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Microorganisms in selected sulphuric springs of the Polish Carpathians

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Microorganisms colonising sulphurous waters were found at the bottoms of the spring niches and along spring outflows. Five springs from the Carpathians and two from the Carpathian Foredeep were selected for investigations. Sulphurous flora is represented mainly by sulphuric bacteria. They occur as individual threads, spider-webs, festoons, encrustations and covers. Their colours may be white, creamy, violet, pink and purple. Altogether 31 microorganism taxa: bacteria (16), cyanoprokaryota (3) and algae (12) were identified and illustrated. The bacterial occurrences have been correlated with physico-chemical state of the waters.

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INTRODUCTION

Bacteria participate in processes associated with a migration of many elements in natural environment, including sulphur, manganese, nitrogen and iodine, a significant part of this migration taking place in groundwater. Particular taxa develop in groundwater under specific conditions determined by thermal regime, redox potential, pH and mineralisation; some require nutritional energy provided by organic matter (Macioszczyk, 1987).

Sulphurous waters are a typical environment of sulphur-fixing bacteria (Strzeszewski, 1913; Topińska-Luchter, 1951; Jarocka and Kłosowska, 1966; Kotełko *et al*., 1979; Spandowska *et al*., 1979) that often take part in hydrogeochemical transformations of sulphur (Rajchel *et al*., 2000*a*, *b*). Denitrifying bacteria may develop in groundwater where sulphates are present but oxygen is lacking. They reduce the sulphates to hydrogen sulphide, evolving H_2S migrating up into zones with available oxygen and facilitating the development of aerobic bacteria. Desulphatising bacteria oxidise hydrogen sulphide and other sulphur compounds into sulphates or sulphuric acid, while thionic bacteria oxidise the reduced sulphur compounds into sulphates or sulphuric acid.

Microorganisms contained in sulphurous waters of southern Poland have been studied by numerous authors: Szafer (1910), Strzeszewski (1913), Namysłowski (1922), Turowska (1934), Topińska-Luchterowa (1951) and others. Only those by Strzeszewski (*op. cit.*) and Turowska (*op. cit.*) discuss the springs in Swoszowice and Rytro, the subject of the research described here.

We have identified the microflora in these sulphurous springs and compared the results with earlier determinations, and attempted to establish links between water chemistry and microorganism species composition and colony form.

METHODS

Samples of microflora were collected in seven springs, during November, February, April and July of 1998/1999. They were taken from the bottoms of the spring niches and from spring outflows, using plastic jars with a capacity of about 120 ml. Simultaneously, water samples for hydrogen sulphide analyses and other standard determinations (Witczak and Adamczyk, 1995) were collected. Water analyses were carried out using the IPC-AES method (Rajchel, 2000). Physico-chemical parameters were measured in each sample (Table 1). The taxonomic study

Table 1

Physico-chemical parameters of water (mg/dm^3) from the springs investigated (after Rajchel, 1998, 2000)

Springs	t [°C]	pH	H_2S	Na^+	K^+	Li^+	Sr^{2+}	Ca^{2+}	Mg^{2+}	Fe^{2+}	Cl^-	HCO_3^-	SO_4^{2-}
Główne	10.2	6.7	62.8	46.80	8.50	0.09	5.97	547.0	92.00	0.01	62.05	564.4	1277.8
Napoleon	10.1	6.8	61.1	72.30	8.90	0.09	4.20	497.0	97.3	0.005	62.05	533.9	1247.8
Jacek	7.8	7.4	12.2	44.60	1.17	0.04	0.80	74.86	15.34	0.02	57.90	246.30	61.62
Paweł	8.8	7.1	15.5	6.06	1.62	0.01	0.20	79.70	28.93	0.01	8.50	362.30	39.62
Jerzy	8.8	7.5	10.2	10.75	1.69	0.02	1.04	51.70	17.84	0.09	3.60	228.10	22.11
Katarzyna	9.3	7.8	10.7	46.40	1.57	0.02	0.40	71.89	13.54	0.01	20.20	362.39	19.60
Stanisław	8.0	7.8	11.2	80.60	2.10	0.04	0.30	17.00	7.90	0.06	6.00	251.30	19.40

was based on live material, using an *ECLIPS 600 Nikon* light microscope. The identification of organisms was made following Häusler (1982), Kramer and Lange-Bertalot (1986*a*, *b*), Wołowski (1998) and Starmach (1966).

