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Chemical Speciation of Heavy Metals in Bioaugmented and Non-Bioaugmented Soils from Taman Beringin Landfill

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Abstract

Heavy metal pollution has led to serious consequences since the dawn of industrialization. The aim of this study is to compare the speciation of heavy metals in bioaugmented and non-bioaugmented soils from Taman Beringin landfill, Malaysia. This is to allow the proper understanding of the specific forms of heavy metals and their mobility in the environment. Sequential extraction process based on Tessier et al. (1979), Tsang et al (2007) and standard ISO 11466 (1995) was performed for the determination of eight heavy metals (Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb) in soils samples. The results revealed that the percentage reduction of the mobile and non mobile forms (F1, F2, F3, F4 and F5) of heavy metals varied for all metals in non- bioaugmented and bioaugmented soils. Additionally, the distribution of the specific form of the metals (for Mn, Co, Ni, Cu and Pb) changed after bioaugmentation at Day 100.

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Introduction

Global industrial revolution has led to the release of unprecedented amount of toxic substances (pollutants) into the environment. These pollutants not only pose threats to human health but also to the environment (air, soil, flora, fauna and water). Major sources of pollutants are attributed to rapid urbanization and increased population growth, invention of new products, methods of waste disposal, agricultural activities such as factory farming, transport and manufacturing industries (Ripin et al., 2014; Dixit et al., 2015; Dhaliwal et al., 2020).

An example of a toxic and recalcitrant pollutant of concern is heavy metals. Heavy metals are group of metals and metalloids with density greater than 4±1 g/cm^3 and typically with atomic number 21 or higher. Examples of heavy metals includes cadmium(Cd), iron(Fe),

cobalt(Co), chromium(Cr), copper(Cu), mercury(Hg), lead(Pb), zinc(Zn), arsenic(As), manganese(Mn), nickel(Ni), selenium(Se) and the platinum group metals, which comprises platinum, palladium, rhodium, ruthenium, osmium, and iridium (Mosa et al., 2015; Wolowiec et al., 2019). There are two major sources through which heavy metals enter into the environment; natural sources and anthropogenic or man-made sources. In nature, trace quantities of some these metal such as Fe, ,Se, Cd, Pb, Cu,, Zn, ,Mn, Ni, V, Cr are some of the essential micronutrients needed by life forms and they are only deleterious in high amounts (Masindi & Muedi, 2018; Singh et al., 2018; Rai et al., 2019).

According to Ripin et al., (2014) and Lai et al., (2011) sources and fluxes of anthropogenically generated heavy metals have received significant attention worldwide. This concern has also been raised in Malaysia. Heavy metal pollution concerns are due to its properties and behavior in the environment. One of the most important properties of these metals which differentiate them from some other toxic pollutants is that they are not biodegradable in the environment. Another problem associated with these metals is their potential for bioaccumulation and biomagnification causing heavier exposure for organisms than is present in the environment alone (Sameera et al., 2011).

Natural degradation of pollutants such as heavy metals is becoming increasingly difficult because of the increased quantity, heterogeneity and increased complexity of pollutants released into the environment (Masindi & Muedi, 2018). Various methods have been employed to achieve heavy metal removal from a given environment (Dhaliwal et al., 2020). These methods include physicochemical methods such as excavation and landfill thermal treatment, acid leaching, electro reclamation, chemical precipitation, electrochemical treatment, reverse osmosis, freeze crystallization, electrodialysis, cementation, bioremediation, chemical oxidation and reduction, ion exchange, filtration, starch xanthate adsorption, solvent extraction (Sinha et al., 2010; Mohammed et al., 2011; Shah, 2014; Li et al., 2019). Compared to other methods, bioremediation is considered the safest and best, it is also relatively low-cost and requires low-technology techniques. Bioremediation techniques also has high public acceptance (because it is safer for the environment) and can often be carried out on site. (Shah, 2014).

In this study, speciation analysis of bioaugmented and non bioaugmented leachate contaminated soil from Taman Beringin landfill site in Malaysia was carried out. This is to ascertain whether or not indigenous microbes found in Taman Beringin landfill site can be used to remove heavy metals such as Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb that were observed in the leachate contaminated soil.

Materials and Methods

Description of the sampled landfill

Taman Beringin landfill is located at North Jinjang which is about 10 km North West of Kuala Lumpur city centre. It received wastes from municipal, commercial, agricultural, recreational, domestic and mixed industrial wastes originating from households and industrial premises (Ashraf et al., 2019). The landfill covers an area of about 16 hectares. The landfill, which is not a sanitary type, started its disposal activities since 1991 and was closed in early 2005 (thus currently inactive) (Ashraf et al., 2019). Ex disposal sites such as Taman Beringin (TB) landfill have been proposed for redevelopment into residential buildings, public parks and commercial premises in the National Urban Policy in Malaysia by

the year 2020 (Simis, Awang & Arifin, 2016). Figure 1 shows the three sampled stations (Inset Figure 1).

