

# From Extremely Acidic to Alkaline – Diversity of Aquatic Invertebrates in Forest Mining Lakes Under the Pressure of Acidification

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## Research Article

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## Abstract

Human activities, including mining industry, have considerably degraded the water habitats worldwide. Acidification has severely affected aquatic environments with acidifying pollutants and constitute threat to freshwater biodiversity. The study area is unique for addressing the effects of mining-related acidification on biodiversity in freshwater ecosystems along a pH gradient (2.4-9.6). Using multivariate ordination techniques, we analysed taxa distribution to determine how variations in invertebrate composition correlated with environmental factors. The results revealed differences in pH of water, salinity indicators, hardness, and content of calcium, nitrites, and iron. The highest iron content, relatively high values of conductivity and chlorides was found in the extremely acidic mining lakes. A clear decreasing tendency was observed for the number of taxa with increasing acidity (Oligochaeta, Chironomidae, Glossiphonidae). The density of Hirudinidae, Lestidae, Libellulidae, Caenidae, Sialidae, Dytiscidae, Helodidae, Hydrophilidae and Polycentropodidae increased along with decreasing pH. Specific communities were found with increasing acidity, therefore a progressive increase in acidity will probably have further influence on biological life and water chemistry. The data yielded offer an opportunity to fill knowledge gaps concerning less-studied aquatic environments and links environmental pollution with communities, which is especially important because forest habitats are especially exposed to different climatic factors and threats.

## Introduction

The implications of anthropogenic pollution such as alkalization and acidification are widely known as one of the main threats to forests, freshwater ecosystems, and biological life <sup>[1, 2]</sup>. These are associated with human activity and considered to be the foremost problems affecting the biodiversity and the functioning of freshwaters and are associated with human activity. The atmospheric deposition of acid components has been reported to have significantly impacted the freshwater habitats in North America and Europe <sup>[3, 4]</sup>, resulting in a huge decreases in pH, and an increase in salinity indicators, and toxic aluminium. The environmental problem of acidification is encountered by organisms at all trophic levels <sup>[5]</sup>. Acidity and alkalinity stress induces multiple adverse effects, which include alteration of soils, stress on forests, and changes in waters-forest interactions, and detrimental effects on aquatic life leading to the disappearance and extinction of sensitive species <sup>[6, 7]</sup>.

The priority directions of research in environmental ecology include biodiversity protection of naturally valuable habitats and aquatic environments, including forest swamps, forest lakes, ponds, and wetlands <sup>[8-10]</sup>. These water environments are of unique value globally <sup>[11, 12]</sup> as the main breeding sites of amphibians and birds <sup>[13]</sup>. The abovementioned habitats are important in practical forest management for the purposes of reducing drought and pollution, mitigating the local floods, and protecting the natural water resources <sup>[14]</sup> as they are essential elements of "small retention" in forest catchments. In addition to their biocenotic and landscape functions, they affect the groundwater levels of adjacent habitats, supply them with water after the periods of drought <sup>[15]</sup>, and increase biodiversity. Moreover, the water from forest lakes increases and diversifies the food base and the thickness of tree trunks <sup>[16]</sup>.

Forest mining lakes are formed due to the intensive activity of the coal mining industry in the basins of land subsidence. Their size and depth vary depending on the size of the subsidence basin area. Polluting discharges originating from coal mines and acid mine drainage often make these lakes highly stressed ecosystems. Because of intensive industrial development in the areas adjacent to forests, many reservoirs disappear due to the drainage melioration and a decrease in groundwater levels in forest catchments. Draining forest areas to meet agricultural and developmental needs and the inflow of pollution are some of the threats to these environments. Forest lakes are also at risk of eradication due to littering and backfilling. The problems associated with the functioning of these lakes and the impact of acidification on the diversity of their fauna have not yet been fully studied what can be identified as a main gap in the literature. There is a complete lack of recent research or review on the biodiversity of benthos along a pH gradient. Recently, some studies were carried out on invertebrates, but they analysed only minimal direct anthropogenic disturbance <sup>[17]</sup>. Overall, studies on the impact of acidity on the taxonomic and functional structure of lake communities remain scarce <sup>[18]</sup>. Most of the previous studies focused on amphibians, fish, and crustaceans and were primarily carried out in acidic wetlands and in flowing waters <sup>[19, 20]</sup> or were experiments performed in the laboratory analysing the physiological reactions of organisms to a decreasing pH <sup>[21]</sup>. In this study, we have broadened the perspective further by assessing the diversity of invertebrate fauna along a gradient of pH in forest mining lakes. The study area is unique for addressing the effects of mining-related acidification on biodiversity in freshwater ecosystems.

The purpose of the research was to fill in the above-mentioned gap in the current knowledge regarding the impact of the environmental conditions and water pH on the structure of invertebrate assemblages in environments remaining under human influence such as coal mining using multivariate analysis techniques. Due to increasing pollution, intensive industrial development, and long-term coal mining activity, the acidification of water environments is a particular threat to biological life. Therefore, in this study, we aimed also to assess

whether there is a significant interaction between extremely acidic, acidic, natural, and alkaline forest lakes and the composition and spatial variability of freshwater invertebrates. Furthermore, we analysed the taxa distribution and similarities among the mining lakes to determine how variations in the benthos composition are correlated with the environmental factors.

## Materials And Methods

### Field study system – study area

The research area included a forest landscape that remains under the influence of anthropopressure and mining lakes formed in the last 90 years, a period during which the coal mining industry caused the largest changes in Poland. In the forest landscape, they are particularly susceptible to acidification because they are supplied with water from forest ditches, rainwaters, meltwaters, and surface runoff water which contains acidic and alkaline substances. They are under influence of atmospheric deposition of acid components, and they location in mining areas results in their exposure to acid mine drainage (Supplementary Fig. S1).

