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APPLICATION OF BIOLOGICAL METHODS TO ASSESS THE TOXICITY OF SOILS CONTAMINATED WITH HEAVY METALS AND THE EFFECTIVENESS OF STABILISATION PROCESSES

Short-term biotests were used to determine the effectiveness of the use of compost and fly ash in the stabilization of heavy metals in contaminated soil. For this purpose, in two independent experiments, either compost (3:1) or fly ash (1:1) were added to soil contaminated with heavy metals. To assess seed germination and root elongation of *Sorghum saccharatum* L., *Lepidium sativum* L., *Sinapis alba* L. after three days a *Phytotoxkit* test was used. Seedling emergence and biomass yield after 21 days were evaluated. Obtained data indicate better practical applicability of the seedling emergence test thanks to the longer duration resulting in more reliable conclusions provided in that test. A short, 3-day test did not confirm any effective stabilising role of theapplied additives. *Sorghum saccharatum* L. turned out to be most sensitive to the altered soil conditions, while *Lepidium sativum* L. was most tolerant.

1. INTRODUCTION

Contamination of soils with heavy metals is a problem currently faced by most developed countries. Contaminants may lead to deformation or even complete degradation of ecosystems. Metals may be emitted from various sources; nevertheless, mechanisms of their accumulation in living organisms and ecosystems are similar. As a result of particulate matter deposition, various metals are accumulated in waters and soils. Certain amounts of these metals are taken up by plants, animals and various aquatic organisms, finally to be accumulated in human organism. This process is a closed cycle, in

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which the circulation of matter results in the risk of contaminants penetration to successive elements of the environment and elements of the trophic chain. This eventually poses an increased potential health hazard to humans [1].

Following the principles of sustainable development, both economic and social aspects should be consistent with the assumptions of environmental protection. The potential use of natural resources should also be ensured for future generations. Despite the implementation of new technologies, which are safer for the environment, many locations had been heavily polluted in the past. For this reason, it is essential to restore the contaminated elements of the environment to their former state to ensure their possibly best quality and functionality. It is particularly important in the case of soils anthropogenically enriched with heavy metals – undegradable mineral xenobiotics, as may be detected in organic contaminants.

According to Polish regulations [2], soils occurring within the zone of industrial impact have to be monitored and subjected to remediation measures if necessary. The most advantageous method of remediation of soils contaminated with heavy metals seems to be based on contaminant stabilisation, resulting in the reduced mobility and bioavailability of metals and thus the limitation of their incorporation in the trophic chain [3]. An obvious advantage of such methods is related to their low costs and rapid effects. For this purpose, various waste materials may be used such as fly ash or composts produced using biodegradable wastes which are not only cheap sources of nutrients but first of all they constitute a valuable absorption and stabilisation matrix [4]. Their application meets the assumptions of the Directive on the landfill of waste [5]. Nevertheless, utilisation of such waste in the stabilisation processes needs to be monitored, which is a key element for the evaluation of contaminant levels, control over their migration and selection of adequate remediation methods.

According to Augustynowicz et al. [6], analyses of chemical properties of contaminated soil do not provide comprehensive information on the potential environmental impact of contamination. This is because they do not reflect the actual response of the populations of various organisms and their associations to xenobiotics or their potential interactions in the environment. El-Alam et al. [7] also expressed an opinion that chemical tests alone may be insufficient to assess biological hazard, which results from a wide spectrum of potentially toxic substances found in the environment. Those authors stated that changes in physicochemical properties of these substances may affect their mobility, bioavailability and biological activity. For this reason, biological, toxicological or ecotoxicological tests are highly recommended in order to evaluate synergistic effects of toxicity of soil contaminants [8, 9]. Oleszczuk and Hollert [10] confirmed applicability of biological methods to assess the potential use of waste for remediation purposes. In turn, van der Vliet et al. [11] reported that biological tests using biotest kits are not only cheap and sensitive but also rapid techniques, which precisely present potential effects resulting from the presence of contaminants in the environment. Thus biological methods may be used in two ways. On the one hand, they may be applied to

detect contaminants and select appropriate remediation, while on the other hand they are useful in environmental biomonitoring as the final stage in the control of remediation operations.

