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Evaluation of heavy metals accumulation by two emergent macrophytes from the polluted soil: an experimental study

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Abstract The concentrations of copper (Cu) and lead (Pb) in, and the biomass of, the different parts of *Persicaria glabra* (Willd.) Gamez and *Juncellus alopecuroides* (Rottb.) C.B.Cl. were evaluated while grown in pots under laboratory conditions. Cu and Pb were added as sulphates (50, 100, 200, 400 mg/kg) to the pots. Heavy metal concentrations in the plants were measured by atomic absorption spectrometry. Results reveal that the biomass of *J. alopecuroides* (particularly roots) was higher than *P. glabra*, and that the growth tendency of macrophytes decreased with increasing heavy metal concentration in the soil, while in *P. glabra*, biomass went on increasing with the increase in copper concentration. Heavy metal accumulation in the roots was more than in aerial parts, and, therefore, barring two exceptions, the transfer factor of heavy metals from roots to aerial parts showed as less than 1, suggesting less transfer of heavy metals from roots to aerial parts. Thus, these macrophytes are efficient accumulators of trace elements, particularly *J. alopecuroides*, which can be recommended for biofiltration of heavy metals from contaminated soils.

Keywords Copper · Lead · *Juncellus alopecuroides* · *Persicaria glabra* · Phytoremediation

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1 Introduction

Anthropogenic activities related to industries have been contributing many hazardous contaminants to the environment, which mostly consist of organic compounds and heavy metals, posing serious risks to the environment and to human health. Over the last few decades, the discharge of heavy metals into the environment (soil, water, and air) has become a critical problem in many countries including India. Heavy metal pollution in the soil is a condition of global concern with regards to its direct/indirect implications on plants, animals, and human health (Bindu et al. 2009).

In recent years, various researchers have worked on the accumulation of heavy metals in plants (Barman et al. 2000; Gupta et al. 2008; Kaer et al. 1998; Sarah et al. 2007; Tiwari et al. 2008), particularly macrophytes, because they grow faster, produce more biomass, and can remediate metals efficiently from the soil (Harvey 1995).

Heavy metals accumulate in different parts of plants depending upon the plant species, soil condition, and the type of heavy metals (Barman et al. 2001; Espinoza-Quinones et al. 2008). Soil parameters (pH, organic matter content, cation exchange capacity, nutrients, etc.) are known to affect the availability of heavy metals for uptake by plants (Ashworth and Alloway 2007; Wu et al. 2004).

The remediation methods require high energy input and expensive machinery (Cunningham and Ow 1996), and at the same time they destroy soil structure and decrease soil productivity. Therefore, phytoremediation is aimed at providing an innovative, economical, and eco-friendly approach in removing toxic metals from the environment by using green plants or to render them harmless (Cunningham and Berti 1993). Some phytoextraction studies have focused on pot experiments (Solhi et al. 2005; Wenzel et al. 2003), and several studies have been suggested that certain accumulating macrophytes concentrate copper and lead (Gupta et al. 2008; Zhuang et al. 2005, 2007).

In the present study, an attempt is made to assess the concentration and distribution of Cu and Pb in the different parts of *Persicaria glabra* (Willd.) Gamez and *Juncellus alopecuroides* (Rottb.) C.B.Cl., and their capacity to accumulate these metals (tolerance level).

2 Materials and methods

2.1 Plant collection

For efficient phytoremediation, it is essential to select the right plant species which could be useful in the accumulation of particular metal contaminant/contaminants. Biological mechanisms of plants, being complex systems, depend on various environmental factors, and their growth in the natural environment is observed on the basis of adaptive morphological features. The plants' growth is dependent on their characters like the nature of the root system (adventitious, fibrous, etc.) and their adaptability to various aquatic conditions (marshy, fringe, emergent, etc.). An expansive root system is a fundamental trait for selecting a plant. Macrophytes like *P. glabra* and *J. alopecuroides* are considered to be most suitable with regard to the above-mentioned characters amongst the available macrophytes which grow in the natural environment of Pune city (India).

2.2 Propagation

The plants were propagated by the vegetative stems cutting method. The cuttings were then grown in sapling trays which were filled with coco-peat in sustainable conditions in the laboratory.