The mining lakes of this study were formed in the basins (troughs) of ground subsidence, which causes the deflection of the external surface layers due to hard coal mining<sup>[22, 23]</sup>. The Upper Silesian Coal Basin is an area that is characterised by the occurrence of numerous anthropogenic reservoirs. This region, with an area of about 5500 km<sup>2</sup><sup>[24]</sup>, is currently perceived as one of the most urbanized parts of Poland and is called the "lake district of Southern Poland" or the "Anthropogenic Lakeland", the subsidence reservoirs are known as "small mining lakes" or "anthropogenic lakes"<sup>[25]</sup>. These lakes form a characteristic element of the industrial landscape<sup>[26]</sup>, where various branches of developing industries, including mining activity, affect the quality of their waters and, consequently, the occurrence of aquatic fauna. Forest mining lakes analysed in this study are located in the forest interior, and are surrounded by full-grown trees and shrubs (Supplementary Figures S3-S6). They differ slightly in their area and depth, and have a different levels of water fluctuation. Bottom sediments of varying types are covered with the allochthonous matter, and remain under the various human activities, but all of them are located in the mining area (Table 1).

Data on freshwater invertebrates from 45 mining lakes were collected from 2016 to 2019 (Fig. 1) between May and October<sup>[27, 28]</sup>. Samples were taken five times from each lake (5×45 mining lakes – 225 water samples and 225 benthos samples, a total of 450 samples). Lakes were classified by pH according to Økland<sup>[29]</sup> Nordstrom et al.<sup>[30]</sup>, and Havas and Hutchinson<sup>[31]</sup> as follows: extremely acidic pH 1.8–5.7 (EAML – extremely acidic mining lakes); acidic pH 5.9–6.6 (ACML – acidic mining lakes); neutral pH 6.8–7.2 (NML – neutral mining lakes), and alkaline pH >7.2 (ALML – alkaline mining lakes).

### Field study and laboratory analyses – invertebrates

Invertebrates were sampled using the quadrat method, which has been widely applied for measuring the diversity and density of invertebrates in lentic environments<sup>[32, 33]</sup>. The sampling area covers eight subsamples (0.25 m<sup>2</sup> each) and is treated as one sample. In forest water bodies, different bottom sediments are covered with leaf deposits, which formed by leaves falling from the riparian trees. Coarse organic matter (primarily whole leaves and leaf fragments) and invertebrates were sampled because they are characteristic of forest water bodies. Material was removed from the quadrat frame, placed in a container, and taken to the laboratory for further processing. Moreover, using a net (0.04 - mm mesh size), we sieved the water column within the frame<sup>[32]</sup>, scraped the bottom, and sampled the leaf deposits, which ensured that a fairly complete collection of invertebrate sample. The samples consisted of organisms that were present in the water column, on the sediment surface, and within the sediment to a depth of 20 cm. After field sampling, the samples were transported to the laboratory in a cold storage container, and preserved in the fridge until further processing<sup>[34]</sup>. In the laboratory, invertebrates were washed using sieves (0.20 - mm diameter mesh), and then all organisms were separated from the samples using a stereoscopic microscope (OLYMPUS SZX16), preserved in 95% ethanol, and identified to family rank<sup>[35, 36]</sup>. The density of invertebrates was estimated as individuals per m<sup>2</sup> (ind./m<sup>2</sup>). The relative abundance of invertebrates [%] was expressed as the percentage composition of specific invertebrate taxa relative to the total number of collected specimens. Communities were analysed using the following parameters: frequency (F%) (constant taxa 100–75.1%, common taxa 75–50.1%, rare taxa 50–25.1%, accidental taxa ≤ 25%); and biodiversity indices (Shannon–Weiner and Simpson: MVSP software 3.13.p. Kovach Computing Services).

### Field study and laboratory analyses – water chemistry

For measuring the physical and chemical parameters linked to water chemistry, water samples were collected on each sampling date (5×45 mining lakes) in 0.5 - L polyethylene bottles and stored at 4°C. In situ measurements of pH, total dissolved solids (TDS), and conductivity were recorded using a portable digital multimeter (HI 9811-5 pH/EC/TDS/°C; Hanna Instruments, USA). Water hardness, and

the content of chlorides (Cl), nitrates (NO<sub>3</sub>), nitrites (NO<sub>2</sub>), phosphates (PO<sub>4</sub>), and the content of ammonia (NH<sub>3</sub>), calcium (Ca), and iron (Fe) were measured in the laboratory using photometers and reagents from Hanna Instruments (spectrophotometric methods), or Merck (colorimetric methods) by following the methodology of Hermanowicz et al. [37] methodology. The total hardness classification proposed by Hermanowicz et al. [37] was adopted, which distinguish (in mg CaCO<sub>3</sub> dm<sup>-3</sup>): soft water (90–180), medium-hard water (180–270), significantly hard (270–360), hard water (360–450), and very hard water (>450).

## Statistical analyses

The indirect (detrended correspondence analysis – DCA and correspondence analysis – CA) and direct (canonical correspondence analysis – CCA) ordination techniques were used to assess the impact of environmental factors on invertebrates (CANOCO 4.5 software). To explore the most significant compositional variations in invertebrate assemblages and relate them to the environmental variables used such as the physico-chemical parameters of the water, the type of substratum, fluctuations in the water level, depth, and lake area and to evaluate the influence of environmental variables on the benthos assemblages, we used Canonical Correspondence Analysis (CCA). To check if the benthos distribution was related to the pH of mining lakes and to group the mining lakes based on taxa distribution, we used Correspondence Analysis (CA) [38]. Both CCA and CA analyses were performed on the biological data after DCA analysis, which assessed the data based gradient length as expressed in the units of standard deviation (SD). Because DCA analysis showed that the gradient length exceeded 3 SD, we performed a unimodal ordination (CA) and a (CCA) with a forward selection. For DCA, CA, and CCA analyses, the data were log-transformed [ $\ln(x + 1)$ ] and centered [38]. The data were also log-transformed to achieved a comparable level of units for all variables. Centering was used to compare whether a given variable reaches a high or low value in a given sample in relation to the entire range of values. During CA and CCA analyzes, scaling was selected: inter-species distances, biplot-scaling and down-weighting rare taxa. The statistical significance of a model was evaluated using the Monte Carlo permutation test (499 permutations). CanoDraw was used to construct the ordination diagram (CANOCO 4.5 software)..

