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# ECOTOXICOLOGICAL INVESTIGATION AND ANALYSIS OF VEGETATION OF HEAVY METAL CONTAMINATED AREA AT KUNSZENTMÁRTON

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**ABSTRACT.** The aim of our study was the examination of vegetation and soil samples of heavy metal contaminated sediment in a post-settled industrial sewage pond-system in Hungary. Due to the disturbance the heavy metal polluted areas have diverse vegetation, and these plants can tolerate higher concentration of heavy metals and they are able to take up them. The accumulated heavy metals could enter the food-chain easily by the process of biomagnification, but these accumulator plants can be applied for phytoremediation. Our paper presents the results of vegetation analysis and heavy metal content of sediment during the period 2003-2005 and the results of ecotoxicological study in 2008. The *Sinapis alba* seed germination and root elongation test which is a simple, economic, quick and efficient test was used to determine the toxic effect of sediment samples. The samples were collected from the units of former secondary Cr contaminated sedimentation pond system of a Hungarian leather factory near the town of Kunszentmárton. The data were compared with on each other in the different periods. The Cr concentration in the sediment samples was between 21-15,000 mg $kg^{-1}$. Based on our studies, it can be stated, that this area has diverse vegetation, high heavy metal content and low toxicity. The heavy metal uptakes by plants depend on the factors of the environment.

**Keywords:** chromium, heavy metal accumulation, phytoremediation

## INTRODUCTION

Nowadays, as a consequence of human activity, the groundwater and soil pollution by heavy metals increasing continuously and it causes huge problems from the point of view of agriculture and human health. Although contamination of the environment with chromium (Cr) is not widespread problem, industrial wastes (electroplating sludge, Cr pigment and tannery wastes, leather manufacturing wastes, by-products of Cr mining and smelting), municipal sewage sludge, fertilizers etc. may contribute to the increase of Cr content in surface soils (Simon et al., 2000, Kabata-Pendias et al., 1992). The most investigated sources are electroplating works, tanneries, pigments, where chromate anion concentration begins with 5-50 $mgkg^{-1}$ and may be attends 200-1000 $mgkg^{-1}$ (Gavris, 2009). The world mean chromium concentration of uncontaminated surface soils is 54 $mgkg^{-1}$. In contaminated sites several hundred or several thousand $mgkg^{-1}$ Cr was found in surface soils, and in a proximity of Cr smelter heaps this value may exceed 10000 mg $kg^{-1}$(Kabata-Pendias and Pendias, 1992, Simon et al., 2000, Lakatos et al., 2008, Csatári et al 2009). At alkaline pH the heavy metal ions can easily adsorb to the small fragment of soil, but the plants can decrease the pH of rhisosphere trough the production of organic acids. The plants of the heavy metal polluted areas can mobilize and uptake the heavy metals in great amounts, and these metals can reach the human organs through the biomagnification process. On the other hand these plants can be applied for phytoremediation of metal polluted sites (Brooks 1998, Keresztúri, 2008). Numerous physical, physico-chemical and chemical methods are used to remove pollutants from the soil, but most of them destroy the physical structure of the soils and changes its chemical composition. Phytoremediation is a novel, environmental sound biotechnology to clean-up contaminated soils or waters with the help of naturally occurring or genetically engineered plants. Phytoremediation is applicable for large surface areas, but the process could be feasible for remediation of agricultural soils which are only moderately polluted (Brooks 1998; Simon 1999). Aquatic macrophytes are widely distributed in various wet environments, from fresh to salt water. They have several characteristic favorable for metal accumulation. However plants have developed a very potential mechanism to combat with such adverse environmental heavy metal toxicity problems, therefore the vegetation of heavy metal polluted sites may be quite diverse (Bricker et al., 2001; Bíró et al., 2007). In terms of biomass, aquatic macrophytes are the predominant organisms on the highly productive, littoral ecosystem, such as wetlands. Rooted species can absorb metals through their roots and rhizomes as well as through their leaves because the latter provide and expanded area to trap particulate matter, sorb metal ions, and accumulate and sequester pollutants (Levine et al., 1990). Macrophytes of heavy metal contaminated soils and wetlands are also stationary and constantly exposed to contaminants such as metals. In comparison with other plant species, wetland macrophytes have been reported to have a larger or comparable capacity for metal accumulation (Csatari et al., 2009). The