2.3 Experimental design

Following successful rooting, the plants were transplanted to 3-kg capacity pots, each filled with a 5:1 mixture of soil (taken from natural habitats) and coco-peat. The soil in the pots then had added to them the desired amounts of Pb and Cu separately, in the form of their sulphates, to get resultant concentrations of 50, 100, 200, and 400 mg/kg in the soil. The plants were allowed to grow for a period of 60 days (flowering stage) along with a group of control plants. The plants were watered to maintain the water level.

2.4 Plant harvesting

The individual plant species, threaded with different concentrationd of Cu and Pb, were harvested after 2 months when they had attained the flowering stage and were used for determination of various parameters. The shoots of the plants were cut 1 cm above the ground level and then the roots were taken out. The shoots and roots were washed with distilled water and weighed.

2.5 Plant analysis

Plant samples were washed thoroughly with de-ionized water to remove surface dust and soil, and further separated into roots, shoots and leaves. The samples were then air-dried, chopped, and further dried for 24 h in oven at 70°C, and weighed with the help of a balance having accuracy up to 0.001 g. The dried plant samples were ground finely, mixed thoroughly, and 1 g of the powder was carefully digested in a borosilicate conical glass flask with 5:1 mixture of HNO_3 and $HClO_4$ (Allen 1989) at 70–80°C on a hot plate. The solutions obtained after digestion were analyzed spectrophotometrically (Perkin-Elmer Model 550 s UV–vis Spectrophotometer; Intertek) for Cu and Pb at wavelengths of 324.7 and 283.3 nm for Cu and Pb, respectively.

2.6 Soil analysis

The soil pH was measured by using pH meter CRISON (microph 2002 model) in a 1:2.5 soil–water suspension. The CEC is measured by summing the cations like calcium, magnesium, and potassium and accounting for the transferable acidity like aluminum, hydrogen, iron, and manganese. The CEC was measured by the methylene blue adsorption method in milliequivalents per 100 g of soil (meq/100 g) (Nevins and Weinttitt 1967). For organic carbon analysis, the Walkley–Black digestion method was adopted, and a conversion factor of 1.724 was used to convert organic carbon to organic matter (Jackson 1962).

2.7 Growth tendency

Growth tendency (GT) is calculated by using an equation which clearly shows the effect of metals on the plants. The higher values of GT are shown for the optimum growth of the plant. Thus, with the help of GT index the threshold level of metal concentrations in the soil can be discovered, beyond which there is a negative effect on plant growth. This further helps in knowing the level of concentration of an element above which the GT declines from the optimum level, and hence it is easy to determine the level of an element concentration above which it is toxic to the plants.

$$GT = \frac{\text{Fresh weight of experimental plant (g)} - \text{Fresh weight of control plant (g)}}{\text{Fresh weight of control plant (g)}} \times 100$$

2.8 Root: shoot transfer factor (TF)

The transfer of an element from roots to shoots (stem and leaves) is quantified by using a transfer factor with the following equation, which helps in comparing the concentrations of an element in the roots and shoots.

$$\text{Root: Shoot (stem and leaves) TF} = \frac{\text{Element on the shoots (stem and leaves) (mg/kg)}}{\text{Element on the roots (mg/kg)}}$$

The TF values of more than 1 indicates significant cross-membrane transport of elements from the roots to shoots in the plants (Sarah et al. 2007).

3 Results and discussion

3.1 Soil properties

The textural characters (proportion of sand, silt, and clay) and a few physico-chemical parameters (pH, organic matter and cation exchange capacity) of the soil are presented in Table 1. Soil pH is an important factor influencing the availability of metals in the soil for plant uptake (Marschner 1995). Under acidic conditions, H^+ ions displace metal cations from the soil components and cause metals to be released from variable-charged clays to which they were chemisorbed (McBride 1994). The retention of elements to soil organic matter (OM) is also weaker at low pH, resulting in more available metal in the soil solution for root absorption, and hence many metals (including Cu and Pb) are readily available in the soil solution under acidic conditions (Blaylock and Huang 2000). Thus, it may be said that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Brown et al. 1994; Salt et al. 1995).

