

## Postprint of Heavy Metal Changes in Fresh Leaves and Litter of *Neolamarckia cadamba* and *Schefflera arboricola* under Sludge Application

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### Abstract

This study conducted large root box experiments with single-species *Schefflera arboricola*, single-species *Neolamarckia cadamba*, and mixed-species *Neolamarckia cadamba* with *Schefflera arboricola*. We tracked and analyzed the Cu, Zn, Cd, and Hg contents in fresh leaves and leaf litter, as well as changes in litter production, for three months (September, October, and November 2020) following surface application of 2% (W/W) sludge. The relationships between heavy metal contents in fresh leaves and leaf litter, and changes in heavy metal return via litter were also analyzed. The results showed that: (1) Under sludge application, the Cu contents in both fresh leaves and leaf litter of *Neolamarckia cadamba* were significantly higher than those of *Schefflera arboricola*, whereas the Zn and Cd contents were significantly lower. (2) The fresh leaf Zn content of *Schefflera arboricola* was lowest in November, while its Hg content was highest in November. (3) The fresh leaf Zn, Cd, and Hg contents of both single-species and mixed-species *Neolamarckia cadamba* were highest in November. (4) The leaf litter Hg content of mixed *Neolamarckia cadamba* increased significantly with prolonged sludge application time, while changes in Cu, Zn, and Cd contents were not significant. (5) The fresh leaf Cd content of *Schefflera arboricola* was significantly positively correlated with leaf litter Hg and Cd contents in both September and November. (6) The leaf litter production and the return amounts of Cu, Zn, Cd, and Hg for *Schefflera arboricola* and *Neolamarckia cadamba* peaked one month (September) and two months (October) after sludge application, respectively. In conclusion, sludge application time had a greater influence on the heavy metal contents in fresh leaves of both species than on those in leaf litter; a positive correlation existed between the fresh leaf Cd content of *Schefflera arboricola* and the Cd and Hg contents in its leaf litter; and the risks of heavy metal pollution from leaf litter were higher for *Schefflera arboricola* at one month (September) and for *Neolamarckia cadamba* at two

months (October) after sludge application. This study provides a reference for sludge utilization in landscaping and the proper disposal of leaf litter.

## Full Text

### Preamble

#### Changes of Heavy Metal in Fresh and Litter Leaves of *Neolamarckia cadamba* and *Schefflera arboricola* under Sludge Application

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**Abstract:** This study conducted a large root box experiment with *Schefflera arboricola* monoculture, *Neolamarckia cadamba* monoculture, and co-planting of *S. arboricola* and *N. cadamba*. The dynamic changes in Cu, Zn, Cd, and Hg content in fresh and litter leaves of *S. arboricola* and *N. cadamba* and their litter production were analyzed for three months (September, October, and November 2020) after the surface application of 2% (W/W) sewage sludge (SS). The relationship between the heavy metal (HM) content of fresh leaf and that of litter leaf and the changes in the HM return amount in leaf litter were further analyzed. The results were as follows: (1) *N. cadamba* had significantly higher Cu content in fresh and litter leaves than that of *S. arboricola*, while having significantly lower Zn and Cd contents. (2) The fresh leaf of *S. arboricola* had the lowest Zn content and the highest Hg content in November. (3) The fresh leaf of *N. cadamba* had the highest Zn, Cd, and Hg contents in November. (4) The Hg content in the litter leaf of co-planted *N. cadamba* increased significantly with the time of SS application, while Cu, Zn, and Cd contents showed no significant change. (5) The Cd content in fresh leaves was significantly and positively correlated with the Hg and Cd contents of leaf litter in *S. arboricola* in both September and November. (6) The highest yield of leaf litter and the highest return amount of Cu, Zn, Cd, and Hg in *S. arboricola* occurred one month after SS application (September), while those in *N. cadamba* occurred two months after SS application (October). In summary, the application time of SS showed a greater effect on the HM contents in fresh leaves of *N. cadamba* and *S. arboricola* than on those in litter leaves. There was a positive correlation between the Cd content in the fresh leaf and the Cd and Hg contents in the litter leaf of *S. arboricola*. The HM pollution risk of the litter leaves of *S. arboricola* and *N. cadamba* was highest in one month (September) and two months after SS application (October), respectively. This study will provide a reference for safe SS utilization and reasonable litter disposal in landscape applications.

