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# DISAPPEARANCE OF AQUATIC BRYOPHYTES RESULTING FROM WATER POLLUTION BY TEXTILE INDUSTRY

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**SUMMARY** — In the years 1981 - 1991 the occurrence of *Fontinalis antipyretica* Hedw. and *Platyhypnidium riparioides* (Hedw.) Dix. was observed in the Włodzica and Piława Rivers (Lower Silesia, Poland), which are polluted by sewage from the textile industry. The specific substances produced by the textile industry include aniline and fuchsine. The chemical characteristics of water from the rivers indicate their eutrophication. The contents of macroelements in mosses is in a high degree related to the content of these elements in the water and correspond to their general increase along the rivers. In sites above the sewage outlets the main shoots of mosses are characterized by larger size while density and biomass of the populations are smaller. Going downstream the size of main shoots of mosses is reduced whereas density and biomass rises. The in vitro experiments showed the toxicity of aniline and fuchsine and defined the tolerance of *F. antipyretica* and *P. riparioides* to these substances. The lethal concentrations  $LC_0$ ,  $LC_{50}$  and  $LC_{100}$  are different for the two investigated species. Higher sensitivity for aniline and fuchsine is manifested by *F. antipyretica*. Concentrations of aniline and fuchsine below  $LC_{50}$  limited growth of the mosses in vitro. The in situ observations compared with the results of the in vitro experiments suggest, that aniline and fuchsine probably are involved in the observed effects of pollution on the local moss populations.

**KEYWORDS** — Aniline, eutrophication, fuchsine, lethal concentration, textile industry sewage, bryophytes, Poland

**ZUSAMMENFASSUNG** — Verschwinden von Wassermoosen unter dem Einfluss der Wasserverschmutzung durch Textilindustrie

Von 1981 bis 1991 wurden Vorkommen von *Fontinalis antipyretica* Hedw. und *Platyhypnidium riparioides* (Hedw.) Dix. in den Flüssen Włodzica und Piława (Niederschlesien, Polen) untersucht. Diese Flüsse sind durch Abwasser aus der Textilindustrie u. a. mit Anilin und Fuchsin verschmutzt. Nach den chemischen Merkmalen ihres Wassers sind die Flüsse eutroph. Der Gehalt der Moose an Makroelementen ist in hohem Grade von den entsprechenden Konzentrationen im Flusswasser abhängig und nimmt im allgemeinen flussabwärts zu. An Untersuchungsstellen oberhalb der Abwasser-Einleitungen sind die Hauptsprosse der Moose länger, und ihre Dichte und Biomasse sind niedrig. Flussabwärts nimmt die Länge der Hauptsprosse ab, während Dichte und Biomasse zunehmen. In-vitro-Experimente zeigten die Toxizität von Anilin und Fuchsin und die Toleranz von *Fontinalis antipyretica* und *Platyhypnidium riparioides* gegenüber diesen Substanzen. Die Letalkonzentrationen  $LC_0$ ,  $LC_{50}$  und  $LC_{100}$  sind für die beiden Moose verschieden. *Fontinalis antipyretica* zeigt gegen beide Stoffe grössere Empfindlichkeit. Sowohl Anilin als auch Fuchsin hemmen schon in Konzentrationen unter  $LC_{50}$  das Wachstum der Moose. Ein Vergleich von Messungen in situ mit den Resultaten der Experimente legt eine Beteiligung von Anilin und Fuchsin an den Auswirkungen der Verschmutzung auf die Moose nahe.

## Introduction

In the years 1981 - 1991 continuous observations of the pollution of the Włodzica and Piława Rivers contaminated by sewage from the textile industry were realised. The situation is worst during dry weather, which has occurred the past few years, when the same quantity of sewage is drained off to a substantially reduced quantity of water. Although initially purified, the sewage has a characteristic colouring caused by the presence of aniline and fuchsine.

The aim of this study was to detect the change in populations of *Fontinalis antipyretica* and *Platyhypnidium riparioides* downstream the Włodzica and Piława Rivers. The effects of aniline and fuchsine on these mosses as potential indicators were studied.

