

Effect of Heavy Metals on the Growth of Spinach

Chunqi Zhai, Yunzhu Tang, Rongfang Feng*

Faculty of Applied Technology, Huaiyin Institute of Technology, Huaian 223001, China

*frfrfrfrf@163.com

Abstract

With the rapid development of industry and agriculture, copper mining, combined with the use of copper-bearing fertilizers and wastewater discharge, has led to a doubling of copper concentrations in farmland soils. When the heavy metal content in soil is too high, the growth and development of plants will be hindered. Heavy metal stress can disturb the physiological function of plants and even lead to plant poisoning. Spinach is a vegetable widely grown all over the world. Spinach is rich in nutrients, vitamins and minerals and has high nutritional value. It is of great theoretical significance and guiding value to explore the effects of heavy metals on the growth of spinach. Certain concentrations of mineral solutions may stimulate spinach growth slightly, but growth slows as concentrations increase. With the increase of mineral concentration, fresh weight and dry weight of spinach decreased, and chlorophyll content increased. Heavy metal detoxification is a necessary step in the phytoremediation process. Plants often employ one of two defense methods to deal with heavy metal toxicity: avoidance or tolerance. Plants use these two methods to keep heavy metal concentrations in their cells below toxicity threshold levels.

Keywords

Heavy Metal; Spinach; Cadmium; Chromium.

1. Introduction of Spinach

Spinach is a nutrient-dense food. It has a lot of nutrients in a small sum of calories. Spinach and other dark, leafy greens are good for your skin, hair, and bones. Protein, iron, vitamins, and minerals are also found in them. Consuming spinach may have health benefits such as improving blood glucose management in diabetics, lowering cancer risk, and enhancing bone health, as well as providing minerals and vitamins that can provide a variety of benefits. Throughout history, spinach has been employed by many cultures, most notably in Mediterranean, Middle Eastern, and Southeast Asian cuisines. It is inexpensive and simple to prepare, so it can be easily introduced into any diet.

2. Uptake and Translocation of Heavy Metals in Plants

Heavy metal mobility, roots intake, xylem loading, root-to-shoot transit, cellular separation, and sequestration are only a few of the mechanisms involved in heavy metal accumulation in plants. Heavy metals are usually found in soil in an insoluble form that plants cannot use. Plants can boost their bioavailability by generating a range of root exudates that can alter the pH of the rhizosphere and increase heavy metal absorption [1]. The accessible metal binds to the plant root and travels through the cell membranes into the plant root. The apoplastic (passive diffusion) and symplastic (active diffusion) pathways are the two most common ways for this to happen (active transport against electrochemical potential gradients and concentration across the plasma membrane). Metal cations carriers or complexing agents mediate the popular

uptake of heavy metals through the symplastic pathway, which is an energy-dependent process [2].

Metal ions can form complexes with chelators like organic acids when they reach root cells. Carbonate, sulfate, and phosphate precipitates are produced and fixed in the extracellular environment (apoplastic cellular walls) or internal regions (symplastic compartments, such as vacuoles) [3]. Metal ions sequestered inside vacuoles may move into the stele and enter the hyphal stream via the root symplasm, where they are then translocated to the shoots via xylem vessels. They are carried and disseminated in leaves by apoplast or symplast, where the ions are stored in external divisions (cell walls) or plants vacuoles, preventing free metal ions from accumulating in the cytoplasm [4].

A variety of molecules, especially metal ion exchangers and complexing agents, are involved in the uptake of heavy metals in plants. These special transporters (carrier protein) either H⁺-coupled carrier proteins are found in the root cell's plasma membrane and are required for heavy metal ion absorption from the soil. They can transport particular metals through cellular membranes and facilitate metal transport from roots to shoots by mediating influx-efflux [5]. Metal transporters discovered so far have been grouped into various families based on sequence homology, including ZIP, HMAs, MTPs, and NRAMPs.

The ZIP family of transporters (ZRT-IRT-like proteins) are engaged in heavy metal deposition activities, such as the absorption and transport of numerous cations (such as F, Mn, and Zinc) from root to shoot. Zn hyperaccumulator *Thlaspi caerulescens* and *Arabidopsis halleri* roots, for example, show higher Zn absorption than non-hyperaccumulator species, which is linked to hyperaccumulator hyperexpression of several ZIP family members [6]. Heavy metal transporter ATPases (HMAs) transporter family P1B-type ATPases are involved in the transfer of heavy metals (such as Zinc, Cd, Co, and Pb) and play an important role in metal regulation and tolerance (Axelsen and Palmgren. By controlling Zn, Cd, Co, and Pb sequestration into the vacuole, HMA3, a vacuolar P1B-ATPase, is implicated in compartmentation [7,8].

