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RELATIONSHIP BETWEEN METAL CONTENTS OF SOIL AND PHYLLOSPHERIC MICRO-ORGANISMS ON UPPER-TISZA AREA

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The aim of study in 2007-2009 were investigated the relationship of soil-plant-metal content in dominant plants and soils on the Tisza river and flood-basin. Between 2007-2009 in soils of flood-basin 20.6-24.6 mg/kg Cr, 0.40-1.15 mg/kg Cd, 28.4-66.7 mg/kg Cu, and 89.7-180.3 mg/kg Zn was found. The metal concentration range in roots of dominant plant species (*Atremisia vulgaris L., Rubus caesius, Solidago canadensis*) found in floodplains were 0.9-4.7 for Cr, 1.6-8.1 for Cd, 22.7-31.5 for Cu, and 106-186 for Zn in mg/kg. The leaves contained 0.7-3.9 Cr, 1.2-1.6 Cd, 0.6-42.8 Cu, and Zn 24.9-203.5 in mg/kg. Our study showed, that the high level of metals as soil contaminations and the high plants metal contents harmful in river compartment. Dominant bacteria were mostly Gram-negative micro-organisms: Pseudomonas, Enterobacter, Bacillus and Coryneform genera. The metal contents and number of bacteria showed positive correlation colonizing the leaves surfaces of plants.

Keywords: micro-organisms, plant surfaces, metal contamination, floodplain, Upper Tisza

INTRODUCTION

Between 2000 and 2004 examinations began at the Hungarian reach of River Tisza, in Bereg Region and along the Tisza floodplains. The aim of this research was to survey the state of heavy metal contamination in catchment areas of Tisza. According to the results soil contamination is one of the degradation factors in this region, since the vegetation also accumulated the contaminants. Balázsy et al. (2007) measured 7.5-46 mg/kg of Cu, 11.3-106.5 mg/kg of Pb, 30.9-267.5 mg/kg of Zn, 0.1-1.33 mg/kg of Cd and 14.5-27 mg/kg of Ni in soil samples, which were taken from the catchment area of Upper Tisza. Considering plant species, the highest cadmium (1.7-11.6 mg/kg) and zinc (113-491 mg/kg) content was found n *Salix* and *Populus* species. According to Csathó (1994) and Kádár (1991) the leaves of plants which grow on soils with high heavy metal content reflects the available metal content in soil with enhanced metal accumulation in plant tissues. Considering inorganic micro-pollutants of the soil-plant system, the most dangerous heavy metals are Cd, Cu, Ni, Hg. Pb and Zn. These metals can be found in the soil of the flood area of River Tisza (Adriano et al., 2003; Prokisch et al., 2005).

Tóth et al. (2005) found a strong positive correlation between increasing heavy metal content in soils and elevated levels of Cd, Ni and Zn in the organs of ragweed plants. They established that in an industrial region, a correlation analysis between the metal concentrations in the roots of ragweed and in the leaves and inflorescence indicated very strong correlations for all the metals studied. All plants in natural habitats have associated epiphytic microflora comprising the so-called phyllosphere (Beattie et al., 1999). The leaf microbial community contains a wide variety of microorganisms, including epiphytes inhabiting leaf surfaces (Kinkel et al. 2000) and endophytes living symptomlessly and intercellularly inside the leaves (Wilson 1995), as well as visible pathogenic organisms (Lappalainen et al. 1999). This community is diverse and dynamic in time and space and its composition is affected by environmental conditions (Kinkel et al. 2000).

It was supposed that the elevated heavy metal content in the soils of River Tisza floodplain is reflected in roots and leaves of dominant plants of a given area. In this research we examined the connections between metal contamination of soils and dominant plant species at 4 locations from Tiszabecs to Tokaj in floodplain areas of River Tisza. The objective of this work was therefore to analyse the microbial communities isolated from the phyllospheres of plants growing in a floodplain ecosystems established under different degrees of heavy-metal contamination.