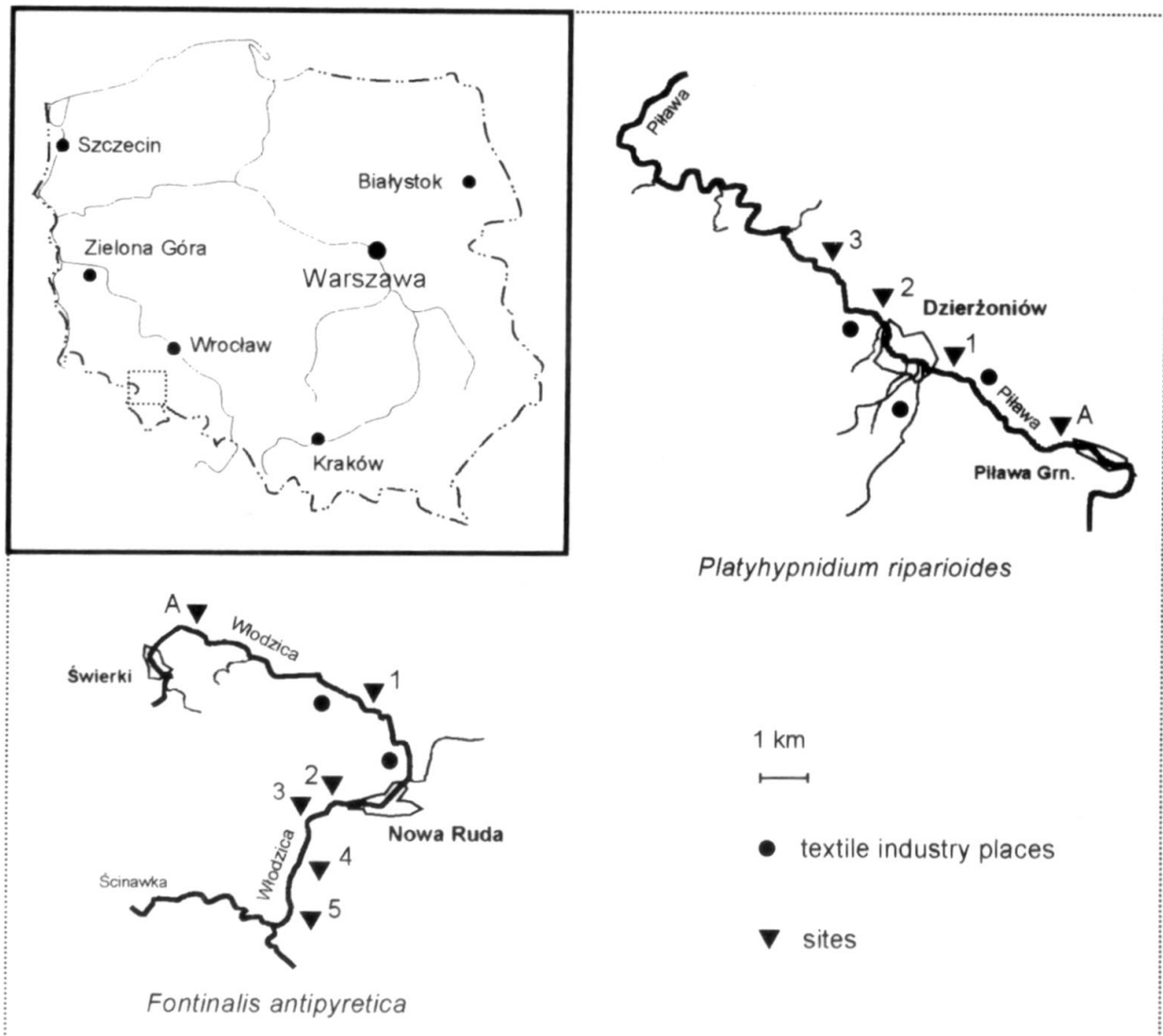
## Materials and Methods

### Description of the study sites

The studied populations are located in the Włodzica River near Nowa Ruda (*Fontinalis antipyretica* Hedw.) and in the Piława River near Dzierżoniów (*Platyhypnidium riparioides* (Hedw.) Dix.) in Lower Silesia (Poland). Permanent plots on which the mosses grow were designated on sections of the rivers. Six sites (A, 1, 2, 3, 4, and 5) were designated for *F. antipyretica*, and four sites (A, 1, 2, and 3) for *P. riparioides* (Fig. 1). The regularly examined sites are in separate sections of the rivers (the sections are several meters long, but not more than twenty meters). One site (A) was appointed above sources of pollution, the others were appointed downstream. From each site five samples of the mosses and water for analysis were taken each year.

### Analysis

Permanent plots of 0.01m<sup>2</sup> were designated in the studied sites within the present moss patches and samples were drawn by means of a random sampling method (Kershaw 1973). Biometrical analysis of the examined mosses was based upon the measurement of characters describing the condition and size of main shoots of mosses and their populations. The following properties



**Figure 1.** Sample sites of *Fontinalis antipyretica* and *Platyhypnidium riparioides* in Włodzica and Piława Rivers (Lower Silesia district).

were chosen to characterize the examined mosses: length of main shoots, number of lateral branches (of any order) [per main shoot], density [number of main shoots (including dead ones) per 0.01m<sup>2</sup>], biomass [dry weight of mosses per 0.01m<sup>2</sup>] and mortality [% dead of all main shoots]. The values of Table 2 are calculated from the ten annual mean values, and these from the measurements performed in five test surfaces.

After harvesting the mosses were washed in distilled water, dried at 65°C, homogenized, and burnt in a muffle furnace at 450°C. The ash was being dissolved in 20% HCl, the extract used for analysis of phosphorus, potassium, calcium, and magnesium. Total nitrogen content in the mosses was determined by acid-base titrimetry after Kjeldahl digestion and distillation (Nowosielski 1968). In the water samples, the following parameters were determined: pH, contents of NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>-3</sup>, K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup>, and BOD<sub>5</sub> [Biochemical Oxygen Demand during five days] (Hermanowicz & al. 1976). All analyses were performed in three replicates. The values of Table 1 and 3 are calculated from the ten annual mean values.

Dilution series of aniline (0-50 mg/dm<sup>3</sup>) and fuchsine (0-10 mg/dm<sup>3</sup>) at intervals of 2.5 mg/dm<sup>3</sup> and 0.25 mg/dm<sup>3</sup> respectively, allowed the experimental determination of LC<sub>0</sub> and LC<sub>100</sub> of *F. antipyretica* and *P. riparioides*. The results made it possible to predict the concentration of LC<sub>50</sub>. For these experiments we used 15 shoot tips of 2 cm length each and water from the 'control' site (A). Experiments were carried out in a greenhouse at 15°C. After 6 weeks the increase in length of main shoot, the number of all new lateral branches (some of the shoot tips were branched at the start of the experiment), and mortality was recorded. Experiments were performed in three replicates.

