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Effect of heavy metals on phytobenthos assemblages in mine pit lakes of Bosnia and Herzegovina

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***Abstract*:**

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The field research was performed during autumn season in 2014, at six mine pit lakes in the territory of Bosnia and Herzegovina. During investigations concentration of certain heavy metals (Al, Cr, Zn, Ni and Fe) was measured and composition of phytobenthos was analysed. Statistically, significant correlation was determined between values of chromium and aluminum (r=0.7685*) and between values of iron and aluminium (r=0.7226*). High values of Pearson's coefficient of correlation and statistical significance are determined between the diversity index (H') and values of zinc (r=-0.8697*). Within the qualitative and quantitative composition of phytobenthos of the explored mine pit lakes, 99 species of cyanobacteria and algae were found altogether. The identified species belong to the following classes: *Cyanophyceae*, *Bacillariophyceae*, *Chrysophyceae*, *Dinophyceae* and *Conjugatophyceae*. Tolerant algal taxa were noted in the explored mine pit lakes listed as follows: *Oscillatoria* sp., *Nitzschia palea*, *Achnanthidium minutissimum*, *Navicula trivialis*, *Brachysira microcephala* and *Ulnaria ulna*. With the aim of improving the status of the mine pit lakes with high concentrations of heavy metals, their restoration is necessary. After the restoration, the mine pit lakes may be used for conservation of wetland diversity.

Key words: heavy metals, mine pit lakes, algae, diversity, restoration

***Apstrakt*:**

Mašić, E., Žero, S., Barudanović, S., Memić, M.: *Efekat teških metala na zajednicu fitobentosa u kopovskim jezerima Bosne i Hercegovine. Biologica Nyssana, 9 (2). Decembar, 2018: 103-118.*

Terenska istraživanja za potrebe ovog rada vršena su tokom jesenje sezone u 2014. godini na šest kopovskih jezera koja se nalaze na području Bosne i Hercegovine. Tokom istraživanja izvršeno je merenje koncentracija odabranih teških metala (Al, Cr, Zn, Ni i Fe) i analiziran je sastav zajednica algi fitobentosa. Statistički značajna korelacija je utvrđena između vrijednosti hroma i aluminijuma (r=0.7685*) i između vrednosti gvožđa i aluminijuma (r=0.7226*). Visoke vrednosti Pearsonovog koeficijenta korelacija i statistička značajnost je utvrđena između indeksa diverziteta i koncentracije cinka (r=-0.8697*). U kvalitativno-kvantitativnom sastavu algi fitobentosa u istraživanim kopovskim jezerima konstatovano je prisustvo 99 vrsta cijanobakterija i algi. Identifikovane vrste pripadaju sledećim klasama: *Cyanophyceae*, *Bacillariophyceae*, *Chrysophyceae*, *Dinophyceae* i *Conjugatophyceae*. Tolerantne vrste algi koje su konstatovane u kopovskim jezerima su: *Oscillatoria* sp., *Nitzschia palea*, *Achnanthidium minutissimum*, *Navicula trivialis*, *Brachysira microcephala* i *Ulnaria ulna*. U cilju poboljšanja statusa kopovskih jezera sa povećanim koncentracijama teških metala, potrebno je izvršiti njihovu restauraciju. Nakon provedene restauracije, kopovska jezera se mogu koristiti za konzervaciju močvarnog diverziteta.

Ključne reči: teški metali, kopovska jezera, alge, diverzitet, restauracija

Introduction

Metals are necessary components of all ecosystems and occur naturally in the earth's crust. They are present in the environment with a wide range of oxidation states and coordination numbers, and these differences are related to their toxicity. Some metals such as copper (Cu) and zinc (Zn) are essential to life, whereas others such as lead (Pb) and mercury (Hg) are not known to perform a useful biochemical function (Pinto et al., 2003).

Some heavy metals such as iron (Fe), copper (Cu) and zinc (Zn) are necessary for the process of photosynthesis (Volland et al., 2014, Shanmugam et al., 2011, Kovacik et al., 2010).

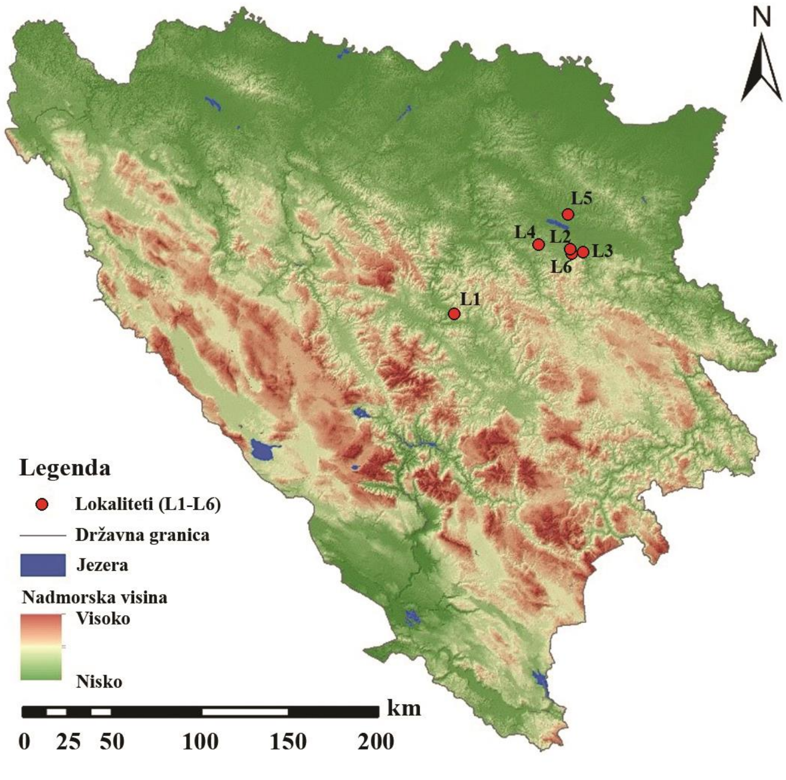

Fig. 1. Distribution map of explored mine pit lakes in Bosnia and Herzegovina

Environmental pollution by metals became extensive as mining and industrial activities increased in the late 19th and early 20th century. The worldwide mine production of Cu, Cd, Pb and Hg was considerable (Pinto et al., 2003).

Metals are considered to be one of the main sources of the environmental contamination since they have a significant effect on its ecological quality (Branković et al., 2013).

These pollutants, ultimately derived from a growing number of diverse anthropogenic sources (industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps, and mining operations) have progressively affected more and more different ecosystems (Macfarlane & Burchett, 2001 in Pinto et al., 2003).