**Key words:** sludge recycling, heavy metal, landscape plant, leaf litter, co-planting

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## Introduction

The scientific disposal of municipal sewage sludge and reduction of environmental risks associated with sludge disposal have become urgent issues in municipal and ecological environmental construction (Chen et al., 2018). Landscape utilization represents an important approach for sludge resource recycling. Sewage sludge is rich in organic matter, N, P, K, and various micronutrients, and can be used as a soil fertilizer to improve soil physical, chemical, and biological properties while promoting plant growth (Bai et al., 2017). However, sludge contains various pollutants, particularly heavy metals, which can affect plant growth to some extent and pose potential contamination risks to landscape soils (Chu et al., 2018). How to reduce the heavy metal pollution risks associated with landscape utilization of sludge has attracted considerable attention.

Plants play an important role in soil heavy metal remediation. Phytoextraction utilizes plants with heavy metal enrichment capacity to absorb heavy metals from soil through their root systems, transfer and store them in aboveground parts, and then remove the heavy metals by harvesting the aboveground biomass. This is an important measure for reducing soil heavy metal pollution and represents a key developing remediation technology (Mohsin et al., 2022; Yang et al., 2022). Rational planting of landscape plants on sludge-amended soils can effectively absorb and transfer heavy metals from sludge, thereby achieving triple benefits of landscape greening construction, sludge resource disposal, and reduction of soil heavy metal pollution risks (Wu et al., 2017, 2021). Leaf litter production is a primary manifestation of plant adaptation to seasonal changes or avoidance of harsh external environments (Liu and Peng, 2010; Yuan et al., 2018). To alleviate the toxicity of harmful substances such as heavy metals in their tissues, plants accelerate the migration of these substances to senescing leaves or accelerate the senescence and abscission of poisoned leaves. Consequently, leaf litter generated during phytoremediation contains certain amounts of heavy metals, which can cause secondary soil pollution if not promptly cleaned up and recovered (Maunoury-Danger et al., 2018; Al Souki et al., 2020). For example, Sun et al. (2011) analyzed heavy metals in the litter layer of different types of plantations and found that Pb, Cd, Cu, and Zn contents in the litter layer of nine plantation types were all higher than those in the soil layer,

indicating that leaf litter poses potential heavy metal pollution risks to soils. Additionally, litter production varies across seasons, and seasonal input of litter affects changes in heavy metal storage in forest soil and hydrological systems; for instance, Cd storage in upstream, midstream, and downstream forest streams was highest during the peak litter production period in autumn (Jiang et al., 2020). Investigating the dynamic changes of heavy metal contents in fresh and litter leaves of landscape plants after sludge application is beneficial for improving heavy metal phytoextraction efficiency and reducing secondary heavy metal pollution risks from litter. Unfortunately, no studies have yet focused on heavy metal changes in fresh and litter leaves of landscape plants under sludge application conditions, nor is it clear what effects sludge has on heavy metals in litter leaves or what relationships exist between heavy metals in fresh and litter leaves.

On the other hand, co-planting can affect heavy metal absorption and accumulation in plants to some extent. For example, co-planting significantly enhanced arsenic (As) and lead (Pb) uptake by brake fern (*Pteris vittata*) (Yang et al., 2017), and co-planting *Schefflera arboricola* with *Bischofia javanica* significantly increased Cd, Ni, and Cu accumulation in *B. javanica* (Lai et al., 2022). Co-planting may improve plant heavy metal extraction efficiency by (1) altering the rhizosphere environment and affecting heavy metal activity, (2) forming a more coordinated root absorption network, and (3) enhancing plant heavy metal tolerance (Wang et al., 2018; Yeboah et al., 2020; Bian et al., 2021). Whether co-planting affects leaf heavy metal contents under sludge application has not been reported. *Neolamarckia cadamba*, a deciduous tree in the Rubiaceae family, offers advantages such as large biomass, long life cycle, and rapid growth rate, demonstrates relatively strong tolerance to sludge, and possesses certain heavy metal accumulation capacity (Chu et al., 2018). *Schefflera arboricola*, a widely planted landscape plant in South China, has been confirmed to have strong heavy metal absorption and enrichment capacity (Sun et al., 2021). These two plant species exhibit substantial differences in growth rate, leaf heavy metal accumulation, and litter production. Co-planting *N. cadamba* and *S. arboricola* can form a synergistic deep-shallow root absorption network, which is beneficial for improving heavy metal removal efficiency (Wu et al., 2021). Based on this, this study selected *N. cadamba* and *S. arboricola* as test plants to conduct a large soil-cultured root box experiment with surface sludge application, aiming to address the following questions: (1) How do heavy metal contents in fresh and litter leaves of the two plants change after sludge application? (2) What relationships exist between heavy metal contents in fresh and litter leaves of the two plants? (3) Does landscape application of sludge create heavy metal pollution risks in litter leaves? (4) How does co-planting the two species affect leaf heavy metal contents?

