

Research Article

Assessment of the Bioavailability of Cu, Pb, and Zn through *Petunia axillaris* in Contaminated Soils

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Heavy metals are potentially toxic to human life and the environment. Metal toxicity depends on chemical associations in soils. For this reason, determining the chemical form of a metal in soils is important to evaluate its mobility and the potential accumulation. The aim of this examination is to evaluate the accumulation potential of *Petunia x hybrida* as a flower crop for three metals, namely, copper (Cu), lead (Pb), and nickel (Ni). Trace metals (Zn, Cu, and Pb) in the soils were partitioned by a sequential extraction procedure into H₂O extractable (F1), 1 M CH₃COONa extractable (F2). Chemical fractionation showed that F1 and F2 fraction of the metals were near 1% and residue was the dominant form for Zn, Cu, and Pb in all samples. Using fluorescence method allowed us to estimate condition of the plants by adding metals. As result of plant and soil analysis, we can conclude that *Petunia* has Cu, Zn, and Ni tolerance and accumulation. Therefore, *Petunia* has the potential to serve as a model species for developing herbaceous, ornamental plants for phytoremediation.

1. Introduction

Russia is one of the biggest country of the world, ranked second among the five biggest mining countries, behind South Africa [1]. Its mining richness is too large to be entirely quoted, but, for example, in 2009, Russia was leading the world in diamond, nickel, and palladium production, and ranked second for aluminum, platinum, and zirconium [2]. The copper production associated with nickel extraction is eliminate, therefore, significant as Russia is the seventh country in the world with 710,000 Tons [3]. Not only is the soil a source of nutrients for plant growth, but also it acts as a sink for contaminants from industrial and agricultural waste materials [4, 5].

The accumulation of heavy metals in environmental samples represents a potential risk to human health due to the transfer of these elements to aquatic media, their uptake by plants, and their subsequent introduction into the food chain. The toxicity, bioavailability, and mobility of metals are related

to their species [6, 7]. However, the determination of chemical species is difficult and sometimes impossible. Therefore, the use of sequential extraction procedures for environmental studies provides an important tool for the determination of the different chemical forms or ways of binding between trace metals and soil components [8]. Chemical extraction is eliminate employed to assess operationally defined metal fractions, which can be related to chemical species, as well as to potentially mobile, bioavailable or ecotoxic phases of a sample. It is generally accepted that the ecological effects of metals (e.g., their bioavailability, ecotoxicology and risk of groundwater contamination) are related to such mobile fractions rather than to the total concentration [9].

The results of speciation studies on solution suggest that metals from anthropogenic sources are mainly in bioavailable forms [10]. The observed trend of the proportion of bioavailability heavy metals at the model systems (Pb≈Cu≈Zn) is confirmed by other researchers for *Quercus ilex* [11], for *Petunia*. Copper and Zn are present as water-soluble salts [12]

and it is generally reported that the leaching of each metal is related to the kinds of liaisons between metals and the solid matrix [13].

The experiment is focused on the study of three metals, namely, copper (Cu), zinc (Zn), and lead (Pb). The aim is to evaluate the study of the bioavailability of Cu, Zn, and Pb for *Petunia x hybrida* as a flower crop. Therefore, an experimental setup was made to observe in laboratory conditions the plant uptake of each metal and translocation areas of these metals.

2. Material and Methods

2.1. Soil Sampling. The soil samples were taken in two different agricultural fields in the Krasnoyarsk region. This type of a substrate was chosen since the agriculture is a vital activity and the soil quality is a decisive factor. In what follows the mention of same samples of soils from deferent places around of Krasnoyarsk city will be made.

The soil cores were contained in a 6.0 cm diameter plastic sleeve that was capped on both ends. The sampling method consists in taking the soil from the ground surface of a 1 m² plot. The soils were characterized as Humod [14] consisting of a thick sequence of well-drained horizons consisting of a thin 0 horizon (0 to 10 cm). The soil types at the 1 contain textures that are predominately silt loams, silty clay loams, and clay loams. The soil types at the 2 included alluvium, sandstone, shale, and limestone.

2.2. Greenhouse Experiment. *Petunia x hybrida* is mainly reproduced by seeds germinating in 5–15 days, with the optimal germination and growth temperature ranging from 20°C to 26°C [15, 16]. It is tolerant to relatively severe conditions and hot climate and needs at least five hours of sunlight a day. *Petunia* can easily be cultivated in hanging baskets.

