STUDY ON COMPETITIVE ABSORPTION BETWEEN Cu^{2+} AND Pb^{2+} IN LETTUCE (*Lactuca sativa* L.) AND SPINACH (*Spinacia oleracea* L.)

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TÓM TẮT

NGHIÊN CỨU SỰ HẤP THỤ CẠNH TRANH GIỮA Cu^{2+} VÀ Pb^{2+} TRÊN CÂY XÀ LÁCH MỠ (*Lactuca sativa* L.) VÀ BÓ XÔI (*Spinacia oleracea* L.)

Trong nghiên cứu này, khả năng tích lũy của đồng và chì từ đất trồng lên cây xà lách mỡ và bó xôi được khảo sát bằng cách gây ô nhiễm đất với từng ion kim loại cũng như hỗn hợp hai ion kim loại trên với các mức hàm lượng khác nhau. Kết quả cho thấy, đồng và chì là các kim loại nặng có tính tích lũy. Mặt khác, khi hai ion kim loại này cùng tồn tại trong đất trồng, chúng đều gây ảnh hưởng đến quá trình hấp thụ và tích lũy của kim loại khác lên cây xà lách mỡ và bó xôi. Cụ thể, đồng ức chế sự hấp thụ và tích lũy của chì trong khi chì lại kích thích sự hấp thụ và tích lũy của đồng từ đất trồng lên hai loại cây được nghiên cứu.

1. INTRODUCTION

Currently, the metal pollution in agricultural products is causing serious impacts on human health and it has been the interest of many scientists. Thus, a number of related studies have been performed in Vietnam and all over the world [1-3]. The results of such studies showed that there was a relationship between the metal content in cultivated environment (soil, water) and metal concentration accumulated in plants. Therefore, to minimize the amount of metals in plants, it is necessary to handle them in the farming environment. However, most of the studies examined the accumulation of each metal from soil or water to plants and proposed solutions to handle such metals in soil and water. Meanwhile, in the polluted soil and water, metals are present and exist simultaneously [4]. This will lead to the possibility of competition among them, causing the state to increase or decrease the level of metal accumulation in the plant. Therefore, the study on competitive absorption among metals in plants is very necessary. Furthermore, the results of such a work will allow predicting the level of metal accumulation in plants from the analysis report of metal content in cultivated environment, without analyzing their content in the plants themselves.

On the other hand, the results of several studies showed that the use of fertilizers, complexing agents or hyperaccumulator plants was able to handle only one or a few metals with certain content. Therefore, in order to propose possible solutions to the problem of metal contaminations in the soil, water, and their spread in plants, it is necessary to get results allowing assessment of competitive absorption among the metals. The results of such a study combined with the results of the analysis of metal content in cultivated environments will allow predicting whether competitive absorption among metals happens or not; which metal is inhibited (i.e. inconsiderable metal accumulation); and which metal is absorbingly stimulated (i.e. a need for handling). This is the basis for the choice of soil treatment, irrigable water or the choice of plants with capacity of absorbing the desired metal to clean up arable land. Moreover, because it costs much money and time to handle soil, water in current conditions, we envisage the results of this kind of research will initially provide the basis for the selection of plant varieties suitable for the soil conditions and current pollution.

2. EQUIPMENTS, INSTRUMENTS AND CHEMICALS

2.1. Equipments and instruments

- Shimadzu Atomic Absorption Spectrometry AA – 7000 Series with hollow cathode lamps of Cu and Pb; λ_{Cu} = 324,64nm, λ_{Pb} = 283,45nm.
- Compressed air and Ar gas systems.
- Drying oven.
- Fisher Science Electric stove, Germany.
- Satorius Analytical Balance measures masses to within 10^{-5}g, Germany.
- pH meter.
- Beakers, hoppers, erlenmeyer flasks, volumetric flasks, graduated cylinders; Germany.
- Pipets, micropipets; England.

2.2. Chemicals

- HNO_3 65% (d=1,35g/ml), $HClO_4$ 70% (d=1,75g/ml); Merck.
- $Cu(NO_3)_2.3H_2O$, $Pb(NO_3)_2$, Kanto Chemical Co., Japan.
- Standards are prepared by serial dilution of single element standards purchased from vendors that provide traceability to National Institute of Standards and Technology (NIST) standards.

3. EXPERIMENTAL

3.1. Field experiment

Empirical model was implemented in Ward 8, Da Lat City, Lam Dong Province – the area of which soil conditions and climate are suitable for the cultivation of lettuce and spinach. Farming period was from March, 2014 to May, 2014.

Lettuce and spinach were grown under cultivation mode which was defined by Lam Dong Province Department of Agriculture and Rural Development [5], with soil contaminated by each metal ion of copper or lead and mixture of these two metal ions at different levels. In control area, these plants were grown in soil uncontaminated.

