UNIVERSIDADE FEDERAL DE LAVRAS

PAULA GODINHO RIBEIRO

STRATEGIES TO IMPROVE REMEDIATION AND ENSURE FOOD SAFETY IN METAL-AFFECTED SITES

LAVRAS-MG

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Thesis presented to the Federal University of Lavras, as part of the requirements of the Graduate Program in Soil Science, area of concentration in Environmental Resources and Land Use, to obtain the title of Doctor.

Luiz Roberto Guimarães Guilherme, PhD
Advisor
Yuncong C. Li, PhD
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RESUMO GERAL

Atividades antrópicas, como descarte inadequado de águas residuárias e mineração estão entre as mais relevantes para o enriquecimento do solo com metais pesados. Para melhorar a remediação do solo e garantir a qualidade dos alimentos em áreas afetadas por metais pesados, foram realizados três estudos. O primeiro estudo avaliou como a exposição ao Zn e, ou, Cd afeta o crescimento de plantas de capim andropogon (*Andropogon gayanus* Kunth) (originalmente encontradas em áreas contaminadas com metais de mineração de zinco). As plantas foram cultivadas por sete semanas em uma solução nutritiva tratada com Zn (142,3–854,0 μM) ou Cd (0,9–13,3 μM) separadamente ou combinada com uma razão molar de 64: 1 (Zn: Cd). Um tratamento controle foi cultivado em uma solução sem Cd. Ao final do experimento, a massa seca da raiz não foi afetada pela adição dos metais e o Zn proporcionou maior massa seca da parte aérea (até 160%) em relação à testemunha. O capim andropogon tolerou melhor os dois metais separadamente do que quando aplicados juntos. O capim andropogon foi tolerante às doses testadas e tem potencial para a recuperação de áreas contaminadas por metais. O segundo estudo avaliou o potencial do HydroPotash (HYP), um novo fertilizante potássico formado a partir da alteração hidrotérmica do feldspato potássico na imobilização de metais pesados em solução aquosa. Os HydroPotashs em pH de suspensão natural removeram 63,8-99,9% de Zn, 20,6-40,7% de Cd e 68,4-99,7% de Pb da solução. O HydroPotash (HYP) também foi testado e comparado com a zeólita (produto comercial) para diminuir os teores de metal solúvel e extraível (DTPA), melhorar a fertilidade e a atividade microbiológica e promover o crescimento de plantas em solos contaminados de uma área de metalurgia de zinco. O experimento teve delineamento inteiramente casualizado, com esquema fatorial 3x4 (amenizantes x doses), utilizando três solos/substratos com diferentes níveis de contaminação ($\text{solo}_{\text{alto}}$, $\text{solo}_{\text{intermediário}}$, $\text{solo}_{\text{baixo}}$) e tratamento controle (solo sem aplicação de amenizantes). As doses testadas foram 15, 30, 60 e 120 Mg ha^{-1}. Os produtos foram incubados com umidade do solo próxima à capacidade de campo por 90 dias. O aumento das doses de HYP resultou na redução efetiva do conteúdo solúvel de Cd, Zn e Pb (até 99,9% de remoção), diminuição da extrabilidade do Cd (até 93%) e anulou o conteúdo de Al trocável. HydroPotash aumentou o pH do solo; capacidade de troca catiônica (CTC); conteúdo disponível de nutrientes; carbono da biomassa microbiana e hidrólise de diacetato de fluoresceína. Os solos foram cultivados com *Andropogon gayanus*, *Eucalyptus grandis* e *Heterocondylus vitalbae* nesta ordem. Todas as espécies testadas começaram a crescer com a aplicação de HYPs a partir das doses de 60 Mg ha^{-1} e 15 Mg ha^{-1}, respectivamente, em $\text{Solo}_{\text{alto}}$ e $\text{Solo}_{\text{intermediário}}$. A massa seca da parte aérea das plantas foi negativamente correlacionada com Cd e Zn fitodisponíveis e positivamente com pH, CTC e conteúdo de Ca. Além de promover o crescimento das plantas, o HydroPotash reduziu a absorção de metais pesados (Cd, Zn) pelas plantas, indicando que tem potencial uso como amenizante em programas de fitoestabilização. O terceiro estudo teve como objetivo avaliar o HYP como um amenizante de solo para imobilizar Cd em solo ácido e reduzir o acúmulo de Cd nos tecidos de arroz. O experimento foi conduzido em casa de vegetação com solo franco-arenoso Cecil (3 mg Cd kg^{-1}) sob dois regimes hídricos: alagamento (4 cm acima da superfície do solo) ou capacidade de campo. Dois materiais hidrotérmicos (HYP-1 e HYP-2) foram comparados com zeólita, K-feldspato + $Ca(OH)_2$ (a matéria-prima usada para produzir HYP), $Ca(OH)_2$ e controle (sem amenizante). HydroPotashs, matéria-prima e $Ca(OH)_2$ aumentaram o pH e reduziram a concentração solúvel de Cd (até 99,7%) após 30 dias de incubação no solo. Em regime de inundação, independentemente dos materiais aplicados, o crescimento das plantas foi favorecido. O HydroPotash reduziu o conteúdo de Cd na biomassa e grãos do arroz em ambos regimes hídricos testados.

Palavras-chave: Metais pesados. Amenizantes do solo. Fitorremediação. Sorção. Qualidade dos alimentos. Imobilização de metais.

GENERAL ABSTRACT

Anthropogenic activities such as inadequate disposal of wastewater and mining are among the most relevant for soil enrichment with heavy metals. To improve soil remediation and ensure food quality in areas affected by heavy metals, three studies were conducted. The first study evaluated how exposure to Zn and/or Cd affects the growth of andropogon grass (*Andropogon gayanus* Kunth) plants (originally found in metal contaminated areas from zinc mining site). Plants were cultivated for seven weeks in a nutrient solution treated with Zn (142.3–854.0 μM) or Cd (0.9–13.3 μM) separately or combined with a molar ratio of 64:1 (Zn:Cd). A control treatment was grown in a solution without Cd. At the end of the experiment, the root dry weight was not affected by the addition of the metals and Zn provided a higher shoot dry weight (up to 160%) relative to control. Andropogon grass tolerated both metals better separately than when applied together. The andropogon grass was tolerant to the doses tested, and it has the potential for recovering metal contaminated areas. The second study evaluated the potential of HydroPotash (HYP), a novel potassium-fertilizer formed from the hydrothermal alteration of K-feldspar in immobilizing heavy metals from aqueous solution. HydroPotashs at natural suspension pH removed 63.8-99.9% Zn, 20.6-40.7% Cd, and 68.4-99.7% Pb from solution. HydroPotash was also tested and compared with zeolite (commercial product) to decrease soluble and extractable (DTPA) metal contents, improve fertility, microbiological activity and promote plant growth in contaminated soils from a zinc-smelting area. The experiment had a randomized design, with a factorial 3x4 (amendments x doses) scheme, using three soils/substrates with differing levels of contamination ($\text{soil}_{\text{high}}$, $\text{soil}_{\text{intermediate}}$, soil_{low}) and control treatment (soil without amendment application). The doses tested were 15, 30, 60, and 120 Mg ha^{-1}. Products were incubated with soil moisture close to the field capacity for 90 days. Increasing HYP doses resulted in effective reduction of the soluble contents of Cd, Zn, and Pb (up to 99.9% removal), decreased Cd extractability (up to 93%), and annulled Al exchangeable content. HydroPotash increased soil pH; cation exchange capacity (CEC); nutrients available content; microbial biomass carbon and fluorescein diacetate hydrolysis. The soils were cultivated with *Andropogon gayanus*, *Eucalyptus grandis*, and *Heterocondylus vitalbae* in this order. All species tested started growing with HYPs application from dose 60 Mg ha^{-1} and 15 Mg ha^{-1}, respectively, in $\text{Soil}_{\text{high}}$ and $\text{Soil}_{\text{intermediate}}$. Plant shoot dry mass was negatively correlated with bioavailable Cd and Zn and positively with pH, CEC, and Ca content. Besides promoting plant growth, HYP reduced heavy metals (Cd, Zn) absorption by plants indicating that it has potential use as an amendment in phytostabilization programs. The third study aimed to evaluate the HYP as a soil amendment to immobilize Cd in acidic soil and reduce the accumulation of Cd in rice tissues. The experiment was carried out in a greenhouse with a Cecil sandy loam soil (3 mg Cd kg^{-1}) under two soil water regimes: flooding (4 cm above the soil surface) or field capacity. Two hydrothermal materials (HYP-1 and HYP-2) were compared with zeolite, K-feldspar + $Ca(OH)_2$ (the raw material used for producing HYP), $Ca(OH)_2$, and control (without amendment). HydroPotashs, raw material, and $Ca(OH)_2$ increased pH and reduced soluble concentration of Cd (up to 99.7%) after 30 days of soil incubation. Under the flooded regime, regardless of the materials applied, plant growth was favored. HydroPotash reduced Cd content in rice biomass and grains in both tested water regimes.

Keywords: Heavy metals. Soil amendments. Phytoremediation. Sorption. Food quality. Metal immobilization

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

Anthropogenic activities such as inadequate disposal of wastewater, application of pesticides and fertilizers, as well as mining are among the most relevant for soil enrichment with heavy metals (ĆWIELĄG-DRABEK et al., 2020; QIN et al., 2021; WUANA; OKIEIMEN, 2011). The contamination of areas nearby mining facilities by heavy metals has been extensively documented (KARACA; CAMESELLE; REDDY, 2018). These are the cases of contamination in the cities of Norilsk (Russia), Kabwe (Zambia), and Kalimantan (Indonesia) that make them some of the most contaminated places on the planet (BLACKSMITH INSTITUTE, 2013). In Brazil, there are also regions with anomalous values of metals resulting from these activities. High concentrations of trace elements in water and sediment have been reported, for example, in Adrianópolis-PR (MELO et al., 2012), Três Marias-MG (TRINDADE et al., 2012) and Sepetiba Bay-RJ (RIBEIRO et al., 2015).

Cadmium (Cd) and lead (Pb) are examples of elements associated with sulfide ores of zinc (Zn) that represent a primary environmental concern (ĆWIELĄG-DRABEK et al., 2020). These metals have no known biological functions and, at moderate/high concentrations, may be toxic to plants, animals, and humans (QIN et al., 2021). The absorption and accumulation of these elements by cultivated plants can result in a risk to human health (RAI et al., 2019). Chronic oral exposure to high levels of these metals can cause, among other effects, damage to the nervous, digestive, and respiratory systems (ATSDR, 2012). The elements Pb and Cd occupy, respectively, the 2nd and 7th positions in the Agency for Toxic Substances & Disease Registry (ATSDR, 2019) Priority List. This list is based on the combination of frequency, toxicity, and potential for human exposure to substances.

In plants, Cd and Pb are shown as non-essential elements and, when in high concentrations, can cause growth inhibition and death (PRASAD, 2004). Plants exposed to Cd show decreased shoot and root growth, as well as chlorosis and photosynthesis suppression (LIN; AARTS, 2012; TRAN; POPOVA, 2013). This element affects plant development by causing reduced chlorophyll content, altered enzyme activity, altered nutrient absorption, respiration inhibition, and oxidative stress (CUI et al., 2013; GILL; KHAN; TUTEJA, 2012; RIAZ et al., 2014). Lead also promotes nutritional disorders and affects plant growth (PRASAD, 2004). This element inhibits respiration and photosynthesis processes and promotes oxidative stress (KABATA-PENDIAS, 2011; POURRUT et al., 2011). Although Zn is a plant nutrient, high levels of this element cause decreased root growth and affect nutrient uptake by the plant, which later presents nutritional deficiency symptoms such as necrosis, chlorosis, and leaf atrophy (HAFEEZ; KHANIF; SALEEM, 2013; ROMEO et al., 2014). Interactions on metal absorption/translocation have been reported in the literature. For example, Pb stimulates Cd's absorption by plants but is antagonistic to Zn in the translocation of roots to the shoots (KABATA-PENDIAS, 2011).

Environmental laws regulate tolerable levels of metals in the environment to contain the contamination, maintain food security, and ensure the functionality of the ecosystem and natural resources for future generations (CONAMA, 2009). In the Brazilian legislation, soil quality indicator values are regulated by Resolution 420 (CONAMA, 2009) and are used to guide the recovery and maintenance of its essential functions. In this resolution, more specifically for the state of Minas Gerais, reference (natural soil) and prevention levels (i.e., levels that maintain essential soil functions) for Cd, Pb, and Zn are respectively 0.4, 19.5, and 46.5; and 1.3, 72, and 300 mg kg^{-1} (CONAMA, 2009; COPAM, 2011). When the soil has elemental concentrations higher than the prevention values, it is considered contaminated (CONAMA, 2009).

For contaminated areas, several remediation, restoration, and rehabilitation techniques are frequently used. By rehabilitation, it is meant the use of techniques to return the area to an appropriate biological state, whereas restoration means operating to ensure the conditions of the area as close as possible to those prevailing before degradation (GASTAUER et al., 2019). Once the reclamation approach has been chosen, it must subsequently select the most appropriate technique, which is made observing the types and concentrations of contaminants present in the area, site characteristics, remediation cost, time, and public acceptance (LIU et al., 2018).

Phytoremediation, which is the use of plants and their associated microbiota in soil decontamination, is a promising technique, especially in Brazil due to the diversity of species and favorable climatic conditions (ANDRADE et al., 2010; MARQUES, 2011). In addition to the low cost, applicable to a wide variety of contaminants, and with good public acceptance, it is applicable on a large scale and aesthetically pleasing (LIU et al., 2018; SARWAR et al., 2017; YAN et al., 2020).

Several phytoremediation techniques are available such as phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation (CUNDI et al, 2016; YAN et al., 2020). Phytoextraction and phytostabilization are based, respectively, on the accumulation of metals in the aerial part of plants and on the immobilization of these elements in the rhizosphere and roots (MAHAR et al., 2016; WONG, 2003). The choice of species used for contaminated soil remediation must therefore be made, considering the purpose of the remediation and the technique employed. In the case of phytoextraction, hyperaccumulating species of metals should be used and in phytostabilization, the ideal is to use plants that retain large amounts of metals in the root system (YAN et al., 2020). In general, fast-growing plants that have a large production of biomass, or species that associate with microorganisms are interesting in containing erosion and stabilizing the site (CHEN et al., 2015; LIU et al., 2017; YAN et al., 2020). In this sense, the use of nitrogen-fixing bacteria and mycorrhizae that improve plant nutrient uptake and modify contaminant availability is especially relevant (KUMAR et al. 2018; SARWAR et al., 2017).

The application of amendments can also improve the phytoremediation process. Soil amendments such as chelating agents (EDTA, DTPA, and citric acid) and organic waste can increase metals' availability to plants (BOLAN et al., 2014; NG et al., 2016) improving phytoextraction. On the other hand, other products can improve the phytostabilization efficiency by immobilizing metals by processes of adsorption, precipitation, and complexation (FORJÁN et al., 2016; FRESNO et al., 2018; YAN et al., 2020). Common amendments used for immobilizing heavy metals include biochar, (BEESLEY et al., 2011; FELLET et al., 2011; PUGA et al., 2015) and silicate minerals such as zeolites (YUNA, 2016).

Another product that has been developed from silicate rocks and with a high potential for use in contaminated areas due to its high cation exchange capacity and buffering pH is hydrothermally-altered feldspar (HydroPotash, HYP) (RIBEIRO et al., 2021). This fertilizer is created by hydrothermal alteration of potassium feldspars mixed with $Ca(OH)_2$ (CICERI et al., 2017a, b). The potassium feldspar has been considered an ineffective source of macronutrients due to its slow dissolution rate (CICERI et al., 2017a). Nevertheless, after hydrothermal alteration, an increased K and other nutrients/beneficial elements such as Si and Ca are observed compared to the syenite, a mineral with a high feldspar concentration and average potassium content of ~ 13% K_2O (CICERI et al., 2017a).

HydroPotash appears as an alternative to the usual potassium fertilizer - KCl and it is considered an environmentally friendly technology - manufactured according to the principles of green chemistry (CICERI et al., 2017a). Tests with HydroPotash as a fertilizer have been carried out, but knowledge of the potential use of this product as an amendment in contaminated areas is still incipient. Therefore, laboratory tests and practical studies should be conducted to evaluate metal sorption capabilities of HydroPotash, clarify its effects in soil fertility and plant growth, as well as its role in the minimization of metal accumulation in aboveground parts and grains of plants cultivated in metal-affected soils.

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SECOND PART – MANUSCRIPTS

MANUSCRIPT 1 Interactions of cadmium and zinc in high zinc tolerant native species *Andropogon gayanus* cultivated in hydroponics: growth endpoints, metal bioaccumulation, and ultrastructural analysis

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Abstract

Cadmium (Cd) and zinc (Zn) toxicity causes physiological disorders and harms plants, interfering with the rehabilitation of areas affected by mining activities. This study evaluated how the exposure to Zn and/or Cd affects the growth of native andropogon grass (*Andropogon gayanus* Kunth) plants originally found in areas contaminated with Cd and/or Zn due to zinc mining activities. Plants were cultivated for seven weeks in a nutrient solution treated with Zn (142.3 – 854.0 µM) or Cd (0.9 – 13.3 µM) separately or combined with a molar ratio of 64:1 (Zn:Cd). A control treatment was grown in a complete Hoagland and Arnon solution (without Cd). Plant height, stem diameter, internode length, dry weight, Cd and Zn concentration and accumulation in shoots/roots, as well as ultrastructure of roots and leaves were analyzed at the end of the experiment. The root dry weight was not significantly affected by the addition of the metals. Moreover, Zn provided higher shoot dry weight (up to 160%) relative to control. Andropogon grass tolerated both metals better separately than when applied together. Transmission electron microscopy analyses showed modifications such as vesiculation and vacuolation in the ultrastructure of andropogon tissues by Cd and/or Zn. The andropogon grass was tolerant to the doses tested, evidencing that it has potential for recovering areas contaminated with Zn and/or Cd.

Keywords: trace elements; mining; phytoremediation; contamination; andropogon grass; hydroponic media

1. Introduction

Mining activities are a basis for economic and industrial development. However, they can generate residues potentially toxic to the environment and to human health, especially in open pit areas (Favas et al. 2011; Martins et al. 2018; Sun et al. 2010; Vareda et al. 2019). Among the pollutants generated, heavy metals (e.g., Zn and Cd) are important soil and groundwater contaminants that may persist in the environment and even enter the food chain (Ali and Khan 2019; Bešter et al. 2013; Huang et al. 2014; Li et al. 2014; Schaider et al. 2014). Cadmium (Cd) intake from contaminated food can affect human health and cause kidney dysfunction, bone problems and cancer (Åkesson et al. 2014; Bishak et al. 2015; Watanabe et al. 2020). Cadmium is a metal found naturally associated with sulfide ores of zinc (Zn), copper, and lead. Due to these associations, mining, especially for Zn, is an important source of this element to the environment (Anju and Banerjee 2011; Ono et al. 2016; Zhang et al. 2012). The main natural source of Zn is the weathering of rocks containing this element. Anthropogenic Zn sources include mining, the use of fossil fuels, inadequate disposal and incineration of waste as well the application of fertilizers (Sindern et al. 2016; Tian et al. 2015). Although Zn is a micronutrient for plants, when in excess, it can become toxic (Alloway 2008). Zinc absorption by plant tissues may be synergistic or antagonistic to that of Cd (Tkalec et al. 2014). When synergistic, this interaction may impair plant development, since the absorbed Cd is not a nutrient, and may be toxic for plants (Asgher et al. 2015).

A powerful technique to observe the effects of potentially toxic metals in plants is transmission electron microscopy (TEM). At the subcellular level, tolerant plants may show accumulation, complexation, and inactivation of metals, while alterations in organelles may indicate increased oxidative stress (Arena et al. 2017; Chang et al. 2020; Chi et al. 2019; Sorrentino et al. 2018).

Due to growing concern about soil quality, much has been discussed about methods for the rehabilitation of contaminated areas (Asad et al. 2019; Bolan et al. 2014; Liu et al. 2018; Mahar et al. 2015; Xiong et al. 2015; Wang et al. 2018; Wiszniewka et al. 2016; Yao et al. 2012). In this sense, phytoremediation stands out as an ecological option with relatively low cost (Ali et al. 2013; Wan et al. 2016). At the beginning of a recovery program based on the revegetation of a contaminated area, it is necessary to select plants tolerant to the contaminants present in the area, and these plants must have the capacity to grow and reproduce in adverse conditions (Gutiérrez et al. 2016; Mingorance et al. 2016). Grasses have great potential, as they present rapid growth, adapt to different soil and climatic conditions, and are appropriate for soil protection (Mahar et al. 2016; Rabêlo and Borgo 2016). Andropogon grass (*Andropogon gayanus* Kunth) is a tall forage grass with high biomass that is native in many Zn mining areas. These characteristics, associated with drought tolerance and good development in acidic and low fertility soils, make it very suitable for the reclamation of many degraded areas (Sousa et al. 2010). Moreover, there is evidence for the potential of this species for the phytoremediation of Cd (Ibigbami et al. 2014) and Zn (Umeoguaju et al. 2016) contaminated areas.

Because contamination in mining environments is most often multielementary, assessing the phytoremediation potential for grasses based on monoelementary systems is not representative of the conditions found in the field. Moreover, it is known that the absorption and subsequent accumulation of Zn in plants are correlated with Cd (Bert et al. 2003). Therefore, the objective of this work was to evaluate the growth of andropogon grasses, autochthonous of zinc mining areas, exposed to both single and combined Cd and Zn contamination in hydroponic media, in a scenario characterized by a higher availability of metals to plants. Thus, we aim at elucidating the metal accumulation capacity of andropogon grass and its limits, as well as the interaction of Zn and Cd in this plant species with potential for recovering abandoned mines.

2. Material and Methods

2.1. Greenhouse Experiment

The experiment was conducted in greenhouse conditions, with natural light and an average temperature of 25 ± 3°C, between May and July. Andropogon grass seeds were collected in an old Zn mining pit in the municipality of Vazante, Minas Gerais, Brazil, one of the major known nonsulfide zinc deposits in the world, where these plants grow naturally (Fig. 1). Seeds were first placed to germinate in commercial substratum, and after 18 days, plants with similar size and shape were selected for further experiments. Seedlings were washed in distilled water and transferred to pots containing 30%, 50%, and 100% Hoagland and Arnon (1950) solutions for acclimatization (each concentration for one week). After the acclimatization period, plants were transferred to 3.5 L polyethylene pots (three plants per pot) containing a complete Hoagland and Arnon solution. Treatments consisted of nutrient solutions with: i) increasing Zn concentrations of 56.9, 142.3, 284.7, 569.3, and 854.0 μM, simulating varying degrees of Zn contamination; ii) increasing Cd concentrations of 0.9, 2.2, 4.4, 8.9, and 13.3 μM, simulating varying degrees of Cd contamination; and, iii) a combination of both Zn and Cd contamination at different concentrations (Zn+Cd) 56.9+0.9, 142.3+2.2, 284.7+4.4, 569.3+8.9, 854.0+13.3 μM, simulating a multielementary contamination with Zn and Cd. These elements were applied as sulphate hydrate forms, $ZnSO_4 \cdot 7H_2O$ and $CdSO_4 \cdot 8H_2O$, respectively for Zn and Cd. The concentrations of Zn and Cd were chosen to be in the range of bioavailable concentrations at the site of plant collection, based on preliminary studies (Carvalho 2010; Carvalho et al. 2013). The control was cultivated only in a complete solution of Hoagland and Arnon (1950) (without Cd and a minimum of 0.76 µM Zn). All single-Cd treatments received the same amount of Zn (0.76 µM Zn), because this element is a nutrient and plants need a minimal amount of Zn to grow without symptoms of Zn deficiency, which could be confused with those of Cd toxicity. Each treatment had four replicates, totaling 64 pots. The nutrient solutions with their respective Cd and Zn doses were renewed every 15 days and the solution volume was controlled daily by the addition of distilled water. The experiment was conducted under constant aeration.

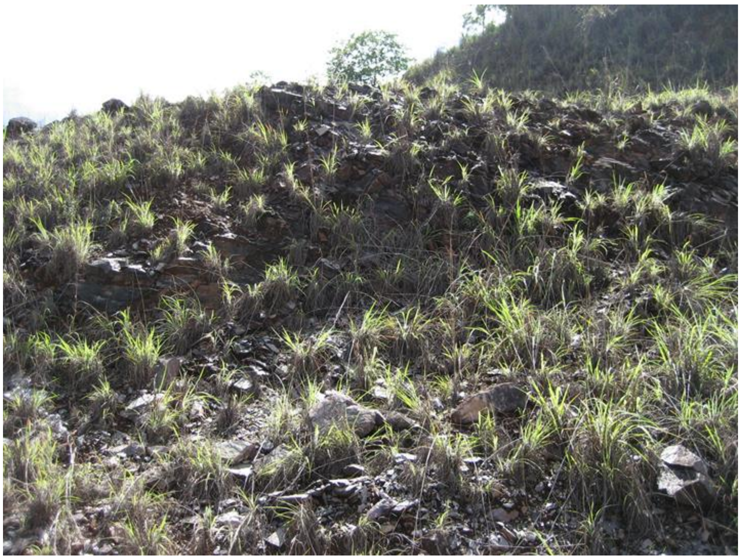

Fig. 1 Andropogon grass growing in a zinc mining area in Vazante, Minas Gerais, Brazil

After seven weeks of exposure, the following endpoints were evaluated: shoot height (from the base of the roots to the highest leaf), stem diameter measured in the middle portion between the first leaf and the base of the roots, and the internode length between the two older leaves. The plants were then removed and washed with distilled water before the separation between the shoot and the root system. The material was placed in an air circulation oven at 65ºC until constant mass, then weighed to obtain the shoot dry weight (SDW) and root dry weight (RDW).

2.2. Determination of Cd and Zn

Shoot and root samples were digested in a microwave oven (MARS-5®, CEM) according to the United States Environmental Protection Agency-USEPA (2007) method 3051-A. Cadmium and Zn concentrations were determined by atomic absorption spectrophotometry, using a Perkin Elmer AAnalyst 800® equipment with flame and graphite furnace atomizers. The control and quality assurance of the Cd and Zn analyses were assessed by using the reference material “Tomato leaves” (SRM 1573a, National Institute of Standards and Technology-NIST). The Cd and Zn recovery was 80 and 104%, respectively. The metal concentrations and accumulation refer to plant dry weight.

2.3. Calculation of Cd and Zn accumulation indices

Bioaccumulation coefficients were calculated in order to assess metal tolerance and the potential use of this species for phytoremediation. The following indices were calculated:

translocation factor (TF) (ratio between the concentration of Cd and Zn in andropogon grass shoots and in roots) and the bioaccumulation coefficient for shoots (BAC_S) and for roots (BAC_R) (ratio between the concentration of Cd and Zn in andropogon grass shoots and roots, respectively, and the concentration of Cd and Zn in hydroponics solution) (Visconti et al. 2019; Zhang et al. 2020).