To test the significance of the differences in the environmental variables on the invertebrate community composition, the diversity indices, the number of taxa, and the density of invertebrates between the 4 types of mining lakes we used the Kruskal-Wallis analysis of variance ANOVA and multiple comparisons post hoc test because the data were found to be of a non-normal distribution (Kolmogorov – Smirnov test for normality) (STATISTICA 12.0).

## Results

### From extremely acidic to alkaline – forest mining lakes and water chemistry

The four groups of mining lakes were found to be similar in their surface (Table 1), depth (1–3m, average 1.5 m), and the plant matter (leaf deposits) that covered the bottom sediments but were different in fluctuations in the water level and human impact. EAML showed large fluctuations of water level and a muddy bottom. In the ACML, we found a muddy bottom and minor fluctuations in the water level. NML were characterised by larger water- level fluctuations compared to the ALML. NML and ALML had sandy-muddy sediments, covered with detritus.

The analysis of the physico-chemical properties showed that the four groups of mining lakes varied significantly in pH, in the salinity indicators, hardness, and in the content of calcium, nitrites, and iron (Table 2). The highest conductivity values, total dissolved solids and chloride content, and hardness were recorded in the ALML in addition to the highest content of ammonia, phosphates, and calcium. NML had a low concentration of the investigated compounds, except for the nitrates, and hardness. The highest content of iron and relatively high values of conductivity and chlorides were found in the EAML (Table 2). The differences in the pH, conductivity, hardness, and the content of TDS, chlorides, nitrites, calcium and iron in water between the studied lakes were statistically significant (Table 2).

### Invertebrate density and diversity along the mining lake gradient

In total, we identified 69994 individuals from 56 taxa (the family level, except the Oligochaeta). The number of taxa ranged from 28 in the EAML to 44 in NML (Table 3). The number of taxa significantly differed between the four groups of mining lakes (ANOVA, K-W:  $H=3$ ,  $(N=225)=7.979$ ,  $p=0.040$ ).

The number of widespread invertebrate taxa (occurring in >50% of lakes) was the smallest in the ALML, while surprisingly, the greatest number of these taxa were found in the ACML (Table 3). The relative abundance of invertebrates was different in mining lakes, with four taxa showing > 10 % abundance in the NML and two showing > 10% abundance in the ACML and ECML. The EAML were inhabited most

abundantly by Oligochaeta, Coleoptera (Dytiscidae), and Chironomidae. The same was also generally true for the ACML; however, in these lakes, gastropods (Lymnaeidae and Planorbidae) also occurred abundantly.

Although diversity indices indicated a rather similar diversity for the studied lakes, the diversity measured by both indices was the lowest in the ALML (Table 3). The differences in diversity indices along the pH gradient were not statistically significant (ANOVA K–W:  $p=0.471$ ).

The mean density of invertebrates was found to be statistically different along the pH gradient (ANOVA, K–W:  $H=3, (N=225)=7.87, p=0.041$ ). The highest density was found in the NML and the lowest in the EAML (Table 3). The densities of Oligochaeta and Chironomidae were the highest at low pH (maximum density: 7240 ind  $m^{-2}$  and 4288 ind  $m^{-2}$ , respectively) (Supplementary Table S7). Their occurrence decreased with decreasing values of pH. The same was also true for the Glossiphonidae and Gastropods (Lymnaeidae and Physidae, maximum density in the ALML: 4440 and 11980 ind/ $m^2$ , respectively). Some taxa, Hirudinidae, Odonata (Lestidae, Libellulidae, Ctenidae), Sialidae, Coleoptera (Dytiscidae, Helodidae, Hydrophilidae) and Polycentropodidae – were found to be the most tolerant to low pH and their density increased along with decreasing pH (Supplementary Table S7).

In CA, the first axis explained 31%, of the overall variability in the community composition of invertebrates, while the second axis 16%. The first ordination axis, with eigenvalue  $\lambda = 0.215$ , significantly ( $p<0.05$ ) differentiated the mining lakes based on the distribution and occurrence of taxa in the water bodies with different pH similarly as cluster analysis (Supplementary Figure S2). The mining lakes with neutral, acidic, and extremely acidic pH were placed in the right part of the ordination diagram, while the alkaline mining lakes were placed in the left part (Fig. 2).

The results of the multivariate CCA analysis indicated that pH, hardness, TDS, and the content of ammonia and calcium were significantly associated with the distribution of the invertebrates in studied mining lakes (statistical significance Monte Carlo test: first axis:  $F=2.545, p=0.0180$ ; all canonical axes:  $F=1.755, p=0.002$ ) (Supplementary Table S8). A group of numerous taxa, including Phryganeidae, Sialidae, Helodidae, and Polycentropodidae, were associated with the lowest values of hardness, ammonia content, and pH (Fig. 3). The occurrence of other taxa such as Limoniidae and Tipulidae, was found to be associated with a higher content of TDS, while that of Naucoridae, Hydrophilidae, and Aeshnidae was associated with a high ammonia content in the water. Other factors, such as lake size, age, fluctuations in the water levels and drainage, were not statistically significant.

