

The Influence of Organic Matter Application Upon The Effectiveness of Reduction of Heavy Metals: Lead (Pb) and Cadmium (Cd) by Hyperaccumulator Plant

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Received: December 25, 2015 / Accepted: March 05, 2018

Abstract

Organic matter is able to bind heavy metals. Soil enrichment with organic matter can reduce the availability of metal species as a result of the complexing of heavy metal free ions. This research examined the chelation of lead (Pb) and cadmium (Cd) waste-source heavy metals by organic matter, and the utilization of hyperaccumulator plants to remediate contaminated lands. The study was aimed to (a) analyze the influence of guano organic matter application to reduce heavy metals, Pb and Cd, and the combined effectiveness with plants; (b) to identify the capability of three plants as hyperaccumulator plants. Guano was used as an organic matter source in soils artificially contaminated with Cd and Pb from industrial solid waste. The research was conducted in two phases. The first research phase used a combination of artificial Pb and Cd with guano. The second Research phase involved two dosages of guano and three concentrations of waste. Three plants were used as hyperaccumulator plants: elephant grass (*Pennisetum purpureum*), chinese cabbage (*Amarantus tricolor*), and water spinach (*Ipomoea aquatica*). The study results indicated that guano had the capability to chelate more than 90% of Pb and a maximum of 61% of Cd, hence reducing the concentration of Pb and Cd. Other results showed that the three hyperaccumulator plants generated different responses toward the addition of heavy metals (Pb and Cd). From the calculation of the Bio-Accumulation Factor (BAF), the value for Cd showed that *P. Purpureum* and *A. Tricolor* had good potential as hyperaccumulators. Only *P. purpureum* had the capacity as a hyperaccumulator for Pb, while *I. aquatica* was not a

hyperaccumulator plant for Cd or Pb.

Keywords: heavy metals; hyperaccumulator; guano; bioaccumulation factor

1. Introduction

In recent times, one of the serious environmental problems is the availability of heavy metals in soils. These metals typically come from waste material of agricultural practices, industries and human activities. The toxicity of heavy metals is another problem that significantly influences ecosystems, nutrition, human health, and environment. The continuous production of pollutants will have an impact on environmental sustainability. As one of the three pillars of sustainability, the environmental pillar influences the other two pillars (economic and social). The ability of both social and economic aspects to support to the expected levels will be hampered as the environmental quality decreases. Thus, pollution should be overcome without posing additional problems or burdens. Removal of persistent heavy metal pollutants is necessary yet very difficult and effective solutions are needed. A number of mechanisms have been suggested to reduce the negative impacts of heavy metals, which include chemical, mechanical and biological remediation.

Nowadays, organic matter is used for reducing bioavailability of heavy metals in soils and for soil improvement. Some researchers have indicated that the amendment of contaminated soils with organic matter reduced bioavailability of heavy metals. Organic amendments enhance bioremediation of heavy metals through various processes including immobilization, reduction, volatilization and rhizosphere modification¹. Soil organic matter is an important component of soil since it has a high surface area, functional groups (carboxyl and phenol groups) which metals can form chemical bonds with, and can form strong complexes with heavy metals. The rate of organic amendment degradation depends on the source and treatment of material prior to the application to soil. For example, application of organic matter from brown coal in soil decreased the bioaccumulation (BI) indexes of Cd, Pb, and Zn with an increase in the brown coal amendment dosage².

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Phytoremediation of heavy metal-contaminated soil is an emerging technology which aims to extract or inactivate metals in soils. It has attracted attention in recent years for the low-cost implementation and environmental benefits. Currently, phytoremediation has become an effective and reasonable technological solution used to reduce metal pollutants from contaminated soil. Phytoremediation takes 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. Many species of plants have been shown to be able to absorb contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. One of phytoremediation categories, phytoextraction, can be used to remove heavy metals from soil using its ability to uptake metals which are essential for plant growth (Fe, Mn, Zn, Cu, Mg, Mo, and Ni). Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se, Hg) can also be accumulated. The combination of utilising organic matter and plants is one method to reduce heavy metal (Bioremediation) and is likely to have a stronger impact than if they were used as independent bioremediation agents. The objectives of this study were: (a) to analyse the influence of organic matter application to reduce heavy metals, Pb and Cd and the combined effectiveness with plants; (b) to identify the capability of three plants as hyper-accumulator plants.

