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Lime-Enhanced Phytoextraction of Copper and Zinc by Land Spinach (*Ipomoea reptans* Poir.) from Tropical Soils Contaminated with Heavy Metals

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Abstract. Phytoextraction and liming were suggested to decrease the concentrations of heavy metals in contaminated soils. This research was to study the effects of liming and phytoextraction by land spinach in lowering the soil concentrations of Cu and Zn in heavy-metal contaminated tropical soils. Soil samples collected from a 22-years-old experimental field one-time amended with heavy-metal-containing waste at 0-60 Mg ha^{-1} were treated with lime at 0-5 Mg ha^{-1} and planted with land spinach in a glass-house experiment. After 4 weeks the soil and plant Cu and Zn were analyzed. The results show that the 60 Mg waste ha^{-1} significantly increased the soil concentrations of Cu and Zn and depressed the land spinach growth. Land spinach slightly lowered the soil concentrations of Cu and Zn in unlimed soils at waste levels $\leq$ 15 Mg ha^{-1}. Liming significantly lowered the soil concentrations of Cu and Zn and improved the growth and Cu-Zn uptake of land spinach but attenuated the effect of land spinach in decreasing the soil concentrations of Cu and Zn. The Cu-Zn uptake of land spinach was well-correlated with the soil concentrations of Cu and Zn.

INTRODUCTION

Phytoextraction was reported to lower heavy metal concentrations in contaminated soils [1-9]. Several heavy metal bio-accumulating plants had been recently reported [1,2, 9-13]. Several plants were reported to stabilize heavy metals in plant roots called phytostabilizers and some transported the most parts of heavy metals to plant shoots called phytoextractors. The basic mechanism is plants extract heavy metals from soil solution and accumulate them in plant roots (phytostabilizers) and/or plant shoots (phytoextractor). The lowering heavy metal levels in soil solution may then drive adsorbed or precipitated heavy metals to release or dissolve into soil solution and be absorbed by plants. The end result expected is the decrease of heavy metals in soils.

Our previous investigation in heavy-metal contaminated tropical soils [8] clearly showed that several plants including lettuce (*Lactuca sativa*), water spinach (*Ipomoea aquatica*), and caisim (*Brassica chinensis var. Parachinensis*) were Cu phytostabilizers and were able to lower the 1 *N* HNO_3-extractable Cu as high as 20% after four weeks growing period in a glass-house experiment. Napier grass (*Pennisetum purpureum*) was also reported to be a Cu and Zn phytoextractor [5]. The accumulations of Cu and Zn in these plants were correlated well with their concentrations in the soils.

The soil levels of heavy metals are also subjected to soil colloid adsorption which may decrease their levels in soil solution [14-28]. The presence of soil colloids may enhance the heavy metal immobilization which may hinder excessive amounts of heavy metal absorption by plants that may depress the plant growth and development. The presence of particular amount of soil colloid negative charges may lower the soil solution heavy metal levels and may stimulate plant roots to absorb lower, non-toxic, and more suitable levels of heavy metals and, therefore, may finally absorb higher amounts of heavy metals. The heavy metals are then temporary pooled in the soil colloid surfaces and to be released and absorbed by plants whenever the heavy metal levels in the soil solution lower due to plant absorption. The enhancement in the adsorption capacity of soil colloids were possible among which by increasing the

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pH of tropical soils by liming [16,17,19,22,23, 29-33]. Our previous investigation showed that the soil concentration and the accumulation of Cu and Zn by napier grass in 20 years old heavy-metal contaminated soils were lower in soils that were also treated with lime than those in soils without lime treatment [5]. Previous report also showed that lime at 5-20 Mg $CaCO_3$ ha^{-1} significantly reduced the soil concentration and plant absorption of Cd by water spinach [33]. Other report *showed* that combined with Ca-bentonite and tobacco biochar, lime treatment significantly immobilized Cd up to 22.0% [22-23].