Statistical analyses were performed using the CSS: Statistica programme (StatSoft Inc. 1991). Data were analysed with ANOVA (Model I). The decision about the null hypothesis (H<sub>0</sub>: m<sub>1</sub> = m<sub>2</sub> = ... = m<sub>n</sub>, where m<sub>1</sub>, m<sub>2</sub> and m<sub>n</sub> - the means of the samples) was made by using F test statistics. For comparison of individual means LSD (Least Significant Difference) was calculated (Woolf 1968). For the determination of LC<sub>50</sub> the probit transformation method was used (Kooijman 1981, Unkelbach & Wolf 1985, Zakrzewski 1991). For the regression analysis of the correlation between aniline or fuchsine concentration and growth or branching respectively, the data were tested by the Kolmogorov-Smirnov procedure (Norcliffe 1986). All data show normal distribution, and the correlation coefficients (r-Pearson) are given in Table 4 (Woolf 1968).

## Results and Discussion

Apart from the characteristic colouring, which gives evidence of aniline and fuchsine presence, the chemical characteristics of water samples from both Włodzica and Piława Rivers indicate eutrophication (Table 1). During the investigated period the concentrations of the investigated variables as well as chemical contamination were in most cases subject to only slight fluctuations. Water samples taken upstream of the sewage outlets from the textile industry were clearly less contaminated.

Further downstream the characteristic colouring remained while the contamination rised. It can be stated that Piława River is more contaminated than Włodzica River, which is expressed in the ion composition and BOD<sub>5</sub> (Table 1). Figure 1 shows only the location of the large textile factories. There are more small factories along both rivers. Their number is much lower for Włodzica River, as well as the number of contaminated tributaries. Domestic sewage is also involved, but of minor importance. In Piława River most parameters of the water show a clear gradient from site 1 to site 3 (Table 1), which is in accordance to the relative position of the large factories and the sample sites (Fig. 1). In contrast, at least sites 4 and 5 in Włodzica River often show lower values of contamination than sites 2 and 3. This suggests a correlation with the distance from the nearest large textile factory and might be some evidence of self-purification of the river.

The content of macroelements in *Fontinalis antipyretica* and *Platyhypnidium riparioides* is correlated to a large extent with the content of these elements in the water (Tables 1 and 3).

| Sites of                          |   | pH        | NO <sub>2</sub> <sup>-</sup> | NO <sub>3</sub> <sup>-</sup> | NH <sub>4</sub> <sup>+</sup> | PO <sub>4</sub> <sup>-3</sup> | K <sup>+</sup> | Ca <sup>+2</sup> | Mg <sup>+2</sup> | BOD <sub>5</sub>      |
|-----------------------------------|---|-----------|------------------------------|------------------------------|------------------------------|-------------------------------|----------------|------------------|------------------|-----------------------|
|                                   |   |           | (mg/dm <sup>3</sup> )        |                              |                              |                               |                |                  |                  | (mg/dm <sup>3</sup> ) |
| <b>Włodzica River</b>             |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| <i>Fontinalis antipyretica</i>    | A | 6.84±0.05 | 0.01±0.001                   | 0.06±0.01                    | 0.14±0.03                    | 0.02±0.01                     | 4.5±0.52       | 20.2±1.31        | 10.7±1.22        | 7.6±1.21              |
|                                   | 1 | 7.03±0.07 | 0.03±0.003                   | 0.08±0.01                    | 0.41±0.05                    | 0.37±0.06                     | 9.7±1.22       | 41.8±5.01        | 18.6±2.16        | 10.9±1.19             |
|                                   | 2 | 7.22±0.04 | 0.04±0.004                   | 0.13±0.03                    | 0.35±0.05                    | 0.62±0.06                     | 12.0±0.97      | 62.1±5.12        | 20.5±2.80        | 15.3±1.42             |
|                                   | 3 | 7.14±0.07 | 0.07±0.005                   | 0.38±0.05                    | 0.51±0.04                    | 0.71±0.07                     | 18.0±1.10      | 80.4±5.50        | 24.8±3.12        | 13.4±1.11             |
|                                   | 4 | 7.80±0.06 | 0.07±0.004                   | 0.51±0.04                    | 0.36±0.02                    | 0.59±0.05                     | 14.3±1.60      | 58.4±7.01        | 19.4±3.10        | 10.5±1.06             |
|                                   | 5 | 7.63±0.04 | 0.06±0.005                   | 0.81±0.06                    | 0.69±0.05                    | 0.60±0.05                     | 11.6±1.18      | 74.9±5.39        | 29.4±4.32        | 16.2±2.89             |
| <b>F-test</b>                     |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| n (sites, replicates)             |   | ← 6,5 →   |                              |                              |                              |                               |                |                  |                  |                       |
| F                                 |   | 10.64     | 8.51                         | 7.61                         | 84.25                        | 17.08                         | 84.27          | 116.31           | 140.28           | 84.39                 |
| Significant at p <                |   | ← 0.05 →  |                              |                              |                              |                               |                |                  |                  |                       |
| LSD                               |   | 0.14      | 0.01                         | 0.07                         | 0.08                         | 0.11                          | 2.12           | 9.58             | 5.46             | 2.98                  |
| <b>K-S test</b>                   |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| n                                 |   | ← 6 →     |                              |                              |                              |                               |                |                  |                  |                       |
| d                                 |   | 0.23      | 0.21                         | 0.25                         | 0.21                         | 0.33                          | 0.16           | 0.20             | 0.21             | 0.17                  |
| Significant at p <                |   | ← 0.05 →  |                              |                              |                              |                               |                |                  |                  |                       |
| <b>Piława River</b>               |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| <i>Platyhypnidium riparioides</i> | A | 7.21±0.12 | 0.02±0.001                   | 0.08±0.01                    | 0.07±0.01                    | 0.04±0.01                     | 8.5±0.78       | 53.2±5.58        | 15.8±1.91        | 9.0±1.13              |
|                                   | 1 | 7.63±0.10 | 0.05±0.003                   | 0.24±0.05                    | 0.53±0.06                    | 0.14±0.03                     | 17.0±1.05      | 64.1±4.42        | 14.3±2.55        | 14.1±1.91             |
|                                   | 2 | 7.82±0.14 | 0.05±0.005                   | 0.64±0.07                    | 0.48±0.06                    | 0.64±0.11                     | 19.6±2.80      | 78.6±6.51        | 21.6±3.71        | 20.6±2.24             |
|                                   | 3 | 7.58±0.20 | 0.09±0.017                   | 0.84±0.05                    | 0.79±0.11                    | 0.82±0.10                     | 25.4±1.64      | 86.7±9.52        | 22.1±3.08        | 19.2±2.71             |
| <b>F-test</b>                     |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| n (sites, replicates)             |   | ← 4,5 →   |                              |                              |                              |                               |                |                  |                  |                       |
| F                                 |   | 9.26      | 11.07                        | 8.46                         | 24.51                        | 153.07                        | 54.32          | 84.56            | 26.31            | 50.24                 |
| Significant at p <                |   | ← 0.05 →  |                              |                              |                              |                               |                |                  |                  |                       |
| LSD                               |   | 0.35      | 0.02                         | 0.12                         | 0.13                         | 0.14                          | 3.32           | 12.86            | 5.48             | 3.96                  |
| <b>K-S test</b>                   |   |           |                              |                              |                              |                               |                |                  |                  |                       |
| n                                 |   | ← 4 →     |                              |                              |                              |                               |                |                  |                  |                       |
| d                                 |   | 0.28      | 0.29                         | 0.23                         | 0.27                         | 0.26                          | 0.21           | 0.20             | 0.29             | 0.25                  |
| Significant at p <                |   | ← 0.05 →  |                              |                              |                              |                               |                |                  |                  |                       |