Speciation of Heavy Metals Using Sequential Extraction Technique

In this study, a sequential extraction process based on Tessier et al. (1979), Tsang et al (2007) and standard ISO 11466 (1995) was performed for the determination of eight heavy metals (Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb) in soils samples. Equal weight of soil samples taken from the three sampled locations were mixed together. An initial weight of 1.0 g was used in the sequential extraction process and all sample analyses were ran in triplicates. The extraction procedures employed are detailed as follows:

Fraction 1 (F1) (Exchangeable metal fraction)

1g sample was extracted with 10 ml of 1M magnesium chloride ($MgCl_2$) at pH 7 for 2 hours at room temperature with continuous agitation.

Fraction 2 (F2) (carbonate bound metal fraction)

The residue from F1 was continuously agitated with 10 ml of 1M sodium acetate (NaOAc) adjusted to pH 5 with acetic acid (HOAc) at room temperature for 5 hours.

Fraction 3 (F3) (Fe-Mn oxides bound metal fraction)

Residue from F2 was mixed with 20 ml of 0.04M Hydroxylammonium hydrochloride (NH_2OH-HCl) in 25% (v/v) HOAc for 6 hours at 96 °C with occasional agitation. After cooling, the sample was diluted to 20 ml with deionised water.

Fraction 4 (F4) (Organic matter and sulphide bound metal fraction)

Residue from F3 was extracted with 3 ml of 0.02M nitric acid (HNO_3) and 5 ml of 30% hydrogen peroxide (H_2O_2) (adjusted to pH 2 with HNO_3), heated to 85 °C for 2 hours, then extracted with another 3 ml aliquot of 30% H_2O_2 (adjusted to pH 2 with HNO_3) at 85°C for 3 hours with intermittent agitation. After cooling 5 ml of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added, with continuous agitation for 30 minutes.

Fraction 5 (F5) (Residual metal fraction)

The residue from F4 was digested with 9 ml of 12 M hydrochloric acid (HCl) followed by 3ml of 15.8 M HNO_3 added dropwise to reduce foam. After that, 5 mL of 0.5 M HNO_3 was added and kept for 16 hours at room temperature to oxidize the organic matter in the soil. The mixture was then heated and maintained for 2 hours before cooling.

Thereafter, samples were centrifuged at 3500 rpm for eight minutes at room temperature for each extraction and the supernatants from each extraction were subjected to ICP-MS analysis. Prior to the start of the next extraction step, 10 ml deionised water was used to wash samples and then the washing solution was discarded after centrifugation. All the experiments were carried out in triplicates to reduce systematic error.

Bioaugmentation Setup

In this study, a blend of a total of fourteen (14) strains of bacteria from TB landfill was used in the bioaugmentation experiment. The following steps were taken to inoculate the blend of microorganisms into the leachate contaminated soil:

a. Leachate contaminated soil from all sampled stations were mixed together and prepared by removing unwanted debris such as stones, plastic, broken glass, grasses etc
b. Next, equal portions of soil in triplicates were set up under different conditions: TA (leachate contaminated soil amended with all the isolated bacteria strains from TB landfill) and TC (control soil which is leachate contaminated soil without any amendment with micro organisms)
c. TA was watered with 200 mL of the inoculum (obtained from equal volumes of pooled discrete species) each containing about 3 x 109 CFU g-1 while TC was not watered with the inoculum
d. After the microbial formula was introduced into the designated microcosm's soils, it marked the start of the bioaugmentation experiment.
e. Thereafter, portions of the soil microcosms were taken from TA and TC every 20 days during the experimental time-course (until 100 days) for heavy metal analysis using sequential extraction procedure and assessment of the total count of microbial population.
f. The soil moisture of the bioaugmentation setup was maintained during the experimental time-course by watering with distilled water. Caution was exercised during watering to avoid leaching of metal contents and contamination of the soil.

Results and Discussion

Chemical Speciation of Heavy Metals in Bioaugmented and Non- Bioaugmented Soils from TB Landfill

The sequential extraction procedure revealed metals in the F1, F2 and F3 which is the mobile fractions or forms of heavy metals in TB landfill. Metals observed in these fractions are bioavailable and potentially toxic. The last two fractions (F4 and F5) are the non mobile fractions or forms of metals observed in TB landfill. Metals extracted in the F4 and F5 are generally considered neither mobile nor bioavailable because they are bound to silicates and primary minerals in uncontaminated soils and sediments (Ashraf et al., 2011) and therefore may not pose immediate threat to the environment.