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heavy metal accumulation of plants depends on the environmental factors, and these can be divided into biological (e.g., species, age, generation) and non-biological (e.g., temperature, season, salinity, pH, metal concentration) groups. Plants have generally little capacity to uptake chromium and to transfer it to aerial parts. In normal conditions, Cr concentration in plants is less than $\mu g g^{-1}$. In plants, which are grown on soils and are treated with tannery waste, concentration may increase significantly (Barcelo and Poschenrieder, 1997). The aim of our work was to examine the vegetation and soil samples of heavy metal contaminated sediment in a post-settled industrial sewage pond-system in Hungary. We examined the toxic effect of the sediment with a *Sinapis alba* seed germination and root elongation test, too. The heavy metal uptake by plants was measured in wetland and in dry period, too.

## MATERIALS AND METHODS

### *Site location*

The sampling area was a former secondary sewage water sedimentation pond system of a Hungarian leather factory near the town of Kunszentmárton (Fig.1). The site of our investigation consists of three straight-line-connected sludge-settling pounds (KM.1., KM.2., KM.3.) The area of the investigated ponds is 1000 $m^2$. The geographic coordination of the examined site: North Latitude 46°50´, and East Longitude 20°15´. The area lies on the flood of the River Körös, and belong to the Körös-Maros National Park. The bottoms of the beds are well insulated with a clay layer; therefore in rainy periods the pond system may be staying under water. In 2003 the ponds were dried up completely, but since 2005 the ponds were filled with water.

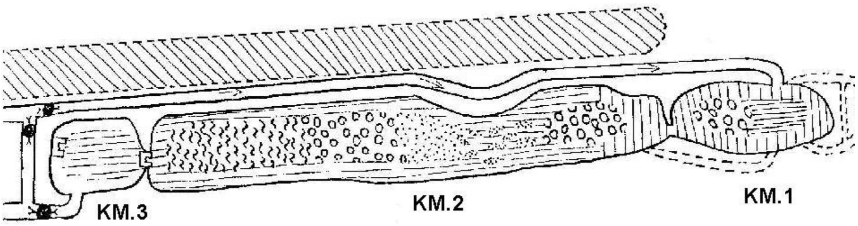


**Fig. 1** The sampling area (Kunszentmárton, Hungary)

### *Field studies*

We compiled the species list of the higher plants which can be found in the investigated site. Plant and sediment samples were collected on the end of summer in 2003, 2005 and 2008. A random design was chosen to obtain a representative sample. For sediment collecting, we used Hargrave sediment sampler equipment. The sampling depth was 0-20 cm in the sediment. The samples were also collected from the rhisosphere of plants in 2008. In 2003 and 2008 the type of the area was terrestrial ecosystem, while in 2005 it was aquatic ecosystem.

