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Effects of Metal Toxicity on the Growth and Photosynthetic Pigment Contents of *Salix purpurea* in Mitrovica, Kosovo

F. Zabërgja Ferati,[a] *M. Kerolli Mustafa,*[b*] and *F. Abazaj*[b]

[a] University of Mitrovica Isa Boletini, Kosovo
[b] International Business College Mitrovica, Kosovo

Abstract

In Mitrovica, Kosovo, mining activity is threatening the environment and living organisms. The major sources of this pollution are the open tailing dumps in the city of Mitrovica, causing contamination of soil, air, and plants. This study determines heavy metal contamination levels in *Salix purpurea* leaf grown in the city of Mitrovica and its surrounding area, where the industrial plants and open tailing dumps of mineral processing are located. Furthermore, the photosynthetic activity of the plant was used to assess the content of heavy metals (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn). The two-year (2019 and 2020) study showed heavy metal upgrade in leaves and chlorophyll. Total chlorophyll, ratios of chlorophyll a over chlorophyll b and total chlorophyll over carotenoids was calculated. The obtained data were analysed using Minitab 19 statistical software. The results indicated that mining activities and the open tailing dumps initiate heavy metal pollution for plants with a high risk of accumulation.

Keywords

Heavy metals, Salix purpurea, photosynthetic activity, photosynthetic pigment, phytoremediation

1 Introduction

The intensive development of industry across our planet has caused major environmental degradation and disruption of the ecological balance. Heavy metal pollution is one of the most serious environmental problems, and is present in many industrial and mining areas around the world.[1–6]

Plants growing in metal-polluted sites can accumulate heavy metals from the soil through the root system,[3] from the air, and water.[11] Although some of the heavy metals, such as Zn, Cu, Mn, Ni, Fe, and Co are essential elements for biochemical and physiological functions in plants, in high concentrations they are phytotoxic and pose an environmental threat.[4–11]

Researchers have reported that some of the direct toxic effects caused by high concentrations of heavy metals in plants include inhibition of photosynthesis, inhibition of cytoplasmic enzymes, and damage to cell structure, as well as many others physiological processes as a result of oxidative stress.[2,7–10,12] The first obvious effects of heavy metals on plants are reduced plant growth, and the appearance of chlorosis and necrosis.

Therefore, numerous studies on metal tolerance and accumulation have been made on willow species, but few studies have focused on *Salix purpurea*.[5,13–15] The concentration of heavy metals in leaves cause chlorophyll biosynthesis,[14] disturbance of metabolic and physiological processes in plants, which often results in losses of vitality and decreases growth.[15]

Furthermore, the impact of heavy metals on the chloroplast ultrastructure is the key in understanding the physiological alterations induced, because of the relationship between chloroplast structure, photosynthetic ability, and plant growth.[13]

The aims of the current work were to: i) determine the levels of concentration of heavy metals in the leaf of *Salix purpurea*; ii) determine and analyse the photosynthetic pigments in the leaf of *Salix purpurea*, including chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids, total chlorophyll (Chl a + Chl b), ratio Chl a/b, and ratio (Chl a + Chl b)/carotenoids; iii) determine metal pollution effects on photosynthetic activity in *Salix purpurea*; and iv) statistical analysis for comparing the heavy metal concentrations in plants in different seasons.

2 Materials and methods

Six sampling sites close to Trepça mining complex were selected in Mitrovica, Kosovo (Fig. 1). These sampling sites have been characterised and evaluated as the most critical points, due to the anthropogenic influences of the discharge of industrial water from mining flotation and open tailing dumps in the Mitrovica Industrial Park.

Each of the selected points has specific characteristics that correspond to the purpose of our research work, and can be evaluated based on the criteria of the methodologies used. Mitrovica is located at lat. 42.53° N and long. 25.52° E in the north of Kosovo. The city is about 508–510 m above sea level. The average monthly temperatures range between 15 and 25 °C. In terms of mineral resources, Mitrovica was one of the most important industrial cities not only in Kosovo, but also in Europe and beyond.

