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Article

Diversity of algal communities in acid mine drainages of different physico-chemical properties

Konrad Wołowski[1*], Łukasz Uzarowicz[2], Magdalena Łukaszek[1] and Barbara Pawlik-Skowrońska[3]

[1] Department of Phycology, Institute of Botany Polish Academy of Sciences, ul. Lubicz 46, 31-512 Kraków, Poland. k.wolowski@botany.pl; m.lukaszek@botany.pl

[2] Faculty of Agriculture and Biology, Warsaw University of Life Sciences SGGW, ul. Nowoursynowska 159, 02-776 Warszawa, Poland. lukasz_uzarowicz@sggw.pl

[3] Department of Hydrobiology, University of Life Sciences in Lublin, Dobrzańskiego 37, Lublin, Poland. pawlik@poczta.umcs.lublin.pl

With 3 tables and 56 figures

Abstract: Algal flora of four post-mining ponds in the area of an abandoned pyrite mine (SW Poland) was examined for the first time. All algal taxa were identified and documented by light microscopy (LM) and scanning electron microscopy (SEM). Two types of waters (on the basis of their properties) were found: (i) strongly acidic with low pH (<3) and high concentrations of sulphates, Al, dissolved silica, and trace metals (e.g. Fe, Mn, Cu, Zn, and Co), as well as (ii) moderately acidic with pH between 4.0 and 4.8, and containing low concentration of ions, Al, dissolved silica, and much lower trace metal concentrations. All together, only 26 taxa (diatoms, filamentous green algae, euglenoids, dinophyte and chrysophyte stomatocysts) were recorded in the investigated acidic waters. Eighteen of them were found for the first time as associated with acid mine drainage (AMD), *Eunotia exigua* (Bacillariophyceae) was the most abundant alga in all studied ponds. The pH of water seemed to be the most important factor influencing species richness of algal communities in the area studied, as the number of taxa was twice as high in the moderately acidic waters (23) compared to the strongly acidic ones (11). The temperature of water, high concentrations of Fe, Al and trace metals, as well as the deficiency of nitrates and phosphates could be other selective factors for algal development in the AMDs studied.

Key words: acidophilus algae, AMD, pyrite mine, extreme habitats.

*Corresponding author

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Introduction

Algae are a group of organisms that take part in colonisation of degraded habitats. They occur in aquatic as well as terrestrial habitats of extreme pH and/or heavy metal pollution (Hargreaves et al. 1975, Round 1981, Albertano 1995, Whiton & Satake 1996, De Nicola 2000, Lopez-Archilla et al. 2001, Pawlik-Skowrońska 2001, Baker & Banfield 2003, Valente & Gomes 2007, Kalinowska & Pawlik-Skowrońska 2008, Kalinowska et al. 2008, Trzcińska & Pawlik-Skowrońska 2008, Wołowski et al. 2008, Turnau et al. 2009). Some algae and cyanobacteria occur in various extreme environments (Seckbach & Oren 2007). Studies on microorganisms occurring in acid mine drainages (AMD) and heavy metal polluted areas are going in various directions, divided into three major lines: taxonomy, physiology of the algae appearing in those environments and the use of algae in the diagnosis and restoration of acid mine drainages. Very few reports on AMD algal communities from various sites, frequently polluted with heavy metals, are available (e.g. Novis & Harding 2007 and references therein; Wołowski et al. 2008, Turnau et al. 2009).

Previously, mainly geological, mineralogical, hydrogeochemical and pedological investigations were carried out in the area of the abandoned pyrite mines in Wieściszowice (Uzarowicz et al. 2008, 2011, Uzarowicz & Skiba 2011). Biocenoses of the acidic ponds and streams in the study area have yet to be examined. The aim of this work was to identify and document algal flora of the acid mine drainages, as well as to determine the influence of water properties on algal communities in the post-mining area in SW Poland.

Materials and methods

The study area was situated in an abandoned pyrite mine, where pyrite occurs in metamorphic chlorite-mica schists (Wieściszowice, Western Sudety Mountains, SW Poland). Pyrite mining started in 1785 (Parafiniuk 1996) when the "Hope" mine was opened. "New Luck" and "Gustav" mines were opened in 1793 and 1796, respectively. Mining operations ceased in 1925. At present, remnants of the abandoned pyrite mines are mine spoils and open pits filled with water, the so-called "Coloured Ponds" (Fig. 1). In the lower part of the "Hope" open pit, the Purple Pond (irregular shape, approximately 100 m long and 2.5 m deep in the central part, Fig. 2) and the Yellow Pond (oval shape, approximately 5 m wide and 10 m long) are situated. Blue Pond is situated in the "New Luck" open pit (oval shape, approximately 30 m wide and 50 m long, Fig. 3) and Green Pond is located in the "Gustav" open pit (oval shape, approximately 5 m wide and 10 m long, Fig. 1). The latter one dries out every summer.

Water samples were collected in the studied area in September 2009 from five sites: (1) Green Pond, (2) Blue Pond, (3) the stream flowing from the adit occurring south of Purple Pond, (4) Purple Pond, and (5) Yellow Pond (Fig. 1). Physico-chemical properties of water (pH, temperature, conductivity, as well as ion content, dissolved silica and selected elements were examined. Ion content was determined by ion chromatography using ICS-2000 (DIONEX) apparatus. Anions were analysed in a 4 mm diameter AS18 analytical column. Cations were analysed in a 5 mm diameter CS16 analytical column. Dissolved silica and selected elements were determined by ICP-AES method using 'Plasma 40' (Perkin Elmer) apparatus. Surface water samples (0–1 m) were collected for phytoplankton and surface sediment samples were taken for phytobenthos analyses. Fresh biological material was taken from the littoral zone of all study sites (0–1 m depth). Phytoplankton was collected using a plankton net (20–25 µm) and phytobenthos was sampled with the use of a water pump. For taxonomical studies, both fresh and fixed (4% formaldehyde) materials were observed using a Nikon ECLIPSE

