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The Phytoremediation Potential of Several Plants in Heavy-Metal-Polluted Tropical Soils

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Abstract. A significant number of plants were suggested potential to alleviate toxic levels of heavy metals in soils through phytoremediation. This research was to study the phytoremediation potential of several plants in heavy-metal polluted tropical soils. Soil samples were collected from experimental plots amended in 1998 with 0, 15, and 60 Mg industrial waste ha^{-1}. The soil samples were planted in a glass-house experiment with 3 different plants i.e caisim (*Brassica chinensis var. Parachinensis*), water spinach (*Ipomoea aquatica*), and lettuce (*Lactuca sativa*). Plant parts (roots and shoots) and soil samples were harvested after a 4-week growth period and analyzed for Zn. The results show that the growth of plants was depressed by the increase in the soil Zn as affected by waste treatment, with water spinach being the most progressive and producing the greatest biomass. All plants significantly decreased the concentration of Zn in soils, with water spinach decreased the most significantly. The absorption of Zn increased with the soil Zn (high linear R^2). Zinc was accumulated greater in plant roots than that in shoots. All plants were good Zn phytostabilizators.

INTRODUCTION

Several methods were proposed to solve the alarming pollution of heavy metals in the soil environment, including the use of physical, chemical, and biological methods [1-9]. The biological method currently developed is the use of several heavy-metal bioaccumulating plants to lower soil heavy-metal concentrations by accumulating the heavy metals in plant roots (phytostabilization) or plant shoots (phytoextraction) or other harvested parts of plants, which are all referred to as phytoremediation [6, 9-11]. High heavy metal absorption and accumulation of heavy metals in plants may eventually lower their concentrations in soils. The effectiveness of phytoremediation were reported higher when combined with chemical methods by also employing soil amendment like lime, organic matter, chelating agents or biochar [1,6-7].

The method of phytoemediation uses plants to extract, neutralize, accumulate, and reduce contaminants like heavy metals from soil, water or air [12]. Phytoremediation is suggested to be an effective and economical strategy for transporting heavy metals from soils [13-14] and useful to reduce the risks associated with heavy metal contaminants through the use of hyperaccumulator plants [15]. The plant species used in phytoremediation must have a rapid growth potential and free of diseases and pests, must be able to compete with less desirable species, must be able to adapt to various soil and climatic conditions, and also must be able to grow in infertile soils [16].

A significant numbers of potential plants were suggested to be phytostabilizators, phytoextractors, or phytoremediators [1,3, 5-6, 9-10, 17-24]. These plants were expected to have hipertolerant characteristics on heavy metals, which mean they are capable of accumulating high concentrations of heavy metals in the root and shoot tissues and, thereby, called hyperaccumulator. Some of these plants may include amaranth, sunflowers, cabbage, chickpea, willows, broccoli, lettuce, and spinach, and several weeds like elephant grass [3,11, 25-28]. For example, our previous investigation showed that the accumulation of Cu or Zn by elephant grass increased with the increase in the soil concentrations of Cu or Zn resulted from a Cu-Zn containing waste amendment [9]. Plant accumulation of Cu and Zn in roots and the whole plant roots and shoots as well as their translocation factors (in general > 1.00) were highly

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correlated with their respective concentrations in soil ($r^2 > 0.90$), which confirm that the root/shoot growth and heavy metal absorption by elephant grass were governed by the concentrations of heavy metals in soils and elephant grass was a Cu and Zn phytoextractor. Investigation in [5] also showed that elephant grass was potential to lower heavy metals in soils.

This research was to evaluate the phytoremediation potential of several plants in heavy-metal-polluted tropical soils. Research on phytoremediation in tropical soils is currently limited. Tropical soils are distinct and significantly different from temperate soils, particularly related to their characteristics and the climate affecting their chemical dynamics and, therefore, may cause different responses of plants.