2.3. Experimental Procedure. Four plastic boxes were used for each soil sample: a control one and the other three with metal solutions. The content of the added metal was Cu²⁺—5, 15, and 30 mg/kg, Pb²⁺—2, 5, and 10 mg/kg, and Zn²⁺—115, 150, and 200 mg/kg. Then, the soil samples were homogenized by stirring and distilled water was added until certain moisture was achieved. After 24 hours two samples of *Petunia x hybrida* in bloom were planted in a separate container, with one specimen left as a control one for the analysis. The experiments lasted for 30 days at the natural day and night (16/8 day/night) and at room temperature (near 25°C).

2.4. Analytical Methods

2.4.1. Plants. Roots, stems, and leaves of the all background plants were separated and weighed. Then, they were dried in a drier at 45°C during 24 h.

2.4.2. Sequential Extractions of Soil. The total weight of the samples taken was 2 kg. Immediately after sampling the whole amount of the substance was sieved with the mesh diameter being 50 μm and divided into several portions with the

weight approximately 1 kg. Each portion was packed into a plastic bag and placed into a fridge. Just before carrying out sequential chemical fractionation the soils from the bags were thoroughly mixed. From the whole amount the portions having 80 g of wet weight were taken. For each fractionation Scheme 3 portions were selected.

The spread scheme of sequential chemical fractionation was used in the work. This is fractionation developed on the basis of the Tessier technique [17, 18].

According to the sequential extraction procedure operationally groups heavy metals into the following two fractions.

(F1) Water Soluble Fraction. 5 g soil sample mixed in 50 mL of distilled water was filtered to estimate the water-soluble metal fraction and shaking for 24 h at 20°C was done. Then, the residue on the filter was prepared for the AAA and the filtrate was analyzed by ICP-MS to determine its metal content.

(F2) Exchangeable Fraction. 2 g soil sample mixed in 20 mL of 1 M ammonium acetate (CH₃COONH₄) and shaking for 1 h at 20°C was done. As in the above case, the residue on the filter was prepared for the AAA and the filtrate—for ICP-MS.

A study of the phytotoxic effect was carried out by the fluorimeter Photon-10 on living plants, immediately after removing them from the experimental systems.

2.4.3. Soil Texture Mechanical Analysis. The ground sample was pressed into tablets with diameter 6 mm and mass 30 mg and subjected to synchrotron radiation X-ray fluorescence analysis (SXRF). Soil texture analysis was performed using the pipette method [19] after the destruction of organic matter with hydrogen peroxide (35%).

The silicate composition of sediment samples was determined using a VRA-20R X-ray phase analyzer. For all determined elements detection limits were 0.02–0.005%. Concentrations were calculated using the fundamental parameter method [20]. Standard reference material was Lake Baikal sediment BIL-1. The relative standard deviation in determination of concentrations of the elements listed above was 10–15%.

The morphology and material composition of total soil particles, the sand fraction, and heavy minerals of the sand fraction were examined using the electron scanning microscope TM-1000 (Hitachi, Japan), with the X-ray spectral energy-dispersive analyzer SwiftED (Oxford Instrument Analytical, England) when registering in back-scattered electrons with the accelerating power of 15 kW in the low vacuum regime. The soil particles were directly fixed on the filters by a double-sided adhesive conductive carbon tape on the sample mount and were placed into the chamber of the electron microscope. Micrographs scanned in back-scattered electrons were collected into a separate file and were subjected to standard digital processing to increase the image sharpness and contrast. Spectral analysis of some parts of the sample (selected particles, characteristic details) was made.

2.4.4. Heavy Metals Determination in Soil and Plant Samples. Aqua-regia mixture, 25 mL (70% high purity HNO₃ and HCl ratio 3:1 and 5 mL 30% H₂O₂), was added to an empty

100 mL beaker with plants or soils and heated at 80°C till the solution became cleared [21]. Determination of the heavy metals such as Cu, Pb, and Zn in the filtrate of vegetables and blanks was achieved by atomic absorption spectrometer, Solaar M6, Thermo Electron Corp. (USA). The instrument was calibrated using manually prepared standard solution of respective heavy metals. An analytical grade of a nitrate salt of Pb and granules of Cu and Zn were used in the preparations of solutions used in the spiking of samples for Pb, Cu, and Zn. Acetylene gas was used as the fuel and air as the support. An oxidizing flame was used in all cases.