Very high concentrations of heavy metals occur close to metal mines and the associated processing plants long after mining ceases (Ciszewski et al., 2013).

Former mine sites contaminate also the aquatic environment either directly from the drainage of adits (Younger, 1997), surface runoff and the spillway of tailing ponds (Marques et al., 2001) or groundwater seepage (Mighanetara et al., 2009 in Ciszewski et al., 2013).

Mine pit lakes are known to be long-term sinks for heavy metals, which affect aquatic biota in a way not proportional to the degree of sediment contamination (Lukin et al., 2003).

The effects are considered to depend on numerous factors such as water and sediment properties, the shape and depth of the reservoir or biosystem productivity (Suchanek et al., 2008 in Ciszewski et al., 2013).

Table 1. Basic characteristics of the explored mine pit lakes

Lake	Mošćanica (L1)	Đuvelika (L2)	Suhodanj (L3)	Ramići (L4)	Mušići (L5)	Sjerkovača (L6)
Area	Zenica	Živinice	Živinice	Banovići	Tuzla	Živinice
Coordinate (N)	44˚10′11.16″	44˚24′14.07″	44°23'34.32"	44˚25′02.60″	44˚31′38.68″	44˚23′52.21″
Coordinate (E)	18˚00′26.67″	18˚35′44.74″	18°38'43.60"	18˚25′36.57″	18˚34′38.81″	18˚36′09.22″
Altitude (m)	672	288	291	380	210	322
Length (m)	200.66	276.76	324.80	644.52	550.17	683.67
Average width (m)	0.066	0.105	0.206	0.157	0.345	0.122
Coastline length (m)	548.39	729.89	1259.32	2015.63	2043.45	2275.30
Total area (ha)	1.32	2.91	6.67	10.14	18.96	8.36

Fig. 2. Mine pit lakes in Bosnia and Herzegovina: a) Mošćanica, b) Đuvelika, c) Suhodanj, d) Ramići, e) Mušići, f) Sjerkovača

The primary effect of heavy metals on the algae is maintained on a biochemical and physiological level, whereupon changes occur on an ecological level. Adverse effect first causes reduction of biomass, and then reduction of diversity (Kalinowska et al., 2008).

Many of these chemicals are toxic to organisms and can lead to rearrangement of the biotic structure of lake ecosystems. Pollutants can release compounds, which assimilated by organisms, can interfere in physiologic processes, such as reproductive aspects, survival and consequently, change the population structure and community structure. Heavy metals accumulated in benthic organisms can also be concentrated in food webs (Klavniš et al., 1998).

Algal communities may be dominated by tolerant species with some diatom taxa forming significant blooms in polluted waters (Chen et al., 2014).

Mine pit lakes are a specific type of water accumulations, caused by the cessation of drainage or exploitation process, and the filling of the final crater with water or by damming the watercourse with mine tailings. These lakes may be formed by subsidence after underground mining (Barudanović & Mašić, 2014, Barudanović et al., 2014, Kamberović et al., 2014).

In the newly established mine pit lakes, microphytes (cyanobacteria and algae) and macrophytes (vascular plants) are the basis for establishing a dynamic equilibrium in the ecosystem. Cyanobacteria and algae are especially prominent in their number. In addition to representing primary producers of organic matter and importance in the food chain, they have the ability to react quickly to changes occurring in the environment (Kamberović & Barudanović, 2012).

Previous researches have determined that the mine pit lakes are colonized by the algae from the phylum Ochrophyta, especially diatoms (Class Bacillariophyceae). Diatoms are sensitive to the many changes taking place in the ecosystems (changes in light intensity, humidity, temperature, oxygen concentration, salinity, pH, concentration of mineral substances and others). They are excellent indicators widely used in biomonitoring of aquatic ecosystems, and mostly during the monitoring of acidification process, eutrophication, water pollution and climate change (Levkov & Krstić, 2002, Kamberović & Barudanović, 2012, Cantonati et al., 2013, Barudanović & Mašić, 2015).

The objective of this paper is: (I) analysis of concentration of heavy metals in water in mine pit lakes, (II) analysis of the phytobenthos assemblages in mine pit lakes and (III) assessment of the effects of certain heavy metals on the diversity of this group of organisms.

Material and methods

Study area

Basic characteristics of the explored mine pit lakes (**Tab. 1)** and the map of their distribution are shown in figure (**Fig. 1**).

The field research was performed during the autumn season in 2014 at six mine pit lakes in the territory of Bosnia and Herzegovina: Mošćanica (**Fig. 2a**), Đuvelika (**Fig. 2b**), Suhodanj (**Fig. 2c**), Ramići (**Fig. 2d**), Mušići (**Fig. 2e**) and Sjerovača (**Fig. 2f**).

The explored mine pit lakes were formed in the craters after the completion of the exploitation and drainage process (Mošćanica, Suhodanj, Sjerkovača, Ramići and Mušići) or subsidence of terrain due to various mining activities (Đuvelika). The littoral zone of the mine pit lakes is overgrown with macrophyte vegetation, while in some mine pit lakes macrophyte vegetation is in the initial phase. The common use of explored mine pit lakes is for recreation and tourism activities, irrigation, a source of industrial water and for educational purposes.

Heavy metals analysis

Water samples were collected in polyethylene bottles, following the ISO standard method (ISO 5667-4:1987). All glass and plastic dishes used for sampling and analysis were washed with 10% HNO_3 and rinsed with redistilled water. The bottles were properly labelled and stored until laboratory analysis. The analysis of water samples was carried out in the next 24 h.

The concentrations of Cr, Zn, Fe and Ni were determined by graphite furnace atomic absorption spectrometry. The concentration of Al was determined by using UV-Vis spectrometry.

All used reagents were of analytical grade. Redistilled water was used throughout the complete experimental work. The concentration of Cr, Zn, Fe and Ni were determined on an electrothermal atomic absorption spectrometer (AA240Z, Varian, Australia) equipped with an autosampler (PSD 120), a graphite furnace (GTA 120), and hollow cathode lamps. The concentration of Al was determined by using a UV-Vis spectrophotometer (Cary® 50, Varian, Australia).

Phytobenthos analysis

Samples of phytobenthos were collected from different types of substrates: epilithon, periphyton and epipelon. Sample from submerged stones was collected by scraping with a scalpel blade or brushing the upper surface of submerged stones. Stones overgrown by filamentous algae or covered by mud were avoided due to possible contamination by non-epilithic diatoms. Periphyton samples contained non-washed parts of submerged macroalgae and macrophytes. Epipelon samples were collected from the uppermost layer of mud with a spoon or pipette aspirator. Portions of the samples were preserved with 4% formaldehyde solutions.

