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Ecotoxicological biotests as tools for continuous monitoring of water quality in dam reservoir

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Abstract: Ecotoxicological biotests were applied in order to evaluate their suitability as early warning systems in the continuous monitoring of lowland shallow dam reservoirs located in Central Europe. The following biotests were used: Daphtoxkit FTMmagna, Algaltoxkit FTM, Ostracodtoxkit F, Phytotoxkit and MARA Test. The experiment was conducted from July 2010 to December 2012 in Goczalkowice Reservoir (the Vistula River, Poland), serving as a model. For the analysis, 41 out of 52 measured water indices were used to assess its toxicity to living organisms. The results of biotests were correlated with 41 hydrochemical indices of water quality. The pattern of relationships among the result of biotest and hydrochemical indices as well as Factor Analysis (FA) and Primary Component Analysis (PCA) revealed that:

- i) signs of ecotoxicity detected with biotests were associated with either low flow periods or spring surface runoff of water;
- ii) single events of increased ecotoxicity in the depression areas behind saddle dam pump stations appeared after high flow periods;
- iii) elevated toxicity was accompanied by high concentrations of dissolved and suspended substances;
- iv) FA and PCA demonstrated correlations among the results of biotests and damming parameters, water conductivity, alkali and transitory metal metals (Ca, Fe, Cu, Zn), and several forms of nitrogen phosphorous and carbon compounds concentration.

The relationships suggest that batteries of biotests may serve as a cost-effective tool for continuous monitoring of water quality in dam reservoirs and can detect effects of extreme hydrologic events, local toxic discharges, and signs of the trophic status of the reservoirs.

Introduction

The condition of water bodies and the biological environment of reservoirs is subject to the regulations of the EU Water Framework Directive (2000), which mandates improving water quality and maintaining the good condition of the natural environment. The high number of chemicals among over 196 million chemical products registered in Chemical Abstracts Services databases in July 2022 (CAS Registry 2022), which are currently produced and used, and many of which are introduced into the environment, caused conventional methods of hydrochemical analyses to be insufficient for rapid and continuous detection and prevention of their environmental toxicity. Commercial biotests, which detect acute and chronic toxic effects of complex chemical mixtures, despite their composition, are modern tools for environmental monitoring (Gagne and Blaise 2005), that act as early warning markers (Maradona et al. 2012). A typical biotest is based on observing physiological processes (motor activity, growth rate, survival rate) in the selected standardized

strain of test organisms exposed to the sample of interest material: water, soil or sediments. Small invertebrates, such as crustaceans, protozoans, algae, yeasts, plant seedlings, bacteria, shellfish, fish and amphibians, are used as the test organisms (Namiesnik and Szefer 2010). Numerous researchers have promoted the usage of biotests in Europe (Blaise and Férard 2006, Persoone et al. 2003, Wadhia and Dando 2009, Palma et al. 2010, Wolska et al. 2010). The advantages of biotests are their broad range sensitivity, technical simplicity, low costs and short time necessary for assay completion (Namiesnik and Szefer 2010). In the last decade, biotests have also been successfully used in Poland to assess water pollution (e.g., Szklarek et al. 2015, Zgórka et al. 2020, Szara-Bak et al. 2021, Szklarek et al. 2021). However, in most studies, the number of biotests used for the simultaneous analysis of water or sediment samples was limited, and the number of tested parameters ranged from a few to a dozen.

To meet the current requirements of environmental quality set forth by the European Community, an interdisciplinary team



of scientists from the University of Silesia, Cracow University of Technology, Institute for Ecology of Industrialized Areas and Institute of Environmental Engineering started the project entitled "Integrated System Supporting Management and Protection of Water Reservoir" (POIG.01.01.02-24-078/09-00: ZiZOZap - Polish acronym) implemented within the governmental Innovative Economy Operational Program and mostly financed by European Union. The model object of the project was Goczalkowice Dam Reservoir (Goczalkowice Reservoir 2006), situated on the Upper Vistula River (Silesia, Poland). One major project activity was water and sediment quality monitoring using selected commercial biotests recommended for these purposes (Namiesnik and Szefer 2010, Persoone et al. 2003).

Goczalkowice Reservoir is the fourth largest among artificial reservoirs in Poland concerning capacity. The reservoir is situated on the Upper Vistula River in an urbanized region, and downstream, three large cities (health resorts) release wastewater into the river. The reservoir is surrounded by agricultural areas. This localization is prone to possible water contamination with a complex mixture of chemical and household waste. The main functions of the reservoir are flood prevention and water supply for nearly 3 million Silesia inhabitants. Goczalkowice Reservoir comprises a part of the Nature 2000 Area, constituting a habitat for about 100 bird species (Nature 2000 Area 2022).

The presented studies aim to characterize the usefulness and efficiency of the battery composed of selected biotests for the monitoring of water quality in dam reservoirs and compare the obtained results with the results of the standard hydrochemical monitoring activities to reveal possible relationships employing advanced statistical analysis (Pejman et al. 2009, Zhengjun and Huili 2010).

Material and Methods

In two stages of the experiment, exploratory and operational monitoring, water and sediment samples were taken 5 to 6 times per year, at 8 sites in the reservoir water body (Z01-Z08) and at 8 sites along the reservoir bank (T01-T08) from July 2010 to June 2011, and at 4 sites in the reservoir water body (Z01, Z05, Z08 and Z09) and 4 sites along the reservoir bank (T04, T06, T08, T05/T12) from July 2011 to December 2012. Points T05/T12 and T08 represent the riverbed of the Vistula River and the Bajerka stream, respectively. This work only considers the results from the measurement points where the classic hydrochemical tests and bio-test were carried out simultaneously. The project followed an extreme flood in May 2010 and covered a flood in September 2010, three high water periods in 2011 and 2012 and a drought from July to November 2012. Sampling sites in the reservoir water body lay along the transects (old river bed) of the Vistula and Bajerka and represent the expected flow trajectory of water masses. Sampling points are characterized in Table 1 and shown on the schematic map in Figure 1 to illustrate the topography of the research area. The text and tables determine the exact dates of sampling. High flood incidents on May 16-26, June 2-6 and September 1-9, 2010, and the low-flow status during drought in late summer of 2012 interfered with the samples collection. Ruttner water sampler and Birge-Ekman grab sampler were used, and the collected samples were thawed and frozen until the analyses were carried on not later than the next day. Several indices, like Secchi disk depth, water and air temperature, specific water conductivity, and water acidity (pH) were measured instantaneously in situ from the board of the experimental boat with appropriate probes. The concentrations of selected chemicals in the originally collected water samples, listed in Table 2, were analyzed under laboratory conditions in the Institute of Environmental Engineering of the Polish Academy of Sciences (Zabrze, Poland). The corresponding median and maximal values of these indices are shown as a background (Tab. 2).

Analytical procedures and methods were accredited (Accreditation Certificate PCA Nr AB 950) and conformed to Decree of the Minister of the Environment from November 15, 2011, on forms and methods of monitoring (Journal of Laws 2011) and Polish Standards PN-EN ISO 17294-1 and PN-EN ISO 17294-2 (Tab. 2; certificates available at: http://www.ipis. zabrze.pl/index.php/en/instytut-2/pm-struktura-instytutu/pmlaboratorium).

The results of hydrochemical analyses were published in several papers (Jabłońska-Czapla et al. 2013, Kostecki et al. 2013). Selected hydrologic indices, such as water inflow and mean damming level for 1, 5 and 30 days (V, V5d, V30d, DL, DL5, DL30) were also regarded. Used indices are listed in the Minister of the Environment decree from May 13, 2009, on form and monitoring methods (Journal of Laws 2009).

In parallel with hydrochemical analyses, the toxicity of environmental samples was tested with a set of five selected commercial biotests.

Model MARA (Microbial Assay for Toxic Risk Assessment Toxicity Test Kit - NCIMB Ltd., the United Kingdom) is based on the array of 11 microorganism species inoculated in a 96-microwell titre plate and incubated for 18 hours with a liquid sample of environmental material in the presence of redox dye. The intensity of the color redox reaction measures metabolic activity of test organisms and their inhibition by the toxins present in the tested material. The pattern of the inhibition may be taken as a fingerprint of a toxicant (Gabrielson et al. 2003, Wadhia and Dando 2009). The system is recommended for Water Framework Directive (Wadhia and Thompson 2007).

