An amino acid fertilizer improves the emergent accumulator plant *Nasturtium officinale* R. Br. phytoremediation capability for cadmium-contaminated paddy soils

Ran Zhang1, Qin Liu2†, Xiangting Xu1†, Ming’an Liao1, Lijin Lin3, Rongping Hu3, Xian Luo1, Zhihui Wang2, Jin Wang2, Qunxian Deng3, Dong Liang2, Hui Xia2, Xiulan Lv2, Yi Tang2 and Xun Wang2

1College of Horticulture, Sichuan Agricultural University, Chengdu, China, 2Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, China, 3Institute of Sichuan Edible Fungi, Chengdu, China

Cadmium (Cd) contamination of paddy soil affects safe crop production. This study aimed to evaluate the effects of plant biostimulant amino acid fertilizer on the phytoremediation capability of an emergent accumulator plant *Nasturtium officinale* R. Br. for Cd-contaminated paddy soils. A pot study was carried out to study the effects of different concentrations of amino acid fertilizer on the Cd accumulation of *N. officinale* grown in Cd-contaminated paddy soil. The amino acid fertilizer increased the biomass of *N. officinale*. The amino acid fertilizer concentration exhibited a quadratic polynomial regression relationship with the root and shoot biomass. The fertilizer also increased the photosynthetic pigment (chlorophyll and carotenoid) contents, peroxidase (POD; EC 1.11.1.7) activity, and catalase (CAT; EC 1.11.1.6) activity of *N. officinale*, but decreased the soluble protein content and had no significant effect on the superoxide dismutase (SOD; EC 1.15.1.1) activity. Furthermore, the amino acid fertilizer increased the Cd content and Cd extraction of *N. officinale*. The shoot Cd extraction increased by 29.06%, 63.05%, 77.22%, and 17.40% at 1500-, 1200-, 900-, and 600-fold dilutions of the amino acid fertilizer, respectively, compared with the control. Moreover, the amino acid fertilizer concentration also exhibited a quadratic polynomial regression relationship with the root Cd content, shoot Cd content, root Cd extraction, and shoot Cd extraction, respectively. The correlation, grey relational, and path analyses revealed that the root biomass, shoot biomass, chlorophyll content, catalase activity, shoot Cd content, and root Cd extraction were closely associated with the shoot Cd extraction. Therefore, the amino acid fertilizer can promote Cd...
uptake and improve the phytoremediation capability of *N. officinale* to remediate Cd-contaminated paddy soils, and 900-fold dilution is the most suitable concentration.

**KEYWORDS**

amino acid fertilizer, cadmium, *Nasturtium officinale*, phytoremediation, physiology
Materials and methods

Materials

*N. officinale* cuttings (10 cm long shoot tips) were collected from the ditch at the Ya’an Campus of Sichuan Agricultural University (29° 59′ N, 102° 59′ E).

Soil samples were collected from the fields around the Chengdu Campus of Sichuan Agricultural University, (30°42′ N, 103°51′ E). The soil samples had no detectable Cd, and their physicochemical properties (Table 1) were the same as the report of Tang et al. (2022).

The water soluble amino acid fertilizer used in this experiment was produced by Shanxi Kingshine Biotechnology Co., Ltd. (China). It contained the total amino acid (various amino acids; ≥ 100 g L⁻¹) and a mixture of copper + iron + manganese + zinc + boron concentration (≥ 20 g L⁻¹).

Experimental design

The experiment was conducted in a greenhouse at the Chengdu Campus of Sichuan Agricultural University. In August 2020, the soil samples were prepared as described by Lin et al. (2020), and 3.0 kg of the soils were put into each plastic pot and treated with the pure analytical Cd chloride (in the form of CdCl₂·2.5H₂O) to make a solution. The water soluble amino acid fertilizer was sprayed in each pot. Because *N. officinale* grew fast, the water depth in the pots was kept at 2 cm above the soil surface during the first week after planting, and adjusted to 5 cm above the soil surface at the following weeks. One week after planting, the leaves of *N. officinale* with uniform thickness were selected. The lower part of cuttings with 5 cm in length was cut into Cd-contaminated soil, and three cuttings were planted in each pot. Because *N. officinale* grew fast, the water depth in the pots was kept at 2 cm above the soil surface during the first week after planting, and adjusted to 5 cm above the soil surface at the following weeks. One week after planting, the leaves of *N. officinale* seedlings were sprayed with the different concentrations of water soluble amino acid fertilizer (0-, 1500-, 1200-, 900-, and 600-fold dilution) until the solution started dripping from the leaves.

