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Original Research

Toxicity Assessment of Water Samples from Rivers in Central Poland Using a Battery of Microbiotests – a Pilot Study

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Abstract

An evaluation of wastewater hazards to aquatic environments with biotests can support traditional monitoring. The conventional classification of surface water is based primarily on chemical and physical analyses. The toxicity of samples from 4 Polish rivers – Pilica, Bzura, Ner and Utrata, which are polluted to different degrees, has been assessed with a battery of biotests composed of representatives of producers (micro – algae – *Pseudokirchneriella subcapitata*, duckweed – *Lemna minor*), consumers (rotifer – *Brachionus calyciflorus*, crustaceans – *Daphnia magna*, *Thamnocephalus platyurus*) and decomposers (bacteria – *Vibrio fisheri*, protozoans – *Spirostomum ambiguum*, *Tetrahymena thermophila*). The physico-chemical characteristics of water also have been performed. No permanent and highly toxic effects were observed. The most toxic effects in spring did not find a confirmation in studies in autumn and vice versa. Most test organisms gave responses. However, it is too early to evaluate the sensitivity of biotests and their usefulness in a monitoring system for rivers. A minimum of one more year of study is needed.

Keywords: microbiotest; river water; battery of tests; sensitivity of species; environmental samples

Introduction

In Poland the control of effluents in water is based on physical and chemical parameters [1]. However, there are too many new substances to investigate their amounts in water. In monitoring stations, mostly well-known toxic substances are determined. Other substances, including new ones, which are not on the obligatory list of controlled parameters, are not examined, mostly because of lack of procedures. Additionally, a large number and diversity of toxic substances potentially present in waters make the analyses more time and cost consuming. In practice, it leads to lowering the number of performed analyses. Another shortcoming is the difficulty in assessing the potential toxic effects on aquatic ecosystems. There is a large amount of chemical data in which different compounds are presented often in different concentrations. Furthermore, there is little knowledge about the toxicity of most chemicals and their interactions in the environment.

To achieve a realistic estimation of the hazard of pollutants, it is necessary to know their toxic effects. The solution is to compare chemical analyses with tests on biological systems (biotests). For the last 20 years biotests have been changed and have optimized their cost-effectiveness. They have been miniaturized and in some cases left to evade the problems of test organisms' culturing. Efforts to achieve the standardization of measurement methods also have been made, which has resulted in many test procedure publications. These modifications have made

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biotests a helpful tool to assess the potential toxic effects on aquatic ecosystems [2].

In studies on surface water or landfill leaches and wastewater discharges, biological analyses have already been used next to chemical ones [1, 3-15]. In earlier studies, the toxicity assessment was made on only one type of organism or on a limited set of test species, e.g. fish/daphnia/bacteria/micro-algae. It is known that each test organism can be sensitive to different chemicals. Therefore, it seems to be necessary for environmental samples which can include a variety of toxic substances, not to use one species but a battery of tests. Such batteries make the opportunity to treat data from the tests as the information about the whole ecosystem, which afterwards makes it easier to assess a real hazard in the environment [16].

The aim of the presented study was to evaluate the usefulness of microbiotests battery in a monitoring system of rivers in Poland.

Materials and Methods

Study Area

The research was conducted in Central Poland, in two districts: Łódzkie and Mazowieckie. Rivers Pilica (P), Bzura (B), Ner (N) and Utrata (U) were chosen for this research because they have been changed by humans differently. Their water contains a broad spectrum of different types of discharges, which can cause diversified toxic effects on test species.

Pilica is the longest (319.0 km) left side tributary of the Vistula. It flows through both investigated regions – Łódzkie and Mazowieckie. The study site has been situated upstream of the biggest hydrotechnical object on the river – the Sulejowski reservoir. Water from the study point has been classified to the third purity class according to an order from the minister of the environment [17].

The Bzura is a 166.2 km-long left side tributary of the Vistula. It flows through both research districts, but in Mazowieckie there is only a short down-river stretch. It is not so polluted like the studied rivers Ner or Utrata, but the whole river has been classified into the fourth and fifth purity classes [17]. Water in the middle stretch contains municipal and industrial pollutants from the upstream-located cities of Łódź and Zgierz. In the down stretch most discharges come from Utrata.

