Exogenous addition of nitrate nitrogen regulates the uptake and translocation of lead (Pb) by Iris lacteal Pall. var. chinensis (Fisch.) Koidz. (Postprint)

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Abstract

Since Pb is a non-biodegradable inorganic pollutant and a non-essential metal, its long-term presence in soil poses a great threat to the environment. Iris lactea Pall. var. chinensis (Fisch.) Koidz., a perennial dense bush herb with high resistance of Pb and wide adaptability, was used in pot experiments to study the effects of exogenous nitrate N (NO-3-N) on the absorption and transportation of Pb and plant growth under different Pb concentrations. Then, the mechanism of NO-3-N affecting Pb and nutrient uptake and transport was explored. The concentration of Pb in the experiment ranged from 0 to 1600 mg/kg, and the added concentration of NO-3-N was 0.0–0.3 g/kg. The results showed that I. lactea was highly tolerant to Pb, and the shoot fraction was more sensitive to varied Pb concentrations in the soil than the root fraction. This protective function became more pronounced under the condition of raised Pb concentration in the soil. When the concentration of Pb in the soil reached 800 mg/kg, the highest Pb content of I. lactea was found under the condition of 0.1 g/kg of NO-3-N addition. When Pb concentration in the soil increased to 1600 mg/kg, the increase in NO-3-N addition promoted Pb uptake by the root. To ensure the well growth of I. lactea and the effect of remediation of Pb-contaminated soil, the recommended concentration of NO-3-N in the soil is 0.1 g/kg. This result provides a theoretical basis for exogenous N regulation of phytoremediation of Pb-contaminated soil.

Full Text

Preamble

Exogenous addition of nitrate nitrogen regulates the uptake and translocation of lead (Pb) by *Iris lactea* Pall. var. chinensis (Fisch.)

Koidz.

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Abstract: Lead (Pb) is a non-biodegradable inorganic pollutant and non-essential metal whose long-term presence in soil poses a great threat to the environment. *Iris lactea* Pall. var. chinensis (Fisch.) Koidz., a perennial dense bush herb with high Pb resistance and wide adaptability, was used in pot experiments to study the effects of exogenous nitrate N (NO_3^--N) on Pb absorption, transportation, and plant growth under different Pb concentrations, and to explore the mechanism by which NO_3^--N affects Pb and nutrient uptake and transport. Experimental Pb concentrations ranged from 0 to 1600 mg/kg, with added NO_3^--N concentrations of 0.0–0.3 g/kg. The results showed that *I. lactea* was highly tolerant to Pb, with the shoot fraction being more sensitive to varying soil Pb concentrations than the root fraction. This protective function became more pronounced as soil Pb concentration increased. At a soil Pb concentration of 800 mg/kg, the highest Pb content in *I. lactea* was found under 0.1 g/kg NO_3^--N addition. When soil Pb concentration increased to 1600 mg/kg, increased NO_3^--N addition promoted Pb uptake by the roots. To ensure healthy growth of *I. lactea* and effective remediation of Pb-contaminated soil, the recommended soil NO_3^--N concentration is 0.1 g/kg. These results provide a theoretical basis for exogenous N regulation in the phytoremediation of Pb-contaminated soil.

Keywords: *Iris lactea*; nitrate nitrogen; plant nutrient; lead accumulation; absorption; transport

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1 Introduction

Lead (Pb) pollutants can enter the soil environment through various pathways, causing severe soil pollution (Sinha et al., 2006). Over the past 50 years, approximately 7.83×10^{5} t of Pb has been discharged into the global environment, resulting in varying degrees of Pb pollution in soils worldwide (Duzgoren-Aydin, 2007). Since Pb is a non-biodegradable inorganic pollutant and non-essential metal, its long-term persistence in soil poses a great threat to the environment (Amari et al., 2017; Gerhardt et al., 2017). Excessive Pb enters the environment and participates in the water-soil-biological system cycle, accumulating in plant roots, stems, leaves, and seeds through plant absorption, and endangering animal and human health through food chain enrichment (Salazar et al., 2016; He et al., 2019). Pb is highly toxic and hazardous to humans and plants even at low concentrations. Excessive Pb in soil may cause stress-induced changes in plants, including growth reduction, decreased biomass, leaf chlorosis, and other physiological and biochemical changes (Chandana et al., 2019).

