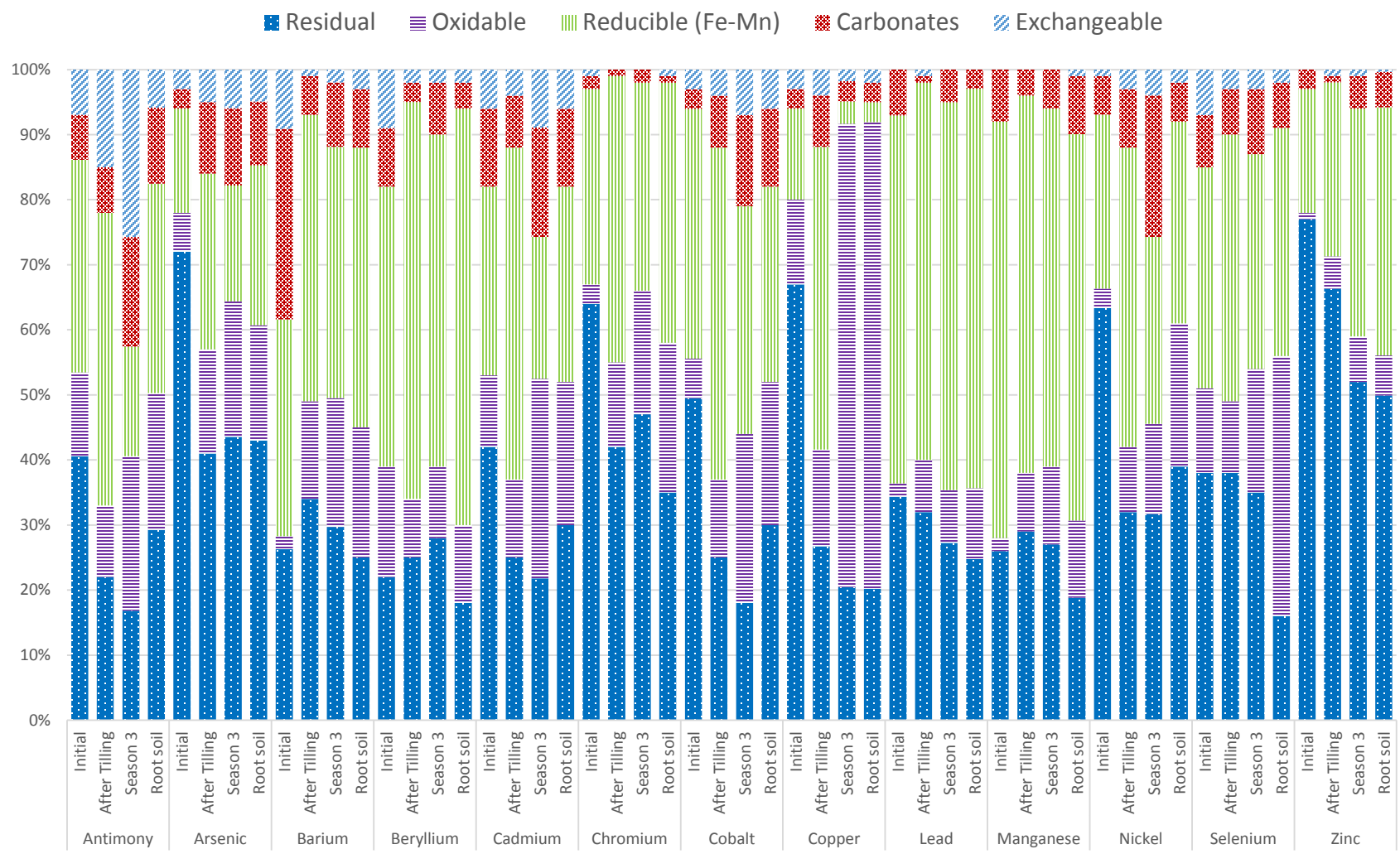
A1. Total metals distribution comparison between soil before and after tilling at slag disposal area



