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INDIRECT EFFECT OF MADE GROUNDS ON THE AQUATIC FLORA AND FAUNA

The presence of made grounds containing industrial waste materials in the natural environment presents a potential ecological threat. Therefore, in the present study we examined the indirect effects of selected made grounds on the aquatic flora and fauna. The subject of the study were aqueous extracts from made grounds containing metallurgical slags and their effect on the survival of the crustacean daphnia (*Daphnia magna*) and the inhibition of growth of the plant duckweed (*Lemna minor*). Due to the fact that duckweed is also used for phytoremediation, the solution after the contact with the plant was assessed for changes of toxicity using the toxicological enzymatic bioassay employing bacteria (*Allivibrio fischeri*). It was found that the extracts of made grounds adversely affect both the survival of the crustaceans and the growth of plants, however, despite the inhibition of the growth of duckweed it has the ability to phytoremediate contaminants present in the made grounds.

1. INTRODUCTION

Made grounds represent anthropogenic layer of a land surface, and often have a thickness of up to a few meters in the cities and even several meters in the industrial areas [1]. Important factors influencing the properties of made grounds are deposition conditions (dry or wet) and a mode of transport (rail, road, pipeline or belt conveyor transport) [2]. They affect the structure of the material, the predictability of the characteristics in the geological area and a number of other physicochemical properties. Clearly the most important factor determining the impact of made grounds on the natural environment is the composition and leachability of pollutants present in a given made ground. In Poland, the degree of contamination of land is assessed in accordance with

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the Regulation of the Minister of Environment of 9 September 2002 on soil quality and soil quality standards [3].

Made grounds occurring in Silesia (which, within the area of Poland is characterized by the highest degree of the urbanization and population density [4]), in a significant number of cases, contain a mixture of metallurgical slag, gravel and other building materials with native soils (mainly sands or clays hauled from other areas). This specific kind of made grounds (containing slag from lead and zinc metallurgy) shows, in terms of its chemical composition, the highest exceedances of the soil quality standards for zinc (Zn) and lead (Pb), with frequently exceeded values for barium (Ba) and arsenic (As), and sometimes for tin (Sn) and copper (Cu). In contrast, the standards set for cadmium (Cd), chromium (Cr) and nickel (Ni) have been rarely exceeded. As evidenced by geological cross-sections, no exceeded standard values for metals were observed in the layers situated directly below the man-made fill layer, regardless of their water permeability, which suggests no noticeable migration of metals from the made grounds containing steel slags. Such a phenomenon could potentially however occur. It should be also taken into account, that the speciation of metals, pH conditions and the presence of neutralizing minerals can reduce mobility of metals from the slags occurring in the made grounds. Such soils are rarely found to have exceeded quality standards set for other pollutants, which could originate from the materials of a man-made fill layer. The exceedances observed in the made grounds for the materials such as oils, petrol, polycyclic aromatic hydrocarbons (PAHs), substances from the group of BETX (benzene, toluene, ethylbenzene and xylenes), result from the on-site pollution of the soil occurring after deposition (levelling) of the made grounds in the area [1, 5].

Because of the diversity and variability of made grounds there is a need for a more detailed assessment of their impact on the environment. Preliminary studies carried out in this field assessing the samples collected from post-industrial areas showed that made grounds exhibit toxicity to the bioluminescent bacteria *Allivibrio fischeri*, which is caused, among others, by heavy metals (mainly zinc) [5]. Therefore, this study assessed the indirect impact of made grounds on the aquatic fauna (crustacean *Daphnia magna*) and flora (duckweed *Lemna minor*) using toxicological analysis. The subject of the study were aqueous extracts of the made grounds containing metallurgical slags. Due to the fact that duckweed is also used for phytoremediation [6–8], the solution before and after the contact with the plant was assessed for the toxicity changes using the Microtox® test by determining the percent inhibition of bioluminescence in *Allivibrio fischeri*.

