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Toxicity evaluation of eluates from waste after thermal conversion of sewage sludge

Jolanta Sobik-Szołtysek, Katarzyna Wystalska*

Faculty of Infrastructure and Environment, Institute of Environmental Engineering, Czestochowa University of Technology, Brzeźnicka 60a, 42-200 Częstochowa, Poland, Tel. +48 34 325 09 17; emails: jszoltysek@is.pcz.pl (J. Sobik-Szołtysek), kawyst@is.pcz.pl (K. Wystalska)

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ABSTRACT

The waste generated in the processes of thermal disposal of sewage sludge introduced into the environment, for example, in the storage process, is subjected to the influence of rainwater, the result of which may be the leaching of many, often hazardous, components therefrom. Hazardous substances in eluates pose a risk of migration thereof into the ground and water environment and can also have a toxic effect on the biosphere. The water leaching test, used in Poland to evaluate environmental risk caused by waste, only shows the capacity to release substances contained in waste under specific conditions without the inclusion of their impact on the biosphere. In contrast, the evaluation of waste bio-toxicity is conducted using bioassays. The undertaken research aimed to examine whether the expansion of environmental risk assessment to include biotests with respect to potentially leachable components from waste would be a better tool for the evaluation of their toxicity. The research included bioassays with the usage of *Lepidium sativum*, and *Avena sativa* plants and the Microtox screening test with the participation of *Vibrio fischeri* bacteria. The analyzed eluates from the ashes obtained through sewage sludge incineration and waste from the treatment of flue gas were characterized by alkaline reaction as well as high levels of chlorides and sulfates. The eluates from fly ashes demonstrated a varied bio-toxicity depending on the type of the test organism. In the case of *Lepidium sativum*, the inhibition of root growth was observed while *Avena sativa* was stimulated. With respect to the *Vibrio fischeri* bacteria, a toxic effect of eluates was not noted. The eluates from waste obtained through the treatment of flue gas had a toxic effect on every biotest. The usage of bioassays as supplementation of chemical research for the assessment of the influence of leachates from waste on the ground and water environment may significantly raise the effectiveness of monitoring and the quality management of water resources.

Keywords: Eluates; Ashes; Toxicity; Biotests; Microtox test

1. Introduction

The formation of sewage sludge is an integral part of the sewage treatment process. The development of methods used to treat sewage translates onto the growth of the quantity of produced sewage sludge. For example, in Poland, in the years 2000–2017, the amount of produced municipal sewage sludge increased by 63%. In accordance with the binding legal norms, the untreated sewage sludge cannot be stored at landfill sites, and their amount is determined by the usage of efficient methods of management. The usage of thermal methods of sewage sludge conversion is one of the more beneficial solutions because it ensures not only a significant reduction of its mass and volume but also the recovery of the energy

* Corresponding author.

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contained therein. At most sewage sludge incineration plants operating in Poland, fluidized bed incineration processes are used. Research is also being conducted on the possibility of the vitrification process usage to dispose of the sludge and ashes formed in this process [1,2]. An adverse phenomenon, accompanying the incineration processes, is the generation of a secondary waste stream, that is, fly ashes and waste formed in flue gas treatment processes. The composition of ashes obtained through incineration is variable, dependent on the properties of the incinerated sewage sludge. Ashes from the incineration of sludge may be appropriately managed [3–5], and the presence of, inter alia, a significant amount of phosphorus therein as a biogenic component creates an alternative direction of their usage [6–8]. However, the frequent presence of heavy metals in ashes may pose a serious ecotoxicological risk in the case of application thereof into the environment [9]. In the storing process, ashes are subjected to the influence of rainwater, the result of which may be leaching of hazardous components therefrom [10]. The aging of ashes (CO_2 absorption) facilitates the leaching of heavy metals, such as Zn and Pb [5]. Hazardous substances present in eluates (including heavy metals) pose a risk of entering the ground and water environment and consequently, they may have a toxic impact on the biosphere by becoming, for example, an element of the human biological life cycle [11]. For this reason, rational waste management, incorporating itself in the closed-loop cycle, should be conducted with the inclusion of ecotoxicological risk assessment, connected with the disposal and conversion of waste [12,13].

The water leaching test, used in Poland to evaluate environmental risk caused by waste, is carried out in accordance with the PN-EN 12457-2 norm [14]. This test only indicates the capacity to release substances contained in waste under specific conditions. However, according to research conducted by Tsiridis et al. [15], it proved to be better than the leaching method called toxicity characteristic leaching procedure method 1311, recommended by the American Environmental Protection Agency [16]. The results of the test are compared with the limit values included in the provisions pertaining to the classification of waste which is acceptable for disposal at landfills of a specific type [17].

The level of leaching of metals from ashes depends on, that is, their content in the analyzed waste, chemical form, the degree of oxidation [11,18,19], and the introduced leaching procedure [15,20–22]. The complex leachate composition, in which many harmful substances appear in trace quantities, does not allow for precise determination which is thus responsible for the highest toxic effect in the environment. It results from the fact that as a mixture they can act additively or synergistically [23,24]. The concentrations of leached heavy metals, determined in eluates, show the level of their content in waste, however, they do not provide information on their impact on the biosphere. Information on the bioavailability of metals may be obtained by using sequential analysis methods. In contrast, bioassays are used to evaluate the biotoxicity of eluates [15,20,21,25]. The usage of bioassays together with the chemical analysis of eluates is deemed as necessary to appropriately assess waste toxicity [26,27]. However, according to Tsiridis et al. [20], a situation may occur where the toxicity of eluates towards selected organisms may be high at a relatively low concentration of metals or vice versa. Therefore, the harmful impact of toxic compounds on organisms may take place at a significantly lower concentration than it would result from chemical analyses. Due to this fact, while determining the bioavailability of xenobiotics, the usage of ecotoxicological tests is already recommended at very low levels thereof [28,29].

Toxicity bioassays may be a tool used for the classification determining whether given ash is a waste or secondary raw material [30]. These tests are characterized by an adequately sensitive toxicological endpoint that may be obtained within a short period (≤24 h) [31]. Live organisms, whose reaction is the basis for the evaluation of the general activity of a given system [32], are used as bioindication markers. As a general rule, to classify waste, it is recommended to use sets of tests [33,34]. The set of toxicity tests should cover test organisms from different trophic levels, as the toxicity of eluates may vary depending on the type of a test organism due to the variable sensitivity of indicator organisms to harmful factors [35]. For this reason, the presented research includes bioassays that use *Lepidium sativum*, and *Avena sativa* plants and the Microtox screening test with the participation of *Vibrio fischeri* bacteria.

The standard *Lepidium* test and the measurement of root growth inhibition is very useful to assess the phytotoxicity of a ground and water medium contaminated by heavy metals [36], and the reactions of this plant, in comparison to other test plants, are deemed to be most representative due to its high sensitivity to the presence of toxic substances [37–39]. This test is recommended due to its simplicity, high sensitivity, speed of execution and efficiency in view of costs [40–43]. It is stated [44] that heavy metal ions have an inhibiting effect on the germination of seeds of *Lepidium sativum*, while root development is influenced by both the ion type and its concentration.

