Open Chem., 2018; 16: 272–282 DE GRUYTER

Research Article Open Access

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Relationships between diatoms and environmental variables in industrial water biotopes of Trzuskawica S.A. (Poland)

Relationships between diatoms and environmental variables

https://doi.org/10.1515/chem-2018-0033
received August 1, 2017; accepted January 27, 2018.

Abstract: The heterogeneous nature and widespread anthropogenic impacts on industrial water biotopes in the Trzuskawica S.A., pose challenges to biomonitoring of this habitat. Generally, the concentration of trace elements in the industrial water biotopes reflects the anthropogenic impacts. With X-ray fluorescence method (TXRF) in waters 17 elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Ba, Pb were revealed. High amounts of Ca, from 300 ppm to 198 ppm in May and from 999 ppm to 231 ppm in September 2015, was determined. A total of 36 diatoms were found in two reservoirs and drainage ditch, but only three taxa: *Cymatopleura radiosa, Navicula upsaliensis* and *Nitzschia angustata* were present in all 7 sampling sites. These species are known to be tolerant to organic pollution, eutrophication, and also characteristic for limestone waters. The results of CVA showed that diatoms in the water reservoir stocked with fish were distinguished by highest species richness. The relationships between diatoms and environmental variables confirm the positive correlation with the currently functioning industrial plant (despite the increased water temperature and large content of trace elements). Our results suggest that, though heterogeneity in both diatoms and selected elements in industrial waters, diatoms can be useful indicators of habitat conditions.

Keywords: diatoms; elements; industrial water biotopes; statistical analysis.

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1 Introduction

Recently the number and importance of activities and exploitation areas increased in many countries. Artificial water biotopes have been created in places where there were opencast mines [1], abandoned granite or limestone quarries [2]. The hydrochemical properties of their waters are mainly determined by regional geochemical background. The knowledge of the microorganisms diversity present in industrial water biotopes of operating plants is generally poor, in spite of their important role in the assessment of the state, dynamics and monitoring of such environment. Anthropogenic activities such as urban, industrial and agricultural changes, increasing consumption of water result in degradation of environment in a wide sense of the term. Diatoms are being used to assess ecological conditions in water biotopes around the world [3]. They respond directly and sensitively to many physical, chemical and biological changes in water ecosystems, such as temperature, nutrient concentrations and others [4]. The heterogeneous nature of industrial environments poses challenges to monitoring industrial waters. Bioassessment of human impacts on industrial water biotopes might be improved by accounting for the

Figure 1: Location of sampling sites: water reservoir stocked with fish (A), reservoir of technological water (B), the drainage ditch of mining area (C).

natural heterogeneity of these systems, which may require more frequent sampling and multiple environmental criteria. For instance, diatom species in habitats with excessive and periodic fine sediment deposition are often dominated by the species with relatively high motility e.g. *Gyrosigma*, *Nitzschia*, *Surirella*. It is difficult to determine which environmental factors are most significant in defining species distribution. Several authors [5,6] indicated that substrate is a critical limiting factor. Diatoms are ecologically diverse and flourish in virtually every industrial water habitat. There is no accurate count of the number of diatom species; however, estimates on the order of 10^4 are often given [7]. Well-developed epiphytic, epipelic, and planktonic diatom assemblages could all potentially be used for bioassessment [8]. Diatoms were used as indictors of various environmental parameters, including salinity, conductivity, phosphorus and water level, and also are involved in the purification of water by oxygenation (photosynthesis) and absorption of heavy metal ions: nickel, lead, zinc and titanium [9].

Beginnings of the limestone industrial production in what is presently Trzuskawica S.A., date back to the year 1910, and it is now one of the largest an industrial plant in Europe producing cement, lime and asphalt mass. According to our knowledge the present studies are one of the first, if not the first of this type. The relationships between diatom species and environmental variables in water biotopes of the functioning industrial plant were studied.

2 Materials and methods

2.1 Samples

Seven water sites for algological, physical and chemical materials were sampled from March till September 2015 in Trzuskawica S.A. located in the southern Poland (Figure 1). Four sites (1-4) from reservoir stocked with fish (A), two (5-6) from reservoir of technological water (B) and one (7)

Figure 2: Study area: water reservoir stocked with fish (A), reservoir of technological water (B), the drainage ditch of mining area (C).

Table 1: Physico-chemical water parameters in two reservoirs (A-B) and drainage ditch (C) in 2015.

Water biotopes	Temperature [°C]	pH	Conductivity [μS cm^{-1}]	Secchi depth [cm]
A	7.4	7.8	767.6	66.3
B	8.7	7.8	711.2	12.8
C	11.2	8.2	865.2	36

from drainage ditch (C) were selected (Figure 2). Diatoms and water samples were collected once every month for seven months, but the limestone rocks were gathered only once. Technological waters used for lime and cement production were discharged into the two reservoirs and mixed with sludge. The limestone rocks were crushed and flooded with water which was subsequently drained by a drainage ditch into the reservoir stocked with fish. Water reservoirs are connected by a drainage ditch to the Bobrza River, a tributary of the Black Nida River. Analysis of water quality and physical parameters from 7 sampling sites is presented in Table 1.