2.4. Ultrastructure Analysis

For TEM analysis, plant tissues of all replicates from control as well as for the lowest and highest doses of the treatments with Cd, Zn and Cd+Zn were analyzed. For this purpose, 0.5 cm^2 andropogon grass leaves and roots fragments were collected. These fragments were immersed in a modified Karnovsky fixing solution [2.5% glutaraldehyde and 2.5% paraformaldehyde] in 0.05 M cacodylate buffer (pH 7.2) + 0.001 M $CaCl_2$ buffer. Samples were cut into 1 mm^2 fragments, washed in phosphate buffer solution three times for 10 minutes and post-fixed in 1% osmium tetroxide in 0.05 M cacodylate buffer for 1-2 hours. They were then washed three times in distilled water and then transferred to a 0.5% uranyl acetate solution for 12 hours at 4°C. After this period, they were again washed in distilled water three times and dehydrated in an acetone gradient (25, 30, 40, 50, 70, 80, 85, 90, and 95% for 10 min followed by three periods of 10 min in 100% acetone). The material was placed in a gradient of acetone and *Spurr* resin 30% for 8 hours, 70% for 12 hours and 100% twice for 24 hours each, and polymerized in an oven at 70°C for 8 hours. After these procedures, the blocks went through the thinning process to remove the excess resin and then ultra-thin sections were obtained with a Leica MT-Ultracut ultra-microtome equipped with a diamond knife. The sections were collected on a copper grid and contrasted in 2% uranyl acetate, followed by 0.2% lead citrate for three minutes each, and, after drying, examined by a Zeiss EM 109 transmission electron microscope (MET) 80 kV.

2.5. Statistical Analyses

The following variables were submitted to analysis of variance (ANOVA) followed by the Dunnett´s test at 5% probability level: Cd and Zn content and accumulation in roots and shoots; shoot and root dry matter; internode length; plant height; and stem diameter. All statistical analyses were carried out using R 3.4.3 (R Development Core Team, 2017). The ANOVA models were fitted using generalized least squares because of the observed heterogeneity of residual variance among treatments (Zuur et al. 2010; Cleasby and Nakagawa

2011). During the exploratory analysis of the data to identify possible statistical issues, as recommended by Zuur et al. (2010), we identified one and three outliers (out of 64 observations) for data on Cd and Zn concentration/accumulation in the roots, respectively. They were removed from the models presented in Figs. 3 and 4, but the original version of the analysis including the outliers is presented in Figures S1 and S2. We compared BAC and TF values using Tukey´s test $p < 0.05$ to observe differences between single-contaminated and combined systems.

3. Results

3.1. Growth parameters

Zinc doses significantly increased andropogon grass height in comparison to the control (up to 22%) except for the dose of 854.0 μM, for which a non-significant effect was observed (Table 1).

For the treatments receiving only Cd, a negative effect on growth was observed only for the highest dose of this element (13.3 μM), with a 55% reduction in growth as compared with the control. In the treatments receiving both Cd and Zn, significant positive effects on SDW were observed for the treatments Zn (56.9) + Cd (0.9) and Zn (142.3) + Cd (2.2). On the other hand, at higher combined doses of Zn and Cd, the effect on shoot dry weight was either null [Zn (284.7) + Cd (4.4)] or negative [Zn (569.3) + Cd (8.9) and Zn (854.0) + Cd (13.3)] (Fig. 2). For plant height, the treatments with combined doses of Zn (284.7) and Cd (4.4) or higher showed significant negative effects on plant height (Table 1). These results indicate that in the presence of Cd, the beneficial effects obtained by increasing the Zn contents were canceled, and the harmful effects of Cd increased. When Cd was applied isolated, 8.9 μM resulted in negative effects on plant growth, whereas when applied with Zn, the dose of 4.4 μM of Cd (~50% less Cd) already resulted in deleterious effects on plants. The individual doses of 0.9 μM of Cd and 569.3 μM of Zn promoted an increase (up to 40%) in stem diameter. For the other treatments, no differences were observed in relation to the control treatment.

Table 1 Growth parameters of Andropogon grass grown in nutrient solution containing different single and combined cadmium and zinc doses. Mean values and standard errors are reported (n = 4).

Treatment	Internode Length	Plant Height	Stem Diam.
μM	cm	m	mm

Control	8.38 (2.66)	1.35 (0.05)	5.11 (0.52)
Zn (56.9) + Cd (0.9)	12.12 (5.57)	1.51 (0.13)	6.75 (1.01)
Zn (56.9)	8.62 (4.27)	1.55 (0.13)*	6.32 (1.55)
Cd (0.9)	3.38 (3.25)	1.54 (0.09)	7.20 (1.33)*
Zn (142.3) + Cd (2.2)	7.25 (2.25)	1.30 (0.13)	5.48 (0.95)
Zn (142.3)	8.75 (2.10)	1.56 (0.14)*	6.11 (0.73)
Cd (2.2)	7.88 (6.64)	1.38 (0.20)	6.61 (1.60)
Zn (284.7) + Cd (4.4)	3.88 (1.44)*	0.92 (0.04)***	4.86 (0.54)
Zn (284.7)	9.00 (5.12)	1.65 (0.13)**	6.57 (1.25)
Cd (4.4)	6.00 (1.29)	1.33 (0.16)	6.88 (0.62)
Zn (569.3) + Cd (8.9)	4.75 (0.96)*	0.77 (0.12)***	4.12 (1.08)
Zn (569.3)	12.75 (4.21)	1.56 (0.17)*	7.16 (0.94)*
Cd (8.9)	4.38 (1.75)*	1.09 (0.14)*	5.01 (0.60)
Zn (854.0) + Cd (13.3)	3.38 (1.80)*	0.65 (0.11)***	3.64 (0.99)
Zn (854.0)	4.88 (1.89)	1.22 (0.05)	5.21 (0.85)
Cd (13.3)	3.75 (1.76)*	0.92 (0.09)***	4.38 (0.95)

Asterisks indicate the significance of the difference between each treatment and the control by the Dunnet's test. * P <0.05; ** P < 0.01; *** P <0.001.

There were no positive effects of treatments on internode length. Reductions were observed for the two higher doses of Cd and for the combined application of Zn (284.7) + Cd (4.4). Similarly, for plant height, the deleterious effects of the combined treatments occurred at lower Cd doses than those with Cd only.

Treatments of Zn alone at 56.9, 142.3, 284.7, 569.3 µM provided higher SDW (up to 160%) relative to the control (0.8 µM Zn). When applied alone, Cd at the doses 0.9, 2.2, and 4.4 µM increased the SDW up to 110% (Fig. 2). In contrast, the 8.9 µM Cd dose had no significant effect relative to the control treatment, and the 13.3 µM dose reduced SDW of approximately 30% (Fig. 2).

For the combined doses, the first two [Zn (56.9) + Cd (0.9) and Zn (142.3) + Cd (2.2)] showed beneficial effects and promoted an increase in plants SDW of 180% and 120%, respectively. However, SDW was reduced (~30%) in the two higher combined doses when compared with the control treatment. Shoot dry weight responded to treatments in a similar way to that observed for other endpoints, with the effect of Cd being more intense when accompanied by Zn in relation to the application of this element individually.

Root development and growth of andropogon grass was not affected by the treatments (Fig. 2).

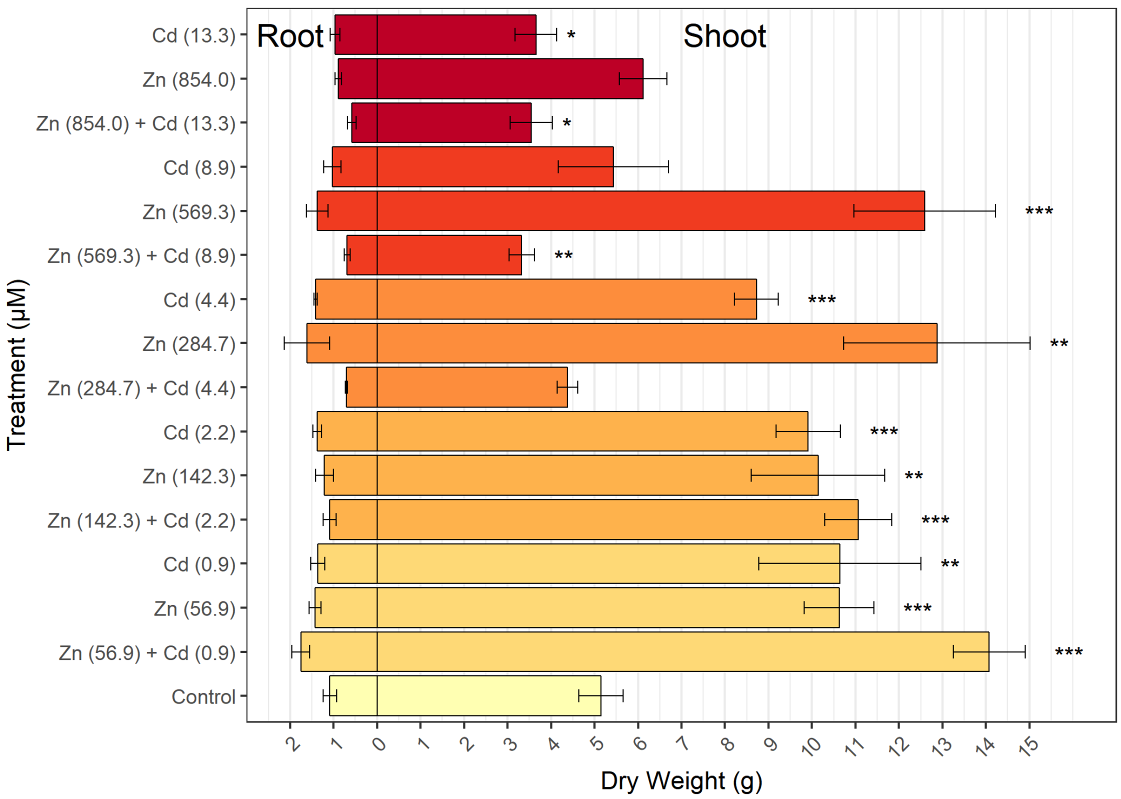

Fig. 2 Effect of Cd and Zn on the dry weight of roots and shoots of andropogon grass. The asterisks next to the bars indicate the significance of the difference between the control and the treatment by Dunnet's test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Mean values and standard errors are shown (n = 4).

3.2. Metal concentrations and accumulation indices

After seven weeks of cultivation, the plants were exposed to a total amount of Cd and Zn, according to the doses applied, as follows: 1.4, 3.5, 7, 12 and 21 mg of Cd and 52.08, 130.34, 260.54, 521.08, 781.76 mg of Zn alone or combined with a molar ratio of 64:1 (Zn:Cd). The total amount was provided with four complete changes of the nutrient solution. The total concentrations of Cd and Zn were higher in roots compared to shoots for all treatments (Fig. 3 and Table S1). Therefore, andropogon grass showed a TF smaller than 1 for both metals (Table 2). The translocation of Cd and Zn to plant shoot reduced when the metals were combined in the doses (4.4 + 284.7 µM) and (8.9 + 569.3 µM). The TF was twice higher for Cd and from 6.5 to ten-fold higher for Zn, in the treatments (4.4 + 284.7 µM) and (8.9 + 569.3 µM), respectively. Cadmium contents in andropogon grass plants increased with increasing the concentration of this element in the solution. A higher content of this element in andropogon grass shoots was observed when it was supplied combined with Zn, except for the 0.9 µM single Cd dose. This is indicated by the high BAC_S values for this element observed in the combined treatments, except for this first dose. No differences were observed for BAC_R between single and combined Cd treatments. The Cd highest concentration was 1083 mg kg^{-1} in the roots and 76.7 mg kg^{-1} in the shoots of the grass at the combined dose Zn (854.0) + Cd (13.3) µM, which represents a total metal stress of 21 mg Cd and 781.76 mg Zn at the end of 45 days of metal exposure.

Similarly, to the treatments with Cd, the addition of Zn in the solution increased the concentration of this element in the roots of andropogon grass (Fig. 3). The Zn concentration in the roots and shoots was higher in all the treatments with Zn alone than in the control group. The BAC_R was higher for the single Zn doses of 56.9, 284.7 and 569.3 µM, but no differences in BACs were observed between single and combined treatments ($p < 0.05$). The Zn concentration was 18,779 mg kg^{-1} in the roots and 2,097 mg kg^{-1} in the shoots of plants grown with single Zn doses of 569.3 and 854.0 µM, respectively.

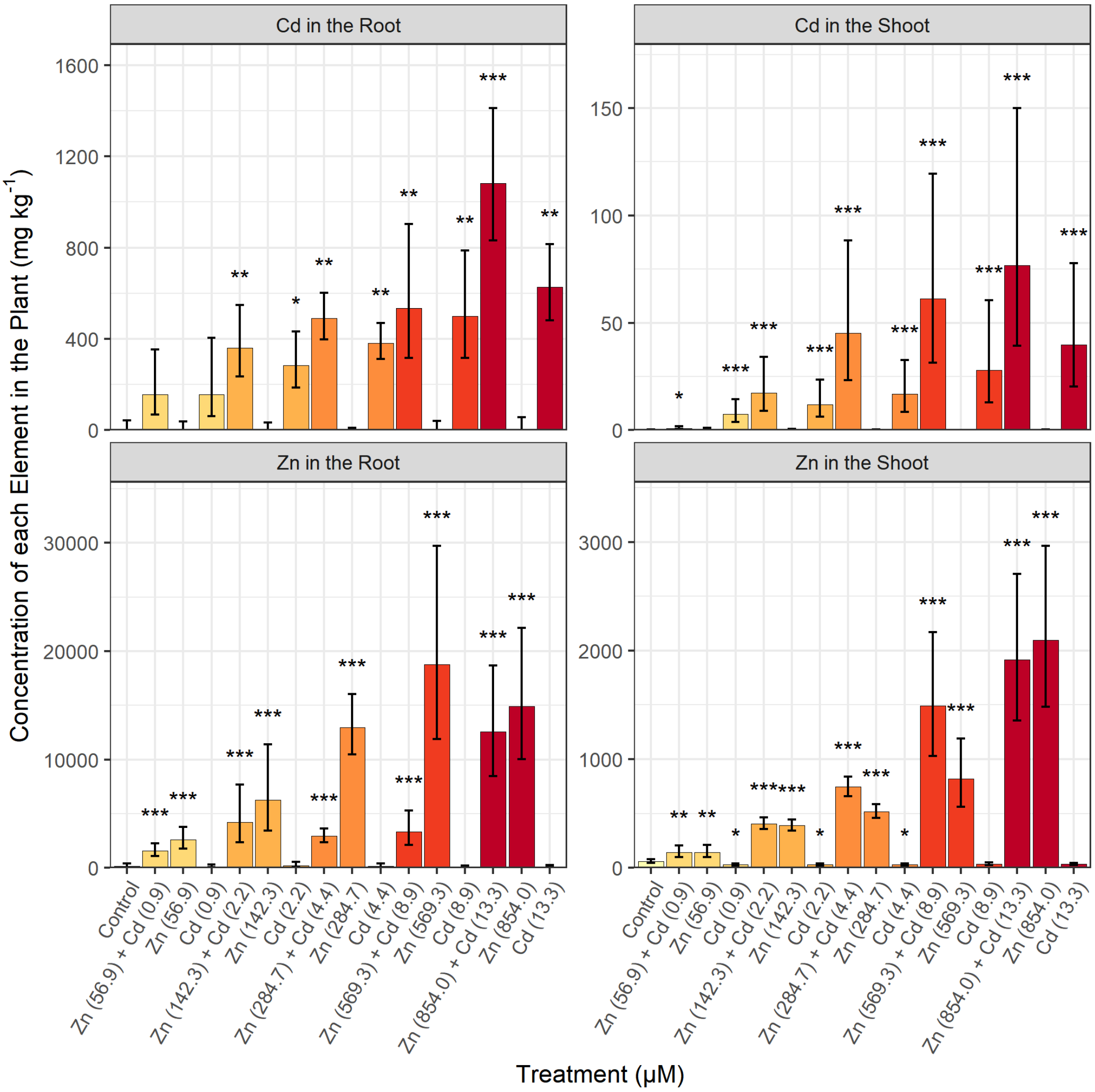

Fig. 3 Concentration of Cd and Zn in shoots and roots of andropogon grass. The asterisks next to the bars indicate the significance of the difference between the control and the treatment according to Dunnet's test. * P <0.05; ** P <0.01; *** P <0.001. Mean values and standard errors are shown (n = 4).

Table 2 Translocation factor (TF) and bioaccumulation coefficient for roots (BAC$_R$) and shoots (BAC$_S$) of andropogon grass grown in nutrient solution treated with single and combined cadmium and zinc doses. Means values and standard error are reported (n = 4).

Treatment (µM)	TF Cd	TF Zn	BAC$_R$ Cd	BAC$_R$ Zn	BAC$_S$ Cd	BAC$_S$ Zn
Control	-	-	-	-	-	-
Zn (56.9)	-	0.06(0.01)a	-	715.93(109.37)a	-	40.77(8.03)
Zn (142.3)	-	0.07(0.02)a	-	704.89(125.08)a	-	42.32(4.09)
Zn (284.7)	-	0.04(0.01)b	-	715.56(93.83)a	-	27.95(1.69)
Zn (569.3)	-	0.05(0.00)b	-	495.26(89.82)a	-	24.22(5.71)
Zn (854.0)	-	0.15(0.03)a	-	279.38(42.85)a	-	37.64(1.46)
Cd (0.9)	0.05(0.02)a	-	1539.18(277.62)	-	74.05 (2.60)a	-
Cd (2.2)	0.04(0.03)a	-	1160.90(266.26)	-	48.22(3.32)b	-
Cd (4.4)	0.05(0.01)b	-	777.50(87.91)	-	33.65(1.86)b	-
Cd (8.9)	0.06(0.01)b	-	558.05(127.73)	-	29.05(4.79)b	-
Cd (13.3)	0.06(0.01)a	-	421.94(60.94)	-	28.62(6.02)b	-
Zn (56.9) + Cd (0.9)	0.01(0.01)b	0.10(0.01)a	2452.44(1463.78)	452.73(103.59)b	12.23(5.74)b	41.11(9.55)
Zn (142.3) + Cd (2.2)	0.05(0.00)a	0.12(0.06)a	1399.29(66.95)	540.40(133.68)a	69.94(3.23)a	43.58(1.13)
Zn (284.7) + Cd (4.4)	0.10(0.02)a	0.26(0.03)a	995.90(111.03)	158.23(11.01)b	92.67(11.82)a	40.28(2.74)
Zn (569.3) + Cd (8.9)	0.12(0.03)a	0.50(0.15)a	534.12(15.01)	95.03(18.58)b	64.04(11.66)a	40.33(2.33)
Zn (854.0) + Cd (13.3)	0.08(0.02)a	0.19(0.06)a	735.77(85.34)	241.90(54.68)a	56.91(13.22)a	37.32(8.07)

Different letters in the same column indicate significant differences between single and combined treatments according to Tukey's test at $p < 0.05$.

Andropogon grass accumulated more Cd and Zn in the roots than in the shoots (Fig. 4 and Table S2). The highest Cd accumulation occurred in the combined dose (854.0) + Cd (13.3) for roots and shoots. However, the Zn levels were higher when the metal was applied alone. Indeed, the accumulation was greater than 12 mg plant^{-1} in the andropogon grass shoots at the last dose. In the roots, the greatest accumulation was about 20 mg plant^{-1} of this element for the dose of 569.3 µM.

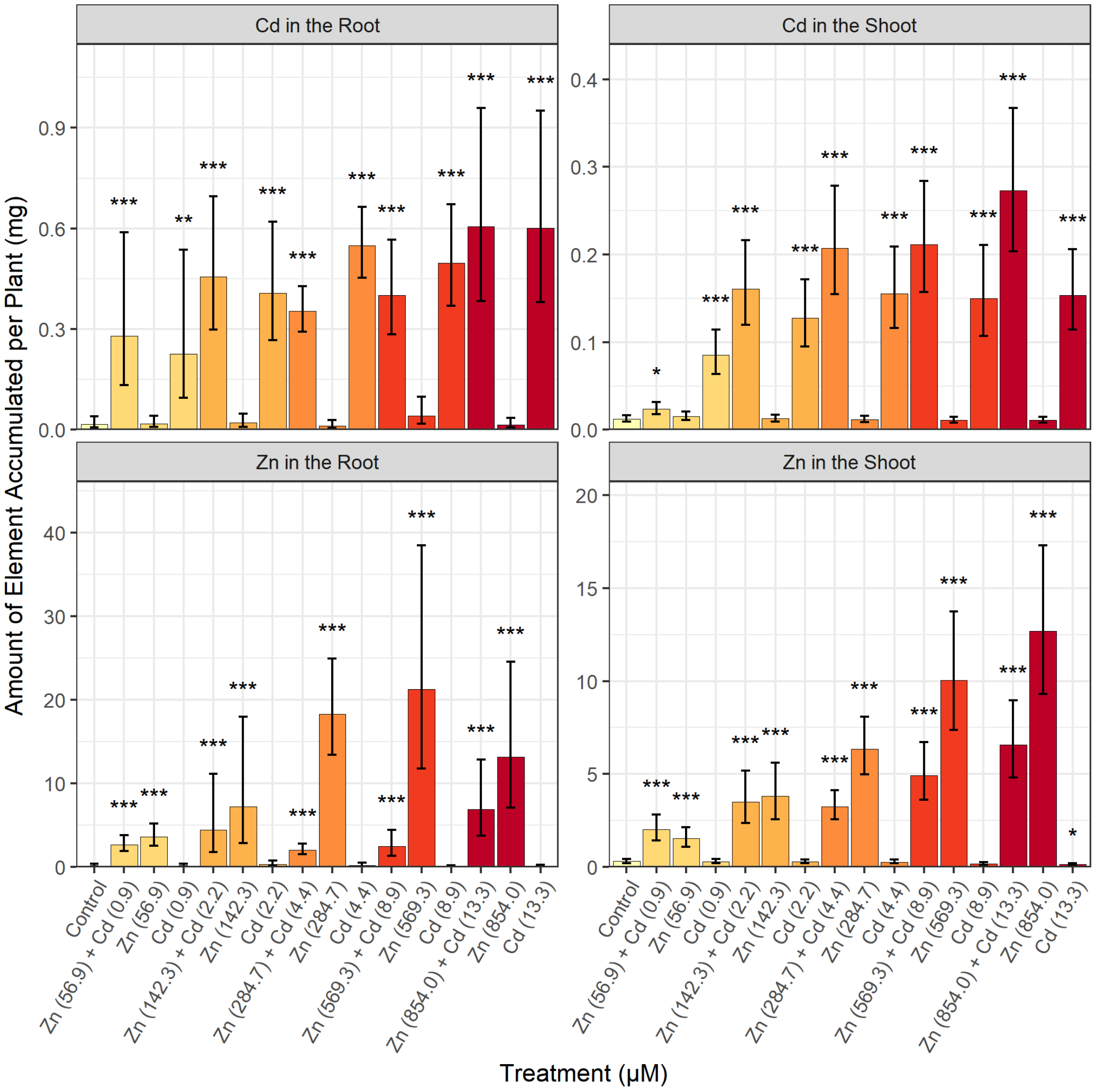

Fig. 4 Cd and Zn accumulation in the shoots and roots of andropogon grass. The asterisks next to the bars indicate the significance of the difference between the control and the treatment according to Dunnet’s test. * P <0.05; ** P <0.01; *** P <0.001. Mean values and standard errors are shown (n = 4).

3.3. Ultrastructure Analysis

Ultrastructural changes were detected by TEM (Figs. 5 and 6). The organelles in the control treatment did not show abnormal features in both leaves and roots (Fig. 6a). However, their structure changed considerably under either Zn stress or Cd stress alone, as compared with the control.

Under Cd stress, starting from 0.9 µM, the root cells exhibited ultrastructural changes such as vesiculation, vacuolation and rugged and withdrawal of the plasma membrane from the cell wall (Figs. 5a, b).

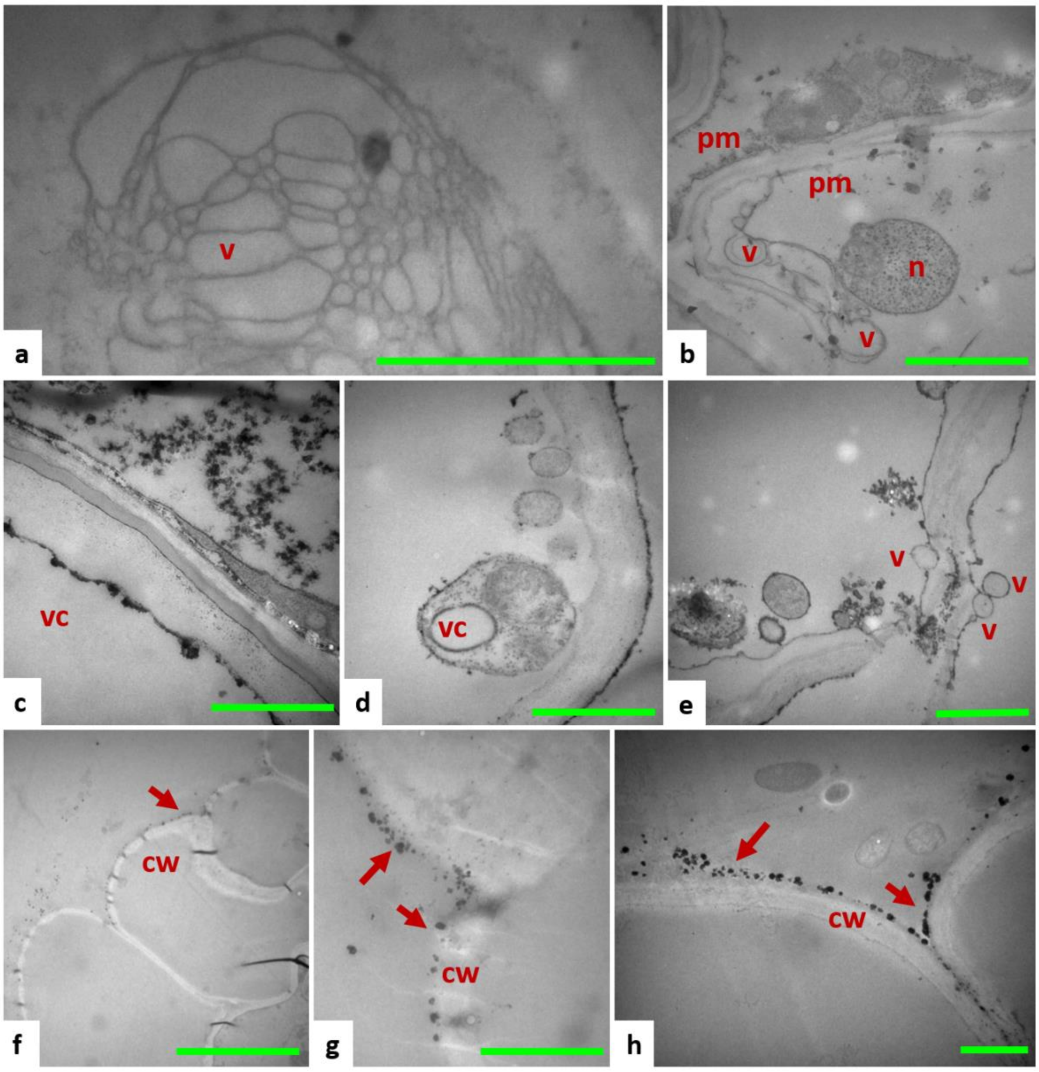

Fig. 5 Root cells ultrastructure of andropogon-grass under Zn and Cd application. (a) 0.9 µM Cd; (b) 13.3 µM Cd; (c, d and e) 56.9 µM Zn + 0.9 µM Cd; (f, g and h) 854.0 µM Zn + 13.3 µM Cd. cw: cell wall; vc: vacuole; n: nucleus; v: vesicles; pm: plasma membrane; red arrows: electron-dense material. Bars = 10 µm.