### *Laboratory studies*

After collecting, the samples were carried to the laboratory of the Department of Applied Ecology (University of Debrecen). We weighed the mass of the different parts of plants. Sediment and plant samples were dried in a drying-oven at 105°C. Then we applied sequential calcinations: at 200°C for 30 min, 250°C for 60 min, then at 300°C for 60 min. again, at 400°C for 30 min, and at the end of the process 500°C for 4 hour. We extracted the metals from ash with cc. $HNO_3$. The elemental composition of the soil and the plant samples was determined by inductively coupled argon plasma emission spectrometry. The samples were prepared after the regulation of the Hungarian National Standard regarding to the F2 biological analyzing toxicology examination prescription. Dried and ground soil sediment sample were mixed with distilled water in a 1:10 ratio on a GFL 3015 shaking-gear one hour long. Then it was left to consolidate and settle. We determined the conducting ability and the pH on a CONSORT C853 appliance, since the complete salt content could influence the outcome of the test. The samples were centrifuged in an IEC CENTRA MP4 set for 5 minutes on 3,000 rpm. Right after, the samples were strained on a filter paper, and the filtrate was used to the test. 3 ml was taken with a pipette from the filtrate mentioned above and then it was poured on a filtrate paper put in a Petri dish. The test was adjusted with 30 and 25 *Sinapys alba* seed.

## RESULTS AND DISCUSSIONS

We measured the pH and conductivity values of the sediment samples, and it can be stated, that those did not report critical effect from the point of view of the plants physiological demand (Table 1).

We collected the sediment samples from 20-30 cm depth and from the rhisosphere of the different plants species. By alkaline pH the heavy metal ions can easily adsorb to the small fragments of soil, but the plants decreased the pH of rhisosphere trough the produce of organic acids (Table 2). The pH values of the sediment from the rhisosphere of *Atriplex hastata* and *Lactuca serriola* were decreased under pH 8. The conductivity values of the soil were between 492-1726 µS. The sediment of the middle basin of the pond-system had to the highest conductivity values.


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The total concentration of Cr measured in the sedimentation pond system of the leather factory far exceeds the concentrations in the control area, since the application of this element in the technology of leather making is of great importance. The heavy metal concentration of the samples can be seen on Figure 3.

*Table 1*

**The conductivity and pH values of the sediment samples in 2008**

| Number of sediment samples | Samples | Conductivity (µS) | pH |
|---|---|---|---|
| **I.** | KM.1 sediment | 550 | 8,01 |
| **II.** | KM.1 sediment from the rhisosphere of *Erigeron canadensis* | 494 | 7,8 |
| **III.** | KM.2 sediment from the rhisosphere of *Atriplex hastata* | 1220 | 7,1 |
| **IV.** | KM.2 sediment from the rhisosphere of *Lactuca serriola* | 1726 | 7,1 |
| **V.** | KM.3 sediment from bottom layer | 640 | 8,28 |
| **VI.** | KM.3 sediment of surface layer | 591 | 8,27 |
| **VII.** | KM.3 sediment from the rhisosphere of *Atriplex hastata* | 492 | 7,5 |

*Table 2*

**Concentration of the aluminium and heavy metals in the sediment samples**

| Samples | | mg*kg-1 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Al | Fe | Mn | Cu | Cr | Pb |
| **2003** | *1. sediment* | 10495 | 17081 | 257 | 9 | 21 | * |
| | *2. sediment* | 24165 | 34668 | 942 | 118 | 15609 | 3 |
| | *3. sediment* | 8766 | 21765 | 339 | 12 | 48 | * |
| **2005** | *2. sediment* | 20044 | 32601 | 645 | 28 | 319 | 37 |
| | *3. sediment* | 27439 | 35464 | 572 | 26 | 326 | * |

The emission of chromium containing industrial wastewater was finished in 1988. Since then stopped the supply of the heavy metals. In comparison with previous studies it can be stated, that the concentration of heavy metals is decreasing continuously, but it is recently still high. The vegetation of the area accumulated and stabilized the heavy metals for many years, and this process yield the heterogeneity of the sediment, too.

