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Phytoremediation Potential of *Helianthus annuus* and *Hydrangea paniculata* in Copper and Lead-Contaminated Soil

Jenna Forte · Samuel Mutiti

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Abstract This study was conducted to assess the hyperaccumulation and phytoremediation potential of copper (Cu) and lead (Pb) in Hardy 'Limelight' Hydrangea (*Hydrangea paniculata*) and the common sunflower (*Helianthus annuus*). The study also investigated the capacity of these two plants to transpire the metals in a temperature-controlled greenhouse. Plants were grown for 4 weeks and periodically watered with known elemental concentrations of copper oxide nanoparticles, copper sulfate, and lead nitrate. Both *H. annuus* and *H. paniculata* accumulated significant amounts of Cu and Pb to be classified as hyperaccumulator species. *H. annuus* took up significant amounts of Cu in the shoots, specifically the leaves (Cu max. = 1368 ppm), and easily translocated it from stem to leaf (translocation factor (TF) ranged from 2.7 to 81.0). Pb was not as easily taken up and translocated (TF = 0.6) as Cu was by this species. *H. paniculata* took up Cu and Pb in high concentrations but preferentially stored more metals in the stems (Cu max. = 1757 ppm; Pb max. = 780 ppm) than in the leaves (Cu max. = 126 ppm; Pb max. = 35 ppm). The translocation ability of *H. paniculata* was much lower for both metals compared to *H. annuus*. Both Cu and Pb transpired from *H. annuus* at concentrations of 0.04 and 0.005 ppm, respectively.

Keywords Phytoremediation · Heavy metals · Sunflower · Hydrangea · Transpiration

1 Introduction

The widespread and deleterious nature of heavy metals is well documented in the literature (Kastratovic et al. 2014; Mudgal et al. 2010; Schmidt 2003). The most common metals in contaminated environments are silver (Ag), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn) (McLean and Bledsoe 1992). Sources of these metals include mines, sewage sludge, wastewater irrigation, paints, pesticides, fertilizers, and coal combustion (Rahman et al. 2013; Tangahu et al. 2011; Garbisu and Alkorta 2003).

Heavy metals are significant pollutants because of their toxicity, resistance to biodegradation, and associated health problems (Kastratovic et al. 2014). In high concentrations, heavy metals are linked to cancer, acute toxicity, and death. Generally, the metals do not undergo biodegradation and can therefore accumulate to high concentrations if the source is not eliminated (Rahman et al. 2013). Among the heavy metals, Cu and Pb are of particular concern due to their ability to bioaccumulate in the food chain and decrease crop production (Huang et al. 1997; Rahman et al. 2013). The major pathways for human exposure include direct contact, consuming

J. Forte (✉) · S. Mutiti
Department of Biological and Environmental Sciences, Georgia College and State University, GCSU CBX 081, Milledgeville, GA 30161, USA
e-mail: jenna.forte@bobcats.gcsu.edu

S. Mutiti
e-mail: samuel.mutiti@gcsu.edu

contaminated water and food, and inhaling airborne contaminated soil particles (Rahman et al. 2013; Tangahu et al. 2011).

In the environment, heavy metals exist as free ions, pure metals, minerals, other compounds, mixtures, and nanoparticles (Fabrega et al. 2011; Schrick et al. 2004; Tangahu et al. 2011; Wigginton et al. 2007). Their toxicity, availability, and reactivity is dependent on their form and existing environmental conditions. Numerous studies have investigated the behavior of most metal forms in the environment, but there is still a lot of research still required to understand the behavior of metals in nanoparticle form (Shah and Belozerova 2008). The technological advancement in nanoparticle science has led to an increase in the use of nanoparticles and, subsequently, a potential increase in the release of these particles into the environment.

Sources of metallic nanoparticles include electronics, fabric manufacturing, and various remediation practices (Duran et al. 2007; Shah and Belozerova 2008). Once in the environment, metallic nanoparticles have a tendency to aggregate preventing any flow through and out of the soil potentially leading to ecotoxicity (Schrick et al. 2004). Many also have antimicrobial properties and can, therefore, inhibit microbial activity in the soil (Jain et al. 2009). Microorganisms play a very important role in maintaining the health of an ecosystem, and should the microorganism community be damaged, the overall health of the environment would degrade as well (Shah and Belozerova 2008). It is, therefore, very important that heavy metal and metallic nanoparticle environmental pollution be mitigated wherever it exists to protect both human and environmental health.