2. MATERIALS AND METHODS

The subject of this study were the samples of 7 made grounds containing metallurgical slags taken from selected areas of the Silesian Voivodeship. According to the Polish soil quality and soil quality standards specified in the Regulation of the Minister of Environment [3], the made grounds samples were analyzed for heavy metals (arsenic, barium, chromium, tin, cadmium, cobalt, molybdenum, nickel, lead, mercury), hydrocarbon impurities (gasoline as the sum of the C6–C12; mineral oil as the sum of the C12–C35 hydrocarbons), aromatic hydrocarbons (benzene, ethylbenzene, toluene, xylene, styrene, and their sum), polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, anthracene, fluoranthene, chrysen, benz(a)anthracene, benzo(a)pyrene, benzo(a)fluoranthene, benzo(ghi)perylene, and their sum) and other impurities.

The concentration of heavy metals was determined in accordance with the Polish norms (Polish Norm 2002: PN-ISO 11466:2002 [9]; Polish Norm 2009: PN-EN ISO 11885:2009 [10]) using the inductively coupled plasma – atomic emission spectrometry method (ICP-AES). Only for mercury cold vapour, the atomic absorption spectrometry was used (CV-AAS) according to the norm (Polish Norm 2009: PN-ISO 16772: 2009 [11]). Hydrocarbon contaminants were determined using chromatographic methods, including the norms (Polish Norm 2013: PN-ISO 22155:2009 [12]; Polish Norm 2008: PN-ISO 18287:2008 [13]).

The toxicity assessment was carried out by an indirect method using the liquid phase obtained after aqueous extraction (with deionized water) of the studied solid samples. The ratio of the mass of a made ground to the volume of deionized water was 200 mg per 1 cm^3. During the extraction, the samples were mechanically mixed at 300 rpm for 10 min using a shaker (type SK-330-PRO, Chemland). In order to remove solids, the aqueous extracts were filtered using 0.45 μm cellulose acetate filter purchased from Millipore. These conditions were adopted based on the results of the previous study [5].

The toxicity of the made ground was evaluated based on selected tests: the survival of the crustacean *Daphnia magna* and the growth of duckweed *Lemna minor*. In addition, the solutions used in the test with duckweed were studied using the enzymatic assay Microtox® employing a strain of luminous marine bacteria *Allivibrio fischeri*.

The survival test with the crustacean *Daphnia magna* was carried out in accordance with the Polish norm (Polish Norm 2003: PN 90C-04610/03 [14]) by recording the mortality of the organisms after 24 and 48 hours of exposure to the aqueous extract of the made ground. The test organisms were obtained from an in-house culture.

The growth test with duckweed *Lemna minor* was performed according to the European Standard 2005: EN ISO 20079:2005 [15] assuming the observation of morphological changes, including the assessment of the number of leaves before and after 7 days. One plant with 2 leaves (fronds) was placed in each extract of the made grounds. Test cultures were run at 25 °C and were continuously illuminated (3000 lx). The test organisms were also obtained from an in-house culture.

The Microtox® bioassay employs a strain of luminous marine bacteria *Allivibrio fischeri*. The exposure of the bacteria to toxic substances leads to changes in the metabolic processes, which simultaneously causes changes in the intensity of the light emitted by the microorganisms [16]. The study was conducted using the MicrotoxOmni system in the Microtox Model 500 analyser (Modern Water, Inc.) which can be used as both an incubator and a photometer. After 5 min of exposure, the percent inhibition of bioluminescence was measured against a blank sample (2% NaCl). The analysis using the Microtox® test was carried out for the aqueous extracts of the made grounds before and after the contact with duckweed after 7 and 14 days.

The effect of the toxicity (%) was determined according to equation:

$$E = \frac{E_K - E_T}{E_K} \times 100\%$$

where: E_K – the effect observed in a blank sample, E_T – the effect observed for a test sample.

Depending on the given test, the effect was measured by decrease in bioluminescence (Microtox® test) or organism viability (*Daphnia magna* test) and leaf growth (*Lemna minor* test).

The toxicity of the samples was categorised according to the classification system, which has been commonly applied by many researchers [16, 17] and is based on the observed effect in the indicator organisms being employed (Table 1).