Ner is the right-side tributary of the river Warta. It is 125.9 km long and flows only through the Łódzkie district. It is strongly polluted with municipal and industrial wastewater from Łódź and the whole river has been classified into the fourth and fifth purity clasess [17]. Two sampling sites were located near Łódź, upstream and downstream of the wastewater treatment plant outflow, and the second two – further downstream.

Utrata is a 76.5 km long right-side tributary of the river Bzura and it flows only through the Mazowieckie district. Quite like the river Ner it has been used for many years as a wastewater receiver from urban areas near Warsaw. Water from the study point Ua has been classified into the fourth and water from other sampling places to the fifth purity class [17].

Sample Collecting

The water samples were collected in April and in September/October. In spring they were taken at 9 sites. In autumn there were 3 new sampling points (12 sampling points altogether). The samples were taken according to the scheme: water from "a" to "c" was taken from the middle river stretch; samples "d" from the estuary. There was no water from sources of the rivers. The samples were treated immediately after arrival to the laboratory or they were stored not more than a week at 4°C. If the tests were made more than one week after sampling date, they had been frozen till analyses. The waters were not cloudy and colored. Due to that fact and because the aim of the study was to estimate raw water samples, they were not filtered before the toxicity assessment.

Chemical Analyses

At sampling sites all samples were analyzed for pH, conductivity [μS/cm], oxygen content [mg/L] and temperature [°C]. Oxygen content was measured for all samples only in autumn. Analyses for the following compounds were made afterwards: total phosphorus TP [mg/L], phosphate phosphorus PO_4-P [mg/L], total nitrogen TN [mg/L], nitrate nitrogen NO_3-N [mg/L], ammonia nitrogen NH_4-N [mg/L] and the contents of metals [mg/L] – Zn, Ni, Pb, Cd, Cu, Fe, Mg, Ca, Co, Cr. Total phosphorus and phosphate phosphorus were measured using the ascorbic acid method [18]. Total nitrogen was analyzed using persulfate digestion method (method no. 10071) [19]. Nitrate nitrogen was determined using the cadmium reduction method (method no. 8039) [19] and ammonia nitrogen ($N\text{-}NH_4$) – the phenate method [18]. The metal concentrations in samples were determined using atomic absorption spectrometry (AAS). Data from physico – chemical analyses were compared with official data from Voivodship Inspectorate for Environmental Protection (WIOŚ) from two districts: Łódzkie and Mazowieckie. Data about all samples dates from 2005.

Toxicity Tests

Toxicity assessment of water samples was performed using a test battery consisting of 8 species (one species was used in two tests). In order to take the ecological realities into consideration, the battery consisted of organisms belonging to the three trophic levels of aquatic food chains: producers (micro algae and higher plants), consumers (rotifers and crustaceans) and decomposers

(bacteria and protozoans). The whole battery used in this study is listed in Table 1.

- The 72 h growth inhibition test with the green algae *Pseudokirchneriella subcapitata* (renamed *Raphidocelis subcapitata* or *Selenastrum capricornutum*) was performed according to the standard operational procedure of the Algaltoxkit F™[20], which follows OECD Guideline 201 [21] and ISO standard 8692 [22]. There were two versions of this test. The first was made with and the second without the addition of nutrient medium to water samples.
- The 168 h growth inhibition test with *Lemna minor* was performed according to the ISO/WD standard 20079 [23]. The modification is that the test has been carried out in test plates with six immersions, containing 10 ml of samples. Inhibition value was calculated by frond number. Color of fronds as well as the condition and quality of roots was also assessed. Estimation of the morphological condition of duckweed was made using a digital camera connected with the view analysis computer system ImageTool®. This test was performed in two versions like the test with micro algae, with and without nutrient medium.
- The 48 h immobilization test with *Daphnia magna* was performed according to the standard operational procedure of the Daphtoxkit F™ magna [24], which follows OECD Guideline 202 [25] and ISO standard 6341 [26].
- The acute 24 h test determining the mortality of *Brachionus calyciflorus* was performed according to the standard operational procedure of the Rotoxkit F™ [27]. This test was used for spring samples. The 48 h reproduction test with Brachionus *calyciflorus* was performed according to the standard operational procedure of the Rotoxkit F™ chronic [28]. This test was used in autumn.
- The 60 min feeding inhibition test with *Thamnocephalus platyurus* was performed according to the standard operational procedure of the Rapidtoxkit® [29].
- The 24 h mortality test with *Thamnocephalus platyurus* was performed according to the standard operational procedure of the Thamnotoxkit F™ [30].
- The 15 min luminescence inhibition test with *Vibrio fisheri* was performed according to the Microtox Manual [31].
- The 24 h mortality test with *Spirostomum ambiguum* was performed following the method described by Nałęcz-Jawecki [32].
- The 24 h mortality test with protozoan *Tetrahymena thermophila* was performed according to the standard operational procedure of the Protoxkit F™ [33].

