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Investigate the influence of halloysite and activated carbon mixtures in phytostabilization of Pb-contaminated soil with *Lolium perenne* L.

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Abstract: Investigate the influence of halloysite and activated carbon mixtures in phytostabilization of Pb-contaminated soil with *Lolium perenne* L. A pot experiment was conducted to investigate the effects of halloysite and activated carbon mixtures on the biomass and distribution of macronutrients (Mg, K, Ca, Na, P) in ryegrass grown in Pb-contaminated soil. The soil was spiked with four different levels of lead contamination, i.e. 0 (control), 200, 400, 800 mg·kg⁻¹ were applied in an analytical grade Pb(NO₃)₂ solution mixed thoroughly with the soil. Raw halloysite (3%) and activated carbon (1% relative to soil mass) mixtures were used to reduce the effect of soil lead contamination. Ryegrass of the Bokser variety was harvested after 42 days, and soil samples were collected for laboratory tests. The mixture of sorbents applied in the experiment which turned out to be most effective at doses of lead amounting to 400 and 800 mg·kg⁻¹ of soil, with the increase in the yield of ryegrass being the highest. Increasing contamination of soil with lead in the series without the mixture of sorbents increased the contents of phosphorus, sodium, calcium and magnesium in ryegrass. The applied mixture of halloysite and activated carbon changed the macronutrient concentration in ryegrass, with the greatest changes found in that of potassium and sodium.

Key words: activated carbon, halloysite, lead contamination, macroelements, ryegrass, yield

INTRODUCTION

The dynamic development of the modern economy, covering a wide range of various branches of industry, transport or agriculture, as well as progressing

urbanization, are inseparably connected with releasing a significant amount of waste and contaminants into the environment, including heavy metals (Rogula-Kozłowska et al. 2013, Vaverková and Adamcová 2014, Sas et al. 2015). Among the elements which are widely encountered in the natural environment is lead (Pb), which in the case of chemical compounds has two oxidation states, i.e. II and IV (Némethet et al. 2016). Currently, 199 minerals containing this element are known. Contamination with lead is caused, above all, by the rapid development of mining and processing of lead ore and many other colored metals containing lead compounds as admixtures (Alumaa et al. 2002). The content of lead in plants depends both on the degree of soil contamination as well as the type of plant grown (Zhu et al. 2014, Adamcová et al. 2016a, Radziemska et al. 2017). Major toxic effects of Pb in plants are presented in Figure 1. Lead is absorbed into the human body, above all, in the digestive tract, as well as by the respiratory system. Small amounts of the metal can also be absorbed by the skin (Skröder et al. 2016).

In recent years, the amount of publications pertaining to the various phytoremediation techniques has rapidly increased, in connection with which there has been much progress in improving the

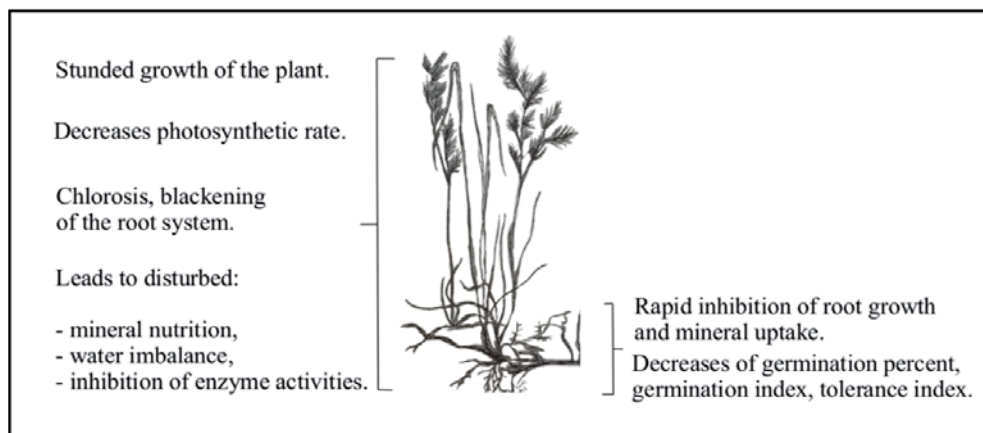


FIGURE 1. Major toxic effects of lead in plants

effectiveness as well as quality of phytoremediation (Pérez-López et al. 2014, Adamcová et al. 2016b). However, despite the undeniable advantages, these methods also have their many imperfections. That is why new materials are continuously being sought to be used as additives aiding soil decontamination processes.