MATERIALS AND METHODS

Sampling sites

I. **Tiszabecs floodplain**. GPS coordinates: 48°11'N, 22°82'E.

The characteristic forest plants of this area were *Salicetum albae-fragili, Salix alba, Salix fragilis, Populus canescens, Cornus sanguinea, Acer negundo, Fraxinus pennsylvanica, Robinia pseudocacia,* and *Populus* species.

In the undergrowth the dominant plants were *Humulus lupulus, Lamium purpureum, Symphytum officinale, Angelica silvestris, Ranunculus repens, Taraxacum officinale, Galium aparin.* The highest number of plants were *Solidago canadensis, Atremisia vulgaris* L. and *Rubus caesius.*

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Soil could be described by the following characteristics; 2 % (m/m) $CaCO_3$, pH_{KCl} 9.81; pH_{H_2O} 5.6, plasticity K_A 60, water holding capacity 45 % (m/m), humus 3.2 % (m/m).

II. **Vásárosnamény floodplain**. GPS coordinates: 48°11'N, 22°33'E. The characteristic forest plants of this area were *Salix alba, Salix fragilis, Populus canescens, Populus nigra,* and *Acer negundo* species.

In the undergrowth the dominant plants were *Humulus lupulus, Lamium purpureum, Symphytum officinale, Angelica silvestris, Ranunculus repens, Taraxacum officinale, Galium aparin.* The highest number of plants were *Solidago canadensis, Atremisia vulgaris* L. and *Rubus caesius.*

Soil could be described by the following characteristics; 2 % (m/m) $CaCO_3$, pH_{KCl} 9.51; pH_{H2O} 5.95, plasticity K_A 58, water holding capacity 38 % (m/m), humus: 2.2 % (m/m).

III. **Dombrád floodplain**. GPS coordinata: 48°24'N, 21°89'E. The characteristic forest plants of this area were *Populus canescens, Robinia pseudacacia, Salix alba, Salis fragilis,* and *Acer negundo* species.

In the undergrowth the dominant plants were *Urtica dioica, Galaium aparine, Ranunculus repens, Symphytum officinale, Gechoma hederacea, Plantago major, Chrysanrhemum vulgare, Rumex patientia, Equisetum aevense.* The highest number of plants were *Solidago canadensis, Atremisia vulgaris* L. and *Rubus caesius.*

Soil could be described by the following characteristics; 2 % (m/m) $CaCO_3$: 2 %, pH_{KCl} 9.11, pH_{H2O} 5.25, plasticity K_A60, water holding capacity 35 % (m/m), humus: 2.1 % (m/m).

IV. **Tokaj floodplain**. The GPS coordinata: 48°12'N, 21°42'E.

The characteristic forest plants of this area were *Fraxinus pennsylvanica, Salix fragilis, Robinia pseudacacia, Cornus sanguine* and *Amorpha fruticosa* species.

In the undergrowth the dominant plants were *Urtica dioica, Galaium aparine, Ranunculus repens, Ficaria verna, Gechoma hederacea, Plantago major.* The highest number of plants were *Solidago canadensis, Atremisia vulgaris* L. and *Rubus caesius.*

Soil could be described by the following characteristics; 2 % (m/m) $CaCO_{3,}$ pH_{KCl} 8.51, pH_{H2O} 6.95, plasticity K_A, water holding capacity 25 %(m/m), humus: 4.2 % (m/m).

Sampling and analysis of soil

The sample collecting was between 2007 and 2009. From every 100 m x 10 m sampling plots 15 soil sub-samples were taken randomly from 20 to 30 cm depth. The total chromium (Cr), cadmium (Cd), copper (Cu), and zinc (Zn) concentrations of the homogenised and air dried soil samples was determined with Lakanen-Erviö method (MSZ-20135:1999) in the certified laboratory of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences.

Sampling and analysis of plant

From the plots, 5-5 dominantly plants were selected. The roots of these plants were dried to constant weight at 70ºC after washing them in water, while the same drying procedure was applied to the leaves, yet without washing. The "total" metal content of the homogenized samples was analysed by X-Ray fluorescent spectrophotometry.