Table 1 Textural and physicochemical properties of the soil used

Soil components	Values
Sand (%)	79.4
Silt (%)	15.9
Clay (%)	4.7
pH	8.2
Organic matter (%)	1.6
Cation exchange capacity (meq/100 kg)	13.3

3.2 Plant response

Plant response to different concentrations of Cu and Pb were evaluated by taking the weights of shoots and roots. It was observed that the weight of the fresh biomass of the macrophytes increased when they were treated with $CuSO_4$ and $PbSO_4$ (Figs. 1 and 2) as compared to those which were not treated. This is mainly due to the fact that Cu and S are essential elements for the plants. *Persicaria glabra* showed an increase in biomass with Cu treatment (increasing up to 400 mg/kg concentration); however, with Pb treatment, it showed an increase in biomass of up to 200 mg/kg and then decreased (Fig. 1). On the other hand, *J. alopecuroides* showed an increase in biomass with Cu treatment up to 200 mg/kg (optimum growth) and beyond that showed a decrease. This indicates that the toxicity of Cu starts from more than 200 mg/kg concentration. However, *J. alopecuroides* when treated with Pb showed a loss in biomass from 100 mg/kg concentration onwards (Fig. 2).

The fresh weights of above- and below-ground parts of macrophytes with different treatments of Cu and Pb are well in conformity with the weight of the total biomass (Figs. 1 and 2). In case of the roots, the weights of the fresh roots of *J. alopecuroides* shows rather similar trends as in the total biomass. but in case of *P. glabra*, it is found that the increase in weight is obvious up to treatment of 50 mg/kg, and beyond that there is not much appreciable increase in the weight in either the Cu or the Pb treatment. It is further found that the weights of the below-ground parts of both the plants show similar trends for Cu and Pb treatments.

The GT studies clearly show that their values increase continuously for *P. glabra* with different concentrations of Cu treatments, while with the Pb treatments, it is found to increase up to the treatment with 200 mg/kg, while with a further increase in the concentration of Pb, the GT decreases (Fig. 3). However, in the case of *J. alopecuroides*, initially there is decrease in GT values for the 50 mg/kg treatment (the growth of the roots is less than in the control plants), but then it increases up to the concentration of 100 mg/kg for Pb and 200 mg/kg for Cu, beyond which concentrations the treatment is toxic.

Fig. 1 Fresh biomass of *Persicaria glabra* under different treatments of Cu and Pb

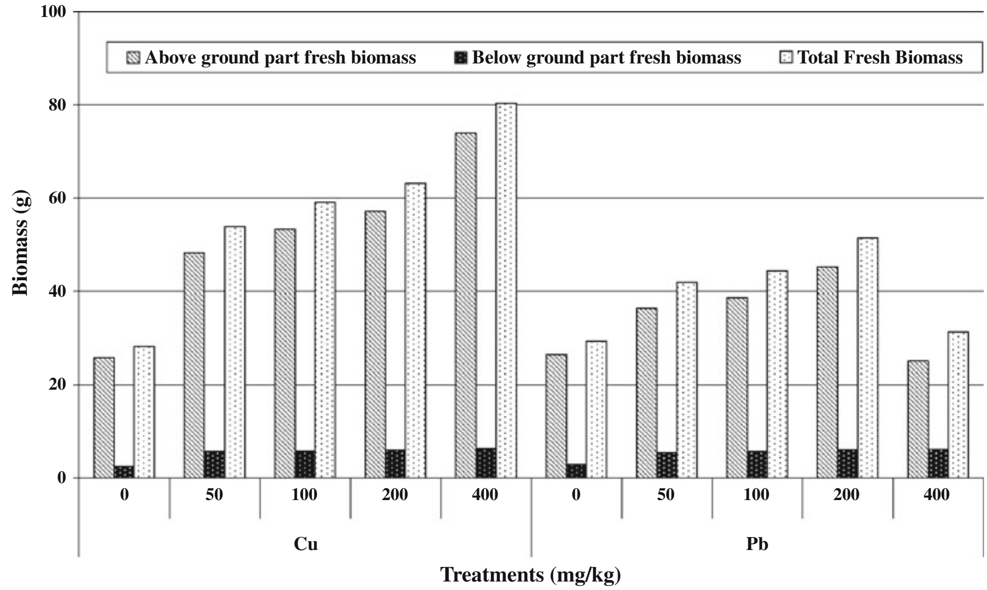
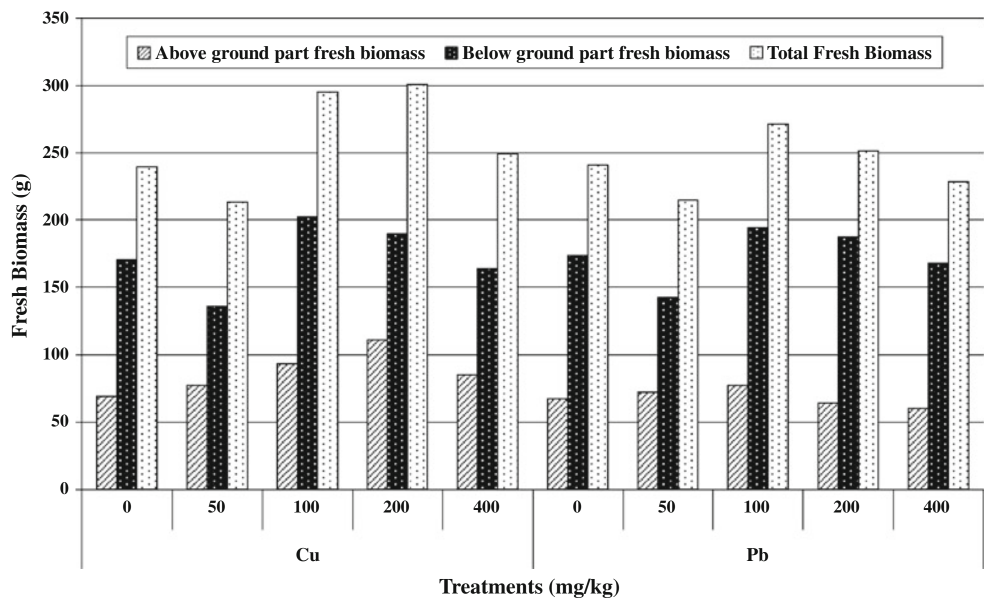


