Phytoremediation of Heavy Metals and PAHs in Alkaline Slag Fill at Wet Meadow Site

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Abstract: The feasibility of using phytoremediation to remediate an alkaline slag filled wet meadow site contaminated with polycyclic aromatic hydrocarbons (PAHs) and heavy metals has been studied. The objective of the present work was to investigate the ability of nine native grasses and trees to survive and remediate PAHs such as benzo(a)pyrene (BaP) and heavy metals such as arsenic (As), chromium (Cr), lead (Pb) and manganese (Mn) existing at the site during the three complete growing seasons. Replicate test plots were prepared by tilling and homogenizing the fill material to approximate depth of approximately 0.3m. Soil samples from each plot were collected before and after preparing the test plots and analyzed for physico-chemical properties and contaminant concentrations. The survival and growth of the plant species were monitored for two growing seasons. Only one plant species out of the total nine initially planted, specifically False Indigo Bush (*Amorpha fruticosa*) (FIB), survived the three growing seasons. Soil sample and plant root and shoot samples were collected at the end of the second and third growing seasons at the plot where FIB was planted. PAHs and heavy metals were analyzed in the soil and plant samples. In addition, sequential extraction procedure was followed to determine the fractionation of the heavy metals in soils before and after planting. The results showed no significant decrease in BaP, As, Cr and Pb concentrations in the soil. In addition, there were no significant changes in heavy metals fractions. However, Mn uptake in roots and shoots was observed with corresponding decrease in soil, at the end of the third growing season. The adaptability and survival of FIB and its high tolerance to harsh site conditions (high pH, fluctuating moisture and contaminant toxicity) demonstrated the potential of this species for its use in the remediation of the study area.

Keywords: Phytoremediation; Plants; Soils; Mixed contamination; Field study; Restoration; Sustainable remediation

Introduction

Throughout the United States and even globally, wetlands are an important resource, but they have been steadily disappearing. Wetlands serve as habitats for threatened and endangered species and are enormous sinks for carbon. Meanwhile, they provide crucial environmental functions including remediating water and mitigating floods. In northwestern Indiana and northeastern Illinois (USA), the Lake Calumet region contains some of the richest of the remaining wetlands. Because of the heavy industrial presence in the surrounding region, a high fraction of these wetlands have been degraded. Many of the wetland sediments and upland areas are contaminated (TCI 2011).

Big Marsh site is one of the largest expanses of wetland within the Calumet region. The site is a 121-hectare open space classified as wooded/marshland without any onsite structures, with 35 hectares of wetland. It falls within the Great Lakes Basin and is hydrologically connected to Lake Michigan through Lake Calumet and the Calumet River. Big Marsh site is relatively level and undeveloped with large areas of open water, degraded wetlands, and upland fill areas covered with invasive species of vegetation (TCI 2011).

Big Marsh site is representative of many other unrestored sites in this region which have been significantly altered by the steel industry and decades of legal and illegal dumping. The site 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 polycyclic aromatic hydrocarbons (PAHs) and heavy metals at slightly higher than permissible limits (IEPA 2011). The site contamination is extended over larger area, but limited to shallow depths (TCI 2011).

Remediation of sites with mixed contamination has been challenging, due to different physico-chemical properties of the contaminants and their interactions with soil and fill materials. Several technologies have been developed for the remediation of contaminated

sites (Sharma and Reddy 2004). However, many of these technologies are applicable for one type of contaminants. In case of mixed contamination, few technologies have been proven to be effective, especially for the sites with larger area and shallow depth contamination. In this context, phytoremediation has great potential to be effective and sustainable for the treatment of contaminated sites with mixed contamination (Reddy and Adams 2015; Cameselle et al. 2013).

A previous study showed that the mixed contamination in the soil had a significant effect on the plant growth (Chirakkara et al., 2014; Chirakkara and Reddy 2015a). The ability of the plants to survive at some sites with high contaminant concentrations and unfavorable environmental conditions (pH, moisture, nutrients) can be limiting factors for successful application of phytoremediation at these sites. Therefore, a site-specific feasibility study is recommended to evaluate the applicability of phytoremediation.

A comprehensive investigation was undertaken to evaluate the feasibility of using phytoremediation at different impact areas at Big Marsh site. Three different areas are identified to represent variable conditions at Big Marsh site: (1) slag disposal area; (2) wet meadow area; and (3) upland area. This paper presents the investigation conducted at wet meadow area at Big Marsh site. The investigations conducted at other two areas (slag disposal area and upland area) are presented by Amaya-Santos (2016) and Reddy et al. (2017). The wet meadow area is located in the southern part of Big Marsh and consists of a thin layer of top soil underlain by slag fill. The area is subjected to cycles of flooding, with surface water inundated over the area. The study conducted included baseline soil characterization, preparation of the area by tilling and homogenization for planting, planting different types of plants, monitoring the survival and growth of the plants and testing of soil and plant samples to evaluate the fate of the contaminants. The results are finally used to assess phytoremediation feasibility at the site.

Research Methodology

Baseline Soil Characterization

The baseline soil sampling was conducted on the site to determine physico-chemical properties of the soils and the contamination present in them. Three soil samples were collected at different locations of the site and these sampling locations were recorded using global positioning system (GPS). Soil samples were oven-dried and then tested for physico-chemical properties and contaminant concentrations using the methods shown in Table 1. Additionally, soil samples were collected at different depths and then their pH was measured to assess the variation of soil pH with depth due to variable fill conditions at the site.

Test Plots

The study area at the site was identified based on the baseline soil characterization results. Two test plots, called experimental plot and adjacent plot, each 15 m x 15 m, were demarcated (Figure 1a). In these plots, soil was tilled and homogenized using mechanized equipment approximately to a depth of 0.3 m. The experimental plot (15m x 15m) was divided into two types of subplots to establish herbaceous plants and woody plants. The subplots intended for planting herbaceous plants were called GP (Grasses and Plugs) subplots, and those used for planting trees and shrubs were called TS (Trees and Shrubs) subplots (Figure 1a). A total number of 5 GP subplots, each 2.4m x 3.7m, were selected. Each of these GP subplots was divided into 6 cells of size 1.2m x 1.2m, and each cell was divided into 16 subcells of size 0.3m x 0.3m in which a plant could be planted (Figure 1b). Similarly, 5 TS subplots, each 3m x 3m each, were selected and each subplot was divided into 4 cells, each of size 1.5m x 1.5m, each cell was used to plant a tree (Figure 1c). One soil sample from each cell of subplots was collected and tested for soil properties and contaminant concentrations, to assess the effects of tilling and homogenizing and serve as the initial

conditions prior to planting. The adjacent plot (15m x 15m) was delineated next to the experimental plot with the purpose of monitoring plant survival and growth characteristics of the grass species. The survival and growth characteristics of plants in the experimental plot and the adjacent plant were similar. Hence the results of monitoring of the plants in the adjacent plot are not presented as there was no soil and plant sampling was performed in the adjacent plot.

