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A Preliminary Study Into the Possibility of $\delta^{13}C$ Being Used as a Sensitive Indicator of the Trophic and Hydrobiological Status of Aquatic Ecosystems

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ABSTRACT

There is a need to search for additional indicators allowing for more accurate identification of both the trophic status of waters as well as its chemical and biological consequences. The work detailed in this paper involved a preliminary analysis pertaining to the possibility of using an isotopic index in association with the values for trophic and saprobic indicators in describing a dam reservoir experiencing a far-reaching eutrophication. The water samples for the physicochemical analysis were collected from three sites along the axis of the dam reservoir in Rzeszów three times during the spring and summer of 2013. The results sustained the classification of the Reservoir's waters as hypertrophic, irrespective of the particular zone sampled. While phytoplankton blooms characterised by reference to the numbers of organisms per unit volume of water were also similar throughout the Reservoir, diversification in terms of taxonomic composition was to be noted, given the occurrence of cyanobacteria among the dominant diatoms in the area close to the dam. This presence was accompanied by enrichment of the Reservoir's suspended organic matter with carbon of the heavier ^{13}C isotope. On this basis, the $\delta^{13}C$ isotopic index can be regarded as a potentially useful indicator allowing for more accurate identification of both the level and the nature of the ongoing trophic degradation in bodies of water.

Keywords: reservoir, trophic status, Carlson indexes, saprobic index, isotopic index

INTRODUCTION

Verification of the trophic status of ecosystems is carried out in line with two groups of criteria, i.e. concentration or index. Where concentration criteria are concerned, the trophic state is determined by reference to the identified concentration ranges of nitrogen and phosphorus compounds, or else chlorophyll *a* [Forsberg & Ryding 1980, Vollenweider & Kerekes 1982, Nürnberg 2001]. The integrated indicators known as TSI (Trophic State Index) [Carlson 1977, Walker 1979] and ITS (Index of Trophical State) [Neverova-Dziopak et al. 2011] were used as well. In these cases, the developers drew on many years of observation to propose simple models by which the advancement of eutrophication could be estimated.

In both cases, the lack of possibility for more-detailed interpretation, especially the distinguishing between various higher trophic states constitutes the limitation. Advanced eutrophication is accompanied by intense blooms of cyanobacteria and other planktonic algae. The classes of algae present depend on many factors, such as temperature, hydrology or solar radiation [Grabowska et al. 2003]. Hence, the proportions of classes present may differ, notwithstanding generally similar values for generalising parameters like chlorophyll *a* [Ostrowska 2013]. Nevertheless, the occurrence or non-occurrence of certain classes of algae relates closely to the subsequent impact on water quality. Reservoirs, especially those suffering degradation, frequently play host to the cyanobacteria capable of producing such varied toxins as microcystins, nodularin, anatoxins, saxitoxins, etc. These are released into the aquatic environment following the death and breakdown of cells [Chorus & Bartram 1999, Boopathi & Ki 2014], often causing a far greater deterioration in water quality than is indicated by the analysis of trophic level.

A helpful supplement to the trophic indicators in the assessed degradation of bodies of water may be the saprobic system, until recently used to assess and classify the purity of surface waters. The saprobic system is based on the assumption that aquatic organisms differ in their tolerances to organic pollutants, to the extent that each species present or absent may assume some indicator value [Lampert & Sommer 2001, Gorzel & Kornijów 2004]. The system has thus been used to assess the degree of contamination of water with the organic matter capable of causing biochemical decomposition.

The practical difficulty in using the saprobic system lies in the absolute necessity that every aquatic organism found in a sample should be identified, which isan extremely difficult, labour-intensive and expensive activity, given the large number of systematic groups to be considered [Klimaszyk & Trawiński 2007, Czerniejewski & Czerniawski 2008].