**Table 1.** Chemical characteristics of water samples 1981 - 1991 (mean ±SD).

| Sites of                          |   | Length of main shoot (cm) | Number of lateral branches | Density (main shoots/0.01m <sup>2</sup> ) | Biomass (g d.wt./0.01m <sup>2</sup> ) | Mortality (%) |
|-----------------------------------|---|---------------------------|----------------------------|---|---------------------------------------|---------------|
| <b>Włodzica River</b>             |   |                           |                            |   |                                       |               |
| <i>Fontinalis antipyretica</i>    | A | 15.7±1.88                 | 4.6±4.27                   | 126.4±15.32                               | 2.54±0.25                             | 8.4±1.65      |
|                                   | 1 | 10.2±1.48                 | 3.3±4.19                   | 176.2±18.54                               | 3.90±0.23                             | 21.4±3.48     |
|                                   | 2 | 8.6±0.71                  | 2.8±3.35                   | 263.5±21.65                               | 4.11±0.28                             | 37.0±3.24     |
|                                   | 3 | 7.5±0.88                  | 3.1±3.57                   | 297.5±13.12                               | 4.80±0.51                             | 38.1±2.94     |
|                                   | 4 | 9.8±1.44                  | 3.8±3.20                   | 241.5±30.12                               | 4.63±0.41                             | 44.1±3.64     |
|                                   | 5 | 9.7±1.79                  | 4.1±4.03                   | 227.1±27.41                               | 4.75±0.53                             | 34.8±3.14     |
| <b>F-test</b>                     |   |                           |                            |   |                                       |               |
| n (sites, replicates)             |   | ← 6,5 →                   |                            |   |                                       |               |
| F                                 |   | 105.12                    | 87.54                      | 46.08                                     | 54.14                                 | 64.38         |
| Significant at p <                |   | ← 0.05 →                  |                            |   |                                       |               |
| LSD                               |   | 2.54                      | 8.18                       | 34.55                                     | 0.27                                  | 5.47          |
| <b>Piława River</b>               |   |                           |                            |   |                                       |               |
| <i>Platyhypnidium riparioides</i> | A | 6.2±0.10                  | 8.8±0.50                   | 132.7±23.45                               | 2.66±0.19                             | 11.5±2.08     |
|                                   | 1 | 5.7±0.23                  | 7.1±0.77                   | 163.9±25.45                               | 3.33±0.08                             | 24.3±4.01     |
|                                   | 2 | 5.0±0.28                  | 7.1±1.09                   | 168.7±16.03                               | 3.12±0.20                             | 37.8±4.16     |
|                                   | 3 | 4.8±0.18                  | 6.9±0.46                   | 202.1±23.70                               | 3.68±0.23                             | 42.1±3.86     |
| <b>F-test</b>                     |   |                           |                            |   |                                       |               |
| n (sites, replicates)             |   | ← 4,5 →                   |                            |   |                                       |               |
| F                                 |   | 15.26                     | 64.12                      | 24.15                                     | 9.46                                  | 45.02         |
| Significant at p <                |   | ← 0.05 →                  |                            |   |                                       |               |
| LSD                               |   | 0.39                      | 1.43                       | 42.57                                     | 0.36                                  | 4.88          |