Table 1. Sampling points in the water body and coastline of Goczalkowice Reservoir considered in the publication

			stline of th s of Vistula				Site	s at water	body and	coves of	the Reser	voir	
ID:	T04	T05	T06	T07	T08	Z01	Z02	Z03	Z05	Z06	Z07	Z08	Z09
GPS °N:	49.9192	49.9079	49.9096	49.9199	49.8896	49.9064	49.9164	49.9284	49.9277	49.9337	49.9352	49.9312	49.9236
GPS °E:	18.7727	18.7618	18.7875	18.8405	18.8492	18.8637	18.8824	18.8890	18.8092	18.8381	18.8569	18.9234	18.8068



Ecotoxicological biotests as tools for continuous monitoring of water quality in dam reservoir

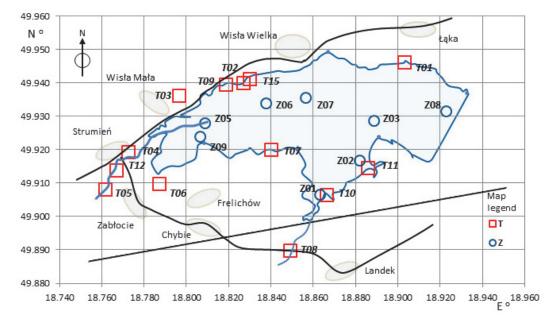


Fig. 1. Location of the sampling points in the water body (Z01–Z09) of Goczalkowice Reservoir and its coast (T01–T15), where the samples of water and sediment were taken during the ZiZOZap Project

Table 2. Names, abbreviations, medial and maximal values of selected indices determined in the reservoir and used for correlation
analysis in the experiment

a) Main hydrological indices

No.	Name of the index determined	Abbrev.	[unit]	Mean	Maximum	Remarks/Standard
I	damming level	DL	[m osl]	254.75	254.90	
П	mean daming level for 30 days	DL30	[m osl]	254.70	254.90	
111	daily water flow	V	[m³ / d]	446262.30	3512336.00	
IV	water flow for 30 days	V30d	[m ³ / 30 d]	18203568.93	56857112.60	

b) Biotests

No.	Name of the index determined in the water sample	Abbrev.	[unit]	Median T-sites	Median Z-sites	Maximum Z & T sites	Remarks/Standard
1	Ostaracodtox test Mortality	OD	[1]	0.10	0.07	1.00	OSTRACODTOXKIT F manual
2	Ostaracodtox test Growth	OG	[1]	0.07	0.18	0.67	OSTRACODTOXKIT F manual
3	MARA test	MARA	[1]	0.01	0.03	0.16	MARA test manual
4	Daphtox test 24 h	D24	[1]	0.00	0.00	0.22	DAPHTOX test F manual
5	Daphtox test 48 h	D48	[1]	0.00	0.05	0.95	DAPHTOX test F manual
6	Algatox test 72 h	Alg	[1]	0.02	-0.03	0.46	ALGALTOX F manual

c) Biophysical and hydrochemical indices

No.	Name of the index determined in the water sample	Abbrev.	[unit]	Median T-sites	Median Z-sites	Maximum Z & T sites	Remarks/Standard
7	air temperature	Ta [°C]		_	19.10	31.30	in situ from the board of boat
8	water temperature	Tw	[°C]	_	17.60	27.00	from the board of boat. Each 1 m of depth
9	weather (5–1; Sun–rain/snow)	W_	[1]	-			cloud cover
10	dissolved oxygen concentration	02	[mg · dcm ⁻³]	_	9.91	17.40	PN-EN- 25814:1999
11	transparency of the water – as Secchi depth	SD	[m]	_	0.80	2.50	in situ. from the board of boat



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12	concentration of ammonium nitrogen	NNH4	[mg · dcm ⁻³]	0.57	0.35	9.06	PN-ISO 5664:2002
13	concentration of nitrite nitrogen	NNO2	[mg · dcm ⁻³]	0.02	0.02	0.14	PN-C-04576-06:1973
14	concentration of nitrate nitrogen	NNO3	[mg · dcm ⁻³]	0.91	1.13	4.27	ISO 7890-1:1986
15	conc. of dissolved organic ntrogen	DON	[mg · dcm ⁻³]	0.62	0.69	1.35	PN-C-04537-02:1989
16	total nitrogen concentration	TDN	[mg · dcm ⁻³]	2.15	2.03	12.64	sum of above [12–15]
17	conc. of orthophosphate phosphorous	PPO4	[mg · dcm ⁻³]	0.04	0.01	0.46	PN-C-04537-02:1989
18	conc. of polyphosphate phosphorous	PnPO4	[mg · dcm ⁻³]	0.09	0.06	0.56	PN-C-04537-06:1991
19	conc. of dissolved organic phosphorous	DOP	[mg · dcm ⁻³]	0.18	0.12	1.57	PN-C-04537-06:1991
20	total phosphorous concentration	TPD	[mg · dcm ⁻³]	0.38	0.20	1.65	PN-C-04537-09:1991
21	nitrogen to phosphorous ratio	N/P	[1]	6.70	10.80	38.20	ratio
22	concentration of inorganic carbon	DIC	[mg · dcm ⁻³]	10.70	11.20	16.20	PB4 – IPIŚ PAN lab standards
23	total conc. of dissolved carbon	TDC	[mg · dcm ⁻³]	19.50	17.90	41.60	PB4 – IPIŚ PAN lab standards
24	chemical oxygen demand (COD)	COD	[mg O ₂ · dcm ⁻³]	21.00	17.00	83.80	PN-ISO 15705:2005
25	5-day biological oxygen demand	BOD5	[mg O ₂ · dcm ⁻³]	5.00	4.60	31.00	DIN EN 1899-1 i 1899-2
26	chloride anion concentration	CI	[mg · dcm ⁻³]	14.60	13.60	62.90	PN-ISO 9297:1994
27	sulfate anion concentration	SO4	[mg · dcm ⁻³]	20.50	19.80	48.30	PN-ISO 9280:2002
28	total water hardness index	HdT	[mval · dm ⁻³]	1.63	1.40	23.19	PN-ISO 6059:1999
29	water alkalinity	ALC	[mval · dm ⁻³]	1.30	1.15	3.00	PN-C-04540-03:1990
30	calcium cation concentration	Са	[mg · dcm ⁻³]	26.30	24.10	42.10	ICP-MS*
31	magnesium cation concentration	Mg	[mg · dcm ⁻³]	3.39	3.04	10.39	ICP-MS
32	sodium cation concentration	Na	[mg · dcm ⁻³]	9.76	8.87	43.10	ICP-MS
33	potassium cation concentration	к	[mg · dcm ⁻³]	2.90	2.87	6.10	ICP-MS
34	water acidity	рН	[1]	7.00	8.00	9.00	PN-C-04540-03:1990
35	specific water conductivity	COND	[mSi · cm ⁻¹]	225.00	199.00	531.00	PB5 – IPIŚ PAN lab standards
36	conc. of soluble substances	SOLUB	[mg · dcm ⁻³]	157.00	140.00	329.00	IPIŚ PAN lab standards
37	conc. of suspended substances	SUSP	[mg · dcm ⁻³]	19.90	13.00	88.00	PN-EN 873:2005
38	iron concentration	Fe	[µg · dcm ⁻³]	106.00	80.00	2146.00	ICP-MS
39	copper concentration	Cu	[µg · dcm ⁻³]	1.49	1.81	12.41	ICP-MS
40	zinc concentration	Zn	[µg · dcm ⁻³]	5.87	4.03	126.64	ICP-MS
41	cadmium concentration	Cd	[µg · dcm ⁻³]	0.03	0.02	15.41	ICP-MS
42	lead concentration	Pb	[µg · dcm-3]	0.16	0.13	7.79	ICP-MS
43	aluminium concentration	AI	[µg · dcm ⁻³]	22.70	23.00	197.50	ICP-MS
44	silica concentration	SiO2	[mg · dcm ⁻³]	4.20	3.35	101.00	PN-C-04567-02:1971
45	chlorophyll a concentration	CHL-a	[mg · dcm ⁻³]	_	16.73	107.80	PN-ISO 10260:2002

Footnote. 48 among 52 determined water quality indices were chosen for further statistical analyses. Each index was characterized by its full name, abbreviation used in the text, unit, the medial value obtained for the T-series sites (T04, T05, T06, T08) and Z-series sites (Z01, Z05, Z08, Z09), the maximal value obtained during the whole period of monitoring, and remark on the method of determination. Abbreviations of the indices names are used in the following text (i.e. NNO₃ for nitrate-nitrogen concentration in the water). * - inductive coupled plasma mass spectrometry method



Daphtoxkit F™magna (MicroBioTests Inc., Gent, Belgium) is a 24 h {D24 – OECD recommendations} or 48 h {D48 – EPA recommendations} assay based on immobility or mortality of the test organisms – a water flea *Daphnia magna* exposed to the sample of water taken from the environment. The standard strain of *Daphnia* is supplied with the kit as dormant eggs (ephippia) and can easily be hatched on demand. The test conforms to ISO standard 6341 and OECD Guideline 202 (available at: https:// www.microbiotests.com/toxkit/freshwater-daphnia-toxicitytest-with-daphtoxkit-f/) (Palma et al. 2010, Persoone et al. 2009).