## 1.1 Experimental Materials

**Test substrates:** The soil was latosolic red soil collected from green spaces in the suburbs of Guangzhou. The sewage sludge was municipal stacked sludge from Qingyuan Lüyou Environmental Protection Technology Co., Ltd., Guangdong Province. Both materials were air-dried naturally and passed through a 1 cm mesh sieve to remove debris before use. Basic chemical properties are shown in Table 1 .

**Test plants:** The experiment selected fast-growing arbor *Neolamarckia cadamba* seedlings and landscape plant *Schefflera arboricola* with consistent growth, no pests or diseases, and approximately 20 cm height. The *N. cadamba* seedlings were 3 months old, and *S. arboricola* seedlings were 5 months old. Both plant species were purchased from Professor Chen Xiaoyang' s research group at the College of Forestry and Landscape Architecture, South China Agricultural University, and the Fangcun Seedling Market in Guangzhou, respectively.

## 1.2 Experimental Design

The experimental treatments were as follows: *S. arboricola* monoculture (S), with 6 *S. arboricola* plants per root box; *N. cadamba* monoculture (N), with 2 *N. cadamba* plants per root box; and co-planting of *S. arboricola* (SNS) and *N. cadamba* (SNN) (S+N), with 3 *S. arboricola* plants and 1 *N. cadamba* plant per root box. A randomized block design was adopted, with plants arranged in each root box according to treatment settings. Each treatment had 5 independent root boxes, with each root box serving as an individual biological replicate. The experiment was conducted in an open field at the Ecological Farm of South China Agricultural University. The experimental soil was filled into root boxes to simulate natural soil layers. Specifically, soil collected from the 30–60 cm layer in the suburbs was first filled as the 30–60 cm layer in the root boxes, watered thoroughly, and allowed to settle naturally for 1 day. Then, soil collected from the 0–30 cm layer was filled as the 0–30 cm layer in the root boxes, watered thoroughly, and allowed to settle and equilibrate for 1 month. Each root box contained approximately 1,000 kg of soil. After soil equilibrium treatment, *N. cadamba* and *S. arboricola* were transplanted into the root boxes according to treatment settings in October 2019 and allowed to grow naturally for 10 months. In August 2020, sludge was applied to the surface of each root box at a rate of 2% (W/W) (the sludge application rate was based on the cumulative limit of  $<30 \text{ t} \cdot \text{hm}^{-2}$  for forest land sludge application specified in standard CJ/T 262-2011, equivalent to a mass ratio of 1.2%–2.1%). During the growth period, water was applied every 3–5 days depending on weather conditions, with 5–10 L per root box each time. After sludge application, litter leaves of test plants were collected daily, and other plant litter and weeds in each partition were cleaned up to ensure that each treatment was not disturbed by other factors.

### 1.3 Sample Collection

**Litter leaves:** One month after sludge application, litter leaves of test plants were collected daily from each root box. After identification and classification, leaves were washed to remove surface soil, placed in nylon mesh bags by treatment, and air-dried naturally. After collection for 1 month, samples were placed in envelopes by treatment and dried to constant weight in a 65°C oven. Considering that *N. cadamba* litter production in December was very low, this experiment mainly collected litter leaves continuously for three months: September, October, and November 2020. Due to the loss of *S. arboricola* litter samples collected in September 2020, relevant data could not be obtained.

**Fresh leaves:** To analyze differences in heavy metal contents between fresh and litter leaves, fresh leaves were also collected during the three months of September, October, and November 2020. On the last day of each month, fresh leaves of *N. cadamba* and *S. arboricola* were collected from each root box. For *N. cadamba*, one fully expanded, healthy green leaf was collected from each of the four directions (east, south, west, and north) in the middle part of the plant. For *S. arboricola*, three fully expanded, healthy green leaves were collected from each of the four directions in the middle part of the plant. Leaves were deactivated and dried for later use.