In view of the above, the aim of the presented investigations was:

- to assess the applicability of selected biological methods in the diagnostics of the toxic effect of heavy metals on plants and to analyse effectiveness of compost for remediation purposes,
- to analyse fly ash as a substance stabilising heavy metals in the soil.

2. MATERIALS AND METHODS

Soil samples for analyses were collected from the topsoil of arable fields located in the vicinity of Huta Miedzi Legnica, a copper smelter and refinery (51°11′52.3″N, 16°05′42.0″E) in the Dolnośląskie province. The long-term operation of the copper smelter resulted in the accumulation of metals, mainly Cu and Zn, in the topsoil in the vicinity of the emitter. The soil contained medium levels of Cu (228.64 $mg \cdot kg^{-1}$) and Zn (127.4 $mg \cdot kg^{-1}$). The contents of metals in the soil were determined by atomic absorption spectrophotometry (AAS) following sample mineralisation in *aqua regia* according to the PN-ISO 11466:2002 standard [12]. In accordance with Polish regulations these soils need to be considered historically contaminated [2]. Following the Systematic classification of soils in Poland [13], the soil used in the experiments was classified as typical lessive soil with the grain size composition of loamy silt (24% of sand, 67% of silt and 9% of clay). In the experiment, compost prepared from biowaste and fly ash, a by-product from the combustion of lignite from the power plants of Zespół Elektrowni Pątnów–Adamów–Konin SA, were used as stabilising materials.

In this study two independent biotests were used: a 3-day Phytotoxkit test meeting the requirements of the PN-EN ISO 11269-1:2013-06 standard, evaluating the rate of seed germination inhibition and root growth, and a 21-day test according to the PN-EN ISO 11269-2:2013-06 standard evaluating the rate of seedling emergence and plant growth inhibition.

Biotest according to the PN-EN ISO 11269-1:2013-06 standard. In order to determine the effect of contaminated soil, compost and fly ash on seed germination a 3-day Phytotoxkit test by Tigret® was used [14]. The experiment was performed independently on a monocotyledonous *Sorghum saccharatum* L. (sorghum) and on dicotyledonous plants: *Lepidium sativum* L. (garden cress) and *Sinapis alba* L. (white mustard). The experimental design covered 5 following combinations: A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1).

The control comprised certified reference soil. The experiment was conducted under controlled conditions at 25 °C and 80% field water capacity in the dark. On plates covered with blotting paper with appropriately prepared soil 10 seeds each of individual plant species were placed. After 3 days of the experiment, images of the plates were recorded using a camera and next shoot length and root growth inhibition were determined using the ImageJ 1.8.0 graphic programme. Additionally, based on the collected data the percentages of seed germination inhibition (*IG*) and root growth inhibition (*IR*) were determined using the following formula:

$$IG\,(IR) = \frac{A - B}{A} \times 100\%$$

where: *A* denotes mean seed germination rate or root length in the control (soil recommended by ISO for toxicity tests with plants), and *B* denotes mean seed germination rate or root length in the tested soil, a mixture of soil and compost, or a mixture of soil and fly ash.

Biotest according to the PN-EN ISO 11269-2:2013-06 standard. The effect of the addition of compost and fly ash to soil contaminated with heavy metals on the emergence rate and further plant growth was evaluated using the biotest following the requirements of the PN-EN ISO 11269-2:2013-06 standard [15]. For this purpose, a pot experiment was established, comprising 5 combinations in 6 replications. 0.5 dm^3 pots of 9 cm in diameter were filled with soil contaminated with heavy metals with an addition of fly ash from brown coal (lignite) combustion at a 3:1 (T1) or 1:1 (T2) ratio (w/w) or soil contaminated with heavy metals with an addition of compost from organic waste at a 3:1 (T3) or 1:1 (T4) ratio (w/w). The control comprised soil contaminated with heavy metals with no additives (T0). The experiment was run under controlled greenhouse conditions at 60% field water capacity and at 25 °C.

