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RESEARCH ARTICLE

Effects of cadmium on mercury accumulation and transformation by *Arundo donax* L.

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Abstract

High biomass energy plants are a promising alternative to hyperaccumulators for the remediation of heavy metals (HMs). *Arundo donax* L. (*A. donax*) is a rapidly growing rhizomatous grass with high biomass production. However, the feasibility of using *A. donax* for the phytoremediation of combined mercury (Hg) and cadmium (Cd) pollution under neutral conditions is unclear. In this study, a hydroponic experiment was performed to investigate the impact of Hg–Cd stress on the growth and physiological properties of *A. donax* and HMs accumulation and transformation. Either single Hg or Cd stress slightly enhanced stem height, fresh biomass, and chlorophyll content, whereas combined Hg–Cd stress reduced these parameters. Furthermore, combined Hg–Cd stress increased the leaf content of malondialdehyde in *A. donax*, indicating that the combined pollution aggravated oxidative stress in *A. donax*. Hg volatilization was observed during the 10-day experiment, implying that a portion of the Hg^{2+} was transformed into Hg^0 by *A. donax*. The bioaccumulation factor (BAF) values of *A. donax* were far greater than 1 for both Hg and Cd, whereas the translocation factor (TF) values were less than 1, indicating that phytovolatilization and phytostabilization rather than phytoextraction contributed to the remediation of Hg and Cd by *A. donax*. The solution pH decreased at the beginning of the experiment, suggesting that acidic root exudates of *A. donax* facilitated the accumulation and transformation of Hg under neutral conditions. Overall, the effects of Cd on Hg accumulation and transformation by *A. donax* followed the rule of "low promotion and high inhibition." This study demonstrates that *A. donax* is a potential candidate for the phytoremediation of combined Hg–Cd pollution under neutral conditions.

Keywords *Arundo donax* L · Mercury · Cadmium · Combined pollution · Volatilization

Introduction

Mercury (Hg) is a toxic heavy metal (HM), which is introduced to the environment through natural processes and anthropogenic activities (Wang et al. 2021). Hg pollution has become a global environmental problem nowadays. For example, in the Wanshan Mercury Mine District, which is located in the Pacific Rim Mercury Mineralization Zone and is the largest historic Hg-producing district in Guizhou Province, China, long-term mining and smelting activities have produced approximately 125.8 million tons of mine waste (Yin et al. 2016), and the contents of Hg in the atmosphere, water, and soil all far exceed background values in the province (Li et al. 2008; Zhang et al. 2010). Hg can be accumulated by aquatic organisms, plants, and human beings, posing a significant threat to ecosystem function and human health (Barone et al. 2021; Lominchar et al. 2019; Raj et al. 2020a, b; Yabanli et al. 2014; Yozukmaz et al. 2018). Feasible remediation technologies for Hg contamination are urgently needed.

Current remediation strategies for heavy metal (HM)-polluted soils include physicochemical and biological techniques. Among biological techniques, phytoremediation is an eco-friendly and low-cost technology that utilizes plants to absorb pollutants from contaminated water and soil. However, most hyperaccumulators have shallow root systems and low biomass production, and attention is increasingly turning toward fast-growing energy plants with high

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biomass production, such as *Miscanthus* × *giganteus*, *Sorghum dochna*, *Ricinus communis* L., *Jatropha curcas*, and *Arundo donax* L. var.versicolor Kunth (*A. donax*) (Pandey et al. 2016). These plants are HM tolerant and highly adaptable. After remediation, the aerial portions of the plants are generally used as biofuels to meet energy demands. Therefore, energy plants can provide not only biomass yield but also ecosystem services for pollution remediation. This study focuses on *A. donax*, a tall perennial rhizomatous grass with fast growth, high biomass, high adaptability, and strong tolerance to various HMs, such as Pb, Cd, Mn, Cr, Hg, As, Zn, and Cu (Cristaldi et al. 2020; Liu et al. 2019; Zhang et al. 2021).

In general, the mechanisms of phytoremediation can be classified into five main pathways: plant extraction, plant degradation, plant stabilization, plant volatilization, and plant filtration. HMs are absorbed by plant roots and transported to aboveground shoots or leaves via the xylem (Zhang et al. 2021). Hg generally exists in elemental (Hg^0), ionic (Hg^{2+}), hydroxide ($Hg(OH)_2$), sulfide (HgS), and methyl (MeHg) forms in the environment, and transformations between these forms can occur under certain conditions. Hg^{2+} is the predominantly bioavailable species for plant uptake (Heaton et al. 2005; Raj and Maiti 2020) and is readily adsorbed by plant roots. However, only a small proportion of this Hg^{2+} transported to the aerial parts of the plant; thus, it predominantly accumulates in roots (Chen and Yang 2012). In general, acidic conditions facilitate HM accumulation in plants because most HMs are transformed into insoluble forms under neutral and weakly alkaline conditions. However, in a field trial study in the Guizhou mining area, a karst mountainous region with a soil pH around 7.0, we found that the content of Hg in the aerial parts of *A. donax* could reach 4.16 mg kg^{-1}, with a yield of approximately 4.0 t (dry weight) ha^{-1} $year^{-1}$ under neutral conditions. How does *A. donax* accumulate, translocate, and transform Hg from soil neutral conditions?