### 1.4 Index Determination

**Dry weight of litter leaves:** The mass of dried litter leaves collected monthly from each root box was weighed using a 0.01 g precision balance. Litter yield per plant = total mass of *N. cadamba*/*S. arboricola* litter in a root box ÷ number of *N. cadamba*/*S. arboricola* plants in that root box.

**Heavy metal contents in litter and fresh leaves:** Various heavy metal contents in sludge were all below the limits for landscape use. Since the total Pb, Cr, Ni, and As contents in the test soil and sludge were relatively low, while total Cu, Zn, Cd, and Hg contents were relatively high, this experiment mainly analyzed Cu, Zn, Cd, and Hg contents in fresh and litter leaves. After drying, plant leaves were crushed and passed through a 60-mesh sieve for later use. Crushed leaf samples (0.5 g) were weighed into microwave digestion vessels, 5 mL of nitric acid was added, and microwave digestion was performed sequentially at 120°C-160°C-180°C. After cooling, the digestion solution was diluted to 25 mL with distilled water. Cu, Zn, and Cd contents in the digestion solution were determined by atomic absorption flame photometry, and Hg content was determined by atomic fluorescence spectrophotometry. The ratio of heavy metal content in leaves was calculated to understand the transfer of heavy metals from fresh leaves to litter leaves: leaf heavy metal content ratio = litter leaf heavy metal content ÷ fresh leaf heavy metal content. The return amount of heavy metals in litter leaves was calculated to assess secondary heavy metal pollution from litter: heavy metal return amount in litter leaves = dry weight of litter leaves × heavy metal content in litter leaves.

## 1.5 Data Processing and Statistical Analysis

All data were organized using Microsoft Excel 2016. Normality and homogeneity of variance were tested using the “shapiro.test” and “kruskal.test” functions in the “stats” package of R software (version 4.20). For non-normal or heteroscedastic data, logarithmic or square root transformations were performed to meet analysis prerequisites. One-way ANOVA was then conducted using the “agricolae” package in R software, with Duncan’s multiple comparison test ( $\alpha = 0.05$ ). The “t.test” function in the “stats” package was used for T-tests. Pearson correlation tests were performed and correlation heatmaps were plotted using the “corrplot” package in R software. Histograms were plotted using the “ggplot2” package in R software. Data in figures and tables are presented as mean  $\pm$  standard error ( $n = 5$ ).

## 2 Results and Analysis

### 2.1 Changes in Heavy Metal Contents in Fresh Leaves

Cu contents in fresh leaves of *N. cadamba* were significantly higher than those of *S. arboricola* across different months ( $P < 0.05$ ), while Cd and Zn contents were significantly lower ( $P < 0.05$ ) (Figure 1 [Figure 1: see original paper]). One month after sludge application (September), leaf Hg content in *N. cadamba* was significantly lower than that in *S. arboricola* ( $P < 0.05$ ). Compared with their respective monocultures, co-planting significantly increased Zn content in *S. arboricola* in September and October, and significantly increased Cd content in *S. arboricola* two and three months after sludge application (October and November) (by 139.90% and 44.72%, respectively). Cu content in leaves of co-planted *S. arboricola* increased significantly over time, while Cu content in monocultured *S. arboricola* showed no significant change. Zn contents in leaves of both monocultured and co-planted *S. arboricola* were lowest in November, while Hg contents were highest in November, with no significant change in Cd content. Zn, Cd, and Hg contents in leaves of both monocultured and co-planted *N. cadamba* were highest in November.

### 2.2 Changes in Heavy Metal Contents in Litter Leaves

Heavy metal contents in litter leaves of the two plant species under different treatments are shown in Figure 2 [Figure 2: see original paper]. Cu contents in *N. cadamba* litter leaves were significantly higher than those in *S. arboricola* in both October and November, while Zn and Cd contents were significantly lower, with no significant difference in Hg content between the two species. Compared with their respective monocultures, co-planting had no significant effect on Cu and Hg contents in litter leaves of either species. However, co-planting significantly increased Zn content in *S. arboricola* litter leaves in November (by 30.10%,  $P < 0.05$ ) and significantly increased Cd content in *S. arboricola* litter leaves in October and November (by 33.10% and 94.48%, respectively,  $P < 0.05$ ). Cu contents in litter leaves of both monocultured and co-planted *S. arboricola*

decreased significantly with extended sludge application time ( $P < 0.05$ ), while Zn, Cd, and Hg contents showed no significant changes over time. Hg content in litter leaves of co-planted *N. cadamba* increased significantly with extended sludge application time ( $P < 0.05$ ), while Cu, Zn, and Cd contents showed no significant changes.