The test plants included monocotyledonous *Sorghum saccharatum* L. (sorghum) and dicotyledonous *Sinapis alba* L. (white mustard). The experiment was conducted simultaneously for both tested plant species. To each pot, 10 seeds of the selected species were sown. The experiment was performed in triplicate. After 21 days, the number of emerged seedlings in pots was recorded, after which plants were cut and fresh weight of shoots of the tested plants (T1–T4) was compared with the fresh weight of shoots from control plants (T0).

The effect of compost and fly ash on the emergence and growth of higher plants in contaminated soil was investigated using one-way analysis of variance at the significance level $\alpha = 0.05$. The analysis of variance provided the least significant differences, while homogeneous groups were identified using Tukey's test.

3. RESULTS

3.1. PHYTOTOXKIT TEST (PN-EN ISO 11269-1:2013-06)

The results of the phytotoxkit test concerning the effect of compost and fly ash added to soil contaminated with heavy metals on seed germination inhibition in *Sinapis alba* L. are given in Fig. 1. The data showed that the smallest number of *Sinapis alba* L. seeds germinated on soil contaminated with heavy metals with compost added at a 3:1 ratio (w/w) (A4), resulting in the greatest percentage seed germination inhibition rate of 6.8%. In turn, on soil contaminated with heavy metals with no additives (A1) the number of germinated seeds was greater than that for reference soil, which was expressed in the negative percentage seed germination rate at –1.7%.

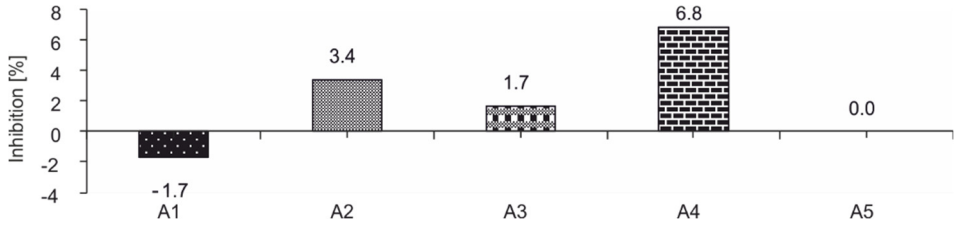

Fig. 1. Mean percentage inhibition of seed germination (*IG*) in white mustard (*Sinapis alba* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

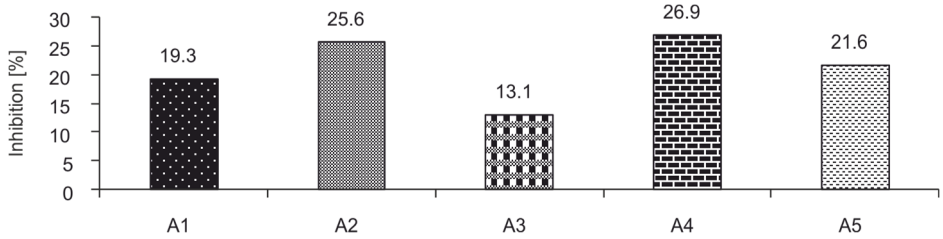

Fig. 2. Mean percentage inhibition of root growth (*IR*) in white mustard (*Sinapis alba* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

Data given in Fig. 2 concerning the effect of compost and fly ash added to soil contaminated with heavy metals on root growth inhibition showed the greatest inhibition rate of root growth in *Sinapis alba* L. in plants grown on soil contaminated with heavy metals with the addition of fly ash and compost at a 3:1 ratio (w/w) (A2 – 25.6% and A4 – 26.9%). In turn, the lowest inhibition rate for root growth in *Sinapis alba* L.

was recorded in the soil contaminated with heavy metals with fly ash added at a 1:1 ratio (w/w) (A3 – 13.1%).