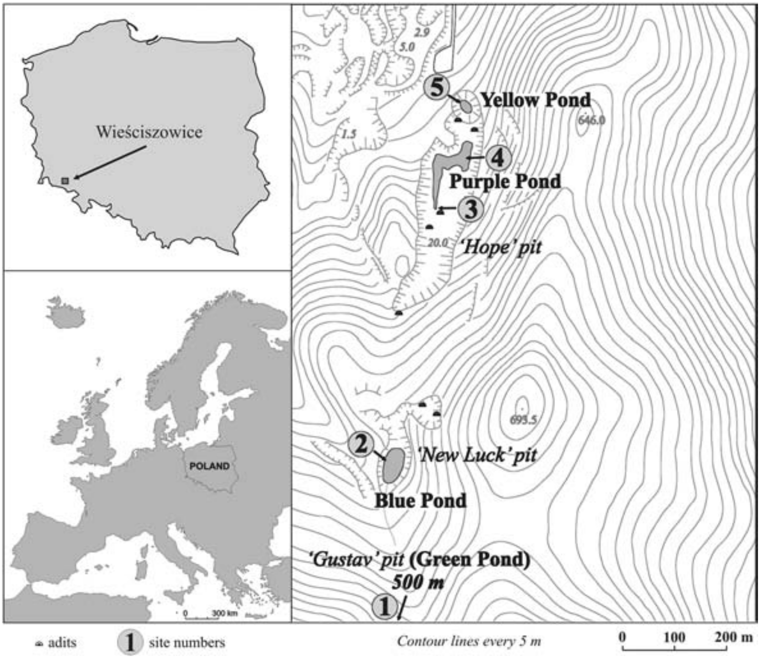


Fig. 1. Location of the study area; point numbers as in the text.

600 light microscope (LM) with Nomarski differential interference contrast (DIC). Samples were collected into 100 ml plastic containers and stored under refrigeration. Samples of Bacillariophyceae were prepared according to the procedure of Bozolla & Russell (1992) and examined using a Hitachi S-4700 scanning electron microscope (SEM). Abundance of a species, expressed in terms of the number of its specimens, was estimated using the four degree scale as follows: +: single specimen (species occurred only as single specimen at least on one slide); ++: rare (species occurred up to 6 specimens, on almost every slide); +++: frequent (species occurred on every slide but not on all visible fields); ++++: abundant (species occurred on every slide and visible on all fields).

Results

Physico-chemical characteristics of waters

The investigated surface waters occurring in the area of abandoned pyrite mines can be divided into two groups: (i) extremely acidic waters (pH 2.7–2.8) of the "Hope" open pit (i.e. waters of Purple Pond, the stream flowing from the adit occurring south of the Purple Pond, as well as the waters of Yellow Pond) and (ii) moderately acidic waters (pH 4.0–4.8) of Green Pond and Blue Pond. The acidity was caused by the presence of sulphuric acid, which is a product of pyrite oxidation. The extremely acidic waters of Purple Pond, the stream flowing from the adit occurring south of Purple Pond and Yellow Pond had typical features of an acid mine drainage (Johnson 2003, Nordstrom

Fig. 2. The Purple Pond.

2011). They were characterized by higher concentrations of H^+, Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, and F^- (Table 1). They also contained large amounts of Al, dissolved silica, and trace metals (e.g. Fe, Mn, Cu, Zn, and Co; Table 2). The concentrations of ions and elements in the waters of Blue and Green Ponds were much lower than in Purple Pond (e.g. the amount of Fe was about 700 times lower in Green Pond and 1400 times lower in Blue Pond). The waters studied also contained different amounts and forms of dissolved nitrogen compounds. Nitrates were detected only in Blue Pond. Phosphates were not detected in the studied waters (Table 1). The temperature of all waters studied ranged from 11 to 20°C (Table 1). The lowest water temperature (11°C) was recorded only in the outflow from the adit occurring south of Purple Pond (site 3).

Both groups of waters represented two fairly different environments taking into account their properties. Differences between them were caused by the way water was supplied. The more acidic Purple and Yellow Ponds were located in a closed open pit supplied with rainwater washing the outcrops of pyrite-bearing schists. They were also supplied by extremely acidic waters coming from neighbouring adits (Fig. 1). The water in Blue Pond came mainly from rainwater, as well as from the stream whose waters were not so acidic (pH 4.6). The Green Pond was supplied mainly by rainwater.

Fig. 3. The Blue Pond.

Algal identification and analysis

Generally, the algal species richness in the AMDs studied was very low. In total, only 26 taxa of mainly eukaryotic algae were detected during the growing season in the five studied sites. They belonged to various classes such as: Bacillariophyceae (15 taxa), Chlorophyceae (5 taxa), Euglenophyceae (2 taxa), Dinophyceae (1), and unidentified coccal forms (Table 3). Moreover, 2 types of stomatocysts of Chrysophytes were found. The most frequently found species were *Eunotia exigua*, *Frustulia crassinervia*, *Euglena mutabilis* and *Chlamydomonas acidophila*. More taxa (11 and 14) occurred in two ponds (site no. 1 and 2, respectively) with moderately acidic waters (pH >4), whereas in the extremely acidic waters (pH <3) of sites no. 3, 4, and 5 fewer taxa (5, 11, and 3, respectively) were found. All of observed taxa were alive. Bacillariophyceae, except for one taxon (i.e. *Eunotia exigua*), were found only in the moderately acidic Blue and Green Ponds. Filamentous algae (Chlorophyceae) were found only in the least acidic Blue Pond and the most acidic Purple Pond, including the stream flowing from the adit occurring south of Purple Pond. All taxa are briefly described below and original documentation included for each (Figs 4–56). The list is arranged according to the taxonomical system of van den Hoek et al. (1995). Abundance of the species expressed in terms of number of specimens was estimated using the 4 degree scale (Table 3). Taxa reported for the first time from AMD are marked with "*".

Table 1. Properties of the investigated waters and content of ions in waters.

