STUDY ON THE CADMIUM TOLERANCE MECHANISM OF *VALLISNERIA NATANS*

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(Received 13th Oct 2025; accepted 23rd Dec 2025)

Abstract. *Vallisneria natans* is an ideal species to restore Cd-polluted waters due to its fast growth. To understand its tolerance mechanism, the Cd stress responses and subcellular distribution were investigated in this study. *V. natans* was cultured hydroponically with Cd concentrations of 0.0, 0.3, 0.6, 0.9,1.2 and 1.5 mg/ L for 21 days. The results showed that the total chlorophyll (Ct), chlorophyll a (Ca), chlorophyll b (Cb), carotenoids (Cc), *Fv/Fm*, *Fv/Fo*, *Y(II)*, *qP* and photosynthetic electron transport rate (ETR) decreased significantly with increasing Cd concentration, while *qN* increased. Cd severely damaged the photosynthetic mechanism and inhibited electron transfer efficiency. Malondialdehyde (MDA) increased and catalase (CAT) decreased with increasing Cd concentration, while glutathione (GSH), superoxide dismutase (SOD) and peroxidase (POD) first increased and then decreased. *V. natans* alleviated oxidative damage by increasing antioxidant enzyme activity to remove excess H_2O_2 in cells. Proline, soluble sugar, and soluble protein were all higher than the control. Cd was mainly localized in the cell wall fraction (31%-50%) and the soluble fraction (37%-53%). *V. natans* responded to Cd stress through photosynthetic adaptation, antioxidant defense, osmotic regulation, and subcellular compartmentalization, thus it can be used for the phytoremediation of Cd polluted water.

Keywords: *Cd stress, Vallisneria natans, photosynthetic property, antioxidant system, osmoregulation, subcellular distribution*

Introduction

Water Cd pollution from metal mining is a common environmental problem in southern China (Ji et al., 2018). Many submerged macrophytes can accumulate heavy metals from water and sediment, and different species exhibit varying tolerances to these metals (Upadhyay et al., 2014). The high biotoxicity of Cd causes severe damage to plant nuclei, chloroplasts, and endoplasmic reticulum, thereby preventing normal development and growth (Yang et al., 2018). Current research on submerged macrophytes focuses on phytoaccumulation of heavy metals, physiological and biochemical effects, and ultrastructural changes (Ferreira et al., 2015; Borisova et al., 2017; Liu et al., 2017).

Vallisneria natans, a perennial submerged macrophyte, is distributed in lakes in southern China, and can serve as a pioneer species for aquatic ecological reconstruction (Gao et al., 2022). The dynamic changes and interactions within the photosynthetic system of *V. natans* under Cd stress have not been fully elucidated. The synergistic regulation between its antioxidant defense system and osmoregulation system requires further investigation. This study systematically analyzed the changes in photosynthetic pigment content, chlorophyll fluorescence characteristics, antioxidant substance levels, osmoregulatory substance content, and the subcellular distribution of Cd in *V. natans*. It aimed to provide a comprehensive understanding of the tolerance mechanism of *V. natans* to Cd stress.

DOI: http://dx.doi.org/10.15666/aeer/2402_25232533

Materials and methods

Experimental design

V. natans and bottom mud were collected from unpolluted waters in Nanchang, China. All macrophytes were trimmed to 15 cm in leaf length and 3 cm in root length, rinsed gently to remove surface attachments, and planted in plastic boxes (21 cm×12 cm×7 cm) with a mud thickness of 5 cm. They were pre-cultured with tap water in glass tanks for two weeks. Then different concentrations of $CdCl_2$ solution (0.3 mg/L, 0.6 mg/L, 0.9 mg/L, 1.2 mg/L and 1.5 mg/L) were applied for a 21-days stress treatment. These concentrations were selected based on previous study, inducing observable physiological and biochemical changes without causing acute toxicity (Singh et al., 2010). Each treatment was conducted with three replicates. Six plants were cultured in each glass tank (three for physiological and biochemical test and three for subcellular distribution).

Measuring method

The photosynthetic pigment was determined using ethanol acetone method (Gao et al., 2019a). The measurement method of leaf chlorophyll fluorescence characteristics was included in the literature (Gao et al., 2019b).

SOD, POD, CAT, MDA and GSH were determined using the nitrogen blue tetrazolium colorimetric method, the guaiacol colorimetric method, the thiobarbituric acid colorimetric method, the ultraviolet spectrophotometry and the thiol colorimetric method, respectively (Anderson, 1985; Chen et al., 2014).

The proline content, soluble sugar content and soluble protein content were determined using the acidic ninhydrin colorimetric method, anthrone colorimetric method, and the Coomassie Brilliant Blue staining method, respectively (Ge et al., 2017; García et al., 2022). Subcellular distribution was described by Weigel and Jäger (1980).

Statistical analysis

All data were expressed as mean ± standard error. The significant differences in different treatment groups were determined by one-way analysis of variance. Duncan's post hoc test was used for multiple comparisons. Statistical analyses were performed using SPSS 21.0, diagrams were made using Origin 2022.