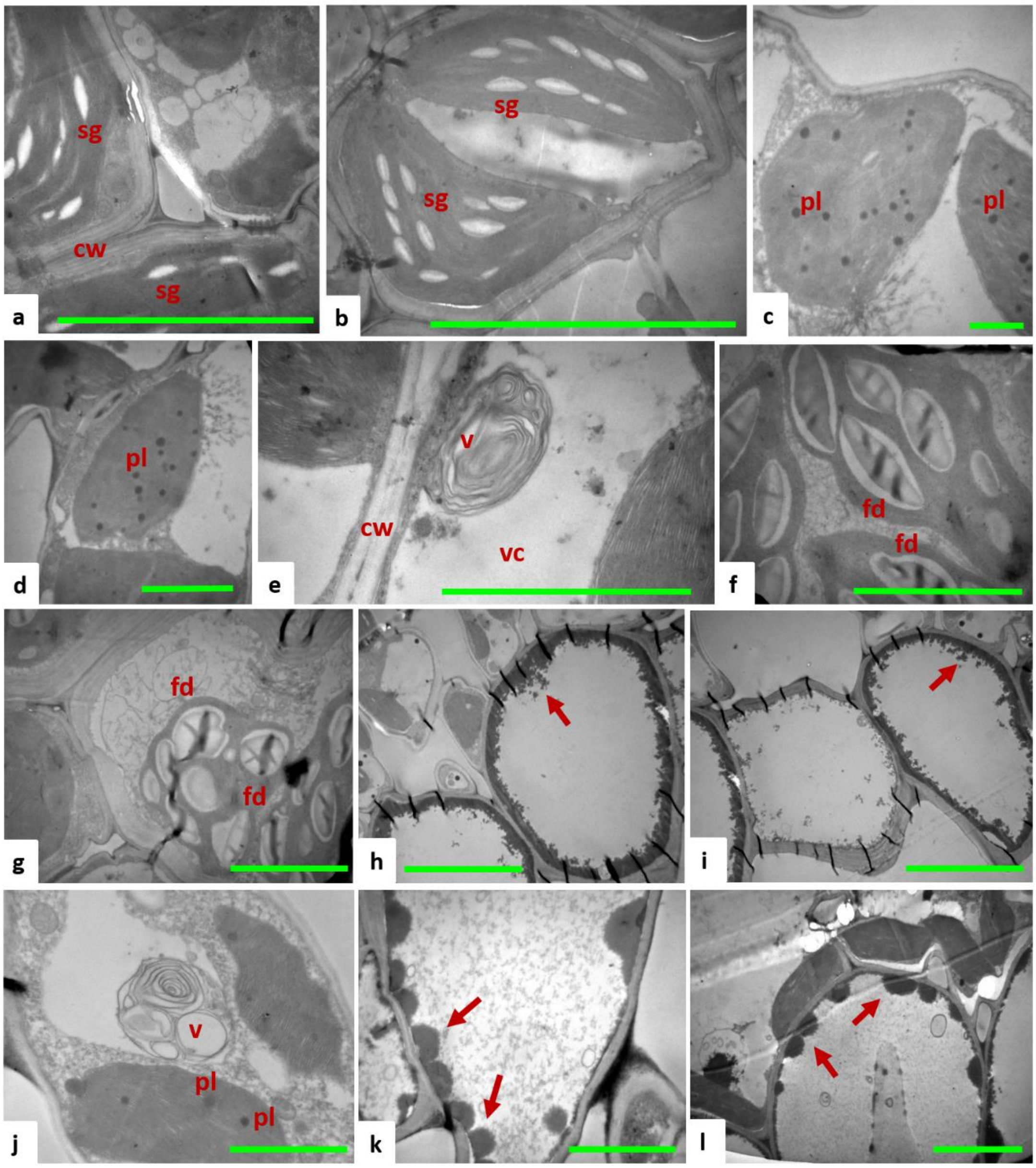

Fig. 6 Leaf cells ultrastructure of andropogon-grass under Zn and Cd application. (a) Control; (b) 854.0 µM Zn; (c) 0.9 µM Cd; (d, e, f and g) 13.3 µM Cd; (h and k) 56.9 µM Zn + 0.9 µM Cd; (i, j and l) 854.0 µM Zn + 13.3 µM Cd. cw: cell wall; vc: vacuole; n: nucleus; sg: starch grains; pl: plastoglobuli; v: vesicles; fd: floridean-like starch grains; red arrows: electron-dense material. Bars = 10 µm.

However, there were different responses of cell roots to Zn stress alone or Cd stress alone as compared to the combined application of these metals. Changes in the organelles structure were more evident when Zn was combined with Cd (Figs. 5c – h). The same ultrastructural modifications caused by the single application of Zn and Cd, were observed also when Zn and Cd were applied in combination at Zn (56.9) + Cd (0.9), e.g., vesiculation (Figs.

5d, e), vacuolation (Figs. 5c – e), as well as rugged and withdrawal of the plasma membrane from the cell wall (Figs. 5c – e). At the higher combined dose, i.e. Zn (854.0) + Cd (13.3), the root cells showed electron-dense material deposits in cell wall, which could be related to the deposition of Zn and/or Cd (Figs. 5g, h).

An increase in starch accumulation in the leaves was observed at 854.0 μM of Zn (Fig. 6b). Under the Cd treatments (starting from the concentration of 0.9 μM), numerous osmiophilic plastoglobuli were observed in the stroma as well as vesicles in the cells (Figs. 6c, d). At 13.3 μM of Cd, chloroplasts with floridean-like starch grains were observed (Figs. 6f, g).

When Zn and Cd were combined, it was possible to observe many vesicles (Fig. 6j), vacuoles with electron-dense material in the form of clumps or globules (Figs. 6k, l), and numerous plastoglobules in the chloroplasts (Fig. 6j), both at the combined treatments of Zn (56.9) + Cd (0.9) and Zn (854.0) + Cd (13.3).

4. Discussion

Zinc plays several roles in plants, including important functions in enzymes activity and protein synthesis (Broadley et al. 2012; Cakmak 2000), as well as carbohydrates and auxins metabolism (Alloway 2008). Thus, plants with Zn deficiency present symptoms such as shorter internodes and decreased growth (Broadley et al. 2007). These functions partly explain the increase in growth, stem diameter, and internode length observed in this study. The Zn toxicity in plants is quite variable, depending on the element bioavailability and differences in metal tolerance between species (Tsonev and Lidon 2012). The main visual toxicity symptoms are, in general, growth reduction, necrosis, chlorosis and foliar atrophy, which reflect a series of nutritional disorders (Cunha et al. 2008; Hafeez et al. 2013; Romeo et al. 2014). In the present work, the Zn treatments did not affect negatively the evaluated endpoints but, on the contrary, this element promoted andropogon grass growth. The content of Zn was higher than 16,000 mg kg^{-1} in andropogon grass roots at the 569.3 μM dose. Similar contents were found by Carvalho et al. (2013) for *G. claussenii* roots exposed to Zn-rich nutrient solutions. These authors observed a value higher than 16,000 mg kg^{-1} of Zn in this species (considered a Cd- and Zn-hyperaccumulator) exposed to 3,000 μM of $ZnSO_4$ in solution for 30 days. Indeed, it is worth of mentioning that the andropogon grass tested in our study is found growing together with *G. claussenii* in the same Zn mining pit in Vazante, Minas Gerais, Brazil (Amaral 2013).

Plants exposed to high Zn concentrations have been reported to present modifications in their ultrastructure (Azzarello et al. 2012) similar to those found in our study. The Zn-induced alterations in the cell wall of cell roots were of the same type as those observed by Jia et al. (2016) in *Sorghum bicolor*. In relation to the increase in starch accumulation observed in leaves at 854.0 μM of Zn, it might be due to the inhibition of phloem loading, as described by Djebali et al. (2005), as a symptom of toxicity. Despite the observed modifications in the ultrastructure of root and leaf cells in the presence of Zn, a reduction of growth was not observed, thus making this grass species tolerant to Zn concentrations up to the maximum concentration tested (854.0 μM).

Plants exposed to Cd showed decreases of root and shoot growth, as well as chlorosis and suppression of photosynthesis (Lin and Aarts 2012; Tran and Popova 2013). This element affects plant development by reducing chlorophyll content, altering enzymatic activity and nutrient absorption, inhibiting respiration and increasing oxidative stress (Cui et al. 2013; Gill et al. 2012; John et al. 2009; Lagriffoul et al. 1998; Riaz et al. 2014). Even at low concentrations, such as 1 μM, this element may negatively influence dry matter production in hydroponic system, as observed by Dias et al. (2013) using plants of *Lactuca sativa*, which was not the case for andropogon grass in this study. In fact, Cd alone affected SDW negatively only at the highest dose tested (13.3 μM), while providing a stimulation on plant growth at lower doses (until 8.9 μM). Because of its toxicity, even at low concentrations, Cd might activate plant defense responses against stresses, which may maintain its normal growth or even potentiate it as a way of tolerating this stress. This stimulatory effect at low doses can be justified by the hormesis effect (Jia et al. 2013). Liu et al. (2015), using the hyperaccumulator *Lonicera japonica* Thunb., verified increases in plant height at doses of 22.2 and 44.48 μM Cd.

At the ultrastructural level, starting from 0.9 μM of Cd, the root and leaf cells exhibited modifications indicating toxicity damages. Alterations in the cell wall of root cells and the increased number of plastoglobuli in chloroplasts in leaves indicated increased oxidative stress. These same symptoms were observed by Jiao et al. (2012) in *Populus euramericana* and by Djebali et al. (2005) in tomato.

The lower Zn and Cd combined doses also promoted an increase in dry weight of andropogon grass. However, from the Zn (284.7) + Cd (4.4) μM dose, there was a decrease in growth, which may be due to the higher Cd absorption and toxicity in plants, since these metals may have a synergistic absorption mechanism (Kabata-Pendias and Szteke 2015). This is evidenced by the higher uptake and accumulation of Cd in the shoots in the combined doses, compared with the treatment with single Cd doses. Conversely to what was observed here, some studies have shown that with the increase of Zn and Cd combined doses, there is a reduction of the negative effect of Cd suggesting that Zn may protect the plant from the oxidative stress and the formation of free radicals promoted by Cd (Aravind and Prasad 2003; Hussain et al. 2016). These different effects can be explained by the higher or lower ratio between Cd and Zn in the solutions (Tkalec et al. 2014).

Although most plants have low tolerance to Cd, some species such as *Thlaspi caerulescens* and *Arabidopsis halleri* are known hyperaccumulators of this metal (Verbruggen et al. 2013). The addition of Zn in these plants makes them more tolerant and increases their Cd accumulation (Guimarães et al. 2008). In the present work, with the increase of the Zn and Cd combined doses, a reduced growth of the plants was observed. However, they absorbed and accumulated more Cd. Besides, although there was a negative effect in the dry weight compared to the control in the two highest combined doses, the growth reduction was about 30%, while the metal concentration in the roots practically doubled. Also, even with the absorption of 587 mg kg^{-1} of Cd in the single dose, there was no effect of this element on the andropogon grass dry weight.

The BAC_S in both single or combined treatments varied from 24.22 to 43.58 for Zn and 12.23 to 92.67 for Cd. For roots, BAC_R values were higher, varying from 95 to 715 for Zn and 421.94 and 2452 for Cd. Other studies in hydroponic conditions showed high bioaccumulation factors for these metals. For example, the BAC for leaves of H. *verticillata* varied from 387.72 to 1084.86 in plants exposed to Cd for 21 days (Zhang et al. 2020). These values are not frequently observed in studies with soils (Amaral, 2013; Visconti et al. 2019), as soil plays an important role in the adsorption of heavy metals, reducing the bioavailable fraction of metals for plants. In studies conducted in soils with high content of metals, plants need concentrating high amounts of metals in the shoots in order to show positive BAC_S. Andropogon grass growing in high contaminated mining soils (1069 mg Zn kg^{-1} and 4.48mg Cd kg^{-1}) showed BACs of 2.4 for Zn and 3.4 for Cd (Amaral 2013).

In the Zn single treatments, with the increase of the doses there was a higher absorption and accumulation of this element in the shoots, without, however, reducing the dry weight. This fact reinforces the tolerance of andropogon grass to high doses of this metal. This high tolerance was also reported in studies of Amaral (2013) and Umeoguaju et al. (2016), but the mechanisms of metal tolerance for this species is still unclear. In this work, we have showed that one mechanism andropogon grass uses to cope with high metal contents in the media is the retention of metal in the roots, which is in accordance with the results of Amaral (2013). In addition to the metal exclusion behavior, we have also observed electron-dense materials that may be the result of metal compartmentalization, as observed by Amaral (2013), even though we could not assess the chemical composition of those particles, as performed in that study. Using scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDS), Amaral (2013) observed an accumulation of Zn in intercellular spaces and formation of crystals in andropogon grass shoots, in addition to high levels of Zn in the superficial layer as well as accumulation of Zn and Cd in intercellular spaces in the roots.

Currently, several phytoremediation techniques such as phytoextraction, phytostabilization, phytodegradation, phytovolatization, and rhizodegradation are available (Cundi et al. 2016). Phytoextraction and phytostabilization are the most applied techniques (Mahar et al. 2016). Phytoextraction programs use hyperaccumulator plants to accumulate metals in the aerial parts and phytostabilization use plants to immobilize these elements in the rhizosphere and roots (Wong 2003). Hyperaccumulator is a species capable of absorbing and accumulating, in the aerial part, concentrations greater than 100 mg kg^{-1} of Cd and 10,000 mg kg^{-1} of Zn (Baker et al. 1994). Although andropogon grass does not accumulate these amounts of metals (Table S3), this grass showed a good dry weight production even at the highest combined dose treatment Zn (854.0) + Cd (13.3), totaling 4.125 g dry weight. Amaral (2013) analyzed the natural levels of Cd and Zn in andropogon grass collected in the same zinc mining pit. The values varied in the root and shoot, respectively, from 350 to 5,306 mg kg^{-1} and 215 to 6,801 mg kg^{-1} for Zn and 3 at 40 mg kg^{-1} and 3 to 28 mg kg^{-1} for Cd. The highest soil levels of Zn and Cd found in the area were respectively 10,681 mg kg^{-1} and 161 mg kg^{-1} (Amaral 2013).

Our study suggests that although this grass cannot be classified as hyperaccumulator and be suitable for phytoextraction programs, its ability to tolerate high Cd and Zn concentrations, combined with its ability to produce large amounts of biomass make it suitable for phytostabilization, especially because as a grass it may promote great soil cover decreasing leaching and erosion (Mahar et al. 2016; Visconti et al. 2019). The Cd and Zn concentration and accumulation were higher in the roots compared with the shoots for all treatments. This is in accordance with the behavior reported for some tropical forage grasses (Ng et al. 2016) and species tolerant to these elements (Mohtadi et al. 2012). In fact, the restriction of metal translocation (all andropogon grass TF values were below 1) is a mechanism utilized by metal-excluder plants (Baker 1981) and is suitable for phytostabilization plans as it aims at inactivating heavy metals avoiding their entrance in the food chain (Mahar et al. 2016). As a final remark, it is important to understand that although phytostabilization is a technique focusing on risk minimization (Mahar et al. 2016), environmental issues such as grazing and leaching of contaminants after plant death should be further addressed (Bolan et al. 2011). In order to avoid these problems, a regular and effective monitoring of the area is required (Bolan et al. 2011).

5. Conclusions

The native andropogon grass evaluated in this study revealed patterns that suggest its high Zn nutritional requirement. The presence of Cd in low doses improves the growth of andropogon grass. Modifications in the ultrastructure of root and leaf cells in the presence of combined Zn and Cd without relevant reduction of growth were observed.

Andropogon grass showed characteristics typical of metal-excluder plants, as it accumulated high amounts of metals but presented a TF<1. This behavior combined with high biomass production as well as a great tolerance to Cd and Zn make this grass species a promising candidate for phytostabilization programs of Zn- and Cd-polluted sites. However, further studies are needed to better understand the role and behavior of this species at the field scale, especially concerning environmental risks associated with phytostabilization, such as root turnover and grazing.

In conclusion, this study provides relevant information on the growth of andropogon grass in different contamination scenarios, which can be applied in the recovery of several Zn mining degraded areas worldwide.

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Supplementary material

Table S1 Concentration of Cd and Zn in shoot and root of andropogon grass.

Treatment (μM)	Cd root (mg kg^{-1})	Cd Shoot (mg kg^{-1})	Zn root (mg kg^{-1})	Zn shoot (mg kg^{-1})
Control	4.36 [0.44 – 43.0]	0.18 [0.09 – 0.34]	143 [54.6 – 376]	57.9 [43.1 – 77.9]
Zn (56.9)	3.73 [0.38 – 36.8]	0.51 [0.26 – 0.99]	2581 [1773 – 3757]***	144 [99.5 – 210]**
Zn (142.3)	3.42 [0.35 – 33.7]	0.23 [0.12 – 0.45]	6265 [3451 – 11373]***	389 [341 – 444]***
Zn (284.7)	0.93 [0.09 – 9.15]	0.15 [0.07 – 0.28]	12955 [10461 – 16043]***	517 [459 – 584]***
Zn (569.3)	4.06 [0.41 – 40.0]	0.06[0.03 – 0.11]	18779 [11869 – 29711]***	819 [563 – 1191]***
Zn (854.0)	5.78 [0.59 – 56.9]	0.13 [0.07 – 0.26]	14901 [10027 -22144]***	2097 [1484 – 2963]***
Cd (0.9)	156 [60.2 – 405]	7.40 [3.79 – 14.5]***	113 [42.9 – 296]	28.6 [21.3 – 38.5]*
Cd (2.2)	283 [186 – 432]*	12.0 [6.13 – 23.4]***	203 [77.3 – 533]	28.0 [20.8 – 37.6]*
Cd (4.4)	382 [311 – 469]**	16.8 [8.58 – 32.7]***	148 [56.3 – 388]	30.2 [22.4 – 40.6]*
Cd (8.9)	499 [317 – 787]**	27.9 [12.9 – 60.4]***	74.8 [28.5 – 196]	33.9 [24.0 – 47.7]
Cd (13.3)	626 [481 – 816]**	39.8 [20.4 – 77.8]***	88.2 [33.6 – 232]	34.4 [25.6 – 46.3]
Zn (56.9) + Cd (0.9)	155 [68.0 – 354]	0.89 [0.45 – 1.73]*	1557 [1070 – 2267]***	143 [98.5 – 207]**
Zn (142.3) + Cd (2.2)	360 [236 – 548]**	17.4 [8.93 – 34.1]***	4232 [2331 – 7682]***	405 [355 – 462]***
Zn (284.7) + Cd (4.4)	489 [399 – 601]**	45.3 [23.2 – 88.4]***	2924 [2361 – 3621]***	745 [660 – 841]***
Zn (569.3) + Cd (8.9)	534 [315 – 903]**	61.1 [31.3 – 119]***	3329 [2104 – 5266]***	1493 [1027 – 2171]***
Zn (854.0) + Cd (13.3)	1083 [831 – 1410]***	76.7 [39.3 – 150]***	12559 [8451 – 18663]***	1917 [1357 – 2708]***

The asterisks next to the bars indicate the significance of the contrast between the control and the treatment according to Dunnet's test. * P <0.05; ** P <0.01; *** P <0.001. Means values and confident interval at 95%.

Table S2 Accumulation of Cd and Zn per plant of andropogon grass

Treatment (µM)	Cd root (mg plant^{-1})	Cd Shoot (mg plant^{-1})	Zn root (mg plant^{-1})	Zn shoot (mg plant^{-1})
Control	0.02 [0.01 – 0.04]	0.01 [0.01 – 0.02]	0.16 [0.06 – 0.40]	0.30 [0.21 – 0.44]
Zn (56.9)	0.02 [0.01 – 0.04]	0.02 [0.01 – 0.02]	3.64 [2.56 – 5.17]***	1.53 [1.09 – 2.14]***
Zn (142.3)	0.02 [0.01 – 0.05]	0.01 [0.01 – 0.02]	7.20 [2.89 – 18.0]***	3.80 [2.58 – 5.61]***
Zn (284.7)	0.01 [0.01 – 0.03]	0.01 [0.01 – 0.02]	18.3 [13.4 – 24.9]***	6.35 [4.99 – 8.08]***
Zn (569.3)	0.04 [0.02 – 0.10]	0.01 [0.01 – 0.01]	21.3 [11.76 – 38.47]***	10.1 [7.37 – 13.7]***
Zn (854.0)	0.02 [0.01 – 0.04]	0.01 [0.01 – 0.01]	13.18 [7.07 – 24.6]***	12.7 [9.32 – 17.3]***
Cd (0.9)	0.23 [0.10 – 0.54]**	0.09 [0.06 – 0.11]***	0.16 [0.07 – 0.41]	0.30 [0.21 – 0.43]
Cd (2.2)	0.40 [0.27 – 0.62]***	0.13 [0.10 – 0.17]***	0.30 [0.12 – 0.74]	0.29 [0.20 – 0.41]
Cd (4.4)	0.55 [0.45 – 0.66]***	0.16 [0.12 – 0.21]***	0.22 [0.09 – 0.55]	0.27 [0.19 – 0.39]
Cd (8.9)	0.50 [0.37 – 0.67]***	0.15 [0.11 – 0.21]***	0.08 [0.03 – 0.21]	0.18 [0.12 – 0.27]
Cd (13.3)	0.60 [0.38 – 0.95]***	0.15 [0.11 – 0.21]***	0.09 [0.04 – 0.24]	0.13 [0.09 – 0.19]*
Zn (56.9) + Cd (0.9)	0.28 [0.13 – 0.59]***	0.02 [0.02 – 0.03]*	2.68 [1.89 – 3.81]***	2.01 [1.44 – 2.82]***
Zn (142.3) + Cd (2.2)	0.46 [0.30 – 0.70]***	0.16 [0.12 – 0.22]***	4.47 [1.79 – 11.15]***	3.50 [2.37 – 5.17]***
Zn (284.7) + Cd (4.4)	0.35 [0.29 – 0.43]***	0.21 [0.15 – 0.28]***	2.06 [1.52 – 2.81]***	3.25[2.56 – 4.14]***
Zn (569.3) + Cd (8.9)	0.40 [0.28 – 0.57]***	0.21 [0.16 – 0.28]***	2.45 [1.35 – 4.43]***	4.92 [3.60 – 6.72]***
Zn (854.0) + Cd (13.3)	0.61 [0.38 – 0.96]***	0.27 [0.20 – 0.37]***	6.91 [3.71 – 12.89]***	6.57 [4.82 – 8.94]***

The asterisks next to the bars indicate the significance of the contrast between the control and the treatment according to Dunnet’s test. * P <0.05; ** P <0.01; *** P <0.001. Means values and confident interval at 95%.

