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Diversity of algae in a thallium and other heavy metals-polluted environment

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Abstract – Thallium (Tl) compounds are extremely toxic to living organisms, including algae, but there is a dearth of basic information regarding the mechanisms of action of Tl in the environment and its effects on algae in natural conditions. This study examined algal diversity in an environment highly polluted by Tl. Graniczna Woda stream is contaminated by Tl and other heavy metal compounds (Cd, Pb and Zn). There we found 66 algae taxa representing five phyla, among which euglenophytes prevailed. We found that euglenophytes, including *Phacus* species, can survive and can show high species diversity in the presence of high Tl concentrations. The fact that these small organisms covered only by a pellicle are able to thrive in such inhospitable habitat, shows a great plasticity of these organisms. It is unclear whether the algae of Graniczna Woda stream have a broad tolerance to harmful conditions or rather represent new varieties/clones that evolved in metal-contaminated waters and are adapted to this environment.

Key words: Euglenophytes / Cd / Zn / Pb / thallium / water pollution

Introduction

Thallium (Tl), a metallic element placed in group 13 (formerly 3A) of the periodic table on account of its high density (> 11.6 g.cm^{-3}), is classified as a heavy metal. Its name, from Greek, is connected with the green spectral line that identified this element (Fleischer, 1997). Tl in the environment exists at two oxidation states: the more stable Tl(I) (*e.g.,* Tl_2O) and the less stable Tl(III) (*e.g.,* $Tl(OH)_3$). The natural background level of Tl in the Earth's crust is put at 0.85 mg.kg^{-1}, and its minerals, associated with K or S, are quite rare but widespread. Its mean concentration in natural water systems is 10 ng.dm^{-3} (Kabata-Penidias and Mukherjee, 2007). Under the quality standards of both the United States Environmental Protection Agency (EPA) and the Polish Ministry of the Environment, the maximum permitted concentration of Tl in surface waters is 2 µg.dm^{-3}. Anthropogenic sources of Tl in the environment are related mainly to coal combustion and ferrous or nonferrous smelting (Peter and Viraraghavan, 2005). Commercial use of Tl is associated with the manufacture of refractive glass and formerly for pesticide production, and it is used as a radioisotope for scintigraphy (Kabata-Pendias and Mickherjee, 2007). Both Tl(I) and Tl(III) compounds are readily soluble and therefore bioavailable. Tl compounds are extremely toxic to living organisms. Its toxicity to mammals is higher than that of Hg, Cd and Pb (Peter and Viraraghavan, 2005; Babula *et al*., 2008). Up to 1984, however, Tl was not considered an environmental pollutant. In Poland, research on the effects of Tl on biota started in the late 1990s, focused on plants and small mammals occurring in the vicinity of Olkusz, a highly industrialized area in southern Poland (*e.g.*, Dmowski *et al*., 1998; Wierzbicka *et al*., 2004).

As with other heavy metals, the effect of Tl on organisms can be explained by its binding to –SH groups of cysteine residues in proteins, leading to changes in the activity of a broad range of enzymes. The most pronounced toxic effect of the Tl ion is related to the similarity of Tl(I) to the potassium ion, due to their similar chemical structure and properties. Monovalent Tl disrupts K-controlled activity of enzymes and membrane processes such as the mitochondrial respiratory chain, and also stabilization of ribosomes (Léonard and Gerber, 1997; Arzate and Santamaria, 1998; Peter and Viraraghavan, 2005).

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Fig. 1. Oxbow of Graniczna Woda stream in the former Dęby Boruszowickie Reserve.