Result and discussion

Effects of Cd stress on plant growth parameters

No significant differences were detected between low-moderate Cd concentration (0.3-0.6 mg/L) and the control group (CK) in the growth parameters of *V. natans* (*Table 1*). When Cd concentration was 0.9 mg/L, the plant height decreased 5.7% compared with the CK. The fresh weight significantly decreased only at 1.5 mg/L Cd. The dry weight and root length significantly decreased at 0.9 mg/L Cd.

Effects of Cd stress on the photosynthetic system

The total chlorophyll (Ct), chlorophyll a (Ca), chlorophyll b (Cb), and carotenoids (Cc) significantly decreased with increasing Cd stress (*Table 2*). Under 1.5 mg/L Cd treatment, they were only 30.0%, 41.3%, 43.6%, and 34.4% of the CK, respectively. The decline in photosynthetic pigment content directly impaired the ability of *V. natans* to capture and convert light energy, thereby inhibiting photosynthesis.

Table 1. *Effects of Cd stress on the growth parameters of V. natans*

Concentration (mg/L)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Root length (cm)
0	25.31±1.22^{a}	2.43±0.33^{a}	0.43±0.04^{a}	7.53±0.58^{a}
0.3	24.83±1.92^{ab}	2.36±0.53^{a}	0.41±0.02^{ab}	7.26±0.43^{a}
0.6	24.56±0.84^{ab}	2.28±0.26^{a}	0.39±0.03^{bc}	7.33±0.38^{a}
0.9	23.87±0.91^{b}	2.31±0.24^{a}	0.39±0.02^{bc}	6.71±0.36^{b}
1.2	22.69±0.64^{c}	2.20±0.36^{a}	0.35±0.01^{c}	6.45±0.21^{b}
1.5	16.26±0.58^{d}	1.63±0.15^{b}	0.26±0.01^{d}	5.26±0.27^{c}

Note: Different lowercase letters in each column indicate significant difference ($p < 0.05$) in growth parameters

Table 2. *Effects Cd stress on the photosynthetic pigment of V. natans*

Concentration (mg/L)	Ca (mg/g FW)	Cb (mg/g FW)	Cc (mg/g FW)	Ct (mg/g FW)
0	1.496±0.062^{f}	0.936±0.023^{a}	0.225±0.014^{a}	2.433±0.026^{a}
0.3	1.238±0.023^{e}	0.792±0.022^{b}	0.199±0.023^{a}	2.031±0.024^{b}
0.6	1.057±0.031^{d}	0.697±0.044^{c}	0.171±0.019^{b}	1.755±0.031^{c}
0.9	0.891±0.019^{c}	0.516±0.037^{d}	0.146±0.014b^{c}	1.407±0.031^{d}
1.2	0.795±0.022^{b}	0.479±0.026^{e}	0.125±0.016^{c}	1.274±0.027^{e}
1.5	0.448±0.016^{a}	0.387±0.018^{f}	0.098±0.031^{d}	0.836±0.033^{f}

Fv/Fm and *Fv/Fo* significantly decreased with increasing Cd stress. The fluctuation amplitude of *Fv/Fo* was larger than that of *Fv/Fm*. Under 1.5 mg/L Cd exposure, *Fv/Fm* and *Fv/Fo* decreased by 35.21% and 65.57%, respectively, compared to the CK (*Fig. 1*). The significant decrease indicated an obstruction in photosynthetic pigment synthesis or damage to the PSII structure, which was consistent with the effects of Pb stress on *V. natans* (Gao et al., 2019c).

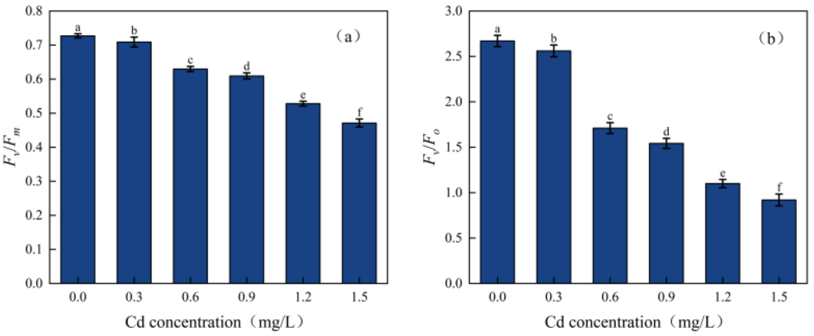

Figure 1. *Effects of Cd stress on Fv/Fm and Fv/Fo. Note: Different lowercase letters indicate significant differences (p <0.05) among different treatments. The same below*

Y(II) significantly decreased with increasing Cd stress. It indicated that Cd damaged the photosynthetic electron transport chain of PSII and inhibited the efficient conversion of light energy to chemical energy. Consequently, the actual photosynthetic efficiency of

V. natans was reduced, which might inhibit the entire photosynthetic process and impair material synthesis and energy metabolism. *Y(NO)* increased with increasing Cd stress. When photosynthetic electron transport was inhibited, excess excitation energy could not be dissipated through normal photosynthetic pathways. This lead to the accumulation of harmful substances, such as reactive oxygen species (ROS), consequently inducing photodamage. The rise in *Y(NO)* was precisely a manifestation of this aggravated photodamage. *Y(NPQ)* increased with increasing Cd stress, but no significant further increase was observed after the Cd concentration reached 1.2 mg/L (*Fig. 2*). This indicated that the intensity of Cd stress had exceeded the regulatory capacity of *V. natans'* photoprotective mechanism.

