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Assessment of using bentonite, dolomite, natural zeolite and manure for the immobilization of heavy metals in a contaminated soil: The Copșa Mică case study (Romania)

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ABSTRACT

The aim of study was to assess the effectiveness of organic and inorganic amendments to immobilize cadmium (Cd), lead (Pb) and zinc (Zn) in soil and reduce their availability in a contaminated soil. We present the results of a field experiment organised in the Copșa Mica area in which different types of amendments were applied: Na-bentonite, dolomite, natural zeolite and manure. The effectiveness of applied amendments was assessed using single extraction of metals from soil (using DTPA-$CaCl_2$-TEA at pH 7.3 or NH_4NO_3) and metal accumulation in plants. All treatments produced significant increases of soil pH, but the best results were observed after applications of dolomite and Na-bentonite. After two years from applications all amendments decreased the metal (Cd, Pb or Zn) availability compared to the control, but the magnitude of effects depends on each metal and amendment. The addition of dolomite and Na-bentonite reduced significantly the concentrations of Pb and Zn in plants, but this reduction is not enough to produce healthy food or fodder. The application of manure led to a significant increasing of biomass yield comparing with control, even if the extractability of metals in manure treated plots was moderate. The results demonstrate the high potential of Na-bentonite and dolomite to reduce the availability and possible toxicity of heavy metals in contaminated soils but more attention should be paid to the transitory liming effect and metal remobilizing over time.

1. Introduction

Heavy metal contamination is increasingly becoming an important threat for soils quality worldwide (Houben et al., 2013; Cui et al., 2016). Generally, contaminants may adversely affect the water - soil – plant – animal system, as well as directly and indirectly influence human health (Adriano, 2001; Guo et al., 2006; Radziemska, 2018). According to the data collected, between 2011 and 2012, by the European Soil Data Center of the European Commission (using EOINET-SOIL), the total number of identified sites contaminated by point pollution was 2.5 million. The main contaminants were mineral oil and heavy metals, and their contribution is around 60% to soil contamination (Panagos et al., 2013). Unlike organic contaminants, heavy metals in soils are not affected by microbial and chemical degradation and therefore the total concentrations and toxicity of metals persist in soils for a long period of time after their input (Koptsik, 2014; Houben et al., 2013). The human and environmental risks posed by contaminants are related not only to their total concentrations, but also to their mobility (Cui et al., 2016). Alloway (2013) mentioned that the mobility of these metals in soil depends strongly on soil characteristics such as soil pH and texture, the type and quantity of oxyhydroxides, and the organic matter, carbonate, phosphate and clay contents, which are the main soil constituents responsible for the sorption of toxic metals.

Heavy metal contamination in croplands has been a serious concern because of its high human health risk through soil-food chain transfer. The presence of heavy metals in croplands can enhance the dietary exposure through soil-plant-food chain transfer and give rise to toxic effects in humans (Bian et al., 2014).

Davari et al. (2015) referred that a series of measures have been suggested to remediate contaminated soils, including excavation, stabilization, vitrification, oxidation/reduction, membrane filtration, ion exchange, bioremediation, and phytoremediation. The technical solutions for the treatment of contaminated sites are usually costly,

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ecologically unsafe and in many cases not feasible in practice. Among the above-mentioned measures methods, in-situ immobilization is one of the key techniques used for remediation (Friesl-Hanl et al., 2009).

Because of high costs, the application of traditional remediation technologies is possible only in accidental pollution cases, on small areas. On larger areas, where land is cultivated and the contamination is historical, produced over a long period of time, it is necessary to find alternative solutions that are less expensive and more effective in reducing the negative effects of pollution. The immobilization of heavy metals is a promising in-situ alternative to the traditional remediation measures used reducing the environmental health risks posed by heavy metal soil contamination. The immobilization methods are based on the simple principle that a decrease in the concentration of the metals' mobile forms, whether through adsorption, complexation and/or precipitation reactions will render the metals less available for plant uptake, leaching to groundwater and animal and human intake (Derakhshan Nejad et al., 2018).

The addition of immobilizing amendments is a promising and suitable technique for the remediation of contaminated soils, even if it does not reduce the total content of contaminants (Burgos et al., 2010). Numerous natural materials and organic substances, waste products of agriculture and industry have been frequently used as amendments for immobilization of metals. Among the natural materials with high sorption capacity are clay minerals, oxide and hydroxides of Al, Fe and Mn, zeolites (Geebelen et al., 2002; Puschenreiter et al., 2005; Peter et al., 2011; Zhang et al., 2011; Ulmanu et al., 2011; Radziemska, 2018). Additionally, organic amendments such as manure, compost, bio-solids and bio-solids compost may effectively reduce the availability of heavy metals, due to their high contents of organic matter and associated improvement of the biochemical properties of contaminated soils (Marques et al., 2008; Katoh et al., 2016). Many studies reported that metals bioavailability may be varied under different environmental conditions and soil processes (leaching, weathering, ageing, acidification, redox reactions, etc.). This is the reason why it is very important to assess the long-term effectiveness of amendments use for heavy metals immobilization. Also, in situ immobilization strategies must take into account both the sustainability of the soil treatment and its effect in reducing the magnitude of exposure (Mench et al., 2006 quoted by Lee et al., 2011, Brown and Chaney, 2016).

