Mercury uptake by *Paspalum distichum* L. in relation to the mercury distribution pattern in rhizosphere soil

Su Xu 1 · Ping Gong 1 · Wen Ding 1 · Shengchun Wu 1,2 · Xinwei Yu 3 · Peng Liang 1,2

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**Abstract**

*Paspalum distichum* L. was tested to evaluate their phytoremediation capacity for Hg contaminated soil through analyzing the dissipation of Hg in soil through a greenhouse study by using self-made rhizos box. Original soil samples were collected at Hg mining site with serious Hg contamination and a control site, respectively. Planting of *P. distichum*. L. for 60 d. Soil and plant samples were collected from four periods (0 d, 20 d, 40 d, and 60 d) and soil samples were collected from five different rhizosphere distance in horizontal direction (0–2 cm, 2–4 cm, 4–6 cm, 6–8 cm, 8–10 cm). The results showed that the presence of *P. distichum*. L. significantly accelerated the Hg dissipation in soil compared with control. Hg concentration in the rhizospheric soil was affected by the plant growth period and the distance to the plant roots. The closer of soil to the root of *P. distichum*. L., the lower mercury concentration in soil. During the 60-day growing period, the concentrations of total Hg (THg) and methylmercury (MeHg) reduced by 45% and 64%, respectively, in the rhizosphere (0–2 cm) of Hg contaminated soil. However, MeHg concentration was increased near the roots (0–4 cm) during the initial growing period (0–20 d), which may be attributed to the influence of root exudates. Root is the major part for Hg accumulation in *P. distichum*. L. The low ratio between Hg concentrations in underground and aboveground tissues indicated that it seemed difficult for Hg translocation from root to shoot. The highest THg (9.71 ± 3.09 μg·g⁻¹) and MeHg (26.97 ± 0.98 ng·g⁻¹) value in root of *P. distichum*. L. were observed at the 20th day when *P. distichum*. L. grown in Hg contaminated soil. The results of chemical fractions analyses showed that elemental Hg and residual Hg were the two major speciations followed by organic bound Hg in the Hg contaminated soil, which indicated the high bioavailability and ecological potential risk of Hg in Hg contaminated soil.

**Keywords** *Paspalum distichum* L. · Rhizobox · Mercury · Phytoremediation

**Introduction**

The high toxicity of mercury (Hg) is illustrated by its long residence times in the atmosphere, rapid bioaccumulative natures and wide distribution on the earth (Fitzgerald et al. 1998). The environmental problem of Hg pollution in soil is pretty serious to the safety of the local agricultural products. Anthropogenic Hg pollution has been brought about by the chloralkali process, cement production, mining and smelting, artisanal small-scale gold mining, coal burning, and oil refining, which together emit huge quantities of Hg to the environment (O'Connor et al. 2019). It is estimated 250–1000 Gg amount of Hg mass accumulated in soils (Obrist et al. 2018). Wanshan is a hot spots in China since the local environment were contaminated by emission and leaching of Hg from large quantities of mine waste (Feng & Qiu, 2008; Li et al. 2012; Qiu et al. 2009; Yin et al. 2017).

A worldwide concern in soil Hg remediation has been increasing at an accelerating tempo in recent years (Wang et al. 2020). Phytoremediation is an environmentally friendly technology as among top international priorities, it is relatively cheaper than physical or chemical treatments (Ashraf et al. 2019). Plants can reduce the concentrations or toxic effects of contaminants on account of their various natural features...
through accumulating heavy mental by their tissues to inactivate them Abhilash et al. 2008; Gomes et al. (2016). The uptake of Hg by plants depends upon the plant species, Hg bioavailability in soil and soil properties. Limnocharis flava L. was found in floodplains in America to facilitate the Hg removal and its removal efficiency unexpectedly up to 52% (Anning et al. 2013). Jatropha curcas L. were known as a pioneer with a contribution toward accumulating Hg in soils from the El Alacran mine, located in northeast Colombia (Marrungo-Negrete et al. 2015). In addition to the phytoremediation, the accumulation of Hg by crops also poses a serious threat to human health. Methylmercury (MeHg) is considered as a highly toxic form of Hg which can be transformed easily to human beings and some other wildlife. A lot of work so far has focused on the rice (Oryza sativa L.) with its enormous potential to accumulate MeHg than inorganic Hg (Meng et al. 2011; Zhang et al. 2019).