TABLE 1 The physicochemical properties of soil (Tang et al., 2022).

| Soil type     | pH value | Total N content (g kg⁻¹) | Total P content (g kg⁻¹) | Total K content (g kg⁻¹) | Alkali hydrolysis N content (mg kg⁻¹) | Available P content (mg kg⁻¹) | Available K content (mg kg⁻¹) | Total Cd content (mg kg⁻¹) |
|---------------|----------|--------------------------|--------------------------|--------------------------|--------------------------------------|-------------------------------|-----------------------------|-----------------------------|
| Fluvic         | 7.09     | 1.50                     | 0.76                     | 18.02                    | 94.82                                | 63.30                         | 149.59                      | Not detected                |

The root bioconcentration factor (BCF) was estimated as the root Cd content/soil bioavailable Cd concentration; the shoot bioconcentration factor (BCF) was estimated as the shoot Cd content/soil bioavailable Cd concentration; the translocation daily, with water depth being maintained at 5 cm above the soil surface.

Determination of parameters

In October 2020 (thirty days after the first spraying phase), the fourth and fifth mature leaves were collected by cutting with scissors from each *N. officinale*. The leaf samples were then used for the photosynthetic pigments (chlorophyll $a$, chlorophyll $b$, and carotenoid) contents, antioxidant enzymes [peroxidase (POD; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1), and catalase (CAT; EC 1.11.1.6)] activities, and soluble protein content determinations.

The photosynthetic pigments were extracted using the acetone-ethanol extraction method, and their contents were determined according to the methods by Hao et al. (2004). Similarly, the SOD, POD, and CAT activities were determined as described by Lin et al. (2020) and Hao et al. (2004). The Coomassie brilliant blue method was adopted for the soluble protein content determination, as described by Lin et al. (2020) and Hao et al. (2004). The determination method details of photosynthetic pigment content, antioxidant enzyme activity, and soluble protein content were described in Supplementary Material.

Subsequently, whole plants were harvested and treated as described by Lin et al. (2020). Briefly, the plants were dried, and their dry weight biomass was weighed using an electronic balance. The dried plant tissues were then finely ground and digested as described by Lin et al. (2020) for the Cd content determination, using an ICP6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA). Additionally, the soil samples were collected from each pot and pre-treated as described by Lin et al. (2020) for determining the soil pH value and bioavailable Cd concentration. The soil pH value was determined using a pH meter in soil-water solution (soil: water 1:2.5), while the soil bioavailable Cd concentration was determined using an ICP6300 ICP spectrometer after extraction with DTPA-TEA (Bao, 2000). The determination method details of plant Cd content, soil pH value, and soil bioavailable Cd concentration were described in Supplementary Material.

The root bioconcentration factor (BCF) was estimated as the root Cd content/soil bioavailable Cd concentration; the shoot bioconcentration factor (BCF) was estimated as the shoot Cd content/soil bioavailable Cd concentration; the translocation...
factor (TF) was estimated as the shoot Cd content/root Cd content (Rastmanesh et al., 2010), while the Cd extraction was calculated as plant Cd content × plant biomass (Zhang et al., 2010).

**Statistical analysis**

All data were analyzed using SPSS 20.0.0 software (IBM, Chicago, IL, USA). Data were normalized and subjected to a homogeneity test, followed by a one-way analysis of variance and Duncan’s Multiple Range Test (\( P < 0.05 \)). Moreover, the quadratic polynomial regression relationship between amino acid fertilizer concentration and root biomass, shoot biomass, root Cd content, shoot Cd content, root Cd extraction, or shoot Cd extraction was analyzed using regression analysis. Pearson’s correlation was used to determine the relationships among the different indicators. The grey associations of the plant biomass, Cd content, root Cd extraction, photosynthetic pigment content, antioxidant enzyme activity, soluble protein content, soil pH value, and soil bioavailable Cd concentration with the shoot Cd extraction were evaluated via the grey relational analysis, according to Tang and Feng (2006); Wang (2019), and Ma et al. (2022). These associations were also subjected to path analysis, according to Tang and Feng (2006). The details of grey relational and path analyses were described in Supplementary Material.

**Results**

**Biomass of *N. officinale***

Compared with the control, the amino acid fertilizer increased the root biomass at the 1200- and 900-fold dilution, but its concentration at the 1500- and 600-fold dilution had no significant effects (\( P > 0.05 \); Figures 1A, B). The shoot biomass increased by 22.71%, 34.80%, 40.85%, and 13.24%, respectively, at the amino acid fertilizer concentrations of 1500-, 1200-, 900-, and 600-fold dilution, respectively, compared with the control. Moreover, the regression analysis showed that the amino acid fertilizer concentration exhibited a quadratic polynomial regression relationship with both the root biomass (x: amino acid fertilizer concentration; y: root biomass; \( y = -4.043E^{-5}x^2 + 7.573E^{-5}x + 0.103 \), \( R^2 = 0.513 \), \( P = 0.013 \)) and shoot biomass (x: amino acid fertilizer concentration; y: shoot biomass; \( y = -1.841E^{-7}x^2 + 0.596 \), \( R^2 = 0.741 \), \( P = 0.000 \)).