In order to mitigate heavy metal pollution in the soil, techniques including fixation, containment, leaching, and soil excavation, among many others, have been used (Tangahu et al. 2011). However, these methods are not cost-effective and could potentially result in damage to soil structure and fertility (Adesodun et al. 2010; Garbisu and Alkorta 2003). A number of cost-effective and eco-friendly techniques are available and being used to remediate and control the toxicity of heavy metals in the environment. One of these methods, phytoremediation, is the use of plants to remediate pollution in contaminated soils and water (Schmidt 2003). The process involves (1) phytoextraction, which is the use of plants to remove contaminants from the soil by absorption into the plant used; (2) phytostabilization, which involves immobilizing contaminants in place by trapping or transforming them into less toxic forms; (3) phytovolatilization, which is a process in which plants volatilize the contaminants; and (4) rhizofiltration, which is the use of roots to remove contaminants from moving water (Chaney et al. 1997). Phytoextraction is most commonly used to remediate heavy metals due to its ability to remove relatively larger quantities of metals from contaminated soil without degrading the soil's structure or fertility (Adesodun et al. 2010). For phytoextraction to work, plants need to easily translocate metals from soil through its root/shoot portions to storage structures. Phytoextraction relies on the selective uptake of metals essential for plant growth (iron (Fe), manganese (Mn), Zn, Cu, magnesium (Mg), molybdenum (Mo), and Ni) and those with unknown biological function (Cd, Cr, Pb, Ag, Se, and Hg) (Tangahu et al. 2011).

For plants to be effectively used in phytoremediation, they need to be hyperaccumulators with ideally a large biomass production. Hyperaccumulators are plants that have the ability to accumulate high concentrations of heavy metals in their aboveground portions without the adverse effects of heavy metal toxicity (Adesodun et al. 2010; Mudgal et al. 2010). Approximately 400 plant species from 45 families have been reported as hyperaccumulators including various species of sunflower (for certain metals) and hydrangea (for aluminum (Al)) (Baker et al. 2000; Prasad and Freitas 2003; Jansen et al. 2002; Kochian et al. 2002; Rahman et al. 2013). Rahman et al. (2013) found the common sunflower, *Helianthus annuus*, to be a hyperaccumulator of heavy metals. However, Hamvumba et al. (2014) showed that *H. annuus* did not accumulate any detectable amounts of Pb in the aboveground portions of the plant when grown in Pb-contaminated soils from a mine site in Kabwe, Zambia, where *H. annuus* grows as a weed (Leteinturier et al. 2001). Their study also reported that this plant did not grow in highly contaminated soils in their pot experiment. These contradicting results call for more research on the hyperaccumulation abilities of this species of sunflower. *H. annuus*, a member of the Asteraceae, grows in a wide range of soils throughout the world. It is cultivated because the seeds are edible and can be used to produce sunflower oil. It has a large aboveground biomass potential with the stem of the flower growing as high as 3 m and the flower head reaching 30 cm in diameter (Adesodun et al. 2010).

The Hardy 'Limelight' Hydrangea, *Hydrangea paniculata*, has been known to hyperaccumulate Al in

Fig. 1 Plants sealed under a plastic bag to allow the collection of transpiration for metal analysis

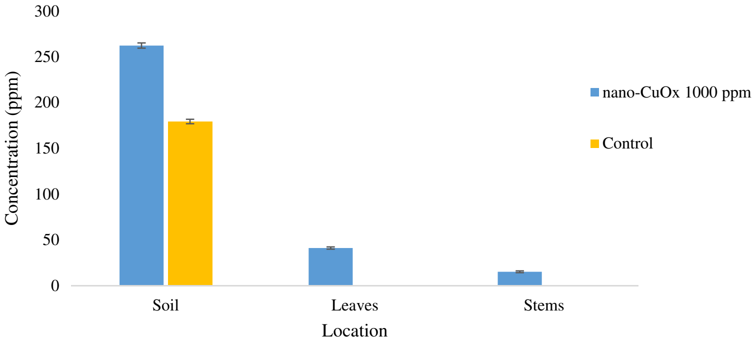
the roots and shoots with leaf Al concentrations reaching up to 3000 ppm (Kochian et al. 2002). It is grown primarily as an ornamental plant. *H. paniculata*, a member of the Hydrangeaceae, can reach a height of 5 m and a diameter of 4 m (Missouri Botanical Garden 2016). To the author's knowledge, there is no known literature investigating *H. paniculata*'s ability to take up and translocate heavy metals other than Al. With its ability to take up as much as 3000 ppm Al from contaminated soils, further research needs to be conducted on *H. paniculata*'s ability to take up other metals. The primary goal of this project was to investigate and compare the potential to take up heavy metals from soils contaminated weekly with 1000 and 10,000 ppm concentrations of Cu or Pb solutions (metal solutions added separately, one metal per plant) in *H. annuus* and *H. paniculata*. The secondary goal was to compare the translocation and potential for transpiration of these two metals within and between the plants. Comparison of heavy metal uptake and transpiration of metals by both *H. annuus* and *H. paniculata*, as well as comparisons of contaminants' translocation ability through these plants, was conducted.