Remediation methods for heavy metal pollution in soil mainly include physical methods (e.g., landfilling and replacement with uncontaminated soil) and chemical methods (e.g., chemical fixation, leaching, and extraction) (Park and Son, 2017; Liu et al., 2018). These methods are inefficient, costly to operate, and may damage the original soil biological environment. Due to the decreased mobility of Pb in soil, tolerant plants may concentrate Pb in their roots, though studies have shown that the ability to translocate Pb into aboveground parts may be very low (Salazar and Pignata, 2014). In this context, testing whether metal-tolerant plant species can accumulate Pb from soil into their roots or translocate it into shoots becomes highly significant. In recent years, in situ remediation of contaminated soils using heavy metal hyperaccumulator plants has been accepted as a very promising method. Phytoremediation is characterized by high efficiency, no secondary pollution, long-lasting effects, low cost, and easy application (Chaney et al., 2005; Han et al., 2008). For example, *Helichrysum microphyllum* subsp. *tyrrhenicum*, an endemic plant species in Sardinia and Corsica, Europe, exhibits good tolerance to heavy metals such as zinc (Zn), Pb, and cadmium (Cd), and was used as a pioneer species for mine soil remediation by Boi et al. (2021).

Several exogenous substances can affect heavy metal uptake and transport by plants, with the most studied being chelating agents and fertilizers (Vassil et al., 1998; Shen et al., 2002). Nitrogen (N) fertilizer mainly affects heavy metal activity through the acidification and alkalization effects of NH_4^+-N and NO_3^--N in the rhizosphere (Zeng et al., 2020). When NH_4^+-N is absorbed, plants secrete H^+, causing rhizosphere acidification; when NO_3^--N is absorbed, plants secrete OH^-, causing rhizosphere alkalization (Wallace, 1979). Pb contents in maize roots and shoots treated with NH_4^+-N were significantly higher than those treated with NO_3^--N, with an increase of about 20%, and NH_4^+-N fertilizer application can increase Pb content in plant tissues (Lou et al., 2005).

Some studies have reported that NO_3^--N can promote heavy metal uptake and transport by plants. Nitrate fertilization has been shown to increase Zn hyperaccumulation in *Nocceaea caerulescens* (J. Presl & C. Presl) F.K. Mey (Schwartz et al., 2003; Monsant et al., 2008; Xie et al., 2009).

I. lactea is a perennial herb distributed in the northern temperate regions of Asia, Europe, and North America, with wide distribution in northwestern, northeastern, and northern China. As a long-lived ornamental plant, *I. lactea* exhibits strong tolerance and uptake capacity for Pb (Bai et al., 2008; Ayyasamy et al., 2009). Yuan et al. (2018) found that Pb content in the shoots (leaf and stem) of *I. lactea* was 983 mg/kg, with a transport coefficient greater than 1, indicating its potential to accumulate high amounts of Pb in its tissues. Han et al. (2008) speculated that *I. lactea* can sacrifice part of its cells to adsorb Pb, thus reducing Pb toxicity and ensuring normal plant growth and development. Hydroponic studies showed that Pb contents in the aerial parts and roots of *I. lactea* were 1109 and 2408 mg/kg, respectively, exceeding the hyperaccumulator standard (1000 mg/kg), suggesting that *I. lactea* application for Pb-contaminated soil remediation is feasible (Han et al., 2013). However, the remediation capacity of *I. lactea* under exogenous fertilizer addition based on soil culture has apparently not been investigated, as previous studies on Pb enrichment in *I. lactea* were conducted in hydroponics. Under different culture conditions, Pb accumulation and translocation to shoots, accumulation mode, and detoxification mechanisms of plants differ significantly. Pb accumulation in *I. lactea* under soil cultivation was significantly reduced compared with solution culture, especially in shoots, possibly related to low Pb water solubility and less plant-available Pb in soil (Zhuang et al., 2000). In conclusion, while NO_3^--N can significantly promote heavy metal uptake by plants, the remediation potential of *I. lactea* in Pb-contaminated soils remains unclear. The present study investigated the effects of exogenous NO_3^--N on Pb absorption, transportation, nutrient accumulation, and growth of *I. lactea* under different Pb concentrations, with results that may provide sustainable management and remediation strategies for Pb-contaminated soils.