**Photosynthetic pigment content in *N. officinale* leaves**

The chlorophyll (chlorophyll \( a \) and \( b \)) content in *N. officinale* leaves increased with the different concentrations of amino acid fertilizer compared with the control (Figure 2A). The 1500-, 1200-, 900-, and 600-fold dilutions of amino acid fertilizer increased the chlorophyll content by 25.44%, 34.21%, 49.71%, and 12.87%, respectively, compared with the control. Additionally, the carotenoid content in *N. officinale* leaves by 56.49%, 81.47%, and 61.26% at the 1500-, 1200-, and 900-fold dilutions of amino acid fertilizer, respectively, compared with the control. However, the amino acid fertilizer concentration at the 600-fold dilution had no significant effects (\( P > 0.05 \)) on the carotenoid content (Figure 2B).

**Antioxidant enzyme activity and soluble protein content of *N. officinale* leaves**

The POD activity of *N. officinale* leaves increased at the 1200- and 900-fold dilutions of amino acid fertilizer compared with the control, while the 600- and 1500-fold dilutions of amino acid fertilizer had no significant effects (\( P > 0.05 \)) (Figure 3A). Conversely, the different concentrations of amino acid fertilizer

---

**FIGURE 1**

Biomass of *N. officinale*. (A) root biomass; (B) shoot biomass. Values are means (± SD) of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan’s Multiple Range Test, \( P < 0.05 \)). The dashed line represents the curve of quadratic polynomial regression relationship between the amino acid fertilizer concentration and root biomass or shoot biomass.
had no significant effects ($P > 0.05$) on the SOD activity of $N.\ officinale$ leaves (Figure 3B), but increased the CAT activity (Figure 3C). The CAT activity was the highest at the 900-fold dilution of amino acid fertilizer, followed by the 1200-, 1500-, and 600-fold dilutions, while the control had the least CAT activity. The soluble protein content in $N.\ officinale$ leaves decreased at the different dilution folds of amino acid fertilizer, with 900-fold dilution having the least soluble protein content, followed by the 1200-, 1500-, and 600-fold dilutions (Figure 3D).

![Figure 2: Photosynthetic pigment content in $N.\ officinale$ leaves. (A) chlorophyll content; (B) carotenoid content. Values are means ($\pm$ SD) of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan’s Multiple Range Test, $P < 0.05$).](image)

![Figure 3: Antioxidant enzyme activity and soluble protein content of $N.\ officinale$ leaves. (A) POD activity; (B) SOD activity; (C) CAT activity; (D) soluble protein content. Values are means ($\pm$ SD) of three replicates. Different lowercase letters indicate significant differences among the treatments (Duncan’s Multiple Range Test, $P < 0.05$).](image)
Cd content, bioconcentration, and transport of *N. officinale*

The root Cd content in *N. officinale* increased only at 1500-, 1200-, and 900-fold dilutions of amino acid fertilizer compared with the control (Figure 4A). The 1200- and 900-fold dilutions of amino acid fertilizer increased the shoot Cd content by 20.92% and 25.81%, respectively, while the 1500- and 600-fold dilutions had no significant effects (*P* > 0.05; Figure 4B). Furthermore, a quadratic polynomial regression relationship existed between the amino acid fertilizer concentration and both the root Cd content (x: amino acid fertilizer concentration; y: root Cd content; $y = -8.282E^{-7}x^2 + 0.004x + 25.191$, $R^2 = 0.539$, $P = 0.010$) and shoot Cd content (x: amino acid fertilizer concentration; y: shoot Cd content; $y = -6.705E^{-6}x^2 + 0.012x + 27.078$, $R^2 = 0.506$, $P = 0.014$). Moreover, the amino acid fertilizer had no significant effects (*P* > 0.05) on the root BCF of *N. officinale* (Figure 4C). The shoot BCF of *N. officinale* increased at the 1200- and 900-fold dilutions of amino acid fertilizer, while the 1500- and 600-fold dilutions had no significant effects (*P* > 0.05).
significant effects ($P > 0.05$) compared with the control (Figure 4D). The TF of N. officinale was increased at the 1200-, 900-, and 600-fold dilutions of amino acid fertilizer, but decreased at the 1500-fold dilution compared with the control (Figure 4E).

**Cd extraction by N. officinale**

Compared with the control, the 1500-, 1200-, 900-, and 600-fold dilutions of amino acid fertilizer increased the root Cd extraction by 20.62%, 52.89%, 59.85%, and 6.10%, respectively, and also increased the shoot Cd extraction by 29.06%, 63.05%, 77.22%, and 17.40%, respectively (Figures 5A, B). Moreover, a quadratic polynomial regression relationship was observed between the amino acid fertilizer concentration and both the root Cd extraction ($x$: amino acid fertilizer concentration; $y$: root Cd extraction; $y = -1.264E^{-6}x^2 + 0.003x + 2.581$, $R^2 = 0.519$, $P = 0.012$) and shoot Cd extraction ($x$: amino acid fertilizer concentration; $y$: shoot Cd extraction; $y = -1.087E^{-5}x^2 + 0.021x + 15.923$, $R^2 = 0.621$, $P = 0.003$).