2. Methods and materials

2.1 Sample and sample preparation

The research was conducted in two phases. The first phase was carried out in a laboratory to test the capability of guano in binding heavy metals Cd and Pb through the use of artificial metal namely, CdCl_2 and PbSO_4 solution. Guano, an organic matter source, was taken from Mataram, West Nusa Tenggara Barat, Indonesia (Table 1). The research deployed a Complete Random Design Guano treatment ($G_1=100$ gram and $G_2=200$ gram) with the addition of heavy metal ($\text{Pb}_1=15$ ppm; $\text{Pb}_2=25$ ppm; $\text{Cd}_1=1$ ppm; $\text{Cd}_2=2$ ppm). The condition of Guano was maintained at 80-90% field capacity.

Metal Analysis of Pb and Cd was conducted at H+15 and H+30 incubation by collecting guano samples of 1-2 g per vase. Samples were taken by using Atomic Absorption Spectrophotometer (AAS). The percentage of bound efficiency by guano was calculated at the end of incubation (H+30) by calculating the ratio of heavy metal concentration at the chelate and metal concentration applied.

The second phase of the research was conducted in the glass-house in vivo with substances such as industrial paper waste as the source of Pb and Cd, guano as chelating agent and three indicator plants. The aims of the second research phase were to identify whether organic matter combinations (guano) with plants is more effective in

reducing the level of pollution and to check the capability of each plant as a hyperaccumulator plant.

Procedure

The research was designed using a split plot in which elephant grass (*Pennisetum purpureum*), chinese cabbage (*Amarantus tricolor*), and water spinach (*Ipomoea aquatica*) were the main plots. The sub plots were the combination of Pb and Cd with the rate of waste consisting of L_0 = without waste; $L_1 = 1.26 \text{ kg.pot}^{-1}$; $L_2 = 1.40 \text{ kg.pot}^{-1}$; and guano comprising G_0 = without guano and $G_1 = 100 \text{ g}$.

Guano as the organic matter source was taken from Mataram Nusa Tenggara Barat, Indonesia (Table 1). Sifted to <2 mm, sterilised to remove pathogens inside the guano. Inceptisol was dried and sifted to 2 mm, put into polybags up to 8 kg at the condition of 80-90% field capacity. Paper waste (contains 15.45 ppm Pb and 7.05 ppm Cd) was prepared by weighing according to treatment and then mixed with soil and guano. Plants are normally harvested at the age of 30 days.

Table 1 Chemical characteristics of the soil, and organic matter (guano)

Characteristic	Soil	Guano
pH (H ₂ O)	6.83	4.21
pH (KCl)	4.86	6.03
C Organic (%)	2.00	10.43
N (%)	0.11	3.43
C/N	18	3.04
Pb ($\mu\text{g.g}^{-1}$)	3.05	0.75
Cd ($\mu\text{g.g}^{-1}$)	0.55	0.55

Soil and plant Analysis

Plants were collected, washed, weighed, cut into pieces, and dried at 60°C to reach constant weight. Content of Pb and Cd in the soil and plant samples were determined using AAS. To evaluate the plant capability as a hyperaccumulator plant, the value of the BAF was calculated as a ratio of heavy metal content in a plant to its total content in a soil

3. Results and discussion

3.1 Chelating Cd and Pb

Guano has the capability to chelate and to reduce Pb concentration. The weight of guano used does not influence the capability to chelate Pb, although it does when chelating Cd. The more guano is in use, the more Cd is chelated (Tabel 2). The larger chelate by guano resulted in decreasing available heavy metal concentration.