2 Methods

Organic *H. annuus* seeds (Seeds of Change) were acquired from a local store while *H. paniculata* plants were propagated in the lab from local plants. For this study, two trials were conducted in a temperature-controlled greenhouse. In the first trial, 20 pots were filled with topsoil (Earthgrow) and *H. annuus* seeds were planted in each pot, one seed per pot. A 1000 ppm Cu concentration liquid mixture of copper oxide nanoparticles (nano-CuOx) and water was prepared. Ten sunflower plants were watered with 100 mL of this mixture on a weekly basis (for 4 weeks) to simulate periodic exposure. The remaining 10 plants were left uncontaminated (watered with regular tap water) and used as controls. One hundred milliliters was selected because under the experiment, this was the amount of water that saturated the soils two thirds of the way without leakage at the bottom of the pot. The 100 mL liquid was always evenly distributed around the plant and top of the pot. In the real world, it would represent contaminated water that would pollute the soils of interest. The selected metal concentrations for the treatment all fall within the range of values found in the soils and water in mining areas of Zambia (Ikenaka et al. 2010; Sracek et al. 2012).

In the second trial for the sunflowers, a 10,000 ppm Pb metal concentration mixture was prepared using lead nitrate ($Pb(NO_3)_2$) and a 10,000 ppm Cu metal concentration mixture using nano-CuOx was also made. Initially, there were 10 replicates of $Pb(NO_3)_2$ and 10 replicates of nano-CuOx-treated plants. However, three of the plants intended for the nano-CuOx treatment were converted to a 10,000 ppm copper sulfate $Cu(SO_4)$ treatment. This allowed us to investigate how nano-CuOx uptake differed from $Cu(SO_4)$ uptake in

Fig. 2 Concentration of metals in the soil, leaves, and stems in the 1000 ppm nano-CuOx and control treatments



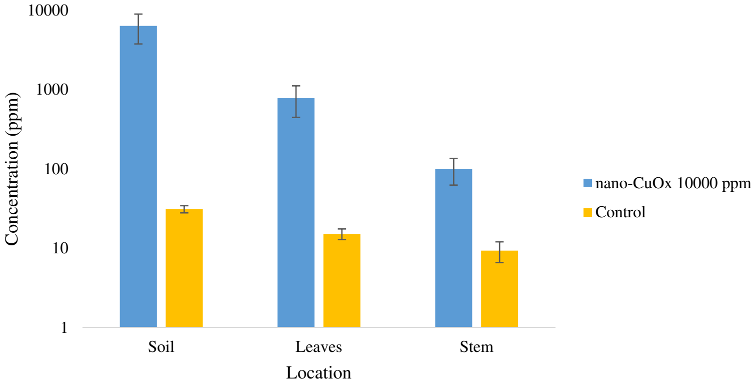


Fig. 3 Concentration of metals in the soil, leaves, and stems in the 10,000 ppm nano-CuOx and control treatments

H. annuus while still retaining an adequate number of replicates for each treatment.

Nine *H. paniculata* branches from three plants were propagated in a separate set of pots with each pot containing 1 kg of potting soil (one branch per pot). Three *H. paniculata* treatments were set up as follows: 10,000 ppm $Pb(NO_3)_2$, 10,000 ppm nano-CuOx, and control treatments. Each treatment had a total of three pots with only one plant per pot. Just like in the first trial, 100 mL of either the 10,000 ppm $Pb(NO_3)_2$, 10,000 ppm CuOx, or tap water was used to water the plants (according the treatment setup) once a week for 4 weeks.

Plants in each pot were sealed under a plastic bag to allow the collection of transpiration for metal analysis (Fig. 1). At the end of the 4-week period, the soil, stems, and leaves were collected from each of the plants and dried in a Binder oven at 75 °C for at least 12 h. Due to the short duration of the experiment, plants did not develop large root masses and, therefore, root analysis was not performed in this study. All stems and leaves were crushed to analyze the internal portion as well as the surface of the plant for heavy metals. The samples (soil, stems, and leaves) were analyzed using an Olympus Delta X-ray Florescence Analyzer for heavy metal content.

Transpiration was collected from the plastic coverings on the plants, which were checked daily to assure no cross-contamination by the leaves or soil (Fig. 1). The lip provided by the careful folding of the bag around the pot allowed for collection of any transpiration that fell from the inside of the bag (Fig. 1). The transpiration was collected in plastic vials once a week. Transpiration was analyzed for heavy metal content using a flame atomic absorption spectrophotometer (AAS).