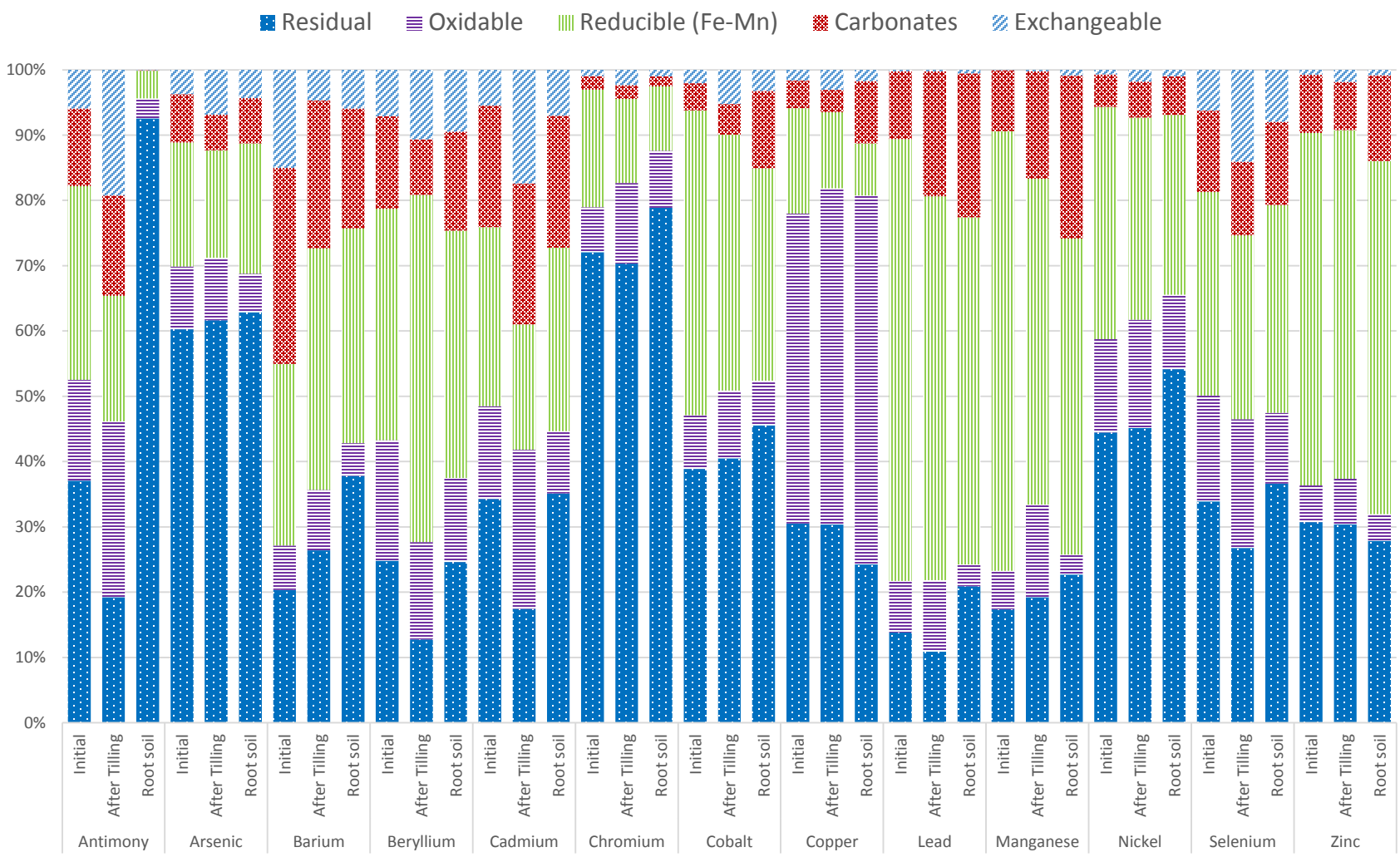
A2. Total metal distribution comparison between soil after tilling (AT) and soils at surviving plant plots at the end of the third season, Switchgrass (SWG), Little Bluestem (LBS) and Yellow Cone Flower (YCF) at slag disposal area



