Enhancing Cleanup of Heavy Metal Polluted Landfill Soils and Improving Soil Microbial Activity Using Green Technology with Ferrous Sulfate

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***Abstract*- Landfills have led to some of the most intense battles over pollution that has ever been seen. With the population skyrocketing worldwide, these landfills will only become more of a public issue as time goes on. Heavy metals from several sources especially in landfills are an increasingly urgent problem because of its contribution to environmental deterioration and intensive degradation of soil microbial biodiversity. Despite the arguments over landfills in general, few or no effort was undertaken to clean up contamination of heavy metals in abandoned landfills. In our study new methods were proposed using a green technology or phytoremediation with ferrous sulfate in enhancing cleanup of heavy metal polluted landfill soils. Composite soil samples were collected near an open abandoned dump site in Cabanatuan City, Nueva Ecija, Philippines. Three rates of sulfur: 0, 40 and 80 mmol kg^{-1} as ferrous sulfate (26% S) was thoroughly mixed with the soil. Four healthy seedlings of mustard (Brassica juncea, L) were transplanted to each pot. Soil pH showed a decreasing trend for soils treated with 0 and 80 mmol kg^{-1} of sulfur (S) after 15 days (8.12 to 7.38) and after 25 days (8.56 to 7.78). Application of ferrous sulfate significantly enhanced microbial activities in contaminated soils. Average respiration rate in soil with 0 mmol kg^{-1} S was about 2.0 mg kg^{-1} CO_2-C compared with 19.0 mg kg^{-1} CO_2-C for soils amended with 80 mmol S kg^{-1}. Although dry matter yield and uptake of heavy metals by mustard were somewhat variable with S application, solubility of copper (Cu), zinc (Zn) and manganese (Mn) in soils was significantly ($p \leq 0.001$) increased with S application. Our study has demonstrated the beneficial outcome of green technology in combination with ferrous sulfate in cleaning up heavy metals contamination in landfills and at the same time improving soil microbial biomass following phytoremediation.**

Keywords- Landfills; Heavy Metals; Mustard; Ferrous Sulfate; Phytoremediation; Solubility; pH

I. INTRODUCTION

The environmental problems caused by landfills are numerous. While there are many problems with landfills, the negative effects are most commonly placed into two distinct categories: atmospheric effects and hydrological effects. While these effects are both of equal importance, the specific factors that drive them are important to be understood on an individual basis. The widespread pollution of soils due to heavy metals from several sources especially in landfills is an increasingly urgent problem because of its contribution to environmental deterioration and intensive degradation of soil microbial biodiversity. Soil enzyme activities are likely to be inactivated under unfavorable conditions. In addition to various unfavorable conditions, the soil environment is also subject to anthropogenic alterations. The severest of such alterations is the introduction of persistent toxics such as heavy metals [1-2].

Heavy metal contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil, traffic, and agricultural chemicals for plant protection. Metals can exist in the soil solutions as free cations (e.g. Cu^{2+}, Cd^{2+}, Zn^{2+}), as soluble complexes with organic or inorganic ligands (e.g. $ZnCl^{+}$, $CdCl_3^{-}$, metal citrates) and associated with colloidal materials. Severe heavy metal contamination in soil may cause a variety of problems, including the reduction of yield and metal toxicity of plants, animals and humans. It is known that high concentrations of heavy metals in soils have harmful effects on soil enzymes biodiversity, especially in soils where organic matter content is low or has declined. The decontaminations of these soils by engineering methods is highly costing project. Other remediation strategies may include dilution techniques, chemical stabilization, soil washing, thermal desorption and phytoremediation.

Phytoremediation is an emerging green technology that employs the use of higher plants for the cleanup of contaminated soils [1]. The use of plants to extract toxic metals from contaminated soils has emerged as a cost-effective, environment-friendly clean up alternative [2-5]. Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation, phytoremediation may provide an attractive alternative for the cleanup of heavy metal contaminated soils. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contaminant

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degradation abilities of the entire plant body. Nevertheless, a major problem still could hinder plant remediation efficiency because some of the metals are immobile in soils and their phytoextraction rates are limited by solubility and diffusion to the root surface. To overcome this problem, we have added ferrous sulfate to lower soil pH [6-8]. The use of ferrous sulfate has been suggested to decrease soil pH and increase solubility of heavy metals in soils [9-11].