The Microtox screening test is applied in the evaluation of an environmental condition within the scope of assessment of the degree of the negative impact of a wide range of pollutants, especially as the first indicator showing the presence of pollutants in the analyzed ecosystem. It detects over 1300 chemical compounds, including PAH, PCB, active substances of herbicides, pesticides, and explosives, as well as heavy metals [45–47]. The basic advantages of the Microtox screening test are repetitiveness, speed, and ease of use of the test system [48], and its sensitivity is generally comparable to the sensitivity of higher organisms, such as crustaceans and fish. The Microtox test may be also used to evaluate chronic toxicity [49]. Another favorable feature is connected with minimum requirements pertaining to the size of the sample in comparison to other standard tools for bioassays [50].

The results of the previously conducted research [25,51] confirm the correlation between the chemical characteristic of eluates from ash and their toxicity observed while carrying out biotests. However, other research within this scope [15,20,21,27,30] did not confirm those correlations and showed non-conformity of ecotoxicological tests with findings from physicochemical analyses of eluates. The usage of bioassays in connection with the required chemical tests of harmful substances significantly increases the efficiency of monitoring of the environment [52].

The evaluation of eluates exclusively in light of ecotoxicity may lead to findings on the lack of toxicity, while the

connection of evaluation in the direction of ecotoxicity and chemical characteristics allows for the recognition of eluates as toxic [53].

For this reason, the aim of the presented research was linked to the toxicity evaluation of eluates from ash after the incineration of sewage sludge by means of the leaching test and selected biotests. Moreover, the correlation was checked between chemical characteristics and biotoxicity of the analyzed eluates.

2. Materials and methods

2.1. Solid samples

For the purpose of research, fly ash (3 samples – A1, A2, A3) was used, which was taken from the fluidized bed installations operating in Poland that incinerate municipal sewage sludge and waste from flue gas treatment processes (2 samples – W1, W2). Sewage sludge comes from the treatment of municipal sewage that is supplied to the treatment plant through a combined sewer system. Before incineration in the fluidized bed furnace, sewage sludge is dried to a dry matter content of approximately 35% for ashes denoted as A1 and A2, and approximately 60% for A3. Drying time is determined by the specific characteristics of the equipment used in this process. As a product of transformation at 850–900°C, ash does not contain moisture, although storage conditions may cause a small amount of moisture to appear. Before the experiments, the ashes were dried at 105°C to eliminate this effect.

The content of metals in solid samples (after prior mineralization by means of Berghof high-pressure microwave digester) and in eluates prepared therefrom, was determined by the spectrophotometric method, by using the inductively coupled plasma emission spectrometer (ICP-OES) Thermo Elemental IRIS INTREPID II XSP DUO. Cu, Zn, Pb, Cr, Ca, Na concentrations were determined in the solid samples and eluates. Other parameters of water eluates, that is, pH, total dissolved solids (TDS), and EC were determined by using a multiparameter meter made by HANNA INSTRUMENTS - modelHI 9828. The concentration of chlorides and sulfates were determined by means of the RIGOL L-3000 liquid ion-exchange chromatography system HPLC. The concentrations of metals obtained in the ICP analysis were converted from mg dm^{-3} to mg kg^{-1}.

2.2. Leaching tests

For the purposes of chemical analyses and biotests, eluates from solid samples were prepared according to the PN-EN 12457-2 norm [14]. In compliance with the norm, 100 g of a solid sample and 1 L of distilled water was mixed in closed glass vessels for 24 h at 10 rpm, at room temperature. The obtained eluate was centrifuged and additionally filtered through filter paper.

2.3. Toxicity tests

The toxicity of eluates prepared from solid samples was studied by using *Lepidium sativum*, and *Avena sativa* plant organisms and *Vibrio fischeri* bacteria. The *Lepidium sativum* test was carried out on the basis of the methodology described by [54]. The test was conducted on water extracts (eluates) from solid samples and the control sample (distilled water). Twenty seeds of *Lepidium sativum* were placed on Petri dishes with filter paper, impregnated with 5 ml of every eluate. A Petri dish with filter paper impregnated with 5 ml of distilled water was used as a control. The dishes were placed in an incubator, at a constant temperature and air humidity, appropriate for seed germination.

Test performance conditions were as follows:

- test type: static
- temperature: 25°C
- humidity: 40%–50%
- test vessel: 9 cm diameter culture dish, Whatman N° 3 filter paper
- light: no
- test volume: 5 ml/dish
- N^0 seeds: 20/dish
- replicate: 3
- test duration: 48 h

In the case of the test carried out with *Avena sativa*, 5 seeds were used which were treated in the same manner as *Lepidium sativum* seeds, only extending the duration of incubation to 144 h. After completing the tests, the number of germinated seeds was calculated and the length of the longest root was measured. These data were used to calculate the percentages of relative seed germination (RSG) (1), relative root growth (RRG) (2) and the germination index (GI) (3) on the basis of equations given by Walter et al. [54].

$$\mathrm{RSG}(\%) = \frac{\text{number of seeds germinated in extract}}{\text{number of seeds germinated in control}} \times 100 \quad (1)$$

$$\mathrm{RRG}(\%) = \frac{\text{mean root length in extract}}{\text{mean root length in control}} \times 100 \quad (2)$$

$$\mathrm{GI}(\%) = \frac{\mathrm{RSG} \times \mathrm{RRG}}{100} \quad (3)$$

Toxicity tests with the luminescent strain of marine bacteria *Vibrio fischeri* were performed by means of the Microtox analyzer (Model 500). The research included the performance of screening tests, having the duration of exposure of 5 and 15 min, on the basis of the standard procedure provided by the producer (81.9% Screening Test). Due to the fact that the pH range recommended by the manufacturer is 6–8, it was established that pH in the experiment would be maintained at a constant level equal to 7.0. When required, pH was corrected by using HCl 0.1 mol dm^{-3} or NaOH 0.2 mol dm^{-3}. The toxicity of eluates was analyzed on the basis of a luminescence measurement with respect to the indicator bacteria *Vibrio fischeri* that emit light as a result of metabolic processes. The disturbance of metabolic processes, resulting from contact with a sample, causes changes in the light emission intensity of these organisms [55]. The presence of toxic substances in the studied sample leads to inhibition and reduction of bioluminescence of the bacteria [56,57]. The degree of change of light output in

relation to the control sample is directly proportional to the toxicity level in the test samples.

2.4. Statistical analysis

IBM SPSS Statistics 25 package was used for the purpose of statistical analysis. Spearman's correlation analysis was implemented to evaluate the importance of mutual dependencies between the content of metals, chlorides and sulfates, and the indicators RSG, RRG and GI with respect to test plants *Lepidium sativum* and *Avena sativa*. The Wilcoxon test was used to check whether there are significant statistical differences between the loss of luminescence of the *Vibrio fischeri* bacteria, and the content of the analyzed metals, chlorides and sulfates in eluates with reference to two periods of incubation time (screening test of 5 and 15 min). The mean and standard deviation were calculated during the statistical analysis of findings. It was determined that the statistically significant level is the value $p < 0.05$.