Aided phytostabilization is a technique which relies on the application of heavy metal immobilizing additives to soil along with adequately chosen plant species (Radziemska et al. 2014). Plants play a very important role in the phytostabilization technique, as they cause the physical stabilization of soils with a dense root system, contributing to a decrease in wind and water erosion, and leading to the precipitation of harmful ions, thus limiting their bioavailability (Ali et al. 2013). Knowledge of the micro- and macroelement contents in plants growing in areas contaminated by heavy metals is very important.

Halloysite is a clay mineral within the kaolinite subgroup belonging to the group of aluminosilicates containing double-

layered packets of the 1 : 1 build type; in the present experiment, the mineral came from the deposits of the mine Kopalnia Haloizytu Dunino LLC. Halloysite is characterized by excellent sorption properties as well as a chemically active surface, which it owes to a high specific surface area (Afshar and Ghaee 2016). It is most common in nature as a rock-forming mineral and is one of the main components of the Earth's crust. The mineral occurs in two varieties of hydrohalloysite ($\text{Al}_4[\text{Si}_4\text{O}_{10}](\text{OH})_8 \cdot 4\text{H}_2\text{O}$) and halloysite ($\text{Al}_4[\text{SiO}_4](\text{OH})_8$). It is composed of approx. 45% crystalline silica and 40% alumina; the remaining components are water and trace amounts of oxides, such as: TiO_2 , Al_2O_3 , FeO , MgO , CaO , Na_2O , K_2O (Cravero et al. 2016). Between the layers are water molecules which create an empty space; the distances between the planes are 10 and 7 Å respectively for the hydrated and dehydrated forms of halloysite. Active carbons are some of the most commonly applied adsorbents in many branches of industry (Lladó et al. 2016). Their adsorption properties result from the microporous structure

and developed specific surface area. The specific area of activated carbons can be from a few hundred up to 2,000 $\text{m}^2\cdot\text{g}^{-1}$; carbon with a specific surface area of 856.05 $\text{m}^2\cdot\text{g}^{-1}$ was used in the present experiment.

Works regarding the use of halloysite and activated carbon in many processes connected with environmental engineering and protection can be found in literature (Horel and Schiewer 2016, Radziemska et al. 2016a, b, c, Zhang et al. 2016). In the carried out experiment, a mixture of these two sorbents was used in order to improve the immobilizing properties of lead contamination of soil used to grow ryegrass; next, plant yield and macronutrient contents were determined in the plant material.

MATERIAL AND METHODS

Plant growth experiment

This study was conducted in a greenhouse of the Department of Environmental Improvement of the Warsaw University of Life Science – SGGW. The soil used in this study was sampled in an uncontaminated agricultural area from the sub-soil found in the layer between 10 and 30 cm below the surface.

Stones, sticks and roots were manually removed. The soil texture was dominated by 2.0–0.05 mm fractions (85%), 0.05–0.002 mm (15%), and 0.002 mm fractions (1.8%). The soil was acidic (pH 5.1), contained 8.12 $\text{g}\cdot\text{kg}^{-1}$ of total organic carbon, and found to have a hydrolytic acidity of 35.25 $\text{mmol}\cdot\text{kg}^{-1}$, sum of exchangeable bases (Ca^{2+} , Mg^{2+} , K^+ , Na^+) 61.13 $\text{mmol}\cdot\text{kg}^{-1}$, total nitrogen 1.12 $\text{g}\cdot\text{kg}^{-1}$, organic carbon 7.32 $\text{g}\cdot\text{kg}^{-1}$, extractable phosphorous 40.12 $\text{mg}\cdot\text{kg}^{-1}$, extractable potassium 8.45 $\text{mg}\cdot\text{kg}^{-1}$, extractable magnesium 30.01 $\text{mg}\cdot\text{kg}^{-1}$, nickel 5.02 $\text{mg}\cdot\text{kg}^{-1}$, copper 7.22 $\text{mg}\cdot\text{kg}^{-1}$, zinc 32.21 $\text{mg}\cdot\text{kg}^{-1}$ and lead 14.11 $\text{mg}\cdot\text{kg}^{-1}$.