Enrichment coefficient (EC), the ability of the plant to take up the heavy metal from the soil, was calculated for each plant using Eq. 1. If the value is greater than 1, it indicates high phytoremediation potential. The formula for the EC was taken from Adesodun et al. (2010)

$$EC = [\text{element}]\text{shoot}/[\text{element}]\text{soil} \qquad (1)$$

Translocation factor (TF) was also calculated using Eq. 2 (Kastratovic et al. 2014; Wu and Sun 1998). TF is the capability of the plant to move a metal throughout

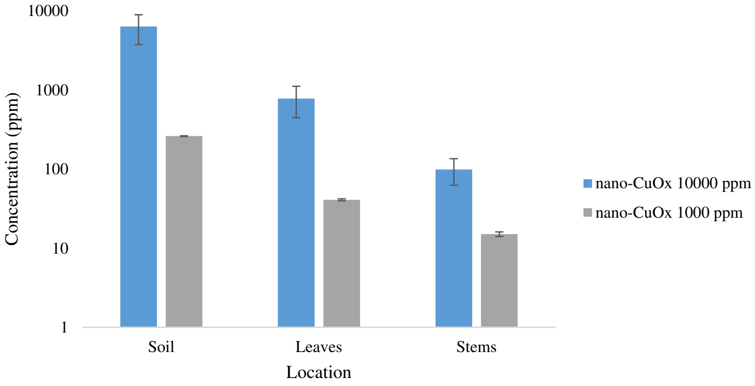


Fig. 4 Concentration of metals in the soil, leaves, and stems in the 10,000 and 1000 ppm nano-CuOx treatments

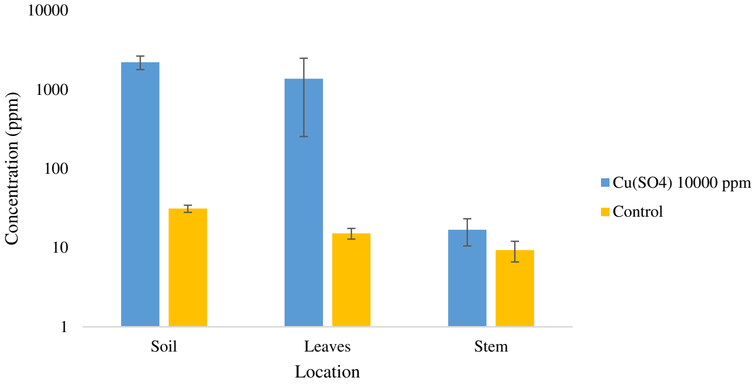


Fig. 5 Concentration of metals in the soil, leaves, and stems in the 10,000 ppm $Cu(SO_4)$ and control treatments

the plant. The greater the value, the more ease with which the plant moves the metal.

$$TF = [\text{metal}]\text{leaf} / \ [\text{metal}]\text{stem} \qquad (2)$$

Statistical analysis was performed using one-way ANOVA to compare the different treatments, concentrations, and accumulation locations in both *H. annuus* and *H. paniculata*. Student's *t* test with unequal variance was also used to compare transpiration of the different metals.

3 Results

3.1 *H. annuus*

The sunflowers treated with 1000 ppm of nano-CuOx had higher concentrations of Cu in the leaves and stems compared to plants in the control treatment. At the end of the growing period, the soil had reached a concentration of 262 ppm while the highest metal uptake in the shoots was observed in the leaves, which contained just over 40 ppm (Fig. 2).

Sunflowers treated with 10,000 ppm nano-CuOx solution took up considerably more Cu than plants in the 1000 ppm treatment. The soils in this treatment had an average Cu concentration of 6379 ppm, with the leaves taking up 783 ppm and the stems taking up 99 ppm (Fig. 3).

Between the two nano-CuOx treatments, there was a statistically significant difference between the 1000 and 10,000 ppm treatments ($p = 0.046$). This was consistent throughout the shoots of the plant (Fig. 4). Leaves always had higher metal uptake than the stems.

Compared to the controls, results from the $Cu(SO_4)$ treatment showed substantial Cu uptake (control = 15.2 ppm; treatment = 1368.0 ppm) in the leaves while the stems showed no statistically significant difference (Fig. 5).

