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Supplementary material

SUPPLEMENTARY MATERIAL TO

# Investigation of soil properties influence on the heavy metals sorption by plants and possibilities for prediction of their bioaccumulation by response surface methodology

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## PROCEDURE FOR PREPARATION AND CONTAMINATION OF SOIL BY Pb AND Cd

The process of soil contamination and preparation is done according to the procedure that has not been used before (no literature data). Calculated amount of the lead salt and/or cadmium was dissolved in a 250 mL volumetric flask by distilled water, corresponding to one kilogram of soil contamination. Soil in the pot was watered with the prepared solution and then mixed with a spatula. Correspondingly, nutrient content was increased through fertilization by adding NPK fertilizer in the amount of 0.5 and 1 g kg$^{-1}$ of soil (≈ 50 and 100 g m$^{-2}$). After drying under ambient conditions, the soil was watered with 200 mL of pure water and mixed with a spatula. The same procedure was repeated four times within 15 days for the purpose of adsorption of heavy metals (Pb and Cd) and their even distribution in the soil. pH values of the soil samples were measured after the preparation and respectively adjusted in the three ranges: 5.5–6.0, 6.5–7.0 and 7.5–8.0. The land is contaminated as shown in Table S-I.

### *Materials and chemicals used for the procedure*

Subsequent materials and chemicals were used for the aforementioned procedure:

– commercially procured compost with the content of elements as declared by the manufacturer: As < 3 mg kg$^{-1}$, Cd < 0.02 mg kg$^{-1}$, Cu < 10 mg kg$^{-1}$, Hg < < 0.1 mg kg$^{-1}$, Pb < 1.5 mg kg$^{-1}$, Zn < 100 mg kg$^{-1}$;

– seedlings of lettuce commercially procured at the marketplace in Kruševac, Serbia;

– $Pb(CH_3COO)_2$ (Merck, *p.a.*), for the soil contamination by lead;

– $Cd(NO_3)_2$ (Merck, *p.a.*), for the soil contamination by cadmium;

– $Al_2(SO_4)_3 \cdot 8H_2O$ (Sigma Aldrich, ACS reagent, ≥ 98 % pure) for pH adjustment value of the soil;

– $K_2CO_3$ (Fluka Chemie GmbH, ≥ 99.0 % pure) for pH adjustment value of the soil;

– artificial fertilizer NPK 5-20-30 + 26 $SO_3$ (Petrohemija, Novi Sad, Serbia);

– 45 pots without holes for water drainage, each containing 1 kg of commercially procured compost;

– concentrated nitric acid – $HNO_3$ (Fluka, ultra-pure), for the preparation of solutions for pH adjustment.

TABLE S-I. The content of heavy metals in contaminated soil

| Sample No. | Contaminant | $c_{Pb}$ and $c_{Cd}$ in compost, mg kg$^{-1}$ |
|---|---|---|
| 1 | No contaminant | – |
| 2 | $Pb^{2+}$ | 50 |
| 3 | $Pb^{2+}$ | 100 |
| 4 | $Pb^{2+}$ | 150 |
| 5 | $Pb^{2+}$ | 200 |
| 6 | $Pb^{2+}$ | 250 |
| 7 | $Pb^{2+}$ | 500 |
| 8 | $Pb^{2+}$ | 1000 |
| 9 | $Cd^{2+}$ | 1 |
| 10 | $Cd^{2+}$ | 3 |
| 11. | $Cd^{2+}$ | 6 |
| 12. | $Cd^{2+}$ | 12 |
| 13. | $Cd^{2+}$ | 25 |
| 14. | $Pb^{2+}$ + $Cd^{2+}$ | 200 + 6 |

### *Cultivation of lettuce samples*

Seedlings of lettuce were planted in the previously prepared and labeled pots with contaminated soil at a depth of 3–5 cm. Pots with seedlings were placed near the window at room temperature, exposed to the daylight and regularly watered every two days. During growth, the changes in lettuce were monitored.

### *Sample analysis, preparation and examination*

After four weeks, lettuce leaves were harvested, packed and labeled separately. Leave samples that have been previously measured, were treated with 1 % HCl, and washed 3–4 times with distilled water. The samples were then dried in air for 48 h, followed by oven drying at 60 °C for 6 h. After drying, leave samples were measured again for later calculation of element content in fresh leaves. Dried leaves were smashed into powder by grinder and then dissolved in acid using following digestion procedure: 1 g of the dried powdered leaves were placed in an Erlenmeyer flask and 15 ml of concentrated $HNO_3$ was added. The flask was then placed in an automatic shaker and stirred for 6 h at room temperature. Afterwards, 3 ml of 70 % $HClO_4$ was added and the solution was heated while stirring at 80 °C, for 30 min.

After cooling down to room temperature, samples were filtered in vacuum through PTF filter with a pore size of 50 µm. The filtrate was diluted to a concentration required for measurement in the ICP-MS device.