MATERIALS AND METHODS

The Samples of Heavy-Metal-Polluted Soils

Soil samples were collected from the experimental field plots set up in 1998 located in Sidosari, Natar South Lampung, Indonesia (5°20'14.1"S 105°14'39.2"E) (**Figure 1**) [29]. Soil samples were taken compositely from 5 points each plot in experimental plots treated with only industrial waste at control level (0 Mg ha^{-1}), low level (15 Mg ha^{-1}), and high level (60 Mg ha^{-1}) as reported by [17]. The soil sampling was conducted using a Belgian auger from 0 – 15 cm. The soil samples were air-dried, ground to pass a 2-mm sieve, and mixed thoroughly before being used in this experiment. The soil sample water content was determined to set oven-dry basis (105^{O}C 24 hours) weighing. Selected initial properties of the soil and industrial waste used are listed in **Table 1** [30].

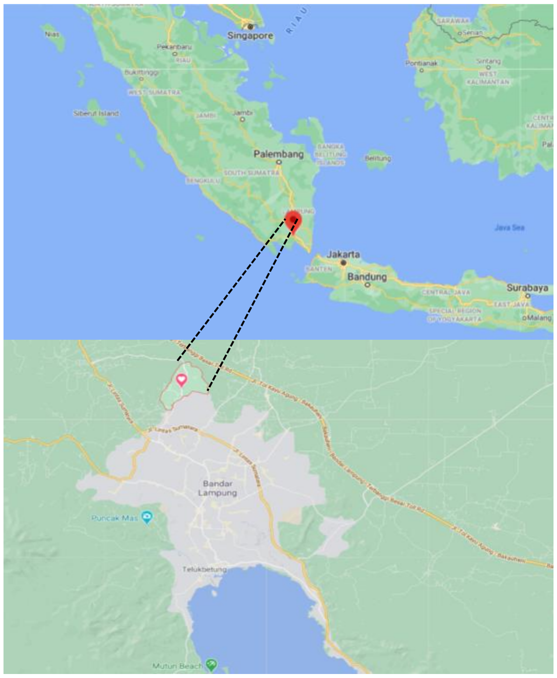

FIGURE 1. The google location of the experimental plots set up in 1998 [17].

The Glass-House Experiment

The glass-house experiment was conducted in the College of Al-Madani in Bandar Lampung, Indonesia. The experiment was conducted using a 200 g of soil sample (105^{O}C, 24 hours oven-dry equivalent) as a planting medium. Plants including caisim (*Brassica chinensis var. Parachinensis*), water spinach (*Ipomoea aquatica*), and lettuce (*Lactuca sativa*) were planted in the potted soil samples after addition of water to 40% (soil field-water moisture capacity). Plants were allowed to grow at the soil field-water capacity regulated by capillary water from a common water reservoir beneath the planting medium. All experimental units were conducted in triplicates.

The Plant and Soil Analyses

Plant shoots and roots were harvested separately and weighed for wet-weight and oven-dry weight (60^{O}C, 3 x 24 hours) and analyzed for plant Zn at the end of a four-week growing period. The soil samples were also taken for Zn analysis using 1 *N* HNO_3 involving the use of the iCE 3000 Atomic Absorption Spectrophotometer (AAS). In addition to soil Zn, soil total-N (using the method of Kjeldahl), organic C (using the method of Walkley and Black), and soil pH (1:2, pH electrode) were also determined. Soil analyses were conducted before and after planting.

TABLE 1. Selected initial properties of the soil and industrial waste used in this research.