### 2.3 Correlation Analysis between Heavy Metal Contents in Litter Leaves and Fresh Leaves

Correlations between heavy metal contents in fresh and litter leaves of *S. arboricola* are shown in Figure 3 [Figure 3: see original paper]. Cu contents in fresh leaves showed no significant correlation with any of the four heavy metal contents in litter leaves across different months. Zn content in fresh leaves in September was significantly negatively correlated with Zn content in litter leaves in September ( $r = -0.669$ ;  $P = 0.035$ ;  $t = -2.544$ ). Zn and Hg contents in fresh leaves in September were significantly positively correlated with Hg content in litter leaves in October, and Cd content in fresh leaves in September was significantly positively correlated with Hg content in litter leaves in November ( $r = 0.722, 0.647, 0.740$ ;  $P = 0.018, 0.043, 0.014$ ;  $t = 2.951, 2.398, 3.113$ ). Zn content in fresh leaves in September was extremely significantly positively correlated with Cd content in litter leaves in November, and Cd content in fresh leaves in September was extremely significantly positively correlated with Hg content in litter leaves in October and November ( $r = 0.806, 0.849, 0.740$ ;  $P = 0.005, 0.002, 0.014$ ;  $t = 3.853, 4.551, 3.113$ ). Zn and Hg contents in fresh leaves in October were significantly positively correlated with Hg content in litter leaves in October ( $r = 0.680, 0.687$ ;  $P = 0.031, 0.028$ ;  $t = -0.997, 1.492$ ). Cd content in fresh leaves in November was significantly positively correlated with Cd and Hg contents in litter leaves in November ( $r = 0.644, 0.730$ ;  $P = 0.045, 0.016$ ;  $t = 2.379, 3.025$ ). Correlation results among different heavy metal contents in litter leaves of *S. arboricola* in the same month showed that Zn content in litter leaves in September was significantly positively correlated with Cd and Hg contents ( $r = 0.764, 0.660$ ;  $P = 0.010, 0.038$ ;  $t = 3.353, 2.483$ ). Cu content was extremely significantly positively correlated with Zn, Cd, and Hg contents, and Hg content was extremely significantly positively correlated with Cd content ( $r = 0.946, 0.909, 0.863, 0.932$ ;  $P = 0.000, 0.000, 0.001, 0.024$ ;  $t = 8.264, 6.161, 4.838, 7.252$ ). Cu content was significantly positively correlated with Hg content in both October and November ( $r = 0.682, 0.647$ ;  $P = 0.030, 0.044$ ;  $t = 2.639, 2.387$ ).

Correlations between heavy metals in fresh and litter leaves of *N. cadamba* are shown in Figure 4 [Figure 4: see original paper]. Cd content in fresh leaves in September was extremely significantly negatively correlated with Hg content in litter leaves in September, and Hg content in fresh leaves in September was extremely significantly negatively correlated with Cu content in litter leaves in September ( $r = -0.819, -0.851$ ;  $P = 0.004, 0.002$ ;  $t = -4.040, -4.591$ ). Hg content in fresh leaves in September was significantly negatively correlated with

Zn content in litter leaves in September ( $r = -0.656$ ;  $P = 0.030$ ;  $t = -2.456$ ). Hg content in fresh leaves in September was extremely significantly positively correlated with Hg content in litter leaves in October ( $r = 0.869$ ;  $P = 0.001$ ;  $t = 4.957$ ). Cu content in fresh leaves in October was significantly positively correlated with Cu content in litter leaves in November ( $r = 0.734$ ;  $P = 0.016$ ;  $t = 3.053$ ). Cd content in fresh leaves in November was significantly negatively correlated with Cd content in litter leaves in November ( $r = -0.658$ ;  $P = 0.039$ ;  $t = -2.473$ ). Correlation results among different heavy metals showed that Zn content in litter leaves in September was extremely significantly positively correlated with Cd content ( $r = 0.771$ ;  $P = 0.009$ ;  $t = 3.425$ ). Cu content was extremely significantly positively correlated with Zn content in November ( $r = 0.874$ ;  $P = 0.001$ ;  $t = 5.098$ ).