Thus, there is a need to look for and hopefully identify additional indicators allowing for more accurate identification of both the trophic status, as well as its chemical and biological consequences. In this regard, the analysis of stable isotopes is a tool which is increasingly often used in assessing the quality of changes in the aquatic environment and their dynamics, among others of carbon (C), expressed as the ratio of heavier to lighter content relative to the standard ($\delta^{13}C$) in individual components of the environment [Koszelnik 2009]. This value depends on the original carbon source, as well as the environmental carbon cycle, including the metabolism engaged in by indicator organisms [Gu et al. 1999]. Thus, for example, Lehmann et al. [2004] report $\delta^{13}C$ enrichment in particulate organic carbon (POC) from Lake Lugano during summer production.

The aim of the work detailed here has been the successful running of a preliminary analysis regarding the possibility of isotopic indices being usefully set against values for trophic and saprobic indicators in a dam reservoir undergoing far-reaching eutrophication.

RESEARCH AREA AND METHODOLOGY

The researched Rzeszów Reservoir came into existence in 1974, thanks to the damming of the River Wisłok at a point 63+760 km along its course. The Reservoir is supplied by two main tributaries, i.e. the Wisłok and the Strug, and its main purpose has been to allow for proper operating of the water supply to the city of Rzeszów. However, given the location on the outskirts of such a large city, a vital role as a sports and recreation lagoon has also been served [Bartoszek et al. 2015]. The morphometric parameters of the Reservoir in 2014 are as shown in Figure 1. The overall volume is seen to have decreased by 0.7 million m^3 over the 40-year period, with major silting having taken place, and gradual development of new land surface in the upper zone in particular. The attempts to improve the reservoir's utility were made in 1986–87 and 1995–97, entailing the work to deepen the part next to the dam, while also achieving a narrowing through partial backfill on the right part of the bank also just by the dam. In each case, approximately 250,000–300,000 m^3 of sediment were dredged [Bartoszek et al. 2015].

The Rzeszów Reservoir has a 2025 km^2 watershed in foothill areas that are largely agricultural, though the upper parts are forested, while the middle part also has industrial centres (glassworks, tanneries and refineries). The reservoir can thus be regarded as under strong anthropogenic pressure associated with local agriculture that causes severe erosion of the land. Wastes of various kinds are also discharged in the area, while other kinds of diffuse pollution also occur [Koszelnik 2007, Gruca-Rokosz et al. 2009].

The water samples were taken from three sites along the axis of the reservoir (Fig. 1), three times during the spring-summer period of 2013. Temperature (T_w), pH and dissolved oxygen (OS) were measured *in situ* with a Hach Lange HQ40D meter. Total organic carbon (TOC) and total nitrogen (TN) was determined using a TOC-VCPN analyzer (Shimadzu), phosphate phosphorus ($P\text{-}PO_4^{3-}$) and chlorophyll *a* spectrophotometrically (Aquamate, Thermo Spectronic) using filtered samples of water following reaction with

Figure 1. Locations of the sampling points in the Rzeszów reservoir and its morphometric parameters

ammonium molybdate and hot extraction with ethanol, respectively. Total phosphorus (TP) was determined analogously, but in non-filtered samples of water that were mineralized (with H_2SO_4 and peroxodisulfate). The trophic status of the water was approximated by reference to Carlson indices (TSI_{TP} and TSI_{Chla}) [Carlson 1977].

Total suspension (TS) and $\delta^{13}C$ in POC were analysed following filtration using Whatman glass microfibre filters. The filters were dried at 50°C and – prior to the analysis for stable isotopes – exposed to fuming concentrated HCl for 72 h in an exicator, in order to remove carbonates. The filters were analysed using a DELTAPlus isotopic ratio mass spectrometer (Finnigan Mat, Germany) coupled with an elemental analyser (Flask 1112, ThermoQuest, Italy). The $\delta^{13}C$ values were expressed per mil (‰), and set against PDB standards, as follows:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \cdot 1000 \quad (1)$$

where: *R* denotes ^{13}C:^{12}C.

Calibration was achieved using the NBS22 standard for $\delta^{13}C$. The standard deviations associated with the isotopic analyses were lesser than 0.1‰ (n = 10).