Ostracodtoxkit F (MicroBioTests Inc.) is a 6 day growth inhibition {OG} and mortality {OM} test using omnivorous ostracod *Heterocypris incongruens* (Crustacea). Tested animals are supplied as dormant eggs, hatched 3 days before the test and next exposed directly to the analyzed sediments or soil for 6 days in a culture media (Chial et al. 2003, Torokne and Toro 2010, Blaise et al. 2004). The test conforms to ISO standard 14371:2012 (available at: https://www.microbiotests.com/ toxkit/freshwater-sediment-toxicity-test-with-ostracodtoxkit-f/).

Algaltoxkit \mathbf{F}^{TM} (MicroBioTests Inc.) is a growth inhibition {ALG} assay conducted on green microalgae *Selenastrum capricornutum* (first renamed as *Raphidocelis subcapitata* and presently as *Pseudokirchneriella subcapitata*). After deimmobilization, the algae are cultured for 72 hours in the presence of water sample taken from the environment. A decrease in optical density (at 670 nm) is an index of algal growth inhibition caused by toxins. The test adheres to OECD and ISO Guidelines (available at: https://www.microbiotests.com/toxkit/freshwater-algae-toxicity-test-with-algaltoxkit-f/) (Vandenbroele et al. 2000, Wielen van der and Halleux 2000, Lucivjanska et al. 2000, Daniel et al. 2004, Baudo et al. 2004, Moser et al. 2009).

Phytotoxkit (MicroBioTest Inc.) is a 72 h seed germination and early growth test with 3 higher plants (monocotyl sorgo, *Sorghum saccharatu* and dicotyls: garden cress, *Lepidium sativum* and white mustard, *Sinapis alba*). Seeds of test plants are sown on the samples of soil or sediments. The decreased germination and root growth in comparison to the control substratum are observed in the cultivation chambers. The test is in analogy to ISO standard 11269-1 (available at: https://www.microbiotests. com/toxkit/phytotoxicity-test-with-phytotoxkit-solid-samples/) (Vliet van der et al. 2012, Baran and Tarnawski 2013).

The obtained results were elaborated as described in the test manuals. The results of ecotoxicological analyses were compared with the abovementioned hydrochemical analyses using a correlation matrix for each case (Table. 2). Principal Component Analysis and Factor Analysis of selected results using the Statistica 10 package were performed as well.

Results

Variability of the biotest results – the basis of the early warning system

Temporal and spatial pattern of ecotoxicity revealed by biotest

Despite noticeable physiologic differences and incompleteness within the matrix of obtained results, biotest applied in the experiment may be ranked concerning their sensitivity in the following order: Ostracodtoxtest (mortality) > Phytotoxtest (root elongation) > Ostracodtoxtest (growth rate) > 48 h Daphtoxtest (mortality/immobilization) > MARA > Algaltoxtest (culture growth) > Phytotoxtest (seed germination) > 24 h Daphtoxtest (mortality/immobilization). Taking into account the criterion of the trophic level of the organisms used in the biotests, the ranking of their sensitivity will be as follows: consumers – omnivorous *H. incongruens* (Ostracodtoxtest – mortality) > producers (Phytotoxtest – root elongation) > consumers – omnivorous *H. incongruens* (Ostracodtoxtest – growth rate) > consumers – phytophagic and detritophagic (48 h Daphtoxtest – mortality/immobilization) > decomposers – bacteria (MARA test) > producers (Algaltoxtest – culture growth) > producers (Phytotoxtest – seed germination) > consumers – phytophagic and detritophagic (24 h Daphtoxtest – mortality/immobilization).

The results showed that the extended exposition of test organisms to the analyzed waters and sediments has more significant effects. In some cases (i.e. for the growth rate in Ostacodtoxtest and culture density in Algaltoxtest), paradoxical effects of exposure were observed as an enhanced vital function of test organisms compared to the effects of control/reference material. The early warning features of environmental risk using the biotests obtained from the three year research are illustrated as spatial and temporal variability of the median and maximum results of the 48-hour Daphtoxtest. The early warning features of environmental risk using the biotests obtained from the three year research are the most evidently illustrated to highlight spatial and temporal variability of the median and maximum results of the 48-hour Daphtoxtest (Fig. 2).

Higher ecotoxicity revealed by all the tests was observed in the samples from the reservoir coastline in the sites T07, followed by T02 and T06 (stagnating water beneath pump station near saddle dams and small, local tributary) and T08 (bed of the Bajerka stream/mill-race – an important tributary). High toxicity was also observed in the water body of the reservoir at the sites Z01 (the deep end of the Bajerka cove near the mouth of the stream and damp pump station), Z05 (in the mouth of the Vistula River) and Z09 (stagnating water in the southern backwater of the Vistula, near saddle dam pump station) – for all locations see Figure 1.

The incidents of enhanced toxicity occurred regularly in the periods of low flow and drought (2010.07.06–2010.08.06; 2011.06.13; 2011.10.05–2011.11.16; 2012.07.17–2012.11.24), except for the slightly increased toxicity revealed by Daphtoxtest, Ostacodtoxtest and MARA test in water samples from the Vistula mouth and bed (Z05, T05/T12) and near the saddle dam pump station (T04) after spring high water/surface runoff (2011.04.27; 2012.04.26) and floods (in 2010.09.12 and 2011.08.12). An example and, at the same time, the most representative temporal and spatial pattern of toxicity detected by the Ostracodtoxtest is presented in Figure 3.

Relations between the results of biotests and hydrochemical indices

To determine the relationships between the results of biotests and hydrochemical indices of water quality, several statistical approaches were applied. Matrices of correlation (Factor Analysis) between the results of any single biotest and the 41 hydrologic and hydrochemical water quality parameters were analyzed. Principal Component Analysis was also applied.

Factor analysis

Factor Analysis and Principal Component Analysis were conducted separately for the T (coastal) and Z (main water body)

sites of the reservoir and both of them together. 41 hydrochemical and hydrologic parameters were considered, including damming level, water inflow, air and water temperature, Secchi disk visibility, oxygen and chlorophyll concentrations (see Table 2).

Mortality and growth inhibition of ostracods in Ostracodtoxtest (OD, OG) was weakly but positively (|r| > = 0.25) correlated (Tab. 3) with DL30, W_ (index of sunny weather), TDC, ALC, COND, Fe, Cu, Zn, SiO2 and negatively correlated with NNO3, NNO2 and pH. In the coastline sites (T), mortality was also positively correlated with Tw, SOLUB, SUSP, and Cd, while within the basin of the reservoir (Z) positively with COD, BOD5, and K, but negatively with Ta, TDN. These T site's relationships with Tw, DL30 and W_ suggest an abundance of temperature-dependent processes in the shallow water near the coast. Additionally, in site Z (in the water body of the reservoir), positive correlations of OD and OG with DL, DL30, BOD, Cd, and Pb were observed. The results of the MARA test were, in general, correlated positively with the results of the Daphtoxtest, NNH4, NNO2, TDN, SO4, ALC, Ca, Mg and COND. These were similar to the general pattern observed for OD and OG – as ammonia (NNH4), alkaline metal (ALC), and conductance (COND) increased ecotoxicity index and an increase in NNO3 and pH lowered it (for OD and OG). In the basin of the reservoir (Z sites), the results of MARA were also correlated positively with DL, V, V30d, Ta, NNH4, NNO2, PnPO4, TDP, and HdT, which resembled relationships among OG and hydrologic indices (DL, V, V30d) in the same site types (Z).

The ecotoxicity shown by Daphtotoxtest (D24 and D48) was positively correlated with the MARA test, Ta, Na and Cl results. In the water body of reservoir (Z sites), the results of Daphtoxtest were also positively correlated with V, NNH4, TDN, PPO4, PnPO4, TDP, and BOD5, which was similar to relations affecting MARA test in Z sites.