Paspalum distichum L. is a gramineae species of perennial weeds (Alcantara et al. 2016) and it has been identified as a great phytoremediation capability for Pb, Zn, and Cu (Shu et al. 2002). Our previous study has showed that P. distichum. L could accumulate MeHg in roots and exert a potential role in phytoremediating MeHg contaminated soil due to its abnormal uptake capacity to MeHg (Liang et al. 2016), five kinds of protein in root of P. distichum. L were response under Hg stress in the growth substrate (Ding et al., 2019). However, the effective distance of Hg uptake pattern of the dissipation gradient within the rhizosphere of this plant and remains unknown. It was hypothesized that P. distichum. L could be used as a potential Hg accumulator in Hg contaminated soil and the accumulation procedure would be presented in this study. Therefore, in this paper, a special rhizobox made by our team was used to separate roots of P. distichum. L and soil in an attempt to: (1) identify the effective Hg absorption area in the rhizospheric soil in term of dissipation gradient along with the greenhouse study; (2) compare Hg absorption intensity and transport characteristics of Hg among roots, stems, and leaves in P. distichum L; (3) evaluate the phytoremediating effects of P. distichum L on the different forms of Hg in different soils.

Materials and methods

Preparation of two kinds of natural soil

Soils used in this experiment were collected from an artificial Hg contamination area in Gouxi village, China (109°10′E, 27°33′N) as Hg contaminated soil (GXS); meanwhile a soil sample was collected from Hangzhou, China (119°44′E, 30°15′N) as reference soil (HZS) in Mar 2018. Both kinds of soil was fully mixed before planting and then freeze-dried and sieved (2 mm mesh) before detection. Surface soil was collected in agriculture sites in each sites.

Preparation of plants

P. distichum. L seeds were collected from a typical compact fluorescent lamp manufactures area in Gaohong town, northwestern Zhejiang province, China.

Experiment design

In order to separate the root system and soil, a rhizobox system was designed to achieve this demand (Fig. S1). The rhizobox is a transparent rectangular container which is made of toughened glass (L × W × H = 25 cm × 10 cm × 15 cm); and two nylon nets (mesh size, 30 µm) were installed in the middle of rhizobox. Each rhizobox containing 2.0 kg soil about 10 cm high (Fig. S1). Four P. distichum. L seeds were sown in the central compartment. All the rhizoboxes were arranged randomly in the greenhouse in Zhejiang A&F University (Hangzhou, China) with temperature control (25–30°C) and with normal illumination. The water holding capacity in rhizobox was maintained to 70% via dairy ultrapure water. Soil subsamples were taken from each rhizobox in accordance with the distance (0–2 cm, 2–4 cm, 4–6 cm, 6–8 cm, 8–10 cm) from nylon nets. P. distichum. L was collected and divided into three parts: root, stem, and leave. All soil and plants samples were freeze-dried, weighed and prepared for THg and MeHg analyses.

Chemical analyses

THg analysis

Soil subsamples (0.2 g) were digested with 5mL aqua regia (HCl:HNO3=3:1) in a 25 mL glass vial at 95 °C for 6h, while plant samples (0.2 g) with 5mL concentrated HNO3 (65% Suprapur®, Merck) for 4h. The volume was added Milli-Q water (18.2 Ω) after cooling to indoor temperature. Then the solution was filtered through 0.45 µm filter membrane. In the end, BrCl was utilized to oxidize the digested solutions and SnCl2 and NH2OH-HCl were used to reduce (Bloom and Fitzgerald 1988). Dual amalgamation cold vapor atomic fluorescence spectroscopy (CVAFS) (Brooks Rand Instruments, Seattle, WA) is an effective way to analyze THg of these samples.