Coexisting HMs can synergistically, additively, or antagonistically affect HMs accumulation and translocation in plants. For example, Zn increases the uptake, translocation, and accumulation of Cd by *Brassica juncea* (Du et al. 2020; Kutrowska et al. 2017). Conversely, As decreases the accumulation and translocation of Zn in *Pteris vittata* (Zhang et al. 2014). Hg inhibits the uptake and translocation of As but enhances the toxicity of As(III) in rice seedlings (Ren et al. 2014). The interaction effects of coexisting HMs depend on many factors, such as plant genotypes and ecotypes and metal concentrations and types (Abid et al. 2019). Hg and Cd are main group IIB elements and thus have similar chemical properties. In fact, Cd is frequently found in Hg ore bodies and usually coexists with Hg in mercury mining areas (Chang 2019). However, little information is available about the effect of Cd on the accumulation, translocation, and transformation of Hg in plants.

HMs uptake and accumulation negatively affect plant physiological processes, including plant growth, photosynthesis, and the antioxidant system (Chen and Yang 2012; Pu et al. 2019; Raj and Maiti 2020). Plant cells have developed mechanisms to resist HMs stress. Superoxide dismutase (SOD) is an important antioxidant enzyme that can eliminate reactive oxygen species (ROS) induced by metal stress to prevent excessive oxidation of membrane lipids. Glutathione (GSH), the main non-protein mercaptan in plants, is an indicator of plant antioxidant capacity. GSH can also effectively eliminate ROS, lipid peroxides (LOOHs), and other harmful molecules to maintain normal cell growth and development (Mwamba et al. 2016). However, the effects of Hg–Cd stress on the growth and physiological properties of plants, including *A. donax*, are unclear.

To address these gaps, a hydroponic experiment was performed at pH 7.0 in this study to fulfill the following objectives: (i) understand the effects of combined Hg–Cd stress on the growth and physiological properties of *A. donax*; (ii) determine the accumulation, translocation, and transformation characteristics of Hg in *A. donax* under neutral conditions in the presence or absence of Cd; and (iii) propose feasible mechanisms for the phytoremediation of Hg by *A. donax*.

Materials and methods

Experimental design

Seedlings of *A. donax* were collected from the seedling breeding base of our research group in Nanjing, Jiangsu Province, China. Healthy *A. donax* seedlings of equal size were selected, purged, and cultured in Hoagland's nutrient solution. After acclimation for 2 weeks, the vigorous seedlings were transferred to glass pots containing 400 mL of 0.25-strength Hoagland's nutrient solution at pH 7.0. The outside of the glass pot was wrapped with aluminum foil to protect against light.

To understand the Hg accumulation and translocation capabilities of *A. donax* under combined Hg–Cd stress conditions, nine treatments in which the seedlings were exposed to nutrient solution containing $Hg(NO_3)_2$ (0, 0.5 or 2 mg·L^{-1}) and/or $Cd(NO_3)_2$ (0, 0.5 or 2 mg·L^{-1}) were established: ① CK; ② Hg1: 0.5 mg L^{-1} $Hg(NO_3)_2$; ③ Hg2: 2 mg L^{-1} $Hg(NO_3)_2$; ④ Cd1: 0.5 mg L^{-1} $Cd(NO_3)_2$; ⑤ Cd2: 2 mg L^{-1} $Cd(NO_3)_2$; ⑥ Hg1Cd1: 0.5 mg L^{-1} $Hg(NO_3)_2$ + 0.5 mg L^{-1} $Cd(NO_3)_2$; ⑦Hg2Cd2: 2 mg L^{-1} $Hg(NO_3)_2$ + 2 mg L^{-1} $Cd(NO_3)_2$; ⑧Hg1Cd2: 0.5 mg L^{-1} $Hg(NO_3)_2$ + 2 mg L^{-1} $Cd(NO_3)_2$; ⑨ Hg2Cd1: 2 mg L^{-1} $Hg(NO_3)_2$ + 0.5 mg L^{-1}

$Cd(NO_3)_2$. The solution pH was maintained at 7.0 by adjustment with 0.01 M NaOH or HCl.

The phytovolatilization of Hg was quantified by enclosing the plant in a gastight compartment (Fig. 1). The experimental unit consisted of one air pump, one gastight volatilization chamber, and a Hg trap. The Hg vapor released from the plant was trapped in 150 mL of 5% $KMnO_4$ dissolved in 1 M H_2SO_4. The plant was placed in a plant growth chamber at an average temperature regime of 25/15 °C (day/night) without supplementary illumination for 10 days. Each treatment had three replicates.

After the 10-day hydroponic experiment, the *A. donax* seedlings were harvested, washed thoroughly with ultrapure water, and separated into roots and aerial parts. The principal root length, stem height, and fresh weight of each plant were determined, and the roots and aerial parts from each treatment were freeze dried and ground to powder for total Hg and Cd analyses.

