

ARSENIC AND COPPER UPTAKE BY CABBAGES GROWN ON POLLUTED SOILS

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ABSTRACT

Cabbages (*Brassica Juncea (L.) Czern*) were grown in pot experiments on typical unpolluted and polluted soils with concentration changing from 20.50 - 50.00 mgAs/kg and 156.00 - 413.00 mgCu/kg dry soil. The results demonstrate the elevation of As and Cu in soil may lead to increased uptake by these cabbages subsequent entry into human food chain. It was found 11.84 - 32.12 mgAs/kg and 46.86 - 94.47 mgCu/kg dry leaves. It has tendency increase uptake and accumulation of Cu in cabbage tissue with increasing cultivated time, whereas, it was found accumulation of As in cabbages tissue decreased with time prolonging. The quantity of As and Cu in these cabbages, were significant higher than 0.2 mgAs/kg and 5.0 mgCu/kg fresh vegetable, the permissible limit concentration in fresh vegetable, (FAO/WHO, 1993). This indicated that human may As and Cu exposure occur through eating these vegetables.

1. INTRODUCTION

Terrestrial plants are able to accumulate metals to a substantial extent but survive the stress to differing degrees of vitality. The principle processes for transport of metals from soil to plant root are convection (mass flow) and diffusion. Metal ion uptake can be passive (apoplastic) or active (symplastic). Crop species and cultivars differ widely in their ability to absorb, accumulate and tolerate heavy metals, and they show a range of different mechanisms for protecting themselves against metal uptake. Types of metal tolerance mechanism in plants include selective uptake of ions, decreased permeability of membranes immobilization of ions in the roots removal ion from metabolism by storage in fixed or insoluble forms, *etc.* Plant uptake metal depends on the concentration and speciation of the metal in soil, the movement of the metal from the bulk soil to the root surface, the transport of the metal from root surface into the root by crossing the membrane of epidermal cells and its translocation from root to the leaf. Each of these processes strongly affects whether a potential toxic element reaches the food chain and each process is strongly affected by the chemical speciation of the element [1].

Copper is a required element for plant growth, as its serves an important role in plant structure and function. However, increased concentration of Cu in soils can lead to toxic effects in plants. Sometimes, plant test is used to recognize incipient Cu toxicity in soils. For that reason some authors established an upper critical level value for Cu toxicity in plant tissue.

Arsenic (As) is ubiquitous the environment and is derived from both natural and anthropogenic sources. As is a nonessential element for plants and inorganic As species are generally highly phytotoxic. As (V) acts as a phosphate analog and can disrupt phosphate metabolism, whereas

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As (III) reacts with sulfhydryl groups of enzymes and tissue proteins leading to inhibition of cellular function and death [8].

The aim of this study was to quantify the relative availability of As and Cu from soil to cabbage uptake and accumulation of these metals in different part of cabbage as well as plant age.

2. MATERIALS AND METHODS

2.1 Soil characterization

Agricultural soil was collected from an agricultural site located around Ho Chi Minh city. The physical-chemical properties of the soil was measured and listed in table 1.

Table 1: Selected physical-chemical properties of the soil.

Soil properties	Values
Clay (%)	13.52
Silt (%)	28.48
Sand (%)	58.00
pH	7.47
OM (%)	4.0
Total N (%)	0.3
Total P (%)	0.4
K ₂ O (%)	0.4
Cu (ppm)	12.36
As (ppm)	4.49