Toxicity Assessment System

The data has been expressed as percentage effects (PE), depending on the effect criterion of the respective assay.

Table 1. Characteristics of the battery of test-organisms used for toxicity assessment.

Trophic level	Organisms	Test name	Endpoint	Test duration	Type of test
Producers	Micro – algae				
	Pseudokirchneriella subcapitata	Algaltoxkit ®	growth inhibition	72 h	chronic
	Duckweed				
	Lemna minor		growth inhibition	168 h	chronic
Consumers	Rotifers				
	Brachionus calyciflorus	Rotoxkit F Acute®	mortality	24 h	acute
		Rotoxkit F Chronic®	reproduction	48 h	chronic
	Crustaceans				
	Daphnia magna	Daphtoxkit F magna®	immobilisation	48 h	acute
	Thamnocephalus platyurus	Rapidtoxkit®	feeding inhibation	60 min	acute
		Thamnotoxkit F®	mortality	24 h	acute
Decomposers	Bacteria				
	Vibrio fisheri	Microtox®	luminescence inhibition	15 min	acute
	Protozoans				
	Spirostomum ambiguum	Spirotox	morphological deformations	24 h	acute
	Tetrahymena thermophila	Protoxkit F®	growth inhibition	24 h	acute

The toxicity data has been classified according to the hazard classification system for natural water [34]. It can be used for samples from rivers, streams, lakes, etc. The 1st step of the analysis assumes testing only undiluted samples with a biotests battery. The classification system ranks samples into one of the following 5 hazard classes on the basis of the highest toxic response shown by at least one of the organisms.

Class 1 No acute hazard – PE<20%; none of the tests shows a toxic effect.

Class 2 Slight acute hazard – PE<50%; a toxic effect is reached in at least one test, but the effect level is below 50%.

Class 3 Acute hazard – PE<100%; the PE is reached or exceeded in at least one test, but the effect level is below 100%.

Class 4 High acute hazard PE=100%; the PE is reached in at least one test.

Class 5 Very high acute hazard – PE=100%; the PE is reached in all tests.

Results

The physical and chemical characteristics of waters are presented in Table 2. The physico-chemical analyses of samples from Ner (Nc) made in spring showed a high level of conductivity. A high concentration of substances stimulating eutrophication also was observed. That was mostly TN in samples from middle stretch of Ner (Nc) and Bzura (Bb) and down a stretch of the Utrata (Ud) but also TP in water from the middle stretch of the Utrata (Uc). Except from Cd, Fe and Ca, no metals were found in water.

The toxicities of the samples from spring are summarized in Table 3. In any bioassays in spring, no very

Table 2. Characteristics of sampling sites with physical and chemical parameters.

