**PhD work**

# BIOACCUMULATION OF TOXIC METALS IN VEGETABLE SPECIES: A POT EXPERIMENT

Emese Sipter[1], Rita Auerbach[1], Katalin Gruiz[1] and Gabriella Mathe-Gaspar[2]
[1]Budapest University of Technology and Economics, Department of Agricultural Chemical Technology
[2]Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences

1111 Budapest, St. Gellert Sq. 4., HUNGARY
Phone/Fax +36-1-463-2347
sipter@mail.bme.hu

Keywords: bioaccumulation factor, heavy metal, pot experiment

## Introduction

Mining activities can generate a huge amount of waste materials in the environment. These contain different toxic metals (arsenic, cadmium, mercury, lead, zinc and copper) depend on the ores. The bioaccumulation factors (BAFs) are one of the most important input variables in human health risk assessment. BAFs are derived from experimental data based on potted plants and artificial pollution with salts.

The investigated area is the Toka-valley situated in North-East-Hungary, where the former lead-zinc mine and the flotation plant have polluted the area. There are several pollution sources: the opened tailing dump, the waste rock heaps, area of the flotation plant, the Toka-creek (which flow along the whole area), the sediment of reservoirs (what provide water for the technology and contain the contaminated sediment) and the flooded vegetable gardens which are under cultivation.

## Materials and methods

In the experiment two soils were used. One was collected from the vegetable garden in the village (M45). The other soil sample (M45+m) was a mixture of reference soil (80%) and tailing material from the tailing dump (20%). Four plant species (sorrel, parsley, carrot and chive) were studied in these experiments. These plants are the most common vegetables in Hungary with similar ecological demand. Sorrel and chive are leafy vegetables, carrot and parsley are root vegetables, but leaves of parsley are also consumed.

36 black plastic pots were filled with 1000 g air-dried soil. There were three replications of each sample in a randomized block design. Vegetable seeds were sowed into the pots: sorrel (*Rumex acetosa*) (100 seeds), parsley (*Petroselinum crispum*) (100 seeds), carrot (*Daucus carota*) (100 seeds) and chive (*Allium schoenoprasum*) (50 seeds). The germination ability had defined in test before the experiment and the sowing number of seeds calculated. The water holding capacity of the soils was measured and the water supply was determined as 70% of the saturation. The evaporated water was supplemented at every 2$^{nd}$ day. Nitrogen and phosphorus were added as 3-3 ml 50 g/l $(NH_4)_2HPO_4$ solution per pots after each harvesting.

The plant fresh weight and toxic metal content was measured after harvesting:

- chive on the 43$^{rd}$, 65$^{th}$, 86$^{th}$, 106$^{th}$ day,
- sorrel on the 65$^{th}$, 86$^{th}$, 106$^{th}$ day,
- parsley and carrot on the 86$^{th}$, 106$^{th}$ day.

The seedling number was determined on the 6$^{th}$, 8$^{th}$, 10$^{th}$, 13$^{th}$, 15$^{th}$, 17$^{th}$, 20$^{th}$ day.

The experiment was carried out in climate chamber for 106 days with 400 W/m$^2$ light and 24°C for 16 hours and darkness and 16°C for 8 hours.

Soil samples were collected from the pots. The samples were dried at 40°C for 36 hours and sieved through a 2 mm nylon mesh. A 0,1 g subsample was digested with 3 ml *agua regia* ($HNO_3$:HCl=1:3) in closed teflon bombs by microwave digestion. The total arsenic, cadmium, copper, mercury, lead and zinc content were measured by inductively coupled-mass spectrometry (ICP-MS) by EPA 6020 standard.

Vegetable samples were washed with distilled water after harvesting to remove all fine soil particles. Carrots and parsleys were separated into roots and stem at the shoot-root junction. The samples were dried at 40°C for 36 hours, then a 0,3g subsample was digested with 8 ml *agua regia* ($HNO_3$:HCl=1:3). The total arsenic, cadmium, copper, mercury, lead and zinc content were measured by inductively coupled-mass spectrometry (ICP-MS) by EPA 6020 standard.