Table 2 Concentration of Pb and Cd (soluble form) in guano

Treatment	Concentration (ppm)		Chelate by guano (%)	
	G1	G 2	G1	G2
Pb1	0.17 a	0.41 a	98.87	97.27
Pb2	0.17 a	0.52 a	99.32	97.92
Cd1	0.51 b	0.39 a	49.00	61.00
Cd2	0.26 a	0.29 a	87.00	86.00

Higher chelation of Pb (>90%) indicated no desorption by guano up to the 30th day of incubation. Naturally, the metal absorption process by organic matter has a certain absorption time and at the same time concurrent desorption processes will likely return the metal that was chelated by organic matter. Research³ has suggested that desorption process of metal Pb does not start until the 32nd day of incubation. Metal absorption by strong organic matter will likely reduce the solubility of a number of metals. Metals are specifically bound by functional groups of organic matter, particularly COOH, phenolic, and thiol-SH. Soft acid metals (Cd²⁺, Hg²⁺) bound with soft ligands (thiol); hard acid metals (Fe³⁺, Mn²⁺) with hard base ligands (OH⁻, COO⁻); borderline acids (Cu²⁺, Zn²⁺, Pb²⁺) will form complexes with weak or strong bonds⁴.

3.1 Plant Biomass

Application of guano organic matter in the soil reduces the concentration of heavy metals (Cd and Pb) either in the soil or in the plant tissue through a metal bonding mechanism with functional groups while increasing soil organic matter content at the same time. Research results showed that the addition of guano increased growth in all plants from 29.61% (*P. purpureum*) to 88.85% (*A. Aquatica*) than that without guano (Fig 1). The results were in line with several previous studies by researchers⁵ which indicated an increase in biomass by the addition of compost on mine-spoiled soil contaminated by heavy metals (Cu, Pb, Cd and Zn). The addition of organic matter enhanced the chemical, physical and biological soil properties.

Figure 1 shows how plants produced less biomass on the heavy metal contaminated soil when no organic matter was added and compares with the ones grown with organic matter. The application of organic matter to the soil has the highest impact on the rhizosphere zone. After the application of organic matter, generally numerous changes are detected in the chemical (e.g., pH level, organic acids, composition of nutrients) and biological soil properties. Chemical changes in the soil due to the addition of organic matter and plant growth can affect the transformation, mobility and bioavailability of metals⁶. Other research⁷ results showed that compost and vermicompost treatments

had significant effect on the physical and chemical properties of soil such as EC, pH, organic matter, macro and micronutrients content. Compared to the un-amended soil, soil treated with organic amendments showed apparent increases of organic matter, total N, pH, EC and available macroelements (P, K, Ca and Mg).

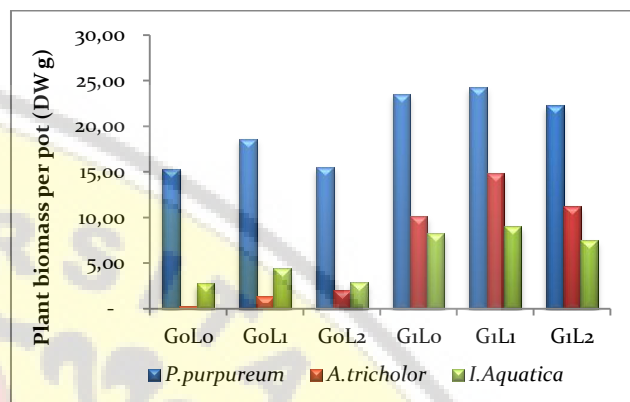


Figure 1 Plant biomass at harvest. Soil mixed with organic matter guano (G1);without guano (Go);without waste (Lo) and with waste application (L1 and L2) on three indicator plants.

