Correlation between Environmental Pollution and Metals Accumulation in *Salix alba* L. (Fam. *Salicaceae*)

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Summary

Heavy metals are considered hazards because of their toxicity to environment and human life. Considering the fact that industrial area of Kosovo Thermal Power Plants (KTPP) is one of most polluted areas in Kosovo, this study describes an investigation of environmental pollution along the river stream of Sitnica, which passes near the KTPP and accumulation of selected heavy metals (As, Cd, Ni, Pb and Cr) in water, soil and willow bark samples (*Salix alba*, L. Fam. *Salicaceae*). Samples were analyzed using ICP-MS (inductively coupled plasma mass spectrometry), and the results were obtained from 30 water samples, 30 soil samples, and 30 willow bark samples, approximately along the 35 km of river stream of Sitnica, in both directions from the source of pollution.

According to the obtained results, it was determined that the content of these pollutants in the study area differ among elements and samples, and they can cause harmful effects in plants, animals and humans. Chromium, nickel and lead in water samples were above target values, and in some cases above intervention values. While in soil samples chromium was within permissible limits, maximum concentrations of arsenic, cadmium and lead were above intervention values. In willow bark samples concentration of nickel was detected in the upper allowed limit. Cadmium and chromium mostly were above permissible limits. Results indicated a possible threat to environment pollution and impact of the industrial sector in the environment, and if adequate protection measures are not taken, degradation of living environment will continue.

Key words

heavy metals, analysis, accumulation, environmental pollution

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Introduction

Species belonging to genus *Salix*, Fam. *Salicaceae*, traditionally were used in medicine, especially for rheumatic and muscular diseases. The painkiller medical effect of leaves and barks from plants belonging to genus *Salix* are described in the Ebers Papyrus in Ancient Egypt, then in Greek civilization, and finally in the 19th century the active extract of the bark, called salicin, was isolated to its crystalline form. Willow is known to possess antipyretic, analgesic, anti-rheumatic, anti-inflammatory, astringent and antiseptic effects (Barnes et al., 2007). Even today willow is used in preparation of some herbal forms, which are used in medicine and they are related to the above-mentioned medical effects. A considerable number of studies showed the medical effect of willow bark, which supports its use in traditional medicine or in the pharmaceutical industry (Barnes *et al.*, 2007).

Heavy metals can be found generally at trace levels in soil and vegetation, and at very low concentrations they are essential for life because they play important roles in metabolic processes in cells. However, these have a toxic effect on organisms at high concentrations. Heavy metal toxicity has an inhibitory effect on plant growth, enzymatic activity, stoma function, photosynthesis activity, accumulation of other nutrient elements and also damages the root system (Onderet *et al.*, 2007).

Industrial area of Kosovo Thermal Power Plants (KTPP) is one of the main problems in terms of environmental pollution in Republic of Kosovo (Zeneli *et al.*, 2013) and during the combustion process of coal, fly ash and bottom ash are released, which are the main components of the environment pollution of this area (Daci, 1985). Analysis of the emission of the flying ash from KTPP during 2005 year has exceeded EU standard by 400-500% (Pietarila and Varjoranta, 2005). Heavy metals occur in soil, surface water and plants (Larisonet *et al.*, 2000). They are not biodegradable and they have long biological half-lives and ability to accumulate.

The river spring of Sitnica is in the region of Ferizaj municipality and river has a length of about 78 km. In Mitrovica region Sitnica joins the Iber river with an average flow 13.8 m^3/s. Along river Sitnica flow several rivers, some of them with industrial content (Graqanka, Drenica) or household residue that contribute to pollution of river, before and after Sitnica passes near KTPP. The willow plant (genus *Salix*, Fam. *Salicaceae*, dominantly *Salix alba* L.) grows nearby water, and it is present along the river stream of Sitnica.

