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The distribution and enrichment characteristics of copper in soil and *Phragmites australis* of Liao River estuary wetland

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Abstract The aims of the present investigation were to reveal the distribution and enrichment characteristics of copper in soil and *Phragmites australis* of Liao River estuary wetland. The concentrations of copper in root, stem, leaf, and ear of *Phragmites australis* as well as in soil were determined to study the absorption capacity of copper by wild *Phragmites australis* of Liao River estuary wetland. The study was carried out at test pool of the Shenyang Agricultural University, and the experimental materials (soil, irrigating water and *Phragmites australis*) were derived from Liao River estuary wetland. The concentrations of copper in soil and *Phragmites australis* were 16.4441 to 49.0209 mg/kg and 0.8621 to 89.5524 mg/kg, respectively. The results indicated that the enrichment coefficients of copper in different tissues of *Phragmites australis* changed with the growth of *Phragmites australis*. The results revealed that the enrichment coefficients of copper in the whole *Phragmites australis* were greater than 1 at each growing stage of the *Phragmites australis*. The results also showed that the transfer coefficients of *Phragmites australis* to copper changed with the growth of *Phragmites australis*. The results revealed that the *Phragmites australis* had a good removal effect on copper from soil and had some characteristics of copper hyperaccumulator.

Keywords Enrichment coefficient · Transfer coefficient · Migration regulation · Hyperaccumualtor

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Introduction

Wetland is one of the most important ecosystems. With the rapid development of industry, livestock and poultry industries, agriculture, and fishery, wetland has been seriously threatened by heavy metals pollution. In recent years, the scholars have paid close attention to the wetland protection, particularly, with regard to the heavy metal pollution to wetland (Li et al. 2008; Sarkar et al. 2011; Naseh et al. 2012; Salamat et al. 2014; Wei et al. 2014; Choo et al. 2015; Cheng et al. 2015; Gao et al. 2016a, b; Yin et al. 2011).

Liao River estuary wetland has total area of about 128,000 ha (Fig. 1). The geographic coordinates of the Liao River estuary wetland are $121.5^{\circ}-122^{\circ}$ E, $40.75^{\circ}-41.17^{\circ}$ N. It had been reported that the Liao River estuary wetland had been found to be polluted by heavy metal pollutants. Guo et al. studied the removal efficiency of Cu, Zn, Pb, and Cd in different growth stages of *Phragmites australis* of Liao River estuary wetland

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(Guo et al. 2012). Zhang et al. made a complete health risk assessment of mercury, arsenic, chrome, and lead pollution by using Philippine clams and human health risk assessment model in different areas around Bohai Bay (Zhang et al. 2012). Yang et al. evaluated the distribution and accumulation of Cd, Pb, Cu, and Zn in water of Liao River estuary wetland by using fuzzy comprehensive evaluation method and statistics method (Yang et al. 2012). Hui et al. researched the distribution and accumulation of Cd, Cu, Pb, and Zn in soil of Liao River estuary wetland by using set pair analysis and comprehensive evaluation model (Hui et al. 2016). Wen et al. investigated the profile distributions and toxic risks of Cd, Cu, Pb, and Zn in soil of the Yellow River Delta of China by using index of geo-accumulation, correlation analysis, and principal component analysis (Wen et al. 2017). The contents of Cu, Zn, Cr, Pb, and Ni in sediment of the Yellow River Delta of China were determined to investigate their spatial and temporal distributions, sources, and ecotoxities (Bai et al. 2016). With the rapid development of industry, livestock and poultry, agriculture, and fishery, heavy metal pollution had become a serious threaten to wetland (Salamat et al. 2014; Choo et al. 2015; Gao et al. 2016a, b; Glazatov et al. 2014; Gao et al. 2008; Deng et al. 2004; Sharma et al. 2018; Zhang 2007; Hao et al. 2012). Copper is a necessary element for plants, and a small amount of copper can meet the needs of the plant growth. A large number of copper may result in crop quality and soil quality reduction (Pan et al. 2000). The excessive copper in soil leads to increased absorption and accumulation of copper in crops, which can cause serious damage to human health through food chain (Ma et al. 2013; Xu et al. 2006; Zhang et al. 2010; Loland and Singh 2004). The study showed that the content of copper in rice seedling increased with copper concentration of solution under hydroponic conditions, while the trend did not appear in the root system of rice (Si et al. 2007). Therefore, it is of great significance to study the absorption and transformation characteristics of plants to copper. The aims of the present investigation were to reveal the distribution and enrichment characteristics of copper in soil and *Phragmites australis* of Liao River estuary wetland. The concentrations of copper in root, stem, leaf, and ear of *Phragmites australis* as well as in soil were determined to study the absorption capacity of copper by wild *Phragmites australis* of Liao River estuary wetland. The results indicated that the enrichment coefficients of copper in different tissues of *Phragmites australis* changed with the growth of *Phragmites australis*. The results revealed that the enrichment coefficients of copper in the whole *Phragmites australis* were greater than 1 at each growing stage of the *Phragmites australis*. The results also showed that the transfer coefficients of *Phragmites australis* to copper changed with the growth of *Phragmites australis*. The results revealed that the *Phragmites australis* had a good removal effect on copper from soil and had some characteristics of copper hyperaccumulator.