A comparison of all three Cu treatments showed a substantial difference in Cu uptake between the 1000 and 10,000 ppm treatments (Fig. 6). The 10,000 ppm treatment contained 741.5 ppm higher Cu in the leaves

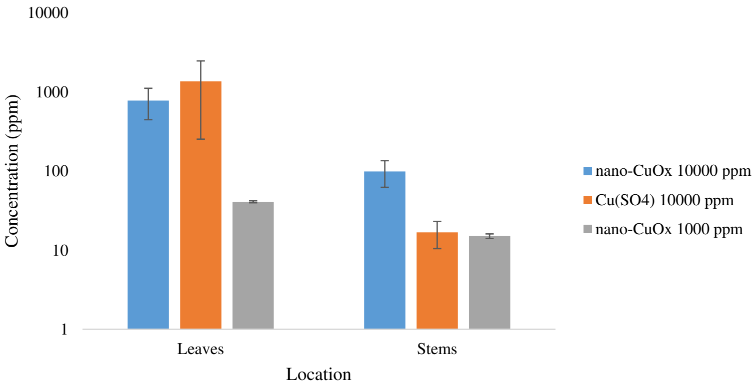


Fig. 6 Concentration of metals in the leaves and stems in the 10,000 ppm nano-CuOx, 10,000 ppm $Cu(SO_4)$, and 1000 ppm nano-CuOx treatments

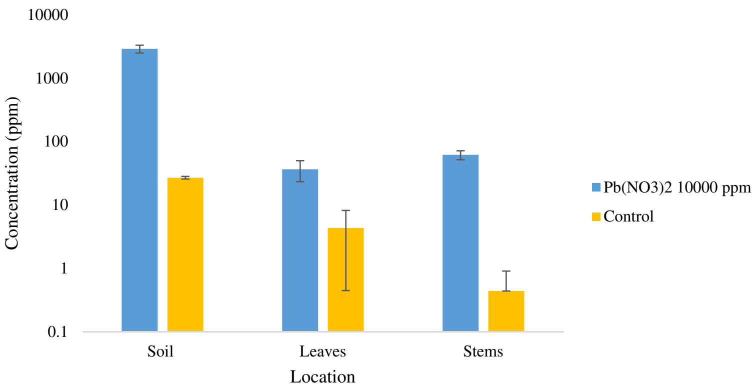


Fig. 7 Concentration of metals in the soil, leaves, and stems in the 10,000 ppm $Pb(NO_3)_2$ and control treatments

and 84.1 ppm higher Cu in the stems compared to the 1000 ppm treatment. There was, however, no statistically significant difference between the two 10,000 ppm treatments. The highest concentrations of Cu in the stems were observed in the 10,000 ppm nano-CuOx treatment, while stems of the 10,000 ppm $Cu(SO_4)$ and 1000 ppm nano-CuOx had similar uptake concentrations (Fig. 6).

The final Pb concentration in the soils for the 10,000 ppm $Pb(NO_3)_2$ treatment was 2912 ppm. The shoots of the plants took up an average of 62 ppm in the stems and 37 ppm in the leaves (Fig. 7). Transpiration collected from *H. annuus* after the 1000 ppm nano-CuOx treatment contained 0.04 ppm of Cu (range = 0.02–0.04 ppm) (Fig. 8a). The amount of Cu in the transpiration was approximately 0.01% of the Cu concentration in the leaves. Figure 8b shows the Pb concentrations present in the transpiration from *H. annuus* after the 1000 ppm treatment. There is about 10 times as much Cu coming out in the transpiration as

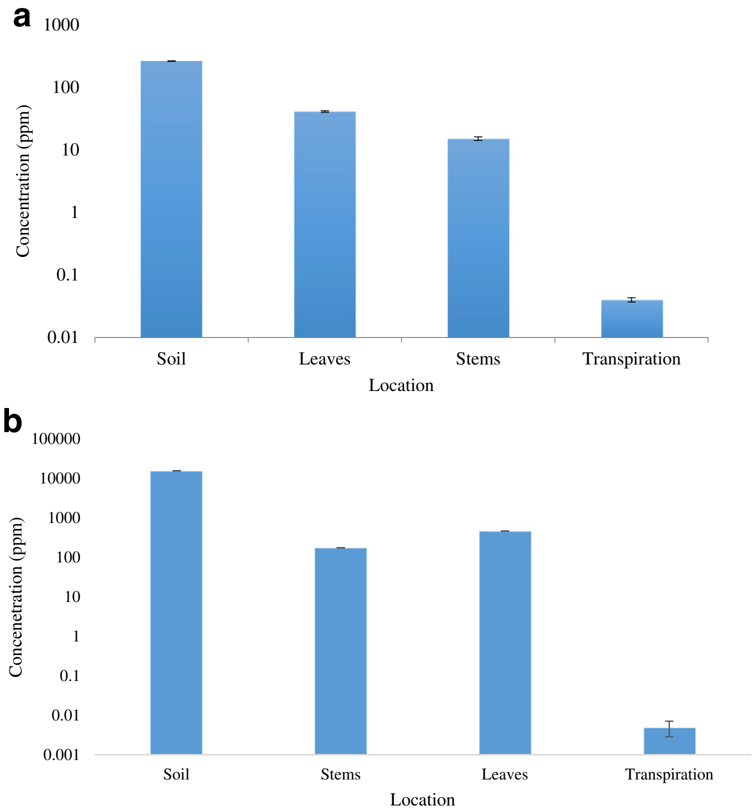
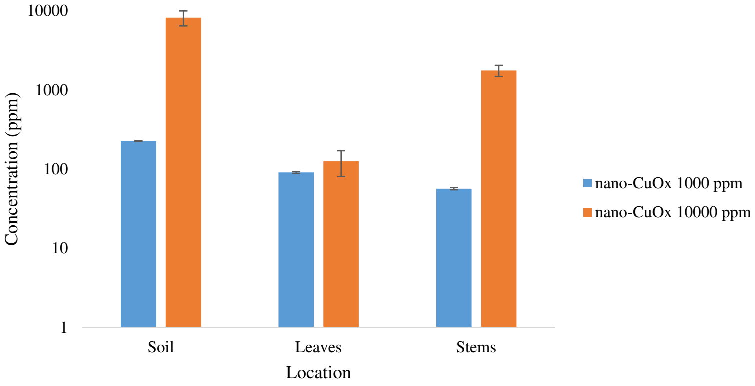