Bolan et al., 2014 considered that more field studies are required to demonstrate the value of immobilizing soil amendments to remediate contaminated soils and these had to include measurements of plant-water relations and studies at the root-soil interfaces.

The main objective of our research was to assess the feasibility of soil remediation for the Copșa Mică area by means of using inorganic amendments (bentonite, zeolite and dolomite) or organic amendments (manure). The effectiveness of amendments was monitored over a two year period following treatments, assessing differences in the availability of heavy metals in control and treated soils under field conditions. The availability of metals in control and treated soil was examined using chemical extraction tests (single extraction) and metal accumulation in plants grown on these soils.

2. Materials and methods

2.1. Study site and experimental design

The study site (46°6′40.1″ N, 24°12′ 50.1″ E) is located in Sibiu County, around the most important factory for processing non-ferrous ores – Copșa Mică, Romania. The mismanagement of the non-ferrous metallurgical activities of the industrial platform, without taking in account the environmental damage, lead to a historical accumulation of heavy metals (Cd, Zn and Pb) in the soil. Data from previous studies indicate that the polluted area, assessed during 1991–1993, (21,875 ha), was very close to that reported for 2005, (22,565 ha). This difference of only 690 ha, showed a slight increase in the polluted area in the time since the year 1991 to 2005, which demonstrates a systematic reduction in the pollution intensity over time (Lăcătușu and Lăcătușu, 2010). Damian et al. (2018) mentioned that the concentrations of metals in soils from Copșa Mică exceed the maximum allowed limit for soils, according to the Romanian legislation. There were identified areas where the concentration of Pb is up to 5000 mg kg^{-1}, Zn up to 8591 mg kg^{-1}, Cu up to 199 mg kg^{-1} and of Cd to 175 mg kg^{-1}.

Pollutant emissions (with a high concentration of SO_x) from the ore processing industry have also decreased soil structural stability and soil pH. Natural sheet, rill and gully erosion processes have been intensified due to the lithological susceptibility of land to erosion.

The decrease of the industrial platform's activities triggered a large scale migration towards the rural areas surrounding Copşa Mică. Because a complex action for remediation of contaminated lands is not possible (neither practical, nor financially due to the extensive affected area), the most appropriate approach would be to change the type of land use (for biofuel cultivation, afforestation, etc.). Small landowners in the neighbouring villages still use the land for subsistence agriculture, even though it is contaminated with heavy metals.

The agricultural use of the land presents a risk for population health and also reduces the available options for soil remediation.

At the setting up the measures for reducing the effects of soil contamination at Copsa Mica, within the RECARE project, the specific conditions of the area (soil type, land use, pollution type, social aspects, etc.) were taken into account, but also the availability of landowners to apply these measures. An experimental field was set-up in 2015 in order to study the effects of amendments used for in-situ remediation of heavy metal contaminated soils. The selection of amendments was based on previous results from pot experiments which showed a high potential for reduction of metal (Cd, Cu, Zn and Pb) transfer into the food chain via crops (maize), by use of bentonite, natural zeolite and liming materials (Vrînceanu et al., 2010). When selecting the measures proposed for testing in the experimental field an important contribution was also made by the representatives of the local community from Copsa Mica, mainly landowners and land users.

The experimental field is located in the highly polluted area, 800 m away from the source of contamination. Prior to the experiment, the land in this area was being used as grassland. The main characteristics

Table 1
Main characteristics of the soil from the experimental field before application of amendments.

	pH	[a]C_{org}	N	[b]CEC	[c]$P_{available}$	[d]$K_{available}$	[e]Cd	[e]Pb	[e]Zn
		g kg^{-1}	g kg^{-1}	mmol kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil	5.17 (0.08)	10.3 (0.3)	0.94 (0.06)	131 (5)	22.1 (1.3)	102 (4)	13.7 (0.5)	359 (26)	713 (28)

Standard deviation in parenthesis (n = 4).
[a] Organic carbon.
[b] Cation exchange capacity.
[c] Available phosphorus.
[d] Available potassium.
[e] Cd, Pb and Zn contents extractable with *aqua regia*.