HMA4, another member of the family, is involved in long-distance Zn and Cd translocation from root to shoot. HMA4 overexpression boosted metal tolerance by increasing Cadmium and Zinc efflux from the roots symplasm into the vascular system. Metal transporter proteins (MTPs), which are involved in the movement of metals (such as Zinc and Nickel) toward internal compartments and extracellular space, are another type of transporters that strictly control metal homeostasis. MTP1, a vesicles Zn²⁺/H⁺ antiporter that is found on both the vacuolar and plasma membranes, is implicated in both Zn accumulation and tolerance [9].

In *Thlaspi goesingense*, MTP members are also engaged in Ni vacuolar storage. Many heavy metal ions, notably Cu²⁺, Mn²⁺, Co²⁺, Fe²⁺, and Cd²⁺, are transported by normally resistant associating macrophage proteins (NRAMPs) [10,11]. Fe and Mn transport is mediated by AtNRAMP1, which is found in the plasma membrane. The tonoplast proteins NRAMP3 and NRAMP4 mediate the export of accumulated Fe from the cell in sprouting seedling.

In addition to metal ion transporters, complexing agents like as organic compounds and amino acids function as metal ligands to facilitate heavy metal ion chelation. Citrate, for example, is a strong chelating agent for Fe and Ni in the xylem, whereas histidine may also chelate Ni [12].

3. Effect of Biogas Fertilizer/Passivator on Cd Content in Spinach

Table 1 shows the influence of biogas fertilizer / Passivator on the content of heavy metal Cd in spinach.

It is possible to see the influence of biogas fertilizer / Passivator on the heavy metal Cd content in spinach roots. The content of heavy metal Cd in spinach roots after application of biogas fertilizer + passivator was lower than in the CK group after the test, with a reduction range of 20.00% to 34.50%, indicating that biogas fertilizer + passivator reduced the content of heavy

metal Cd in spinach roots planted in soil. The application of biogas fertilizer + passivator had a significant effect on the heavy metal Cd content in the roots of spinach planted in soil, according to the findings. ; The use of biogas as a fertilizer The content of heavy metal Cd in the roots of soil-planted spinach increased by 0.005 mg/kg in the treatment group compared to the CK group, showing that the application of biogas fertilizer did not lower the content of heavy metal Cd in the roots of soil-planted spinach. The content of heavy metal Cd in spinach roots from small to large was zd3 zd1 ZD2 CK ZF in these five treatments. The amount of heavy metals in spinach roots in the group with biogas fertilizer + passivator and the group with biogas fertilizer + passivator was found to be similar using LSD multiple comparative analysis The content of heavy metal Cd in spinach roots treated with CK was significantly higher than that in those treated with biogas fertilizer. Heavy metal Cd content was significantly higher in spinach roots treated with CK than in those handled with biogas fertilizer, heavy metal Cd content in green beans root system of the zd3 group (biogas fodder + calcium and phosphate fertilizer) has been considerably higher than that of the zd1 group [13].

Table 1. Effects of biogas fertilizer/passivator on the content of heavy metal Cd in spinach

	CK	ZF	ZD1	ZD2
Root	0.206	0.210b	0.180b	0.135a
Stem	0.160d	0.005c	0.080b	0.045a
Leaf	0.160e	0.033d	0.020c	0.035a
Total	0.340d	0.310c	0.250b	0.208a

The content of heavy metal Cd in the roots of soil-planted spinach was lowest when biogas fertilizer + calcium magnesium phosphate fertilizer was applied, according to the above test results and significance analysis results. It is possible to see the effect of Passivator on the content of heavy metal Cd in spinach stems. The content of heavy metal Cd in spinach stems after application of biogas fertilizer or biogas fertilizer + passivator was lower than that in the CK group after the test, with a reduction range of 21.43 percent to 38.57 percent, indicating that the application of biogas fertilizer or biogas fertilizer + passivator reduced the content of heavy metal Cd in spinach stems planted in soil. The use of biogas fertilizer or biogas fertilizer + passivator had a significant effect on the heavy metal Cd level in the stem of spinach planted in soil, according to the findings [14].

Among them, the content of heavy metal Cd in the stem of spinach treated with biogas fertilizer was 0.015mg/kg lower than that in the CK group, indicating that biogas fertilizer reduced the content of heavy metal Cd in the stem of spinach; compared to the ZF group, the three treatment groups with biogas fertilizer + passivator decreased by 0.005mg/kg 0.12mg / kg, indicating that biogas fertilizer + passivator reduced the heavy metal Cd content in spinach stems from small to large was zd3 ZD2 zd1 ZF CK in these five treatments.