Fig. 8 **a** Transpiration from *H. annuus* in the 1000 ppm nano-CuOx treatment. **b** Concentration of Pb in the transpiration from *H. annuus* in the 1000 ppm treatment

Fig. 9 Concentration of Cu in the soil, leaves, and stems in the 1000 and 10,000 ppm nano-CuOx treatments



Pb. All the transpiration results reported above do not account for any potential attachment of the metals to the collection bags and could be slightly lower than what was transpired.

3.2 *H. paniculata*

In the 1000 ppm nano-CuOx treatments, the soil had a final Cu concentration of 226 ppm while the *H. paniculata* leaves and stems had concentrations of 91 and 57 ppm, respectively. A different pattern in the shoots was seen in the 10,000 ppm nano-CuOx treatment where the concentration of Cu in the stems was higher than that in the leaves (Fig. 9).

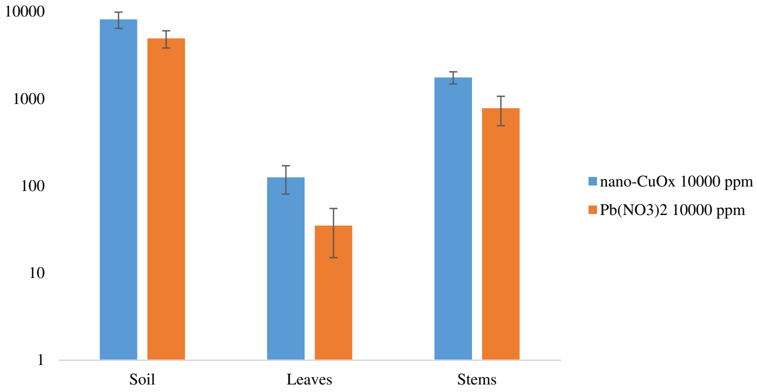
Pb concentrations in the soil at harvest was 4935 ppm for the 10,000 ppm $Pb(NO_3)_2$ treatment. The stems of *H. paniculata* took up 780 ppm, and the leaves took up 36 ppm. All concentrations were statistically significantly higher in the 10,000 ppm $Pb(NO_3)_2$ treatment than in the control treatment ($p < 0.01$). The amounts of Pb taken up in this treatment were similar to those taken up by plants in the 10,000 ppm nano-CuOx treatment (Fig. 10).

At the lower concentration treatment of 1000 ppm nano-CuOx, *H. paniculata* took up significantly more Cu in the leaves and stems than *H. annuus* (Fig. 11). For the higher concentration, in the 10,000 ppm nano-CuOx treatment, stems of *H. paniculata* also had more Cu than the stems of *H. annuus* (Fig. 12).

In the Pb treatments, *H. paniculata* and *H. annuus* accumulated similar amounts of Pb except in the stems where *H. paniculata* had significant more Pb than *H. annuus* pots (Fig. 13).

The 10,000 ppm $Cu(SO_4)$ treatment had the highest ECs and TFs, while the 10,000 ppm $Pb(NO_3)_2$ treatment yielded the lowest values (Table 1). *H. paniculata* had higher EC for all treatments but lower TF values for all treatments as well (Table 1). The highest EC was observed in *H. paniculata* in the 1000 ppm CuOx treatment (0.7) while the lowest was in the 10,000 ppm $Pb(NO_3)_2$ treatment of *H. annuus* (0.03). The highest overall TF was for Cu in *H. annuus*, and the lowest was Pb in *H. paniculata*.