All algological samples containing diatoms had a volume of 100 ml and were transported to the laboratory where they were analyzed using a light microscope Nikon Eclipse 600 equipped with a digital camera Nikon Coolpix 4500. Diatoms were identified according to [5,10-13].

Water samples for chemical analysis were collected from flowing water (drainage ditch) and from stagnant water (two reservoirs). Temperature, pH and electrolytic conductivity were measured with the multi measurement device HANNA pH-meter Instruments HI9126 (Table 1). Water transparency was measured using the Secchi disk [14]. All these measurements were done *in situ*. The elemental analysis of waters was carried out using Total reflection X-Ray Fluorescence analysis (TXRF) in X-Ray Methods Laboratory (Institute of Physics, Jan Kochanowski University in Kielce, Poland) in May and September 2015.

2.1.1 TXRF – Total Reflection X-Ray Fluorescence analysis (spectrometer S2 PICOFOX)

Analysis was performed using S2 Picofox spectrometer (Bruker AXS Microanalysis GmbH) equipped with air cooled, 30 W X-ray tube with Mo anode working with parameters: high voltage 50 kV and electron current 600 μA. The measurement system includes a Peltier-cooled XFlash® Silicon Drift Detector with area 10mm^2 and energy resolution < 160 eV at 100 kcps (for Mn Kα line). This device allows to measure the characteristic X-rays of elements from Al (aluminium) to U (uranium), with exception of Zr (zirconium) to Ru (ruthenium). The internal standard containing 100 μL solution with a concentration of selenium equal to 1,961 ppm was added to the analyzed water. Liquid samples were measured directly after adding Se internal standard (500 μL water + 100 μL Se + 1 mL HNO_3) while diatom samples in the amount of 0.2 – 0.3 g were mineralized with 4 mL of high purity HNO_3 and 0.1 mL of 100 μg/g Se standard (or 6 ml of HNO_3 and 0.2 ml Se (100 μg/g)). The sample was further mineralized in microwave digestion system. Next, 5 μL of solution was pipetted into Synsil backing, and this drop was dried in infrared.

Table 2: Element concentrations in two water reservoirs A (sites 2-4) and B (sites 5-6), and drainage ditch C (site 7) determined by TXRF method in ppm units with total experimental uncertainty (in %) (May 18th of 2015).

Element TXRF	Concentration (ppm) ± total experimental uncertainty (%) *LLD (lower limits of detection)					
	site 2	*site 3*	*site 4*	*site 5*	*site 6*	*site 7*
P	10.5 ± 0.3	8.4 ± 0.3	11.9 ± 0.41	11.4 ± 0.4	7.2 ± 0.3	13.9 ± 0.4
S	41.9 ± 0.2	42.1 ± 0.2	43.8 ± 0.3	39.1 ± 0.2	39.8 ± 0.2	38.2 ± 0.2
Cl	97.6 ± 0.3	99.2 ± 0.3	113.9 ± 0.4	65.5 ± 0.3	68.9 ± 0.2	46.8 ± 0.2
K	6.8 ± 0.06	7.1 ± 0.05	8.5 ± 0.07	6.5 ± 0.06	6.5 ± 0.05	2.5 ± 0.05
Ca	249.5 ± 0.3	198.2 ± 0.2	262.4 ± 0.4	288.7 ± 0.3	211.4 ± 0.3	300.03 ± 0.3
Ti	<0.01*	0.03 ± 0.006	0.02 ±0.008	0.2 ± 0.008	0.2 ± 0.009	<0.02*
Cr	<0.008*	<0.009*	<0.01*	<0.01*	<0.008*	0.02 ± 0.005
Mn	0.1 ± 0.002	0.2 ± 0.006	0.1 ± 0.005	0.1 ± 0.005	0.06 ± 0.005	<0.008*
Fe	0.6 ± 0.005	0.3 ± 0.005	0.9 ± 0.005	2.2 ± 0.01	1.7 ± 0.01	0.05 ± 0.002
Ni	0.003 ±0.003	0.009 ±0.002	0.01 ±0.002	0.005 ±0.002	0.01 ± 0.002	<0.005*
Cu	0.006 ±0.003	0.009 ±0.002	0.01 ±0.002	0.005 ±0.002	0.008 ± 0.002	<0.003*
Zn	0.1 ± 0.002	0.04 ± 0.002	0.2 ± 0.002	0.4 ± 0.002	0.1 ± 0.002	0.2 ± 0.002
Br	0.1 ± 0.003	0.1 ± 0.003	0.1 ± 0.003	0.1 ± 0.003	0.1 ± 0.003	0.08 ± 0.002
Rb	<0.003*	<0,003*	<0.003*	0.009 ±0.003	0.006 ± 0.003	<0.003
Sr	0.3 ± 0.003	0.3 ± 0.003	0.3 ± 0.03	0.3 ± 0.003	0.3 ± 0.003	0.3 ± 0.003
Ba	0.05 ± 0.01	0.06 ± 0.01	0.07 ±0.02	<0.04*	<0.03	0.04 ±0.02
Pb	0.006 ±0.003	0.006 ±0.003	0.01 ±0.003	0.04 ±0.003	0.03 ±0.003	0.003 ±0.003

The uncertainty values are the expanded uncertainty with an expansion of level about 95% with factor of k = 2. The value of extended uncertainty is given as a range (± U95%).