Unlike for the heavy metals Cd, Pb, Hg, Ni and Zn, the literature contains little data on the effects of Tl on plants. Only three plant species have been classified as Tl hyperaccumulators, that is, plants able to accumulate >100 mg.kg^{-1} d.w. in natural conditions: *Iberis intermedia* Guersent, *Biscutella laevigata* L. (both from the Brassicaceae family) and *Silene latifolia* Poir. (Caryophyllaceae) (van der Ent *et al.*, 2013). These are all terrestrial species originating from southern France. Studies showed that Tl can accumulate especially in plants of the Brassicaceae family (Leblanc *et al.*, 1999; Al-Najar *et al.*, 2005). There is far less information about aquatic vegetation in Tl-contaminated environments, although these organisms, immersed as they are, are far more exposed to harmful substances. In *Synechocystis*, Avery *et al.* (1991) demonstrated competition between Tl(I) and K ions, and Lustigman *et al.* (2000) showed Tl(I) toxicity to the cyanobacterium *Anacystis nidulans* (Richter) Drouet & Daily and the chlorophyte *Chlamydomonas reinhardtii* P.A. Dang. Ralph and Twiss (2002) reported differential toxicity of Tl to the unicellular chlorophyte *Chlorella*, which depended on the oxidation state of the metal. They observed the same degree of *Chlorella* growth inhibition under treatment with Tl (III) at a dose of 2×10^{-13} [mol.dm^{-3}] (4.1×10^{-2} ng.dm^{-3}) and with Tl(I) at a dose of 10^{-8} [mol.dm^{-3}] (2.0 μg.dm^{-3}). They noted that Tl(III) toxicity was orders of magnitude greater than Tl(I) to this phytoplankton but that the bioavailability of Tl(III) was significantly limited, and pointed to the lack of fundamental information regarding Tl in the environment. In experiments on *Lemna minor* L., Tl(I) induced generation of reactive oxygen species, resulting in damage to DNA and cell proteins (Babić *et al.*, 2009). The intrinsic toxicity of Tl and the mechanism of its transport through the cell membrane were investigated by Turner and Furniss (2012) in the marine alga *Ulva lactuca* L.

Upper Silesia (southern Poland) is a highly contaminated mining and industrial region. Some watercourses in this area, including Graniczna Woda stream, are polluted by heavy metal compounds from chemical plants and mines. Graniczna Woda stream was polluted by chemical plants in the town of Tarnowskie Góry and by zinc smelters in the town of Miasteczko Śląskie (Reczyńska-Dutka, 1986). In our latest work (Augustynowicz *et al.*, 2014), we conducted the *Microtox*® toxicity test on the Graniczna Woda strem and showed that studied water exhibited second class of acute toxicity. In the above-mentioned work, we also found high Tl pollution of the water in Graniczna Woda stream with almost complete absence of higher aquatic plants in its streambed. In the present study, we examined there the diversity of algal flora. In an unpolluted location in this area we had recorded a rich algal flora in an earlier study (Wołowski *et al.*, 2013a).

Material and methods

Study site

Material was obtained from Graniczna Woda stream and its oxbow (Fig. 1) in the former Dęby Boruszowickie Reserve near Tarnowskie Góry (Upper Silesia, Poland: *ca.* 50°30′N/18°49′E). The stream bed is silty. The study

Table 1. Algae taxa occurrence in Dęby Boruszowickie water bodies during sampling with comparison to the species occurrence in Poland (rare – noted up to three times from Poland, often – noted four or more times from Poland).