The bioaccumulation factor was calculated with the following equitation: $BAF=c_{plant}/c_{soil}$

## Results and discussion

*Metal content of soils*

Metal content of soils was determined in the $0^{th}$ day of the experiment (Table 1). Mixing the soil with tailing material (M45+m) principally increased the lead and arsenic content. The zinc and copper content were nearly unchanged, the cadmium content became twofold higher, the mercury content increased threefold. The arsenic and lead content of soils exceeded the Hungarian limit value. The M45 soil sample represents an average garden soil from the village and the mixture can represent an average garden soil from the flooded area.

**Table 1**
**Metal content of soils (mg/kg)**

| | Zn | Cu | As | Cd | Hg | Pb |
|---|---|---|---|---|---|---|
| **M45** | 112.50 | 32.65 | 30.40 | 0.22 | 0.502 | 25.22 |
| **SD** | 3.00 | 0.48 | 1.20 | 0.01 | 0.034 | 2.43 |
| **M45+m** | 152.25 | 33.32 | 173.03 | 0.46 | 1.547 | 386.01 |
| **SD** | 9.88 | 0.31 | 8.02 | 0.08 | 0.170 | 30.51 |
| **Hungarian limitation value** | 200 | 75 | 15 | 1 | 1 | 100 |

*Germination and growth*

The number of germinated seeds was dependent on the toxic metal content; the germination was tardy in soil M45+m. The difference was significant at sorrel samples, tendency could be observed at chive and parsley, in case of carrot there was not any difference in the germination.

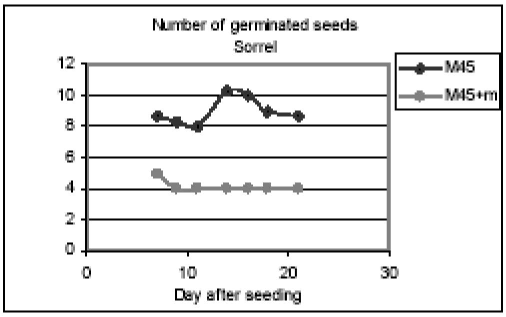


Figure 1. Germination tendency of carrot

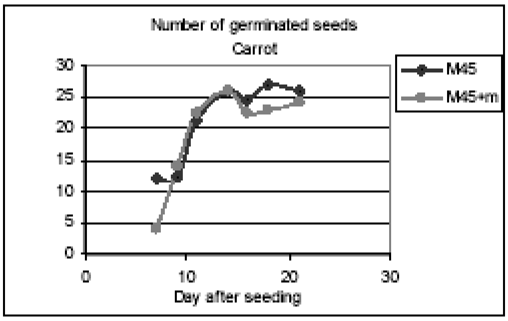


**Figure 2. Germination tendency of carrot**

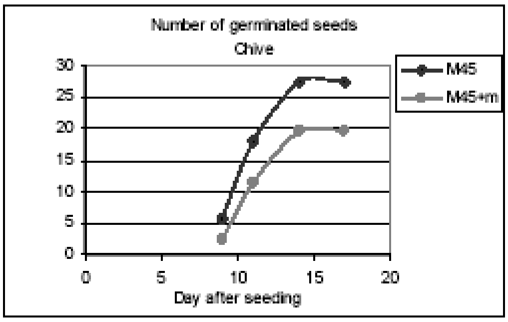


**Figure 3. Germination tendency of chive**

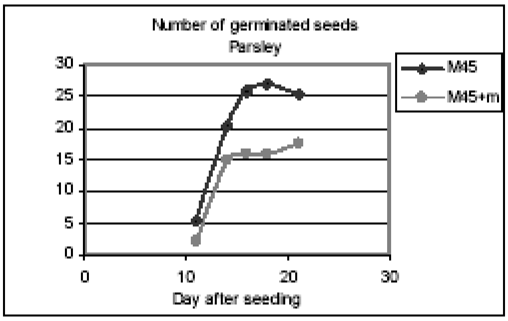


**Figure 4. Germination tendency of parsley**

No definite correlation was found between the biomass production of chive and sorrel and the heavy metal content of the soils (Table 2). The root weight of carrot and parsley were significantly smaller in polluted soil (Table 3, Figure 5). This result shows a discrepancy between the leafy and root vegetables.