Table 1

Toxicity classification system [16, 17]

Effect [%]	Toxicity class
<25	non toxic
25–50	low toxicity
50.1–75	toxic
75.1–100	high toxicity

In this study, we determined also the correlation between the observed effect in the applied toxicological tests and the concentration of selected contaminants in the made grounds. This assessment was aimed to determine the cause of toxicity, as well as the sensitivity of individual indicator organisms to specific contaminants present in the made grounds. The results are the arithmetic average of the four replicates of each experiment. For all the cases assigned error (estimated based on the standard deviation) did not exceed 5% so the results are presented without marking of the ranges of error.

3. RESULTS AND DISCUSSION

The analysed made grounds were characterized by various degrees of contamination in terms of inorganic substances (heavy metals) and organic (gasoline, oil, aromatic hydrocarbons and polycyclic aromatic hydrocarbons) (Table 2). The high combined concentrations of heavy metals were determined in the made grounds 1 (5171 mg/kg dry basis) and 5 (5492 mg/kg dry basis), where it was mainly caused by lead contamination. Contamination by zinc was also found in most of the analysed made ground samples with the highest concentration in the made ground 3 (1750 mg/kg dry basis). The organic substances found in high concentrations in the made grounds 3 and 5 were gasoline, diesel oil and aromatic hydrocarbons (defined as the sum of the compounds). Furthermore, polycyclic aromatic hydrocarbons (also defined as the sum of the compounds) were detected in the made grounds 1 and 6. In the case of 2 out of 7 made ground samples no contamination by the analyzed inorganic and organic substances was found. It should be emphasized that the assessment of the degree of contamination of the made grounds was focused exclusively on the compounds listed in the Regulation of the Minister of the Environment [3].

Table 2

Contamination of the made grounds

	Made grounds						
	1	2	3	4	5	6	7
Depth [m bls]	0.6	0.7	0.7	0.8	0.8	1.5	1.8
Contaminant	Concentration[a] [mg/kg dry basis]						
Arsenic (As)	97	n.d.	n.d.	122	356	n.d.	79
Barium (Ba)	n.d.	n.d.	n.d.	483	n.d.	n.d.	n.d.
Chromium (Cr)	n.d.	n.d.	161	n.d.	n.d.	n.d.	n.d.
Zinc (Zn)	404	n.d.	1750	418	508	n.d.	496
Cadmium (Cd)	n.d.	n.d.	n.d.	6	18	n.d.	n.d.
Copper (Cu)	n.d.	n.d.	138	n.d.	n.d.	n.d.	n.d.
Nickel (Ni)	n.d.	n.d.	138	n.d.	n.d.	n.d.	n.d.
Lead (Pb)	4670	n.d.	648	602	4610	n.d.	2370
Sum of heavy metals	5171	n.d.	2835	1625	5492	n.d.	2945
Gasoline	n.d.	n.d.	16	n.d.	23	n.d.	n.d.
Oil	n.d.	n.d.	424	n.d.	780	n.d.	640
Sum of aromatic hydrocarbons (AHs)	n.d.	n.d.	436	47	800	n.d.	413
Sum of polycyclic aromatic hydrocarbons (PAHs)	20	n.d.	n.d.	n.d.	n.d.	41	n.d.

[a]n.d. – not detected.

The results of the study on the effect of the made grounds extracts on the survival of the crustacean *Daphnia magna* and the growth of duckweed *Lemna minor* and the corresponding toxicity class are presented in Table 3. The tested aqueous extracts affected the indicator organisms to a various extent. The survival of crustaceans only slightly depended on the duration of the test (24 and 48 h). Analysis of the results for the 48-hour exposure time shows that 4 out of the 7 extracts had a toxicological effect on the indicator organisms. In terms of the toxicity classes, the extracts of the made grounds 1 and 3 had a low toxicity, the extract of the made ground 5 was toxic and the extract from the made ground 2 had a high toxicity.

