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THE EFECTS OF HEAVY METALS, PHOSPHATE, LIME AND SAWDUST ON PLANT GROWTH AND HEAVY METAL ACCUMULATION BY LECTTUCE

Nguyen Xuan Cu
Department of Pedology and Soil Environment, Faculty of Environmental Sciences, VNU-University of Science,
334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam
E-Mail: cunx@vnu.edu.vn

ABSTRACT

Heavy metal pollution in soil and water is a global environmental concern. In Vietnam, accumulation of heavy metals in soil, water and plant biomass has been widely reported. Cultivation of crops on contaminated sites may result in both growth inhibition and tissue accumulation of heavy metals with resulting possible risks to human health. In this paper, plant growth inhibition and accumulation of Cu, Pb and Zn by Lettuce (*Lactuca sativa* L.), and the ability of phosphate, lime and sawdust reducing accumulation of heavy metals by plant are evaluated in pot experiments. The heavy metal concentrations in above-ground tissue of Lettuce indicated a high accumulation of heavy metals due to Cu, Pb and Zn stress in soil. The contents of Cu, Pb and Zn in plant were related more closely to heavy metal of EDTA-extractable concentrations in soils. More effects to reduce the accumulation of Cu, Pb and Zn in Lettuce were observed in all experiments with phosphate fertilizer, lime or sawdust applied. However, the application of phosphate fertilizer and lime are more effective than sawdust to reduce Cu, Pb and Zn uptake by Lettuce, especially for Cu and Pb.

Keywords: lettuce, heavy metal accumulation, lime, phosphate, sawdust.

1. INTRODUCTION

Increasing environmental pollution caused by heavy metals, released by industries and agricultural activities, is a major problem in the world. Heavy metal pollution in soil and water is a global environmental concern (Shyama and Somaratne, 2009). The hazardous levels of heavy metals in vegetables grown in peri-urban areas affected by the effluent waste water from cities and industrial areas also reported by Ravi Naidu *et al*. (2003). Singh et al. (2010) concluded that soil, irrigation water and some vegetables from peri-urban sites are significantly contaminated by the heavy metals, i.e., Cu, Pb and Zn. Heavy metals in soils reduce the yield of vegetables because of disturbing the metabolic processes of plants (Ogbodo *et al*., 2006). In Vietnam, the use of wastewater from the city in agriculture irrigation may have significant effects of accumulation of heavy metals in soils and agricultural products (Nguyen Xuan Cu, Le Duc, 1998). Many studies showed hazardous levels of unsafe vegetables in daily meals for residents due to high accumulation of heavy metals such as Cu, Pb, Zn and Cd (Cheang Hong and Nguyen Dinh Manh, 2003; Dang Thi An, Chu Thi Thu Ha, 2005; Ho Thi Lam Tra, 2007).

Heavy metals are toxic elements that can be harmful to plants, although plants usually show ability to accumulate large amounts of heavy metals without visible changes in their appearance or yield. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human consumption. Vegetables, especially those of leafy vegetables grown in heavy metals contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their leaves (Muhammad et al., 2008).

Lettuce is an annual plant most often grown as a leaf eating vegetable. The consumption of Lettuce has become very popular in daily diet of Vietnamese people. It was in this regard that Lettuce which is also one of the vegetables grown for commercial purposes in Vietnam. So that Lettuce was found to be more responsible than other vegetables for the accumulation of heavy metals in humans through the edible portion (Itanna, 2002).

However, the solutions for contaminated soil treatment do not handle effective because the actual pollution levels of heavy metals due to wastewater or fertilizer use often unreasonably low and scattered distribution. It is also difficult to prevent farmers from cultivation on this soil in agricultural production. Use of fertilizers to reduce heavy metal uptake and accumulation in agricultural products are considered towards positive solutions to maintain the production process should easily be accepted by farmers. Organic amendments such as farmyard manure or inorganic additives such as lime, zeolites, and iron oxides where they are found to reduce the transfer of metals into crops (Iwona, 2001).

The main purpose of this study is to examine the impact of the heavy metals (Cu, Pb, Zn) as stress factors to growth and heavy metal concentration by Lettuce, and evaluate the ability to use phosphate fertilizer, lime and sawdust to reduce heavy metal accumulation in plants grown on contaminated soils with different levels of heavy metals.