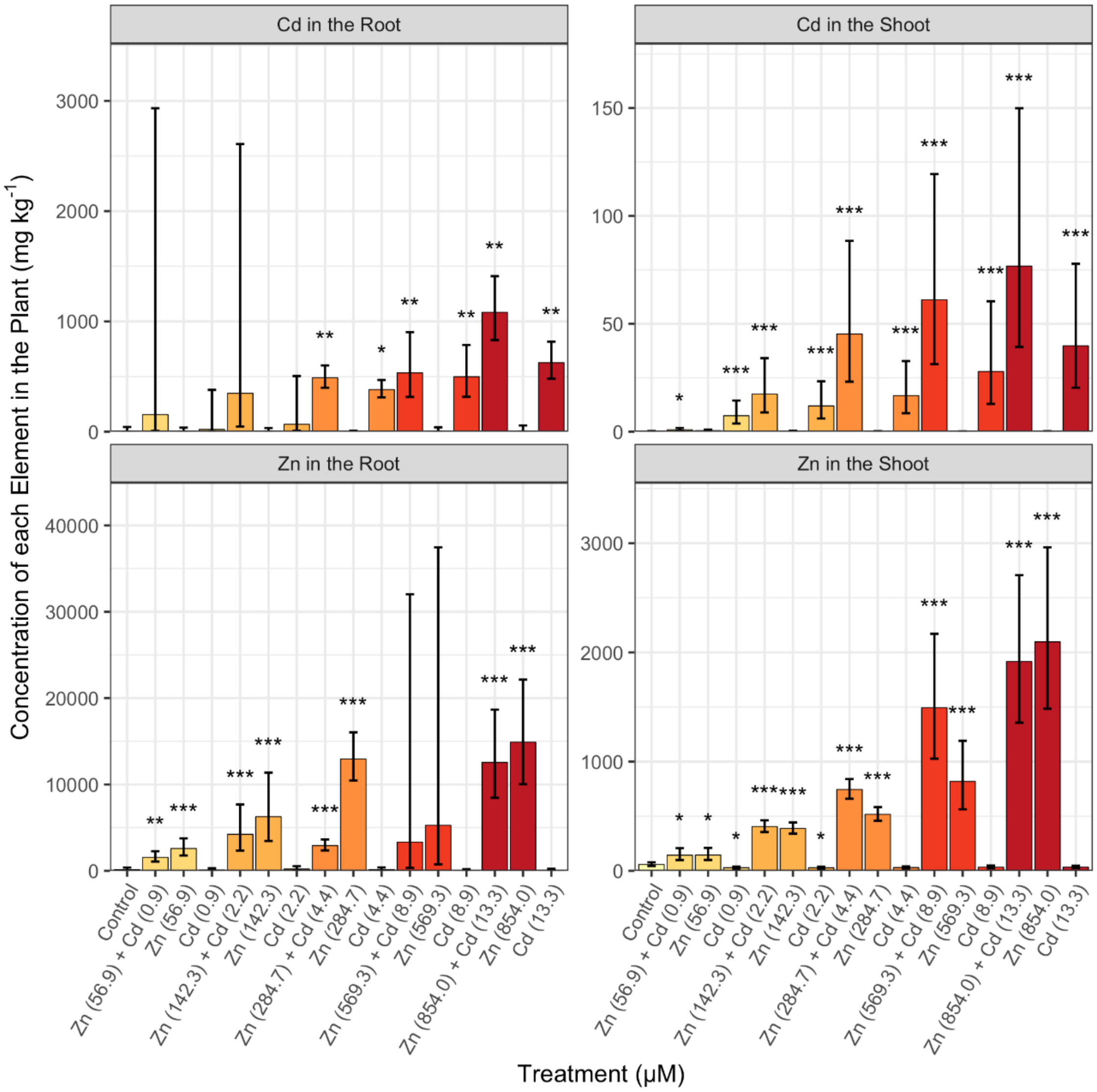

Fig. S1 Original data of concentration of Cd and Zn in shoot and root of andropogon grass before removing outliers

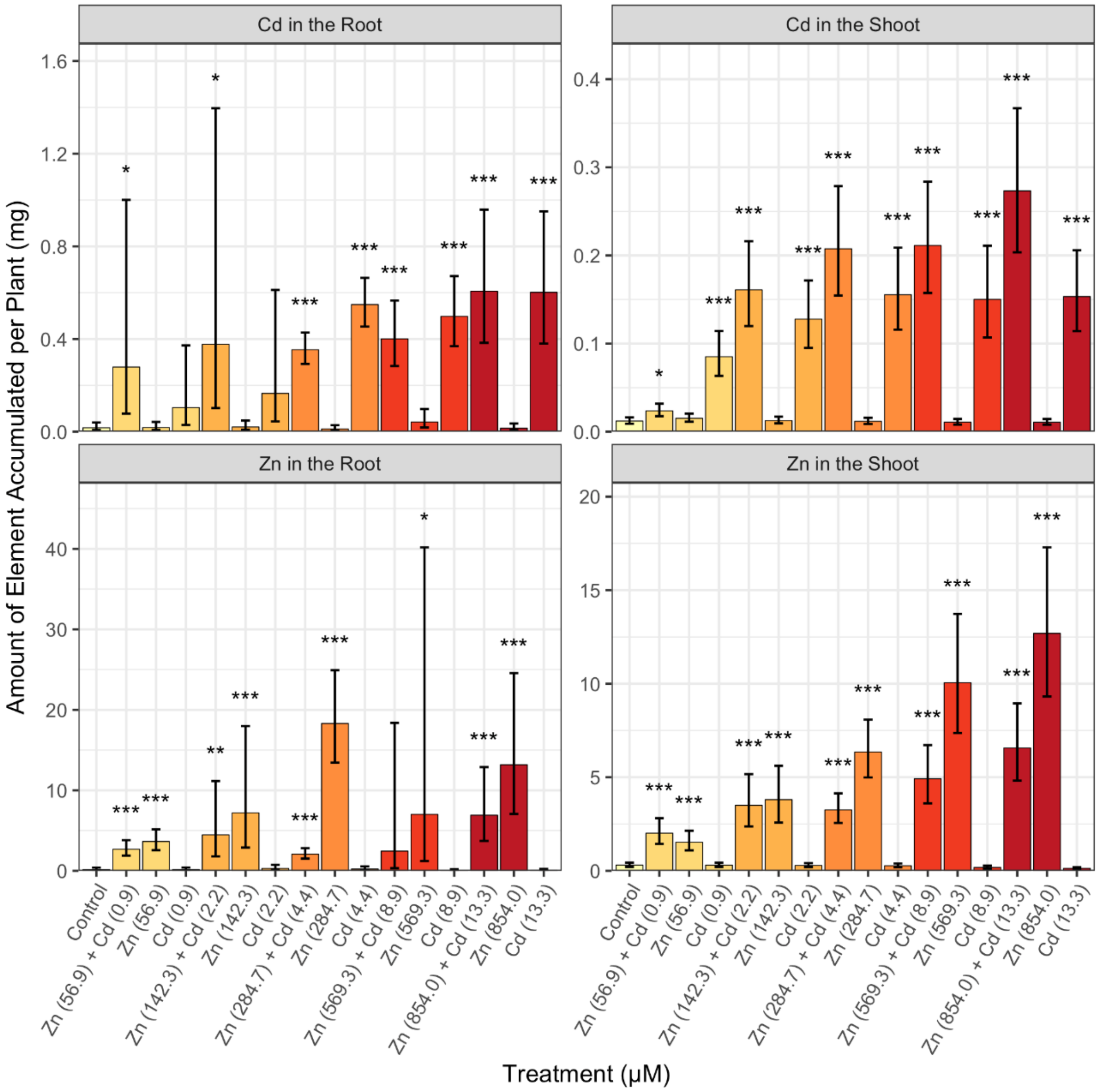

Fig. S2 Original data of accumulation of Cd and Zn per plant of andropogon grass before removing outliers

MANUSCRIPT 2: Hydrothermally-altered feldspar as an environmentally-friendly technology to promote heavy metals immobilization: batch studies and application in smelting-affected soils

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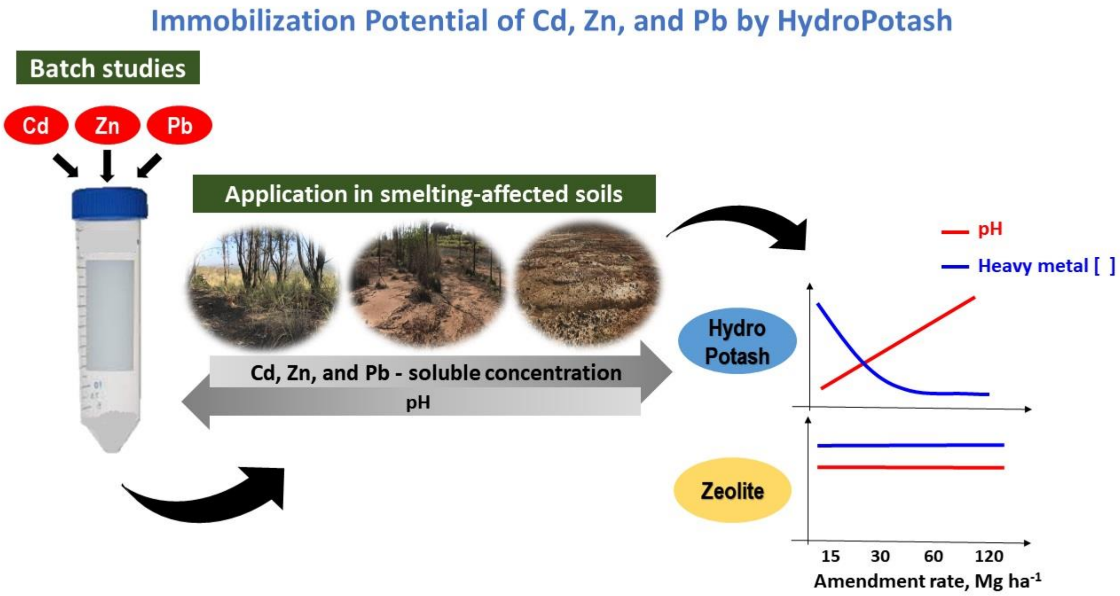
Graphycal abstract



Highlights

Hydrothermally-altered Feldspar (HYP) was effective in immobilizing metals from water

Amending HYPs in contaminated Zn-smelting soils reduces metal mobilization

The HYPs immobilized Cd, Zn, and Pb by precipitation and adsorption

Abstract

Hydrothermally-altered feldspar (HydroPotash, HYP) possesses, among other physicochemical properties, high pH buffering and cation exchange capacity. Therefore, it may potentially remove heavy metals from aqueous solutions and immobilize these metals in contaminated soil. This study aimed to evaluate the capabilities of two types of HydroPotash (HYP-1 and HYP-2) and a zeolite sample (a commercial adsorbent) for immobilizing cadmium (Cd), zinc (Zn), and lead (Pb) from both aqueous solution and contaminated soils from a Zn-smelting area (classified as $\mathrm{soil}_{\mathrm{high}}$, $\mathrm{soil}_{\mathrm{intermediate}}$, and $\mathrm{soil}_{\mathrm{low}}$ based on their level of soluble metal concentration). Sorption studies in natural suspension pH showed that HYPs removed 63.8-99.9% Zn, 20.6-40.7% Cd, and 68.4-99.7% Pb from aqueous solution. In the batch test with controlled pH (at pH 5.5), HYPs sorbed more Cd than zeolite. Analyses of scanning electron microscopy-energy dispersive X-ray spectroscopy after desorption showed the presence of Pb at HYP-2, indicating that this metal was effectively adsorbed. In $\mathrm{soil}_{\mathrm{high}}$ HYPs immobilized 99.9% of Zn, Cd, and Pb after one week of soil incubation with these products. The HYPs immobilization effect persisted up to 84 days of soil incubation with these products. The increased soil pH promoted by HYPs appears to be the main factor controlling metal sorption. In conclusion, HydroPotash can be used as an adsorbent/amendment to effectively immobilize heavy metals in both water and contaminated soils by precipitation and adsorption. Our findings indicate the high potential of this material for Cd, Zn, and Pb stabilization, which is of great relevance when recovering areas affected by mining/smelting activities with multi-element contamination.

Keywords: metal mobility; sorption; cadmium; lead; zinc; soil remediation.

1. Introduction

Mining and smelting activities are crucial for the population and stimulation of economic development, but industrial wastes generated from these operations may directly or indirectly impact soil and water quality in areas nearby mining and smelting regions (Bernhardt and Palmer, 2011; Hudson-Edwards, 2016; Zipper et al., 2016). One of the major concerns in this context is the high heavy metal levels in soil and water surrounding mining/smelting sites (Chen et al., 2018; Hoaghia et al., 2019; Kalyvas et al., 2018; Shen et al., 2019; Zhao et al., 2020). These heavy metals, such as cadmium (Cd), zinc (Zn), and lead (Pb), pose short- and long-term risks to the ecosystem and human health (Ali et al., 2019; ATSDR, 2012).

Many strategies for remediating contaminated soils have been studied worldwide in recent years, including electrokinetic (Sun et al., 2019), vitrification of contaminant sources (Ballesteros et al., 2017), phytoremediation (Zeng et al., 2019), and bioremediation (Zhao et al., 2019). Another promising technique is the immobilization of heavy metals by adding amendments to contaminated soils (Palansooriya et al., 2020). These amendments may reduce metal mobilities in soil by adsorption, precipitation, and ion exchange, thus preventing the leaching of these elements in water or their uptake by plants (Lwin et al., 2018; Mahar et al., 2015). Besides being the use of amendments relevant to decrease the mobility of contaminants, it can also be a source of plant nutrients, which may be released to plants, enhancing plant growth and, as a result, the remediation process (Lopes et al., 2016; Teodoro et al., 2020).

Many amendments such as biochar (Fellet et al., 2011; Yuan et al., 2019), phosphates (Mignardi et al., 2012), zero-valent iron (Tang et al., 2019), limestone (Bade et al., 2012), and industrial by-products (Costa et al., 2020; Martins et al., 2018) have been studied for reducing bioavailability and toxicity of metals in contaminated soils. Due to their abundance and availability, silicate minerals, such as montmorillonite (Ijagbemi et al., 2009), bentonite (Anna et al., 2015; Kumararaja et al., 2017; Sun et al., 2015), and zeolite (Hong et al., 2019; Yuna, 2016) have also been used as adsorbent materials of heavy metals. Of over 40 types of natural zeolites, clinoptilolite is the most commonly used for agronomic purposes, as soil conditioning and soil remediation, immobilizing metals in contaminated soils (Reháková et al., 2004; Yuna, 2016).

Hydrothermally altered feldspar called HydroPotash (HYP), patented and licensed by APT - Advanced Potash Technologies Ltd., is a silicate mineral synthesized through hydrothermal alteration of potassium (K) feldspars plus $Ca(OH)_2$ (Ciceri et al., 2017a, b). It has been marketed as a potassium fertilizer and its efficiency for promoting plant growth has been reported by Ciceri et al. (2019). Feldspar, altered by a hydrothermal process, is considered a green technology since it is produced with globally abundant raw materials (Ciceri et al., 2017a, b). Because of its high pH buffering and cation exchange capacity (CEC), it may have the efficiency to immobilize heavy metals from contaminated sites. Therefore, considering that little is known about the HYP use in such context, studies to assess its effectiveness as heavy metals adsorbent and as an amendment in contaminated soil are of great relevance and required. This study aimed to: 1) evaluate the sorption capacity of Cd, Zn, and Pb in HYPs (HYP-1 and HYP-2) and zeolite in natural suspension pH (without altering pH); 2) assess the adsorption-desorption of Cd, Zn, and Pb on HYPs and zeolite at pH 5.5; and, 3) evaluate the efficiency of these products in immobilizing metals in contaminated soils collected from a smelting site. From that, we intended to evaluate the potential use of these products as immobilization agents in soil-remediation projects and learn how they may act (i.e., only due to an increasing-pH-effect causing precipitation or thru additional adsorption processes when the pH is controlled).

2. Material and Methods

2.1. Adsorbent materials description and characterization

HydroPotash (HYPs) samples were provided by Advanced Potash Technologies Ltd. The detailed preparation of these materials was described by Ciceri et al. (2017a). Briefly, milled feldspars (>80% pure $KAlSi_3O_8$) and $Ca(OH)_2$ were mixed under a hydrothermal process (200-230ºC for 1-2.5 h) and subsequently dried. HydroPotash-1 and HYP-2 were the two types of HYPs used for this study. They were prepared using the same raw material with different temperatures (200ºC for HYP-1 and 230ºC for HYP-2) and residence time (1 h for HYP-1 and 2.5 h for HYP-2) during the hydrothermal process, which may differentiate them in terms of the dominant mineralogical composition. The clinoptilolite (zeolite) was purchased from Celta Brasil Ltd. (Cotia, Brazil).

The pH of materials was determined in a 1:10 product: water ratio after 30 minutes of agitation (Seyfferth et al., 2019) using pH meter (AR60, Fisher, Fisher Scientific, Fairlawn, NJ). The CEC of HYPs and zeolite was determined following ISO 11260 (2011) and Farkas et al. (2005), respectively. The particle size of all materials was determined by laser diffraction following ISO 13320:2009 (2009).

Chemical characterization of products was accessed by wavelength dispersive X-ray fluorescence technique. For preparation, samples of each product were ground, passed through a 150 µm nylon sieve, and mixed with C micropowder (Merck®) using a product: wax ratio 9:1. The product-wax mixture was placed on a tungsten carbide support for pressing in an automatic press machine (Vaneox model – Fluxana®) at a pressure of 25 t cm^{-2}. The pellets have a diameter equal to 4.0 cm and a height of 0.7 cm. After preparation, samples were placed in the sample holder to determine the elements using a WDXRF equipment, Bruker®, model S8 Tiger (Kennewick, WA, USA) equipped with the Geochem software using the Quant-Express analytical curve. The percentages of oxides have been converted to total elemental concentration. The total concentrations of Cd, Zn, and Pb were determined by inductively coupled plasma mass spectrometry (ICP-MS, HP4500 PLUS, Agilent Technologies, Palo Alto, CA, USA) after nitric acid + hydrofluoric acid digestion – modified USEPA 3052 method (USEPA, 1996).

Surface morphology images of all samples (HYP-1, HYP-2, and zeolite) were recorded by Quanta 650FEG scanning electron microscopy (SEM). Also, at the same surface capture by SEM, elemental distribution maps were created using Energy Dispersive X-ray Spectroscopy (EDS) model Oxford.

X-ray diffraction analysis using an equipment Rigaku Miniflex II with Cu K α radiation and a graphite monochromatic beam, in the range of 3 to 50 2θ degrees, step size of 0.02 2θ degree, and counting time of 1 s/step was used to analyze the crystalline phases of the materials. Software Match!3 was used to identify mineral patterns.

2.2. Batch sorption studies of Cd, Zn, and Pb

2.2.1. Sorption of Cd, Zn, and Pb in natural suspension pH

The adsorbent materials described in item 2.1 were firstly tested for the sorption of all evaluated metals (Cd, Zn, and Pb) in natural suspension pH, i.e., without adjusting the pH of the suspension obtained from adsorbents after their contact with the electrolyte solution. For that, sorption reactions were carried out at room temperature (23±2ºC) with the addition of solutions containing 10 mmol L^{-1} of each metal (Cd, Zn, and Pb), which were added from stock solutions of 100 mmol L^{-1} of $Cd(NO_3)_2.4H_2O$, $Zn(NO_3)_2.6H_2O$, and $Pb(NO_3)_2$, prepared in 10 mmol L^{-1} $Ca(NO_3)_2$, used as background electrolyte solution. The concentration of 10 mmol L^{-1} for each metal is considered high and was chosen in this step in order to check the effectiveness of applying the evaluated adsorbent materials as amendments in real Zn-smelting affected soils, where high soluble concentrations of metals are present and also due to the high buffering capacity observed in HYP-1 in previous tests (Fig. S1). The experiments were carried out in a completely randomized design with three replicates. An amount of 0.3 g of HYP-1, HYP-2, and zeolite (naturally dry and at their original granulometry) was added in a 50 mL-polypropylene centrifuge tube with 27 mL of $Ca(NO_3)_2$ (pH = 5.5, 10 mmol L^{-1}). After 24 h of stirring at 120 rpm, samples were centrifuged during 15 minutes using 2,000 rpm, pH was measured, and 3 mL of the stock solutions (100 mmol L^{-1}) of $Cd(NO_3)_2.4H_2O$, $Zn(NO_3)_2.6H_2O$, and $Pb(NO_3)_2$ were added. The samples were then stirred for 24 h, centrifuged again during 15 minutes using

2,000 rpm, and the supernatants collected for further analyses. The pH of supernatant was measured using a pH meter (Metller Toledo, Model Seven Multi, Schwerzenbach), and metal concentrations in the supernatant (Ce) were determined by air-acetylene flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 400). The amount of each metal sorbed (Q) was calculated according to Eq. 1.

$$Q = ((Ci - Ce) * V_{sol} / M_{adsorbent} \text{Eq. (1)}$$

where Q is the amount of each metal sorbed (mmol kg^{-1}), Ci and Ce (mmol L^{-1}) are initial and equilibrium solution concentrations, respectively, V_{sol} (L) is the final volume of solution, and $M_{adsorbent}$ (kg) is the mass of the adsorbent.

2.2.2. Isotherm studies of Cd, Zn, and Pb at pH 5.5

Adsorption experiments were carried out using batch procedures at room temperature (23±2ºC) with three adsorbents (HYP-1, HYP-2, and zeolite), seven metal (Zn, Cd, and Pb) addition rates (0, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.5 mmol L^{-1}), and three replicates. To standardize the particle size, the three adsorbents were grounded in an agate mortar to pass a 50-micron sieve. Then, 0.3 g of samples were weighed into a 50 mL-polypropylene centrifuge tube, and 27 mL of $Ca(NO_3)_2$ (pH = 5.5, 10 mmol L^{-1}) was added into the tube to achieving an adsorbent:solution ratio equals to 1:90. The solution pH was adjusted to 5.5± 0.5 using diluted nitric acid. This pH value for the adsorption reaction was chosen to avoid metal precipitation (Pierangeli et al., 2005, 2007). Samples were shaken at 120 rpm for 12 h per day during ten days until suspension pH values reached stabilization (variation of daily pH reading < 0.2). After pH stabilization, 3 mL of the metal stock solutions containing 0; 0.5; 1.0; 2.5; 5.0, 7.5, and 15 mmol L^{-1} of each element (Cd, Zn, and Pb) were added to the tube. The tubes were prior shaken for 24 h and then centrifuged for 15 minutes using 2,000 rpm. Desorption was performed after the adsorption phase, where 10 mmol L^{-1} $Ca(NO_3)_2$ solution was added to the remaining adsorption solution. Desorption time was also 24 h, similarly to what was described for the adsorption. Metal concentrations on both supernatants (from adsorption or desorption steps) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro - model Blue, Germany), and the amounts of the metals sorbed (Q) were calculated based on the equation (1). Metals present in the entrained solution at the end of adsorption were subtracted in the calculation of the amount desorbed (Araujo et al., 2020).

Data obtained in the adsorption experiments were fitted to the Langmuir and Freundlich equations (Equations 2 and 3). Before that, the linearized forms of Langmuir and Freundlich models (Equations 4 and 5) were used to obtain the model parameters.

$$q = \frac{kbC_e}{1+kC_e} \text{Eq. (2)}$$

$$q = K_f (Ce)^{1/n} \text{Eq. (3)}$$

$$\frac{Ce}{q} = \frac{1}{kb} + \frac{Ce}{b} Eq.\ (4)$$

$$\log q = \log kf + {}^{1}\!/_{n} \log Ce \text{ Eq. (5)}$$

where q is the amount of metal adsorbed (mmol kg^{-1}), Ce is the concentration of adsorbate in the equilibrium solution (mmol L^{-1}), K_f and n are constants of adjustments of the Freundlich model, k is the Langmuir constant, and b is the maximum adsorption capacity (mmol kg^{-1}).

The amounts of Cd, Zn, and Pb that kept adsorbed after the desorption with $Ca(NO_3)_2$ were termed as effectively adsorbed amounts and obtained by the difference between the desorbed (mmol kg^{-1}) from the previously adsorbed amounts (mmol kg^{-1}).

2.2. Incubation experiment

2.3.1. Soil characterization

Three top soils (0-20 cm) were collected from a Smelting Plant area located in Três Marias, State of Minas Gerais, Brazil as follows: a Technosol severely contaminated, located in an area historically used for disposing of metallurgic residues; an Oxisol, collected from a place located close to the industrial zone, currently used as recovery area with eucalyptus and pasture, for soil covering; and an Oxisol from a location outside the industrial sites, with no register of residues disposal apart from the dust deposition of the metals, currently covered with established eucalyptus cultivation acting as wind and dust barrier. Soil samples were well mixed, air-dried, and sieved through 4 mm for pots experiment and 2 mm for soil chemical and physical characterization. The soils were first analyzed for their soluble Cd, Zn, and Pb content extracted with Rhizon samplers (Rhizosphere Research Products, Wageningen, The Netherlands) after 24 h incubation with ultrapure water at field capacity. The soluble levels of Cd, Zn, and Pb were analyzed by ICP-OES (Spectro - model Blue, Germany). Soil solution electrical conductivity (EC) was measured with a pH meter (Metller Toledo, Model Seven Multi, Schwerzenbach) and the ionic strength (I) of the soil solution was estimated from the equation: I (mol L^{-1}) = 0.0127EC (dS m^{-1}) (Griffin and Jurinak, 1973). The soil pH in water, KCl, and $CaCl_2$ was determined as described in Teixeira et al. (2017). The determination of soil texture and organic matter content followed Bouyoucos (1951) and Walkley and Black (1934) methodologies, respectively.

2.3.2. Experimental design

The experiment was carried out in a greenhouse with controlled temperatures (26±3°C). The completely randomized experiment included four replicates, three products (HYP-1, HYP-2, and zeolite), four rates of each product (0.75, 1.5, 3.0, and 6.0% product:soil ratio (w/w), equivalent to 15, 30, 60, and 120 Mg ha^{-1} in 0-20 cm depth), and a control (soil without amendment). The products, were mixed with 3.5 kg soil, packed in the 4-L pots, and incubated for 90 days. During the incubation period, soil moisture was kept close to the water holding capacity by irrigation based on weekly weight changes. The pots were irrigated by hand using only deionized water.

2.3.3. Soil solution and soil analyses

Soil solutions were extracted using one Rhizon (Rhizosphere Research Products, Wageningen, The Netherlands) per pot, installed at a 45° angle. The pots were weighed and standardized for soil moisture a day before each sampling. Soil solutions were collected at 7, 14, 28, 42, 56, 70, and 84 days after starting the incubation using a syringe and stored at 4ºC before being analyzed for Cd, Pb, and Zn using ICP-OES (Spectro - model Blue, Germany). After completing the incubation period, soils were dried and analyzed for pH (1:2.5 soil: water ratio) and CEC (sum of Ca, K, Mg, and H + Al, determined following Teixeira et al. (2017)).

2.3. Statistical analyses

Analysis of variance was performed to verify the effect of products addition, products rate, and their interaction on decreasing soluble metal contents in the contaminated soils. Regression analysis was performed for modeling the effect of each amendment on Cd, Pb, and Zn concentrations. These concentrations were log-transformed before fitting the models for correcting detected violations of both normality and homoscedasticity (Zuur et al., 2010). Those analyses were conducted in the R environment (R Development Core Team, 2016). Comparison of desorbed and effectively adsorbed amounts of Cd, Zn, and Pb between materials and comparison of soil pH and CEC after incubation period between control and amendments were performed using Tukey´s test ($p<0.05$) and Dunnett's test ($p<0.05$), respectively with STATISTICA software (StatSoft, 2011).

3. Results and discussion

3.1. Characterization of adsorbents/amendments

The pH of materials (HYP-1, HYP-2, and zeolite) varied from 8 to 12.6, and their CEC from 530 to 1971 $\text{mmol}_c\ \text{kg}^{-1}$ (Table 1). Based on SEM images with EDS, the main composition of HYP, an aluminosilicate after the hydrothermal process, was still silicon (Si), aluminum (Al), and oxygen (O) (Fig. S2 and S3). Peaks of potassium (K) and calcium (Ca) were observed in all materials.

Mineralogical analysis of X-ray diffraction (XRD) confirmed the presence of clinoptilolite in the zeolite sample (Yang et al., 2010) (Fig. S4). HydroPotash-1 presented microcline, orthoclase, albite, pigeonite, and diopside in the crystalline phase. On the other hand, XRD detected in HYP-2, tobermorite, diopside, albite, pigeonite, plazolite, magnesioferrite, microcline, and orthoclase. These results are consistent with those found by Ciceri et al. (2017a), reporting K-feldspar, plazolite, tobermorite, and albite in hydrothermally-altered feldspar (T = 200ºC; t = 5 h).

Table 1. Physical characteristics, pH, CEC and total elemental analyses of HYPs and zeolite.

Analysis	HYP-1	HYP-2	Zeolite
Particle size (μm)	150	150	45
Color	white	white	beige to slightly greenish
pH	12.6	11.3	8.00
CEC (mmol$_c$ kg^{-1})	1,971	530	1,500
Si (%)	15.9	16.2	29.4
Ca (%)	15.7	13.6	2.20
Al (%)	4.87	4.73	5.48
K (%)	7.08	7.31	2.97
Fe (%)	1.97	2.12	1.12
Na (%)	0.51	0.59	0.14
Ba (%)	0.50	0.50	0.10
Mg (%)	0.22	0.22	0.40
Ti (%)	0.15	0.17	0.11
P (%)	0.05	0.05	0.01
Mn (%)	0.05	0.05	0.02
S (%)	0.01	0.01	0.00
Cd (mg kg^{-1})	0.02	0.02	0.01
Pb (mg kg^{-1})	0.52	0.49	0.58
Zn (mg kg^{-1})	3.71	4.08	0.75

3.2. Sorption in natural suspension pH

The amounts of metals sorbed (adsorbed/precipitated) on the three adsorbents are shown in Table 2. From the initial amount of each metal added (10 mmol L^{-1}), 99.9% Zn, 40.7% Cd, and 99.7% Pb were removed from the solution by HYP-1. HydroPotash-2 and zeolite removed 20.6-68.4%, and 10.5-54.3% of the total amount of metals added, respectively. Cadmium was the metal with the lowest affinity for all adsorbents, a result that was reported also by Costa et al. (2020) on a study evaluating sorption reactions of selected cations on an aluminum-mining by-product. Zeolite showed the lowest sorption values for all metals tested.