Liming was, therefore, expected to enhance the phytoextraction of soil heavy metals by availing heavy metals at particular lower levels and may enable the phytoextraction to progress in lowering heavy metal concentrations in soils. This research aimed to evaluate the effect of liming on heavy metal phytoextraction by land spinach planted in long-time heavy-metal contaminated tropical soils.

MATERIALS AND METHODS

Experimental Design

This research was a glass-house experiment. The experiment involved 3 factors i.e. heavy-metal-containing-waste-amended soils, lime, and plant, arranged in a completely randomized design. Soil samples consisted of those from control plots (without industrial waste amendment), from plots amended with 15 Mg industrial waste ha^{-1}, and from plots amended with 60 Mg industrial waste ha^{-1}. Lime was given at 0 and 5 Mg $CaCO_3$ ha^{-1}. Plant was without plant and with land spinach. All treatments were replicated 3 times.

Soil, Lime, and Plants

Soil samples were taken from a well maintained experiment field in Natar, South Lampung, Indonesia previously reported [31]. The soil in the experimental field was initially characterized by the properties listed in **Table 1**. Soil samples were collected only from plots treated one time with industrial waste in July 1998 or 22 years ago at 0 Mg ha^{-1} (Control Plots), 15 Mg ha^{-1}(low heavy metal plots), and 60 Mg ha^{-1} (high heavy metal plots) (**Table 2**). Some properties of the industrial waste are listed in **Table 1**.

Soil samples (0 – 15 cm) were collected compositely from 5 sampling points in each plot and combined for all replications for the same treatment plot. After sampling, the soil samples were air-dried, ground to pass a 2-mm sieve, and thoroughly mixed for the glass-house experiment. Lime was $CaCO_3$. Plant was land spinach (*Ipomoea reptans* Poir).

TABLE 1. Several initial properties of the soil and industrial waste used in this research [17].

Materials	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[b]				7.30		754	44.6	2.44	0.12

[a]Sandy Clay Loam; [b]metal-wares industrial waste

TABLE 2. The heavy-metal contaminated soils used in this research[a].

Soil Symbol	Industrial Waste	Lime	Organic Compost
	………….…………. Mg ha^{-1}		……………………..….
S_0	0	0	0
S_1	15	0	0
S_2	60	0	0

[a]Taken in July 2019 from experimental plots set in July 1998 [31]

Planting and Observation

A 200 g of air-dry soil sample (oven-dry equivalent 105^{O}C 24 hours) was used as the planting medium. After being mixed with lime and capillary-watered to the soil field-water capacity, 2 seedlings of land spinach prepared 2 weeks earlier were planted. The soil water content was maintained capillary at the soil field capacity during plant growth.

Soil and Plant Harvest and Analyses

The plant biomasses were harvested at the end of a 4-week growing period. Plants were cut at the soil surface. The plant shoots and roots were cleaned up with tap water to free the biomasses from the soil masses. After drying in a 60^{O}C-oven for 3 x 24 hours, the dry-weight of shoots and roots was determined using an analytical balance. Soil samples were also harvested for soil analyses on heavy metal status. The Cu and Zn concentrations in plant root and shoot as well as in soil samples were determined with the methods described in [8]. The soil pH (water 1:2) and Walkley and Black Organic C were also determined.

RESULTS AND DISCUSSION

The Changes in the Soil Concentrations of Cu and Zn by Lime Treatment

As expected, in the absence of land spinach, lime significantly decreased the soil concentrations of Cu and Zn particularly at waste levels ≤ 15 Mg ha^{-1} (**Table 3**, **Table 4**, and **Table 5**). It is consistent with the previous report that lime at 5-20 Mg $CaCO_3$ ha^{-1} significantly reduced the soil and plant absorption of Cd by water spinach [33]. Copper and Zn were probably adsorbed by soil colloids that may have had more exchange sites at the increasingly soil pH (**Table 6**) [5, 29-33]. The presence of 1:1 soil clay minerals and organic matter in this tropical soil may have enabled this process to occur [22-24]. Higher concentration of OH^- ions may have also caused the occurrence of precipitation reaction lowering the soil concentrations of Cu and Zn [29]. This phenomenon was not observed at waste level of 60 Mg ha^{-1}, both for Cu (**Table 3**) and Zn (**Table 4**). The increase in the soil exchange sites were probably not sufficient to accommodate high concentrations of Cu, Zn, and other metal cations at high levels of industrial waste.