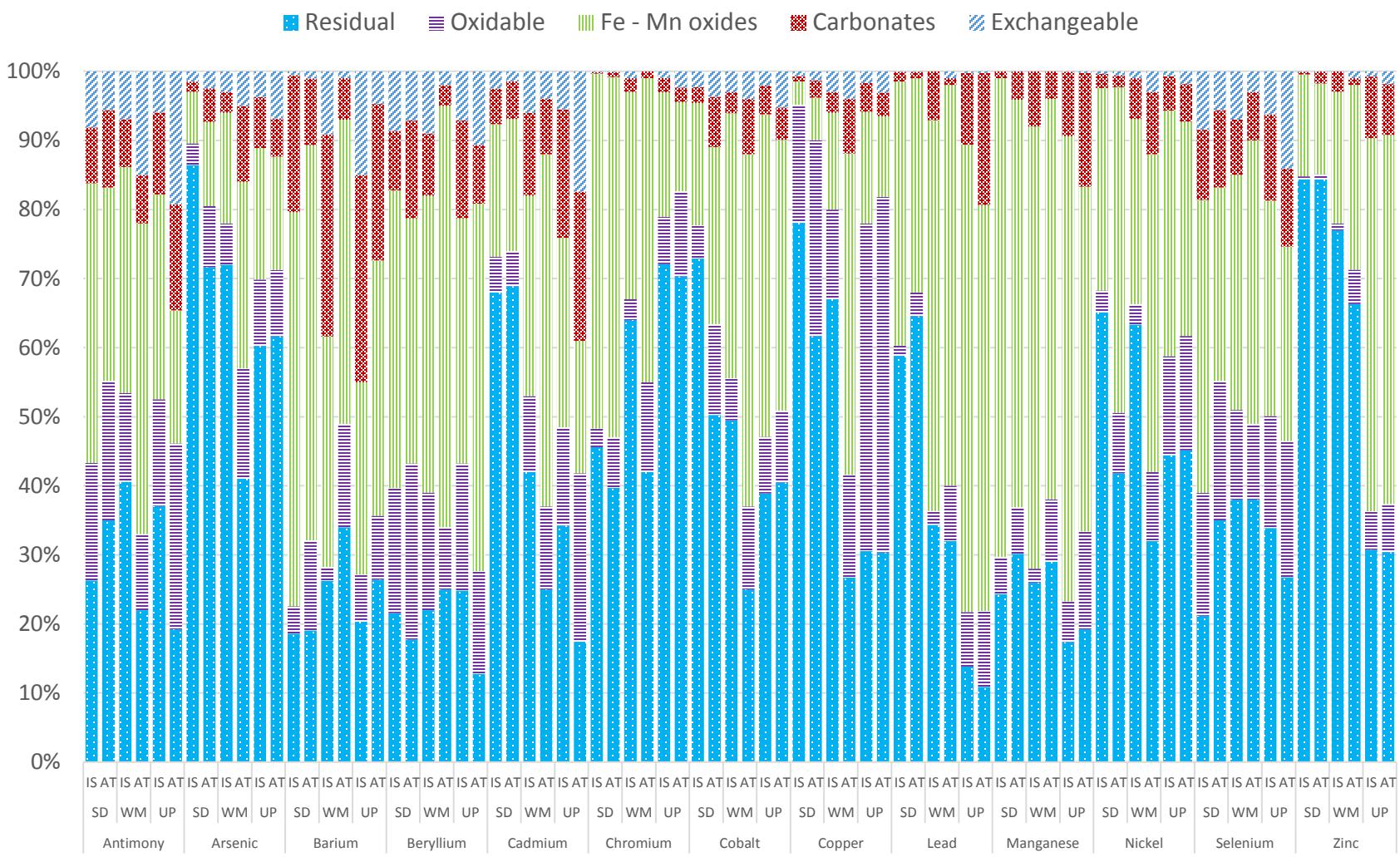
A3. Metal distribution comparison between soils of surviving plant plots, Switchgrass (SWG), Little Bluestem (LBS) and Yellow Cone Flower (YCF), and root soil (R-SWG, R-LBS, R-YCF) at slag disposal area