MeHg analysis

Freeze-dried soil and plant samples were added into a clean 50 mL centrifuge tube with accurately weight of 0.2 g. Soil sample was leached by 1.5 mL 2M CuSO4 solution and 5 mL 5% HNO3 solution, while 5 mL 25% KOH solution was added in
the centrifuge tube of plant samples for about 3 h water bath. Next, samples were added about 10 ml dichloromethane and adequately shaken for 30 min. In order to separate the aqueous layer from the organic layer, the tube was centrifuged for 25 min at 3000 rpm. After that, a vacuum air pump was used to remove the aqueous layer and a new centrifuge tube had been prepared to receive the organic layer. The following step is to add Milli-Q water for about 30 mL and 40 mL. Back extraction used a bamboo in case of bumping of dichloromethane. Temperature of a water bath need to be kept in 45 °C until organic layer disappeared then increased to 80 °C with a purification with N₂ for approximately 8 min. At last, the sample was mixed with Milli-Q water to 45 mL (Liang et al. 1994). After the processing of ethylation, MeHg in sample was quantified by GC-CVAFS (Brooks Rand Instruments, Seattle, WA) (Liang et al. 2000).

Chemical fraction extraction procedure

Sequential extraction was performed according to a modified four stage procedure (Bloom et al. 2003). Five chemical fractionations were extracted sequentially:

F1: 25 ml of pure water was added to the centrifuge tube with 0.5 g soil sample. The suspensions were shaken for 24 h at 25 °C and the extract from the solid residue was separated by centrifugation (4000 rpm) and decanted into a borosilicate glass container. The residue was washed by adding 12.5 ml of distilled water, shaken for 15 min at 4000 rpm twice and the supernatant were also decanted into the borosilicate glass container.

F2: 25 ml of the mixture solution (0.1 mol/L CH₃COOH and 0.1 mol/L HCL) was added to the residue from step 1 in the centrifuge tube. The suspensions were shaken for 24 h at 25 °C and the extract from the solid residue was separated by centrifugation (4000 rpm) and decanted into a borosilicate glass container. The residue was washed by adding 12.5 ml of the mixture solution (0.1 mol/L CH₃COOH and 0.1 mol/L HCL), shaken for 15 min at 4000 rpm twice and the supernatant were also decanted into the borosilicate glass container.

F3: 25 ml of 1 mol/L KOH was added to the residue from step 2 in the centrifuge tube. The suspensions were shaken for 24 h at 25 °C and the extract from the solid residue was separated by centrifugation (4000 rpm) and decanted into a borosilicate glass container. The residue was washed by adding 12.5 ml of 1 mol/L KOH, shaken for 15 min at 4000 rpm twice and the supernatant were also decanted into the borosilicate glass container.

F4: 25 ml of 12 mol/L HNO₃ was added to the residue from step 3 in the centrifuge tube. The suspensions were shaken for 24 h at 25 °C and the extract from the solid residue was separated by centrifugation (4000 rpm) and decanted into a borosilicate glass container. The residue was washed by adding 12.5 ml of 12 mol/L HNO₃, shaken for 15 min at 4000 rpm twice and the supernatant were also decanted into the borosilicate glass container.

Determination of other parameters

The fresh soil sample was extracted with Milli-Q water (soil:water = 1:5) to analyze the dissolved organic carbon (DOC) with a total organic carbon analyzer (Shimadzu TOC-Vcph, Japan) (Subasinghe et al. 2009). The centrifugal supernatant was also used for the determination of pH.

Quality control of sample analysis

The quality of THg and MeHg in soils and P. distichum L were controlled by sample blanks, instrument blanks, certified reference materials (CRMs), and duplicate samples. The relative standard deviations of duplicate sample analyses were < 20% for THg and MeHg analysis. Moreover, GSS-27 for THg in soil, CRM 580 for MeHg in soil, and GSB-11 for THg in plant were used to ensure recoveries as much as possible (92.6%–104% for THg and 86.3%–96.3% for MeHg).

Data analyses

Multiple-way analyses of variance (ANOVA) were used to investigate the differences of Hg concentrations and pH value. The distribution gradient of these indicators was performed using Sigma plot 14.0.