TABLE 3. The influence of waste, lime, and land spinach on the concentrations of Cu in waste-amended soils.

Waste	Lime	Without Land Spinach[a]	With Land Spinach[a]
.... Mg ha^{-1}		**............** mg kg^{-1} **............**	
0	**0**	33,1 d	30,2 b
	5	29,1 c	31,0 b
15	**0**	25,6 b	24,9 a
	5	23,0 a	24,7 a
60	**0**	65,1 e	64,8 c
	5	63,9 e	64,4 c
HSD 5%		2,3	2.3

[a]Different lowercases in one column show a significant difference at 5% HSD test

Effect of Land Spinach on Soil Concentrations of Cu and Zn

The presence of land spinach lowered the concentrations of Cu and significantly decreased the soil Zn at control soil (**Table 3**, **Table 4** and **Table 5**). The decreases were greater in the control soils than those in soils treated with ≥ 15 Mg waste ha^{-1} (**Table 7**). The phenomenon was related to the inhibited growth of land spinach with increasing heavy metals originated from waste amendment. Heavy metals were in general reported to be detrimental to plants [5,8,29]. Previously reported [5,8] that the growth of several plants including water spinach, caisim, lettuce, and also napier grass were inhibited by the increasing levels of Cu and Zn. The root/shoot ratio of napier grass increased and correlated well with the increase in soil Cu or Zn concentrations, indicating the negative effect of heavy metal on the growth of plants [5].

The lowering effect of land spinach on soil concentrations of Cu and Zn was attenuated by liming (**Table 3**, **Table 4** and **Table 7**). In soils with lime treatment, the presence of land spinach significantly increased the soil concentrations of Cu and Zn in the control soils. Land spinach lowered the effect of lime and in general increased the concentrations of soil Cu and Zn at all waste levels.. The excretion of H^+ and organic acids by land spinach plant roots may have probably caused the soil concentrations of Cu and Zn higher than that in soils not planted with land spinach.

TABLE 4. The influence of waste, lime, and land spinach on the concentrations of Zn in waste-amended soils.

Waste	**Lime**	**Without Land Spinach**[a]	**With Land Spinach**[a]
.... Mg ha^{-1}		.... mg kg^{-1}	
	0	27,0 c	22,3 b
0		B	A
	5	20,8 b	23,5 b
		A	B
	0	20,2 b	19,4 a
15		A	A
	5	17,4 a	19,3 a
		A	A
	0	44,2 d	43,4 c
60		A	A
	5	42,1 d	42,7 c
		A	A
HSD 5%		2,3	

[a]Different characters in one column or one line indicate a significant difference at 5% HSD test; upper-case letters indicate line comparison, lower-case letters indicate column comparison.

TABLE 5. Analysis of variance on the effects of waste, lime and land spinach on Cu, Zn, and pH of waste-amended soils.

Treatment Factor[a]	**Cu**	**Zn**	**pH**
W	**	**	ns[b]
L	**	**	**
S	ns	ns	ns
WL	ns	ns	ns
WS	ns	ns	ns
LS	**	**	ns
WLS	ns	*	ns

[a]W = waste, L = lime, S = land spinach; *Significantly different at 5% and ** at 1%; [b]ns non-significant

TABLE 6. The influence of waste, lime, and land spinach on the pH of the waste-amended soils.