Physiological and biochemical measurements

The chlorophyll content of the leaves was determined using a SPAD-502 instrument (Konica Minolta, Japan). The relative chlorophyll content was determined from the dual-wavelength optical absorbance (at 620 and 940 nm) of the leaf samples. The average value of 5 selected points on each leaf was calculated to reflect the chlorophyll content of the whole leaf (Sun et al. 2021).

Malondialdehyde (MDA) content was measured using the thiobarbituric acid method (Jiang et al. 2022). Fresh leaves (~ 0.50 g) were crushed in a mortar, and 2 mL of 0.05 M (pH 7.8) phosphate buffer was added. The liquid mixture was centrifuged at 4500 rpm for 10 min, and the supernatant (MDA extract solution) was retained. Next, 2 mL of the MDA extract solution and 3 mL of 5% trichloroacetic acid solution were heated in a boiling water bath for 10 min and centrifuged at 4500 rpm for 10 min. The MDA content was determined by measuring the absorbance at 532 nm and 600 nm in a spectrophotometer (Shanghai Precision Instrument Co., Ltd., model v-5800).

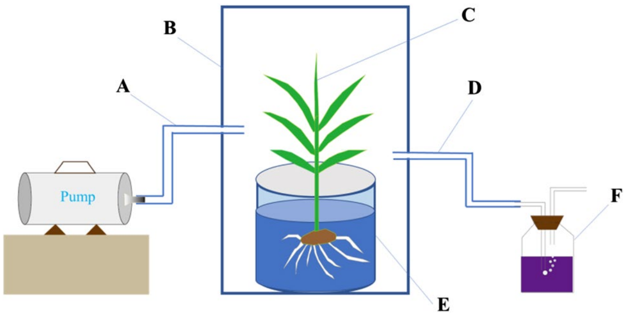

Fig. 1 Experimental apparatus used to capture volatilized Hg from *Arundo donax* L. **A** Air inlet; **B** gastight shoot compartment (4.5 L); **C** *Arundo donax* L. seedling; **D** air outlet; **E** glass pot; **F** permanganate acid trap

SOD activity was assayed following Shah et al. (Shah and Nahakpam 2012). Fresh leaves (~ 0.30 g) were homogenized in 5 mL of ice-cold 50 mM phosphate buffer at pH 6.5 and centrifuged at 10,000 g for 20 min at 0 to 4 °C. The absorbance of the supernatant was used for the measurement of SOD activities, which was determined in an ELISA reader (Beijing Perlong New Technology Co., Ltd., DNM-9602) at 450 nm.

GSH content was determined according to the method of Xun et al. (Xun et al. 2017). Briefly, leaf samples (~ 0.50 g) were homogenized in 5% trichloroacetic acid and centrifuged at 15,000 rpm for 10 min. Two milliliters of the supernatant were combined with 2.6 mL of 150 mM NaH_2PO_4 buffer and 0.2 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and GSH content was determined by measuring the absorbance of the mixture at 412 nm in a spectrophotometer (Shanghai Precision Instrument Co., Ltd., model v-5800). Three individual replicates were performed for each plant sample.

Hg and Cd analysis

The total Hg concentration in the plant was determined following the method of Zhu et al. (Zhu et al. 2021). Roots or aerial parts (~ 0.20 g) were mixed with 5 mL of HNO_3 and 3 mL of H_2O_2 in a digestion vessel, heated at 140 °C for 4 h and diluted to 25 mL with ultrapure water. The total Hg concentration in the permanganate acid trap was assayed as follows: 5 mL of solution from the trap was mixed with 0.5 mL of HNO_3 and 0.3 mL of H_2O_2 in a digestion tube, heated in a boiling water bath for 1 h and diluted to 10 mL. The Hg concentration in the digestion was determined by atomic fluorescence spectrophotometry (Model AFS-930 Jitian, Beijing, China).

The total Cd concentration in the plant was determined using the method of Zhou et al. (Zhou et al. 2020). Briefly, plant samples (~ 0.20 g) were digested with 6 mL of HNO_3 and 2 mL of H_2O_2 in a digestion vessel heated at 105 °C for 7 h and then diluted to 25 mL with ultrapure water. The Cd concentration in the digestion was determined by atomic absorption spectrophotometry (Varian SpectrAA 240Z or 220FS; Varian, Palo Alto, CA).

Replicates, blanks, and reference materials GBW10048 (celery) were included for quality control throughout the analytical procedure. About 95.7–101% of Cd and 94.6–98.5% of Hg in GBW10048 were recovered.

The phytoextraction ability of *A. donax* was assessed using the bioaccumulation factor (BAF), which is the ratio of the concentration of metals in the plant to the concentration in the environment. When the BAF is greater than 1, the plant is considered as an accumulator. The translocation factor (TF), which is the ratio of the metal concentration in the aerial parts to the metal concentration in the roots, was used to evaluate the ability of the plant to transfer metals from the roots to the aerial parts (stems and leaves).

Data analysis

Results obtained for the *A. donax* experiment were expressed as mean ± Standard Deviation (SD). Differences were tested for significance by analysis of variance (ANOVA). Subsequent multiple comparisons among means were examined by the least significance difference (LSD) test ($p < 0.05$) in SPSS 20.0. All graphics were generated using Origin 8.0.

