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Effect of Heavy Metals on Plant Growth and Ability to Use Fertilizing Substances to Reduce Heavy Metal Accumulation by *Brassica Juncea* L. Czern

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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 humans health. In this paper, plant growth inhibition and accumulation of Cu, Pb and Zn by *Brassica juncea* L. Czern are studied in pot experiments.

The heavy metal concentrations in above-ground tissue of *Brassica juncea* indicated a possible accumulation of heavy metals due to plant uptake by the high levels of Cu, Pb and Zn 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 *Brasica juncea* was observed in all experiments with phosphate fertilizer, lime or sawdust was applied. However, the application of phosphate fertilizer and lime are more effective to reduce Cu, Pb and Zn uptake by *Brassica juncea* than sawdust. Phosphate fertilizer was founded most effective to reduce Pb accumulation in plant, whereas lime for Zn.

Keywords: brassica juncea, heavy metal accumulation, lime, phosphate, sawdust.

I. 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 R et al., 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). *Brassica juncea* L. Czern has the capacity to take up and accumulate heavy metals such as Cd, Cu, Ni, Zn, Pb and Se to high levels ((Shyama R et al., 2009; Blaylock et al., 2000). In recent years, the use of waste water from the city in agriculture irrigation have made significant accumulation of heavy metals in soils and in agricultural products in Vietnam (Nguyen Xuan Cu and Le Duc, 1988; Nguyen Xuan Cu, 2008). 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 and Chu Thi Thu Ha, 2005; Ho Thi Lam Tra, 2007).

Generally green vegetables, i.e., *Brassica juncea* L. Czern are very common use in daily diet of Vietnamese people. *Brassica juncea* is a plant with high capable of accumulation of heavy metal elements, and then affect human health through pathway of bio-accumulation (Ravi Naidu et al., 2003). However, the solutions for 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 these land in practice.So agricultural products containing high concentrations of heavy metals have been used in the daily diet of the people. 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 manue or inorganic additives such as lime, zeolites, and iron oxides where they are found to reduce the transfer of metals into crops (Iwona Grabowska, 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 uptake of *Brassica juncea* L. Czern, and determine 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.

II. Materials and Methods

The pot experiments were carried out at the green house of Vietnam Academy of Agricultural Sciences in Hanoi, Vietnam. The soil and fertilizers are mixed and contaminated by heavy metals in different rates and place in pot experiments (5kg soil/pot), left it overnight and then sown seeds of vegetables (*Brassica juncea* L. Czern), 30 seeds/pot. After 15 days of sowing pruning conducted to ensure appropriate density for

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growing plants (10 plants/pot). The soils used in the experiments is Red river alluvial soil collected at a vegetable growing areas of Thanh Tri district, Hanoi, Vietnam. Some properties of soil is as follows: pH (KCl) 6.15; SOM 2.44 %; CEC 23.46 Cmol/kg; total N 0.22 %, total P_2O_5 0.19 %, total K_2O 1.02 %; total of Cu 21.29 ppm, Pb 57.16 ppm and Zn 7.24 ppm.

The treatments were contaminated with heavy metals at levels of 50 ppm, 100 ppm, 200 ppm for Cu and Pb; and 100 ppm, 300 ppm, 500 ppm for Zn. The phosphate fertilizer at the rates of 17ppm, 26ppm and 34 ppm P_2O_5 (equivalance 40 kg, 60 kg and 80 kg P_2O_5/ha); lime use at the rates of 435ppm, 870ppm and 1305 ppm $CaCO_3$ equivalance (1 ton, 2 tons and 3 tons $CaCO_3$/ha); while sawdust applied at 6522ppm, 9783ppm and 13044ppm (equivalance 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 *Brassica juncea*. The fertilizers used in the forms of urea (NH2)2CO; potassium chlorua KCl, superphosphate and lime (CaO). Chemical contaminants $Cu^{2}+$, $Pb^{2}+$ and $Zn^{2}+$ is added as the chemicals of $CuSO_4.5H_2O$, $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$. Control treatment fertilized with $(NH_2)_2CO$ and KCl at the rates of 33ppm N and 13ppm K_2O (75 kg N and 30 kg K_2O/ha).

Plant growth and yield (above ground), and the contents of heavy metals determined for *Brassica juncea* in response to Cu, Pb and Zn stress. Plants were observed for their growth and harvested after 45 days. Heavy metal contents (Cu, Pb and Zn) in soil and plant (above ground) were estimated using Atomic Absorption Spectroscopy; extractions to assess mobilization of $Zn^{2}+$, $Pb^{2}+$and $Cu^{2}+$ in EDTA solution.