**Soil pH value and bioavailable Cd concentration**

The 1500-, 1200-, and 900-fold dilutions of amino acid fertilizer decreased the soil pH value, while the 600-fold dilution had no significant effects ($P > 0.05$) compared with the control (Figure 6A). Conversely, the soil bioavailable Cd concentration increased at the different concentrations of amino acid fertilizer compared with the control (Figure 6B).

**Correlation analysis**

The root Cd content, shoot Cd content, root Cd extraction, and shoot Cd extraction had a significant ($P < 0.01$) positive correlation with the root biomass, shoot biomass, chlorophyll content, carotenoid content, POD activity, CAT activity, and soil bioavailable Cd concentration, while exhibited a significantly ($P < 0.01$) negative correlation with the soluble protein content and soil pH value (Table 2). The shoot Cd content and shoot Cd extraction positively correlated ($0.01 \leq P < 0.05$) with the SOD activity, unlike the root Cd content and root Cd extraction ($P > 0.05$). There was also a positive correlation between the shoot Cd extraction ($P < 0.01$) and the root Cd extraction. The root Cd extraction and shoot Cd extraction were positively correlated ($P < 0.01$) with the root Cd content and shoot Cd content. Contrarily, the soil bioavailable Cd concentration negatively correlated ($P < 0.01$) with the soil pH value.

**Grey relational and path analyses**

The grey relational analysis showed that the shoot Cd extraction correlated with the biomass, plant Cd content, root Cd extraction, photosynthetic pigment content, antioxidant enzyme activity, soluble protein content, soil pH value, and soil bioavailable Cd concentration (Figure 7). Among these indicators, the root biomass, shoot biomass, chlorophyll content, CAT activity, shoot Cd content, and root Cd extraction had grey correlation coefficient values higher than 0.60, with the shoot Cd extraction. Therefore, the root biomass, shoot biomass, chlorophyll content, CAT activity, shoot Cd content, and root Cd extraction were closely associated with the shoot Cd extraction.
The direct path coefficients of the shoot biomass and shoot 
Cd content were higher than 0.45 compared to the path 
coefficient values (absolute) of the other indicators. This 
indicates that the shoot biomass and shoot Cd content directly 
affected the shoot Cd extraction (Figure 8; Table S1). Moreover, 
the indirect path coefficients of root biomass, chlorophyll 
content, CAT activity, and root Cd extraction were higher 
than 0.95, indicating their indirect positive effects on the shoot 
Cd extraction.

**Discussion**

Amino acids contain carbon, nitrogen, and other elements, 
which provide nutrients for plants and serve as the carbon 
skeleton for energy metabolism in the human body (Song 
et al., 2012). The application of amino acids has been reported 
to promote the growth and increase the biomass of lettuce under 
hydroponic conditions (Khan et al., 2019). Similarly, an amino 
acid liquid fertilizer (made of pig hairs) also increased the yield 

| Indicator          | Root biomass | Shoot biomass | Chlorophyll content | Carotenoid content | POD activity | SOD activity | CAT activity | Soluble protein content | Root Cd content | Shoot Cd content | Root Cd extraction | Shoot Cd extraction | Soil bioavailable Cd concentration | Soil pH value |
|--------------------|--------------|---------------|---------------------|-------------------|--------------|--------------|--------------|------------------------|----------------|-------------------|---------------------|---------------------|-------------------------|--------------|
| Root biomass       |              | 0.934**       |                     |                   |              |              |              |                        |                 |                   |                      |                      |                         |              |
| Shoot biomass      |              | 0.897**       |                     |                   |              |              |              |                        |                 |                   |                      |                      |                         |              |
| Chlorophyll content|              | 0.891**       |                     |                   |              |              |              |                        |                 |                   |                      |                      |                         |              |
| Carotenoid content |              | 0.781**       | 0.899**             | 0.884**           |              |              |              |                        |                 |                   |                      |                      |                         |              |
| POD activity       | 0.950**      | 0.887**       | 0.901**             | 0.703**           |              |              |              |                        |                 |                   |                      |                      |                         |              |
| SOD activity       | 0.452        | 0.528*        | 0.488               | 0.411             | 0.41         |              |              |                        |                 |                   |                      |                      |                         |              |
| CAT activity       | 0.893**      | 0.971**       | 0.94**              | 0.928**           | 0.888**      | 0.506        |              |                        |                 |                   |                      |                      |                         |              |
| Soluble protein content | -0.895** | -0.975** | -0.990** | -0.882** | -0.904** | -0.572** | -0.984** |                        |                 |                   |                      |                      |                         |              |
| Root Cd content    | 0.704**      | 0.835**       | 0.874**             | 0.937**           | 0.701**      | 0.453        | 0.923**      | -0.876**               |                 |                   |                      |                      |                         |              |
| Shoot Cd content   | 0.936**      | 0.908**       | 0.919**             | 0.775**           | 0.942**      | 0.543*       | 0.924**      | -0.922**               | -0.817**         |                   |                      |                      |                         |              |
| Root Cd extraction | 0.976**      | 0.962**       | 0.949**             | 0.873**           | 0.944**      | 0.484        | 0.961**      | -0.949**               | 0.839**          | 0.968**          |                      |                      |                         |              |
| Shoot Cd extraction| 0.964**      | 0.977**       | 0.974**             | 0.855**           | 0.944**      | 0.537*       | 0.970**      | -0.972**               | 0.842**          | 0.976**          | 0.989**             |                      |                         |              |
| Soil bioavailable Cd concentration | 0.773** | 0.916** | 0.907** | 0.862** | 0.745** | 0.606* | 0.905** | -0.926** | 0.798** | 0.754** | 0.826** | 0.854** |
| Soil pH value      | -0.859**     | -0.933**      | -0.909**            | -0.941**          | -0.783**     | -0.352       | -0.925**     | 0.897**                | -0.847**         | -0.806**         | -0.905**            | -0.891**            | -0.856**                |              |