Results

THg and MeHg concentrations in P. distichum. L

Figure 1 shows THg concentration in different part of P. distichum. L that planted in two kinds of soil. The original THg concentration in root, stem, and leaf of P. distichum. L was only 0.453±0.079, 0.043±0.019, and 0.064 ± 0.034 mg·kg⁻¹, respectively. THg in all of tissues of P. distichum. L grown in GXS increased on the 20th day immediately and decreased subsequently after 20th day (Fig. 1a). The maximum THg concentrations in root, stem, and leaf of P. distichum. L were 9.71 ± 3.09, 1.27 ± 0.71, and 3.42 ± 0.61 times higher than the original value. In addition, THg concentrations in root were significantly higher than those in stem and leaf (p < 0.05). As for HZS, THg in root of P. distichum. L was also increased along with the growing period when compared with the original value. However, no significant difference (p > 0.05) was observed for THg in root, stem, and leaf among the four sampling period (Fig. 1b).
MeHg concentrations in different tissues of *P. distichum* L. were shown in Fig. 2. At the initial stage, MeHg concentrations in root, stem, and leaf were 3.48 ± 0.913, 0.997 ± 0.240, and 1.09 ± 0.686 ng·g⁻¹. As same as THg, MeHg concentration in different tissues also increased immediately during the first 20 days and decreased subsequently. The highest MeHg concentration was observed in root of *P. distichum* L with 27.0 ± 0.98 ng·g⁻¹ for GXS, which was 7.75 times higher than the original value. However, no significant change was observed for MeHg in shoot of *P. distichum* L in GXS during the sampling period. MeHg concentrations in roots were significantly higher than stern and leaf (p < 0.01). As for *P. distichum* L grown in HZS, MeHg concentration in root also increased to the highest value at the 40th day with 6.65 ± 0.085 ng·g⁻¹, which was 1.91 times higher than the original value. At the end of growing period, MeHg concentration in roots decreased to 10.4 ± 6.50 and 4.17 ± 0.23 ng·g⁻¹ for GXS and HZS, respectively. From the 20th day to 60th day, the reduction of MeHg concentration in roots for GXS were 61%, while THg was lowered by 75.6%.

**Speciation of Hg in soil**

Figure 3 shows the temporal change of THg concentration in soil. There is a significant reduction of THg in GXS (Fig. 3a). With the lasting of the greenhouse study, the concentration of THg decreases and the great rate of decline appeared near the root. At the 60th day of 0–2 cm, the concentration of THg reduced from the initial concentration of 197.86 ± 34.43 mg·kg⁻¹ to 107.86 ± 58.91 mg·kg⁻¹ in GXS. In comparison, the difference between the maximum concentration of THg (0.15 ± 0.02 mg·kg⁻¹) and the minimum value (0.09 ± 0.02 mg·kg⁻¹) is very weak for HZS.

MeHg concentration in soil increased in the 20th day and decreased subsequently during the following growing period (Fig. 4). In the 20th day, the maximum MeHg concentration for GXS was 27.7 ± 4.97 ng·g⁻¹ and appeared at 0–2 cm respectively and there was a downward trend with the distance gradients increasing. In comparison, the variation of MeHg
The concentration of MeHg in HZS was increasing along with the growing period, and reached the highest value of 1.03 ± 0.60 ng·g⁻¹ on 60th day. However, the minimum value (0.18 ± 0.32 ng·g⁻¹) appeared in no plant control.

Chemical fractions of Hg in soil were shown in Fig S2. It was noted that the chemical fractions for Hg in two soils were different. For GXS, residual Hg, element Hg, and organo-chelated Hg were the three major fractions. Bioavailable Hg, including water soluble Hg and simulated gastric acid Hg accounted for less than 5% of total Hg. In comparison, residual Hg and element Hg were the major fractions of Hg in HZS. The other three speciations accounted for less than 1% of total Hg. Along with the growing periods, bioavailable Hg increased gradually in GXS and especially near the plant roots (0–2 cm), while organo-chelated Hg decreased firstly and increased subsequently. In contrast, residual Hg and element Hg increased in the 20th day and decreased gradually. However, few organo-chelated Hg was observed for HZS and element Hg increased in the 20th day for HZS.