**Table 2.** Characters of populations and individuals of *Fontinalis antipyretica* and *Platyhypnidium riparioides* sampled 1981 - 1991 (mean ±SD).

| Sites of                          |           | N         | P         | K         | Ca        | Mg        | Ash       |
|-----------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| (% dry weight)                    |           |           |           |           |           |           |           |
| <b>Włodzica River</b>             |           |           |           |           |           |           |           |
| <i>Fontinalis antipyretica</i>    | A         | 1.61±0.04 | 0.16±0.02 | 0.46±0.04 | 0.41±0.02 | 0.15±0.02 | 9.9±0.27  |
|                                   | 1         | 2.21±0.06 | 0.15±0.02 | 0.55±0.03 | 0.30±0.02 | 0.16±0.01 | 11.9±0.45 |
|                                   | 2         | 1.93±0.06 | 0.20±0.01 | 0.42±0.05 | 0.50±0.04 | 0.70±0.01 | 10.3±0.51 |
|                                   | 3         | 1.84±0.05 | 0.18±0.01 | 0.60±0.02 | 0.75±0.04 | 0.23±0.02 | 13.5±0.74 |
|                                   | 4         | 2.06±0.07 | 0.24±0.01 | 0.63±0.02 | 0.44±0.03 | 0.16±0.01 | 12.7±0.85 |
| 5                                 | 2.13±0.08 | 0.25±0.02 | 0.68±0.04 | 0.52±0.04 | 0.27±0.02 | 11.9±1.07 |           |
| F-test                            |           |           |           |           |           |           |           |
| n (sites, replicates)             |           | ← 6, 5 →  |           |           |           |           |           |
| F                                 |           | 132.05    | 54.21     | 35.64     | 18.46     | 42.08     | 167.91    |
| Significant at p <                |           | ← 0.05 →  |           |           |           |           |           |
| LSD                               |           | 0.09      | 0.03      | 0.08      | 0.06      | 0.02      | 1.26      |
| K-S test                          |           |           |           |           |           |           |           |
| n                                 |           | ← 6 →     |           |           |           |           |           |
| d                                 |           | 0.17      | 0.19      | 0.17      | 0.25      | 0.35      | 0.22      |
| Significant at p <                |           | ← 0.05 →  |           |           |           |           |           |
| <b>Piława River</b>               |           |           |           |           |           |           |           |
| <i>Platyhypnidium riparioides</i> | A         | 2.02±0.06 | 0.38±0.02 | 0.28±0.02 | 0.94±0.05 | 0.23±0.01 | 11.9±0.49 |
|                                   | 1         | 2.41±0.11 | 0.48±0.03 | 0.36±0.03 | 1.18±0.04 | 0.28±0.02 | 13.3±0.66 |
|                                   | 2         | 2.38±0.01 | 0.41±0.02 | 0.38±0.03 | 1.42±0.03 | 0.26±0.02 | 15.0±0.56 |
|                                   | 3         | 2.70±0.11 | 0.46±0.03 | 0.40±0.08 | 1.36±0.05 | 0.21±0.02 | 16.8±0.78 |
| F-test                            |           |           |           |           |           |           |           |
| n (sites, replicates)             |           | ← 4, 5 →  |           |           |           |           |           |
| F                                 |           | 70.25     | 94.02     | 18.04     | 21.04     | 65.60     | 94.38     |
| Significant at p <                |           | ← 0.05 →  |           |           |           |           |           |
| LSD                               |           | 0.05      | 0.09      | 0.08      | 0.08      | 0.03      | 1.08      |
| K-S test                          |           |           |           |           |           |           |           |
| n                                 |           | ← 4 →     |           |           |           |           |           |
| d                                 |           | 0.25      | 0.23      | 0.29      | 0.23      | 0.19      | 0.18      |
| Significant at p <                |           | ← 0.05 →  |           |           |           |           |           |

**Table 3.** Macroelements and ash in mosses sampled 1981 - 1991 (mean ±SD).

According to Gaudet (1974) an increase in content of chemical elements in the water induces an increase of content of these elements in plants. Most distinct differences in the content of macroelements in the investigated mosses is found between the sites situated before the sewage outlets and after these.

As it could have been assumed in accordance with Samecka-Cymerman & Sarosiek (1985), the moss populations in the two rivers, originating from different sites of different water contamination differ with respect to biological parameters (Table 2). In the two sites placed before the sewage outlets the populations are characterized by longer main shoots and stronger lateral branch development, while the density, biomass, and mortality are lower. Going downstream, the size of the mosses is reduced, whereas the population density, biomass production, and mortality rise. However, the size of *F. antipyretica* individuals in greater distance from the sewage source (sites 4 and 5) is slightly larger, and population density, biomass, and mortality are lower. In the lowest site of *P. riparioides* (3) the length of main shoots and number of lateral branches is minimal, and density, biomass, and mortality show their maximum.

