Effect of Lignin and Commercial Plant Extract on the Fractionation of Heavy Metals in Multi-Metal Contaminated Soil

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***Abstract*—Soil stabilization is a remediation method that involves the application of stabilizing materials to the contaminated soil to reduce heavy metal mobility in the soil by changing their forms from mobile to a less mobile form. The heavy metals can be categorized into five fractions, in decreasing mobility order, exchangeable (Ex), carbonate-bound (Carb), iron-manganese bound (FeMnO), organic (Org), and residual (Res) fraction. This study attempts to evaluate the lignin and a commercial plant extract as stabilizing materials for heavy metal immobilization, specifically on copper (Cu) and Nickel (Ni). Lignin showed significant effect on the heavy metal mobility as observed from the change in distribution of the heavy metal fractions. The FeMnO bound Cu in samples treated by lignin was reduced by approximately 23% while the Org Cu increased by 23%. The Carb Ni slightly reduced from 3.71% to 1.47% while the FeMnO and Org Ni increased by about 8% and 9% respectively. The FeMnO Cu in the samples treated by plant extract slightly reduced by 1.81%–3.56%, with increasing reduction as the concentration increases. The Pearson correlation analysis suggest that the increase in pH reduces the mobility of heavy metals by reducing the Carb fraction of the heavy metals (Cu: r=−0.872; Ni: r=−0.915). The soil organic matter (OM) could be the main factor for heavy metal immobilization as OM has strong, positive correlation with the Org fraction of the heavy metals (Cu: r=0.942; Ni: r=0.938). In short, lignin was found to have better stabilizing effect than the plant extract.**

***Keywords*—heavy metal immobilization, lignin, plant extract, soil remediation**

I. Introduction

Heavy metal contamination in the environment raises concerns around the globe as it threatens the food security and the safety and health of the people. Human activities cause soil heavy metal pollution due to pesticide and fertilizer application at agricultural lands, wastewater irrigation, landfill leachate, accidental spills during waste handling, mining activities and et cetera [1]. The pollution could affect soil fertility and functionality of the land [2]. Heavy metals such as copper (Cu) and nickel (Ni) bioaccumulates in crops following the plant's mineral and nutrient uptake from the soil and at high concentration levels, it could cause health implications as people consume the crops. Excessive levels of Cu in the body could lead to implications such as anemia, arthritis, headaches and migraines, and kidney disorders [3]. Ni, on the other hand, could cause kidney diseases, cardiovascular, and respiratory complications [4]. Hence, soil remediation towards heavy metal contamination especially in agricultural lands is crucial to maintain food safety for the health and safety of the people. Soil stabilization is a soil remediation technique where stabilizing material(s) is/are added to immobilize the contaminants in the contaminated soil. Soil stabilization is an in-situ remediation method. It is simple, cost-effective, and less time consuming as laborious work such as excavation of land and transportation of the contaminated soil is avoided. Soil stabilization involves the application of soil amendments or stabilizing materials to contaminated soil to reduce the mobility of the heavy metals. Heavy metals may present in different forms with different mobility and availability to the living organisms in the natural environment. In the study of their mobility, the heavy metal forms are often categorized into 5 different fractions, the exchangeable (Ex), carbonate bound (Carb), iron-manganese oxide bound (FeMnO), organic (Org), and residual (Res) fractions. The Ex and Carb fractions are considered to be the mobile fractions of the heavy metals as they are easily released into the environment. The Ex fraction mobilizes readily with free ions while the Carb fraction mobilizes under slightly acidic condition. The stabilizing effect of the materials is observed when the mobile fractions are reduced as they change to lower mobility fractions (FeMnO, Org, and Res).