Fig. 2 Fresh biomass of *Juncellus alopecuroides* under different treatments of Cu and Pb



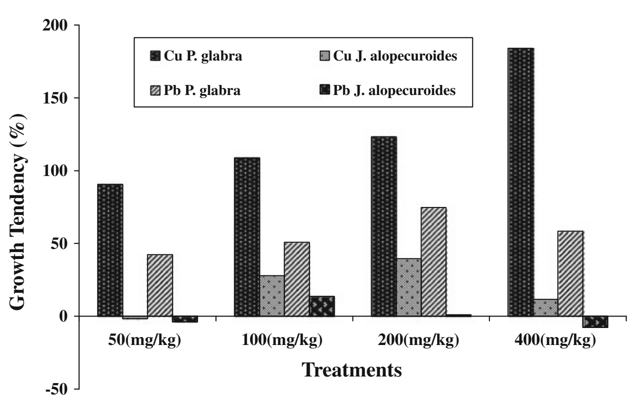


Fig. 3 Growth tendency of macrophytes grown in the different treatments

3.3 Bioaccumulation of Cu and Pb by the macrophytes

Plants pump water, solutes, and organic matter from the surrounding medium as a part of their natural physiological processes. This process can be potentially explored in stabilizing, removing, or breaking down the contaminants from the soil (Robinson et al. 2003). Such a phytoextraction process that uses hyper-accumulator plants to remove metals from the soil stands out among other forms of phytoremediation of heavy metals-contaminated soils (Khan et al. 2000).

Roots absorb the nutrients along with the heavy metals from the soil via the plasma membrane, probably involving cationic channels such as calcium channels. Roots are

Table 2 The mean and standard deviation of Cu and Pb concentration in different parts of *P. galabra* and *J. alopecuroides* under Cu and Pb treatments (mg/kg)

Treatments	*P. galabra*			*J. alopecuroides*	
	Con. stem	Con. roots	Con. leaves	Con. roots	Con. leaves
Cu					
0					
Mean	41.0	60.3	19.0	69.3	50.3
SD	7.0	13.1	2.0	2.2	7.4
50					
Mean	175.3	318.0	123.0	329.0	85.0
SD	2.5	8.7	8.2	2.0	5.3
100					
Mean	221.3	322.0	149.0	340.0	122.0
SD	18.6	4.6	23.6	6.6	6.1
200					
Mean	261.3	451.0	167.0	352.0	195.0
SD	15.5	6.6	20.1	5.6	4.4
400					
Mean	351.0	755.0	306.3	769.0	290.0
SD	9.5	9.6	52.0	11.5	11.5
Pb					
0					
Mean	0.0	0.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0
50					
Mean	14.0	29.0	9.7	31.0	16.0
SD	4.6	3.0	2.1	3.6	2.0
100					
Mean	28.0	50.0	19.0	79.0	15.0
SD	6.6	3.0	1.0	5.3	2.6
200					
Mean	47.0	124.0	30.0	218.0	31.0
SD	3.5	11.5	9.5	3.5	2.6
400					
Mean	59.3	217.0	49.0	397.0	50.0
SD	0.6	3.0	5.6	2.6	4.6