N = 15. **: Correlation is significant at the 0.01 level (2-tailed test). *: Correlation is significant at the 0.05 level (2-tailed test).
of cowpea (Wang et al., 2019). Therefore, the application of amino acids promotes plant growth and improves crop yield. In this study, the amino acid fertilizer increased the biomass of *N. officinale*, consistent with previous reports by Khan et al. (2019) and Wang et al. (2019). This indicates that amino acid fertilizer could promote the growth of *N. officinale* by enhancing the synthesis of hormones in plants, thereby promoting the nutrient absorption. Amino acids also induce the expression of certain transporters, making it easier for plants to absorb and utilize nutrients (Sharma and Dietz, 2006). The amino acid fertilizer used in this experiment contained copper, iron, manganese, zinc, and boron, which could have also promoted the growth of *N. officinale*.

The application of amino acids can also promote the chlorophyll synthesis, thereby increasing the chlorophyll content and improving the photosynthetic efficiency of crops.
(Yao et al., 2021). Cheng et al. (2012) reported that amino acid foliar fertilizer increased the chlorophyll content of strawberries, thus improving its net photosynthetic rate and photochemical energy conversion efficiency. The addition of exogenous proline also increased the chlorophyll a and chlorophyll b contents in pigeon pea under Cd stress (Hayat et al., 2021). In this study, the amino acid fertilizer increased the chlorophyll and carotenoid contents in N. officinale. Under Cd stress, the Cd ions inhibit the chlorophyll synthase by replacing the central magnesium ions in chlorophylls, thereby reducing the magnesiuim absorptive capacity of plants (Jamers et al., 2009; Willows, 2019). Thus, the amino acid fertilizer may have reduced the inhibitory effect of Cd on chlorophyll synthase (Yao et al., 2021), thereby increasing the photosynthetic pigment content in N. officinale.

Under heavy metal stress, ROS are generated in plants, causing damage to plant DNA, proteins and lipids (Hernández-Jiménez et al., 2002; Orhan et al., 2004). The SOD, POD, and CAT are the main cellular enzymes that protect plants against the ROS damage (Hernández-Jiménez et al., 2002; Orhan et al., 2004; Zhang et al., 2021). A previous study has shown that amino acids increased the activity of antioxidant enzymes in soybean (Teixeira et al., 2017). Proline, a multifunctional molecule involved in ROS tolerance and scavenging, increased the activity of antioxidant enzymes in pigeon pea under Cd stress (Hayat et al., 2021). In this experiment, the amino acid fertilizer increased the POD and CAT activities of N. officinale, thus improving its resistance to Cd stress. However, the amino acid fertilizer had no significant effect on the SOD activity of N. officinale, suggesting that the SOD of N. officinale was not sensitive to the amino acid fertilizer. The amino acid fertilizer decreased the soluble protein content in N. officinale, possibly because the proteins chelated with Cd (Zhang, 2007), thus, further improving the resistance of N. officinale to Cd.