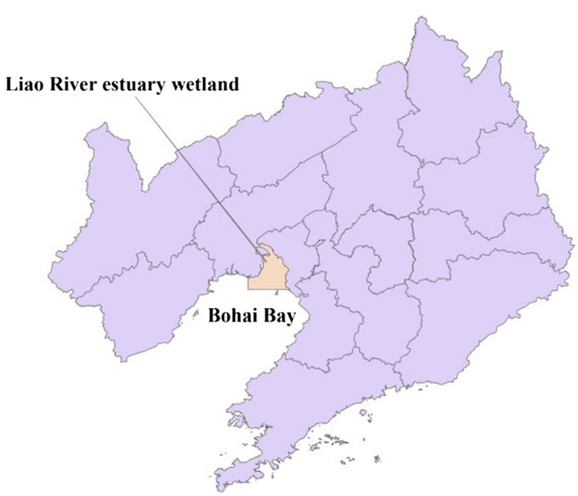


Fig. 1 Liao River estuary wetland in Liaoning province

Materials and methods

The study was carried out at test pool of the Shenyang Agricultural University. The experimental materials (soil, irrigating water, and *Phragmites australis*) were derived from Liao River estuary wetland. The experimental water came from the sewage outfalls of the paper mill near the Liao River estuary wetland. The concentration of copper in experimental water was 0.6 mg/L, pH = 7.6, and the CODcr concentration of experimental water was 300 mg/L. The experimental soil was the undisturbed soil of Liao River estuary wetland. The soil type was meadow soil and pH = 8.51. The soil bulk density was 1.03 g/cm^3 and the organic matter content of soil was 1.05%. The *Phragmites australis* was transplanted from Liao River estuary wetland in 2009. The rhizome of *Phragmites australis* with no diseases and pests was dug from Liao River estuary wetland. The rhizome of *Phragmites australis* was cut into about 0.2-m section with at least three buds in it. The rhizome of *Phragmites australis* was transplanted to test pool of Shenyang Agricultural University to ensure the survival of *Phragmites australis*. About 1250 *Phragmites australis* were transplanted into each test pool. The *Phragmites australis* is planted with both row spacing and line spacing at the length of 0.04 m. According to the Specifications for Irrigation Experiment of China (SL13–2015), the growth period of *Phragmites australis* was divided into seedling stage, leaf stage, jointing stage, heading flowering period, and mature period. The specific process of this experiment was as follows. Chemical oxygen demand (CODcr) was an important index to measure organic matter content in water. The use of papermaking wastewater promoted the growth of *Phragmites australis* and improved the physical and chemical properties of soil. The irrigating waters with three types of CODcr concentrations were prepared for experimental use by mixing the papermaking wastewater with tap water. The CODcr concentrations of irrigating water were 50, 175, AND 300 mg/L, respectively. The irrigating waters with three types of CODcr concentrations were injected at the initial stage of each growth period of *Phragmites australis* and then the tap water was injected to maintain the water depth of 0.1 m above the soil surface to ensure the growth of *Phragmites australis*. The control groups were established in each growth period of *Phragmites australis* and the control group was irrigated with tap water. The CODcr concentration of tap water was 0 mg/L. Five soil samples were taken at each testing pool. Soil samples were collected at five depths of 0–5, 5–10, 10–20, 20–40, and 40–60 cm and then stored in polyethylene containers. The soil samples were ground after drying in shade naturally and passed through 0.149-mm sieve. The concentrations of copper in soil were measured by the mixed acid digestion method with HNO3–HClO4–HF (Fig. 2). Five *Phragmites australis* samples were randomly selected in each growth stage. The *Phragmites australis* samples (root, stem, leaf, and ear) were rinsed three times with tap water and then with distilled water. The concentrations of copper in *Phragmites australis* were determined as previously described (Shao et al. 2010) by inductively coupled plasma-mass spectrometry (ICP–MS) (Figs. 3, 4, 5, and 6). The quantification limit for copper was 0.1880 mg/kg.