2. MATERIALS AND METHODS

The pot experiments were carried out at the green house of Vietnam Academy of Agricultural Sciences, Hanoi, Vietnam. The soil and fertilizers are mixed and contaminated by heavy metals with different rates and place in pot experiments (5kg soil/pot), left it overnight and then sown

seeds of Lettuce, 30 seeds/pot. After 15 days of sowing pruning conducted to ensure appropriate density for growing plants (10 plants/pot). The soils used in the experiments is Red river alluvial soil collected at a vegetable growing area of Thanh Tri district, Hanoi, Vietnam. Some properties of soil is as follows: pH (KCl) 6.15; SOM 2.44 %; CEC 23.46 Cmolc/kg; total N 0.22 %, total P_2O_5 0.19 %, total K_2O 1.02 %; total of heavy metal of Cu 21.29 ppm, Pb 57.16 ppm and Zn 7.24 ppm.

The treatments were contaminated with heavy metals at levels of 0 ppm, 50 ppm, 100 ppm, 200 ppm for Cu and Pb; and 0 ppm, 100 ppm, 300 ppm, 500 ppm for Zn. The phosphate fertilizer at the rates of 0ppm, 17ppm, 26ppm and 34 ppm P_2O_5 (equivalance 0 kg, 40 kg, 60 kg and 80 kg P_2O_5/ha); lime use at the rates of 0 ppm, 435ppm, 870ppm and 1305 ppm $CaCO_3$ equivalance (accounting for 0 ton, 1 ton, 2 tons and 3 tons $CaCO_3$/ha); while sawdust applied at 0 ppm, 6522ppm, 9783ppm and 13044ppm (accounting for 0 ton, 10 tons, 15 tons and 20 tons/ha).

The treatments were arranged individually responsible for determining the influence of the levels of added Cu, Pb, Zn, phosphate, lime and sawdust on the growth, yield and heavy metal accumulation in plant of Lecttuce. The chemical contaminants Cu^{2+}, Pb^{2+} and Zn^{2+} is added as the chemicals of $CuSO_4.5H_2O$, $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$. The base fertilizers use in the forms of urea $(NH_2)_2CO$; potassiumchlorua KCl, superphosphate and burned lime (CaO) were applied at the rate of 33ppm N and 13ppm K_2O (accountinh for 75 kg N and 30 kg K_2O/ha).

The plant were observed for their growth against stress of Cu, Pb, Zn and fertilizers, and above ground biomass of Lettuce harvested after 45 days. Heavy metal contents (Cu, Pb and Zn) in soil and plant were estimated by using Atomic Absorption Spectroscopy; extractions to assess mobilization of Zn^{2+}, $Pb^{2+}$and Cu^{2+} in EDTA solution.

3. RESULTS AND DISCUSSIONS

3.1. Effect of Cu, Pb, Zn on the growth and accumulation of heavy metals by Lettuce

3.1.1. Effect of Cu on the growth and Cu accumulation by Lettuce

The results on the effects of added Cu on growth and Cu accumulation in Lettuce in the experiments are presented in Table-1. These data in Table-1 shows the rate Cu applied has been obvious influence to Lettuce growth. The height of plants decreases from 11.4 cm in the control (without Cu added) to 11.0 cm; 9.6 cm and 6.9 cm when the rates of Cu applied increased, respectively from 0 ppm to 50 ppm, 100 ppm and 200 ppm. Thus in the highest amount of Cu applied at 200 ppm, Lettuce plant height was reduced about 39% compared to control.

Table-1. Effect of Cu application on plant growth and Cu accumulation by Lettuce.

Cu added (ppm)	Plant height		Yield		Cu content in plant	
	cm	%	g/pot	%	ppm	%
0	11.4	100	93.8	100	2.25	100
50	11.0	97	82.2	88	3.04	135
100	9.6	85	73.8	79	3.33	148
200	6.9	61	53.6	57	426	189

The amount of Cu not only reduces plant height but also strongly affect the yield of Lettuce. Even at low rate of 50 ppm vegetable yield reduced 12%, and at the rate of 100 ppm the yield decreased 29%, and at the rate 200 ppm the yield decreased 43% compared to control.

Other than the influence of Cu applied on the growth of Lettuce, the amount of Cu also increased significantly the amount of Cu accumulation in plants. According to these results, when the rate of Cu increased from 0 ppm to 50 ppm, 100 ppm and 200 ppm, the contents of Cu in plants also increased corresponding from 2.25 ppm to 3.04 ppm, 3.33 ppm and 4.26 ppm; or Cu contents in plants increased respectively 35%, 48% and 89% compared to control.