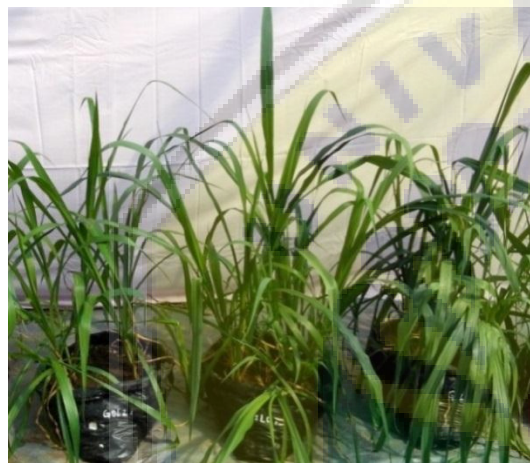
Increasing the amount of waste yielded lower plant biomass in both cases with and without organic matter, excluding the *A. Tricholor* plant. The existence of heavy metals in the soil solution can inhibit the absorption of other nutrients. Plant nutrient and Cd compete for the same transporter, and therefore the presence of Cd results in mineral nutrient deficiency⁸. The partitioning of Cd to different plant organs played a pivotal role in the toxicity of Cd to plants. The amount of Cd which accumulates in the plant was limited by several factors including: 1) Cd bioavailability within the rhizosphere; 2) Rates of Cd transport into roots via either the apoplastic or symplastic pathways; 3) The proportion of Cd fixed within roots as a Cd- phytochelatin complexes and accumulated within the vacuole; and 4) Rates of xylem loading and the translocation of Cd⁶. Visible symptoms of toxicity vary for each plant species and even for individual plants. Yet, most common and nonspecific symptoms were chlorosis, interveinal chlorosis, necrosis, stunted growth, shorter root length, and narrow leaves⁹.



(a)



(b)



(c)

Figure 2 (a) water spinach (*Ipomoea aquatica*); (b) chinese gabbage (*Amarantus tricolor*), and (c) elephant grass (*Pennisetum purpureum*) at 30th days. Control pot (GoLo) and treatment by added guano (GoL1).

The addition of guano also increased levels of soil organic C by 9.29% to 28.19% (Fig 3), hence improving plant growth although the soil was added with waste containing heavy metals Cd and Pb.

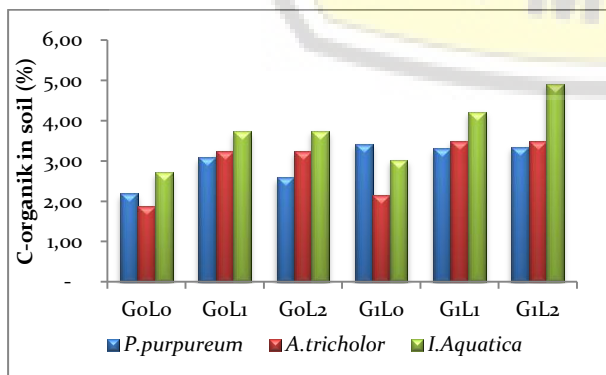


Figure 3 C-organic concentration in soil. Soil mixed with organic matter guano (G1) and without guano (Go) and application of waste contain Cd and Pb (L1 and L2) on three indicator plants.

3.2 Concentration of Cd and Pb in soil

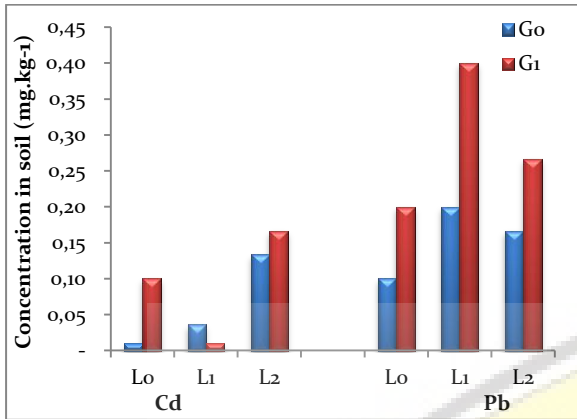
3.2.1 Cd in soil

The major factors which governed Cd speciation, absorption and distribution in soils were pH level, soluble organic matter content, hydrous metal oxide content, clay content and type, and the presence of organic and inorganic ligands. Cd concentration in the soil was the greatest with the addition of sewage sludge with indicator plant *I.aquatica* compared to the other two plants, namely $0.47 \mu\text{g.g}^{-1}$. The level of Cd concentration of soil is due to abnormal plant growth (Fig 2a), so the concentration in the plant tissue and the absorbance is also low (Fig 3a).