2.2 Experiment setup

Air-dried soil was amended with salt of arsenic (NaAsO₂) and copper (CuSO₄.5H₂O) as solution at different concentrations: 20.5, 25, 35 and 50 ppmAs³⁺; 156, 179, 249 and 413 ppmCu²⁺. 1.5 kg amended soil was placed in 20 cm diameter plastic pots (2 L). Four replicated were prepared per each treatment. After 4 weeks equilibrium, cabbages seeds (*Brassica juncea*) were sown directly in pots. After germination, excess seedlings were removed to keep 5 plants per pots. Soils were irrigated daily to maintain the moisture content at 80% of field moisture capacity with fertilized N, P, K (16:16:8) solution rate 100 kgN/ha to approach optimum yield.. Control treatments were used agricultural soil without contaminated As and Cu. Cabbages were grown under average temperature 30°C, 12 h of light. Metal concentrations in plant were determined at Day 30, 45 and 60 days after sowing. Leaves were separated from roots and all plant parts were washed with tap water to remove adherent soil particles and rinse with distilled water, dried at 60°C for 48 hrs and ground in an agate mortar and pestle to pass a 0.85 mm (20 meshes) sieve.

2.3 Elemental analysis

Cabbages analysis for As and Cu were accomplished by digesting in microwave (MarsX). The content of As and Cu, were determined by GF-AAS (GBC-Ultra Z) and Flame AAS (GBC-Avanta).

3. RESULTS AND DISCUSSION

Significant amount these metals of As and Cu in soil was not available (table 2 and table 3)

indicating that only small portion of the total may be reached to the cabbages.

Cabbage grows well in tropical area, after sowing 3 days the crops started germinating. At the early stage, cabbage grew very well even in the high As and Cu contaminated soil. After 20 days germinated, visible damage was most obvious in the youngest leaves whereas the primary leaves and the second pair showed invisible and more evident as plant grew to maturity.

Metals content in plant tissue in this study is not a fixed entity, but varies from month to month, day to day even from hour to hour as well as differing between the various parts of plant itself. The rate movement among tissues varies greatly depending on the plant organ, its age and element evolved.

3.1 Arsenic concentration and distribution in *Brassica juncea*

The results of experiment showed that cabbage could grow in soils contaminated from 20.5 to 50 mgAs/kg. Toxic effect of As inhibited uptake and metabolic activity, which leads to suppressed plant growth and slow development. Visible toxic symptoms are stunted and blackened roots, dried leaf margins. Table 2 presented the accumulation of As in cabbage tissue grown on polluted soil at different concentrations

Arsenic considerable as toxic are listed 5 - 10 mg/kg dry leaves [6]. The observation data from these experiments are greater than the list, 11.84 - 32.12 mgAs/kg dry leaves.

The concentration of As in cabbage tissue grown on polluted soils were much higher in comparison with the control plant, concentration of As in plant increased with increasing As concentration in soil. Significantly decrease arsenic concentrations in roots and leaves of cabbages grown on soils containing 50 mgAs/kg dry soil (Fig. 1). It might be due to plant genotype that have low uptake at quite high external concentrations of the element. There was no significant different of As concentrations in cabbages grown on control treatments as time increasing but significant decreasing As concentration in roots and leaves of cabbages as time increasing.

Table 2: Total and availability of soil arsenic concentration, accumulation of As in roots and leaves of cabbage (mean values, n = 4).

Trtm.	As in soil		Roots As			Leaves As		
	mg kg ⁻¹ dry weight		mg kg ⁻¹ dry weight			mg kg ⁻¹ dry weight		
	Total	Avai.	D 30	D 45	D 60	D 30	D 45	D 60
Contr.	4.41	0.37	2.42	2.55	2.29	0.91	1.23	1.26
No. 1	20.50	2.06	26.71	21.99	20.28	14.13	13.27	11.84
No. 2	25.00	2.84	48.21	38.22	27.44	25.59	23.58	18.49
No. 3	35.00	6.84	57.22	45.81	37.28	32.12	26.65	21.77
No. 4	50.00	9.18	42.22	36.75	28.26	16.00	14.58	12.48

Trtm.: Treatment, Avai.: Availability, D30, D45, D60 Day 30, Day 45, Day 60; Contr.: Control soil.