Site code	Pollution class	Polluting substances*	Polluting substances determined in spring and their classification**	Polluting substances determined in autumn and their classification**
P	III	color, O_2, BZT5, CHZT-Mn, ChZT-Cr	Fe(III)***, TN(II), Cd(II), Ca(II)	Fe(II), Ca(II)
Ba	V	color, ChZT-Mn, N-Kjeldahl, PO_4, TP	not performed	TN(IV), TP(III), NH_4(III), Fe(III), conductivity(II), NO_3(II), Cd(II), Ca(II)
Bb	IV	color, O_2, ChZT-Cr, TC org., NO_3, TN, Hg	TN(III), Fe(III), NO_3(II), Ca(II)	NH_4(III), Fe(III), conductivity(II), O_2(II), TP(II), TN(II), Cd(II), Ca(II)
Bd	V	TC org., PO_4, Se	Fe(III), TN(II), Ca(II)	conductivity(III), O_2(III), Fe(III), TP(II), PO_4(II), Ca(II)
Na	IV	color, BZT5, ChZT-Cr, NH_4, N-Kjeldahl, NO_2, Pb, Hg	Fe(III), conductivity(II), O_2(II), TN(II), Cd(II), Ca(II)	TP(III), Fe(III), conductivity(II), TN(II), Ca(II)
Nb	V	color, NH_4, N-Kjeldahl, NO_3, NO_2, TN, PO_4, TP	not performed	TN(IV), TP(III), conductivity(II), O_2(II), NO_3(II)
Nc	V	color, BZT5, ChZT-Cr, TC org., NH_4, N-Kjeldahl, NO_3, NO_2, TN, PO_4, TP, Pb, Hg, Fe	TN(IV), conductivity(III), Fe(III), NH_4(II), NO_3(II), Ca(II)	TN(IV), conductivity(III), TP(III), Fe(III), NO_3(II), Ca(II)
Nd	V	color, NH_4, N-Kjeldahl, PO_4,	not performed	conductivity(III), TP(III), TN(III), Fe(III), NO_3(II), Cd(II), Ca(II)
Ua	IV	color, BZT5, ChZT-Cr, TC org., N-Kjeldahl(V), PO_4, TP, conductivity, Mn, Se, Fe(V)	Fe(III), conductivity(II), TN(II), Cd(II), Ca(II)	O_2(V), TP(III), Fe(III), conductivity(II), TN(II), Ca(II)
Ub	V	N-Kjeldahl, PO_4, conductivity, Se, Fe	Fe(III), conductivity(II), TN(II), Ca(II)	O_2(IV), conductivity(III), TP(III), TN(III), Fe(III), Cd(II), Ca(II)
Uc	V	O_2, ChZT-Cr, NH_4, N-Kjeldahl, NO_2, PO4, TP, conductivity	TP(III), Fe(III), TN(II), NH_4(II), Ca(II)	O_2(V), TP(V), TN(IV), conductivity(III), Fe(III), NO_3(II), Ca(II)
Ud	V	NH_4, N-Kjeldahl, NO_2, PO_4, TP, Se	TN(III), Fe(III), conductivity(II), NO_3(II), Ca(II)	TP(V), TN(IV), conductivity(III), O_2(III), Fe(III), Ca(II)

Explanations: * Substances deciding on pollution classes according to WIOŚ data, ** Substances detected in the study that exceed 1st pollution class, ***classes are given in brackets, O_2 – content of oxygen dissolved in water, BZT5 – biological demand for oxygen, ChZT-Mn – Chemical demand for oxygen (permanganate method), ChZT-Cr – Chemical demand for oxygen (chromate method), TC org. – Total organic carbon, N- Kjeldahl – Nitrogen determined with Kjeldahl's method, TN – Total nitrogen, TP – Total phosphorus

high or high hazards of waters were observed. Most of them belonged to the 1st and 2nd class of toxicity. Over 50% toxic effect was observed only in the Rapidtoxkit® in two cases – samples Na and Uc. *T. platyurus* in the Rapidtoxkit® test was the most sensitive species, which responded to samples from the middle stretch of Ner (Na) and Utrata (Uc) and from an estuary of the Bzura (Bd). A sensitive species was also *D. magna*, which reacted to water from the middle stretch of Utrata (Uc).

Tests with plants were performed in two versions; with and without the addition of a nutrient medium. The aim of such an operation was finding out if there were compounds that stimulate growth of producers and how it affects the toxicity of samples. The test with micro algae *P. subcapitata* showed a very high inhibition of growth (over 40%) in samples, where no nutrient medium was added. In the test with *L. minor,* such an effect was not observed.