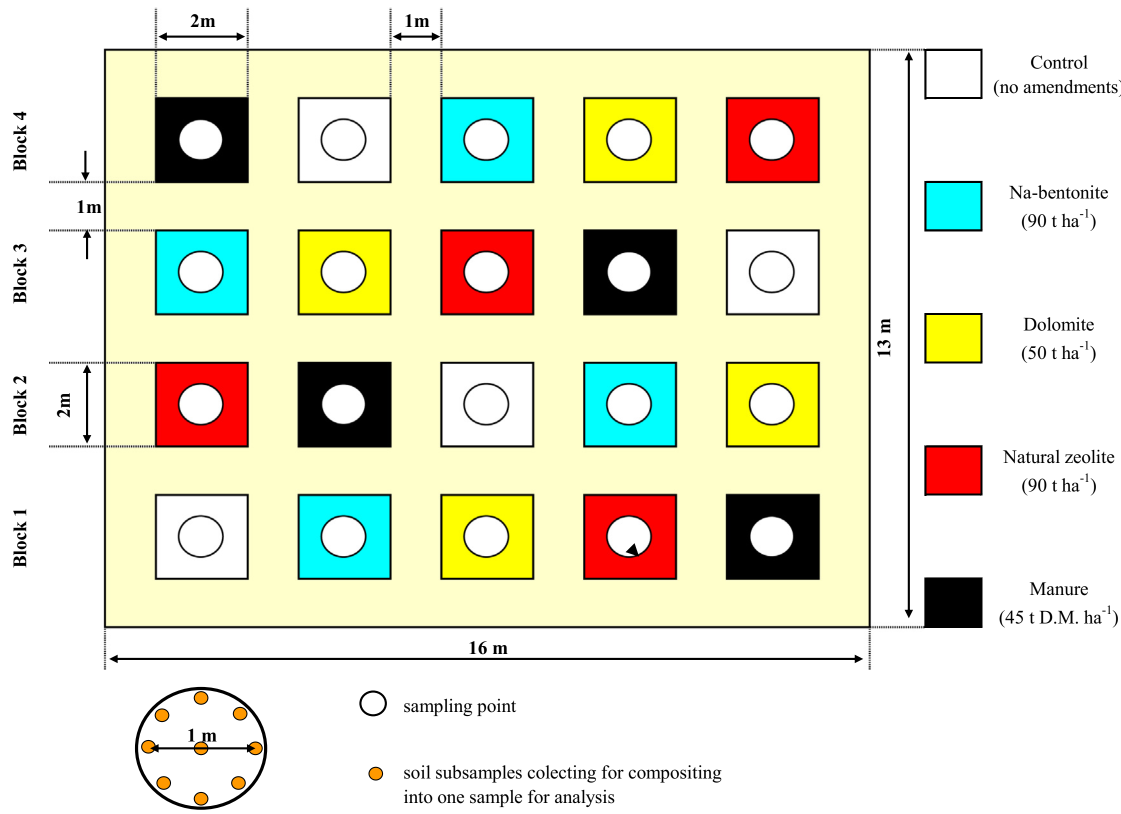

Fig. 1. Experimental layout (randomized block design) of the treatment plots in RECARE field from Copșa Mică.

of the soil from the experimental field, before application of amendments, are presented in Table 1.

The experiment was carried out in a Randomized Complete Blocks Design (RCBD) with 20 experimental plots organised in 4 blocks (Fig. 1). Four amendments were applied: bentonite, dolomite, manure and natural zeolite. The experimental field also included four control plots (without treatment). The amendments were applied only once in October 2015 and the following rates were used: 90 t ha^{-1} of Na-bentonite, 50 t ha^{-1} of dolomite, 90 t ha^{-1} natural zeolite and 45 t ha^{-1} of manure dry matter and the application rates of amendments were comparable with those used in previous studies regarding soil remediation in Copșa Mică area. Amendments were first hand-scattered over the surface and then mixed with the top soil (0–20 cm) in 2 × 2 m plots.

A mix of perennial grasses and straw cereals, belonging to the Leguminosae (*Trifolium pratense*) and Gramineae family (*Dactylis glomerata*, *Lolium perenne*, *Agropyron repens*), were sown at a ratio of 30 kg ha^{-1} in each plot.

2.2. Soil amendments

The Na-bentonite was purchased from SC Bega Minerale Industriale SA, Aghires –Gurasada branch (grain size 0.16 mm, pH 9.81, 0.073 mg kg^{-1} Cd, 20.2 mg kg^{-1} Pb and 15.1 mg kg^{-1} Zn). The dolomite (micrite dolomite lime) - double carbonate of calcium and magnesium (commercial name DEL-CA-MAG) was provided by SC Exploatarea Miniera Harghita SA from Delnita, Harghita County, Romania. The chemical characteristics indicated a highly alkaline reaction (pH 9.51) and high contents of CaO (28.2%), MgO (19.8%).

The natural zeolite was purchased from SC Zeolites Production SA, Rupea Brasov (pH 9.20, CEC 200 meq/100 g, 0.049 mg kg^{-1} Cd, 9.3 mg kg^{-1} Pb and 6.0 mg kg^{-1}Zn). The studied zeolite was composed mainly of clinoptilolite (90%).