DESCRIPTION OF THE SPRINGS

The following springs from the Carpathians (Fig. 1) were selected for microbiological investigations: "Jacek" at Lipnica Wielka (Orawa), "Paweł" at Polichty near Ciężkowice, "Katarzyna" at Rytro near Stary Sącz, "Jerzy" at Wierchomla near Krynica and "Stanisław" at Łabowa near Krynica. Two springs are situated in the Carpathian Foredeep: "Główne" and "Napoleon" at Kraków-Swoszowice (Rajchel, 1998, 2000; Rajchel and Rajchel, 1999; Rajchel *et al*., 2000).

The "**Jacek**" spring at Lipnica Wielka is situated in the right, steep bank of the Lipniczanka stream and its small, right tributary, some 1.2 km upstream from the bridge across the former stream. It is a fracture spring with a diameter of about 0.25 x 0.35 m and a depth of 0.05 m, in an outcrop of thick-bedded sandstone that limits the niche from the slope. Incrustations and festoons of white desulphurising bacteria mark the streambed (Fig. 2a).

The "**Katarzyna**" spring at Rytro is sited in the scarp of the terrace, on the right bank of the Wielka Roztoka stream, *ca*. 300 m below a footbridge, opposite the last bus stop. Water

Fig. 1. The geological structure of the Carpathians and the Carpathian Foredeep (after Poprawa and Nemčok, 1988–1999; changed, simplified) and the location of analysed springs (after Rajchel, 2000)

Fig. 2. **a** — sulphuric bacteria with fringe-like appearance from the "Jacek" spring at Lipnica; **b** — violet mats of sulphuric bacteria in the "Katarzyna" spring at Rytro; **c** — violet mats of sulphuric bacteria in the "Paweł" spring at Polichty; **d** — sulphuric bacteria resembling thin filaments or spider-webs in the "Paweł" spring at Polichty; **e** — the outflow of the "Główne" spring at Swoszowice covered by purple sulphuric bacteria

flows from the fissure down the steep bank, covered by a thick layer of white bacteria, into an oval niche (Fig. 2b) with a diameter of 0.35 m and a depth of about 0.04 m, which has retreated because of backwards erosion. The niche bottom is covered with encrustations of white bacterial colonies.

The "**Jerzy**" spring at Wierchomla flows out from the large niche in the Izwór stream valley, on its left bank, *ca*. 1.8 km from the church, at the foot of a hill slope covered with a beech forest. The niche is oval with a diameter of 1.3 m, 0.5 m deep, its banks being lined with sandstone fragments and timbered. Water overflows the linings, leaving at the bottom of the spring niche a violet-coloured deposit of sulphurous bacteria, while bacterial colonies form white incrustations along the watercourse.

The "**Stanisław**" spring at Łabowa is found in a forest on the southern slope of Sapalska Hill, *ca*. 500 m from the forest edge. Its oval niche is 1.3 m in diameter and 1 m deep, the banks being lined with sandstone blocks up to a ground level. The stone lining is covered up to the water table with violet deposits composed of sulphurous bacteria.

The "**Paweł**" spring at Polichty is located on Sucha Hill, on the right bank of a stream. The niche is oval, 1.2 x 1.5 m across, and 0.3 m deep. Continuous, violet deposit of sulphurous bacteria cover its bottom (Fig. 2c), while the entire niche, filled with water, is covered with white bacteria that form a network of densely spaced (Fig. 2d) veinlets, 0.5 m long. Such veinlets have so far not been found anywhere else in the Carpathians.

The "**Główne**" spring at Kraków-Swoszowice is situated in the park, NW of the bath building, where the ground surface begins to slope toward the Wilga River valley. The spring takes the form of an artesian well, 10.2 m deep, lined with blocks of the Szydłowiec sandstone, situated inside a small wooden house resting on a stone foundation. Water flows out through a pipe, then along an open ditch and a short watercourse, emptying into the Wilga. The pipe is covered with thick encrustations of white sulphurous bacteria, while the bacteria deposited along the watercourse are purple (Fig. 2e).

The "**Napoleon**" spring at Kraków-Swoszowice is situated north of the "Główne" spring on the other side of the tarmac Swoszowice–Kraków road, at the bottom of a sandy gully. It is a natural outflow of sulphuric water from the mouth of a collapsed adit. The intake is in the form of a concrete ring, partly hidden inside the ground, and the water flows out from below the ring and down a narrow watercourse to the Wilga. White encrustations and purple covers of sulphurous bacteria can be seen along the stream.