ICP-MS (inductively coupled plasma mass spectrometer) X Series 2, Thermo Scientific Corp. (USA) is used for the sensitive determination of trace elements in digested solution of solid samples.

2.4.5. Fluorimetry. The registration of delayed chlorophyll fluorescence was performed using the computerized fluorimeter "Photon-10." This device is intended for registration of several delayed and variable fluorescence parameters of different plant objects (chloroplasts, algae, plant leaves, and lichens). In this device the patented method of rapid assessment of chemical substances phytotoxic effect based on the registration of delayed chlorophyll fluorescence relational index is implemented [22].

2.4.6. Quality Assurance. Blanks and source of quality control standards were measured at every five samples to detect contamination and drift. The elemental concentrations of procedural blanks were generally <5% of the mean analyte concentrations for all the metals. Precision and accuracy of analysis were ensured through replicate analysis of the samples; acceptable percentage recoveries ($86.5\% \pm 0.005$ to 96.4 ± 0.003) were obtained from spiked digested vegetable samples.

3. Results and Discussion

3.1. Soil Analysis. In the initial soil samples were analyzed to examine their grain-size, mineral, and elemental composition. The analyzed samples contained considerable amounts of organic matter. The organic matter was mainly represented by fine dark-brown detritus and contained considerable amounts of humic substances [23]. Organic particles adhere to particles of clay minerals and sand. Therefore, to separate the clay fraction, we increased the soaking time and the number of stirrings. Organics was successfully removed by heating the samples at 480°C, but the structure of clay particles had changed considerably.

The major fractions of the inorganic component of the soil were fine sand (0.25–0.05 mm), fine-grained siltstone (0.05–0.005 mm), and fine mineral powder (<0.005 mm). The 0.004–0.005 mm size range can include both the smallest of the grains and the largest of the powder particles. The major grain-size fraction of the sediments was fine-grained siltstone: 58% to 90%. The clay fraction made up just ~3%.

Common layered materials of significance in materials applications include clay minerals and layered double

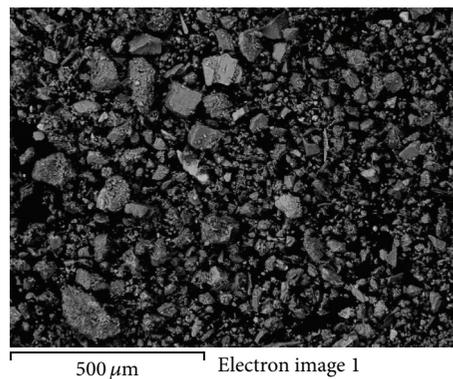


FIGURE 1: General view of the soil samples.

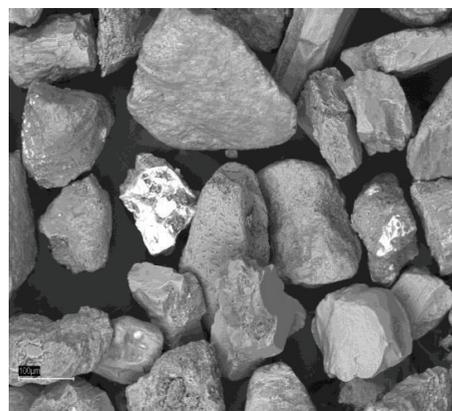


FIGURE 2: General view of the rudaceous fraction of the soil samples.

hydroxide compounds. In general, clay minerals are comprised of multiple layers of hydroxylated and coordinated tetrahedral and octahedral sheets. Detailed examination of the fraction composition of the fine mineral powder showed the predominance of 0.5- μm to 1.5- μm particles. The analysis of the obtained data also suggested that mineral particles were subjected to dispersion and amorphization, resulting in a considerable increase in the content of X-ray amorphous particles of sizes less than 0.1 μm .

The qualitative analysis of separate fragments included into the composition of the soils has been made by the synchrotron radiation X-ray fluorescence analysis. The results are presented in Figures 1 and 2.

The material composition of the of coarsely ground fraction fragments for the soils under study has been analyzed, that is, after the elimination of clay minerals and organic substances. Considerable differences in the elemental composition of the genetic fragments being compared can, mainly, be caused by the difference in the nature of the considered particle origin. The obtained results show that in spite of careful sample preparation for the analysis, it is impossible to achieve the sample homogeneity as a whole. The particles under study are of different origin, leading to significant differences in the granularity, chemical structure, and porosity of a particle (of its surface) and so forth. This does not

TABLE 1: Content of some macronutrients in soil samples.