Results and discussion

Effects of Hg and Cd on plant growth and biomass

No symptoms of toxicity in *A. donax*, such as leaf fall, necrosis, or chlorosis, were observed in any of the treatments during the experiment (Table 1). When *A. donax* was exposed to single Hg or Cd, the stem height, fresh biomass, and chlorophyll content increased as the solution concentration of Hg or Cd increased from 0.5 to 2.0 mg L^{-1}, suggesting that *A. donax* is highly tolerant of Hg and Cd and that single Hg or Cd stress may even stimulate growth. These findings are consistent with those of Cristaldi et al. (Cristaldi et al. 2020). The growth-promoting effect of single Hg or Cd exposure on *A. donax* may be due to the hormesis effect, which has been observed in many plants (Ozturk et al. 2010). However, compared with the control, the roots of *A. donax* exposed to single Hg or Cd were 34.4–45.4% or 9.8–16.9% shorter, respectively. In general, roots are the main interface for ion exchange between plants and the environmental medium, and they are the most sensitive organ to the stress of HMs in the environment. Hg or Cd stress may have inhibited mitosis in root tip meristematic cells, leading to chromosomal aberrations and reduced root growth due to asymmetry among the root epidermis, cortex cells, and cell gap (He et al. 2010). The negative effects of Hg stress on root growth were much greater than those of Cd stress.

Compared to the single Hg or Cd stress treatments, the combined Hg–Cd stress treatments significantly reduced the stem height, root length, and fresh biomass of *A. donax*, indicating that the combination aggravated the toxic effects of these HMs on plant growth. These results are similar to those of Ren et al. (2014). Co-exposure to Hg and Cd increases the toxicity of these HMs via effects on the plant cell membrane and transporters that reduce nutrient transport and inhibit plant growth (Cargnelutti et al. 2006). With the exception of the Hg1Cd1 treatment, all combined Hg and Cd treatments significantly reduced *A. donax* leaf chlorophyll content compared with the control and single Hg or Cd stress treatments, consistent with a previous report by Pu et al. (2019). These findings suggest that Hg and Cd co-exposure within a certain concentration range can promote chlorophyll generation in *A. donax* leaves in accordance with the rule of “low promotion and high inhibition”; that is, low concentrations have positive effects, while high concentrations have negative effects (Guo et al. 2019).

Accumulation of Hg and Cd in A. donax

Hg accumulation characteristics

The contents of Hg in the roots and aerial parts of *A. donax* are shown in Fig. 2. The Hg content ranged from 12.47 ± 2.54 to 35.57 ± 1.76 in the roots and from 7.72 ± 2.20 to 26.27 ± 4.65 mg kg^{-1} in the aerial parts of *A. donax*, indicating that the roots of *A. donax* had a much higher capacity for Hg accumulation than the aerial parts. Furthermore, the accumulation of Hg in *A. donax* roots and aerial

Table 1 Growth characteristics and chlorophyll content of *Arundo donax* L. in the different treatments after 10 days

Treatments	Stem height cm	Root length cm	Fresh biomass g	Chlorophyll content mg g^{-1}
CK	9.93 ± 0.51^{ab}	6.10 ± 3.15^{a}	2.81 ± 1.69^{ab}	38.86 ± 0.76^{d}
Hg1	10.00 ± 6.76^{ab}	4.00 ± 1.32^{ab}	2.09 ± 1.84^{ab}	46.68 ± 0.82^{b}
Hg2	14.50 ± 9.96^{a}	3.33 ± 1.89^{ab}	4.35 ± 1.73^{a}	46.11 ± 1.17^{b}
Cd1	10.5 ± 0.87^{ab}	5.07 ± 4.18^{a}	3.30 ± 0.84^{ab}	43.79 ± 0.64^{bc}
Cd2	14.50 ± 9.50^{a}	5.50 ± 0.87^{a}	3.66 ± 2.51^{ab}	43.36 ± 0.84^{c}
Hg1Cd1	8.67 ± 4.25^{ab}	2.87 ± 0.32^{ab}	2.41 ± 1.52^{ab}	52.84 ± 1.45^{a}
Hg1Cd2	5.47 ± 3.70^{ab}	1.93 ± 0.55^{b}	1.69 ± 0.71^{b}	36.19 ± 0.26^{e}
Hg2Cd1	4.37 ± 3.09^{b}	1.27 ± 1.01^{b}	1.91 ± 1.07^{ab}	40.13 ± 0.24^{d}
Hg2Cd2	3.73 ± 3.09^{b}	0.93 ± 0.42^{b}	1.28 ± 1.13^{b}	36.70 ± 0.59^{e}

Data represent average mean from three repetitions. Different letters in the same column are significantly different at $p < 0.05$