The soil pH value is an important factor affecting the migration and availability, adsorption-desorption, dissolution-precipitation, and other reactions of heavy metal ions in soil (Yu et al., 2016). At higher pH values (pH > 6.0), the soil Cd exists in the form of insoluble compounds resulting from complexation, chelation, and precipitation, which reduces its availability in the soil. However, at lower pH values, the complexed forms of Cd are easily converted into the bioavailable Cd, increasing the bioavailable Cd in the soil (Dou et al., 2020). In this study, the amino acid fertilizer decreased the soil pH value, thereby increasing the soil bioavailable Cd concentration. Therefore, the soil bioavailable Cd concentration negatively correlated with the soil pH value, and this increased the absorption of soil Cd by N. officinale. The decrease in soil pH may have been due to the secretion of organic acids by the roots of N. officinale, induced by the amino acid fertilizer, which increased the soil bioavailable Cd concentration (Montiel-Rozas et al., 2016). Thus, the amino acids indirectly correlate with the soil Cd concentration and the Cd accumulation in plants. High histidine contents in plants grown in nickel-containing medium improved their tolerance to nickel stress, indicating that histidine may be related to plant resistance against nickel stress (Kramer et al., 1996; Kerkeb and Kramer, 2003). Moreover, aspartic acid can chelate the Cd, lead, and zinc, reducing their toxicity (Bottari and Festa, 1996). Cysteine synthesis also improved the Cd tolerance in Arabidopsis and tobacco (Dominguez-Solis et al., 2004; Ning et al., 2010).

In this study, the amino acid fertilizer increased the Cd content, shoot BCF, TF, and Cd extraction of N. officinale. These results correlate with the increased soil bioavailable Cd concentration, suggesting that amino acid fertilizer could improve the Cd phytoremediation capability of N. officinale. Furthermore, correlation analysis showed that the Cd contents and Cd extractions of roots and shoots positively correlated with the root biomass, shoot biomass, chlorophyll content, carotenoid content, POD activity, CAT activity, and soil bioavailable Cd concentration, and negatively correlated with the soluble protein content and soil pH value. The root and shoot Cd extractions had positive correlations with the root and shoot Cd contents. These results suggest that the amino acid fertilizer improves the phytoremediation capability of N. officinale by enhancing its physiological resistance and promoting its growth. Additionally, the grey relational analysis showed that the root biomass, shoot biomass, chlorophyll content, CAT activity, shoot Cd content, and root Cd extraction were closely associated with the shoot Cd extraction. The path analysis further demonstrated that the shoot biomass and shoot Cd content had the direct effects on the shoot Cd extraction, while the root biomass, chlorophyll content, CAT activity, and root Cd extraction had the indirect effects on the shoot Cd extraction.

Conclusion

The amino acid fertilizer increased the biomass, photosynthetic pigment content, POD activity, and CAT activity of N. officinale, but decreased the soluble protein content, thus promoting N. officinale growth under Cd-contaminated soil. The amino acid fertilizer also increased the Cd content and Cd extraction of N. officinale, and promoted the Cd transport from the roots to shoots. A quadratic polynomial regression relationship existed between the amino acid fertilizer concentration and the root biomass, shoot biomass, root Cd content, shoot Cd content, root Cd extraction, and shoot Cd extraction, respectively. Notably, the root biomass, shoot biomass, chlorophyll content, CAT activity, shoot Cd
content, and root Cd extraction were closely associated with the shoot Cd extraction. Thus, the amino acid fertilizer could improve the phytoremediation capability of *N. officinale* to remediate the Cd-contaminated paddy soils, and 900-fold dilution was the most suitable concentration. Replicating this experiment in different settings would help validate the potential of amino acid fertilizer in enhancing the phytoremediation efficiency of Cd-contaminated paddy soils by *N. officinale*.

**Data availability statement**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**Author contributions**

RZ, QL, and XX: Investigation, Data curation, Writing-Original draft preparation. M’al, RH, XL, ZW, JW, QD, DL, HX, XLL, YT, and XW: Investigation, Data curation. LL: Conceptualization, Methodology. All authors contributed to the article and approved the submitted version.

**References**

Bao, S. (2000). *Soil and agricultural chemistry analysis* (Beijing, China: China Agriculture Press).

Bottari, E., and Festa, M. R. (1996). Asparagine as a ligand for cadmium (II), lead (II) and zinc (II). *Chem. Speciat. Bioavailability*. 8, 75–83. doi: 10.1080/095442399.1996.11083272

Chen, C., Chen, T., Lo, K., and Chiu, C. (2004). Effects of proline on copper transport in rice seedlings under excess copper stress. *Plant Sci.* 166 (1), 103–111. doi: 10.1016/j.plantsci.2003.08.015

Cheng, X., Feng, X., Zhang, Z., Shen, M., and Wang, L. (2012). Effects of “AI strong” amino-acid fertilizer on photosynthetic efficiency and yield of strawberry in plastic tunnels. *J. Fruit Sci.* 29 (5), 883–889. doi: 10.11392/cj.fski.gzh.2012.93.032

Domínguez-Solis, J. R., López-Martín, M. C., Ager, F. J., Ynsa, M. D., Romero, L. C., and Gómez, V. (2004). Increased cysteine availability is essential for cadmium tolerance and accumulation in Arabidopsis thaliana. *Plant Biotechnol. J.* 2, 469–476. doi: 10.1111/j.1467-7652.2004.00092.x

Dus, W., An, Y., Qin, Li., Lin, D., Zeng, Q., and Xia, Q. (2020). Advances in effects of soil pH on cadmium form. *Soils* 52 (3), 439–444. doi: 10.13758/j.cnki.tr.2020.03.002

Grzegórska, A., Rybaczczyk, P., Rogala, A., and Zaborckii, D. (2020). Phytoremediation—from environment cleaning to energy generation—current status and future perspectives. *Energize* 13 (11), 2905. doi: 10.3390/en13112905

Hao, Z. B., Cang, J., and Xu, Z. (2004). *Plant physiology experiment* (Harbin, China: Harbin Institute of Technology Press).