The manure was provided by a small dairy farm from case study area (pH 8.97, 1.59 mg kg^{-1} Cd, 106 mg kg^{-1}Pb and 420 mg kg^{-1} Zn).

2.3. Sample collection

One composite soil sample (9 subsamples) was collected from each plot (see Fig. 1) on two occasions: 14 September 2016 and 15 July 2017. The soil samples were collected from a 0–20 cm soil depth. The soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to the chemical analysis. Soil samples were sieved to pass through a 0.2-mm sieve for pseudo-total concentrations of heavy metals and soil organic carbon.

Plant samples were collected in the second year at 19 July 2017. From each plot plants samples (aerial part) consisting of a mixture of plants from the plot area were collected.

2.4. Chemical analysis

Soil pH was measured using the potentiometric method (1:2.5 w/v, soil: water). The soil organic carbon content (SOC) was determined on 0.2 mm grounded soil samples using dichromate oxidation followed by titration with ferrous ammonium sulphate (Walkley and Black, 1934).

The available phosphorus and potassium in soil were extracted with ammonium acetate lactate (AL extractable) at pH 3.75 (Romanian Standard STAS 7184/19-82 based on the Egner–Riehm-Domingo method, Egner et al., 1960) and analyzed by flame photometry (for potassium content) and UV/Vis spectrometry (for phosphorus content).

The pseudo-total concentrations of Cd, Pb and Zn were determined only in the soil samples, collected before the application of treatments, by atomic absorption spectrometry, after extraction by the aqua regia – microwave digestion method. Microwave digestion was performed using 10 mL of aqua regia (7.5 mL HCl and 2.5 mL HNO_3) at 140 °C for 30 min., method developed according to SR ISO 11466:1999. A certified soil reference material (ERM–CC141) was used to ensure the accuracy of the analytical data. The average recovery values of Cd, Pb and

Zn in the reference soil were: 83.5%% for Cd, 101.6% for Pb and 106% for Zn.

A wide range of extractants, including DTPA, EDTA, NH_4NO_3, $CaCl_2$, etc., are used to assess the availability of metals in soil but there is no fully satisfactory extractant for all soil-plant system (Lin and Zhou, 2009; Madejón et al., 2018). We selected for our experiments two extractants: solution (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA-triethanolamine adjusted to pH 7.3) and NH_4NO_3 (1 M).

The chemical extraction with buffered solution of DTPA-TEA-$CaCl_2$ was initially developed to identify the available micronutrients in the soils. Simultaneously, it is being widely used in heavy metal extraction (Lindsay and Norvell, 1978) and became a standardized method (SR ISO 14870:2002). The extraction with solution of NH_4NO_3 (1 M) is frequently used to estimate the exchangeable fraction of metal in soil, an important pool regarding their toxicity, lability, and bioavailability (Usman et al., 2006).

DTPA-extractable heavy metals were extracted from soil (10 g) with 20 mL of extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA-triethanolamine adjusted to pH 7.3), according to SR ISO 14870:2002. Extracts were centrifuged at 7000 rpm, filtered on a 0.2 μm Whatman filter. Clear solutions of DTPA-extracted samples were analyzed for their heavy metal content by means of the flame AASpectrometer GBC Avanta 932AA.

NH_4NO_3-extractable heavy metals were extracted from soil (10 g) with 20 mL of extracting solution (NH_4NO_3 1 M). Extracts were centrifuged at 7000 rpm, filtered on a 0.2 μm Whatman filter. Clear solutions of NH_4NO_3-extracted samples were analyzed for their heavy metal content by means of flame AASpectrometer GBC Avanta 932AA.The plant samples were washed with tap water first and then with deionized water in order to remove soil particles. Afterwards the washed samples were dried (60 °C, 72 h), grounded and then digested with nitric acid in a microwave digestion system. The metal content was measured using atomic absorption spectrometry (Flame GBC 932AA or Graphite furnace GBC SavanatAAZ).

2.5. Statistical analysis

One-way ANOVAs were performed to assess whether significant differences of all variables existed between the treatments; Tukey's HSD test was applied to assess significant differences of all variables between individual treatments ($p < 0.05$). Data from the field experiment were analyzed using STATISTICA CSS (Complete Statistical System by StatSoft, Tulsa, OK, USA).

3. Results and discussion

In our study, the effectiveness of in situ immobilization of heavy metals was assessed using chemical extraction tests (single extraction of metals with different extracting solutions) and metal accumulation in plants. The goal of this experiment was not to remove the metals (decreasing the contamination degree), but to reduce the available fractions of metals by changing some soil characteristics (pH and cation exchange capacity).

3.1. Effects of amendments on soil pH

Soil pH is an important factor that affects the behaviour of metals in soil and affects directly and indirectly their availability and toxicity to plants (Radziemska, 2018).

The soil of the experimental field is acid, as shown in Fig. 2, the mean soil pH of the control plots was 5.18 (in 2016) and 5.47 (in 2017). Compared to the control, all treatments elevated the soil pH.

