

AIDED PHYTOSTABILIZATION OF COPPER CONTAMINATED SOILS WITH *L. PERENNE* AND MINERAL SORBENTS AS SOIL AMENDMENTS

Maja RADZIEMSKA¹

Warsaw University of Life Sciences – SGGW, Warszawa, Poland

Abstract

The present study was designed to assess phytostabilization strategies for the treatment of soil co-contaminated by increasing levels of copper with the application mineral amendments (chalcedonite, zeolite, dolomite). From the results it will be possible to further elucidate the benefits or potential risks derived from the application of different types of mineral amendments in the remediation of a copper contaminated soil. A glasshouse pot experiment was designed to evaluate the potential use of different amendments as immobilizing agents in the aided phytostabilization of Cu-contaminated soil using ryegrass (*Lolium perenne* L.). The content of trace elements in plants and total in soil, 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 mineral amendments to the soil, as well as increasing concentrations of copper. The greatest average above-ground biomass was observed for soil amended with chalcedonite. In this experiment, all analyzed metals accumulated predominantly in the roots of the tested plant. In general, applying mineral amendments to soil contributed to decreased levels of copper concentrations.

Keywords: aided phytostabilization, metal-contaminated soil, risk minimization, *Lolium perenne* L.

¹ Corresponding author: Warsaw University of Life Sciences - SGGW, Faculty of Civil and Environmental Engineering, Nowoursynowska st 159, 02-776 Warszawa, Poland, e-mail: maja_radziemska@sggw.pl, tel. +48225935370

1. INTRODUCTION

Contamination of soil with the heavy metals has become a serious problem throughout the world, causing the reduction of agricultural yield and harmful effects on human health by entering the food chain [27, 11, 1]. Copper is introduced into the soil and ground water through various products of human activity, including: fertilizers, fungicides and pesticides, sludge, pig wastes, emissions from municipal wastes, mining and residues from metalliferous mines [9, 2, 4]. Despite being an essential element and acting as a co-factor in numerous enzymes, at higher concentrations, copper is highly toxic to plants and microorganisms [22, 6]. In fact, one of the most complicated issues for environmental engineering is to detoxify soils polluted with copper [16].

Main principles of phytoremediation are (1) improvement of the topsoil quality as an important ecological factor (2) phytoimmobilization of the heavy metals, (3) phytoextraction of heavy metals into roots and shoots of the plants, (4) minimization of seepage water formation by evapotranspiration and new soil formation [33].

Phytostabilization is a simple, cost-effective and less environmentally invasive approach to stabilize and reduce the bioavailability of contaminants by using plants [28]. It is important to mention that phytostabilization is not a technology for real clean-up of contaminated soils, but a management strategy for inactivating (stabilizing) heavy metals with a labile pool in excess leading to biological effects [7, 19]. Aided phytostabilization is a technique which relies on the application of metal immobilizing additives (mineral/organic) to the soil, along with appropriately selected plant species. It is important to gain an understanding of the chemical composition of plants growing on the contaminated areas [38].

In connection with the above, studies were assumed whose aim was to determine the influence of chalcedonite, zeolite, and dolomite applied to soil contaminated with copper on the effectiveness of the process of aided phytostabilization by determining: mobility and redistribution of copper on *L. perenne* and the concentration of copper in soil.

2. MATERIALS AND METHODS

2.1. Experiment set-up

The experiments were conducted in 5.0 kg polyethylene pots using a non-contaminated soil collected at a depth of about 0-20 cm in the agricultural area. The soil was characterized for several parameters (Tab. 1). The methods used

for the determination of organic matter content in the soil Tiurin's method have been described by [17]. The ammonium content of the soil was determined with an ammonia electrode as ammonium formed by Kjeldahl digestion [5]. The Kappen method [12] was employed to estimate the hydrolytic acidity (HAC) and total exchangeable bases (TEB- K^+ , Na^+ , Ca^{2+} , and Mg^{2+}) of the used soil. Cation exchange capacity (CEC) from the formula: $CEC = HAC + TEB$ and percentage base saturation (V) from the formula: $BS = 100 \cdot TEB / CEC^{-1}$. Soil pH (with a glass electrode using a 1:1 sample/water ratio). Electrical conductivity (EC) measured with a HandylabpH/LF 12 conductometer (Schott, Germany), in a 1:2 soil/deionized water suspension (w/v). Phosphorus and potassium content – Egner-Riehm method [28], and magnesium content – atomic absorption spectrometry method following extraction using the Schachtschabel method [14].