This experiment was performed by mixing adsorbents with 10 mmol L^{-1} of Cd, Zn, and Pb without adjusting suspension pH. The values of suspension pH after adding HYP-1, HYP-2, and zeolite and before adding stock solutions of metals are also presented in Table 2 as pH before reaction. They were 11.5, 10.9, and 8.6 for HYP-1, HYP-2, and zeolite, respectively (Table 2). Given the high pH value obtained, metals (Cd, Zn, and Pb) added into these solutions would be predominantly precipitated instead of adsorbed (He et al., 2016). Precipitation could be initiated by ions leached from the sorbents (mostly hydroxide) (Pohl, 2020). This is especially relevant for HYPs, which have $Ca(OH)_2$ as raw material for synthesis. Also, these pHs are greater than the first hydrolysis constants (pK_1) of Pb (7.7), Zn (9.0), and Cd (10.1), indicating that these metals should be hydrolyzed in solution (McBride, 1989; Pierangeli et al., 2005). When the metals precipitate as hydroxides, a pH drop will be expected because of the consumption of solution hydroxide. This could partly explain the lower values of pH in solutions after reactions/sorption compared with the situation before (Table 2). The pHs for HYP-2 and zeolite solutions were reduced by 4.6 and 2.7 units, respectively, after adding Pb. The same marked decrease was observed after Cd sorption onto HYP-1 (3.9 units), HYP-2 (3.4 units), and after Zn sorption onto HYP-2 and zeolite.

Table 2. Sorption of Zn, Cd, and Pb by adsorbents in natural suspension pH. Mean values and standard errors (in parentheses) (n = 3).

Samples	**pH before reaction**[a]	**pH after reaction**			**Sorption**[b] **%**		
		Zn	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**
HYP-1	11.5 (0.01)	10.2 (0.13)	7.60 (0.05)	11.1 (0.05)	99.9 (0.01)	40.7 (1.20)	99.7 (0.0
HYP-2	10.9 (0.01)	7.10 (0.16)	7.50 (0.02)	6.30 (0.01)	63.8 (0.38)	20.6 (1.57)	68.4 (4.1
Zeolite	8.6 (0.30)	7.10 (0.02)	7.50 (0.03)	5.90 (0.18)	21.0 (0.54)	10.5 (0.44)	54.3 (10.

[a] pH at 24h after reaction with $Ca(NO_3)_2$ (pH = 5.5, 10 mmol L^{-1}) – suspension pH.

[b] Element adsorbed in relation to the initial amount of each metal added.

Sorption/desorption reactions at pH 5.5

3.3.1 Sorption isotherm

Based on the values of the determination coefficient (R^2), the Freundlich model fitted better ($R^2 = 0.83$-0.93) the data for Zn and Cd sorption by the three amendments than the Langmuir model ($R^2 = 0.14$-0.37) except for Zn adsorption by zeolite, which was well fitted with both Freundlich model ($R^2 = 0.93$) and Langmuir model ($R^2 = 0.92$) (Table 3). Similar results were reported for the adsorption of Zn by zeolite (Mirjavadi et al., 2019) and Cd by K-feldspar (Aşçı et al., 2008), and by synthetic minerals (Chen and Shi, 2017). Lead sorption by the three amendments fitted well to both the Freundlich model (R^2 = 0.90-0.92) and Langmuir model (R^2 = 0.89-0.99).

Table 3. Langmuir and Freundlich isotherm parameters after adsorption of Cd, Zn, and Pb metals onto HYP-1, HYP-2, and zeolite (n = 3).

Adsorbate	Adsorbent	Langmuir Isotherm			Freundlich Isotherm		
		b^a (mmol kg^{-1})	K_l	R^2	n	K_f	R^2
Zn	HYP-1	203 (6.57)	0.17	0.14	1.26	25.4	0.83
	HYP-2	148 (15.01)	0.30	0.23	1.31	30.2	0.87
	Zeolite	43.8 (3.11)	2.36	0.92	1.77	30.6	0.93
Cd	HYP-1	-8.25 (2.80)	-0.66	0.22	0.71	17.3	0.90
	HYP-2	49.1 (9.30)	0.46	0.32	1.23	14.4	0.92
	Zeolite	37.5 (3.64)	0.42	0.37	1.24	10.6	0.91
Pb	HYP-1	44.4 (6.44)	4.59	0.89	2.04	37.6	0.90
	HYP-2	98.3 (2.55)	7.94	0.90	2.05	104	0.92
	Zeolite	165.6 (4.75)	119	0.99	1.52	1,481	0.91

[a] Mean values and standard errors (in parentheses) (n = 3).

The Freundlich model assumes that the heavy metal is adsorbed in a heterogeneous surface (Javadian et al., 2013), differently from the Langmuir model, which represents monolayer adsorption into a homogeneous surface (Mirjavadi et al., 2019). From the Freundlich model is possible to calculate the K_f, which predicts the affinity between adsorbent and adsorbate (Giannakopoulou et al., 2012; Sposito, 1989). Among the adsorbents in our study, zeolite showed the highest K_f values for the adsorption of Zn (30.6) and Pb (1,481). For Cd adsorption, the highest K_f values were found with HYP-1 (17.3) and HYP-2 (14.4), which were 36-63.1% higher than with zeolite (10.6).

Compared with other materials, zeolite sorbed the highest amount of Zn in all doses tested, except for the dose 1.5 mmol L^{-1} (Fig. 1). HydroPotash-1, HYP-2, and zeolite showed similar Cd sorption capacities at the doses between 0.05 and 0.75 mmol L^{-1}, whereas HYP-1 had the highest Cd sorption at the highest dose (1.5 mmol L^{-1}). The order of Pb sorption capacity was zeolite>HYP-2>HYP-1. The high sorption capacity of zeolite led the Pb concentration in the equilibrium solution to be close to zero. The selectivity and preference of Pb sorption by zeolite have been described (He et al., 2016; Li et al., 2018; Li et al., 2019; Liu et al., 2019), and it is probably due to the lower dehydration energy and smaller hydrated radii of Pb (He et al., 2016).

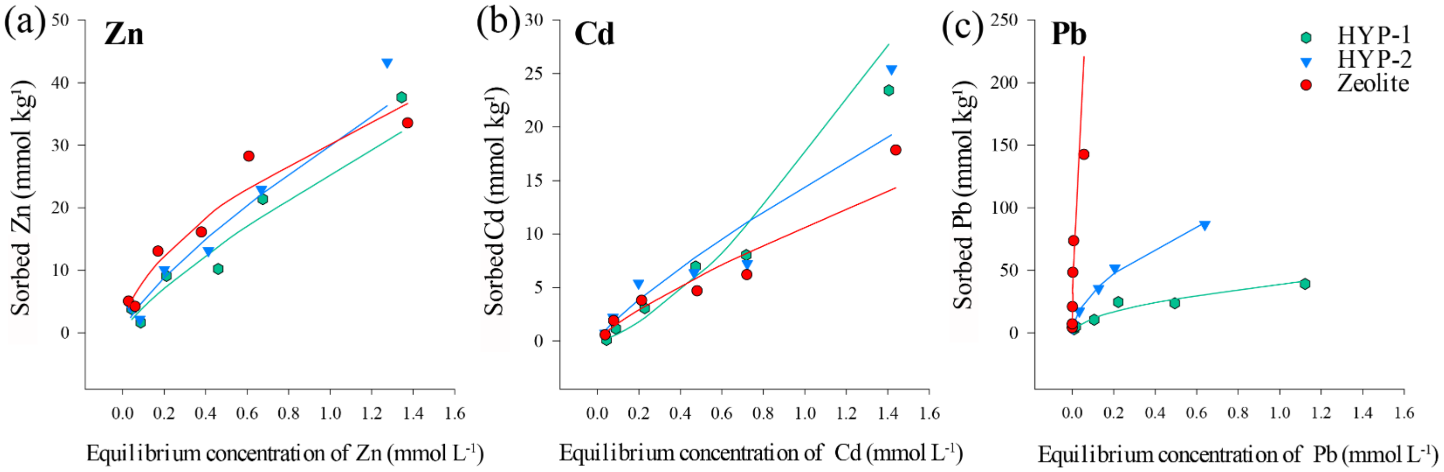


Fig. 1. Zinc (a), Cd (b) and Pb (c) sorbed (mmol kg^{-1}) as a function of equilibrium concentrations of metals (mmol L^{-1}). The points indicate data obtained experimentally, and the lines, provided by the Freundlich model.

After sorption, the pH of solutions was measured and the highest pH observed was 5.79 (Table S1). At this pH, precipitation of tested metals is not expected (Pierangeli et al., 2005, 2007; Marchi et al., 2015; Pohl, 2020). This scenario is different from the test of sorption at natural suspension pH that leads to overestimating the amount of metals adsorbed due to precedence of precipitation processes over any other sorption mechanism. A drop in solution pH was observed after Pb sorption into materials, especially in the last two doses (Table S1). Under low pHs of final solutions, the specific adsorption or inner-sphere complex should have occurred (Sposito, 1989). In this case, Pb displaced H$^+$ from the hydroxyl groups, decreasing the solution pH.

3.3.2 Desorption results

The amounts of metals desorbed from amendments were positively correlated to the initial concentrations of these elements previously adsorbed (Table 4). Higher amounts of Zn were desorbed from zeolite than from HYPs, indicating that the affinity between Zn and the surface of HYPs was stronger than with zeolite. The weaker retention of metal by the adsorbents is possibly due to water molecules between Zn and surface functional groups (Sposito, 1989). The zeolite desorbed 44.5 and 212% more Cd than HYP-2 and HYP-1, respectively. However, there was very low (<0.06 mmol kg^{-1}) desorption of Pb from zeolite for treatments with low doses (0.25 mmol kg^{-1}), and the maximum desorption (0.98 mmol kg^{-1}) of Pb from zeolite occurred with the highest dose.

Table 4. Cadmium, zinc, and lead desorbed and effectively adsorbed amounts by HYP-1, HYP-2, and zeolite. Mean values and standard errors (in parentheses) (n = 3).

Desorbed amounts (mmol kg^{-1})*									
Adsorbate	Cd			Zn			Pb		
Initial dose (mmol L$^{-1)}$	HYP-1	HYP-2	Zeolite	HYP-1	HYP-2	Zeolite	HYP-1	HYP-2	Zeolite
0.00	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a
0.05	0.18 (0.02)a	0.25 (0.01)a	0.54 (0.01)b	0.17 (0.01)a	0.12 (0.06)b	0.46 (0.01)b	0.05 (0.01)a	0.03 (0.01)b	0.01 (0.00)b
0.10	0.36 (0.04)a	0.52 (0.01)a	1.04 (0.03)b	0.25 (0.01)a	0.44 (0.02)b	0.93 (0.01)c	0.11 (0.04)a	0.04 (0.01)b	0.02 (0.00)b
0.25	0.81 (0.02)a	1.36 (0.03)b	2.29 (0.07)c	0.69 (0.05)a	1.17 (0.02)b	2.19 (0.04)c	0.51 (0.01)a	0.23 (0.04)b	0.06 (0.00)c
0.50	1.62 (0.05)a	2.61 (0.04)b	4.25 (0.02)c	1.25 (0.13)a	2.33 (0.07)b	3.90 (0.04)c	0.96 (0.05)a	0.86 (0.26)a	0.15 (0.01)b
0.75	1.98 (0.13)a	3.70 (0.09)b	5.94 (0.12)c	1.95 (0.13)a	3.32 (0.04)b	5.42 (0.07)c	1.81 (0.09)a	1.45 (0.07)b	0.30 (0.02)c
1.50	3.45 (0.26)a	7.46 (0.09)b	10.78 (0.15)c	3.20 (0.12)a	6.68 (0.02)b	8.79 (0.20)c	3.43 (0.03)a	4.46 (0.16)b	0.98 (0.04)c
Effectively adsorbed amounts (mmol kg^{-1})									
Adsorbate	Cd			Zn			Pb		
Initial dose (mmol L^{-1})	HYP-1	HYP-2	Zeolite	HYP-1	HYP-2	Zeolite	HYP-1	HYP-2	Zeolite
0.00	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a	0.00 (0.00)a
0.05	0.00 (0.01)a	0.49 (0.05)a	0.04 (0.01)a	3.64 (0.07)a	4.03 (0.05)a	4.59 (0.02)a	2.87 (0.23)a	3.86 (0.02)a	4.02 (0.03)b
0.10	0.80 (0.08)a	1.71 (0.17)a	0.87 (0.12)a	1.36 (0.01)a	1.74 (0.04)a	3.28 (0.01)b	4.93 (0.73)a	6.46 (0.15)b	7.09 (0.04)c
0.25	2.26 (0.23)a	4.05 (0.15)a	1.50 (0.88)b	8.42 (0.06)a	8.96 (0.03)b	10.84 (0.17)c	9.96 (0.70)a	17.16 (0.25)b	20.86 (0.21)c
0.50	5.35 (0.15)a	3.78 (0.18)a	0.43 (0.04)b	8.97 (0.25)a	10.81 (0.17)b	12.20 (0.49)b	23.65 (1.12)a	34.56 (1.51)b	48.14 (0.63)c
0.75	6.05 (0.32)a	3.52 (0.21)a	0.55 (0.34)b	19.44 (0.49)a	19.60 (0.32)a	22.81 (0.51)c	21.90 (3.06)a	50.39 (1.11)b	73.25 (0.34)c
1.50	19.87 (0.08)a	17.99 (3.47)a	7.07 (0.90)b	34.47 (0.43)a	36.60 (0.81)a	24.77 (1.56)b	35.69 (3.36)a	82.11 (1.93)b	141.65 (0.67)c

[*] Different letters in the same line indicate significant differences between materials for each metal according to Tukey's test ($p<0.05$).

In addition to desorption, in mmol kg^{-1}, it is necessary to take into account the amount of metals effectively adsorbed (mmol kg^{-1}), determined after discounting the amount desorbed, to infer about the ability of each material to keep metals bound to the binding sites. In general, zeolite showed a greater amount of Zn effectively adsorbed, even desorbing greater amounts of this element, except in the highest dose, for which HYPs were superior. Regarding the amount effectively adsorbed of Cd, both HYPs, but especially HYP-1, were more effective than zeolite. In the highest dose (1.5 mmol L^{-1}), HYP-1 and HYP-2 effectively adsorbed 181 and 155% more Cd, respectively, than zeolite (Table 4). Zeolite showed the highest amount of Pb effectively adsorbed (141.65 mmol kg^{-1}), which was expected due to the greater amount of metals adsorbed and less desorbed.

Scanning electron microscopy was performed only for HYPs and zeolite samples after adsorption and desorption of Pb (Ci = 1.5 mmol L^{-1}), where the highest amount of metal effectively adsorbed was found (Table 4). In all materials, the presence of Pb was detected in SEM-EDS after adsorption and desorption in 1.5 mmol Pb L^{-1} (Fig. 2, S5, and S6). The elemental composition of the analyzed sample for HYP-2 was 19.9% O, 11.3% Si, 3.6% Al, 2.0% Pb, 1.6% K, and 1.4% Ca. Despite a possible overestimation by this surface analysis technique, SEM-EDS indicates that HYP-2 may have effectively adsorbed 20,000 mg Pb kg^{-1}, which was similar to the amount calculated 17,013 mg Pb kg^{-1} (82.11 mmol kg^{-1}) (Table 4). In addition to the presence of Pb in the sample, it should be emphasized that it had a homogeneous distribution and a strong relationship with the distribution of Si, O, and Al (Fig. 2). As observed in the characterization of HYP-2 (Fig. S4), Ciceri et al. (2017a) also detected tobermorite ($Ca_5Si_6O_{16}(OH)_2\cdot 4H_2O$) with isomorphic incorporations in the hydrothermal material. These tobermorites act as an ion exchanger and can adsorb heavy metals (Guo and Shi, 2017). The main mechanisms of Pb adsorption described by Guo and Shi (2017) for both tobermorite and Al-substituted tobermorite are surface complexation and uptake in the interlayer with main functional group hydrocerussite ($Pb_3(CO_3)_2(OH)_2$) after adsorption.

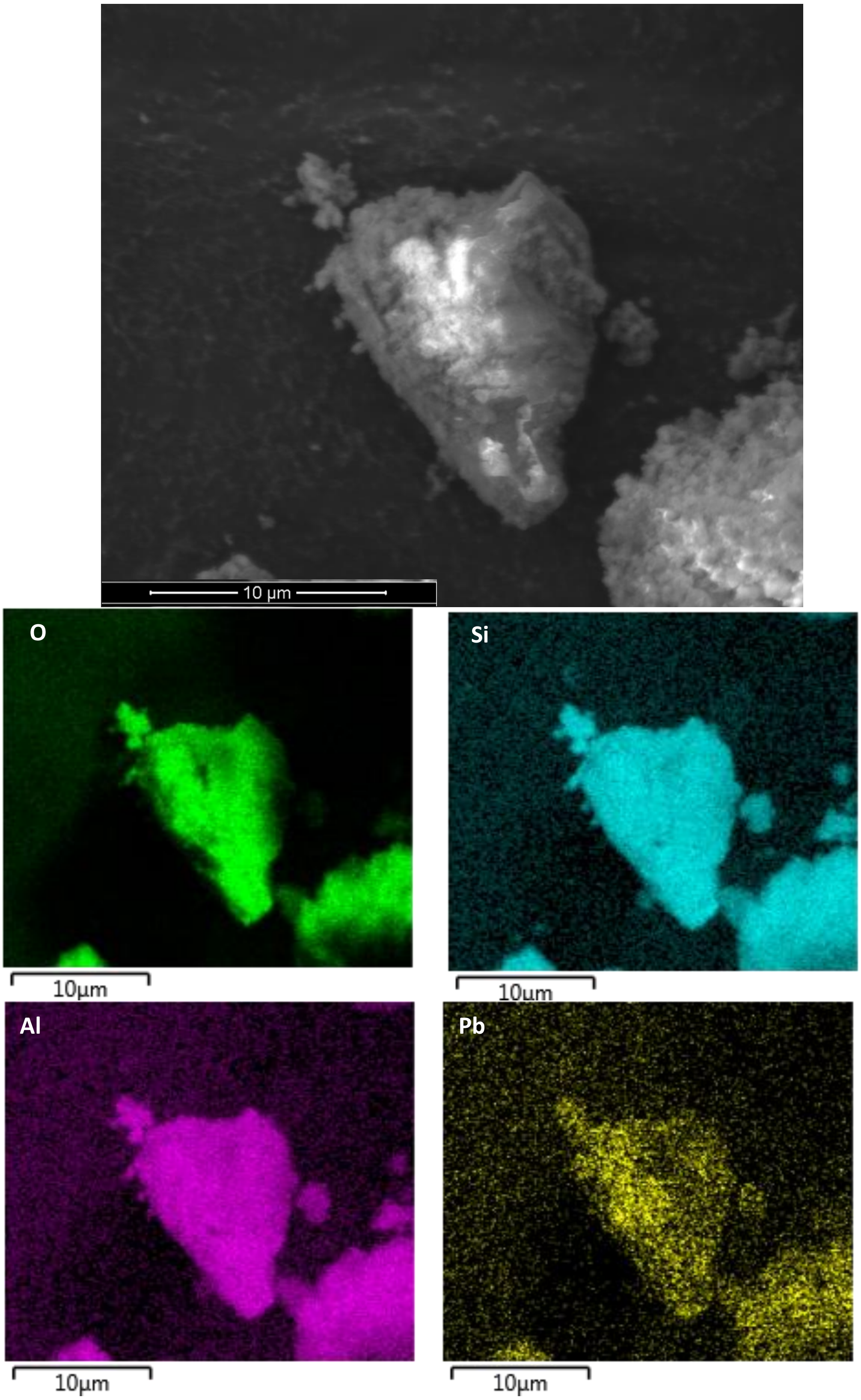


Fig. 2. Scanning electron microscopy and elemental mapping images of HYP-2 after Pb adsorption and desorption (Ci = 1.5 mmol L^{-1}).

3.3. Soil solution assessments from incubation experiment

The soils were classified regarding their contamination, based on the Cd, Zn, and Pb levels in soil solution, as follows: Soil$_{high}$ (Technosol); Soil$_{intermediate}$ (Oxisol from recovery area) and Soil$_{low}$ (Oxisol covered with established eucalyptus) (Table 5). In addition to metal solubility, soils varied in their basic properties and showed 23-61% sand, 17-64% silt, 13-45% clay, 4.5-7.7 pH (pH in H_2O), and 0.71-17.85 dS m^{-1} EC (Table 5). The mobility of heavy metals in the soil is directly related to pH, ionic strength, oxidation potential, texture, moisture, and ion interaction (Kabata-Pendias, 2011; McBride, 1989), thus the effects of materials were evaluated in each specific soil condition.

Table 5. Characterization of the soils collected in the zinc smelting area, Três Marias, Minas Gerais State, Brazil.

Properties	**Soil$_{high}$**	**Soil$_{intermediate}$**	**Soil$_{low}$**
pH (H_2O)	4.5	5.5	7.7
pH ($CaCl_2$)	4.5	5.4	7.5
pH(KCl)	4.1	5.2	7.6
Organic matter (g kg^{-1})	0.06	0.69	2.47
Clay (%)	13	45	22
Silt (%)	64	16	17
Sand (%)	23	39	61
Cd (mg L^{-1})	17	34	0.2
Zn (mg L^{-1})	14,866	535	3.6
Pb (mg L^{-1})	0.36	0.03	0.01
Electrical conductivity (dS m^{-1})	17.85	2.71	0.71
I*est* (mmol L^{-1})[a]	226	34	9.0

[a] estimated ionic strength of the soil solution.

The contents of Cd, Zn, and Pb, extracted at 7, 42, and 70 days of incubation, are presented for soil$_{high}$, soil$_{intermediate,}$ and soil$_{low}$ in Fig. 3, 4, and 5, respectively. The other extractions can be found in the supplementary material (Fig. S7, S8, and S9). In general, the effect of products was the same during all experiment. This is relevant since HYPs effectively immobilize metals in the first week and the effect remains until the last soil solution extraction - after 84 days of soil incubation with products.

In soil$_{high}$, the immobilization effect increases with increasing doses of HYPs, which is not observed in the treatments that received zeolite (Fig. 3). HydroPotash promoted an effective reduction of the soluble contents of metals (up to 99.9% removal) compared with control ($p<0.001$). In $\text{soil}_{\text{intermediate}}$, HYPs immobilized almost 100% of the Cd and Zn present in the soil solution in the first dose (Fig. 4). In soil_{low}, the HYPs reduced Cd and Zn soluble concentration compared with the control, except for Pb, which tended to increase with the two highest doses of HYPs applied (Fig. 5). An increase of soluble or extractable content of Pb in the soil after application of amendments have been reported (Aragón et al., 2019; Bade et al., 2012; Penido et al., 2019) and some of the reasons suggested are dissolution of Pb-organo complex and the oxidation of PbS (Bade et al., 2012) and ion competition (Aragón et al., 2019).