II. Literature Review

Through literature review, lignin was shown to be an affordable and efficient adsorbent for heavy metal removal from aqueous solution [5]. Mohammadabadi and Javanbakht [6] synthesized biocomposites made up of lignin extracted from barley straw and found that they performed well as biosorbent for Pb adsorption. A previous study also discovered that lignin was able to stabilize Cd and Pb in both alkaline and acidic soil [7]. Plant extracts were also researched for their various applications in heavy metal remediation. For instance, one study was able to combine plant extracts directly with lead and cadmium ions to form complexes for heavy metal removal from the solution [8]. There were also studies that produced nanoparticles using plant extracts for the heavy metal removal purposes. In one study, coconut husk extract was used to produce iron oxide nanoparticles and was found to have good adsorption for

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calcium (Ca) and cadmium (Cd) from aqueous solution [9]. In another study, the extract of the leaves of a flowering plant was used to produce nanoparticles that was able to remove Cr and Cd from solutions [10]. One of the main compositions of the plant extract is kelp. Sodium alginate, a common compound found in kelp and seaweeds was also found to be capable in immobilizing heavy metals in soil [11]. From the literature review, at the time of this study, there were no known studies on the immobilization of Cu and Ni in soil using lignin. Moreover, the commercial plant extract used in this study was mainly promoted as a product for crop production improvement with no known studies on its effect on the heavy metals. Hence, lignin and the commercial plant extract was studied for their potential as stabilizing materials for heavy metal immobilization in contaminated soil. The goal of this study is to study the effect of lignin and plant extract on the mobility of heavy metals in multi-metal contaminated soil. In addition, the correlation relationship between the soil properties and heavy metal fractions were also evaluated.

III. Materials and Methods

The materials used in the research of the current paper includes:

1) Lignin, kraft (analytical grade, Sigma-Aldrich)
2) Commercial plant extract
3) Copper (II) sulphate ($CuSO_4$, analytical grade, ChemSoln)
4) Nickel (II) chloride ($NiCl_2$, analytical grade, R&M Chemicals)
5) Sodium acetate (NaOAc, analytical grade, Bendosen)
6) Acetic acid (HOAc, analytical grade, HmbG Chemicals)
7) Hydroxylamine hydrochloride (NH2OH-HCl, analytical grade, HmbG Chemicals)
8) Hydrogen peroxide (H_2O_2, 30%, analytical grade, HmbG Chemicals)
9) Ammonium acetate (NH_4OAc, analytical grade, Bendosen)
10) Nitric acid (HNO_3, 65%, analytical grade, R&M Chemicals)
11) Hydrochloric acid (HCl, 37%, analytical grade, J. T. Baker)

A. Preparation of Contaminated Soil

The contaminated soil used for this study was prepared in the laboratory using metal solutions of the target heavy metals, Cu and Ni. Planting black soil (Lotus's Black Soil, Lotus's) was first sieved to <2 mm particle size to ensure homogeneity throughout the soil. Then, metal solutions, $CuSO_4$ and $NiCl_2$ were added to the soil to contaminate the soil with Cu and Ni, each at a concentration of 500 mg/kg soil. The soil was then mixed thoroughly for 1 month to ensure ample contamination in the soil, also maintain 70% moisture content throughout the incubation period.

B. Application of Stabilizing Materials

For each pot, approximately 100 g of contaminated soil was treated with lignin at 10% (labelled as LN10) and plant extract at varying dosage, 1%, 5%, and 10% (labelled as TM1, TM5, and TM10, respectively). A control with no treatment (Control) was also prepared. Each treatment was carried out in duplicates. The pots were kept at room temperature and at a moisture content of 70% throughout the experimental period. The soil physiochemical properties and heavy metal concentrations of the treated and untreated soils were measured after one month.

C. Soil Physiochemical Properties

Several soil characteristics were measured throughout the study period, before the treatment, one week, and one month after the treatment. The soil pH and electrical conductivity (EC) were determined using the USEPA 9045d method. Distilled water was added to the soil at a soil-to-water ratio of 1:5 and was mixed thoroughly for approximately 5 min. The suspension was then left to settle before being measured for its pH and EC using the Hanna instruments 2211 pH meter and Eutech CyberScan PCD 640 meter kit, respectively. The estimated organic matter (OM) content in the soil was determined via the loss-on-ignition method. Soil samples oven-dried to constant weight at 150 °C underwent combustion at 550 °C for 3 h in the muffle furnace. The organic matter percent is determined using Eq (1), where *OM* refers to the soil organic matter content, $m_{oven\text{-}dried}$ refers to the weight of soil measured after oven drying, $m_{combustion}$ refers to the weight of soil measured after the furnace combustion process.