The strong alkaline reaction of -bentonite and dolomite induced a significant increase in soil pH ($p < 0.05$). Two years after amendments' application, the highest values of pH were measured in plots treated with dolomite (7.18) and bentonite (6.83). Radziemska (2018) also

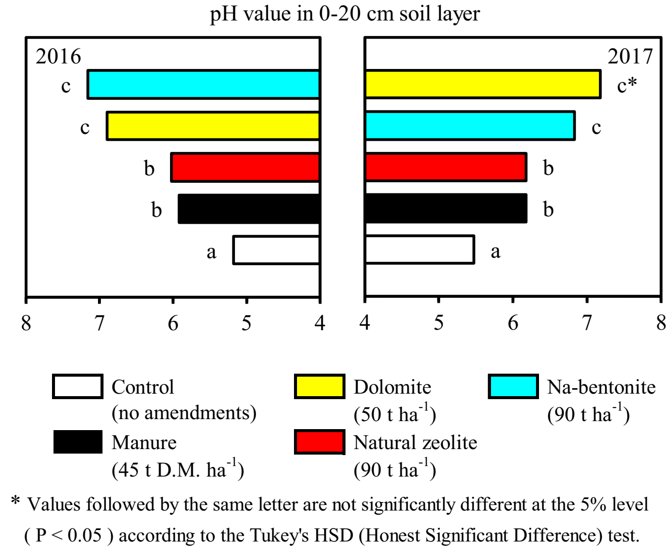

Fig. 2. Changes in soil pH in the 0–20 cm layer from non-amended control soil and amended soils.

found that the addition of dolomite increased the pH value of soil and favours the formation of oxides and carbonate precipitates, which decreased metals availability.

The pH values for all treated soils were constant in these two years of experiment, even if two years from amendments application could be considered a monitoring period too short for relevant conclusions. Lombi et al. (2003) noticed that the immobilizations effects by increasing of pH under the influence of amendments were not assured for a long time in remediation treatments. Madejón et al. (2009) reported that the effect of sugar beet lime and biosolid compost applications induced changes in pH lasted minimum 2 years after the last amendment application, under field conditions. Based on the long-term monitoring of the experimental site in Guadiamar valley, Madejón et al. (2018) noticed that the necessity for re-treatment of the contaminated soil was amendment and element dependent.

Increasing the pH is frequently used to remediate metal contaminated soils, as the majority of those elements are less mobile in alkaline conditions (Adriano, 2001; Friesl et al., 2006; Teng et al., 2015; Burgos et al., 2010). The increase of pH in the contaminated soil is going to ensure the optimal conditions for plants development and at the same time will reduce the mobility of heavy metals from the soil solution, through different processes like selective sorption or the precipitation of metals as oxides, hydroxides, carbonates and phosphates (McBride, 1987; Naidu et al., 1994; Cárcamo et al., 2012).

3.2. Effects of amendments on availability of metals in soil

All amendments caused a reduction of DTPA-extractable concentrations of Cd, Pb and Zn compared to the control, but the magnitude of effects depends on each metal and amendment (Fig. 3). In both years, the addition of 50 t ha^{-1} dolomite reduces statistically significant the DTPA-extractable Cd concentration in soil from 10.5 mg kg^{-1} Cd (control) to 8.5 mg kg^{-1} Cd (2016) and from 11.2 mg kg^{-1} Cd (control) 8.3 mg kg^{-1} Cd (in 2017). The same trend was noticed for DTPA-extractable Pb concentrations in soil. The dolomite had the best suitable effect on enhancing the Zn immobilization in soil compared with manure and natural zeolite. Compared with the control, the addition of dolomite led to a decrease of the DTPA-extractable Zn concentrations in soil by 52.2% in 2016 and 69.5% in 2017.

Some authors considered that the DTPA is most suitable for calcareous soils, as it is buffered at pH 7.3 and therefore prevents $CaCO_3$ from dissolution and release of occluded metals. According to Lindsay