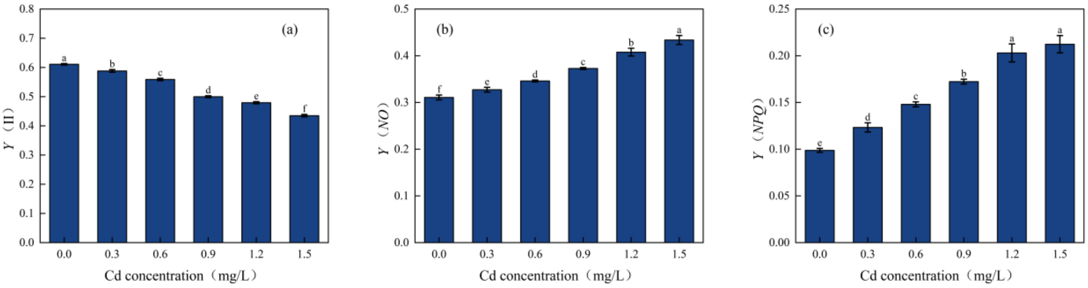

Figure 2. *Effects of Cd stress on Y(II), Y(NO)and Y(NPQ)*

As shown in *Fig. 3*, *qP* decreased with increasing Cd stress, and was 57.21% lower than the CK when the Cd concentration reached 1.5 mg/L. This indicated that high concentrations of Cd inhibited the activity of the photosynthetic reaction centers, thereby reducing the proportion of light energy used for photochemical reactions and severely suppressing photosynthetic activity. Consequently, the efficiency of subsequent processes in photosynthesis, such as carbon assimilation, was impaired, ultimately hindering the material synthesis, growth, and development of *V. natans*. *qN* showed an opposite trend. It reached its maximum at a Cd concentration of 1.5 mg/L, which was 2.12 times that of the CK. This indicated that as Cd stress intensified, the portion of light energy received by *V. natans* that could not be used for normal photochemical reactions increased with increasing Cd stress. *V. natans* dissipated the excess light energy as heat by enhancing enhanced non-photochemical quenching (NPQ) to alleviate the photodamage to PSII (Gao et al., 2019b).

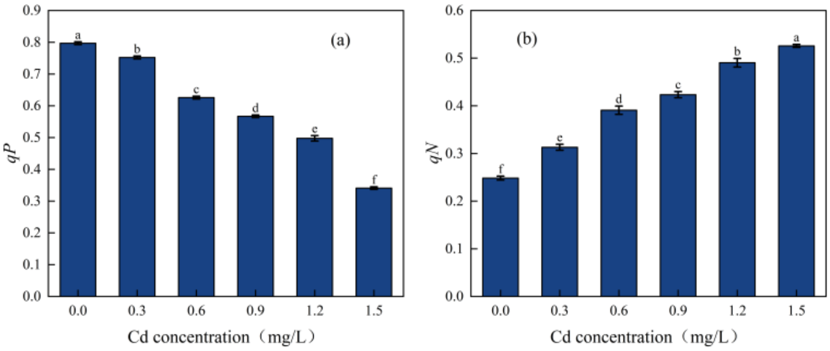

Figure 3. *Effects of Cd stress on qP and qN*

The relative electron transfer rate (ETR) of *V. natans* initially increased rapidly and then declined gradually with increasing photosynthetic active radiation (PAR). ETR reached its maximum at a PAR of 200 $\mu mol\cdot m^{-2}\cdot s^{-1}$. When Cd concentration was 1.5 mg/L, ETR reached a peak of 9.78 $\mu mol\cdot m^{-2}\cdot s^{-1}$, indicating a significant inhibition of electron transport in *V. natans* leaves (*Fig. 4*). Inhibition of electron transport lead to reduce synthesis of ATP and NADPH, thereby curbing CO_2 fixation in the Calvin cycle and resulting in a decreased photosynthetic rate (Xue et al., 2018).