In autumn the high level of conductivity was observed not only in the samples from Ner (Nc, Nd) [Table 2], but also from Bzura (Bd) and from Utrata (Ub, Uc, Ud). In all cases it was higher than in spring. In all waters, also a big quantity of eutrophication stimulants was reported. Except from the control water from Pilica, the level of TP was high. The biggest amount of it was in samples Uc and Ud. It was three times bigger than in other waters. Also the concentration of TN was the lowest in water from Pilica and high in other samples; the highest in Utrata (Ub, Uc, Ud), Bzura (Ba) and Ner (Nb, Nc). All samples from Utrata, especially Ua and Uc, and sample from the Bzura (Bd) had low oxygen content. In other samples from Bzura (Ba, Bb) the concentration of $N\text{-}NH_4$ was higher than in other rivers. As in spring, no metals were found in water, except from Cd, Fe and Ca.

The toxicities of the samples from autumn are summarized in Table 4. Autumn's bioanalyses showed that like in spring most of the waters belonged to the 1st and 2nd class of toxicity. However, organisms different than in spring responded. The highest, 100% effect, was observed in the Spirotox test in waters from Bzura (Ba) and Utrata (Uc). *B. calyciflorus* in the test Rotoxkit F™ chronic also responded to the samples Ba and Uc with almost 50% effect. The crustacean *T. platyurus* was sensitive to the water from Utrata (Uc) too. In these samples, where no nutrient medium was added, the growth of *P. subcapitata* was strongly inhibited. In a version with nutrient medium, except from the low toxic sample Nc, all waters were not toxic or like in Ub, Uc and Ud even high stimulation of growth was observed. In tests without the addition of nutrient medium the growth of *L. minor* was slightly inhibited except from the samples from the middle stretch of the river Ner (Na, Nb, Nc). However, in tests with nutrient medium no toxic effects were observed.

Table 3. Results of toxicity tests performed in spring.

Sample	P.s.		L.m.		B.c	D.m.	T.p.	T.p.	V.f.	S.a.	T.t.
	72h-%E		168h-%E		24h-%L	48h-%E	60min-%E	24h-%L	15min-%E	24h-%E	24h-%E
	N(-)	N(+)	N(-)	N(+)							
P	88	-8	21	12	0	0	14	0	-5	0	-3
Ba	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Bb	92	11	-5	-9	0	45	8	3	-19	0	10
Bd	89	-4	-7	-2	0	0	31	0	-12	0	8
Na	80	0	17	9	0	0	56	0	-2	0	1
Nb	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Nc	44	0	12	1	0	0	15	3	-20	0	-2
Nd	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Ua	82	-6	2	10	0	30	22	0	-19	0	7
Ub	87	-5	10	16	0	35	14	5	-18	0	11
Uc	84	5	1	1	0	40	53	0	-23	0	12
Ud	92	-4	0	7	0	0	17	0	-8	0	13

Explanations: E – effect, L – lethal, P.s. – *Pseudokirchneriella subcapitata* (E – growth inhibition), L.m. – *Lemna minor* (E – growth inhibition), B.c. – *Brachionus calyciflorus* (mortality), D.m. – *Daphnia magna* (E – immobilization), T.p. – *Thamnocephalus platyurus* (E – feeding inhibition/mortality), V.f. – *Vibrio fisheri* (E – luminescence inhibition), S.a. – *Spirostomum ambiguum* (E – morphological deformations), T.t. – *Tetrahymena thermophila* (E – growth inhibition), N(-) – samples without nutrient medium; N(+) – samples with nutrient medium; NP – not performed

Discussion

Information about classes and parameters that decided the classes of rivers were taken from WIOŚ while the physical and chemical analyses were made to support the bioassay and provide reliable information about actual river pollution. Therefore, it was possible to check if the eventual toxicity of sample was connected with exceeding levels of a monitored factor at the time of sampling. The analyses show that there are not significant differences between the data received in this research and that from WIOŚ.

The high level of substances stimulating eutrophication is typical for all studied middle stretches of rivers and afterwards for their estuaries, except from Pilica. The analyses show that stretches of rivers which had been receivers of wastewater from urban areas – Utrata (the whole river) and Ner (at points Nb, Nc, Nd) – are in bad condition. Mostly the values of TP, TN, conductivity and oxygen content are responsible for this. However, downstream, if there is not a new source of pollution, the water is slightly cleaner like in Bzura, where we observed the worst water quality in autumn. The reason for such a situation might be a lower water level than in spring, which could cause higher concentrations of monitored compounds.