**Table 2**
**Yield of sorrel and chive (g)**

| | 43rd day | 65th day | 86th day. | 106th day | ∑ |
|---|---|---|---|---|---|
| **sorrel** | | | | | |
| M45 | | 3.117 | 3.094 | 4.328 | 10.539 |
| M45+m | | 3.855 | 3.262 | 3.525 | 10.642 |
| **chive** | | | | | |
| M45 | 1.203 | 1.072 | 0.939 | 0.784 | 3.998 |
| M45+m | 0.375 | 1.119 | 1.111 | 0.631 | 3.236 |

**Table 3**
**Yield of carrot and parsley in the 106th day (g)**

| | **carrot** | | **parsley** | |
|---|---|---|---|---|
| | root | shoot | root | shoot |
| M45 | 12.148 | 5.793 | 4.496 | 5.225 |
| M45+m | 8.759 | 5.024 | 3.066 | 5.466 |

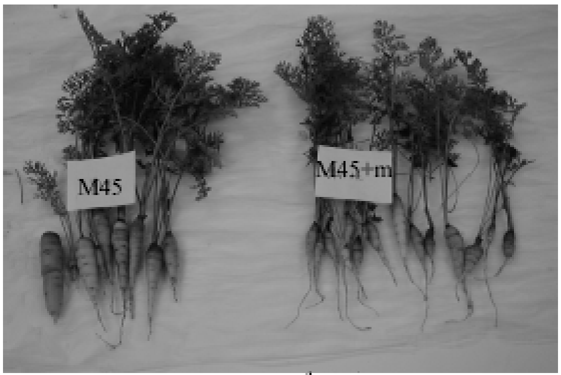

Figure 5. Carrot in the 106th day after harvesting

*Metal content of plant*

The mercury concentration was immeasurable in every species. The Hungarian safety value is set for different type of foods. These are the following for dried vegetables: As 2.0, Hg 0.05, Pb 2.0, Cd 0.5 mg/kg. The arsenic concentration was high in the root of parsley. The lead concentration exceeds the safety value in nearly every case. The cadmium concentration was higher than the safety value in the plants grown in polluted soil. For copper and zinc there are not any safety values in Hungary.

**Table 4**
**Metal content of plants in the 106$^{th}$ day (mg/kg dry weight)**

| | As | Cd | Cu | Pb | Zn |
|---|---|---|---|---|---|
| **Carrot$_{root}$** | | | | | |
| M45 | n.d. | 0.06 | 3.68 | 0.32 | 16.59 |
| M45+m | 1.21 | 0.38 | 5.39 | 7.72 | 27.82 |
| **Carrot$_{shoot}$** | | | | | |
| M45 | 0.18 | 0.11 | 5.95 | 0.96 | 27.25 |
| M45+m | 0.72 | 1.71 | 6.35 | 10.06 | 57.33 |
| **Parsley$_{root}$** | | | | | |
| M45 | n.d. | 0.03 | 8.06 | n.d. | 28.22 |
| M45+m | 5.82 | 0.54 | 8.77 | 13.17 | 49.96 |
| **Parsley$_{shoot}$** | | | | | |
| M45 | n.d. | n.d. | 5.65 | n.d. | 34.08 |
| M45+m | n.d. | 0.21 | 5.52 | 6.33 | 95.09 |
| **Sorrel** | | | | | |
| M45 | n.d. | 0.16 | 4.89 | 2.11 | 43.18 |
| M45+m | n.d. | 1.27 | 9.67 | 8.57 | 139.87 |
| **Chieve** | | | | | |
| M45 | n.d. | 0.07 | 10.5 | 1.69 | 76.92 |
| M45+m | n.d. | 1.13 | 9.78 | 3.73 | 151.12 |

Important pollutant elements of investigated soil (M45+m) were the hardly available and slowly translocating elements: As and Pb, which concentration was highest in the root of parsley (As: 5.82 and Pb: 13.17 mg/kg dry weight). In case of root vegetables the metal concentration was higher in the root of parsley than in the root of carrot. The rate of metal concentration for parsley and carrot varied between 1.42 and 4.81, the biggest was in the concentration of arsenic. The mobile metals (Cd and Zn) translocated into the shoot except the cadmium in parsley. Root of parsley took up more metals than carrot.

Cadmium content increased at a higher rate in both species compared to zinc content. Increase of cadmium content of sorrel was higher than parsley (sorrel: 7.45, parsley 4.80 times). Change of zinc concentration did not differ (sorrel: 3.30, parsley 2.90).