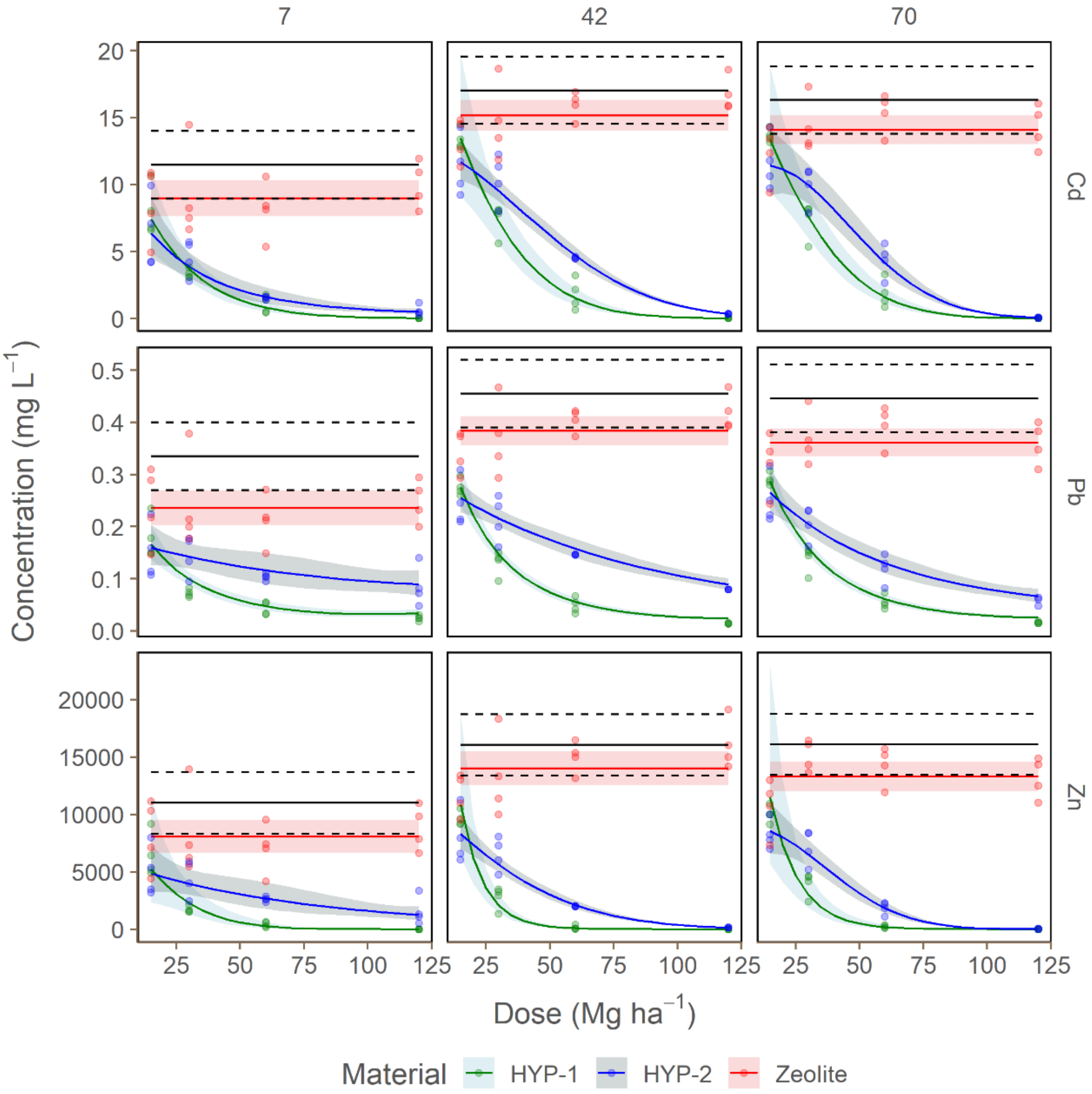


Fig. 3. Heavy metals concentration (mg L^{-1}) in soil solution after 7, 42, and 70 days of $\text{soil}_{\text{high}}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid black line represents

the mean for the control without amendment application, while the black dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

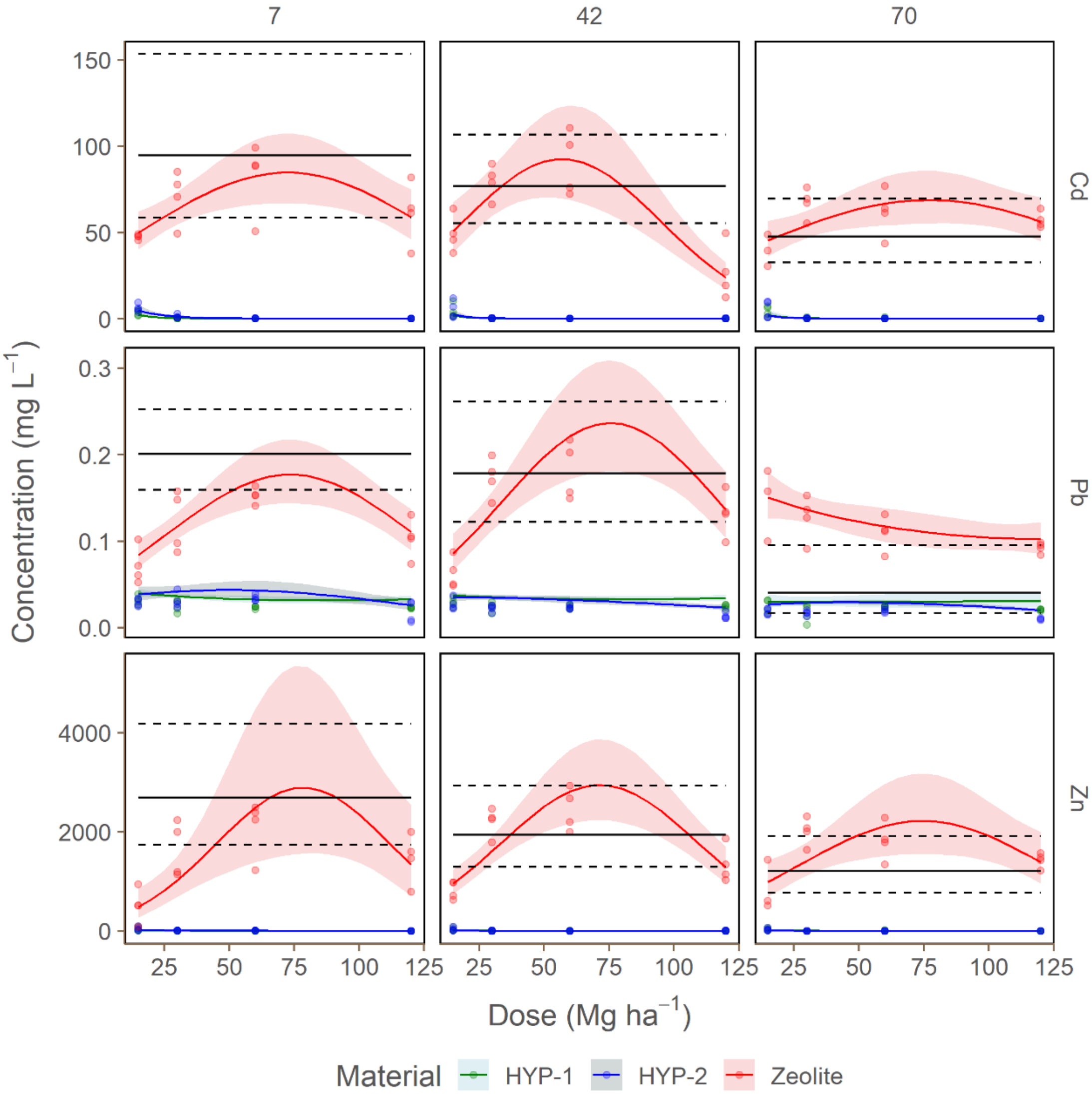


Fig. 4. Heavy metals concentration (mg L^{-1}) in soil solution after 7, 42, and 70 days of soil$_{\text{intermediate}}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid black line represents the mean for the control without amendment application, while the black dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

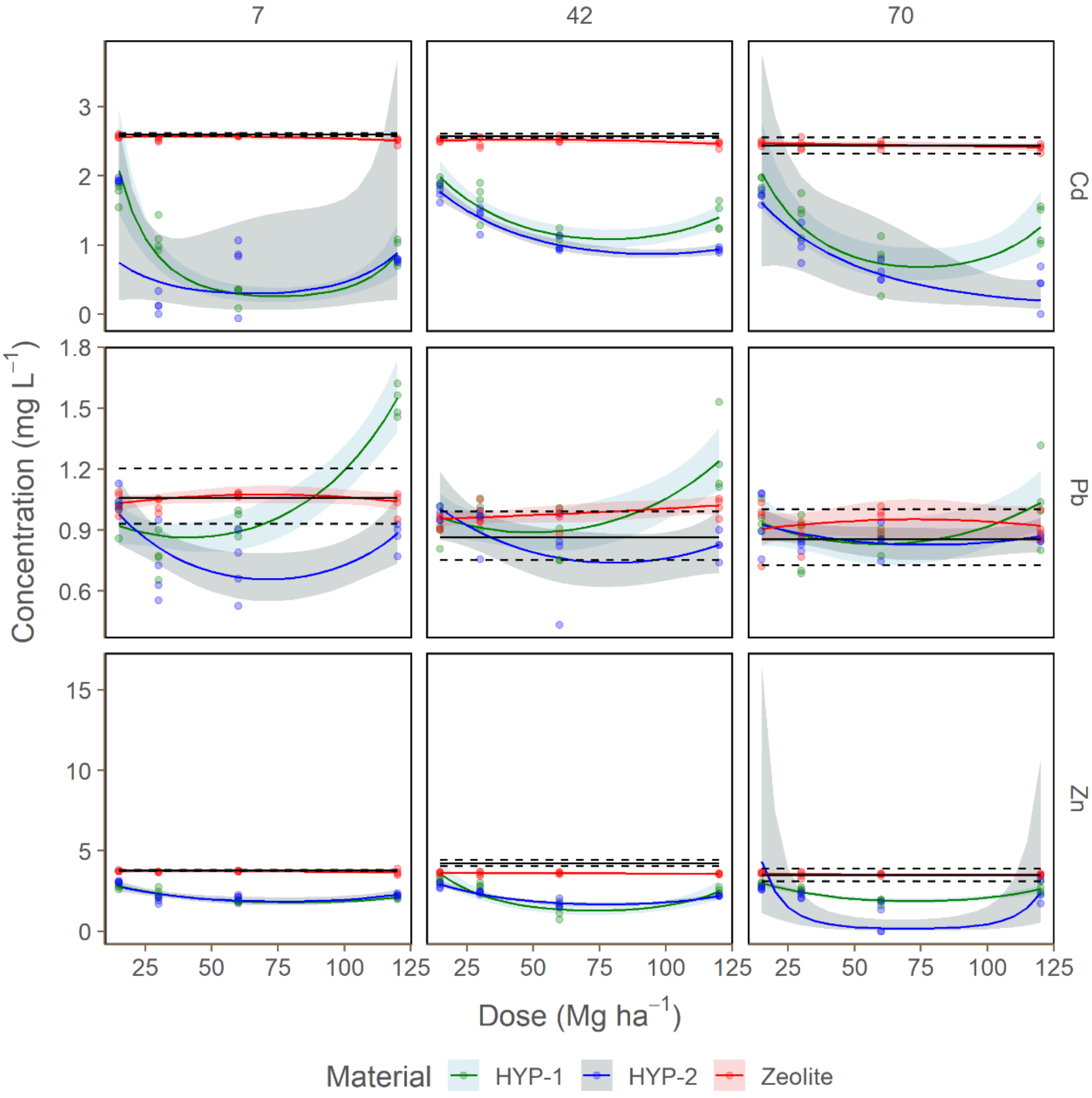


Fig. 5. Heavy metals concentration (mg L^{-1}) in soil solution after 7, 42, and 70 days of soil$_{\text{low}}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid black line represents the mean for the control without amendment application, while the black dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

Soil pH is a particular determinant for the availability of metals in soils (Bradl, 2004), as increasing soil pH may promote precipitation of metals as carbonates, hydroxides, and phosphates (Kabata-Pendias, 2011). The increase in soil pH appears to be the main reason for decreasing soluble metal content in the studied soils, with a pH value above 7 as a determinant for the drastic decrease in metals content compared with the control (Table 6). This is easily observed by comparing the decreasing trend of Cd, Zn, and Pb in the solution of $soil_{high}$ and $soil_{intermediate}$ (Fig. 3 and 4) with the increase in soil pH after the incubation period (Table 6). In the first dose of $soil_{intermediate}$, there was already an increase in soil pH to values above 7.4 in soils treated with HYP-1 and HYP-2, whereas for $soil_{high}$ this was only observed for these products at the highest dose. This possibly explains the drastic reduction of metals concentration in the solution already in the first dose in $soil_{intermediate}$ and the decrease in their content with increasing doses in the $soil_{high}$. Other reasons influencing these dose-effects are the possibility of ionic competition observed in $soil_{high}$ (higher electrical conductivity compared with $soil_{intermediate}$) and the fact that $soil_{intermediate}$ is a clayey soil and clay could be a source of negative charge when pH increases.

Soil pH is essential for increasing the CEC of soils with variable charges because as pH increases, the number of negative charges on the colloids increase. Thus, in addition to the precipitation effect, increasing soil pH may increase variable charges, allowing additional retention of cationic metals. All products (in the last two doses) increased CEC in all soils (Table 6).

The most significant difference between pH in H_2O and KCl was found in the $soil_{high,}$ followed by $soil_{intermediate}$ and $soil_{low}$ (Table 5). A greater difference between these two pH (Δ pH) values indicates the predominance of negative charges in the soil, while similar values suggest that the pH of the soil is close to the PZC (point of zero charge) (Naidu et al., 1994). In $soil_{low}$, with a pH of 8 and lower Δ pH (close to PZC point), the reduction of soluble metal content may be explained by specific adsorption, which confirms that HYPs have other mechanisms that are not pH-dependent to adsorb heavy metals, in accordance to the results found in adsorption tests at pH 5.5.

Table 6. Soil pH and CEC after soil incubation with amendments for 90 days. Mean values and standard errors (in parentheses) (n = 4).

Product	Dose (Mg ha^{-1})	pH			CEC^{a}		
		Soil$_{\text{high}}$	Soil$_{\text{intermediate}}$	Soil$_{\text{low}}$	Soil$_{\text{high}}$	Soil$_{\text{intermediate}}$	Soil$_{\text{low}}$
Control	0	4.8 (0.10)	6.6 (0.13)	8.1 (0.02)	9.4 (0.44)	3.7 (0.25)	3.8 (0.19)
HYP-1	15	5.5 (0.02)*	7.8 (0.21)*	8.1 (0.19)	10.9 (0.22)	4.8 (0.31)	4.0 (0.12)
HYP-1	30	5.9 (0.03)*	8.3 (0.08)*	8.7 (0.08)*	14.6 (0.46)*	5.6 (0.67)*	4.8 (0.17)*
HYP-1	60	6.6 (0.10)*	8.5 (0.03)*	8.9 (0.05)*	18.1 (0.55)*	7.2 (0.13)*	5.2 (0.28)*
HYP-1	120	7.8 (0.15)*	8.8 (0.03)*	9.4 (0.03)*	16.5 (0.64)*	7.6 (0.38)*	5.6 (0.12)*
HYP-2	15	5.4 (0.05)*	7.4 (0.12)*	8.1 (0.24)	10.7 (0.28)	4.8 (0.18)	4.8 (0.26)*
HYP-2	30	5.8 (0.05)*	7.8 (0.23)*	8.7 (0.03)*	13.9 (0.54)*	4.8 (0.69)	4.7 (0.23)*
HYP-2	60	6.3 (0.05)*	8.4 (0.05)*	9.2 (0.00)*	16.8 (0.13)*	6.9 (0.18)*	5.1 (0.04)*
HYP-2	120	7.7 (0.16)*	8.6 (0.14)*	9.7 (0.03)*	17.5 (0.36)*	6.6 (0.52)*	5.7 (0.05)*
Zeolite	15	4.8 (0.03)	5.8 (0.05)*	8.2 (0.07)	8.6 (0.59)	4.5 (0.38)	4.4 (0.07)
Zeolite	30	4.8 (0.03)	5.9 (0.19)*	8.2 (0.05)	10.1 (0.58)	5.1 (0.19)	5.3 (0.08)*
Zeolite	60	5.0 (0.12)	6.2 (0.18)	8.2 (0.03)	11.4 (0.42)*	5.8 (0.33)*	5.9 (0.23)*
Zeolite	120	5.1 (0.09)	6.6 (0.28)	8.1 (0.04)	11.4 (0.37)*	7.3 (0.44)*	7.8 (0.26)*

[a]Cation exchange capacity at pH = 7.

*Different to control group according Dunnett´s test ($p<0.05$).

Soil leaching results were a key part of the present study in order to help with a better understanding of the dominant sorption processes in the soil environment. In the case of HYPs, these materials are buffered by their minerals and pH whereas zeolite presents surface charges that act in non-specific adsorption. In a contaminated environment such as smelting-affected soils, heavy metal non-specific adsorption by zeolite may generate a transitory state, which is reversible and dependent on the soil buffering capacity. Conversely, HYPs promoted a suitable environment for heavy metals sorption by two concomitant sorption mechanisms, precipitation, and adsorption.

Several types of amendments have been studied for the remediation of contaminated areas. The product choice must be made observing, among others, the price, efficiency, safety, and compliance with legal requirements (Mench et al., 2007). HydroPotash fits in the green-chemistry principles, including the use of a safe solvent (water) and no waste or by-product derived from its production (Ciceri et al., 2017a). This product has no pathogens, and its production process can be monitored to prevent metal contamination, common problems observed in wastes used for metals immobilization, such as sewage sludge (Alvarenga et al., 2015; Forján et al., 2016). In addition, the application of HYPs decreased heavy metals concentrations to values below the critical levels adopted in Brazil for water effluents by

Normative 430/2011 (CONAMA, 2011), which are 0.2 mg Cd L^{-1}, 0.5 mg Pb $L^{-1,}$ and 5 mg Zn L^{-1}.

The present study showed the potential of HYPs in heavy metal immobilization in laboratory and greenhouse studies. As a fertilizer, this product may release nutrients (Ciceri et al., 2017a, 2019) helping plant establishment in contaminated environments. The use of HYP will have additional advantages (compared with other amendments, such as red mud, a very effective adsorbent studied by Costa et al. (2020)) for phytoremediation studies, for example, since in addition to toxicity, the lack of nutrients can be a problem. Amendments efficiency requires holistic evaluation, including their investigation in plant environments in order to clarify the changes in the soil properties and plant growth to establish bioremediation strategies. These and other subjects need to be addressed in further studies with HYPs with soil cultivation and field application.

4. Conclusions

Hydrothermally-altered feldspar (HydroPotash) showed the ability to immobilize heavy metals in both aqueous solution and smelting-affected soils. The mechanisms for immobilizing metals by HydroPotash materials were precipitation by increasing soil/solution pH and adsorption, which was proven by the batch test with adjusted pH (5.5). The zeolite tested in the present study had significant lower efficiency for immobilizing heavy metals compared with the HYPs in the soil incubation experiment.

Declaration of competing interest

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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Supplementary material

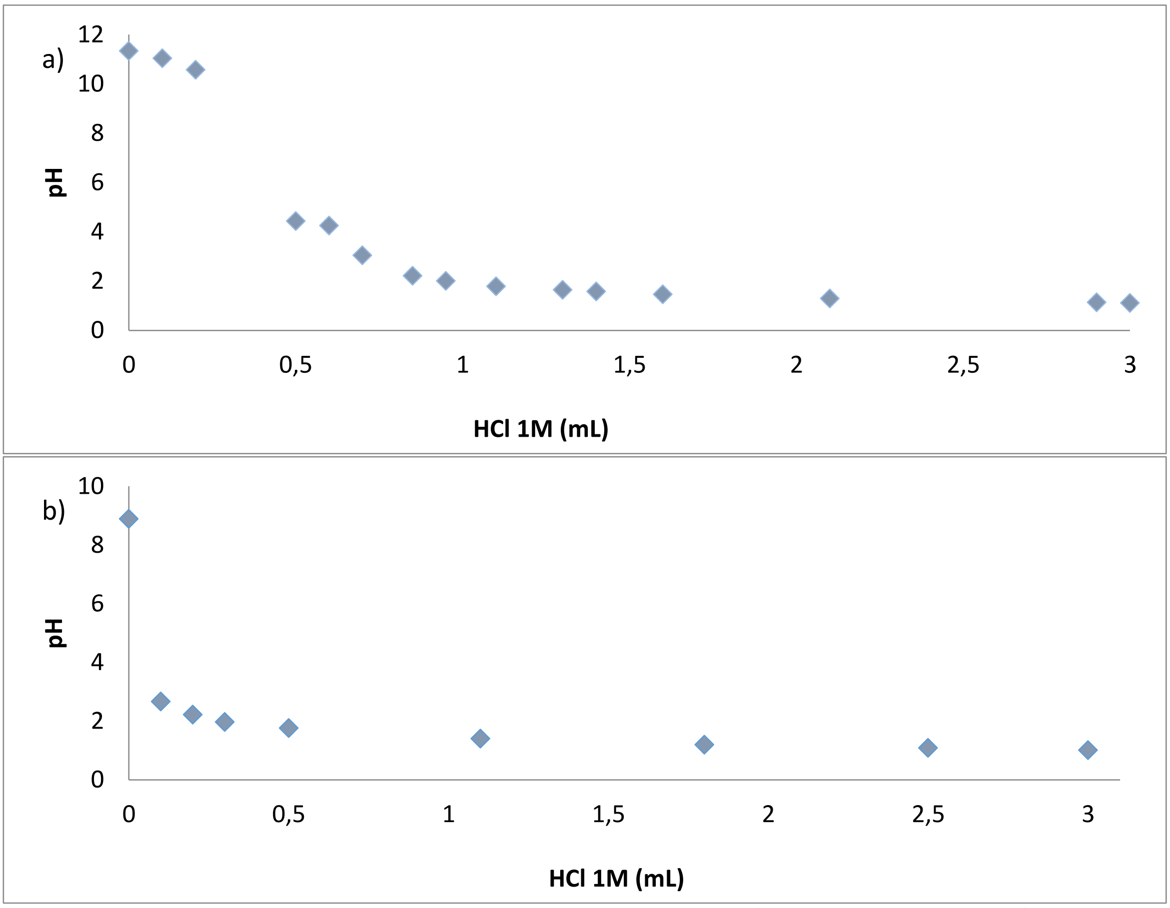

Fig. S1. Titration of HYP-1 (a), and zeolite (b) in 0.5 mol L^{-1} NaCl with 1 mol L^{-1} HCl.

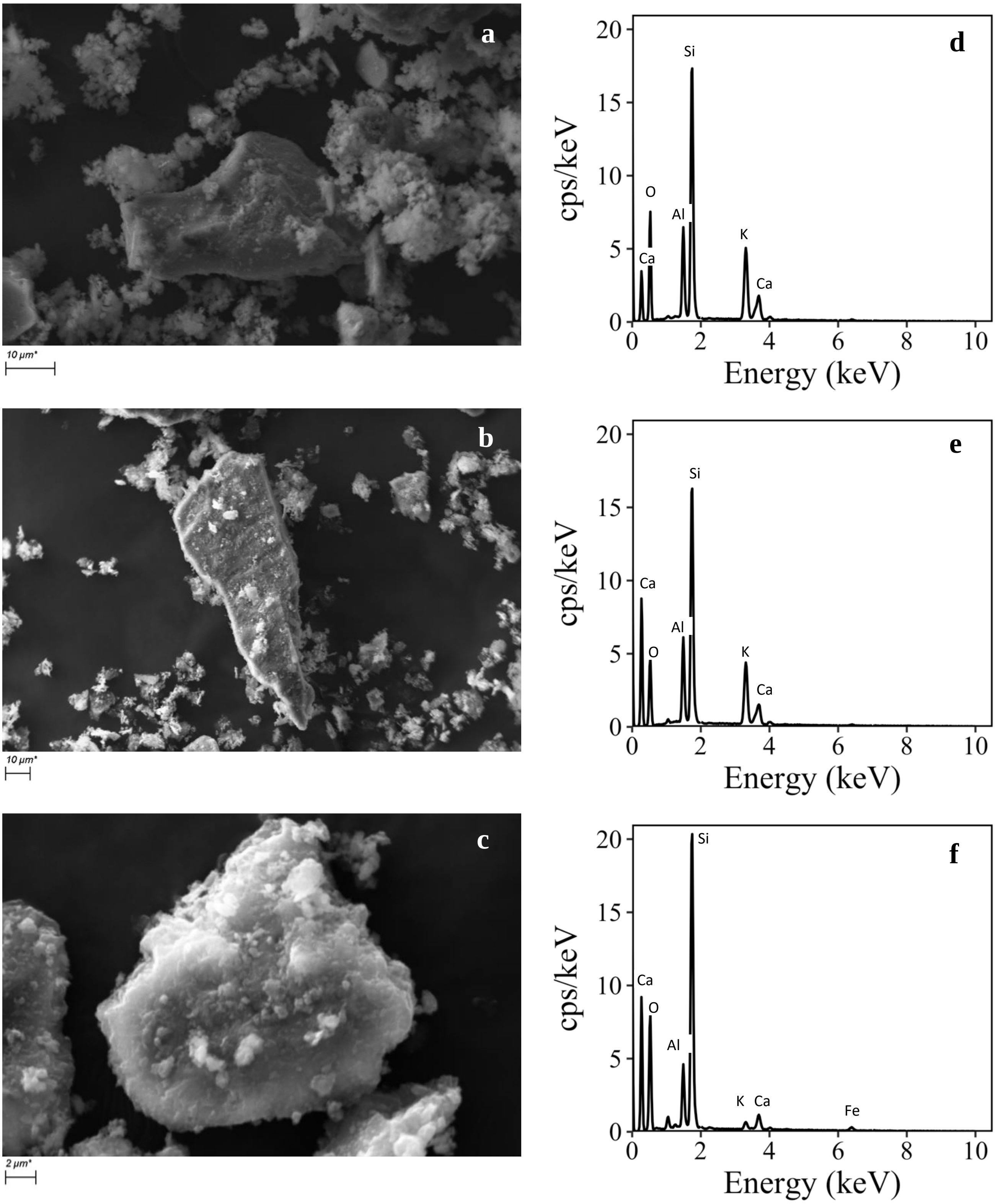

Fig. S2. SEM images of HYP-1 (a), HYP-2 (b), and zeolite (c); EDX spectrum of HYP-1 (d), HYP-2 (e), and zeolite (f) obtained from the surface of the solids shown in a, b, and c, respectively.

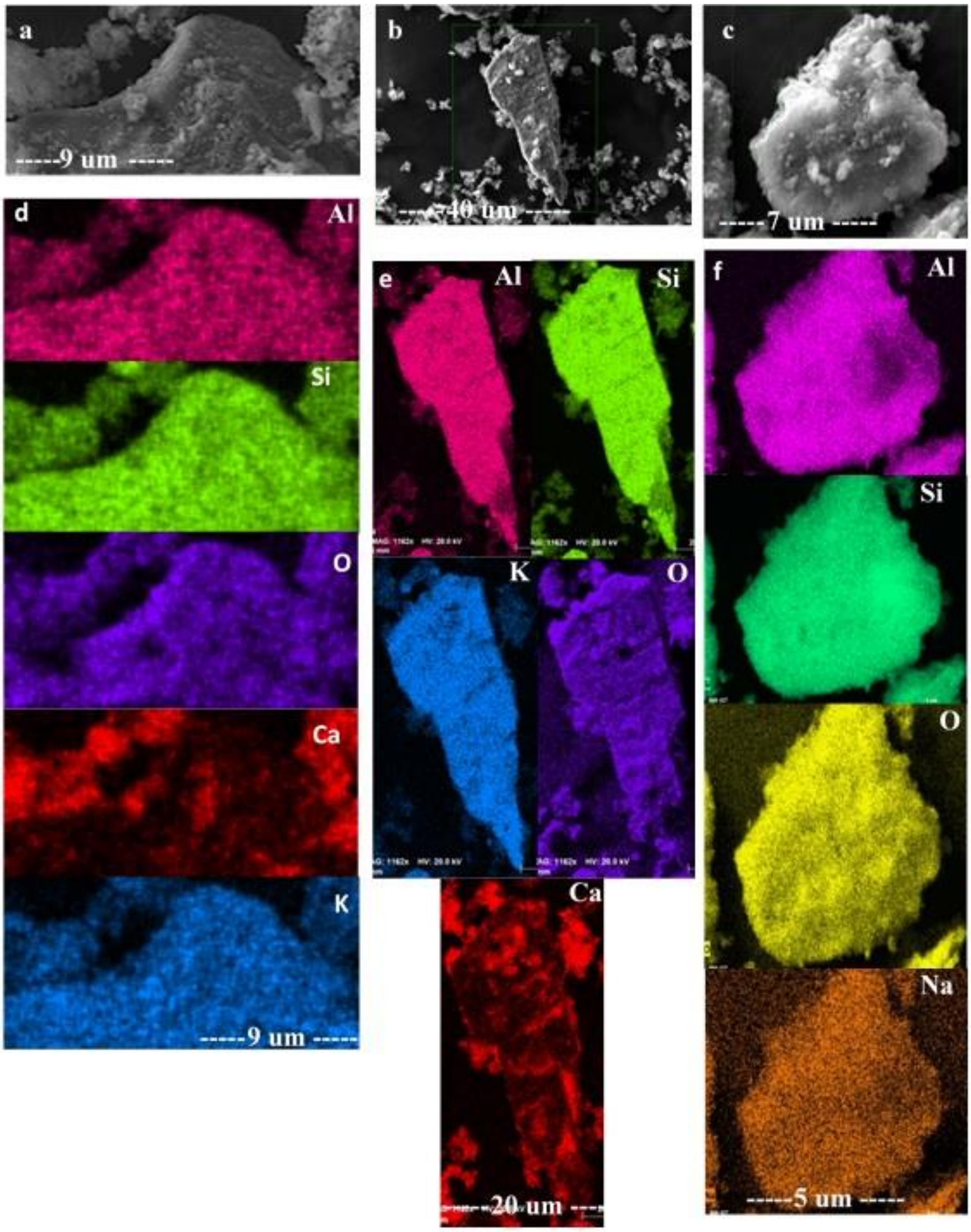

Fig. S3. Elemental mapping of HYP-1 (d), HYP-2 (e) and zeolite (f) from the surface of the solids shown in a, b and c, respectively.