**pH value and DOC concentration in soil**

The pH value and DOC concentration of two kinds of soil were show in Fig S3 and S4. During the experiment period, pH value of GXS and HZS decreased from 8.35 ± 0.02 and 6.74 ± 0.04 to 8.02 ± 0.03 and 6.34 ± 0.13 respectively. This might attribute to the organic acid from root exhaust during plant growth period. In addition, DOC concentration of GXS was higher than those in HZS, but there is no significant trend for DOC concentration in these two kinds soil.

**Discussions**

Accumulation characteristics of THg and MeHg in different parts of *P. distichum* L

For the terrestrial plant, Hg in root was hard to transport to the aboveground. Only 0.45–0.65% of Hg could transport to the aboveground in willows (Greger et al. 2005). However,
Lominchar et al. (2015) reported that wetland plant, i.e., *Typha domingensis* had a strong ability for Hg accumulation from sediment. Castro et al. (2009) also reported that monocots plant showed higher Hg retention in the belowground organs while the dicots presented a more pronounced translocation to the aboveground. In this study, the ratio of THg between shoot (stem and leaf) and root increased from 12.9% to 28.0% and 17.1% at the 20th day for GXS and HZS respectively (Table 1), which indicated that Hg could transport from root to shoot for *P. distichum*. L, although Hg concentration in shoot were significantly lower than root. Growth periods also affect THg accumulation in *P. distichum*. L obviously.

There is no significant change observed for MeHg concentrations in stem and leaf during the growing period for the shoot part. This demonstrated that MeHg is not easier to be transported from root to shoot and MeHg were easier to be stored in shoot than root, since root MeHg concentration decreased subsequently. Previous studies showed that MeHg is easily accumulated in seeds of *Oryza sativa* L. (Meng et al. 2011; Zhang et al. 2019), and even higher than that in soil (Meng et al. 2011). However, MeHg concentrations in shoots of *P. distichum*. L were significantly lower than MeHg in roots and soil in this study, which may be due to the difference of growing environment and growth cycles. The production of MeHg in flooding soil were higher than drying soil since the anaerobic condition were beneficial for MeHg formation (Yin et al. 2020). Water holding capacity in soil in this study were around 70%, MeHg formation in soil was therefore limited. In addition, peak values of MeHg in rice seeds were frequently observed in the last growing period, along with the drying of flooding environment. However, the growth cycles of *P. distichum*. L were only 60 days and lack of the period from flooding to drying.

Bioavailability of Hg in the substrate and the characteristics of plant have been demonstrated could affect the phytoremediation procedure of Hg contaminated soil or sediment (Lomonte et al. 2010), although Hg is known to be relatively immobile since it can be bound strongly with soil particles and organic matter (Liao et al., 2009). However, growth cycles might be another factor influence Hg accumulation in plant since it is considerable for the phytoremediation engineering. Previous studies reported that THg in *Cyrtomium macrophyllum* (Makino) Tagawa and *Jatropha curcas* L. increased gradually along with the growing period (Marrugo-Negrete et al. 2015). However, the highest of THg and MeHg concentration of *P. distichum*. L in this study were shown in 20th day and they decreased subsequently after 20th day. This indicated that *P. distichum*. L could accumulate Hg immediately with high efficiency. Based on the result of this study, the harvesting of *P. distichum*. L during the phytoremediation could reduce to 20 days. This was indeed beneficial for reducing the phytoremediation cycles.

### The reduction of THg in soil during the phytoremediation period

THg concentration in the substrate during the phytoremediation process was affected by different factors, e.g., accumulation ability of plant, the additives in the soil, and the evaporation of Hg from the substrate. In this study, the accumulation amount of Hg were calculated by production of the THg concentration in plant and plant biomass (Table 2). The amount of Hg in root were relatively higher than those in shoot. The accumulation amount did not increase along the growing period. Table 3 shows the reduced amount of Hg in soil before and after experiment. There were 2.65 and 0.084 g Hg in soil reduced, which accounted for 30.3% and 57.4% of the original amount of Hg in GXS and HZS respectively. However, the contribution of phytoaccumulation only accounted for no more than 0.1% of the reduction. Most of Hg from soil would be reduced through evaporation (Gworek et al, 2020). More interesting, the reduced amount of Hg decreased along with the distance from the root (Table 3). These phenomenon indicates that the accumulation of Hg by *P. distichum*. L has an effect on the dissipation of Hg in soil.