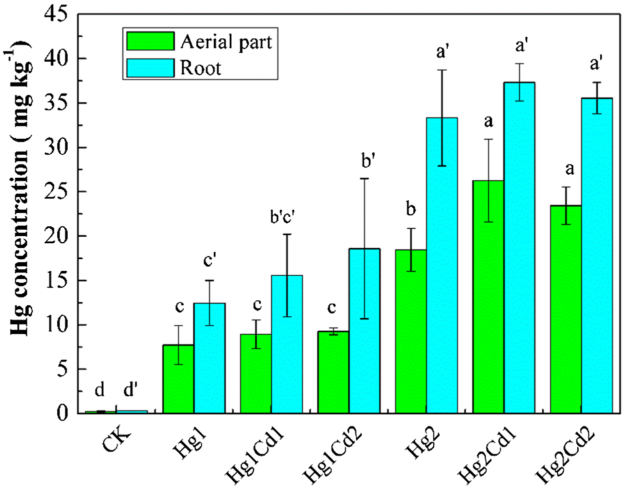

Fig. 2 Mass fractions of Hg in the aerial parts and roots of *Arundo donax* L. in the different treatments (bars with different letters are significantly different at $p < 0.05$)

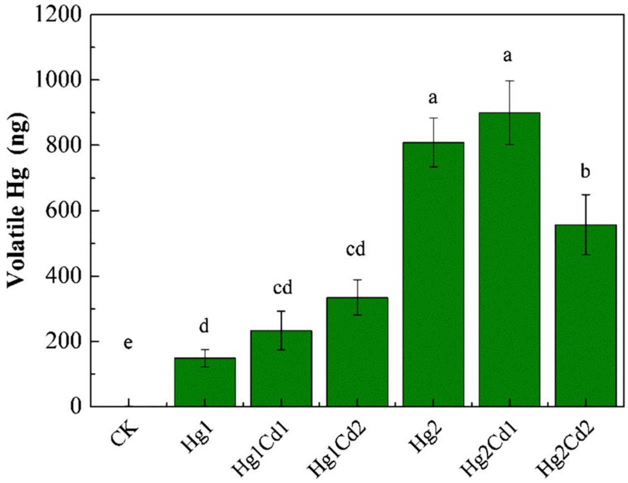

Fig. 3 Mass of volatile Hg captured from *Arundo donax* L. in the different treatments (bars with different letters are significantly different at $p < 0.05$)

parts increased with increasing Hg concentration from 0.5 to 2.0 mg L^{-1} in both the single Hg and combined stress treatments. These results indicate that *A. donax* has high Hg accumulation efficiency, in accordance with previous studies (Chen and Yang 2012). Roots are the main pathway for HM absorption by plants, and the well-developed root systems of *A. donax* can release large amounts of root exudates to facilitate HM accumulation and translocation. Previous studies have shown that HMs are readily adsorbed by and primarily accumulate in roots, with only 1.20 to 3.80% transported aboveground (Chen and Yang 2012; Liu et al. 2020; Ren et al. 2014). The process of translocation from roots to aerial parts generally involves several steps, including symplastic absorption in roots, root sequestration, xylem loading, and storage in leaf epidermal and mesophyll cells. In this study, the accumulation of Hg in the aerial parts accounted for 32.98–44.27% of the total Hg accumulation by *A. donax*, which indicates that *A. donax* has rather high translocation efficiency. *A. donax* is rich in cellulose and has hollow stems, which may facilitate the translocation of Hg from the roots to the aerial parts by xylem loading (Zhang et al. 2021).

The most striking result was the generation of volatile Hg, as shown in Fig. 3. Volatile Hg was detected in all Hg stress treatments at the end of the experiment. The mass of volatile Hg ranged from 1.81 ± 0.36 in Hg1 (0.5 mg L^{-1} Hg) to 899.90 ± 97.73 ng in Hg2Cd1 (2 mg L^{-1} Hg and 0.5 mg L^{-1} Cd). Thus, *A. donax* can accumulate Hg from the environment and convert ionic Hg into gaseous Hg. Photochemical reduction has been proposed as an important route of transformation of Hg^{2+} to Hg^0 (Ma et al. 2015), but Hg reduction and volatilization in plants can also be mediated by enzymatic reduction (Haque et al. 2010). The glass pots were protected from light by aluminum foil throughout the experiment, thus excluding photochemical reduction as the mechanism of volatile Hg generation. Consequently, the formation of volatile Hg in our experiment can be attributed to enzymatic reduction.

Effect of Cd accumulation on Hg uptake by A. donax

As shown in Fig. 4, *A. donax* also accumulated Cd. The concentration of Cd ranged from 3.15 ± 0.94 to 22.74 ± 0.84 mg kg^{-1} in the roots and from 1.25 ± 0.19 to 7.74 ± 1.83 mg kg^{-1} in the aerial parts. Similar to Hg, the accumulation of Cd was much higher in the roots than in the