Naaz and Pandey (2010) also reported Lettuce accumulated heavy metals in their shoot at high concentrations (Zn, 21.6 and Cu 14.76 mg/kg dry weight) after irrigation with undiluted industrial waste water (Naaz and Pandey, 2010). Use of industrial waste water on agricultural lands is not found suitable without proper treatment. It could be injurious to plants growth and may be a potential threat to food web.

3.1.2. Effects of Pb application on plant growth and accumulation of Pb by Lettuce

The finding in Table-2 shows the rate of Pb application clearly influence the growth and Pb accumulation by Lettuce plant. The height and yield of plant are reduced sharply while the Pb content in plants has increased.

At the applied rate of 50 ppm Pb, the plant height decreased by 12%, whereas at the rate of 100 ppm Pb the height of plant reduced by 21%, and at 200 ppm Pb the plant height decreased 41% compared to the control.

The effect of Pb application on yield of Lettuce also occur in similar trends as the plant height but much stronger. Even at the rate of 50 ppm Pb, yields of Lettuce were down 33% compared to control. When the applied rate 100 ppm Pb, the yield of Lettuce reduced 59% and at the rate of 200 ppm Pb the yield was reduced 71% compared the control.

Table-2. Effect of Pb application on plant growth and Pb accumulation by Lettuce.

Pb added (ppm)	Plant height		Yield (g/pot)		Pb contents in plant	
	cm	%	g/pot	%	ppm	%
0	11.4	100	93.8	100	0.12	100
50	10.0	88	71.7	77	0.17	142
100	8.9	79	38.1	41	0.23	192
200	6.7	59	26.7	29	0.30	250

In contrast to the effect of Pb to plant growth, the rate of Pb application significantly increased Pb accumulation in vegetables. At the rate of 50 ppm, the total content of Pb in plant increased 142%, and at the rate of 100 ppm the content of Pb accumulation in vegetables increased 192%. But at the rate of 200 ppm, the content of Pb in plant increased 250% compared to the control. At the high rate of 200 ppm Pb, Lettuce had the appearance of lead poisoning symptoms, such as plant growth slowly, small leaves and dark green. Accumulation of heavy metals by crop plants from contaminated soils may pose health risks. The introduction of Pb into the food chain may affect human health and thus, studies concerning Pb accumulation in vegetables have increasing importance (Adu *et al*., 2012).

The high content of Pb in Lettuce was reported by Sardar Khan *et al.* (2008) in a greenhouse pot experiment using lettuce (*Lactuca satuva* L.) as a representative vegetable was conducted to assess the concentrations of heavy metals in vegetables grown in wastewater-contaminated soils. The plant shoots were also highly contaminated with heavy metals, particularly Pb (2.3-5.3 mg/kg dry weight). This study highlights the potential health risks associated with cultivation and consumption of leafy vegetables on wastewater-contaminated soils (Sardar *et al*., 2008).

3.1.3. Effect of Zn application on growth and Zn accumulation by Lettuce

The data presented in Table-3 shows the effects of Zn on growth and Zn accumulation in Lettuce plant. However, the level of affect is less than that of Cu and Pb. At the rate of 300 ppm Zn, the plant height just reduced 11% and even at very high rate 500 ppm Zn application, the plant height only reduced 17% compared to the control.

Similarly, application of Zn only reduces a little Lettuce yield even at the high rate of application. For example at the rate 300 ppm and 500 ppm Zn, the Lettuce yield decreased respectively 20% and 31% compared to the control. Event at the low rate of 100 ppm, Zn has the possitive effect of increasing the growth of Lettuce: The height and yield increased only 5% and 3 % respectively compared to control. This can be explained by the role of Zn as a trace elements and it may contribute to stimulating the growth of Lettuce in this soil.

Other than the effects of Zn on growth of Lettuce, the content of Zn in plant always tightly correlated with the amount of Zn application to soils. When the rate of Zn application increased from 0 ppm to 100 ppm, 300 ppm and 500 ppm, the total content of Zn in plant also increased from 5.02 ppm to 6.98 ppm, 7.08 ppm and 9.76 ppm respectively. The increase is about 39%, 41% and 94% compared to control.