3.2. Elemental analysis

At the end of the growth period, the plants were carefully removed from the soil. The leaves were cleaned and washed properly, then they were dried at 60°C in the drying oven to constant weight. The dried leaf samples were homogenized separately in a porcelain mortar. The homogenized leaf samples were also digested (HNO_3 and $HClO_4$, 25:10mL) [6]. The clear digested liquid was filtered through filter paper and the contents of Cu^{2+}, Pb^{2+} in the filtrate were determined using the flame atomic absorption spectrophotometer (F-AAS).

Excel 2010 software was applied to create the database and some diagrams.

4. RESULTS AND DISCUSSION

4.1. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of lettuce and spinach grown in individual metal contaminated soil

The results obtained from the research model of accumulation of each heavy metal ion from soil to plants showed that copper and lead were cumulative metals. When we increased their amounts in soil, the levels of their hoardings in examined vegetables were increased. The obtained copper and lead contents in edible parts of lettuce and spinach grown in corresponding metal contaminated soils are presented in Table 1, Table 2, Figure 1 and Figure 2.

Table 1. Concentration of Cu^{2+} in Cu^{2+} contaminated soil and in edible parts of lettuce and spinach grown in this soil

Entry	Concentration of Cu^{2+} in soil (mg/kg of dried soil)	Concentration of Cu^{2+} in lettuce (mg/kg fresh vegetable)			Concentration of Cu^{2+} in spinach (mg/kg fresh vegetable)		
		Range	Average	STDV	Range	Average	STDV
1	50	3.39 ÷ 3.99	3.78	0.34	2.92 ÷ 3.47	3.16	0.28
2	100	4.40 ÷ 4.98	4.69	0.29	4.96 ÷ 5.83	5.28	0.48
3	200	5.54 ÷ 6.42	6.02	0.44	6.18 ÷ 7.02	6.53	0.44
4	300	6.11 ÷ 6.97	6.48	0.45	6.54 ÷ 7.39	7.06	0.45
5	400	6.34 ÷ 7.37	6.81	0.52	7.01 ÷ 8.09	7.49	0.55

Copper content in lettuce which was planted in soil contaminated by 50 ppm of Cu^{2+} [7] was 3.78ppm (Entry 1, Table 1), within the authorized limit of the Ministry of Health [8]. When we doubled the level of copper in soil (100ppm), the concentration of this ion in the vegetable was 4.69ppm (i.e. an increase by 1.24 times, Entry 2, Table 1). When the level of copper in soil was increased by 8 times to 400ppm, the copper content in the vegetable was increased by 1.8 times to 6.81ppm (Entry 5, Table 1), exceeding approximately 1.36 times of the permitted limit.

In addition, the results revealed that the accumulation of Cu^{2+} in lettuce leaves was higher than that of Pb^{2+}. At an equipvalent level, i.e. using soil contaminated by the heavy metal content of 100 ppm, the difference was clear (Cu^{2+}: 4.69mg/kg of fresh vegetable vs Pb^{2+}: 0.41mg/kg of fresh vegetable; Entry 2, Table 1 and Entry 7, Table 2). Increasing the amounts of these two ions in soil to 200ppm led to the fact that lead in the vegetable was 1.49mg/kg of fresh vegetable while the accumulation of copper was 6.02mg/kg of fresh vegetable (i.e. 4.04 times higher, Entry 8, Table 2 and Entry 3, Table 1).

The results presented in Table 1 also showed that the accumulation of copper in spinach was higher than the accumulation of this ion in lettuce (approximately 1.06 times). However, spinach accumulated lead lower than lettuce did (about 2.75 times). This result proved that the biological features of each plant had an dramatically effect on the accumulated level of heavy metal ions from soil to plant.

Table 2. Concentration of Pb^{2+} in Pb^{2+} contaminated soil and in edible parts of lettuce and spinach grown in this soil

Entry	Concentration of Pb^{2+} in soil (mg/kg of dried soil)	Concentration of Pb^{2+} in lettuce (mg/kg fresh vegetable)			Concentration of Pb^{2+} in spinach (mg/kg fresh vegetable)		
		Range	Average	STDV	Range	Average	STDV
6	70	0.17 ÷ 0.20	0.19	0.02	0.11 ÷ 0.14	0.12	0.02
7	100	0.36 ÷ 0.45	0.41	0.05	0.20 ÷ 0.25	0.22	0.03
8	200	1.39 ÷ 1.65	1.49	0.14	0.39 ÷ 0.47	0.43	0.04
9	300	2.05 ÷ 2.51	2.31	0.24	0.63 ÷ 0.73	0.67	0.05
10	400	2.84 ÷ 3.31	3.02	0.25	0.82 ÷ 0.97	0.89	0.08