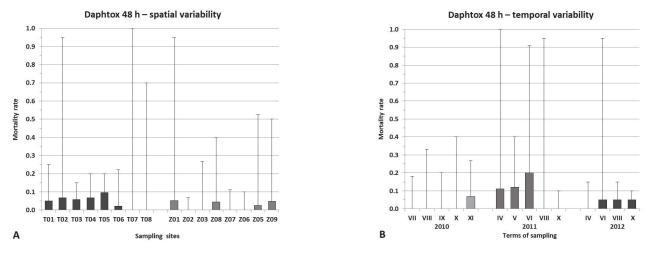


Fig. 2. Results for the Daphtoxkit F 48 as an index of early warning system indicator – mortality rate [0–1] of *Daphnia magna* after 48 h exposition to water samples from Goczalkowice Reservoir expressed as median value (bars) and maximal value (whiskers): A) spatial variability (sampling site codes on the abscissa) – the median for the site for 2010–2012, and B) temporal variability (terms of sampling – Latin numbers of months and years on the abscissa) – the median for the particular month from all sites. Y-axis represents the tested organisms' mortality rate (0–1 range). Critical level of significant ecotoxicity above mortality rate higher than 0.20, as stated in the Daphtox test manual

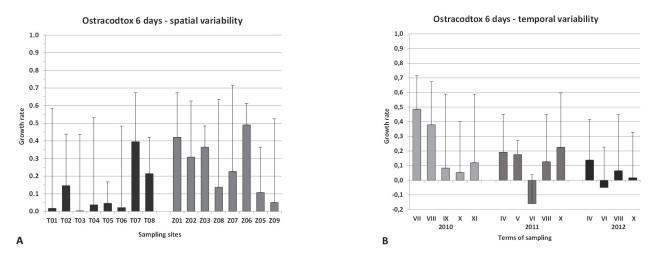


Fig. 3. Spatial (A) and temporal (B) pattern of sediment toxicity revealed as a reduction of the growth rate (control value/sample) of ostracods exposed to the sediments from Goczalkowice Reservoir for 6 days in Ostracodtoxkit F test expressed as a rate [0–1]. Ostracod body size should be measured unless the mortality rate exceeds P < 0.30. Abbreviations and explanations as in Tab. 2 and Fig. 2</p>

Table 3. Results of Factor Analysis – tables of correlation coefficients among results of three biotests and all analyzed variables for T and Z sites in the Goczalkowice reservoir

		Tsit	es (T04, T(T sites (T04, T05/T12, T06, T08)), T08)				Zs	Z sites (Z01, Z05, Z08, Z09)	Z05, Z08, 2	(60Z		
Variable		Standard		Correlatic	Correlation coeficient value	nt value		N S S	Standard		Correlation	Correlation coeficient value	ent value	
	Mean	Deviaton	QO	90	MARA	D24	D48	Mean	Deviation	QO	90 0	MARA	D24	D48
ЪГ	254.69	0.20	0.11	-0.05	-0.03	0.17	0.16	254.60	0.25	0.16	0.33	0.34	-0.24	0.20
DL30	254.64	0.18	0.28	-0.08	0.10	0.26	0.20	254.58	0.23	0.24	0.38	0.23	-0.24	0.06
>	881268	1100707	-0.29	0.12	-0.15	60.0-	-0.07	672921	874818	-0.01	0.19	0.35	-0.15	0.30
V30d	21988904	14142197	-0.25	0.18	-0.07	-0.03	-0.05	18978692	12565459	0.00	0.28	0.30	-0.28	0.24
Та	12.94	7.77	-0.15	0.18	-0.04	0.25	0.29	14.46	6.70	-0.31	-0.24	0.26	-0.09	0.30
Τw	15.84	6.38	-0.17	0.26	0.06	0.20	0.17	17.47	5.49	-0.22	-0.06	0.06	-0.17	0.11
×	4.03	0.74	0.23	0.27	-0.08	-0.09	-0.04	4.22	0.75	0.08	0.34	-0.42	-0.46	0.06
OD	0.282	0.280	1.00	0.18	-0.06	0.03	0.13	0.160	0.212	1.00	0.51	0.01	0.21	-0.10
90	0.042	0.364	0.18	1.00	0.10	0.10	0.04	0.182	0.227	0.51	1.00	-0.29	-0.11	-0.03
MARA	0.048	0.110	-0.06	0.10	1.00	0.25	0.01	0.037	0.040	0.01	-0.29	1.00	0.27	0.27
D24	0.038	0.074	0.03	0.10	0.25	1.00	0.88	0.012	0.030	0.21	-0.11	0.27	1.00	0.15
D48	0.122	0.244	0.13	0.04	0.01	0.88	1.00	0.145	0.217	-0.10	-0.03	0.27	0.15	1.00
02								10.30	2.74	-0.07	-0.09	-0.16	-0.05	0.14
SD								0.77	0.36	-0.08	-0.11	-0.10	-0.19	0.17
NNH4	1.44	1.97	0.22	0.14	0.37	0.10	0.07	0.31	0.16	0.00	-0.15	0.50	0.15	0.29
NNO2	0.03	0.03	-0.27	0.06	0.36	-0.11	-0.20	0.02	0.01	-0.25	-0.44	0.31	-0.05	0.09
NNO3	0.98	0.92	-0.28	-0.04	0.15	-0.25	-0.31	1.13	0.80	-0.25	-0.25	-0.09	-0.24	0.13
DON	0.68	0.32	-0.14	0.25	0.09	-0.21	-0.18	0.79	0.23	0.16	0.15	-0.10	0.13	0.11
TDN	3.13	2.52	0.05	0.13	0.36	-0.04	-0.08	2.25	0.76	-0.21	-0.25	-0.01	-0.18	0.24
PPO4	0.069	0.097	0.13	0.08	-0.10	-0.15	-0.22	0.014	0.016	0.11	-0.21	-0.15	0.06	0.25
PnPO4	0.100	0.099	0.02	0.05	0.03	-0.01	-0.18	0.040	0.030	-0.01	-0.24	0.25	0.05	0.45
DOP	0.327	0.319	-0.29	-0.02	0.00	0.02	0.05	0.140	0.130	-0.03	-0.05	0.71	0.06	0.13
TDP	0.496	0.340	-0.23	0.01	-0.02	-0.03	-0.06	0.192	0.133	-0.02	-0.13	0.73	0.08	0.25
N/P	9.5	7.2	-0.04	0.19	0.22	-0.12	-0.14	16.2	9.0	-0.05	0.04	-0.46	-0.03	-0.17
TDC (CT)	21.9	6.8	0.33	0.00	0.24	0.06	0.09	18.9	3.2	0.28	0.32	-0.12	0.09	-0.33

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Pd

Ecotoxicological biotests as tools for continuous monitoring of water quality in dam reservoir

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0.16 -0.06	0.32 0.10	0.11 -0.44	0.00 -0.45	0.03 -0.14	0.12 -0.13	0.02 -0.14	0.13 -0.08	0.10 -0.29	0.23 -0.14	-0.13 0.14	0.05 -0.21	0.14 -0.19	0.11 0.01	-0.10 -0.13	0.01 -0.06	-0.19 -0.14	-0.24 -0.07	-0.15 -0.16	-0.04 0.10	
-0.17	0.01	-0.14	0.14	0.35	0.41	0.35	0.17	-0.13	-0.01	-0.16	0.07	-0.09	-0.05	-0.18	-0.04	-0.10	-0.04	-0.10	0.19	0 1 4
0.42	0.31	-0.17	0.06	-0.07	0.01	-0.07	0.03	-0.19	0.13	-0.18	0.11	-0.24	0.20	0.20	0.55	0.45	0.42	0.45	0.42	
0.34	0.42	-0.07	0.09	0.03	0.15	0.02	0.13	-0.11	0.28	-0.21	0.20	-0.08	-0.03	0.06	0.53	0.24	0.16	0.30	0.40	90.0-
6.2	1.8	5.9	3.8	0.23	0.18	4.4	0.49	3.28	0.70	0.56	36	39	13.70	101	0.60	5.53	0.028	0.408	1.13	202
17.8	4.8	16.4	19.7	1.48	1.14	24.6	3.08	10.05	2.87	8.15	203	147	16.27	126	1.63	6.26	0.039	0.335	2.46	30.8
-0.03	0.07	0.39	0.08	0.01	0.24	0.16	0.20	0.36	-0.01	-0.16	0.20	0.24	0.18	-0.04	-0.11	-0.02	00.00	0.20	0.09	
0.02	0.12	0.28	0.11	-0.07	0.21	0.15	0.22	0.22	-0.02	-0.08	0.14	0.17	0.15	0.06	-0.15	-0.03	0.05	0.23	0.07	
0.08	0.13	0.29	0.49	0.02	0.35	0.35	0.51	0.20	0.16	-0.29	0.30	0.35	0.08	0.01	-0.06	0.19	-0.06	-0.03	0.23	
0.13	0.13	0.00	-0.12	0.00	-0.09	-0.12	0.02	-0.15	-0.02	0.09	-0.07	-0.12	0.32	0.10	0.06	0.14	0.01	-0.03	-0.07	
-0.08	-0.02	0.24	-0.08	0.20	0.29	-0.08	0.22	0.18	-0.02	-0.43	0.29	0.27	0.46	0.28	0.30	0.35	-0.16	0.03	0.24	
6.2	2.2	10.9	8.0	3.60	0.47	5.9	1.97	6.88	1.13	0.49	06	52	19.35	385	1.94	21.67	2.560	1.600	3.62	
20.1	4.6	19.6	22.0	2.38	1.42	28.2	4.05	12.80	3.04	7.40	256	163	22.38	250	1.88	12.92	0.526	0.726	4.89	
COD	BOD5	Ū	S04	НdТ	ALC	Ca	Mg	Na	¥	Ηd	COND	SOLUB	SUSP	Fe	Cu	Zn	Cd	Pb	SiO2	CHL-a

Abbreviations as in Tab. 2. Statistically significant values of r p < 0,05 when |r| > 0.30| for n > 40

Principal Component Analysis

PCA was conducted for five biotests (D24, D48, MARA, OD, OG) and 38 or 41 environmental indices (Tab. 4). Among many factors distinguished by PCA (43 for combined T & Z sites, 35 and 26 for separate Z and T sites, respectively), only 4 factors fulfilling Cattell's criterion and explaining more than 7% of overall variation were analyzed. These four factors determined about

52–56% of overall variability. Factor 1, calculated independently either for coastal (T) and main body (Z) sites or for both (T and Z), determined about 20% of total results variability, while factor 2 from 12 to 15%, factor 3–8–10%, and factor 4–7–9%. Despite the dataset being taken for analyses (T and Z sites, either together or separately), factors 1, 2 and 3 revealed similar patterns of relationships and determined about 45% of the variability (Tab. 4).