The content of heavy metal Cd in spinach stems treated with biogas fertilizer or biogas fertilizer + passivator was significantly higher than that in the CK group, the content of heavy metal Cd in spinach stems treated with biogas fertilizer + passivator was significantly higher than that in the biogas fertilizer group, the content of heavy metal Cd in spinach stems treated with biogas fertilizer + passivator was significantly higher than that in the biogas fertilize the zd3 group (biogas fertilizer + calcium magnesium phosphate fertilizer) and the ZD2 group (biogas fertilizer + red mud) had no significant differences (P > 0.05). According to the above test findings and significance analysis, the concentration of heavy metal Cd in the stem of spinach

planted in soil is lowest when biogas fertilizer + calcium magnesium phosphate fertilizer is applied.

The Biogas Fertilizer's Impact The effect of passivator on the content of heavy metal Cd in spinach leaves can be seen that the content of heavy metal Cd in spinach leaves after harvest in the four treatments with biogas fertilizer or biogas fertilizer + passivator is lower than that in the CK group, with a reduction range of 50.00% to 66.67%, indicating that biogas fertilizer or biogas fertilizer + passivator reduces the content of heavy metal Cd in spinach leaves. The application of biogas fertilizer or biogas fertilizer + passivator had a significant influence on the content of heavy metal Cd in spinach leaves planted in soil, with biogas fertilizer being applied to 40% of the harvested spinach.

When compared to the CK group, the content of heavy metals in the leaves fell by 0.015 mg/kg, demonstrating that using biogas fertilizer reduced the content of heavy metals D in spinach leaves. The level of heavy metal Cd in spinach leaves fell by 0.005 0.015mg/kg in the three treatment groups with biogas fertilizer + passivator compared to the ZF group, showing that using biogas fertilizer + passivator reduced the content of heavy metal Cd in spinach leaves. The heavy metal Cd level in spinach leaves ranged from small to big in these five treatments: zd3 ZD2 zd1 ZF CK. The heavy metal Cd concentration in spinach leaves treated with biogas fertilizer or biogas fertilizer + passivator was substantially greater than in the control group, according to the findings [15].

The content of heavy metal Cd in spinach leaves treated with biogas fertilizer and passivator was significantly higher than that of spinach leaves treated with biogas fertilizer; the content of heavy metal Cd in spinach leaves treated with zd3 (biogas fertilizer + calcium magnesium phosphate fertilizer) was significantly higher than that of zd1 (biogas fertilizer + zeolite) and ZD2 (biogas fertilizer + red mud). It may be concluded from the above test results and significance analysis that when biogas fertilizer + calcium magnesium phosphate fertilizer is used, the content of heavy metal Cd in spinach leaves decreases [16].

4. Effect of Biogas Fertilizer/Passivator on Cr Content in Spinach

Table 2 shows the influence of biogas fertilizer / passivator on the content of heavy metal Cr in spinach. Heavy metal Cr content in spinach was D3 ZD2 ZD1 ZF CK in these five treatments, ranging from small to large. The content of heavy metal Cr in spinach treated with biogas fertilizer or biogas fertilizer + passivator was significantly higher than that in the CK group; the content of heavy metal Cr in spinach treated with biogas fertilizer was significantly higher than that in the CK group; the content of zd1 + Mg (zd1 + zd2.05) was significantly higher than that of ZD2 + Mg (zd1 + zd2.05) (Applying Biogas Fertilizer).

Table 2. Effect of biogas fertilizer/passivator on heavy metal Cr level in spinach

	CK	ZF	ZD1	ZD2	ZD3
Root	66.75%d	77.16%e	55.00%b	60.98%c	44.69%a
Stem	20.48d%d	20.21%b	18.58%c	15.66%b	16.26%a
Leaf	20.55%e	16.22%dc	15.25%cb	15.59%b	5.97%aa
Total	92.65d%c	89.60%	80.97%	80.50%b	67.55%

The heavy metal Cr concentration of red mud) spinach was significantly greater than that of the zd1 group (biogas fertilizer Plus zeolite). According to the findings of the aforementioned tests and significance analysis, the concentration of heavy metal Cr in soil planted spinach is lowest when biogas fertilizer + calcium magnesium phosphate fertilizer is used. The content of heavy metal Cr in spinach roots after harvest of the three treatments with biogas fertilizer + passivator

is lower than that of the CK group, with a reduction range of 60% to 12.04 percent, indicating that the application of biogas fertilizer + passivator reduced the content of heavy metal Cr in the roots of spinach planted [17].