The soil was spiked in the laboratory with increased doses of lead (0, 200, 400, and 800 $\text{mg}\cdot\text{kg}^{-1}$), introduced in the form of chemically pure aqueous solutions of lead nitrate [$\text{Pb}(\text{NO}_3)_2$] salt. Polyethylene pots were filled with 5 kg of soil, and mixed with halloysite 3% (v/v) and activated carbon 1% (v/v). Soils without lead and amendments (0.0%) were designated as the control. Scanning electron microscope (SEM) images of raw halloysite and activated carbon are shown in Figure 2. Basal fertilization of a macro- and micronutrient fertilizer mixture (200 mg per pot)

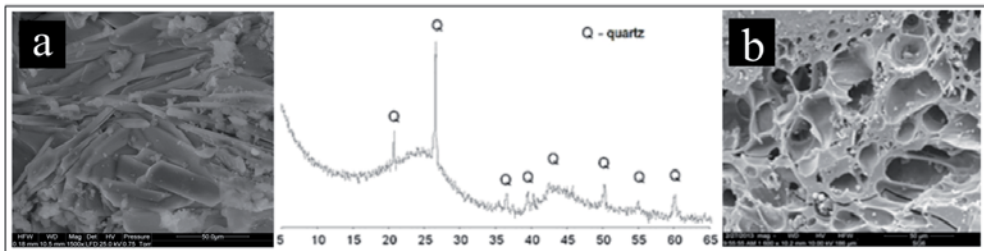


FIGURE 2. The SEM images of raw halloysite (a) and SEM images and EDX pattern of activated carbon (b) samples

containing: N – 26%, P_2O_5 – 12%, K_2O – 26%, B – 0.013%, Cu – 0.025%, Fe – 0.05%, and Mn – 0.025%, was applied to all pots. The plants were watered every other day with Milli-Q water to 60% of the maximum water holding capacity of the soil by adding deionized water. The ryegrass (cv. Bokser) was harvested after 42 days and soil samples were collected.

Analytical determinations

The harvested plants were dried at 60°C. After oven drying, the plants were weighed and powdered using an analytical mill (Retsch type ZM 300, Hann, Germany) preceding the chemical analyses. The samples were kept at an ambient temperature until analysis. The plant samples were subjected to mineralization in condensed sulfuric acid with hydrogen peroxide as the catalyst. The content of the following elements was determined in the obtained extracts: phosphorus measured colorimetrically using the vanadium-molybdenum method; sodium (Na), calcium (Ca) and potassium (K) contents obtained by the atomic emission spectrometry (AES) method; and magnesium (Mg) assayed by the atomic absorption spectrometry (AAS) method (Ostrowska et al. 1991).

The following parameters were analyzed in the soil before the experiment was carried out: soil pH – by the potentiometric method in an aqueous KCl solution with a concentration of $1 \text{ mol} \cdot \text{dm}^{-3}$, using a digital pH-meter (Model pH/LF 12, Schott, Germany); hydrolytic acidity (HAC) and the total exchange bases Ca^{2+} , Mg^{2+} , K^+ , Na^+ (TEB) – by the Kappen method (Ostrowska et al. 1991); the content of total organic carbon (TOC) according to Tiurin's method following the hot

digestion of soil samples with $K_2Cr_2O_7$ and H_2SO_4 in the presence of Ag_2SO_4 as a catalyst and the titration of excess $K_2Cr_2O_7$ with $FeSO_4/(NH_4)_2SO_4 \cdot 6H_2O$ (Mocek and Drzymała 2010); and finally the available phosphorus (P) and potassium (K) – by the Egner–Riehm method and available magnesium (Mg) – by the Schachtschabel method (Lityński et al. 1976).