The highest As concentration was found at Day 30, the lowest concentration was found at Day 60, perhaps due to the “exclusion strategy and dilution effect” caused by toxic action of As, after 30 days cabbages limited accumulation in roots and limited translocation to the leaves and biomass production during period. Arsenic located in the roots higher than in leaves as expect.

There were significant correlations between soil As availability and As cabbage tissue ($R^2 = 0.96 - 0.99$) (Fig. 2).

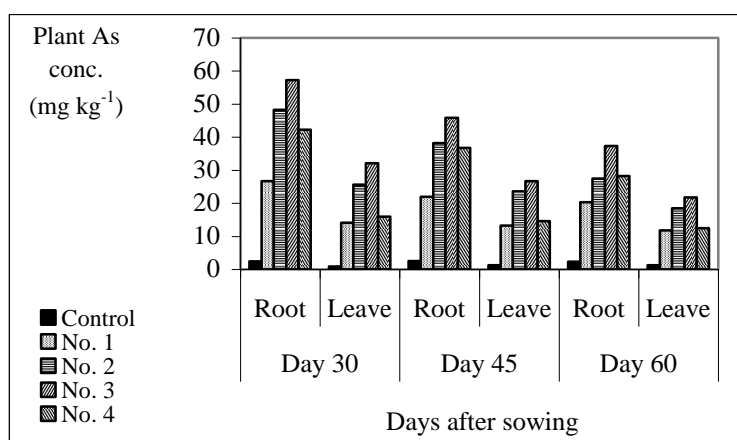


Fig. 1: Distribution of arsenic concentration in root and leaf of cabbage grown on control and polluted soil at different harvested time.

Arsenic has been reported to trigger the formation of phytochelatins in plants [5, 9]. Phytochelatins is As-binding peptides with SH-groups, leading to inhibition of cellular function and death. Phytochelatins are considered to be part of detoxifying mechanism of higher plants, immobilized metals are less toxic than free ions [4, 12, 9].

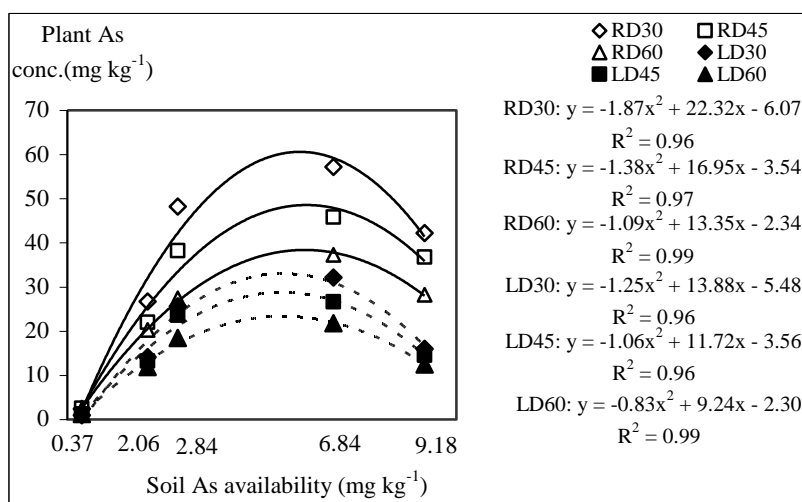


Fig. 2: Relationship of soil As availability and plant As concentration at different harvested time. RD30, 45 and 60: root Day 30, 45 and 60; LD 30, 45 and 60: leaf Day 30 45 and 60.

3.2 Copper concentration and distribution in *Brassica juncea*

Both shoot and root growth of cabbage decreased with increasing Cu concentration in soil. The injury was initiated root system, browning the root increased with increasing Cu in growth

medium, at higher Cu treatment (249.53 mg/kg dry soil) the root showed dark brown, stunted growth and inhibition of root elongation.

Concentration of Cu in soil was as low as 156.54 mg/kg, the cabbage growth significantly retarded in comparison with control pot. On pots which had received high doses of copper salt exhibit photo-inhibition and chlorosis in cabbage leaves.

