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APPLICATION OF THE MICROTOX SYSTEM TO ASSESS THE TOXICITY OF MADE GROUNDS

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Abstract

Selected made grounds were subjected to toxicity analysis with the use of the Microtox bioassay employing a strain of luminous marine bacteria *Vibrio fisherii*. The toxicity assessment was carried out by analysing the liquid phase obtained after aqueous extraction of the studied solid samples. The conditions of the extraction process, i.e. sample preparation and duration of the process, were selected in the preliminary study. The effect of the exposure time for the extract on the bioluminescence inhibition value was also studied. The native soils situated below the layer of made grounds were analysed for their toxicity in a comparative manner. The following conditions for the toxicity analysis of the made ground were determined: the ratio of a sample mass to the volume of deionised water 200 mg/1cm^3, the extraction time of 10 min, and the exposure time of 5 min. It was found that the toxicity of made ground depended on the type of the sample, and the value of this parameter may be associated with the presence of various heavy metals and organic contaminants. No toxic interaction was found between the made ground and the native soil.

Streszczenie

Wybrane grunty nasypowe poddano analizie toksyczności przy pomocy biotestu Microtox wykorzystującego luminescencyjny szczep bakterii morskich *Vibrio fisherii*. Ocenę toksyczności dokonano poprzez analizę fazy ciekłej uzyskanej po ekstrakcji wodnej badanych próbek stałych. W zakresie badan wstępnych dobrano warunki prowadzenia ekstrakcji tj. sposób przygotowania próbki oraz czas procesu. Badano także wpływ czasu ekspozycji ekstraktu na wartość inhibicji bioluminescencji bakterii. Porównawczo przeprowadzono analizę toksyczności gruntów rodzimych znajdujących się pod warstwą gruntu nasypowego. Określono następujące warunki analizy toksyczności gruntów nasypowych tj. proporcja naważki próbki gleby do objętości wody zdejonizowanej 200 mg/1 cm^3, czas ekstrakcji 10 min, a czas ekspozycji 5 min. Stwierdzono, że toksyczność gruntów nasypowych zależała od rodzaju analizowanej próbki, a wartość tego parametru może być związana z obecnością różnych metali ciężkich lub zanieczyszczeń organicznych. Nie zaobserwowano toksycznego oddziaływania pomiędzy gruntem nasypowym i gruntem rodzimym.

Keywords: **Made grounds, Microtox test, Toxicity.**

1. INTRODUCTION

Modern environmental analysis has been increasingly using bioassays [1-9] in which various indicator organisms, including bacteria [1], freshwater- and salt-water crustaceans [2] and plants [3] are employed. The toxicity of a sample, which is determined by a selected bioassay, indicates a cumulative impact of various types of pollution on the individual elements of the environment. An example of a commonly used bacterial bioassay is Microtox [7-13].

The Microtox bioassay was developed in the late seventies by Beckman, as a quick toxicity test [10]. It is an alternative to the tests employing higher aquatic organisms such as fish [9]. This bioassay has been started to be used on a larger scale in toxicity studies in the early eighties. It was then adopted as a criterion for toxicity assessment in many countries, including Australia, the United States, Canada, Spain, the United Kingdom, Germany, Sweden, the Netherlands and Poland [10]. The subject of the toxicity assessment can be both a sample of water, waste water and bottom sediments [7-9, 11-13]. Noteworthy, it can be found in the literature that bioassays have been increasingly used in an innovative manner. For example, in [12] we have reported on successful application of the Microtox bioassay for the toxicity assessment of solid (ash) and liquid (tar) by-products generated during the gasification of sewage sludge. In this case, however, it was necessary to develop dedicated procedures for sample preparation prior to the toxicological examination.

The aim of this study was to determine the suitability of the selected bacterial bioassay Microtox for the toxicity assessment of selected non-engineered made grounds.

Made grounds represent anthropogenic layer of a land surface, and often have a thickness of up to a few metres in the cities and even several meters in the industrial areas [14-17]. Made grounds occurring in Silesia, 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 very 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 [14]. It should be also taken into account, that the speciation of metals, the 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.

2. METHODS

The subject of this study were the samples of made grounds taken from selected areas of the Silesia region. General characteristics of the made grounds are presented in Table 1. The studied made grounds differed mainly in terms of the zinc and lead content. The determinations of metals present in soils or made grounds were carried out by atomic absorption spectrometry after digestion. Comparative analysis was performed also for the native soil underlying the layer of made ground 3. This made ground was found to contain no heavy metals.

