STUDY ON THE ACCUMULATION OF ZINC FROM SOIL TO THE BIOMASS OF LETTUCE (*Lactuca sativa* L. var. *capitala* L.)

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Abstract

The transport of heavy metals from soil to plants is a complex process depending on many factors. This study investigated the accumulated level of zinc – a heavy metal which plays an important role in the growth of plants and animals at a certain content and causes some diseases in these candidates at high concentrations – from soil contaminated by this element to lettuce. The results of this study showed that zinc can accumulate at high level in biomass of lettuce and zinc content in leaf was higher than its content in root. On the other hand, this investigation proved that the accumulating process of zinc from soil to lettuce was influenced by some factors including: soil pH, type and amount of mineral fertilizer used in cultivation and the presence of other heavy metals in soil.

Keywords. Heavy metal pollution, accumulation of zinc, lettuce

1. INTRODUCTION

Soil pollution by heavy metals is great concern to public health (Goyer, 1996) [1]. The main reasons causing this environmental problem are mining and smelting of metal ores, industrial emissions and applications of insecticides and fertilizers. These activities have all contributed to elevated levels of heavy metals in the environment (Alloway, 1995) [2]. Cultivated in metal polluted areas, agricultural products can accumulate heavy metals at high levels and cause impact directly on the health of consumers through a food chain.

Differing than other toxic heavy metals, zinc plays an important role in the growth and development of plants as well as human health. Shortage or excess of zinc is causing adverse effects. For plants, zinc deficiency makes plants grow poorly thereby reducing productivity. However, zinc excess causes the loss of chlorophyll disease affecting the photosynthetic process of plants [3]. For humans, zinc is extraordinarily useful in biological systems. It is involved in many biochemical processes that support life and required for a host of physiological functions including normal immune function, sexual function, neurosensory function such as cognition and vision. But the most common effects associated with long-term, excessive zinc intakes have included sideroblastic anamenia, hypochromic microcytic anaemia, leukopenia, lymphadenopathy, neutropenia, hypocupraemia and hypoferraemia [4].

Therefore, determining the accumulation of zinc from soil to plant will provide the basis for the addition of this essential elements in farming at suitable dose to ensure the development of the plant but not harmful to consumer health. On the other hand, the content of zinc accumulate in plants can be influenced by many factors such as mode of cultivation, using mineral fertilizers and competition with other heavy metals in soil. Thus, this study is performed in order to evaluate the accumulation of zinc from contaminated soil on lettuce biomass and investigate the factors such as the amount of lime, fertilizer regime, the competitive absorption of other metal ions on this process.

2. EXPERIMENTAL

2.1. Field experiment

Empirical model included three areas:

- The research model of accumulation of zinc from soil to plants: lettuce was grown under cultivation mode as in reality, but the soil was contaminated by zinc at different levels.

- The research model of cultivation mode to zinc accumulation from soil to plants: the soil was contaminated by zinc at 100 ppm, 100ppm and the added amounts of lime as well as N, P, K fertilizers were changed.

- Control experiment: lettuce was grown under the same conditions as models mentioned above in soil uncontaminated.

The soil used in this experiment was a topsoil (0-20 cm) collected from an uncontaminated vegetable field in Ward 8, Dalat city. The soil was air – dried, passed through 2mm sieve and spiked with different zinc doses by spraying of $Zn(NO_3)_2$ solution. Fifteen kilograms of the prepared soil were placed in each pot for growing lettuce. Seeds of lettuce were germinated in plastic trays and the seedlings were transplanted after two weeks, into individual pots in research model. Lettuce was grown under cultivation mode which was defined by Lam Dong Province Department of Agriculture and Rural Development [5].

2.2. Elemental analysis

At the end of the growth period, lettuce was carefully removed from the soil. The leaves and roots were separated, cleaned and washed properly, then they were dried at 60 °C in the drying oven to constant weight. The dried samples were homogenized separately in a porcelain mortar. The homogenized samples were also digested (HNO₃ and HClO₄, 25:10 mL) [6]. The clear digested liquid was filtered through filter paper and the contents of zinc 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.

3. RESULTS AND DISCUSSION

3.1. Accumulation of zinc biomass of lettuce grown in this metal contaminated soil

The results obtained from the research model of accumulation of zinc from soil to lettuce are presented in table 1.

The results showed that levels of zinc accumulation on lettuce biomass were very high. When we increased zinc amounts in soil, the levels of its hoardings in biomass of lettuce were increased. The dependence of the zinc content in leaf and root of lettuce and zinc content in soil can be expressed through these equations:

- Leaf: y =
$$13.151\ln x - 53.483$$
 (R² = 0.8612)
- Root: z = $8.10^{-6}x^2 + 0.0182x + 3.8696$
(R² = 0.9844)

where x is the content of zinc in soil (mg.kg⁻¹ of dried soil), y is the content of zinc in lettuce leaf (mg.kg⁻¹ of fresh vegetable), and z is the content of zinc in root of lettuce (mg.kg⁻¹ of fresh vegetable).