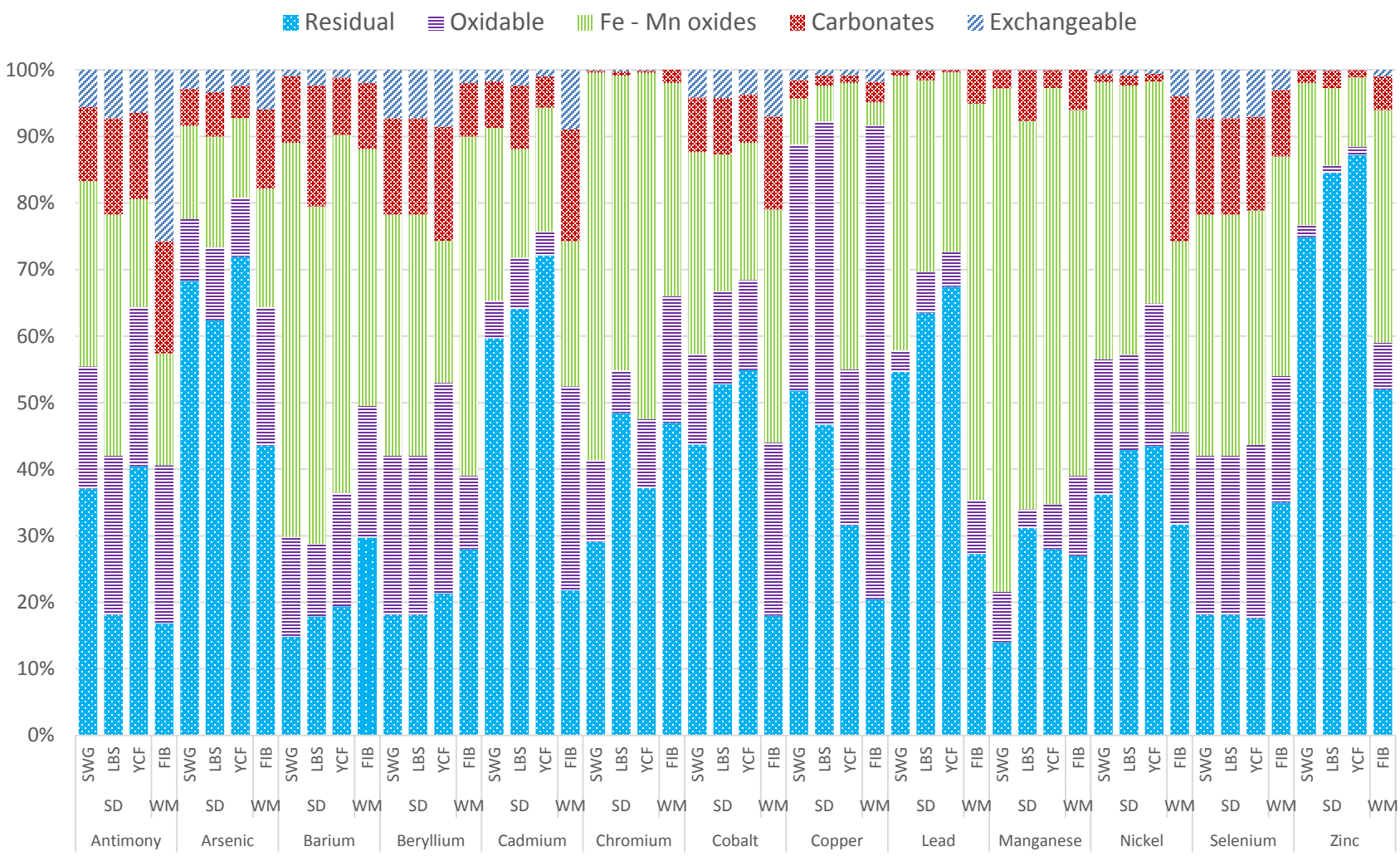
A4. Total metal distribution comparison between soil before and after tilling, at the end of the third season and root soil at FIB plot at the wet meadow area