Table 3

Effect of the made grounds extracts on the indicator organisms

Test	Test duration	Made grounds extracts						
		1	2	3	4	5	6	7
		Effect [%] (toxicity class)						
Survival with *Daphnia magna*	24 hours	15 (–)	100 (+++)	5 (–)	5 (–)	45 (+)	5 (–)	5 (–)
	48 hours	40 (+)	100 (+++)	30 (+)	15 (–)	75 (++)	15 (–)	20 (–)
Growth with *Lemna minor*	7 days	50 (+)	100 (+++)	50 (+)	0 (–)	0 (–)	50 (+)	100 (+++)

(-) non-toxic, (+) low toxicity, (++) toxic, (+++) high toxicity

The analyzed extracts of the made grounds adversely affected the growth of the aquatic plant, since the toxicological effect was found in 5 out of 7 tested extracts. In the case of four extracts (made grounds 1–4), the toxicity class was the same as in the survival test with crustaceans, which can suggest similar sensitivity of the indicator organisms. High toxicity to duckweed was found for the extracts of the made grounds 2 and 7.

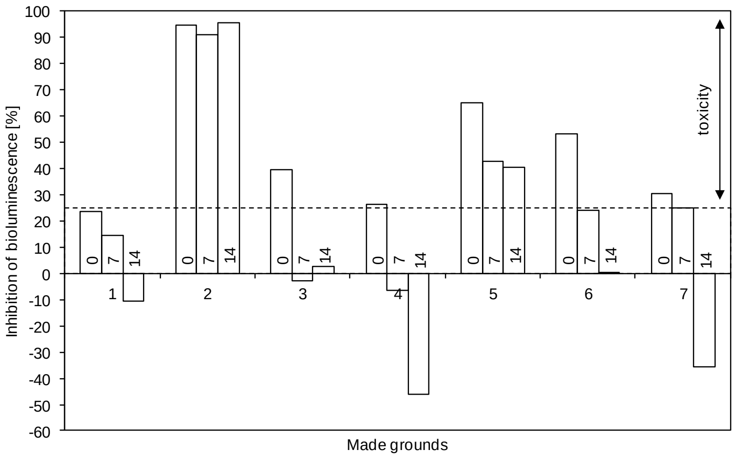

Fig. 1. Changes in the inhibition of bacterial bioluminescence for the extracts of the made grounds before and after various contact times (0, 7 and 14 days) with duckweed