Significant differences were found between *Fontinalis antipyretica* and *Platyhypnidium riparioides* with respect to their sensitivity against aniline and fuchsine in the experiments (Table 4). Greater sensitivity is manifested by *F. antipyretica* similar to their reaction to phenol and glycol (Samecka-Cymerman 1983, Sarosiek & Samecka-Cymerman 1987). The following

| Concentration of aniline / fuchsine (mg/dm <sup>3</sup> ) | <i>Fontinalis antipyretica</i> |                                       |                                 | <i>Platyhypnidium riparioides</i> |                                       |                                 |
|---|--------------------------------|---------------------------------------|---------------------------------|-----------------------------------|---------------------------------------|---------------------------------|
|   | Mortality (%)                  | Increase in length of main shoot (cm) | Number of new lateral branches  | Mortality (%)                     | Increase in length of main shoot (cm) | Number of new lateral branches  |
| 0.0 / 0.00  | 1.8/ 2.2                       | 6.24/7.32                             | 7.03/3.62                       | 2.4/ 3.1                          | 4.12/6.84                             | 3.03/2.84                       |
| 2.5 / 0.25  | 3.4/ 2.4                       | 6.03/6.08                             | 7.54/3.48                       | 4.8/ 2.8                          | 4.07/6.12                             | 3.71/2.74                       |
| <b>LC<sub>50</sub></b> 5.0 / 0.50                         | <b>7.6/</b> 3.9                | 6.11/5.67                             | 7.24/2.87                       | 7.1/ 3.8                          | 4.19/6.20                             | 3.28/2.63                       |
| <b>LC<sub>0</sub></b> 7.5 / 0.75                          | 9.7/ 6.3                       | 6.17/5.82                             | 5.81/2.45                       | <b>14.0/</b> 4.5                  | 4.20/5.94                             | 3.31/2.21                       |
| 10.0 / <b>1.00LC<sub>0</sub></b>                          | 17.1/ <b>12.5</b>              | 5.84/5.80                             | 2.24/2.48                       | 19.3/ 5.0                         | 4.04/5.97                             | 3.40/1.83                       |
| 12.5 / 1.25   | 26.7/ 19.1                     | 4.11/5.89                             | 1.03/1.17                       | 25.0/ 5.7                         | 3.87/5.61                             | 2.83/1.79                       |
| 15.0 / <b>1.50LC<sub>0</sub></b>                          | 32.2/ 27.4                     | 3.61/5.31                             | /0.83                           | 31.1/ <b>13.5</b>                 | 3.71/5.60                             | 2.14/1.80                       |
| 17.5 / 1.75   | 44.8/ 39.5                     | 3.10/4.18                             |                                 | 30.0/ 17.2                        | 2.94/5.34                             | 1.31/1.54                       |
| 20.0 / 2.00   | 59.1/ 41.2                     | 1.28/4.11                             |                                 | 35.4/ 19.8                        | 2.68/5.29                             | 0.78/1.48                       |
| 22.5 / 2.25   | 62.2/ 40.0                     | /3.73                                 |                                 | 43.1/ 20.5                        | 2.57/5.30                             | /0.82                           |
| 25.0 / 2.50   | 88.1/ 43.3                     | /3.78                                 |                                 | 43.0/ 23.8                        | 1.54/5.35                             | /0.94                           |
| <b>LC<sub>100</sub></b> 27.5 / 2.75                       | <b>100.0/</b> 47.8             | /3.36                                 |                                 | 48.2/ 22.0                        | /5.09                                 |                                 |
| 30.0 / 3.00   | / 51.8                         | /2.09                                 |                                 | 57.6/ 20.0                        | /4.98                                 |                                 |
| 32.5 / 3.25   | / 57.1                         | /0.61                                 |                                 | 59.8/ 27.1                        | /4.90                                 |                                 |
| 35.0 / 3.50   | / 69.2                         |                                       |                                 | 79.5/ 30.6                        | /4.14                                 |                                 |
| 37.5 / 3.75   | / 80.1                         |                                       |                                 | 83.9/ 36.1                        | /4.02                                 |                                 |
| <b>LC<sub>100</sub></b> 40.0 / 4.00                       | / 92.1                         |                                       |                                 | <b>100.0/</b> 33.0                | /3.74                                 |                                 |
| 42.5 / 4.25   | / 94.0                         |                                       |                                 | / 41.2                            | /2.64                                 |                                 |
| 45.0 / 4.50   | / 94.0                         |                                       |                                 | / 40.7                            | /2.19                                 |                                 |
| 47.5 / 4.75   | / 98.2                         |                                       |                                 | / 39.5                            | /1.67                                 |                                 |
| 50.0 / 5.00   | / 98.4                         |                                       |                                 | / 45.7                            | /1.70                                 |                                 |
| / <b>5.25LC<sub>100</sub></b>                             | <b>/100.0</b>                  |                                       |                                 | / 48.0                            | /1.64                                 |                                 |
| / 5.50  |                                |                                       |                                 | / 48.6                            | /1.68                                 |                                 |
| / 5.75  |                                |                                       |                                 | / 48.2                            | /1.43                                 |                                 |
| / 6.00  |                                |                                       |                                 | / 54.8                            | /1.40                                 |                                 |
| / 6.25  |                                |                                       |                                 | / 60.0                            | /1.43                                 |                                 |
| / 6.50  |                                |                                       |                                 | / 62.5                            |                                       |                                 |
| / 6.75  |                                |                                       |                                 | / 61.0                            |                                       |                                 |
| / 7.00  |                                |                                       |                                 | / 73.8                            |                                       |                                 |
| / 7.25  |                                |                                       |                                 | / 84.2                            |                                       |                                 |
| / 7.50  |                                |                                       |                                 | / 93.1                            |                                       |                                 |
| / <b>7.75LC<sub>100</sub></b>                             |                                |                                       |                                 | <b>/100.0</b>                     |                                       |                                 |
| F-test  |                                |                                       |                                 |                                   |                                       |                                 |
| n (sites, replicates)                                     | 12, 3/22, 3                    | 9, 3/14, 3                            | 6, 3/7, 3                       | 17, 3/32, 3                       | 11, 3/26, 3                           | 9, 3/11, 3                      |
| F   | 26.31/64.15                    | 15.34/54.07                           | 14.07/42.74                     | 64.52/28.69                       | 8.46/24.41                            | 25.80/33.17                     |
| Significant at p <  | ← 0.05 →                       |                                       |                                 |                                   |                                       |                                 |
| LSD   | 3.42/ 4.13                     | 0.44/ 0.26                            | 0.17/ 0.20                      | 5.61/ 4.03                        | 0.38/ 0.21                            | 0.19/ 0.31                      |
| K-S test  |                                |                                       |                                 |                                   |                                       |                                 |
| n   | 9/17                           | 9/14                                  | 6/7                             | 13/25                             | 11/26                                 | 9/11                            |
| d   | 0.15/0.17                      | 0.29/0.16                             | 0.26/0.23                       | 0.13/0.11                         | 0.25/0.21                             | 0.24/0.16                       |
| Significant at p <  | ← 0.05 →                       |                                       |                                 |                                   |                                       |                                 |
| Correlation   |                                |                                       |                                 |                                   |                                       |                                 |
| n   | 9/17                           | 9/14                                  | 6/7                             | 13/25                             | 11/26                                 | 9/11                            |
| r-Pearson   |                                |                                       |                                 |                                   |                                       |                                 |
| r   | 0.94/0.93                      | -0.91/-0.94                           | -0.91/-0.97                     | 0.93/0.90                         | -0.90/-0.96                           | -0.85/-0.97                     |
| Significant at p <  | ← 0.05 →                       |                                       |                                 |                                   |                                       |                                 |
| Regression equation                                       | y=3.39x+0.83/<br>y=4.49x+3.45  | y=-0.24x+7.10/<br>y=-1.61x+7.17       | y=-0.54x+8.53/<br>y=-1.91x+3.85 | y=2.60x+1.44/<br>y=2.74x+3.13     | y=-0.10x+4.64/<br>y=-0.94x+7.02       | y=-0.13x+3.91/<br>y=-0.80x+2.87 |