Waste	**Lime**	**Without Land Spinach**[a]	**With Land Spinach**[a]
.... Mg ha^{-1}			
0	**0**	5,09 a	5,09 a
	5	6,56 b	6,45 b
15	**0**	5,15 a	5,25 a
	5	6,21 b	6,47 b
60	**0**	5,10 a	4,94 a
	5	6,26 b	6,43 b
HSD 5%		0,35	

[a]Different characters in one column indicate a significant difference at 5% HSD test

Copper and Zinc Uptake by Land Spinach

The influence of lime on Cu and Zn uptake of land spinach grown in waste amended soils is given in **Table 8**, **Table 9**, and **Table 10**, respectively. The accumulations of Cu in plant roots, shoots, and the whole plant were all slightly higher in limed treated soils at all levels of waste except in roots at 15 Mg waste ha^{-1}. Similar phenomenon was observed for Zn. This data indicate that the presence of lime tended to increase the bioavailability of Cu and Zn in waste amended soils. The decrease in the soil concentrations of Cu and Zn (**Table 3** and **Table 4**) by liming seems to have availed the soil heavy metals at suitable concentrations for better absorption by water spinach.

TABLE 7. The changes in heavy metal concentrations in waste-amended soils by lime and land spinach.

Waste	Lime	Water Spinach ΔCu^a	Water Spinach ΔZn^a
…… Mg ha^{-1}	……	…… %	……
0	0	- 8.76	-17.4
	5	+ 6.53	+ 13.0
15	0	-2.73	-3.96
	5	+ 7.39	+ 10.9
60	0	-0.46	-1.81
	5	+ 0.78	+ 1.43

[a]ΔM = [M (planted) – M (not planted)] x 100%; M is heavy metal

TABLE 8. The influence of waste and lime on Cu uptake by land spinach grown in waste-amended soils.

Waste	Lime	Root + Shoot[a]	Root[a]	Shoot[a]
…... Mg ha^{-1}	…...	…………………….......	μg $plant^{-1}$	....
0	0	0,66 ab	0,34 ab	0,33 a
	5	1,50 c	0,83 b	0,67 ab
15	0	1,36 abc	0,81 b	0,54 ab
	5	1,48 bc	0,68 ab	0,80 b
60	0	0,63 a	0,29 a	0,34 a
	5	1,26 abc	0,65 ab	0,61 ab
HSD 5%		0,83	0,51	0,36

[a]Different lowercases in one column show a significant difference at 5% HSD test

TABLE 9. The influence of waste and lime on Zn uptake by land spinach grown in waste-amended soils.

Waste	Lime	Root + Shoot[a]	Root[a]	Shoot[a]
…... Mg ha^{-1}	…...	....	μg $plant^{-1}$	....
0	0	0,37 a	0,17 a	0,20 a
	5	0,43 a	0,27 a	0,16 a
15	0	0,51 a	0,27 a	0,24 a
	5	0,54 a	0,28 a	0,26 a
60	0	0,36 a	0,21 a	0,16 a
	5	0,56 a	0,35 a	0,22 a
HSD 5%		0,30	0,18	0,14

[a]Different lowercases in one column show a significant difference at 5% HSD test

TABLE 10. Effects of waste and lime on several growth-properties and heavy-metal uptake of land spinach grown in waste-amended soils.

Treatment Factor[a]	Dry Weight			Cu Uptake			Zn Uptake		
	Root + Shoot	Root	Shoot	Root + Shoot	Root	Shoot	Root + Shoot	Root	Shoot
W	ns[b]	ns	ns	ns	ns	ns	ns	ns	ns
L	**	*	*	*	ns	*	ns	ns	ns
WL	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a]W = waste, L = lime; *Significantly different at 5% and ** at 1%; [b]ns = non-significant

The higher soil concentrations of Cu and Zn in soils caused by waste amendment tended to increase the Cu and Zn uptake at 15 Mg waste ha^{-1} but to decrease them at 60 Mg ha^{-1} (**Table 8** and **Table 9**), in line with the changes in land spinach biomasses (**Table 11**). It is logical that the total uptake of heavy metal is proportional to the total plant biomass. The higher concentrations of soil Cu and Zn also tended to decrease Cu uptake in limed soils (**Table 8**) and give no effect on Zn uptake (**Table 9**) in line with the decrease in plant biomass (**Table 11**). This phenomenon is clearly caused by the toxicity of Cu and Zn at high levels of waste. The suggestion is shown by the high correlation between the plant Cu and Zn uptake with the soil concentrations of Cu ($R^2 = 0.87*$) and ($R^2 = 0.78*$) (**Figure 1**).