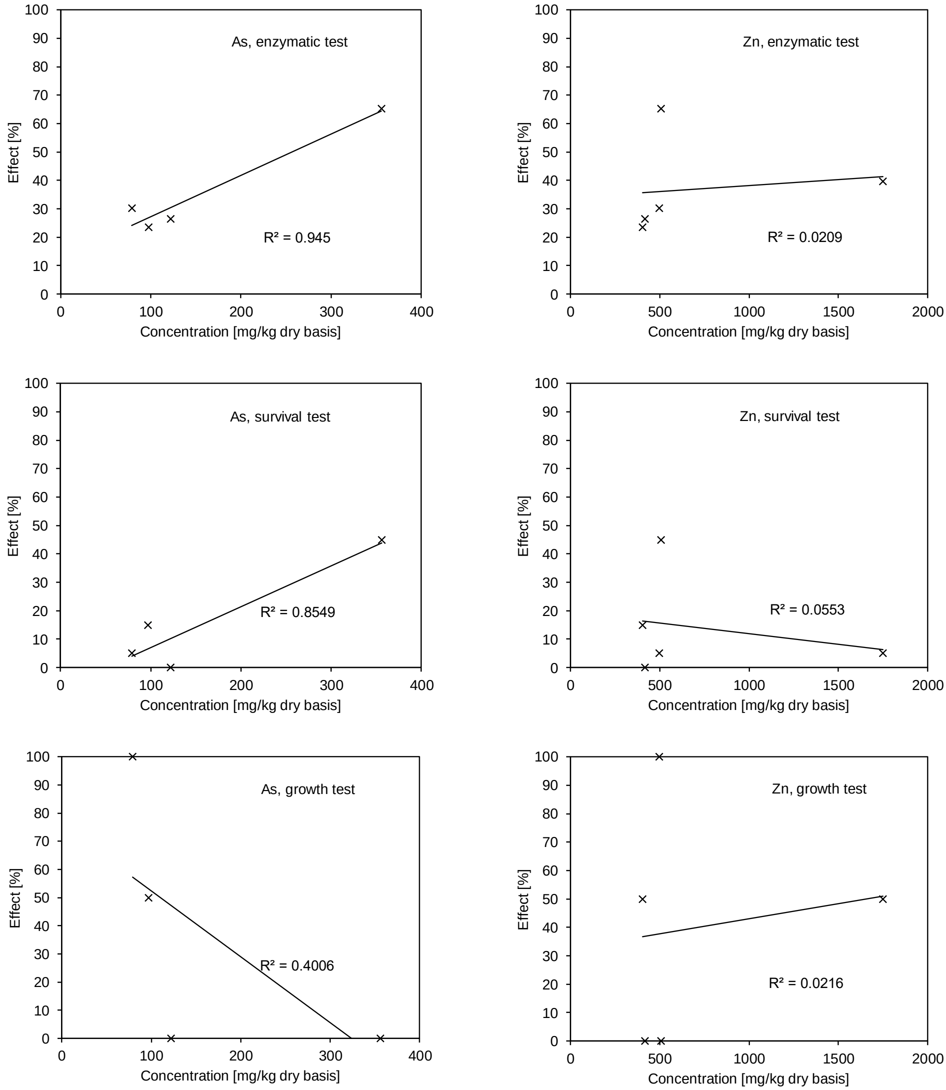

Fig. 2. Relationship between the observed toxicological effect
and the concentration of As (left column) and Zn (right column) in the made grounds

The determined high toxicity of the extract from the made ground 2 to both indicator organisms is surprising due to the fact that the analysed contaminants were not detected in this made ground (Table 2). As was mentioned above, the assessment of the degree of contamination of the made grounds was aimed at compounds listed in the Regulation of the Minister of the Environment [3]. For this reason, it can be assumed that the toxicity of this made ground was caused by different substances than those listed in Table 2.