capable of accumulating significant quantities of heavy metals and simultaneously restrict their translocation to the shoots (Lane and Martin 1977). The retention of metals (particularly Pb) in roots involves binding to the cell wall and extracellular precipitation which is deposited in the cell wall. At low concentrations, metals can move through root tissue, mainly via the apoplast, and radially through the cortex where they accumulate near the endoderm. The endoderm acts as a partial barrier to the translocation of metals through the roots to the shoots. This may be one of the reasons for the much greater accumulation of metals in roots than in shoots (Jones et al. 1973; Verma and Dubey 2003). The concentrations of Cu and Pb in the different parts of both the macrophytes which were treated with the different concentrations of Cu and Pb treatment were determined (Table 2), and their distribution in the form of percentage is graphically represented in Figs. 4 and 5.

The present study clearly demonstrates that root and shoot tissue of *J. alopecuroides* show higher concentrations of Cu and Pb in roots than in shoots. Similarly *P. glabra* also shows increases in concentrations of Cu and Pb in roots compared with the stem. In the case of *J. alopecuroides*, there is more accumulation of Pb and Cu in the roots than in the leaves. The concentration of Pb is seen to be very high in the roots of both plants, and it increases with the treatments of higher concentrations, suggesting that Pb is poorly translocated from roots to shoots and hence accumulates in the roots.

Plants differ in their ability to accumulate heavy metals (Cordwell et al. 2002). Their roots accumulate higher

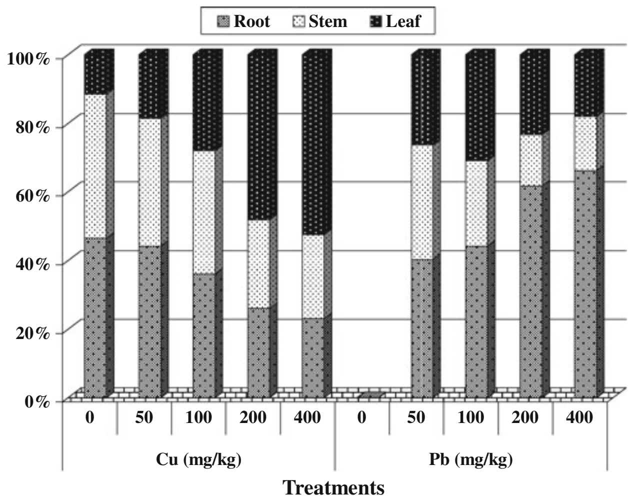


Fig. 4 Distribution of metals in roots, stem and leaves of *P. glabra* grown in the different treatments

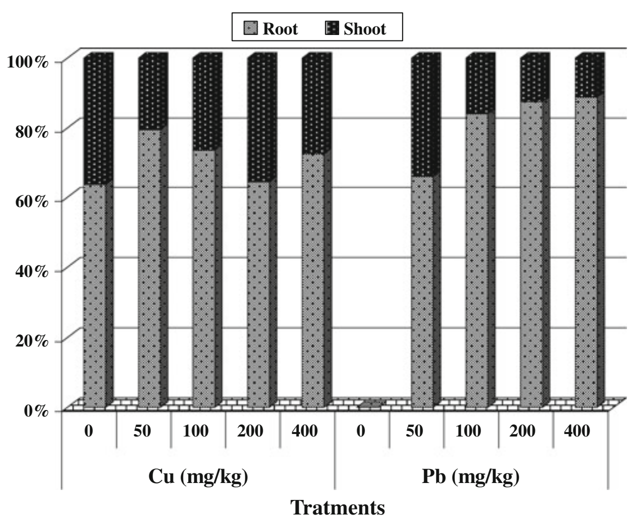


Fig. 5 Distribution of metals in roots and leaves of *J. alopecuroides* grown in the different treatments

concentrations of metals than their shoots, indicating limited mobility of the metals inside the plants, and thus plants immobilize the metals in their roots (Ye et al. 2001).

