

The Effect of Cadmium, Copper, and Lead on *Brassica juncea* in Hydroponic Growth Medium

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ABSTRACT

This study measured the accumulation of cadmium (Cd), lead (Pb), and copper (Cu) in *Brassica juncea* grown using the hydroponic method in a water environment contaminated with these heavy metals. The accumulated metal content in each part of the plant was monitored after one, three, and six weeks of exposure. The concentrations of Cd, Cu, and Pb in the biomass of *B. juncea* were determined using atomic absorption spectroscopy. The results showed that heavy metal pollution in water caused heavy metal accumulation in vegetable biomass. Pb tended to accumulate lower vegetable biomass than Cu and Cd. The metal accumulation level in Cu and Cd was in the order of roots > stems > leaves, while with Pb, the concentration accumulated in roots > leaves > stems. The translocation factors of Cu, Cd, and Pb from shoots to stems and shoots to leaves were less than 1.

Keywords: Accumulation, *Brassica juncea*, cadmium, copper, lead, translocation factor

INTRODUCTION

The contamination of heavy metals from the environment into food can result from industrial or agricultural activities, vehicle exhaust, or contamination during food processing and

storage. Such contamination and its effects on human health are major challenges in many countries (Anwar et al., 2016). Vegetables have high amounts of vitamins, minerals, fibers, and antioxidants, making them valuable human food (Gupta et al., 2021). The quality of vegetables is highly dependent on the quality of the growing environment. Heavy metal contamination of

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vegetables may also occur due to irrigation in environments with heavy metal pollution.

Hydroponic vegetable cultivation is growing in interest worldwide due to the high quality of vegetables grown in this manner and the ability to control the vegetables' composition. The hydroponic technique involves soil-free gardening in which plants grow in nutrient-rich water without soil, gravel, rockwool, coconut fiber, or sawdust base (Sharma et al., 2018). The hydroponic technique is a relatively easy and clean method that reduces or eliminates pesticide use because crops do not incur soil-borne diseases or insect and pest infestations (AlShrouf, 2017; Wang et al., 2017). However, the quality of hydroponic vegetables depends largely on water quality. If the water is contaminated, pollutants can accumulate in agricultural products. Other factors include vegetable species, soil composition, growth stages, atmospheric and geographic conditions, and the types of metals present (Dulama et al., 2012; Radulescu et al., 2010, 2013).

Previous studies have documented that heavy metals are toxic and persistent and have the ability to bioaccumulate in aquatic atmospheres (Bai et al., 2018). Agriculture's increasing demand for water requires research on how to use other water sources and treated wastewater (TWW). TWW is rich in nutrients but may also contain various heavy metals (Mourato et al., 2015). Plants can uptake, transfer, and accumulate heavy metals in the growth stage because plants have a higher absorption capacity than mature plants (Souri et al.,

2019). Additionally, roots have a higher concentration of heavy metals than other tissues of plants due to their direct contact with heavy metals in the environment (Mohtadi et al., 2012).

This study aimed to evaluate the effects of the accumulation of copper (Cu), cadmium (Cd), and lead (Pb) in the leaves, stems, and roots of *Brassica juncea* grown using the hydroponic technique.

MATERIALS AND METHODS

Vegetable and Conditional Experiments

In this study, young *B. juncea* were collected from a vegetable farm in the VI district of Ho Chi Minh City, Vietnam. The vegetables selected were 5.0 ± 0.5 cm in height and 4.0 ± 0.2 g in weight. First, 192 individual young *B. juncea* were kept under static hydroponic conditions in 48 tanks ($30 \text{ cm} \times 20 \text{ cm} \times 16 \text{ cm} = 9.6 \text{ L}$). Every tank contained four individual young *B. juncea*. The nutrient solution was purchased from Hydroponic Garden Company Limited (Vietnam), which composition was as follows: 2 mM calcium nitrate [$\text{Ca}(\text{NO}_3)_2$], 3 mM potassium nitrate (KNO_3), 0.50 mM magnesium nitrate (MgSO_4), 1 mM ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), 1 μM potassium chloride (KCl), 25 μM boric acid (H_3BO_3), 20 μM sodium ferric ethylenediaminetetraacetate [$\text{Fe}(\text{Na})\text{-EDTA}$], 2 μM zinc sulfate (ZnSO_4), 2 μM manganese(II) sulfate (MnSO_4), and 0.1 μM ammonium heptamolybdate [$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$]. After five days of acclimation, each tank was exposed to one

of four simulated heavy metal contamination levels. All experiments were triplicated ($n = 3$).