In order to determine diatoms to the species level, permanent diatom slides were prepared. Permanent slides were prepared after chemical treatment of samples of phytobenthos using sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid ($C_2H_2O_4$). The cleaned valves are then mounted in a special mountant (Canada balsam) with a high refractive index in order to make it easier to see surface ornamentation such as striae and other characteristic structures (Hustedt, 1930).

Five permanent slides have been prepared for each sample and a total 400 valves were counted to asses relative abundance. All slides were scanned for taxa with low relative abundances.

Light microscope observation was conducted using a Best Scope 2020 microscope. Species composition and quantitative relationship of diatoms are estimated from the permanent slides under 1000x magnification. Species abundance of diatoms are estimated on a five-degree scale as follows: 1-rare (single valve or frustule), 2-sparse (up to 10% of the sample), 3-frequent (11-15% of the sample), 4-very frequent (51-75% of the sample), 5-common (in more than 75% of the sample). The relative abundance for the cyanobacteria and algae (except diatoms) assess was carried out by assigning them numbers from 1 to 3 (1-rare, 2-frequent and 3-numerous). The sampling of the cyanobacteria and algae was carried out at the same places where the water for heavy metals had been sampled. On each lake, one sample of water and phytobenthos were collected. The type of the sample was dependent on the current characteristics of the lake bottom. Cyanobacteria and algae identification were mainly based on: Hustedt, 1930, Krammer & Lange-Bertalot, 1981-1991, Lange-Bertalot, 1993, 2001, Cvijan & Blaženčić, 1996, Lange-Bertalot & Metzeltin, 1996, Krammer, 1997a,b., 2000, 2001, 2003, Reichardt, 1999, John et al., 2003, Bey & Ector, 2013a-f, Hofman et al., 2013, John, 2015, Cantonati et al., 2017, and a number a specialized references listed with respective taxa: Wojtal, 2003, Witkowski et al., 2004, Schmidt et al., 2004, Wojtal & Kwandras, 2006, Wojtal, 2009, Wojtal et al., 2011, Levkov et al., 2010, Levkov & Ector, 2010, Cantonati et al., 2010, Levkov & Williams, 2012, Pavlov et al., 2013, Pavlov & Levkov, 2013, Bucko et al., 2013, Lange-Bertalot & Wojtal, 2014, Pavlov et al., 2016.

The nomenclature of the cyanobacteria and algae are adjusted according to the following internet base: Guiry & Guiry (2018).

Data analysis

In order to determine variables important for the number of species, i.e. determining the correlation between dependent (diversity index) and the independent variables (concentration of heavy metals) Pearson's coefficient of simple linear correlation (r) was used. Pearson's coefficient of correlation between analysed variables was tested using Student t-test at a significance level of $p<0.05$. A univariate statistical analysis was performed using the software package PAST v.3.15 (Hammer, 2017).

In order to analyse the diversity of cyanobacteria and algae in the studied mine pit lakes α-diversity is selected or diversity within the investigated samples. α-diversity is expressed through the Shannon diversity index (Shannon & Weaver, 1949).

For differentiation of samples, a method of Cluster analysis was used, and in order to understand the differentiation of phytobenthos community in relation to the gradient of the measured chemical parameters PCO analysis was used. The ordination was conducted on the Bray-Curtis similarity matrix of species data (Legendre & Legendre, 1998, Kamberović et al., 2016). The data were transformed by square root to restrict the influence of abundant taxa on the results. A multivariate statistical analysis was performed using the software package PRIMER v6 (Anderson et al., 2008).

Results

Concentration of selected heavy metals in mine pit lakes

Results of concentration measurements of selected heavy metals are presented in **Tab. 2.**

The highest concentrations of aluminium (Al) were measured in the mine pit lakes of Đuvelika (49 μg/L), Mušići (37 μg/L) and Mošćanica (37 μg/L) (**Tab. 2**).

The highest concentrations of chromium (Cr) were measured in the mine pit lake of Đuvelika (4.92 μg/L). Concentration of zinc (Zn) was in the range of 1.17 to 6.49 μg/L. High concentrations of nickel (Ni) were measured in the mine pit lake of Mošćanica (57 μg/L) and Mušići (43 μg/L).

Iron (Fe) was not detected by using graphite furnace atomic absorption spectrometry in the mine pit lakes of Sjerkovača, Ramići and Mušići. Obtained concentration of iron in the mine pit lakes of Đuvelika is 6.90 μg/L and Mošćanica is 6.40 μg/L.

High values of Pearson's correlation coefficient obtained between Cr and Al ($r=0.7685^*$; $p<0.05$), Fe and Al ($r=0.7226^*$; $p<0.05$) and Fe and Cr ($r=0.6883$) may indicate a strong chemical relationship between these parameters. There is a significant correlation at the $p<0.05$ level between concentrations of chromium and aluminium, and iron and aluminium. Moderate values of Pearson's correlation coefficient are obtained between Ni and Al ($r=0.4360$), Fe and Ni ($r=0.4371$), Ni and Zn ($r=-0.4111$) and Zn and Cr ($r=0.3634$). Weak values of Pearson's correlation coefficient are obtained between Zn and Al ($r=0.2390$) and there was no

Table 2. The obtained concentrations of heavy metals in mine pit lakes

Indicator	Al	Cr	Zn	Ni	Fe
Unit	μg/L	μg/L	μg/L	μg/L	μg/L
Mošćanica	37	0.71	1.38	57	6.40
Suhodanj	18	0.50	1.41	2.47	0.50
Sjerkovača	29	0.61	6.49	2.96	ND
Đuvelika	49	4.92	4.33	13.30	6.90
Ramići	28	0.55	1.17	4.71	ND
Mušići	37	0.63	1.47	43	ND

*ND – not detected by the used determination technique.

Table 3. Correlation between measured heavy metals and level of significance

	Al	Cr	Zn	Ni	Fe
Al	1.00	.	.	.	.
Cr	0.7685*	1.00	.	.	.
Zn	0.2390	0.3634	1.00	.	.
Ni	0.4360	-0.117	-0.4111	1.00	.
Fe	0.7226*	0.6883	0.0547	0.4371	1.00

*Correlation is significant at the p<0.05 level.