18 healthy leaf samples were randomly collected from the 25 m^2 plots into sterile containers and total bacteria counts were determined. For qualitative and quantitative analyses of the bacteria populations of the dominant plant leaves, 18 leaves of five randomly collected plants were used in triple replications for each sampling area. The leaf samples were each placed in a Universal sterile bottle containing 100 ml sterile distilled water with acid-washed quartz and shaken for 30 min on a horizontal shaker at 150 rpm. A dilution series was prepared. Population densities were determined in terms of CFU (Colony Forming Unit), according to Lappalainen et al. (1999), for aerobic heterotrophic bacteria contents of the plant leaves. Samples (0.1 cm^3) of the original suspension and subsequent dilutions were inoculated onto the surfaces of plates of Nutrient agar (0.5 g beef extract, 1 g yeast extract, 5 g NaCl, 5 g peptone, 15 g agar, 1000 ml distilled water, Merck) to count the number of CFU cm^{-2}. Three replicate plates were prepared for each dilution per sample. Plates were incubated at 26 °C with incubation times (48 h) that were suitable for the growth of bacteria respectively. It was possible to distinguish and enumerate the predominant genera of aerobic heterotrophic bacteria. The representative colonies of the predominant microbial communities were subcultured and identified to generic and species levels.

Statistical analysis

Statistical analysis of the experimental data was made with SPSS 14.0 software, using analysis of variance with Tukey's b-test. The statistical significance was defined at $P<0.05$.

RESULTS AND DISCUSSION

On the basis of the limit values for metal soil contamination (6/2009. (IV.14.) KvVM-EüM-FVM regulation), and according to metal concentrations which are considered to be contaminated (Alloway, 1990), soils of the examined floodplains were not contaminated with chromium, cadmium, copper and zinc (Table 1.). Cadmium concentrations in soil samples from Dombrád slightly exceeded the limit values for this metal.

Table 1.

Chromium, cadmium, cooper and zinc concentrations (mg/kg) of soils from floodplains. Means are the averages of three years (2007–2009).

Sampling sites	Heavy metals			
	Cr	Cd	Cu	Zn
Tiszabecs	24.0b	0.48a	41.8b	89.7a
Vásárosnamény	20.6a	0.40a	66.6c	110ab
Dombrád	24.6b	1.15b	28.4a	180c
Tokaj	21.6a	0.43a	30.1a	122b
Average	23.5b	0.61a	41.7c	125d

Statistical analysis was done by ANOVA with Tukey's b-test.
Means within the rows followed by the same letter are not statistically significant at P<0.05.

Chromium concentrations in soil samples from Tiszabecs and Dombrád are significantly higher than chromium content in the soil samples from Vásárosnamény and Tokaj. The largest amount of cadmium and zinc was measured in the samples from Dombrád, while the largest quantities of copper could be found in the soil samples from Vásárosnamény. Differences are significant (Table 1).

The metal concentrations of investigated plant's leaves were statistically different in investigated areas (Table 2). In plants, zinc and copper were found in largest quantities. Chromium concentration of plants in non-polluted soils is between 0.02 and 0.2 mg/kg (Csathó, 1994; Kádár 1995; Keresztúri et al., 2003; Pitchel et al., 2000). Considering our results, chromium content of all plant roots and leaves exceeded these aforementioned values in all sampling sites (Table 2). Significantly largest amount of chromium was measured in *Solidago canadensis*' leaves in samples from Dombrád. Cadmium concentration of plants from non-polluted soils is usually 0.3-0.5 mg/kg (Simon, 1999, 2003). Considering our results, cadmium content of all plant leaves exceeded the aforementioned values in all sampling sites (Table 2).

Table 2.

Chromium, cadmium, cooper and zinc concentrations (mg/kg) in leaves of dominant plant species. Means are averages of three years (2007–2009).