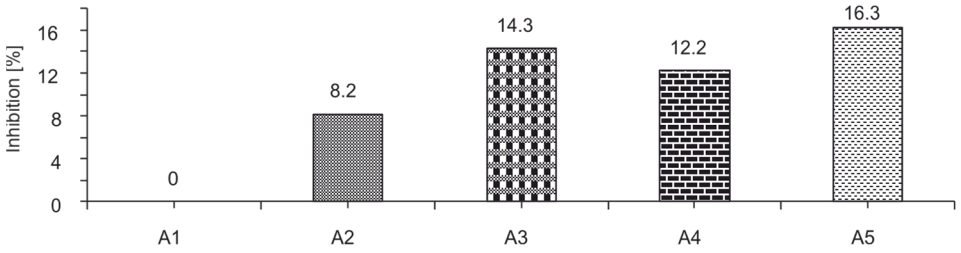

Fig. 3. Mean percentage inhibition of seed germination (*IG*) in sorghum (*Sorghum saccharatum* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

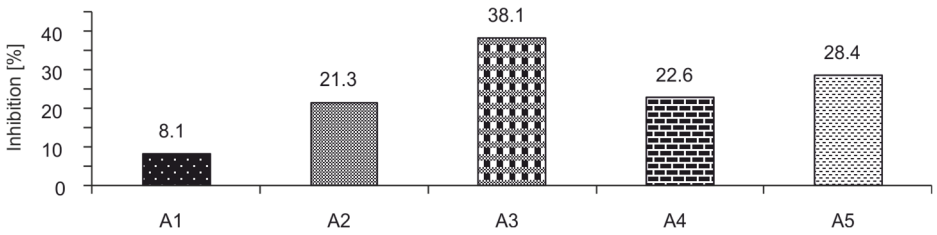

Fig. 4. Mean percentage inhibition of root growth (*IR*) in sorghum (*Sorghum saccharatum* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

Figures 3 and 4 present the effect of contaminated soil and applied compost and fly ash on the inhibition of seed germination and root growth in *Sorghum saccharatum* L. Heavy metals found in the soil did not inhibit seed germination (A1 – 0%). In contrast, the addition of fly ash or compost markedly reduced seed germination (8.2–16.3%), while a more negative effect was observed for compost (12.2–16.3%). Increasing amounts of fly ash or compost in the mixture with soil contributed to a lower number of germinated seeds in *Sorghum saccharatum* L. (fly ash 8.2–14.3%, compost 12.2–16.3%) (Fig. 3).

Soil contamination with heavy metals reduced root growth in *Sorghum saccharatum* L. by 8.1%. Applied stabilisers contributed to increased inhibition of root growth in that plant (21.3–38.1%). At the same time, it was observed that a greater content of compost or fly ash in the mixture with soil contaminated with heavy metals to a greater extent inhibited root growth in *Sorghum saccharatum* L. (for A3 – 38.1% and A5 – 28.4, respectively, Fig. 4).

In turn, lack of any effect of the tested soil and the applied addition of compost or fly ash on seed germination in *Lepidium sativum* L. is evidenced by data presented in

Fig. 5. Compared to the number of germinated seeds on the reference soil a larger number of seeds germinated on the soil from the experimental combinations (A1–A5), as evidenced by the negative values (from –1.9 to –9.3%). The largest number of germinated seeds was found in soil contaminated with heavy metals (A1) and with fly ash added at a 1:1 ratio (w/w) (A3).