Plant Selection and Planting

Plants were selected based on their phytoremedial properties and the site soil conditions. A total of 9 native species that included 5 species of grasses and plugs and 4 species of trees and shrubs were chosen (Table 2).

The GS subplots were divided into 6 cells, and each cell was used to plant a particular type of grass species except the sixth cell which was used to plant all 5 grass species together to assess any potential synergistic effects. A total of 16 samples of the same species were planted at each cell, and 3 samples of each species were planted at the mixed plant cell. A total of 95 grass samples of each type were planted within the experimental plot, and additional 50 grass samples of each type were planted in the adjacent plot (Table 2).

The TS subplots intended for planting trees and shrubs were divided into 4 cells. Each cell was planted with four samples of selected TS species. In total, 20 samples of each woody species (trees and shrubs) were planted within the experimental plot (Table 2). No woody samples were planted in the adjacent plot. The pictures of the experimental plot before, during and after soil preparation and planting can be seen in Figure 2.

Watering and Monitoring

After soil preparation and planting, the test plots were watered twice a week throughout summer months (June to August) and monitored weekly for survival, quality of leaves and pests infection during the first growing season. For the adjacent plot, only survival of plants was monitored. Table 3 shows the rating system created and used for monitoring purposes.

During the second growing season, the test plots were monitored bi-weekly during the summer. No additional water or pest control was performed 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.

Termination Sampling

By the end of the second growing season, only FIB (woody plant) survived and all sampling was done at the FIB plots. Soil samples were collected from each FIB plot. All soil samples were kept on ice during the day. Vegetative biomass, divided into above-ground (leaves and shoots) and below-ground (roots), was taken from one representative sample plant at each of FIB plots. All the samples were transported back to the lab, weighed, and oven–dried. Contaminant concentration analysis was performed on all soil and vegetative samples.

At the end of the third growing season, a terminal sampling was performed. Soil samples were collected from all FIB plots. Soil samples from the root zone were separately collected. Vegetative samples consisting of roots, leaves and shoots of FIB were also collected. Additionally, two grab samples of invasive vegetation (Phragmites) at the site were collected to assess their uptake of the contaminants, if any. Physico-chemical properties of soils and the concentrations of PAHs and heavy metals in soil and plant biomass samples were analyzed using the methods listed in Table 1.

Soil and Plant Sample Testing Procedures

Soil samples were tested for physico-chemical properties, specifically the pH, electrical conductivity (EC), organic content (OC), oxidation–reduction potential (ORP), water holding capacity (WHC), grain size distribution (GSD), and exchangeable nutrients content. These tests were conducted according to the standard ASTM procedures (ASTM 2015). The soil pH and ORP were measured according to the ASTM D4972 using an Orion Model 720-A pH/ISE meter. Water content was measured according to ASTM D2216. Organic content was determined using ASTM D2974. 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 D422. To analyze exchangeable nitrogen, 1 gram of soil was mixed and shaken with 10 mL of 2M KCl solution for 1 hour (Xu et al., 2013). The filtered extractant was analyzed using Spectronic Genesys Spectrophotometer, following the procedure reported by Sattayatewa et al. (2011). To measure the exchangeable phosphorus, 1 gram of soil was mixed and shaken with 1 M ammonium acetate for 1 hour. 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.

Soil and vegetative samples acid digested and analyzed for heavy metal concentrations using EPA method SW6020 with inductively coupled plasma mass spectrometry (ICP/MS), and PAHs were analyzed using EPA method SW8270C with gas chromatography mass spectrometry (GC/MS) (USEPA 1986). In order to assess the uptake by the plant, the percentage plant contaminant uptake is defined as follows:

$$\%\ contaminant\ uptake = \frac{Contaminat\ mass\ in\ the\ plant}{Initial\ contaminant\ mass\ in\ the\ soil} \cdot 100$$

Sequential extraction was performed following the procedure developed by Tessier et al. (1979) with slight modifications to determine the speciation or fractionation of heavy

metals in the soil samples collected (Amaya-Santos 2016). Extracts from sequential extractions were analyzed for heavy metal concentrations with ICP/MS using EPA method SW6020 (USEPA 1986). Benzo(a)pyrene (BaP) was used as a representative target PAH contaminant in this study due its known carcinogenic and mutagenic potential. Among the heavy metals, arsenic (As), chromium (Cr), lead (Pb) and manganese (Mn) were used as the representative or target heavy metal contaminants in this study. To minimize analytical testing, the target contaminants (BaP, As, Cr, Pb and Mn) were analyzed in multiple samples, whereas entire list of USEPA heavy metals and PAHs were analyzed in selected samples.

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

Results and Discussion

Baseline Soil Characterization

The baseline soil characterization results are shown in Table 4. The average pH of the surface soil at the beginning of this study was 7.29. The ORP is negative, which indicates reducing conditions in the soil. The soil was predominantly sandy soil with low organic content. The grain size distribution of the soil is shown in Figure 6. Based on the additional samples collected at different depths, the soil pH was 11 at 36 cm depth and ranged from 9 to 10 near the surface. Table 5 shows the concentrations of PAHs and heavy metals found initially in the soil at the experimental area.

Soil Characterization after Tilling

Soil properties after tilling and homogenization are shown in Table 4. After tilling, the pH of the soil was 10, which is about 2.5 units higher than the surface soil as found in the baseline condition. This increase of pH is likely due to mixing of high pH slag and fill materials underlying the thin surface top soil, during tilling and homogenization. The organic content in the soil increased from 2.56% to 5.48% after tilling. As no organic amendments were added to the soil, the increase in organic content is attributed to variable organic content of the topsoil at the site. The ORP increased one order of magnitude, resulting in highly reductive conditions. The exchangeable phosphate and nitrate decreased by 83% and 52%, respectively, probably due to mixing with underlying slag fill materials. The water holding capacity did not change, likely due to the predominant presence of the sand fraction, which increased after tilling.