	Site no.				
	1	2	3	4	5
Properties of waters:					
pH	4.0	4.8	2.8	2.7	2.8
Temperature (°C)	15	16	11	20	19
Conductivity ($\mu S \cdot cm^{-1}$)	234	186	2870	2860	1848
Content of ions (mg·dm-3):					
H^+	0.11	0.02	1.53	1.83	1.75
Ca^{2+}	5.62	14.57	222.52	201.67	110.79
Mg^{2+}	6.37	6.92	162.76	136.08	47.31
Na^+	4.70	3.35	4.90	6.16	4.85
K^+	1.06	1.01	0.49	1.50	1.23
NH_4^+	0.85	0.04	0.56	0.61	0.68
HCO_3^-	3.64	3.44	38.43	38.87	36.77
SO_4^{2-}	91.3	75.6	1949.80	1953.40	921.80
Cl^-	3.91	2.78	3.31	3.04	3.56
NO_3^-	bdl	0.20	bdl	bdl	bdl
PO_4^{3-}	bdl	bdl	bdl	bdl	bdl
F^-	0.28	0.25	6.48	5.54	1.20

bdl: below detection limit; the limit for NO_3^- and PO_4^{3-} is 0.0025 and 0.01 $mg \cdot dm^{-3}$, respectively.

Bacillariophyceae

Cyclotella radiosa (Grunow) Lemmerm. Figs 4, 5

Valves circular, ca. 20 µm in diameter, striae on each valve extended to valve centre, 16 in 10 µm. From Poland reported once by Bennin (1926). Alkaliphilous, euplanktonic species (www.eecrg.uib.no/Homepages/Teaching/cyclorad.htm). Reported for the first time from AMD. Accessory specimen.

Fragilaria crotonensis Kitton Figs 6–8

Valves ca. 61 µm long, 3 µm wide, 16 striae per 10 µm.

Earlier reported from Poland very frequently (Siemińska & Wołowski 2003). Cosmopolitan species, oligotrophic to weakly mesotrophic waters, mainly occurring at pH >7 (Krammer & Lange-Bertalot 1986). Reported for the first time from AMD.

Staurosirella pinnata sensu Hust. Fig. 9

Valve 6 µm long, 4 µm wide, 13 striae per 10 µm.

Table 2. Content of the selected elements and dissolved silica in waters (in mg·dm^{-3}).

Constituent	Site no.				
	1	2	3	4	5
Fe	0.12	0.06	66.00	85.89	32.59
Al	4.89	1.29	47.66	46.28	18.61
SiO_2	15.31	14.01	32.09	38.51	25.25
Mn	0.22	0.10	1.47	1.28	0.31
Cu	0.02	0.01	0.76	0.79	0.18
Zn	0.09	0.02	0.29	0.24	0.08
Pb	0.01	<0.01	0.01	0.01	<0.01
Co	0.01	0.01	0.19	0.17	0.05
Cd	<0.01	<0.01	0.01	0.01	<0.01
As	<0.01	<0.01	<0.01	<0.01	<0.01
Sb	<0.01	<0.01	<0.01	<0.01	<0.01

Earlier reported from Poland very often, as *Fragilaria pinnata* (Siemińska & Wołowski 2003), and earlier synonym. Common in circumpolar Arctic lakes and ponds, where it is one of the taxa used as an indicator of prolonged ice cover, short growing season and high alkalinity (Paull et al. 2008 and references therein). Reported for the first time from AMD.

***Punctastriata linearis** D.M.Williams & Round — Figs 10–12

Valve 6–8 µm long, 4–5 µm wide, 12 striae per 10 µm.

Reported for the first time from Poland. Widespread species, though its exact microhabitat is not known (Round et al. 1990). Reported for the first time from AMD.

***Eunotia praerupta** Ehrenb. — Figs 13–15

Valve 11–19 µm long, 3–5 µm wide, 11–26 striae per 10 µm.

Earlier reported from Poland very frequently (Siemińska & Wołowski, 2003). Cosmopolitan, common in North Europe, found in various types of waters (Krammer & Lange-Bertalot 1991). Reported for the first time from AMD.

Eunotia exigua (Bréb.) Rabenh. — Figs 16, 17 and 46

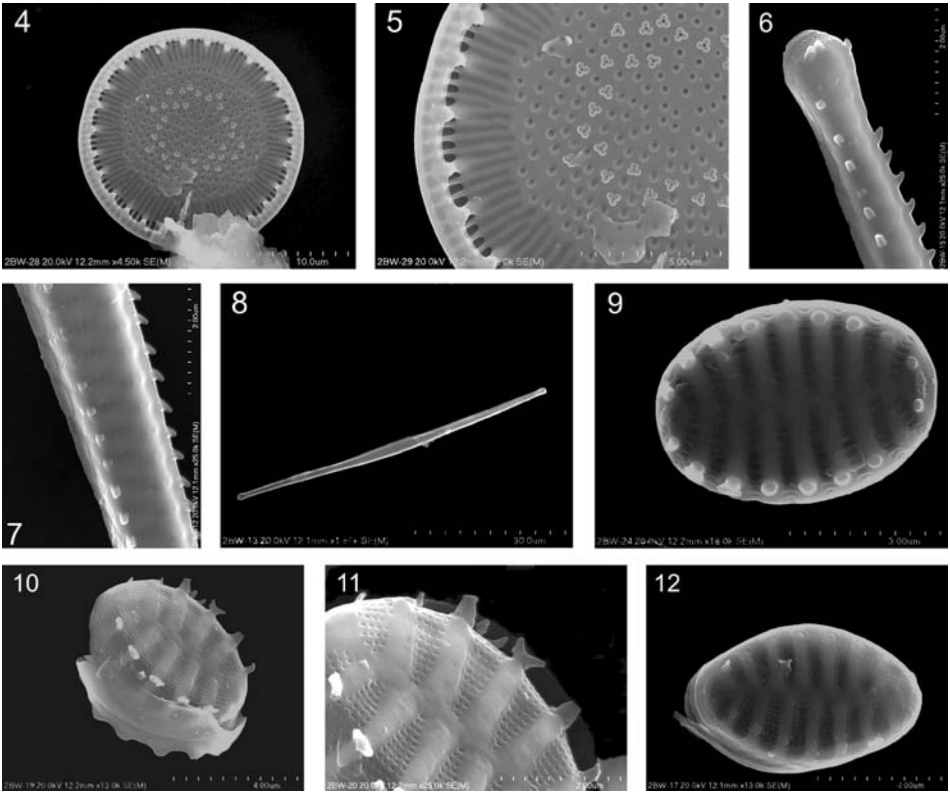
Valve 13–21 µm long, 3–5 µm wide, 24–30 striae per 10 µm.

Earlier reported from Poland very frequently (Siemińska & Wołowski 2003). Extremely oligotrophic species, reported from acidic habitats. One of the taxa that can survive in acid mine drainage with high concentrations of nutrients and heavy metals (Whitton & Diaz 1981). Associated with AMD.