Table 1. Selected physico-chemical parameters of soil used for pot experiment

Soil parameter	Value
pH	4.92
Hydrolytic acidity ($mmol \cdot kg^{-1}$)	31.21
Sum of exchangeable bases Ca^{2+} , Mg^{2+} , K^+ , Na^+ ($mmol \cdot kg^{-1}$)	61.10
Cation exchange capacity ($mmol \cdot kg^{-1}$)	94.20
Base saturation (%)	65.20
Total N ($g \cdot kg^{-1}$)	1.22
Organic carbon ($g \cdot kg^{-1}$)	7.42
N- NH_4^+ ($mg \cdot kg^{-1}$)	20.32
N- NO_3^- ($mg \cdot kg^{-1}$)	10.01
Extractable P ($mg \cdot kg^{-1}$)	43.20
Extractable K ($mg \cdot kg^{-1}$)	8.72
Extractable Mg ($mg \cdot kg^{-1}$)	31.2
Cu ($mg \cdot kg^{-1}$)	8.20

Soil/amendments mixtures were placed into pots and sown perennial ryegrass (*Lolium perenne* L.) variety Bokser on the surface. The plants were watered every other day to 60% of the maximum water holding capacity of the soil by adding deionized water. Soil was artificially polluted with aqueous solutions of copper in the form of copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$, produced at the POCh, Gliwice) in the following doses (mg/kg of soil): 0 (control), 150, 300, and 600, and was fertilized with amacro- and micronutrient fertilizer mixture (g/kg) containing N-26%, P_2O_5 -12%, 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 two weeks before using as a growth experiment. Mineral amendments (chalcedonite, zeolite, dolomite) was mixed in with the soil in the amount of 3.0% (each). Soils without copper and amendments (0.0%) were designated as the control. At the

end of the experiment (approx. 45 days after seed sowing), plants were harvested, weighed and separated into above-ground parts and roots.

2.2. Plant and soil elemental analysis

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 ZM300, Hann, Germany) and kept at ambient temperature prior to the chemical analyses. A representative subsample was mineralized in nitric acid (HNO₃ p.a.) with a concentration of 1.40 g/cm and 30% H₂O₂ using a microwave oven (Milestone Start D, Italy). After filtration, the digestion products were adjusted to 100 mL volume with deionized water. Extracts were analyzed for total copper concentrations were determined by Atomic Absorption Spectrometry method using an iCE-3000 spectrophotometer (Thermo Scientific, USA). Copper in soil was analyzed using U.S. EPA method 3051.

2.3. Statistical analysis

Experiments were performed in triplicates and the values presented as the means ± standard deviation. The data were analysed using Statistica software (version 10.0, San Diego, CA, USA). Significant differences ($p < 0.05$) between the mean values of different treatments were compared and evaluated using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

The effects of chalcedonite, zeolite, and dolomite on the biomass production of *Lolium perenne* L. grown in a copper contaminated soils are shown in Fig. 1. In our study, the biomass of plants in the control series (without the addition of amendments to copper-contaminated soil) decreased by 9% in the treatment with the lowest copper dose (150 mg/kg soil) to 40% in the treatment with the highest copper dose (600 mg/kg soil). The results of this study and published data [20, 35, 39] suggest that copper's adverse influence on crop yield is determined not only by copper concentration in soil, but also by the applied soil amendments. The greatest average above-ground biomass was observed in cases of amending soil with chalcedonite (38%) and zeolite (43%) as compared to not applying any of these substances to the soil. Several authors have reported the effectiveness of lime materials to enhance revegetation, or increase plant survival and growth on contaminated soils by copper [32, 15]. The growth of rye seedlings, in particular the above-ground parts of the analyzed plants, was also stilted under exposure to increasing doses of copper [13]. In the work of Wyszowski and Radziemska [36, 37, 35], Radziemska and Mazur [20] zeolite

increased the average yield of aerial parts of oats, spring barley, spring rapeseed and maize grown in soil polluted with heavy metals. There are some reports indicating that rice seed biomass increases in bentonite-treated soil [29].