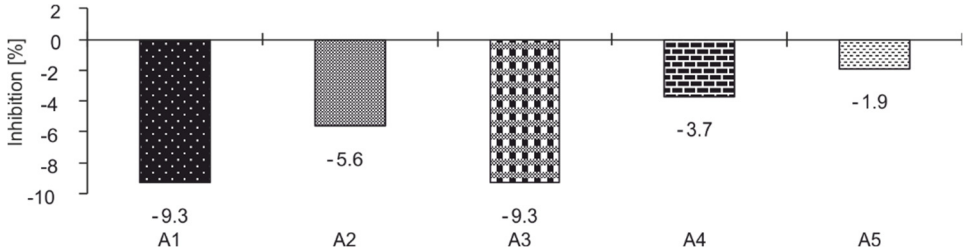

Fig. 5. Mean percentage inhibition of seed germination (*IG*) in garden cress (*Lepidium sativum* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

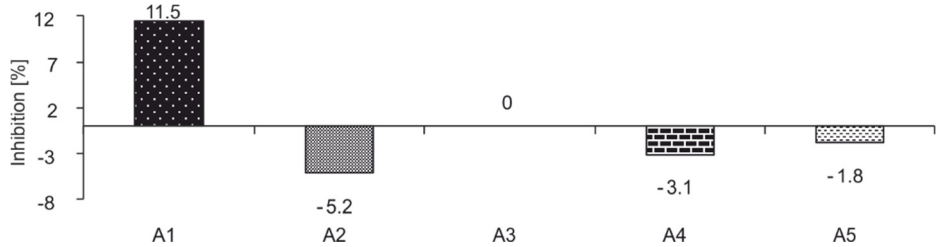

Fig. 6. Mean percentage inhibition of root growth (*IR*) in garden cress (*Lepidium sativum* L.) depending on applied additives; A1 – contaminated soil, A2 – contaminated soil + fly ash (3:1), A3 – contaminated soil + fly ash (1:1), A4 – contaminated soil + compost (3:1), A5 – contaminated soil + compost (1:1)

Data presented in Fig. 6 show that compost and fly ash added both at a 3:1 and 1:1 ratios had no negative effect on root elongation in *Lepidium sativum* L. The positive effect of tested mixtures (A2–A5) needs to be stressed here, as in comparison to plants grown on the control soil they had longer roots, which was expressed by negative percentage values of root growth inhibition (from –1.7 to –5.5%). Only in the case of soil contaminated with heavy metals root growth inhibition was recorded (A1 – 11.5%).

3.2. THE EMERGENCE AND EARLY PLANT GROWTH TEST (PN-EN ISO 11269-2:2013-06)

Figure 7 presents data concerning the effect of contaminated soil and its mixtures with compost and fly ash added at various ratios on early growth and development of *Sorghum saccharatum* L. Fly ash added at various proportions (T1 and T2) to a limited

extent influenced a reduction of biomass in the tested plants, by 33 and 43% in relation to the plants from the control. An opposite trend was observed in plants grown on contaminated soil with compost added to the soil at a 3:1 (T3) and 1:1 ratios (w/w) (T4) It had a significant effect on an increase of biomass in *Sorghum saccharatum* L. by 66%.

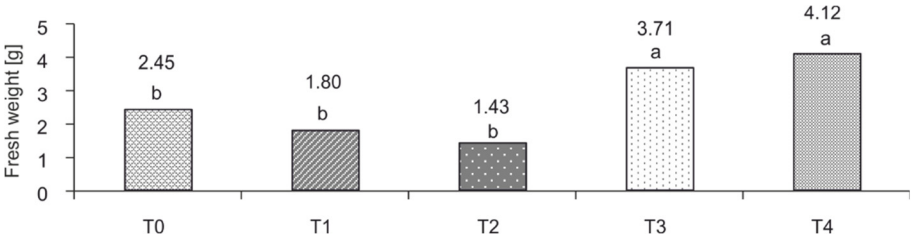

Fig. 7. Mean fresh weight of shoots culture of sorghum (*Sorghum saccharatum* L.) obtained after 21-day culture on heavy metals contaminated soil (T0) with additives: fly ashes 3:1 (T1), fly ashes 1:1 (T2), compost fertilizer 3:1 (T3), compost fertilizer 1:1 (T4)