The elements usually are divided into several groups: Macro elements: C, H, O, P, K, N, S, Ca, Mg; Micro elements: Fe, Mn, Zn, Cu, B, Mo, Cl and Ni; Helpful or Useful elements: Co, Na, Si, Al, La, Ti, Ce, Se, V; Toxic elements: Cr, Cd, U, Hg, Pb, As. Different plant cultures grown in contaminated soil with metals and metalloids indicated significant difference in the rate of the metal accumulation, absorption and their distribution and also showed significant difference in the heavy metal concentration in different parts of plant. Within the tree, lead, chromium and copper are usually immobilized in the roots, while cadmium, nickel and zinc are more easily transported to shoots, but the different soil properties in different sites and the different sampling times and climatic conditions in the individual experiments should be taken into account (Tlustošet *et al*., 2007).

Plants can accumulate trace elements in their tissues due to their great ability to adapt to variable chemical properties of the environment. Thus, plants are intermediate reservoirs through which trace elements from soils, and partly from waters and air, move to man and animals (Kabata - Pendias, 2011). Though the heavy metals as Cd, Pb and Ni are not essential for plant growth, they are readily taken and accumulated by plants in toxic forms. The concentrations of elements in *Salix* spp. varied between plant parts and between species, particularly where As concentrations decreased in the following order: leaves >branch bark >stem bark >wood and Cd concentrations: leaves >wood = roots (Tlustoš *et al.*, 2007).

Heavy metals above the certain concentrations turn into toxins and have a damaging effect on the plants. This research provides an overview of environmental pollution and on bioaccumulation of heavy metals in water, soil and willow bark samples, along the approximately 35 km of river stream of Sitnica in both direction from area of Kosovo Thermal Power Plant (source of pollution). This is a first report that presents such data for this area, especially for willow.

Material and Methods

The annual average temperature in the study area is 10.9°C, the average annual rainfall is 638.3 mm and the annual average insulation is 2,140 hours per year. The wind predominantly blows from north and northeast with an average velocity of 3 m/s (data from Kosovo Meteorological Institute).

During autumn 2016, 30 samples of water from river Sitnica, 30 samples of soil next to the river stream and 30 willow bark samples were collected. Fifteen samples (for each group of samples: water, soil, bark) were taken from starting point near the city of Lipjan approximately 17 km from KTPP up to close to source of pollution (sample one: λ Longitude 21 o 6' 41.864" and φ Latitude 420 30'51.67") and 15 another samples starting between two Thermal Power Plants, up to city of Vushtrri, approximately 15 km from the source of pollution (Sample 30: λ - Longitude 20057'14.357" and φ – Latitude 420 49' 29.0712") The average distance between samples was 1 km and samples points are shown in Figure 1. Water samples were marked as W_1 -W_{30} (W - water), soil samples as S_1-S_{30} (S-soil) and willow bark samples as P_1-P_{30} (P-plant).

One liter of water was taken from river in the area approximately up to 0.5 m from river side and up to 0.5 m depth, packed in polyethylene plastic bottle and placed in freezer. Four soil samples were taken in around area 1 - 2 m from the willow tree, approximately up to 30 - 40 cm depth, mixed and 2 kg of soil samples were placed in plastic bags. Willow bark samples from *S. alba* were taken from the trees located at 3 m average distance from the river, at the height of 1.5 - 2 m from the ground, and 200 - 250 g was packed in the paper bag.

To 45 mL of water sample in microwave digestion vessels 4 ± 0.1mL concentrated nitric acid and 1 mL concentrated hydrochloric acid were added and the mixture was placed in microwave. After the cooling the digest was filtered and in volumetric flask was filled up to the mark (100 mL) with deionized water, and determination of elements was performed according to International Standard ISO 11885:2010.