Table 1: Concentration of zinc in contaminated soil and in biomass of lettuce grown in this soil

Concentration of zinc in soil (mg.kg ⁻¹ of dried	Concentration of zinc in lettuce (mg.kg ⁻¹ of fresh vegetable)		
soil)	Leaf	Root	
50	7.04±0.78	5.32±0.39	
100	7.64±0.73	4.49±0.37	
200	8.43±0.84	6.25±0.36	
300	$15.01{\pm}1.51$	10.45±0.66	
400	19.75±2.01	14.38±0.71	
600	29.89±3.12	18.91±1.02	
800	30.82±3.18	23.81±1.96	
1000	43.95±4.41	29.37±1.81	
1200	45.81±4.63	32.76±2.38	
1500	44.59±3.85	50.38±2.67	

When zinc content in soil ranged from 50 to 200 ppm, the accumulation of zinc in biomass of lettuce changed slowly but at higher levels of pollution (above 300ppm), the concentration of zinc in root and leaf lettuce increased significantly. This can be caused by defense mechanism of plant living in polluted environment. At low polluted levels, plants absorb heavy metals initiatively and control heavy metals at nontoxic concentration to cells. However, when the polluted level rises and exceeds the tolerance ability of plants, plants absorb passively heavy metals [7]. Specifically, at a certain level of pollution, the plant cannot absorb heavy metals anymore. For example, when zinc level in soil ranged from 1000 to 1500 ppm, the accumulation of this metal in leaves was almost unchanged.

3.2. Effects of cultivation mode on zinc accumulation from soil to lettuce biomass

3.2.1. Effect of lime

Before seeding or planting, farmers often use lime to reduce soil acidity as well as to sterilize soil. This activity can change soil pH so this can effect on the accumulation of heavy metals from soil to plants. The results of this research were presented in table 2.

When pH was raised from 6 to 7, there was a decrease in this heavy metal uptake from soil to lettuce. From this results, it is evident that pH soil has a profound effect on the metal uptake by the plants. At low pH values, the heavy metal ions show greater cation exchange capacity (CEC) and become more available in the aqueous medium thereby making the metal to be more bioavailable to the plant. pH dramatically effects the CEC of soil by limiting the available exchange sites at low pH. H⁺ binds to soil particles tighter than other cations, thus, any metal bound to a soil particle will get booted off in the presence of excess H^+ . At low pH, H^+ is in excess and replaces all other cations on the micelle, thus making them bioavailable. At high pH, cations are less bioavailable because they have less competition from H⁺ for available binding sites. Many cations bind to free hydroxyl groups (OH⁻) and form insoluble hydrous metal oxides, which are unavailable for uptake. This result is also consistent with the conclusions of While et al. (1979) [8] for that liming is considered the most effective method to reduce the absorption of heavy metals and reduce toxicity of these metals for plants.

But at 7.5 pH value, the concentration of zinc in leaf and root increased. This result is due to when pH > 7, the mobility of zinc increased, involving the formation of zincate $[Zn(OH)_4] - zinc$ compounds

where zinc exists as an anionic component. This causes an increase in zinc content in easily digestible format, leading to that the absorption from the soil to the plants becomes easier. Therefore, it should be noted that using lime in a certain amount minimizes the possibility of accumulation of heavy metals and ensure growth rate of plants.

Table 2: The concentration of zinc in biomas	s of
lettuce when soil pH was changed by using li	ime

The amount of lime (kg per	Corresponding soil pH	Concentration of zinc in biomass (mg.kg ⁻¹ of fresh vegetable)		
pot)		Leaf	Root	
0	6	7.64±0.73	4.49±0.37	
1.5	6.5	5.43 ± 0.55	6.64±0.53	
3.5	7	4.92±0.51	8.52±0.49	
6.0	7.5	6.13±0.35	9.05±0.56	

3.2.2. Effect of N, P, K fertilizers

N, P, K fertilizers are commonly used during cultivation to provide nutrients to plants. In order to investigate the influence of these mineral fertilizers to zinc accumulation, we fixed zinc concentration in soil at 100 ppm, pH = 6 and changed every single fertilizer N, P, K at different levels. The results of this investigation were presented in table 3.