$$OM = (m_{oven\text{-}dried} - m_{combustion}) / m_{oven\text{-}dried} \times 100\% \quad (1)$$

D. Extraction of Heavy Metals from Soil Samples and Analysis

The different fractions of Cu and Ni were extracted via sequential extraction procedure. The sequential extraction procedure extracts the different heavy metal forms from a single sample in a sequential manner using different reagent(s) under different conditions. The sequential extraction procedure used in this study is the modified Tessier method where the heavy metals were extracted into five different fractions (Ex, Carb, FeMnO, Org, Res) [12, 13]. Table 1 shows the reagents used, period and condition for each extraction. The concentration of Cu and Ni in the different fractions was analyzed via the Agilent Technologies 200 Series atomic absorption spectroscopy (AAS).

E. Statistical Analysis

Pearson correlation analysis was carried out using the SPSS Statistics 26 (IBM) to determine the relationship between the soil properties and heavy metal fractions. The strength of the correlation between variables is indicated by the value of the correlation coefficient, r, where $0 \leq |r| < 0.4$ represents weak correlation, $0.4 \leq |r| < 0.7$ represents moderate correlation, $0.7 \leq |r| \leq 1$ represents strong correlation [14].

Table 1. Reagents used, extraction period and condition for each heavy metal fraction via the modified Tessier Method [15, 16].

Heavy metal fraction	Reagent	Extraction condition
Exchangeable	8 mL of 1 M NaOAc.	1 h of continuous agitation under room temperature.
Carbonate	8 mL of 1 M NaOAc, adjusted to pH 2 with HOAc	5 h of continuous agitation under room temperature.
Iron-manganese bound	20 mL of 0.04 M NH_2OH-HCl in 25% HOAc.	6 h of occasional agitation at 96°C.
Organic	• 3 mL of 0.02 M HNO_3 and 5 mL of H_2O_2, adjusted to pH 2 using HOAc. • 3 mL of pH 2 30% H_2O_2. • 5 mL of 3.2 M NH_4OAc in 20 % HNO_3.	2 h of occasional agitation at 85°C, another 3 h of occasional agitation at 85°C after addition of the acidified H_2O_2. After cooling, add in the 3.2 M NH_4OH-HCl and have 30 min of continuous agitation at room temperature.
Residual	20 mL of aqua regia, a mixture of HCl and HNO_3 of 3:1 ratio.	Continuous stirring at approximately 110 (near boiling state) for approximately 30 min to 1 h until solution decolorizes or emission of fumes stops.

IV. Result and Discussion

A. Effect of Stabilizing Materials on Soil Physiochemical Properties

Table 2 shows the physiochemical properties of the soil samples. The addition of lignin to the soil samples significantly increased the soil pH from pH 7.34 to pH 9.90 while the samples treated by the plant extract increased slightly to pH 7.78–8.14. The pH rise caused by plant extract is observed to slightly increase with its increasing dosage concentration. The soil electrical conductivity (EC) also follows similar trends as the soil pH. The application of lignin raised the soil EC from 3.129 to 10.86 ms/cm while the plant extract merely slightly raised the soil EC from 3.129 to 3.243–3.369 ms/cm. The soil EC usually are measured to estimate the salinity of the soil. However, it is actually dependent other factors such as the soil moisture content and cation exchange capacity (CEC) [15]. Here, the soil EC of the samples treated by lignin is very high and indicates that the soil may not be productive for plant growth [16]. The organic matter content of the soil can be seen to increase in the LN10 samples, from 12.12% to 18.21%. The organic matter content in the samples treated by the plant extract only faced very little changes, slightly decreasing from 12.12% to 11.44%–11.80%. In samples treated by the lignin treatment increased the CEC from 1.477 meq/100g soil to 1.988 meq/100g soil. The CEC of the TM1 samples increased to 1.632 meq/100g soil while the CEC in the TM5 and TM10 samples decreased to 1.225 meq/100g soil and 1.295 meq/100g soil respectively.