RESULTS

About 31 taxa of microorganisms: Schizomycetes (16), Cyanophyceae — Cyanoprokaryota (3), Euglenophyceae (5), Bacillariophyceae (4) and Chlorophyceae (3) were identified in the seven springs investigated. Sulphuric bacteria belonging to genus *Chromatium* (Fig. 3a–d, j and l) occur abundantly and frequently in five of these springs. This type of bacteria occurs throughout the year in the "Główne" and the "Napoleon" spring at Swoszowice. During the summer and hot autumns of 1999 and 2000 these bacteria thrived as pinkish-red or pinkish-purple deposits. These springs have a high concentration of hydrogen sulphide, which favours the development of *Chromatium* species. In both these springs *Chromatium weissei* (Fig. 3c), *Ch. okenii* (Fig. 3j) occur abundantly. Also *Chlorobium vibrioforme* (Fig. 3h and i) formed masses here during the summer but it did not occur in the remaining springs investigated. More algal taxa occur in the "Napoleon" spring than in the "Główne" spring: *Pseudanabaena constricta* (Fig. 4j), *Lyngbya aerugineo-coerulea* (Fig. 4i), *Navicula* sp. div., *Nitzschia linearis* (Fig. 5h), *Pinnularia viridis* (Fig. 5f), *Euglena mutabilis* (Fig. 5b), *E. viridis* (Fig. 5a) and *Lepocinclis ovum* (Fig. 5c) while *Euglena viridis* and *E. retronata* (Fig. 5d) developed abundantly during August 2000.

The taxa *Chromatium gracile*, *Ch. weissei* and *Thiocapsa roseopersicina* (Fig. 3k) occur as masses in the "Paweł" spring at Polichty, colouring the sediment pinkish-violet. *Achroonema spiroideum* (Fig. 4k) and *Chlorochromatium aggregatum* (Fig. 3m), which were not observed in the other springs, developed here.

In the springs "Jerzy" at Wierchomla, "Katarzyna" at Rytro, and "Jacek" at Lipnica Wielka filamentous bacteria of the genus *Thiothrix* developed well. *Thiothrix nivea*, *Th. tennuissima*, *Beggiatoa alba* and *B. minima* occurred abundantly. These bacteria turn the sediments white. *Chromatium weissei* and *Ch. vinosum*, which also developed abundantly among filamentous bacteria in the "Jerzy" spring during the summer, coloured the surface of the spring bottom whitish-pink.

Only 5 taxa were recognised in the "Stanisław" spring of these *Thiocapsa roseopersicina* (Fig. 3k) occurred abundantly, colouring the sediments violet-pinkish. Only in this spring were a few specimens of the colourless euglenophyte *Entosiphon sulcatus* (Fig. 5e) were found.

Among the springs investigated, only the "Główne" and "Napoleon" at Swoszowice, and "Katarzyna" at Rytro, have been investigated earlier. Strzeszewski (1913) gave a list of taxa from the "Napoleon" and "Główne" springs where many *Chromatium* taxa occurred. From the "Główne" spring he reported *Ch. minus* and *Thiothrix nivea*, *Th. nivea* var. *verticillata*, *Beggiatoa alba*, *B. leptomitiformis*, *Lamprocystis roseopersicina*, and several taxa of cyanobacteria (5), diatoms (13), and green algae (2), which have not been found during this study. He found a similar, but more diverse bacterial and algal flora in the "Napoleon" spring. A lower number of organisms in those springs was noted 20 years later by Turowska (1934), who also found *Chromatium okenii* fo *minutissima* and *Thiocapsa agilissima*. She listed taxa of cyanoprokaryota (*Anabaena constricta, Oscillatoria geminata* var. *sulphurea, Oscillatoria trichoides*), and green algae *Stigeoclonium subsecundum*, *St. pygmaeum*, *Ulothrix subtilis*, *Oedogonium* sp. which have not been observed in this study.

The present list of taxa (Table 2) shows only 11 taxa of bacteria and 11 taxa of other organisms, in the "Główne" and "Napoleon" springs, fewer than reported by Strzeszewski (1913, about 50 taxa), and Turowska (1934, about 19 taxa). This is probably connected with regulation of the outflow of the springs and local extinction of some populations. During Strzeszewski's (1913) investigation, a small equalising tank