Table 3. Microorganisms and their abundances at all investigated sites (sites ordered from the highest to the lowest pH).

Taxa	**Site no.**				
	2	**1**	**5**	**3**	**4**
Unidentified coccal forms	+				+
Stomatocysts – Chrysophyceae					
Stomatocyst 146	++++				
Stomatocyst 134	++++				
Bacillariophyceae					
Cyclotella radiosa	+				
Eunotia arcus		+++			++
E. exiqua	++++	++++	++++		++++
E. paludosa		++			++
E. praerupta		++++			++
Fragilaria crotonensis	+				
Frustulia crassinervia		++++			
Gomphonema parvulum	+				
Pinnularia borealis var. borealis	++	++			
P. brebissoni var. minuta		+			
P. divergentissima		+++			
P. permicrostauron		+++			
P. silvatica		++			
Punctastriata linearis	++				
Staurosirella pinnata	++				
Dinophyceae					
Parvonidium umbonatum	++				
Euglenophyceae					
Euglena mutabilis		+++	+++	+++	++++
Lepocinclis ovum			++		++
Chlorophyceae					
Chlamydomonas acidophila	++				
Cylindrocapsa sp.				+	+
Klebsormidium flaccidum	++			++	++
Mougeotia sp.	+			+	+
Rhizoclonium sp.				+	+
TOTAL	**14**	**11**	**3**	**5**	**11**

+: single specimen; ++: rare; +++: frequent; ++++: abundant



Figs 4–12: 4–5. *Cyclotella radiosa*; 6–8. *Fragilaria crotonensis*; 9. *Staurosirella pinnata*; 10–12. *Punctastriata linearis*.

***Eunotia arcus** Ehrenb.* — Figs 18, 19

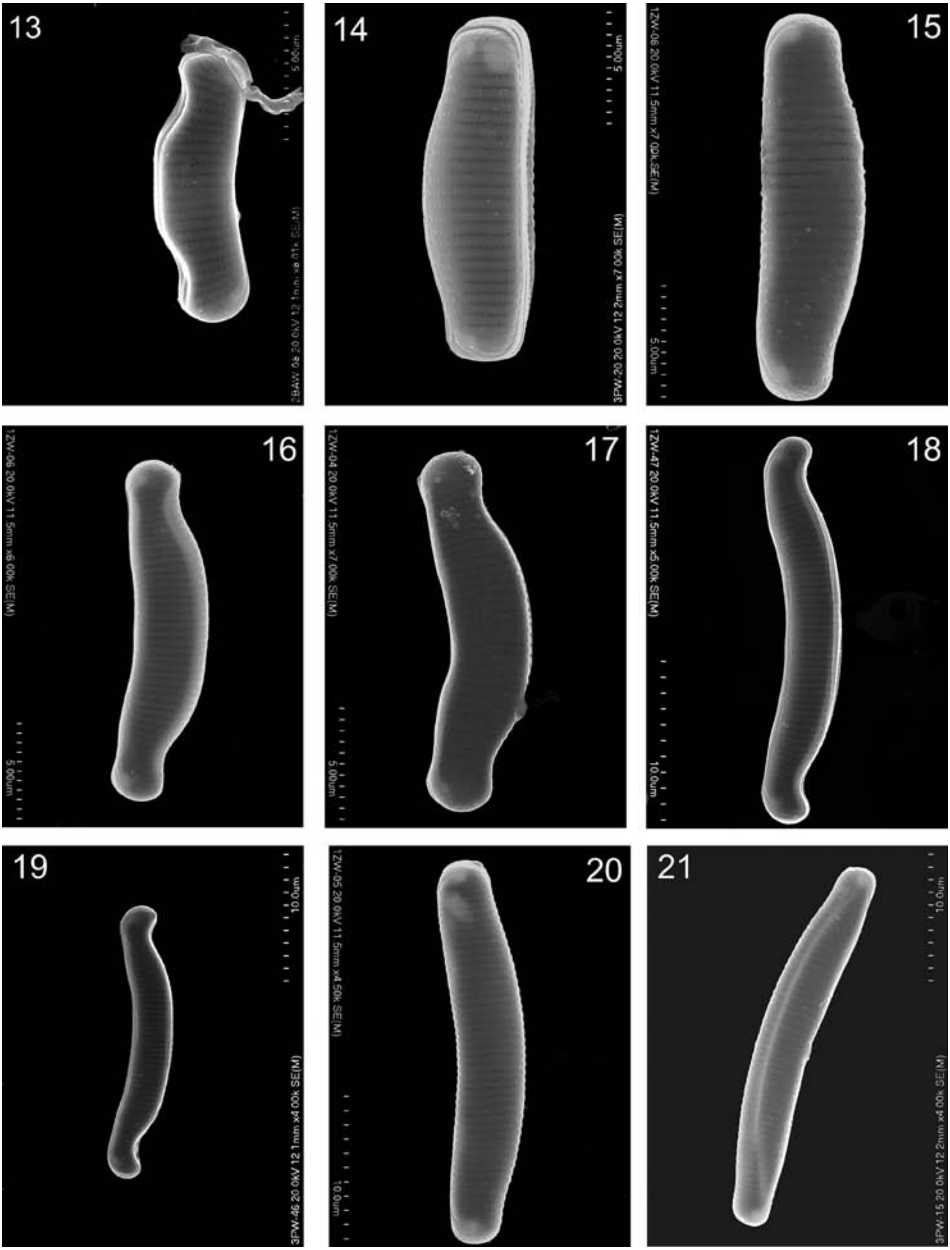
Valves 21–24 µm long, 23 µm wide, 23–26 striae per 10 µm.

Earlier reported from Poland very often (Siemińska & Wołowski 2003). Freshwater alga, occurring in water bodies as well as in wet, moist places (van Dam et al. 1994). Reported for the first time from AMD.

***Eunotia paludosa** Grunow* — Figs 20, 21

Valves 27–34 µm long, 4–5 µm wide, 17–19 striae per 10 µm.

Earlier reported from Poland very often (Siemińska & Wołowski 2003). Acidobiontic, freshwater species, occurring in wet, moist and occasionally dry places (van Dam et al. 1994). Reported for the first time from AMD.