Results indicated different characters of species for element uptake.

*Bioaccumulation factor*

**Table 5**
**Bioaccumulation factor**

| Species | Day of sampling | As | | Cd | | Pb | |
|---|---|---|---|---|---|---|---|
| | | **M45** | **M45+m** | **M45** | **M45+m** | **M45** | **M45+m** |
| **Chive** | 43 | 0.000 | 0.014 | 0.22 | 67.82 | 0.000 | 0.012 |
| | 65 | 0.000 | 0.012 | 0.55 | 125.95* | 0.043 | 0.005 |
| | 86 | 0.015 | 0.011 | 1.95 | 5.09 | n.d. | n.d. |
| | 106 | 0.007 | 0.013 | 0.32 | 2.92 | 0.079 | 0.009 |
| **Sorrel** | 65 | 0.003 | 0.012 | 0.83 | 2.68 | 0.084 | 0.013 |
| | 86 | 0.002 | 0.007 | 0.74 | 3.68 | 0.044 | 0.018 |
| | 106 | 0.014 | 0.006 | 0.83 | 3.21 | 0.099 | 0.028 |
| **Parsley$_{shoot}$** | 106 | 0.010 | 0.009 | 0.00 | 0.55 | 0.000 | 0.015 |
| **Parsley$_{root}$** | 106 | 0.001 | 0.032 | 0.09 | 1.09 | 0.000 | 0.031 |
| **Carrot$_{root}$** | 106 | 0.000 | 0.007 | 0.27 | 0.77 | 0.010 | 0.018 |

The calculated bioaccumulation factors were very low for arsenic (0-0.032) and for lead (0-0.099). Bioaccumulation factors for cadmium were significantly higher (0-5.09), the consumption of these plants can cause very high human health risk. The bioaccumulation factors for cadmium in chive were extremely high on the 43rd and 65th sampling day. These can show: what are the riskiest dates for harvesting and consuming the vegetables; and which vegetable can be grown in these soils.

The BAF values are dependent on the soil parameters (pH, humus content, metal concentration). It is known that BAF values decreased with increasing metal contamination in soil, especially for the mobile metals, cadmium and zinc (Lise et al.). In this experiment the higher metal concentration in soil causes bigger BAF, the possible reason is that the plants growth was slow in the contaminated soil during the first two harvesting.

The two types of vegetables (root and leafy) are differing, but we found differences also in the group, between the carrot and parsley.

## Conclusion

The aim of this experiment was to investigate the variance of bioaccumulation factors and to try to make fast screening tests to measure the toxicity of toxic metal polluted soils. Our pot experiment represented the average vegetable gardening habits in a Hungarian village; soil samples represented a reference soil and a mixture soil in the investigated area. The harvesting day of vegetables represent the average vegetable consumption in Hungary. In the process of human health risk assessment the concentration in plants is calculated with the BAF values. These values are differentiated between root and leafy vegetables. The results show that the BAF depends on the harvesting day, the type of vegetables and the contamination of soil.

## Acknowledgements

Work was supported by the National Research and Development Program (NKFP-3/002/2001) and the Hungarian Scientific Research Fund (OTKA T 042778).

## References

1. Dudka, S. et al: 1996, Transfer of cadmium, lead and zinc from industrially contaminated soil to crop plants: a field study. Environmental Pollution 94, 181-188
2. Finster, M.E. at al: 2004 Lead levels of edibles grown in contaminated residental soils: a field survey, Sci Total Environ, 320, 245-257
3. Gondek, K., Filipek-Mazur, B.: 2003 Biomass yields of shoots and roots of plants cultivated in soil amended by vermicomposts based on tannery sludge and content of heavy metals in plant tissues. Plant Soil Environ 49, 402-409
4. Gruiz, K., Horváth, B., Molnár, M., Sipter, E.: 2000 When the chemical time bomb explodes, Contaminated Soil 2000, Thomas Telford Publishing, London, 662-670
5. Hungarian Quality Criteria for Soil, 2002, Ministry of Environment
6. Hungarian Safety Value for Food, 1999, Ministry of Health
7. Lise, S.P. et al: 2002, Uptake of trace elements and PAHs by fruit and vegetables from contaminated soils. Environ. Sci Technol 36, 3057-3063