The overall goal of this study was to demonstrate the beneficial outcome of green technology in combination with ferrous sulfate to cleanup soil contaminations in abandoned landfills and at the same time to improve soil microbial biomass following phytoremediation. The objectives of the study were to assess the efficacy of green technology in cleaning up metal contaminations in soils in combination with soil application of ferrous sulfate, determine the ability of sulfur application in enhancing the uptake of heavy metals by mustard, and quantify soil microbial activities following phytoremediation of soils contaminated with heavy metals with or without ferrous sulfate using a Solvita Soil Life test kit.

II. MATERIALS AND METHODS

A. *Soils and Site Description*

Composite soil samples were collected near an open abandoned landfill site (15.5^{o}N latitude; 120.9^{o}W – 121.0^{o}W longitude) in Cabanatuan City, Nueva Ecija, Philippines (Fig. 1). Soil pH of the soil was about 8.4 and contained 650; 62; 8.2; 7.7; 2,130 and 43 mg kg^{-1} of Co; Ni; Zn; Mn; Zn and Cr, respectively. About a kilogram of soils were placed in plastic pots (20 cm diameter $\times$ 20 cm height).

B. *Plants and Cultivation*

Four healthy seedlings of mustard (Brassica juncea, L) were transplanted to each pot. Water was added on the soil at about 60% of the water holding capacity and this level of soil moisture was kept during the whole period of the experiment. All the cultural practices needed to optimally grow mustard were followed for this study. After growing for 15 days and 25 days, respectively, mustards were harvested accordingly. For each harvest date (i.e., at 15 days and at 25 days), aboveground biomass of mustard was assessed for the total plants grown per pot.

C. *Experimental Design, Treatments and Data Analyses*

There were two stages in this work. First stage was the greenhouse study on cleaning up of metal contamination in soil using phytoremediation. The second stage of this study was on assessing the post effect of phytoremediation on soil microbial activities. Detailed descriptions of the methods and materials in the first stage of study were as follows. About 0.13 g of urea (45%N) was added to each pot. Three rates (0, 40 and 80 mmol kg^{-1}) of ferrous sulfate ($FeSO_4$) were thoroughly mixed with the soil. Pots were arranged in the greenhouse following the principle of two-factor experimental design. The main treatment was the sulfur application and harvest time as the sub-treatment effect. Each treatment was replicated three times (27 pots). The data were analyzed following the principle of a two-way ANOVA using PROC GLM [12].

D. *Sampling and Analysis of Soils and Plant Tissues*

Immediately after removing all mustard plants from each pot (i.e., at 15 days and at 25 days after planting), soils were taken from each pot. These soils were analyzed for Mehlich-1 extractable heavy metals and soil microbial respiration following cleanup using green technology or phytoremediation. Concentrations of heavy metals from soils and plants were analyzed using an Inductively Coupled Plasma Spectroscopy. Soil pH was measured with subsamples using a 1:2 of soil: water ratio by pH meter. Soil microbial activities were analyzed using a Solvita Soil Life Kit. The Solvita Soil Life Kit test was used to quantitatively describe the soil respiration rating from no soil microbial activity to unusually high soil microbial activity. The aboveground biomass of mustard was ground to pass through 1-mm mesh screen in a Wiley mill. Ground samples were digested in an auto-block using a mixture of nitric and perchloric acid and were analyzed for tissue Cu, Mo, Ni, Zn, Fe and Mn concentrations using an Inductively Coupled Plasma (ICP) spectroscopy. Nutrient uptake of mustard tissues was calculated using equation 1 for the aboveground uptake.

$$NU_{Cu, Mo, Ni, Zn, Fe, Mn} = [CN_{Cu, Mo, Ni, Zn, Fe, Mn}] \times DMY_{Aboveground} \quad (1)$$

where, NU = nutrient uptake (kg ha^{-1}), CN = concentration of heavy metals (g kg^{-1}), $DMY_{Aboveground}$ = dry matter yield (kg ha^{-1}).