Type of fertilizer	Content compared to standard amount [5]Amount of fertilizer in pot (kg)	1 1110 1111 01	Zinc content in biomass (mg/kg ⁻¹ of fresh vegetable)		
		Leaf	Root		
N	0.5	0.72	4.23±0.56	3.46±0.19	
	1	1.44	6.26±0.54	4.23±0.31	
	1.5	2.16	6.52±0.66	4.96±0.34	
Р	0.5	3.00	6.62±0.65	4.12±0.24	
	1	6.00	5.47±0.37	3.49±0.25	
	1.5	9.00	5.53 ± 0.42	6.02 ± 0.48	
K	0.5	0.45	7.38±0.72	5.82±0.43	
	1	0.90	6.96±0.63	5.13±0.41	
	1.5	1.35	5.22±0.46	4.75±0.28	
control	0	0	7.41±0.69	4.49±0.25	

Table 3: Effect of type and amount of mineral fertilizers on the accumulation of zinc

These results showed that, by adding N, P or K fertilizer in soil, the zinc content in biomass of lettuce was lower compared to the control.

Specifically, using N fertilizer in cultivation reduced 15.52 % zinc content in leaf and decreased 5.79 % zinc content in root. The presence of P fertilizer

reduced the amount of zinc in leaf about 26.18 % and 22.27 % in root. Meanwhile, using K as a fertilizer reduced 6.07 % zinc accumulation on leaf and raised 12.48 % zinc accumulation in roots. Thus, the use of N, P, K fertilizers in the process of cultivation can reduce the zinc accumulation levels on crops. When adding mineral fertilizers, plants grow better and the rapid growth of crops reduced the increase amount of zinc in plants due to the phenomenon of "diluting the concentration" [9]. Besides, the results of this survey showed that the accumulation of zinc in biomass of lettuce in case of having added P fertilizer was lower than in case of having added N or K fertilizer. The results of Zhang and Shan (2001) [10], Oyedele (2006) [11] proved the ingredient of P fertilizer not only included nutritional elements but also contained other metals such as Cd, Pb or Hg. The presence of these metals in phosphate fertilizer has caused the phenomenon of competition in transport process of zinc from soil to plant, reducing the accumulation of zinc in plants. This result proved that fertilizer could also limit the accumulation ability of heavy metals from soil to plants. However, while the heavy metal acts as a trace element essential for the growth of plants such as zinc, using mineral fertilizers in farming can reduce absorbing efficiency of this nutritional element.

3.3. Effects of other heavy metal ions on zinc accumulation from soil to lettuce biomass

Heavy metal contaminated soil often contains more than one heavy metal (Prashant Srivastava et al., 2005) [12]. Among heavy metals, copper is an essential element for the growth of plants which was commonly added during cultivation, while lead is a common toxic heavy metal in polluted soil. Within the limit of this article, we investigated the effect of copper and lead on zinc absorption from soil to biomass of lettuce. The results were presented in table 4.

Heavy metals content in soil (mg.kg ⁻¹ dried	biomass (m	ion of zinc in g.kg ⁻¹ of fresh etable)	Heavy metals content in soil (mg.kg ⁻¹ dried	Concentration of zinc in biomass (mg.kg ⁻¹ of fresh vegetable)	
soil)	Leaf	Root	soil)	Leaf	Root
Zn 100 + Cu 100	6.76 ± 0.62	4.23±0.31	Zn 100 + Pb 100	4.66 ± 0.48	4.38±0.24
Zn 100 + Cu 200	5.18 ± 0.52	3.58±0.21	Zn 100 + Pb 200	3.76±0.28	3.25±0.22
Zn 100 + Cu 300	4.53±0.46	3.47±0.26	Zn 100 + Pb 300	3.12±0.23	2.78±0.16
Zn 100 + Cu 400	3.32±0.34	3.06±0.18	Zn 100 + Pb 400	2.82±0.21	2.47±0.17

Table 4: Effects of copper and lead on zinc accumulation from soil to lettuce biomass

The obtained results showed that the presence of either copper or lead reduced the absorption of zinc from soil to lettuce, where the effect of lead was higher than that of copper. The order of ionic radius of these metal ions is $Pb^{2+} > Cu^{2+} > Zn^{2+}$ so the affinity of these ions towards cationic absorption center on plant cells decreases following this order. Thus, lead and copper will be absorbed dominantly into cationic absorption center, then limit the transport of zinc through cell membrane and reduce the amount of zinc in the plant cells. The effect of lead on the zinc accumulation is stronger than that of copper because lead affinity towards cationic absorption center is higher than copper affinity.

4. CONCLUSIONS

Lettuce can accumulate zinc from the soil at

high level, so this kind of vegetable may be additional source of micronutrients in the daily diet. However, we should note that zinc concentration in leaf can make serious harm when the lettuce is planted in contaminated soil.

Cultivated mode has great influence on the accumulation of zinc from soil to plans. Using lime before planting and mineral fertilizers at a suitable amount in farming can reduce the zinc content in lettuce. On the other hand, the presence of essential elements (copper) and toxic heavy metals (lead) reduces the concentration of zinc in biomass of lettuce. The results obtained in this study provide the importance data to investigate the heavy metal absorption from soil to plants and contribute proposing effective solutions for the treatment of the pollution by heavy metals in farming environment.

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