* Corresponding author: Mihone Kerolli-Mustafa, PhD
Email: m.kerolli@ibcmitrovica.eu

Unfortunately, these mining operations have resulted in negative environmental impacts.[16,17] Fig. 1 presents the six sampling sites: three sampling sites along the riverbed of Trepça River (S1–S3) (close to Trepça Mine Flotation), and three sampling sites along the riverbed of Sitnica River (S4–S6) very close to Mitrovica Industrial Park (Zink smelter, Lead Battery Factory, and open tailing dumps of Zn Electrolysis Process and waste containing heavy metals).

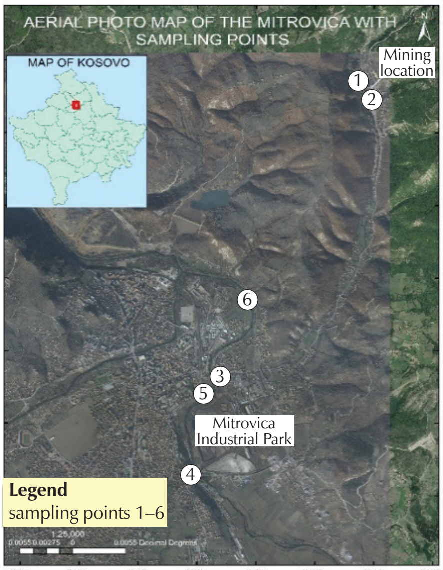

Fig. 1 – Sampling locations

2.1 Plant sampling and preparation

The plant was sampled and prepared according to the procedure of ICARDA's manual and *Estefan et al.*[19] In order to determine the concentrations of elements (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn), leaves from the willow material (*Salix purpurea*) were collected in June 2019 and September 2020. All samples were taken in triplicate.

Material in the amount of about 100 g fresh weight was placed in polypropylene vials. After being transported to the laboratory, the experimental material was dried in an electric dryer for 24 h at a temperature of 70 °C, after which the dry samples were ground to a powder for 3 min in a Wiley mill. The material, as three representative samples, (0.5 g each), was mineralised in a closed microwave system (BERGHOF Speed wave MWS-3+) using 3 ml concentrated HNO_3, 3 ml 30 % H_2O_2, and 0.5 ml concentrated HCl. All experiments were done in triplicate, and data are presented as mean ± standard deviation.

The means of element content in the plant samples were determined using inductively coupled plasma – optical emission spectrometry (ICP–OES Optima 2100 DV, Perkin-Elmer). Certified reference materials NIST 1575a (Pine Needles) and NCS DC 73350 (Leaves of Poplar) for plants were analysed to test the accuracy of the applied method for the determination of total metal concentrations in investigated samples.

Soil samples at 20 cm depth and plants were collected in the same area and neutral zones, and analysed at the same locations. The results for metal accumulation efficiency of soil and plants, the Bioconcentration factor (*BCF*) and Translocation factor (*TF*), were recently published.[18] These results were used as a comparison to the current research on analysing the effects of metal toxicity on the growth and photosynthetic pigment contents of *Salix purpurea*.

2.2 Photosynthetic pigments

The middle part of the leaf of *Salix purpurea* was taken, excluding its main stem. Pigments were extracted by grinding freshly sampled leaves (0.5 g). For each sampling point, a fourth leaf was taken, and put in the dark for 24 h in an 80/20 % (v/v) acetone/water solution containing $MgCO_3$ (0.5 % w/v), at room temperature. The pigments were extracted from samples taken in June 2019, September 2019, June 2020, and September 2020. Photosynthetic pigments of all samples were extracted in triplicate to minimize experimental errors. Spectrophotometric measurements were performed at wavelengths of 663 nm for chlorophyll a (Chl a), 644 nm for chlorophyll b (Chl b), and at 470 nm for carotenoids. These analyses were determined using UV-Vis spectrophotometer (4802H UV/Vis Double Beam Spectrophotometer). Pigment contents were calculated in mg *per* g of dry leaf weight (*DW*) by applying the absorption coefficient equations: Eqs. (1)–(3).[21]