2.1 Plants and soil sampling

I. lactea seeds and soil samples were obtained from the Yanqing Experimental Base of Beijing Oasis Technology Co., Ltd., China. Soil samples were sieved to a particle size of <2 mm to exclude coarse debris and air-dried at 22°C for 1 week. Soil properties are shown in Table 1 .

2.2 Pot experiment

Plants were grown in a plant cultivation room (25°C (\$$\pm1°C)/12hlight, 22°C(\pm1°C)/12hdark, 63\{2\}O\{2\}$\$ and repeated washing with deionized water, *I. lactea* seeds were mixed with river sand sterilized at 105°C and refrigerated at 4°C. After two months, seeds were disinfected with 0.5% NaClO for 20 min, washed with tap water, and germinated in a continuous dark incubator at 25°C using the sand bed tissue culture method. After germination, seeds were irrigated with 1/4 nutrient solution (formula shown in Table S1), which gradually increased from 1/2 concentration to full strength. During the culture period, nutrient solution pH was adjusted to approximately 6.0.

Pb was added as $Pb(CH_3COO)_2 \bullet 3H_2O$ at concentrations of 0 (Pb0), 800 (Pb1), and 1600 (Pb2) mg/kg. N fertilizer was added as $NaNO_3$ at concentrations of 0.0 (N0), 0.1 (N1), 0.2 (N2), and 0.3 g/kg (N3) (Table 2). A total of 12 treatments were established (Table 2), each replicated three times. Phosphate and potash fertilizers were added as $KH_2(PO_4)_3$, with P_2O_5 and K_2O concentrations of 0.20

and 0.25 g/kg, respectively. Soil was mixed with Pb and base fertilizer, loaded into plastic pots (12.0 cm diameter × 13.5 cm height) to a bulk density of 1.4 g/cm^3. After greenhouse culture for 2 weeks, seedlings with uniform growth trend and 5 cm height were transplanted to pots (six seedlings per pot) for 8 weeks and watered appropriately to maintain 70% field capacity.

2.3 Sample collection

After 45 days of treatment, plants were harvested for roots and shoots, and potted soil samples were collected simultaneously. Plant samples were treated at 105°C for 15 min, dried at 75°C to constant weight, and weighed. Samples were ground and sieved to determine nutrient element and Pb contents. Soil samples were ground and sieved after air-drying to determine soil pH, nutrients, and Pb contents.

2.4 Measurements

Pb forms in soil were determined using the modified BCR (Bureau of Reference, European Community) three-step sequential extraction procedure (Rauret et al., 1999; Bao, 2000) (Table 3). Plant Pb content was determined by HNO_3-$HClO_4$ digestion and inductively coupled plasma atomic emission spectrometry (Perkin Elmer, Waltham, USA). Total N, phosphorus (P), and potassium (K) contents were determined using the Kjeldahl method, vanadium molybdenum yellow colorimetry, and flame photometry after H_2SO_4-H_2O_2 digestion (Stanisław et al., 2017), respectively. Biomass was determined by drying plants and weighing with a 1/10,000 balance. The Pb transfer coefficient was calculated as:

Transport coefficient = aboveground Pb content / belowground Pb content

2.5 Statistical analysis

Data were processed using SPSS software package v.24.0, and results were visualized using Origin Pro v.8.5. Variance analysis was used to assess the significance of relationships between different NO_3^--N amounts and Pb adsorption by *I. lactea* (F test with significance threshold of 0.05). Differences between treatments were tested using the LSD (least significant difference) method at a significance level of 0.05.

3.1 Biomass

Under Pb0 treatment, *I. lactea* shoot biomass showed an overall decreasing trend with increasing NO_3^--N concentration, with no significant differences among CK, N2, and N3 treatments, but significantly lower than N1 treatment (Fig. 1 [Figure 1: see original paper]). Shoot biomass under Pb1 treatment was slightly higher than under Pb0 and Pb2 treatments, while root biomass decreased with increasing NO_3^--N concentration. Shoot biomass decreased significantly under Pb2 treatment, while root biomass showed no significant difference at N0 and N1 concentrations but was substantially higher than at N2 and N3 concentrations. The root/shoot ratio increased with increasing NO_3^--N concentration under Pb0 and Pb2 treatments, but decreased initially then slowly increased under Pb1 treatment (Table S1).