The application of biogas fertilizer and passivator had a significant effect on the decrease of Cr content in spinach roots; however, the application of biogas fertilizer increased the content of heavy metals in spinach roots by 0.038 mg/kg when compared to the CK group, indicating that biogas fertilizer could not reduce the content of heavy metals Cr in spinach roots planted in soil. Heavy metal content ranged from zd1 to ZD2 to ZF CD5 in spinach roots. The amount of heavy metal Cr in spinach roots was substantially greater in the zd3 group (biogas fertilizer + calcium magnesium fertilizer) than in the other treatment groups.

Heavy metal Cr content in spinach roots of the CK, zd1 (biogas fertilizer + zeolite), and ZD2 (biogas fertilizer + red mud) groups was significantly higher than that of the ZF; there was no significant difference between the CK, zd1 (biogas fertilizer + zeolite), and ZD2 (biogas fertilizer + red mud) groups ($P > 0.05$). According to the findings of the aforementioned tests and significance analysis, the amount of heavy metal Cr in the root of spinach planted in soil is lowest when biogas fertilizer + calcium magnesium phosphate fertilizer is used.

The content of heavy metal Cr in spinach stems after harvest of the four treatments with biogas fertilizer or biogas fertilizer + passivator is lower than that of the CK group, with a decrease range of 9.3 percent to 74.65 percent, indicating that the application of biogas fertilizer or biogas fertilizer + passivator reduces the content of heavy metal Cr in spinach stems. The use of biogas fertilizer or biogas fertilizer + passivator had a significant impact on the content of heavy metal Cr in the stems of spinach planted in soil, according to the findings [18].

The content of heavy metal Cr in the stem of spinach treated with biogas fertilizer was 0.040mg/kg lower than that in the CK group, indicating that biogas fertilizer reduced the content of heavy metal Cr in the stem of spinach planted in soil; among them, the three treatment groups with biogas fertilizer + passivator decreased by 0.176mg/kg 0.281mg/kg compared to the ZF group, indicating that biogas fertilizer + passivator decreased the content of The heavy metal Cr content in spinach stems ranging from small to large was zd3 ZD2 zd1 ZF CK in these five treatments.

The content of heavy metal Cr in spinach stem treated with biogas fertilizer or biogas fertilizer + passivator was significantly higher than that in the CK group, according to LSD multiple comparative analysis; the content of heavy metal Cr in spinach stem treated with fertilization + passivator was significantly higher than that treated with biogas fertilizer; and the content of heavy metal Cr in spinach stem treated with fertilization + passivator was significantly higher than that treated with biogas fertilizer. The content of heavy metal Cr in spinach stems from the zd3 group (biogas fertilizer + calcium magnesium phosphate fertilizer) was significantly higher than that of the zd1 group (biogas fertilizer + zeolite) and the ZD2 group (biogas fertilizer + red mud); the content of heavy metal Cr in spinach stems from the ZD2 group (biogas fertilizer + red mud) was significantly higher than that of the zd1 group According to the findings of the aforementioned tests and significance analysis, the concentration of heavy metal Cr in the stem of spinach planted in soil is lowest when biogas fertilizer + calcium magnesium phosphate fertilizer is used.

Table 3 shows that the content of heavy metal Cr in spinach leaves after harvest of the four treatments with biogas fertilizer or biogas fertilizer + passivator is lower than that in the CK group, with a decrease range of 9.30 percent to 74.65 percent, indicating that the application of biogas fertilizer or biogas fertilizer + passivator reduces the content of heavy metal Cr in spinach leaves. The use of biogas fertilizer or biogas fertilizer plus passivator had a significant effect on the content of heavy metal Cr in spinach leaves planted in soil, according to the findings.

The content of heavy metal Cr in spinach leaves treated with biogas fertilizer was 0.128mg/kg lower than that in the CK group, indicating that biogas fertilizer reduced the content of heavy metal Cr in spinach leaves; the three treatment groups with biogas fertilizer + passivator decreased by 0.176mg/kg-0.281mg/kg when compared to the ZF group, indicating that biogas fertilizer + passivator reduced the content of heavy metal Cr in t Heavy metal Cr content in spinach leaves from small to large in these five treatments was ZD: ZD2 zd1 ZF CK The level of heavy metal Cr in spinach leaves treated with biogas fertilizer or biogas fertilizer + passivator was substantially greater than in the CK group, according to the findings.