2.2 Data analysis

Diatoms data were presented in three ways: a) relative abundance within a sample (species abundance/total abundance x 100), b) mean species relative abundance across all sites where present, and c) species frequency across all studied water biotopes. Two approaches to examine the relationship between water chemistry of the sampling sites and sampling time of water, diatoms and limestone rocks in the three industrial water biotopes (A-C) in the Trzuskawica S.A. were used. The first approach was an indirect analysis of changes in water chemistry while collecting materials from sampling sites. The principal component analysis (PCA) was performed. The second approach was a direct analysis on the relationship between the element concentrations and the changes in months (May, June, September) and sampling sites (A, B, C). Canonical variate analysis (CVA) was applied as the discriminant analysis [15]. The importance of these variables in relation to months and sampling sites was tested using a Monte Carlo permutation test and CVA was done with Canoco v. 5.0 statistic software test [16].

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

We identified a total of 36 diatoms from 24 genera, the vast majority of which were typically epilithic or epiphytic forms. The most species belong to genera *Amphora*, *Cymatopleura*, *Eunotia*, *Gomphonema*, *Navicula* and *Nitzschia* which are characteristic for waters rich in calcium carbonate (Table 4). Only 16 species were common (i.e., present at $^{<}$ 10% relative abundance in at least one sample) and 10 species were reached > 20% relative abundance in at least one sample.

The pH range was 7.4–11.2 for the analyzed sites. In the reservoirs A and B the waters pH was slightly alkaline, whereas the high pH 11.2 was recorded in the drainage ditch (C) due to large amounts crashed limestone (Table 1). There was relatively high variation of conductivity in the industrial water biotopes, ranging from 711 µS cm^{-1} (reservoir of technological water B) to 865 µS cm^{-1} (drainage ditch C). The diatoms were dominated by neutrophilous

Table 3: Element concentrations in two water reservoirs A (sites 1-4) and B (site 5), and drainage ditch C (site 7) determined by TXRF method in ppm units with total experimental uncertainty (in %) (September 22nd 2015).

Element TXRF	Concentration (ppm) ± total experimental uncertainty (%) *LLD (lower limits of detection)					
	site 1	*site 2*	*site 3*	*site 4*	*site 5*	*site 7*
P	12.3 ±0.4	11.8 ±0.4	7.4 ±0.5	7.2 ±0.4	44.9 ±0.8	10.6 ±0.4
S	32.9 ±0.3	43.9 ±0.3	45.1 ±0.36	42.8 ±0.3	44.5 ±0.4	40.08 ±0.3
Cl	4.4 ±0.1	0.6 ±0.1	< 0.26*	0.4 ±0.1	<0.4*	<0.2*
K	6.7 ±0.08	6.8 ±0.08	8.5 ±0.08	8.01 ±0.08	21.8 ±0.1	4.8 ±0.07
Ca	239.6±0.4	272.4 ±0.4	231.9 ±0.4	226.8 ±0.4	999.4 ±1.3	285.3 ±0.4
Ti	0.07 ±0.01	0.04±0.01	0.1 ±0.01	0.2 ±0.01	3.5 ±0.03	<0.02*
Cr	<0.01*	<0.01*	<0.01*	<0.01*	0.1 ±0.01	<0.01*
Mn	0.01 ±0.005	0.02±0.006	0.01 ±0.005	0.01 ±0.006	1 ±0.01	<0.01*
Fe	0.2 ±0.006	0.6±0.009	0.3 ±0.009	0.08 ±0.006	32.4 ±0.07	0.02 ±0.003
Ni	0.009 ±0.003	<0.006*	<0.006*	<0.006*	0.04 ±0.006	<0,006*
Cu	0.1 ±0.003	0.02 ±0.003	0.01±0.003	0.008 ±0.003	0.06 ±0.003	0.03 ±0.003
Zn	0.08 ±0.003	0.05 ±0.003	0.04±0.003	0.04 ±0.003	1 ±0.009	0.1 ±0.003
Br	0.1 ±0.003	0.1 ±0.003	0.1 ±0.003	0.08 ±0.003	0.09 ±0.003	0.08 ±0.003
Rb	0.02 ±0.003	0.01 ±0.003	0.01±0.003	0.01 ±0.003	0.1 ±0.003	<0.003*
Sr	0.3 ±0.003	0.3 ±0.003	0.3 ±0.003	0.3 ±0.003	0.7 ±0.006	0.3 ±0.003
Ba	<0.05*	<0.05*	<0.06*	0.09 ±0.02	<0.08*	<0.05*
Pb	0.08 ±0.002	<0.006*	<0.006*	0.006 ±0.003	0.6 ±0.006	<0.006*

The uncertainty values are the expanded uncertainty with an expansion of level about 95% with factor of k = 2. The value of extended uncertainty is given as a range (± U95%).

and alkaliphilous, mesotraphentic and eutraphentic, ß-mesosaprobous (Table 4).