Copper in leaves of cabbages grown on polluted soil was range from 46.86 to 94.47 mg/kg dry weight (Table 3), significantly higher than healthy plant from 5 to 20mg.kg⁻¹ dry weight [6]. It was found Cu accumulation in the roots was higher than in the leaves. The Cu concentration in plants had a tendency to increase with the age of plant, the highest copper concentration was found at Day 60 (Fig. 3).

Table 3: Total and availability of soil copper concentration, accumulation of Cu in roots and leaves of cabbage (mean values, n = 4).

Trtm.	Cu in soil mg kg ⁻¹ dry weight		Roots Cu mg kg ⁻¹ dry weight			Leaves Cu mg kg ⁻¹ dry weight		
	Total	Avai.	D 30	D 45	D 60	D 30	D 45	D 60
Contr.	12.36	2.36	5.81	11.35	45.23	18.31	12.17	20.97
No. 1	156.48	17.88	20.63	57.10	68.69	46.86	53.97	85.07
No. 2	179.60	26.70	23.52	75.73	92.66	49.12	61.22	92.84
No. 3	249.53	51.60	24.98	78.31	98.18	63.04	68.67	92.60
No. 4	413.75	116.76	60.68	120.21	130.87	78.59	82.89	94.47

Trtm.: Treatment, Avai. Availability, D30, D45, D60 Day 30, Day 45, Day 60; Contr.: Control soil.

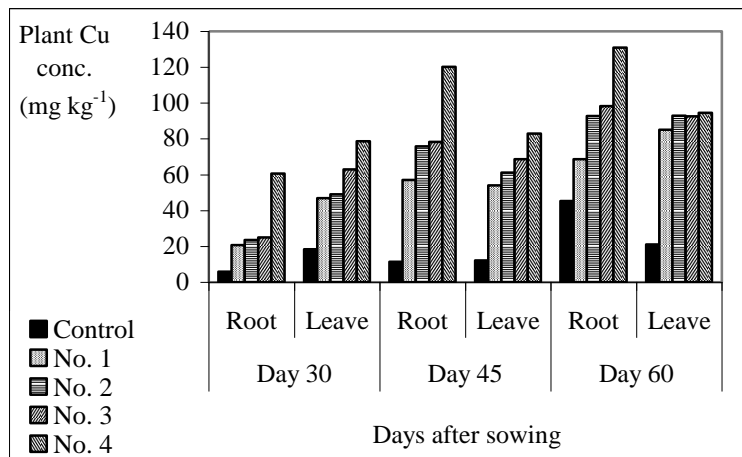


Fig. 3: Distribution of copper concentration in root and leaf of cabbage grown on control and polluted soil at different harvested time.

At Day 30, concentration of Cu in roots was less than in leaves; this indicated that Cu is mobile elements in cabbage. From 30 to 45 days, the visible damage appeared obvious in the leaves; it

seemed cabbage limited translocation of Cu from roots to shoots. Significantly increasing amount of Cu in the roots at Day 45 whereas not much increasing in case of leaves. However, no such accumulation in roots has been found. There were not much different Cu concentration between roots and leaves at Day 60 unless in the cabbages grown on pots received highest dose of Cu, this indicated that transfer of Cu to the top of plant depended upon the function of the roots, when to tolerance mechanisms in the root zone become overload; Cu is translocated by both the xylem and phloem up to the leaves. Copper contents in roots of cabbages were strongly correlation with soil Cu availability ($R^2 = 0.91 - 0.95$), the level of correlations soil copper availability and total copper in leave of cabbages decreased from 0.96 to 0.76. (Fig. 4).