Taxa	October 2008	May 2009	July 2010	June 2011	October 2011	Reported from Poland
Cyanophyta						
Cyanophyceae						
Oscillatoria sp. Vaucher ex Gomont	+					Often
Phormidium sp. Kützing ex Gomont	+					Often
Planktothrix agardhii (Gomont) Anagnostidis and Komárek				+		Often
Heterokontophyta						
Bacillariophyceae						
Achnanthes sp. Bory de Saint-Vincent				+		Often
Cyclotella sp. (Kützing) Brébisson		+				Often
Eunotia bilunaris (Ehrenberg) Schaarschmidt		+				Often
Eunotia exigua (Brébisson ex Kützing) Rabenhorst		+				Often
Eunotia sp. Ehrenberg	+					Often
Gomphonema parvulum (Kützing) Kützing		+		+		Often
Navicula sp. Bory de Saint-Vincent			+			Often
Nitzschia obtusa W.Smith	+			+		Often
Nitzschia palea (Kützing) W.Smith	+		+	+	+	Often
Pinnularia cf. *ferrophila* K. Krammer				+		Rare
Pinnularia nodosa (Ehrenberg) W.Smith		+				Often
Pinnularia viridis (Nitzsch) Ehrenberg	+	+	+	+	+	Often
Heterokontophyta						
Xanthophyceae						
Characiopsis subulata var. *ensiformis* (Hermann) Lemmermann	+					
Tribonema sp. Derbès & Solier	+					Often
Euglenophyta						
Euglenophyceae						
Euglena agilis H.J. Carter		+		+		Often
Euglena archaeoviridis B. Zakrys and P.L. Walne			+			Rare
Euglena archaeoplastidiata M. Chadefaud	+	+			+	Rare
Euglena hemichromata Skuja		+		+		Often
Euglena mutabilis F. Schmitz	+		+	+	+	Often
Euglena sp. Ehrenberg			+	+		Often
Lepocinclis spirogyroides Marin & Melkonian	+					Often
Euglena viridis (O.F. Müller) Ehrenberg		+	+	+		Often
Lepocinclis fusca (Klebs) Kosmala and Zakrys	+					Often
Lepocinclis acus (O.F.Müller) Marin & Melkonian				+		Often
Lepocinclis oxyuris (Schmarda) Marin and Melkonian			+			Often
Lepocinclis ovum (Ehrenberg) Lemmermann		+				Often
Monomorphina pyrum (Ehrenberg) Mereschkowsky			+	+		Often
Petalomonas mediocanellata Stein				+		Rare
Phacus acuminatus Stokes	+			+	+	Often
Phacus angustus Drezepolski	+					Rare
Phacus caudatus Hübner	+	+	+	+	+	Often
Phacus curvicauda Svirenko	+			+	+	Often
Phacus ichthydion Pochmann		+				Rare
Phacus indicus Skvortzov		+				Rare
Phacus inflexus (I. Kiselev) Pochmann	+					Rare
Phacus longicauda var. *tortus* Lemmermann		+				Often
Phacus orbicularis Hübner	+					Often
Phacus obolus Pochmann		+				Rare
Phacus parvulus Klebs		+	+	+		Often
Phacus pleuronectes (O.F. Müller) Nitzsch	+					Often
Phacus pusillus Lemmermann		+				Rare
Phacus unguis Pochmann		+				Rare
Trachelomonas bacillifera Playfair		+	+			Often
Trachelomonas cervicula Stokes		+				Often
Trachelomonas hispida (Perty) F.Stein	+	+			+	Often
Trachelomonas oblonga Lemmermann		+				Often
Trachelomonas perforata Awerinzew		+				Rare
Trachelomonas volvocinopsis Swirenko		+				Often

Table 1. (*Contd.*)

Taxa	October 2008	May 2009	July 2010	June 2011	October 2011	Reported from Poland
Chlorophyta *Chlorophyceae*						
Chlamydomonas sp. Ehrenberg			+	+		Often
Desmodesmus armatus (R.Chodat) E.Hegewald	+				+	Often
Desmodesmus sp. (R.Chodat) S.S.An, T.Friedl and E.Hegewald	+	+	+	+		Often
Oedogonium sp. Link ex Hirn	+					Often
Scenedesmus ecornis (Ehrenberg) Chodat	+					Often
Scenedesmus cf. *armatus* (R.Chodat) R.Chodat	+					Often
Scenedesmus intermedius Chodat	+					Often
Scenedesmus sempervirens Chodat	+					Rare
Scenedesmus sp. Meyen	+			+		Often
Stigeoclonium tenue (C.Agardh) Kützing		+				Often
Ulothrix sp. Kützing		+		+		Often
Chlorophyta *Trebouxiophyceae*						
Microthamnion kuetzingianum Nägeli ex Kützing		+				Often
Chlorophyta *Ulvophyceae*						
Ulothrix sp. Kützing		+		+		Often
Chlorophyta *Zygnematophyceae*						
Closterium moniliferum Ehrenberg ex Ralfs	+					Often
Closterium ehrenbergii Meneghini ex Ralfs	+					Often

stream is in the Stoła River basin, which has the annual discharge of 1.46 $m^3.s^{-1}$ (Program of the Environmental Protection for the Tarnowskie Góry community). Part of the oxbow borders the peat bog. The fieldwork was done during the 2008, 2009, 2010 and 2011 vegetation seasons, together with studies on the occurrence of carnivorous *Utricularia* in this area. Samples for taxonomic research were taken once a year (except in 2011 when sampling was done twice) using a plankton net (0.25 mm mesh) and a big pipette (phytobenthos). The samples were placed in 35 ml plastic containers and refrigerated. Fresh samples or samples fixed with 4% formaldehyde were observed with a Nikon ECLIPSE 600 light microscope with Nomarski phase contrast. The collected water samples were transported to the W. Szafer Institute of Botany (Polish Academy of Sciences) in Cracow, where they were analyzed as previously described (Wołowski *et al*., 2011).