In this study, in order to gain an understanding of the mechanism by which bioremediation of heavy metals in leachate contaminated soil from TB landfill is reduced, sequential extraction procedures was used to identify changes associated with metal speciation in bioaugmented soil. The results of the sequential extraction obtained at Day 100 (which marked the end of the bioaugmentation) were compared to Day 0 i.e prior to the addition of the microorganism (at the beginning of the bioaugmentation) and with control soils. Detailed discussion of each metal and their speciation before and after bioaugmentation is given in subsequent sections.

The percentage reduction of the heavy metals at Day 100 compared to Day 0 was calculated using the following formula:

$$\text{Removal \%} = [(Co - Cf)/ Co] \times 100 \quad \text{.....equation 1}$$

Where Co and Cf are the initial and final concentration (µg/g) of metal in soil, respectively (Salehzadeh, 2013).

(a) Chromium

The distribution of Cr in TB landfill indicates that its content in various fractions varied in the microcosm at the beginning of the experiment (in non bioaugmented soil at Day 0), F4 and F5 had the highest concentration at 1.610 µg/g and 6.640 µg/g, respectively while F1, F2 and F3 were 0.060 µg/g, 0.070 µg/g and 0.700 µg/g of Cr, respectively. The F4 and F5 recorded the highest amount of Cr and thus the immobile fraction predominates over the mobile fraction in non bioaugmented soil at Day 0 (0.360 µg/g in F4 and 0.400 µg/ in F5) and in bioaugmented soil at Day100 (0.690 µg/g in F4 and 1.000 µg/g in F5). Furthermore, concentration of Cr was found to have decreased in bioaugmented soil at Day 100 (0.690 µg/g in F4 and 1.000 µg/g in F5) across all fractions. The highest percentage removal was observed in F1 in bioaugmented soil at Day 100.

Similar to this study, Jeyasingh and Philip (2005) reported that indigenous soil microbial community were able to reduce approximately 97% of hexavalent Cr (VI) (mobile and toxic form of Cr) in contaminated soil. Also Bader et al., (1999) reported that reduction in Cr concentration in contaminated sites is due to widespread Cr (VI) reducing bacteria in such soil and thus decontaminating polluted sites as seen in this study.

A decrease in percentage distribution of Cr in bioaugmented soil at Day 100 (except in F2 and F4) was observed compared to its distribution in non bioaugmented soil at Day 0. Cr decreased from 73.3% to 63.92% in F5, 7.71 to 7.56% in F3 and 0.66% to 0% in F1. The influence of microorganisms in contaminated soil was also observed to decrease Cr distribution in F1, F2, F3 in control soil (Insert figure 2).

(b) Manganese

High concentration of Mn was observed in TB landfill. Sources of manganese in MSW can be traced to the disposal of bottle caps, cosmetics, insecticides, paints and pigments along with domestic garbage (Kanmani and Gandhimathi, 2013). The mobile form of Mn was highest with F1, F2, and F3 having 6.560 µg/g, 25.180 µg/g and 22.940 µg/g, respectively in non bioaugmented soil at Day 0. Additionally, mobile form of Mn still dominated over the immobile form even in bioaugmented soil at Day 100. According to Nadaska (2010) and Habibah et al (2014), Oxide-Mn is readily reduced to available forms and is an important source of Mn for plants and so elevated levels observed in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100 is easily bioavailable in the environment. The removal efficiency in bioaugmented soil at Day 100 was about 49% in F1, 71% in F2 and 64% in F3. Reduction in Mn immobile forms was at lower rate in F4 (19.802%) as compared to other fractions. In F5, a 76.120% reduction was also observed

Mn distribution and speciation before and after remediation in TB landfill revealed that Mn reduced from 33.74% to 28.85% in F2 and 22.67% to 6.03% in F5. However, an increase in the percentage of F3 and F4 occurred in bioaugmented soil at Day 100. Addition of the microbial formula could have altered the distribution of Mn in soil (Insert figure 3).

(c) Cobalt

Speciation of Co revealed that the most dominant fraction was in the immobile form in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100. Additionally, complete reduction (100%) of Co in F1, F2 and F4 was observed in soil with added microorganisms after Day 100.

The percentage distribution of Co in the various chemical fractions was different from all other metals in TB. Co distribution was reduced from 31.3% to 25% in F2 with a

corresponding increase in the percentage distribution in F5 from 57.39% to 75%.The bioaugmentation of soil reduced the bioavailable form of Co in TB landfill. Complete reduction of Co in control soil can be attributed natural bioattenuation phenomenon (Emenike et al., 2016) (Insert figure 4).

In comparison to other metals in this study, low levels of cobalt recorded maybe due to the fact that typically, MSW landfills such as TB may not receive cobalt containing waste such as fossil fuel and waste combustion, aircraft exhausts, cobalt containing alloys, copper and nickel smelting and refining wastes, cobalt chemicals and fertilizers derived from phosphate rocks which have been observed to be the primary source of anthropogenically induced cobalt in soils (Sternenberger, 2007).