Hayat, K., Khan, J., Khan, A., Ullah, S., Ali, S., Salabuddin, et al. (2021). Ameliorative effects of exogenous proline on photosynthetic attributes, nutrients uptake, and oxidative stresses under cadmium in pigeon pea (Cajanus cajan L.). *Plants* 10 (4), 796. doi: 10.3390/plants10040796

Hernández-Jíménez, M. J., Lucas, M. M., and Felipe, M. (2002). Antioxidant defence and damage in senescing lupin nodules. *Plant Physiol. Biochem.* 40 (6–8), 645–657. doi: 10.1016/S0981-9428(02)00142-5

He, X., Zhang, J., Ren, Y., Sun, C., Deng, X., Qian, M., et al. (2019). Polyaspartate and liquid amino acid fertilizer are appropriate alternatives for promoting the phytoextraction of cadmium and lead in *Salicornia nigrum L.* *Chemosphere* 237, 124483. doi: 10.1016/j.chemosphere.2019.124483

Hu, M., Yuan, J., and Yang, X. (2011). Effects of temperature on purification ability of a planted floating bed system treating eutrophic water. *Acta Scientiae Circumstantiae* 31 (2), 283–91.

Jammers, A., Lenjou, M., Deraedt, P., Bockstaele, D. V., Blust, R., and Coen, W. (2009). Flow cytometric analysis of the cadmium-exposed green alga *Chlamydomonas reinhardtii* (Chlorophyceae). *Eur. J. Physiol.* 44 (4), 541–550. doi: 10.1007/s00424-009-1182-4

Kerke, L., and Kramer, U. (2003). The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol.* 131 (2), 716–724. doi: 10.1104/pp102.016686

Khan, S., Yu, H., Li, Q., Gao, Y., Sallam, B. N., Wang, H., et al. (2019). Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. *Agronomy* 9 (5), 266. doi: 10.3390/agronomy9050266

Khodamoradi, K., Khoshgoftarmanesh, A. H., and Maibody, S. (2017). Root uptake and xylem transport of cadmium in wheat and triticate as affected by exogenous amino acids. *Crop Purifure Sci.* 68, 415–420. doi: 10.1071/CP17061

Kramer, U., Cotter-Howells, J. D., Charnock, J. M., Baker, A., and Smith, A. (1996). Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379 (6566), 635–638. doi: 10.1038/379635a0

Liao, R., and Zhu, J. (2002). Amino acid promotes selenium uptake in medicinal plant *Plantago asiatica* *Physiol. Mol. Biol. Plants* 28, 1005–1012. doi: 10.1007/s11298-002-01196-2

Lin, I., Luo, L., Liao, M., Zhang, X., and Yang, D. (2015). Cadmium accumulation characteristics of emerged plant *Nasturtium officinale* r. *Environ. Yangtze Basin* 24 (4), 684–689. doi: 10.11870/cjepyhyh201504021

Lin, L., Wu, C., Jiang, W., Liao, M., Tang, Y., Wang, J., et al. (2020). Graining increases cadmium accumulation in the post-grafting generations of the potential cadmium-hyperaccumulator *Solanium poireticum* *Chem. Ecol.* 36 (7), 685–704. doi: 10.1080/02311830.2020.1760853

Liu, W., Shu, W., and Lan, C. (2003). *Viola baoshanensis*—a new cadmium hyperaccumulator. *Sci. Bulletin.* 48 (19), 2046–2049. doi: 10.3321/j.issn:0021-074X.2003.19.009

Ma, Q., Fasih, U. H., Muhammad, F., Muhammad, A., Noman, S., Wu, J., et al. (2022). Selenium treated foliage and biochar treated soil for improved lettuce

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Supplementary Material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1003743/full#supplementary-material
alternative amendments on the (im)mobilization and phytoavailability of Cd and
quantitative design, statistical analysis and modeling
of nickel in roots of the hyperaccumulator
higher plants. Research progresses of amino acids uptake, transport and their biological roles in
thresholds for paddy soil by species-sensitivity distribution. Rev. Environ. Contamination Toxicol.
doi: 10.1073/pnas.97.9, 4956–4960.