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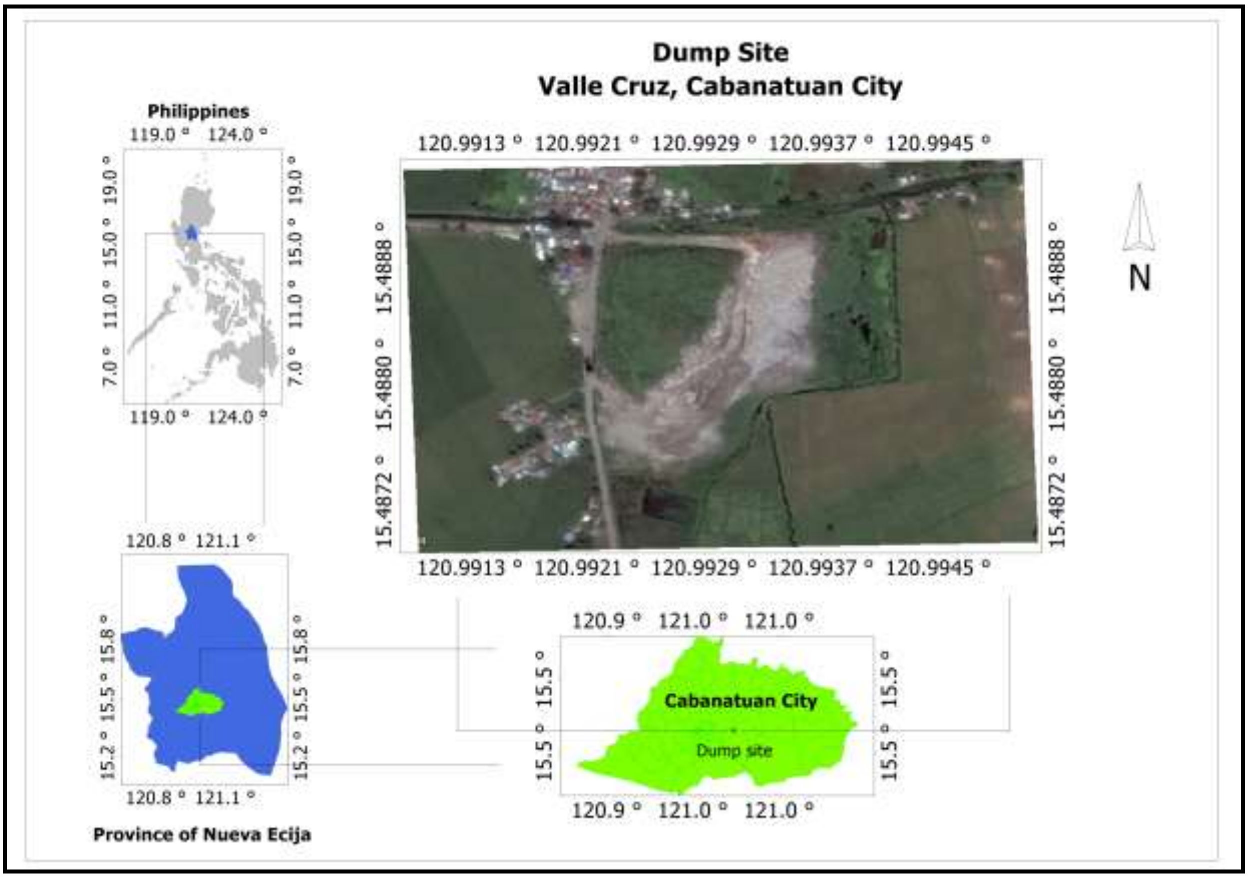

Fig. 1 Sampling Site Location: Dump Site at Valle Cruz, Cabanatuan City, Philippines

III. RESULTS

A. Aboveground Biomass and Soil pH

Aboveground biomass of mustard varied significantly ($p \leq 0.05$) with the interaction of sulfur application and harvest time while soil pH varied widely ($p \leq 0.001$) with sulfur treatment and harvest time (Table 1). The aboveground biomass of mustard when averaged across sulfur applications had an increase of about 199% between 15 days and 25 days after transplanting (DAT). The greatest aboveground biomass (averaged across harvest time: 15 and 25 DAT) of about 1487.5 kg ha^{-1} was from 80 mmol S kg^{-1} while the lowest aboveground biomass production was from 0 mmol S kg^{-1} (1106.7 kg ha^{-1}).