Figs 13–21: 13–15. *Eunotia praerupta*; 16–17. *E. exigua*; 18–19. *E. arcus*; 20–21. *E. paludosa*.

***Pinnularia divergentissima** (Grunow) Cleve* Figs 22–24

Valves 30 µm long, 6 µm wide, 15 striae per 10 µm.

Earlier reported from Poland as rare; found in the Tatra Mts (Bílý 1941) and in the vicinity of Cracow (Rumek 1946). Freshwater alga, acidophilous, occurring in wet, moist and temporarily dry places (van Dam et al. 1994). Reported for the first time from AMD.

Pinnularia brebissonii var. ***minuta*** Krammer Figs 25, 26

Valves ca. 32 µm long, 7 µm wide, 15 striae per 10 µm.

Unreported from Poland. Common in acidic to neutral mountain waters, frequently found in alkaline waters. The species is tolerant of a wide range of ecological factors (Krammer 2000). Reported for the first time from AMD.

Pinnularia permicrostauron Krammer & Metzeltin Fig. 27

Valves 56.0–57.5 µm long, 9.0–9.5 µm wide, 12 striae per 10 µm.

Reported for the first time from Poland. Rare, earlier reported only from northern Finland. According to Krammer (2000) distinguished from *P. microstauron* by size, parallel sides and the long broad ends. Reported for the first time from AMD.

Pinnularia silvatica Petersen Fig. 28

Valves 15.0–16.5 µm long, 4.0–4.2 µm wide, 20 striae per 10 µm.

Earlier reported from Poland frequently as *Navicula falaisiensis* (Siemińska & Wołowski 2003). Probably cosmopolitan and common; resistant to various types of pollution. Reported for the first time from AMD.

Pinnularia borealis var. ***borealis*** Ehrenb. Fig. 29

Valves 30.0–33.0 µm long, 8.0–9.0 µm wide, 5 striae per 10 µm.

Earlier reported from Poland very often (Siemińska & Wołowski 2003). Cosmopolitan and common worldwide; resistant to various types of pollution (Krammer 2000). Reported for the first time from AMD.

Frustulia crassinervia (Bréb.) Lange-Bert. & Krammer Figs 30–32, 44

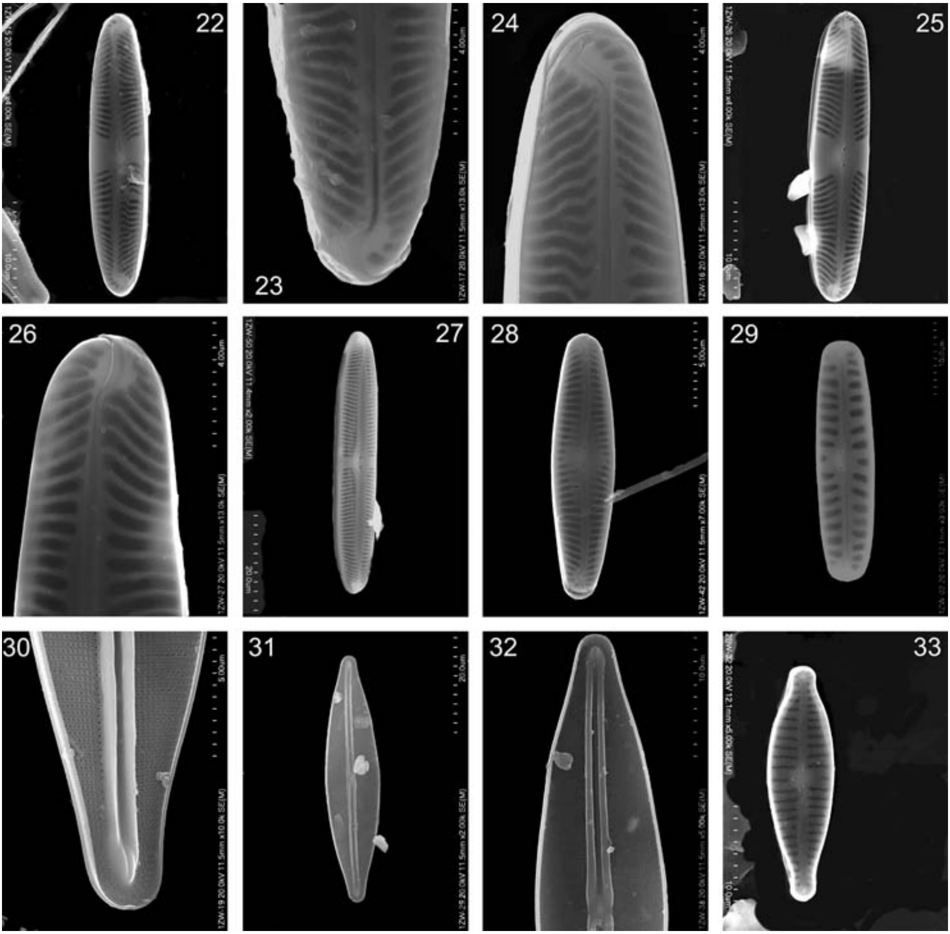
Valves 49–52 µm long, 10–11 µm wide, 32 striae per 10 µm.

Earlier reported from Poland several times as *Navicula crassinervia* (Siemińska & Wołowski 2003). Cosmopolitan, common in acidic, oligotrophic to mesotrophic waters (Krammer & Lange-Berthalot 1986). Associated with AMD.

Gomphonema parvulum (Kütz.) Rabenh. Fig. 33

Valves 19.0–20.0 µm long, 5.0–6.0 µm wide, 12 striae per 10 µm.

Earlier reported from Poland very often from various types of waters (Siemińska & Wołowski 2003). Cosmopolitan and worldwide; resistant to various types of pollution (van Dam 1994). Reported for the first time from AMD.



Figs 22–33: 22–24. *Pinnularia divergentissima*; 25–26. *P. brebissonii* var. *minuta*; 27. *P. permicrostauron*; 28. *P. silvatica*; 29. *P. borealis* var. *borealis*; 30–32. *Frustulia crassinervia*; 33. *Gomphonema parvulum*.