3. Results

3.1. Characteristics of solid samples

The content of selected metals in ashes and waste from the treatment of flue gas is presented in Table 1.

Fly ashes from the fluidized bed incineration of sewage sludge (A1, A2, A3) were characterized by a high content of Cu, Zn and Pb. These metals are part of the substance group that is seen as the cause of the toxicity of a wide array of materials [15,51,58,59]. During wastewater treatment, heavy metal ions are mainly adsorbed on the surface of suspensions and on activated sludge flocs. During the biochemical treatment of sediments, only a small portion of metals can be released to the supernatant liquid, while a significant amount remains in the sludge. The processes of drying and incineration of sewage sludge do not fundamentally modify the metal content in the sludge, especially during incineration at temperatures of up to 900°C. Due to the decrease in organic matter content, higher metal concentrations are quantified in the ashes. The highest concentrations of Cu, Zn, Pb and Cr were found for the substrate A2. The high content of heavy metals found in this substrate may be caused by the fact that the wastewater discharged into the treatment plant is produced in a large industrial agglomeration. The highest concentrations of Cu, Zn, Pb and Cr were indicated in the substrate A2, which may be caused by high participation of industrial sewage delivered to a sewage treatment plant, from which the sludge incinerated at a fluidized bed incineration plant originates. The fly ashes were also characterized by a high content of Ca, which may be the result of CaO usage in the hygienization of sewage sludge. In ashes deriving from the process of waste gas treatment (W1, W2), very high concentrations of Na were indicated, exceeding 2,00,000 mg kg^{-1} in the W2 material. Higher content of Ca, Zn, Pb and Cu were determined in the W1 substrate in comparison to the W2 material that, in contrast, was characterized by a higher content of Cr. The high concentration of Na in the W1 and W2 waste is determined by the usage of its compounds ($NaHCO_3$) in the processes of flue gas treatment.

3.2. Characteristic of eluates

Eluates formed from fly ashes (EA1, EA2, EA3) and waste from the process of flue gas treatment (EW1, EW2) were subjected to the physicochemical analyses, the results of which are presented in Tables 2 and 3. The obtained findings were compared with the limit values included in the national provisions allowing for the disposal of waste at landfills of a given type [17].

All eluates were characterized by an alkaline reaction within the range pH = 9.35 ÷ 11.51, which is connected with the presence of Ca in the analyzed samples [15]. The level of pH of eluates is the most significant factor influencing their toxicity, inter alia, as a result of the possibility of metal transformation into forms available to organisms [21,22]. Important parameters of eluate quality are chlorides, sulfates and chromium [59]. The conducted analysis of eluates showed that the authorized concentrations of sulfates in EW1 and EW2 water extracts were exceeded, which confirms the classification of the waste as hazardous (Table 2). In the case of these eluates, the concentrations of chlorides did not exceed the limit values. In EA1, EA2, EA3 eluates, the concentrations of chlorides and sulfates were indicated at a lower level, not exceeding the limit values.

EW1 and EW2 eluates were characterized by high conductivity values at the level of 54.5 ÷ 70.3 mS cm^{-1}. In terms of the eluates from fly ashes (EA1, EA2, EA3), the conductivity

Table 1
Content of selected metals in ashes from the incineration of sewage sludge and waste from the processes of flue gas treatment

Metal mg kg^{-1}	Fly ash and waste samples				
	A1	A2	A3	W1	W2
Cu	601.6 ± 22.6	696.4 ± 29.5	890.7 ± 16.8	241.8 ± 2.5	39.2 ± 20.6
Zn	5,002 ± 183	6,180 ± 20	5,685 ± 25	1,769 ± 60	22 ± 12
Pb	181 ± 17	350 ± 38	171 ± 36	177 ± 7	78 ± 35
Cr	56.1 ± 16.2	903.8 ± 63.9	80.8 ± 50.2	<0.005	148.8 ± 17.4
Ca	1,30,400 ± 3444	1,03,400 ± 768	1,62,100 ± 454	42,420 ± 274	1,839 ± 147
Na	<0.005	<0.005	<0.005	1,99,800 ± 7965	2,75,100 ± 6,232

A1, A3, A3 – fly ashes.
W1, W2 – waste from the processes of flue gas treatment.

Table 2
Selected properties of eluates from ashes and waste from flue gas treatment

Parameter	Unit	Fly ash and waste samples					Permissible contents[a]	Permissible contents[b]
		EA1	EA2	EA3	EW1	EW2		
pH	(–)	11.51	9.35	9.81	10.80	10.30	–	–
Chlorides	mg kg^{-1}	66	56	30	10,000	11,000	15,000	25,000
Sulfates	mg kg^{-1}	12,000	9,500	9,900	4,50,000	4,30,000	20,000	50,000
Conductivity	mS cm^{-1}	2.2	1.6	1.6	54.5	70.3	–	–
TDS	mg kg^{-1}	11,300	7,900	8,100	2,72,400	3,51,700	60,000	1,00,000

EA1, EA2, EA3 – eluates from fly ashes.
EW1, EW2 – eluates from waste from flue gas treatment.
[a]Acceptable boundary limits for leaching concerning storage of waste other than dangerous and neutral waste calculated per dry matter of waste [17, Appendix 3].
[b]Acceptable boundary limits for leaching concerning storage of dangerous waste calculated per dry matter of waste [17, Appendix 5].

value was within the range of 1.6 to 2.2 mS cm^{-1}. According to the literature [21], conductivity values reaching 10 mS cm^{-1} indicated potential phytotoxic impact of leachates. In the case of EW1 and EW2 eluates, this level was significantly exceeded. The observed dependencies confirmed the indicated TDS values (Table 2).

The analysis of metal concentrations in eluates did not show that the limit values provided for in the provisions of the law were exceeded [17]. Water extracts made from substrates from the process of flue gas treatment (EW1 and EW2) were characterized by the highest concentrations of Na, which are not regulated by provisions. In the case of Ca content, its level was dependent on the technology of sewage sludge and flue gas treatment.

3.3. Biotest with plants

The results of biotests carried out with the use of *Lepidium sativum* and *Avena sativa* showed that EW1 and EW2 eluates were characterized by high toxicity, because no germination of test plants was found as opposed to EA1, EA2 and EA3. This is likely to have been caused by the high contents of chlorides, sulfates and sodium in EW1 and EW2 eluates compared to the concentrations of these components in EA1, EA2 and EA3. Analysis of the contents of heavy metals determined in the EW1 and EW2 eluates revealed no effect of these elements on the germination of test plants because in most cases, their contents were lower than in EA1, EA2 and EA3. The observed total lack of germination of both test plants in the case of EW1 and EW2 eluates was also not caused by the effect of the pH of these eluates (pH was 10.8 for EW1 and 10.30 for EW2), because for EA1, with a higher pH value (11.51), germination and growth of the test plants was obtained. The obtained findings in the form of RSG and RRG indicators, calculated in accordance with Eqs. (1) and (2), are shown in Figs. 1 and 2.