Soil pH decreased in all the treatments with application of ferrous sulfate when compared to the control (Table 1). Soil pH when averaged across harvest time (15 DAT and 25 DAT) was in the order: 0 mmol S kg^{-1} (8.34) > 40 mmol S kg^{-1} (7.69) > 80 mmol S kg^{-1} (7.58).

TABLE 1 ABOVEGROUND BIOMASS OF MUSTARD, PH AND RESPIRATION RATE IN CONTAMINATED SOILS AS AFFECTED BY APPLICATION OF FERROUS SULFATE

Sulfur (mmol kg^{-1})	**Aboveground Biomass (g pot^{-1})**	**Aboveground Biomass (kg ha^{-1})**	**pH**	**CO_2-C Respiration Rate (mg kg^{-1})**
	15 Days After Transplanting			
0	0.28	559.33	8.12	2.00
40	0.40	802.00	7.53	2.00
80	0.37	746.67	7.38	6.00
	25 Days After Transplanting			
0	1.32	1654.00	8.56	6.00
40	0.70	1418.07	7.86	14.00
80	1.11	2228.40	7.78	19.00
Sources of Variations	**F-values**			
Sulfur (S)	1.68ns	1.68ns	90.20***	31.75***
Harvest (H)	36.63***	36.63***	62.14***	121.00***
S × H	3.45*	3.45*	0.37ns	10.75***

ns – not significant; * – significant at $p \leq 0.05$; *** – significant at $p \leq 0.0001$

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B. Heavy Metal Uptake and Solubility of Heavy Metals in Soils

Uptake of mustard for selected heavy metals as affected by the different levels of ferrous sulfate additions is shown in Table 2. Uptake of Cu, Mo, Zn, Fe and Mn were significantly affected by the interaction effects of harvest time and ferrous sulfate addition. Uptake of mustard for Cu, Zn, Fe and Mn were increased by 227%, 201%, 422% and 148%, respectively while a significant reduction in the uptake of Mo (-50%) and Ni (-67%) between the 15 days and 25 days after transplanting (Table 2). Overall, sulfur application resulted in increased uptake of mustard for Co, Mo, Ni, Zn, Fe and Mn. Between 0 and 80 mmol S kg^{-1}, average uptake of Co was increased from 0.01 to 0.02 kg ha^{-1}, 0.007 to 0.01 kg ha^{-1} for Ni, 0.067 to 0.070 kg ha^{-1} for Zn, 0.068 to 0.124 kg ha^{-1} for Fe and 0.020 to 0.030 kg ha^{-1} for Mn.

Solubility of heavy metals especially copper, zinc and manganese in the soils following harvest of mustards was significantly increased with ferrous sulfate application (Fig. 2). Soils with 80 mmol S kg^{-1} had the greatest concentrations of extractable Ni (10.7 mg kg^{-1}), Mn (10.9 mg kg^{-1}) and Ni (0.09 mg kg^{-1}).

TABLE 2 UPTAKE OF MUSTARDS FOR SELECTED HEAVY METALS AS AFFECTED BY DIFFERENT LEVELS OF FERROUS SULFATE ADDITIONS

Sulfur (mmol kg^{-1})	Copper (kg ha^{-1})	Molybdenum (kg ha^{-1})	Nickel (kg ha^{-1})	Zinc (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})
15 Days After Transplanting						
0	0.016	0.006	0.007	0.067	0.068	0.020
40	0.028	0.002	0.01	0.098	0.126	0.038
80	0.020	0.001	0.01	0.070	0.124	0.030
25 Days After Transplanting						
0	0.100	0.011	0.003	0.307	0.648	0.090
40	0.046	0.003	0.012	0.142	0.284	0.039
80	0.069	0.004	0.003	0.256	0.375	0.088
Sources of Variations	**F-values**					
Sulfur (S)	2.52ns	1.68ns	90.20***	31.75***	4.18*	2.65ns
Harvest (H)	43.27***	36.63***	62.14***	121.00***	54.96***	31.06***
S ×H	6.17*	3.45*	0.37ns	10.75***	8.27**	7.64**

ns – not significant; * – significant at p ≤0.05; ** – significant at p ≤0.001; *** – significant at p ≤0.0001