**Table 4.** Experiments *in vitro* with aniline and fuchsine (mean values).

concentrations of LC<sub>50</sub> were calculated by means of probit transformation method (Fig. 2a, 2b and 3a, 3b):

*Fontinalis antipyretica*

17.03 mg aniline per dm<sup>3</sup>  
and 2.22 mg fuchsine per dm<sup>3</sup>

*Platyhypnidium riparioides*

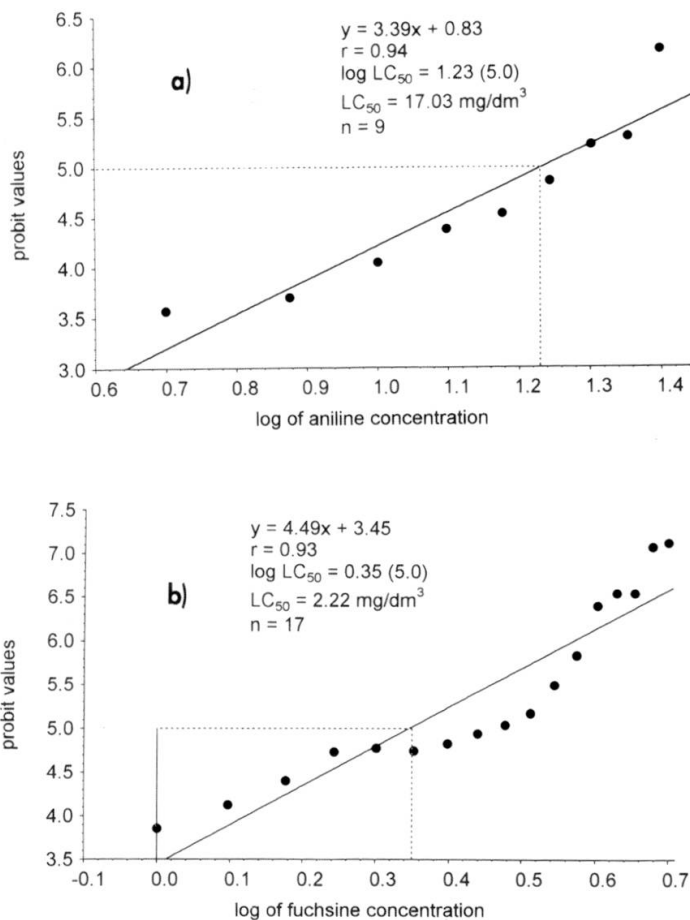
23.48 mg aniline per dm<sup>3</sup>  
and 4.81 mg fuchsine per dm<sup>3</sup>

The rise of aniline and fuchsine concentrations results in a decrease of growth of main shoots and of lateral branch development. In *F. antipyretica*, the induction of lateral branches ceases at concentrations below LC<sub>50</sub>, i.e. 15 mg aniline per dm<sup>3</sup>. At concentrations above 22.5 mg per dm<sup>3</sup> the main shoots cease to grow. Similarly, the induction of lateral branches in main shoots ceases at concentrations of 1.75 mg fuchsine per dm<sup>3</sup> and more, and growth stops from a concentration of 3.5 mg per dm<sup>3</sup> onward. The ceasing of lateral branch development and of the growth of main shoots in *P. riparioides* occurs at concentrations much higher than in the case of *F. antipyretica*. The thresholds lie at 22.5 mg aniline per dm<sup>3</sup> and 2.75 mg fuchsine per dm<sup>3</sup> for lateral branch development, and 27.5 mg aniline per dm<sup>3</sup> and 6.5 mg fuchsine per dm<sup>3</sup> for the growth of main shoots. The *in vitro* experiments revealed specific reactions of the considered mosses in relation to their sensitivity to aniline and fuchsine. The results obtained *in vitro* proved that the examined mosses are less sensitive to aniline and fuchsine than algae (Meinck & al. 1968).