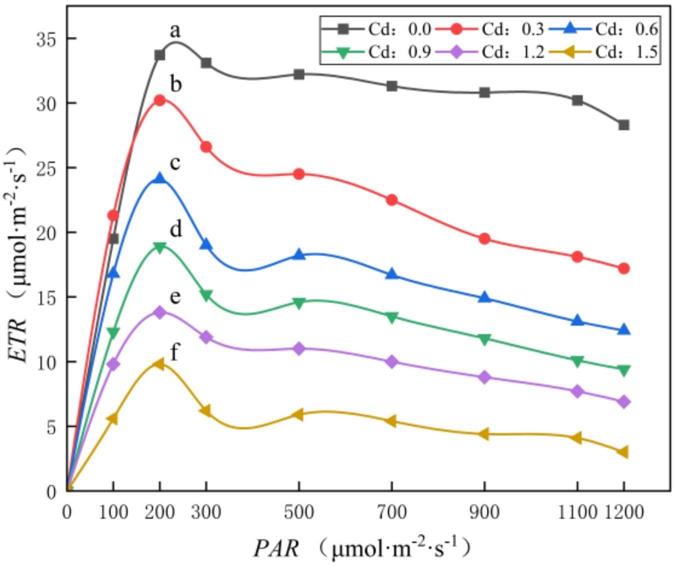

Figure 4. *Effects of Cd stress on the rapid light curves*

Effects of Cd stress on antioxidant system

MDA significantly increased with increasing Cd stress. Under 1.5 mg/L Cd stress, it reached 3.12 times that of the CK (*Fig. 5*). MDA is a terminal product of lipid peroxidation which reflects the degree of cellular oxidative damage (Deng et al., 2014).

After entering *V. natans* cells, Cd induced oxidative stress through disruption of the electron transport chain and excessive ROS generation. When the ROS production rate exceeded the scavenging capacity of the plant's antioxidant system, it attacked unsaturated fatty acids in the cell membrane, thereby initiating lipid peroxidation.

GSH first increased and then decreased. It reached peak at approximately 164.3% of the CK under 1.2 mg/L Cd (*Fig. 5*). Under low-concentration Cd stress, *V. natans* enhanced its antioxidant capacity by inducing elevated GSH to mitigate Cd-induced oxidative stress, which was an adaptive defense mechanism against heavy metal stress. When Cd concentration further increased to a certain threshold (＞1.2 mg/L), its toxicity surpassed *V. natans*' compensatory capacity to counteract stress by increasing GSH synthesis. This might inhibit the activity of enzymes involved in GSH synthesis or accelerate GSH consumption. GSH still exceeded the CK at 1.5 mg/L Cd. It indicated that *V. natans*' antioxidant system remained active. Chronic high-concentration Cd exposure was likely to cause more severe physiological metabolic damage.

DOI: http://dx.doi.org/10.15666/aeer/2402_25232533

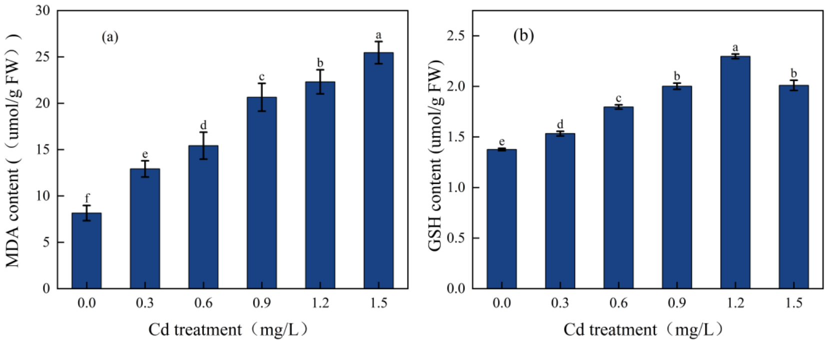

Figure 5. *Effects of Cd stress on MDA and GSH*

SOD, acting as the first line of defense in the antioxidant system, is responsible for dismutating·O_2^- into H_2O_2 and O_2. In this study, it showed a trend of first increasing and then decreasing. It peaked at 1.61 times the CK at a Cd concentration of 0.9 mg/L (*Fig. 6*), indicating that low to medium concentrations of Cd could induce SOD synthesis to enhance ROS scavenging capacity (Bouchama et al., 2023).

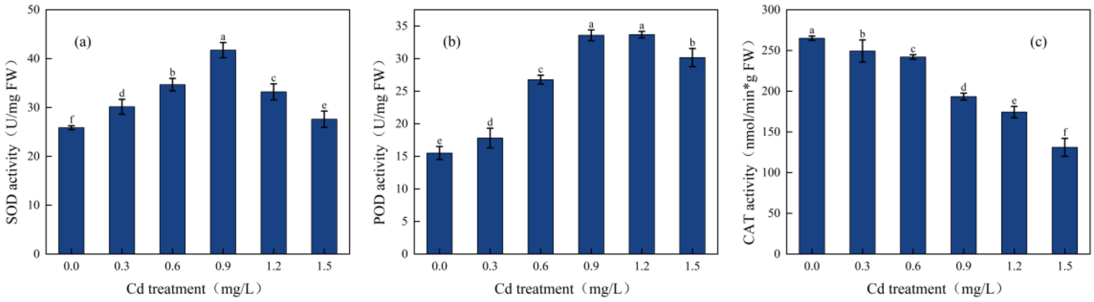

Figure 6. *Effects of Cd stress on SOD, POD and CAT*

Under high Cd stress (1.5 mg/L), SOD decreased to a level close to that of the CK. This could be attributed to several factors: the high Cd concentration may disrupt the spatial structure of SOD and inhibit its catalytic activity; alternatively, the excessive accumulation of ROS might exceed the scavenging capacity of SOD, leading to the oxidative degradation of the enzyme; Cd may suppress the expression of genes related to SOD synthesis (Zulfiqar et al., 2022).