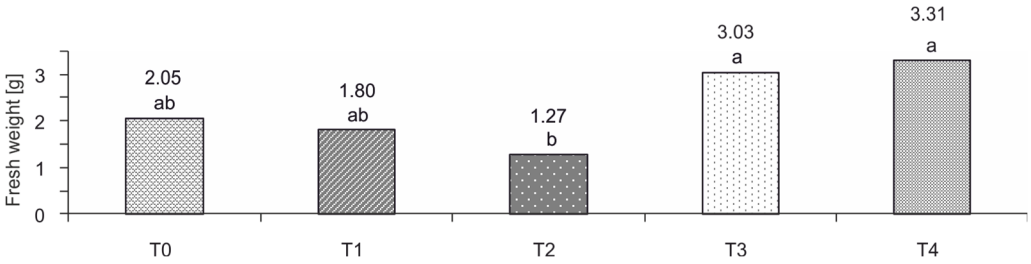

Fig. 8. Mean fresh weight of shoots of white mustard (*Sinapis alba* L.) obtained after 21-day culture on heavy metals contaminated soil (T0) with additives: fly ashes 3:1 (T1), fly ashes 1:1 (T2), compost fertilizer 3:1 (T3), compost fertilizer 1:1 (T4)

A similar effect like that recorded for *Sorghum saccharatum* L., was also observed in *Sinapis alba* L. (Fig. 8). Fly ash added to soil at a 3:1 and 1:1 ratios (w/w) caused a reduction in plant mass by 12% and 38%, respectively, when compared to the decrease recorded for plants grown on the control, i.e., contaminated soil (T0 – 2.05 g). In turn, the application of compost resulted in shoot biomass increase by 32% and 37%, respectively, after 21-day culture of *Sinapis alba* L. on the T3 and T4 soils in comparison to the biomass of plants grown on soil T0.

4. DISCUSSION

The germination index (*IG*) test is particularly popular, being used in studies concerning the assessment of compost maturity [16, 17]. Justification for the application of this test was stressed by Bae et al. [18] who reported that germination as the first stage of seed contact with the surrounding environment is a good indicator of their sensitivity

to changing environmental conditions. According to Cesaro et al. [19], germination tests provide an immediate picture of phytotoxicity, which may change at a later stage of plant growth. Nevertheless, Kranner and Colville [20] stated that the degree, to which toxicity of metals inhibits germination, may vary depending on individual elements and plant species, with some species tolerating levels of heavy metals, which are toxic to other, more sensitive species. According to Márquez-García et al. [21], the effect of any metal on germination depends on its capacity to penetrate the seed coat and reach the embryo tissues. This means that depending on the anatomy and structure of the seed coat the same level of metal may cause different effects in individual plant species.

Genetic variation in plants and thus different responses to variable environmental conditions were confirmed in this study. The used test plants responded differently, with *Sorghum saccharatum* L. proving to be most sensitive. That plant showed poorest germination, produced shortest roots and the biomass of its aboveground parts was small. The greatest sensitivity of *Sorghum saccharatum* L. in relation to other test plants was reported in their studies e.g. Mamindy-Pajany et al. [22]. In contrast, *Sinapis alba* L. or *Lepidium sativum* L. showed a much better response to the provided conditions, which was particularly evident in relation to the application of fly ash mixed with soil at a 1:1 ratio (w/w). Addition of fly ash contributed to better root growth in *Sinapis alba* L. and better seed germination in *Lepidium sativum* L. In this context, a weakly manifested stabilising function of compost or fly ash needs to be underlined. The assumption was to ensure effective immobilisation of heavy metals in the soil so that they exerted no toxicity towards test plants. However, this was not fully confirmed, since plants grown on soil contaminated with heavy metals generally showed better germination and produced longer roots. A lack of precise results in this respect needs to be interpreted in a multifaceted manner, taking into consideration plant physiology, biogeochemistry of metals and finally the individual, chemical character of applied stabilisers.