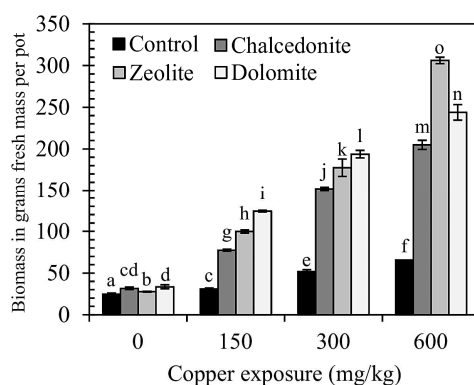


Fig. 1. Effect of copper and various mineral sorbents on the aboveground biomass of *Lolium perenne* L., in grams fresh mass per pot. Error bars are \pm standard error (n=3).

Bars indicated by the different letter are significantly different ($P < 0.05$) according to Tukey's test

The chemical mobility and uptake of metal ions by the plants can be affected by microbial activities, rhizospheres and roots [18]. The chelator compounds inside plant tissue play an important role in the toleration, sequestration and transportation of contaminants [30]. Plants used in the phytostabilization technique should store trace elements in root tissues and be characterized by a limited ability of translocating them to the above-ground parts [40]. As shown in Fig. 2, the accumulation of copper content was much higher in roots than in shoots, and this major advantage indicates the potential of *Lolium perenne* L. as a phytostabilizer. The normal concentration of copper in plant material ranges from 5 to 25 mg/kg [2]. In the control, copper concentrations (in mg/kg dry weight) varied between 7.61-55.10 in shoots and between 24.53-306.14 in roots. Data found in literature confirm the existence of a correlation between total copper content in soil and its concentration in plants [34]. According to Tiecher et al. [31] copper accumulation occurred predominantly in the young grapevine roots, with low translocation to the shoots. In the control series (without additives), a positive correlation between the copper content in tested plant and the increasing contamination of soil with copper occurred. The highest reduction of copper contents (39%) - was observed in the above-ground parts of the tested plant species grown in soil containing dolomite, as compared to the uncontaminated soil. Compared with the control treatment, the chalcedonite and zeolite treatments significantly increased copper concentration in plant roots as compared to pots to which additives had not been added. These results were

similar to another experiment conducted by Radziemska et al. [25, 23] where the heavy metal contents in the above-ground parts of maize, Indian mustard, and ryegrass were affected by the application of mineral sorbents (i.e. zeolite, halloysite, limestone, chalcedonite and dolomite).

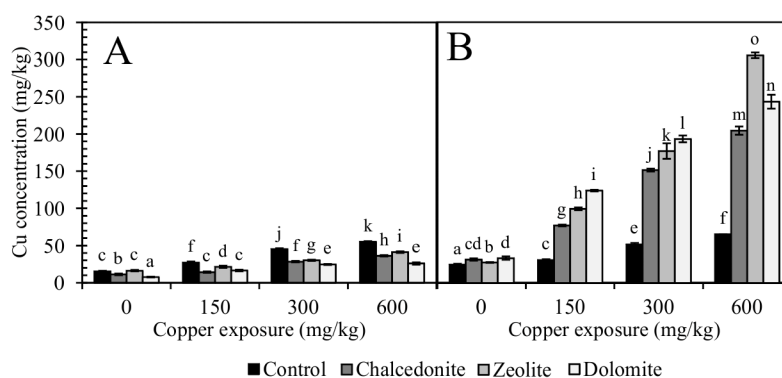


Fig. 2. Copper concentration (mg/kg, dry weight basis) in the above-ground part (a) and roots (b) of *Lolium perenne* L. at the end of the trial. Error bars are \pm standard error ($n = 3$). Bars marked with different letters differ significantly for the same Cu exposure ($P < 0.05$) according to the Duncan test