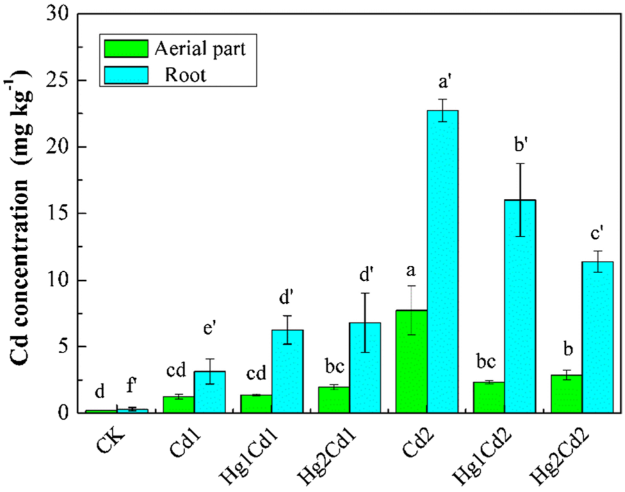

Fig. 4 Mass fractions of Cd in the aerial parts and roots of *Arundo donax* L. in the different treatments (bars with different letters are significantly different at $p < 0.05$)

aerial parts of *A. donax*, and increasing the solution concentration of Cd enhanced the accumulation of Cd in both the roots and aerial parts of *A. donax*. However, the accumulation and translocation efficiency of Cd were much lower than those of Hg, indicating that *A. donax* prefers to take up Hg rather than Cd. These findings are also consistent with those of Cristaldi et al. (2020).

To further confirm the effect of Cd on Hg uptake, accumulation, and transformation in *A. donax*, four different combination treatments (Hg1Cd1, Hg2Cd2, Hg1Cd2, and Hg2Cd1) were analyzed in this study. As shown in Fig. 2, the presence of Cd enhanced the accumulation of Hg in the roots and aerial parts of *A. donax* under both low and high Hg stress. Furthermore, the accumulation of Hg in roots increased as the concentration of Cd increased from 0.5 to 2.0 mg L^{-1} under both low and high Hg stress (Fig. 2). Increasing the Cd concentration also promoted the accumulation of Hg in the aerial parts under high Hg stress but not low Hg stress.

In addition, the presence of Cd impacted Hg volatilization by *A. donax*. Under low Hg stress, the mass of volatile Hg increased slightly as the concentration of Cd increased (Fig. 3). Under high Hg stress, a low concentration of Cd promoted Hg volatilization, whereas a high Cd concentration significantly inhibited the Hg volatilization process (Fig. 3). Similar "low promotion and high inhibition" effects of coexisting HMs on Hg volatilization in plants have been observed in previous studies (Guo et al. 2019) and have been attributed to changes in plant enzyme activities induced by the combined stress. Another possibility is that coexisting HMs compete for HM transporters in roots (Du et al. 2020), thus inhibiting their accumulation and translocation compared with single HM stress. Saifullah et al. (2014) reported that phytochelatins (PCs) induced by Cd stress in roots bind Cd^{2+} and prevent Cd translocation from the roots to the aerial parts. However, when Zn^{2+} and Cd^{2+} are both present, Zn^{2+} competes with Cd^{2+} for PC binding, thus decreasing Cd-PC formation and promoting Cd translocation in plants. Zn, Cd, and Hg are all main group IIB elements and thus have similar accumulation and translocation properties in plants. Therefore, in *A. donax*, Hg stress likely induces the production of PCs to bind Hg^{2+}, but the formation of Hg-PCs is reduced in the presence of Cd, which competes with Hg for PC binding. As a result, Cd promotes Hg accumulation and translocation in *A. donax*.

Changes in pH during the experiment

In general, Hg^{2+} is the most bioavailable form of Hg for uptake and accumulation in plants, but Hg^{2+} is susceptible to inert precipitate formation when the environmental pH is ≥ 7.0. To determine whether *A. donax* can accumulate Hg under neutral conditions, the pH of the culture solution was adjusted to 7.0 at the beginning of the experiment, and the pH value in the control treatment was monitored daily. As shown in Fig. 5, the solution pH decreased from 7.0 to 6.1 ± 0.07 on day 1 but increased from 6.1 ± 0.07 on day 2 to 7.4 ± 0.3 on day 10. Plants typically release large amounts of root exudates such as organic acids, amino acids, and fatty acids, which can decrease solution pH (Shi et al. 2011), enhance HM activity, and stimulate HM accumulation in plants (Ubeynarayana et al. 2021). *A. donax* is a perennial plant with a well-developed root system that can release acidic exudates during growth, resulting in a decrease in solution pH. In addition to acidic root exudates, plants can release alkaline root exudates such as alkaloids. Arundamine, donaxine, and N,N-dimethyltryptamine have been identified as the major alkaloids released by *A. donax* roots (Khuzhaev et al. 1994). Cano-Ruiz et al. (2021) also reported that soil pH increased significantly after planting *A. donax* on farmland for 2 years. In the present study, the release of acidic root exudates initially decreased the solution pH and promoted the uptake and accumulation of Hg by *A. donax* under neutral and weakly alkaline conditions. During the remainder of the incubation period, the generation of alkaline root exudates increased the solution pH.