Results and discussion

The average concentrations of copper in *Phragmites australis* and soil

The highest average concentration of copper in soil was 49.0209 mg/kg under the condition that the CODcr concentration of irrigating water was 50 mg/L. Its corresponding growth stage of *Phragmites australis* was heading flowering period. The lowest average concentration of copper in soil was 16.4441 mg/kg in the control group. Its corresponding growth stage of *Phragmites australis* was heading flowering period.

The highest average concentration of copper in root of *Phagmites australis* was 89.5524 mg/kg under the condition that the CODcr concentration of irrigating water was 300 mg/L. Its corresponding growth stage of *Phragmites australis* was jointing stage. The lowest average concentration of copper in root of *Phragmites australis* was 11.3485 mg/kg in the control group. Its corresponding growth stage of *Phragmites australis* was mature period.

The highest average concentration of copper in stem of *Phragmites australis* was 6.5232 mg/kg under the condition that the CODcr concentration of irrigating water was 300 mg/L. Its corresponding growth stage of *Phragmites australis* was jointing stage. The lowest average concentration of copper in stem of *Phragmites australis* was 0.8621 mg/kg in the control group. Its

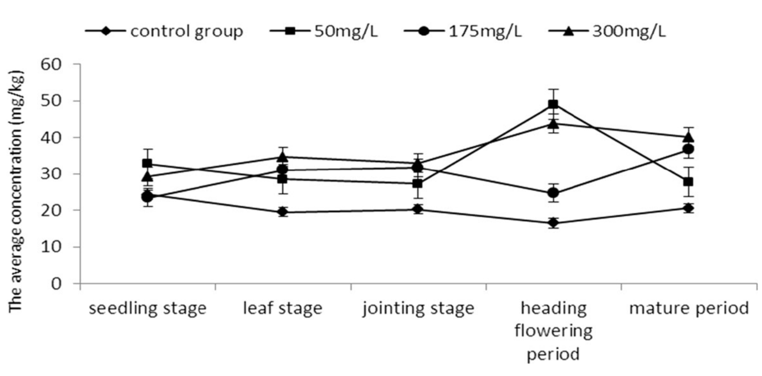
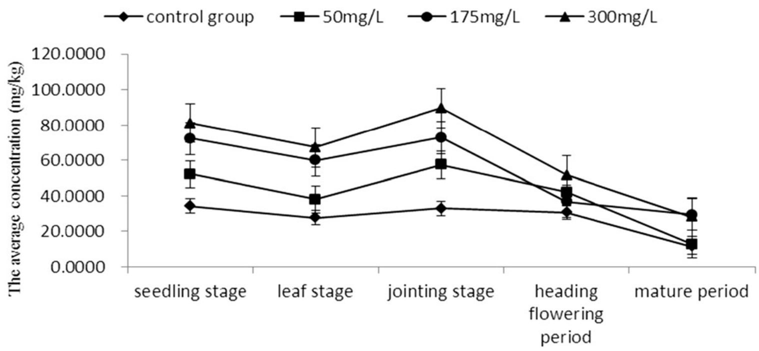


Fig. 2 The average concentration of copper in soil

Fig. 3 The average concentration of copper in root of *Phragmites australis*



corresponding growth stage of *Phragmites australis* was heading flowering period.

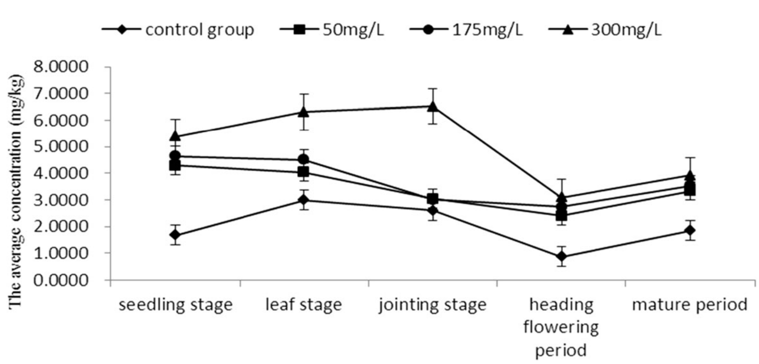
The highest average concentration of copper in leaf of *Phragmites australis* was 10.7213 mg/kg under the condition that the CODcr concentration of irrigating water was 300 mg/L. Its corresponding growth stage of *Phragmites australis* was seedling stage. The lowest average concentration of copper in leaf of *Phragmites australis* was 2.0522 mg/kg in the control group. Its corresponding growth stage of *Phragmites australis* was heading flowering period.