Laboratory equipment was acid-washed (10% HNO_3) and rinsed with deionized water. Ultra-pure water (Millipore System, USA) of $0.055 \mu\text{S} \cdot \text{cm}^{-1}$ resistivity was used for preparing the solutions and dilutions. Each sample was measured in three replications.

Statistical analysis

The test results were analyzed statistically with the Statistica package (StatSoft 2010) using two-factor analysis of variance (ANOVA). The means and standard deviations (\pm SD) of the three replications are reported.

RESULTS AND DISCUSSION

Biomass response of ryegrass to Pb-contamination and application of amendments

Plant biomass (dry mass) was measured at the end of the experiment and has been shown in Figure 3. The above-ground parts of ryegrass grown in soil without the addition of the mixture of halloysite and activated carbon were characterized by high sensitivity to soil contamination with lead. In the pot, to which the highest dose of lead was applied ($800 \text{ mg} \cdot \text{kg}^{-1}$ of soil), a significant decrease in plant yield occurred as compared to the control se-

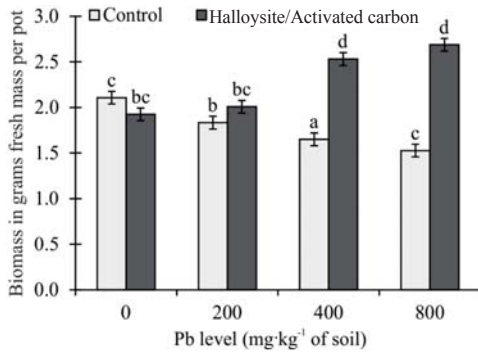


FIGURE 3. Effect of lead and halloysite/activated carbon mixture on the aerial biomass of ryegrass (*Lolium perenne* L.). Error bars are \pm standard error ($n = 3$). Bars marked with different letters differ significantly for the same lead exposure ($P < 0.05$) according to the Duncan test

ries. Lower crop quality when lead bioavailability increased in soils was reported by Yongsheng et al. (2011), Rodriguez et al. (2015) and Blanco et al. (2016). When found in excessive amounts in soil, lead negatively influences the fundamental life processes of plants, causing, among others, disruptions in photosynthesis, cell division, nitrogen metabolism and water balance (Smolders et al. 2015, Dikilitas et al. 2016). Plants exposed to the effects of lead exhibit stunted growth caused by disturbances in the functioning of biological membranes; however, plants have different defense mechanisms protecting them from the stress caused by the presence of lead in the environment (Břendová et al. 2016). Several authors (Haussling et al. 1998, Rout and Das 2003) reported that application of lead inhibits the growth of Norway spruce, and this effect is related to the decreased uptake of K^+ , Ca^{2+} , Mg^{2+} , Na^+ and Mn^{2+} . The mixture of sorbents in the pots in which the soil had not been contaminated with lead compounds caused a slight (9%) decrease in plant yield.

However, in the case of greater doses of this contaminant, significant increases in the yield of ryegrass were observed. The mixture of halloysite and activated carbon turned out to be most effective for the highest doses of lead contamination (400 and 800 $mg \cdot kg^{-1}$ of soil); in these cases, the increase in plant yield was the highest and resulted in respective 53 and 76% increases as compared to the control series. These results indicate that the addition of the mixture of halloysite and activated carbon stimulates plant growth, and hence affects the phytostabilization process. In another experiment carried out by Radziemska et al. (2016b), the introduction of both raw as well as modified halloysite to soil which had been contaminated with nickel caused an increase in the yield of Indian mustard. The application of inorganic amendments to soil alleviates the toxic effect of heavy metals on soils and, at same time, influences crop yield (Friesl et al. 2003). In a study on reducing the effects of tri- and hexavalent chromium on plant biomass and development, zeolite and especially calcium oxide (CaO) proved to be the most effective minerals amendments, particularly for oat and spring barley (Wyszkowski and Radziemska 2010, 2013a). Mallampati et al. (2012) investigated the use of a nanometallic Ca/CaO dispersion mixture for the immobilization of lead-contaminated soil. The authors' results suggest this to be a suitable treatment for the gentle immobilization of polluted soil with lead.