III. Results and Discussion

a) *Effect of Cu, Pb, Zn on growth and their accumulation in Brassica juncea*

i. *Effect of Cu on the growth and Cu accumulation in Brassica juncea*

The research results on the effects of applied Cu on plant growth and Cu accumulation in plants of *Brassica juncea* are presented in Table 1. The data in Table 1 show the amount of added Cu quite markedly influence the plant height. When the amount of added Cu increased to 50 ppm, 100 ppm and 200 ppm as the height of the plant decreases significantly corresponding to 23%, 25% and 36% compared to the control.

Table 1 : Effect of added Cu on growth and Cu accumulation in *Brassica juncea* (fresh weight)

Added Cu (ppm)	Plant height		Yield		Content of Cu in plant	
	cm	%	g/pot	%	ppm	%
0	19.5	100	70.3	100	2.75	100
50	15.0	77	55.9	80	3.17	115
100	14.7	75	50.0	71	5.21	190
200	12.5	64	33.1	47	8.46	308

The amount of added Cu not only reduces plant height but also affect the yield of plants. At the rates of 50 ppm, 100 ppm and 200 ppm the vegetable yields decreased 20%; 29% and 53% respectively to the control. Such changes tend to yield of plants are also similar to the height of plants, however the level of influence is much higher, especially at the rate of 200 ppm Cu.

The effect of added Cu to soil on Cu accumulation in plants is also significant. The results showed when the rate of added Cu increased from 0 to 50 ppm; 100 ppm and 200 ppm the content of Cu in plants have increased 15%, 90% and 308% respectively. Thus at the rate of 200 ppm, Cu accumulation in plants increased markedly. This result is consistent with a decline in the growth of vegetables has been discussed above. We can say that cultivation of crops in or close to contaminated sites may result in both growth inhibition and tissue accumulation of heavy metals, with resulting possible risks to humans or livestock health if these products are ingested.

The similar results of growth inhibition and accumulation of Cu, Pb and Zn for *Brassica* species were reported by Carmina Gisberta et al. (2004). The research of Naiyanan and Banchagan (2006) on copper removal from soil by Brassica juncea (L.) Czern also showed the maximum concentrations of copper in *Brassica juncea* (L.) Czern was 3,771 mg/kg (dry weight) in the pots experiment with 150 mg Cu/kg soil. The statistical analysis indicated that copper accumulations between shoots and roots of *Brassica juncea* (L.) Czern were not significantly different when Cu was added lower than 50 mg/kg. However, in the experiment amended with 100, 150 and 200 mg Cu/kg, copper concentration in the roots was greater than those in the shoots. The highest accumulation efficiency of *Brassica juncea* (L.) Czern was 1.61% in the pot with 150 mg Cu/kg soil.

ii. *Effect of Pb on the growth and Pb accumulation in Brassica juncea*

The effect of added Pb to plant growth and Pb accumulation in plants are presented in Table 2. The height of plant decrease from 19.5 cm to 14.5 cm, 13.4 cm and 11.0 cm when the rates of added Pb increase from 0 ppm to 50 ppm, 100 ppm and 200 ppm, respectively. That is calculated to reduce the plant height corresponding to 25%, 31% and 44% compared to the control. Similarly, the yield of plant also tends to decrease rapidly with increasing the rates of Pb; i.e., at the rates of 50ppm, 100ppm and 200 ppm Pb the yield corresponding reduction of 27%, 51% and 56% compared to the control.

Table 2: Effect of the added Pb to plant growth and Pb accumulation in plant (fresh weigh)

Added Pb (ppm)	Plant height		Yield		Content of Pb in plant	
	cm	%	g/pot	%	ppm	%
0	19.5	100	70.3	100	0.17	100
50	14.7	75	37.0	53	0.96	565
100	13.4	69	34.7	49	1.67	982
200	11.0	56	31.0	44	1.79	1053