- Control sample: Only nutrient solution
- EXP 5 sample: Add 5 mg L⁻¹ each of Cd, Pb, and Cu into the nutrient solution
- EXP 10 sample: Add 10 mg L⁻¹ each of Cd, Pb, and Cu into the nutrient solution
- EXP 20 sample: Add 20 mg L⁻¹ each of Cd, Pb, and Cu into the nutrient solution



Figure 1. The tank of the experiment

The tanks were in a room with the temperature set at $30 \pm 2^\circ\text{C}$ by an air conditioner, and they received sunlight all day (Figure 1). Every six days, 100% of each tank's water was replaced to ensure consistency and that the nutrient solution's acidity remained at 5.5. Hydroponics experiments solution samples for total Cd, Pb, and Cu analysis were collected twice a week and analyzed by flame atomic

absorption spectroscopy (ZA-3000, Hitachi, Japan) to check nominal concentrations of solutions. If the three metals measured and nominal concentrations were significantly different, the experiment solutions were adjusted to match the nominal concentration.

Sampling and Measurements

Sampling. On days 7, 21, and 42 of exposure, 20 plants from each treatment were randomly harvested and washed carefully in bidistilled water. All harvested plants were drained in room temperature for 2 hours and were weighed to determine the mass of plant. And then, all harvested plants were separated into roots, stems, and leaves. The roots were rinsed carefully for 15 min to remove the adsorbed metals on the surfaces of the root using a 10 mmol L⁻¹ disodium ethylenediaminetetraacetate solution (Merck, Germany), followed by washing them again in bi-distilled water. The collected vegetable tissue samples were dried in an oven at 105°C for 24 h and then ground into a fine powder in a coffee grinder (HC-600, HeyCafé, Taiwan) before the Cd, Pb, and Cu concentrations were analyzed. Changes in the studied heavy metals content in the roots, stems, and leaves within six weeks from the time the vegetables were grown in contaminated water were monitored.

Cd, Pb, and Cu Analysis. To determine the concentrations of Cd, Pb, and Cu, a wet digestion method was used to digest 1 g of the powdered sample in a closed vessel using 4 ml of concentrated nitric acid (HNO₃, Merck, Germany) and 2 ml of concentrated hydrogen peroxide (H₂O₂,

Merck, Germany). The closed vessels were then heated to 85°C for 30 min until preliminary mineralization was achieved. Next, the obtained solution was diluted to 25 mL using deionized water. Cd, Pb, and Cu concentrations were assayed using flame atomic absorption spectroscopy (ZA-3000, Hitachi, Japan). The method's detection limits were 3.6 µg kg⁻¹ for Cd, 25 µg kg⁻¹ for Pb, and 3.5 µg kg⁻¹ for Cu. The method's quantification limits were 11.8 µg kg⁻¹ for Cd, 83 µg kg⁻¹ for Pb, and 11.8 µg kg⁻¹ for Cu. The assay recovery was approximately 92.3% for Cd, 92.6% for Pb, and 97.1% for Cu. The relative standard deviation (RSD) was approximately 0.79% for Cd, 0.54% for Pb, and 3.55% for Cu.

Calculation and Statistical Analysis

The translocation factor (TF) indicates the ability to transfer metals in a plant from its roots to its stems and leaves. Following Deng et al. (2007), the TF of metal from a plant's roots to stems or leaves was calculated as follows: $TF = \text{leaves' or stems' metal concentration} / \text{roots' metal concentration}$.