Seedling roots are the first contact surface with toxic substances, thus a reduction of root growth is typically the first visual manifestation of metal phytotoxicity and their inhibitory effect on root elongation is greater than on shoot growth [23]. The reported lack of a negative effect of heavy metals on plants may suggest that in the soil metals are mostly found in sparsely soluble and biologically inactive combinations. Moreover, a measurement taken after 3 days using the Phytotoxkit test needs to be treated as relatively unreliable in view of the short exposure time of seeds to soil conditions and the complete contact with available heavy metal forms. At the same time, as indicated by the results from this study a more dangerous effect on germinating seeds was found for an excessive alkaline reaction of soil caused by the applied fly ash, with the response being immediate and dramatic, as evidenced by markedly reduced germination and root elongation. A similar effect of applied fly ash on the inhibition of seed germination and root elongation was observed by Samaras et al. [24]. Those authors reported that excessive alkalinity of fly ash, exceeding the level optimal for appropriate plant growth, may result in inhibition of their development. Another interesting finding was also connected

with a lack of an advantageous effect of compost on seed germination and root elongation, particularly since literature data [25] stress the advisability of such an additive. A probable cause for the weak positive effect of compost on plants manifested in the results of the biotest according to the PN-EN ISO 11269-1:2013-06 standard, was connected with its short-term exposure in the soil (3 days). A longer contact of compost with soil (21 days) specified using the biotest according to the PN-EN ISO 11269--2:2013-06 standard showed a positive effect of this additive on the biomass of test plants, thus confirming not only its fertilising effect but also the stabilising role exerted in this experiment.

5. CONCLUSIONS

Analyses confirmed that biological methods provide a suitable tool for the assessment of the potential impact of soil contamination with heavy metals and may be helpful in the evaluation of effectiveness of contaminant stabilisation. Recorded data indicate greater suitability of the biotest based on the PN-EN ISO 11269-2:2013-06 standard, since the results obtained after a longer period of soil contact with the stabilising substances seem more reliable, particularly in relation to the soil environment enriched with different substances. These experiments showed the greatest sensitivity to variable soil conditions in *Sorghum saccharatum* L., with *Lepidium sativum* L. being least sensitive. The substances applied in the form of compost and fly ash showed limited stabilising effect in relation to heavy metals contained in the soil, which was particularly evident in the period of seed germination and root elongation. The positive impact of compost was manifested in the greater biomass of test plants after 21 days of the experiment.

REFERENCES

[1] ROSTAŃSKI A., CABAŁA J., SŁOTA M., *Metal-bearing areas as a source of threats to the environment*, [In:] M. Wierzbicka (Ed.), *Ecotoxicology: Plants, Soil, Heavy Metals*, Warsaw University, Warsaw 2015, 522–544 (in Polish).

[2] The Act of 11 July 2014, *Environmental Protection Law*, Journal of Laws of 2014, item 1101 (in Polish).

[3] SARWAR N., IMRAN M., SHAHEEN M.R., ISHAQ W., KAMRAN A., MATLOOB A., REHIM A., HUSSAIN S., *Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives*, Chemosphere, 2017, 171, 710.

[4] TSANG D.C., YIP A.C., OLDS W.E., WEBER P.A., *Arsenic and copper stabilisation in a contaminated soil by coal fly ash and green waste compost*, Environ. Sci. Poll. R, 2014, 21 (17), 10194–10204.

[5] Council Directive 1999/31/EC on the landfill of waste, Article 5.

[6] AUGUSTYNOWICZ J., TOKARZ K., BARAN A., PŁACHNO B.J., *Phytoremediation of water polluted by thallium, cadmium, zinc, and lead with the use of macrophyte Callitriche cophocarpa*, Arch. Environ, Con. Tox., 2014, 66 (4), 572.

[7] EL-ALAM I., VERDIN A., FONTAINE J., LARUELLE F., CHAHINE R., MAKHLOUF H., SAHRAOUI A.L.H., *Ecotoxicity evaluation and human risk assessment of an agricultural polluted* soil, Environ. Monit. Assess., 2018, 190, 738, 1.