Table 2. Soil physiochemical properties of different samples

Sample ID	Treatment dosage (%)	pH	EC (ms/cm)	OM (%)	CEC (meq/100g soil)
Control	0	7.73 (±0.045)	3.129 (±0.201)	12.12 (±0.103)	1.477 (±0.067)
LN10	10	9.90 (±0.066)	10.86 (±0.197)	18.21 (±1.185)	1.988 (±0.062)
TM1	1	7.70 (±0.129)	3.243 (±0.182)	11.80 (±0.449)	1.632 (±0.434)
TM5	5	7.91 (±0.019)	3.369 (±0.106)	11.44 (±0.210)	1.225 (±0.076)
TM10	10	8.14 (±0.046)	3.334 (±0.165)	11.73 (±0.059)	1.295 (±0.087)

B. The Effect of Stabilizing Materials on the Mobility of the Heavy Metals

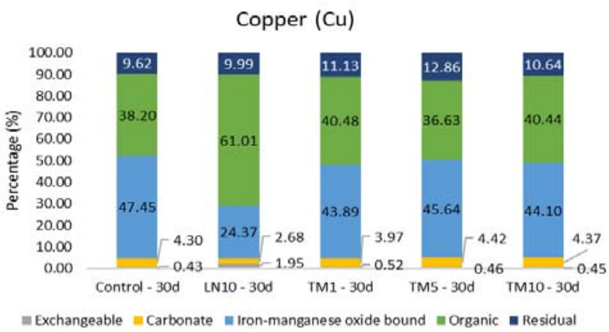

Fig. 1. Fractionation of Cu detected in the soil samples at the end of the treatment.

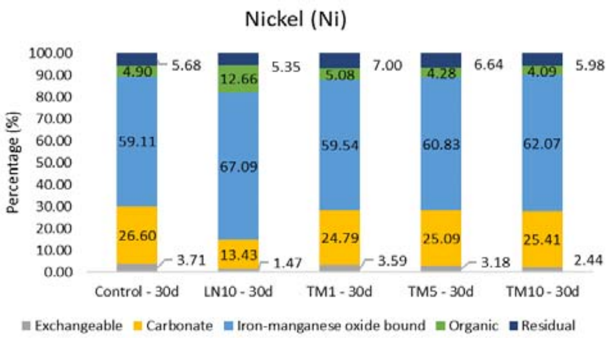

Fig. 2. Fractionation of Ni detected in the soil samples at the end of the treatment.

Fig. 1 and Fig. 2 show the distribution of Cu and Ni, respectively, in the soil samples across the five fractions. Table 3 shows the Pearson correlation coefficient, *r*, computed between the soil properties and the different fractions of Cu and Ni.

1) Copper (Cu)

Although there was not much changes in the mobile fractions of Cu in the treated samples, the reduction of mobility of Cu can still be observed. There was a significant decrease of FeMnO Cu in the LN10 samples, with a reduction of approximately 48.6% relative to the control. The FeMnO Cu in samples treated by TM were also reduced, with differences of 1.81%–3.56% as compared to the control. On the other hand, the org Cu increased in both the LN10 and TM samples from 38.2% to 61.0% and 40.4%–40.5%, except for TM5 where there is a slight decrease in its percentage (36.6%). These results show the transformation of Cu from a higher mobility fraction to a lower mobility fraction in the treated samples, especially in the LN10 samples. The Cu in the LN10 samples mainly changes from FeMnO fraction to the Org fraction. While the effect is not as evident, the TM samples also demonstrated the changes of FeMnO Cu to Org and Res Cu where slight increase in the latter fractions were observed.