The rootly, leafy, and stemly vegetable Cu, Cd, Pb concentrations were plotted against the Cu, Cd, Pb concentrations in nutrient solution, and a linear regression

analysis was performed on the data to obtain the linear regression equation and R-squared.

All experiments were triplicated ($n = 3$), and the experimental data were presented in average \pm standard deviation (SD). The analytical procedure was validated based on the Certified Reference Materials (white cabbage - trace elements, BCR[®]-679). The RSD of each analysis was found within $\pm 2.4\%$ of the certified values. The one-way analysis of variance (ANOVA) was utilized to evaluate the significant differences between the lead content in particular vegetables and contaminated soil. Statistical significance was evaluated via the student's *t*-test with a *p*-value < 0.005 .

RESULTS AND DISCUSSION

Effect of Cd, Pb, and Cu Concentration in the Nutrient Solution on the Growth Rate of *Brassica juncea*

The plant mass of the control and treatment plants is shown in Table 1. The toxicity of Cd, Pb, and Cu metals affected exposure plants' growth, and the absorption of these metals consequently depends on metabolic needs. The group of plants exposed to heavy metal showed clearly the changed metabolic

Table 1
Effect of Cd, Cu, and Pb on the growth of *B. juncea* over time

Treatments	Mass of plant (g)		
	Day 7 th	Day 21 st	Day 42 nd
Control	10.94 \pm 0.35	25.29 \pm 0.69	41.45 \pm 0.49
EXP 5 treatment	10.25 \pm 0.39	24.35 \pm 0.85	40.89 \pm 0.82
EXP 10 treatment	9.95 \pm 0.31	21.04 \pm 0.74	34.99 \pm 0.87
EXP 20 treatment	9.88 \pm 0.36	18.73 \pm 0.76	27.01 \pm 0.69



Figure 2. Effect of Cu, Cd, and Pb to plant experiment

demands compared to the control group plants. As a result, the treatment plants reduced biomass yields compared to the control plants (Figure 2). For example, after 42nd days of treatment, the biomass yields of the EXP 5 sample, EXP 10 sample, and EXP 20 sample treatments were reduced by 88.3, 71.7, and 63.2% compared to control plants (Table 1).

The Accumulate Level of Cd, Cu, and Pb in the Biomass of *Brassica juncea*

The contents of Cu, Cd, and Pb accumulated in the biomass of *B. juncea* collected from each field after each week are presented in Figures 3, 4, and 5. Over time, the extent of accumulated studied metals in the roots, stems, and leaves of *B. juncea* increased.

Copper, this metal tends to accumulate in roots higher than in stems and leaves (Figure 3). In general, the copper content in the roots was, on average, 12.49 times higher than the copper in the stems and 16.59 times higher than the copper in the leaves. This result is consistent with the judgment of Feigl et al. (2013), under the stress of Cu, *B. juncea* and *Brassica napus* accumulated more Cu in roots than in shoots for 7 and 14 days in hydroponic solution when the amount of copper in the nutrient solution increased the amount of copper accumulated in the parts of *B. juncea*. On average, when doubling and quadrupling the amount of copper in the nutrient solution, the copper amount in the roots increased by 2.78 and

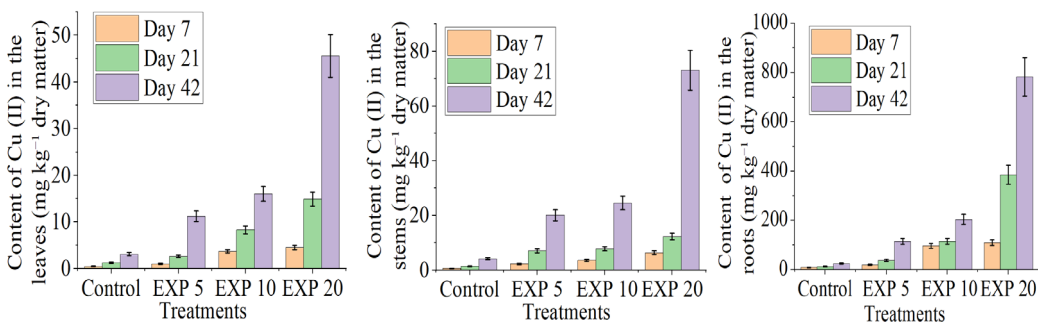


Figure 3. The accumulation of copper in the biomass of *B. juncea*

7.01 times, respectively. Meanwhile, the copper content in the stems increased by 1.22 and 2.92 times, respectively, and the content of this metal in the leaves increased by 2.77 and 4.71 times, respectively.