Table 1.
The characteristics of the made grounds

Made grounds	Depth [m bls]	Metals	
		Zinc (Zn)	Lead (Pb)
		Maximum allowable concentrations [mg/kg DW]	
1	0.6	404	4670
2	0.8	418	602
3	1.8	496	2370
4	0.8	508	4610

DW – Dry Weight

The toxicity of the ground was assessed with the use of the Microtox bioassay, which was mentioned in the Introduction section, employing a strain of luminous marine bacteria *Vibrio fisherii*. 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 [13]. The study was conducted using the MicrotoxOmni system in the Microtox Model 500 analyser purchased from Tigret sp. z o.o (Poland), which can be used as both an incubator and a photometer. The toxicity assessment was carried out by analysing the liquid phase obtained after aqueous extraction (with deionised water) of the studied solid samples. The conditions of the extraction process, i.e. sample preparation and duration of the process, were selected in this study. During the extraction, the samples were mechanically mixed at 300 rpm/min using a shaker from Labor System (Wroclaw, Poland). Next, the suspension of rehydrated bacteria was added to water extracts, which were already filtered using 0.45 μm cellulose acetate filter purchased from

Millipore (Warsaw, Poland). After 5 and 15 minutes of exposure the percent inhibition of bioluminescence was measured against a blank sample (2% NaCl). The toxicity of the samples was categorised according to the classification system, which has been commonly applied by many researchers [11-13] and is based on the magnitude of the observed effect in the indicator organisms being employed (Table 2).

The results presented in this paper are arithmetic means of the four replicates of each experiment.

Table 2.
Samples toxicity classification system [11-13]

Effect, %	Toxicity class
<25	non toxic
25-50	low toxic
50.1-75	toxic
75.1-100	highly toxic

3. RESULTS

The value of the bioluminescence inhibition was dependent on both the sample preparation method and aqueous extraction time. The increasing proportion of soil sample mass to extractant volume (deionised water) corresponded to increased value of the bioluminescence inhibition (Fig. 1. a). A similar observation was made when the extraction time for soil samples increased (Fig. 1. b). The duration of the exposure of the extract (5 or 15 min) had no significant effect on the value of the determined parameter (Fig. 2). The difference between the results of the analyses did not exceed 5%.

Taking into account the obtained results, the following analysis conditions were assumed for further study: the ratio of the mass of a soil sample to the volume of deionised water of 200 mg/1cm^3, the extraction time of 10 min, and the exposure time of 5 min. In the further part of this study, a comparative assessment of the toxicity of selected made grounds was carried out (Fig. 3).

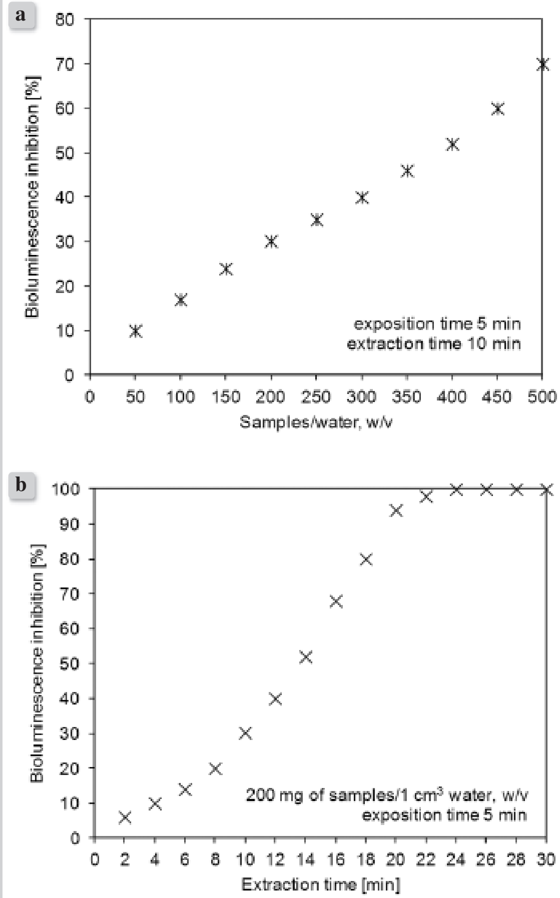

Figure 1.
Impact of the preparation methods of grounds samples (a) and water extraction time (b) on bioluminescence inhibition (made grounds 3)