Plant samples	Sampling sites				Average
	Tiszabecs	Vásárosnamény	Dombrád	Tokaj	
	Cr				
Rubus caesius	0.70a	0.90a	1.10a	0.90a	0.90a
Solidago canadensis,	0.90a	1.10a	3.90b	1.20a	1.75b
Artemisia vulgaris L	0.80b	0.90b	1.10b	0.40a	0.80a
	Cd				
Rubus caesius	1.20a	1.21a	1.20a	1.20a	1.20a
Solidago canadensis,	1.20a	1.40b	1.60c	1.60c	1.35a
Artemisia vulgaris L	1.60b	1.60b	1.50b	1.20a	1.47a
	Cu				
Rubus caesius	2.01b	3.12b	7.60c	0.60a	3.33a
Solidago canadensis,	42.8b	42.0b	13.2a	12.6a	27.8b
Artemisia vulgaris L	42.1b	40.3b	12.3a	13.1a	26.9b
	Zn				
Rubus caesius	24.9a	25.0a	26.1a	187b	65.8a
Solidago canadensis,	81.0a	106a	191b	203b	145b
Artemisia vulgaris L	73.0a	78.2a	95.3a	201b	112b

Statistical analysis was done by ANOVA with Tukey's b-test.
Means within the rows followed by the same letter are not statistically significant at P<0.05.

Cadmium content was similar in *Rubus caesius* leaves at all investigated sampling sites (Table 2). Compared to Tiszabecs *Solidago canadensis* leaves were found significantly higher cadmium quantities in samples from Vásárosnamény, Dombrád and Tokaj. In *Artemisia vulgaris* L. leaves were accumulated the significantly highest cadmium quantities from Tiszabecs, Vásárosnamény, and Dombrád sampling sites. Copper accumulates mainly in plant roots, while in the aboveground plant organs it can be found in relatively small amounts (Várallyay, 2001; Simon, 1999). Copper concentrations in *Solidago canadensis* and *Artemisia vulgaris* L. leaves were higher than of *Rubus caseius*. This was significant for all sampling sites. The largest concentrations of zinc were measured in the samples from Tokaj. Differences are significant (Table 2).

It was found that the total microbial colonisation of the *Rubus caesius* leaf surfaces was similar in all sampling sites (Table 3).

Table 3.

Total phyllosphere bacterial population densities of leaf surfaces of dominant plant grown on sampling areas. Means are averages of three years (2007 – 2009)

Plant species	Sampling areas			
	Tiszabecs	Vásárosnamény	Dombrád	Tokaj
Rubus caesius	31x10^5 a	27x10^5 a	41x10^5 b	32x10^5 a
Solidago canadensis	29x10^5 a	30x10^5 a	47x10^5 b	27x10^5 a
Artemisia vulgaris L.	35x10^5 a	34x10^5 a	42x10^5 b	31x10^5 a

Statistical analysis was done by ANOVA with Tukey's b-test.
Means within the rows followed by the same letter are not statistically significant at P<0.05.

Similar results were obtained of *Solidago canadensis* and *Artemisia vulgaris* L. leaves surfices. The study showed that the increased levels of metals found in the leaves of *Solidago canadensis* plants grown in the Dombrád areas with elevated chromium, cadmium and zinc contents, had allowed the emergence of different phyllospheric bacterial communities (Table 3).

The results showed a positive correlation coefficient (r) between the densities of the phyllosphere bacterial communities and the chromium and cooper content ($p = 0.05$) in the *Rubus caseius* leaves (Table 4), although the relationship was weak in the case of cadmium and zinc.

Table 4.

Correlation coefficient (r) between heavy metal (Cr, Cd, Cu and Zn) contents (at $p = 0.05$, $n=9$) in the leaves of plants grown at different floodplain areas and the phyllospheric bacterial populations densities. Means are averages of three years (2007 – 2009).