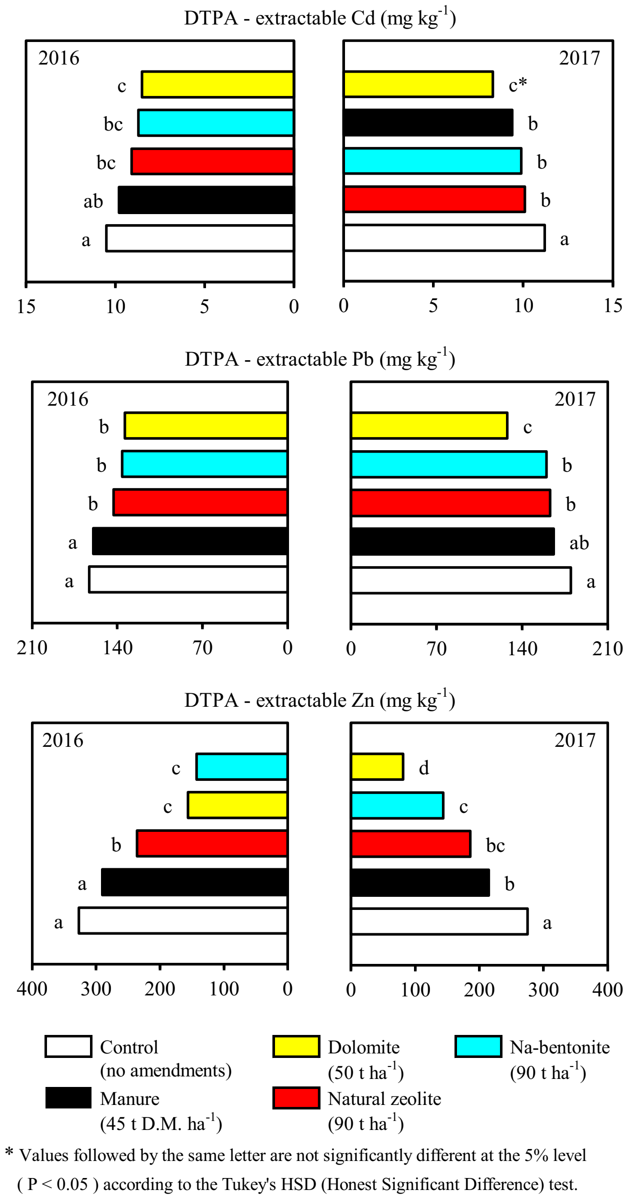

Fig. 3. Changes in Cd, Pb and Zn concentrations extracted with DTPA-$CaCl_2$-TEA buffered solution from non-amended control soil and amended soils.

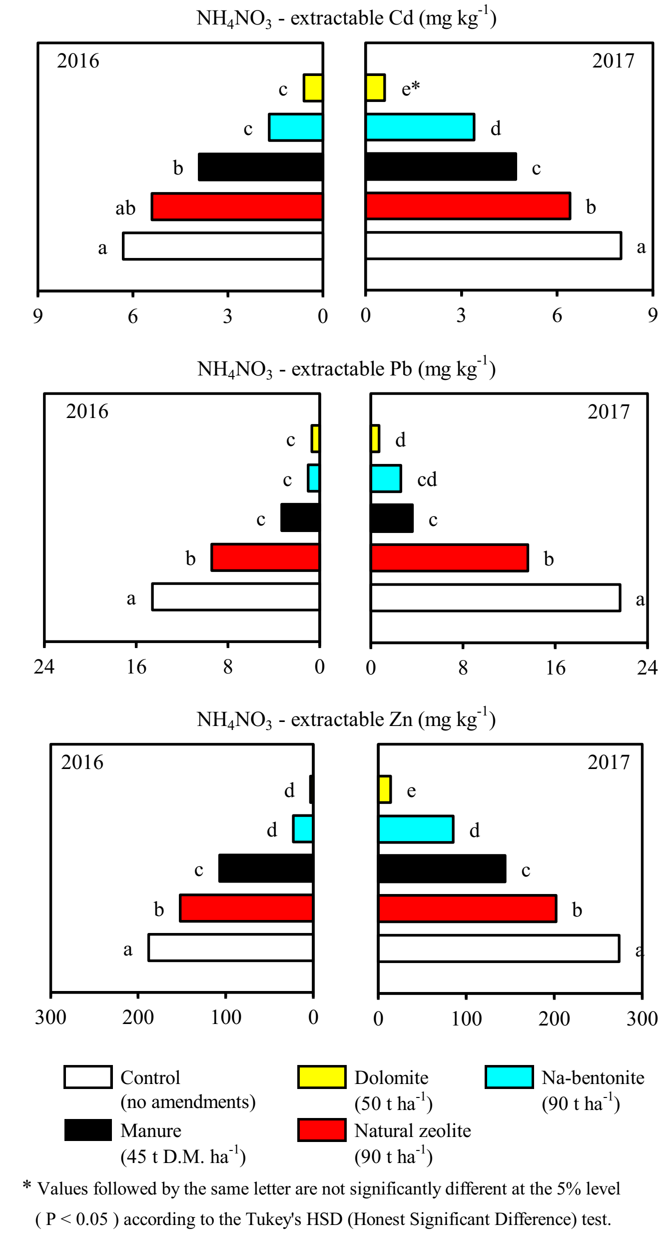

Fig. 4. Changes in Cd, Pb and Zn concentrations extracted with NH_4NO_3 (1 M) solution from non-amended control soil and amended soils.

and Norvell, 1978 quoted by Lin and Zhou (2009) the DTPA extractant contains (1) a high concentration of $CaCl_2$, of which Ca^{2+} may exchange rapidly with bivalent cations, especially Cd and Pb in the case of the acidic soil, (2) a high chloride concentration, which complexes these two elements, and (3) triethanolamine (TEA), which is protonated at pH 7.3 and could exchange H^+ with cations from the exchange sites. The results obtained from these extraction studies are useful for assessing the effectiveness of the soil treatments.