The accumulation of Cd in parts of *B. juncea* is lower than that of copper. When the Cd content in the nutrient solution was 5 mg/L, the amount of Cd accumulated in the roots was 18.15 times higher than its content in the stems and 15.24 times higher than in the leaves. At a content of 10 mg L⁻¹ and 20 mg L⁻¹ Cd in a nutrient

solution, the amount of Cd in the roots was, on average, 6.59 times higher than the amount of Cd in the stems and 6.1 times higher than its content in the leaves (Figure 4). Jiang et al. (2001) reported that cadmium could accumulate in *Allium sativum* by hydroponically cultivated. In the group exposed to Cd at 0.01 M, Cd accumulation in plants was 1,826-fold higher than in the control group. Later, Barazani et al. (2004) reported that Cd was tolerant and hyperaccumulation in *Allium schoenoprasum*.

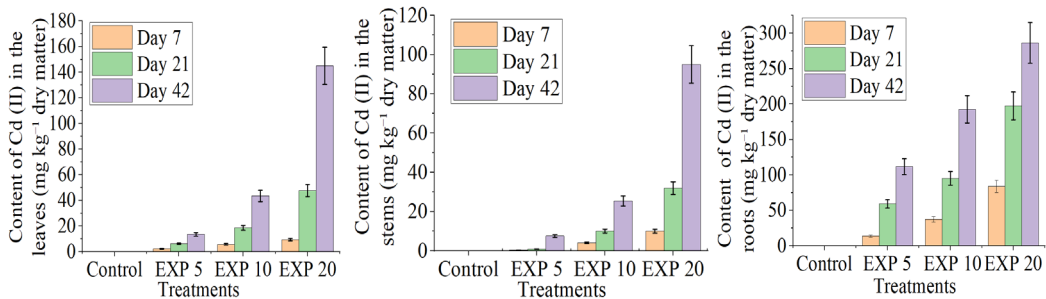


Figure 4. The accumulation of cadmium in the biomass of *B. juncea*

Unlike Cu and Cd, the amount of Pb accumulated in the roots of *B. juncea* is relatively large and much higher than the amount of lead in the stems and leaves. The heavy metals concentrations in the *B. juncea* tissue in this research were similar to the Zhou et al. (2016) reported that heavy metals concentrations in the edible tissue of different vegetable species were different. Because of the binds of Pb to cell walls and root surfaces, therefore, it reduced the transfer of Pb from roots to shoots or leaves (Cobb et al., 2000). Similarly, in this study,

Pb accumulation was high in the roots compared to other tissue.

At the 5 mg L⁻¹ content of the studied metal in the nutrient solution, the amount of lead accumulated in the roots of *B. juncea* was twice as high as cadmium and copper. At 10 mg/L concentration, the amount of lead accumulated in the roots was 1.44 and 1.80 times higher than that of copper and cadmium. These value at 20 mg L⁻¹ metal contamination in the nutrient solution is 1.54 and 3.03, respectively (Figure 5).