Accumulation macroelements in plant

Lead blocks the access of many ions to their absorption sites on the roots, thus inhibiting their uptake (Godbold and

Kettner 1991). In most cases Pb blocks the entry of cations (K^+ , Ca^{2+} , Mg^{2+}), and anions (NO_3^-). Lead influences the overall distribution of nutritional elements within the different organs of the plant. In *Cucumis sativus* seedlings Pb decreased uptake of Ca, K, Mg and in *Zea mays* the uptake of Mg, Ca, K and P (Walker et al. 1997). Magnesium is an element that is essential to the proper functioning of all plant species. Moreover, the absorption of a series of nutrients by the roots takes place thanks to special channels the functioning of which depends on this element (Jariene et al. 2016). Approximately 10–20% of magnesium taken up by plants is stored as a specific component of chlorophyll. In addition to this, the nutrient is a component of a structural element of the cell wall (pectin). The competition of magnesium with other cations for uptake by plants ranges, from the highest to the lowest, as follows $K^+ > NH_4^+ > Ca^{2+} > Na^+$ (Péñalosa et al. 1995). The content of magnesium in the above-ground parts of ryegrass was shaped by the dose as well as the addition of the mixture of halloysite and activated carbon to the soil (Fig. 4). In the series, to which reactive materials had not been introduced, a positive correlation between increasing doses of lead in the soil and the content of magnesium was observed. The application of the mixture of halloysite and activated carbon lead to a positive influence on the average content of magnesium in the above-ground parts of the test plant, causing a 13% increase of the above-mentioned element as compared to the control series. In the pot with the lowest dose of lead (200 $mg \cdot kg^{-1}$ of soil), the sorbent mixture resulted in the highest increase of magne-

sium content in the above-ground parts of ryegrass. In a study on reducing the effect of nickel contamination on the accumulation of magnesium, zeolite and especially modified halloysite proved to be the most effective substances, particularly for maize (Radziemska et al. 2013). Similar data have been reported by Leszczyńska and Kwiatkowska-Malina (2012) who in a study of soil contaminated with zinc, lead and cadmium noted that the application of dolomite increased the magnesium content of winter wheat.

Potassium is essential to the process of creating amino acids and proteins, and thus the conversion of nonorganic nitrogen to its organic forms. Moreover, it increases the utilization of nitrogen by plants (Manning et al. 2017). The introduction of increasing doses of lead as well as sorption materials to the soil significantly affected the content of potassium in the above-ground parts of ryegrass (Fig. 4). In the control series (without the addition of halloysite/activated carbon mixture), increasing soil contamination with lead resulted in decreased potassium contents in the above-ground parts of the tested plant by 23% in pots with the highest dose of contamination. The application of a mixture of sorbents to soil contaminated with lead resulted in a significant increase (by 14%) of the average potassium content as compared to the control series. However, increasing contamination of soil with lead resulted in the successive decrease in the content of the above-mentioned nutrient in the above-ground parts of plants in pots with the addition of halloysite and activated carbon.

The applied dose of lead as well as the addition of the halloysite and acti-

vated carbon mixture influenced the calcium content in the above-ground parts of ryegrass (Fig. 4). Calcium determines the proper growth and development of plants (Janet et al. 2016). It decreases the ability of plants to take up minerals from the soil, which leads to disturbances in basic metabolic processes leading to the inhibition of growth. It regulates the take up of mineral salts by the plant roots, has a beneficial effect on transpiration processes, and prevents the accumulation of nitrates. According to Falkowski et al. (2000), excessive amounts of calcium in plants lead to restricted take up of magnesium, thus the significance of the Ca : Mg ration. Calcium deficiencies in plants can facilitate the adsorption of toxic compounds, particularly aluminum. In the carried out experiment, in the series without the addition of sorbents, increasing lead contamination caused a significant increase in calcium content in the above-ground parts of the test plant. The application of a mixture of halloysite and activated carbon had a negative influence on the average calcium content in the above-ground parts of ryegrass, causing a 9% decrease in the average content of the analyzed element. The most significant decrease in calcium content was observed in soil to which the lowest dose of lead $200 \text{ mg}\cdot\text{kg}^{-1}$ was applied. Tlustoš et al. (2006) claims that the use of calcium oxide as a soil amendment increased calcium levels in spring wheat plants grown in soil contaminated with heavy metals.