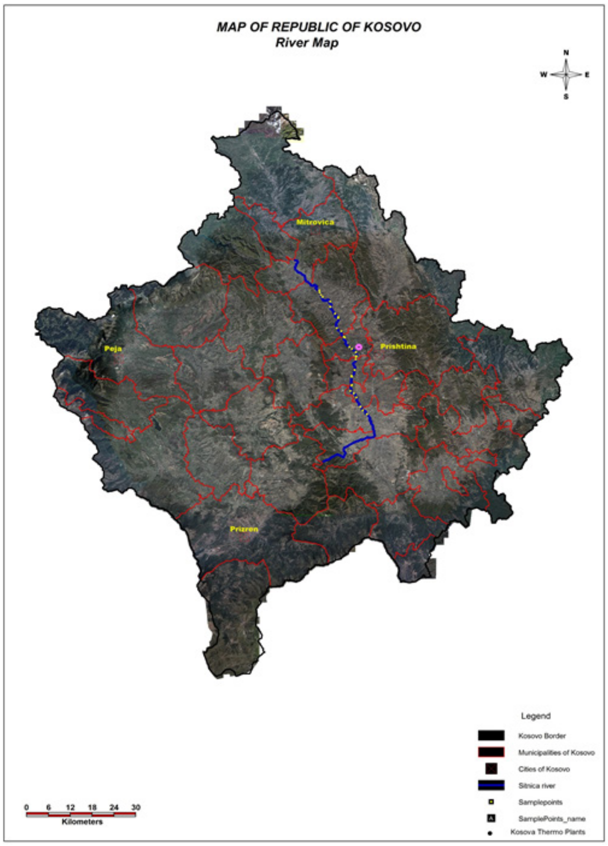

Figure 1. Samples points along the river stream of Sitnica

Soil samples were air dried. After milling (Retsch mill) 2.5 g±1 mg of soil sample was weighed, wet digested with 10 mL aqua regia (mixture of concentrated nitric acid and concentrated hydrochloric acid, in a molar ratio of 1:3) and shaken carefully. After 20 min the vessel was closed and solution was heated in microwave at 170°C and 30 bar pressure (1 min), 170°C and 30 bar pressure (10 min), 170°C and 30 bar pressure (10 min) and for 30 min (3x10 min) at 100°C. After vessels were cooled to room temperature the solution was filtered and diluted with deionized water up to 100 mL in volumetric flask.

Bark samples were initially thoroughly washed with deionized water, to remove any particles or surface impurities attached to the samples, and were dried in thermostat for 24 hours at 65°C. Samples were minced using a mixer grinder and digested in acid solution using a microwave digestion system. To 500 mg of sample, in microwave digestion vessel, 6 mL of concentrated nitric acid and 2 mL concentrated hydrogen peroxide were added. The solution was shaken carefully. After 20 min the vessels were closed and heated in microwave digestion at temperature of 145°C and 30 bar pressure (5 min), 170°C and 30 bar pressure (10 min), 190°C and 30 bar pressure (15 min), and then for 20 minutes (2x10 min) at 100°C. After vessel have cooled to room temperature the solution was filtered and diluted with deionized water up to 50 mL in volumetric flask.

Metal contents were measured by inductively coupled plasma optical emission spectrometry using Perkin-Elmer Optima 2100DV and it was set up and optimized according to the manufacturer's recommended procedures. Blank samples were prepared under the same conditions.

Regulation for heavy metals content in water, in the Republic of Kosovo are based on EU Directives, but for the purpose of this research results were compared with literature data, Dutch Target and Intervention Values (2000), Parameters of Water Quality (Environmental Protection Agency, 2001) and with metals concentrations in other rivers in Kosovo as control values. The intervention values indicate that the concentration levels of the contaminants in the soil above which the functionality of the soil for human, plant and animal life is seriously impaired or threatened. Concentrations in excess of the intervention values correspond to-serious contamination. The target values indicate the soil quality required for sustainability or, expressed in terms of remedial policy, the soil quality required for the full restoration of the soil's functionality for human, animal and plant life. Target values were based on standards for surface waters (Friday, 1998). Regardless, the target values are based on ecological risks and background concentrations and the intervention values are based on human health and ecological risks (Rijkswaterstaat, 2014).

The heavy metal Transfer Factor (TF) from soil to plant was established using the formula: TF = Mp/Ms (Mp = metal concentration in plant mg/kg; metal concentration in soil mg/kg).

Results and discussion

There is substantial concern regarding heavy metals because of their threat to environment and human life. Samples of water, soil and willow bark along the river stream of Sitnica, which passes near the KTPP, were investigated for accumulation of As, Cd, Ni, Pb and Cr. Obtained results of concentrations of these heavy metals in the samples are presented in Table 1 and Figure 2.