 Table 4. Results of Principal Component Analysis – tables of principal factor 1 and 2 coordinates of variables for T and Z sites in Goczalkowice reservoir – ranged from minimal to maximal values

	T Sites (T04,	T05, T06, T08)			Z Sites (Z01, Z	Z05, Z08, Z09)	
Variable	Factor 1 scores	Variable	Factor 2 scores	Variable	Factor 1 scores	Variable	Factor 2 scores
COND	-0.935	DL	-0.788	DL	-0.629	Cu	-0.755
Mg	-0.904	V30d	-0.750	V30d	-0.615	Zn	-0.712
ALC	-0.894	TDP	-0.685	V	-0.553	Pb	-0.696
SOLUB	-0.886	DOP	-0.681	PnPO4	-0.534	Cd	-0.611
TDC (CT)	-0.878	V	-0.665	SD	-0.510	OG	-0.603
NNH4	-0.861	Pb	-0.579	DL30	-0.461	DL	-0.583
CI	-0.828	DL30	-0.564	D48	-0.421	DL30	-0.559
Na	-0.815	Та	-0.505	W_	-0.407	CHL-a	-0.550
TDN	-0.812	Cd	-0.481	TDP	-0.357	SiO2	-0.508
Ca	-0.703	Tw	-0.386	PPO4	-0.335	V30d	-0.497
SiO2	-0.696	SiO2	-0.293	NNH4	-0.327	OD	-0.456
К	-0.674	D24	-0.220	TDN	-0.317	Mg	-0.434
SO4	-0.558	NNH4	-0.189	pН	-0.316	ALC	-0.410
NNO2	-0.486	D48	-0.188	NNO3	-0.294	V	-0.394
MARA	-0.430	SUSP	-0.176	DOP	-0.207	DOP	-0.386
SUSP	-0.425	Fe	-0.169	Та	-0.200	W_	-0.370
Zn	-0.402	BOD5	-0.164	O2	-0.162	TDP	-0.347
N/P	-0.379	ALC	-0.156	Cd	-0.133	к	-0.336
Fe	-0.322	Ca	-0.146	MARA	-0.122	TDC (CT)	-0.323
DON	-0.293	PPO4	-0.145	Cu	-0.076	COD	-0.314
PPO4	-0.279	TDN	-0.143	SiO2	-0.044	NNH4	-0.306
BOD5	-0.269	Mg	-0.142	Tw	-0.029	COND	-0.290
NNO3	-0.268	Zn	-0.137	OG	0.003	Fe	-0.288
OD	-0.260	NNO3	-0.129	Pb	0.028	MARA	-0.275
COD	-0.211	W	-0.094	SUSP	0.079	SO4	-0.256
D48	-0.191	Cu	-0.082	OD	0.108	BOD5	-0.220
D24	-0.171	OG	-0.072	Fe	0.168	HdT	-0.171
DL30	-0.118	TDC (CT)	-0.035	DON	0.185	Tw	-0.124
Pb	-0.074	PnPO4	-0.017	BOD5	0.191	Та	-0.118
Tw	-0.066	COND	-0.011	Zn	0.207	Са	-0.109
Cu	-0.047	HdT	0.009	D24	0.208	02	-0.078
Та	-0.027	MARA	0.023	CHL-a	0.242	SUSP	-0.061
HdT	-0.025	NNO2	0.025	NNO2	0.278	D48	0.016
OG	-0.015	OD	0.043	COD	0.373	DON	0.016
DL	0.011	pН	0.068	N/P	0.390	PPO4	0.032



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W_	0.048	Na	0.114	Са	0.392	рН	0.032
TDP	0.085	SOLUB	0.123	HdT	0.476	PnPO4	0.072
DOP	0.103	CI	0.149	ALC	0.568	D24	0.076
V30d	0.123	SO4	0.204	Mg	0.597	NNO2	0.107
Q (V)	0.161	COD	0.222	COND	0.697	CI	0.125
Cd	0.165	К	0.271	К	0.701	Na	0.145
PnPO4	0.234	DON	0.410	SO4	0.707	SOLUB	0.192
рН	0.526	N/P	0.568	SOLUB	0.720	SD	0.237
				TDC (CT)	0.755	TDN	0.261
				Na	0.879	NNO3	0.306
				CI	0.924	N/P	0.346

Abbreviations as in Tab. 2

Factor 1 was weakly and negatively correlated with (|r| > 0.25; presented in the rank of decreasing value) COND, ALC, Mg, TDC, NNH4, Na, SOLUB, Cl, Ca, K, SO4, NNO2, MARA, SUSP, OD, and D24, but positively correlated with pH. When calculated for sites Z, factor 1 showed these relations in a reverse manner - as positive correlations, at the same time being negatively correlated with hydrologic parameters DL, V30d, V, DL30 as well PnPO4 and SD. These relationships seemed consistent with the previous observation that toxicity increases during low damming - low flow periods. Factor 2 showed negative correlations with DL, V30d, TDP, V, DL30, DOP, Pb, Cd, and SiO2 affecting the results of MARA, OD, D24 and OG only in sites Z. Positive correlation between Factor 2 and N/P ratio was characteristic in all sites (T and Z). The above relationships among OD, OG, V30d and DL30 may reflect the described earlier effects of floods and spring surface runoff. Factor 3, affecting results of OD, revealed a negative correlation with Tw, Ta, DOP, V30d, Ca, V, SO4 and a positive correlation among Fe, Zn, and Cu. Positive correlations of factor 3 with SiO₂, PPO4, and PnPO4 were also demonstrated in T sites. These relationships may reflect the effects of low flow in cold months of the year. Factor 4 revealed concordant relationships among D24 and D48 tests and thermal conditions - Ta, Tw.

Discussion

Biotests are the modern tools used for monitoring environmental quality and possess numerous advantages compared to traditional chemical analyses. They are relatively cheap, do not need large quantities of analyzed environmental material, and are readily available since indicatory organisms are stored in cryptobiotic form, and many samples may be analyzed at the same time (Mankiewicz-Boczek et al. 2008). Moreover, the tests are technically simple, time-saving and relatively fast-lasting from one day to a week, reproducible, repeatable and replicable under laboratory conditions (Namiesnik and Szefer 2010). Their great advantage relies on the registration of actual toxic effects independently of the chemical composition of environmental media and all the possible interactions (Latif and Licek 2004, Kahru et al. 1999). This feature solves the problem of environmental pollution caused by a high number of chemical compounds produced, sold, used and finally introduced into the environment, representing a tiny percentage of over 196 million substances registered in the Chemical Abstracts Service database CAS, July 2022 (CAS Registry 2022). Most of these substances interact with organisms at the NOEL (No Observed Effect Level) level for a long time. Notwithstanding that biotests cannot substitute for precise chemical analyses with particular methods, they may reduce the necessity to apply complex and expensive chemical methods only to the cases when toxic effects have occurred in the environment (Gabrielsson et al. 2003, Namiesnik and Szefer 2010, Latif and Licek 2004).