The data from toxicity tests has been expressed as percentage effects (PE) and classified according to the hazard classification system for natural water [30]. The 1st step of the analysis assumes testing only undiluted samples with biotest battery. The classification system ranks these undiluted samples in 5 hazard classes after determination of the percentage effect obtained with each of the tests. If the percentage effect amounts to less than 20% there is not any hazard of water sample. The PE in range 20-50% in at least one test from the battery classifies the sample to the second – slight acute hazard class. If one test reaches or exceeds PE 50%, but the effect level is below 100%, the sample is classified to the third acute hazard group. When one test and all tests exceed the 100% effect, such a sample is respectively in high acute hazard and in very high acute hazard class. Depending on the situation, the samples from the third and upper classes should be treated further. In biotests that resulted in a more than 50% effect in the screening, the tests with dilution series should be performed. However, in this study the 2nd step was not performed for any sample. The assumption of the research was that samples were tested only in screening system. Moreover, the method is cheaper than testing with dilution series. The second point is that no very high toxicity samples were expected. No physico-chemical data has been found that could witness to very high toxic of treated rivers. Screening was used here as a system only to find and catch toxic water from a large group of environmental samples.

Table 4. Results of toxicity tests performed in autumn.

Sample	P.s.		L.m.		B.c	D.m.	T.p.	T.p.	V.f.	S.a.	T.t.
	72h-%E		168h-%E		48h-%E	48h-%E	60min-%E	24h-%L	15min-%E	24h-%E	24h-%E
	N(-)	N(+)	N(-)	N(+)							
P	98	-16	4	-20	1	0	0	3	-1	0	0
Ba	87	2	24	14	46	0	23	0	0	100	4
Bb	89	10	12	22	14	0	0	0	-11	0	24
Bd	82	2	18	12	3	0	0	0	-1	0	5
Na	78	-6	-14	-13	11	0	14	0	-13	0	0
Nb	64	-1	-28	-13	-7	0	5	0	-14	0	-4
Nc	61	27	-14	-16	-9	0	0	0	-12	0	-6
Nd	9	-9	2	-11	6	0	10	0	-5	0	-7
Ua	78	-19	4	4	6	0	0	0	-12	0	13
Ub	65	-42	21	8	6	0	2	3	-13	0	10
Uc	27	-25	23	10	45	0	2	3	-15	100	5
Ud	38	-28	21	8	4	0	12	3	-18	0	3

Explanations: E – effect, L – lethal, P.s. – *Pseudokirchneriella subcapitata* (E – growth inhibition), L.m. – *Lemna minor* (E – growth inhibition), B.c. – *Brachionus calyciflorus* (E – reproduction), D.m. – *Daphnia magna* (E – immobilization), T.p. – *Thamnocephalus platyurus* (E – feeding inhibition and mortality), V.f. – *Vibrio fisheri* (E – luminescence inhibition), S.a. – *Spirostomum ambiguum* (E – morphological deformations), T.t. – *Tetrahymena thermophila* (E – growth inhibition), N(-) – samples without nutrient medium; N(+) – samples with nutrient medium

Tests with plants were performed in two versions; with and without nutrient medium. In the test with *P. subcapitata* a very high inhibition of growth for all samples was observed in the version without nutrient additions, but the enormous PE is not a reason to classify them as a group with high acute hazard [3]. Tests with algae are performed typically according to the standards with nutrient medium addition, because study classification is based on this variant. The first reason for not adding nutrients was to find out if there is a possibility to determine with phytotest a content of compounds which stimulate growth of producers in environmental samples. The second is how eutrophication stimulants affect the toxicity of samples. Like in studies in Greece [3] results prove that poor growth in the non-enriched water samples was mainly due to the nutrient characteristics of the water and less to the presence of toxicants. However, it cannot be excluded that the reason for the poor growth of micro-algae are due to unfavorable environmental factors, because the high level of eutrophication stimulants can hide the real toxicity to producers. Therefore, in such samples it might be necessary to do tests with dilution series to find out if it is toxic or not [5]. In autumn samples, the lowest inhibition effect was observed indeed for waters which have the highest level of TN (Uc, Ud) and the effect increases with a parallel decreasing level of TN in other samples. At the same time in samples Uc, Ud in variant with the addition of nutrient growth stimulation was observed. In the second test with producers (*L. minor*), no inhibition effects for samples without nutrients were observed. There were no such "clear" differences between samples with and without the addition of nutrient medium. None of them were toxic to *L. minor*.