2) Nickel (Ni)

The mobile Ni in samples treated by lignin has reduced significantly. The mobile Ni in the LN10 samples is observed to have reduced by 51% relative to the control. The FeMnO and Org Ni in the LN10 samples has increased by an approximate of 8% for both fractions from the control (59.1% to 67.1% and 4.9% to 12.7% respectively). This signifies the changes of the heavy metal form from higher to a lower mobility and thus showing good immobilizing effect of the lignin towards Ni. In the TM samples though, not much difference was observed in the fractionation of Ni when compared to the control, indicating possible weaker stabilizing effect of the plant extract towards Ni in the soil.

C. The Effect of Soil Properties on the Mobility of the Heavy Metals

The soil pH plays a major role in heavy metal immobilization. When the soil pH rises, the H+ ions dissociate from functional groups such as carboxyl and hydroxyl groups, leaving them free to bind with the metal cations and form metal precipitates [17]. In this study, the reduced mobile fractions of Ni in the LN10 samples can be explained by the soil pH was raised by the addition of lignin [18]. He *et al*. [7] also showed similar findings in which the amount of adsorbed Cd is higher when the soil pH was increased. This also corresponds to the results from the correlation analysis in Table 3 where the soil pH was found to have strong, negative correlation with the Carb fraction of both Cu and Ni. Other than that, the soil organic matter also plays a part in soil stabilization. The heavy metals in the soil can be immobilized by the presence of the organic matter in the soil by adsorption or by formation of complexes of lower mobility [16]. The organic matter in the soil transforms into humic substances that can complex with contaminants and thus reduces their mobility in the environment [19]. The addition of lignin into the soil can promote the formation of humic substances as lignin is oxidized by the microorganisms into polyphenols and quinone-like substances which are precursors for the formation of humic substances [19]. For this reason, the increment in the Org Cu and Ni observed in the LN10 samples could be explained by the increased soil organic matter content from the addition of lignin. Furthermore, it also agrees with the correlation analysis where the soil organic matter displayed strong, positive correlation with the Org fraction of both Cu and Ni in Table 3. The soil CEC measures the presence of exchangeable cations such as Na+, Ca+, Mg+, Al+ in the soil where the cations serve as exchangeable sites for the heavy metals. Higher CEC in the soil would indicate that there are more available sites and encourages cation exchange of the heavy metals to occur. From Table 2, LN10 samples had higher CEC than the TM samples which could also be one of the reasons contributed to the better stabilizing effect displayed by lignin. The correlation analysis in Table 3 shows that the CEC displayed strong, positive relation with the Org fraction of both Cu and Ni while strong, negative relation is observed with Carb fraction of both heavy metals and FeMnO fraction of Cu. This may suggest that the immobilization of Cu and Ni mainly occurs at the surface of the organic matter rather than precipitation or sorption at the iron and manganese oxides in the soil. It can also be observed from Table 3 that the Res fraction of both Cu and Ni does not have strong correlation with any of the soil properties. This is because the Res fraction of the heavy metals is those that are bounded in the crystalline structure of the minerals and can only be released by highly concentrated acids. Hence, this fraction is very stable and does not show much changes in the soil samples.

Table 3. Pearson correlation coefficient, *r*, value between soil properties and fractions of heavy metals

	Cu					Ni				
	Ex	**Carb**	**FeMnO**	**Org**	**Res**	**Ex**	**Carb**	**FeMnO**	**Org**	**Res**
pH	**0.924****	**−0.872****	**−0.962****	**0.942****	−0.354	−0.652**	**−0.915****	0.602**	**0.938****	−0.395*
EC	**0.941****	**−0.92****	**−0.975****	**0.958****	−0.368	−0.611**	**−0.931****	0.569**	**0.972****	−0.385*
Organic matter (%)	**0.895****	**−0.933****	**−0.967****	**0.943****	−0.410*	−0.577**	**−0.903****	0.495**	**0.955****	−0.386*
CEC	0.696**	**−0.852****	**−0.798****	**0.756****	−0.107	−0.171	**−0.732****	0.118	**0.761****	−0.109*