At present, biotests were applied either for the detection of the effects of a particular chemical agent under laboratory conditions (Nałęcz-Jawecki et al. 2010) or to assess the overall toxicity of material taken from the environment (Chial et al. 2003, Manusadzianas et al. 2003, Persoone et al. 2003, Latif and Licek 2004, Czerniawska-Kusza et al. 2006, Blaise and Ferard 2006, Fai and Grant 2010, Palma et al. 2010, Torokne and Toro 2010, Heisterkamp et al. 2021, Szara-Bąk et al. 2021, Szklarek et al. 2021). Several studies focused on the relationships among the chemical composition of environmental samples or model laboratory mixtures and their effects revealed in biotests (Kahru et al. 1999, Olkova and Berezin, 2021). In other research, biotest results were compared with chemical analyses in highly contaminated environmental matrices such as wastewaters (Manusadzianas et al. 2003, Czerniawska-Kusza et al. 2006, Cloete et al. 2017, Zgórska et al. 2020). In most of the cited studies, tested samples produced significant toxicity due to the high content of noxious substances. In our study, the tested material from Goczalkowice Reservoir did not contain high levels of toxic substances since the overall quality of the water was relatively good (corresponding to the I or II class of water quality according to unpublished results gathered by the team of the Institute for Environmental Engineering). In a few cases, sediments and water collected from the near shore areas showed increased toxicity in biotests. A low concentration of toxic agents, which was also confirmed by chemical analyses, may not result in high values of correlation coefficients suggesting causal relationships between the content of toxic compounds and the results of biotests. Thus, in our experiment, the effects observed with the used microbiotest did not fall into the toxicity classification system proposed by Persoone and co-authors (2003).

Moreover, in several cases, the observed vital functions of organisms constituting biotest used in our experiments were stimulated compared to the control. These kinds of results may reflect the hormetic effects of low contamination with particular unidentified substances (Calabrese 2004), which are noxious at higher doses but may be stimulating or even necessary at low doses, according to the basic rule of Shelford. The other explanation is that particular test species used in the biotest supplied additional nutrients (minerals for algae or prey/bacteria for filter feeders), which may enhance their growth and survival.

Sensitivity of the biotests

The results demonstrated that the duration of exposure to environmental material enhanced test sensitivity in an obvious manner. Thus, in this respect, the biotest used in the present experiment may be ranged in order: Ostracodtoxtest (mortality) > Phytotoxtest (root elongation) > Ostracodtoxtest (growth rate) > 48 h Daphtoxtest (mortality) > MARA > Algaltoxtest (culture growth) > Phytotoxtest (seed germination) > 24 h Daphtoxtest (mortality). The battery of microbiotests used in this experiment reflects the idea of testing various trophic levels of the environment (Szklarek et al. 2021), where Phytotoxtest, Algaltoxtest represents producers, Daphtoxtest – phytophagic consumers, Ostracodtoxtest – omnivorous consumers, and MARA test – microbial decomposers.

In the tested material from the water body of the reservoir, no significant effects of typical toxins, such as heavy metals (Cd, Pb) or transitory metals (Fe, Cu, Zn), have been determined based on chemical analysis as well as the results of biotests, which was further confirmed by analysis of correlation and PCA. The results of the chemical analysis do not show the presence of polycyclic hydrocarbons and other organic toxins from the list of priority substances (analyses in unpublished data; Institute of Environmental Engineering, 2010-2013). The presence of algal toxins is also excluded since, during the whole project, no algal bloom was observed. Local physical conditions (pO2, pH, temperature) at the sampling sites could not affect the result of biotests conducted under stable and optimal laboratory conditions. However, some of the substances present in the samples may stimulate the growth of green algae in Algaltoxtest, acting as fertilizers.

Effects of extreme hydrologic events on biotests

High values of biotest results indicating elevated water toxicity were observed either near selected point sources of contamination – saddle dam pump stations (T04, T06) or in the sites representing main tributaries (T08, T012/T05, Z05). In the sites representing the Vistula river bed (T12/T05, Z05), incidents of elevated ecotoxicity indicated by OG, MARA and D24 were related to periods of floods or high flow. In the coastline sites and sites of stagnant water (T04, T06, Z09, Z01), high values of OD, OG, and MARA tests are attributed to low flow and drought periods. The results of OD and MARA tests in some coastal sites (T04, T06) may also reflect water contamination caused by spring surface runoff of slowly decomposing winter deposits.

In conclusion, the battery of biotests may reflect extreme hydrologic events affecting water quality in characteristic sites of the examined reservoir.

Relations revealed by Factor Analysis

The results of Factor Analysis reveal a negative correlation between mortality and growth inhibition of ostracods (OD and OG) with the 30-day mean damming level (DL30), total dissolved carbon (TDC) and water alkalinity (ALC), conductivity and concentration of transitory metal ions (Fe, Cu, Zn), which may reflect effects of contaminations carried with inflowing water. However, the opposed relations among damming and water flow indices (DL30, V30d) and ecotoxicological indices (OD, OG) - negative in coastal (T) while positive in the main basin (Z) sites, together with an inverse relation to the temperature (Ta, Tw) - suggested that near the coastline (T sites) occurred a detrimental temperature and low follow the dependent process, whilst in the open water body of reservoir (Z sites) occurred an opposing, water quality improving natural processes related to high flow.

The results of the MARA test were, in general, correlated positively with the results of the Daphtoxkit test, increased levels of nitrogen compounds (NNH_4 , NNO_2 , TDN), alkali substances (ALC, Ca, Mg) and water conductivity (COND). These were similar to the general pattern of increased ecotoxicity registered by OD and OG, correlated with ammonia, alkaline metals and water conductivity concentrations and reduced ecotoxicity by elevated NNO3 and pH. In the basin of the reservoir (Z), positive correlations of MARA results with DL, V, V30d, and Ta have resembled relationships among OG and hydrologic indices (DL, V, V30d) in the same type sites (Z).

Relationships among the Daphtoxtest results (D24 and D48) and environmental indices: Ta, Na and Cl in the coastal sites (T) and with V, NNH4, TDN, PPO4, PnPO4, TDP, BOD5 in the water body of reservoir (Z) were similar to relations affecting MARA test.

Described relationships between ecotoxicity in Ostracodtoxtest (OD, OG) and MARA test and indices of alkalinity (ALC, Ca, Mg), water conductivity (COND) and concentration of transitory metals acting as microelements (Fe, Cu, Zn) suggest more complex determinants of water quality in relatively non-polluted dam reservoirs than those based on the presence of highly toxic substances. Moreover, the relation of Ostacodtoxtest, MARA, and Daphtoxtest results with hydrologic variables (DL30, V30d, DL, V) showed possible effects of local processes depending on the release of noxious agents from the reservoir bottom during long-lasting changes (decrease followed by an increase) of damming level (remobilization of contaminants through flood/high flow events).

Relations revealed by PCA

Relations revealed by Primary Component Analysis as Factor 1 correlations among increased indices total content of solutes, alkalinity (COND, ALC, Mg, Na, SOLUB, Cl, Ca, K, SO4, SUSP), damming level (DL; V30d; V; DL30) and ecotoxicity (MARA, OD, D24,) seemed to be consistent with the previous conclusions that water quality depends in an opposite manner on the hydrology and processes occurring in the main basin and near the coastline of the reservoir. Biotests' results worsen within the main reservoir basin during low damming – low flow periods, while improving in the coastal sites (Fig. 4).



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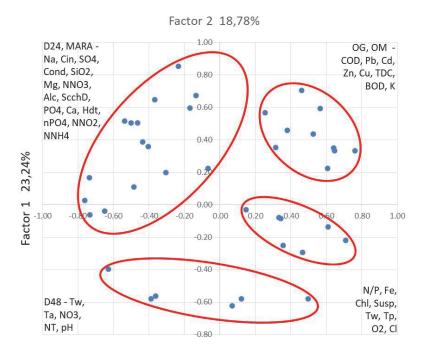


Fig. 3. Results of Principal Component Analysis – a graph of the configuration of points representing variables in the system of the first two factor axes (principal components) for the results of microbiotests and selected hydrochemical indices (abbreviations of variables as in Table. 2 and the text)

Factor 2 showed that in Z sites, high values of damming (DL, DL30, V, V30d) and TDP, DOP, Pb, Cd, SiO2 are associated with an improvement of ecotoxicological indices (MARA, OD, D24 and OG). A positive correlation between Factor 2 and the N/P ratio was characteristic in all sites (T and Z). The above relation may reflect the described earlier effects of floods. Factor 3 – revealing association among high V, V30d, Ta, Tw, DOP, Ca, SO4 and low ecotoxicity in Ostracodtoxtest (OD) may reflect adverse effects of low flow in cold months of the year on water quality. Factor 4 revealed concordant relations among D4, D48 tests and thermal conditions – Ta, Tw.

The results of detailed Factor Analysis and PCA demonstrated that the reservoir system is highly complex. None of the investigated indices alone was decisive for the overall status of the reservoir and ecotoxicity revealed with biotests. Moreover, the causal relationships seemed to be fluent and site-dependent – opposite near the coastline and in the water body of the reservoir. None of the biotests is specific to any of the hydrochemical variables analyzed. The biotests may, however, reflect the hydrological status of the reservoir. Moreover, as shown by FA and PCA, in the absence of toxic compounds, the results of biotests may also detect the level of minerals and microelements which contribute to the general trophy of the reservoir. These features suggest that the battery of the biotest may serve as a universal tool for continuous monitoring.