The biotoxicity tests performed with respect to EA1, EA2 and EA3 eluates showed differences in their impact on particular test plants. These eluates proved to be less toxic to *Avena sativa* in comparison to *Lepidium sativum*. With respect to both test plants, the RSG indicator of EA2 and EA3 eluates exceeded the value by 100%, indicating a stimulating effect. In contrast, with regard to the EA1 eluate, this indicator was

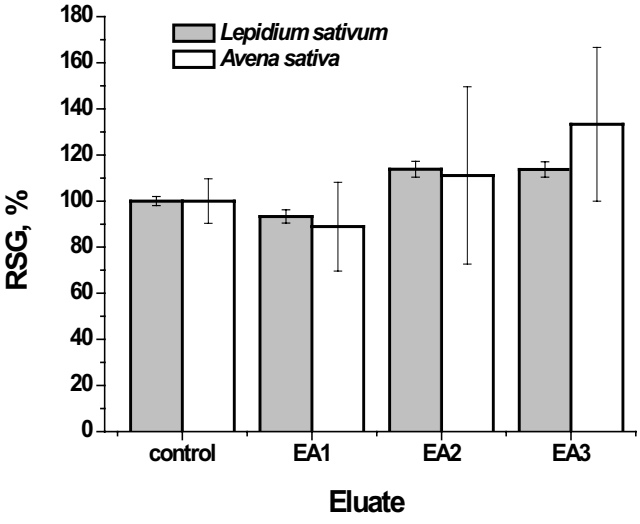

Fig. 1. RSG indicator for the biotest with *Lepidium sativum* and *Avena sativa*.

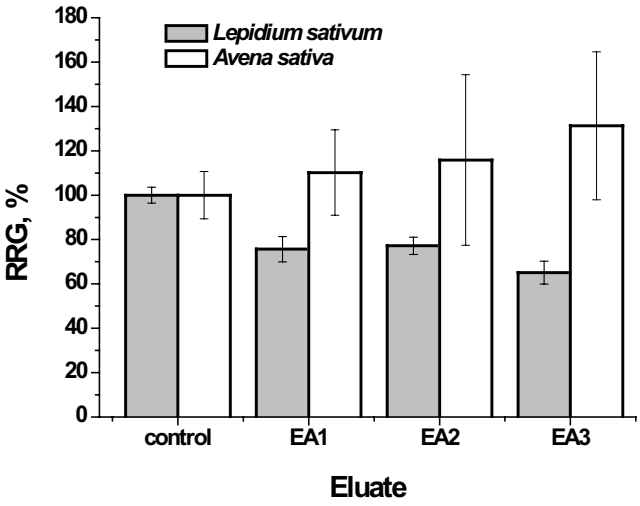

Fig. 2. RRG indicator for the biotest with *Lepidium sativum* and *Avena sativa*.

equal to 93.33% for *Lepidium sativum*, and 88.9% for *Avena sativa*. The analysis of the characteristics of eluates (Table 3) shows differences in Cu and Cr contents in the EA1 eluate compared to EA2 and EA3. This may have been caused by the RSG value for this eluate (below 100%).

In the case of RRG, a clear difference was observed in its value between the plants used in biotests. For *Lepidium sativum*, the value of RRG did not exceed 78%, whereas, for *Avena sativa*, it was over 100%. Similarly, as in the case of the RSG index, the highest values of RRG (max. stimulation) obtained for *Avena sativa* were obtained for the EA3 eluate, which was characterized by the highest content of Cu and Cr. Therefore, the EA1, EA2, EA3 eluates allow *Lepidium sativum* to germinate, however, they have an inhibiting effect on root growth. According to many authors [60–62], cytotoxic compounds present in eluates may inhibit the meristematic cell division processes of roots, causing the inhibition of their growth.

The GI was calculated on the basis of the obtained RRG and RSG values according to Eq. (3). The findings are presented in Fig. 3.

The value of the GI confirmed the observed dependencies. It did not achieve the value of the control sample (100%) with respect to *Lepidium sativum*, and its maximum value was obtained in relation to the EA2 eluate (87.87%). A different dependency was observed in the case of *Avena sativa*, where the GI value for EA2 and EA3 eluates significantly exceeded the value regarding the control sample. It confirms the stimulating effect of these eluates. By comparing the GI values for *Avena sativa* and *Lepidium sativum* in the case of the EA1 eluate, the lowest values of this indicator were obtained, which suggests the highest toxicity of this eluate in relation to the test plants.

3.4. Biotests with Vibrio fischeri bacteria

The research was conducted with *Vibrio fischeri* bacteria using the Microtox test. During the study, a screening test was performed in accordance with the standard procedure of the manufacturer. The analysis of the findings (Fig. 4) indicates that a substantial toxicity effect (level of luminescence reduction over 50%) was observed after the lapse of 5 min only in the case of EW1 and EW2 eluates. The extension of exposure time to 15 min caused a further increase in the toxicity of these eluates. In contrast, EA1, EA2, EA3 eluates did not show a toxic effect because the luminescence of the test bacteria increased both after 5 and 15 min of exposure. This effect may be caused by the presence of substances in eluates that create good conditions for the development of the test bacteria. The Microtox test procedure adjusts the pH to the required level (pH 6–8) to exclude any possible effect of the pH on the test results. Therefore, this factor did not affect the results obtained for toxicity to the test organism. The high toxicity of EW1 and EW2 was probably due to their high content of Na, chlorides and sulfates.

In the case of eluates EA1, EA2 and EA3, the contents of Na and Ca are at levels that do not cause a toxic but rather stimulating effect. For these eluates, the hormesis process was likely to have occurred [63]. In the case of the EA1, EA2, EA3 eluates, the phenomenon of hormesis likely occurred [63]. A stimulating effect might have occurred as a result

Table 3
Content of selected metals in eluates from ashes and waste from flue gas treatment

Metal	Fly ash and waste samples					Permissible contents[a]	Permissible contents[b]
mg kg^{-1}	EA1	EA2	EA3	EW1	EW2		
Cu	<0.005	4.76 ± 0.74	4.65 ± 0.09	<0.005	1.41 ± 0.92	50	100
Zn	1.72 ± 0.03	0.33 ± 0.25	4,14 ± 0.07	0.72 ± 0.15	0.83 ± 0.25	50	200
Pb	2,22 ± 0.21	2.54 ± 1.27	3.73 ± 3.09	1.74 ± 0.66	3.80 ± 1.32	10	50
Cr	0.07 ± 0,03	4.55 ± 2.44	5.56 ± 0.55	<0.005	2.33 ± 0.21	10	70
Ca	7,727.67 ± 568.92	1,922.33 ± 36.5	2,725.33 ± 54.5	3,999 ± 147.96	45.23 ± 37.25	–	–
Na	267.53 ± 3.47	121.73 ± 4.55	56.97 ± 24.59	1,93,333.3 ± 65,064.07	2,63,333.3 ± 25,166.11	–	–

[a]Acceptable boundary limits for leaching concerning storage of waste other than dangerous and neutral waste calculated per dry matter of waste [17, Appendix 3].
[b]Acceptable boundary limits for leaching concerning storage of dangerous waste calculated per dry matter of waste [17, Appendix 5].

of the activity of nutrients or toxic substances but which were present in sublethal concentrations. Tsiridis et al. [20] indicated that a high concentration of Ca may increase the hormesis effect and reduce the toxicity of various metals, which was confirmed in relation to EA1, EA2 and EA3 eluates that were characterized by a high content of this element. This is confirmed by the observed differences in the luminescence effect of bacteria for these eluates. The greatest effect of stimulation was obtained for EA1, characterized by the highest Ca content in the group of the eluates discussed.