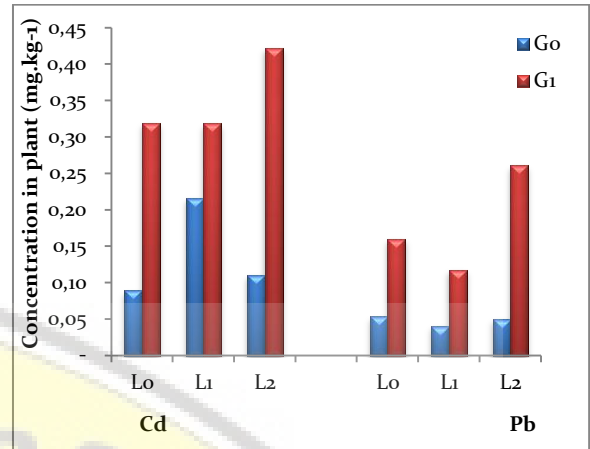
Based on the analysis, the addition of guano organic matter in the soil increased Cd concentration in the soil or in the tissue of the three plants (Figs 4, 5 and 6). Chelating metal with guano was not effective during plant growth as the plant was still taking-up Cd and translocating it into plant tissue. In addition, the surface of plant roots affected metal absorption. In plants with abnormal growth, the root absorption became lower due to heavy metals. Two mechanisms are responsible for metal transport from the bulk soil to plant roots: 1) convection or mass flow, and 2) diffusion.

Some methods based on the application of chelating agents (organic or inorganic) have shown an opposite effect of increasing heavy metals solubility¹⁰. Organic material affects contaminant concentrations, including heavy metal concentrations through a number of processes, such as immobilisation, reduction, evaporation or modification of the plant rhizosphere. Increasing the retention of metals in the soil resulted from increased surface electric charges and the presence of metal binding particles¹¹. Other research results¹² indicated a decrease in bioavailability of Cd, Pb and Zn (to 50%)with the addition of sewage sludge in the contaminated soil.

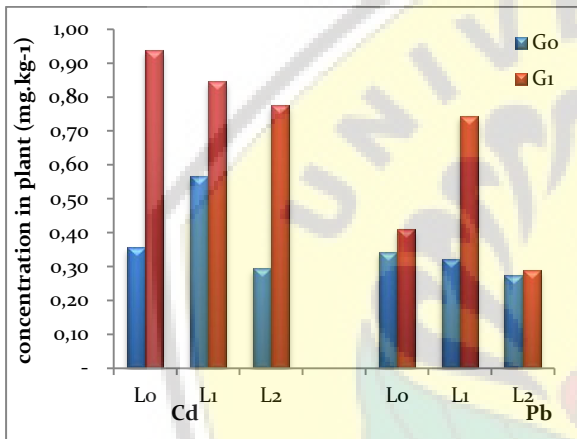
Three main processes control the flow of metals in soils such as (i) removal of metals from the soil solution by sorption onto soil particles, (ii) release of the metal from the soil particle to the soil solution (desorption), and (iii) precipitation–dissolution of the metal as an independent phase in the soil matrix.