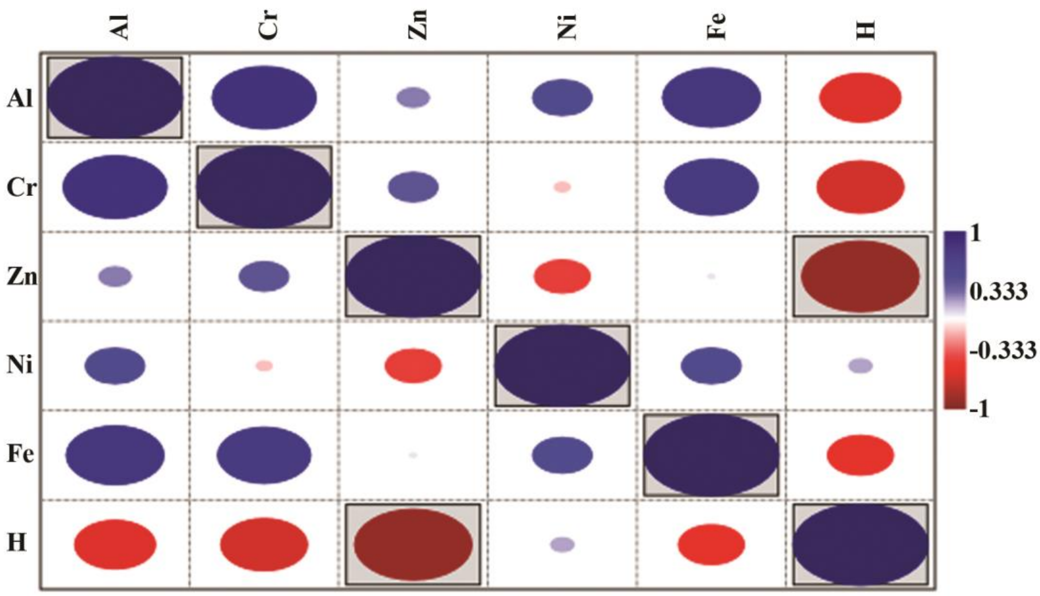

Fig. 3. Correlation plot between heavy metals and diversity index (H')

significant correlation between Ni and Cr (r=-0.117) and Fe and Zn (r=0.0547).

Correlation between measured heavy metals in mine pit lakes and level of significance are presented in **Tab 3**.

Correlation between diversity index and heavy metals

Diversity index (H') are in correlation with concentrations of zinc (Zn), chromium (Cr), aluminium (Al) and iron (Fe). Concentration of nickel (Ni) is slightly correlated with diversity index. High value of Pearson's correlation coefficient obtained between diversity index and concentration of zinc (r=-0.8697*; p<0.005). This correlation is significant at the p<0.05 level. Moderate and negative values of Pearson's correlation coefficient obtained between diversity index and concentration of chromium (r=-0,6408), aluminium (r=-0.5968), iron (r=-0.4852) and weak and positive Pearson's correlation coefficient obtained between diversity index and concentration of nickel (r=0.1715). This correlation is not significant at the p<0.05 level.

Table 4. Algal and cyanobacterial taxa composition and diversity presented as abundances (1-5) and frequency (F) in studied mine pit lakes

	Mine pit lakes						
Taxon	**L2**	**L6**	**L1**	**L4**	**L5**	**L3**	**F**
Cyanophyceae							
Oscillatoria sp. 1	1	1	1	1	1	1	6
Merismopedia punctata Meyen	.	.	1	.	.	.	1
Phormidium sp. 1	.	.	1	.	.	.	1
Chroococcus minutus (Kützing) Nägeli	.	.	.	1	.	.	1
Anabaena sp.2	.	.	.	.	1	.	1
Dinophyceae							
Peridinium bipes Stein	.	.	1	.	.	.	1
Ceratium hirundinella (O.F.Müller) Dujardin	.	.	.	.	1	.	1
Chrysophyceae							
Dinobryon divergens O.E.Imhof	.	.	.	1	.	.	1
Bacillariophyceae							
Navicula sp. 1	2	2	2	3	1	1	6
Nitzschia palea (Kützing) W. Smith	4	1	1	1	.	1	5
Nitzschia sp. 1	4	1	1	1	.	1	5
Achnanthidium minutissimum (Kützing) Czarnecki	1	1	1	.	1	4	5
Navicula trivialis Lange-Bertalot	2	1	.	2	.	1	4
Brachysira microcephala (Grunow) Compère	.	1	5	.	1	1	4
Ulnaria ulna (Nitzsch) Compère	.	1	.	1	2	1	4
Navicula radiosa Kützing	.	.	3	1	1	1	4
Hantzschia amphioxys (Ehrenberg) Grunow	1	1	.	.	.	1	3
Nitzschia commutata Grunow	1	.	1	.	.	1	3
Nitzschia recta Hantzsch ex Rabenhorst	1	.	.	1	.	1	3
Discostella stelligera (Cleve & Grun.) Houck & Klee	.	5	1	.	1	3	3
Gomphonema acuminatum Ehrenberg	.	1	.	.	1	1	3
Encyonopsis microcephala (Grunow) Krammer	.	.	2	.	1	1	3
Cymbella sp. 1	.	.	.	1	1	1	3
Luticola mutica (Kützing) D.G. Mann	1	1	.	.	.	.	2
Caloneis bacillum (Grunow) Cleve	.	1	.	.	.	1	2
Cymbella affinis Kützing	.	1	.	.	.	1	2
Gomphonema truncatum Ehrenberg	.	1	.	.	1	.	2
Pinnularia gibba Ehrenberg	.	1	.	.	.	1	2
Cymbopleura amphicephala (Nägeli) Krammer	.	.	1	.	1	.	2
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	.	.	1	.	1	.	2
Eunotia minor (Kützing) Grunow	.	.	1	.	1	.	2
Encyonopsis sp. 1	.	.	1	1	.	.	2
Denticula kuetzingii Grunow	.	.	3	.	.	1	2
Navicula viridula (Kützing) Ehrenberg	.	.	1	1	.	.	2
Pinnularia major (Kützing) Rabenhorst	.	.	1	1	.	.	2
Epithemia sorex Kützing	.	.	.	1	4	.	2
Cocconeis placentula Ehrenberg	.	.	.	1	.	1	2
Fragilaria vaucheriae (Kutzing) J.B. Petersen	.	.	.	1	1	.	2
Navicula erifuga Lange-Bertalot	.	.	.	2	.	.	2
Pantocsekiella ocellata (Pantocsek) K.T.Kiss & E.Ács	.	.	.	3	1	.	2
Pinnularia obscura Krasske	.	.	.	1	.	1	2
Pinnularia viridis (Nitzsch) Ehrenberg	.	.	.	1	.	1	2
Placoneis placentula (Ehrenberg) Mereschkowsky	.	.	.	1	.	1	2
Craticula cuspidata (Kützing) D.G.Mann	.	.	.		1	1	2
Cyclotella meneghiniana Kützing	.	.	.		1	1	2
Cymatopleura solea (Brébisson) W.Smith	.	.	.	.	1	1	2
Encyonema ventricosum (C.Agardh) Grunow	.	.	.	.	1	1	2
Amphypleura pellucida (Kützing) Kützing	.		.	.	1	1	2
Gomphonema sp. 1	.		.	.	1	1	2
Gyrosigma sciotense (Sullivan & Wormley) Cleve	2	.	.	.	.	.	1
Hantzschia sp. 1	1		.	.	.	.	1
Tryblionella angustata W.Smith	1	.	.	.	.	.	1
Achnanthes sp. 1	.	.	1	.	.	.	1
Denticula tenuis Kützing	.	.	1	.	.		1
Diploneis sp. 1	.	.	1	.	.		1
Eucocconeis flexella (Kützing) Meister	.	.	1	.		.	1

Continuation of the table 4.