The PAHs concentrations of the soil are shown in Table 5. The target contaminant BaP was analyzed in 4 composite soil samples, whereas all PAHs were analyzed in only one sample. No significant differences were found in BaP concentration values before and after the soil tilling ($p<0.05$). Heavy metal concentrations can also be seen in Table 5. As and Pb concentrations in the soil decreased after tilling ($p<0.05$). The differences found in As and Pb concentrations after tilling may be due to spatial variability of contamination. For the baseline conditions, the As and Pb concentrations were 7±0.69 mg/kg and 111±24.30 mg/kg, respectively, while the concentrations of Cr and Mn were 36 mg/kg and 1400 mg/kg, respectively. After tilling, concentrations of Pb and As decreased, while the concentrations of Cr and Mn increased. These results are attributed to mixing of the top soil with underlying slag fill materials and heterogeneous distribution of the contaminants in the soils at the site. The flooding cycles and the variable moisture conditions of the site may have contributed to spatial variability of the contaminant concentrations in the study area.

248 The results of heavy metals fractionation in the soil are shown in Table 6. The results
249 for target heavy metals As, Cr, Pb and Mn are plotted in Figure 7. These results show that As
250 and Cr predominantly exist in the residual fraction, while more than 50% of the Pb and Mn
251 exists as the Fe/Mn-oxides bound fraction. On the other hand, the exchangeable fraction,
252 which is the most bioavailable fraction in the soil is very low for all the metals. Due to tilling,
253 Fe/Mn- oxides fraction of As and Cr increased, while fractions of Pb and Mn remained the
254 same. Organic fraction of all heavy metals increased for all heavy metals after tilling, while
255 carbonates–bound fraction increased for As and decreased for Pb and Mn, and remained the
256 same for Cr.
257
258 ***Plant Monitoring***
259 Figures 3 and 4 show the survival and growth of CGS (a grass species) and FIB (a woody
260 species), respectively, during the monitoring period. Figure 5 shows the monitoring results
261 for the first and second growing seasons based on the monitoring scheme presented in Table
262 3. During the first growing season, all the species showed high survival rates. However, a
263 sharp decrease is found at the second season for all the species, with exemption of FIB. The
264 plant survival rates found in the adjacent plots and in the mix plant subcell were similar to
265 those in the experimental plots. Invasive plant species were also not observed in the test plots,
266 highlighting the harsh conditions for the establishment of any plants on the site. The plants
267 survival and leaf quality assessment (Figure 5b) was performed on woody species, only
268 during the first growing season, with the aim of carrying out a detailed monitoring of their
269 development and growth and their adaptation to the site conditions. As it can be observed,
270 FIB reached the best performance out of all the selected species. It should be noted that
271 intensive plant monitoring was performed during the first growing season, and the plants
272 were watered weekly during the summer, allowing the soil moisture to remain constant. The

regular watering and availability of nutrients present in the potting soil with the plants may have helped the plants to survive during the first year. During the second season, on the contrast, no watering or pest control was performed, and the potting soil nutrients progressive consumption may have affected the plant survival rates. No monitoring was performed during the third growing season. However, field observations made during the terminal sampling showed that all species planted, except FIB, survived at the end of the experiment.

None of the plant species except FIB survived by the end of the third growing season. However, some signs of stress were observed in FIB during the terminal sampling. The roots of the FIB appeared restricted to the original planting zone and appeared to grow laterally without penetrating into the underlying slag material and showed a high density of nodules.

The topsoil has near neutral pH as compared to underlying highly alkaline slag fill. Due to tilling, the topsoil and underlying slag fill were mixed, raising the pH of the soil in the tilled depth (0.3m). The high pH of the soil was likely the main reason limiting the plant survival and growth. According to the United States Department of Agriculture (USDA) plants database, all of the plant species used in this study require an optimum pH ranging from slightly acidic to neutral (USDA, 2016). In this regard, FIB appeared to have tolerance to wider pH range.

Additionally, the drought or flood tolerance of the plant species was low as the site was subjected to cycles of submerged and dry conditions. The drastic variations in soil moisture in the experimental area could have also contributed to limited survival and growth of plants. The soil in the study area experienced long periods of drought followed by flooding periods, which may have caused the deterioration of the plants especially the phenotype, which is not acclimated to this type of moisture variations.

Other factors may have also contributed to the limited plant growth, such as macronutrients deficiency (Pulford 1991). High concentration of contaminants may have also

caused phytotoxicity, leading to poor survival of the plants selected in this study. Previous studies obtained in lab scale pointed phytotoxicity of mixed contaminants (PAHs and heavy metals) resulting in poor survival and growth of plants (Chirakkara and Reddy 2015a,b; Shahid et al. 2014). On the other hand, FIB, like other species of the family *Fabaceae*, possesses properties that make it resistant to heavy metal pollution. The advantages of this plant 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 may be the key reason for low phytotoxicity (Chaudri et al., 2000). In addition, FIB can tolerate a wide range of soil moisture, and is able to survive in saturated or very wet soil and also under prolonged periods of drought (Hong et al. 2010). This adaptability, together with the capacity of fixing nitrogen, can explain the suitability of FIB to survive the conditions of the study area. Overall, FIB showed extraordinary resistance to the severe conditions of the experimental area and thrived by the end of the third season. As a result, the fate of the contaminants in the soil in the FIB plots and within the FIB plants was further investigated.

Fate of PAHs

The PAH concentrations in the soil at the FIB plot are summarized in Table 5. The target PAH contaminant selected for this study was benzo(a)pyrene (BaP), which was analyzed with enough replicates to perform statistical analysis (the average and standard deviation results are shown in the table). Results for the baseline soil (see Table 5) reveal high concentrations of PAHs as compared to the soil samples taken after two growing seasons. The results of BaP in the baseline 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 baseline 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 7 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 PAHs are not sorbed or degraded by the plant. The results in the present study are consistent with Chekol et al. (2002) and Yan (2012) in that the plants did not affect the dissipation of organic contaminants such as pyrene or trinitrotoluene (TNT) in the soil.

No detailed phytoremediation studies have been reported investigating degradation of PAHs using FIB. However, several studies have been reported on phytoremediation using legume species. Fu et al. (2012) investigated alfalfa for phytoremediation of BaP in a PAH – contaminated soil and found 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 phenanthrene, 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.

Fate of Heavy Metals

Heavy metal concentrations in the bulk soil in the FIB subplot are summarized in Table 5. The target contaminants (As, Cr, Pb and Mn) were analyzed with enough replicates to perform statistical analysis (average and standard deviation are shown in the table). These statistical analysis results show a high spatial variability in terms of heavy metals distribution 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 that decreased slightly ($p<0.05$). These results show that very little mobilization of the heavy metals occurred; they remained in the soil in spite of 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 shown a different behavior, and its concentration tends to decrease when compared to the unplanted tilled soil.