3.2 Effect of exogenous NO_3^--N on soil pH

Soil pH generally showed a decreasing trend with increasing exogenous NO_3^--N concentration across different Pb treatments (Fig. 2 [Figure 2: see original paper]), though variations existed among soils with different Pb treatments. Under Pb0 treatment, NO_3^--N addition at all concentrations reduced soil pH to the same extent. Under Pb1 treatment, NO_3^--N at N2 and N3 concentrations reduced soil pH similarly. Under Pb2 treatment, NO_3^--N at N1 concentration did not significantly affect soil pH, while N2 and N3 concentrations similarly reduced soil pH.

3.3 Uptake and transport of N by I. lactea

Exogenous NO_3^--N addition had no significant effect on N content in *I. lactea* roots in soil without Pb contamination (Table S2). Under Pb0 treatment, shoot N content showed an increasing then decreasing trend with increasing NO_3^--N concentration, reaching maximum accumulation of 1.94 mg/plant at N1 concentration, which was 29.33% higher than the treatment without NO_3^--N (Table S3). Under Pb1N2 treatment, the highest shoot N accumulation reached 1.88 mg/plant, with remaining treatments ranked as Pb1N1 > Pb1N0 > Pb1N3. Under Pb2 treatment, exogenous NO_3^--N addition had a significant positive effect on shoot and root N contents, with the highest shoot N accumulation (1.16 mg/plant) occurring under Pb2N0 treatment, significantly higher than other treatments. Shoot and root N contents across NO_3^--N treatments followed the order Pb2N0 > Pb2N1 > Pb2N2 > Pb2N3, showing significant inhibition of N translocation to shoots with increasing NO_3^--N concentration (Table S4). Root N uptake did not differ extensively with increasing NO_3^--N concentration.

3.4 Chemical form distribution of Pb in the soil

In all treatments, Pb mainly existed in reducible and residual states, accounting for more than 40% and 30% of total Pb, respectively (Fig. 3 [Figure 3: see original paper]). The proportion of different Pb forms from high to low was reducible > residual > acid soluble > oxidizable. With increasing exogenous Pb, the proportion of acid soluble Pb increased while reducible and oxidizable Pb proportions decreased, with residual Pb showing no obvious trend. Under Pb1 treatment, exogenous NO_3^--N addition had no significant effect on acid soluble, reducible, and residual Pb proportions, but oxidizable Pb proportion significantly increased under Pb1N2 treatment.

3.5 Absorption and transport of Pb by I. lactea

Under Pb1 and Pb2 treatments, root Pb concentrations reached up to 1744.09 and 3893.24 mg/kg, respectively, while shoot Pb concentrations reached 111.92 and 284.30 mg/kg, respectively. Pb uptake and accumulation in *I. lactea* were mainly concentrated in roots, with root Pb content 9.6–22.1 times higher than shoot content (Fig. 4 [Figure 4: see original paper]).

Under Pb1 treatment, root Pb concentration increased initially then decreased with increasing NO_3^--N concentration (Fig. 5a [Figure 5: see original paper]), reaching a maximum of 1744.09 mg/kg and highest accumulation of 0.06 mg/plant under Pb1N1 treatment, which was drastically higher than other treatments. Transport coefficients ranked as Pb1N0 > Pb1N2 > Pb1N3 > Pb1N1, all less than 0.1 (Table S5).

Under Pb2 treatment, exogenous NO_3^--N addition promoted Pb absorption in roots (Fig. 5b [Figure 5: see original paper]) but had no significant effect on shoot Pb concentration. However, with increasing NO_3^--N concentration, the transport coefficient decreased linearly, following the order Pb2N0 > Pb2N1 > Pb2N2 > Pb2N3.