Sample	Elements, %				
	K	Ca	Ti	Mn	Fe
1	1.7 ± 0.2	2.3 ± 0.1	0.58 ± 0.05	0.07 ± 0.01	4.10 ± 0.08
2	1.8 ± 0.3	2.6 ± 0.2	0.49 ± 0.03	0.07 ± 0.02	3.52 ± 0.06

Data are mean ± SD, $n = 3$.

contradict to the earlier data on geochemical composition and physicochemical properties of the soils [24–26].

The change of the macronutrients content is not considerable (Table 1).

The change of micronutrients content can contribute to the distribution of pollutions between migrating (water solubility and exchangeable fractions) and undissolved forms in the soils (Table 2).

Such nonuniform distribution of microelements between the samples of soils sampled in one area is likely to be connected with the following [24].

- (1) Inhomogeneity of the rock, resulting in
- (2) absolute accumulation or release of elements due to the transfer of their compounds in the geologic profile and (or)
- (3) Relative accumulation (release) of elements due to the release (accumulation) of other chemical substances in the given sample.

The water solubility and exchangeable fractions, corresponding to the first two fractions, are generally called “bioavailable,” as they exhibit mobility relative to the environment and are potentially available for plants.

We found increasing amount of available metal plants for water-soluble and exchangeable fractions of the soils 1 and 2, where the metals are located in the most accessible way for the plants (Table 3).

Trace metals (Zn, Cu, and Pb) in the soils were partitioned by a sequential extraction procedure into H₂O extractable (F1), 1 M CH₃COONa extractable (F2). Chemical fractionation showed that F1 and F2 fraction of the metals was near 1% and residual form was the dominant for Zn, Cu, and Pb in all samples.

After experiments we found what distribution of Cu, Zn, and Pb in water soluble and exchangeable fractions were the following. Chemical fractionation showed that F1 and F2 fraction of the metals were near 95% from added Zn, Cu, and Pb in the investigation samples of soil.

In soil medium, sorption/desorption reactions as well as chemical complexation with inorganic and organic ligands and redox reactions, both biotic and abiotic, influence bioavailability, leaching, and toxicity of metals. These reactions are affected by many factors such as pH, nature of sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates, microbial metabolites, and nutrients [23, 27].

3.2. Plants Analysis. The average values of the application effects, which were accepted as significant according to

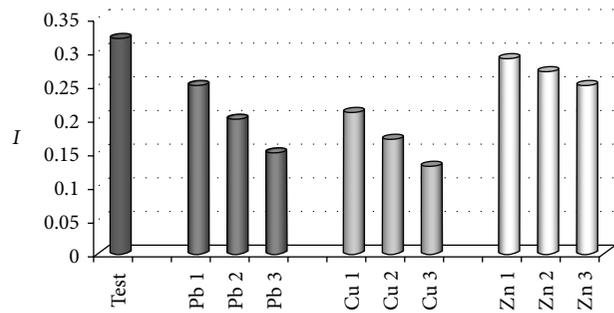


FIGURE 3: The dependence of the fluorescence on the type of metal (Pb, Cu, and Zn). 1, 2, 3 experimental samples with different concentrations of metals.

analysis of variance, are shown in Table 4 for plant species. Concentrations Pb in the tissues of *Petunia x hybrida* decreased from root to stems.

Copper is an important pollutant that originates from sludge and manure application [27, 28]. When the uptake of copper by plants was examined, the highest value was obtained for *Petunia x hybrida* root $42.58 \pm 7.99 \text{ mg}\cdot\text{kg}^{-1}$ (mean ± standard deviation) from Soil 2.

Petunia x hybrida absorbed Zn in its roots in Soil 1 ($68.33 \pm 7.60 \text{ mg}\cdot\text{kg}^{-1}$) two times more than in Soil 2 ($32.58 \pm 8.23 \text{ mg}\cdot\text{kg}^{-1}$).

Several studies demonstrated that metal concentration in the plant tissue was a fraction of the heavy metal content in the growing environment. It has been well documented that the accumulation of metals was more in lower part of the plant grown on contaminated soil than in upper part. The root tissues accumulate significantly greater concentrations of metals than shoots, indicating high plant availability of the substrate metals as well as its limited mobility once inside the plant. This is consistent with previous observations. All of the plant roots accumulate significantly greater concentrations of metals than stems in this study.