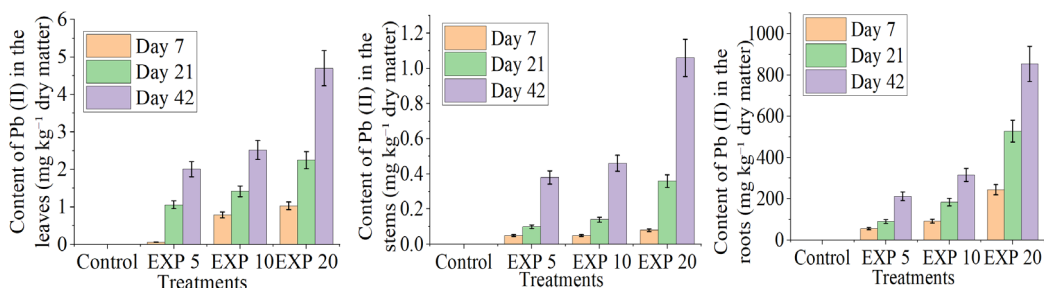


Figure 5. The accumulation of lead in the biomass of *B. juncea*

In general, the accumulated studied heavy metals in the roots were much higher than in the shoots and leaves because the roots were in direct contact with the nutrient solution. For copper, this vegetable's order of accumulation on biomass was roots > stems > leaves. Meanwhile, the order of accumulation for cadmium and lead was roots > leaves > stems. According to Soudek et al. (2009), the accumulation of heavy metals was predominantly in the root system, so its transport from roots to the bulbs was rather low. The heavy metal concentration in leaves resulted from the heavy metal absorption by roots, which then was transferred to leaves via stems. There were significantly different heavy metal levels in the different vegetable tissue, which linked to the characteristics of the plants and different heavy metals (Tom et al., 2014).

The accumulation of studied heavy metals in the biomass of *Brassica juncea* follows the order: Cd < Cu < Pb. This result proves that *B. juncea* has better Cd tolerance than Cu and Pb.

The Translocation Factors of *Brassica juncea* Grown in Polluted Condition by Cu, Cd, and Pb

The translocation of Cu in *B. juncea* clones is shown in Table 2. Under 5 mg L⁻¹ Cu exposure, the stems and leaves TFs for Cu ranged from 0.114 to 0.186 and from 0.052 to 0.098, respectively. These results show that Cu tends to be accumulated in the roots rather than the stems and leaves. At 20 mg L⁻¹ Cu exposure, the stems and leaves TFs of vegetable tissues were slightly increased. The order of TF values follows stems > leaves. In General, the TFs of vegetable tissues increased with time proceeds, whereas they decreased with increases in Cu concentration in the nutrient solution. The different TFs for Cu in *B. juncea* tissues showed different absorbing capacities and affinities for Cu. The decrease in TFs of vegetable tissues in *B. juncea* parts can be an informative sub-lethal response, indicating increased Cu availability and potential Cu stress in the *B. juncea*. Plants also can translocate and store micronutrients in their environment. Furthermore, toxic elements can have similar absorption, translocate, and storage mechanisms.

Table 2

The translocation factors (TFs) of *B. juncea* with 5, 10, and 20 mg L⁻¹ of Cu treatment in hydroponic culture for six weeks

Days	The concentration of Cu (II) in nutrient solution (mg L ⁻¹)					
	5		10		20	
	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs
7 th	0.114	0.052	0.037	0.038	0.058	0.041
21 st	0.186	0.069	0.068	0.072	0.084	0.047
42 nd	0.195	0.098	0.120	0.079	0.093	0.058

Compared to Cu exposure, the TF of Cd in *B. juncea* is larger than (Table 3). The stems and leaves TFs for Cd ranged from 0.025 to 0.332 and from 0.103 to 0.507, respectively. These results show that Cd tends to be accumulated in the roots rather than in other parts. The order of TF for

Cd values in *B. juncea* was the following: leaves > stems. Perhaps Cd has similar properties to Ca, so *B. juncea* cannot recognize its toxicity. From there, Cd was absorbed and transported to the stems and leaves of *B. juncea*.

Table 3

The translocation factors of *B. juncea* with 5, 10, and 20 mg L⁻¹ of Cd treatment in hydroponic culture for six weeks

Days	The concentration of Cd (II) in nutrient solution (mg L ⁻¹)					
	5		10		20	
	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs
7 th	0.025	0.167	0.025	0.155	0.091	0.112
21 st	0.071	0.103	0.106	0.196	0.129	0.242
42 nd	0.090	0.121	0.166	0.226	0.332	0.507

The translocation of Pb in *B. juncea* clones is shown in Table 4. Similar to the case of Cu and Cd exposure, the stems TFs for Pb of *B. juncea* are higher than leaves TFs. However, the translocation factors of Pb in the stems and leaves of *B. juncea* are much lower than that of Cu and Cd. Therefore, it may indicate that *B. juncea* can

recognize toxic of Pb so that they can limit absorption and transport to stems and leaves.