(d) Nickel

Ni was not detected in F1 and F2 in TB landfill and therefore the immobile form was more dominant especially in the residual fraction in non bioaugmented soil at Day 0. The only mobile form of this metal observed was associated with the Fe-Mn oxide bound metal fraction at 0.430 µg/g. In bioaugmented soil at Day 100, the immobile form of Ni was still observed to be prevalent. Further, in bioaugmented soil at Day 100, the percentage reduction of Ni concentration in the F3 was over 97%, thus eliminating the potential risk of this metal in the environment. Similarly, over 70% reduction in the immobile form of Ni was observed after Day 100 of bioaugmentation (Insert figure 5).

The chemical fraction of Ni changed in bioaugmented soil at Day 100 due to the addition of microbial formula to soils. Reduced percentage of Ni was observed in F3 from 14.04% to 1.47% and an increase in F5 (from 79.53% to 91.18%) was observed in bioaugmented soils at Day 100. Ni immobilization was observed in bioaugmented and control soils due to microbial action in soil.

(e) Copper

Similar to Ni, immobile forms of Cu were most prevalent in non bioaugmented soil at Day 0. The mobile form of Cu was still dominant over the immobile form even in bioaugmented soil at Day 100 with 3.010 µg/g in F4 and 4.780 µg/g in F5. In the mobile form, concentration of Cu was 0.040 µg/g, 0.110 µg/g and 0.570 µg/g in F1, F2 and F3 at Day 0, respectively. By Day 100, 100% reduction in F1 and F2 of Cu was observed while over 85% reduction in F3 was recorded thus making the immobile form still prevalent. Because the concentration of Cu was majorly associated with the F4 and F5, Cu in TB landfill may not pose immediate threat to the environment.

The percentage distribution of Cu showed there is a difference in the distribution of Cu in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100. Cu was majorly found in the F5 in non bioaugmented soil at Day 0. After bioaugmentation at Day 100, Cu was majorly found in F4 and this could be because Cu was completely reduced in F1 and F2 (Insert figure 6).

(f) Zinc

Similar to Ni, the immobile form of Zn predominate over all other fractions in non bioaugmented soil at Day 0 and in bioaugmented soil at Day 100. Zn concentration in mobile form was 0.250 µg/g and 2.520 µg/g in F2 and F3, respectively. A complete reduction of Zn in F2 was observed after Day 100 treatment while almost 70% reduction was observed in F3.

Similar to Ni, percentage distribution of Zn showed that in non bioaugmented soil at Day 0, Zn was mostly associated with F5 (65.29%). However, after bioaugmentation at Day 100, the pattern of distribution changed and thus Zn became mostly associated with F3 (43.56%). Complete reduction in percentage distribution of Zn in F2 was recorded bioaugmented soil at Day 100. This can be attributed to the addition of microbial formula in soil (Insert figure 7).

(g) Lead

Pb concentration in microcosm at the beginning of the experiment at Day 0 was observed to be highest in the immobile form with F4 and F5 having 0.790 µg/g and 1.980 µg/g, respectively. A contrast in the concentration of Pb in F1, F4 and F5 was observed in microcosm with microbial formula after Day 100 where an increase in Pb concentration occurred. Although metals confined in the residual fractions (immobile forms) are not usually expected to be released over short period of time under the conditions usually encountered in nature, Pb observed in TB may still pose immediate threat to the environment. This is because of its increased concentration in F1 observed after Day 100 of bioaugmentation. This result agreed with other studies by Kulikwoska and kluimuik (2008) who reported that there was no decrease in Pb concentration compared to other metals because Pb was observed to form very stable complexes with humic acid.

Pb distribution was in the order F5>F3>F4>F2>F1 however, a decrease in F2 (from 2.05% to 1.09%) and F3 (26.41% to 6.56%) was observed (Insert figure 8).

Conclusions

The chemical speciation of heavy metals in non-bioaugmented and bioaugmented soils Taman Beringin landfill revealed that the percentage reduction of the mobile and non mobile forms (F1, F2, F3, F4 and F5) varied for all metals and the distribution of the specific form of metals changed after bioaugmentation at Day 100. In addition, the percentage of metal distribution before (at Day 0) and after bioaugmentation (Day 100) highlighted that mobility of Mn, Co, Ni, Cu and Pb were reduced. However, Cr and Zn remained highly mobile after bioaugmentation at Day 100. These results indicate that microorganisms are able to reduce the mobility of most metals, however it will not completely eliminate the risk and the toxicity that maybe caused by these metals.

This study highlights the need for proper assessment of ex disposal sites such Taman Beringin landfill before they are redeveloped into residential buildings, public parks and commercial premises as proposed in the National Urban Policy in Malaysia by the year 2020.

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