The heavy metals concentrations in stems and leaves of the FIB are presented in Table 7. The concentrations of heavy metal in the plant 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, Cr, Pb and Mn were detected in the roots of FIB at concentrations 5 mg/kg, 10 mg/kg and 480 mg/kg, respectively. Although concentrations of Pb and Mn were found in the root biomass, the proportion of contaminant mass uptake from the soil was very low (Table 8).

The fractionation of heavy metals in the soil in the FIB plot are shown in Table 6, and the fractionation of the target heavy metals are plotted in Figure 7. Two different tendencies are observed on the fractionation of the target heavy metals. The residual fraction of As and Cr reduced after tilling the soil, and the metals tend to be retained in the organic and reducible fractions. The more bioavailable fractions (exchangeable and carbonates) also tend to increase, although the proportion of contaminant uptake by the plant was very small (Table

8). The highest percentages of Pb and Mn, on the other hand, tend to remain retained in the fraction bounded to Fe/Mn-oxides (reducible fraction). The 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.

In the soil–water environment, the chemical form of a metal determines the biological availability and chemical reactivity such as sorption/desorption and precipitation/dissolution. The mobility of heavy metals in the soil can be affected by the pH and local equilibriums or kinetic limitations (Tack and Verloo, 1995; Villén – Guzmán et al., 2015). Among the variables, soil pH is the most important as it controls the solubility of metal hydroxides, carbonates and phosphates (Clemente et al., 2003; Carrillo – González et al., 2006). Soil moisture regime can also affect the transformation rate of heavy metals (Zheng and Hang 2011; Li et al 2015). The latter study found that when the factors, high pH and wetting–drying cycles are combined, the available fractions of metals decrease. It appears that the moisture cycles along with the high pH conditions in the study area could have contributed to low mobilization of metals.

The results of this study show that Pb and Mn in the root of the FIB (10 mg/kg and 480 mg/kg, respectively) could be from Fe/Mn-oxides fractions present in the soil (Figure 7). Both Fe and Mn are two essential micronutrients for the development of the plant, but their bioavailability is subjected to the chemical conditions in the environment. Under an oxidizing atmosphere and alkaline pH, these metals exist as insoluble oxides, making them 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. When there is oxygen deficiency in the environment, the redox potential changes, and NO_3^-, Mn and Fe serve as alternative electron acceptors for microbial respiration, transformed into their reduced ionic species. Therefore, this could have increased the solubility and availability of Mn and Fe (Rengel, 2000).

Although some amount of Mn and Pb are found to undergo uptake by the plant (FIB), 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 indicates the existence of some mechanism whereby the plant assimilates the metals, but they remain 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, 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 4) 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 phytoremediation 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 (Gawronski 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 8).

The results of the present study are consistent with those reported by 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 metal.

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.

Root Soil Characterization

Root zone soil collected from FIB plots was tested for physico-chemical properties and the results are shown in Table 4. These results can be compared with the bulk soil surrounding

448 the roots (season 3 soil results). As it can be observed, after 3 growing seasons, the pH of the
449 soil decreased in both the bulk soil and the root zone soil, with no significant differences
450 between them. This decrease of pH in the soil could be mainly due to the weatherization of
451 the surface, caused by the moisture–drying cycles at the study area. The organic content in
452 the root–zone soil is 100% higher than the organic content in the bulk soil. This is due to the
453 presence of humic acids, roots exudates and biological activity that occur in the root system.
454 The nitrogen content of the bulk soil at the end of the experiment was higher than that of it
455 before and after tilling, but lower that the nitrogen content in the root–zone soil. The higher
456 presence of organic content and the presence of nitrogen fixing symbiont mycorrhiza in the
457 root system may be the reason for this increase of exchangeable nitrogen in the soil. The
458 presence of phosphate, however, did not increase significantly throughout the experiment,
459 indicating a low availability of this nutrient in the soil at the site.
460
461 **Practical Implications**
462 The harsh conditions of the site were exacerbated by tilling and homogenizing. The topsoil
463 was mixed up with the underlying highly alkaline slag fill, drastically increasing the soil pH.
464 Based on these results, it is advisable to mix the topsoil layer without mixing the deeper soil
465 and fill materials or neutralize soil pH before planting. Furthermore, in order to improve the
466 success of the phytoremediation, it would be important to evaluate the soil conditions and
467 contaminant concentrations in the soil after tilling and homogenization, instead of baseline
468 soil conditions, as significant changes could occur to the soil that can affect plant survival and
469 growth.
470 Due to the low survival of all the plant species except FIB, it is recommended to
471 establish the necessary initial conditions for a better survival and growth of the selected
472 species. This could be accomplished by amending the soil with organic-rich material such as

compost or biochar, in order to buffer pH and the toxicity of the heavy metals and provide nutrients to the plants. Therefore, the addition of organic amendment in the experimental area is highly recommended.

The high tolerance of FIB to site harsh conditions makes it ideal to use for phytoremediation of sites with similar characteristics. In addition, legumes have potential to be applicable due to their capacity to survive and resist heavy metal toxicity.

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

Conclusions

Field investigation revealed only one out of nine selected 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 PAHs (as reflected by BaP) by the surviving FIB was not observed. PAHs were also not assimilated by the plant. The presence of FIB did not affect the mobility and speciation of heavy metals in the soil. Only decrease of initial Mn occurred. Mn was also detected in roots and shoots of FIB, indicating that there exists assimilation of this metal 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 metals were taken up 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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References

Amaya-Santos, G. (2016). Field-scale phytoremediation of mixed contaminated site in Chicago, USA. MS Thesis, University of Illinois, Chicago.

ASTM (2015). Annual Book of Standards, Volume 04.08 Soil and Rock, ASTM International, West Conshohocken, PA.

Brookes P C (1995). The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils. 19: 269 – 279.

Cameselle C, Chirakkara RA, Reddy KR (2013). Electrokinetic – enhanced phytoremediation of soils: status and opportunities. Chemosphere 93(4): 626 – 636.

Carrillo – González R, Šimůnek J, Sauvé S, Adriano D (2006). Mechanisms and Pathways of Trace Element Mobility in Soils. Advances in Agronomy 91: 111-178.

Chaudri A M., Allain C. M. G, Barbosa – Jefferson V L., Nicholson F A., Chambers B J., McGrath S P (2000). A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. Plant and Soil 221: 167 – 179.

520 Chekol T, Vough R L, Chaney R L (2002). Plant-Soil Contaminant Specificity Affects
521 Phytoremediation of Organic Contaminants, International Journal of
522 Phytoremediation, 4:1, 17-26.