## Discussion

In this study, we defined the tolerance limits of various aquatic invertebrates in acidic and alkaline waters in order to predict how acidification alters their distributions. Research revealed that impoverished water quality due to acidification is followed by a decrease in diversity and a general shift in the community structure from acid-sensitive taxa to more acid-tolerant taxa in affected lakes<sup>[39]</sup>. However, some investigations conducted in the 1980s, have found no effect of pH on invertebrates (e.g., Simpson et al.<sup>[40]</sup>; Winterbourn and Collier<sup>[41]</sup>). Research on the structure of benthos communities along a pH gradient is very scarce. Moreover, there is a lack of such research in the forest mining lakes. Because fewer works have been carried out analysing the effects of acid stress on invertebrates, to the best of our knowledge, our paper presents the first quantitative data from a field study in forest mining lakes along a gradient of pH.

In the global range, forest lakes provide habitats for rare, protected, and endemic species. In industrial areas, their role is even more significant because they are valuable water habitats for birds, amphibians, and invertebrate fauna. The reservoirs that have been created as a result of human activity usually have a poor fauna<sup>[42]</sup>; however, because of location in forests and isolation from other water environments, mining lakes are habitats of diverse snail fauna, including species that only occur sporadically in other types of reservoirs<sup>[43]</sup>. Our study revealed diverse invertebrate communities and a total of 56 taxa ranging from 28 in the EAML to 44 in the NML. However, the diversity when measured by the diversity indices, the diversity of invertebrates was not found to be significantly different between the four groups of mining lakes studied.

We found significant differences in the density of invertebrates. We identified taxa whose densities tended to decrease with a decreasing pH values (Glossiphonidae, Gastropods: Lymnaeidae and Physidae, Chironomidae and Oligochaeta). However, we also found taxa that were more tolerant to low pH and their density increased along with decreasing pH; therefore, it can be concluded that there is a straightforward relationship between the acid conditions and the presence/absence of specific invertebrates in relation to acidification<sup>[44]</sup>. Considering the interaction of acidifying pollutants with water environments, it seemed appropriate to assess how significant these interactions are in the context of invertebrates. Benthos belong to a generally abundant group seen in waters affected by human activity. However, there are many invertebrates that are sensitive to acidification, and some even disappear at pH values as high as 6.0<sup>[45-47]</sup>.

In the Extremely acidic lakes most abundantly by Dytiscidae, Chironomidae and Oligochaeta. The same was also generally true for ACML in the case of Oligochaeta and Chironomidae; however, gastropods also occurred abundantly in these lakes (ACML). Generally, the relative abundance of taxa that are highly sensitive to low pH decreases at pH 6.5 with only a few present below pH 6.0<sup>[48]</sup>, whereas the occurrence of taxa that are less sensitive to low pH is relatively high at pH 6.0 and such taxa are only occasionally found below pH 5.5. While acid-sensitive species may disappear at moderate levels of acidification, acid-tolerant taxa may appear or even increase in abundance, thereby resulting in a small or no overall decrease in biodiversity in aquatic habitats<sup>[49]</sup>. Although Gammarus sp., mosquito larvae, Leptophlebiidae, and Pisidium can tolerate very low pH<sup>[50]</sup>, we did not find them in EAML.

Although we found numerous occurrences of some taxa with increasing acidity, this was not the case for snails. They were the most abundant in lakes having a pH range of 6.0–6.7, and were also found in EAML. Mollusks need for calcium to build their shell, and thus, pH is an essential factor in molluscan ecology<sup>[51]</sup>. Changes seen in periphyton composition and abundance along with decreasing pH are also significant because it is the main source food for mollusks. This could be the reason for the low abundance of some invertebrates, including snails. On the other hand, other invertebrates such as crayfish are also absent in acidic waters, and their absence is not always linked with the calcium concentration in water as a limiting factor but rather with the biology of biocalcification<sup>[52, 53]</sup>.

Invertebrate communities found in the EAML and ACML were clearly different from those in the NML and ALML. The lowest density of invertebrates was found in extremely acidic waters (mean 770 ind m<sup>-2</sup>), whereas the highest was found in the alkaline lakes (mean 1682 ind m<sup>-2</sup>). Among the lakes with alkaline waters, the highest density was found with the high abundance of Physidae snails (maximum density: 11 980 ind m<sup>-2</sup>). A similar number of Odonata occurred in mining lakes. Heteroptera was the least numerous in the EAML and ALML. In aquatic habitats with a very acidic water, benthic fauna is dominated by Chironomus sp. and water beetle, as found in the study of Wickham et al.<sup>[54]</sup> in the acidic reservoir (pH 3.2). This finding was also confirmed for a similar pH by Rodrigues and Scharf<sup>[55]</sup> in the case of chironomids, which were the most abundant of the insects. The results obtained by Raddum and Sæther<sup>[56]</sup> showed the lowest abundance of chironomids in Norwegian lakes with pH ranging from 4.4 to 6.2; however, the number of species decreased with decreasing pH, with the lowest number being found in a humic acid lake. According to Mossberg and Nyberg<sup>[57]</sup>, in lakes with different values of pH, the composition of benthos is very much the same; however, they found that chironomids clearly exhibited a tendency to occur in assemblages consisting of only one genus (Chironomus sp.) in acid lakes. This thesis was also confirmed by Økland and Økland<sup>[51]</sup> in acidic Norwegian lakes. Our results showed that the composition of benthos differed between the four groups of mining lakes.

We found oligochaetes to be tolerant of high pH (maximum density 7240 ind/m<sup>2</sup>); however, they were also abundant in the EAML (maximum density 1164 ind/m<sup>2</sup>). As was shown by Crisman et al.<sup>[58]</sup> in Florida, their occurrence decreased significantly in acidic lakes, while the proportion of chironomids increased. However, we cannot confirm these results because we found the density of chironomids to be the highest in the ALML (mean density 1254 ind m<sup>-2</sup>) and in the EAML (mean density; 682 ind m<sup>-2</sup>). The density of other annelids (Glossiphoniidae) decreased with the decreasing values of pH, while Erpobdellidae were the most abundant in the EAML. In Norway, leeches mainly occurred at a pH >5.5; however, they were also found in environments with pH ≥ 4.2 in Sweden<sup>[59]</sup>. We did not find more recent studies in the literature on the occurrence of leeches in acidic environments.