Effects of Hg and Cd on the physiological properties of A. donax

Antioxidant reactions are an important means of detoxification for plants under HM stress. To maintain normal physiological activities, plants generate a series of reactive oxygen species (ROS) in response to HM stress, such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\cdot$), and superoxide anions (O_2^-). However, ROS can also lead to lipid peroxidation of cell membranes, damage to individual organelles,

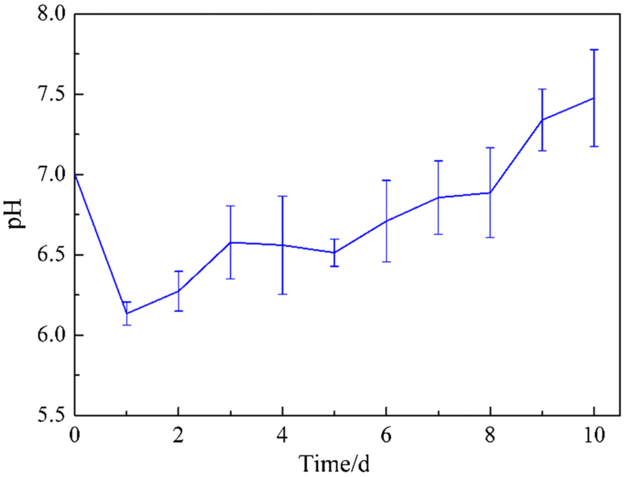

Fig. 5 pH in Hoagland's nutrient solution after planting *Arundo donax* L

and plant death (Xun et al. 2017). MDA, SOD, and GSH are frequently used as indicators of antioxidant levels in plants under HM stress. As shown in Table 2, compared with the control, both single and combined Hg and Cd stress significantly increased *A. donax* leaf MDA content. Furthermore, the MDA content increased as the concentrations of coexisting Hg and Cd increased, in agreement with previous studies (Jiang et al. 2022; Mao et al. 2022). Single Hg stress resulted in slightly higher MDA content than single Cd stress, indicating that the toxicity of Hg in *A. donax* is higher than that of Cd. MDA is the final product of lipid peroxidation in plants, and its content usually reflects the degree of oxidative stress and the level of tolerance under adverse conditions. Our results indicate that combined Hg-Cd stress intensifies membrane lipid peroxidation in *A. donax*.

Leaf SOD activity was significantly higher in *A. donax* under either single or combined Hg and Cd stress than in the control treatment. Under single Hg or Cd stress, SOD activity increased with increasing Hg or Cd concentration; by contrast, SOD activity decreased with increasing Hg and Cd concentrations in the combination treatments. SOD is an important antioxidant enzyme that can eliminate ROS to prevent overoxidation of membrane lipids. In general, increases in HM stress induce elevated SOD content to eliminate ROS and alleviate plant damage (Kou et al. 2022). Compared with the control or single Hg or Cd stress, coexisting Hg and Cd stress aggravated the damage to the antioxidant system of *A. donax* and decreased SOD activity, consistent with the results of Xun et al. [21].

Table 2 Physiological properties of the leaves of *Arundo donax* L

Treatments	MDA content nmol g^{-1}	SOD activities U g^{-1}	GSH content μg g^{-1}
CK	26.56 ± 1.57^{d}	153.60 ± 1.83^{f}	159.46 ± 2.16^{d}
Hg1	36.92 ± 4.21^{c}	180.29 ± 2.78^{c}	169.22 ± 2.64^{ab}
Hg2	39.55 ± 6.26^{bc}	191.53 ± 1.82^{b}	172.99 ± 3.57^{a}
Cd1	34.92 ± 1.51^{c}	187.35 ± 0.52^{b}	162.98 ± 3.45^{cd}
Cd2	35.88 ± 5.44^{c}	202.61 ± 2.20^{a}	164.78 ± 1.12^{c}
Hg1Cd1	43.18 ± 0.68^{bc}	189.22 ± 4.53^{b}	166.43 ± 2.09^{bc}
Hg1Cd2	43.73 ± 2.24^{b}	162.72 ± 4.48^{e}	166.58 ± 3.40^{bc}
Hg2Cd1	45.67 ± 3.50^{ab}	170.62 ± 2.57^{d}	171.57 ± 0.72^{a}
Hg2Cd2	51.60 ± 2.06^{a}	158.49 ± 2.45^{e}	169.09 ± 1.00^{ab}

Data represent average mean from three repetitions. Different letters in the same column are significantly different at $p < 0.05$

Compared with the control, all treatments increased GSH content. Furthermore, either single or combined Hg stress induced higher GSH content than Cd stress. These results indicate that GSH and SOD play similar roles in resisting oxidative damage. GSH is the main non-protein mercaptan in plants and is used as an indicator of plant antioxidant capacity (Abid et al. 2019). GSH can effectively eliminate ROS, lipid peroxides (LOOHs), and other harmful molecules to maintain normal cell growth and development. GSH is also a precursor of PCs, which chelate HMs to form complexes for transport to the vacuole for storage (Dago et al. 2014).