Table 2

Abundance of microflora in the springs investigated

Taxa	Springs/Sites						
	"Główne" Swoszowice	"Napoleon" Swoszowice	"Jerzy" Wier-chomla	"Katarzyna" Rytro	"Paweł" Polichty	"Stanisław" Łabowa	"Jacek" Lipnica Wielka
Schizomycetes							
Achroonema spiroideum Skuja	–	–	–	–	1	–	–
Beggiatoa alba Trevisan	–	–	–	1–2	–	–	1
*Beggiatoa minima*Vinogradskij	–	–	2	1–2	–	–	2
Chlorochromatium aggregatum Lauterborn	–	–	–	–	1	–	–
Chlorobium vibrioforme Pel's	3	3	–	–	–	–	–
Chromatium gracile Strzeszewski	1	–	–	–	2	1	–
Chromatium weissei van Niel	3	3	1–3	–	3	–	–
Chromatium vinosum Vinogradskij	+	2	1	–	+	–	–
Chromatium minus Vinogradskij	2	–	–	–	–	–	–
Chromatium okenii Perty	2	2	–	–	–	–	–
Thiocapsa roseopersicina Vinogradskij	+	–	–	–	+	3	–
Thiocystis violacea Vinogradskij	+	–	1–2	–	–	–	–
Thiothrix annulata Molisch	–	–	2	–	–	1	–
Thiothrix minutissima Upehof	–	+	–	2	–	–	2
Thiothrix nivea Vinogradskij	–	1	3	3	–	–	3
Thiothrix tenuissima Vinogradskij	1–3	1	1–3	2	–	–	2–3
Cyanophyceae — Cyanoprokaryota							
Pseudanabaena constricta (Szafer) Lauterborn	1	1	–	–	–	1	–
Lyngbya aerugineo-coerulea (Kützing) Gomont	–	1	–	–	–	–	–
Oscillatoria tenuis Agardh	–	+	–	1	–	–	–
Euglenophyceae							
Entosiphon sulcatus Stein	–	–	+	–	–	+	–
Euglena mutabilis Schmitz	–	1	+	–	+	–	–
Euglena retronata Johnson	–	3	–	–	–	–	–
Euglena viridis Ehrenberg	–	1	1	–	–	–	–
Lepocinclis ovum Minkiewicz	–	+	–	–	+	–	–
Bacillariophyceae							
Gomphonema parvulum (Kützing) Grunov	–	1	–	–	–	–	–
Navicula sp. div.	1	1	–	–	–	1	–
Nitzschia linearis W. Smith	–	1	1	–	+	–	–
Pinnularia viridis Ehrenberg	–	1	1	–	+	–	1
Chlorophyceae							
Klebsormidium tribonematoideum Hindák	–	–	–	1	–	–	–
Microthamnion kützingianum Nägeli	–	–	–	+	–	–	–
Stigeoclonium tenue Kützing	–	–	–	–	1	–	1
Number of taxa (Σ)	11	18	12	7	11	6	7

+ — single, 1— sparse, 2 — frequent, 3 — very frequent

was lined with wood and overgrown by vascular plants, giving good conditions for the development of cyanobacteria and diatom species. At present, the tank is covered with concrete, there are no plants, and the H_2S concentration is similar to that in the spring, limiting the development of algae. We have found some taxa which were not observed (or recognised) during the earlier investigations: individual specimens of *Euglena viridis*, *E. mutabilis*, *E. retronata* and *Lepocinclis ovum*.

Comparing our results with those of Turowska (1934) on the "Katarzyna" spring it can be seen that the occurrence of bacteria in this spring is almost the same as it was 65 years ago. She found *Beggiatoa leptomitiformis*, *B. arachnoidea* and a few taxa of *Oscillatoria* which the present authors did not find i.e. *Oscillatoria kützingii*, *O. limnetica* and *O. geminata* var. *sulphurea*. Neither we nor Turowska found species of *Chromatium* in the spring.

CONCLUSIONS

Altogether 16 taxa of bacteria, 3 taxa of cyanoprokaryota and 12 taxa of algae were found in the springs investigated. Sulphuric bacteria are the most frequently occurring organisms