Materials	Soil Fractions (Hydrometer)			pH 1:2 (H_2O)	Org. C (Walkley and Black)	Heavy Metals (DTPA)			
	Sand	Silt	Clay			Cu	Zn	Pb	Cd
	……….…… % ……........….				g kg^{-1}	…………..……. mg kg^{-1} ……..………			
Soil[a]	41.2	26.0	32.8	5.11	1.28	2.51	1.31	0.13	0.01
Waste				7.30		754	44.6	2.44	0.12

[a]Sandy Clay Loam [30]

Plant Zn was determined as followed, a 1 g of oven-dried and finely ground plant tissue was put in a porcelain crucible and placed in a furnace, heated at 300^{O}C for 2 hours and then at 500^{O}C for 4 hours, after which the plant sample was let to reach room temperature. The plant sample was wetted with several drops of distilled water and treated with 10 ml of 1 *N* HCl and put on a hot plate and let to gently boil. After cooling, the soluble plant tissue ash was filtered into a 100 ml volumetric flask. The crucible was then rinsed with 10 ml 1 *N* HCl and about 50 ml distilled water on the filter paper into the volumetric flask. Distilled water was added to dilute the filtrate to 100 ml. The filtrate was gently shaken before analyzed for Zn using flame AAS at λ = 213.9 nm [17].

Soil Zn was determined as followed, 2 g (105^{O}C, 24 hours oven-dry equivalent) of soil sample was put into an extracting bottle and 20 ml 1 *N* HNO_3 was added. The mixture was then put in an end-to-end shaker for 2 hours. After filtration, the supernatant was then determined for Zn concentration using flame AAS at λ = 213.9 nm [31].

RESULTS AND DISCUSSION

The Changes in Soil and Plant Zn

As expected, all plants evaluated in this research significantly lowered the concentration of soil Zn (Table 2). Water spinach showed the greatest alleviation effect on soil Zn. The decreases in soil Zn range between (-16.0) – (-38.5) % by water spinach, which are higher compared to those by caisim that range between (-8.90) – (-29.8) % and the lowest by lettuce that range between (-7.8) – (-23.7) %. This data clearly shows that water spinach was the most effective of the three plants in lowering the concentrations of soil Zn. These observations are in accordance with the decrease in soil Cu reported by [17] by these plants which also showed that water spinach lowered the soil concentration of Cu more significantly compared to the other two plants.

The effectiveness of each plant in lowering the soil Zn was dependent on the concentrations of soil Zn as affected by waste amendment (**Table 2**). In general, the percentage of the soil Zn decrease was lower at higher level of waste. For example, the decrease of soil Zn by water spinach was (-38.5), (-21.6), and (-16.0%) in soil amended with 0, 15, and 60 Mg waste ha^{-1}, respectively. Similar phenomena were observed in soils planted with caisim and lettuce (**Table 2**). The presence of higher Zn at higher waste levels seemed to inhibit the Zn absorption by all plants. As suggested by some researchers [31-33], high concentrations of heavy metals may disturb and inhibit the growth of plants. Investigation in [32] showed that the excess of Cu in soils shows a cytotoxic role causing stress and injury to plants which may cause growth retardation. Exposure of plants to high Cu may stimulate oxidative stress that causes disturbance of metabolic pathways and damages to macromolecules and reduces growth of some plants. The phytotoxicity of Zn is also indicated by the decrease in plant growth and development as well as the induction of oxidative damages in some plants.

The uptake of Zn by all plants actually increased with the increase in soil concentration of Zn (**Figure 2**). For example, the uptake of Zn by water spinach from soil with 0, 15, and 60 Mg waste ha^{-1} were 1.821, 1.926, and 2.096 mg kg^{-1}, respectively, which increases with the increase in the soil Zn concentration. Similar trend was observed in soils planted with caisim or lettuce (**Figure 2**). Regression analysis shows that the plant Zn uptake and soil Zn were also positively and linearly well-correlated with high correlation coefficient with $R^2 = 0.96$* in soils planted with caisim, $R^2 = 0.95$* in soils planted with water spinach, and 0.64* in soils planted with lettuce (**Figure 3**). The finding in [9] also shows a similar trend in the heavy-metal contaminated soils planted with elephant grass; plant accumulation of Cu and Zn in roots and the whole plant roots and shoots were highly correlated with their respective concentrations in soils ($r^2 > 0.90$). However, the increase in plant Zn uptake was not enough to significantly lower the concentration of soil Zn with the increase in waste levels, particularly at high levels of waste. It is interesting to note that the Zn uptake of water spinach is shown higher than those of caisim and lettuce at all levels of waste, underlining the fact that water spinach was the most effective heavy-metal extractor among the three plants. No data for lettuce at the highest level of waste. Lettuce was dead at high waste level (**Figure 2**).