Conclusions

The battery of biotests applied for continuous monitoring of water quality in relatively unpolluted dam reservoirs can detect and localize incidental contamination and may be used as a cost-effective early warning tool.

Incidental toxic effects revealed by the biotest could be attributed to extreme hydrologic events and overall water quality in the reservoir. However, they could be regarded as the results caused by unknown or not analyzed physicochemical or biological factors.

Signs of ecotoxicity revealed by the biotest battery in Goczalkowice Reservoir correlate with a set of hydrochemical indices representing a concentration of nontoxic or low toxic chemical compounds, which are decisive for the mineralization, hardness and conductivity of water as well as general trophy of the reservoir. These results suggest that biotests might help monitor the trophic status of dam reservoirs.

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References

- Baran, A. & Tarnawski, M. (2013). Phytotoxkit/Phytotestkit and Microtox[®] as tools for toxicity assessment of sediments. *Ecotoxicology and Environmental Safety*, 98, pp. 19–27.
- Baudo, R., Sbalchiero, A. & Beltrami, M. (2004). Test di Tossicita acuta con *Daphnia magna* (Acute toxicity test with *Daphnia magna*). *Biologi Italiani*, 6, pp. 62–69.



- Blaise, C., Gagné. F., Chèvre, N., Harwood, M., Lee, K., Lappalainen, J., Chial, B., Persoone, G. & Doe, K. (2004). Toxicity assessment of oil-contaminated freshwater sediments. *Environmental Toxicology*, 19, 4, pp. 267–273.
- Blaise, C. & Férard, J-F. (2006). Microbiotests in aquatic toxicology: the way forward. [in:] *Environmental Toxicology*, Kungolos, A., Brebbia, C.A., Samaras, C.P. & Popov, V. (Eds.). UK WIT Press, Southampton, pp. 339–348.
- Calabrese, E.J. (2004). Hormesis: A revolution in toxicology, risk assessment and medicine. *EMBO Reports*, 5, Suppl 1, pp. S37–S40.
- CAS Registry. (2022). CAS REGISTRY[®]. A division of the American Chemical Society. (https://www.cas.org/cas-data/cas-registry (14.07.2022))
- Chial, B.Z., Persoone, G. & Blaise, C. (2003). Cyst-based toxicity tests. XVIII. Application of ostracodtoxkit microbiotest in a bioremediation project of oil-contaminated sediments: sensitivity comparison with *Hyalella azteca* solid-phase assay. *Environmental Toxicology*, 18, 5, pp. 279–283.
- Cloete, Y.C., Shaddock, B.F. & Nel, A. (2017). The use of two microbiotests to evaluate the toxicity of sediment from Mpumalanga, South Africa. *Water SA*, 43, pp. 409–412. DOI:10.4314/wsa.v43i3.05
- Czerniawska-Kusza, I., Ciesielczuk, T., Kusza, G. & Cichoń, A. (2006). Comparison of the Phytotoxkit Microbiotest and chemical variables for toxicity evaluation of sediments. *Environmental Toxicology*, 21, pp. 367–372.
- Daniel, M., Sharpe, A., Driver, J., Knight, A.W., Keenan, P.O., Walmsley, R.M., Robinson, A., Zhang, T. & Rawson, D. (2004). Results of a technology demonstration project to compare rapid aquatic toxicity screening tests in the analysis of industrial effluents. *Journal of Environmental Monitoring*, 6, pp. 855–865.
- EU Water Framework Directive. (2000). Directive 2000/60/EC of the European Parliament and of the Council of October 23, 2000 establishing a framework for Community action in the field of water policy. *Official Journal L*, 327, 22/12/2000, pp. 1–73.
- Fai, P.B. & Grant, A. (2010). An assessment of the potential of the microbial assay for risk assessment (MARA) for ecotoxicological testing. *Ecotoxicology*, 19, 8, pp. 1626–1633.
- Gabrielson, J., Kühn, I., Colque-Navarro, P., Hart, M., Iversen, A., Mckenzie, D. & Möllby, R. (2003). Microplate-based microbial assay for risk assessment and (eco)toxic fingerprinting of chemicals. *Analytica Chimica Acta*, 485, pp. 121–130.
- Gagne, F. & Blaise, C. (2005). Review of biomarkers and new techniques for in situ aquatic studies with bivalves, [in:] *Environmental Toxicity Testing*, Thompson, K.C., Wadhia, K. & Loibner, A.P. (Eds.). Blackwell Publishing Ltd., Oxford, pp. 206–228.
- Goczałkowice Resorvoir. (2022). The Goczałkowicki reservoir (the so-called Goczałkowickie Lake). (http://web.archive. org/web/20140828020743/http://www.gocz.pl:80/content/ view/63/39 (14.07.2022)). (in Polish)
- Górecki, T. & Heba El-Hussieny, M. (2010). Total Parameters as a Tool for the Evaluation of the Load of Xenobiotics in the Environment. [in:] *Analytical Measurements in Aquatic Environment*, Namiesnik, J. & Szefer, P. (Eds.). CRC Press, Taylor & Francis Group, Boca Raton, pp. 223–241.
- Heisterkamp, I., Ratte, M., Schoknecht, U., Gartiser, S., Kalbe, U. & Ilvonen O. (2021). Ecotoxicological evaluation of construction products: inter-laboratory test with DSLT and percolation test eluates in an aquatic biotest battery. *Environmental Science Europe*, 33, pp. 1–14. DOI:10.1186/s12302-021-00514-x
- Jabłońska-Czapla, M., Kowalski, E. & Mazierski, J. (2013). The role of point and non-point water pollution in metal deposits dispersion in Goczalkowice water reservoir, [in:] *Current issues in water treatment and distribution*, Zimoch, I. & Sawiniak, W. (Eds.). Institute of Water and Wastewater Engineering, Silesian University of Technology, Gliwice, pp. 47–57. (in Polish)

- Journal of Laws. (2009). Regulation of the Minister of the Environment of May 13 2009 on the forms and methods of monitoring surface and groundwater bodies, Journal of Laws of the Republic of Poland 2009 No. 81, item 685, (https://dziennikustaw.gov.pl/ DU/2009/s/81/685 (14.07.2022)). (in Polish)
- Journal of Laws. (2011). Regulation of the Minister of the Environment of November 15, 2011 on the forms and methods of monitoring surface and groundwater bodies, Journal of Laws of the Republic of Poland 2011 No. 258, item 1550, (https://dziennikustaw.gov. pl/DU/2011/s/258/1550 (14.07.2022)). (in Polish)
- Kahru, A., Põllumaa, L., Reiman, R. & Rätsep, A. (1999). Predicting the toxicity of oil-shale industry wastewater by its phenolic composition. *Alternatives to Laboratory Animals*, 27, pp. 359–366.
- Kielka, E., Siedlecka, A., Wolf, M., Stróżak, S., Piekarska, K. & Strub, D. (2018). Ecotoxicity assessment of camphor oxime using Microtox assay – preliminary research. E3S Web of Conferences 44, 00066. DOI:10.1051/e3sconf/20184400066
- Kostecki, M., Kernert, J., Nocoń, W. & Janta-Koszuta, K. (2013). Seasonal and spatial variability of selected hydrochemical indices in Goczalkowice Reservoir. [in:] *Current issues in water treatment and distribution*, Zimoch, I. & Sawiniak, W. (Eds.). Institute of Water and Wastewater Engineering, Silesian University of Technology, Gliwice, pp. 93–103. (in Polish)
- Latif, M. & Licek, E. (2004). Toxicity assessment of wastewaters, river waters, and sediments in Austria using cost-effective microbiotests. *Environmental Toxicology*, 19, 4, 302–309.
- Lucivjanska, V., Lucivjanska, M. & Cizek, V. (2000). Sensitivity comparison of the ISO Daphnia and algal test procedures with Toxkit microbiotests. [in:] New Microbiotests for Routine Toxicity Screening and Biomonitoring, Persoone, G., Janssen, C. & De Coen, W. (Eds.). Kluwer Academic/Plenum Publishers, New York, pp. 243–246.
- Mankiewicz-Boczek, J., Nałęcz-Jawecki, G., Drobniewska, A., Kaza, M., Sumorok, B., Izydorczyk. K.M., Zalewski, M. & Sawicki, J. (2008). Application of a microbiotests battery for complete toxicity assessment of rivers. *Ecotoxicology and Environmental Safety*, 71, 3, pp. 830–836.
- Manusadzianas, L., Balkelyte, L., Sadauskas, K., Blinova, I., Põllumaa, L. & Kahru, A. (2003). Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests and correspondence between toxicity and chemical-based indices. *Aquatic Toxicology*, 63, 1, pp. 27–41.
- Maradona, A., Marshall, G., Mehrvar, M., Pushchak, R., Laursen, A.E., Mccarthy, L.H., Bostan, V. & Gilbride, K.A. (2012). Utilisation of multiple organisms in a proposed early-warning biomonitoring system for real-time detection of contaminants: preliminary results and modeling. *Journal of Hazardous Materials*, 219–220, pp. 95–102.
- Moser, H., Angrick, M. & Römbke, J. (2009). *Ecotoxicological Characterisation of Waste: Results and Experiences of an International Ring Test*, Springer, Stuttgart, 2009.
- Nałęcz-Jawecki, G., Wadhia, K., Adomas, B., Piotrowicz-Cielak, A.I. & Sawicki, J. (2010). Application of microbial assay for risk assessment biotest in evaluation of toxicity of human and veterinary antibiotics. *Environmental Toxicology*, 25, 5, pp. 487–494.
- Nature 2000 Area. (2022). Central Register of Forms of Nature Protection. GDOŚ, (https://crfop.gdos.gov.pl/CRFOP/widok/ viewnatura2000.jsf?fop=PL.ZIPOP.1393.N2K.PLB240001.B (14.07.2022)). (in Polish)
- Olkova, A. & Berezin, G. (2021). "Battery of bioassays" for diagnostics of toxicity of natural water when pollution with aluminum compounds. *Journal of Ecological Engineering*, 22, 2, pp.195–199. DOI:10.12911/22998993/131029
- Palma, P., Alvarenga, P., Palma, V., Matos, C., Fernandes, R.M., Soares, A. & Barbosa, I.R. (2010). Evaluation of surface water quality



