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The application of the germination index in the assessment of the phytotoxicity of bottom sediments from the Rybnik Reservoir

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Abstract: The aims of the study were to assess the phytoxicity of bottom sediments collected from the Rybnik Reservoir. The water reservoir in Rybnik is located in the Silesian Voivodeship. The reservoir constitutes a part of the technological chain of Elektrownia Rybnik S.A. as a direct receiver of industrial and rainwater sewage, sewage from a water treatment plant, blowdowns from cooling towers, and as an essential source of cooling water. Sediment samples were collected with an Eckman sampler from 33 locations. The toxicity of bottom sediments was determined using the Phytotoxkit direct contact test, carried out for 3 plants: *Sorghum saccharatum, Sinapis alba* and *Lepidium sativum.* On the basis of the data received, the germination index (GI) was calculated. We found the mean value of the germination index indicated the dominance of the inhibitory effect of bottom sediments on plant growth. *Sorghum saccharatum* was the most sensitive to pollutants in sediments, while *Lepidium sativum* was the least sensitive. The Phytotoxkit is a good tool for assessing the toxicity of bottom sediments.

Keywords: bottom sediment, phytotoxicity, germination index, Phytotoxkit

INTRODUCTION

In urban areas, the composition of bottom sediments depends on natural factors (the type of foundations that build the catchment, land geomorphology, climatic conditions) and, to a large extent, anthropogenic factors (the method of the management and use of the catchment, the type and amount of pollutants introduced) (Tarnawski & Michalec 2006, Aleksander-Kwaterczak 2007, Szalińska 2011, Szarek-Gwiazda 2013). The most common forms of anthropopressure include industrial and municipal sewage discharges to waters as well as dust and gas pollution in the atmosphere (Kostecki 2004, Dmitruk et al. 2013, Ciszewski et al. 2013, Baran & Tarnawski 2015). The migration of pollutants in the aquatic environment occurs under the influence of transport and sedimentation processes (Jasiewicz & Baran 2006, Jancewicz et al. 2012). The structure of sediments makes them a natural geosorbent, i.e. a place where the accumulation of migrating pollutants takes place (Förstner & Salomons 2010, Szarek-Gwiazda 2013, Baran & Tarnawski 2013, 2015). Currently, according to numerous studies, classic chemical methods do not provide sufficient information on the potential hazard resulting from the presence of pollutants in bottom

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sediments, for which biotests are a useful tool, the use of which allows a comprehensive assessment of bioavailability, toxicity and interoperability (Latif & Licek 2004, Mankiewicz-Boczek et al. 2008, Mamindy-Pajany et al 2011, Baran & Tarnawski 2013). A biological response of a test organism is measured as the result of the combined effect, including antagonism and synergism, of the mixture of all potential pollutants contained in the sediment (Wadhia & Thompson 2007, Wolska et al. 2007, Lopez-Rondan et al. 2012). The necessity to analyse a large number of environmental samples induced the popularisation of miniaturised toxicity tests – microbiotests, which are easy to carry out, characterised by their simplicity of observation, repeatability of results, and the fact that they do not require expensive laboratory equipment (Smreczak & Maliszewska-Kordybach 2003, Wolska et al. 2007, Oleszczuk 2008). The Phytotoxkit is a root biotest for chronic toxicity testing. It is based on three plants: monocotyledonous *Sorghum saccharatum* as well as dicotyledonous *Sinapis alba* and *Lepidium sativum*, whose germinating seeds, as a result of contact with substances contained in sediments, show specific reactions: a lack of germination or a reduction of root length. Inhibition of reactions or vital functions of the test organisms indicates the presence of harmful factors or units in the environmental matrices (Wadhia & Thompson 2007, Łaszczyca et al. 2012). Moreover, it is worth noting that plants are important components of ecosystems; they are the primary food producers and, therefore, it is important to identify the magnitude of the toxic effects on these organisms (Czerniawska-Kusza et al. 2006, Garcia-Lorenzo et al. 2009, Czerniawska-Kusza & Kusza 2011, Baran & Tarnawski 2013).