## 2.4 Changes in Litter Leaf Yield

Litter leaf yield of *S. arboricola* showed a decreasing trend with extended sludge application time (Table 2). Litter leaf yield of monocultured *S. arboricola* was lowest in November, significantly lower than that in October (decreased by 82.1%,  $P < 0.05$ ). Litter leaf yields of both monocultured and co-planted *N. cadamba* were highest in October (increased by 223.7% and 169.7% compared with September, respectively) and lowest in November (decreased by 72.1% and 72.6% compared with September, respectively).

## 2.5 Changes in Heavy Metal Return Amount in Litter Leaves

Cu, Zn, Cd, and Hg return amounts in *N. cadamba* litter leaves were significantly higher than those in *S. arboricola* across different months (Table 3). Compared with their respective monocultures, co-planting significantly increased Cd return amount in *S. arboricola* in November (by 71.23%,  $P < 0.05$ ) and significantly decreased Hg return amount in *N. cadamba* in October (by 27.27%,  $P < 0.05$ ). The four heavy metal return amounts in litter leaves of monocultured *S. arboricola* (S) and Hg return amount in litter leaves of monocultured *N. cadamba* (N) were highest in September. Cu, Zn, Cd, and Hg return amounts in litter leaves of either monocultured or co-planted *N. cadamba* were highest in October.

## 3 Discussion and Conclusion

Different plants exhibit different heavy metal absorption capacities in sludge-amended soils. In previous studies, we noted that compared with *N. cadamba*, the root system of *S. arboricola* had stronger Zn and Cd absorption capacity but weaker Cu absorption capacity (Wu et al., 2021). Numerous studies have found that differences in root heavy metal absorption affect aboveground heavy metal accumulation (Chu et al., 2018; Mohsin et al., 2022; Yang et al., 2022). This study further revealed that Zn and Cd contents in both fresh and litter leaves of *N. cadamba* were significantly lower than those of *S. arboricola*, while Cu content was significantly higher, indicating that differences in root heavy

metal absorption capacity affect not only heavy metal contents in fresh leaves but also those in litter leaves.

This study also noted that Zn, Cd, and Hg contents in fresh and litter leaves of *S. arboricola* and Cu, Zn, and Cd contents in fresh and litter leaves of *N. cadamba* did not show obvious dynamic changes with sludge application time. Only Cu content in *S. arboricola* litter leaves decreased significantly, while Hg content in *N. cadamba* litter leaves increased significantly. These differences may be related to the toxicity of different elements and plant detoxification mechanisms (Cui et al., 2013). Unlike Cd and Hg, which have direct toxic effects on plants, Zn and Cu are essential micronutrients for plant growth and only become toxic at high concentrations (Sturikova et al., 2018; Kumar et al., 2021). Plants transfer harmful elements such as Cd and Hg to litter leaves to alleviate their toxicity (Li et al., 2016). However, for nutrient elements like Zn and Cu, plants reabsorb and reuse them before leaf senescence and abscission (Killingbeck, 1986; Aerts, 1996; Yan et al., 2016). In this study, the high Zn concentration in fresh leaves of *S. arboricola* may have adverse effects similar to Cd and Hg, leading to its transfer to litter leaves to reduce toxicity to fresh leaves. In contrast, Cu content in fresh leaves had not yet reached toxic concentrations and needed to be reabsorbed and reused. Peng et al. (2023) reported that different types of forests have different resorption effects on Cr, Cd, and Pb, and the dynamic changes of Cr, Cd, and Pb in leaves are correlated to some extent, leading to differences in heavy metal contents between fresh and litter leaves in different forest types. This study further analyzed correlations between heavy metals in fresh and litter leaves to explore the specific reasons for changes in heavy metal contents in litter leaves. Interestingly, Cu contents in fresh leaves of *S. arboricola* in different months did not affect heavy metal contents in litter leaves, whereas Cd contents in fresh leaves in different months had prominent positive effects on Cd and Hg contents in litter leaves. This indicates that changes in leaf Cu content had not yet triggered the transfer of heavy metals to litter leaves in *S. arboricola*, whereas Cd content triggered detoxification mechanisms and induced the transfer of Cd and Hg to litter leaves to alleviate toxicity. Unlike *S. arboricola*, correlations between heavy metals in fresh and litter leaves of *N. cadamba* were weaker. The main reason may be that, compared with *S. arboricola*, *N. cadamba* has larger biomass and stronger heavy metal dilution effects (Chu et al., 2017), and its leaf heavy metal concentrations had not yet reached levels that trigger detoxification mechanisms.