Fig. 10 Concentration of Pb in the soil, leaves, and stems in the 10,000 ppm nano-CuOx and 10,000 ppm $Pb(NO_3)_2$ treatments



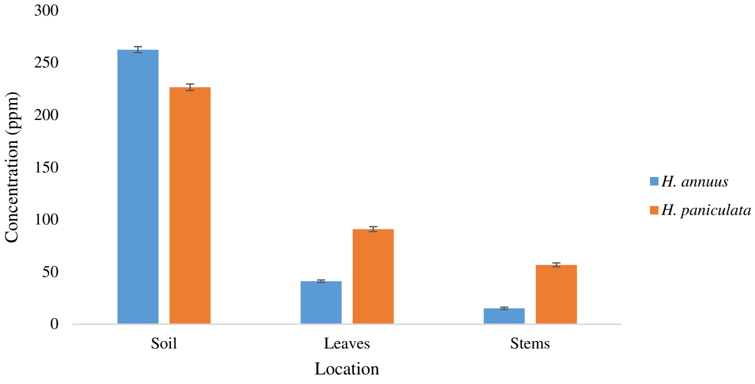


Fig. 11 Concentration of Cu in the soil and in the leaves and stems of *H. paniculata* and *H. annuus* in the 1000 ppm nano-CuOx treatment

4 Discussion

4.1 *H. annuus*

H. annuus accumulated significant amounts of Cu in the shoots, over 1000 ppm in some portions, which confirms its classification as a hyperaccumulator of Cu (Baker and Brooks 1989). The highest TFs were observed in Cu uptake at high contamination levels (compared to the relatively lower levels). This could mean that even though the plant is able to take up Cu from the soils and move it up the plant at all concentrations, the rate of translocation is increased when the concentrations are very high. The EC values also suggest that the relative uptake of the metal from the soils decreases at higher concentrations. This could be to protect the plant by limiting uptake and quickly moving the metal into the leaves for either storage or detoxing (Chaney et al. 1997).

Copper was more easily taken up and translocated by *H. annuus* in the form of $Cu(SO_4)$ than as nano-CuOx (Table 1). However, the difference in the amount of Cu taken up was not significantly different between the two forms. This plant, therefore, can be used for remediation in places where either form of the metal is present, especially when liquid effluent is the source of contamination (which would be similar to the experimental setup in this present study).

Relative to Cu, Pb was not taken up as much from the soils and was also not easily translocated within *H. annuus*. The distribution of Pb within the plant was the opposite of what was observed with Cu. Stems had relatively higher Pb concentrations than the leaves. Together with the lower TFs, this suggests *H. annuus* does not move Pb as easily within the plant and/or prefers to store the metal in the stems rather than in the leaves. It is most likely that the plant preferentially moves Cu through the plants because Cu is an essential nutrient while Pb lacks an essential function in plants. Therefore, Pb does not require translocation to any specific part of the plant. Copper is an essential micronutrient for growth and development in a plant, playing a large role in photosynthesis, pigment synthesis, and other metabolic mechanisms (Fernandes and Henriques 1991;

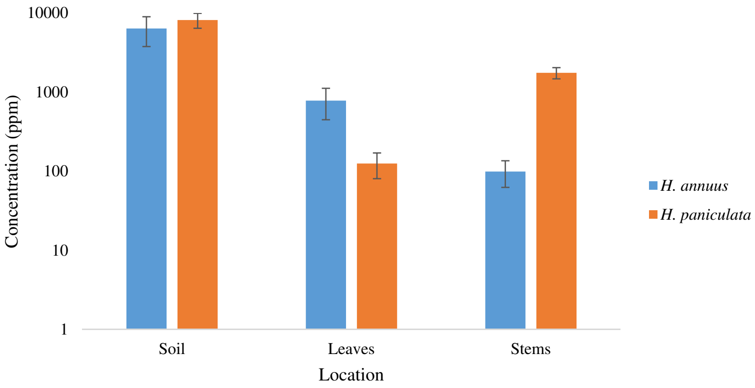


Fig. 12 Concentration of Cu in the leaves and stems of *H. paniculata* and *H. annuus* in the 1000 ppm nano-CuOx treatment

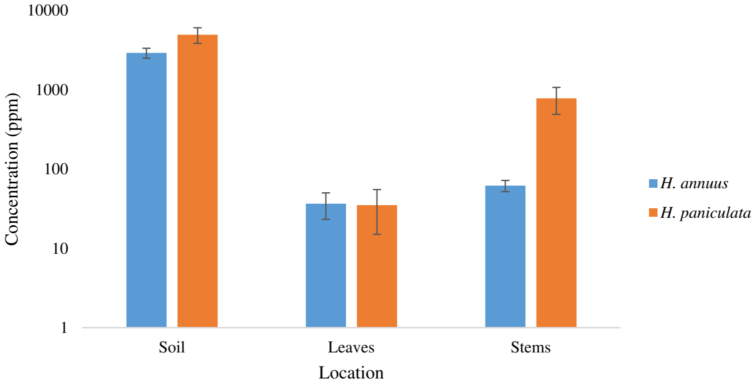


Fig. 13 Concentration of Pb in the leaves and stems of *H. paniculata* and *H. annuus*

Yruela 2009). Due to the fact that photosynthesis occurs in the leaves, the plant would partition a higher amount of Cu in the leaves than it would in the stems. The plant must have dedicated Cu transporters to move Cu into storage components once it enters the plant, most likely to reduce its toxic effects at high concentrations.