Chemical analysis of water samples

Inductively coupled plasma mass spectrometry (ICP-MS) (ELAN 6100, Perkin Elmer) (PN-EN ISO 9963-1:2001) as well as titration methods (PN-ISO 9297:1994; PN-EN ISO 17294-1:2007) were applied to measure the chemical composition of samples. The spectrometer was calibrated to the ICP multi-element standard (Merck).

Results

Water chemistry analyses showed the following average amounts of elements ($mg.dm^{-3}$): inorganic C 78.17, N 7.41, P 0.45, S 151.03, K 36.07, Fe 0.86, Mg 8.71, Mn 0.26, Ca 123.10. The amounts of heavy metals and metalloids such as Cr, Ni, Cu, Zn, Ag, Hg, As and Se did not exceed the relevant norms. For Cd (0.06 $mg.dm^{-3}$) the upper limit was exceeded by a factor of 16 (Polish standard) or 40 (US EPA standard), for Pb (0.05 $mg.dm^{-3}$) by a factor of 7 (US EPA standard) and for Zn (1.06 $mg.dm^{-3}$) by a factor of 10 (US EPA standard). The concentration of Tl (0.24 $mg.dm^{-3}$) exceeded the surface-water limits most spectacularly: by a factor of 120 under both Polish and US EPA standards (Rozporządzenie Ministra Środowiska z dn. 9 listopada 2011 r.; US EPA Water Quality Standards, 2013). In the group of biogenic substances, the upper limits were exceeded for NO_3^- at 7.15 $mg.dm^{-3}$ (Polish standard ≤ 5 $mg.dm^{-3}$) and for PO_4^{3-} at 0.45 $mg.dm^{-3}$ (Polish standard ≤ 0.31 $mg.dm^{-3}$). Other water parameters were as follows: pH 7.0–7.5, electrical conductivity 1.04 $mS.cm^{-1}$ and redox potential (Eh) 258 mV.

We identified 66 algal taxa representing five phyla (Table 1). The euglenophytes of Graniczna Woda stream showed great diversity, including 9 species of *Euglena*, 5 of *Trachelomonas*, 15 of *Phacus*, 4 of *Lepocinclis*, and one each of *Monomorphina* and *Petalomonas* (see Fig. 2). Among the other algal groups were 3 taxa of Cyanophyceae, 2 of Xanthophyceae, 14 of Bacillariophyceae and 15 of green algae (Chlorophyceae). The euglenophytes dominated among the observed algae in every sampling year, and they also were the group with the highest diversity, numbering 37 taxa. Some of them are taxa rarely noted from Poland: *Euglena archaeoviridis* B. Zakrys & P.L. Walne, *Euglena archaeoplastidiata* M. Chadefaud, *Petalomonas mediocanellata* Stein, *Phacus angustus* Drezepolski, *Phacus ichthydion*