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

In overall, the lignin had a more significant influence on the mobility of the heavy metals as compared to the plant extract. Besides the soil properties discussed previously, the functional groups present in the stabilizing materials can also influence the material's capacity to bind and immobilize the heavy metals. A study suggested that carboxyl, lactonic, and phenolic hydroxyl functional groups can be found in lignin [11]. These functional groups contain oxygen which can react with the metal ions in the soil to form metal complexes that have lower mobility. This could explain the higher stabilizing effect demonstrated by lignin in this study. In a multi-metal contaminated soil, competitive behavior between the metal ions can happen and should be taken into consideration when analyzing the materials' stabilizing potential. In this study, competition between Cu and Ni ions for binding sites at the soil surface may have influenced the immobilizing effect of the stabilizing materials. From the results observed, lignin has a selective immobilization for Ni ions over the Cu ions. In one study, the simultaneous adsorption of coal fly ash in a binary system containing Cu and Ni ions was found to be ineffective as compared to the single system due to selective adsorption behavior of the material towards the metal ions [20]. Lekgoba *et al*. [20] also found that the maximum adsorption of Ni occurs at a higher pH condition as compared to Cu which occurs at around neutral pH. This may explain the better stabilizing effect of Ni as compared to Cu in this study as the soil condition is alkaline at a higher pH.

D. Cost Comparison between Lignin and Plant Extract

The commercial plant extract costs approximately RM310 for 1 L while the lignin costs approximately RM375 for a 100 g bottle [21, 22]. Assuming that the treatment needs an application dosage of 10% for a pot of 100 g soil, the treatment with the plant extract would cost RM3.10 (10 mL for 100 g soil) while the lignin would cost RM37.50. According to the experiment of this study, the lignin treatment would cost significantly more than the plant extract. However, as mentioned previously, the lignin treatment works significantly better than the plant extract when both are at similar application dosage. Higher application dosage of the plant extract may be needed to have better stabilizing effect on the heavy metals which would then increase the cost of the treatment. The study on both stabilizing materials, in particularly the commercial plant extract, for their application in the area of soil remediation is still immature. Hence, this simple cost comparison should only be taken as reference at the surface level.

V. Conclusion

Lignin was able to reduce the mobile fractions of Ni when added to the multi-metal contaminated soil. Although the percentage of mobile Cu in the soil did not show obvious change after the lignin treatment, the lignin still demonstrated stabilizing effect on Cu as the transformation of FeMnO Cu to Org Cu can be observed. Similar transformation was also observed in the soils treated by the plant extract, though there were less obvious changes as compared to the lignin. The stabilizing effect of the plant extract towards Ni was less noticeable as the fractionation of Ni in soils treated by the plant extract were almost similar to the control sample. The Pearson correlation analysis revealed that the pH had strong, negative correlation with Carb fraction of both Cu and Ni which suggest the increase in pH reduces the mobility of heavy metals. The organic matter content and CEC showed strong, positive correlation with the Org fraction of the heavy metals. This could indicate that the increase in CEC could be mainly caused by the increase of organic matter and that the stabilizing effect of the materials was driven by the presence of organic matter. The residual fraction is known to be very stable and inert to changes which does corresponds to the correlation analysis as it does not have strong correlation to any of the soil properties. In overall, lignin was found to have greater stabilizing effect on the heavy metals while the effect of the plant extract is less evident. The stabilizing effect of lignin towards Ni was more apparent as compared to Cu. Lastly, the organic matter is likely to be the main contributor to the stabilizing effect of the materials used in this study.

For future projects, the study and monitoring of the functional groups in both the material and soil is recommended as it may be useful to understand how the functional groups may change as the material interacts with the heavy metals in the soil. Moreover, modifications to the stabilizing materials such as changing its form, molecular structure or chemical properties might change their behavior towards the heavy metals and can be potentially viable for further exploration.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Lee Li Na conducted the research and wrote the paper. Dr Matsura Ibrahim is the co-researcher. Assoct. Prof. Dr Guo Xinxin is the main supervisor and Asst. Prof. Dr Chng Lee Muei is the co-supervisor of the research and advises on the research direction and writing of the paper. Prof. Ir. Dr Ng Choon Aun and Prof. Dr Mohammed JK Bashir are co-researchers under the same funding. All authors had approved the final version.

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