$$\text{Chl a}\left(\text{mg g}^{-1}\ DW\right) = \\ = \left[10.3 \cdot OD_{\text{at 663 nm}} - 0.918 \cdot OD_{\text{at 644 nm}}\right] \cdot V \cdot \frac{100}{FW} \cdot DW \quad (1)$$

$$\text{Chl b}\left(\text{mg g}^{-1}\ DW\right) = \\ = \left[19.7 \cdot OD_{\text{at 644 nm}} - 3.87 \cdot OD_{\text{at 663 nm}}\right] \cdot V \cdot \frac{100}{FW} \cdot DW \quad (2)$$

$$\text{Carotenoids}\left(\text{mg g}^{-1}\ DW\right) = \\ = [4.75 \cdot OD_{\text{at 470 nm}} - 0.226\left(\text{Chl a} + \text{Chl b}\right) \cdot V \cdot \frac{100}{FW} \cdot DW \quad (3)$$

DW, *FW*, *OD*, and *V*, are dry leaf weight, fresh leaf weight, optical density, and volume of sample, respectively.

2.3 Statistical analysis

The first stage in analysing the heavy metal concentrations in leaves was to describe the ranges of concentrations of the elements, and to obtain an indication of the accumulation of these metals in plants in different seasons and in different locations. Considering the data-dependence of our research, the visualization of concentration data by parallel coordinates was used for the data set. The parallel coordinate methodology is known as the methodology for unambiguous visualization of multivariate data and relations.[20] The display of observations was achieved by marking the value of each dimension (concentration) at the corresponding axis (samples), and connecting the values belonging to the same observation with a line. Each line represents a sample, and the parallel coordinate plot establishes the trends between the different samples against concentration and month.

In this study, the matrix plot correlation analysis was also used to measure the degree of linear association between pigments of *Salix purpurea*. Histograms were used to examine the distribution of values of each variable. The parallel coordinate plot and the matrix plot correlation were created using Minitab 19 Statistical Software. Statistical comparison of obtained data was performed by two-paired *t*-test at a significance level of $p = 0.05$.

3 Results and discussion

Eight hazardous heavy metals (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) were studied and determined in *Salix purpurea* leaves in June 2019 and September 2020 (Table 1). The results of heavy metal concentrations in leaves present the uptake and accumulation patterns of selected metals (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn). Mostly, the higher uptake of metals in the leaves of *Salix purpurea* was recorded in the area close to mining flotation (S2 and S5) and Mitrovica Industrial Park. The mean of total contents of eight heavy metals in all six leaf samples were in the range of: As 0.02–19.38 mg kg^{-1}, Cd 1.74–29.59 mg kg^{-1}, Co 2.5–3.8 mg kg^{-1}, Cr 1.21–2.13 mg kg^{-1}, Cu 8.81–40.47 mg kg^{-1}, Ni 5.11–26.38 mg kg^{-1}, Pb 6.85–762.21 mg kg^{-1}, and Zn 59.52–1394.03 mg kg^{-1}. As may be seen from Table 1, the metal concentrations in samples 2 and 5 were higher than were those in all other samples. By comparing the results with the limited values (values of *Salix purpurea* grown in non-contaminated location and from the contaminated locations),[22] the obtained results were quite high and above the limits in the areas close to flotation and Mitrovica Industrial Park. The leaf samples collected in September 2020 showed lower values compared to those sampled in the drier season, however the results still revealed high concentrations above the limit values. The statistical results presented in Fig. 2 also confirm a similar trend, suggesting that the industrial activities and open tailing dumps in Mitrovica present a serious threat to the environment and human health in that area.