TABLE 11. The effect of waste and lime on the land-spinach biomass grown in waste-amended soils.

Waste	Lime	Root + Shoot[a]	Root[a]	Shoot[a]
.... Mg ha^{-1}		.... g $plant^{-1}$		
0	0	0,25 ab	0,09 a	0,16 ab
	5	0,75 c	0,38 ab	0,38 bc
15	0	0,62 bc	0,29 c	0,33 abc
	5	0,80 c	0,33 bc	0,47 c
60	0	0,17 ab	0,05 c	0,12 a
	5	0,55 abc	0,22 abc	0,33 abc
HSD 5%		0,42	0,20	0,23

[a]Different characters in one column indicate asignificant difference at 5% HSD Test

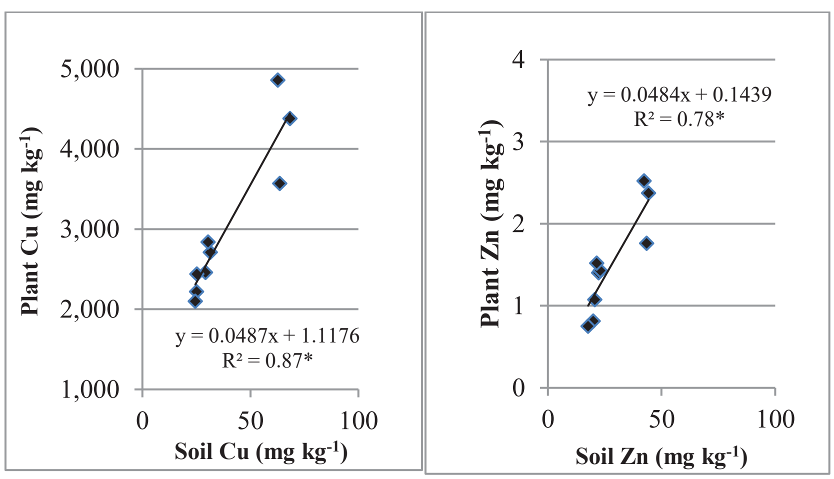

FIGURE 1. The correlation between heavy metal uptake by land spinach and their concentrations in waste-amended soils.

Copper and Zn were with some exceptions mostly accumulated in roots than that in shoots of land spinach as indicated by their translocation factors (**Table 12**). The translocation factor of Cu ranges between 0.67 – 1.18 and that for Zn ranges from 0.59 – 1.18. The TF < 1.00 indicated that land spinach is not a good phytoextractor but a phytostabilizer.

TABLE 12. The translocation factors of Cu and Zn in land spinach planted in waste-amended tropical soils.

Waste	Lime	TF[a] Cu	TF[a] Zn
…… Mg ha^{-1}	……		
0	**0**	0.92	1.18
	5	0.81	0.59
15	**0**	0.67	0.89
	5	1.18	0.93
60	**0**	1.17	0.76
	5	0.93	0.77

[a]TF = [Uptake M (Shoot) – Uptake M (Root)] * 100%; M is heavy metal

CONCLUSIONS

The amendment with industrial waste at 60 Mg waste ha^{-1} significantly increased the soil labile Cu and Zn and depressed the land spinach growth. Land spinach slightly decreased the soil levels of Cu and Zn in unlimed soils at waste levels ≤ 15 Mg ha^{-1}. Liming significantly decreased the soil levels of Cu and Zn and improved the growth and Cu-Zn uptake of land spinach but attenuated the effect of land spinach in decreasing the soil concentrations of Cu and Zn. The Cu-Zn uptake of land spinach was well-correlated with the soil levels of Cu and Zn.

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