Jiang et al. (2020) found that heavy metal return risks were high during peak litter fall periods, and heavy metal return risks from litter production in most plants were mainly affected by seasonal changes. Notably, in this study, the timing of heavy metal return risks from litter leaves differed between *S. arboricola* and *N. cadamba*. *S. arboricola* produced large amounts of litter leaves within one month after sludge application (September), resulting in high heavy metal return risks, whereas *N. cadamba* produced more litter leaves and exhibited high heavy metal return risks in the second month after sludge application (October). Considering that (1) the evergreen shrub *S. arboricola* does not have seasonal

leaf abscission, (2) sludge compost has high nutrient and salt contents and is prone to ammonia volatilization in the short term (Xu et al., 2015), and (3) *S. arboricola* has a relatively short stature, we speculate that salt stress and ammonia toxicity generated shortly after sludge application were important causes of massive leaf abscission and heavy metal return risks in *S. arboricola*. In contrast, *N. cadamba* has a tall stature and large biomass, making it less susceptible to salt stress and ammonia toxicity from sludge. However, it exhibits seasonal leaf abscission (mainly in October in South China), so its heavy metal return risks occurred primarily during the leaf-fall season. These results suggest that to reduce secondary heavy metal pollution risks from litter leaves when using landscape plants to remediate sludge-amended soils, attention should be paid not only to seasonal changes in litter production but also to massive leaf abscission caused by direct sludge toxicity.

Intercropping between plant species not only makes full use of spatial structure, soil fertility, and light conditions but also enables synergistic extraction of multiple heavy metals from soil by various plants, representing a typical phytoremediation measure (Zeng et al., 2018; Zeng et al., 2019). Unlike previous findings that co-planting can promote heavy metal absorption in plants (Yang et al., 2017), this study found that co-planting increased Zn and Cd contents in fresh and litter leaves of *S. arboricola* in the short term but had no significant effect on leaf heavy metal contents of *N. cadamba*. This implies that co-planting *S. arboricola* with *N. cadamba* is beneficial for improving the heavy metal extraction capacity of *S. arboricola* but also promotes the transfer of heavy metals from fresh leaves to litter leaves. Based on the analysis of root heavy metal absorption, changes in heavy metal contents in litter leaves, and correlations between heavy metals in fresh and litter leaves, we speculate that the effects of co-planting on changes in Zn and Cd contents in fresh and litter leaves of *S. arboricola* are related to the Zn and Cd absorption capacity of *S. arboricola* roots. However, the mechanisms by which co-planted *N. cadamba* promotes Zn and Cd absorption in *S. arboricola* roots require further investigation. Future research should focus on changes in rhizosphere physiological ecology after co-planting the two species, such as whether rhizosphere organic acid contents and abundance of plant growth-promoting bacteria are altered.

In summary, this study found that under sludge application conditions, Zn and Cd contents in fresh and litter leaves of *S. arboricola* were significantly higher than those of *N. cadamba*, while Cu content was significantly lower. With extended sludge application time, Cu content in *S. arboricola* litter leaves decreased significantly, while Hg content in *N. cadamba* litter leaves increased significantly. Cd and Hg contents in litter leaves of *S. arboricola* in different months were greatly affected by Cd content in fresh leaves. Heavy metal return risks from litter leaves of *S. arboricola* and *N. cadamba* were high within the first and second months after sludge application, respectively. Co-planting *N. cadamba* with *S. arboricola* increased Zn and Cd contents in fresh and litter leaves of *S. arboricola*. Considering the limited standards for landscape utilization of sludge (GB/T 23486-2009), this study specifically selected sludge with

relatively low heavy metal contents. The results provide a reference for the safe landscape utilization of current sludge and reasonable disposal of litter leaves. Future research could further explore the effects of sludge with higher heavy metal contents to more comprehensively understand heavy metal changes in fresh and litter leaves under sludge application.

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