Metals	Plant species		
	Rubus caseius	*Solidago canadensis*	*Artemisia vulgaris L.*
chromium	0.69	0.98	0.84
cadmium	-0.64	0.44	0.64
copper	0.76	-0.45	0.07
zinc	-0.07	0.39	-0.71

Positive correlation coefficient (r) between the densities of the phyllosphere bacterial communities and the chromium content ($p = 0.05$) in the *Solidago canadensis* and *Artemisia vulgaris* L. leaves (Table 4), although the relationship was weak in the case of cadmium cooper and zinc. On the leaf surfaces, the correlation between chromium contents and aerobic heterotrophic bacterial population densities was high.

Our results demonstrated that, among the population diversity of bacteria of the dominant plants phyllosphere in the floodplain ecosystems, plant leaf surfaces were dominantly colonised by aerobic heterotrophic bacterial populations related to *Pseudomonas syringae, Pantoea agglomerans*, *P. putida*, *Bacillus cereus* and *Corynebacterium striatum* (Table 5). These results showed no statistically significant differences between the population densities and diversities of epiphytic aerobic heterotrophic bacterial total microbial populations on the leaf surfaces, in relation to the metal contents of the leaves.

Table 5.
The attendance (%) of detachable aerobic heterotrophic bacteria, in the phyllosphere of dominant three plant grown at four investigated floodplain sites. Means are averages of three years (2007 – 2009).

	Sampling area			
	Tiszabecs	Vásárosnamény	Dombrád	Tokaj
Pantoea agglomerans	11	15	14	9
Brenneria sp.	5	7	4	5
Pseudomonas syringae	31	35	38	40
P. fluorescens	8	9	1	2
P. chlororaphis	1	1	1	2
P. putida	8	7	9	12
Bacillus sp.	-	-	2	1
Micrococcus sp.	1	1	-	-
Xanthomonas maltiphilia	1	1	-	-
Bacillus cereus	9	7	7	6
B. subtilis	2	1	-	-
Corynebacterium striatum	4	2	4	5
Total genera	7a	7a	5a	5a
Total related species	11a	11a	9a	9a

Statistical analysis was done by ANOVA with Tukey's b-test.
Means within the rows followed by the same letter are not statistically significant at P<0.05.

Concerning the population diversity of microorganisms, Gram-negative aerobic heterotrophic bacteria showed the most frequent occurrence on the leaf surfaces. Brunel et al. (1994) reported that the endospore-forming *B. cereus* and *B. subtilis* were components of the microbial contents of the phyllosphere of *Quercus ilex*. Similarly, the results of the present study arein accordance with those of Tanpraset and Reed (1998), who identified the bacterial contaminants of strawberry runner explants. Microbial biofilms have been demonstrated in the phyllospheres of terrestrial plants (Morris et al., 1998). It is assumed that biofilms promote metabolic and genetic exchange between microorganisms (Davey et al., 2000), influence the phenotypic plasticity of epiphytic microorganisms and influence the stress resistance of plants (Lindow et al, 2003). Microbial ecologists have made a huge effort to investigate microbial diversity in the phyllosphere and to studyi biological interactions between microbial species, but interactions between the leaf surfaces, which form the habitat, and the microorganisms have rarely been analysed (Krimm et al., 2005).

CONCLUSIONS

In addition to the effects of a Romanian mining accident a flood occurred in River Tisza by March 23, 2000, which led to significantly increased amounts of zinc and copper in floodplain soils. On the basis of the limit values for soil metal contamination (6/2009. (IV.14.) KvVM-EüM-FVM regulation) and according to metal concentrations which are referred to be under the background level (Alloway, 1990) soils of the examined floodplains were not contaminated with chromium, cadmium, copper and zinc. The cadmium concentration in soil samples from Dombrád exceeded the limit values for soil contamination.