The highest average concentration of copper in ear of *Phragmites australis* was 14.6200 mg/kg under the condition that the CODcr concentration of irrigating water was 50 mg/L. The lowest average concentration of copper in ear of *Phragmites australis* was 5.3100 mg/kg in the control group. Their corresponding growth stages were mature period and heading flowering period respectively. Figures 3, 4, 5, and 6 also indicated that the CODcr concentration of irrigating water significantly increased the copper concentration in different tissues of *Phragmites australis*. The results provided theoretical foundation for the phytoremediation of *Phragmites australis* on copper pollution.

Enrichment coefficient

Enrichment coefficients reflected the enrichment degree of heavy metals in plants (Yan et al. 2016; Pan et al. 2010; Gao et al. 2016a, b; Ji et al. 2015; Grisey et al. 2012; Sarkar et al. 2011; Ghassemzadeha et al. 2008; Zhang et al. 2013). Zhang et al. investigated the levels, sources, and toxic risks of Al, As, Cd, Cr, Cu, Ni, Pb, and Zn in the Yellow River Delta of China by using enrichment factor, toxic units, toxic risk index, and principal components analysis (Zhang et al. 2016). Lu et al. assessed the spatial and temporal distributions of heavy metals (As, Ni, Cr, Zn, Pb, Cd, and Cu) and the potential risk to the *Tamarix chinensis* Wetland (Lu et al. 2016). The enrichment coefficients for the root (ECR) and for the stem (ECS) and for the leaf (ECL) and for the ear (ECE) of *Phragmites australis* to copper were defined as follows. ECR = the average concentration of copper in root of *Phragmites australis*/the average concentration of copper in soil; ECS = the average concentration of copper in stem of *Phragmites australis*/the average concentration of copper in soil; ECL = the average concentration of copper in leaf of *Phragmites australis*/the average concentration of

Fig. 4 The average concentration of copper in stem of *Phragmites australis*



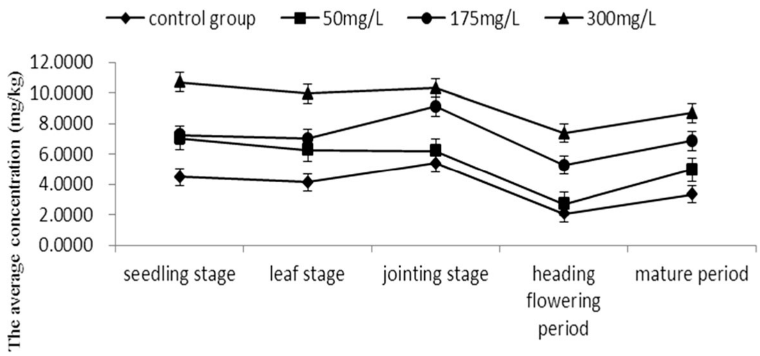


Fig. 5 The average concentration of copper in leaf of *Phragmites australis*

copper in soil; ECE = the average concentration of copper in ear of *Phragmites australis*/the average concentration of copper in soil.

The highest enrichment coefficient of *Phragmites australis* to copper appeared at seedling stage under the condition that the CODcr concentration of irrigating water was 175 mg/L. The highest enrichment coefficient of *Phragmites australis* to copper was 3.0845. The lowest enrichment coefficient of *Phragmites australis* to copper appeared at heading flowering period under the condition that the CODcr concentration of irrigating water was 50 mg/L. The lowest enrichment coefficient of *Phragmites australis* to copper was 0.0489 (Table 1).

The enrichment coefficients of copper by root of *Phragmites australis* were greater than 1 at seedling stage, leaf stage, jointing stage, and heading flowering period, except for the enrichment coefficient at heading flowering period under the condition that the CODcr concentration of irrigating water was 50 mg/L. Although the enrichment coefficients of copper by root of *Phragmites australis* were less than 1 at mature period, the enrichment abilities of copper by root of *Phragmites australis* were higher than that of leaf, stem, and ear of *Phragmites australis* (Table 1). The reason for this phenomenon was that the root endothelial tissue of *Phragmites australis* prevented copper from root to the leaf, stem, and ear of *Phragmites australis*.