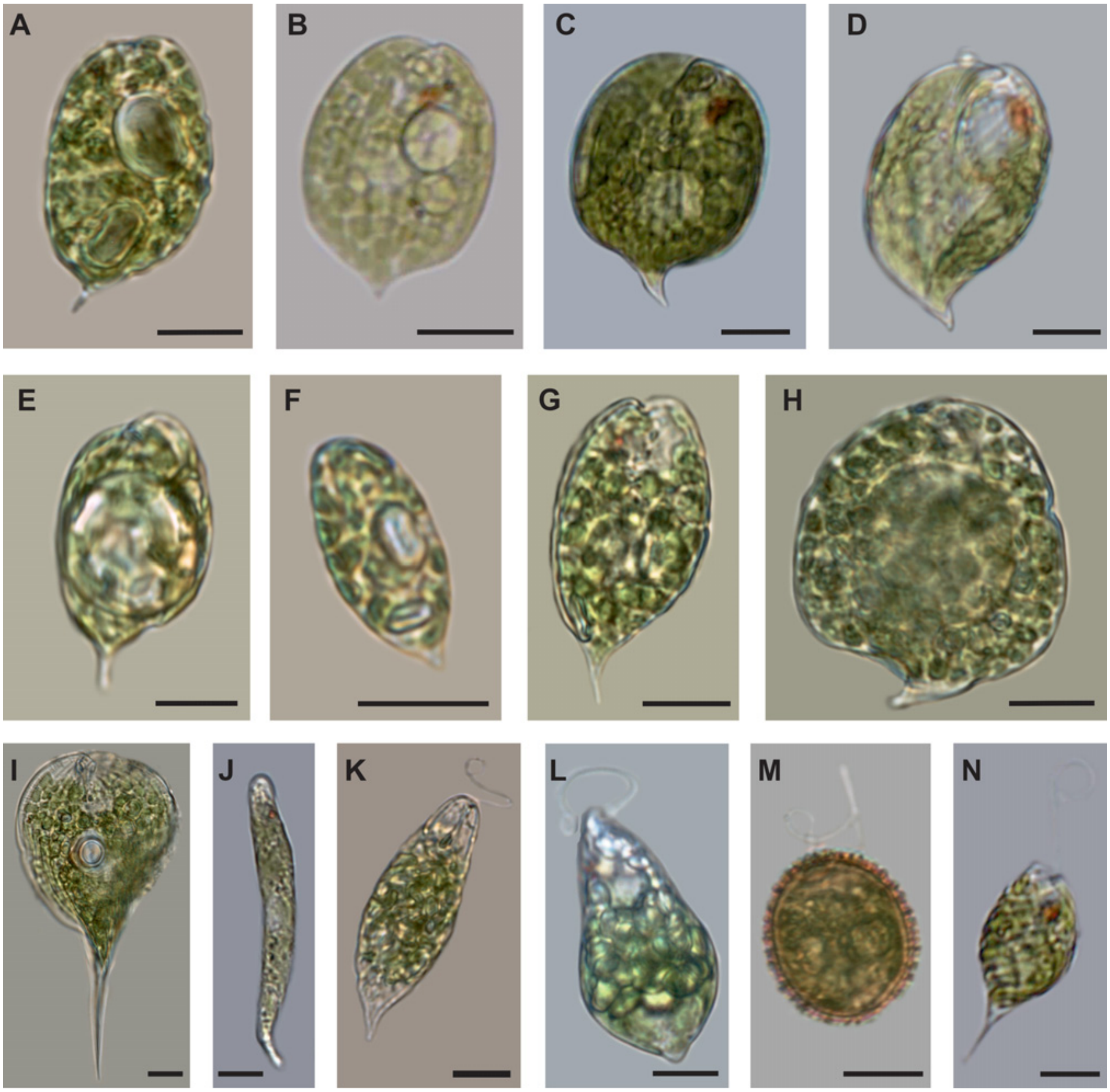

Fig. 2. Euglenophytes documented in samples from Graniczna Woda stream: (A) *Phacus indicus*, (B) *Phacus acuminatus*, (C) *Phacus alatus*, (D) *Phacus curvicauda*, (E) *Phacus obolus*, (F) *Phacus parvulus*, (G) *Phacus caudatus*, (H) *Phacus unguis*, (I) *Phacus longicauda* var. *tortus*, (J) *Euglena mutabilis*, (K) *Euglena hemichromata*, (L) *Euglena archaeoviridis*, (M) *Trachelomonas bacillifera*, (N) *Monomorphina pyrum*. Scale bar = 10 μm.

Pochmann, *Phacus indicus* Skvortzow, *Phacus inflexus* (Kiselev) Pochmann, *Phacus obolus* Pochmann, *Phacus pusillus* Lemmermann, *Phacus unguis* Pochmann and *Trachelomonas perforata* Awerinzew. The other determined algal taxa are often reported from contaminated waters.

In the area we examined, the water chemistry analyses clearly indicated contamination with biogenic compounds and heavy metals (Tl, Cd, Zn and Pb). This is the first time so many *Phacus* taxa (15) have been recorded in such a contaminated habitat; *Phacus caudatus* Hübner, *Phacus curvicauda* Svirenko and *Phacus parvulus* Klebs were recorded repeatedly throughout the study. Among the euglenas, *Euglena mutabilis* F. Schmitz, *Euglena viridis* (O.F. Müller) Ehrenberg, *E. archaeoplastidiata* Chadefaud and *Euglena agilis* H.J. Carter were constant in the study area. *E. agilis* occurred en masse in the pallmeloid stage at sampling time in summer 2011. *Trachelomonas hispida* (Perty) F. Stein was noted in almost every studied sample. Diatoms were equally represented, and *Nitzschia palea* (Kützing) W. Smith and *Pinnularia viridis* (Nitzsch) Ehrenberg were noted in every sample. Among the 15 Chlorophyceae taxa the one most commonly noted was *Desmodesmus* sp. We observed that the shape and arrangement of the chloroplasts in *E. viridis* and *E. mutabilis* varied and did not always fit the classical description, and that the dimensions of euglenophyte specimens were at their lower limits.