TABLE 2. The changes in the concentration of Zn in contaminated soil by phytostabilization.

Waste Level	Before Planting	After Planting	Δ Zn^a	
… Mg ha^{-1} …	…………………... mg kg^{-1}	……..……….		… % …
		Caisim		
0	46.0	32.3	- 13.7	- 29.8
15	58.2	46.9	- 11.3	- 19.4
60	81.0	73.8	- 7.2	- 8.9
		Water Spinach		
0	46.0	30.3	- 17.7	- 38.5
15	58.2	45.6	- 12.6	- 21.6
60	81.0	68.0	- 13.0	- 16.0
		Lettuce		
0	46.0	35.1	-10.9	- 23.7
15	58.2	47.7	- 10.5	- 18.0
60	81.0	74.7	- 6..3	- 7.8

$^a\Delta$Zn = Zn (After Planting) – Zn (Before Planting); %ΔZn = 100 x ΔZn/Zn (Before Planting)

All data shown above show that water spinach was able to grow well in the Zn polluted tropical soils (**Table 2**, **Figure 2**). This plant showed high adaptation power to environmental pressure such as heavy metals as it was proven from its adaptability to contaminated tropical soils and showed no toxicity symptom. According to [34], plants with high biomass and rapid growth such as water spinach tended to have better absorption and translocation of metals. Conversely, observation showed that lettuce demonstrated the symptoms of heavy metal toxicity in the form of chlorosis at 4 days after planting and died 2 weeks after planting in soil treated with 60 Mg waste ha^{-1}, which suggests that lettuce was not suitable for phytoremediation agent in soils with high heavy metals. As that the biomasses of all plants, the soil organic C and total N were also affected by soil Zn; increased at lower concentration but were lowered by high concentrations of Zn (**Table 3**). The presence of more heavy metals like Zn in soils may have depressed the ability of plant roots to produce organic exudates such as organic acids. High soil Zn and other heavy metals may also lower the soil microbial population and activity, and soil enzymatic properties, that may lower organic C.

The Plant Phytoremediation Characteristics

The behavior of each plant in accumulating Zn from soils was closely related to their root and shoot growth properties. As also shown by [17] in the case of Cu, in general, the presence of Zn in soils stimulated plant-mass distribution to the roots, which was normal for plant roots in responding to the environmental stresses like high level of Zn in soil (**Table 4**). For example, the root-to-shoot ratio (RSR) of caisim grown in the control soil was 0.40 while that in the highest Zn-containing waste was 0.50 (**Table 4**). Lettuce also showed similar behavior, the RSR in soils with 0 and 15 Mg waste ha^{-1} were 0.50 and 0.75, respectively (**Table 4**). This phenomenon was also previously reported for napier grass (*Pennisetum purpureum*) grown in these soils [9], the RSR of napier grass increased with the increase in soil Cu and Zn concentrations. However, this phenomenon was not observed for water spinach, which showed decreasing RSR with the increase in soil Zn concentration (**Table 4**). Water spinach was observed to be more tolerant to high level Zn as shown by higher Zn uptake (**Figure 2**) and their effect on soil ΔZn (**Table 2**) than the other two plants. However, the growth of all plants (roots and shoots) was significantly depressed by the increasing soil Zn (**Table 4**).