Table 1. Min. and max. values of selected heavy metals in water, soil and willow bark samples

Parameters	Water samples (mg/mL)		Soil samples (mg/kg dry mass)		Plant samples (mg/kg dry mass)	
	Min. & max. values	Mean	Min. & max. values	Mean	Min. & max. values	Mean
As	0.0019 - 0.022	0.01	20.29 - 78.36	37.67	< 0.002mg/kg	-
Cd	< 0.0001 mg/mL	-	9.83 - 22.23	14.85	0.10 - 4.49	0.63
Ni	0.029 - 0.078	0.049	66.74 - 129.59	95.04	6.49 - 10.09	7.70
Pb	0.299 - 0.642	0.47	84.20 - 667.97	185.22	< 0.00 1 mg/kg	-
Cr	0.006 - 0.037	0.011	18.06 - 35.85	24.27	0.85 - 1.89	1.29

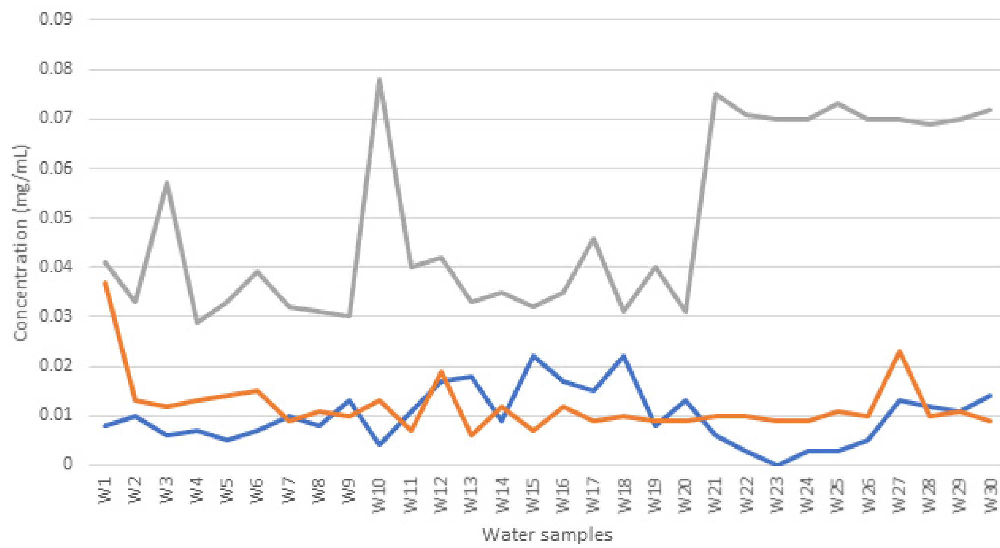

Figure 2. Concentrations of metals in water samples along the river stream of Sitnica (mg/L)

Arsenic is very toxic and according to the WHO Guidelines, the industrial effluents contribute in a considerable extent in the presence of arsenic in water. The mean detected concentration for arsenic was 0.01 mg/L (ranging from 0.0019 mg/L to 0.022 mg/L), which is below intervention value for arsenic based on Dutch Target and Intervention Values (2000).

Cadmium is also highly toxic, hence there are severe restrictions on its concentration in water (0.005 mg/L). Cadmium was not detected in water sample. The limit of detection for cadmium with this method was lower than 0.001 mg/L.

Nickel is another toxic and harmful element, which is of moderate concern because of possible carcinogenicity as far as humans are concerned; it also has variable harmful effects on aquatic life. It is toxic to plant life, too, and is a hazard to fish (Environmental Protection Agency, 2001). Its content ranged from 0.029 mg/L to 0.078 mg/L. These detected values are above target value (0.0021 mg/L), but in limits of intervention value (0.075 mg/L). The maximum detected value was in sample 10, which is near the entering point in area of KTPP. But in north direction from point 21 the values were higher continuously all to the ending point of sampling. Nickel content in source of Sitnica reported by Shala *et al.* (2015) was in range 0.015-0.033 mg/L and lower compared with obtained results in KTTP polluted area.

Lead is the most often analyzed heavy metal. During this research it was detected in only four water samples with concentrations ranging from 0.299 mg/L to 0.642 mg/L, which are above permissible value according to WHO standards (0.05 mg/L) (Nazir *et al.*, 2015). In other samples the concentration of lead detection was under the limit of detection (< 0.001 mg/L).