These results are consistent with other studies that demonstrate substantial levels of heavy metals in soil and plants.[18] The study reported that the results of heavy metal concentrations in soil and plant bioconcentration and translocation factors present the uptake and accumulation patterns of heavy metals, such as As, Cd, Co, Cr, Cu, Ni, Pb, and Zn. The higher uptake of metals from the soil and *Salix purpurea* plant was recorded in the area close to mining flotation and Mitrovica Industrial Park, which corresponds with the results of the current study. The concentration of heavy metals in soil samples were found in the range of: As 48.91–881.26 mg kg^{-1}, Cd 5.48–238.57 mg kg^{-1}, Co 17.83–31.25 mg kg^{-1}, Cr 34.98–168.56 mg kg^{-1}, Cu 58.93–943.8 mg kg^{-1}, Ni 82.86–282.33 mg kg^{-1}, Pb 359.26–4662.22 mg kg^{-1}, and Zn 386.21–4482.26 mg kg^{-1}.[18] The results obtained from the roots, stems, and leaves of *Salix purpurea* presented the similar trend: As 1.09 to17.75 mg kg^{-1}, Cd 2.67 to 37.2 mg kg^{-1}, Co 3.37 to −17.54 mg kg^{-1}, Cr 1.95 to 9.67 mg kg^{-1}, Cu 5.95 to 32.9 mg kg^{-1}, Ni 4.74 to 61.25 mg kg^{-1}, Pb 5.98 to 1986 mg kg^{-1}, and Zn 1095.86 to 3045.65 mg kg^{-1}.[18]

Parallel coordinates is a very popular multivariate visualization technique that can present the direct analysis of multivariate relationships in data used on metal concentrations in *Salix purpurea* for different seasons. The used technique showed a compact two-dimensional representation of data representing the metal concentrations with coordinates (metal concentrations in Samples S1–S6) by points in

Table 1 – Heavy metal concentrations (mg kg^{-1}) in leaves of *Salix purpurea*

Samples	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
June 2019								
S1	0.17 ± 0.01	2.01 ± 0.05	2.50 ± 0.40	1.21 ± 0.05	19.68 ± 0.02	5.33 ± 0.37	13.18 ± 0.05	219.66 ± 0.03
S2	19.38 ± 0.04	9.12 ± 0.30	3.80 ± 0.20	2.13 ± 0.50	40.47 ± 0.14	25.51 ± 0.22	762.21 ± 0.12	1147.63 ± 0.30
S3	0.05 ± 0.20	4.75 ± 0.70	3.20 ± 0.02	1.52 ± 0.21	12.72 ± 0.17	6.36 ± 0.06	13.68 ± 0.21	364.74 ± 0.12
S4	0.03 ± 0.06	1.74 ± 0.11	3.70 ± 0.21	1.74 ± 0.07	8.81 ± 0.40	9.74 ± 0.12	6.85 ± 0.16	59.52 ± 0.25
S5	8.03 ± 0.40	16.18 ± 0.17	3.50 ± 0.60	1.96 ± 0.18	13.06 ± 0.12	26.38 ± 0.15	100.02 ± 0.06	1394.03 ± 0.24
S6	0.02 ± 0.20	29.59 ± 0.08	2.8 ± 0.17	1.61 ± 0.15	16.80 ± 0.32	5.11 ± 0.21	8.88 ± 0.27	585.00 ± 0.08
September 2020								
S1	0.28 ± 0.05	1.20 ± 0.02	3.80 ± 0.60	1.04 ± 0.30	11.30 ± 0.03	6.39 ± 0.13	24.49 ± 0.06	805.50 ± 0.05
S2	16.38 ± 0.06	9.93 ± 0.50	3.40 ± 0.19	1.75 ± 0.12	16.83 ± 0.23	22.62 ± 0.16	570.30 ± 0.25	2300.00 ± 0.02
S3	0.09 ± 0.05	10.61 ± 0.80	3.60 ± 0.12	1.89 ± 0.17	9.47 ± 0.11	5.28 ± 0.03	17.14 ± 0.14	161.00 ± 0.21
S4	0.02 ± 0.13	2.32 ± 0.21	3.68 ± 0.18	1.06 ± 0.03	7.84 ± 0.36	6.52 ± 0.24	8.61 ± 0.28	157.00 ± 0.15
S5	7.97 ± 0.30	16.70 ± 0.25	3.55 ± 0.40	1.06 ± 0.15	17.57 ± 0.21	27.16 ± 0.25	131.40 ± 0.18	1903.00 ± 0.15
S6	0.04 ± 0.25	22.09 ± 0.06	3.89 ± 0.15	1.65 ± 0.12	15.83 ± 0.18	6.22 ± 0.32	8.74 ± 0.28	501.60 ± 0.50
Limit values	1–1.70	0.05–0.20	0.02–1	0.10–5	5–30	0.10–5	5–10	27–150