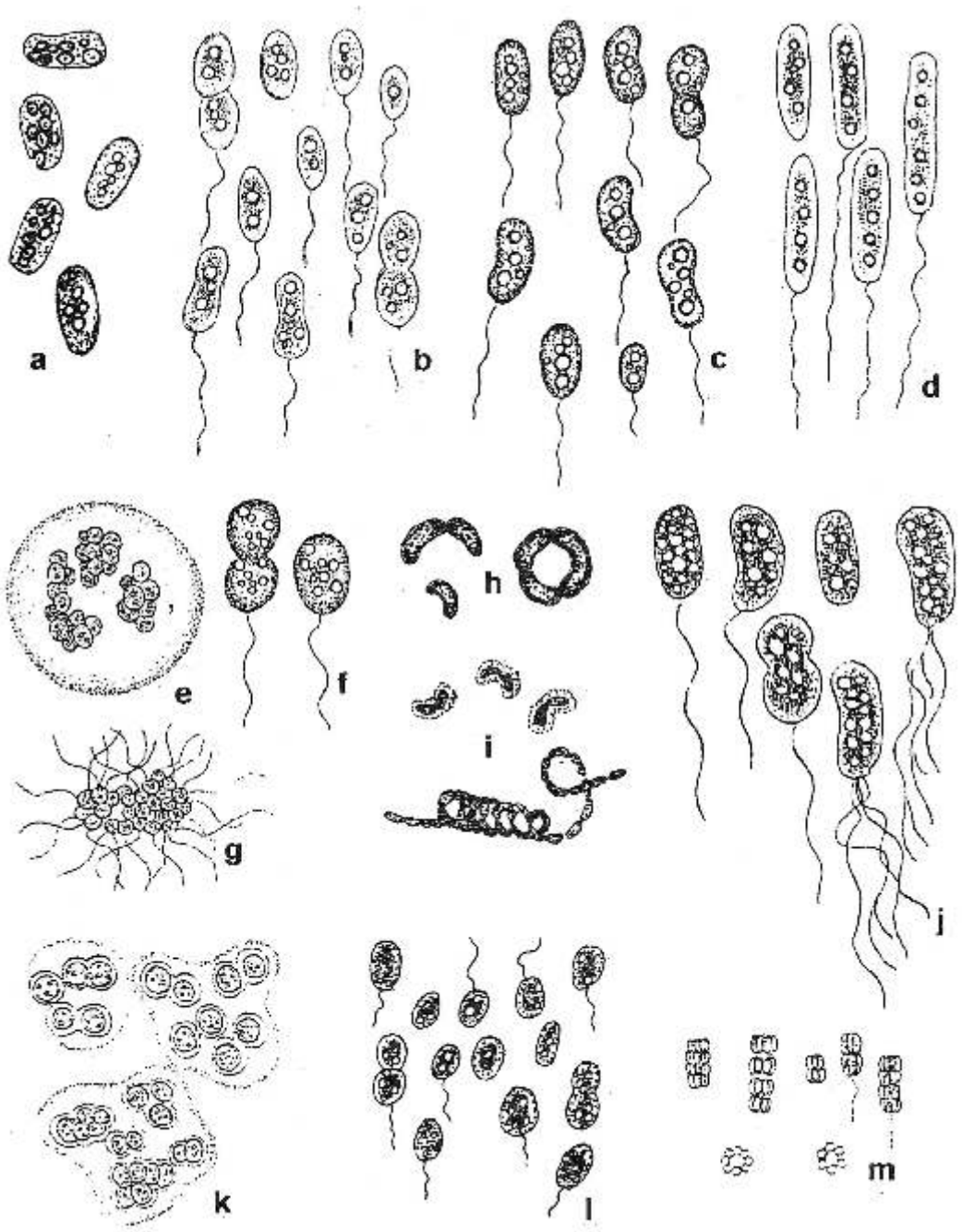

Fig. 3. **a**, **b** — *Chromatium vinosum* (2400 x), **c** — *Chromatium weissei* (1200 x), **d** — *Chromatium gracile* (4000 x), **e**–**g** — *Thiocystis violacea* (**e**, **g** — 650 x, **f** — 2400 x), **h**, **i** — *Chlorobium vibrioforme* (4000 x), **j** — *Chromatium okenii* (1200 x), **k** — *Thiocapsa roseopersicina* (1000 x), **l** — *Chromatium minus* (1400 x), **m** — *Chlorochromatium aggregatum* (1700 x); **a**–**b** — original, **c**–**m** — after Häusler (1982)

in the springs (Table 2). Usually they cover a hard surface forming thin filaments resembling a spider web or a soft coat plait.

Taxa of the genus *Chromatium* (Fig. 3a–d, j and l) were found in five springs out of the seven. They proliferated during the summer and autumn, giving an overall pinkish-red colour to the surface of leaves, twigs and stones. They were observed frequently and in abundance throughout the year in the "Główne" and "Napoleon" springs at Swoszowice. *Thiothrix* and *Beggiatoa* taxa occur abundantly in the "Katarzyna" and "Jacek" springs, forming a greyish-white filamentous mat. Interesting taxa of *Chlorochromatium aggregatum* and *Chlorobium vibrioforme* (Fig. 3h and i) were occasionally found during summer at the "Paweł" and "Główne" springs.