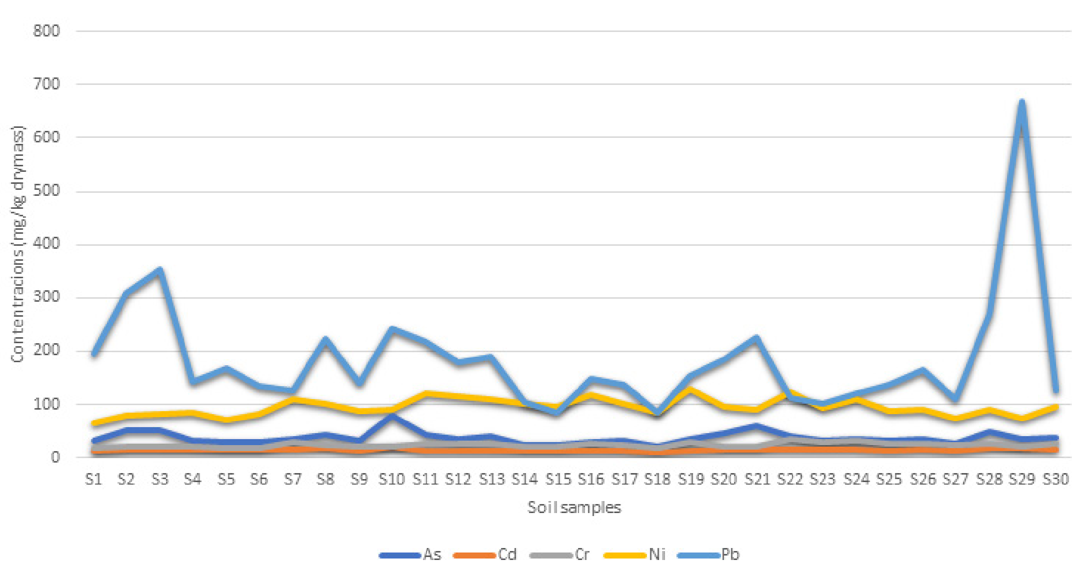

Figure 3. Concentrations of metals in soil samples along the river stream of Sitnica (mg/kg dry mass)

Chromium is toxic, it is considered that the element is carcinogenic (at high concentrations), and it can act as a skin irritant (Environmental Protection Agency, 2001). The values in all water samples ranged from 0.006 mg/L to 0.037 mg/L. Maximum detected concentration of chromium (0.03 mg/L) was higher than intervention value, and in all samples concentration was above target value (0.0025 mg/L).

The concentration of heavy metals in soil depends on many factors. Soil pollution by heavy metals is a critical environmental concern due to its adverse ecological effects. Heavy metals occur naturally at low concentration in soils but are considered soil contaminants at high concentration due to their acute and chronic toxicity (Han *et al.*, 2013). Pollution occurs when an element or a substance is present in greater than natural (background) concentrations as a result of human activity and has a net detrimental effect on the environment and its components. Thus, from a plant, animal, and human health perspective, soils are not considered polluted unless a threshold concentration exists that begins to affect biological processes (Kabata-Pendias, 2011).

All results from soil samples along the river stream of Sitnica were expressed on mg/kg dry mass basis as shown in Figure 3.

The toxicity of arsenic in soils could be overcome by several ways, depending on arsenic pollution sources and on soil properties (Kabata-Pendias, 2011). Arsenic values were ranging from limit of target value of 20.29 mg/kg) to max. detected value of 78.36 mg/kg. Compared to Dutch Target and Intervention Values (2000) it is above intervention value (55 mg/kg) and the results are higher than obtained results in Anadrinia region (Kosovo) where the arsenic values ranged from 16.49 mg/kg to 62.44 mg/kg (Shehu *et al.*, 2016)

Cadmium concentrations ranged from 9.83 mg/kg, which is almost equal with the highest value (9.36 mg/kg) for Anadrinia region presented by Shehu *et al.*, 2016), to 22.23 mg/kg (above intervention value) (Dutch Target and Intervention Values (2000). Cadmium is considered as being one of the most ecotoxic metals that exhibits adverse effects on all biological processes in humans, animals and plants. This metal reveals its great adverse potential to affect the environment and the quality of food (Kabata-Pendias, 2011).