Chrysophyceae – stomatocysts

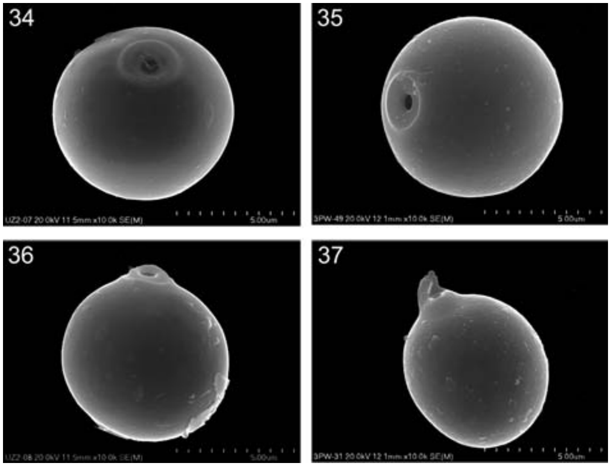
***Stomatocyst 146**, Zeeb & Smol (in Pienitz et al. 1992) Figs 34, 35

Biological affinity: unknown.

SEM description: smooth and spherical stomatocyst, 6.4–7.7 µm in diameter. The collar is short and 2.1–2.7 µm in diameter. The pore is regular, 0.5–0.7 µm in diameter. Reported for the first time from AMD.

***Stomatocyst 134**, R.J.Duff & Smol (in R.J.Duff et al. 1992) Figs 36, 37

Biological affinity: unknown.



Figs 34–37: 34–35. Stomatocysts no. 146 Zeeb & Smoll, various specimens; 36–37. Stomatocyst no. 134 Duff & Small.

SEM DESCRIPTION: smooth and oval stomatocyst, 5.97 µm wide and 7.1 µm long. The collar is conical, 1.5 µm in diameter with characteristic hooked projection (broken on the presented image, Fig. 7.3). The pore has 0.45–0.65 µm in diameter. Reported for the first time from AMD.

Dinophyceae

Parvodinium umbonatum F.Stein — Fig. 40

Cells broadly ovoid in ventral view, slightly dorso-ventraly flattened 18–22 µm long epitheca, longer than hypotheca.

Reported from Poland as quite frequent, as *Peridinium umbonatum* (Siemińska & Wołowski 2003). Widely distributed, common in small water bodies and lakes (Starmach 1974). Reported for the first time from AMD.

Euglenophyceae

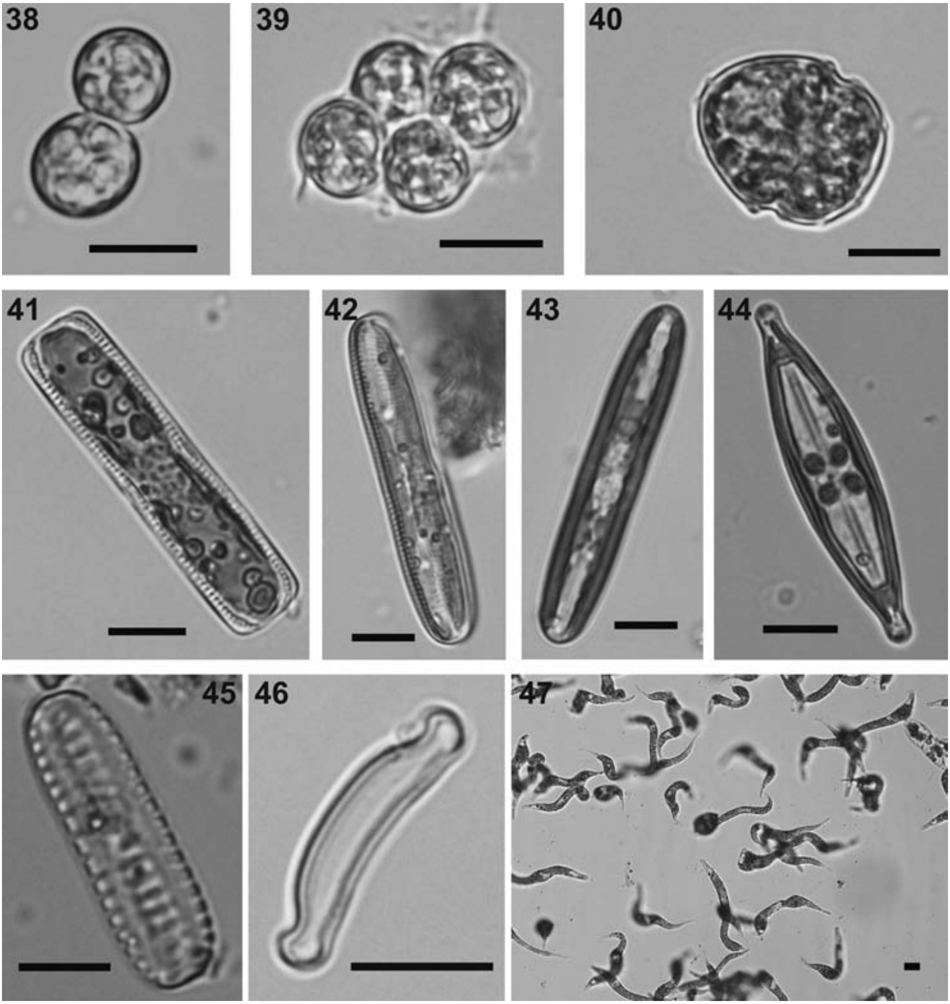
Euglena mutabilis F.Schmitz — Figs 47–50

Cells 5–10 wide, 83–125 µm long, narrowly cylindrical, slender or elongate spindle-shaped.

Earlier reported from Poland as quite common (Siemińska & Wołowski 2003). Probably cosmopolitan. Occurs in acidic water bodies, resistant to various types of pollution as well as to a heavy metal contamination, acidophilous (Wołowski 2011; Novis & Harding 2007). Associated with AMD.

Lepocinclis ovum (Ehrenb.) Lemmerm. — Fig. 51

Cells 22 µm wide, 30 µm long, broadly ovate, posterior end with short blunt tail-piece.



