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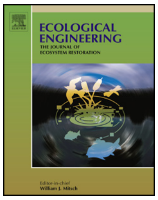

Potential of using immobilizing agents in aided phytostabilization on simulated contamination of soil with lead

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ABSTRACT

Lead (Pb) is one of the key heavy metals which have a significant influence on the individual components of the natural environment. A glasshouse pot experiment was designed to evaluate the potential use of different amendments as immobilizing agents in the aided phytostabilization of Pb-contaminated soil, using *Lolium perenne* L. The research aimed to determine the influence of Pb in doses of 0, 100, 200, 400 and 800 mg/kg of soil, as well as diatomite, chalcedonite, dolomite, limestone, and activated carbon amendments on the content of trace elements in the above-ground parts and roots of *L. perenne.* The study utilised analysis of variance (ANOVA), principal component analysis (PCA) and Factor Analysis (FA). The content of trace elements in plants, pseudo-total and extracted by 0.01 M $CaCl_2$, were determined using the method of spectrophotometry. All of the investigated element contents in the tested parts of *L. perenne* were significantly different in the case of applying reactive amendments to the soil, as well as increasing concentrations of Pb. The greatest average above-ground biomass was observed when diatomite and chalcedonite were amended into the soil. Activated carbon, limestone and chalcedonite caused significant increases of Pb concentrations in the roots.

1. Introduction

Large areas of Earth throughout the world are becoming more and more contaminated with heavy metals by various kinds of anthropogenic activities. Heavy-metal contamination of soil is one of the main global environmental problems, with Poland being no exception (Dąbrowski et al., 2016; Gusiatin and Kulikowska, 2016; Radziemska and Fronczyk, 2015). The permanent nature of heavy metal contamination as well as their inclusion into the food chain are very dangerous. Along with the development of civilization and anthropogenic activities, the level of contamination with heavy metals has intensified (Pietrzykowski et al., 2014; Santos et al., 2016; Vaverkova and Adamcova, 2014; Vymazal et al., 2010). The phenomenon is especially observable in soils and in groundwater (Fronczyk et al., 2016). Human activities, e.g. coal combustion, industrial, mining and agriculture are responsible for a significant accumulation of trace elements in the soil environment (Mazur et al., 2013; Sas et al., 2015; Rogula-Kozłowska et al., 2013). As a result of surface run-off, wind and leaching processes, heavy metals can be distributed large distances throughout the natural environment. Carried by the wind, dust particles can settle on plants, including crops, causing the introduction of dangerous heavy metals into the food chain. Moreover, groundwater may become contaminated due to surface run-off and the leaching of contaminants into deeper layers of the soil profiles (Fronczyk et al., 2014). Limiting the exposure of pathways will help to decrease environmental risks.

A properly planned method of phytoremediation can lead to an increase in the functionality of soil by restricting the mobility of heavy metals and influencing biological processes taking place in the soil. In answer to the rising threat to the soil environment, a series of phytoremediation technologies of contaminated soils have been developed, including, e.g. rhizofiltration, rhizodegradation, phytoextraction, phytovolatization and phytostabilization (Ali et al., 2013). Each of these techniques is based on using the physiological response of a plant organism (accumulation, hyperaccumulation, adsorption and immobilization) to the presence of heavy metals in the environment.

Amongst the methods used for heavy metal contaminated soil management, aided phytostabilization is attractive. It is defined as the immobilization of contaminants in the soil as a result of their absorption and accumulation in the roots, adsorption on the sur-

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face of the roots, or transformation within the rhizosphere into compounds characterized by low solubility (Pérez-López et al., 2014). Vegetation cover minimizes the wind dispersion of heavy metals and water migration through the soil resulting from evaporation (Arienzo et al., 2004; Xue et al., 2016). The method of phytostabilization is not based on removing contaminants from the contaminated area but merely limiting their mobility in the environment, thus preventing them from entering further elements of the food chain. It is a management strategy for stabilizing potentially toxic contaminants (Ali et al., 2013). As a result of plants exhibiting an influence on the soil environment, bioavailable forms of metals can be transformed into less readily available ones. Areas which are especially problematic are places with high soil acidity in which the germination of seeds and growth of plants is more difficult (Alvarenga et al., 2008). It is there that techniques of aided phytostabilization, which are based on using various kinds of soil additives, such as: organic matter, lime, nano-hydroxyapatite, fertilizers or various kinds of mineral materials, may be suitable for application (Jin et al., 2016; Radziemska et al., 2013). Literature (Santibáñez et al., 2008; Arienzo et al., 2004; Pichtel and Salt, 1998) reports *Lolium perenne* L. to be a suitable species for the revegetation of metal-contaminated soils from metallurgical sites, metalliferous wastes, and mine tailings. Furthermore, ryegrass does not present mechanisms of metal hyperaccumulation during metal uptake and is recommended as a valuable tool for bioavailability assessment (Lambrechts et al., 2011).There have been several studies inliterature reporting the potential of ryegrass in phytoremediation processes, as confirmed by the research of authors, such as: Jin et al. (2016), Alvarenga et al. (2009), Pichtel and Salt (1998).

The phytostabilization process could be improved by the application of reactive materials which decrease the availability of heavy metals in acidic contaminated soils (Radziemska et al., 2013, 2014). Seeking alternatively cheap reactive materials that are easily accessible and do not require complicated and expensive treatment has gained a lot of interest. Removing heavy metals from soils using clay minerals has been the topic of many studies, but the current state of knowledge regarding the topic of using the reactive materials tested by the authors is low.