3.1.4. The relationship between the content of Cu, Pb and Zn in soil and in Lettuce plant

The concentration of heavy metals in soil and their contents in Lettuce have close positive relationship. The correlation between the concentration of Cu^{2+}, Pb^{2+}, Zn^{2+} in soils and their contents in Lettuce are the same trend, i.e., when the concentration of heavy metals in soil increase the accumulation in plants also increase (Table-4).

Table-3. Effect of Zn application on plant growth and Zn accumulation by Lettuce.

Zn added (ppm)	Plant height		Yield		Zn content in plant	
	cm	%	g/pot	%	ppm	%
0	11.4	100	93.8	100	5.02	100
100	12.0	105	96.4	103	6.98	139
300	10.1	89	74.5	80	7.08	141
500	9.4	83	65.0	69	9.76	194

Table-4. The relationship between the content of Cu, Pb and Zn in soil and in Lettuce plant.

Added Cu, Pb or Zn* (ppm)	Cu content (ppm)		Pb content (ppm)		Zn content (ppm)	
	In soil	In plant	In soil	In plant	In soil	In plant
0 (0)	13.38	2.25	1.88	0.12	7.24	5.02
50 (100)	15.52	3.04	4.10	0.17	17.81	6.98
100 (300)	27.20	3.33	11.23	0.23	75.20	7.08
200 (500)	51.10	4.26	18.27	0.30	112.83	9.76

*The numbers in parentheses are added Zn

3.2. Effect of phosphate fertilizer, lime and sawdust on the growth and accumulation of Cu, Pb, Zn by Lettuce

3.2.1. Effect of phosphate fertilizer on growth and accumulation of Cu, Pb, Zn by Lettuce

Effects of phosphate fertilizer on growth and accumulation of Cu, Pb, Zn in Lettuce are presented in Table-5. The results showed that phosphate fertilizer promotes effective growth of Lettuce, the plant height and yield increased significantly when the rates of phosphate fertilizer increased. At the rate 34 ppm (80 kg P_2O_5/ha), the plant height increased 2, 82cm (29%) and the yield increased 11.53g/pots (16%) compared to the control without phosphate application.

Phosphorus is an important nutrient for plant. However, phosphate fertilizers usually have efficiency at relatively high rate of application because of high fixation capacity of phosphorus in soil. In these results, the rate of phosphate use should be higher than 60 kg P_2O_5/ha. This is consistent with the research of Nguyen Xuan Cu (2002) pointed out that with most soil types in Vietnam, phosphate fertilizer only have high effect on crops when the amount of fertilizer significantly higher phosphorous absorption capacity of the soil (Nguyen Xuan Cu, 2002).

Phosphate fertilizer not only increases productivity of Lettuce but also reduce the accumulation of heavy metals in plant. When increasing the amount of phosphate fertilizer to 17 ppm, 26 ppm and 34 ppm, the contents of Cu in Lettuce plant decrease corresponding to 2%; 5% and 54% compared to the control. Similarly, Pb contents decreased 9%; 26% and 35%; and Zn contents also decreased 1%; 4% and 6%. Thus phosphate fertilizer works very well to reduce the accumulation of Cu and Pb in Lettuce plant, especially at the rate higher 26 ppm (60 kg P_2O_5/ha equivalence). But this effect is not significant for Zn.

3.2.2. Effects of liming on growth and accumulation of Cu, Pb, Zn in Lettuce

Lime not only reduces the acidity of the soil, changing the composition of cations in the soil solution and the soil adsorption complex, but also affecting the mobility of nutrients in soil. For Cu, Pb and Zn, liming reduces the mobility of these elements. This is also reflected in the results of liming on growth and accumulation of heavy metals in Lettuce plant are presented in Table-6.

The height and yield of Lettuce plant have the lowest values at the control (without lime), while the highest value was observed at the liming rate 1305 ppm (equivalance 3 tons $CaCO_3$/ha). In general, lime has more impact on yield of Lettuce than the height of the plants. For example, liming at 3 tons/ha, the plant height increased only 18% while the yield increased 21% compared to the control. However, at the liming rates over 2 tons/ha an increase in plant height also slowed down, while the increase in yield was maintained at a higher level.

The accumulation of heavy metals in Lettuce also significantly affected by lime application. When liming increased to 435 ppm, 870 ppm and 1305 ppm, Cu content in plant reduced correspondingly to 63%; 74% and 79% compared to the control; Pb content in plant reduced 22%; 35% and 43%, while the content of Zn in plant only reduced 6%; 12% and 16%. This result indicates the effect to minimize absorption of Zn in Lettuce plant due to liming is relatively lower than Cu and Pb.