The water for phytoplankton analysis was collected using a suitable sampler in July 2013. The quantitative and qualitative analyses of phytoplankton were carried out using a reversed microscope and cylindrical sedimentation chambers (Utermöll method) [Picińska-Fałtynowicz & Błachuta 2012]. The hydrobiological classification was based on saprobic zones, which correspond to specific numerical values of the saprobic index (IS). IS phytoplankton was determined using the Pantle and Buck method (1955), based on the assessment of the group of observed indicator species [Czerniejewski & Czerniawski 2008].

RESULTS AND DISCUSSION

Water quality and trophic status

The results of the physicochemical analyses of the waters studied are as presented in Table 1. Only a slight variability in the ranges of all indicators was observed, the calculated coefficient of variance (CVP) each time falling below 8%.

The reservoir water was well-oxygenated during the period of study. The values for oxygen saturation (OS) with respect to Tw indicated levels are close to full saturation and even supersaturation. The measured pH of water was in the range 8.18–8.44. Such slight alkalisation of water may be presumed to reflect the excessive development of phytoplankton [Bartoszek et al. 2017]. The average concentration of $P\text{-}PO_4^{3-}$ was about 0.03 mg · dm^{-3}, while the value for TP was about 0.15 mg ·dm^{-3}. Slight spatial differentiation is to be observed, with an downward trend for the contents of forms of phosphorus in Reservoir water along the axis from the inflow (St. 3) in the direction of the dam (St. 1). However, no significant spatial

Table 1. Selected physicochemical parameters of water in the Rzeszów reservoir

Site	Scope	Tw	pH	OS	$P\text{-}PO_4^{3-}$	TP	TN	TOC	TS	Chl *a*
		°C	-	%	mg·dm^{-3}					µg·dm^{-3}
St. 1	Minimum	16.2	8.18	91.7	0.005	0.127	0.77	5.75	12.5	13.6
	Maximum	26.1	8.42	106.6	0.060	0.141	1.79	11.3	25.0	53.1
	Average	20.9	-	99.8	0.023	0.136	1.35	8.24	20.0	32.9
St. 2	Minimum	16.3	8.31	84.7	0.010	0.145	0.82	6.11	25.0	18.5
	Maximum	26.1	8.44	113.2	0.053	0.166	1.68	9.25	40.0	46.9
	Average	20.8	-	99.0	0.026	0.156	1.32	8.19	33.3	30.0
St. 3	Minimum	16.3	8.30	87.4	0.016	0.147	0.88	7.06	20.0	21.0
	Maximum	26.1	8.43	111.5	0.108	0.176	1.64	9.86	32.5	71.6
	Average	20.7	-	99.8	0.049	0.163	1.33	8.72	25.0	54.3

differentiation was observed in the case of either TN, the concentrations of which varied from 0.77 to 1.79 mg · dm^{-3} or TOC (5.75- 11.3 mg · dm^{-3}). The highest content of suspended matter (TS) was each time present in the transitional zone of the reservoir (St. 2), the average value being 33.3 mg · dm^{-3}. Marked differentiation was observed in the case of Chl *a*, with the back zone (St. 3) reporting the highest average value of 54.3 µg · dm^{-3}, while the transition zone (St. 2) had the lowest (30.0 µg · dm^{-3}).

The afore-mentioned recorded values, and especially TP concentrations above 0.1 mg · dm^{-3}, result in the classification of the waters under study as hypertrophic [Vollenweider & Kerekes 1982]. This status is also confirmed by the values of the Carlson index, TSI_{TP}, which exceed 70 (Table 2). Equally, in line with the TSI_{Chla} index, the obtained values in the range 50–70 are indicative of eutrophic waters.