Among the algal group, Cyanoprokaryota (3 taxa), Euglenophyceae (5), Bacillariophyceae (4) and Chlorophyceae (3) were found. All of them occurred as individual specimens. Only *Euglena viridis* and *E. retronata* (Fig. 5a and d) formed a light green bloom during the autumn of 2000 in the "Napoleon"

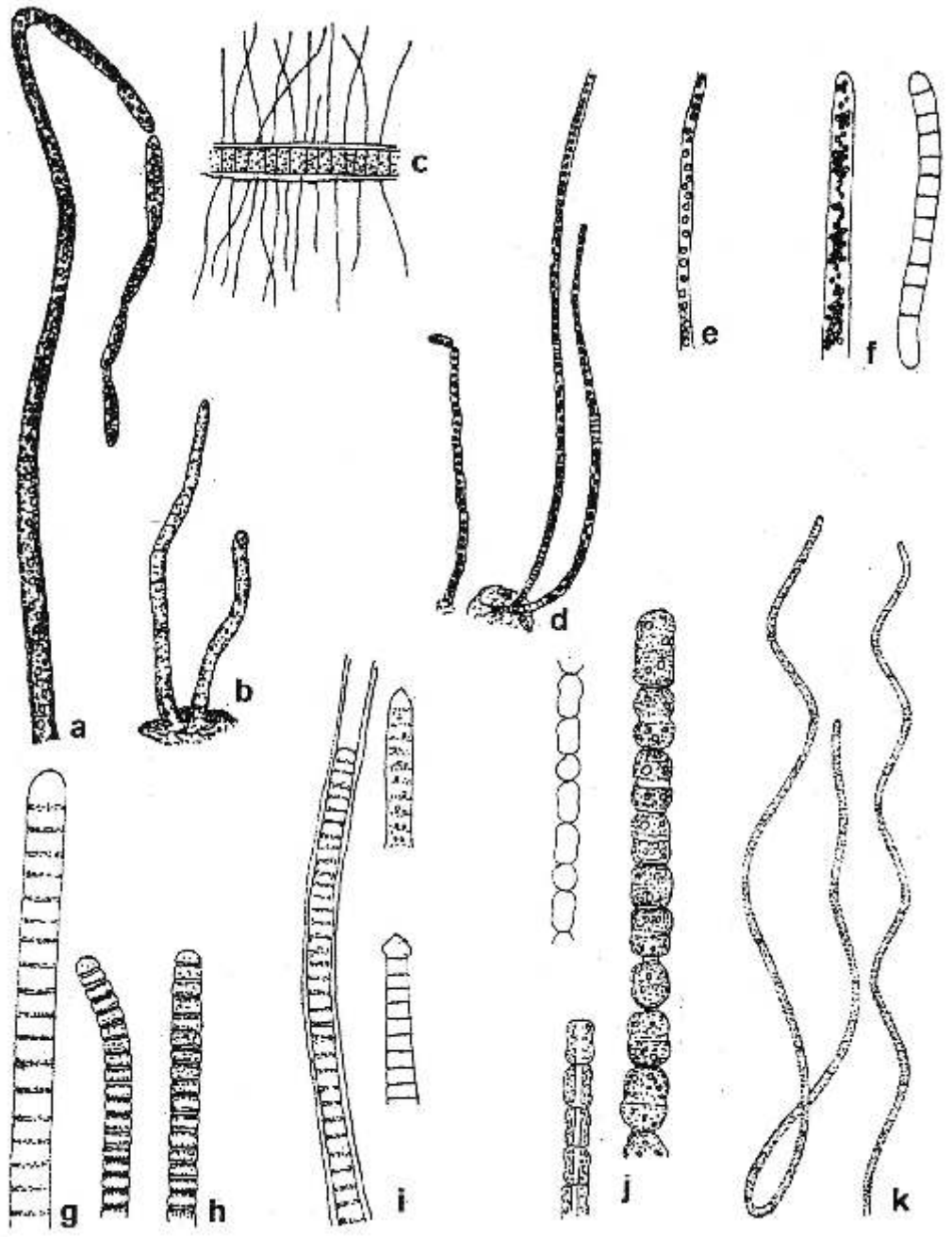

Fig. 4. **a**–**c** — *Thiothrix nivea* (**a**, **b** — 1000 x, **c** — 60 x), **d** — *Thiothrix annulata* (250 x), **e** — *Beggiatoa minima* (1000 x), **f** — *Beggiatoa alba* (900 x), **g**, **h** — *Oscillatoria tenuis* (**g** — 2000 x, **h** — 1000 x), **i** — *Lyngbya aerugineo-coerulea* (1000 x), **j** — *Pseudanabaena constricta* (1000 x), **k** — *Achroonema spiroideum* (2000 x); **a**–**c**, **k** — after Häusler (1982), **d**, **f** — after Huber-Pestalozzi (1938), **e** — original, **g**, **h** — after Starmach (1966)

spring. Most of the algal taxa occurred only at spring outlets, this being connected with a lower sulphur concentration in the water.