Taxon	L2	L6	L1	L4	L5	L3	F
Nitzschia amphibia Grunow	.	.	1	.	.	.	1
Caloneis fontinalis (Grunow) Cleve-Euler	.	.	.	1	.	.	1
Craticula ambigua (Ehrenberg) D.G. Mann	.	.	.	1	.		1
Cyclotella sp. 1	.	.	.	1	.		1
Cymatopleura elliptica (Brébisson) W. Smith	.	.	.	1	.		1
Frustulia vulgaris (Thwaites) De Toni	.	.	.	1		.	1
Gomphonema parvulum (Kützing) Kützing	.	.	.	1	.	.	1
Gyrosigma obtusatum (Sulivan & Wormley) C.S.Boyer	.	.	.	1	.	.	1
Nitzschia sp. 2	.	.	.	1	.	.	1
Planothdium lanceolatum (Brébis. ex Kütz.) Lange-Bert.	.	.	.	1	.	.	1
Rhopalodia gibberula (Ehrenberg) Otto Müller	.	.	.	1	.	.	1
Tryblionella debilis Arnott ex O'Meara	.	.	.	1		.	1
Rhopalodia gibba (Ehrenberg) Otto Müller	.	.	.	.	4	.	1
Epithemia turgida (Ehrenberg) Kutzing	.	.	.	.	2	.	1
Ulnaria capitata (Ehrenberg) Compère	.	.	.	.	2	.	1
Cocconeis pediculus Ehrenberg	.	.	.	.	1	.	1
Amphora ovalis (Kützing) Kützing	.	.	.	.	1	.	1
Cymbella neolanceolata W.Silva	.	.	.	.	1	.	1
Cymbella proxima Reimer	.	.	.	.	1	.	1
Gomphonema dichotomum Kützing	.	.	.	.	1	.	1
Halamphora sp. 1	.	.	.	.	1	.	1
Halamphora veneta (Kützing) Levkov	.	.	.	.	1	.	1
Nitzschia sigma (Kützing) W.M.Smith	.	.	.	.	1	.	1
Placoneis clementis (Grunow) E.J.Cox	.	.	.	.	1	.	1
Placoneis gastrum (Ehrenberg) Mereschkowsky	.	.	.	.	1	.	1
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	.	.	.	.	1	.	1
Cymatopleura solea var. *apiculata* (W.Smith) Ralfs	.	.	.	.	.	2	1
Cymbella cistula (Ehrenberg) O. Kirchner	.	.	.	.	.	1	1
Eunotia sp. 1	.	.	.	.	.	1	1
Fallacia pygmea (Kützing) Stickle & D.G.Mann	.	.	.	.	.	1	1
Fragilaria sp. 1	.	.	.	.	.	1	1
Neidium affine (Ehrenberg) Pfitzer	.	.	.	.	.	1	1
Nitzschia gracilis Hantzsch		.		.	.	1	1
Nitzschia sigmoidea (Nitzsch.) W. Smith	.	.	.	.	.	1	1
Nitzschia tabellaria (Grunow) Grunow	.	.	.	.	.	1	1
Pinnularia borealis Ehrenberg	.	.	.	.	.	1	1
Sellaphora pupula (Kützing) Mereschkowsky	.	.	.	.	.	1	1
Surirella angusta Kützing	.	.	.	.	.	1	1
Ulnaria delicatissima (W. Smith) Aboal & P.C.Silva	.	.	.	.	.	1	1
Conjugatophyceae							
Spirogyra sp. 1	1	.	1	.	.	1	3
Mougeotia sp. 1	.	.	1	1	1	.	3
Cosmarium sp. 1	.	.	1	.	1	.	2
Pleurotaenium trabecula Nägeli	.	.	.	.	.	1	1
Number of species (S)	**14**	**16**	**28**	**34**	**41**	**45**	
Shannon diversity index (H')	**1.0738**	**1.1086**	**1.3808**	**1.5004**	**1.5665**	**1.6306**	

Results of the correlation between heavy metals and diversity index are shown in **Fig. 3.**

Heavy metal pollution alters algal diversity and also community structure. Different species may be more or less tolerant of pollution and some may dominate in a polluted environment. The analysis determined that high concentrations of heavy metals in the investigated mine pit lakes significantly affect the diversity of algae and colonisation of these artificial lakes. Correlation analysis has shown that the mine pit lakes with a higher value of concentration of heavy metals have a smaller number of species and vice versa.

Diversity of cyanobacteria and algae of the explored mine pit lakes

Within the qualitative and quantitative composition of phytobenthos of the explored mine pit lakes, 99 species of cyanobacteria and algae were found altogether. The identified species belong to the following classes: *Cyanophyceae* (5), *Bacillariophyceae* (87), *Chrysophyceae* (1), *Dinophyceae* (2) and *Conjugatophyceae* (4) (**Tab. 4**).

Dominant algal taxa were noted in the explored mine pit lakes listed as follow: *Oscillatoria* sp. 1, *Navicula* sp. 1, *Nitzschia palea*, *Nitzschia* sp. 1, *Achnanthidium minutissimum*, *Navicula trivialis*,