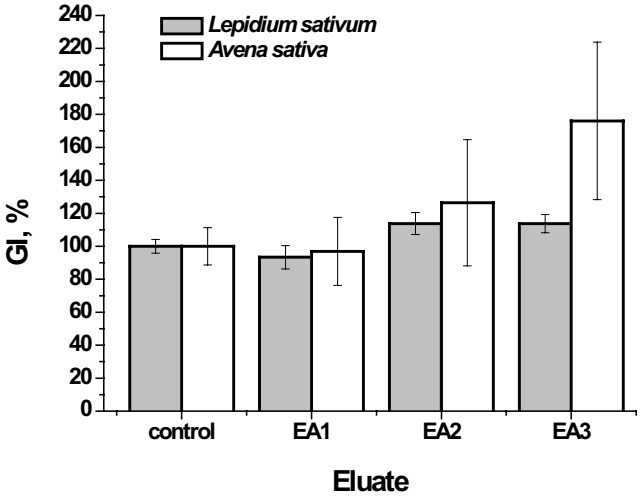

Fig. 3. Germination indicator (GI) for the biotest with *Lepidium sativum* and *Avena sativa*.

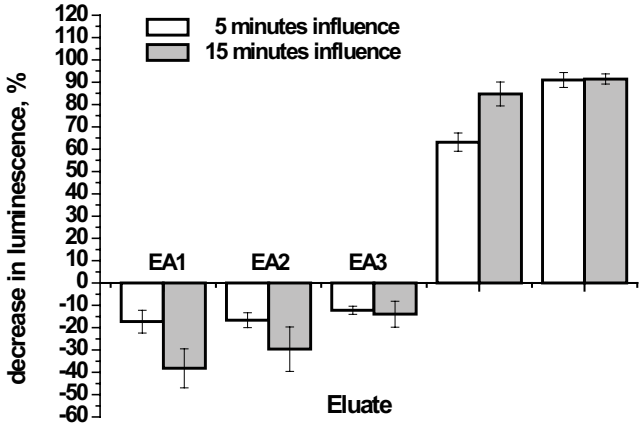

Fig. 4. Change of the *Vibrio fischeri* bacteria luminescence after 5 and 15 min of exposure of the studied eluates.

3.5. Statistical analysis

To evaluate the existence of dependencies between the content of the analyzed metals, chlorides and sulfates and the values of the RSG, RRG and GI indicators of the studied test plants (*Lepidium sativum* and *Avena sativa*), the Spearman's correlation test was used. The results of the test are presented in Tables 4 and 5.

The results of the test showed significant statistical relations between the content of Cu, Cr and Na in eluates and the value of indicators depicting the germination and root growth capacity (Table 4) of the test plants. The increase of the content of Cu and Cr in eluates increased the value of the RSG and GI indicator. This dependency was not observed with respect to the RRG indicator in the case of testing with *Lepidium sativum* because at a specific concentration of heavy metals, germination of seeds is possible, while the root growth is inhibited. It is confirmed by data from literature, where, that is, Gondek et al. [64] showed the dependency of metal impact (Cr, Ni, Cu, Zn) on the inhibition of root growth. The content of Na proved to be a significant statistical factor (strong correlation) because when the content of this element in eluates was increased it resulted in the reduction of values of

Table 5
The relation between the content of chlorides and sulfates and the indicators RSG, RRG and GI for the biotest with *Lepidium sativum* and *Avena sativa*

Indicator	Chlorides		Sulfates	
	Lepidium sativum	*Avena sativa*	*Lepidium sativum*	*Avena sativa*
RSG	–0.85***	–0.89***	–0.89***	–0.87***
RRG	–0.65**	–0.9***	–0.78**	–0.77**
GI	–0.76**	–0.93***	–0.92***	–0.86***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 4
Relation between the content of metals and the indicators RSG, RRG and GI for the biotest with *Lepidium sativum* and *Avena sativa*

Metal	RSG		RRG		GI	
	Lepidium sativum	*Avena sativa*	*Lepidium sativum*	*Avena sativa*	*Lepidium sativum*	*Avena sativa*
Cu	0.75**	0.51*	0.27	0.61*	0.58*	0.57*
Zn	0.14	0.37	0.04	0.4	–0.03	0.4
Pb	0.05	0.09	–0.04	0.24	0.04	0.12
Cr	0.73**	0.71**	0.41	0.63*	0.66**	0,74**
Ca	–0.002	0.1	0.27	0.19	0.02	0.08
Na	–0.9***	–0.85***	–0.63*	–0.91***	–0.79**	–0.89***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

RSG, RRG and GI indicators especially in the case of RRG in relation to *Avena sativa* (very strong correlation >0.9).

Statistical analysis was also performed with regard to the impact of chlorides and sulfates in eluates on the RSG, RRG and GI indicators in view of both test plants (Table 5).

All the obtained correlations have a negative value which means that the increase of the content of chlorides and sulfates results in lower values of germination and growth indicators. The strongest relation (very strong correlation >0.9) pertained to the impact of sulphate concentration on the GI value for *Lepidium sativum* and of chloride concentration on the GI value for *Avena sativa*.

The Wilcoxon test was used to perform statistical analysis of dependencies between the loss of luminescence and the content of the analyzed metals, chlorides and sulfates in eluates. The findings from testing are presented in Table 6.

The findings obtained through the statistical analysis showed that Na was the only element in the group of the analyzed metals that was characterized by a strong correlation between its concentration and the inhibition of the *Vibrio fischeri* bacteria luminescence. It is confirmed by the toxic influence of this component of the eluate. A stronger correlation occurred in the case of the 5 min exposure. A strong correlation was also observed in the case of dependencies between the content of chlorides and sulfates in eluates and the inhibition of *Vibrio fischeri* luminescence at the exposure time of 5 min. Reduction of correlation strength was also noted when exposure time was increased. Analysis by means of the Wilcoxon test did not show the occurrence of statistically significant differences between the two time periods of luminescence.

The statistical analysis was also used to determine the relations between the inhibition of *Vibrio fischeri* luminescence and the values of indicators depicting the germination and growth capacity of *Lepidium sativum* and *Avena sativa* test plants (Table 7).