523 Chirakkara R A and Reddy K R (2014). Phytoremediaton of mixed contaminated soils –
524 effects of initial concentration. Geotech Spec Publ 241:1-10.

525 Chirakkara R A and Reddy K R (2015a). Biomass and chemical amendment for enhanced
526 phytoremediation of mixed contaminated soils. Ecological Engineering 85 (2015):
527 265 – 274.

528 Chirakkara, R.A., and Reddy, K.R. (2015b). "Plant species identification for
529 phytoremediation of mixed contaminated soils." J. Hazardous, Toxic and Radioactive
530 Waste, 19(4), 04015004

531 Clemente R, Walker D J, Roig A, Bernal M P (2002). Heavy metal bioavailability in a soil
532 affected by mineral sulphides contamination following the mine spillage at
533 Aznalcóllar (Spain). Biodegradation 14: 199 – 205.

534 Fu D, Teng Y, Luo Y, Tu C, Li S, Li Z, Christie P (2012). Effects of alfalfa and organic
535 fertilizer on benzo[a]pyrene dissipation in an aged contaminated soil. Environ. Sci.
536 Pollut. Res 19: 1605 – 1611.

537 Gawronski S W and Gawronska H (2007). Plant taxonomy for phytoremediation in:
538 Advanced science and technology for biological decontamination of sites affected by
539 chemical and radiobiological nuclear agents pp, 79 – 88. Marmiroli N, Samotokin B
540 and Marmiroli M (eds). Springer P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

541 Giller K E, McGrath S P, Hirsch P R (1989). Absence of nitrogen fixation in clover grown on
542 soil subject to long term contamination with heavy metals is due to survival of only
543 ineffective Rhizobium. Soil Biology and Biochemistry 21: 841 – 848.

544 Hong E, Ketterings Q, McBride (2010). Manganese. Fact Sheet 49. Agronomy Fact Sheet
545 Series. Cornell University Cooperative Extension.
546 http://nmsp.cals.cornell.edu/publications/factsheets/factsheet49.pdf.

547 IEPA Illinois Environmental Protection Agency Web (2011). Tiered Approach to Corrective
548 Action Objectives (TACO) - Illinois EPA - Bureau of Land. Illinois.

549 Kabata - Pendias A and Pendias H (2001). Trace elements in soils and plants, 3rd Ed CRC
550 Press, Boca Raton, FL.

551 Li Z, Wu L, Zhang H, Luo Y, Christie P (2015). Effects of soil drying and wetting – drying
552 cycles on the availability of heavy metals and their relationship to dissolved organic
553 matter. J. Soils Sediments 15: 1510 – 1519.

554 Lorenz S E, McGrath S P, Giller K E (1992). Assessment of free – living nitrogen fixation
555 activity as a biological indicator of heavy metal toxicity in soil. Soil Biology and
556 Biochemistry. 24: 601 – 606.

557 Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010). Metal tolerance and
558 hyperaccumulation: costs and trade-offs between traits and environment. Environ Exp
559 Bot 68:1–13.

560 Piechalak A, Tomaszewska B, Baralkiewiez D, Maleka A (2002). Accumulation and
561 detoxification of lead ions in legumes. Phytochemistry 60: 153 – 162.

562 Pulford I D (1991). Nutrient provision and cycling in soils in urban areas. In: Soils in the
563 urban environment. Oxford: Blackwell; p. 119– 38.

564 Rascio N and Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do
565 they do it? And what makes them so interesting? Plant Sci 180:169–181.

566 Reddy, K.R. and Adams, J.A. (2015). Sustainable Remediation of Contaminated Sites.
567 Momentum Press, New York,

568 Reddy, K.R., Amaya-Santos, G. and Cooper D.E. (2017). Field-scale phytoremediation of
569 mixed contaminants in upland area of Big Marsh site, Chicaago, USA. Indian
570 Geotechnical Journal.

571 Rea, P.A. (2012). Phytochelatin synthesis: of a protease a peptide polymerase made.,
572 Physiologia Plantarum, 145: 154-164.

573 Rengel, Z (2000). Uptake and transport of manganese in plants. In: A. Sigel, H. Sigel (ed.)
574 Metal Ions in Biological Systems. Marcel Dekker, New York, pp: 57-87.

575 Sattayatewa, C, Arnaldos M, Pagilla K, (2011). Measurement of organic nitrogen and
576 phosphorus fractions at very low concentrations in wastewater effluents. Water
577 Environ. Res. 83 (8) (2011): 675–683.

578 Seo K W, Son Y, Rhoades C C, Noh N J, Koo J K, Kim J (2008). Seedling Growth and
579 Heavy Metal Accumulation of Candidate Woody Species for Revegetating Korean
580 Mine Spoils. Restoration Ecology 16: 702 – 712.

581 Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E (2014). Heavy – Metal –
582 Induced reactive oxygen species: phytotoxicity and phyisicochemical changes in
583 plants. Reviews of Environmental Contamination and Toxicology 232.

584 Sharma H D and Reddy K R (2004). Geoenvironmental Engineering: Site Remediation,
585 Waste Containment, and Emerging Waste Management Technologies. John Wiley &
586 Sons, Hoboken, NJ (2004).

587 Shi X, Zhang X, Chen G, Chen Y, Wang L, Shan X (2011). Seedling growth and metal
588 accumulation of selected woody species in copper and lead/zinc mine tailings. Journal
589 of Environmental Sciences 23 (2) 266 – 274.

590 Smith KE, Schwab AP, Banks MK (2008) Dissipation of PAHs in saturated, dredged
591 sediments: a field trial. Chemosphere 72:1614–1619.

592 Tack F and Verloo M G, (1995). Chemical speciation and fractionation in soil and sediment
593 heavy metal analysis: a review. International Journal of Environmental Analytical
594 Chemistry, 59(2): 225 – 238.

595 TCI (Terracon Consultants, Inc.) (2011). Phase I Environmental Assessment: Big Marsh,
596 South Stony Island Avenue between 110th Street and 116th Street, Chicago, Cook
597 County, Illinois. March 25, 2011. Terracon Project No.: A2117000-Task 2b. 732 p.
598 Prepared for the Chicago Park District.

599 Tessier A, Campbell PGC, Bisson M (1979) Sequential Extraction Procedure for the
600 Speciation of Particulate Trace Metals. Analytical Chemistry 51 (7): 844 – 850.

601 USEPA (United States Environmental Protection Agency) (1986) Test methods for
602 evaluating solid waste laboratory manual physical/chemical methods, SW-846, 3rd
603 Ed., US Environmental Protection Agency Office of Solid Waste and Emergency
604 Response, Washington, D.C.