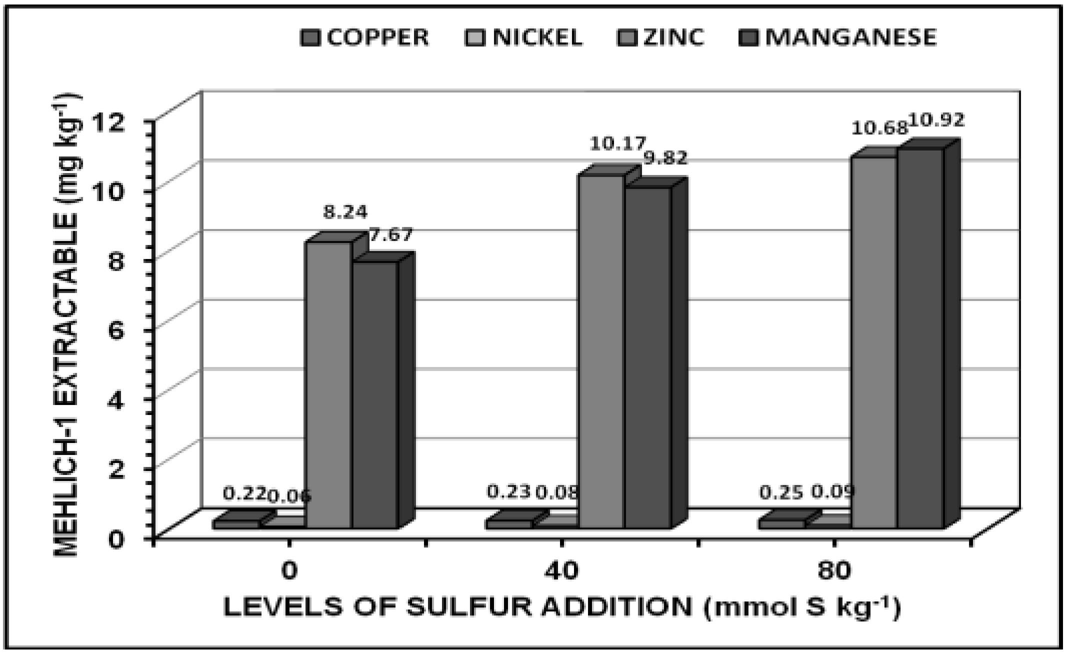

Fig. 2 Concentrations of Mehlich-1 extractable heavy metals in contaminated landfill soils applied with ferrous sulfate

C. Microbial Activity

Microbial respiration rate in landfill soils following harvest of mustard was significantly affected by ferrous sulfate additions (p≤0.0001), harvest time (p≤0.0001) and the interaction effects of sulfur addition and harvest time (p≤0.0001). Average respiration rate (mg kg^{-1} CO_2-C) in soil with 0 mmol kg^{-1} ferrous sulfate was about 2.0 mg kg^{-1} CO_2-C compared with 19.0 mg kg^{-1} CO_2-C for soils amended with 80 mmol kg^{-1} of ferrous sulfate (Table 1). The respiration rate when averaged across sulfur applications had an increase of about 294% between 15 DAT (3.3 mg kg^{-1} CO_2-C) and 25 DAT (13.1 mg kg^{-1} CO_2-C) after transplanting (Table 1). Soils applied with 80 mmol kg^{-1} of ferrous sulfate resulted in 200% increase in respiration rate when compared with the control during the first 15 days and about 216% increase in respiration rate after 25 days of transplanting when compared with the control soils (Table 1).