Some of the relationships between invertebrates and environmental variables, such as the content of nutrients (nitrites, nitrates, and phosphates) and iron, were nonsignificant. It is likely that this lack of response occurred because most benthic invertebrates are habitat generalists in anthropogenic reservoirs. Benthos can routinely endure pronounced and unpredictable changes in the environment; hence, they probably possess potential durability which makes them resistant to most natural variations in the habitat conditions. The physiological effects of high pH (>9) on aquatic life have not been studied much because such pH of the water is less common in aquatic habitats<sup>[60]</sup>. High values of pH were not found in leaf-fed forest lakes, but productive agricultural lakes can have quite high pH values however they were not studied to date in the context of the invertebrate community composition. In many forest lakes, we observed high values of pH. In alkaline conditions, we found 40 taxa with high densities of some groups, whereas Wickham et al.<sup>[54]</sup> recorded a total of 37 insect genera. Increase in pH is usually associated with increasing numbers of taxa and individuals. Acidification is responsible for decreasing phosphorus input, increasing humic inputs and increasing the mobilization of toxic heavy metals. A study by Courtney and Clements<sup>[61]</sup> showed that chronic metal pollution might have resulted in communities that are tolerant of metal but more sensitive to acidic pH. Although our findings only partly reflected this generalization, we confirm the statement that acidic habitats generally have fewer taxa and lower taxa densities.

Polluting discharges from coal mines and acid mine drainage have a higher acidity, and high concentrations of iron and sulphates<sup>[62, 63]</sup>. In this study, we found extremely acidic water, high conductivity, and high content of chlorides and iron in some of the examined mining lakes

which are located in the mining area (Table 2). Acid mine drainage are toxic to benthic invertebrates and affects communities to ones dominated by a few tolerant species, they impair ecosystem processes (e.g., decomposition) [64-66]. Because of the increased pollutants emission, there is a systematic increase in the areas under the influence of acidification. This is a global trend that is visible in many regions of the world [48] and also in Poland. The effect of this trend are manifested by forests that are dying and affect the functioning of environments, thus causing changes in their structure due to the elimination of taxa that are sensitive to low pH, as found in our study: Odonata, Ephemeroptera, Trichoptera, and snails (Supplementary Table S7). According to Nisbet and Evans [67], it seems that, due to their forest locations, water environments are particularly vulnerable to acidification because of the weak capacity of these lakes. It is of particular importance because the impact of acidification manifests itself within a short period of time due to the high degree of sensitivity of different groups of freshwater invertebrates [64, 68].

## Conclusions And Recommendations

The present study is a novel contribution to ecological and environmental research, as it specified the impact of water pH on aquatic invertebrates, and enabled the identification of taxa that are characteristic of extremely acidic to alkaline waters. We found a specific composition of invertebrates along with increasing acidity. Therefore, it can be concluded that a progressive increase in acidity will have further influence on biological life and on water chemistry, because we found significant differences in the pH, salinity indicators, hardness, and the content of calcium, nitrites, and iron. The results showed the visible distinctiveness of invertebrate fauna in the NML. Variations in invertebrate composition correlated with the environmental factors such as the content of ammonia, calcium and TDS, pH, and hardness. Our research contributes to raising awareness on human disturbance such as coal mining and its influence on aquatic biota. The results of the study may be useful for organizations involved in spatial planning, because the reservoirs formed in the forests are an important element in the landscape of industrial areas. Furthermore, our findings can also be used in implementing the long-term management and remediation strategies for mine water pollution. In water management, it is advised to limit the urbanization of the shore zone of forest lakes, in order to prevent the deterioration of the ecological functions of water and ecosystems. While working on the recreational development of forest areas associated with water environments, we recommend ensuring the protection of the natural values and elements of the hydrographic system against solid and liquid pollutions. We also suggest carrying out specific water chemistry research on acidification in water environments and assess their distance from mine locality before using forest lakes for recreation or tourism.

## Declarations

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### Author Contributions

AS conceived study; AS, AC performed research; AC, NKT analyzed and interpreted data; AC statistically analyzed data; AS, AC wrote manuscript and prepared final version of the figures; AS, AC, NKT edited manuscript

### Competing interests

The authors declare no competing interests.

### Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

### Founding

This research was founded by University of Silesia

### Compliance with ethical standards

All experiments comply with the current Polish laws.

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## Tables

**Table 1.** The characteristics of the forest mining lakes; ALML -Alkaline mining lakes, NML- neutral mining lakes, ACML- acidic mining lakes, EAML- extremely acidic mining lakes, leaf deposits- leaves fallen from the riparian trees.

	EAML	ACML	NML	ALML
Area in ha	10-24	15-29	14-59	2 - 60.5
Depth in m	1,5-2.5	1-3	0.8-3	0.8-2.5
Age	1970-1998	1973-1995	1970-1982	1920-1976
Sources of water supply	atmospheric precipitation, surface runoff, groundwater, water from forest ditches			
Fluctuation in water level	Moderate or high fluctuations	small fluctuation or no fluctuation	small	no
Bottom sediments	Muddy bottom covered with leaf deposits and tree branches	Sandy or muddy sediments covered with leaf deposits and small branches	Sandy- muddy sediments covered with leaf deposits,	Muddy sediments covered with leaf detritus
Human impact present	recreation	3 mining lakes used in fish management, stocked with fry fish for recreational fishing	fish management in 5 mining lakes, stocked with fry fish for recreational fishing	recreation
	saline mining waters	recreation	recreation	municipal sewage, saline mining waters,
	acid mine drainage runoff waters	acid mine drainage runoff waters	runoff waters	runoff waters from industrial areas
Fish stocking	no	<i>Ctenopharyngodon idella</i> , <i>Leuciscus idus</i> , <i>Silurus glanis</i> ,  <i>Abramis brama</i> , <i>Carassius carassius</i> ,  <i>Esox lucius</i>	<i>Ctenopharyngodon idella</i> , <i>Hypophthalmichthys molitrix</i> ,  <i>Leuciscus idus</i> , <i>Silurus glanis</i> ,  <i>Abramis brama</i> , <i>Carassius carassius</i> ,  <i>Esox lucius</i> ,  <i>Rutilus rutilus</i>	no
Drainage	no	no	5 mining lakes during fish trapping and refilling within a 1 week time	no
Permanency	permanent except 3 temporary mining lakes	permanent except 2 temporary mining lakes	permanent	permanent