605 USDA (United States Department of Agriculture) (2016). NRCS (Natural Resources
606 Conservation Service) National Plants Material Center. Beltsville, MD.
607 http://plants.usda.gov/java/ Accessed on June 22, 2016.

608 Villén – Guzmán M, Paz-García J M, Amaya – Santos G, Rodríguez – Maroto J M., Vereda –
609 Alonso C, Gómez – Lahoz C (2015) Effects of the buffering capacity of the soil on
610 the mobilization of heavy metals. Equilibrium and kinetics. Chemosphere 131: 78 –
611 84.

612 Yan L (2012). The use of plants, including trees, to remediate oil contaminated soils: a
613 review and empirical study. MSc Degree Thesis in Forest Ecology and Management.
614 University of Helsinki.

615 Xu G, Wei L L, Sun J N, Shao H B, Chang S X (2013). What is more important for
616 enhancing nutrient bioavailability with biochar application into a sandy soil: Direct or
617 indirect mechanism? Ecological Engineering, 52: 119 – 124.

618 Zhao X, Liu J, Xia X, Chu J, Wei Y, Shi S, Chang E, Yin W, Jiang Z (2014). The evaluation
619 of heavy metal accumulation and application of a comprehensive bio – concentration
620 index for woody species on contaminated sites in Hunan, China. Environmental
621 Science and Pollution Research 21: 5076 – 5085.

622 Zheng S and Hang M (2011). Effect of moisture regime on the redistribution of heavy metals
623 in paddy soil. Journal of Environmental Sciences, 23 (3): 434 – 443.
624

625 Table 1. Test methods for characterization of soil

Soil parameter	**Testing method/reference**
pH	ASTM D4972
Oxidation-reduction potential (ORP)	ASTM D4972
Electrical conductivity	ASTM D4972
Water content	ASTM D2216
Organic content	ASTM D2974
Grain size distribution	ASTM D422
Exchangeable nitrate	Sattayatewa et al. (2011)
Exchangeable phosphorous	Sattayatewa et al. (2011)
Water holding capacity	ASTM D2980
Heavy metals	USEPA method SW020 (USEPA 1986)
Polycyclic aromatic hydrocarbons	USEPA method SW8270 (USEPA 1986)

626

627

628 Table 2. Species selected for phytoremediation at wet meadow area

Type	**Scientific Name**	**Common Name**	**Sample ID**	**Number of Samples**	
				Experimental Plot	**Adjacent Plot**
Grasses and Plugs	*Asclepias incarnata*	Swamp milkweed	SMW	95	50
	Cassia hebecarpa	Wild Senna	WSA	95	50
	Deschampsia caespitosa	Tufted hair grass	THG	95	50
	Solidago graminifolia	Common grass-leaved goldenrod	CGG	95	50
	Spartina pectinata	Prairie cord grass	PCG	95	50
Trees	*Acer saccharinum*	Silver maple	SMP	20	0
	Quercus bicolor	Swamp white oak	SWO	20	0
Shrubs	*Amorpha fruticosa*	False indigo bush	FIB	20	0
	Cornus stolonifera	Red-osier dogwood	ROD	20	0

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631
632 Table 3. Monitoring rating system
633

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.)

634
635

636 Table 4. Soil characterization before, after and at the end of the third growing season
637

Soil Parameter	Baseline 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	-

638 ORP=Oxidation-reduction potential; OC=Organic content; EC=Electrical conductivity;
639 MC=Moisture content; WHC=Water holding capcity
640

641

642 Table 5. Contaminant concentrations in soils

Contaminant	Concentration (mg/kg – dry soil) Baseline 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

643 Target contaminant concentrations Average±SD (number of samples) Before tilling – Season 3,
644 Respectively:
645 a. BaP: 0.4±0.7 (5); 0.1±0.04 (4); 0.1±0.02 (7); 0.1±0.02 (6)
646 b. As: 7±0.69 (5); <DL(3) ±0.7 (4); 4.6±2.75 (7); 4±0.99 (6)
647 c. Cr: 36 (1); 60±18 (4); 62±16 (7); 68±14 (6).
648 d. Pb: 111±24 (5); 59±13 (7); 51±15 (7); 53±19 (6).
649 e. Mn: 1400 (1); 8150±353 (2); 8457±1827 (7); 6767±1015 (6).

Table 6. Percentage of metal fractionation from sequential extraction in False Indigo Bush plot soil

	Baseline 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.

Table 7. Contaminant concentration in leaves and stems of False Indigo Bush

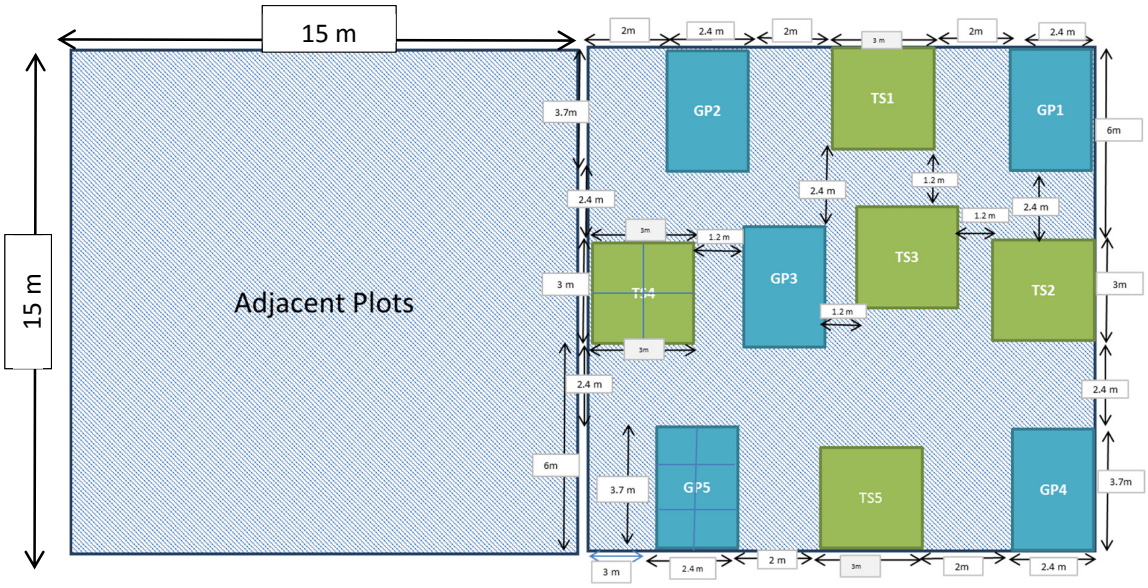
Contaminant	Concentration (mg/kg) Season 2[a]	Season 3
PAHs		
Acenaphthene	<DL (0.3)	
Acenaphthylene	<DL (0.3)	
Anthracene	<DL (0.3)	
Benz(a)anthracene	<DL (0.3)	
Benzo(a)pyrene[b]	<DL (0.3)	<DL (0.03)
Benzo(b)fluoranthene	<DL (0.3)	
Benzo(g,h,i)perylene	<DL (0.3)	
Benzo(k)fluoranthene	<DL (0.3)	
Chrysene	<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 and leaves.
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).
f. Mn: 66.3±25.7 (10); 72±15.2 (4).