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IV. DISCUSSION

Our results have demonstrated the beneficial outcome of green technology in combination with application of sulfur (as $FeSO_4$) in cleanup of contaminated landfill soils while at the same time improving soil microbial biomass following phytoremediation. Phytoremediation when supplemented with sulfur had enhanced the uptake and phytoextraction of heavy metals by mustard as shown by overall data trend in our study. Someone may think that we have a short study; results however, suggest the positive effect of sulfur addition during phytoremediation. In our present study, application of sulfur led to substantial increases in the aboveground biomass mustard and uptake of Ni, Zn and Fe.

The use of plants to extract toxic metals from contaminated soils has emerged as a cost-effective, environment-friendly clean up alternative. Since plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering practices that rely on intensive soil manipulation, phytoremediation may provide an attractive alternative for the cleanup of heavy metal-contaminated soils in abandoned landfills. Heavy metals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavy metals contaminated environment. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contaminant degradation abilities of the entire plant body. Nevertheless, a major problem still could hinder plant remediation efficiency because some of the metals are immobile in soils and their phytoextraction rates are limited by solubility and diffusion to the root surface. Our approach of solubilization of soil-bound metals by artificial soil acidification was using elemental sulfur. Tichy et al. [11] suggested the use of elemental sulfur to gradually solubilize heavy metals in soils. Microbial oxidation of elemental sulfur introduced into soil leads to the formation of sulfuric acid [13] and hence a decrease in soil pH and an increase in the soluble fraction of heavy metals. Sulfur treatments in our study led to a decrease of soil pH and an increase in soluble Zn and Mn in the soil. The average pH of soils with no sulfur application was about 8.34. Soil pH with sulfur was 7.69 and 7.58 for 40 and 80 mmol S kg^{-1}, respectively. The increase in metal solubility with decreasing pH is in good agreement with the findings of other authors to which soil pH is a key factor governing the solubility of Zn and Cd in soils [14-16].

Application of ferrous sulfate during phytoremediation could enhance solubility of heavy metals and microbial activities in contaminated soils. In our study, the solubility of copper, zinc and manganese in abandoned landfill soils was significantly increased with ferrous sulfate application following phytoremediation. In recent years, phytoremediation has been considered as a cost-effective approach to remediate soil contaminated by heavy metals. The success of phytoremediation depends on several factors, e.g., plant must produce sufficient biomass while accumulating a high concentration of certain metal. In the process of phytoremediation, the bioavailability of heavy metals is an important factor [17]. There are two main approaches that have been used to increase the bioavailability of heavy metals in soils and the mobility of heavy metals within the plants by lowering soil pH and adding synthetic chelates such as ethylenediaminetetra acetic acid (EDTA). Kayser et al. [10] demonstrated that application of ferrous sulfate increased zinc solubility in the soil and utilization by plants. Results of our study were quite similar with the findings of Kaya et al. [18] who reported that application of ferrous sulfate resulted in decrease in soil pH and increased in the concentration of nutrients available to plants, such as zinc, copper and manganese.

Our study may have demonstrated the process of phytodegradation or phytotransformation which is a type of phytoremediation whereby plants can breakdown organic contaminants by internal metabolic processes where plants hydrolyze the organic compounds into smaller units that can be absorbed by the plants. These smaller molecules may be used as metabolites by the plant as it grows, while incorporating into the plant tissues. The findings of this present study confirmed the research results of Uera et al. [5] and Paz-Alberto et al. [4] who reported that phytoremediation as a green technology could be used to treat and clean up soil contaminated with heavy metals, toxic organic compounds and other environmental contaminants. Phytoremediation is a relatively low cost, safe, efficient and environment-friendly technology. Nevertheless, a major problem still could hinder plant remediation efficiency because some of the metals are immobile in soils and their phytoextraction rates are limited by solubility and diffusion to the root surface. To overcome this problem, we have added ferrous sulfate to lower soil pH. The use of ferrous sulfate in our study resulted in decrease of soil pH and increase in solubility of heavy metals in landfill soils.