POD showed a similar trend to SOD, peaking at 2.17 times of the CK at 0.9-1.2 mg/L Cd. It can catalyze the oxidation of various substrates using H_2O_2. It may be related to the demand for H_2O_2 scavenging. When SOD declines, POD may partially compensate for its function, thereby reducing H_2O_2 accumulation. POD also participates in physiological processes such as lignin synthesis, and its higher activity under high concentrations may be related to cell wall reinforcement, indirectly enhancing its ability to retain Cd (Zou et al., 2018).

CAT significantly decreased with increasing Cd stress, and was only 49% of the CK at 1.5 mg/L Cd. CAT, which is primarily located in peroxisomes and catalyzes the rapid removal of H_2O_2, may show higher sensitivity to Cd stress compared to SOD and POD. The sensitivity of CAT to heavy metals may be related to the susceptibility of its heme cofactor to Cd damage, or the inhibition of CAT gene expression by Cd to reduce its synthesis (Molina et al., 2008).

Effects Cd stress on osmoregulatory substance

Proline, soluble sugar, and soluble protein all first increased and then decreased (*Fig. 7*). After entering the cell, Cd may disrupt the selective permeability of the cell membrane, leading to intracellular water loss. Meanwhile, the substantial accumulation of proline can help maintain osmotic balance by increasing cytoplasmic concentration, thereby reducing the damage to cellular structure (Wang et al., 2021). The protective effect of proline on enzyme molecules and biomembranes can, to some extent, alleviate Cd-induced protein conformational damage and membrane lipid peroxidation to a certain degree. Proline decreased at 1.5 mg/L Cd, but its was still significantly higher than the CK. This may be because the high concentration of Cd inhibits the activity of enzymes involved in proline synthesis, or the rate of proline consumption exceeds its synthesis rate. At this point, the osmotic adjustment capacity of *V. natans* has begun to decline, yet it still relied on the remaining proline to maintain basic physiological function.

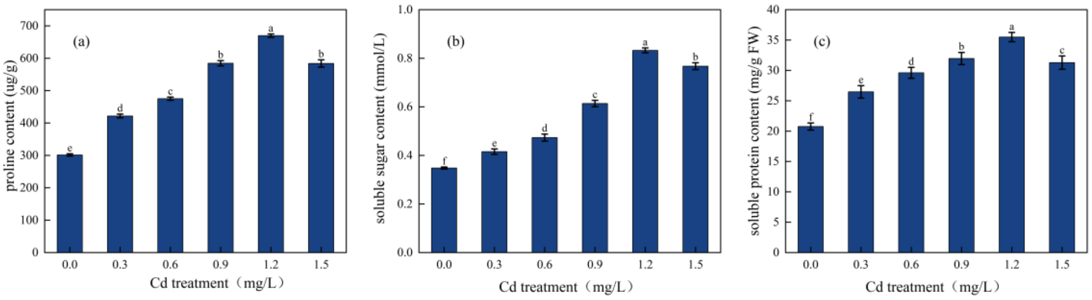

Figure 7. *Effects of Cd stress on proline, solube sugar and solube protein*

Soluble sugar and soluble protein both peaked at 1.2 mg/L Cd, reaching 2.39 times and 1.71 times of the CK, respectively, which indicated that both the osmotic adjustment and antioxidant defense systems in *V. natans* were activated fully at this concentration (Ge et al., 2017). High Cd concentrations likely diminish the supply for soluble sugar synthesis by impairing photosynthesis and respiration. The disruption of ribosomal structure and activation of proteases by heavy metals may lead to both the inhibition of soluble protein synthesis and the acceleration of its degradation. The decrease in soluble sugars and proteins at 1.5 mg/L Cd revealed the limitations of the adaptation mechanisms in *V. natans*. It indicated that the osmotic regulation and defense systems were unable to counter effectively the intense stress, and irreversible damage to cellular structures and metabolic functions began to occur.

Subcellular distribution of Cd in V. natans leaves

Cd was localized primarily in the soluble fraction and the cell wall fraction, which together accounted for over 80% of the total (*Fig. 8*). The proportion of Cd in the cell wall fraction increased significantly with increasing Cd concentration. It accounted for 31% at 0.3 mg/L Cd, which increased to 50% at 1.5 mg/L Cd. The proportion of Cd in the soluble fraction exceeded 50% at Cd concentrations (0.3-0.6 mg/L), but it decreased to 37% at 1.5 mg/L Cd. The proportion of Cd in organelles remained consistently low and showed a decreasing trend with increasing stress.