Table 8. 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



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 (GP) 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 1. Plots and subplots delineation layout.

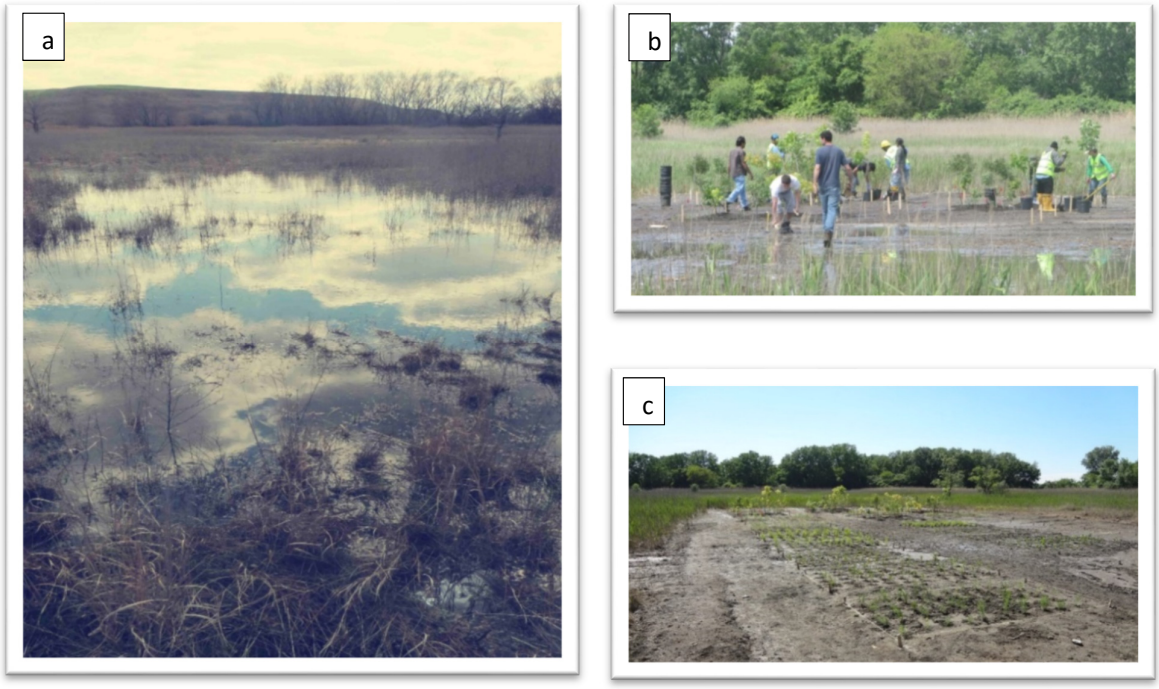


Figure 2. Experimental area. (a) Before any treatment. (b) Planting after tilling and homogenization. (c) After planting

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

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

Figure 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 2nd Season f. End of 3rd season

Figure 4. Monitoring of False Indigo Bush (FIB) at the experimental plot TS1

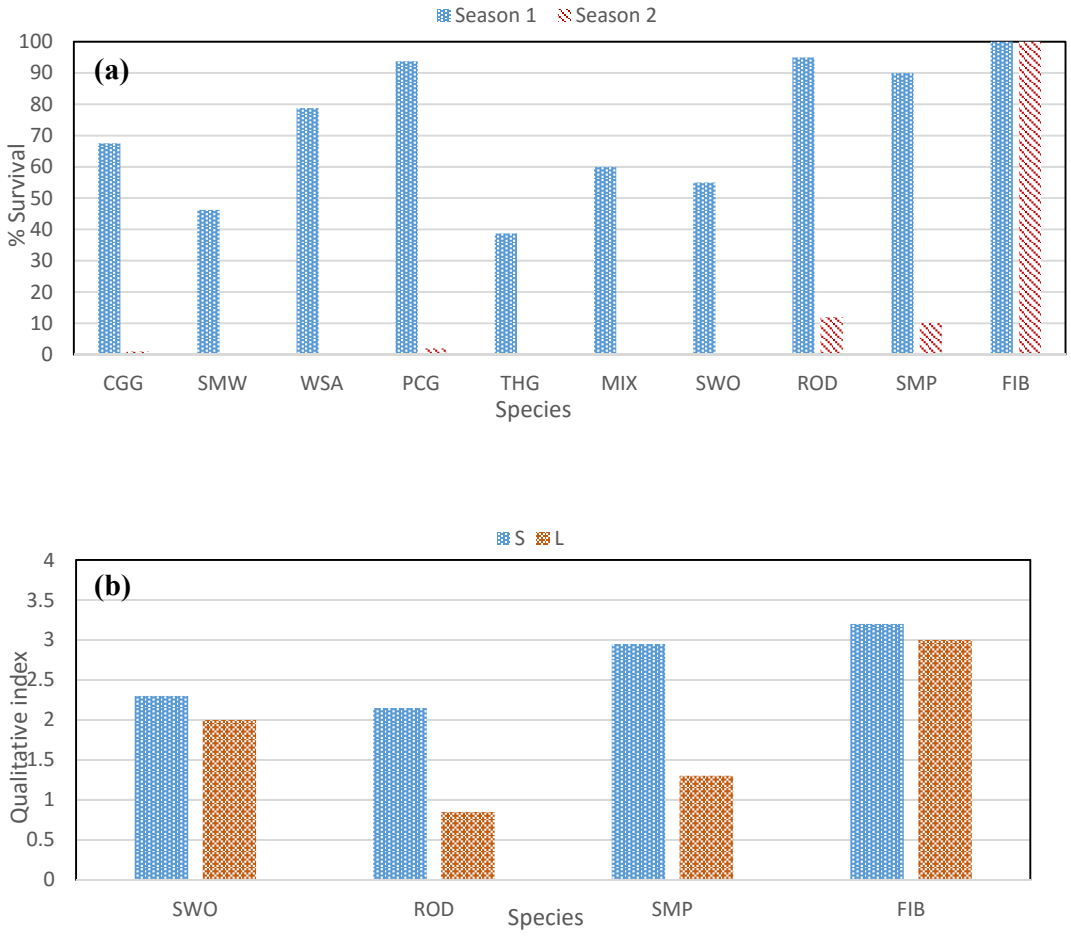


Figure 5. Monitoring rating results (a) Plant survival in the experimental plots at the end of the first and second growing season. (b) Plant survival and leaf quality in grass and trees at the end of the first growing season.