The application of a mixture of halloysite and activated carbon to soil contaminated with lead influenced the sodium content in the above-ground part of the test plant (Fig. 4). Lead contamina-

tion of soil in the control series contributed to the accumulation of sodium in the above-ground parts of ryegrass. The carried out studies show that using a mixture of halloysite and activated carbon had a significant influence on the average sodium content in the above-ground parts of the test plant, causing a nearly twofold increase in the contents of the given element. The results of this research were partly confirmed by the experiments of other authors. The addition of halloysite and zeolite to nickel-contaminated soil also contributed to the accumulation of sodium in maize (Radziemska et al. 2013). Zeolite significantly shaped the content of phosphorus, calcium, sodium, magnesium and potassium in the grain, straw and roots of oat (Wyszkowski and Radziemska 2013b).

As shown in Figure 4, the accumulation of phosphorous in ryegrass was influenced by the lead concentration in the soil and the introduced mixture of halloysite and activated carbon. The $800 \text{ mg}\cdot\text{kg}^{-1}$ dose of lead caused a 24% increase in phosphorus content in the above-ground parts of the tested plant in relation to the control series (without lead and sorbents). The applied additives had a negative effect on the contents of this component, which was also especially visible in pots with the highest doses of contaminants 400 and $800 \text{ mg Pb}\cdot\text{kg}^{-1}$. Paivoke (2002) and Akinci et al. (2010) observed a negative correlation between phosphorous uptake and the concentration of lead in soil. Similarly, Azmat et al. (2009) also found an inverse relation between Pb^{2+} and Ca^{2+} ion accumulation, attributed to their ionic similarity which allows lead to replace calcium during specific physiological processes.