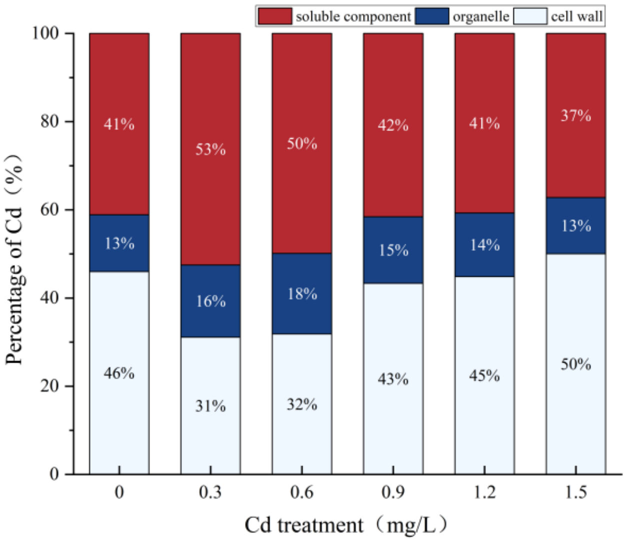

Figure 8. *Subcellular distribution of Cd*

The cell wall’s adsorption of heavy metals mainly depends on functional groups in their components, such as carboxyl and hydroxyl in cellulose, hemicellulose, and pectin. These groups can bind with Cd through ion exchange and complexation, thereby reducing its transport into the cell (Ramos et al., 2002). Pectin, a major polysaccharide component of the cell wall, possesses free carboxyl groups with the strongest affinity for heavy metal ions, serving as the key binding site for Cd immobilization (Hao et al., 2015). Under high-concentration Cd stress, the proportion of Cd in the cell wall increased significantly. This may be related to the increased pectin content or enhanced exposure of pectin carboxyl groups in *V. natans*. This adaptive adjustment can effectively reduce the concentration of free Cd in the cytoplasm, thereby alleviating damage to organelles. When the Cd concentration exceeds the plant’s self-regulatory capacity, the binding sites on the cell wall may become saturated, leading to more5 Cd entering the cell interior.

In the soluble fraction, Cd exists primarily in chelated form. It binds to small organic molecules (e.g., organic acids, amino acids) or macromolecular chelators (e.g., phytochelatins, metallothioneins) in the cytoplasm, thereby reducing the toxicity of free Cd ions (Fu et al., 2011). Cd stress can induce the synthesis of phytochelatins (PCs) in plants, and the PCs content shows a positive correlation with the amount of Cd accumulation (Sylwia et al., 2010). The high proportion of Cd under low Cd stress may be related to the fixation of Cd in the cytoplasm by the synthesis of chelating agents (such as PCs). The decreased proportion under high Cd stress may be due to the enhanced sequestration by the cell wall, which reduces the total amount of Cd entering the cytoplasm, or high concentration of Cd inhibits the synthesis ability of PCs. Cd in soluble components may be isolated through vacuolar compartmentalization (Wang et al., 2022).

As the core sites of cellular metabolism, organelles like chloroplasts and mitochondria are particularly sensitive to heavy metals. Substantial Cd influxing into organelles can impair normal cellular function by disrupting thylakoid membranes, inhibiting enzymatic reactions, and interfering with energy metabolism.

DOI: http://dx.doi.org/10.15666/aeer/2402_25232533

In this study, the low level and declining trend of the Cd proportion in organelles indicated that *V. natans* effectively reduces Cd allocation to organelles through cell wall retention and cytoplasmic chelation. This is a crucial safeguard for maintaining its basic physiological functions. This result was consistent with studies on terrestrial plants such as rice and wheat (Li et al., 2011; Liu et al., 2014). This result was consistent with studies on terrestrial plants such as rice and wheat, and confirmed a widespread protective mechanism in plants that reduced the allocation of heavy metals to organelles. Given their aquatic habitat with more direct Cd exposure, the low Cd accumulation in organelles of submerged plants may represent a long-term adaptation to the aquatic environment.

Conclusions

By elevating antioxidant enzyme activities and GSH content, *V. natans* enhanced the scavenging capacity for intracellular excess H_2O_2, thus mitigating oxidative damage. When the Cd concentration exceeded 1.2 mg/L, the antioxidant defense capacity gradually declined, the content of osmotic adjustment substances decreased, and the photosynthetic system was significantly impaired. The majority of Cd was in the cell wall and soluble fractions. The proportion in the cell wall increased with increasing Cd stress. This may represent a crucial detoxification strategy in *V. natans* to reduce Cd bioavailability by cell wall retention. *V. natans* exhibited good tolerance to Cd stress. It can be used for ecological restoration of Cd polluted water.

Acknowledgements. This study was supported by Jiangxi Provincial Technology Department (20232BAB215011, 20242BAB25200), Graduate Innovation Special Fund Project of Jiangxi Province (YC2024-S877).

REFERENCES

[1] Anderson, M. E. (1985): Determination of glutathione and glutathione disulfide in biological samples. – Methods in Enzymology 113: 548-555. https://doi.org/10.1016/S0076-6879(85)13073-9.