The highest average annual temperature of water was recorded in the drainage ditch (C), while the lowest in the water reservoir stocked with fish (A). The Secchi depth was the highest (66.3 cm) in reservoir A, and the lowest (12.8 cm) in reservoir B.

Using TXRF method 17 elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Ba, and Pb in two reservoirs (A and B) and drainage ditch (C) were determined in May 2015 (Table 2). For diatoms development main organic elements (C, N, P, S, Si) and microelements (Cu, Mn, Se, Zn, Mo, B) are important. However, in studied industrial waters not all the elements mentioned above were detected. Among organic elements only P and S were found. The highest P concentration (13.9 ppm) was in site 7 (drainage ditch C), while in site 6 (reservoir B) was the lowest (7.2 ppm). Whereas the highest S concentration (42.1 ppm) was in site 3 (reservoir A), while in site 7 (drainage ditch C) was the lowest (38.2 ppm). Among microelements only Cu, Mn and Zn were found. The highest Cu concentration (0.01 ppm) was in site 4 (reservoir A), while in site 7 (drainage ditch C) was the lowest (0.003 ppm). Whereas the highest Mn concentration (0.02 ppm) was in site 3 (reservoir A), while in site 7 (drainage ditch C) was the lowest (0.008 ppm). The highest Zn concentration (0.4 ppm) was in site 5 (reservoir B), while in site 3 (reservoir A) was the lowest (0.04 ppm). Generally, the highest elements concentration was in water reservoirs A and B. The average values of elements concentration were detected in the drainage ditch water (C). The nine elements namely: Ca, Fe, K, Mn, Ni, P, S, Sr, Zn, were identified in the limestone rocks and in the reservoirs water (A, B, C).

Using TXRF method 17 elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Ba, and Pb in two reservoirs and drainage ditch were determined in September 2015 (Table 3). The highest P concentration (44.9 ppm) was in site 5 (reservoir B), while in site 4 (reservoir A) was the lowest (7.2 ppm). Whereas the highest S concentration (45.1 ppm) was in site 3 (reservoir A), while in site 1 (reservoir A) was the lowest (32.9 ppm). Among microelements only Cu, Mn and Zn were found. The highest Cu concentration (0.1 ppm) was in site 1 (reservoir A), while in site 4 (reservoir A) was the lowest (0.008 ppm). Whereas the highest Mn concentration (1 ppm) was in site 5 (reservoir B), while in site 7 (drainage ditch C) was the lowest (<0.01 ppm). The

Table 4: Ecological characteristic of diatoms acc. [5] most often found in Trzuskawica S.A in 2015. Explanation: sampling *i.e.*: water biotops 1-7 (see Figs 1-2). pH (R): acidophilous (2), circumneutral (3), alkaliphilous (4), alkalibiontic (5). Salinity (H): fresh (1), fresh brackish (2), brackish fresh (3). Nitrogen uptake metabolism (N): nitrogen-autotrophic taxa (1), nitrogen-autotrophic taxa (2). Oxygen requirements (O): continuously high (1), fairly high (2), moderate (3). Saprobity (S): oligosaprobous (1), p-mesosaprobous (2), α-mesosaprobous (3). Trophic state (T): oligotraphentic (1), oligo-mesotraphentic (2), mesotraphentic (3), meso-eutraphentic (4), eutraphentic (5), oligo-to eutraphentic (7).