The samples from tested rivers were generally not toxic or low toxic to organisms. Additionally, no permanent toxicity was detected. The toxicity of spring samples differed from autumn samples taken from the same place. There were attempts made to point out which organisms from the battery seem to be the best tool in analyses of surface and wastewater, to next construct a battery consisting only of these organisms. The reason is that each test organism is sensitive to various toxins, but the most sensitive species all together can supply sufficient information about a hazard to the environment of various pollutants [16]. Some authors show *V. fisheri* to be the most sensitive and useful organism [7, 10]. Others point out that *T. platyurus* [3, 13] or *D. magna* [9, 12] could be. However, there is not a general agreement about which organisms should be used. In this study it is also not possible yet. *T. platyurus* in the Rapidtoxkit® and *D. magna responded* only in spring screening. In autumn no water was toxic to them. S. *ambiguum* in Spirotox and *T. platyurus* in the Thamnotoxkit F™ reacted conversely only in autumn. No samples were toxic to bacteria *V. fisheri* and protozoan *T. thermophila* in spring and in autumn. Changing Rotoxkit F Acute® to Rotoxkit F Chronic® in autumn screening caused the change in sensitivity of *B. calyciflorus,* which was the most sensitive species then. It seems to be necessary to use more chronic tests in analyses of surface water. Such tests might be helpful to assess sublethal effects caused by toxins, which are present even in low concentrations.

There were no significant differences in the amount of chemical compounds monitored in spring and in autumn that could influence the organisms. Probably other substances are responsible for observed toxic effects, but they are different in spring and in autumn samples. That might be a reason why samples from the same place were found to be toxic in spring but not toxic to some organisms in autumn. However, if the studied rivers are receivers of municipal and industrial wastewater why is their water only low toxic? It is possible that the persistence substances are cumulating in sediments where they can reach high concentrations, like a.o. metals. Chemicals bound to the sediments may persist long after the actual discharge has stopped [35]. And their mobility and bioavailability depend on multiple factors, for example reaction, organic matter content, oxidation and biological activity [36-39]. The issue of metal contents in sediments from studied rivers and soils from first floodplains in their valleys is being investigated in a separate study [40]. Lower metals concentrations in river water arise due to the lack of conditions that stimulate leaching metals from sediments and soils (a.o. the acid reaction and low organic matter content). Concentrations of metals are indeed higher in sediments and soils than in water samples [40]. The presence of Fe and Ca in water is typical of all tested rivers. The content of these metals probably is naturally high and even low concentrations in discharges can affect purity class markedly.

The most toxic river stretch is the middle Utrata in point Uc. Its waters were harmful to two organisms in spring and two others in autumn screening. Generally the toxicity of middle stretch of Utrata and also toxicity of other rivers in several cases confirms the right choice of Pilica as a reference river. But after one year it is not possible to affirm if toxicity data from other samples should be compared with the data from Pilica to eliminate the background of natural compounds. No toxic effects in water from Pilica in any tests from the battery were observed. But there were a lot of nontoxic samples from other rivers too, although they have been much more polluted. Such a situation shows that the classification which was based only on the concentration of chemical compounds might not present a real hazard of pollution, which could contain new unknown and unmonitored chemicals. A reverse situation can take place: the water, which is chemically clear, might be very toxic to the test organisms. Due to the impossibility of looking for every new compound, the usage of biotests might be justified.

The research will be continued because it is too early to evaluate the usefulness of biotests in a monitoring system of rivers. In the next season a.o. tests with dilution series will be performed and more chronic tests will be made.

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