Our results indicated that the soil pH increases after applications of dolomite and -bentonite which led to a greater retention of metals in the soil solid phase. While the liming effect may be responsible for the increased metal retention of the dolomite, bentonite might fix the heavy metals both through increased pH and by long-term diffusion into clay mineral layers (Lahori et al., 2017).

After 2 years from application, the dolomite had a positive effect on immobilization of all studied metal, thus leading to a reduction in the amount of metals, easily available to plant roots, which is in agreement with the results of Trakal et al. (2011), who noticed a decreasing of the metal uptake efficiency of willow after dolomite application. The results obtained in 2017 indicated that the dolomite application led to lower content of exchangeable zinc, of 19.6 times compared with the control (274 mg kg^{-1} Zn).

Comparing with DTPA, NH_4NO_3 is a mild extractant, that can extract only the easily exchangeable metals fractions from soil and this is the reason why the concentrations of NH_4NO_3-extractable metals in soils are lower than concentrations of DTPA-extractable metals. Because the availability of metals is related to soil pH, the effects of treatments on pH are reflected on metals availability assessed by NH_4NO_3-extractable metal fractions.

As shown in Fig. 4, in both experimental years, NH_4NO_3-extractable concentrations of Cd, Pb and Zn were decreased significantly with the application of amendments and they followed the order (highest to lowest): control > natural zeolite > manure > bentonite > dolomite.

The values of exchangeable Pb content in dolomite treated soil was 30.8 times lower than values of exchangeable Pb (extracted with NH_4NO_3 1 M) content in control plots. After two years from the addition of bentonite, the Cd, Pb and Zn contents of NH_4NO_3-extractable forms in soils decreased statistically significant ($p<0.05$) from 8.0 mg kg^{-1} Cd (control) to 3.4 mg kg^{-1} Cd, from 21.6 mg kg^{-1} Pb (control) to 2.6 mg kg^{-1} Pb (treated soil) and from 274 mg kg^{-1} Zn (control) to 85 mg kg^{-1} Zn. By using the natural zeolites (90 t ha^{-1}) the values of heavy metal contents in mobile forms from soil decreased significantly compared to the control, but the decreases are lower than those noticed after treatment with bentonite and dolomite. These results are consistent with Száková et al., 2007, who observed that the lowest effect on the extractability was reported for the application of the natural zeolite, where the decrease did not exceed 30% of the extractability in the control experiment. Usman et al., 2005 reported similar results from a study regarding the effects of clay minerals on immobilization of heavy metals.

The addition of manure enhances the formation of stable complexes with organic compounds, improving the heavy metals immobilization processes in soil and leads to a decreasing of metals available amounts in soil. Due to the addition of manure, collected from a local farm, 38–41% of NH_4NO_3 - extractable Cd, 77–83% of NH_4NO_3 - extractable Pb and 43–47% of NH_4NO_3 - extractable Zn were immobilised compared to control (Fig. 4). But, some authors noticed that these amendments could not be effective in the long-term and periodic re-applications may be recommended (Madejón et al., 2009; Cui et al., 2016).

3.3. Effects of treatments on dry biomass yield and on metal concentrations in plants

The application of the amendments increased dry biomass yield, but there are no statistically significant differences between the effects of treatments compared to the control (Fig. 5.). The highest average

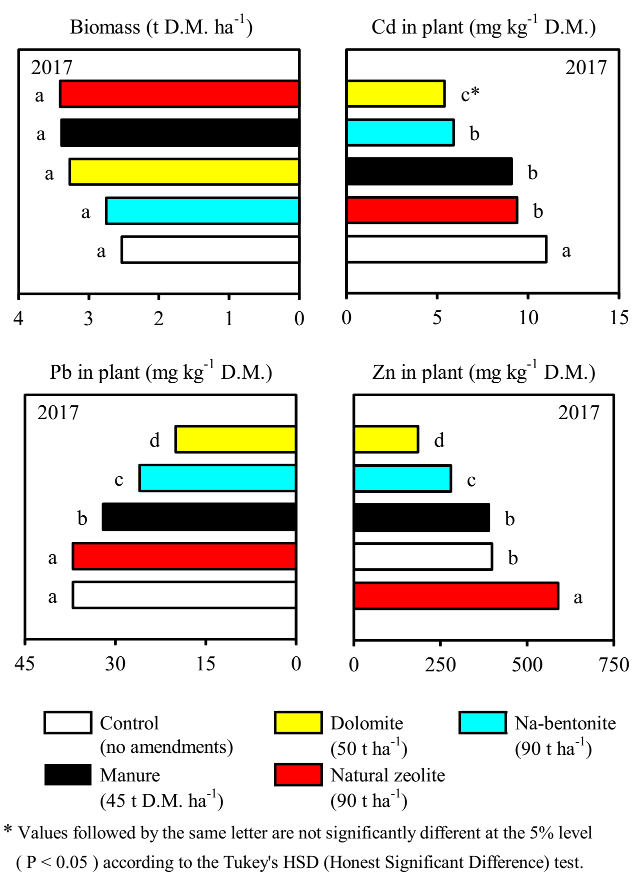

Fig. 5. Effects of immobilization amendments on dry biomass of grass and metals concentrations in above ground parts of plants grown on non-amended control soil and amended soils.

above-ground biomass was observed after the application of natural zeolite (90 t ha^{-1}) and manure (45 t ha^{-1}).