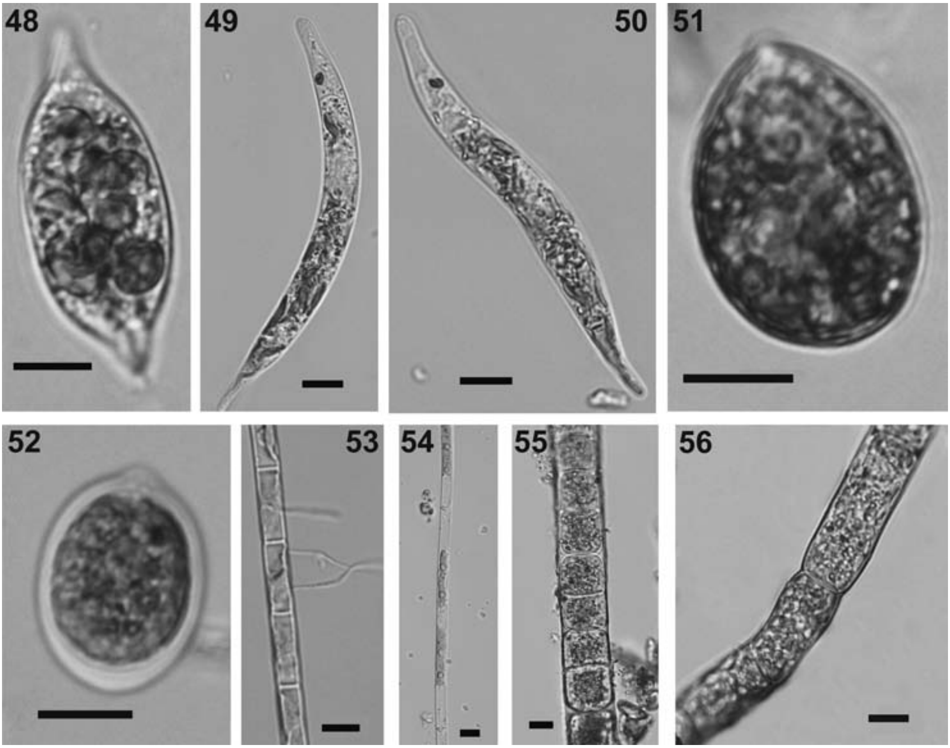
Figs 38–47: 38–39. Unidentified coccal forms; 40. *Parvodinium umbonatum*; 41–43. *Pinnularia* conf. *permicrostauron* in various positions; 44. *Frustulia crassinervia*; 45. *Pinnularia borealis* var. *borealis*; 46. *Eunotia exigua*; 47. *Euglena mutabilis*; Scale bar = 10 µm.

Earlier reported from Poland quite frequently (Siemińska & Wołowski 2003). Usually occurs in pools, puddles, ditches, fish ponds as well as in the littoral zone of lakes (Wołowski 2011). Reported for the first time from AMD.

Chlorophyceae

Chlamydomonas acidophila Negoro Fig. 52

Cells 3–5 µm wide, 6–10 µm long, elliptical to obovoid, broadly rounded.



Figs 48–56: 48–49. *Euglena mutabilis*; 51. *Lepocinclis ovum*; 52. *Chlamydomonas acidophila*, 53. *Klebsormidium flaccidum*; 54. *Mougeotia* sp., 55. *Rhizoclonium* sp.; 56. *Cylindrocapsa* sp.; Scale bar = 10 µm.

Earlier reported from Poland quite frequently (Siemińska & Wołowski 2003). Usually occurs in pools, puddles, ditches, fish ponds as well as in the littoral zone of lakes. In the British Isles recorded from highly acidic mine waters associated with pyrite oxidation (Hargreaves et al. 1975). Associated with AMD.

Klebsormidium flaccidum (Kütz.) P.C.Silva, Mattox & W.H.Blackw. Fig. 53

Filaments long, cells cylindrical to barely-shaped, 5–7 µm wide, 1 to 3 times longer than wide (9–11µm long).

Earlier reported from Poland as quite common. Probably cosmopolitan taxon, occurring as fealty mats or mucilaginous masses (Rindi 2011) on soils, rocks, artificial substrates as well as in the freshwater environments (Lockhorst 1996, after Nagao et al. 2008, Trzcińska & Pawlik-Skowrońska 2008). Associated also with AMD.

Mougeotia sp. C.Agardh Fig. 54

Filaments simple, cells cylindrical 6–7 µm wide, 50–56.5 µm long, chloroplasts with 2 pyrenoids.

Earlier reported from Poland as quite common (Siemińska & Wołowski 2003). Reminds *Mougeotia viridis* (Kütz.) Wittr. which according to Johnson (2002), is one of the most abundant *Mougeotia* species, often in ditches, meadow and moorland pools (pH 4.5–7.5). Reported for the first time from AMD.

Rhizoclonium sp. Kütz. Fig. 55

Filaments uniseriate and unbranched 10–12 µm wide, 50–55 µm long.

Probably associated with AMD. Benthic *Rhizoclonium tortuosum* (Dillwyn) Kütz. was recorded in mine outflows (southern UK) containing high amounts of heavy metals (Pawlik-Skowrońska et al. 2007)

Cylindrocapsa sp. Reinsch Fig. 56

Cells 18–24 µm wide, 1–2.5 µm times longer than wider.

Probably associated with AMD.

Unidentified coccal forms Figs 38, 39

Cells 8–9.5 µm in diameter usually grouped in 2 or 4 cells.

Resembles *Achromatium volutans* (Hinze) C.B.Niel or *Thiosphaerella amylifera* (cf. Häusler 1982). A similar form was observed in a Portuguese mine associated with AMD (Wołowski et al. 2008).