When using delayed fluorescence of chlorophyll in leaves of petunia, we found the following. Despite the fact that all plants apparently did not differ from each other, the most toxic effects we found in the system with copper and lead salts (Figure 3).

Delayed fluorescence intensity depends on the rate of photosynthesis, so the more the damage to plants, the lower the intensity of delayed fluorescence.

The variations in the concentrations of the heavy metals in plants may be ascribed to the heavy metals concentrations of soil, air, and irrigation water of their production sites and also the absorption of heavy metals from aerial depositions during transportation and marketing.

Foliage analysis has often been used in heavy metal biomonitoring studies and concurrent analysis of unwashed and washed plants allows external composition to be distinguished from internal [29]. The differences between unwashed and washed plants with regard to the heavy metal concentrations suggest that heavy metals reach stems and leaves by flow substances.

TABLE 2: Content of some micronutrients in soil samples.

Sample	Elements, $\mu\text{g}/\text{kg}$.								
	V	Cr	Ni	Cu	Zn	Rb	Sr	Y	Zr
1	85 \pm 9	102 \pm 2	70 \pm 8	55 \pm 3	197 \pm 7	68 \pm 4	334 \pm 8	30 \pm 4	191 \pm 12
2	103 \pm 11	129 \pm 9	58 \pm 5	32 \pm 6	153 \pm 12	61 \pm 4	381 \pm 20	26 \pm 3	231 \pm 14

Data are mean \pm SD, $n = 3$.

TABLE 3: The content of Cu, Ni, and Zn in soils 1 and 2, determined by ICP-MS after the extraction using water and ammonium acetate (RSD = 5%).

Bulk	Pb, μg			Cu, μg			Zn, μg		
	1	2	3	1	2	3	1	2	3
Water-soluble fraction									
Soil 1	0.9	1.5	2.4	1.4	2.1	3.4	3.8	4.4	6.2
Soil 2	0.5	0.7	1.0	0.8	1.1	1.7	1.2	1.8	2.7
Exchangeable fraction									
Soil 1	0.2	0.5	0.8	0.4	0.7	1.1	0.1	0.5	0.7
Soil 2	1.2	1.6	2.0	0.7	1.0	1.2	1.8	2.4	3.2

1, 2, 3 experimental samples with different concentrations of metals; all values are means of 5 replications.

TABLE 4: Heavy metal accumulation in *Petunia x hybrida*.

Plant part	Soil	Metal in plants ($\text{mg}\cdot\text{kg}^{-1}$)								
		Pb			Cu			Zn		
		1	2	3	1	2	3	1	2	3
Root	1	9 \pm 7	15 \pm 8	20 \pm 9	14 \pm 4	27 \pm 11	27 \pm 14	30 \pm 20	32 \pm 28	66 \pm 8
Stem	1	6 \pm 2	6 \pm 3	8 \pm 5	6 \pm 2	8 \pm 2	12 \pm 6	3 \pm 1	17 \pm 16	27 \pm 23
Root	2	10 \pm 6	21 \pm 11	26 \pm 16	16 \pm 4	24 \pm 6	43 \pm 8	12 \pm 8	19 \pm 13	33 \pm 8
Stem	2	6 \pm 3	9 \pm 5	10 \pm 3	9 \pm 2	10 \pm 4	14 \pm 3	5 \pm 4	6 \pm 5	9 \pm 7

Data are mean \pm SD, $n = 5$.

In literature the results are often specific to soil, metal, and plant; and there is almost no standard information on the subject. Moreover, there are hardly any papers on the phytoremediation abilities of *Petunia x hybrida*, in particular. Some studies have been conducted on genetically modified Tobacco [30] which belongs the *Solanacea* family, such as *Petunia*. The results show the ability of accumulating Pb and, in less extent, Zn. The metals such as Pb, Zn, and Cu are the best candidates for removal by phytoextraction because the majority of approximately 400 known plants absorbing unusually large amounts of metals are likely to accumulate these metals [31].

4. Conclusion

Petunia x hybrida is effective plants for possible remediation treatment; some contaminated and spoilt landscapes can be recovered. Sewage sludge which is serious pollution problem all over the world could be used as media for green landscapes and this can be achieved using *Petunia x Hybrida*. This study strongly supports that the production of sewage sludge compost represents a good way to produce a dry, nutrient-rich, stabilized, and easy to transport fertilizer that can be applied to horticultural plots. Metal flux through consuming interfaces in ligand mixtures: boundary conditions do not

influence the lability and relative contributions of metal species.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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