Between 2000 and 2004, the largest copper content was measured in Vásárosnamény (66.6 mg/kg). This value is similar to copper content (58 mg/kg) measured by Boyko et al. (2007) in the floodplain of Upper Tisza. Zinc concentration of soil samples significantly decreased in relation to the largest zinc concentration (452 mg/kg) which was measured just after the mining accident. According to our measurements, the largest zinc concentration was 185 mg/kg. In spite of the similar physicochemical properties of the soil, chromium and zinc content is larger on the floodplain soil of Dombrád, which can be found on a low-lying area, farther from the mouth of River Szamos (Sucharev, 2007). This probably can be explained by the large amount of contaminating elements which are released by the non-ferrous metallurgical companies. In accordance with the results of Sucharev (2007), largest amounts of chromium, cadmium and zinc were measured in the soil samples from Dombrád. Soils can be contaminated with chromium through industrial activities.

Chromium content of plants on non-polluted soils is between 0.02-0.2 mg/kg (Csathó, 1994; Kádár 1995; Keresztúri et al., 2003; Pitchel et al., 2000). Considering our results, chromium content of all plant roots and leaves exceeded the aforementioned values in all sampling sites.

As cadmium does not move in the soil, it can critically accumulate in the upper soil layers. Generally, cadmium content of soils is in linear relation with the cadmium content of plants (Kabata-Pendias et al., 1992; Simon, 1999). In the examined floodplains of Upper Tisza, accumulation of cadmium could be mainly detected in plant roots. According to Kabata-Pendias et al., (1992), average cadmium content of plant leaves is 0.05-0.2 mg/kg, referring to dry matter content. Multiples of these values were measured in dominant plants of floodplains.

Copper accumulates mainly in plant roots, while in the aboveground plant organs it can be found in relatively small amounts (Várallyay, 2001). In several cases, our results support the data of other scientists. However, on the floodplains of Vásárosnamény and Tiszabecs, copper concentration in the leaves of *Solidago canadensis* and *Artemisia vulgaris* L. exceeded the upper value of 5-30 mg/kg concentrations, which were determined by Kabata-Pendias et al., (1992) referred to dry matter content. In case of air pollution zinc accumulates in plant shoots, while in case of soil pollution it accumulates in roots (Simon, 1999; Csathó, 1994; Kádár, 1995; Várallyay, 2001).

Zinc accumulated mostly in plant roots in the examined floodplains, except the floodplain area of Tokaj. In Tokaj, zinc concentration in the leaves of all three plants was significantly higher than zinc concentration of the roots. Zinc concentration in plant leaves exceeded the upper value of 27-150 mg/kg, which were determined by Kabata-Pendias et al., (1992) referred to dry matter content.

Considering the river section of Upper Tisza between Tiszabecs and Tokaj, chromium, cadmium (except of Dombrád area), copper and zinc concentrations of soil samples did not exceeded the limit values of metal soil contamination, which was determined by 6/2009. (IV.14.) KvVM-EüM-FVM regulation. However, chromium, cadmium and copper concentrations (in Tiszabecs and Vásárosnamény), and zinc concentrations (in Tokaj) in plant leaves were larger than metal contents in plants of non-contaminated soils, which was determined by Kabata-Pendias et al., (1992).

In this study, the most common aerobic heterotrophic bacterial isolates found were *Pseudomonas syringae and Pantoea agglomerans*. In addition, *Rhizobium radiobacter*, *Brenneria sp.*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, *Xanthomonas maltiphilia*, the endospore-forming *Bacillus cereus* and *B. subtilis* and *Corynebacterium striatum* were identified. Gram staining supported the classification of the bacterial isolates into Gram-negative and Gram-positive bacteria. Throughout three years of investigation, made during the annual growing period, the density and diversity of Gram-negative aerobic heterotrophic bacteria in the phylloplane of dominant plants increased progressively and to a greater extent than was the case with Gram-positive bacteria. The most common aerobic heterotrophic bacterial populations were related to *P. syringae*, *P. putida*, *B. cereus* and *C. striatum*.

According to Tóth D. M. et al (2005) the metals accumulated by the plants have given grounds to different phyllosphere microbial communities at the sites with different metal contents. It can be supposed that the metal excess found in soil originates from the water contamination of Tisza river. Soil contamination was reflected in the vegetation of floodplains. The metal contents and number of bacteria showed positive correlation colonizing the leaves surfaces of plants.

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