Discussion

Reports on microalgal flora in acidic habitats associated with weathering zones of pyrite-bearing rocks are scarce (e.g. Shubert et al. 2001, Novis & Harding 2007 and references therein). However, several bacteria and archaea species have been documented from acid mine waters (Nordstrom 2011 and references therein). Our study revealed essential differences in algal communities composition inhabiting acid mine drainages (AMDs) with extremely acidic (pH <3) and moderately acidic (pH 4–5) waters. The most often encountered representatives of algae in these types of biotopes were: diatoms (*Eunotia* and *Pinnularia*), euglenoids (*Euglena*, *Phacus* and *Lepocinclis*), green algae (*Chlamydomonas*) and filamentous green algae (*Klebsormidium*, *Mougeotia*) (Novis & Harding, 2007). We found most of them in the investigated AMDs. *Eunotia exigua*, *Frustulia crassinervia* and *Euglena mutabilis* were the abundant occurring species. However, other species of *Eunotia* and *Pinnularia* which until now have not been associated with AMD also occurred together with the taxa mentioned above. Certain species found during the study i.e. *Klebsormidium flaccidum*, *Mougeotia* sp. and *Pinnularia borealis* are known for their high metal and acid tolerance and have been reported from many extremely acidic biotopes (Fisher et al. 1998, Novis & Harding 2007, Kalinowska et al. 2008, Wołowski et al. 2008, Turnau et al. 2009). It is very interesting that among 26 reported taxa, 18 were found for the first time in AMD. Furthermore, we found Bacillariophyceae such as *Cyclotella radiosa*, *Fragilaria crotonensis* and *Staurosirella pinnata* classified previously as alkalophilous (van Dam et al. 1994). During taxonomical examination we noted two morphotypes of Chrysophyte stomatocysts which occurred in abundance in acidic waters, but have not been reported earlier from environments similar to those investigated. The occurrence of *Parvodinium umbonatum* also attracted our attention, as reported from AMD for the first time.

Euglena mutabilis and *Eunotia exigua* were the most common acidophilous species, as they occurred in all four studied sites (Table 3). *Euglena mutabilis* occurred in high abundance in site 4 and *Eunotia exigua* in sites 1, 2, 4, and 5. A similar composition of algal flora was observed in São Domingos AMD in Portugal (Wołowski et al. 2008), where *Eunotia exigua* and *Pinnularia acoricola* occurred as dominant components. However, the last species was not found in AMD in Wieściszowice.

Our study revealed that number of taxa present decreased with the decrease of pH, so species richness was higher at pH 4.8 in Blue Pond (14 taxa) than in Green Pond at pH 4.0 (11 taxa) (Table 3). The latter pond contained the majority of diatoms present in all ponds in Wieściszowice. The results obtained suggest that pH is the primary selective factor for algal microfloral inhabitants. However, it should be taken into account that high concentrations of Fe, Al, and trace metals (e.g. Cu, Zn, Mn) may also exert a selective effect (Kalinowska et al. 2008). This may explain the lowest algal richness and abundance in Purple Pond, characterised by the lowest pH (Table 1) and the highest amounts of trace metals (Table 2). This is consistent with the report of Pawlik-Skowrońska et al. (2007) which showed that in heavy metal polluted waters of river estuaries in the UK, the algal macroflora was limited to several taxa (with *Rhizoclonium tortuosum* (Dillwyn) Kütz. occurring among others). It is known that filamentous green algae may develop in heavy metal polluted habitats regardless of pH. For instance, *Stigeoclonium tenue* (C. Agardh) Kütz. was found to be abundant in heavy metal polluted streams with circum neutral waters (Pawlik-Skowrońska 2001), and *Klebsormidium* spp. and *Microspora* sp. occur both in neutral and acidic terrestrial and aquatic environments (Shubert et al. 2001, Trzcińska & Pawlik-Skowrońska 2008). Soils of mining dumps containing high concentrations of heavy metals, regardless of pH were also inhabited by a low number (11–18 taxa) of microalgae (Shubert et al. 2001, Trzcińska & Pawlik-Skowrońska 2008), such as *Klebsormidium* spp, *Stichococcus bacillaris* (Nägeli) Nägeli, and *Pinnularia* sp. It is worth mentioning that some unidentified diatoms and stomatocysts were found not only in the waters investigated, but also in acidic soils developed in the weathering zone of pyrite-bearing rocks in Wieściszowice (Ł. Uzarowicz, personal communication, not published). The high heavy metal resistance of algae can result from the limited intracellular transport of metals at low pH (Skowroński et al. 1991, Pawlik et al. 1993). Moreover, some eukaryotic algae possess an effective mechanism of heavy metal detoxification using metal-complexing thiol oligopeptides such as glutathione and phytochelatins (Skowroński et al. 1998, Pawlik-Skowrońska 2001, 2003).

Another selective factor for algal microflora in the area studied is the water temperature. During our investigation there was an opportunity to study two sites: Purple Pond (site 4) and the stream flowing from the adit occurring south of Purple Pond (site 3), whose waters had almost the same pH and water chemistry, but with differing temperatures (Table 1 and 3). We suggest that the temperature influenced the composition and the abundance of algal flora in the two sites, as more taxa were found in warmer waters (site 4) than in colder ones (site 3).

Very acidic AMDs are poor in bioavailable biogenic nutrients (e.g. Shubert et al. 2001, Novis & Harding 2007, Nordstrom 2011 and references therein) and this can be another selective factor for algae. Low amounts of nitrates and lack of dissolved phosphates in the waters investigated, despite the abundance of macroelements such as Ca and Mg (Table 1), may explain why algal communities in the AMDs are poorer than the communities occurring in Polish circum neutral surface waters that are rich in nutrients (Starmach 1989). Observations similar to those shown in the present study were carried out on heavy metal polluted soils with neutral pH, where filamentous Chlorophyceae were able to grow at higher nutrient contents (Trzcińska & Pawlik-Skowrońska 2008). As shown in this paper, acidotolerant and metalotolerant algal communities may exist under very extreme environmental conditions, however there are still a lot of questions concerning their ecophysiology.

Conclusions

Structure of algal communities occurring in AMDs from the area of abandoned pyrite mines was dependent on the physico-chemical properties of localities. The pH of water seems to be the most important factor influencing a variety of algal communities, and more algae species occurred in moderately acidic waters (pH >4) than in strongly acidic ones (pH <3).

Taxonomic and environmental studies help us to recognize that all 18 taxa described for the first time from AMD of Wieściszowice (*Cyclotella radiosa*, *Fragilaria crotonensis*, *Staurosirella pinnata*, *Punctastriata linearis*, *Eunotia praerupta*, *E. arcus*, *E. paludosa*, *Pinnularia divergentissima*, *P. brebissonii* var. *minuta*, *P. permicrostauron*, *P. silvatica*, *P. borealis* var. *borealis*, *Gomphonema parvulum*, *Parvodinium umbonatum*, *Lepocinclis ovum*, *Mougeotia* sp. and two stomatocysts (Stomatocyst 134 and 146) should be treated as more ubiquitous than previously believed.

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