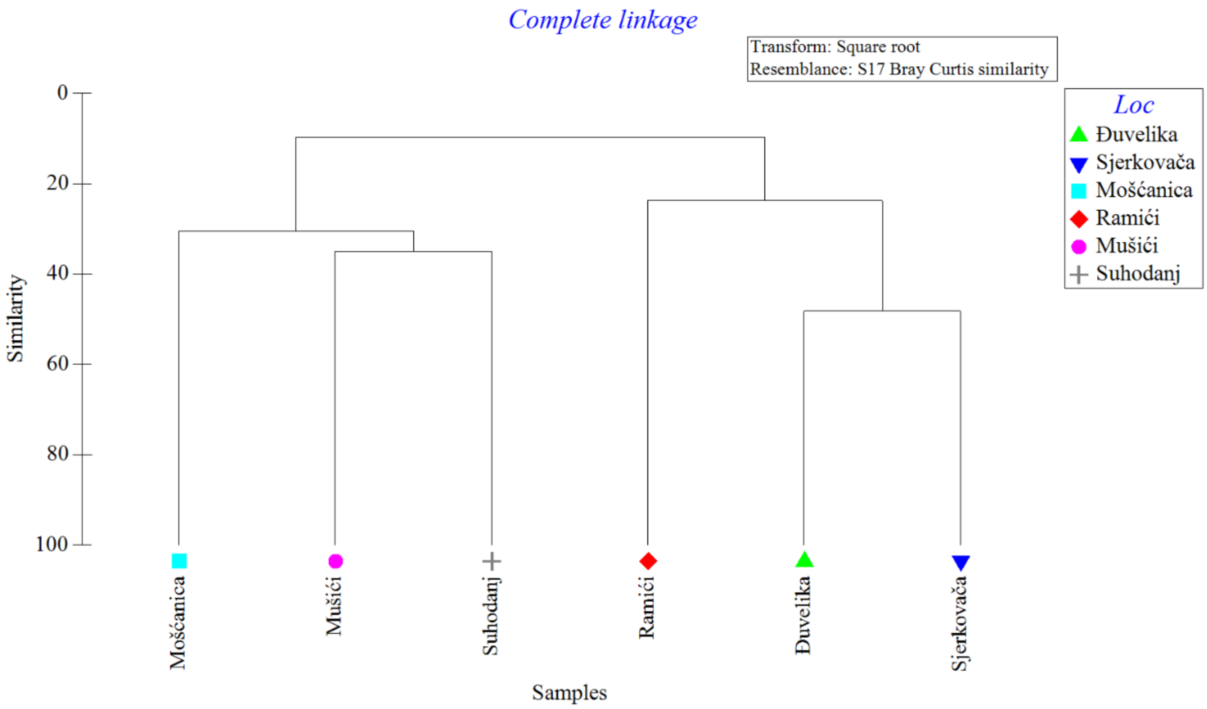

Fig. 4. Cluster differentiation of the analysed communities of the phytobenthos

Brachysira microcephala, *Ulnaria ulna*, *Navicula radiosa*, *Hantzschia amphioxys*, *Nitzschia commutata*, *Nitzschia recta*, *Discostella stelligera*, *Gomphonema acuminatum*, *Encyonopsis microcephala*, *Cymbella* sp. 1, *Gyrosigma sciotense*, *Rhopalodia gibba*, *Epithemia turgida*, *Ulnaria capitata* and *Cymatopleura solea* var. *apiculata*.

Upon the diversity level analysis (Shannon's Index) it may be concluded that the phytobenthos samples collected from the mine pit lakes of Đuvelika (1.0738), Sjerkovača (1.1086) and Mošćanica (1.3808) have a low diversity level. In these lakes, high concentrations of heavy metals were measured.

The phytobenthos samples collected from the mine pit lakes of Suhodanj (1.5665), Mušići (1.5665) and Ramići (1.6306) have a higher diversity level. In these lakes, lower concentrations of heavy metals were measured.

In order to differentiate the composition of the phytobenthos, the method of cluster analysis was used (Bray-Curtis similarity).

Figure 4 presents a clear differentiation of phytobenthos community into two groups:

- Group I (samples from Mošćanica, Mušići and Suhodanj) and
- Group II (samples from Ramići, Đuvelika and Sjerovača).

The left side of the chart contains phytobenthos samples collected from the mine pit lakes of Ramići, Đuvelika, and Sjerkovača. In these lakes, high concentrations of zinc, chromium, iron and aluminium were measured. The right side of the chart contains phytobenthos samples collected from the mine pit lakes of Mošćanica, Mušići and Suhodanj. In these lakes, high concentrations of nickel and aluminium were measured.

Results of PCO differentiation of the analysed communities of the phytobenthos in relation to the gradient of the measured chemical parameters were shown in **Fig. 5.**

PCO analysis showed similar differentiation as a cluster analysis. The first PCO axis explained 35.5% of total variation, while the second PCO axis explained 25% of the total variation. On the first PCO axis, mine pit lakes from the first Cluster group were distributed (Mošćanica, Mušići and Suhodanj), and on second PCO axis mine pit lakes from the second group were distributed (Ramići, Đuvelika and Sjerovača). Mine pit lakes from second group were characterised with higher concentrations of heavy metals, especially Al, Cr, Fe and Zn, while the mine pit lakes from first group were characterized by lower concentrations of heavy metals. Higher value of nickel was measured in the mine pit lakes of Mošćanica 57 μg/L and Mušići 43 μg/L.

Tolerant species of cyanobacteria and algae on high heavy metal concentrations which is noted in mine pit lakes were: *Oscillatoria* sp., *Nitzschia palea*, *Achnanthes minutissima*, *Navicula trivialis*, *Brachysira microcephala* and *Ulnaria ulna*. Finding of this species in mine pit lakes with high heavy metal concentrations correspond with literature data (Chen et al., 2014, Cantonati et al., 2014).