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using an ecotoxicological approach: a case study of the Alqueva Reservoir (Portugal). Environmental Science and Pollution Research, 17, 3, pp. 703-716. DOI:10.1007/s11356-009-0143-3

- Pejman, A.H., Nabi Bidhendi, G.R., Karbassi, A.R., Mehrdadi, N. & Esmaeili Bidhendi, M. (2009). Evaluation of spatial and seasonal variations in surface water quality using multivariate statistical techniques. International Journal of Environmental Science and Technology, 6, 3, pp. 467-476.
- Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K.C., Moreira-Santos, M., Vollat, B., Törökne, A. & Han, T. (2009). Review on the acute Daphnia magna toxicity test - Evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowledge and Management of Aquatic Ecosystems, 393, pp. 1-29.
- Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L. & Kolar, B. (2003). A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environmental Toxicology, 18, pp. 395-402.
- Szara-Bak, M., Baran, A., Klimkowicz-Pawlas, A., Tkaczewska, J. & Wojtasik, B. (2021). Mobility, ecotoxicity, bioaccumulation and sources of trace elements in the bottom sediments of the Rożnów reservoir. Environmental Geochemistry Health, 43, pp. 4701-4718. DOI:10.1007/s10653-021-00957-4
- Szklarek, S., Stolarska, M., Wagner, I. & Mankiewicz-Boczek, J. (2015). The microbiotest battery as an important component in the assessment of snowmelt toxicity in urban watercourses - preliminary studies. Environmental Monitoring Assessment, 187, 16, pp. 1-12. DOI:10.1007/s10661-014-4252-1
- Szklarek, S., Kiedrzyńska, E., Kiedrzyński, M., Mankiewicz--Boczek, J., Mitsch, W.J. & Zalewski, M. (2021). Comparing ecotoxicological and physicochemical indicators of municipal wastewater effluent and river water quality in a Baltic Sea catchment in Poland. Ecological Indicators, 126, pp. 1-12. DOI:10.1016/j.ecolind.2021.107611
- Törökne, A. & Toro, K. (2010). Evaluation of the toxicity of river and creek sediments in Hungary with two different methods. Environmental Toxicology, 25, 5, pp. 504-509.

- Vandenbroele, M.C., Heijerick, D.G., Vangheluwe, M.L. & Janssen. CR (2000). Comparison of the conventional algal assay and the Algaltoxkit F microbiotest for toxicity evaluation of sediment pore waters. [in:] New Microbiotests for Routine Toxicity Screening and Biomonitoring, Persoone, G., Janssen, C. &, De Coen W. (Eds.). Kluwer Academic/Plenum Publishers, New York, pp. 261-268.
- Vliet Van der, L., Velicogna, J., Princz, J. & Scroggins, R. (2012). Phytotoxkit: a critical look at a rapid assessment tool. Environmental Toxicology and Chemistry, 31, 2, pp. 316-323.
- Wadhia, K. & Thompson, K.C. (2007). Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive. Trends in Analytical Chemistry, 26, 4, pp. 300-307.
- Wadhia, K. & Dando, T.R. (2009). Environmental toxicity testing using the Microbial Assay for Risk Assessment (MARA). Fresenius Environmental Bulletin, 18, 2, pp. 213-218.
- Wielen Van der, C. & Halleux, I. (2000). Shifting from the conventional ISO 8692 algal growth inhibition test to the Algaltoxkit F microbiotest. [in:] New Microbiotests for Routine Toxicity Screening and Biomonitoring, Persoone, G., Janssen, C. & De Coen, W. (Eds.). Kluwer Academic/Plenum Publishers, New York, pp. 269-272. DOI:10.1007/978-1-4615-4289-6 44
- Wolska, L., Kochanowska, A. & Namiesnik, J. (2010). Application of Biotests - chapter 9. [in:] Analytical Measurements in Aquatic Environment, Namiesnik, J. & Szefer, P. (Eds.). CRC Press, Taylor & Francis Group, Boca Raton, pp. 189-223.
- Zgórska, A., Bondaruk, J., Dudziak, M. & Hamerla, A. (2020). Impact of industrial discharge on aquatic ecosystems of the Kłodnica River with reference to Water Framework Directive objectives. Polish Journal of Environmental Studies, 29, 4, pp. 2945–2953. DOI:10.15244/pjoes/112931
- Zhengjun, W. & Huili, G. (2010). Evaluating the effectiveness of routine water quality monitoring in Miyun reservoir based on geostatistical analysis. Environmental Monitoring and Assessment, 160, pp. 465-478. DOI:10.1007/s10661-008-0711-x

Biotesty ekotoksykologiczne jako narzędzie do ciągłego monitoringu jakości wody w zbiornikach zaporowych

Streszczenie: Celem pracy była analiza zastosowania biotestów ekotoksykologicznych do oceny ich przydatności jako systemów wczesnego ostrzegania w ciagłym monitoringu nizinnych, płytkich zbiorników zaporowych zlokalizowanych w Europie Środkowej. Zastosowano następujące biotesty: Daphtoxkit FTMmagna, Algaltoxkit FTM, Ostracodtoxkit F, Phytotoxkit i MARA Test. Badania prowadzono od lipca 2010 do grudnia 2012 roku na Zbiorniku Goczałkowickim (Wisła, Polska), który pełnił funkcję modelu badawczego. Do analizy wykorzystano 41 z 52 zmierzonych wskaźników wody celem oceny jej toksyczności na organizmy żywe. Wyniki biotestów skorelowano z 41 hydrochemicznymi wskaźnikami jakości wody. Schemat zależności między wynikiem biotestów i wartościami wskaźników hydrochemicznych oraz wyniki analizy czynnikowej (FA) i analizy składowych pierwszorzędowych (PCA) wykazały, że:

- i) oznaki ekotoksyczności wykryte za pomocą biotestów były związane albo z okresami niskiego przepływu, albo z wiosennym spływem wód powierzchniowych;
- ii) po okresach wzmożonych przepływów wystąpiły pojedyncze przypadki zwiększonej ekotoksyczności w obszarze obniżenia tamy bocznej za przepompowniami zapory;
- iii) podwyższonej toksyczności towarzyszyły wysokie stężenia substancji rozpuszczonych i zawieszonych; iv) FA i PCA wykazały korelacje między wynikami biotestów i parametrami piętrzenia, przewodnością wody, me-
- talami alkalicznymi i przejściowymi (Ca, Fe, Cu, Zn) oraz kilkoma grupami związków azotu, fosforu i węgla.

Uzyskane wyniki analizy sugerują, że baterie biotestów mogą służyć, jako efektywne, nisko kosztowe narzędzie do ciągłego monitorowania jakości wody w zbiornikach zaporowych i mogą wykrywać negatywne skutki ekstremalnych zdarzeń hydrologicznych, lokalnych zrzutów zanieczyszczeń oraz zmian stanu troficznego zbiorników. Wyniki sugerują, że biotesty mogą pomóc w ciągłym monitorowaniu poziomu troficznego zbiorników zaporowych.