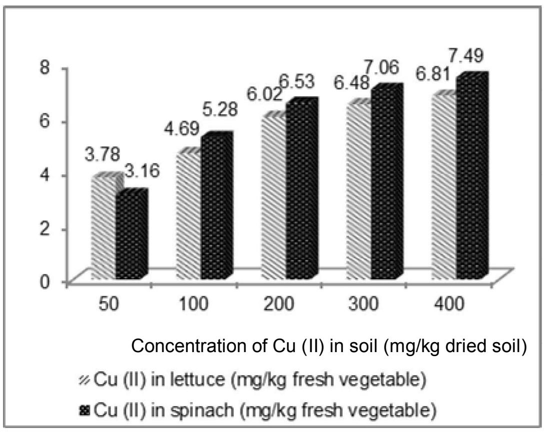

Figure 1. Cu^{2+} concentrations in soil and in edible parts of lettuce and spinach grown in this soil

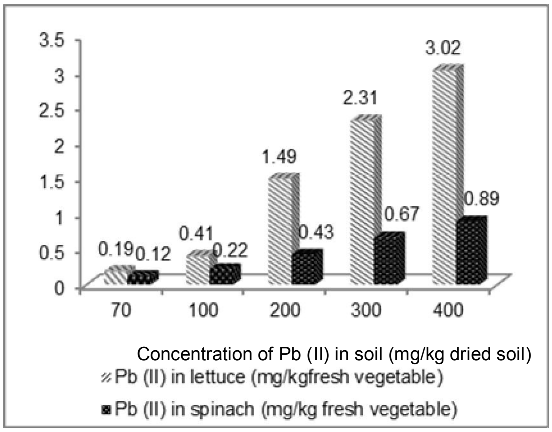

Figure 2. Pb^{2+} concentrations in soil and in edible parts of lettuce and spinach grown in this soil

4.2. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of lettuce and spinach grown in soil contaminated 7by mixtures of these metal ions

The study on the competition between copper and lead in lettuce and spinach showed that when both metals were present in soil, they influenced to each other in the process of absorption and hoarding in these plants. The results of our work are given in Table 3, 4, 5, 6.

Table 3. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of lettuce grown in soil contaminated by mixture of these metals at equivalent levels

Entry	Cu^{2+} content in soil[a]	Pb^{2+} content in soil[a]	Concentration of Cu^{2+} in lettuce[b]			Concentration of Pb^{2+} in lettuce[b]		
			Range	Average	STDV	Range	Average	STDV
11	100	100	5.11 ÷ 5.66	5.45	0.30		-	
12	200	200	5.82 ÷ 6.49	6.13	0.34	0.99 ÷ 1.11	1.05	0.07
13	300	300	6.52 ÷ 7.59	7.01	0.54	1.53 ÷ 1.92	1.71	0.20
14	400	400	7.05 ÷ 8.02	7.59	0.50	2.28 ÷ 2.73	2.47	0.23

a: mg/kg of dried soil *b: mg/kg of fresh vegetable*

When soil was contaminated by copper and lead with the same amounts, lead stimulated the absorption of copper in lettuce. In soil with only copper contamination at a level of 100ppm, the cumulative copper content in lettuce was 4.69mg/kg fresh vegetable (Entry 2, Table 1). Meanwhile, in the presence of lead with the equivalent level, the cumulative copper content was increased by 16.2% to 5.45 mg/kg fresh vegetable (Entry 11, Table 3).

On the other hand, the results of this study also revealed that when soil had the presence of both copper and lead at similar levels, Cu^{2+} inhibited the uptake and accumulation of Pb^{2+} by lettuce. When soil was polluted by Pb^{2+} at a level of 100 ppm, the cumulative lead content in lettuce was 0.41 mg/kg of fresh vegetable, but in the presence of copper at that level the lead concentration in lettuce was not observable (Entry 7, Table 2 and Entry 11, Table 3). Besides, when we used soil with only lead contamination at a level of 300 ppm, the content of lead in lettuce was 2.31 mg/kg of fresh vegetable (Entry 9, Table 2). However, in the presence of copper with equivalent level, the cumulative lead content was decreased by 25.97% to 1.71 mg/kg of fresh vegetable (Entry 13, Table 3).

Table 4. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of spinach grown in soil contaminated by mixture of these metals at equivalent levels

Entry	Cu^{2+} content in soil[a]	Pb^{2+} content in soil[a]	Concentration of Cu^{2+} in spinach[b]			Concentration of Pb^{2+} in spinach[b]		
			Range	Average	STDV	Range	Average	STDV
11	100	100	5.62 ÷ 6.34	5.93	0.37	-	-	-
12	200	200	6.50 ÷ 7.62	7.05	0.56	-	-	-
13	300	300	7.30 ÷ 8.27	7.92	0.54	0.23 ÷ 0.27	0.25	0.02
14	400	400	7.56 ÷ 8.93	8.17	0.70	0.49 ÷ 0.58	0.53	0.05

a: mg/kg of dried soil *b: mg/kg of fresh vegetable*

The results of the competitive absorption between copper and lead from soil to spinach when they co-existed in soil with the same amount are illustrated in Table 4. Here again, lead stimulated the absorption of copper while copper inhibited the accumulation of lead by spinach.