The amount Pb applied to soil rapidly increased Pb accumulation in plants. The lowest content of 0.17 ppm Pb in plant is observed in the control (without Pb applied), and the highest value of 1.79 ppm Pb in the treatment with 200 ppm Pb applied. The results show that Pb accumulation in plants increase more than 5; 9 and 10 times compared to the control, corresponding to the amount of added Pb at 50 ppm, 100 ppm and 200 ppm. These results also show the ability uptake and accumulation of Pb in plant increase quickly and it decrease when the rates of added Pb higher than 100 ppm. The similar results of effects of Pb on plant growth and accumulation was reported by John et al (2009) that indicated the exposure of *Brassica juncea* to Cd and Pb results is an decrease in growth and *Brassica juncea* can be used as a heavy metal accumulator in heavy metal affected soils.

iii. *Effect of added Zn on plant growth and Zn accumulation in Brassica juncea*

The effect of Zn on plant growth and its accumulation in plant are presented in Table 3. At the rate of 100 ppm Zn, the plant height virtually unchanged comparing to the control treatment. However, at the rate of 300 ppm Zn, the height of plant has fallen by 14%, while at the rate of 500 ppm Zn reducing plant height by 20% compared to control. This results show that Zn only influence on plant growth at the high rates above 300 ppm. For the yield, low adding rate at 100 ppm Zn increase yield 8%, but at 300 ppm the yield decreased 9%, while at 500 ppm the yield dropped 38% compared to the control. The results show that Zn only discernible impact on the growth of *Brassica juncea* as high adding rates exceeded 300 ppm.

Table 3 : Effects of the adding Zn to plant growth and Zn accumulate in plants (fresh weigh)

Added Zn (ppm)	Plant height		Yield		Content of Zn in plant	
	cm	%	g/pot	%	ppm	%
0	19.5	100	70.3	100	9.05	100
100	19.7	101	75.7	108	18.06	200
300	16.7	86	64.1	91	20.40	225
500	15.5	80	43.8	62	22.82	252

The contents of Zn in plants increase coresponding to 100%; 125 % and 152 % when the adding rate of added Zn increase to 100 ppm; 300 ppm and 500 ppm compared to the control, respectively. So it can be said that the contents of Zn in soils is closed relationship with Zn accumulation in plants.

The rates of added Cu, Pb and Zn have significant influence on growth and yield of *Brassica juncea*, in which the influence of Pb is the most evident, followed by the effects of Cu and the lowest impact of Zn. Overall, the growth and yield of *Brassica juncea* decrease when the amount of added heavy metal increase. At 100 ppm, Pb reduce yield 51%, while Cu only reduces yield by 29%. For Zn, the amount of added 300 ppm decreased yield 9% and at very high rate 500 ppm Zn reduces yield 38%. At low amounts of added 100ppm, Zn influence on the productivity of vegetables is not clear.

iv. *The relationship between the concentration of Cu^2+, Pb^2+ and Zn^2+ mobilization in soil and plants*

The relationship between concentration of Cu^2+, Pb^2+, Zn^2+ in soil and in plants is presented in Table 4. The correlation between the of mobilization Cu^2+ in soil and Cu content in plants are represented by equations of YCu=0.15x+0.868 (R^2=0.99). While the correlation equation between mobilization Pb2+ in soil and Pb contents in plants can be in the form Y_{Pb}=0.72lnx-0.182 (R^2 = 0.97). But the relationship between mobilization Zn^2+ in soil and Zn contents in plants is represented through equation Y_{Zn}=4.39lnx+2.299 (R^2=0.87). In general, concentrations of heavy metals in soil are closely correlated with their contents accumulated in vegetables.

Table 4 : Relationship between concentrations of Cu^{2+}, Pb^{2+}, Zn^{2+} in soil and in plants (fresh weight)

Added Cu, Pb or Zn* (ppm)	Cu (ppm)		Pb (ppm)		Zn (ppm)	
	Cu^{2+} in soil	Cu in plant	Pb^{2+} in soil	Pb in plant	Zn^{2+} in soil	Zn in plant
0 (0)	13.38	2.75	1.88	0.17	7.24	9.05
50 (100)	15.52	3.17	4.10	0.96	17.81	18.06
100 (300)	27.20	5.21	11.23	1.67	75.20	20.40
200 (500)	51.10	8.46	18.27	1.79	112.83	22.82