Table 2 indicated that the enrichment coefficients of copper by the whole *Phragmites australis* were greater than 1 at each growth stage of *Phragmites australis* under any irrigation condition. The highest enrichment coefficient of copper by the whole *Phragmites australis* was 3.5778 and the lowest enrichment coefficient of copper by the whole *Phragmites australis* was 1.0993. It was clear that the *Phragmites australis* had a good removal effect on copper from soil and had some characteristics of copper hyperaccumulator (Nie et al. 2016; Wang et al. 2017). In most cases, the CODcr concentration of irrigating water greatly promoted the absorption of copper by *Phragmites australis* (Table 2). This result also indicates that we should try a similar catalyst in the study of remediation for heavy metal pollution so as to improve the removal efficiency of heavy metal pollution. The investigation of these works is left for future work.

Transfer coefficient

The transfer coefficient estimated the ability of the transfer heavy metals from roots to shoots (Zhao et al. 2014; Ku et al. 2014; Soriano-Disla et al. 2014; Saha

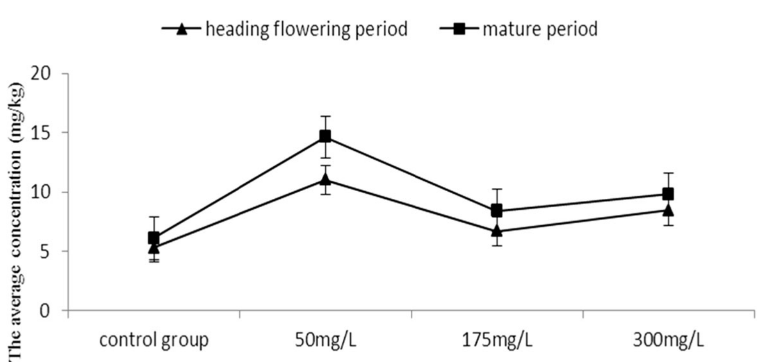


Fig. 6 The average concentration of copper in ear of *Phragmites australis*

Table 1 Enrichment coefficients of *Phragmites australis* to copper

Enrichment coefficient	CODcr	Seedling stage	Leaf stage	Jointing stage	Heading flowering period	Mature period
ECR	0	1.4121	1.4250	1.6247	1.8708	0.5509
	50	1.5855	1.3303	2.1050	0.8560	0.4603
	175	3.0845	1.9265	2.3006	1.4936	0.8013
	300	2.7734	1.9408	2.7177	1.1798	0.7035
ECS	0	0.0690	0.1532	0.1287	0.0524	0.0897
	50	0.1305	0.1415	0.1116	0.0489	0.1200
	175	0.1984	0.1446	0.0946	0.1110	0.0961
	300	0.1840	0.1822	0.1980	0.0705	0.0979
ECL	0	0.1850	0.2119	0.2673	0.1248	0.1620
	50	0.2145	0.2202	0.2275	0.0551	0.1789
	175	0.3093	0.2248	0.2872	0.2136	0.1864
	300	0.3606	0.2874	0.3133	0.1683	0.2168
ECE	0	0	0	0	0.3229	0.2967
	50	0	0	0	0.2248	0.5268
	175	0	0	0	0.2709	0.2288
	300	0	0	0	0.1929	0.2441

et al. 2016; Wei et al. 2014; Li et al. 2008). Transfer coefficient of *Phragmites australis* to copper = the sum of the average copper concentration in leaf, stem, and ear of the *Phragmites australis*/the average concentration of copper in root of the *Phragmites australis*.

Table 3 also indicated that the irrigating waters with three types of CODcr concentrations promoted the migration of copper from root to leaf, stem, and ear of *Phragmites australis* to some extent. The results showed that the irrigating water with CODcr concentration of 50 mg/L was most favorable for the transfer of copper from root to leaf, stem, and ear of *Phragmites australis* at seedling stage, leaf stage, and mature period. However, the irrigating water with CODcr concentration of 175 mg/L was most favorable for the transfer of copper from root to leaf, stem, and ear of *Phragmites australis* at heading flowering period. It was very strange that the irrigating water with CODcr concentration of 0 mg/L was most favorable for the transfer of copper from root to leaf, stem, and ear of *Phragmites australis* at jointing stage. The results also showed that the transfer coefficients of *Phragmites australis* to copper changed with the growth of *Phragmites australis*. The transport capacities of *Phragmites australis* to copper were showed as follows: mature period > heading flowering period > leaf stage > jointing stage > seedling stage. The highest transfer coefficient of *Phragmites australis* to copper appeared at mature period. The highest transfer coefficient of *Phragmites australis* to copper was 1.7938 under the condition that the CODcr concentration of irrigating water was 50 mg/L. The lowest transfer coefficient of *Phragmites australis* to copper appeared at