(a)



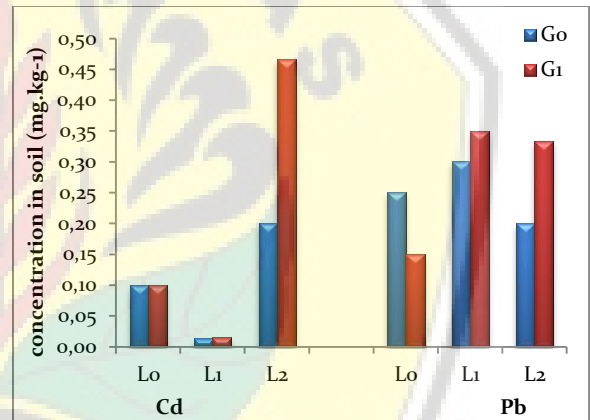
(b)



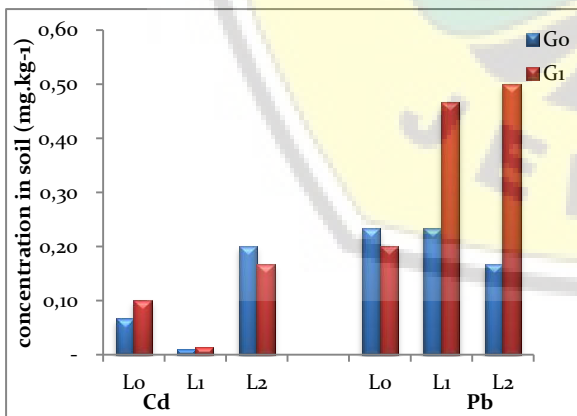
(b)

Figure 4 Concentration of Cd and Pb in soil (a) and in *Pennisetum purpureum* (b)

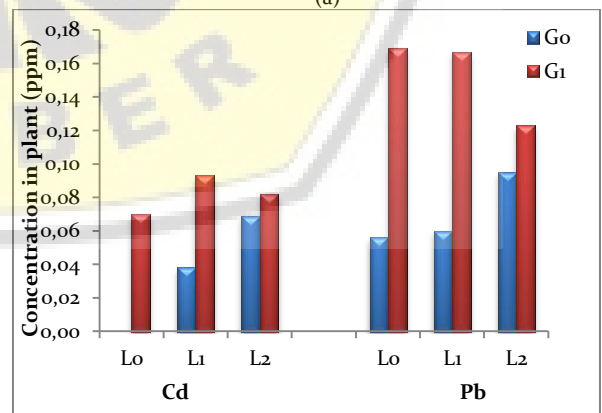
Figure 5 Concentration of Cd and Pb in soil (a) and in *Amarantus tricolor* (b)



(a)



(a)



(b)

Figure 6 Concentration of Cd and Pb in soil (a) and in *Ipomoea aquatica* (b)

3.2.2 Cd in plant

Plants are affected by a wide range of pollutants such as Pb, Cd Ni, Cr and others that vary in concentration, speciation, and toxicity. Figure 4b, 5b and 6b show the respective heavy metals concentrations in the biomass from the three plants (Elephant grass, chinese cabbage, and water spinach). The cadmium concentrations ranged between 0.10 to 0.94 $\mu\text{g}\cdot\text{g}^{-1}$ in *P. Purpureum*; 0.11 – 0.42 $\mu\text{g}\cdot\text{g}^{-1}$ in *A. tricolor*; 0.038 to 0.093 $\mu\text{g}\cdot\text{g}^{-1}$ in *I. aquatica* and these values were higher than the recommended WHO/FAO¹³ guidelines of 0.05 to 0.10 $\mu\text{g}\cdot\text{g}^{-1}$.

Cd Concentration in *P.purpureum* is higher than that in *A.tricolor* and *I.aquatica*. Root exudates have an important role in the acquisition of several essential metals. In addition, some plants can regulate metal solubility in the rhizosphere by exuding a variety of organic compounds from the roots. The complex metal ions of the root exudates keep them in solution available for uptake into the roots¹⁴. The plant roots also exudates to stabilize, demobilize and bind the contaminants in the soil matrix, thereby reducing their bioavailability. These are all termed the phytostabilization process. Certain plant species have been known to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone¹. Some grass species, for instance, can exude from roots a class of organic acid called siderophores (mugineic and avenic acids). In the present study, Cd was discovered to be particularly mobile in the soil-plant continuum.