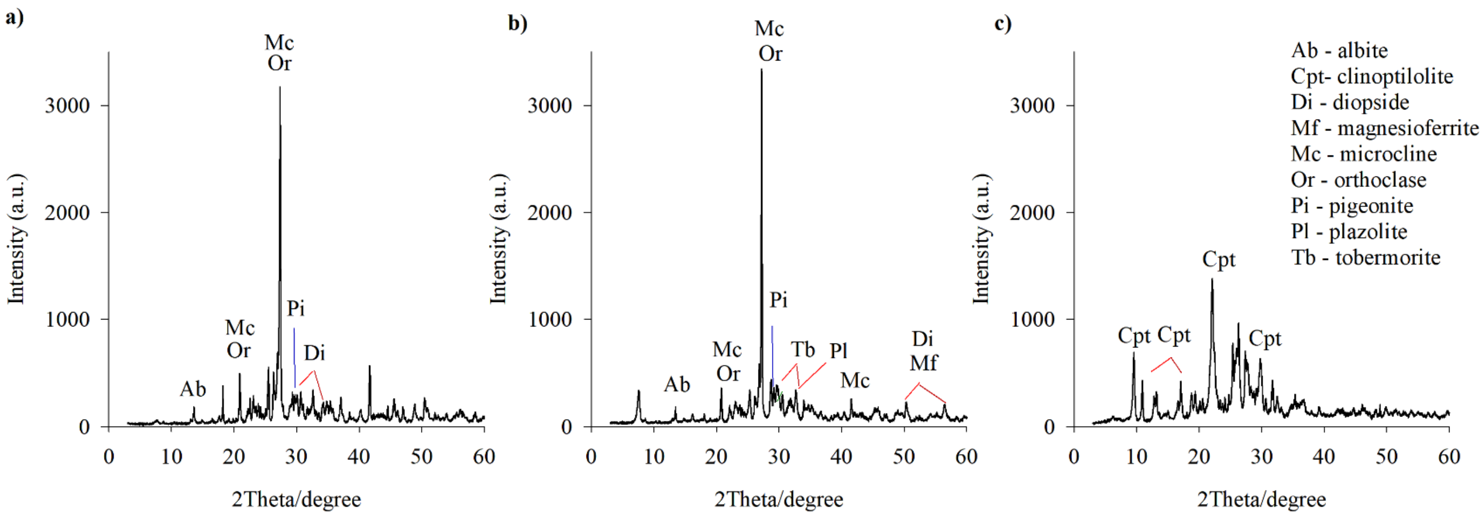

Fig. S4. X-ray diffraction pattern of a) HYP-1, b) HYP-2, and c) zeolite.

Table S1. pH of equilibrium solution before metals addition, after sorption and after desorption process. Means and standard errors (in parentheses) (n = 3).

Material	Dose (mmol L^{-1})	Cd			Pb			Zn		
		Before	Sorption	Desorption	Before	Sorption	Desorption	Before	Sorption	Desorption
HYP-1	0.05	5.25 (0.15)	5.43 (0.14)	5.89 (0.10)	5.26 (0.12)	5.42 (0.04)	5.84 (0.03)	5.26 (0.02)	5.53 (0.03)	6.08 (0.01)
	0.10	5.34 (0.10)	5.54 (0.03)	5.91 (0.06)	5.46 (0.16)	5.74 (0.28)	5.94 (0.12)	5.21 (0.04)	5.40 (0.00)	5.92 (0.02)
	0.25	5.44 (0.08)	5.49 (0.06)	5.98 (0.02)	5.33 (0.02)	5.14 (0.03)	5.77 (0.07)	5.35 (0.09)	5.45 (0.05)	5.92 (0.04)
	0.50	5.69 (0.08)	5.62 (0.03)	6.12 (0.03)	5.67 (0.03)	5.12 (0.02)	5.78 (0.03)	5.36 (0.10)	5.43 (0.11)	5.97 (0.08)
	0.75	5.45 (0.10)	5.40 (0.06)	5.88 (0.05)	5.30 (0.20)	4.78 (0.09)	5.58 (0.09)	5.47 (0.10)	5.48 (0.09)	5.96 (0.03)
	1.50	5.31 (0.18)	5.27 (0.17)	5.80 (0.15)	5.36 (0.10)	4.68 (0.05)	5.53 (0.02)	5.28 (0.09)	5.39 (0.02)	5.93 (0.01)
HYP-2	0.05	5.32 (0.01)	5.52 (0.04)	6.02 (0.05)	5.29 (0.05)	5.40 (0.06)	5.93 (0.04)	5.34 (0.01)	5.60 (0.04)	6.23 (0.01)
	0.10	5.31 (0.07)	5.48 (0.08)	6.01 (0.06)	5.39 (0.04)	5.67 (0.22)	6.06 (0.03)	5.27 (0.01)	5.55 (0.05)	6.07 (0.04)
	0.25	5.33 (0.03)	5.50 (0.05)	6.06 (0.02)	5.42 (0.04)	5.27 (0.02)	5.79 (0.03)	5.32 (0.03)	5.51 (0.02)	6.07 (0.00)
	0.50	5.32 (0.01)	5.48 (0.02)	6.00 (0.00)	5.51 (0.05)	5.16 (0.03)	5.59 (0.08)	5.37 (0.01)	5.55 (0.05)	6.17 (0.02)
	0.75	5.29 (0.02)	5.43 (0.04)	5.94 (0.00)	5.50 (0.08)	5.12 (0.04)	5.57 (0.03)	5.25 (0.03)	5.40 (0.00)	5.98 (0.01)
	1.50	5.34 (0.03)	5.42 (0.02)	5.94 (0.02)	5.38 (0.05)	4.86 (0.02)	5.39 (0.01)	5.35 (0.01)	5.46 (0.02)	6.06 (0.03)
Zeolite	0.05	5.69 (0.03)	5.73 (0.03)	5.82 (0.03)	5.55 (0.03)	5.66 (0.07)	5.76 (0.03)	5.52 (0.05)	5.57 (0.07)	5.67 (0.06)
	0.10	5.50 (0.13)	5.58 (0.11)	5.67 (0.06)	5.65 (0.02)	5.75 (0.02)	5.83 (0.01)	5.50 (0.06)	5.58 (0.06)	5.65 (0.04)
	0.25	5.67 (0.08)	5.79 (0.05)	5.87 (0.08)	5.53 (0.05)	5.59 (0.04)	5.71 (0.03)	5.47 (0.08)	5.50 (0.06)	5.63 (0.04)
	0.50	5.48 (0.04)	5.56 (0.03)	5.72 (0.01)	5.52 (0.17)	5.46 (0.11)	5.55 (0.110	5.39 (0.10)	5.40 (0.10)	5.58 (0.10)
	0.75	5.44 (0.14)	5.56 (0.10)	5.90 (0.12)	5.57 (0.10)	5.53 (0.08)	5.65 (0.07)	5.39 (0.22)	5.50 (0.12)	5.67 (0.06)
	1.50	5.55 (0.03)	5.61 (0.02)	5.86 (0.00)	5.58 (0.04)	5.18 (0.02)	5.52 (0.04)	5.65 (0.07)	5.62 (0.06)	5.81 (0.07)

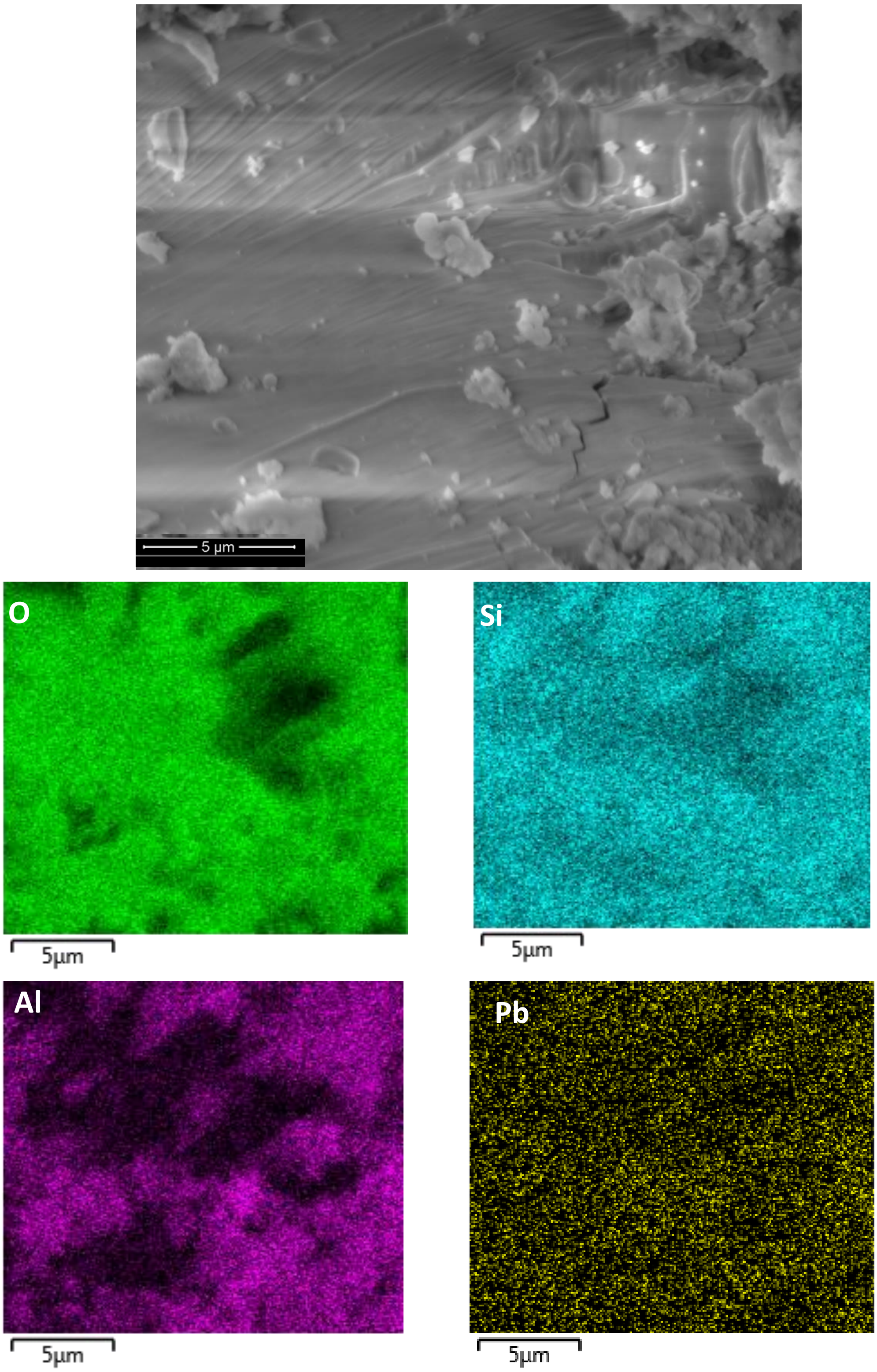

Fig. S5. SEM and elemental mapping images of HYP-1 after Pb adsorption and desorption (Ci = 1.5 mmol L^{-1}).

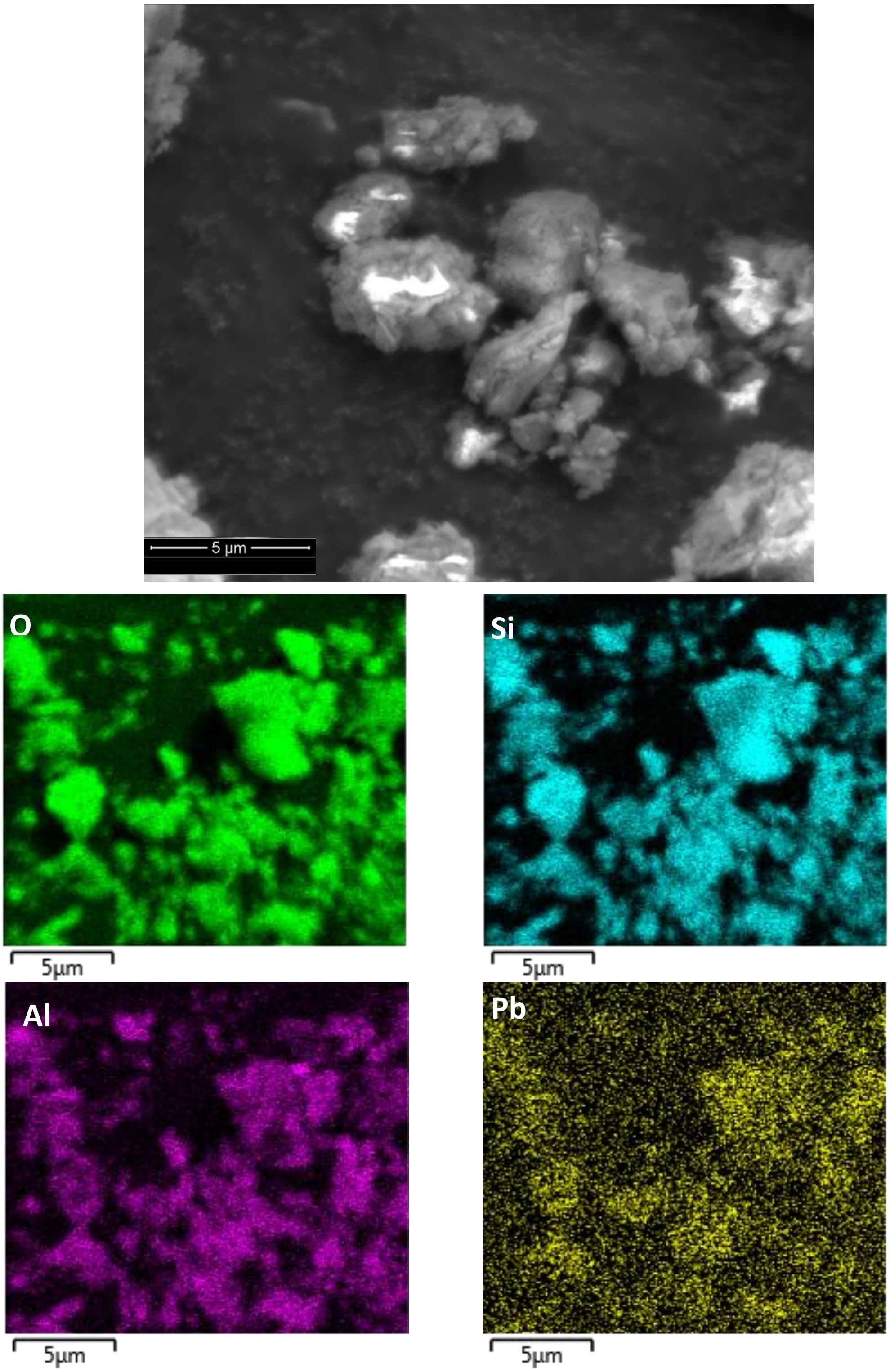

Fig. S6. SEM and elemental mapping images of zeolite after Pb adsorption and desorption (Ci = 1.5 mmol L^{-1}).

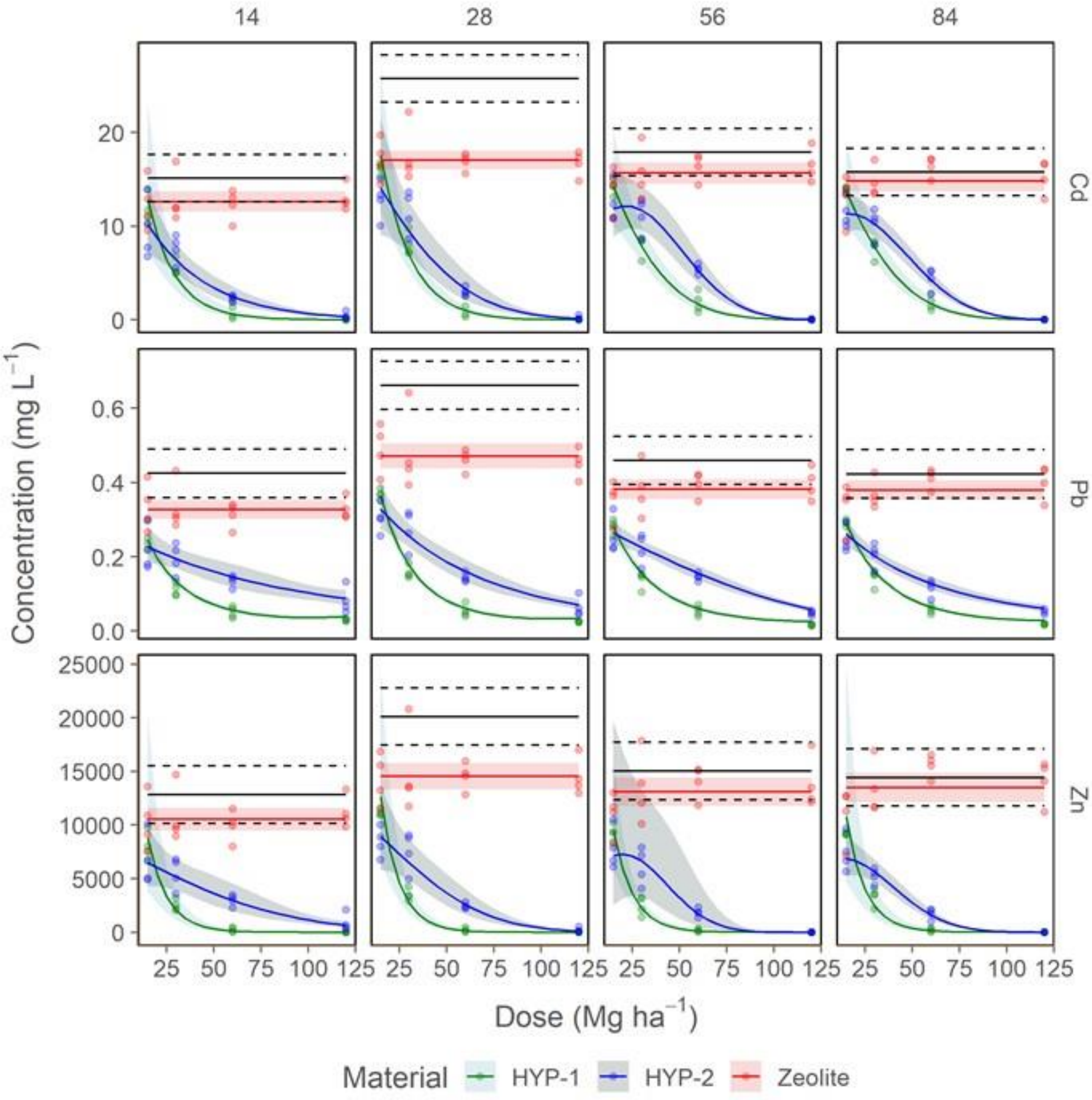

Fig. S7. Heavy metals concentration (mg L^{-1}) in soil solution after 14, 28, 56, and 84 days of soil$_{high}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid black line represents the mean for the control without amendment application, while the black dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

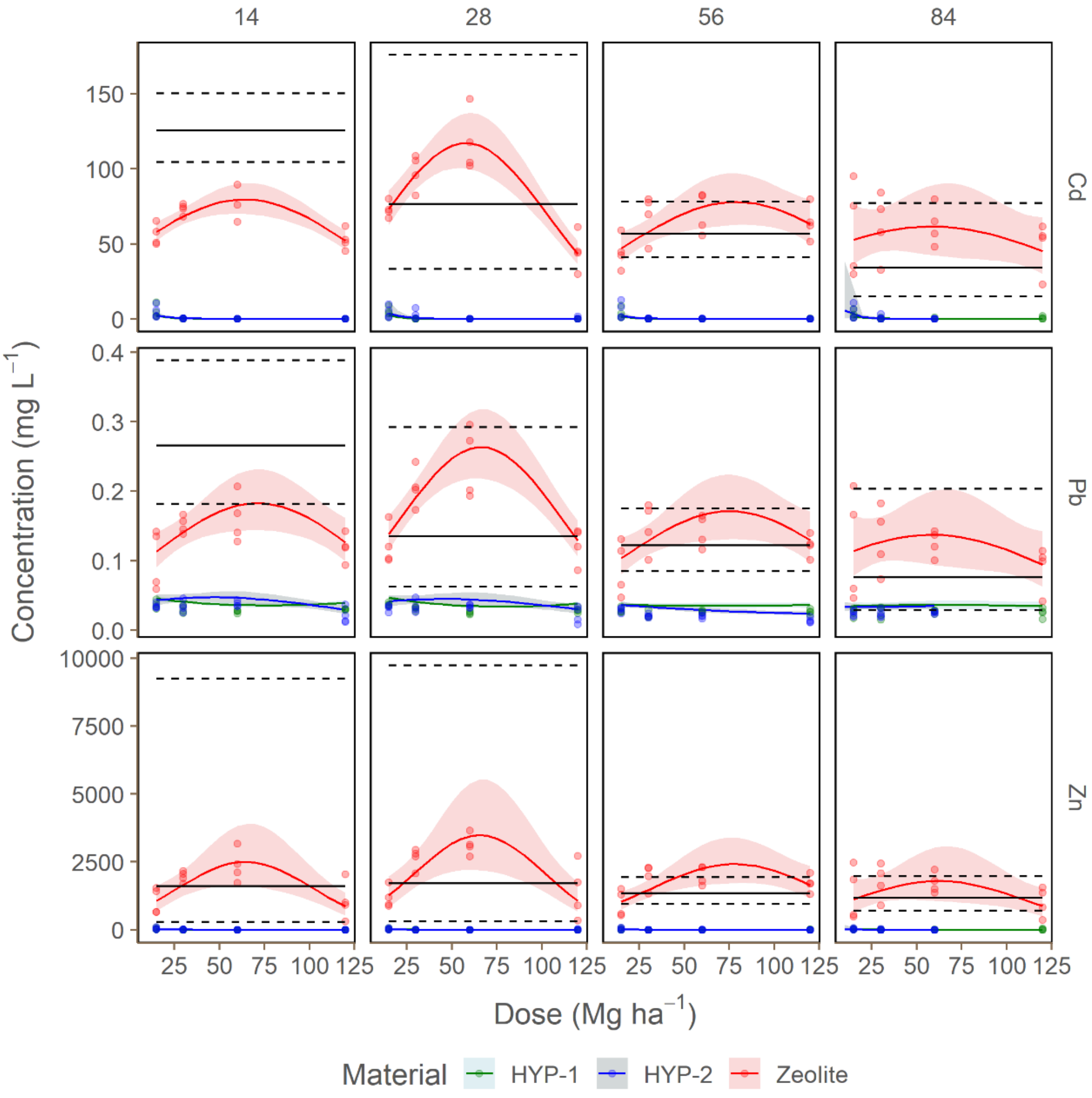

Fig. S8. Heavy metals concentration (mg L^{-1}) in soil solution after 14, 28, 56, and 84 days of soil$_{\text{intermediate}}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid black line represents the mean for the control without amendment application, while the black dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

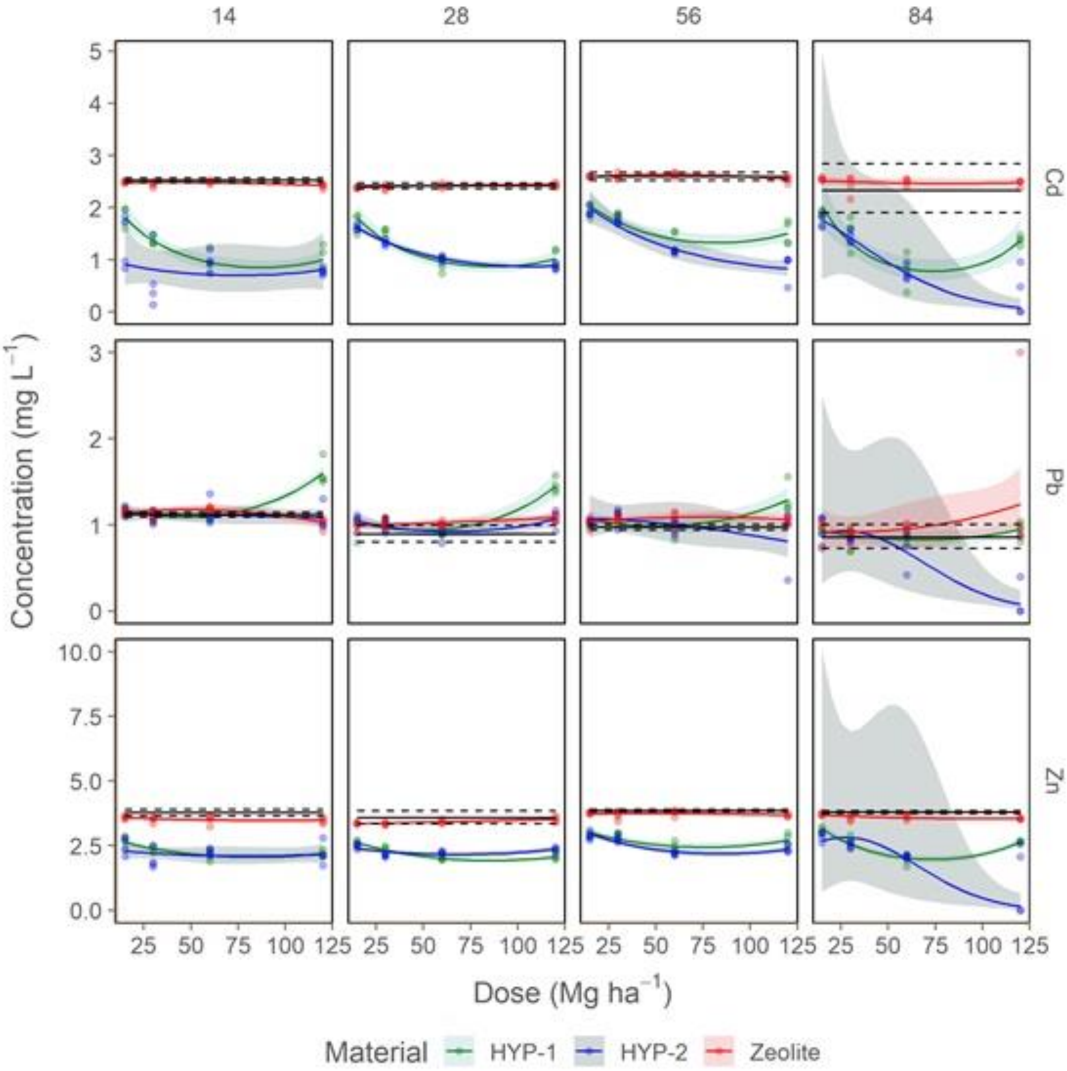

Fig. S9. Heavy metals concentration (mg L^{-1}) in soil solution after 14, 28, 56, and 84 days of soil$_{low}$ incubation with amendments. Solid lines represented the fitted values for each model, and the shaded areas represent their respective 95% confidence interval. The solid blue line represents the mean for the control without amendment application, while the blue dashed lines represent its 95% confidence interval. Non-overlapping confidence intervals indicate significant differences between treatments.