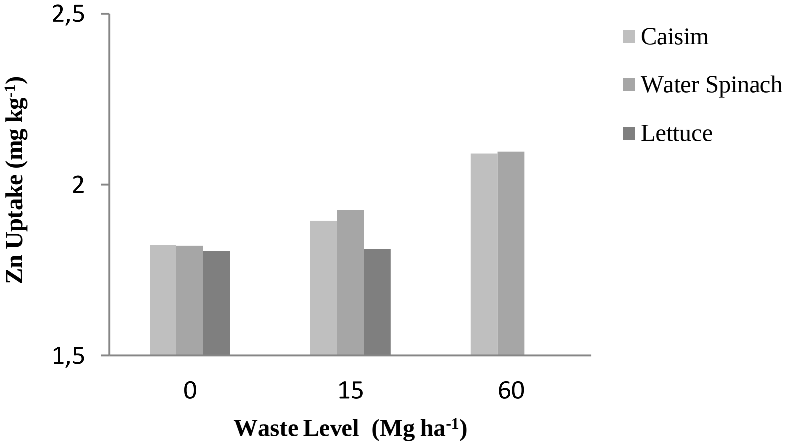

FIGURE 2. The uptake of Zn by several plants from heavy-metal polluted soils

(lettuce was dead at high level of Zn).

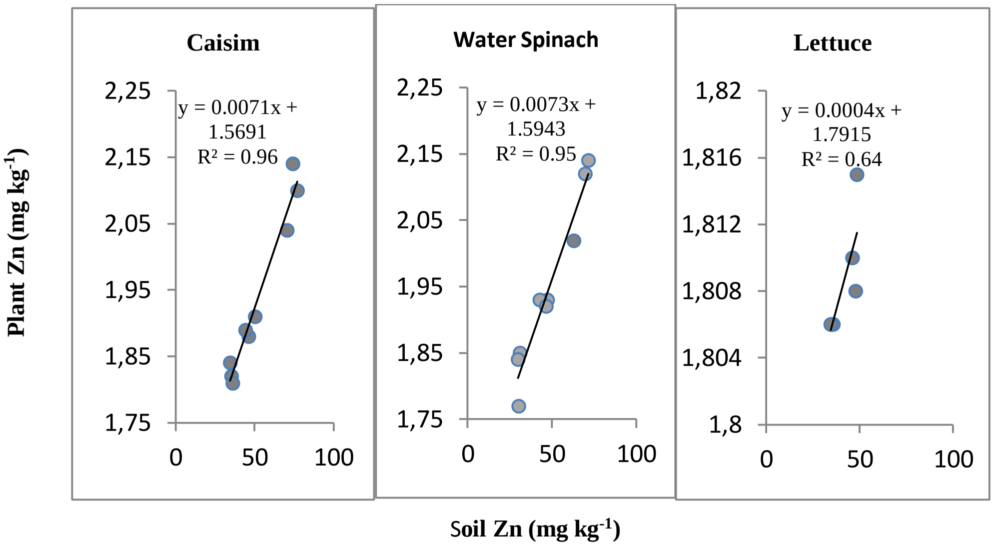

FIGURE 3. The relationship between plant Zn and soil Zn (lettuce was dead at high level of Zn).

Table 3. The changes in soil organic C and total N by different plants.

Waste Levels .. $Mg\ ha^{-1}$ ..	Before Planting	After Planting With Caisim	Water Spinach	Lettuce[a]
		Organic C ($mg\ kg^{-1}$)		
0	14.8	11.8	11.7	12.5
15	14.6	14.5	13.9	14.2
60	14.4	13.5	12.8	13.3
		Total N ($mg\ kg^{-1}$)		
0	1.8	1.1	1.2	1.0
15	1.6	1.3	1.2	0.9
60	1.6	1.3	1.3	1.2

[a]lettuce was dead at high level of Zn

TABLE 4. The growth properties of plants as affected by waste level.