Soils with sulfur treatments in our study had the greatest concentrations of extractable Cu, Zn and Mn. Our study has shown the beneficial effect of sulfur application when combined with phytoremediation. One approach to enhance phytoavailability of metals is the solubilization of soil-bound metals by artificial soil acidification. Tichy et al. [11] suggested the use of sulfur to gradually solubilize heavy metals in the soil to be superior than application of organic agents, like EDTA. An alternative approach to solubilize metals is the application of organic agents that form soluble complexes with heavy metals. Walace and Wallace [19] described the solubilizing effect of the synthetic chelate and their effects on micronutrient and heavy metals uptake. Some authors, however, reported that chelating substances such as EDTA did not enhance [20] or rather reduced plant uptake [21]. Considerable debates exist on the issue whether heavy metals were taken as metal complexes or only after first being split from the chelate [22]. A problem associated with the use of EDTA in chelate-assisted phytoextraction is its low biodegradability [22]. The low biodegradability of chelating agent can be a major obstacle for phytoremediation operations. This calls for more efficient soil amendment. Wenger et al. [22] described the ideal amendment. An ideal amendment would not only solubilize heavy metals in the soils, but also facilitate their uptake and translocation within the plants and at the same time mitigate toxic metal effect in plant tissues and should be sufficiently biodegradable to

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limit the risk of metal leaching into the subsurface. Sulfur treatments that led to a decrease of soil pH and an increase in soluble Cu, Ni, Zn and Mn in our study could be a better option in improving the efficiency of phytoremediation or phytoextraction of heavy metals from contaminated landfill soils.

Another interesting result of our study is on the enhancement of microbial activities in contaminated soils following phytoremediation, which is due to the application of sulfur ($FeSO_4$). Our results have shown that sulfur supply can significantly enhance the quantities of soil microorganism in landfills. This is probably related to the soil pH decrease, which can be favorable and propitious to the growth of fungi. Average respiration rate in soil with 0 mmol kg^{-1} of ferrous sulfate was about 2.0 mg kg^{-1} CO_2-C compared with 19.0 mg kg^{-1} CO_2-C for soils amended with 80 mmol kg^{-1} of ferrous sulfate. Similar results were reported by Zhao et al. [23]. From their study about the effects of sulfur fertilization on soybean root, leaf traits and soil microbial activity, their results showed that application of elemental sulfur significantly increased the number of soybean side roots by 8.6%, dry weight by 6.6% and the number of soil microorganisms (bacterium, fungi and actinomycetes). Sulfur supply promoted the growth of soybean, increased the yield and enhanced soil microbial activity. Zheng et al. [24] also reported that sulfur application could stimulate crop growth and had positive influence on microbial activities of the soil. Sulfur addition may increase the effective sulfur content in the soil that can promote the microbial conversion of organic matter, and then microbial biomass increased.

V. SUMMARY AND CONCLUSIONS

Important findings from our greenhouse study are summarized below. Soil pH decreased in all the treatments with application of ferrous sulfate when compared to the control. Solubility of heavy metals especially copper, zinc and manganese in the soils following harvest of mustards was significantly increased with ferrous sulfate application. Application of ferrous sulfate significantly enhanced microbial activities in contaminated soils. Average respiration rate in soil with 0 mmol kg^{-1} of ferrous sulfate was about 2.0 mg kg^{-1} CO_2-C compared with 19.0 mg kg^{-1} CO_2-C for soils amended with 80 mmol kg^{-1} of ferrous sulfate.

Our study has demonstrated the beneficial outcome of green technology in combination with ferrous sulfate in cleaning up heavy metals contamination in landfills and at the same time improving soil microbial biomass following phytoremediation. However, additional research is still needed to better understand the long-term effects of green technology in combination with sulfur during phytoremediation. Several factors must be considered in order to accomplish successful remediation result. The most important factor is the suitable plant species, which can be used to uptake the contaminant efficiently by acting both as accumulators and excluders. Finally, this study will pave the way for a larger and more comprehensive exploration of phytoremediation application in the management of toxic and hazardous wastes emanating from landfill operations. Research is in progress to screen plants for hyperaccumulation of heavy metals while advancement in molecular studies will improve efficiency of phytoremediation in contaminated sites.

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