Lead (Pb) pollution of soil, primarily originating from smelting and mining processes, fertilizers, and pesticides, has been a major concern in the recent years. In the 20th century, lead was commonly applied in the manufacturing of water pipes, batteries, paints, cables and for enriching engine fuels. The element is still used in industry, as a result of which it makes its way into the environment along with the emissions of industrial dusts and improperly managed industrial waste. The average Pb content in the Earth's crust is estimated as being 15 mg/kg (Kabata-Pendias, 2011). In Poland, the highest amount of lead amounting to over 1000 mg/kg soil occurs in soils from areas of concentrated mining, processing and smelting of zinc-lead ore. The lead content in the Eastern part of the country is lower than 13 mg/kg, whereas in the western part, it falls within the range of 9–25 mg/kg. In soils of the Carpathian and Sudety Mountains, the concentration of lead exceeds 13 mg/kg, and in some areas – 50 mg/kg. Lead in soil solutions may easily move downwards from upper surfaces leading to the pollution of groundwater (Alumaa et al., 2002). Several Pb species, such as Et_2MePb^+, Me_2Pb^{2+}, Me_3Pb^+, Et_3Pb^+, have been reported to be absorbed by plants through roots or by foliage from the atmosphere (van Cleuvenbergen and Adams, 1991). The accumulation of Pb in the grain of crops from agricultural soils is a major concern due to its high mobility and toxicity (Zhu et al., 2014). Lead may accumulate in the human body as a result of its higher intake by plants, causing acute and chronic poisoning (Aziz et al., 2016; Xia et al., 2016).

The hypothesis for this study is that the application of soil amendments for Pb immobilization when growing perennial ryegrass are an effective phytostabilization technique. The novelty of this study is in the assessment of the usefulness of previously not applied additives in processes of lead immobilization in soils contaminated with this element.

The study aim of the conducted research was the comprehensive presentation of issues connected with supporting the phytostabilization process of heavy metals by various reactive materials. In this study, perennial ryegrass (*Lolium perenne* L.) was tested as a potential metal-tolerant plant to be used in the reactive amendment aided phytostabilization of Pb-contaminated soil. Plant biomass growth, as well as above-ground tissue, root, and soil trace element contents were measured.

2. Materials and methods

2.1. Plant growth experiment

Soil/reactive materials mixtures were placed into 5.0 kg PVC pots and sown perennial ryegrass (*Lolium perenne* L.) cv. Bokser on the surface. The plants were watered every other day with distilled water to 60% of the maximum water holding capacity of the soil by adding deionized water. The plants were harvested after 40 days, and soil were collected.

Soil was artificially polluted with aqueous solutions of lead in the form of lead nitrate ($Pb(NO_3)_2$) salt and was fertilized with a macro- and micronutrient fertilizer mixture (g/kg) containing N-26%, K_2O-26%, B-0.013%, Cu-0.025%, Fe-0.05%, Mn-0.25%, and Mo-0.20%. The soil samples were thoroughly mixed and were allowed to stabilize under natural conditions for three weeks before using as a growth experiment. The experiment was assessed with two factors and three replication. The first factor was the addition of increased doses of lead to soil i.e. 0 (control), 200, 400, and 800 mg/kg. The second factor consisted of the addition of five reactive materials, i.e., diatomite, chalcedonite, dolomite, sand, limestone, and activated carbon (3.0% w/w). Soils without lead and amendments (0.0%) were designated as the control. Each treatment was replicated thrice. Pb was selected as a target contaminant as it is the most common contaminant found in subsurface soils.

2.2. Soil characterization

The soil was collected from the top layer (0–20 cm) from a non-contaminated site in the agricultural area. The soil was the following physicochemical properties: pH 4.92; hydrolytic acidity (mmol/kg) 31.21; sum of exchangeable bases Ca^{2+}, Mg^{2+}, K^+, Na^+ (mmol/kg) 61.10; cation exchange capacity (mmol/kg) 94.20; base saturation (%) 65.20; total N (g/kg) 1.22; organic carbon (g/kg) 7.42; $N\text{-}NH_4{}^+$ (mg/kg) 20.32; $N\text{-}NO_3{}^-$ (mg/kg) 10.01; extractable P (mg/kg) 43.20; extractable K (mg/kg) 8.72; extractable Mg (mg/kg) 31.2; Pb (mg/kg) 16.38; Cu (mg/kg) 8.20; Ni (mg/kg) 4.10; Zn (mg/kg) 23.22; Mn (mg/kg) 208.3.

2.3. Plant analysis

At the end of the experiment harvested plants were divided into shoot and root parts. Plant material was carefully washed with deionized and further ultrapure water to remove soil particles, and then air-dried at room temperature. Before analysis, the plants were powdered using an analytical mill (Retsch type ZM 300, Hann, Germany) and kept at ambient temperature prior to the chemical analyses. The roots and shoots were oven-dried at 55 °C to a stable weight, and the dry biomass was recorded. A representative subsample was mineralized in nitric acid (HNO_3 p.a.) with a concentration of 1.40 g/cm and 30% H_2O_2 using a microwave oven (Milestone Start D, Italy). After filtration (Whatman, Sigma-Aldrich,