Previous publications have confirmed the above-mentioned interpretation arrived at on the basis of a short period of study. Evaluations of the trophic status on the basis of concentration criteria or trophic indexes have all been indicative of advanced eutrophication in Rzeszów Reservoir [Gruca-Rokosz 2013, Bartoszek et al. 2018]. Indeed, according to Gruca-Rokosz [2013], the average annual concentrations of nitrogen, phosphorus and chlorophyll *a* in the Reservoir's waters over the 2009–2011 period are sufficient to qualify it as hypertrophic. Only the concentration of Chl *a* at St.3 site was such as to point merely to eutrophy. In turn, the values for Carlson trophic indices determined for Rzeszów Reservoir have indicated hypertrophy in the case of the phosphoric version (TSI_{TP}), and eutrophy in the case of the version based on chlorophyll (TSI_{Chla}). An assessment made by Bartoszek et al. [2018], based on the phosphoric and chlorophyll indices, also showed hypertrophic and eutrophic status of the water (respectively).

Table 2. Average values of Carlson's trophic indexes (TSI), N:P ratio for water and $\delta^{13}C$ for suspension of the Rzeszów reservoir

Site	N:P	TSI_{TP}	TSI_{Chla}	$\delta^{13}C$
	-	-	-	‰
St. 1	9.8	75	63	-27.3
St. 2	8.4	77	63	-27.9
St. 3	8.2	78	68	-28.4

The above-mentioned results indicate that a high content of total phosphorus in the reservoir waters was not accompanied by a commensurately high content of chlorophyll *a*. Furthermore, the average values of the N:P ratio did not exceed 10 at any of the sites, suggesting that a relative lack of nitrogen might inhibit the progress of the internal production process in this Reservoir. Some of the nutrients were used by luxuriant emergent vegetation in the littoral zone. In fact, competition for phytoplankton here may be provided by water chestnut (*Trapa natans*) – a plant enjoying legal protection in Poland, which covers a large part of the Reservoir surface, most especially near the dam [Kukuła & Bylak 2017].

Isotopic composition of the suspension

The values for $\delta^{13}C$ determined in the POC taken from the sites studied were progressively higher along the reservoir, at: -28.4 ‰ (St. 3), -27.9 ‰ (St. 2) and -27.3 ‰ (St. 1) (Table 2). POC in the sediment collected next to the dam (St. 1) was most enriched in the heavier ^{13}C isotope. Such enrichment is to be observed in the context of algal-bloom conditions in summer [Lehmann et al. 2004]. Differences obtained for $\delta^{13}C$ may also suggest a slightly different kind of primary production processes along the reservoir, i.e. in its riverine and lacustrine zones.

Biological parameters

Table 3 presents the results of hydrobiological tests of water. The results obtained for the saprobic index values are indicative of the β-mezosaprobic zone, i.e. water hardly contaminated by organic matter. Generally, no differences in the IS values were found between the selected water-intake points, which may suggest that – given the short retention time (of 0.8 d) – the flow of water through the reservoir is not associated with any decrease in the level of pollution. Nevertheless, the largest numbers of producers and smallest numbers of reducers were reported from a near-dam zone (Site 1), where the producer/reducer ratio equal to 50.3 contrasted with the values for the remaining sites of 8.3 and 7.5. A greater share of producers in biocoenosis occurs as a result of self-purification processes that should entail a reduction in saprobility [Lampert & Sommer 2001].

Diatoms were the dominant algae at all research sites (Fig. 2). These were mainly species from Cyclotella, Navicula and Nitzschia genera. In the majority of dam reservoirs, cyanobacteria and green algae dominate in the summer period [Ostrowska 2013]. Diatoms are more abundant in waters subject to constant mixing, there from mainly in flowing waters. In the analysed case, the dominance of diatoms may in part reflect the high flow characterising the water, as well as its turbidity, as confirmed by the TS values up to 40 mg · dm^{-3}. Diatoms require less light than other photosynthesising organisms, and this is most likely a decisive factor in the dominance of this type of algae. The studies on bioindicators have shown that

Table 3. Results of hydrobiological research of water

Site	Saprobic index	Numer of organism per 1 cm^3	Primary producers / Decomposers
St. 1	2.08	13244	12986
			258
St. 2	2.13	12829	11453
			1376
St. 3	2.03	13158	11610
			1548

diatoms are among the best indicators of the state of the environment [Panek 2011b]. They react *inter alia* to the changes in trophic conditions and the conditions relating to the contamination with organic matter, which allows them to be used in monitoring surface waters in areas experiencing varying degrees of anthropogenic transformation [Panek 2011a]. Cyanobacteria were observed mainly in the lower part of the Reservoir near the dam (St.1). Mass development of these organisms is considered one of the signs of progressing eutrophication [Szymański et al. 2013]. At St.2 site, the numbers of cyanobacteria in the water were lower, while at the St. 3 site in the vicinity of the inflow, they were not present at all. In order to develop, these organisms require a large amount of biogenic substances (especially phosphorus) to be present in water [Pac 2012].