The aims of the study were to assess the phytoxicity of bottom sediments collected from the Rybnik Reservoir (Silesian area) and to evaluate the spatial distribution sediment toxicity using the Phytotoxkit microbiotest.

MATERIALS AND METHODS

Study area

The water reservoir in Rybnik is located in the Rybnik Coal Basin in the Silesian Voivodeship (Fig. 1). It was formed in 1972 as a result of the damming of the Ruda River, a right-bank tributary of the Oder. The total capacity of the reservoir amounts to 24 mln m^3, while its average depth is 5.4 m (Tab. 1) (Wiechuła et al. 2005).

Fig. 1. *Localization of the Rybnik Reservoir*

Table 1
Characteristic parameters of the Rybnik Reservoir

Parameter	Value
Storage reservoir [mln m^3]:	
total	24
flood storage	1.5
usable storage	4.3
dead storage	18.2
Lenght of reservoir [km]	4.5
Area of the reservoir [ha]	555
main reservoir	444
The dam earth embankment [m]	
lenght	975.00
maximum height	12.00
Outlet facilities:	
spillway	2 × 5.0 m max. outflow 60 m^3·s^{-1}
bottom river outlet	3 × 1.8 × 1.8 m max. outflow 39 m^3·s^{-1}

The area around the reservoir is characterised by great diversity, from recreational development with dispersed urban development, through numerous industrial and commercial areas, dumping grounds and heaps, up to a small number of areas used for agriculture and forestry. The reservoir constitutes a part of the technological chain of Elektrownia Rybnik S.A. as a direct receiver of industrial and rainwater sewage, sewage from a water treatment plant, blowdowns from cooling towers, and as an essential source of cooling water (Baran & Tarnawski 2015, Baran et al. 2016). There is a strong emission of dust and gas pollution in the Silesian Voivodeship, accounting for 19% of the total emissions in Poland. The highest point sources of atmospheric air pollution are CHP plants (EC Nowa – Dąbrowa Górnicza, Chorzów ELCHO, Będzin), power plants (Rybnik, Jaworzno III, Łagisza, Łaziska, Halemba), Częstochowa steelworks, coke-oven plants (Przyjaźń Dąbrowa Górnicza) and the Arcelor Mittal plant in Dąbrowa Górnicza. Pollutants transported in the atmosphere and introduced with wet atmospheric precipitation constitute a significant source of pollution in the area (Jancewicz et al. 2012). According to atmospheric chemistry studies from the IMGW-PIB station in Katowice (Muchowiec) and Racibórz, "acid rain", i.e. precipitation below pH 5.6 indicating the natural acidity level of rainwater, showing the content of strong mineral acids, was reported in the case of 35% of samples in 2015. Sulphates, chlorides, ammonium nitrogen, general nitrogen, calcium and chlorine were found at the highest concentrations. The analysis of the content of pollutants accumulated in the bottom sediments of reservoirs located in the Silesian Voivodeship showed high levels of trace elements and persistent organic pollutants (Stan środowiska, 2016).

Sample collection

Sediment samples were collected in July – August 2017 with an Eckman sampler from 33 set locations. Based on our previous studies, three zones were determined in the reservoir (Baran et al. 2016). The sediment samples were collected from inlet (backwater), middle and outlet (near to dam) zone. In each zone, 12, 10 and 11 samples of the upper layer of bottom sediments (0–15 cm) were collected respectively. The sediments were put in polyethylene containers and transported to the laboratory. The sediment samples were refrigerated in darkness until analysed.