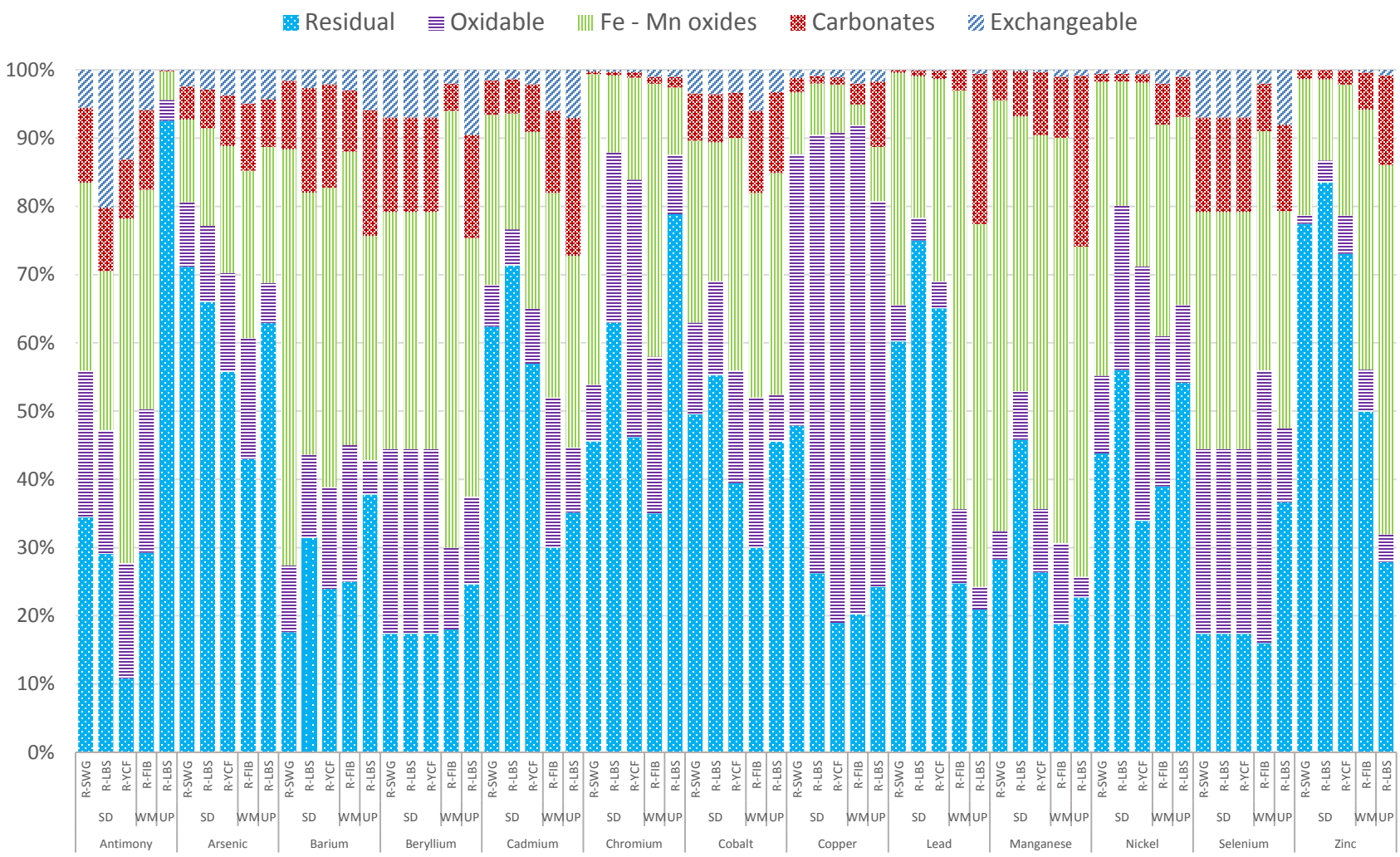
A5. Total metals fractionation in soil before, after tilling and in root soil of LBS at the upland area



A6. Total metals distribution comparison between soil before tilling (BT) and after tilling (AT) at the three experimental areas: slag disposal (SD), wet meadow (WM) and upland (UP)



A7. Total metal distribution comparison of the different surviving plots soil at the experimental areas: slag disposal (SD) and wet meadow (WM) at the end of the third growing season



A8. Total metal distribution comparison of the root zone soil at the different surviving plots at the three experimental areas: slag disposal area (SD), wet meadow area (WM) and upland area (UP) at the end of the third growing season

APPENDIX B

VITA

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EDUCATION	BSc. Environmental Sciences, University of Málaga, Málaga, Spain, June 2012
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HONORS	Andalucia Tech Scholarship for the Incorporation of Undergraduate Student to Research Groups at the University of Málaga, Málaga, Spain. November 2012
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PUBLICATIONS	Amaya-Santos, G., and Reddy, K. R. (2017). "Field Evaluation of Switchgrass (Panicum virgatum) to Phytoremediate Mixed Contaminants at Slag-Fill Site". Geotechnical Frontiers, Orlando, FL, March 12-15.
	Villen-Guzman, M., Paz-Garcia, J. M., Rodriguez-Maroto, J. M., Garcia-Herruzo, F., Amaya-Santos, G., Gomez-Lahoz, C., Vereda-Alonso, C. (2015). "Scaling – up the acid – enhanced electrokinetic remediation of a real contaminated soil". *Electrochimica Acta*, 181:139-145.
	Villen-Guzman, M., Paz-Garcia, J. M., Amaya-Santos, G., Rodriguez-Maroto, J. M., Gomez-Lahoz, C., (2015). "Effects of the buffering capacity of the soil on the mobilization of heavy metals. Equilibrium and kinetics". *Chemosphere*, 131:78-84.