The analysis of correlation showed the occurrence of negative relations between the inhibition of the *Vibrio fischeri* bacteria luminescence after 5 and 15 min and RSG, RRG and GI indicators with respect to both test plants. The increase of values of RSG, RRG and GI indicators influences the conditions favourable for the development of bacteria and thus the limitation of luminescence inhibition, probably as a result of hormesis. The strongest correlation was noted with respect to *Avena sativa* after 5 min of exposure. The results of the statistical analysis confirmed the lack of toxicity of EA1, EA2, EA3 eluates in relation to both the *Vibrio fischeri* bacteria and the plants used in biotests.

Table 6
Relation between the content of heavy metals, chlorides, sulfates in eluates and the luminescence of the *Vibrio fischeri* bacteria after 5 and 15 min of exposure

Parameter	Decrease in luminescence after 5 min of exposure	Decrease in luminescence after 15 min of exposure
Cu	–0.36	–0.09
Zn	–0.2	–0.11
Pb	0.18	0.15
Cr	–0.45	–0.26
Ca	–0.28	–0.48
Na	0.77**	0.58*
Chlorides	0.76**	0.57*
Sulfates	0.75**	0.62*

*p < 0.05; **p < 0.01; ***p < 0.001

4. Conclusion

Characteristics of the pollution type present in waste does not fully reflect the toxic impact of those pollutants on live organisms. Therefore, it is important to supplement chemical analyses with bioassays. Such tests are a measure of impact of various types of pollutants on particular elements of the environment and allow for both risk assessment, meaning the probability of harmful influence of a given factor on a live organism, and the determination of its toxicity. Chemical characteristic of eluates only covers the determination of concentration of selected components, however, according to literature [15], part of toxic influences may be an effect of the presence of substances appearing in low concentrations, and the toxicity of multicomponent mixtures does not always reflect the amount of toxic components

Table 7
The relation between the inhibition of the *Vibrio fischeri* bacteria luminescence after 5 and 15 min of exposure and RSG, RRG and GI indicators for the biotest with *Lepidium sativum* and *Avena sativa*

Indicator		Decrease in the luminescence of the *Vibrio fischeri* bacteria after 5 min of exposure	Decrease in the luminescence of the *Vibrio fischeri* bacteria after 15 min of exposure
RSG	*Lepidium sativum*	–0.74**	–0.62*
	Avena sativa	–0.87***	–0.72*
RRG	*Lepidium sativum*	–0.64*	–0.76**
	Avena sativa	–0.63*	–0.57*
GI	*Lepidium sativum*	–0.72**	–0.67**
	Avena sativa	–0.84***	–0.67**

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

contained therein. Biotoxicological tests of direct contact allow for the simultaneous determination of the harmful impact of all harmful substances contained in the tested material on selected live organisms. They simultaneously include interactions taking place among all elements of the studied system. The toxic effect of a material is connected with the type of the test organism and its sensitivity to particular pollutants, including heavy metals.

The findings obtained during the tests evaluating the impact of eluates from wastes from fluidised bed incineration of sewage sludge on the ground and water environment allow for the formulation of the following conclusions.

- Eluates, which were classified as hazardous on the basis of chemical tests due to the fact that the authorised sulphate concentrations were exceeded (EW1 and EW2), were toxic to the test plants and bacteria. Their phytotoxic impact is also proven by the high values of conductivity. In relation to the test plants (*Lepidium sativum* and *Avena sativa*) it was total toxicity manifested as the lack of seed germination and growth. Toxicity to *Vibrio fischeri* bacteria was high (over 50% of luminescence inhibition) where the highest values were observed with respect to the EW2 eluate, which was connected with the high content of Na.
- In tests with the plants, seed germination and root growth were noted with regard to EA1, EA2 and EA3 eluates for both test plants, where these eluates were less toxic to *Avena sativa* in comparison to *Lepidium sativum*. The RSG indicator for EA2 and EA3 exceeded the value of 100%, which proves their stimulating effect. The analysis of the RRG indicator for *Lepidium sativum* results in findings that all eluates from ashes allow for the germination of this plant, simultaneously inhibiting the root growth. In contrast, in the case of *Avena sativa*, all eluates showed a stimulating effect in relation to root growth. The most favourable values of the RSG, RRG and GI indicators were observed with respect to EA2 and EA3 eluates, which were simultaneously characterized by the lowest concentrations of chlorides, sulfates and TDS as well as Na and Ca.
- By comparing the GI values for *Lepidium sativum* and *Avena sativa* in the case of the EA1 eluate, the lowest values of this indicator were obtained, which indicates the highest toxicity to the test plants. This eluate was characterized by the highest content of Ca, which had impact on the pH value. Due to the fact that pH correction was conducted in the Microtox test to neutral values, an inverse dependency was observed – the EA1 eluate showed the lowest toxicity to *Vibrio fischeri* bacteria from all that were tested.
- During the Microtox test, a significant toxic effect occurred after 5 min in the case of EW1 and EW2 eluates, and the extension of exposure time caused a further increase of toxicity. In contrast, toxic effects of eluates from ashes were not observed – in fact, an increase of bacteria luminescence was noted, probably due to the presence of substances facilitating their development, for example, Ca (hormesis).
- All the studied eluates were characterized by alkaline reaction. During the Microtox test, the pH of eluates was corrected to neutral, which could have had impact on the bioavailability of particular components and eliminated the toxic effect of this parameter, leading to false toxicity test results with respect to bacteria. For this reason, findings obtained by means of this test should be interpreted with utmost caution.
- The lack of germination observed for both test plants in the case of EW1 and EW2 eluates was probably not caused by the effect of the pH of these eluates (which was 10.8 for EW1 and 10.30 for EW2), because the higher pH value (11.51) measured in EA1 allowed for germination and growth of the test plants.

In conclusion of the results obtained, it was found that toxic factors in the eluates analyzed were high concentrations of chlorides, sulfates and the Na content rather than the concentration of heavy metals and pH. Furthermore, the results of the research presented in this paper confirmed that the physicochemical and physical characteristics of the eluates in terms of their potential toxicity are consistent with the results of the biotests. The eluates obtained from the waste from flue gas cleaning processes (EW1, EW2), characterized by a high concentration of sulfates, Na, high pH and high conductivity value, were toxic for plants (no germination) and bacteria. However, the eluates prepared from fly ashes after incineration of sewage sludge showed a stimulating effect on test organisms. This confirms a correlation between the chemical characteristics of the eluates and the effects obtained for the biotests. It therefore appears that the use of biological tests in addition to chemical examinations to assess the environmental impact of eluates from waste can be an excellent supplementation to their chemical characterization and thus improve the effectiveness of monitoring and quality management of the ground and water environment.