There are few diatom taxa relative to other freshwater springs (Namysłowski, 1922; Wołoszyńska, 1922; Foged, 1951; Round, 1960). Among other taxonomical groups: *Vaucheria*, *Tribonema*, *Mougeotia*, *Spirogyra*, *Drapanaldia* and *Batrachospermum* frequently occur. There is no doubt that characteristic groups of algae develop in different types of springs, e.g. in springs with high concentrations of sulphides the community of algae consists of *Lyngbya* and *Phormidium* species; in oligohaline springs community of *Cladophora-Cocconeis-Enteromorpha* species usually occur (Kawecka and Eloranta, 1994).

In the springs investigated, when the H_2S concentration reached about 7 mg/dm^3, a bacterial flora of *Thiothrix* and *Beggiatoa* occurred at the bottom and around the rim and outlet of the spring as single, very tiny filaments covering the surfaces

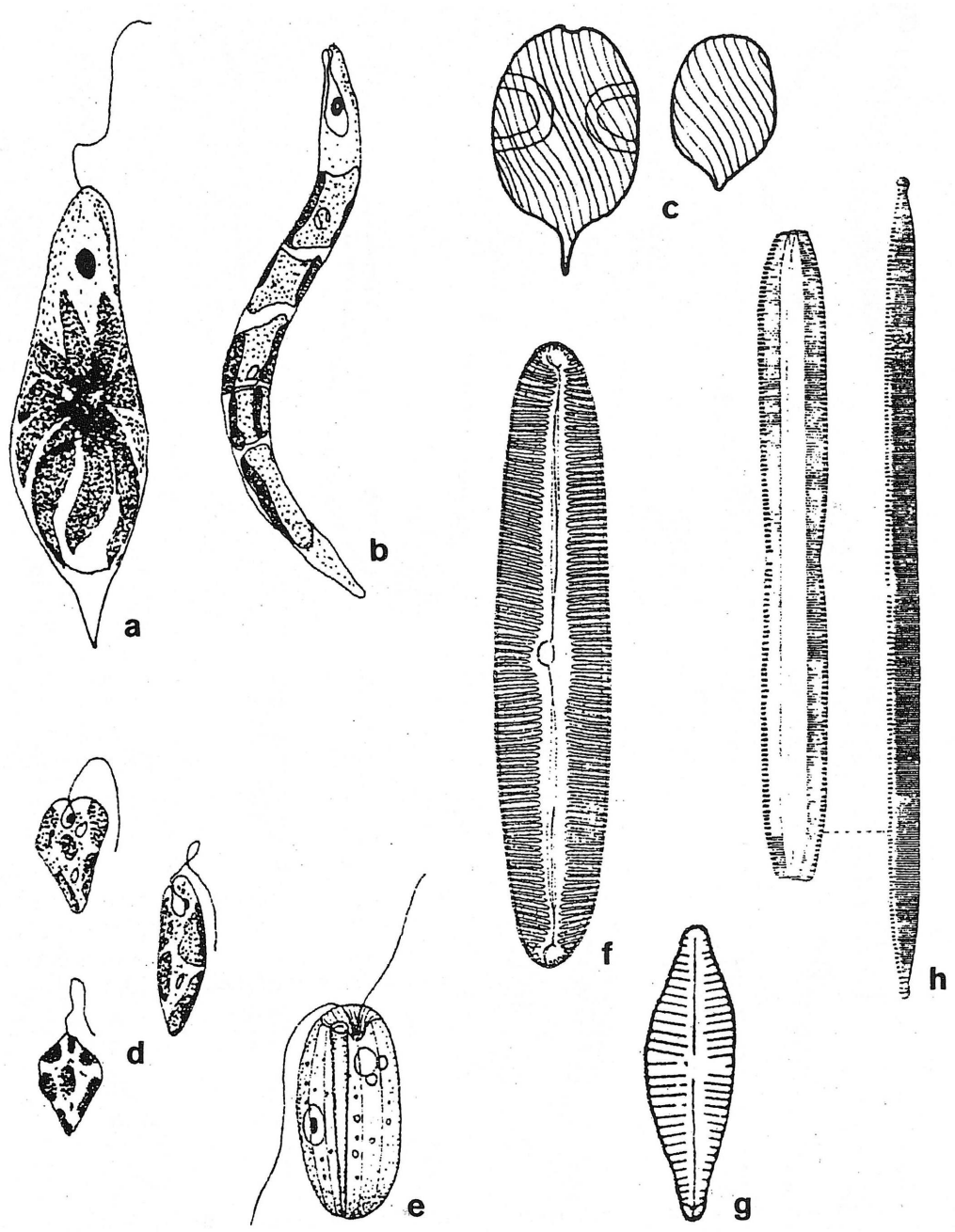

Fig. 5. **a** — *Euglena viridis* (2000 x), **b** — *Euglena mutabilis* (2000 x), **c** — *Lepocinclis ovum* (1500 x), **d** — *Euglena retronata* (1000 x), **e** — *Entosiphon sulcatus* (1000 x), **f** — *Pinnularia viridis* (1000 x), **g** — *Gomphonema parvulum* (2000 x), **h** — *Nitzschia linearis* (1000 x); **a**, **b**, **e** — after Wołowski (1998), **c** — after Starmach (1983), **f**–**h** — after Siemińska (1964)