Further factors favouring cyanobacterial blooms include the sun exposure and a higher temperature of water that lasts for a longer period of time. The most favourable conditions for development could therefore be present in the near-dam zone, given the slower flow of water there. Cyanobacteria may develop in the circumstances of nitrogen deficiency, i.e. where the values for

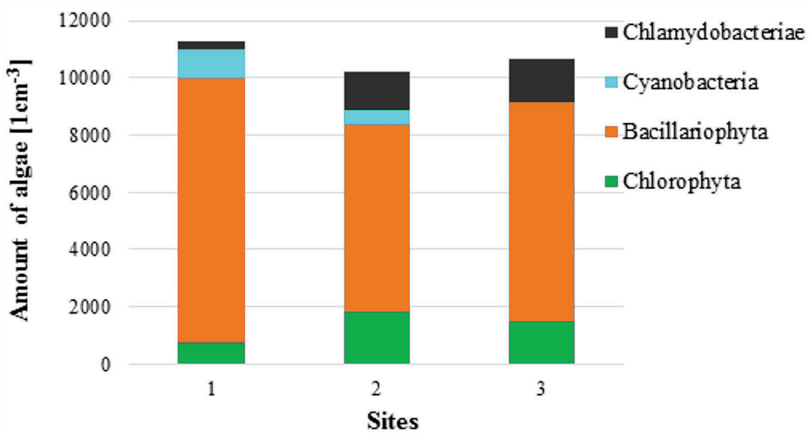

Figure 2. Plankton occurring in water in July 2013 at individual research sites

the ratio of nitrogen to phosphorus in water are low, for example owing to the ability of certain species to bind free atmospheric nitrogen [Kobos 2009]. The cyanobacteria found in the Reservoir belonged to the Microcystis, Oscillatoria, Aphanothece and Romeria types, among which strains capable of producing toxins are present [Mazur-Marzec et al. 2009]. At the same time, these cyanobacteria lack heterocysts, ensuring that their development depends on aquatic concentrations of nitrogen [Bergman et al. 1997].

An isotopic index of trophic status

Graphical analysis of spatial distributions associated with the indicators examined (Fig 3) indicates that the isotopic composition of POC did not reflect variability characterising TN and Chl *a*. TSI_{Chl} also varied across a range not affecting the $\delta^{13}C$ parameter (Fig. 3A and B). This was not the case for TP, the N:P ratio, TSI_{TP} and the hydrobiological indicators. It was at site St. 1, identified as having the largest population of blue-green al-