4.1 Variation of nitrogen on soil pH

Selection of appropriate N fertilizer forms and application methods can serve as a strategy to control heavy metal absorption by crops and improve agricultural product safety (Hamlin and Barker, 2006). Chemical N fertilizer effects on soil physicochemical properties are mainly manifested through soil acidity changes, attributable to two aspects. First, pH change is caused by N form transformation and migration. When NH_4^+-N is applied, soil pH can be significantly reduced over relatively short periods through nitrification, and NO_3^--N produced in this process or applied as fertilizer can leach base ions, further acidifying soil (Williams et al., 1987). Second, rhizosphere pH changes result from plant absorption of different N forms and secretion of H^+ or OH^-. When different N fertilizers are applied, distinct changes occur in the plant rhizosphere environment, with root systems secreting different ions: H^+ secretion after NH_4^+-N uptake causes rhizosphere acidification, while OH^- secretion after NO_3^--N uptake results in rhizosphere alkalization (Wallace et al., 1979). Different N fertilizer forms have different effects on soil acidification and rhizosphere environment, thus affecting heavy metal adsorption in soils (Lou et al., 2005).

4.2 Effect of nitrate nitrogen addition on lead content

Previous studies have reported different Pb forms in soil. Rosik-Dulewska and Karwaczyńska (2004) found that Pb enters soil mainly in residual form, while Lee et al. (2015) showed Pb predominantly in carbonate and reducible fractions. This study found that Pb mainly exists in reducible state after entering soil, though residual state also accounts for a large proportion, with reducible and residual Pb content comprising >78% of the total. Differences among studies may be related to soil type. There is a very significant positive correlation between Pb contents in *I. lactea* roots and shoots and various Pb forms in soil, with the most significant correlation occurring with acid soluble Pb (Table S6). Stepwise regression analysis between lead content in *I. lactea* roots and shoots and soil acid soluble Pb content yielded the following equations: $Y_1 = -222.58 + 18.99X$, $R^2 = 0.944$, $P < 0.05$; $Y_2 = -3.85 + 1.24X$, $R^2 = 0.810$, $P < 0.05$, where Y_1 and Y_2 are lead contents (mg/kg) in shoot and root, respectively, and X is acid soluble lead content (mg/kg). These results confirm that Pb absorbed by *I. lactea* roots and shoots transformed into acid soluble Pb in soil. Exogenous NO_3^--N addition positively impacted Pb forms in soil.

4.3 Effect of nitrate nitrogen addition on absorption and transport of lead

The low Pb content in *I. lactea* shoots does not stress normal plant growth, yet the amount of Pb absorbed far exceeds the tolerance of most plants to Pb in nature (Yuan et al., 2015). Moreover, Pb addition did not affect the content and distribution proportion of various Pb forms in soil (Wang et al., 2018). Therefore, the direct effect of NO_3^--N may be responsible for increased Pb absorption in *I. lactea* roots. It is presumed that increased NO_3^--N concentration introduces more anion charge to soil, prompting greater anion uptake by plants, which requires more cation uptake for balance, thereby facilitating cation membrane transport processes and increasing Pb content absorbed by plant roots. Additionally, exogenous NO_3^--N addition promotes organic acid synthesis in soil, which may combine with Pb and assist in Pb translocation and accumulation (Monsant et al., 2010).

Studies have proven that most NO_3^--N absorbed by plant roots must be reduced and utilized by plants, while a small portion is stored in plant vacuoles for ion balance and osmotic regulation. When soil NO_3^--N exceeds the upper limit of nitrogen required during plant growth, it may cause ion imbalance and inhibit absorption of other essential elements, thus affecting plant growth (Kastl et al., 2015). In this experiment, under Pb2 treatment, exogenous NO_3^--N addition substantially inhibited normal aboveground growth of *I. lactea* but had relatively small impact on roots, partly due to Pb concentration stress but more significantly due to inhibitory effects of exogenous NO_3^--N addition on *I. lactea* growth.

4.4 Relationship between nutrient absorption and transport of lead

Shoot N content in *I. lactea* negatively correlated with shoot P content, shoot and root Pb contents, and four Pb forms, while positively correlating with root P content and shoot and root dry weights (Fig. 6 [Figure 6: see original paper]).

Root N accumulation correlated with shoot dry weight. With NO_3^--N addition, P accumulation in roots increased considerably, with most P remaining in roots, while N was transported to aboveground parts in large quantities, contributing to plant dry weight. A small amount of N promoted plant dry weight and Pb absorption, but as NO_3^--N concentration increased, Pb absorption capability of *I. lactea* decreased.