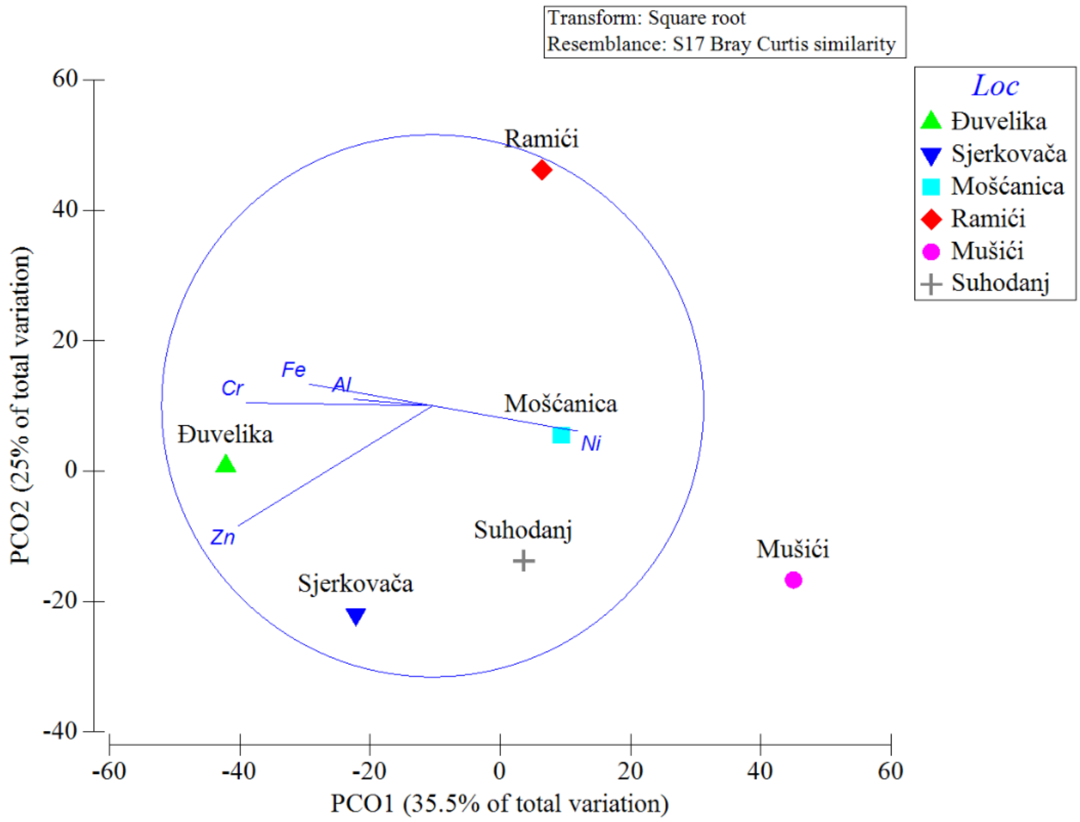

Fig. 5. PCO differentiation of the analysed communities of the phytobenthos

Discussion

The negative effects of heavy metals on the structure and patterns of phytobenthos have been studied by many authors (Wołowski et al. 2013; Pandey et al., 2014; Płachno et al. 2015).

The most evident effect of heavy metals in water is reduced biodiversity level (Hernandez & Pastor, 2008).

Based on examination of the latest scientific literature, it may be observed that high concentrations of heavy metals in water have adverse effects on structure and patterns of the living world. All benthic organisms may be directly and/or indirectly impacted by heavy metals in water (Kiffney & Clements, 1996), substratum (Chapman et al., 1998) and food resources (Farag et al., 1998 in Chiba et al., 2011).

In terms algae of phytobenthos, heavy metals cause oxidative stress (Pint et al., 2003) or various morphological abnormalities typical for diatoms (Falasco et al., 2009; Pandey et al., 2014).

Selective pressures also lead to the development of metal resistance in diatoms living in contaminated water. Therefore, these species, owing to their capability to survive in these extreme conditions, may be used successfully as resources for biomonitoring of polluted waters in terms of presence of heavy metals (Levkov & Krstić, 2002; Cattaneo et al., 2011; Pandey et al., 2014; Cantonati et al., 2014,).

First studies of mine pit lakes in Bosnia and Herzegovina were carried out on a wider area of Tuzla region (Kamberović & Barudanović, 2012; Kamberović et al., 2014).

Authors were studying abiotic and biotic characteristics of mine pit lakes with special emphasis on colonization of new water bodies and a possibility of establishing wetland ecosystems. Following mine pit lakes were explored: Sjerkovača, Ramići, Mušići, Suhodanj, Požar, Šićki Brod and Brestovica. These lakes were colonized with cyanobacteria and algae, and wetland vegetation was also established.

Authors Kamberović & Barudanović (2012) determined the presence of 73 species of cyanobacteria and algae. The largest numbers of species belong to the class *Bacillariophyceae* (48 taxa), while representatives from the classes *Cyanophyceae*, *Dinophyceae*, *Zygnematphyceae* and *Chlorophyceae* appear with lower values. Dominant species which are determined in all explored mine pit lakes where listed as follows: *Synedra ulna*, *Mougeotia* sp. and *Cymbella affinis*. Besides them, it is important to highlight the presence of the following species: *Navicula cuspidata*, *Rhopalodia gibba*, *Navicula radiosa*, *Cocconeis placentula* etc.

The explored mine pit lakes were colonised with wetland vegetations. Association *Typhetum latifoliae* G. Lang 1973 were confirmed in the coastal part of mine pit lakes Suhodanj, Mušići and Šićki Brod, while association *Phragmitetum australis* Schmale 1939 were confirmed in coastal part of mine pit lakes Šićki Brod and Ramići (Kamberović et al., 2014).

In the mine pit lakes Suhodanj, Požar and Šićki Brod hard oligo-mesotrophic waters were confirmed with benthic vegetation of algae from genus *Chara* (Natura code 3140), while in mine pit lakes Mušići and Ramići habitat of eutrophic lakes with *Magnopotamion* or *Hydrocharition* - vegetation type were confirmed (Natura code 3150). The presence of wetlands vegetation has not be confirmed in mine pit lakes Sjerovača and Brestovica (Kamberović & Barudanović, 2012).

The concentration of heavy metals in mine pit lakes in Bosnia and Herzegovina has not been carried out. Only basic physical and chemical parameters were measured. Based on that measurement, it can be concluded that mine pit lakes in our area were slightly neutral to slightly alkaline. pH values in the mine pit lakes in the wider area of Tuzla region varied from 7.8 to 8.3. In the water of the investigated mine pit lakes, average values of oxygen and low nutrient concentrations were measured (Kamberović & Barudanović, 2012, Kamberović et al., 2014).

Authors Wołowski et al. (2013) have determined the presence of 96 algae taxa in the area of Poland (Jeleniak Mikuliny Reserve). Freshwater habitats were characterized by a high concentration of heavy metals. Identified species belong to the following classes *Cyanophyceae*, *Bacillariophyceae*, *Chrysophyceae*, *Raphidophyceae*, *Xanthophyceae*, *Cryptophyceae*, *Dinophyceae*, *Euglenophyceae*, *Chlorophyceae* and *Zygnematophyceae*.

In similar freshwater habitats in the area of Graniczna Woda stream (southern Poland), Płachno et al. (2015) have identified the presence of 66 algal taxa, and the most numerous were algal species from phylum Euglenophyta (37 taxa) and species with small number belong to the class as follows *Cyanophyceae*, *Xanthophyceae*, *Bacillariophyceae* and *Chlorophyceae.* The most numerous species from phylum Euglenophyta in Graniczna Woda stream were *Euglena archaeoviridis*, *Euglena archaeoplastidiata*, *Petalomonas mediocanellata*, *Phacus angustus, Phacus ichthydion*, *Phacus indicus*, *Phacus inflexus*, *Phacus obolus*, *Phacus pusillus*, *Phacus unguis* and *Trachelomonas perforata*. In the investigated freshwater habitats high concentration of heavy metals (Tl, Cd, Zn and Pb) have been measured.