Species	Sampling	R	H	N	O	S	T
Amphora copulata (Kutzing) Schoeman & Archibald	2, 3, 4, 5	4	2	2	2	2	5
Caloneis schumanniana (Grunow) Cl.	5, 6	5	2	1	2	1	3
Cocconeis placentula Ehr.	1, 6	4	2	2	3	2	5
Craticula cuspidata (Kutzing) Mann in Round	5, 6, 7	4	2	2	3	3	5
Cyclotella radiosa (Grunow) Lem.	2, 3, 4	4	2	1	2	2	5
Cymatopleura solea (Brêb.) W. Smith	1,2,3,4,5,6,7	4	2	2	3	2	5
Cymbella cymbiformis Agardh	4, 5, 6	3	2	1	1	1	2
Cymbella lanceolata var. *lanceolata* (Agardh) Agardh	4, 5	4	2	1	1	2	7
Cymbopleura inaequalis (Ehr.) Krammer	4, 6	5	2	1	1	1	3
Ellerbeckia arenaria (Moore ex Ralfs) Crawford	2, 3	4	1	1	1	1	1
Eunotia arcubus Norpel & Lange-Bertalot	3, 4, 5, 6	3	1	1	-	1	2
Eunotia glacialifalsa Lange-Bertalot in Krammer & Lange-Bertalot	3, 4, 6	2	1	1	1	1	2
Fragillaria parasitica var. *parasitica* (W. Smith) Grun. in Van Heurck	4	4	2	1	1	2	4
Gomphonema acuminatum var. *acuminatum* Ehr.	4, 5, 6	4	2	1	2	2	5
Gomphonema pala Reichardt	4, 5, 6, 7	4	2	1	2	2	4
Gyrosigma obtusatum (Sullivant & Wormley) Boyer	6, 7	4	2	2	1	2	5
Navicula radiosa Kutzing	4,	3	2	2	2	2	4
Navicula upsaliensis (Grun.) Peragallo	1,2,3,4,5,6,7	4	2	-	-	2	-
Nitzschia angustata (W. Smith) Grun. in Cl. & Grun.	1,2,3,4,5,6,7	3	2	1	1	1	3
Nitzschia brevissima Grunow in Van Heurck	5, 6, 7	3	3	-	3	2	5
Nitzschia denticula Grunow in Cl. & Grun.	5, 6, 7	4	2	1	1	2	3
Pinnularia viridis (Nitzsch) Ehr.	4, 5, 6	3	2	2	3	2	7
Ulnaria biceps (Ehrenberg) Compere	4	4	2	-	-	-	5

highest Zn concentration (1 ppm) was in site 5 (reservoir B), while in site 1 (reservoir A) was the lowest (0.08 ppm). Generally, the highest elements concentration was in water reservoirs A and B. The average values of elements concentration were detected in water of the drainage ditch (C) water of mining area.

Element concentrations in two water reservoirs and drainage ditch (in ppm units) using TXRF method were measured in 6 sites (site 2-7) in May and in 6 sites (site 1-5, 7) in September 2015. In May for a technical reason it impossible to carry on the collection from the site 1, whereas in September for the same reason there was no collection from the site 6.

The elements Ca and S in water are used to build the diatoms silica cell walls. The highest Ca concentration (300 ppm) was in site 7 (drainage ditch C) in May, whereas in September the highest (999,4 ppm) was in site 5 (reservoir B), while in site 3 (reservoir A) was the lowest (198 ppm) in May, but in September in site 4 (reservoir A) was the lowest (226.8 ppm). The highest S concentration (43.8 ppm) was in site 4 (reservoir A) in May, whereas in September the highest (45.1 ppm) was in site 3 (reservoir A), while in site 7 (drainage ditch C) was the lowest (38.2 ppm) in May, but in September in site 1 (reservoir A) was the lowest (32.9 ppm).

The P element plays a major role in the structural framework of DNA, RNA, ATP, and the phospholipids, which form cell membranes. Low phosphate levels are an important limit for growth in aquatic systems. However, Sr and alkaline earth metals, are highly reactive chemicals. In groundwater Sr combines the calcium ions, and forms co-precipitates with calcium minerals such as calcite and anhydrite at an increased pH.

Figure 3 presents striking differences for all sites in the elemental concentration for the May, June and September 2015. Samples collected in May are characterized by the

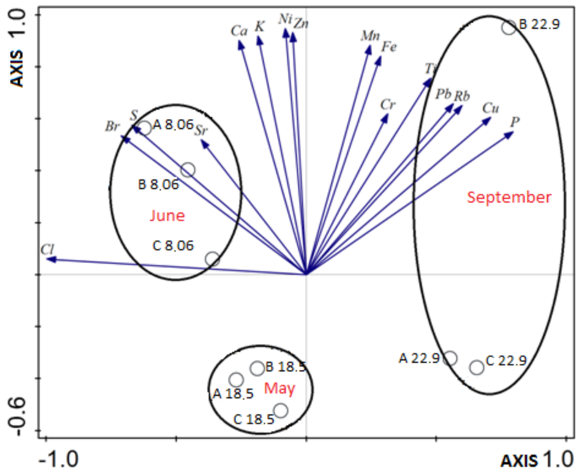

Figure 3: Principal components analysis (PCA), changes in the content of elements in studied water biotopes in the month (May, June and September).