**The numbers in parentheses are added Zn*

In contrast, the rates of adding heavy metals Cu, Pb, Zn are rapidly increasing their accumulation in plant of *Brassica juncea*, especially at the high rates of application. At the rates of 100 ppm and 200 ppm, Cu content accumulated in vegetables increased respectively 90% and 208%, while Pb contents increase corresponding 882% and 953% compared to the control. For Zn, when the rates of application increased to 100; 300 and 500 ppm the contents of Zn accumulation in vegetables increase respectively to 100% , 125% and 152% compared to the control. That is also an evidence for the high ability of heavy metals extraction of *Brassica juncea*. Ebbs et al. (1997) also remarked the *Brassica* spp. were the effective plant in removing Zn, Cu and Cd from the contaminated soil.

b) Effect of phosphate fertilizer, lime and sawdust on the growth and accumulation of Cu, Pb, Zn in Brassica juncea

i. *Effect of phosphate fertilizer on growth and Cu, Pb, Zn accumulation in plants*

The results showed that phosphate fertilizer has significant influence on growth and yield of *Brassica juncea*. The plant height increased from 14.7 cm in the control (without phosphorus fertilizer) to 16.3 cm at the rate of 17 ppm P_20_5; 17.4 cm at the rate 26 ppm P_20_5 and 19.1 cm at the rate 34ppm P_2O_5. Similarly, the yield also increased respectively from 50.0 to 53.1; 57.3 and 65.5 g/pot (Table 5). Thus in all rates of phosphate fertilizer, the plant height and yield are higher than the control treatment (without phosphate fertilizer). In particular, *Brassica juncea* have the best growth at the rate 34ppm, equivalence to 80 kgP_2O_5/ha.

Table 5 : Effects of phosphate fertilizer on growth and accumulation of Cu, Pb, Zn in plant (fresh weight)

Added phosphate (ppm P_2O_5)	Plant height (cm)	Yield (g/pot)	Contents 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
17	16.3	53.1	5.10	98	1.42	85	19.63	96
26	17.4	57.3	4.78	92	1.02	61	17.97	88
34	19.1	65.5	4.46	86	0.96	58	17.35	85

Adding phosphate also have a significant impact to the heavy metal accumulation in plants. At the rate 17ppm P_2O_5, the contents of Cu, Pb and Zn in plant has declined 2%, 15 % and 4 %; at the rate 26ppm P_2O_5, the contents of Cu, Pb and Zn in plants decreased 8%, 39 % and 12 %; and at the rate 34ppm P_2O_5, the contents of Cu, Pb and Zn in plants decreased 14 %, 42 % and 15 %, respectively, compared to control. It can be said that application of phosphate fertilizer can reduce the accumulation of Cu, Pb, Zn in plants of *Brassica juncea*. When the amount of phosphate fertilizer increase the contents of heavy metals in plants decrease, in which the affects of phosphate fertilizer on Pb uptake by plants is the most evident. In this study, the rate 34ppm (80 kgP_2O_5/ha) is considered as best performance in reducing uptake ability and accumulation of heavy metals in plant of *Brassica juncea*. Phosphate decreased heavy metals uptake by vegetables and markedly promoted growth of vegetables was also reported by Tan Wan-Neng et al. (Tan Wan-Neng et al., 2011).

The phosphorus fertilizer is capable of reducing the concentration of heavy metals Cu, Pb and Zn in plants can be explained through the fixation processes between heavy metals and phosphate, and the results it can reduce uptake ability and accumulation of heavy metals in plants. Moreover, the presence of the cations come from phosphate fertilizer (especially Ca^{2+}, Mg^{2+}) also have the effect limiting uptake of heavy metals by plants due to the ion antagonist. In addition, the rapid growth of crops also contribute to reducing the contents of heavy metals in plants due to the phenomenon of "diluting".

ii. *Effects of liming on growth and Cu, Pb, Zn accumulation in plants*

In agricultural production, lime is considered as an effective fertilizer reduce soil acidity and provide some nutrients for crops. The effect of lime to promote growth and reduce the accumulation of heavy metals in *Brassica juncea* is also observed in this study.

The results showed that when the rate of liming increased from 0 to 435ppm, 870ppm and 1305ppm $CaCO_3$ increase the plant height from 14.7 cm to 15.9 cm, 16.4 cm and 18.2 cm; and the yield also increased from 50g to 51.8 g, 56.8 g and 62.5 g/pot, respectively (Table 6). The plant height and yield reached the highest value at the rate of 1305ppm (3 tons $CaCO_3$/ha). However, compared with phosphate fertilizer mentioned above, lime has less effect on the growth of *Brassica juncea*.