5 Conclusions

This study investigated the effects of exogenous NO_3^--N addition on Pb and nutrient uptake and transport, as well as *I. lactea* growth. The results found that exogenous NO_3^--N mainly affected Pb content in *I. lactea* roots. Under different Pb concentrations, NO_3^--N application inhibited Pb transport from roots to shoots, with higher Pb concentrations producing more significant effects. This may be due to the ion balance effect caused by NO_3^--N addition on *I. lactea* root absorption. In soils with certain degrees of Pb pollution, heavy metal mobility in plants can be improved by adjusting soil nutrition levels. Exogenous NO_3^--N addition reduces soil pH and affects the distribution and composition of different Pb forms in soil, as NO_3^- combines with base ions, leading to reduced soil pH through leaching. The demand for NO_3^--N in *I. lactea* is very low. In soils polluted with different Pb concentrations, exogenous NO_3^--N addition can promote *I. lactea* growth, but there is a limit to the effective concentration range. When NO_3^--N concentration is 0.1 g/kg, the promotional effect is optimal.

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Appendix

Table S1 Effect of NO_3^--N on root/shoot ratio of *Iris lactea* in Pb-contaminated soil

Pb treatment	NO_3^--N treatment	Root/shoot ratio
Pb0	N0	0.31$±0.02*c*||*Pb*0|*N*1|0.35±0.01*b*||*Pb*0|*N*2|0.42±0.03*a*||*Pb*

Note: N0, 0.0 g/kg; N1, 0.1 g/kg; N2, 0.2 g/kg; N3, 0.3 g/kg; Pb0, 0 mg/kg; Pb1, 800 mg/kg; Pb2, 1600 mg/kg. The abbreviations are the same in the following tables.

Table S2 Effect of NO_3^--N on N, P, and K uptake and transportation of *Iris lactea* in Pb-contaminated soil

Nutrient element	Pb treatment	NO_3^--N treatment	Content in shoot (g/kg)	Content in root (g/kg)
N	Pb0	N0	20.82$±1.52*c*|9.57±1.02*a*||*N*|*Pb*0|*N*1|20.49±1.36*b*|10.46±0.33*b*||*N*|	

Note: Different lowercase letters within the same nutrient element and lead treatment indicate significant differences among different nitrate treatments at P<0.05 level. Mean±SD.

Table S3 Effect of NO_3^--N on N, P, and K accumulation of *Iris lactea* in Pb-contaminated soil

Nutrient element	Pb treatment	NO_3^--N treatment	Accumulation in shoot (mg/plant)	Accumulation in root (mg/plant)
N	Pb0	N0	1.50$±0.06ab	0.42±0.05a
N	Pb0	N1	1.74±0.10a	0.46±0.05a
N	Pb0	N2		

Note: Different lowercase letters within the same nutrient element and lead treatment indicate significant differences among different nitrate treatments at P<0.05 level. Mean±SD.

Table S4 Effect of NO_3^--N on transfer coefficients of N, P, and K of *Iris lactea* in Pb-contaminated soil

Pb treatment	NO_3^--N treatment	N transfer coefficient	P transfer coefficient	K transfer coefficient
Pb0	N0	0.20$±0.01c	0.12±0.01b	0.15±0.01c
Pb0	N1	0.21±0.01bc	0.12±0.01b	0.15±0.01c
P				

Table S5 Effect of NO_3^--N on transfer coefficients of Pb of *Iris lactea* in Pb-contaminated soil

Pb treatment	NO_3^--N treatment	Pb transfer coefficient
Pb1	N0	0.064$±0.001a
Pb1	N1	0.058±0.001d
Pb1	N2	0.062±0.001b
P		

Table S6 Correlation between Pb forms in soil and contents of Pb in shoot and root of *Iris lactea*

Index	Acid soluble state	Reducible state	Oxidizable state	Residual state
Pb content in root	0.972**	0.900**	0.946**	0.877**
Pb content in shoot	0.889**	0.859**	0.897**	0.875**

Note: **, P<0.01 level.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv –Machine translation. Verify with original.