We measured high concentration values in the case of Fe, Cr and Mn in the samples. The Cr concentration in the sediment samples was between 21-15.000 mg $kg^{-1}$. The maximum value was 15000 mg $kg^{-1}$ and measured in the sampling site 2$^{nd}$ (KM.2.). Previous studies showed that the concentration of chromium can exceed the 10 000 mg $kg^{-1}$ value (Simon et al., 2000, Lakatos et al., 2008). 10594 mg $kg^{-1}$ concentration of chromium was measured in the soil of a leather tannery district in Italy (Bini et al., 2007), and these also verify that the chromium concentration of such soils may reach so high values. In the concentration of Cu and Pb the concentration values were low in the sediment samples (in the case of Pb the concentration were below the detection limit in the 1, 3 and the 2 samples of sediment). In contrarily we measured high Pb and Cu concentration values in the plant samples. This points to the fact that the accumulation capacity of plants decreased the metal concentration of the surrounding sediment. We proved that the examined site is polluted strongly with heavy metals therefore this site is ideal for study and application of phytoremediation processes and methods.

*Table 3*

**Results of seed germination and roots elongation test in 2008**

| | | 30 seeds | | 25 seeds | |
|---|---|---|---|---|---|
| | | **%** | **toxicological categories** | **%** | **Toxicological categories** |
| **Samples** | I. | 94,8 | non-toxic | 212,9 | Stimulate |
| | II. | 124,2 | stimulate | 119,2 | Stimulate |
| | III. | 100,8 | non-toxic | 132,3 | Stimulate |
| | IV. | 84,1 | slightly-toxic | 171,9 | Stimulate |
| | V. | 91,6 | non-toxic | 101,2 | non-toxic |
| | VI. | 76,1 | slightly-toxic | 147,1 | Stimulate |
| | VII. | 71,5 | slightly-toxic | 76,5 | slightly-toxic |

The results of seed germination and roots elongation test in 2008 are included in Table 3. In the first test 30 while in the second test 25 *Sinapis alba* seeds were placed in a petry-dish. The roots of the seedlings were longer (22mm) than in the first case. It can be explained by the lower number of seeds. From the sediment samples 3 were slightly-toxic, 3 were non-toxic and 1 was stimulative. In the second test 5 were stimulative, 1 was non-toxic and 1 was slightly-toxic. The sediment samples from the 3$^{rd}$ part of pond system had not toxic effects, but the soil from the *Atriplex hastata*s rhizosphere show slight-toxicity according to both of the tests. In our opinion, it can be explained with allelopatic interactions. Numerous plant species use this method in competition for different nutrient sources. It is possible, that the heavy metal stress induces, or increases the allelochemical production of plants. Our ecotoxicological study was compared with the results of previous studies (Lakatos et al 1999, Keresztúri 2004). It can be ascertained, that the sediment of sampling area does not show toxicological effects and danger. It may be concluded that the toxic Cr(VI) form is reduced to moderately- or non-toxic Cr(III) form.

### *Vegetation studies*

We found some plant species with extreme (ranges between 3000-4000 mg $kg^{-1}$) chromium accumulation capacity. For example in the case of *Aster punctatus* the Cr concentration in the stem was 9750 mg $kg^{-1}$ in 2003. The Mn concentration values in the examined plant samples exceeded the limit of toxic level. We measured extremely high Mn values in the leaves of *Phragmites australis* (7599 mg $kg^{-1}$). On the basis of average concentration values measured in different plant organs the *Epilobium parviflorum*, the *Solanum dulcamara*, the *Aster punctatus*, the *Lactuca serriola*, the *Typha angustifolia* and the *Phragmites australis* seemed to be the best Mn accumulator species. The concentration values of Cu were below the limit of phytotoxic level (150-400 mg $kg^{-1}$) in the sediment samples of examined site, but in the most cases of examined plant samples we measured Cu concentration values over the toxic 20-30 mg $kg^{-1}$ level. On the basis of average concentration values measured in different plant organs the best Cu accumulator in 2003 seemed to be the *Phragmites australis*, *Erigeron canadensis* and the *Aster punctatus.* On the basis of average concentration values the *Bidens tripartitus*, *Phragmites australis*, *Sparganium erectum*, *Typha angustifolia*, and the *Xanthium strumarium* can be characterised with good Cu accumulation capacity in 2005. In contrarily the *Populus alba* excluded the surplus Cu.