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References

[1] G. Borowski, Application of vitrification method for the disposal of municipal sewage sludge, Annu. Set Environ. Prot., 15 (2013) 575–583.
[2] K. Wystalska, J. Sobik-Szołtysek, J. Bień, Vitrification and devitrification of ash after sewage sludge combustion, Annu. Set Environ. Prot., 15 (2013) 181–191.
[3] J.A. Cusidó, L.V. Creades, Environmental effects of using clay bricks produced with sewage sludge: leachability and toxicity studies, Waste Manage., 32 (2012) 1202–1208.
[4] J.-S. Li, Q. Xue, L. Fang, C.S. Poon, Characteristics and metal leachability of incinerated sewage sludge ash and air pollution control residues from Hong Kong evaluated by different methods, Waste Manage., 64 (2017) 161–170.
[5] W.Y. Lin, W.C. Ng, B.S.E. Wong, S.L. Teo, G.D. Sivananthan, G.H. Baeg, Y.S. Ok, C.H. Wang, Evaluation of sewage sludge incineration ash a potential land reclamation material, J. Hazard. Mater., 357 (2018) 63–72.
[6] L. Fang, L. Jiang-shan, M.Z. Guo, C.R. Cheeseman, D.C.W. Tsang, S. Donatello, C.S. Poon, Phosphorus recovery and leaching of trace elements from incinerated sewage sludge ash (ISSA), Chemosphere, 193 (2018) 278–287.

[7] Q. Zhang, J. Hu, D.-J. Lee, Y. Chang, Y.-J. Lee, Sludge treatment: current research trends, Bioresour. Technol., 243 (2017) 1159–1172.
[8] R. Kleemann, J. Chenoweth, R. Clift, S. Morse, P. Pearce, D. Saroj, Comparison of phosphorus recovery from incinerated sewage sludge ash (ISSA) and pyrolysed sewage sludge char (PSSC), Waste Manage., 60 (2016) 201–210.
[9] Y. Pan, Z. Wu, J. Zhou, J. Zhao, X. Ruan, J. Liu, G. Qian, Chemical characteristic and risc assessment of typical municipal solid waste incineration (MSWI) fly ash in China, J. Hazard. Mater., 261 (2013) 269–276.
[10] M. Cobo, A. Gálvez, J.A. Conesa, C. Montes de Correa, Characterization of fly ash from a hazardous waste incinerator in Medellin, Colombia, J. Hazard. Mater., 168 (2009) 1223–1232.
[11] D. Vamvuka, J. Hahladakis, D. Pentari, Leaching of toxic elements from lignite and agroresidue ashes in cultivated soils of Crete, Energy Fuels, 19 (2005) 807–812.
[12] R. Weltens, K. Deprez, L. Michiels, Validation of Microtox as a first screening tool for waste classification, Waste Manage., 34 (2014) 2427–2433.
[13] P. Hennebert, Proposal of concentration limits for determining the hazard property HP 14 for waste Rusing ecotoxicological tests, Waste Manage., 74 (2018) 74–85.
[14] Polish Norm, PN-EN 12457-2, Characterization of Waste – Leaching – Compliance Test for Leaching of Granular Waste and Sludges – Part 2: One Stage Batch Test at a Liquid to Solid Ratio of 10 l/kg for Materials with Particle Size below 4 mm (Without or With Size Reduction).
[15] V. Tsiridis, P. Samaras, A. Kungolos, G.P. Sakellaropoulos, Application of leaching tests for toxicity evaluation of coal fly ash, Environ. Toxicol., 21 (2006) 409–416.
[16] USEPA, Method 1311, Toxicity Characteristic Leaching Procedure (TCLP), Publication SW 846: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, 1992. Available at: www.epa.gov/epaoswer/hazwaste/test/pdfs/1311.pdf.
[17] Regulation of the Minister of Economy of 16 July 2015 on the Acceptance of Waste for Landfill, Journal of Laws 2015 No. 0, Item 1277 (in Polish).
[18] A.V. Filgueiras, I. Lavilla, C. Bendicho, Chemical sequential extraction for metal partitioning in environmental solid samples, *J. Environ. Monit.*, 4 (2002) 823–857.
[19] K. Yin, W.P. Chan, X. Dou, F. Ren, V.W.-C. Chang, Measurements, factor analysis and modeling of element leaching from incineration bottom ashes for quantitative component effects, J. Cleaner Prod., 165 (2017) 477–490.
[20] V. Tsiridis, M. Petala, P. Samaras, A. Kungolos, G.P. Skellaropoulos, Environmental hazard assessment of coal fly ashes using leaching and ecotoxicity tests, Ecotoxicol. Environ. Saf., 84 (2012) 212–220.
[21] V. Tsiridis, M. Petala, P. Samaras, G.P. Skellaropoulos, Evaluation of interaction between soil and coal fly ash leachates using column percolation tests, Waste Manage., 43 (2015) 255–263.
[22] S. Stiernström, A. Enell, O. Wik, K. Hemström, M. Breitholtz, Influence of leaching conditions for ecotoxicological classification of ash, Waste Manage., 34 (2014) 421–429.
[23] S. Parvez, C. Venkataraman, S. Mukherji, Toxicity assessment of organic contaminants: evaluation of mixture effects in model industrial mixtures using 2n full factorial design, Chemosphere, 73 (2008) 1049–1055.
[24] R.J. Slack, J.R. Gronow, N. Voulvoulis, Household hazardous waste in municipal landfills: contaminants in leachate, Sci. Total Environ., 337 (2005) 119–137.
[25] G. Skodras, M. Prokopidou, G.P. Sakellaropoulos, Leaching and toxicity behavior of coal-biomass waste cocombustion ashes, Environ. Toxicol., 21 (2006) 317–323.
[26] A. Kuczyńska, L. Wolska, J. Namieśnik, Application of biotests in environmental research, Crit. Rev. Anal. Chem., 35 (2005) 135–154.
[27] N. Lapa, R. Barbosa, M.H. Lopes, B. Mendes, P. Abelha, I. Gulyurtlu, J.S. Oliveira, Chemical and ecotoxicological characterization of ashes obtained from sewage sludge combustion in fluidized-bed reactor, J. Hazard. Mater., 147 (2007) 175–183.
[28] K. Wadhia, K.C. Thompson, Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive, Trends Anal. Chem., 26 (2007) 300–307.
[29] M.K. Banks, K.E. Schultz, Comparison of plants for germination toxicity tests in petroleum-contaminated soils, Water Air Soil Pollut., 167 (2005) 211–219.
[30] J. Römbke, T. Moser, H. Moser, Ecotoxicological characterization of 12 incineration ashes using 6 laboratory tests, Waste Manage., 29 (2009) 2475–2482.
[31] G. Rosen, A. Osorio-Robayo, I. Rivera-Duarte, D. Lapota, Comparison of bioluminescent dinoflagellate (QwikLite) and Bacterial (Microtox) rapid bioassays for the detection of metal and ammonia toxicity, Arch. Environ. Contam. Toxicol., 54 (2008) 606–611.
[32] G. Persoone, B. Marsalek, I. Bilinova, A. Törökne, D. Zarina, L. Manusadzianas, G. Nalecz-Jawecki, L. Tofan, N. Stepanova, L. Tothova, B.A. Kolar, A practical and user friendly toxicity classification system with microbiotests for natural waters and wastewaters, Environ. Toxicol., 18 (2003) 395–402.
[33] L. Wolska, A. Sagajdakow, A. Kuczyńska, J. Namieśnik, Application of ecotoxicological studies in integrated environmental monitoring: possibilities and problems, Trends Anal. Chem., 26 (2007) 332–344.
[34] K. Deprez, J. Robbens, I. Nobels, C. Vanparys, G. Vanermen, K. Tirez, L. Michiels, R. Weltens, Discriset, A battery of tests for fast waste classification – application of tests on waste extracts, Waste Manage., 32 (2012) 2218–2228.
[35] S. Manzo, F. De Nicola, F. De Luca Picione, G. Maisto, A. Alfani, Assessment of the effects of soil PAH accumulation by a battery of ecotoxicological tests, Chemosphere, 71 (2008) 1937–1944.
[36] J. Sobik-Szołtysek, K. Wystalska, A. Grobelak, Effect of addition of sewage sludge andcoal sludge on bioavailability of selected metals in the waste from the zinc and lead industry, Environ. Res., 156 (2017) 588–596.
[37] I. Czerniawska-Kusza, G. Kusza, The potential of the Phytotoxkit microbiotest for hazard evaluation of sediments in eutrophic freshwater ecosystems, Environ. Monit. Assess., 179 (2011) 113–121.
[38] A. Trojanowska, Application of Phytotoxkit microbiotest for hazard assessment of bottom sediments in the eutrophic dam reservoir, Limnol. Rev., 10 (2010) 173–180.
[39] J. Mankiewicz-Boczek, G. Nałęcz-Jawecki, A. Drobniewska, M. Kaza, B. Sumorok, K. Izydorczyk, M. Zalewski, J. Sawicki, Application of a microbiotests battery for complete toxicity assessment of rivers, Ecotoxicol. Environ. Saf., 71 (2008) 830–836.
[40] H. Ko, K. Kim, H. Kim, C. Kim, C.M. Umeda, Evaluation of compost parameters and heavy metals contents in composts made from animals mature, Waste Manage., 28 (2008) 813–820.
[41] H. Miaomiao, L. Wenhong, L. Xinqiang, W. Donglei, T. Guangming, Effect of composting process on phytotoxicity and speciation of copper, zinc and lead in sewage sludge and swine manure, Waste Manage., 29 (2009) 590–597.
[42] M. Gao, F. Liang, A. Yu, B. Li, L. Yang, Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratio, Chemosphere, 78 (2010) 614–619.
[43] K.A. Gyekye, An assessment of toxic in urban soils using garden cress (*Lepidium sativum*) in Vasileostrovsky Ostrov and Elagin Ostrov, Saint Petersburg, Russia, 2013, J. Geogr. Geol., 5 (2013) 63–70.
[44] V.L. Pavel, D.L. Sobariu, M. Diaconu, F. Stătescu, M. Gavrilescu, Effects of heavy metals on *Lepidium sativum* germination and growth, Environ. Eng. Manage. J., 12 (2013) 727–733.
[45] S. Parvez, C. Venkataraman, S. Mukherji, A review on advantages of implementing luminescence inhibition test (*Vibrio fischeri*) for acute toxicity prediction of chemicals, Environ. Int., 32 (2006) 265–268.
[46] A. Baran, M. Tarnawski, Phytotoxkit/Phytotestkit and Microtox® as tools for toxicity assessment of sediments, Ecotoxicol. Environ. Saf., 98 (2013) 19–27.