3.4 Root: shoot, (stem and leaves) transfer factor (TF)

Transfer factor showed lower transportation of Cu and Pb from roots to shoots (Figs. 6 and 7). The same result has been obtained in *Eichhornia crassipes* by Victor and Ishola (2007). Lower accumulation of metals in leaves than in roots can be related to the protection of photosynthesis from toxic levels of trace elements (Baker 1981; Landberg and Greger 1996; Peverly et al. 1995). Heavy metal concentrations in plant tissues of experimental sets showed different capacities of metal accumulation by the plants.

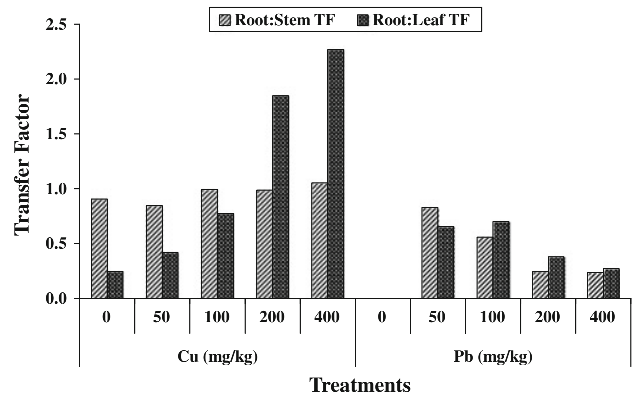


Fig. 6 Root:stem and leaf transfer factor (TF) of *P. glabra* grown in the different treatments (mg/kg)

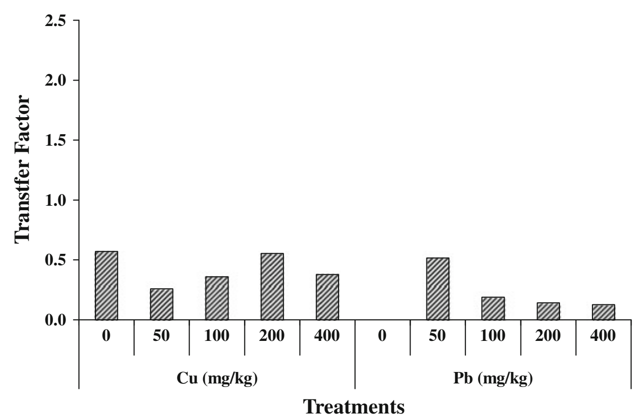


Fig. 7 Root:leaf transfer factor of *J. alopecuroides* grown in the different treatments (mg/kg)

Data obtained indicate that roots mainly retain accumulated metals. Root:shoot TF of macrophytes did not exceed 1, indicating that these macrophytes were not efficiently translocating the elements to the aerial portions of the plants. It is further observed that the transfer factor is increasing for Cu and decreasing for Pb with the increasing concentration of treatments to the soil (Figs. 6 and 7). Although the TFs for Cu and Pb were always less than 1, the TF was greater in *P. glabra* than in *J. alopecuroides*. This may be due to physically absorption at the extracellular negatively charged sites on the root cell walls and not on the total biomass of plants. This cell wall-bound fraction cannot be translocated to the shoots, and therefore this may explain the decrease in root:shoot transfer in *J. alopecuroides* despite higher metal concentrations in the roots.

4 Conclusions

Results from this research based on fresh weight data provided information on the absorption and accumulation of Cu and Pb by macrophytes under experimental conditions in the laboratory. The study demonstrated that *P. glabra* and *J. alopecuroides* could extract copper and lead from contaminated soil with a greater extraction of copper. Therefore, the macrophytes proved to have the ability to phytoextract copper and lead with good efficiency. The biomass growth of *J. alopecuroides* was almost double of *P. glabra*, while it also accumulated high concentrations of heavy metals in different portions. This resulted in maximum removal of heavy metals by *J. alopecuroides*. The transfer factor study revealed a higher accumulation of heavy metals in plant roots and their lower translocation from roots to shoots (stem and leaves). Consequently, the results confirm that macrophytes are efficient accumulators of trace elements, thus enhancing soil quality. These macrophytes, particularly *J. alopecuroides*, can be recommended for the biofiltration of heavy metals from contaminated soil, but there needs to be further extensive long-term studies to find out their suitability for different regions.

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