The total content of heavy metals in the soil is, in addition to pH, one of the most important factors influencing the absorption of heavy metals and their content in a plant. In the presented research, the pH of soil solutions increased following the addition of mineral sorbents (control: 5.71 ± 0.05 ; chalcedonite: 6.23 ± 0.05 ; zeolite 6.78 ± 0.04 ; dolomite: 7.12 ± 0.05). In the series lacking mineral additives, increasing doses of copper contamination caused a successive increase in the pH of soil. The greatest increase was 0.89 pH units, observed after dolomite was added to the soil. The best growth in trace elements contaminated soils occurred at pH 6.0 to 5.5 - the reason might be the only slight solubility of toxic metals in the soil solution under these conditions [10]. Metal solubility and mobility increased as pH decreased [8]. As reported by Wyzkowski and Radziemska [37], the addition of zeolites and CaO reduced copper solubility in the soil by forming stable complexes. Soil copper concentrations have been presented in Fig. 3. In this study, the application of zeolite and chalcedonite led to a significant decrease in total copper concentrations in soil as compared to the control pots. This suggests that soils treated with the application of mineral sorbents exhibit a greater ability to desorb copper from the soil in comparison to soil without amendments. In an earlier experiment conducted by Radziemska and Mazur [21], the addition of

zeolite to soil contaminated with heavy metals caused a significant decrease of copper in the soil.

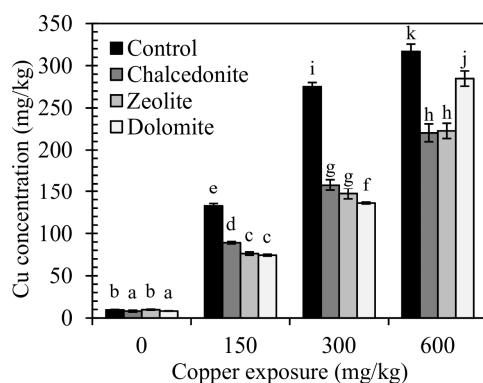


Fig. 3. Cu concentrations in soil with the different soil treatments (mean±SD, n=3). Values in columns marked with the same letter do not differ significantly (Duncan test, P>0.05)

4. CONCLUSIONS

Phytostabilization assisted by mineral sorbents was used to immobilize a copper-contaminated soil in a pot experiment. The present study clearly showed that soil aided phytostabilization techniques can be useful for alleviating the adverse effects of copper deposition in the food chain. The efficiency of phytostabilization depends on the plant species and soil amendments used. In the presented research, the greatest average above-ground biomass of *Lolium perenne* L. was observed in cases of amending soil with zeolite. Copper accumulated in the roots, thereby reducing its toxicity to the aerial parts of the tested plant. The application of dolomite to soil contaminated with copper contributed to a significant increase in pH values. The application of zeolite and chalcedonite turned out to be the most effective when it came to reducing total copper content in the soil.

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WSPOMAGANA FITOSTABILIZACJA GLEBY ZANIECZYSZCZONEJ MIEDZIĄ
Z WYKORZYSTANIEM *L. PERENNE* I SORBENTÓW MINERALNYCH
JAKO DODATKÓW

Streszczenie

Niniejsze badania zostały przeprowadzone w celu oceny procesu wspomaganej fitostabilizacji gleby zanieczyszczonej wzrastającymi dawkami miedzi przy zastosowaniu dodatków mineralnych (chalcedonit, zeolit, dolomit). Na podstawie otrzymanych wyników badań można wyjaśnić korzyści jak i potencjalne zagrożenia wynikające ze stosowania różnego rodzaju dodatków mineralnych w celu immobilizacji zanieczyszczenia gleby miedzią. Eksperyment wazonowy został przeprowadzony z wykorzystaniem rośliny testowej jaką była życica trwała (*Lolium perenne* L.) odmiany Bokser. Zawartość miedzi w poszczególnych organach rośliny (część nadziemna, korzenie) oraz w glebie określono przy wykorzystaniu metody spektrofotometrii. Koncentracja miedzi w poszczególnych organach *L. perenne* zależała od rodzaju wprowadzonego do gleby dodatku mineralnego oraz dawki miedzi. Największy przyrost biomasy zaobserwowano w przypadku dodatku do gleby chalcedonitu. W przeprowadzonym eksperymencie miedź gromadziła się głównie w korzeniach rośliny testowej. Zastosowanie dodatków mineralnych miało tendencję do zmniejszania całkowitej zawartości miedzi w glebie, w porównaniu do kontroli.

Keywords: wspomagana fitostabilizacja, zanieczyszczenie gleby metalami ciężkimi, minimalizacja ryzyka, *Lolium perenne* L.

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