Table 2 The enrichment coefficients of whole *Phragmites australis* to copper

Enrichment coefficient	CODcr	Emergence stage	Leaf-expansion stage	Jointing stage	Heading to flowering stage	Mature stage
Phragmites australis	0	1.7276	1.7901	2.0207	2.3709	1.0993
	50	1.9984	1.6920	2.4441	1.1848	1.2860
	175	3.5778	2.2959	2.6824	2.0891	1.3126
	300	3.3190	2.4104	3.2290	1.6115	1.2623

Table 3 Transfer coefficients of *Phragmites australis* to copper

Growing stage of *Phragmites australis*	CODcr (mg/L)	Transfer coefficient
Seedling stage	0	0.1798
	50	0.2176
	175	0.1644
	300	0.1985
Leaf stage	0	0.2563
	50	0.2719
	175	0.1917
	300	0.2419
Jointing stage	0	0.2438
	50	0.1611
	175	0.1660
	300	0.1881
Heading flowering period	0	0.2673
	50	0.3842
	175	0.3987
	300	0.3659
Mature period	0	0.9956
	50	1.7938
	175	0.6380
	300	0.7942

jointing stage. The lowest transfer coefficient of *Phragmites australis* to copper was 0.1611 under the condition that the CODcr concentration of irrigating water was 50 mg/L. The reason for this phenomenon was that the accumulation of copper in stem and leaf of *Phragmites australis* was not as fast as that of stem and leaf growth at jointing stage (Wang et al. 2016; Lin et al. 2014).

The enrichment ability of copper in root of *Phragmites australis* was the highest during the whole growth period of *Phragmites australis*. This was mainly due to the fact that the rhizomes and adventitious roots of *Phragmites australis* were very well-developed. In addition, the root tissue of *Phragmites australis* secreted phytosidemphores, which promoted the absorption of copper by root of *Phragmites australis* (Römheld 1991). The accumulation capacity of copper in root of *Phragmites australis* was the lowest at mature stage, which attributed to the relatively stable physiological metabolism in the late growth stage of *Phragmites australis* and the migration of copper from root to stem, leaf, and ear. The enrichment ability of copper in stem of *Phragmites australis* was the highest at leaf stage, which attributed to the transport of copper from root to stem. The enrichment ability of copper in stem of *Phragmites australis* was the lowest at heading flowering period. The phenomenon was caused by the dilute effect, namely the accumulation ability of copper in stem decreased with the increase of *Phragmites australis* biomass. The enrichment ability of copper in leaf of *Phragmites australis* was the highest at jointing stage. This was mainly due to the fact the leaves biomass of *Phragmites australis* increased sharply at jointing stage. The enrichment ability of copper in leaf of *Phragmites australis* was the lowest at heading flowering stage. This was mainly due to the fact that the metabolism of *Phragmites australis* slowed down. The enrichment ability of copper in ear of *Phragmites australis* was high at mature period and was low at heading flowering period, which attributed to the physiological metabolism of different tissues of *Phragmites australis* at different growth stages.

Conclusions

The study had showed that *Phragmites australis* played an important role in removing the copper pollution from soil of Liao River estuary wetland. It is clear that the distribution and accumulation of heavy metals in *Phragmites australis* and soil of Liao River estuary wetland is a complex process. The changes of composition and pH of irrigating water will affect the distribution and accumulation of heavy metals in *Phragmites australis* and soil. In addition, the distribution and accumulation of heavy metals in *Phragmites australis* and soil of Liao River estuary wetland is also affected by many natural factors. Therefore, how to further improve the enrichment ability of *Phragmites australis* to heavy metals in Liao River estuary wetland will be the focus of future research. It is well known that the Liao River estuary wetland has a variety of wetland plants, such as *Phragmites australis* and Suaeda glauca and so on. Therefore, synergistic or antagonistic effects of different heavy metals on different wetland plants need to be analyzed. In addition, the interaction of different heavy metals and the absorption mechanism of *Phragmites australis* to different heavy metals should be further studied in heavy metal compound pollution area.

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