**Table 2.** The water chemistry parameters summary; TDS-Total dissolved solids, \*- statistically significant results (post hoc tests  $p < 0.05$ ). ALML -Alkaline mining lakes, NML- neutral mining lakes, ACML- acidic mining lakes, EAML- extremely acidic mining lakes; EC- Conductivity; Hard – water hardness; Conductivity in  $\mu\text{S}/\text{cm}$ , Hardness in  $\text{mgCaCO}_3/\text{dm}^3$ , other parameters in  $\text{mg}/\text{dm}^3$ .

	ALML			NML			ACML			EAML			ANOVA
	min-max	mean	SD	min-max	mean	SD	min-max	mean	SD	min-max	mean	SD	
pH	7.5-9.6	8.4	0.62	6.8-7.2	6.9	0.16	6.0-6.7	6.2	0.18	2.4-5.6	4.5	1.01	100.63 p=0.000*
EC	350-6340	1827.8	1827.0	150-1700	605.8	495.2	140-750	301.4	109.9	140-4140	1301.9	1560.8	29.79 p=0.0000*
TDS	170-3670	893.7	906.9	60-850	300.0	247.2	75-370	155.5	56.97	60-2090	643.9	776.04	27.28 p=0.0000*
Cl	35-930	146.9	229.3	6-182	42.3	46.6	8.0-86	27.0	20.18	13-895	178.0	295.8	27.81 p=0.0000*
NO3	0-45.2	5.58	8.44	0-15	6.74	4.5	0-23.1	9.03	9.1	0-50	11.09	14.54	6.38 p=0.0944
NO2	0-0.31	0.058	0.05	0-0.3	0.08	0.1	0-0.8	0.05	0.2	0.002-0.09	0.03	0.02	15.23 p=0.0016*
NH3	0.03-39	2.82	8.59	0.05-10	1.42	2.6	0.05-25	1.6	4.8	0.04-8	0.71	1.72	6.05 p=0.1090
PO4	0.03-2.46	0.44	0.52	0.04-0.8	0.26	0.19	0.05-1	0.33	0.2	0-0.82	0.27	0.23	4.12 p=0.2481
Hard	98-2482.5	609.2	726.4	45.2-678	249.3	182.9	71.4-317.9	125.4	55.3	58.9-789.4	237.2	170.29	27.47 p=0.0000*
Ca	36-314	114	81.4	14-187	66.0	49.4	15-81	42.2	18.6	0-190	35.61	40.08	36.81 p=0.0000*
Fe	0.07-6.5	1.06	1.64	0.09-3.7	1.25	1.17	0.35-5.6	1.5	1.5	0.3-25.7	10.5	10.07	13.83 p=0.0031*

**Table 3.** Mean density of benthic invertebrates and diversity indices in forest mining lakes; ALML -Alkaline mining lakes, NML- neutral mining lakes, ACML- acidic mining lakes, EAML- extremely acidic mining lakes, \* mean density ind (individuals) m<sup>2</sup>.

	ALML	NML	ACML	EAML
Number of taxa	37	37	38	27
Number of taxa per lake	7-17	3-23	6-26	7-18
Mean density of benthos *	1682	1320	1175	770
Mean density of benthos per lake *	196-6487	550-2612	160-2646	139-2723
Taxa present in >50% of mining lakes	Oligochaeta Glossiphonidae Erpobdellidae Dytiscidae Hydrophilidae Chironomidae Lymnaeidae Planorbidae	Oligochaeta Glossiphonidae Erpobdellidae Coenagrionidae Caenidae Dytiscidae Hydrophilidae Chironomidae Ceratopogonidae Lymnaeidae Planorbidae Physidae	Oligochaeta Glossiphonidae Erpobdellidae Asellidae Coenagrionidae Dytiscidae Helodidae Chironomidae Halplidae Hydrophilidae Chironomidae Ceratopogonidae Tabanidae Lymnaeidae Planorbidae	Oligochaeta Erpobdellidae Asellidae Dytiscidae Helodidae Hydrophilidae Chironomidae Ceratopogonidae Tabanidae Lymnaeidae Planorbidae
Mean Simpson diversity index	0.18	0.85	0.77	0.86
Simpson diversity index per lake	0.123-0.854	0.103-0.873	0.269-0.843	0.639-0.829
Mean Shannon- Wiener diversity index	1.99	2.33	2.16	2.39
Shannon- Wiener diversity index per lake	0.346-2.204	0.282-2.308	0.703-2.077	1.317-1.996
Relative abundance-taxa occurred > 10 %	Oligochaeta Chironomidae Physidae	Oligochaeta Chironomidae Lymnaeidae Planorbidae	Oligochaeta Planorbidae	Dytiscidae Chironomidae