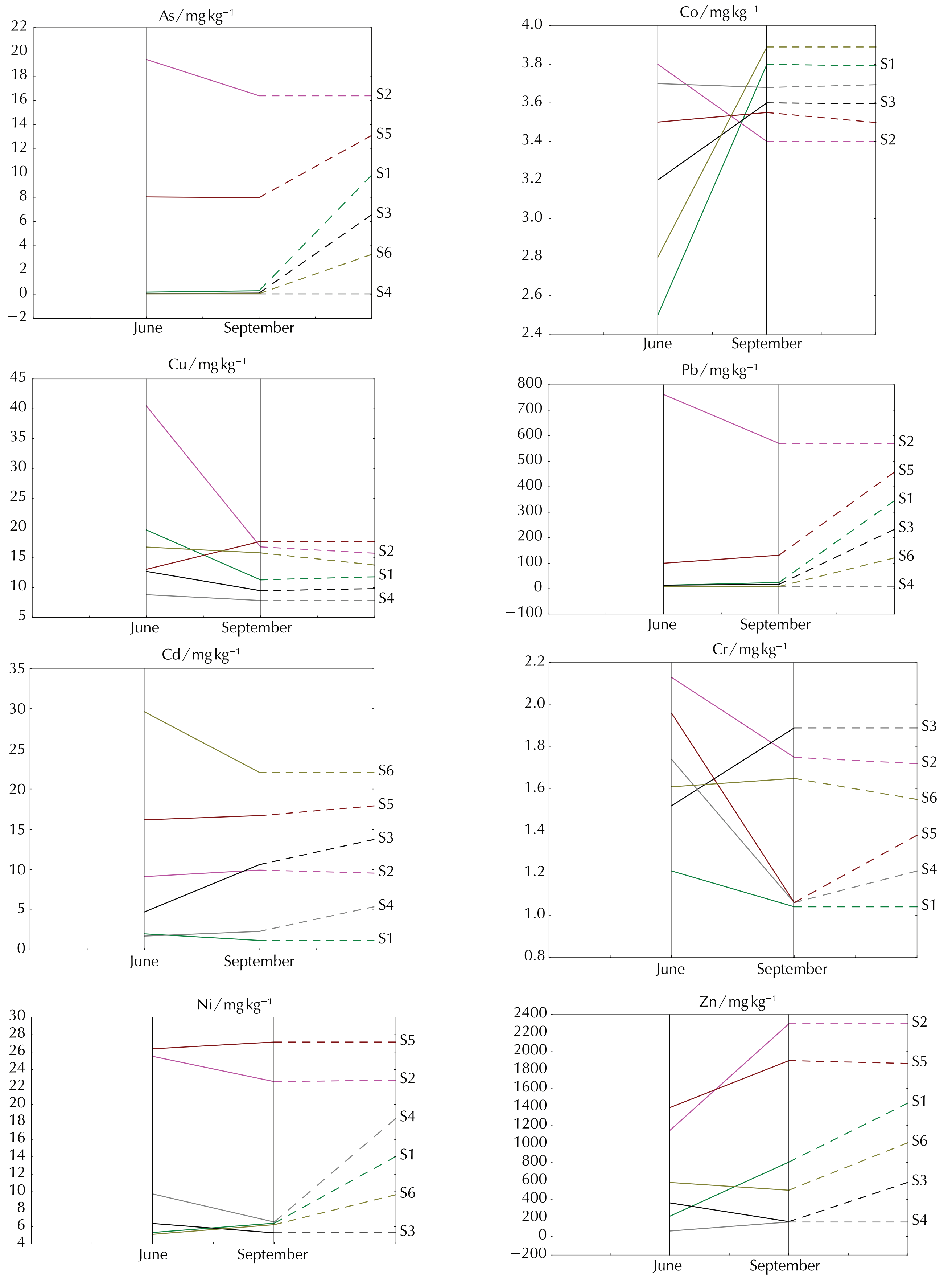

Fig. 2 – Parallel coordinate plot of heavy metal concentrations in leaves of *Salix purpurea* from Mitrovica, Kosovo

time (June–September) parallel axes, joined with a polyline (Fig. 2). The parallel coordinate plots correspond with the leave's heavy metal uptake trends presented in Table 1.