The high diversity of euglenoids in Graniczna Woda stream indicates high tolerance on Tl by these algae. Years of research on euglenophytes have shown that they are remarkably tolerant to various kinds of pollution with heavy metals such as Fe, Zn, Cu, Cd, Mn, Pb, Ni and Al (Walne & Kivic, 1990).

They have also been found in waters polluted with diesel oil (Dennington et al., 1975), phenol (Pawlitz & Werner, 1978) and herbicides and insecticides (Poorman, 1973; Butler, 1977), and can survive in highly radioactive water (Lackley, 1968 in Płachno et al., 2015).

Euglenophytes are also found living under very high salinity, for example, in Great Salt Lake (Jones, 1944 in Płachno et al., 2015).

It is generally known that acidophilic *Euglena mutabilis* and *Euglena gracilis* are able to grow in highly polluted habitats. Species *Euglena mutabilis* colonizes highly acidified waters, tolerates pH of ca. 1 and can be dominant among the eukaryotes in habitats such as the metal-contaminated ponds of the Smoking Hills region of the Canadian Arctic and acidic post-mining ponds contaminated with heavy metals (Wołowski et al., 2008., 2013).

Autors Płachno et al. (2015) have determined that the shape and arrangement of the chloroplasts in *Euglena viridis* and *Euglena mutabilis* varied and did not always fit the classical description, and that the dimensions of euglenophyte specimens were at their lower limits.

In the studied freshwater habitats authors have found the presence of 15 species of the genus *Phacus* as follows *Phacus caudatus*, *Phacus curvicauda* and *Phacus parvulus* were recorded repeatedly throughout the study. Following species from genus Euglena were constant in the study area: *Euglena mutabilis*, *Euglena viridis*, *Euglena archaeoplastidiata* and *Euglena agilis* (Bartosz et al., 2015).

In different freshwater habitat in the area of Egypt (El-Farafra Oasis, Western Deset, Egypt) which are contaminated with heavy metals, Wołowski et al. (2017) have identified the presence of 20 species from the phylum Euglenophyta. In investigated freshwater habitats authors have measured high concentration of heavy metals as follows Sr, B, Li, Fe, Mn, Zn and Cu. Determined species belong to the genera *Peranema* (1), *Euglena* (4), *Euglenaformis* (1), *Euglenaria* (2), *Discoplastis* (1), *Lepocinclis* (4), *Phacus* (6) and *Trachelomonas* (1).

Some cyanobacteria of the genera *Oscillatoria*, *Phormidium*, *Plectonema* and *Schizothrix* are often abundant in alkaline waters polluted by heavy metal compounds. Golden algae are more sensitive to both eutrophication and metal contamination (Cattaneo et al., 2008).

Among the 15 *Chlorophyceae* taxa found in Graniczna Woda stream one of the most commonly noted was *Desmodesmus* sp. (Płachno et al., 2015).

Several studies on metal polluted rivers have shown that diatoms respond to perturbations not only at the community level through shifts in dominant taxa (Hirst et al., 2002), changes in diversity (Leland & Carter, 1984 in Medley & Clements, 1998), but also at the individual level with alteration in cell wall morphology (Chen et al., 2014).

In the presence of different types of environmental stressors, diatoms can produce frustules that are deformed in different ways (anomalies mainly in symmetry, striation pattern, raphe course and structure) (Falasco et al., 2009). However, the exact physiological processes causing diatom teratologies are yet mostly unsolved (Morin et al., 2012).

The causes of these deformations are diverse, but the most common appears to be due to toxic concentrations of trace elements. Cell size reduction (Cattaneo et al., 1998) and frustule deformations have been associated with high metal concentrations, commonly related to high concentrations of copper and zinc (Ruggiu et al., 1998 in Chen et al., 2014).

That means that diatoms teratology is considered to be one of the most significant indicators of metal pollution (Falasco et al., 2009; Lavoie et al., 2012; Cantonati et al., 2014).

Other causes, such as silicon limitation and extreme pH are also indicated for such abnormalities (Chen et al., 2014). Diatoms metal response models are difficult to establish because metal contamination is frequently associated with acidic environments (Dixit et al. 1991).

While high metal concentrations may cause a marked increase in the relative abundance of metal-tolerant species, such as *Achnanthidium minutissimum*, *Fragilaria tenera*, *Gomphonema parvulum*, *Eolimna minima* and *Nitzschia palea*, diatom biomass is apparently not affected (Cantonati et al., 2014).

Nitzschia palea is known to tolerate heavy metals and it is often designated as a characteristic species in streams and lakes with heavy metal contamination. Nutrient enrichment should prohibit the development of *Achnanthidium minutissimum* but favour the growth of *Nitzschia palea* in the metal-polluted waters. The dominant species should be co-tolerant to metals and nutrients. The phenomena of multiple tolerance and co-tolerance have been reported in some algae (Chen et al., 2014).

There are mostly negative opinions about the effects of heavy metals on planktonic organisms. It seems that algae populations can quite easily adjust to long-lasting contamination. However, there are other factors that may explain the existence of microorganisms inhabiting contaminated water. One is the presence of high concentrations of Ca and Mg cations. It is known that these cations can limit the penetration of high amounts of Zn and Pb into cells (Ciszewski et al., 2013). In fact, high nutrient loading would protect some algae from heavy metal toxicity (Chen et al., 2014).

The effect is less drastic in benthic diatoms because, in thick biofilms, metals are mostly assimilated by the cells present in the upper part, which reduces the exposure of the underlying diatom assemblage (Cantonati et al., 2014).

High concentration of heavy metals has direct toxic effects on diversity and abundance of benthic invertebrates (Clements, 1994).

Indirect effects include modification of species interaction (Clements, 1999) and reduction in food quality (Chiba et al., 2011, Carlise, 2000).

Many studies have been developed in concentrations of heavy metals as well in aquatic macroinvertebrate population and in cyanobacteria and algae assemblages through the establishment of bioindicators. This approach reflects a new trend in conservation worldwide because it uses a methodology that integrates low cost and high efficiency and accuracy (Chiba et al., 2011).

Conclusion

High concentrations of heavy metals in the water of the mine pit lakes may have adverse effects on the structure and patterns of the living world. Adverse effects of the presence of heavy metals in water are primarily observed with photosynthetic organisms, including cyanobacteria and algae, as well as other groups of organisms. In terms of photosynthetic organisms, heavy metals cause various morphological and physiological changes. The most prominent effect of heavy metals in water is the reduction of the biodiversity level. With the aim of improving the status of the mine pit lakes with high concentrations of heavy metals, their restoration is necessary. After the restoration, the mine pit lakes may be used for conservation of wetland diversity.

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