Nickel toxicity is generally seen in soil irrigated with waste water. Nickel is absorbed easily and rapidly by plants (Güne *et al.*, 2004). Nickel concentrations in all samples were above target value (35 mg/kg), ranging from 66.74 mg/kg to 129.59 mg/kg, but below intervention value (210 mg/kg). Average value reported by Cicek and Koparal (2004) for nickel in surrounded area of Thermal Power Plant Tunçbilek was 25 mg/kg, which is below the average value (95.04 mg/kg) from samples around KTPP.

Lead in the environment comes mainly from anthropogenic sources (mines, metallurgical plants etc.). Lead recorded concentrations (84.20 mg/kg to 667.97 mg/kg), were ranging from limits of target value (85 mg/kg) and above intervention value (530 mg/kg). These results are much higher than in Anadrinia region presented in range from 16.27 mg/kg to 42.58 mg/kg (Shehu *et al.*, 2016). There was also deference in lead concentration between sample points regarding the distance form source of pollution. For example, in the sample point S3, which is located south from KTPP, the lead concentration was 353.71 mg/kg, whereas in the S29, which is the point north from KTPP, the lead concentration was 667.97 mg/kg.

Chromium concentrations in all samples were below target value (100 mg/kg), ranging from 18.06 mg/kg to 35.85 mg/kg. These values were higher compared to average value (15 mg/kg) presented by Cicek and Koparal (2004). Shehu *et al.* (2016) presented higher values for chromium in range from 55.99 mg/kg to 166.79 mg/kg for Andrinia region.

Different vegetable species accumulate different metals depending on environmental conditions, metal species and plant available forms of heavy metals (Lokeshwari and Chandrappa, 2006).

The results for willow bark samples along the river stream of Sitnica are presented in Figure 4.

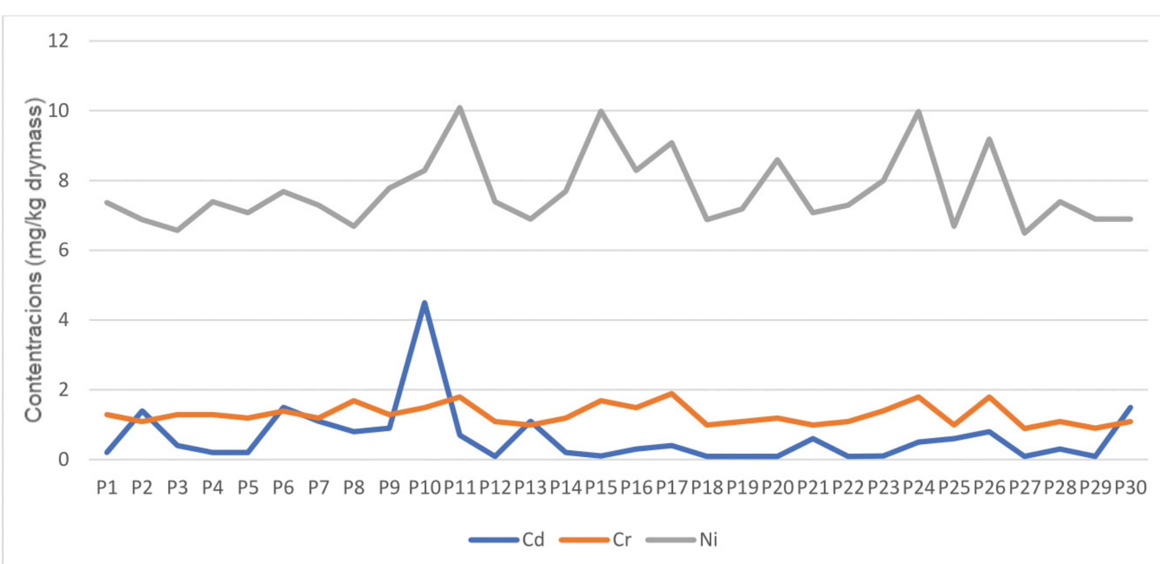

Figure 4. Concentrations of metals in willow bark samples along the river stream of Sitnica (mg/kg dry mass)

Studies of the uptake and chemical behavior of nickel in plants are related mainly to its toxicity having possible implications with respect to animals and human. Restricted growths of plants and injuries caused by an excess of nickel were observed for quite a long time (Kabata - Pendias, 2011). Nickel concentrations (ranged from 6.49 mg/kg to 10.09 mg/kg) were within the upper permissible limit of nickel in plants recommended by WHO (10 mg/kg) (Ogundele *et al.*, 2015).