Discussion

Previously we studied algal flora in the Jeleniak-Mikuliny Reserve, situated in the same large forest complex as Dęby Boruszowickie. The area of the Jeleniak-Mikuliny reserve consists of two shallow, overgrown water ponds lying in the lowland between two sand dunes. In this reserve, we found 96 algal taxa [Cyanophyceae (4) Bacillariophyceae (20), Chrysophyceae (1), Raphidophyceae (1), Xanthophyceae (1), Cryptophyceae (2), Dinophyceae (1), Euglenophyceae (24), Chlorophyceae (19), Zygnematophyceae (27)] and 11 morphotypes of chrysophyte stomatocysts (Wołowski *et al*., 2013a). The algal flora of polluted Graniczna Woda stream was poorer, with 66 species. It is known that heavy metal pollution alters algal diversity and also community structure. Different species may be more or less tolerant to pollution and some may dominate in a polluted environment (Say and Whitton, 1980; Whitton *et al*., 1981; Podda *et al*., 2013; Trzcińska and Pawlik-Skowrońska, 2013). Some cyanobacteria of the genera Oscillatoria, *Phormidium*, *Plectonema* and *Schizothrix* are often abundant in alkaline waters polluted by heavy metal compounds (Say and Whitton, 1980; Whitton *et al*., 1981). Thus we might expect such taxa to be very abundant in the alkaline, metal-polluted water of Graniczna Woda stream, but we observed *Oscillatoria* sp. and *Phormidium* sp. during only one season. The low occurrence of filamentous cyanobacteria may also be related to a lack of N limitation (as the mean N: mean P was 16.4:1 which is very close to the Redfield ratio of 16:1) (see Reynolds, 1984), or to the presence of other organic compounds that were present in the studied water.

The high diversity of euglenoids in Graniczna Woda stream indicates high tolerance of 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 (Albergoni *et al*., 1980; Tam *et al*., 1981; Fasulo *et al*., 1983; Walne and Kivic, 1990). They have also been found in waters polluted with diesel oil (Dennington *et al*., 1975), phenol (Pawlitz and Werner, 1978) and herbicides and insecticides (Poorman, 1973; Butler, 1977), and can survive in highly radioactive water (Lackley, 1968). Euglenophytes are also found living under very high salinity, for example, in Great Salt Lake (Jones, 1944).

Several features help euglenophytes to survive in an unfavorable environment, such as fast reproduction (division), formation of cysts (Hindák *et al*., 2000) and mixotrophy. Euglenophytes thrive very well in eutrophic water and as a consequence they are often used as bioindicators of water contamination (Starmach, 1983; Sládeček and Sládečková, 1996; Wołowski, 1998, 2011, Wołowski and Hindák, 2005). They usually inhabit α-mesosaprobic and polysaprobic waters.

The contamination of the studied stream with organic phosphates and nitrates explains the abundant occurrence of euglenophytes. It is known that high concentrations of heavy metals limit a site's availability to different groups of organisms but in this case they apparently had no major effect on the algal taxa that occurred in Dęby Boruszowickie.

It is generally known that acidophilic *E. mutabilis* (Lane and Burris, 1981) and *Euglena gracilis* (Cook, 1968) are able to grow in highly polluted habitats. *E. 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 (Tam *et al*., 1981; Havas and Hutchinson, 1983) and acidic post-mining ponds contaminated with heavy metals (Wołowski *et al*., 2008, 2013b). In the investigated area of Dęby Boruszowickie, *E. mutabilis* was present but did not develop en masse. Probably its growth was limited by the high pH (7–7.5) of the water. Our results confirm the higher tolerance spectrum of *E. mutabilis*. Presumably the very low pH of post-mining ponds also limits the growth of euglenophytes, such as *Phacus* species, which prefer slightly acidic or neutral water.

We found no literature data about the occurrence of these *Phacus* species in waters contaminated with heavy metals, including Tl. *Ph. caudatus* and *Ph. curvicauda* are common, well-known species that occur in waters polluted with organic compounds. There are somewhat fewer records published for *Ph. parvulus*, which occurred in Graniczna Woda stream throughout the studied period. That this small organism covered only by a pellicle can thrive in such an inhospitable habitat is an intriguing finding.

Future studies should determine whether the algae of Graniczna Woda stream accumulate heavy metals or possess physiological adaptations endowing them with a mechanism to effectively exclude harmful heavy metals.

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