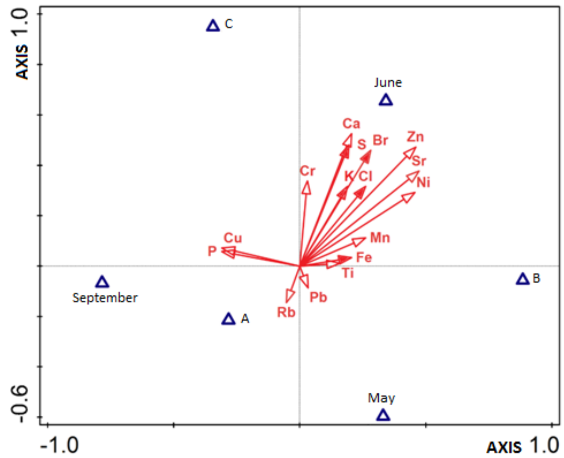

Figure 4: Discriminant analysis (CVA, Canonical Variate Analysis) of data on changes in the content of elements in the studied water biotopes and in the studied months (May, June, September). Elements, which significantly differentiate the test set indicates full returns vectors. It explain 70.5 % of the total variability of harvest.

lowest level of all 17 elements in two reservoirs and drainage ditch. In June in drainage ditch we observed higher level of Cl, and in remaining sites higher level of S, Br and Sr. In September, in drainage ditch and in reservoir A we did not find such high levels of elements concentration, however, in drainage ditch were large amount of Mn, Fe, Cr, Ti, Pb, Rb, Cu and P.

The discrimination analysis (DA) shows that S, Br, K, Cl and Fe differentiate statistically water biotopes studied (Figure 4). The variables explain 70.5% of the total variance of these elements concentration. In June contents of these elements were highest in reservoir with technological water (B) and in drainage ditch (C), while in September the lowest in reservoir stocked with fish (A) were recorded. Two discriminatory analysis, which separately considered the time of testing and the sampling sites were performed. First analysis considering the sampling sites showed the significance of differentiation in Cr, P and Rb content. These elements together account for 45.9% of the total variability. Higher content of these elements were found in the reservoir with technological water (B), while the Cr (the second ordination axis) was also higher in a drainage ditch (C). The elevated levels of S and Cl in reservoir stocked with fish (A) was not considered significant (Figure 4). The second discriminant analysis present that in May, June and September the collected samples contained different amounts of elements concentration. The S and Cl were significant elements in June sampling, whereas in September were significant amounts of P and Pb (Figs 5-6). None of the elements was significant for May sampling. Elements significantly differentiating studied data explain 75.5% of the total variability.

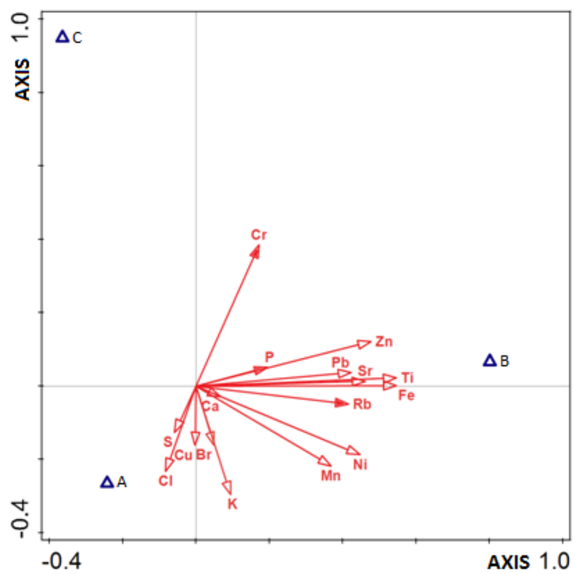

Figure 5: Discriminant analysis (CVA) of data on changes in the content of elements in the studied water biotopes: water reservoir stocked with fish (A), reservoir of technological water (B), and the drainage ditch of mining area (C). Elements, which significantly differentiate the test set indicates full returns vectors. It explain together 45.9 % of the total variability of harvest.

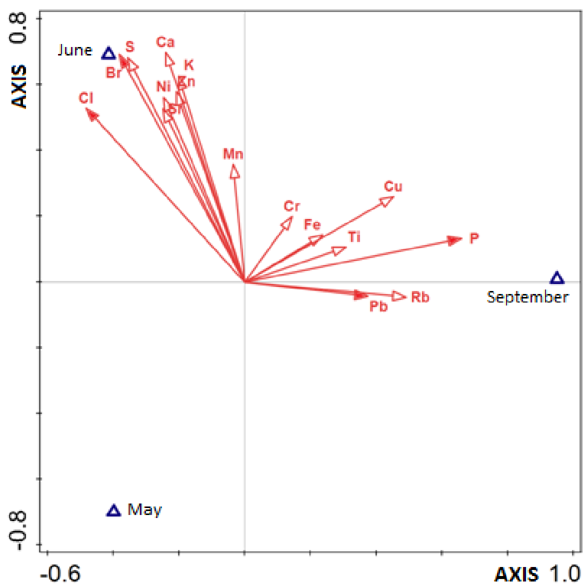

Figure 6: Discriminant analysis (CVA) of data on changes in the content of elements in the studied months (May, June and September). Elements, which significantly differentiate the test set indicates full returns vectors. It explain together 75.5 % of the total variability of harvest.