Photosynthesis is the process in which green plants use solar energy to produce carbohydrates from carbon dioxide (CO_2) and water (H_2O) in the presence of chlorophyll.[20-24] Plants are also characterized by their affinity to absorb and uptake metals through their root system and other organs. The main means of metal entry into plants is by absorption from the soil through the roots. Accumulation differs for each metal, plant type, organ, soil type, and season.

The willow has a deep root system compared to other trees, which can act as a biological filter. *Unterbrunnera et al.*[24] reported that *Salix purpurea* is able to accumulate heavy metals and present an environmentally stable answer for phytoremediation.

The effects of heavy metals on plants result in structural damage, inhibition of growth, physiological, biochemical activity, and plant functioning. These measurements revealed how the growth and photosynthetic pigment content of the plant in the surrounding area were affected by the presence of metals. Chlorophyll is essential for carrying out the process of photosynthesis; this is shown in Table 2. In sample 2, where the heavy metal pollution is lower, it was observed that other photosynthetic pigments in the willow material were at their highest level in comparison to other selected samples. The results showed that the sample location and the distance from the pollution hotspots (Trepça mining area including flotation, Mitrovica Industrial Park, and open tailing dumps) was affecting the level of heavy metal accumulation.[16]

Table 2 – Photosynthetic pigment content of *Salix purpurea* leaves in mg *per* gram dry leaf weight

Samples	Chl a / mg g^{-1}	Chl b / mg g^{-1}	Carotenoids / mg g^{-1}
S1	6.62 ± 0.61	2.37 ± 0.22	1.19 ± 0.07
S2	4.78 ± 0.29	1.48 ± 0.23	0.92 ± 0.07
S3	3.32 ± 2.46	1.17 ± 0.87	0.67 ± 0.50
S4	3.52 ± 0.29	0.99 ± 0.11	0.87 ± 0.04
S5	3.65 ± 0.03	0.99 ± 0.04	0.87 ± 0.02
S6	3.90 ± 0.45	1.19 ± 0.21	0.78 ± 0.03

Table 3 presents the results of total chlorophyll, ratios of chlorophyll a over chlorophyll b, and total chlorophyll over carotenoids. Considering the fact that the presence of heavy metals supports the degradation of chlorophyll and supports the attack of chloroplast, the amount of chlorophyll is affected significantly by mining activities. Due to anthropogenic activity, the content of total chlorophyll, ratios of chlorophyll a over chlorophyll b and total chlorophyll over carotenoids differed (Table 3). The results revealed lower values in June 2019 and June 2020. Furthermore, depending on the time and distance between the

Table 3 – Total chlorophyll, ratios of chlorophyll a over chlorophyll b and total chlorophyll over carotenoids