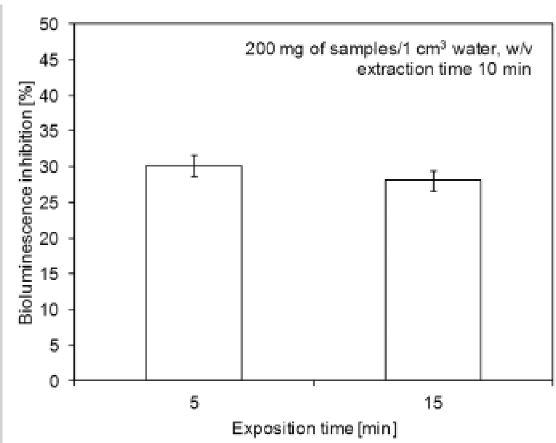

Figure 2.
Impact of the exposition time on bioluminescence inhibition (made grounds 3) (mean values, ± standard deviation)

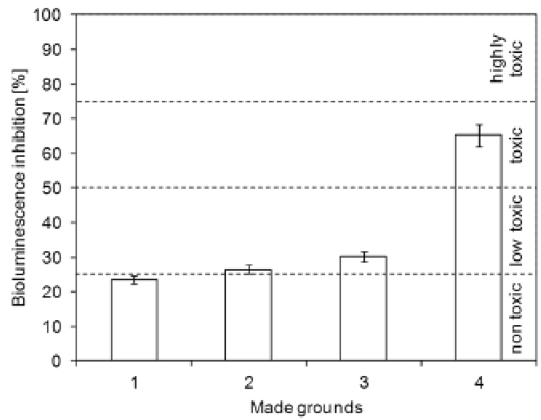

Figure 3.
Toxicity of the made grounds (mean values, ± standard deviation)

Based on the results of the analyses, it was demonstrated that the investigated made grounds affect the intensity of bioluminescence to a various. According to the toxicity classification scheme (Table 2) it was determined that made ground 1 is not toxic, made grounds 2 and 3 have a low toxicity, and made ground 4 is toxic. Taking into account the same source of Zn and Pb in the samples (a fraction of the same slag), it can be excluded that the presence of these metal affected the toxicity of these samples. The toxicity of the made grounds can be attributed to the presence of other heavy metals (except for Zn and Pb). Also, it cannot be excluded that this parameter was affected by organic pollutants. The previous literature studies on this topic [14, 15] have demonstrated that the made grounds contain mainly exceeded concentrations of selected heavy metals as compared to relevant quality standards of soil [18]. Therefore, the continuation of this research will focus on statistical evaluation of the interdependence between the concentration of inorganic contaminants (heavy metals), and the value of the bioluminescence inhibition of specific made grounds. For cognitive reasons this assessment may also be extended to include organic pollutants e.g. polycyclic aromatic hydrocarbons.

In the final stage of the study, the toxicity of the made grounds and the underlying native soil was compared (Fig. 4). The analysis showed that the two samples were not toxic. The toxic effect of the native soil was lower than observed for the made grounds. This observation corresponds to the results of the previous studies [14, 15], in which the geological cross-sections were found to have exceeded metals concentrations only in the made ground layer but never in the layers situated directly beneath them, which indicates no noticeable metal migration from the made grounds. This was observed regardless of the permeability of each layer.

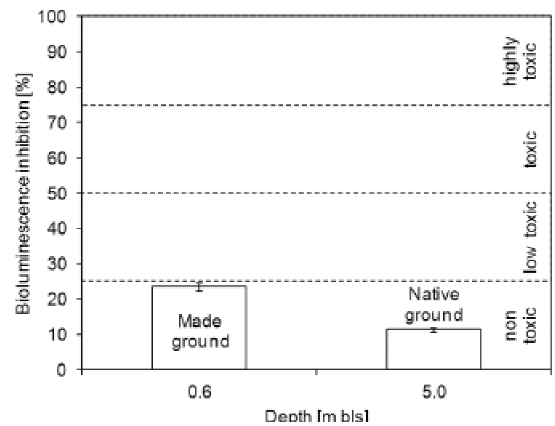

Figure 4.
Comparison of toxicity o the selected made and native grounds (made grounds 1) (mean values, ± standard deviation)

Based on the results of the study it can be concluded that the assessment of the toxicity of made grounds using the Microtox test may be helpful in terms of the environmental risk assessment associated with this type of waste. This method is competitive with multi-target instrumental analysis for the characterization of samples for various types of contaminants.

4. CONCLUSION

Based on the obtained results it can be concluded that:

- The developed extraction procedure allows for the toxicity assessment of made grounds using the Microtox test. This procedure was also successfully used to evaluate the toxicity of the native soil situated beneath the layer of the made grounds.
- It was found that the toxicity of the made grounds depended on the type of the sample, and that the value of this parameter may be associated with the presence of various heavy metals and organic contaminants, which will be discussed in further publications.
- Comparative analysis of the made grounds and the underlying native soil showed no interaction between these layers.

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