Phytotoxkit biotest

The toxicity of bottom sediments was determined using the Phytotoxkit direct contact test, carried out in accordance with the ISO procedure and standard (Phytotoxkit, 2004, ISO 18763:2016). The test was carried out for 3 plants: *Sorghum saccharatum* (monocotyledonous), *Sinapis alba* and *Lepidium sativum* (dicotyledonous). The sediment samples were placed in the bottom part of 21 cm × 15.5 cm × 0.8 cm test plates and covered with a paper filter. At a distance of approx. 1 cm from the top edge of the filter, 10 seeds of the same plant species were placed. The plates were closed with covers and incubated in a vertical position at 25°C in the dark for 72 hours. After this time, pictures were taken and then, using the Image J image analysis programme, the number of seeds germinated was determined and the root length was measured. On the basis of the data received, the germination index (GI) was calculated according to the following formula:

$$GI = \frac{GsLs}{GcLc} \times 100\%,$$

where Gs and Ls are the seed germination [%] and root elongation [mm] for the sample, and Gc and Lc are the corresponding control values. GI values within the range of 90–110% were classified as "no effect/non-toxic", GI values < 90% were classified as inhibition, and GI values > 110% were classified as stimulation (Beltrami et al. 1999, Czerniawska-Kusza & Kusza 2011, Baran & Tarnawski 2013).

Statistical analysis

The results were expressed as mean ± standard deviation (SD), minimum and maximum values and coefficient of variation (CV%). Pearson's correlation matrix was used to explore the possible relationships between plants. The differences between the means were detected by ANOVA and Tukey's test to be at a significance level of 0.05. All statistical analyses were preformed using Microsoft Office Excel and STATISTICA 12.0 software.

RESULTS AND DISCUSSION

Table 2 presents the germination index of three plant species exposed to the tested sediment. The value of the germination index was in the range from 22 to 117% for *Sinapis alba*, from 32 to 158% for *Lepidium sativum,* and from 6 to 110% for *Sorghum saccharatum*. In general, the mean and low spatial variability of bottom sediment toxicity, indicated with GI, was exhibited. This parameter indicated the highest spatial differentiation for *S. saccharatum* (CV = 55%) and the lowest for *S. alba* (CV = 28%) (Tab. 2, Fig. 2). For each of the plants, the mean value of the germination index indicated the dominance of the inhibitory effect of the tested sediment on plant growth.

Table 2
Germination index values (GI) for sediment samples

Plants	Mean	Median	SD	Minimum	Maximum	CV%*
Sinapis alba	79 b**	80	21	22	117	26
Lepidium sativum	84 b	84	32	32	158	38
Sorghum saccharatum	57 a	67	31	6	110	55

* CV% – variation coefficient.

** Means followed by the different letters in line indicate significant differences at α ≤ 0.05 according to the t-Tukey test.