3.2.3 Pb in soil

In soils, Pb may be in the form of free metal ion, complex with inorganic constituents (e.g., HCO_3^- , CO_3^{2-} , SO_4^{2-} , and Cl^-), or may exist as organic ligands (e.g., amino acids, fulvic acids, and humic acids). Alternatively, Pb may be absorbed onto particle surfaces (e.g., Fe-oxides, biological material, organic matter, and clay particles)⁸. For Pb concentrations, the values ranged from 0.27 to 0.74 $\mu\text{g}\cdot\text{g}^{-1}$ in *P. Purpureum* were higher than the WHO/FAO recommended value of 0.30 $\mu\text{g}\cdot\text{g}^{-1}$ while Pb concentration in *A. Tricolor* and *I. aquatica* was lower than WHO/FAO standard. Application of guano increased the concentration of Pb in soil and in the tissue of the three plants (Fig 4a, 5a and 6a). However, based on the analysis of the experiment, the availability of Pb concentration in the soil was still low. The initial concentration of total Pb in soil was 3.05 $\mu\text{g}\cdot\text{g}^{-1}$. Yet, at the end of the experiment, the availability of Pb concentration in the soil for the three plants ranged from 0.10 to 0.50 $\mu\text{g}\cdot\text{g}^{-1}$.

Heavy metal-contaminated soil from organic wastes which contains 15.45 $\mu\text{g}\cdot\text{g}^{-1}$ was not entirely available or mineralized. Some factors such as pH, competing cations, plant species, and cation exchange capacity may act individually or in combination with one another or even alter soil behaviour of the Pb present, as well as the rate of uptake by plants. In the research, part of the Pb present in

the soil solution is absorbed into the roots, and then becomes bound to carboxyl groups or still in organic form in the waste. Pb uptake by plants was low in the present study, as it is typical for this relatively immobile metal. Previous research results¹⁵ indicated the application of composted sewage sludge in contaminated soil lowered the concentration of heavy metals Cd, Cu, Pb and Zn in plant tissue to up to 80%.

3.3 Potential use of hyperaccumulator plant for phytoremediation

Phytoremediation is an emerging technology that employs the use of higher plants for the clean-up of contaminated environments. The effectiveness of phytoremediation methods depend on many factors, mainly on the bioavailability of pollutants to the roots of the plants and the processes of absorption, translocation and accumulation of contaminants by plants. The characteristic related to hyperaccumulator plant is the Bio-Accumulation Factor (BAF), that is, the concentration of heavy metal in plant divided by that in soil which should be higher than 1.

The value of BAF is one indicators of plant's ability to absorb metal elements. The greater the value of BAF, the greater the plant's ability in accumulating metals in the plant tissue.. BAF value for Cd in elephant grass and chinese cabbage plants are more than 1, with the concentration of Cd in tissue ranging from 0.11 to 0.94 $\mu\text{g}\cdot\text{g}^{-1}$ or more than 1% of total metal concentration in the soil. For economical phytoextraction grounds, plants should be able to accumulate at least 1% of the total heavy metal content in the soil into their dry shoot biomass.

A large dose of waste added to the soil mixed with guano lowers BAF value compared to that of no addition of guano (Figure 8). It showed that up to 30 days, the addition of guano in the soil with elephant grass and chinese cabbage is not optimal to bond Cd. In addition, the low BAF value could be due to the decomposition of organic waste which contains Cd since the treatment is not mineralized. Thus, the added guano did not bind Cd or desorb otherheavy metals from added waste. Cd concentration in plant tissue is the uptake of Cd from the ground. Other research results¹⁶ showed the addition of domestic sewage sludge which contains N could lower the value of the transfer factor (TF) in the three plants with the harvesting age of 6 weeks.

In contrast to water spinach, physiologically, plants do not show normal growth, and BAF value is low. In the research, water spinach was not a hyperaccumulator plant. However, other research results¹⁷ indicated that the potential use of water spinach as Cd phytoextraction were due to the behaviour of water spinach in regard to Cd accumulation, tolerance, and translocation to shoots.