		Jun 2019	Sep 2019	Jun 2020	Sep 2020
	Sample	Concentration / mg g^{-1}			
Chl a + Chl b	S1	2.80 ± 0.15	8.99 ± 0.83	3.77 ± 0.35	7.57 ± 0.40
	S2	2.15 ± 0.08	6.26 ± 0.52	2.25 ± 0.20	6.72 ± 0.27
	S3	1.83 ± 0.34	4.49 ± 3.33	1.47 ± 1.09	5.25 ± 0.97
	S4	2.21 ± 0.14	4.51 ± 0.40	2.16 ± 0.43	6.16 ± 0.41
	S5	1.91 ± 0.03	4.65 ± 0.08	1.81 ± 0.03	4.79 ± 0.08
	S6	1.89 ± 0.08	5.09 ± 0.66	2.18 ± 0.28	4.50 ± 0.19
Chl a / Chl b	S1	3.37 ± 1.43	2.79 ± 0.03	2.81 ± 0.04	3.36 ± 0.11
	S2	3.05 ± 0.57	3.22 ± 0.13	3.24 ± 0.10	3.06 ± 0.02
	S3	3.25 ± 1.29	2.84 ± 0.24	2.87 ± 0.34	3.24 ± 0.18
	S4	3.09 ± 2.98	3.54 ± 0.18	3.59 ± 0.06	3.04 ± 0.21
	S5	3.24 ± 0.97	3.65 ± 0.13	3.73 ± 0.20	3.26 ± 0.19
	S6	2.76 ± 1.35	3.27 ± 0.22	3.27 ± 0.14	2.72 ± 0.09
(Chl a + Chl b) / Carotenoids	S1	6.22 ± 2.43	7.55 ± 0.24	7.54 ± 0.20	6.12 ± 0.14
	S2	5.81 ± 3.64	6.81 ± 0.89	5.92 ± 0.41	5.69 ± 0.32
	S3	5.71 ± 1.87	6.70 ± 0.42	6.68 ± 0.55	5.58 ± 0.28
	S4	5.97 ± 1.65	5.18 ± 0.42	5.26 ± 0.11	5.85 ± 0.13
	S5	5.45 ± 0.98	5.33 ± 0.21	5.32 ± 0.24	5.42 ± 0.19
	S6	4.47 ± 1.74	6.52 ± 0.66	6.60 ± 0.23	4.44 ± 0.26

Fig. 3 – Matrix plot of *Salix purpurea* photosynthetic pigments

samples, the rates of chlorophyll vary: the minimum total chlorophyll value of 1.47 ± 1.09 was recorded in S3. In addition, the matrix plot (Fig. 3) was used to present the correlation of S1 to S6 in 2019 and 2020, which indicated higher correlation meaning that the plants were adapting easily to the pollution. These correlations in chlorophyll concentrations can be identified as indicators of environmental pollution in Mitrovica, Kosovo.

4 Conclusion

The environmental pollution in Mitrovica, Kosovo, is highly due to mining activities. The measurements of heavy metal concentrations in leaves of *Salix purpurea* and its photosynthetic pigment content show high levels of metal concentration and bioaccumulation. The application of statistical analysis of matrix plots and parallel coordination plots supports the fact that heavy metals are more concentrated close to the mining smelting area, but it has an impact on the surrounding area as well. The establishment of regular monitoring programs is an urgent need for Kosovo. The most important and cheap procedure is the application of phytoremediation as a method of great interest. The results also indicate that there is an increasing need for further research, mainly focused on the mechanisms of remediation process.

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SAŽETAK

Utjecaj toksičnosti metala na rast i sadržaj fotosintetskog pigmenta biljke *Salix purpurea* na području Mitrovice, Kosovo

Flora Zabërgja Ferati,[a] *Mihone Kerolli Mustafa*[b*] i *Flamur Abazaj*[b]

U Mitrovici (Kosovo), rudarske aktivnosti ugrožavaju okoliš i žive organizme. Glavni izvori onečišćenja su otvorena jalovišta u gradu Mitrovici, koja uzrokuju onečišćenje tla, zraka i biljaka. U ovom radu odredila se koncentracija teških metala u listovima biljke *Salix purpurea* ubranim na području grada Mitrovice i okolici, gdje se nalaze industrijska postrojenja te otvorena jalovišta. Na temelju fotosintetske aktivnosti biljke procjenjivao se udio teških metala (As, Cd, Co, Cr, Cu, Ni, Pb i Zn) u biljci. Dvogodišnja istraživanja (2019. i 2020.) pokazala su povećanje udjela teških metala u listovima i klorofilu. Izračunat je ukupni klorofil, omjer klorofila a i klorofila b te omjer ukupnog klorofila i karotenoida. Dobiveni podatci analizirani su primjenom programskog paketa *Minitab 19* namijenjenog statističkoj obradi podataka. Provedena istraživanja ukazala su da rudarske aktivnosti i otvorena jalovišta povećavaju udio teških metala u biljkama, odnosno da dolazi do njihove akumulacije.

Ključne riječi

Teški metali, Salix purpurea, fotosintetska aktivnost, fotosintetski pigment, fitoremedijacija

[a] *University of Mitrovica Isa Boletini, Kosovo*
[b] *International Business College Mitrovica, Kosovo*

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