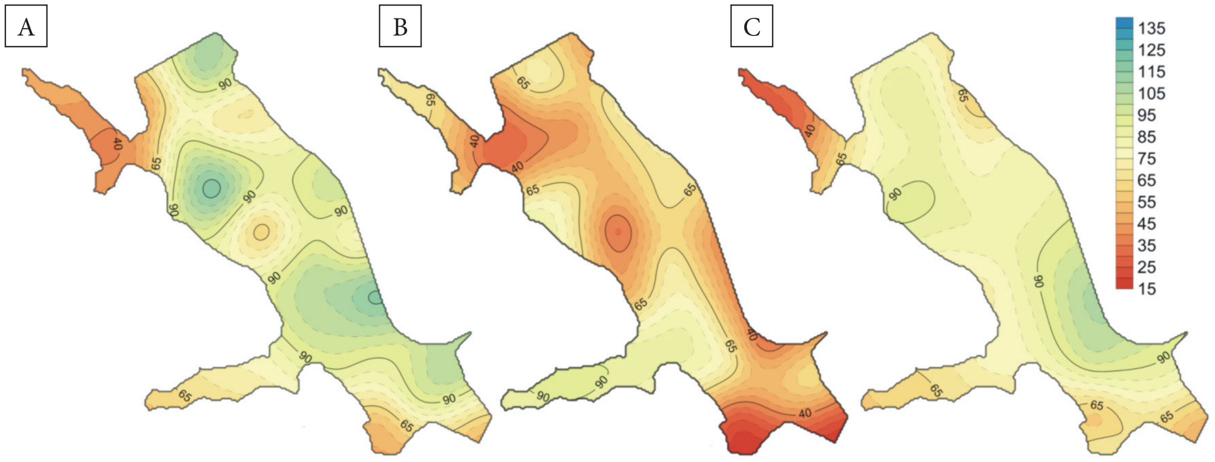

Fig. 2. *Spatial distribution phytotoxicity (GI) of bottom sediments: A) Lepidium sativum; B) Sorghum saccharatum; C) Sinapis alba*

The monocotyledonous plant *S. saccharatum* was indeed the most sensitive to pollutants present in the sediments. The calculated germination index for *S. saccharatum* indicated that as much as 85% of the sediment samples inhibited the growth of this plant (GI < 90%), while 15% of the samples showed no stimulation or inhibition. This result is in agreement with the works by Czerniawska-Kusza et al. (2006), Czerniawska-Kusza & Kusza (2011), Mamindy-Pajany et al. (2011), Baran & Tarnawski (2015) who reported that *S. saccharatum* is the most sensitive species for identifying phytotoxic sediment samples compared to *L. sativum* and *S. alba*. Among dicotyledonous plants, *S. alba* turned out to be a more sensitive one since up to 73% of the sediment samples were observed to inhibit its growth. Neither positive nor negative reactions of *S. alba* were found in 24% of the sediment samples, while stimulation of plant growth was observed in 3% of the samples. *L. sativum* was the least sensitive to the bottom sediment from the Rybnik reservoir. Inhibition of plant growth was observed in 52% of the sediment samples, whereas stimulation was reported in 15% of the samples. In the study, a significant correlation between the growth inhibition of L. sativum and the growth inhibition of *S. alba* and *S. saccharatum* ($r = 0.36$, $r = 0.43$, $p < 0.5$) was found, so it would seem that the correlation analysis showed a similar sensitivity to toxicants in the sediments. However, the observed relationship between the response of the test organisms was statistically insignificant, as it explained only 13% and 18% of variations, respectively.

Figure 3 presents the values of the germination index of plants depending on the characteristic zone in the reservoir. Three main zones were selected: inlet (backwater zone, I), middle (II), outlet (dam, III). It is interesting that GI values grouped in such a way indicated the inhibitory effect of sediments on the growth of the test plants. Regardless of the plant tested, the sediment samples collected in the inlet zone (I) and then in the outlet zone (dam, III) were characterised by the highest toxicity. The sediment samples from the middle (II) part of the reservoir showed the least negative impact on plant growth.

Our previous studies found that the most polluted sediments are found at points in the inlet zone and near the dam (outlet zones) and close to dam (Baran et al. 2016). The pollution of sediments by metals and PAHs is associated with the discharge of cooling water from the power plant and with an inflow of contaminated water of the Ruda River and long-range transport.