with a white spider-web-like pattern. At higher concentrations of H_2S the white colonies of bacteria look like fringes or thin mats. Among these *Chromatium* sp. or *Thiocapsa* sp. locally developed, giving a pinkish colour to some parts of the sediments. In spring, when the concentration of H_2S was higher than 50 mg/dm^3, pink mats of bacteria (*Chromatium weissei*, *Ch. vinosum*) cover the surface of the spring bottom as in the "Główne" and "Napoleon" at Swoszowice. The pinkish-violet bacteria represented by *Thiocapsa roseopersicina* occur at the bottom of the "Stanisław" spring at Łabowa and the "Paweł" spring at Polichty. It was observed that sulphuric bacteria in the springs were rather stagnant throughout the year (Table 1). White filamentous and violet coccal bacteria occurred all the year round in equal amounts. Pink and purple bacteria developed well in late spring and early autumn. The colour and shape of agglomerated bacteria can indicate the level of hydrogen sulphide in a spring (Table 1 and 2).

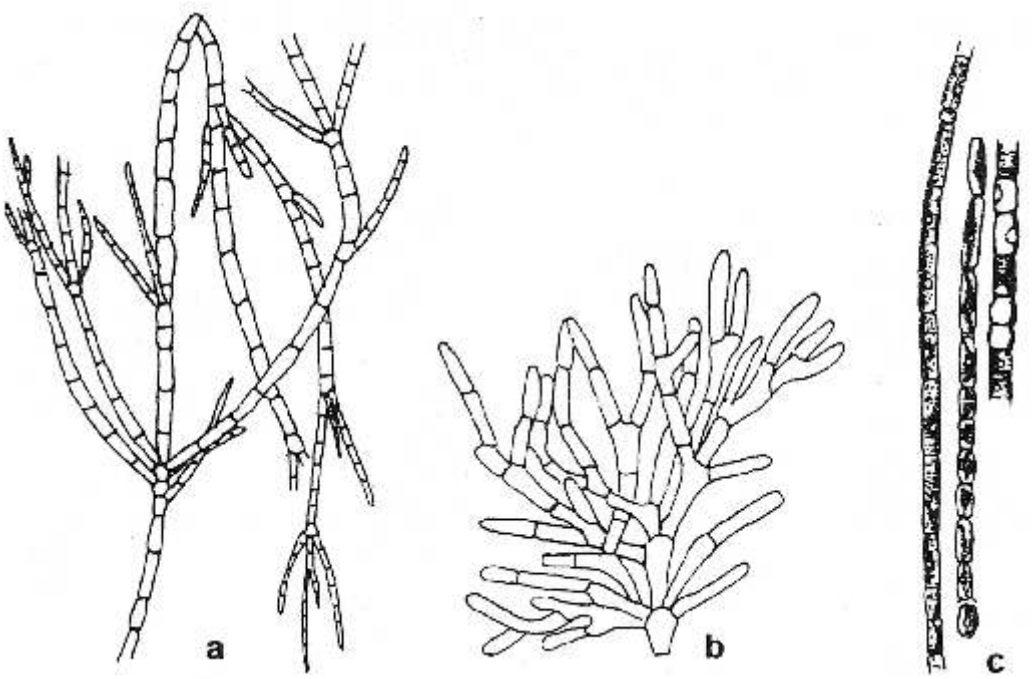

Fig. 6. **a** — *Stigeoclonium tenue* (500 x), **b** — *Microthamnion kützingianum* (500 x), **c**— *Klebsormidium tribonematoideum* (1200 x); **a**, **b** — after Starmach (1972), **c** — after Hindák (1996)

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