All treatments are focused on reducing the toxic effects of high level heavy metals in soils, but only additions of some amendments like manure or natural zeolite, tested for immobilization of heavy metals, improved the soil fertility with positive effects on plant growth.

Using additives like bentonite or dolomite should be carefully considered, because some authors noticed that extensive increase of pH values of soil can lead to the immobilization of essential nutrients (Puschenreiter et al., 2005).

Plant metal uptake is one of the most accepted measures of metal availability. The majority of field trials included measurements of plant metal concentrations as a means to demonstrate the effectiveness of the amendments in reducing the toxicity for plant.

The average contents of Cd, Pb and Zn in aboveground part of plants collected from the control plots were: 11.0 mg kg^{-1} DW Cd, 37 mg kg^{-1} DW Pb, 399 mg kg^{-1} DW Zn. Compared to the control, the contents of Cd, Pb and Zn decreased statistically significant ($p<0.05$) to 5.4 mg kg^{-1} DW Cd, 20 mg kg^{-1} DW Pb and 185 mg kg^{-1} DW Zn in plants grown in soil treated with dolomite (Fig. 5).

The addition of bentonite (90 t ha^{-1}) significantly reduced the plant uptake of Cd, Pb and Zn as compared with control, by 46.4% for Cd, 29.7% Pb and 29.8% Zn.

Radziemska (2018) reported that addition of dolomite, halloysite and chalcedonite significantly reduced the Pb, Cd and Zn contents in above ground parts of *F. rubra* by 25–40%, 45–58% and 2–11%. Similar results were also obtained by Lin and Zhou (2009) and Woldetsadik et al. (2016) who noticed that alkaline amendments have a high potential to reduce the availability of metals in contaminated soils.

The application of natural zeolite (90 t ha^{-1}) had no significant effects on Pb accumulation in plant tissues; moreover, it seems to enhance the uptake of Zn in plant. The highest value of zinc content (589 mg kg^{-1} DW) was observed to plant grown of soil treated with natural zeolite, which represented an increase with 47.6% of Zn content in control plants. These findings need further long-term studies to understand the behaviour of natural zeolite for the immobilization of heavy metals in field conditions.

The addition of 45 t ha^{-1} of manure dry matter, led to an increase of biomass yield by 34.8% from control. Although addition of manure had no significant effect on biomass yield, the contents of Cd and Pb in plants were significantly decreased (Fig. 5). The plant concentrations of Pb in all treatments were below the threshold for green fodder (40 mg kg^{-1}) according with Directive 2002/32/EC on undesirable substances in animal feed (European Parliament and Council, 2002).

Even if soil amendments like dolomite and bentonite significantly reduced the Cd and Zn uptake from the soil, the metal contents in plant tissues are still higher. The dolomite caused a reduction of 50% of Cd content in plant comparing with control, but Cd concentration still exceeded the threshold (1 mg kg^{-1}) for animal feed (European Parliament and Council, 2002).

4. Conclusions

The main findings of this study comparing the effectiveness of four amendments aiming to reduce the negative effects of soil contamination from a non-ferrous ore-processing plant in Romania, through the immobilization of heavy metals were the following:

(i) inorganic amendments were effective in significantly decreasing metal availability through the effects on soil pH; dolomite and bentonite have highly potential as amendments in terms of decreasing the availability of Cd, Pb and Zn;
(ii) organic amendments in the form of farm manure decreased significantly metal availability in soil, due to the binding of metals to organic matter as metal-organic complexes;
(iii) although tested amendments significantly reduced the availability

of metals, this reduction was not sufficient to produce uncontaminated food or fodder; cadmium concentration in grass still amounted to 5.4 mg kg^{-1} in the case of the most efficient amendment (dolomite).

(iv) by reducing metal toxicity and improving soil fertility, the treatments, especially natural zeolite and manure, resulted in the development of a permanent vegetation cover, even if they did not change significantly biomass yield;

(v) nevertheless, despite the promising results, the immobilization effects of tested amendments need to be further assessed in the future, especially the case of historical contaminated areas, under field conditions; more attention should be paid to the transitory liming effect and metal remobilizing over time because our results indicated that the repeated application of amendment could be essential for decreasing the environmental risks.

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