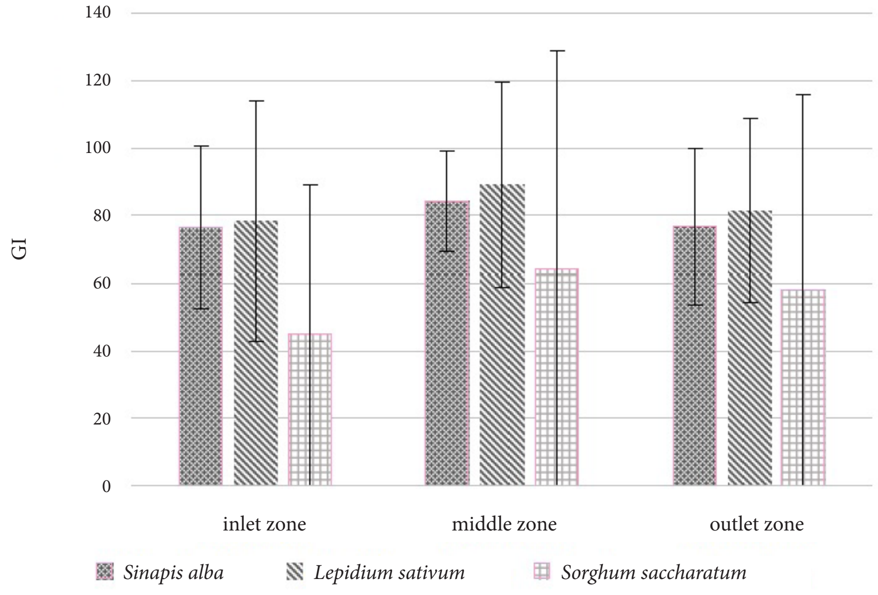

Fig. 3. *Germination index of plants depending on the zone in the reservoir*

Specific currents are formed in the reservoir as a result of discharge water from a power plant. These currents cause the formation of the discharge area, a zone of significantly increased levels of metals in sediments (Kostecki 2004, Kostecki & Kowalski 2004). Other sources of sediment contamination include: treated industrial sewage emitted by the Rybnik power plant, municipal sewage, rain wastewaters, sewage from the water treatment plant, cooling tower blowdowns, and dry precipitation (Loska &Wiechuła 2003, Baran et al. 2017).

The toxicity of the analysed sediments against test plants can be also caused by numerous chemical factors. Many authors point to heavy metals contained in sediments as a basic factor inhibiting the growth of test plants (Valerio et al. 2007, Czerniawska-Kusza & Kusza 2011). In the studies conducted by Kostecki & Kowalski (2004) and Baran et al. (2016), it was shown that the bottom sediments of the Rybnik Reservoir are polluted with heavy metals. However, Potential Ecological Risk Index (PERI) values suggested that 70% of the samples exhibited a low ecological risk from metal pollution, while 24% of the samples had severe or serious risk. Low or no toxicity of sediments from heavy metals may be caused i.a. by their presence in a very stable and insoluble form, a high content of organic matter or clay fraction as well as a neutral or a slightly alkaline reaction (Baran & Tarnawski 2013, 2015). The studies conducted by Gong et al. (2001) and Czerniawska-Kusza et al. (2006) also confirmed that the false assessment may be caused by sediments rich in organic matter, which can hide the inhibitory effect of pollutants on test plants. The lack of significant dependencies between the content of metals in the sediments and the response of test organisms also suggest that other factors, such as arsenic, PAHs, PCBs and dioxins, may be present in the sediments, determining their toxicity (Urbaniak et al. 2013, Baran et al. 2017). In the study of Baran et al. (2017), it was found that individual PAHs, such a NAP, PHE, FLT, PYR, BAA, CHR, BAP, in sediments from the Rybnik Reservoir indicated a higher possibility of the occurrence of an adverse ecological effect. In addition, the specific interactions occurring between pollutants may also result in antagonistic or synergistic effects, the prediction of which may be challenging (Kabata-Pendias & Pendias 1999, Baran & Tarnawski 2015, Czarniewska-Kusza et al. 2006, Simeonov et al. 2007).

CONCLUSIONS

1. The tested monocotyledonous (*S. saccharatum*) and dicotyledonous plants (*S. alba and L. sativum*) reacted differently, reflecting their own physiological mechanisms of reaction to stress factors. However, for each of the plants, the mean value of the germination index indicated the dominance of the inhibitory effect of bottom sediments on their growth.
2. *Sorghum saccharatum* was the most sensitive to pollutants in sediments, while *Lepidium sativum* was the least sensitive.
3. Sediments in the inlet zone of the reservoir showed the highest toxicity level for the test plants.
4. Phytotoxkit is a good tool for assessing the toxicity of bottom sediments. However, for this assessment to be comprehensive and reliable, it is necessary to use biotest batteries consisting of organisms belonging to different species and trophic levels.

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