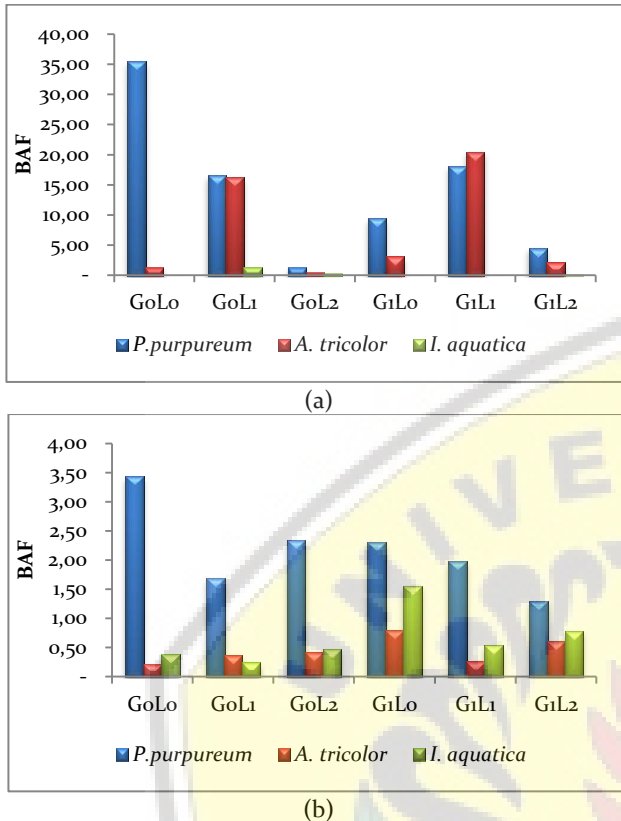


Figure 8 (a) Value of BAF Cd and (b) BAF Pb for elephant grass, chinese cabbage and water spinach

Based on BAF value for Pb, only elephant grass showed BAF value of more than 1. From Figure 2c, elephant grass growth is good through additional treatment of waste. Differences in the ability to transfer heavy metals in plants are strongly influenced by the characteristics and the physiological aspects of plants. It also indicates several mechanisms which function as plant systems to reduce the metal content through excessive metal excretion⁹. Furthermore, plants are perhaps capable of removing heavy metals from leaves and fruits and lowering the heavy metal content to reduce the toxicity of heavy metal⁸.

Differences of each crop in the BAF pattern on Pb and Cd is affected by the characteristics of each heavy metal, stage of plants growth, plant tolerance to heavy metal, the availability of heavy metal in the soil solution, the persistence of metals in soil and the presence of microorganisms around the plant roots⁹.

The effectiveness of phytoremediation methods depend on many factors, mainly on the bioavailability of pollutants in the roots of the plants and the processes of absorption, translocation and accumulation of contaminants by plants. The bioavailability of metals played a pivotal role in both approaches. Recent studies have shown that the transformation of pollutants in the soil is a dynamic process and metal bioavailability varies with time.

4. Conclusions

The research results showed that guano (organic matter) is capable of chelating more than 90% of Pb and a maximum of 61% of Cd. Combination of guano with plant increases plant biomass and concentration of heavy metals (Cd and Pb) in soil and plant than without guano. All of the three hyperaccumulator plants responded differently toward the addition of heavy metals (Pb and Cd) which contain waste. From the calculation of the Bio-Accumulation Factor, BAF value for Cd, it is obvious that *P. Purpureum* and *A. Tricolor* had the capacity as a hyper-accumulator. In addition, only *P. Purpureum* had the capacity as a hyperaccumulator for Pb. Furthermore, *I. Aquatica* was not considered as hyperaccumulator plant for Cd and Pb.

Acknowledgements

The fundamental research program from the University of Jember supported this research. We thank Mr. Lalu Sukarno for the heavy metals analysis.

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