Another possible explanation for higher concentrations of Cu in the leaves than in the stems would be that as water evaporates from the leaves, they act as a pump to absorb nutrients and other elements from the soil (Tangahu et al. 2011). It is possible that the weight of the metals might have an impact on the movement within the plant. Pb is much heavier than Cu and, therefore, might be harder to move up the plant resulting in lower uptake. *H. annuus* was able to transpire more Cu (over an order of magnitude) than lead through the leaves. Unfortunately, the amount of heavy metals transpired was too low for transpiration to contribute significantly to phytoremediation or be a cause for concern.

Table 1 ECs and TFs for all similar treatments (1000 ppm nano-CuOx, 10,000 ppm nano-CuOx, 10,000 ppm $Pb(NO_3)_2$, 10,000 ppm $Cu(SO_4)$) of *H. annuus* and *H. paniculata*

	EC	TF
H. annuus		
1000 ppm nano-CuOx	0.2	2.7
10,000 ppm nano-CuOx	0.1	7.9
10,000 ppm $Pb(NO_3)_2$	0.03	0.6
10,000 ppm $Cu(SO_4)$	0.6	81.0
H. paniculata		
1000 ppm nano-CuOx	0.7	1.6
10,000 ppm nano-CuOx	0.2	0.07
10,000 ppm $Pb(NO_3)_2$	0.2	0.05

4.2 *H. paniculata*

H. paniculata, like *H. annuus*, also took up enough Cu to qualify as a hyperaccumulator of this metal. However, unlike *H. annuus*, hydrangea also took up Pb in concentrations above the 1000 ppm hyperaccumulator threshold, making it a hyperaccumulator for Pb as well. Compared to *H. annuus*, *H. paniculata* took up relatively more Cu and Pb and had higher EC but lower TF. *H. paniculata* had higher Cu in the stems than in the leaves. The only exception was Cu in the relatively lower concentration treatments (1000 ppm nano-CuOx) where the leaves and the stems had similar concentrations. This suggests that *H. paniculata* easily takes up both Cu and Pb but prefers to store both of them in the stems than in the leaves when the concentration in the plants is high. This is opposite of what was observed with *H. annuus*, which had higher concentrations in the leaves than in the stems. This suggests that *H. paniculata* protects itself from the effects of phytotoxicity by storing excess Cu and Pb in the stems as opposed to the more photosynthetically active leaves. The actual detoxification and chelation mechanisms or proteins for these metals are not clear and need further investigation. More research on why *H. paniculata* preferred storage of both Cu and Pb in its stems is also required.

5 Conclusion

Both *H. annuus* and *H. paniculata* meet the requirements to be classified as hyperaccumulator species for both Cu and Pb. At lower concentrations of Cu contamination, *H. paniculata* is relatively more efficient in taking up and translocating Cu throughout the plant.

However, at high concentrations, the plant is still more efficient in taking up Cu from the soil but is less efficient at translocating it to the leaves compared to *H. annuus*. At high concentrations of Pb, *H. paniculata* again is more efficient in the uptake of Pb from the soil but significantly less efficient at translocating the metal to the leaves. Both these plants are able to translocate Cu and Pb through the plants' system and then transpire some of the metals out. They both can accumulate significant amounts of the Cu and Pb in their stems.

It appears that a combination of these two plants would be good for remediating sites with either Cu or Pb contamination. *H. paniculata* is an easy plant to propagate and grow, can survive without watering for long periods of time, and is an ornamental plant with beautiful flowers. It would be ideal in places where small-scale remediation (while maintaining esthetic beauty) is required. However, before full-scale utilization commences, more research is required to verify results from this study, and to investigate their ability to take up these metals from contaminated field soils (both in the lab and in the field), and when the metals are in insoluble mineral forms. Soils that are periodically contaminated with sewage and/or industrial effluents containing either $Cu(SO_4)$, nano-CuOx, $Pb(NO_3)_2$, or all of them can potentially be remediated using both *H. annuus* and *H. paniculata*. More research is also required on the fate of these metals once they have accumulated in these plants.

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