3.2.3. Effect of sawdust application on the growth and accumulation of Cu, Pb, Zn in Lettuce

Unlike phosphate fertilizer and lime as described above, and it tends to reduce the height and yield of lettuce (Table-7). The reason of this problem may be caused by the high C/N ratio of sawdust causing nutrients competition between soil microorganisms and plant during their decomposition. The results led to a shortage of nutrients for plant growth.

But on the other hand, sawdust can reduce accumulation of heavy metals by Lettuce plants. In the rate of 13044 ppm (20 tons sawdust/ha), the contents of Cu, Pb and Zn in plants decreased 15%; 12% and 9% compared to the control.

Table-5. Effect of phosphate fertilizer on growth and accumulation of Cu, Pb, Zn by Lettuce.

P_2O_5 added* (ppm)	Plant height (cm)	Yield (g/pot)	Content of heavy metals in plant					
			Cu		Pb		Zn	
			ppm	%	ppm	%	ppm	%
0	9.64	73.82	3.33	100	0.23	100	7.08	100
17 (40)	9.87	74.02	3.19	98	0.21	91	7.01	99
26 (60)	10.35	80.26	2.50	75	0.17	74	6.76	96
34 (80)	12.46	85.37	1.52	46	0.15	65	6.68	94

*The numbers in parentheses are kg P_2O_5/ha equivalence

Table-6. Effects of liming on growth and accumulation of Cu, Pb, Zn in Lettuce.

Lime added* (ppm)	Plant height (cm)	Yield (g/pot)	Content of heavy metals in plant					
			Cu		Pb		Zn	
			ppm	%	ppm	%	ppm	%
0	9.64	73.82	3.33	100	0.23	100	7.08	100
435 (1)	10.32	75.94	1.23	37	0.18	78	6.65	94
870 (2)	10.97	81.68	0.85	26	0.15	65	6.20	88
1305 (3)	11.42	89.35	0.71	21	0.13	57	5.92	84

*The numbers in parentheses are tons $CaCO_3$ /ha, equivalence

Table-7. Effect of sawdust application on the growth and accumulation of Cu, Pb, Zn in Lettuce.

Sawdust added* (ppm)	Plant height (cm)	Yield (g/pot)	Content of heavy metals in plant					
			Cu		Pb		Zn	
			ppm	%	ppm	%	ppm	%
0	14.7	50.0	5.21	100	1.67	100	20.40	100
6522 (10)	13.5	47.9	5.04	97	1.55	93	20.05	98
9783 (15)	15.2	49.0	4.95	95	1.50	90	19.57	96
13044 (20)	13.1	45.1	4.42	85	1.47	88	17.51	91

*The numbers in parentheses are tons sawdust/ha, equivalence

4. CONCLUSIONS

Heavy metals (Cu, Pb and Zn) have clearly influenced the growth and accumulation of them in Lettuce plants. At the high applied rates of 200 ppm Cu, the Lettuce yield decreased 43% while the content of Cu in plant increase 89% compared to control. For Pb, at the high rate of 200 ppm, the Lettuce yield decrease 71% and the content of Pb in plant increase 250% compared to the control.

The application of Zn also significantly impact on the growth of Lettuce, especially at the high rates higher than 300 ppm. At the rates 500 ppm Zn, the Lettuce yield decrease respectively 31% and the contents of Zn in Lettuce plant increase 94% compared to the control.

Phosphate fertilizer, lime and sawdust have ability to reduce the accumulation of heavy metals in Lettuce plant. At the high rate of phosphate fertilizer 80 kg P_2O_5/ha, the contents of heavy metals in plants decrease 54% for Cu; 35% for Pb and 6% for Zn compared to the control.

Lime also has an important role to minimize absorption of heavy metals in Lettuce. When the amount of lime rate at 2 tons $CaCO_3$/ha, the content of heavy metals in plants decrease 74% for Cu; 35% for Pb; 12% for Zn compared to control respectively.

The effect of sawdust to reduce heavy metals accumulation in Lettuce plant are less than that of phosphate and lime. Even at the high rate of 20 tons sawdust/ha, the contents of heavy metals in Lettuce plants only reduce 15% for Cu, 12% for Pb and 9% for Zn.

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