*In situ* the populations of these mosses show comparable reactions to stronger pollution, i.e., there is a distinct tendency to reduced main shoot growth and number of lateral branches and to an increased mortality. In the rivers the mosses show additional reactions, i.e. an increase of density and biomass. This is most probably a mere fertilisation effect.

When comparing the measurements *in situ* with the results of the experiments, there are some more factors to be taken into account. Some parameters (e.g., temperature) kept constant during the experiment are subject to variation *in situ*. The experiments were carried out in still water, in contrast to the running water of the rivers. In the experiments aniline and fuchsine were studied separately, whereas in the rivers they occur in combination. No precise information on the content of toxic substances in the river water is available. Therefore, one has to reckon with cumulative, synergetic or antagonistic effects. Long-term effects not revealed by the experiments cannot be excluded neither.

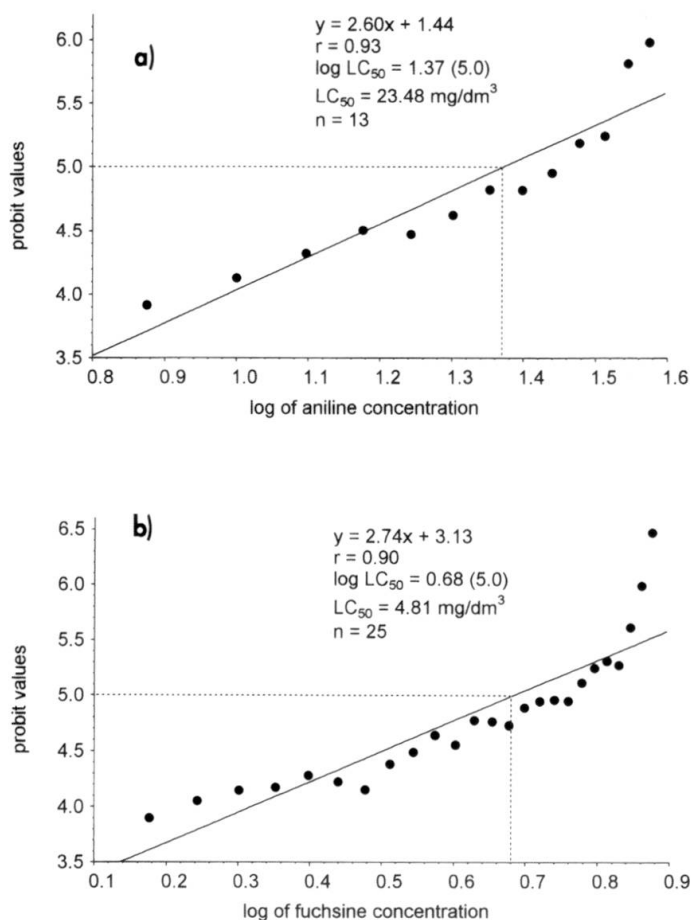
In the immediate vicinity of the waste outlets the total lack of the studied moss species as well as other macrohydrophytes is obvious. The nearest patches of mosses observed are scattered, misshaped, and very variable in size and density of main shoots. The fact that in the rivers mortality rates more or less corresponding to LC<sub>50</sub> of aniline and fuchsine occur, indicates as well that the mean total content of toxic substances is considerably high.



**Figure 2.** Correlation between probit values of *Fontinalis antipyretica* mortality and

a) aniline concentration

b) fuchsine concentration



**Figure 3.** Correlation between probit values of *Platyhypnidium riparioides* mortality and

a) aniline concentration

b) fuchsine concentration

for *Fontinalis antipyretica* and *Platyhypnidium riparioides*. Fuchsine shows a higher degree of toxicity than aniline. The tolerance of the mosses towards these substances is different, *Fontinalis antipyretica* being the more sensitive species.

2. Investigated populations of *Fontinalis antipyretica* present in Włodzica River and *Platyhypnidium riparioides* in Piława River show differences with respect to biological characters and to contents of mineral nutrients. This is due to the different chemism of the water, which is polluted mainly of sewage dumped by textile industry.

3. The mosses both react to increasing water pollution, on the one hand by decreasing growth of main shoots and increasing mortality, and on the other hand by increasing density and biomass.

4. These responses were found in situ as well as experimentally. The observed changes in biological parameters of *Fontinalis antipyretica* and *Platyhypnidium riparioides* are most probably due to sewage of textile industry containing aniline and fuchsine among other substances.

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The proved toxicity of aniline and fuchsine, and the colour of the river water show that both substances could influence the mosses growing in the two rivers. The parallel variation of several moss parameters in the experiments and along the rivers, suggest that at least one of these toxic substances is probably among the causes of the observed differences between different local moss populations.

The study concerns the unfavourable changes of the Włodzica and Piława River environment which are similar for other water ecosystems. Sewage of textile industry causes stress and decline of bryophyte populations, and can be of vital significance for more rare species. Whole populations of more sensitive taxa could be destroyed. Therefore, the results of this type of investigations should be taken into account in active protection of mosses and their biotopes. The present study shows that some production processes are in urgent need of technological innovations better adapted to environmental requirements.

## Conclusions

1. Aniline and fuchsine both are toxic

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