The heavy metal Cr content of spinach leaves treated with biogas fertilizer and passivator was substantially greater than that of spinach leaves treated with biogas fertilizer. The heavy metal Cr content in spinach leaves was substantially greater in the zd3 group (biogas fertilizer + calcium magnesium phosphate fertilizer) than in the zd1 group (biogas fertilizer + zeolite) and D2 group (biogas fertilizer + red mud). Heavy metal Cr levels in spinach leaves were substantially higher in the ZD2 group (biogas fertilizer + red mud) than in the zd1 group (biogas fertilizer + zeolite). According to the findings of the aforementioned tests and significance analysis, the amount of heavy metal Cr in spinach leaves planted in soil is lowest when biogas fertilizer + calcium magnesium phosphate fertilizer is used [19, 20].

Acknowledgments

This study was supported by Jiangsu Innovation and entrepreneurship project of college students No. 202211049134H and 202211049184XJ.

References

- [1] Dalvi AA, Bhalerao SA. Response of plants towards heavy metal toxicity: an overview of avoidance, tolerance and uptake mechanism. *Annals of Plant Sciences*. 2013, 2: 362-368.
- [2] Peer WA, Baxter IR, Richards EL, et al. Phytoremediation and hyperaccumulator plants, in *Molecular Biology of Metal Homeostasis and Detoxification*, (Berlin: Springer), 2005, 299-340.
- [3] Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals-concepts and applications. *Chemosphere*. 2013, 91: 869-881.
- [4] Tong YP, Kneer R, Zhu YG. Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. *Trends in Plant Science*. 2004, 9: 7-9.
- [5] DalCorso G, Fasani E, Manara A, et al. Heavy metal pollutions: state of the art and innovation in phytoremediation. *International Journal of Molecular Sciences*. 2019, 20:3412.
- [6] Assunção A, Martins PDC, De Folter S, et al. Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant Cell Environment*. 2001, 24: 217-226.
- [7] Axelsen KB, Palmgren MG. Inventory of the superfamily of P-type ion pumps in Arabidopsis. *Plant Physiology*. 2001, 126: 696-706.
- [8] Williams LE., Mills RF. P1B-ATPases—an ancient family of transition metal pumps with diverse functions in plants. *Trends of Plant Science*. 2005, 10: 491-502.
- [9] Desbrosses-Fonrouge AG, Voigt K, Schröder A, et al. Arabidopsis thaliana MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Letters*. 2005, 579: 4165-4174.
- [10] Supek F, Supekova L, Nelson H, et al. Function of metalion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. *Journal of Experimental Biology*. 1997, 200: 321-330.
- [11] Bastow EL, Garcia De La Torre VS, Maclean AE, et al. Vacuolar iron stores gated by NRAMP3 and NRAMP4 are the primary source of iron in germinating seeds. *Plant Physiology*. 2018, 177: 1267-1276.

- [12] Krämer U, Cotter-Howells JD, Charnock JM, et al. Free histidine as a metal chelator in plants that accumulate nickel. *Nature*. 1996, 379: 635–638.
- [13] Ma LO, Rao G. Chemical fractionation of cadmium, copper, nickel and zinc contaminated soil. *Journal of Environmental Quality*. 1997, 26: 259-264.
- [14] Rufus LC. Food Safety Issues for Mineral and Organic Fertilizer. *Advances in Agronomy*. 2012, 17: 51-116.
- [15] Sharma A, Nagpal AK. Soil amendments: a tool to reduce heavy metal uptake in crops for production of safe food [J]. *Reviews in Environmental Science and Bio-Technology*, 2018, 17: 187-203.
- [16] Rajaganapathy V, Xavier F, Sreekumar D. Heavy Metal Contamination in Soil, Water and Fodder and their Presence in Livestock and Products. *Journal of Environmental Science and Technology*. 2011, 4 (3): 234-249.
- [17] Larison JR, Likens GE, Fitzpatrick JW, et al. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature: International weekly journal of science*. 2000, 406(6792): 181-183.
- [18] Svensson LM, Christensson K, Björnsson L. Biogas production from crop residues on a farm-scale level in Sweden: scale, choice of substrate and utilisation rate most important Parameters for financial feasibility. *Bioprocess and Biosystems Engineering*. 2006, 29 (7): 137-142.
- [19] Rauret G, Rubio R, LOPEZ-SÁNCHEZ JF. Optimization of Tessier procedure for metal solid speciation in river sediments. *Trends in Analytical Chemistry*. 1989, 36: 69-83.
- [20] Mathieu R, Sónia C, Paula A, et al. Organic wastes as soil amendments - Effects assessment towards soil invertebrates. *Journal of Hazardous Materials*. 2017, 330 (5): 149-156.