[2] Borisova, G. G., Chukina, N. V., Maleva, M. G., Levchenko, U. A. (2017): Accumulation of heavy metals in leaves of submerged hydrophytes (*Elodea Canadensis Michx*. and *Potamogeton perfoliatus* L.) and their responses to the effect of the wastewater of a metallurgical plant. – Inland Water Biology 10(2): 176-181. https://doi.org/ 10.1134/S1995082917020031.

[3] Bouchama, K., Rouabhi, R., Djebar, M. R. (2023): Cadmium phytotoxicity on seed germination, early growth and the differential antioxidant response of *Guaiacol Peroxidasein Phragmites australis* seedling srgans. – Polish Journal of Environmental Studies 32(2): 1531-1544. https://doi.org/10.15244/pjoes/157492.

[4] Chen, Y. H., Liu, X. Y., Wang, M. X., Wang, J., Yan, X. (2014): Cadmium tolerance, accumulation and relationship with Cd subcellular distribution in *Ricinus communis* L. – Acta Sci. Circumstantiae 34(9): 2440-2446. https://doi.org/10.13671//j.hjkxxb.2014.0545.

[5] Deng, G., Li, M., Li, H., Yin, L., Li, W. (2014): Exposure to cadmium causes declines in growth and photosynthesis in the endangered aquatic fern (*Ceratopteris pteridoides*). – Aquatic Botany 112: 23-32. https://doi.org/10.1016/j.aquabot.2013.07.003.

[6] Ferreira, C., Horta, P. A., Almeida, G. M., Zitta, C. S., Oliveira, E. M., Gueye, M. B., Rodrigues, A. C. (2015): Anatomical and ultrastructural adaptations of seagrass leaves: an

evaluation of the southern Atlantic groups. – Protoplasma 252: 3-20. https://doi.org/10.1007/s00709-014-0661-9.

[7] Fu, X., Dou, C., Chen, Y., Chen, X., Shi, J., Yu, M., Xu, J. (2011): Subcellular distribution and chemical forms of cadmium in *Phytolacca americana* L. – Journal of Hazardous Materials 186(1): 103-107. https://doi.org/10.1016/j.jhazmat.2010.10.122.

[8] Gao, G. Q., Jian, M. F., Lu, L., Ji, Y., Wang, X. L., Wang, Y. (2019a): Effects of Cu^{2+} or Cd^{2+} stress on photosynthetic pigment and photosynthetic fluorescence characteristics of *Potamogeton malaianus*. – Chinese Journal of Applied and Environmental Biology 25(3): 517-523. https://doi.org/0.19675/j.cnki.1006-687x.2018.10003.

[9] Gao, G. Q., Wang, X. L., Lu, L., Ji, Y., Li, W., Xiao, L. Y., Wang, Y., Xia, Z. Y. (2019b): Effects of water Cu pollution on growth and chlorophyll fluorescence characteristics of *Vallisneria natans*. – Guihaia 39(2): 209-217. https://doi.org/10.11931/guihaia.gxzw 201806043.

[10] Gao, G. Q., Zeng, K. H., Ji, Y., Li, W., Wang, Y. (2019c): Effects of lead stress on the chlorophyll content and photosynthetic fluorescence characteristics of *Vallisneria natans*. – Applied Ecology Environment Research 17(2): 4171-4181. https://dx.doi.org/10.15666/aeer/1702_41714181.

[11] Gao, G. Q., Yang, W. H., Zeng, K. H., Hu, L., Wang, X. L., Liu, Z. M. (2022): Effects of single and combined of Cu or Zn on the photosynthetic fluorescence characteristics of *Vallisneria natans*. – Applied Ecology Environment Research 20(5): 3927-3938. https://doi.org/10.15666/aeer/2005_39273938.

[12] García de la Torre, V. S., Coba de la Peña, T., Lucas, M. M., Pueyo, J. J. (2022): Transgenic medicago truncatula plants that accumulate proline display enhanced tolerance to cadmium stress. – Frontiers in Plant Science 13: 829069. https://doi.org/ 10.3389/fpls.2022.829069.

[13] Ge, H., Liu, Z., Zhang, F. (2017): Effect of *Rhodopseudomonas palustris* G5 on seedling growth and some physiological and biochemical characteristics of cucumber under cadmium stress. – Emirates Journal of Food and Agriculture 29(11). https://doi.org/10.9755/ejfa.2017.v29.i11.1327.

[14] Hao, X., Li, T., Yu, H., Zhang, X., Zheng, Z., Chen, G., Zhang, S., Zhao, L., Pu, Y. (2015): Cd accumulation and subcellular distribution in two ecotypes of *Kyllinga brevifolia* Rottb as affected by Cd treatments. – Environmental Science and Pollution Research 22(10): 7461-7469. https://doi.org/10.1007/s11356-015-4379-9.

[15] Ji, Y., Wu, P. J., Zhang, J., Zhou, Y. F., Zhang, S. F., Cai, G. T., Gao, G. Q. (2018): Heavy metal accumulation, risk assessment and integrated biomarker responses of local vegetables: a case study along the Le’an river. – Chemosphere 199: 361-371. https://doi.org/10.1016/j.chemosphere.2018.02.045.