[47] E.H. Jho, J. Im, K. Yang, Y.J. Kim, K. Nam, Changes in soil toxicity by phosphate-aided soil washing: Effect of soil characteristics, chemical forms of arsenic, and cations in washing solutions, Chemosphere, 119 (2015) 1399–1405.
[48] J.M. Ribò, K.L. Kaiser, Photobacterium phosphoreum toxicity bioassay. Test procedures and applications, Environ. Toxicol., 2 (2006) 305–323.
[49] J. Jensen, M. Mesman, Ecological Risk Assessment of Contaminated Land. Decision Support for Site Specific Investigations, RIVM Report 711701047, 2006, p. 138.
[50] C.A. Ajuzieogu, L.O. Odokuma, C.C. Blaise, Toxicity assessment of produced water using microtox rapid bioassay, South Asian J. Res. Microbiol., 1 (2018) 1–9.
[51] S. Stiernström, K. Hemstrom, O. Wik, G. Carlsson, B.-E. Bengtsson, M. Breitholtz, An ecotoxicological approach for hazard identification of energy ash, Waste Manage., 31 (2011) 342–352.
[52] B. Kołwzan, W. Kołwzan, A.M. Dziubek, G. Pasternak, Statistical approach to assessing groundwater pollution from gasworks, Environ. Prot. Eng., 37 (2011) 119–126.
[53] R. Barbosa, N. Lapa, D. Boavida, H. Lopes, I. Gulyurtlu, B. Mendes, Co-combustion of coal and sewage sludge: chemical and ecotoxicological properties of ashes, J. Hazard. Mater., 170 (2009) 902–909.
[54] I. Walter, F. Martinez, V. Cala, Heavy metal speciation and phytotoxic effects of Tyree representative sewage sludge for agricultural uses, Environ. Pollut., 139 (2006) 507–514.
[55] S. Ren, P.D. Frymier, Toxicity of metals and organic chemicals evaluated with bioluminescence assays, Chemosphere, 58 (2005) 543–550.
[56] F. Conforti, G. Ioele, G.A. Statti, M. Marrelli, G. Ragno, F. Menichini, Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants, Food Chem. Toxicol., 46 (2008) 3325–3332.
[57] S. Girotti, E.N. Ferri, M.G. Fumo, E. Maiolini, Monitoring of environmental pollutants by bioluminescent bacteria, Anal. Chim. Acta, 608 (2008) 2–29.
[58] S. Stiernström, O. Wik, D. Bendz, Evaluation of frameworks for ecotoxicological hazard classification of waste, Waste Manage., 58 (2016) 14–24.
[59] D. François, C. Criado, Monitoring of leachate at a test road using treated fly ash from municipal solid waste incinerator, J. Hazard. Mater., B, 139 (2007) 543–549.
[60] P. Gong, B.M. Wilke, E. Strozzi, S. Fleischmann, Evaluation and refinement of a continuous seed's germination and elary seedling growth test use in the ecotoxicological assessment of soil, Chemosphere, 44 (2001) 491–500.
[61] M. Gouider M. Feki S. Sayadi, Bioassay and use in irrigation of untreated and treated wastewaters from phosphate fertilizer industry, Ecotoxicol. Environ. Saf., 73 (2010) 932–938.
[62] A.D. Khan, M. Libby, D. Winnick, J. Palmer, M. Sumarah, M.B. Ray, S.M. Macfie, Uptake and phytotoxic effect of benzalkonium chlorides in *Lepidium sativum* and *Lactuca sativa*, J. Environ. Manage., 206 (2018) 490–497.
[63] E.J. Calabrese, L.A. Baldwin, Defining hormesis, Hum. Exp. Toxicol., 21 (2002) 91–97.
[64] K. Gondek, A. Baran, M. Kopec, The effect of low-temperature transformation of mixtures of sewage sludge and plant materials on content, leachability and toxicity of heavy metals, Chemosphere, 117 (2014) 33–39.