Table 6 : The effects of liming on growth and Cu, Pb, Zn accumulation in plants (fresh weight)

Liming (ppm $CaCO_3$)	Plant height (cm)	Yield (g/pot)	Contents 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
435	15.9	51.8	5.01	96	1.60	96	19.46	95
870	16.4	56.8	4.95	95	1.30	78	14.29	70
1,305	18.2	62.5	4.02	77	0.95	57	13.20	65

The effect of the lime on the content of Cu , Pb and Zn in plants also shown clearly. At low rate of 435ppm (1 ton $CaCO_3$/ha), lime has little effect on the contents of Cu, Pb and Zn in plant, but at the high rate of 1305ppm (3 tons $CaCO_3$/ha), the contents of Cu, Pb and Zn in plant have declined sharply by 23%, 43% and 35% respectively compared to the control.

The addition of lime achieved higher plant biomass production, although effects concerning metal bioavailability and accumulation were masked somewhat by pH variability. These results are also consistent with the conclusions of Tan Wan-Neng et al. [14] suggested that liming is considered the most effective method to reduce absorption and reduce harmful of Zn to crops. Tissue metal concentrations of *Brassica juncea* were elevated for Zn, Cu and Pb, especially in leaves of plants from plots with low pH values (Clemente et al., 2005).

iii. *Effect of sawdust amendment on growth and Cu, Pb, Zn accumulation in plants*

Unlike phosphate fertilizer and lime as described above, sawdust tends to reduce the growth and yield of *Brassica juncea*. The causes may be due to the high ratio of C/N in sawdust leading to the nutrient competition between soil microorganisms and plants. The effect of sawdust amendment to heavy metal accumulation in plant is not significant as lime and phosphate fertilizer. At the highest rate of 13044ppm (20 tons/ha), sawdust only reduce heavy metal concentrations in plants about 13% for Cu , and 10% for Pb and 9% for Zn (Table 7). It can be said that sawdust has reduced the largest percentage of Cu, followed by Pb and Zn did not differ significantly.

Table 7 : The effects of sawdust amendment on growth and Cu , Pb and Zn accumulation in plants (fresh weight)

Added sawdust (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
6,522	15.0	49.2	4.99	96	1.56	93	19.23	94
9,783	14.9	51.8	4.61	88	1.55	93	19.01	93
13,044	14.1	47.0	4.53	87	1.51	90	18.57	91

IV. Conclusions

The applied rates of Cu, Pb and Zn have a tremendous impact on the growth and yield of *Brassica juncea*, in which the effect of Pb is highest, followed by Cu and Zn. The plant growth and yield of *Brassica juncea* decrease with the rates of heavy metals increases. At the rate of 100 ppm, Pb reduce yield by 51 %, Cu reduces yield by 29% but the effect of Zn is not clear. The effect of Zn on yield of *Brassica juncea* is only significant at very high rate of 500 ppm Zn where the yield decrease by 38%.

The rates of applied heavy metals (Cu, Pb, Zn) rapidly increase their accumulation in plants, especially at high rates. When applied 100 and 200 ppm, the content of Cu in plants increase 90% and 208% respectively, whereas the content of Pb increase corresponding to 882% and 953% compared to the control. For Zn, the contents of Zn in plants increase by 100%, 125% and 152% compared to control when the rates of applied Zn increase to 100; 300 and 500 ppm respectively.

Phosphate fertilizer not only affect the growth of plants but also effect the accumulation of heavy metals in plants. When the rate of phosphate fertilizer apply at the rates increase to 40 kg, 60 kg and 80 kg P_2O_5/ha the content of Cu in plant decrease corresponding by 2%; 8% and 14%; the contents of Pb in plants decrease 5%;

39% and 42%; and the contents of Zn in plants decrease 4%; 12% and 15% compared to the control.

Lime also has an important role to reduce the heavy metal accumulation in plants. At the liming rates of 1 ton, 2 tons and 3 tons of $CaCO_3$/ha, the content of Cu in plants decrease corresponding to 4%; 5% and 23%; the contents of Pb decrease 4%; 22% and 43%; and the contents of Zn decrease 5%; 30% and 35% compared to the control.

Sawdust amendment to soil has little impact on the growth of *Brassica juncea* but effective in reducing uptake of heavy metals in plants. However, their reduction is not much as applied phosphate fertilizer or lime. At the highest amount of sawdust 30 tons/ha, the contents of Cu, Pb and Zn in plants only reduced 13%; 10% and 9%, respectively, compared to the control.

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