Zhang, X. (2019). Application of grey relation analysis theory to choose high
reliability of the network node. J. Phys. Conf. Series 1237 (3), 032056. doi: 10.1088/
1742-6596/1237/3/032056

Wang, D., Deng, X., Wang, B., Zhang, N., Zhu, C., Jiao, Z., et al. (2019). Effects
of foliar application of amino acid liquid fertilizers, with or without Bacillus
amylophilus flavus SQR9, on cowpea yield and leaf microbiota. PloS One 14 (9), e0222048. doi: 10.1371/journal.pone.0222048

Wang, R., Liu, K., Chen, H., Qi, Z., Liu, B., Cao, F., et al. (2021a). Metabolome analysis revealed the mechanism of exogenous glutathione to alleviate cadmium stress in maize (Zea mays L.) seedlings. Plants 10 (11), 105. doi: 10.3390/plants10110105

Wang, Z., Xia, L., Song, S., Farias, M. E., Li, Y., and Tang, C. (2021b). Cadmium removal from diluted wastewater by using high-phosphorus-cultured modified microbial. Chem. Phys. Lett. 771, 138561. doi: 10.1016/j.cplett.2021.138561

Willows, R. D. (2019). The mg branch of chlorophyll synthesis: Biosynthesis of chlorophyll a from protoporphyrin IX. Adv. Botanical Res. 90, 141–182. doi: 10.1016/bs.abr.2019.03.003

Xiao, R., Guo, D., Ali, A., Mi, S., Liu, T., Ren, C., et al. (2019). Accumulation, ecological-health risks assessment, and source apportionment of heavy metals in paddy soils. A case study in hanzhong, shaanxi, China. Environ. Pollut. 248, 349–357. doi: 10.1016/enivpol.2019.02.045.6

Xie, S., Wang, W., Zhang, F., and Yin, H. (2019). Research progress of plant
biostimulants. Chin. J. Biol. Control 35 (3), 487–496. doi: 10.16460/j.cnki.2095-0390.2019.03.017

Xiong, Y., Yang, X., Ye, Z., and He, B. (2004). Comparing the characteristics of
growth response and accumulation of cadmium and lead by Sedum alfredii hance. J. Northwest Sci Tech Univ. Agric. For. (Nat. Sci. Ed.). 32 (6), 101–106. doi: 10.3321/
issn:1671-9387.2004.06.023

Yang, C., Wei, S., Zhao, Q., Zhang, L., Bao, Y., Gu, P., et al. (2009). Promotion effects of exogenous amino acids on phytoremediation of cd-PAHs contaminated soils by using hyperaccumulator plant Solanum nigrum. Chin. J. Ecol. 28 (9), 1829–1834.

Yao, D., Zhang, S., Zhang, D., and Zhang, Y. (2021). Effects of amino acids on growth and chlorophyll fluorescence parameters of blue green alga Microcystis aeruginosa. J. Dalian Ocean Univ. 36 (3), 446–453. doi: 10.16535/
j.cnki.dlhyxb.2020-177

Yuan, F., Zhu, K., Li, X., Li, G., and Xu, S. (2015). Discuss the water-soluble fertilizers containing amino-acids application briefly. Shandong Chem. Industry. 44 (11) 111–112, 115. doi: 10.3969/j.issn.1008-021X.2015.14.042

Yu, H., Liu, C., Zhu, J., Li, F., Deng, D., Wang, Q., et al. (2016). Cadmium availability in rice paddy fields from a mining area: the effects of soil properties highlighting iron fractions and pH value. Environ. Pollut. 209, 38–45. doi: 10.1016/
jenviron.2015.11.021

Zhang, X. (2007). Studies on the effects of four heavy-metals bioaccumulation in
ganoderma lucidum (Master thesis) (Chinese Academy of Medical Sciences, China: Peking Union Medical College).

Zhang, H., Shao, J., Zhang, S., Zhang, X., and Chen, H. (2019). Effect of phosphorus-modified biochars on immobilization of Cu (II), cd (II), and (as V) in paddy soil. J. Haz. Mat. 390, 121349. doi: 10.1016/j.jhazmat.2019.121349

Zhang, X., Xia, H., Li, Z., and Gao, B. (2010). Potential of four forage grasses in remediation of cd and mn contaminated soils. Biores. Technol. 101 (6), 2063–2066. doi: 10.1016/j.biortech.2009.11.065

Zhang, Y., Ye, Z., and Zhang, Y. (2021). Advances in physiological and molecular mechanism of plant response to cadmium stress. Plant Physiol. J. 57 (7), 1437–1450. doi: 10.11893/CRB20160

Zhang, Y., Zhang, L., Cheng, H., Sun, H., and Cui, X. (2020). Soil cadmium pollution and crop health risk in a mining area in south China. J. Agro-Envi. Sci. 39 (12), 2752–2761. doi: 10.1016/j.57.

Zhao, T., Guo, R., and Lan, Y. (1987). Flora repubica popularis sinicae-crucefove (Beijing. China: Science Press).