[16] Li, D. D., Zhou, D. M., Wang, P., Weng, N. Y., Zhu, X. D. (2011): Subcellular Cd distribution and its correlation with antioxidant enzymatic activities in wheat (*Triticum aestivum*) roots. – Ecotoxicology and Environmental Safety 74(4): 874-881. https://doi.org/10.1016/j.ecoenv.2010.12.006.

[17] Liu, J., Qu, P., Zhang, W., Dong, Y., Li, L., Wang, M. (2014): Variations among rice cultivars in subcellular distribution of Cd: The relationship between translocation and grain accumulation. – Environmental and Experimental Botany 107: 25-31. https://doi.org/10.1016/j.envexpbot.2014.05.004.

[18] Liu, H., Cao, Y., Li, W., Zhang, Z., Jeppesen, E., Wang, W. (2017): The effects of cadmium pulse dosing on physiological traits and growth of the submerged macrophyte *Vallisneria spinulosa* and phytoplankton biomass: a mesocosm study. – Environmental Science and Pollution Research 24(18): 15308-15314. https://doi.org/10.1007/s11356-017-9155-6.

[19] Ramos, I., Esteban, E., Lucena, J. J., Gárate, A. (2002): Cadmium uptake and subcellular subcellular distribution in plants of Lactuca sp. Cd–Mn interaction. – Plant Science 162(5): 761-767. https://doi.org/10.1016/S0168-9452(02)00017-1.

[20] Singh, R., Tripathi, R. D., Dwivedi, S., Singh, M., Trivedi, P. K., Chakrabarty, D. (2010): Cadmium-induced biochemical responses of *Vallisneria spiralis*. – Protoplasma 245(1): 97-103. https://doi.org/10.1007/s00709-010-0146-4.

[21] Sylwia, W., Anna, R., Ewa, B., Stephan, C., Danuta Maria, A. (2010): The role of subcellular distribution of cadmium and phytochelatins in the generation of distinct phenotypes of AtPCS1- and CePCS3-expressing tobacco. – Journal of Plant Physiology 167(12): 981-988. https://doi.org/10.1016/j.jplph.2010.02.010.

[22] Upadhyay, A. K., Singh, N. K., Rai, U. N. (2014): Comparative metal accumulation potential of *Potamogeton pectinatus* L. and *Potamogeton crispus* L.: role of enzymatic and non-enzymatic antioxidants in tolerance and detoxification of metals. – Aquatic Botany 117(5): 27-32. https://doi.org/10.1016/j.aquabot.2014.04.003.

[23] Wang, X. L., Zhang, B. J., Wu, D. S., Hu, L., Huang, T., Gao, G. Q., Huang, S., Wu, S. (2021): Chemical forms governing Cd tolerance and detoxification in duckweed (*Landoltia punctata*). – Ecotoxicology and Environmental Safety 207: 111553. https://doi.org/10.1016/j.ecoenv.2020.111553.

[24] Wang, X. L., Hu, L., Wu, D. S., Huang, T., Zhang, B. J., Cai, G. J., Gao, G. Q. (2022): Large-scale screening and characterization of Cd accumulation and ultrastructural deformation in duckweed. – Science of The Total Environment 832: 154948. https://doi.org/10.1016/j.scitotenv.2022.154948.

[25] Weigel, H. J., Jäger, H. J. (1980): Subcellular distribution and chemical form of cadmium in bean plants. – Plant Physiology 65(3): 480-482. https://doi.org/10.1104/pp.65.3.480.

[26] Xue, Z. C., Li, J. H., Li, D. S., Li, S. Z., Jiang, C. D., Liu, L. A., Wang, S. Y., Kang, W. J. (2018): Bioaccumulation and photosynthetic activity response of sweet sorghum seedling (*Sorghum bicolor* L. Moench) to cadmium stress. – Photosynthetica 56(4): 1422-1428. https://10.1007/s11099-018-0835-3.

[27] Yang, L. P., Zhu, J., Wang, P., Zeng, J., Tan, R., Yang, Y. Z., Liu, Z. M. (2018): Effect of Cd on growth, physiological response, Cd subcellular distribution and chemical forms of *Koelreuteria paniculata*. – Ecotoxicology and Environmental Safety 160: 10-18. https://doi.org/10.1016/j.ecoenv.2018.05.026.

[28] Zou, J. H., Shang, X. S., Li, C. H., Ouyang, J., Li, B. B., Liu, X. J. (2018): Effects of cadmium on mineral metabolism and antioxidant enzyme activities in *Salix matsudana Koidz*. – Polish Journal of Environmental Studies 28(2): 989-999. https://doi.org/10.15244/pjoes/81697.

[29] Zulfiqar, U., Ayub, A., Hussain, S., Waraich, E. A., El-Esawi, M. A., Ishfaq, M., Ahmad, M., Ali, N., Maqsood, M. F. (2022): Cadmium toxicity in plants: recent progress on morpho-physiological effects and remediation strategies. – Journal of Soil Science and Plant Nutrition 22(1): 212-269. https://doi.org/10.1007/s42729-021-00645-3.