## Figures

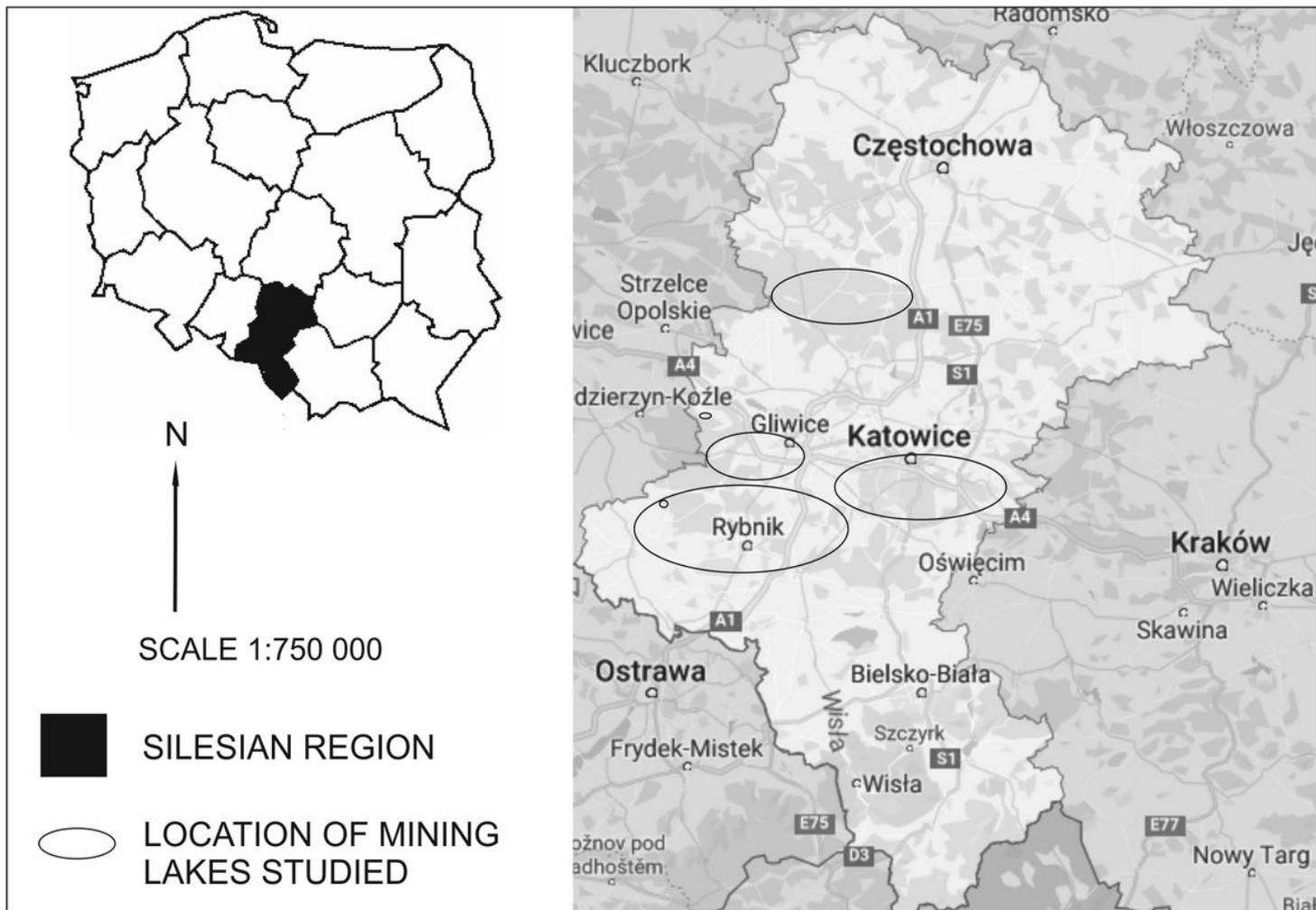
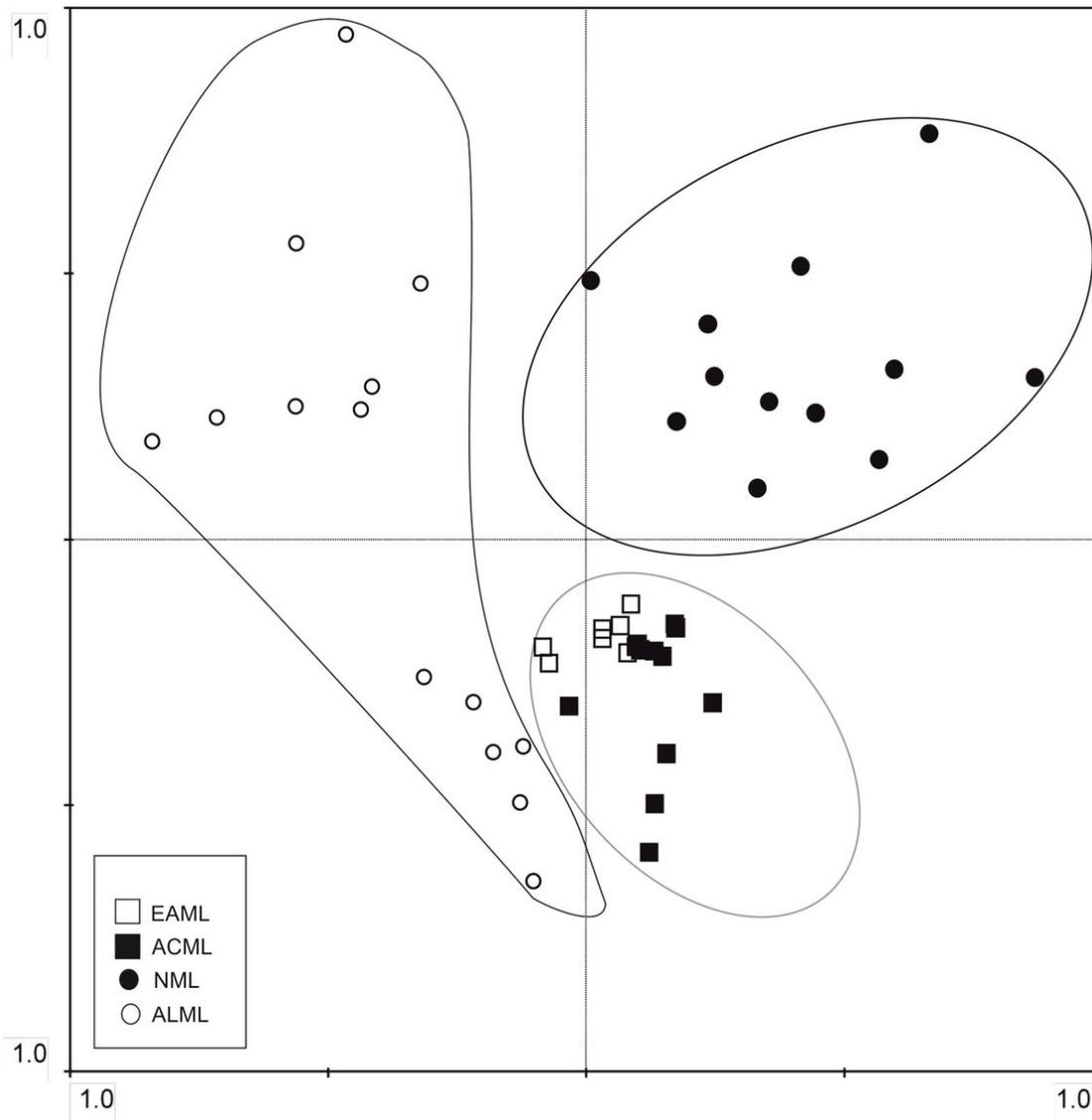