Bioaccumulation and translocation abilities of A. donax

The BAF indicates the ability of the plant to accumulate HMs from the environment. As shown in Table 3, the BAF_{Hg} of the roots and aerial parts of *A. donax* ranged from 16.66 ± 2.71 to 37.18 ± 2.90 and from 9.24 ± 1.22 to 18.56 ± 0.79, respectively. The BAF_{Cd} of the roots and aerial parts of *A. donax* ranged from 5.69 ± 0.40 to 13.60 ± 4.44 and from 1.17 ± 0.05 to 3.93 ± 0.40, respectively. The BAF_{Hg} and BAF_{Cd} were both far greater than 1, suggesting that *A. donax* efficiently accumulates Hg and Cd, primarily in the roots (Chen and Yang 2012). The BAF_{Hg} decreased as the Hg concentration increased. The BAF_{Cd} increased with the concentration of Cd under single stress but decreased with increasing Cd concentration under combined stress. Similarly, Xun et al. (Xun et al. 2017) reported that the BAF_{Hg} of *Cyrtomium macrophyllum* decreased as the soil

Table 3 Bioaccumulation factor (BAF) and translocation factor (TF) of Hg and Cd

Treatments	BAF_{Hg}		BAF_{Cd}		TF_{Hg}	TF_{Cd}
	Aerial part	Root	Aerial part	Root		
Hg1	15.45 ± 4.40^{ab}	24.94 ± 5.08^{bc}	–	–	0.61 ± 0.06^{b}	–
Hg2	9.24 ± 1.22^{c}	16.66 ± 2.71^{d}	–	–	0.56 ± 0.11^{b}	–
Cd1	–	–	2.50 ± 0.38^{c}	6.29 ± 1.88^{c}	–	0.44 ± 0.22^{a}
Cd2	–	–	3.87 ± 0.92^{b}	11.37 ± 0.42^{ab}	–	0.34 ± 0.07^{ab}
Hg1Cd1	17.91 ± 3.24^{a}	31.17 ± 9.28^{ab}	2.75 ± 0.15^{c}	12.53 ± 2.12^{a}	0.59 ± 0.10^{b}	0.22 ± 0.03^{b}
Hg2Cd2	11.72 ± 1.06^{bc}	17.78 ± 0.88^{cd}	1.43 ± 0.18^{d}	5.69 ± 0.40^{c}	0.66 ± 0.09^{b}	0.25 ± 0.05^{ab}
Hg1Cd2	18.56 ± 0.79^{a}	37.18 ± 2.90^{a}	1.17 ± 0.05^{d}	8.00 ± 1.37^{bc}	0.50 ± 0.06^{b}	0.15 ± 0.03^{b}
Hg2Cd1	13.13 ± 2.33^{bc}	18.66 ± 1.05^{cd}	3.93 ± 0.40^{a}	13.60 ± 4.44^{a}	0.70 ± 0.13^{a}	0.32 ± 0.15^{ab}

Data represent average mean from three repetitions. Different letters in the same column represent the significant difference at $p < 0.05$

concentration of Hg increased. In addition, the BAF_{Hg} of *A. donax* was higher than the BAF_{Cd} (Table 3), further indicating that the ability of *A. donax* to accumulate Hg is greater than its ability to accumulate Cd.

The TF indicates the efficiency of translocation of HMs from the roots to the aerial part of the plant (Raj et al. 2020a, b). As shown in Table 3, the TF_{Hg} and TF_{Cd} of *A. donax* were less than 1 in all treatments; furthermore, the TF_{Hg} was higher than the TF_{Cd}, in accordance with the results of Cano-Ruiz et al. (2020). The TF_{Hg} and TF_{Cd} values suggest that phytostabilization rather than phytoextraction should be considered one of the main phytoremediation pathways of *A. donax*. Given the formation of volatile Hg, *A. donax* appears to accumulate and translocate Hg from the roots to the aerial parts and transform Hg^{2+} into volatile Hg. This process might reduce the toxicity of Hg in the plant and result in a high TF value. In fact, the TF_{Hg} was highest in the Hg2Cd1 treatment, whereas the TF_{Cd} was highest in the Cd1 treatment, further confirming previous reports that Cd is less toxic to plants than Hg (Cristaldi et al. 2020).

Conclusion

In this study, single Hg or Cd stress had no obvious effect on *A. donax* growth, whereas Hg–Cd combined stress significantly inhibited *A. donax* growth. The acidic root exudates released from *A. donax* at the beginning of the incubation period quickly decreased the solution pH, which facilitated the accumulation of Hg and Cd in *A. donax*. *A. donax* exhibited high efficiency in accumulating and translocating Hg and Cd, and the accumulation of Hg and Cd was higher in the roots than in the aerial parts. Furthermore, Hg was accumulated and translocated by *A. donax* more readily than Cd. Even in the absence of illumination, Hg^{2+} was transformed into volatile Hg by *A. donax*. Cd exhibited a “low promotion and high inhibition” effect on the accumulation, translocation, and transformation of Hg by *A. donax*. The increases in leaf SOD activity and GSH content indicated high resistance of *A. donax* to both single and combined Hg and Cd stress. Based on the TF values and the formation of volatile Hg, phytostabilization and phytovolatilization should be considered the main phytoremediation pathways for the removal of Hg by *A. donax*. In summary, *A. donax* is a potential candidate for the phytoremediation of combined Hg–Cd pollution even under neutral conditions because of its high accumulation ability and high tolerance to Hg and Cd.

Author contribution Xiuhua Li and Ling Zhao wrote the paper; Ying Teng, Yongming Luo, and Qiguo Zhao conceived and designed the experiments; Xiuhua Li and Ying Teng analyzed the data; Xiuhua Li performed the experiments and collected the data.

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Data availability The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate All the authors approved of participating in this publication.

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Competing interests The authors declare no competing interests.

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