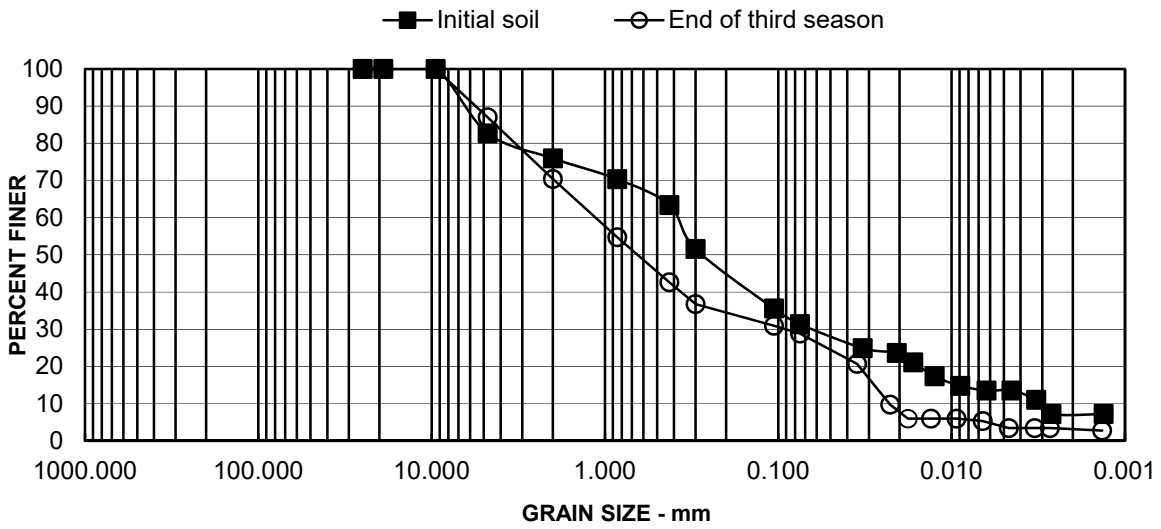


Figure 6. Grain size distribution of soil before tilling and at the end of the third growing season

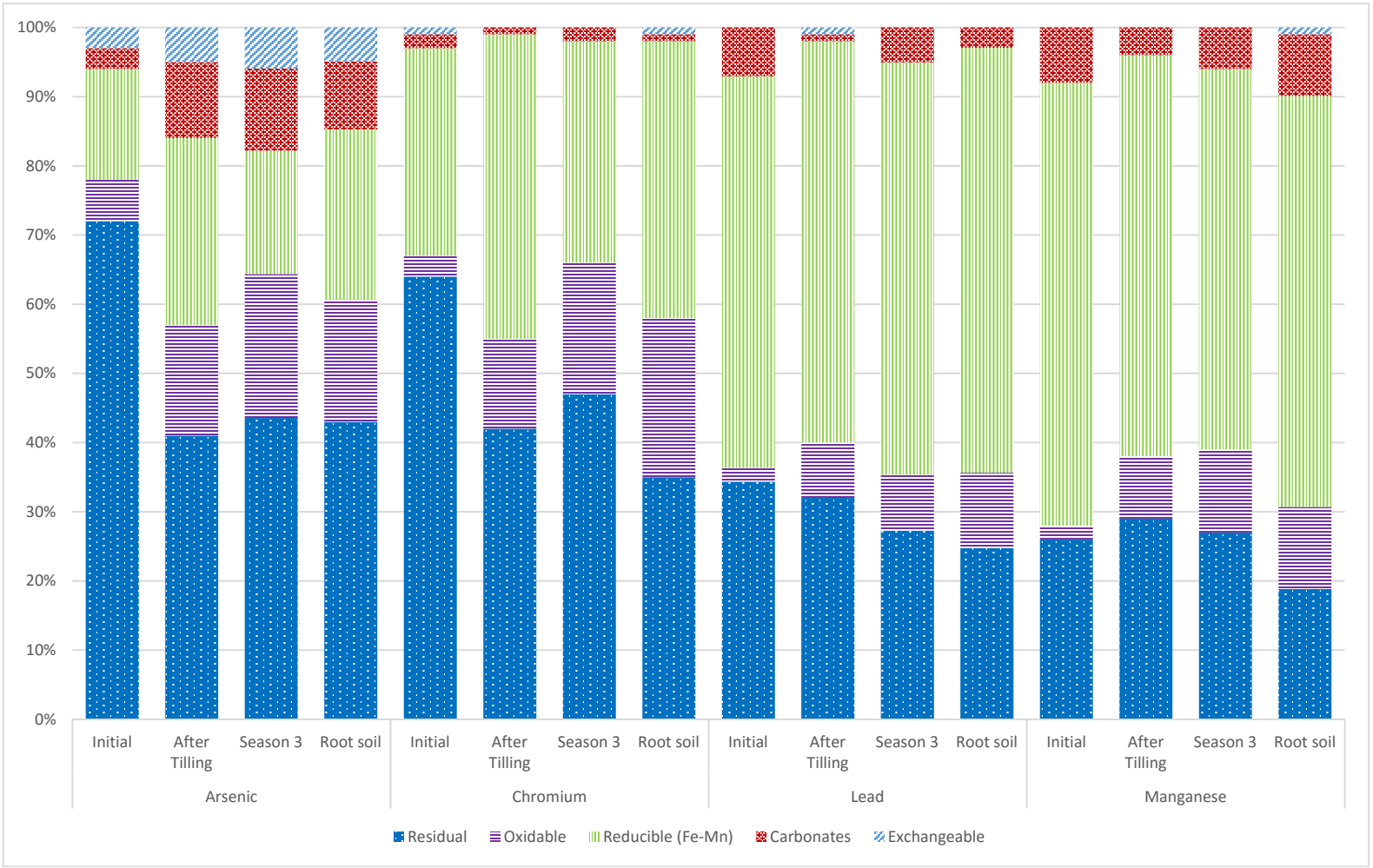


Figure 7. Metal distribution comparison between soil before and after tilling, at the end of the third season and root soil at FIB plot.