Waste Levels .. $Mg\ ha^{-1}$ ..	Growth Properties[a]	Plants Caisim	Water Spinach	Lettuce
0	Root Weight ($g\ pot^{-1}$)	0.04	0.18	0.03
	Shoot Weight ($g\ pot^{-1}$)	0.10	0.46	0.06
	Root/Shoot	**0.40**	**0.39**	**0.50**
15	Root Weight ($g\ pot^{-1}$)	0.03	0.11	0.03
	Shoot Weight ($g\ pot^{-1}$)	0.07	0.33	0.04
	Root/Shoot	**0.43**	**0.33**	**0,75**
60	Root Weight ($g\ pot^{-1}$)	0.03	0.07	0.00
	Shoot Weight ($g\ pot^{-1}$)	0.06	0.24	0.00
	Root/Shoot	**0.50**	**0.29**	-[b]

[a]After [17]; [b]lettuce was dead at high level of Zn

Based on the RSR, water spinach was the most adaptive to the environmental pressure by Zn and other heavy metals as shown by the lowest RSR (**Table 4**). For example, the RSR in soils with 15 Mg waste ha^{-1} were 0.0.33 < 0.43 < 0.75 for water spinach < caisim, and < lettuce, respectively. These values also indicate that lettuce was the most ineffective in responding to the environmental stress by heavy metals. Therefore, lettuce was dead in soil with 60 Mg waste ha^{-1}. According to [34], plants with high biomass and rapid growth tended to have high heavy metal absorption and better metal translocation; which are shown by the highest absorption of Zn by water spinach.

In the process of phytoextraction, the heavy metals in soils are subsequently absorbed by plant roots and transported to plant shoots. Higher accumulation in plant shoots and/or plant roots are expected, preferably in plant shoots (phytoexctraction) rather than in plant roots (phytostabilization). The accumulation of Zn by water spinach shows that it is possible for water spinach to be a phytoremediation agent in Zn-contaminated soils through phytostabilization, in which greater Zn is accumulated in plant roots (**Table 5**). It is in line with [35] stating that plant roots have a role more important than plant shoots in restoring contaminated soils. However, the selection of plants for phytoremediation does not consider only the ability of plant to absorb heavy metals, but also the growth response of plants. To optimize the success of phytoremediation, the selected plants must be fast-growing, have a high ability to absorb metals, and able to provide large biomass [36].

There was a decreasing trend of TF for Zn with the increase in the soil Zn concentration. For example, the TF for caisim were 0.029, 0.030, and 0.026 in soils with 0, 15, and 60 Mg waste ha^{-1} (**Table 5**), which indicate less Zn was accumulated in shoots and conversely higher Zn was accumulated in roots at higher concentration of Zn in soil. The trend was also observed for water spinach (**Table 5**). However, in is clear from **Table 5** that the major part of absorbed Zn by all plants was accumulated in plant roots, underlining that all of the investigated plants were phytostabilizators rather than phytoextractors.

Table 5. The translocation factor of absorbed Zn in plants.

Waste Levels .. Mg ha^{-1}..	Plant Parts	Plants		
		Caisim	Water Spinach	Lettuce
0	Root Zn (mg kg^{-1})	1.770	1.766	1.754
	Shoot Zn (mg kg^{-1})	0.052	0.054	0.052
	TF[1]	**0.029**	**0.031**	**0.030**
15	Root Zn (mg kg^{-1})	1.839	1.873	1.758
	Shoot Zn (mg kg^{-1})	0.056	0.052	0.053
	TF	**0.030**	**0.028**	**0.030**
60	Root Zn (mg kg^{-1})	2.071	2.039	**-**[2]
	Shoot Zn (mg kg^{-1})	0.054	0.057	**-**
	TF	**0.026**	**0.028**	**-**

[1] TF (Translocation Factor) = shoot Zn/root Zn; [2] lettuce was dead at high level of Zn

CONCLUSIONS

The growth of all plants was depressed by the increase in the soil Zn as affected by waste treatment, with water spinach being the most progressive and producing the greatest biomass. All plants significantly decreased the soil Zn with water spinach caused the greatest decrease. The absorption of Zn increased with the soil Zn (high linear R^2). Zinc was accumulated greater in plant roots than that in shoots. All plants were Zn phytostabilizators.

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