Lead adversely affects the process of respiration and metabolism of plants (Paolacci *et al.*, 1997), while several plant species are known to tolerate a high level of arsenic in tissues (Kabata - Pendias, 2011). In analyzed bark samples along the river stream of Sitnica, arsenic and lead were not detected. The limit of detection for arsenic and lead with methods used in this study was 0.002 mg/kg and 0.001 mg/kg, respectively.

Cadmium induces changes in plants at all biochemical, physical and genetic levels, which are responsible for the reduction in the growth of plants (Nouariri *et al.*, 2006). In seven analyzed samples cadmium was not detected, and in others, concentrations of cadmium ranged from 0.10 mg/kg to 4.49 mg/kg, and max. value was higher than in research by Vandecasteele *et al.*, (2002) (1.3–3.6 mg/kg). The permissible limit of cadmium in plants recommended by WHO is 0.02 mg/kg (WHO, 1998).

Content of chromium in plants is controlled mainly by its soluble content in the soil. Content of chromium in plants recently received considerable attention due to the knowledge of its importance as an essential micronutrient in human metabolic processes, but also because of its carcinogenic effects (Kabata-Pendias, 2011). The concentrations of chromium ranged from 0.85 mg/kg to 1.89 mg/kg and in 20 samples were within permissible limit of chromium recommended by WHO for plants (1.30 mg/kg) (Ogundele *et al.*, 2015).

Plants uptake heavy metals from soils through several processes, sush as redox reactions, ionic exchange, precipitation-dissolution, etc. Transmission of metals from soil to plant tissues is studied using an index called Transfer Factor (TF). Higher TF values (≥1) indicate higher absorption of metal from soil by the plant and higher suitability of the plant for phyto-extraction and phytoremediation. On the contrary, lower values indicate poor response of plants towards metal absorption and the plant can be used for human consumption (Rangnekar *et al.*, 2013). If the transfer coefficient of a metal is greater than 0.5, the plant will have a greater chance of the metal contamination by anthropogenic activities (Sajjad *et al.*, 2009). Based on the obtained results, the TF of cadmium from soil to plant were between 0.004 and 0.201, which is quit high compared with TF values for nickel (0.055 – 0.110) and chromium (0.030 - 0.088). TF results with correlation analysis between selected parameters are shown in Table 2.

Correlation analysis shows statistically significant positive correlation between the concentration of arsenic in soil and cadmium in plant (r=0.635), cadmium in soil and cadmium in plant (r=0.561) and nickel in soil and nickel in plant (r=0.339).

Conclusion

In this research, the main goal was to analyze heavy metals concentration in the water, soil and willow bark samples that were collected along the approximately 35 km of the river stream of Sitnica. According to the obtained values, it was determined that the content of these pollutants in the study area differ among elements and samples, and those values indicate a possible threat by the industrial sector to the environment. Chromium, nickel and lead in water samples were above target values, and in some cases above intervention values. Arsenic values were below intervention value. Heavy metals content in soil samples varied. While chromium was within permissible limits, max. concentrations of arsenic, cadmium and lead were above intervention values. In willow bark samples concentrations of nickel was detected in the upper allowed limits. Cadmium and chromium were mostly above permissible limits. TF decreased in the following order: Cd>Ni>Cr. Based on our presented results we came to conclusion that there is positive correlation with high statistical significance between concentration of arsenic, cadmium and chromium in soil and willow bark.

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Table 2. Transfer factor soil to plant and correlations between selected metals

	Cd	Ni	Cr	As /Cd (s/p)	Cd/Cd	Ni/Ni
TF (min -max)	0.004 -0.201	0.055 – 0.110	0.030-0.088	-	-	-
r	-	-	-	0.653	0.561006	0.33946

TF - Transfer factor; r - correlation; (s) - soil; (p) - plant

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