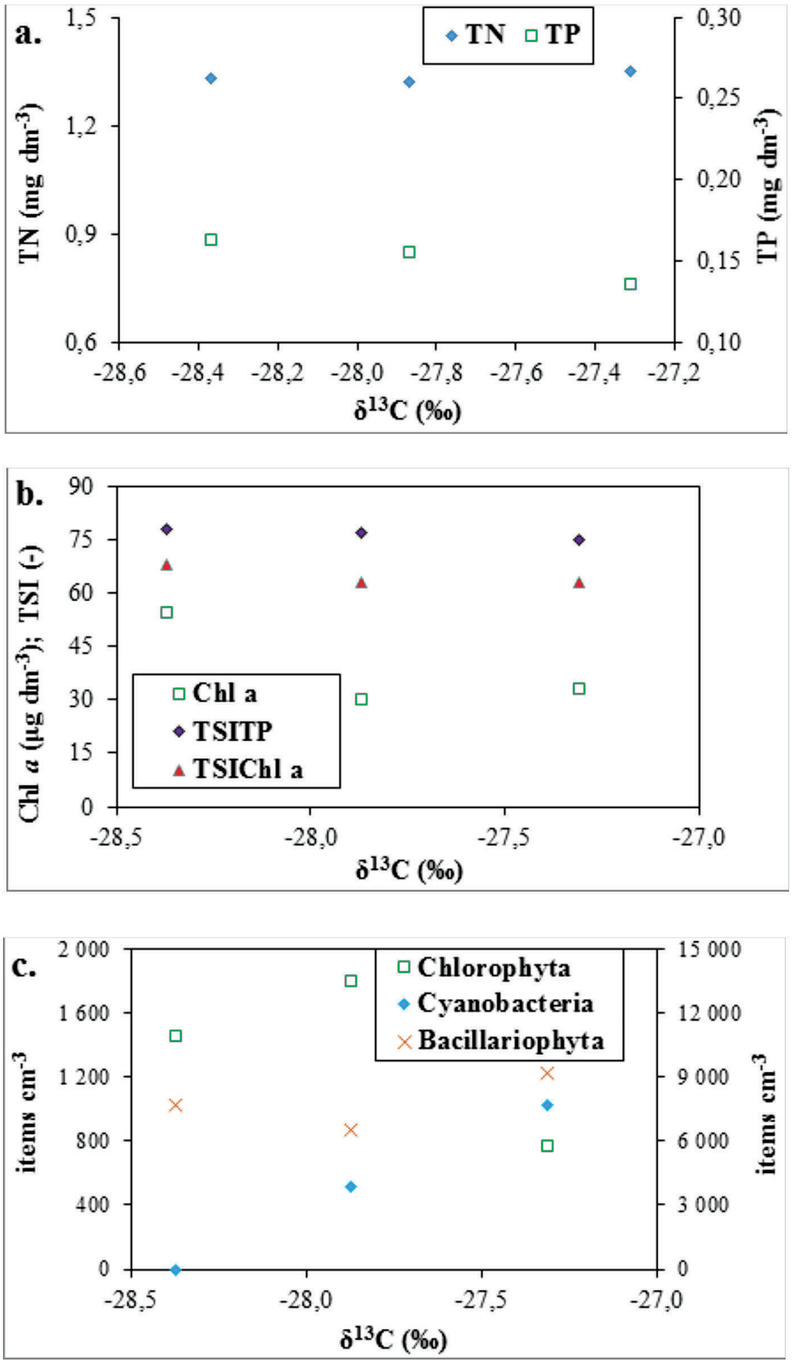

Figure 3. Relations between spatial distribution of selected water parameters and $\delta^{13}C$ values; a. with total nitrogen, total phosphorus, b. with chlorophyll *a*, TSI_{TP}, TSI_{chla}, c. with phytoplankton

gae where the highest $\delta^{13}C$ value was reported. At the same time that site had higher noted concentrations of diatoms and lower concentrations of green algae, compared with other sites (Fig. 3C). The decrease in TP and the resulting increase in N:P seems to exert an indirect impact, by stimulating the growth of specific phytoplankton groups. The situation described may suggest that summer blooms of cyanobacteria achieve greater enrichment of matter produced with the heavier ^{13}C isotope, as they do so potentially poisoning waters with toxins.

CONCLUSIONS

The water of Rzeszów Reservoir was classified as hypertrophic irrespective of zones. The phytoplankton blooms expressed in the form of numbers of organisms per unit volume of water were also shaped at similar levels. However, diversification of taxonomic composition was evident, given the presence of cyanobacteria among the dominant diatoms in the zone next to the dam. This effect was accompanied by the enrichment of organic matter suspended in the reservoir by the heavier ^{13}C isotope. On this basis, the $\delta^{13}C$ isotopic index can be regarded as a useful indicator allowing for more accurate identification of the trophic status and follow-up degradation of bodies of water. Further analyses are necessary in this respect, on the basis of a larger sample of data.

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