Similarly, several previous studies have documented that TFs were less than 1. Majid et al. (2014) studied *Typha angustifolia* and *Phragmites australis* species which TFs of Cu and Pb were 0.12 to 0.87 and 0.33 to 0.42, respectively. Takarina et al. (2017)

showed that TFs of Cu from roots to stems and from roots to leaves in *Rhizopora* sp. were 0.33 and 0.78. Rezapour et al. (2022), the TF values of Cu, Pb, and Cd were 0.72–0.85, 0.09–0.63, and 0.17–0.22,

respectively. The values of TFs of Cu, Pb, and Cd ranged between 0.08 and 0.63 in the lettuce and spinach plants (Eissa & Negim, 2018).

Table 4

The translocation factors of *B. juncea* with 5, 10, and 20 mg L⁻¹ of Pb treatment in hydroponic culture for six weeks

Days	The concentration of Pb (II) in nutrient solution (mg L ⁻¹)					
	5		10		20	
	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs	Roots-stems TFs	Roots-leaves TFs
7 th	0.0009	0.0011	0.0005	0.0085	0.0003	0.0042
21 st	0.0011	0.0117	0.0008	0.0077	0.0007	0.0043
42 nd	0.0018	0.0095	0.0015	0.0080	0.0012	0.0055

Relationship Between Rootly, Leafy, and Stemly Vegetable Cu, Cd, Pb Concentrations and Cu, Cd, Pb Concentrations in Nutrient Solution

A high correlation was observed between Cu, Cd, and Pb concentrations in tissues of *B. juncea* and Cu, Cd, and Pb concentrations in nutrient solution (Table 5). The significant positive correlation between Cu, Cd, or Pb concentrations in tissues of *B. juncea* and Cu, Cd, and Pb concentrations in nutrient solution may indicate that Cu, Cd, and Pb have similar uptake mechanisms. Roots

absorbed trace elements through passive (which was nonmetabolic) and active (which was metabolic) mechanisms. The different uptake mechanisms depended on the kind of element. For example, Pb absorbed mechanisms passively, while Cu absorbed mechanisms actively (Kabata-Pendias, 2011). And then, Cu, Cd, and Pb were probably transported from roots to stems and leaves by similar transporters in the form of compounds or chelate metal complexes (Foy, 1983; Tyler et al., 1989).

Table 5

Correlation coefficients between Cu, Cd, and Pb concentrations in nutrient solution and Cu, Cd, and Pb concentrations in the tissue of *B. juncea* (mg kg⁻¹ dry matter)

Treatments		Roots	Stems	Leaves
Cu	Linear regression	y = 46.444x - 174.5	y = 3.7197x - 4.11	y = 2.3846x - 3.52
	R-squared	R ² = 0.9566	R ² = 0.9352	R ² = 0.9587

Table 5 (Continue)

Treatments		Roots	Stems	Leaves
Cd	Linear regression	$y = 11.317x + 64.835$	$y = 8.9671x - 37.25$	$y = 5.7574x - 21.47$
	R-squared	$R^2 = 0.9782$	$R^2 = 0.9871$	$R^2 = 0.9933$
Pb	Linear regression	$y = 44.412x - 57.125$	$y = 0.0474x + 0.08$	$y = 0.1849x + 0.92$
	R-squared	$R^2 = 0.9672$	$R^2 = 0.9499$	$R^2 = 0.9765$

CONCLUSION

This study investigated the accumulation of heavy metals, including copper, cadmium, and lead, in the different parts of *B. juncea* planted by the hydroponic method. The research results show that the accumulation of these heavy metals in the different parts of *B. juncea* varied over time. However, generally, the results showed the highest concentration of all tested heavy metals in the root, and transport of metals to other plant parts (stems and leaves) was very low.

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