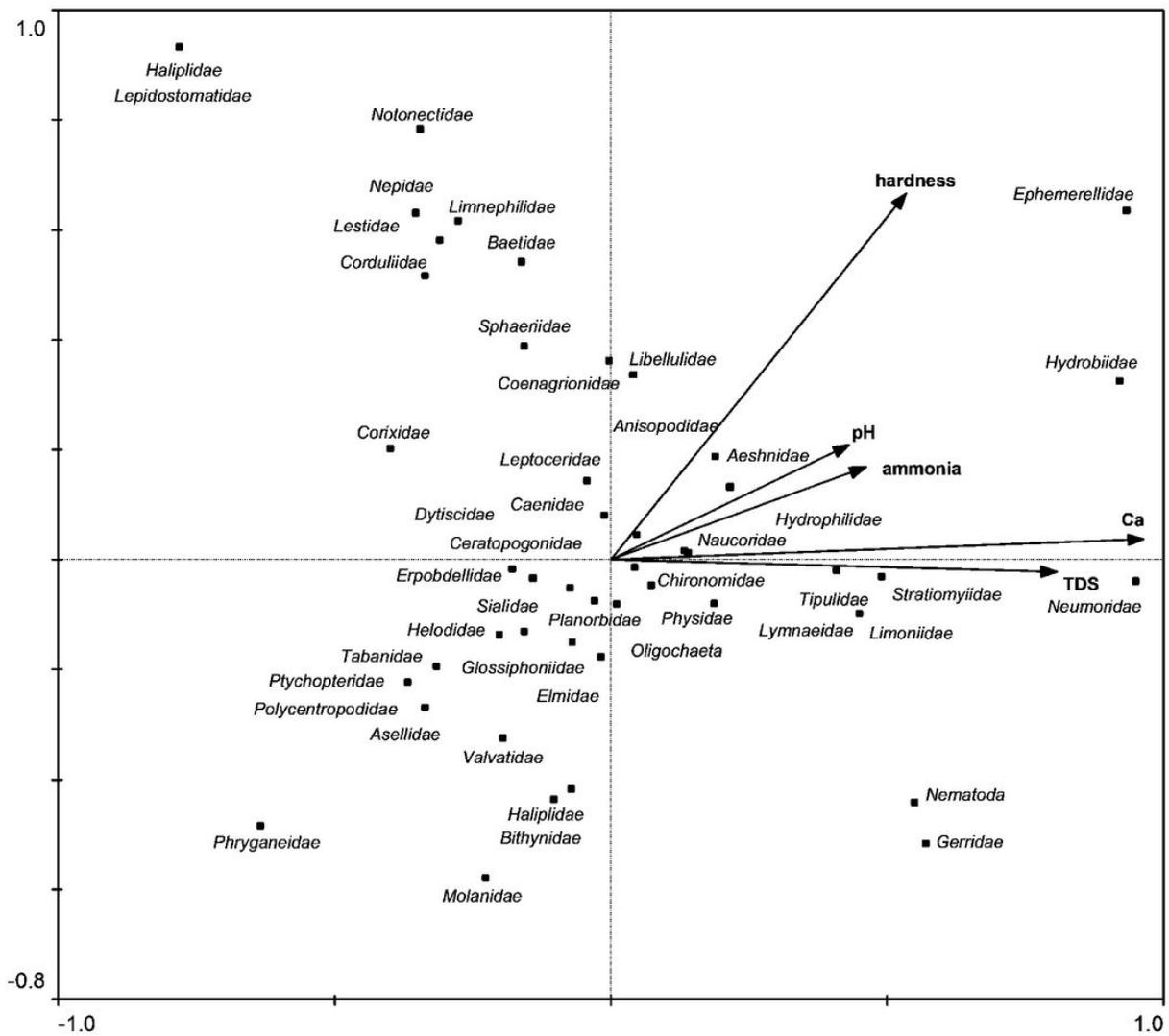
Figure 1

Location of the forest mining lakes in the industrial area of southern Poland. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



**Figure 2**

Correspondence analysis (CA) ordination diagram showing the distribution of the mining lakes based on the benthic invertebrate composition. ALML—Alkaline mining lakes, NML—neutral mining lakes, ACML—acidic mining lakes, and EAML—extremely acidic mining lakes.



**Figure 3**

Canonical correspondence analysis (CCA) ordination diagram for the physico-chemical parameters and aquatic invertebrate taxa of the mining lakes. TDS—Total dissolved solids, ALML—Alkaline mining lakes, NML—neutral mining lakes, ACML—acidic mining lakes, and EAML—extremely acidic mining lakes.

## Supplementary Files

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