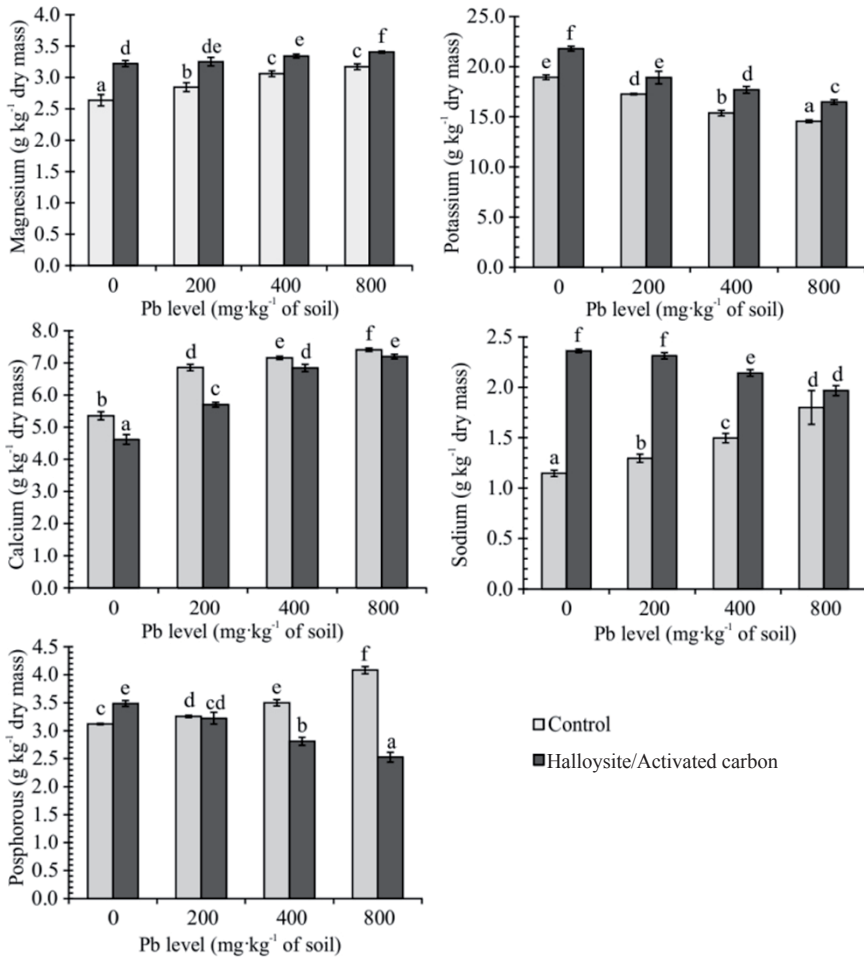


Fig. 4. Magnesium, potassium, calcium, sodium, and phosphorous concentrations in aerial biomass of *Lolium perenne* L. at the end of the experiment. Error bars are \pm standard error ($n = 3$). Bars marked with different letters differ significantly for the same lead exposure ($P < 0.05$) according to the Duncan test

The same relationship was recorded in another experiment conducted by Radziemska et al. (2013), where halloysite in crops subjected to doses of $240 \text{ mg Ni kg}^{-1}$ of soil led to a nearly twofold increase in the phosphorus content of maize in relation to plants without the addition of mineral sorbents. The experiment conducted by Szostek and Ciećko (2015) showed that the application of lime and loam to soil

caused changes in the phosphorus content in the green mass of yellow lupine, narrow-leaf lupine, black radish and alfalfa.

CONCLUSIONS

The effect of soil contamination with lead on the aerial biomass and content of macronutrients (magnesium, potas-

sium, calcium, sodium and phosphorous) depended on the dose of lead and the mixture of halloysite and activated carbon applied to the soil. The mixture of halloysite and activated carbon added to the soil clearly limited the negative influence of lead on the yield of ryegrass. The applied mixture of halloysite and activated carbon turned out to be the most effective when dealing with the doses of lead contamination (400 and 800 mg·kg⁻¹ of soil), for which the increase in plant yield was the highest. Increasing the contamination of soil with lead in the series not containing the mixture of halloysite and activated carbon increased the contents of phosphorus, sodium, calcium and magnesium in ryegrass. Of all the macronutrients, the greatest changes were found in the concentration of calcium in the aerial mass of the tested plant. Increasing doses of lead reduced the concentration of potassium. The applied mixture of halloysite and activated carbon changed the macronutrient concentrations of ryegrass, with the greatest changes found in those of potassium and sodium. Under the influence the mixture of sorbents added to soil contaminated with lead, an increase in the contents of magnesium, potassium and sodium occurred, along with a decrease of calcium content in the plant subject to the studies.

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Streszczenie: Ocena dodatku mieszanki haloizytu i węgla aktywnego w fitostabilizacji gleby zanieczyszczonej ołowiem z wykorzystaniem *Lolium perenne* L. Eksperyment wazonowy został przeprowadzony w celu określenia wpływu mieszaniny haloizytu i węgla aktywnego na wielkość biomasy i zawartość makroskładników (Mg, K, Ca, Na, P) w życicy trwałej uprawianej na glebie zanieczyszczonej ołowiem. Gleba została zanie-

czyszczona czterema różnymi dawkami ołowiu: 0 (kontrola), 200, 400, 800 mg·kg⁻¹ zostały wprowadzone w formie związku Pb(NO₃)₂ cz.d.a., który wymieszano z glebą. Mieszanka surowego haloizytu (3%) i węgla aktywnego (1% w stosunku do masy gleby) została użyta w celu zminimalizowania wpływu zanieczyszczenia gleby ołowiem. Życicę trwałą odmiany Bokser oraz próbki gleby do analiz chemicznych pobrano po 42 dniach wegetacji. Mieszanina sorbentów okazała się być najskuteczniejsza przy zanieczyszczenia gleby ołowiem w dawkach 400 i 800 mg·kg⁻¹ gleby, w tych przypadkach plon życicy trwałej był największy. W serii bez dodatku do gleby mieszaniny sorbentów wzrastające zanieczyszczenie gleby ołowiem wywołało zwiększenie zawartości fosforu, sodu, wapnia i magnezu w życicy trwałej. Aplikacja mieszaniny haloizytu i węgla aktywnego wpłynęła na zmianę zawartości makroelementów w życicy trwałej, największe różnice zaobserwowano w przypadku potasu i sodu.

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