The competitive relationship between Cu^{2+} and Pb^{2+} in absorption and accumulation from soil to lettuce and spinach was confirmed by a research model in which the content of Cu^{2+} in soil was lower than that of Pb^{2+}.

Clearly, Pb^{2+} in soil stimulated the absorption of Cu^{2+} to lettuce. At a level of 100 ppm, in case soil was added copper alone, the cumulative Cu^{2+} content in lettuce was 4.69 ppm (Entry 2, Table 1), but in the presence of Pb^{2+} with the double level, the cumulative Cu^{2+} content was raised to 1.6 times (7.51 ppm, Entry 15, Table 4). In the presence of lead at the concentration of more than 3 times (300 ppm), the level of lead hoarding in vegetable was increased by 1.81 times (Entry 16, Table 4).

Table 5. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of lettuce grown in mixture metal contaminated soils in which the content of Cu^{2+} was lower than that of Pb^{2+}

Entry	Cu^{2+} content in soil[a]	Pb^{2+} content in soil[a]	Concentration of Cu^{2+} in lettuce[b]			Concentration of Pb^{2+} in lettuce[b]		
			Range	Average	STDV	Range	Average	STDV
15	100	200	7.21 ÷ 7.98	7.51	0.41	0.57 ÷ 0.67	0.62	0.05
16	100	300	8.04 ÷ 8.97	8.49	0.47	0.82 ÷ 1.02	0.90	0.10
17	100	400	8.52 ÷ 9.57	8.97	0.54	1.26 ÷ 1.52	1.42	0.14

a: mg/kg of dried soil *b: mg/kg of fresh vegetable*

In addition, the inhibitory effect of Cu^{2+} to Pb^{2+} was confirmed. When soil was polluted by Pb^{2+} at a level of 200 ppm, the content of Pb^{2+} in lettuce was 1.49 mg/kg of fresh vegetable (Entry 8, Table 2). In the presence of Cu^{2+} at a level of 100 ppm, the cumulative lead content was reduced by 58.39% to 0.62 mg/kg of fresh vegetable (Entry 15, Table 4). In soil with only lead contamination at a level of 300 ppm, the cumulative lead content in lettuce was 2.31 mg/kg of fresh vegetable (Entry 9, Table 2). However, in the presence of copper at the concentration of less than 3 times (100 ppm), the cumulative lead content was decreased by 61.04% to 0.90 mg/kg of fresh vegetable (Entry 16, Table 4). These results confirmed the impact of copper on the uptake and accumulation of lead from soil to lettuce.

This relationship between copper and lead also was described clearly in experimental results of spinach.

Table 6. Accumulation of Cu^{2+} and Pb^{2+} in edible parts of spinach grown in mixture metal contaminated soils in which the content of Cu^{2+} was lower than that of Pb^{2+}

Entry	Cu^{2+} content in soil[a]	Pb^{2+} content in soil[a]	Concentration of Cu^{2+} in spinach[b]			Concentration of Pb^{2+} in spinach[b]		
			Range	Average	STDV	Range	Average	STDV
15	100	200	7.12 ÷ 8.37	7.82	0.64	0.27 ÷ 0.32	0.29	0.03
16	100	300	7.64 ÷ 8.73	8.35	0.62	0.48 ÷ 0.59	0.54	0.06
17	100	400	8.25 ÷ 9.56	9.02	0.69	0.68 ÷ 0.78	0.72	0.05

a: mg/kg of dried soil *b: mg/kg of fresh vegetable*

5. CONCLUSION

The results of this study proved that the concentration of heavy metals in soil effected dramatically on the heavy metal pollution in agricultural products. Besides, the absorption and accumulation of heavy metals from soil to plants depended on biological features of each plant. Furthermore, when both copper and lead were added to soil, they effected to each other in the process of absorption and accumulation in the plant. Copper inhibited the uptake and accumulation of lead by lettuce and spinach while lead stimulated the absorption of copper by these plants.

We believe that the finding of this work is the basis for further study on the subject of heavy metals on different crops, opening interdisciplinary research to explain the mechanism of this phenomenon. A similar work on other crops grown in different soil conditions as well as an attempt to propose solutions for the treatment of the pollution by heavy metals in farming environment are under way in our lab.

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NGHIÊN CỨU HOÀN THIỆN PHƯƠNG PHÁP VON......... *(tiếp theo tr. 355)*

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