4 Discussion

Philips [17] and Philips and Rainbow [18] described diatoms as bioindicators of pollution in aquatic environments. These organisms should reflect only contaminants specific to a particular site and found in many different locations ensuring wide geographic relevance [19]. Bioindicators should also be sensitive to specific pollutants and tolerate large concentration of the pollutants in the environment [5]. Mehta and Gaur [20] stated that algae can effectively remove metals from multi-metal solutions and dead cells absorb more metal than live cells. Various pretreatments enhance metal sorption capacity of algae. Algal periphyton has great potential for removing metals from wastewaters. The algae have ability to absorb high concentrations of heavy metals making them a suitable candidate for removing these ions from the wastewaters. Mehta and Gaur [21] suggested that there is a possibility to build an efficient and commercially viable algal technology based upon filling the gap of knowledge concerning metal sorption by algae. The toxic level of heavy metal ions in various algal species can be highly strain specific, which consequently determines the potential remediation capacity using a specific algal strain [22]. However, a heavy metal ion may exhibit a selective interaction with one specific algal strain, in addition to differences between similar species. All heavy metals, including those that are essential micronutrients (e.g., copper, zinc, etc.), are toxic to algae at high concentrations [23].

The concentration of different elements such as: K, Ca, Ti, Mn, Fe, Co, Ni, Br, Sr, Zn, Cu, Pb, and As in *Fucus vesiculosus* samples using energy-dispersive X-Ray fluorescence (EDXRF) were described by Carvalho et al. [24]. The base of this red alga showed higher concentrations for most elements than other parts, except in the case of As. Whereas in our study using X-ray fluorescence method (TXRF) we found 17 elements, but only 11 elements (K, Ca, Ti, Mn, Fe, Ni, Br, Sr, Zn, Cu, Pb) were the same. While in our samples we identified additionally P, S, Cl, Cr, Rb and Ba, but As and Co were only present in Carvalho et al. [24].

Hernández-Àvila et al. [25] concluded that Si element (the main component of diatom frustule) is not capable of effecting anionic exchange, but with an adequate activation can be used as anionic exchangers, however, the diatoms are used in the treatment of wastewater contaminated with heavy metals.

Despite the toxic influence of high concentrations of heavy metals (e.g., Cd, Cu, Zn, Pb, Hg, Cr, As) on diatoms, some of them are able to inhabit degraded and polluted freshwater of marine and terrestrial environments. The effects of metals on diatom communities have been studied in many polluted watersheds as well as in laboratory experiments [26]. However, Panday et al. [27] suggested that to understand responses of algal communities to metals it is necessary to create application of algal criteria for biomonitoring of these pollutants. Diatoms species richness in metal polluted sites is very low in comparison with unpolluted ones. Usually, in freshwaters and soils common are diatoms, however some species of other taxonomic groups (e.g., coccoid green algae) also occur. Our results suggest that the studied two reservoirs and drainage ditch are specific biotopes of increased heavy metals resistance. Mechanisms of their heavy metal resistance or even tolerance are still poorly known. This resistance of diatoms may be a result of limited metal bioavailability and/or intrinsic morphological and biochemical features of organism Two basic mechanisms may be involved: metal avoidance and intracellular metal detoxification [18]. External cell structures or regulated ion transport system may prevent only some diatom/algae from excessive internal metal accumulation.

It is of interest that electrolytic conductivity optima for diatoms ranged from 25.96 µS cm^{-1} to 324.76 µS cm^{-1} [28,29]. Most of the diatoms that exhibited highest affinity towards $Ca(HCO_3)_2$ water type had low (*Diatoma mesodon*) to moderate (*Nitzschia sinuata*) conductivity optima.