The Pb concentration values were considerably low in the sediment samples of the examined site. Contrarily the values of Pb in the plant organs in many cases greatly exceeded the limit of phytotoxic levels (30-300 mg). The accumulated Pb values were higher in 2005 than in 2003 (Table 4).

*Table 4*

**Concentration of heavy metals in different parts of plant species**

| | | mg $kg^{-1}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2003 | | | | 2005 | | | |
| **Plants** | **Fraction** | **Mn** | **Cu** | **Cr** | **Pb** | **Mn** | **Cu** | **Cr** | **Pb** |
| *Aster punctatus* /Asteraceae/ | root | 2803 | 275 | 1915 | 1621 | | | | |
| | stem | 3104 | 256 | 9750 | 500 | | | | |
| | leaf | 2909 | 47 | 2367 | 813 | | | | |
| *Atriplex hastata* /Chenopodiaceae/ | root | 967 | 126 | 15016 | 438 | 643 | 36 | 2016 | 3297 |
| | stem | 560 | 45 | 628 | 498 | 96 | 93 | 293 | 186 |
| | leaf | 1825 | 11 | 223 | 117 | 743 | 123 | 1556 | 744 |
| *Bidens tripartitus* /Cyperaceae/ | root | | | | | 924 | 129 | 606 | 931 |
| | stem | | | | | 371 | 273 | 1656 | 382 |
| | leaf | | | | | 899 | 2440 | 388 | 2194 |
| *Cirsium arvense* /Asteraceae/ | root | 1141 | 133 | 2072 | 567 | 1203 | 16 | 166 | * |
| | stem | 656 | * | 86 | 386 | 114 | 61 | * | 193 |
| | leaf | 297 | 68 | 745 | 152 | 257 | 62 | * | 575 |
| *Epilobium parviflorum* /Onagraceae/ | root | 2677 | 142 | 1644 | 336 | | | | |
| | stem | 4055 | * | 902 | 263 | | | | |
| | leaf | 4011 | * | 142 | * | | | | |
| *Erigeron canadensis* /Asteraceae/ | root | 844 | 251 | 600 | 40 | | | | |
| | stem | 1549 | 213 | 1031 | 144 | | | | |
| | leaf | 3378 | 214 | 770 | 183 | | | | |
| *Lactuca serriola* /Asteraceae/ | root | 1815 | 91 | 1294 | 136 | | | | |
| | stem | 2367 | 6 | 300 | 180 | | | | |
| | leaf | 3940 | 23 | 481 | 372 | | | | |
| *Lycopus europaeus* /Lamiaceae/ | root | 267 | 272 | 321 | 321 | | | | |
| | stem | 548 | 68 | 335 | 335 | | | | |
| | leaf | 568 | 82 | * | * | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Phragmites australis* /Poaceae/ | root | 1092 | 537 | 3564 | 573 | 17901 | 908 | 4027 | 340 |
| | stem | 2579 | 37 | 1861 | 620 | 747 | 685 | 50 | 1557 |
| | leaf | 7599 | 127 | 3024 | 613 | 1965 | 335 | 1140 | 568 |
| *Rumex stenophyllus* /Polygonaceae/ | root | 1840 | 51 | 807 | 278 | | | | |
| | stem | 2416 | * | 1734 | 1070 | | | | |
| | leaf | 2203 | 29 | 578 | 100 | | | | |
| *Solanum dulcamara* /Solanaceae/ | root | 2265 | 160 | 1975 | 181 | | | | |
| | stem | 2847 | * | * | * | | | | |
| | leaf | 4037 | 215 | 712 | 369 | | | | |
| *Sparganium erectum* /Sparganiaceae/ | rhizome | | | | | 2338 | 391 | 2235 | 464 |
| | root | | | | | 1503 | 113 | 1633 | 678 |
| | sprout | | | | | 2750 | 238 | 1675 | 447 |