[8] BEBIANNO M.J., PEREIRA C.G., REY F., CRAVO A., DUARTE D., D'ERRICO G., REGOLI F., *Integrated approach to assess ecosystem health in harbor areas*, Sci. Total Environ., 2015, 514, 92.
[9] ROMANOWSKA-DUDA Z.B., GRZESIK M., HAZEM M., KALAJI H., *Phytotoxkit test in growth assessment of corn as an energy plant fertilized with sewage sludge*, Environ. Prot. Eng., 2010, 36 (1), 73.
[10] OLESZCZUK P., HOLLERT H., *Comparison of sewage sludge toxicity to plants and invertebrates in three different soils*, Chemosphere, 2011, 83 (4), 502.
[11] VAN DER VLIET L., VELICOGNA J., PRINCZ J., SCROGGINS R., *Phytotoxkit: a critical look at a rapid assessment tool*, Environ. Toxicol. Chem., 2012, 31 (2), 316.
[12] Polish Committee for Standardization, PN-EN ISO 11466:2002: *Soil quality – Extraction of trace elements soluble in aqua regia*, Warsaw, Poland, 2002.
[13] *Polish Soil Classification*, Soil Sci. Ann., 2011, 62 (3), 1.
[14] Polish Committee for Standardization, PN-EN ISO 11269-1:2013-06, *Soil quality. Determination of the effects of pollutants on soil flora. Part 1. Method for the measurement of inhibition of root growth*, Warsaw, Poland, 2013.
[15] Polish Committee for Standardization, PN-EN ISO 11269-2:2013-06, *Soil quality. Determination of the effects of pollutants on soil flora. Part 2. Effects of contaminated soil on the emergence and early growth of higher plants*, Warsaw, Poland, 2013.
[16] RADZIEMSKA M., VAVERKOVÁ M.D., MAZUR Z., *Pilot scale use of compost combined with sorbents to phytostabilize Ni-contaminated soil using Lolium perenne L.*, Waste Biomass Val., 2017, 1.
[17] JAKUBUS M., BAKINOWSKA E., *Practical applicability of germination index assessed by logistic models*, Compost Sci. Util., 2018, 1.
[18] BAE J., BENOIT D.L., WATSON A.K., *Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes*, Environ. Poll., 2016, 213, 112.
[19] CESARO A., BELGIORNO V., GUIDA M., *Compost from organic solid waste. Quality assessment and European regulations for its sustainable use*, Res. Cons. Rec., 2015, 94, 72.
[20] KRANNER I., COLVILLE L., *Metals and seeds: biochemical and molecular implications and their significance for seed germination*, Environ. Exp. Bot., 2011, 72 (1), 93.
[21] MÁRQUEZ-GARCÍA B., MÁRQUEZ C., SANJOSÉ I., NIEVA F.J., RODRÍGUEZ-RUBIO P., MUÑOZ-RODRÍGUEZ A.F., *The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes*, Mar. Poll. Bull., 2013, 70, 119.
[22] MAMINDY-PAJANY Y., HAMER B., ROMÉO M., GÉRET F., GALGANI F., DURMIŠI E., HURREL C., MARMIER N., *The toxicity of composted sediments from Mediterranean ports evaluated by several bioassays*, Chemosphere, 2011, 82 (3), 362.
[23] YANG Y., WEI X., LU J., YOU J., WANG W., SHI R., *Lead-induced phytotoxicity mechanism involved in seed germination and seedling growth of wheat (Triticum aestivum L.)*, Ecotox. Environ. Safe., 2010, 73 (8), 1982.
[24] SAMARAS P., PAPADIMITRIOU C.A., HARITOU I., ZOUBOULIS A.I., *Investigation of sewage sludge stabilization potential by the addition of fly ash and lime*, J. Hazard Mater., 2008, 154 (1–3), 1052.
[25] MATTEI P., PASTORELLI R., RAMI G., MOCALI S., GIAGNONI L., GONNELLI C., RENELLA G., *Evaluation of dredged sediment co-composted with green waste as plant growing media assessed by eco-toxicological tests, plant growth and microbial community structure*, J. Hazard Mater., 2017, 333, 144.