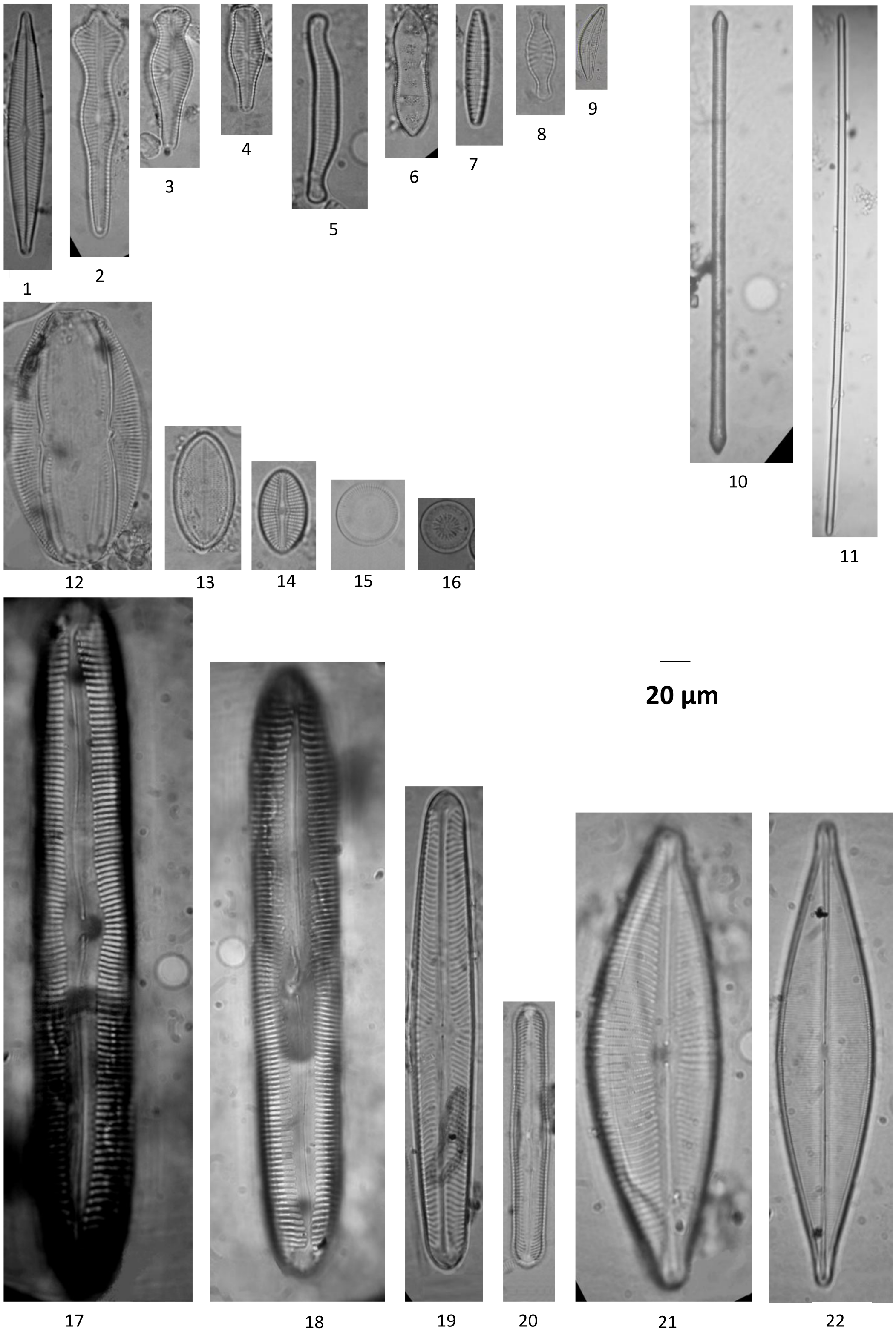

Figure 7: Diatoms in the industrial water biotopes: *Navicula angusta* (1), *Gomphonema acuminatum* (2), *Gomphonema capitatum* (3), *Gomphonema pala* (4), *Eunotia arcubus* (5), *Cymatopleura solea* (6), *Nitzschia denticula* (7), *Planothidium daui* (8), *Cymbopleura ineaqualis* (9), *Ulnaria biceps* (10), *Flagilaria capitata* (11), *Amphora copulata* (12), *Cocconeis placentula* (13), *Cocconeis* sp. (14), *Ellerbackia arenaria* (15), *Cyclotella radiosa* (16), *Pinnularia viridis* (17), *Pinnularia nobilis* (18), *Pinnularia divergens* (19), *Pinnularia gibba* (20), *Cymbopleura inaequalis* (21), *Craticula cuspidata* (22). All LM micrographs, scale bars = 20 µm.

Diatoms that had highest optima for proportion of Na^+ and Cl^- either had very low (*Eunotia flexuosa*) or relatively high conductivity optima. Many diatoms known as acidophilous taxa (*Eunotia rhomboidea, E. paludosa*) had relatively high optima for concentration of Cl^- and K^+ and very low conductivity optima [30].

Our studies indicate the importance from both taxonomical and ecological points of view, since our results showed that there may be new possibilities for using the phenotypic plasticity of diatoms to assess water quality and as potential bioindicators of nutrient availability in industrial ecosystems.

5 Conclusions

Diatoms are an important part of industrial water biotopes, playing diverse roles as powerful and reliable environmental indicators. A total of 36 species of diatoms were found in industrial water biotopes (in two reservoirs and drainage ditch) from Trzuskawica S.A. and 17 elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Ba, Pb using X-ray fluorescence method (TXRF) were revealed. An algological study in Trzuskawica S.A. reported in the present paper was, to our knowledge, the first of its type. The presence or absence and distribution of diatom species in industrial water biotopes is dependent on the water chemistry. It was found that total elemental content occurring in waters was positively correlated with diatom species occurrence. Ecological characteristic of diatoms indicate significant number of species prefering alkaline waters (with a large amount of calcium ions). However, some diatom species which have individual chemical and ecological preferences contrary to accepted standards were observed.

Acknowledgements: We would like to thank Łukasz Lechowicz, Jan Kochanowski University, for help in collecting samples. We are also grateful to Anna Wojciechowska, Mikołaj Kopernik University, for her review of the statistical methods and to Ilona Stabrawa, Jan Kochanowski University, for particle size analysis by TXRF.

Conflict of interest: Authors state no conflict of interest.

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