*Table 5*

**List of plant families on the sampling area**

| Family | 1998 42 species | 2001 46 species | 2003 19 species | 2004 23 species | 2005 21 species | 2006 18 species | 2007 17 species | 2008 27 species |
|---|---|---|---|---|---|---|---|---|
| Alismataceae | | 1 | | 1 | | 1 | | |
| Amaranthaceae | 1 | 1 | | | | | | |
| Apiaceae | 3 | 1 | | | | | | |
| Asteraceae | 11 | 13 | 7 | 3 | 3 | 3 | 5 | 9 |
| Brassicaceae | 2 | | | | | | | |
| Butomaceae | | | | | | 1 | | |
| Capriofoliaceae | 1 | | | | | | | |
| Caryophyllaceae | 1 | | | | 2 | | | |
| Ceratophyllaceae | | | | 1 | | 2 | | |
| Chenopodiaceae | 1 | 3 | 1 | 1 | | 1 | 2 | 2 |
| Convolvulaceae | 1 | 2 | | 1 | 1 | 1 | | |
| Cucurbitaceae | | | | | 1 | 1 | | 1 |
| Cyperaceae | 1 | 1 | | 1 | 1 | 1 | 1 | |
| Dipsacaceae | | | | 1 | | | | |
| Fabaceae | | | | 1 | 1 | | 1 | 1 |
| Iridaceae | | | 1 | | | | | |
| Lamiaceae | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 4 |
| Lemnaceae | | | | 2 | 2 | 1 | | |
| Lythraceae | | 1 | | 1 | 1 | | 1 | |
| Malvaceae | 1 | 1 | | | | | | |
| Oleaceae | | | | 1 | | | | 1 |
| Onagraceae | 1 | 2 | 1 | | | | | 1 |
| Poaceae | 6 | 5 | 3 | 3 | 3 | 2 | 2 | 2 |
| Polygonaceae | 3 | 4 | 2 | | | | 3 | 2 |
| Ranunculaceae | 1 | 2 | | | | | | |
| Rubiaceae | 1 | | | | | | | |
| Salicaceae | | 1 | 1 | | 1 | | | |
| Salviniaceae | | | | 1 | 1 | 1 | | |
| Scrophulariaceae | 1 | | | | | | | |
| Solanaceae | 2 | 2 | 1 | | | | | 1 |
| Sparganiaceae | | | | | 1 | 1 | | |
| Typhaceae | 2 | 2 | | 2 | 1 | 1 | 1 | 1 |
| Urticaceae | | 1 | 1 | 1 | 1 | | | 1 |

Most of the identified plants belonged to the families Asteraceae and Poaceae (Table 5). The dominant plants were: *Atriplex hastata*, *Bidens tripartitus*, *Bolboschoenus maritimus*, *Cirsium arvense*, *Erigeron canadensis*, *Phragmites australis* and *Typha angustifolia*. Besides these dominant species mostly weeds with great water-demand were present. Plants of fresh soils and plants of frequently flooded soils were present in highest numbers in the area. We ascertained that the succession process was directed to the highest degree by the inundation.

## CONCLUSIONS

Results of our examination proved that the vegetation of metal contaminated areas could accumulate heavy metals in great amount without showing any unambiguous syndrome of metal toxicity. We observed that the plants of wetlands could be characterized with more intensive heavy metal accumulation capacity. The distribution of accumulated metals between the different plant organs is greatly affected by the species-specific character of the given plant species and the environmental parameters. The accumulated metals could be enter in the food-chain easily by the biomagnification process. These accumulator plants can be applied for phytoremediation of metal polluted sites. Based on our studies, it can be stated, that this area has diverse vegetation, high heavy metal content and low toxicity. We ascertained that the succession process was directed to the highest degree by the phytoremediation.

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