Excess Cu may replace other metals in metalloproteins or may interact directly with -SH groups of protein [11], Cu induced free radical formation may also cause protein damage. High concentrations of Cu^{2+} may catalyze the formation of the hydroxyl radical from O_2 and H_2O_2 . This Cu^{2+} catalyzed Fenton-type reaction takes places mainly in chloroplasts [10]. The hydroxyl radical may start the peroxidation of unsaturated membrane lipids and chlorophyll and these inhibitory mechanisms might contribute to the observed inhibition of photosynthetic electron transport by excess Cu^{2+} [2].

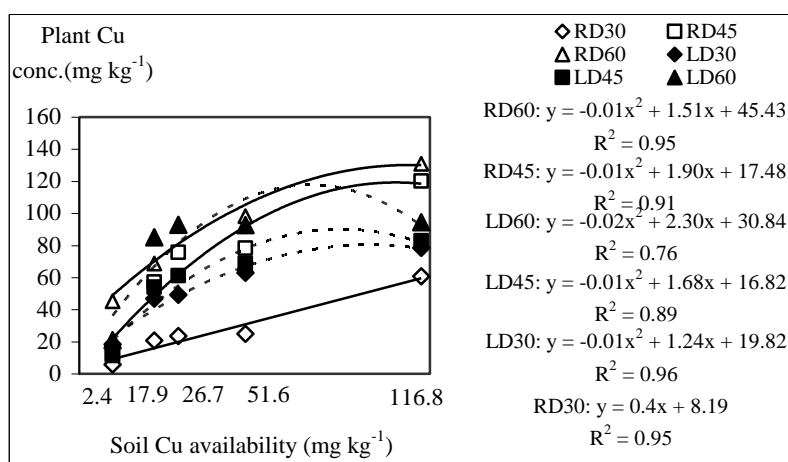


Fig. 4: Relationship of soil Cu availability and plant Cu concentration at different harvested time. RD30, 45 and 60: root Day 30, 45 and 60; LD 30, 45 and 60: leave Day 30 45 and 60.

3.3 Cu and As concentration in fresh cabbage

All findings have shown that elevated levels of As and Cu in soil may lead to increased uptake by cabbages. So crop uptake and translocation to edible tissue are important process in the analysis of metal movement in food chain. There are public health implications if plant foods accumulate high concentration of metals.

The impact of As and Cu contaminated in cabbage on human health is more concern and it may correlate with the frequency of these metals contaminated cabbage consumption. The permissible limit concentration in fresh vegetable established by FAO/WHO (1993) is 0.2 mg of As and 5.0 mg of Cu per kg fresh vegetable. The quantity of As and Cu in these cabbages, were significant higher than those limits, this indicated that human may As and Cu exposure occur through eating these cabbages.

Table 4: As and Cu concentration in edible portion of cabbages.

Trtm	As concentration mg kg ⁻¹ fresh weight			Cu concentration mg kg ⁻¹ fresh weight		
	D 30	D 45	D 60	D 30	D 45	D 60
Contr.	0.09	0.12	0.13	1.83	1.22	2.10
No. 1	1.41	1.33	1.18	4.69	5.40	8.51
No. 2	2.56	2.36	2.12	6.31	6.87	9.28
No. 3	3.21	2.67	1.85	4.91	6.12	9.26
No. 4	1.60	1.46	1.25	7.86	8.29	9.45

Trtm.: Treatment, D30, D45, D60 Day 30, Day 45, Day 60; Contr.: Control soil.

4. CONCLUSIONS

The present of As and Cu in agricultural soil were significantly increased metal uptake by cabbage and its were well high in comparison with those grown on control pot.

Certain amount of As and Cu concentration in soil are rising concern about phytotoxicity.

More Cu was found in the plant with time prolonging; on the contrary, less As in plant tissue was found with increasing cultivated time.

The quantity of As and Cu in edible tissue of cabbages, were significant higher than the permissible limit concentration in fresh vegetable 0.2 mgAs/kg and 5.0 mgCu/kg fresh vegetable (FAO/WHO, 1993). This indicated that human may As and Cu exposure occur through eating these vegetables.

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