### MeHg formation in the rhizosphere of *P. distichum*. L

MeHg concentration in soil increased gradually, which indicated that the planting of *P. distichum*. L could promote MeHg formation in soil and the influence range can reach 4 cm in rhizosphere distance. MeHg could be formed in the reducing conditions that occur in many permanently or periodically flooded soils (O’Connor et al. 2019). The bioavailability of Hg species to methylating microorganisms, pH value, organic matter concentrations, sulfur concentrations are crucial in determining the extent of this conversion (Lei et al. 2019). pH value is a key factor for MeHg formation in soil. The pH value of GXS showed a downward trend with the time increasing (Fig. S3). This is attributed to the acidic root secretions of *P. distichum*. L which lower soil pH and create a favorable circumstance for the formation of MeHg in the soil, especially that around the root of *P. distichum*. L. Studies have shown that the root secretions of plant can change the

| Soil | Period (day) | THg | MeHg |
|------|--------------|-----|------|
| HZS  | 20           | 0.17 ± 0.03 | 0.63 ± 0.13 |
|      | 40           | 0.130.07 | 0.46 ± 0.05 |
|      | 60           | 0.12 ± 0.07 | 0.47 ± 0.10 |
| GXS  | 20           | 0.28 ± 0.11 | 0.21 ± 0.03 |
|      | 40           | 0.06 ± 0.02 | 0.13 ± 0.03 |
|      | 60           | 0.11 ± 0.04 | 0.80 ± 0.19 |

Table 1 Transfer coefficient of THg and MeHg by *P. distichum*. L.
solubility and mobility of the harmful substances in rhizosphere by changing soil pH (Natasha et al. 2020). Therefore, it can be speculated that the acidic root secretion can activate the solubility of Hg, thus promoting the absorption of Hg by *P. distichum*.

Bioavailability of Hg is another key factor for MeHg formation. Generally, sequential leaching provides classification of Hg species in terms of bioavailability or mobility. Researchers usually defined the bioavailability of Hg in different sequential extraction fractions as either high, medium or low. In this study, water soluble Hg and simulated gastric acid Hg were defined as high bioavailability, organo-chelated Hg and element Hg was moderate bioavailability, while residual Hg was low bioavailability in the substrate. During the experiment, high bioavailability of Hg, i.e., water soluble Hg and simulated gastric acid Hg in GXS increased, which indicated that acid root exudation promote the transfer of Hg from low mobility bioavailability to high bioavailability phase. Root exudates facilitate rhizosphere interactions by serving as energy sources for microorganisms and acting as chemical attractants and repellents (Akhami et al. 2017). The rhizosphere is strongly influenced by plant metabolism through the release of carbon dioxide and secretion of photosynthate.

**Conclusion**

The experiment showed that *P. distichum* has strong accumulation ability for both THg and MeHg. Root is the major part for Hg accumulation. During the phytoaccumulation process, peak values of THg and MeHg were observed on the 20th day for GXS and decreased gradually, which indicated that accumulation of Hg by *P. distichum*. L have growth cycles effect. Dispersion gradient effect was observed during the phytoaccumulation process. THg in soil reduced near the roots reduced higher than the far away soil. Therefore, in practice, we can use the *P. distichum*. L to quickly remove the Hg in soil when *P. distichum*. L grows after 20–40 days, especially the farmland soil in abandoned mining areas and the planting density of *P. distichum*. L could be less than 40 cm.

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**Availability of data and materials** All data generated or analyzed during this study are included in this manuscript and its supplementary information files.

**Authors’ contributions** Su Xu, Ping Gong, and Wen Ding analyzed and interpreted the data regarding this experiment. Shengchun Wu, Xinwei Yu, and Peng Liang performed the explanation of the results. All authors read and approved the final manuscript.

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**Declarations**

**Ethical approval** Samples of this study only including soil and plant. Therefore, this manuscript did not require the ethical approval.

**Consent to participate** Not applicable.

**Competing interests** The authors declare that they have no competing interests.

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