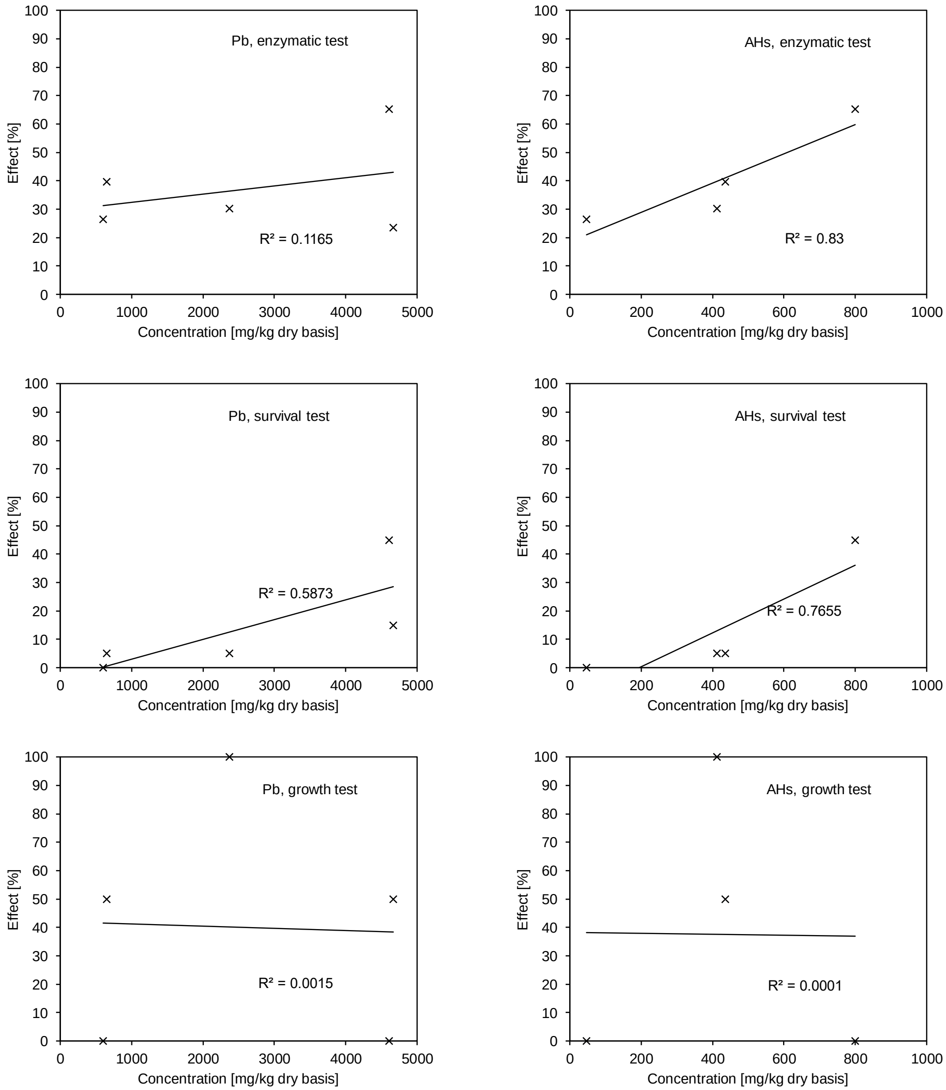

Fig. 3. Relationship between the observed toxicological effect and the concentration of Pb (left column) and aromatic hydrocarbons (AHs) (right column) in the made grounds

Figure 1 shows the changes in the inhibition of bacterial bioluminescence for the extracts of the made grounds before and after 7- and 14-day contact times with duckweed. This study was carried out taking into consideration that duckweed is also applied in phytoremediation [6–8]. The bioluminescence inhibition for the solutions after the contact with duckweed was lower than for the initial solutions. The slight increase in the bioluminescence inhibition was observed only in the case of extract from made ground 2 and 14 day of experiment duration. The reduction of the bioluminescence inhibition indicates the occurrence of the phytoremediation process, but it cannot be excluded that photolysis also influenced this parameter as the experiment was carried out under continuous illumination. After 7-day contact time of the solutions with duckweed, 5 out of 7 the studied extracts were found to be non-toxic. On the other hand, after 14 days, three extracts – 1, 4 and 7 stimulated physiological processes of the bacteria. According to various literature reports, duckweed can be successfully used to remove both inorganic [7, 8] and organic substances [8] from water, which is also confirmed by the results of this study.

The relationship between the observed toxicological effect and the concentration of selected contaminants in the made grounds has also been examined (Figs. 2, 3). The results are presented in Table 2 (concentration of contaminants in the made grounds), Table 3 (toxicological effect determined by the survival test – time 48 h, and in the growth test – 7 days) and in Fig. 1 (toxicological effects observed in the enzymatic assay). The basis of this assessment was the correlation coefficient (R^2) with the assumption that the minimum value of this parameter is 0.60 [18].

The analysis of the relationship of the tested variables showed that the toxicological effect in the enzymatic assay depends both on the concentration of arsenic ($R^2 = 0.94$) and aromatic hydrocarbons ($R^2 = 0.83$). Similar observations were made for the survival test, where also the toxicological effect was related to both the concentration of arsenic ($R^2 = 0.91$) and the concentration of aromatic hydrocarbons ($R^2 = 0.77$). Additionally, slightly weaker relationship between the variables was also observed for the toxicological effect and the concentration of lead ($R^2 = 0.59$). On the other hand, in the case of the growth test clear relationship between the examined variables was not observed.

4. CONCLUSIONS

- The substantial majority of the analysed extracts of the made grounds adversely affected both the survival of the crustacean *Daphnia magna* and the growth of the aquatic plant *Lemna minor*. The observed toxicological effect in the case of crustaceans survival test was related to the pollution of the made grounds mainly by arsenic and aromatic hydrocarbons, whereas in the case of the growth inhibition of the aquatic plants the cause of the toxicity was not identified.
- Despite the growth inhibition of duckweed this plant was found to be able to phytoremediate the contaminants present in the made grounds, which was determined using the enzymatic assay. Thus, this species of plants can be used in the case of the rehabilitation of water reservoirs exposed to surface runoff from areas containing contaminated made grounds.

The present study confirmed the complexity of the problems associated with the occurrence of made grounds in the natural environment, which creates a need for their constant monitoring. There is also a necessity to develop procedures as well as analytical methods for accurate determination of the exposure and for increasing the understanding of mechanisms of the effect of made grounds on the physiological processes of plants and animals.

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