MANUSCRIPT 3: Hydrothermally-altered feldspar reduces metal toxicity and promotes plant growth in highly metal-contaminated soils

Manuscript submitted to Chemosphere

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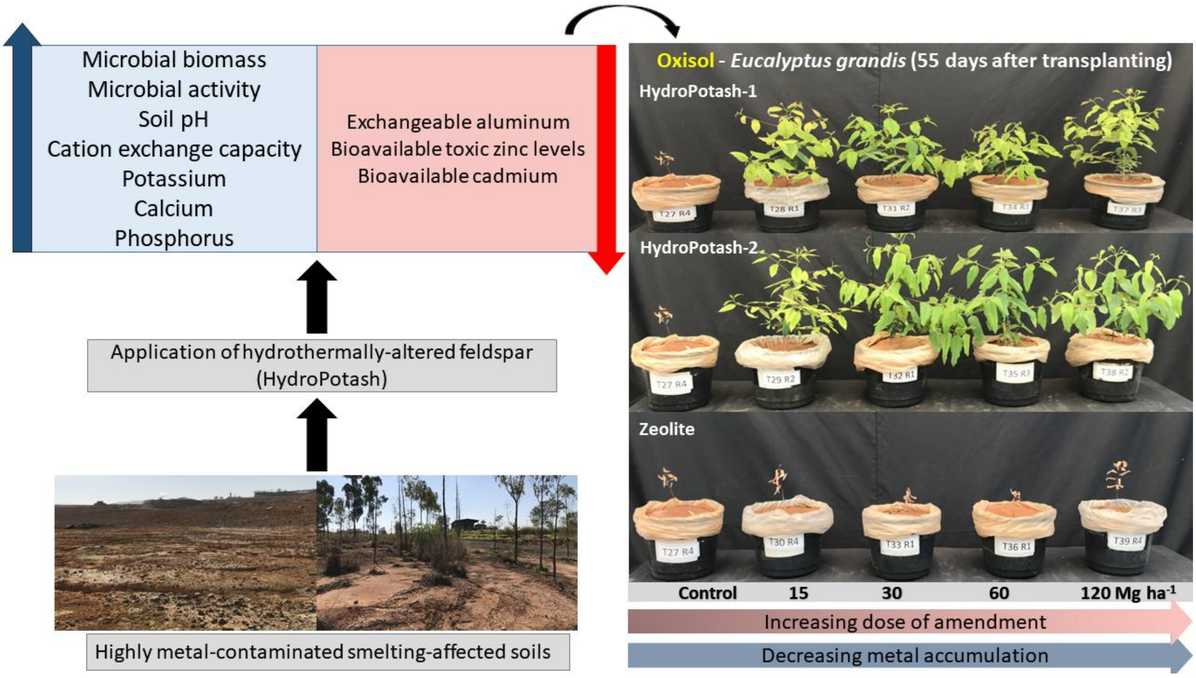
Graphical abstract



Highlights

Hydrothermally-altered feldspar (HydroPotash) decreased phytoavailable Cd in soils

HydroPotash decreased exchangeable Al and increased exchangeable K in soils

HydroPotash promoted K uptake and improved plant growth in smelting-affected soils

The application of HydroPotash increased soil microbial biomass and its activity

Abstract

Novel green technologies for soil remediation have been focusing on altering soil properties and improving soil health. Hydrothermally-altered feldspar (HYP, HydroPotash), recently developed, is being related as both an efficient amendment to immobilize heavy metals in soils and a plant nutrients source, consisting in a promising technology for revegetation of contaminated sites. In order to evaluate the effectiveness of using HYP for phytostabilization programs, two different soils (Technosol and Oxisol) collected from a smelting site were amended with increasing doses of HYPs (HYP-1 and HYP-2): 15, 30, 60, and 120 Mg ha^{-1}. For comparison, a control (soil without amendment) and a soil amended with zeolite (clinoptilolite) were also included as treatments. After 90 days of incubation, HYPs decreased up to 83.8% of Cd availability and reduced exchangeable Al up to 100%. HydroPotash increased pH, cation exchange capacity, and contents of potassium, calcium, and phosphorus, as well as microbial biomass carbon, and fluorescein diacetate hydrolysis of soils. *Andropogon gayanus*, *Eucalyptus grandis*, and *Heterocondylus vitalbae* started growing from the dose of 15 Mg ha^{-1} HYPs in the Oxisol and 60 Mg ha^{-1} HYPs in the Technosol. Principal component analysis indicates that plant shoot dry weight was negatively correlated with extractable Cd and Zn and positively with pH, CEC, and Ca content. Besides promoting plant growth, HYPs reduced heavy metals (Cd and Zn) absorption by plants, indicating that HYP has potential use as an amendment in phytostabilization programs.

Keywords: aided phytostabilization; metal extractability; soil remediation; heavy metals; soil amendments.

1. Introduction

Soil contamination with heavy metals is a worldwide concern. Since these elements may represent a risk to human health and the environment, eliminating and/or mitigating their impact are critical actions for assuring better soil health and food safety (Qin et al., 2021; Rai et al., 2019; Setia et al., 2021).

Many strategies could be adopted in heavy-metal-contaminated soil remediation, including solidification, vitrification, electrokinetics, and soil washing techniques (Khalid et al., 2017; Liu et al., 2018; Yao et al., 2012). Most of them can be employed *in-situ*, which is preferable compared with *ex-situ* techniques, because the soils do not need to be removed from the site for disposal or treatment. In addition, *in-situ* remediation is preferred due to lower costs, less disturbance to the environment, and is the only practical alternative for large contaminated areas (Liu et al., 2018; Majone et al., 2015; Song et al., 2017).

Several physical and chemical remediation techniques could promote rapid soil decontamination. However, in many cases, these interventions do not improve soil fertility, which often inhibits adequate land revegetation. An alternative for improving soil quality is the implementation of techniques such as bioremediation (Cundy et al., 2016) and/or the application of soil amendments (Lwin et al., 2018; Mahar et al., 2015; Martins et al., 2018; Penido et al., 2019). Combining the use of soil amendments with phytoremediation is often the best strategy for recovering soils contaminated by heavy metals (Chen et al., 2000) and have been proved to be efficient with numerous experiments under greenhouse and field conditions (Lan et al., 2020; Madejón et al., 2014; Martínez-Martínez et al., 2019; Martins et al., 2018; Touceda-González et al., 2017). These products (i.e., soil amendments) may promote the immobilization of metals, reducing their availability to plants (Martins et al., 2018; Puga et al., 2015) in a process referred to as phytostabilization, or may increase metals uptake by plants, favoring phytoextraction (Mahar et al., 2016; Ng et al., 2016). The application of amendments can also increase microbial population (Sun et al., 2015), microbial biomass carbon (Al-Wabel et al., 2019), and enzyme activity (He et al., 2019; Wu et al., 2017) in contaminated soils, thus facilitating the revegetation process.

Several organic and inorganic amendments have been widely tested in metal-contaminated soils, especially those based on sustainable technologies (Chen et al., 2018; Elbehiry et al., 2020; Martins et al., 2018; Penido et al., 2019). A novel adsorbent, produced according to green-chemistry principles, with high cation exchange capacity (CEC) and high efficiency to immobilize heavy metals is the hydrothermally-altered feldspar (HYP, HydroPotash) (Ciceri et al., 2017; Ribeiro et al., 2021). This innovative soil amendment immobilized up to 99.9% of cadmium (Cd), zinc (Zn), and lead (Pb) in the soil solution of highly contaminated areas from a zinc-smelting site (Ribeiro et al., 2021). Since it uses K-feldspars ($KAlSi_3O_8$) and calcium hydroxide ($Ca(OH)_2$) as raw materials, HYP contains multiple nutrients/beneficial elements [potassium (K), calcium (Ca), and silicon (Si)] and also possesses characteristics of common soil amendments such as lime and gypsum. Therefore, the application of HYP should not only immobilize heavy metals but at same time also improve the physical, chemical, and biological properties of soils, thereby enhancing the establishment of vegetation in heavy-metal-contaminated sites.

The plant species choice is a key factor for the revegetation success. Plants with high tolerance to heavy metals toxicity, drought, low soil fertility, and extreme pH values are indicated to revegetate mining/smelting areas (Peco et al., 2021). Some promising species are *Andropogon gayannus*, a plant with high Zn tolerance (Ribeiro et al., 2020); *Eucalyptus grandis*, a species used for reforestation of degraded areas (Guedes et al., 2018); and *Heterocondylus vitalbae* (DC.) R. M. King & H. Robins, a species found growing naturally in areas of mining waste deposits from the Fundão dam spill in Mariana-MG, Brazil.

The effects of HYP on soil fertility and plant growth have not been evaluated in highly metal-contaminated smelting-affected soils until now. Thus, the objective of this study was to compare two HYPs (with different chemical properties) and a zeolite (commercial comparative) with respect to their metal sorption capacities and potential for improving plant growth in metal-contaminated soils situated nearby a zinc-smelting facility, an area with a historical attempt of revegetation. The effects of HYPs on soil chemical properties and microbiological attributes, as well as on the accumulation of heavy metals and nutrients by plants were analyzed. With that, the viability of using this novel product as a soil amendment in smelting-affected soils was accessed.

2. Materials and Methods

2.1 Amendments characterization

Two types of HYP (HYP-1 and HYP-2) provided by Advanced Potash Technologies Ltd. were used for this study. The HydroPotash production process allows one to tune the characteristics of the final product in accordance with the desired final use, favoring nutrient availability or adsorption capacity or a balance of both. For the preparation of both HYPs, (>80% pure $KAlSi_3O_8$) and $Ca(OH)_2$ were mixed under a hydrothermal process with different temperatures and residence time (HYP-1: 200ºC for 1 h and HYP-2: 230ºC for 2.5 h) (Ciceri et al., 2017, Ribeiro et al., 2021). A commercial silicate comparative, zeolite, was purchased from Celta Brasil (Cotia, Brazil). These products (HYP-1, HYP-2, and zeolite) were selected because they had the higher sorption capacities compared with other adsorbents in previous batch sorption tests. Scanning electron microscopy images and chemical composition by dispersive wave X-ray fluorescence spectrometry (WDXRF) of the selected materials are shown in Fig. S1 and Table S1 respectively. The products are naturally dry, presenting particle size < 0.150

mm (+95%). The HYP-1, HYP-2, and zeolite had a pH of 12.6, 11.3, and 8.0 and CEC of 1,971, 530, 1,500 $\text{mmol}_c\ \text{kg}^{-1}$, respectively (Ribeiro et al., 2021). A detailed description of the studied materials, including analyses of X-ray diffraction and scanning electron microscopy with energy dispersive X-ray spectroscopy, is shown in Ribeiro et al. (2021).

2.2 Soil sampling and characterization

Soil samples were collected in two different sites nearby a zinc-smelting plant located in the State of Minas Gerais, Brazil. Both sites present soils with high heavy metal levels and without or with sparse plant coverage. The first soil, Technosol (18° 11' 13.2" S, 45° 14' 24.8" W), was collected in an area historically used for disposing of metallurgic residues (Lopes et al., 2021). The second one is an Oxisol (18° 11' 26.4" S, 45° 14' 11.2" W) from an area with dust deposition of metals (Ribeiro et al., 2021).

The soil samples were collected at the 0-0.2 m soil depth, air-dried, and sieved through 4 mm for pot experiments and 2 mm for soil analyses. The contents of soil sulfur (S) (Hoeft et al., 1973), organic matter (Walkley and Black, 1934), as well as particle size (Bouyoucos, 1951) were determined and are showed in Table 1. Total heavy metal concentrations (Cd, Zn, Pb) were quantified using an optical emission spectrometry with inductively coupled plasma (ICP-OES, Spectro - model Blue, Germany) after extraction following the 3051A method (U.S. EPA., 2007). The pH was determined using a 1:2.5 soil:water ratio (Teixeira et al., 2017) and CEC was the sum of Ca and magnesium (Mg) (McLean et al., 1958), K (Mehlich, 1953), and H + aluminum (Al) (Shoemaker et al. 1961). A detailed description of analyses is presented in Table S2.

Table 1. Physical-chemical properties of soils collected in the zinc-smelting area, Três Marias, MG, Brazil.

Properties	**Technosol**	**Oxisol**
pH (H_2O)	4.50	5.50
CEC[a] (mmol$_c$ kg^{-1})	91.6	22.7
Organic matter (g kg^{-1})	0.06	0.69
Clay (%)	13.0	45.0
Silt (%)	64.0	16.0
Sand (%)	23.0	39.0
S (mg kg^{-1})	598	287
Cd[b] (mg kg^{-1})	15.1	62.3
Zn[b] (mg kg^{-1})	6,665	2,261
Pb[b] (mg kg^{-1})	63.9	53.6

[a]CEC = cation exchange capacity at pH 7.0; [b] Total concentration (U.S. EPA., 2007).

2.2 Experimental design

The experiment was conducted in a greenhouse with a temperature of 26±3ºC in a completely randomized factorial design (3 amendments x 4 doses) with four replicates. The three products were HYP-1, HYP-2, and zeolite and the doses tested were 15, 30, 60, and 120 Mg ha^{-1} (equivalent to 26.25, 52.5, 105, and 210 g product per pot). An additional treatment without any amendment application was used as control. Each product was well mixed with 3.5 kg soil by hand and packed and packed into 4L-pots (180 mm-top diameter x 110 mm-basal diameter x 165 mm-height).

The soil field capacity was measured with a preliminary test by saturating the amended soils with deionized water and letting excess water to drain for three days (with a cover at the top to avoid evaporation). Soils with treatments were wetted to field capacity and this soil moisture was maintained during the 90 days of the incubation period. The pots were weekly irrigated to supply the water lost by evaporation considering pot weight changes. In the end of the incubation period the soil was sampled for chemical and microbiological analyses and plant cultivations (Fig. S2).

2.3 Characterization of amended soils after incubation

2.3.1 Microbiological attributes

Firstly, a moist soil sample was collected with a sampler in the 0-8 cm depth from each pot immediately after the end of the incubation period and before plant cultivation. This soil depth was selected for this measurement due to a more significant fraction of active soil microbiota reported by other researchers (Fang and Moncrieff, 2005). We evaluated the microbiological attributes before plant cultivation to avoid possible interference of plants. The microbiological attributes analyzed were: microbial biomass carbon (MBC), determined by the fumigation and extraction method assuming a fumigation and extraction efficiency of 0.45 (Vance et al., 1987); microbial basal respiration (MBR) by the estimation of the CO_2 captured with NaOH during incubation and later titration with HCl (Jenkinson and Powlson, 1976), and fluorescein diacetate hydrolysis (FDA) by the release of fluorescein (Dick et al., 1996). Moreover, qCO_2 (i.e., microbial metabolic quotient) was also derived from the respiration-to-biomass ratio.

2.3.2 Chemical attributes

After being air-dried and homogenized, soil samples were collected and sieved to 2 mm. The concentrations of phosphorus (P) (Mehlich, 1953), Al (McLean et al. 1958) and soil pH, as well as CEC and exchangeable contents of Ca, K, Mg (same methodology described before) were determined. The extractable metal contents - Cd, Zn, and Pb - were determined using a chelating DTPA solution (Lindsay and Norvell, 1978). Metals (Cd, Zn, and Pb) were quantified using an optical emission spectrometry with inductively coupled plasma (ICP-OES, Spectro - model Blue, Germany). The detailed description of soil analyses can be found in Table S2.

2.4 Sequential cultivation

Three different species commonly found growing in mining areas and/or used in phytostabilization programs were selected as testing plants and planted in the smelting-affected soils. These species were cultivated in a chronological sequence in the same pot in the following order; *Andropogon gayannus*, *Eucalyptus grandis*, and *Heterocondylus vitalbae* (DC.) R. M. King & H. Robins. (Fig S2). In all cultivations, soil moisture was maintained nearly at 60% of the field capacity using deionized water irrigation. The soil was dried, homogenized, and the roots removed after each crop (Fig. S2). The detailed preparation for each cultivation is described below.

Seeds of *Andropogon gayanus* Kanth cv Planaltina were conditioned in KNO_3 (0.05%) with aeration at 21ºC for three hours (BRASIL, 2009). After air-drying for a day, 10 cm^3 of andropogon grass seeds were sown in pots at a depth of 0.5-1.0 cm. All treatments received 67 mg P kg^{-1} and 39 mg N kg^{-1} at 10, 18, and 23 days of sowing with a monobasic ammonium phosphate solution. Additional fertilization was applied after 26 days of sowing with 200 mg P kg^{-1}, 50 mg K kg^{-1}, 18 mg N kg^{-1}, 30 mg Mg kg^{-1}, 40 mg S kg^{-1}, and 0.5 mg B kg^{-1} via triple superphosphate, potassium nitrate, magnesium sulfate, and boric acid. After 21 days of seed germination, plants were thinned, leaving only ten plants per pot. *Eucalyptus grandis* seedlings (90-days old) were treated with foliar fertilization of 10 g L^{-1} KCl and 100 g L^{-1} monobasic ammonium phosphate before being transplanted into the pots. No other fertilization was applied in the soils, except for the addition of 0.5 mg B kg^{-1} as boric acid in the moment of transplanting.

Seedlings of *H. vitalbae* (75 days old - 15 in vitro + 60 days of acclimatization in a commercial substrate) were transplanted to the pots, followed by the application of 30 mg N kg^{-1} as ammonium nitrate.

At the end of each cultivation, shoots of *A. gayanus* (45 days after planting), *E. grandis* (55 days after planting), and *H. vitalbae* (45 days after planting) were cut, washed with distilled water, dried for one day under room temperature, and dried in an oven at 65ºC. The plant shoot dry weight of each pot was measured.

2.5 Determination of heavy metals and nutrients in plant

The elemental composition of plant shoot biomass was determinate only for *E. grandis* and *H. vitalbae* because *A. gayanus* did not produce enough material for analyses. Heavy metals (Cd, Zn, and Pb) and nutrients in plant shoots (Ca, K, Mg, P, S, Cu, Fe, and Mn) were extracted following the 3051A method (U.S. EPA., 2007) and analyzed with ICP-OES. The quality assurance of the analyses was assessed using the reference material “Peach leaves” (SRM 1547, National Institute of Standards and Technology-NIST). The elemental recovery in the reference material (certified or provided as additional information in the case of S) varied from 82 to 119%. Bioaccumulation coefficients (the ratio between the concentration of Cd, Zn, and Pb in *E. grandis* and *H. vitalbae* shoots and the total concentration of these metals in soil – Table 1) were calculated for evaluating the phytostabilization potential of these plants (Buscaroli, 2017).

2.6 Statistical analysis

Before one-way analysis of variance at 5% of significance level, data were checked for normality and homogeneity of variances. When necessary, data was log-transformed for correcting detected violations on both ANOVA assumptions (Zuur et al., 2010). Regression analysis was performed for modeling the effect of amendments on K, P, Ca, and Mg extractable concentrations. In addition, treatments (DTPA-extractable concentrations of metals, pH, CEC, microbiological attributes, plant shoot dry weight and elements in plant shoot tissues) were either compared with each other by Tukey´s test ($p<0.05$) and Scott-Knott algorithm ($p<0.05$) or to the control group by Dunnett's test ($p<0.05$). Principal component analyses were performed using package "vegan" (Oksanen et al., 2020) to further analyze the main attributes of amended soils influencing plant growth. All analyses were conducted in R Environment (R Development Core Team, 2016).

3. Results

3.1 Chemical attributes

The addition of zeolite in the tested doses did not reduce Cd, Zn, and Pb DTPA-extractable content in the Technosol and the Oxisol ($p<0.05$) (Table 2). On the other hand, the application of HYPs decreased Cd and Zn extractability in both soils. In the Technosol, HYPs reduced DTPA-extractable Cd by up to 50 and 84% respectively with the application of 60 and 120 Mg ha^{-1} doses, compared with the control. The application of 120 Mg ha^{-1} of HYP-1 and HYP-2 reduced DTPA-extractable Zn by 8.7- and 5.3-fold, respectively, compared with the control. In the Oxisol, the application of 120 Mg ha^{-1} of HYP-2 reduced 56.6% of Cd and the highest reductions of Zn were observed with the application of 120 Mg HYP-1 ha^{-1} (39.7%) and 15 Mg HYP-2 ha^{-1} (42.1%). The Pb DTPA-extractable content was significantly increased with HYPs application, especially in the Oxisol ($p<0.05$) (Table 2).

The HYPs and zeolite increased soil CEC by as much as 97% (Table 2). On the other hand, soil pH increased (up to 3 units) only with HYPs application. The increasing doses of HYPs and zeolite increased Ca and K in the studied soils (Fig. 1). The P levels also increased, though Mg decreased in both soils with the application of increasing doses of HYPs (Fig. 1).

Extractable metal contents and soil fertility attributes after cultivation are shown in Tables S2 and S3.

Table 2. DTPA-extractable concentrations of metals and soil fertility attributes of smelting-affected soils after amended with HYPs and zeolite and incubated for 90 days. Mean values and standard error (n=4).

Technosol

Material	**Dose (Mg ha $^{-1}$)**	**Cd^{a} (mg kg^{-1})**	**Zn^{a} (mg kg^{-1})**	**Pb^{a} (mg kg^{-1})**	**Al^{b} (cmol$_{c}$ kg^{-1})**	**pH^{c,e}**	**CEC^{d,e} (cmol$_{c}$ kg^{-1})**
Control	0	5.79 (0.17)	3575 (146)	0.17 (0.02)	0.44 (0.02)	4.8 (0.10)	9.4 (0.44)
HYP-1	15	4.99 (0.20)	2564 (61.4)*	0.15 (0.03)	0.18 (0.01)*	5.5 (0.02)*	10.9 (0.22)
HYP-1	30	3.73 (0.08)*	1589 (60.9)*	0.08 (0.01)	0.13 (0.01)*	5.9 (0.03)*	14.6 (0.46)*
HYP-1	60	2.24 (0.04)*	785 (38.8)*	0.48 (0.08)*	0.05 (0.01)*	6.6 (0.10)*	18.1 (0.55)*
HYP-1	120	1.23 (0.29)*	411 (46.3)*	1.75 (0.32)*	0.00 (0.00)*	7.8 (0.15)*	16.5 (0.64)*
HYP-2	15	5.19 (0.41)	2678 (197)	0.10 (0.01)	0.17 (0.01)*	5.4 (0.05)*	10.7 (0.28)
HYP-2	30	3.87 (0.02)*	1710 (13.4)*	0.09 (0.01)	0.13 (0.01)*	5.8 (0.05)*	13.9 (0.54)*
HYP-2	60	1.75 (0.06)*	762 (13.8)*	0.16 (0.02)	0.05 (0.01)*	6.3 (0.05)*	16.8 (0.13)*
HYP-2	120	0.97 (0.03)*	672 (15.7)*	0.94 (0.14)*	0.01 (0.01)*	7.7 (0.16)*	17.5 (0.36)*
Zeolite	15	5.21 (0.77)	3354 (427)	0.12 (0.01)	0.42 (0.03)	4.8 (0.03)	8.6 (0.59)
Zeolite	30	5.70 (0.08)	3786 (174)	0.13 (0.01)	0.38 (0.03)	4.8 (0.03)	10.1 (0.58)
Zeolite	60	5.03 (0.28)	3893 (82.2)	0.14 (0.01)	0.39 (0.02)	5.0 (0.12)	11.4 (0.42)*
Zeolite	120	4.35 (0.16)	3062 (160)	0.12 (0.01)	0.40 (0.03)	5.1 (0.09)	11.4 (0.37)*

Oxisol

Material	**Dose (Mg ha $^{-1}$)**	**Cd^{a} (mg kg^{-1})**	**Zn^{a} (mg kg^{-1})**	**Pb^{a} (mg kg^{-1})**	**Al^{b} (cmol$_{c}$ kg^{-1})**	**pH^{c,e}**	**CEC^{d,e} (cmol$_{c}$ kg^{-1})**
Control	0	25.6 (1.70)	579 (29.1)	1.79 (0.71)	0.07 (0.01)	6.6 (0.13)	3.7 (0.25)
HYP-1	15	28.5 (0.81)	456 (39.6)	5.67 (1.27)*	0.07 (0.01)	7.8 (0.21)*	4.8 (0.31)
HYP-1	30	25.5 (1.26)	492 (11.6)	6.79 (1.56)*	0.00 (0.00)*	8.3 (0.08)*	5.6 (0.67)*
HYP-1	60	19.6 (0.28)*	440 (62.1)	4.79 (0.08)*	0.00 (0.00)*	8.5 (0.03)*	7.2 (0.13)*

HYP-1	120	15.3 (0.44)*	349 (15.2)*	4.32 (0.07)*	0.00 (0.00)*	8.8 (0.03)*	7.6 (0.38)*
HYP-2	15	24.5 (0.38)	335 (26.9)*	5.10 (0.26)*	0.04 (0.01)*	7.4 (0.12)*	4.8 (0.18)
HYP-2	30	20.0 (0.46)*	566 (30.2)	5.86 (0.71)*	0.03 (0.01)*	7.8 (0.23)*	4.8 (0.69)
HYP-2	60	12.5 (0.27)*	394 (7.66)*	4.23 (0.08)*	0.03 (0.01)*	8.4 (0.05)*	6.9 (0.18)*
HYP-2	120	11.1 (0.49)*	403 (35.1)*	5.87 (1.27)*	0.00 (0.00)*	8.6 (0.14)*	6.6 (0.52)*
Zeolite	15	28.2 (1.18)	645 (66.3)	2.08 (0.99)	0.07 (0.01)	5.8 (0.05)*	4.5 (0.38)
Zeolite	30	27.1 (0.81)	649 (21.5)	0.29 (0.01)	0.07 (0.01)	5.9 (0.19)*	5.1 (0.19)
Zeolite	60	26.4 (1.15)	601 (30.5)	0.35 (0.04)	0.06 (0.01)	6.2 (0.18)	5.8 (0.33)*
Zeolite	120	28.1 (0.92)	691 (94.9)	0.53 (0.23)	0.06 (0.01)	6.6 (0.28)	(0.44)*

[a]DTPA extraction; [b]KCl 1 mol L^{-1}, [c]pH in water (1:2.5); [d]Cation exchange capacity; [e]data taken from Ribeiro et al. (2021).

*Different to control group according Dunnett´s test ($p<0.05$).

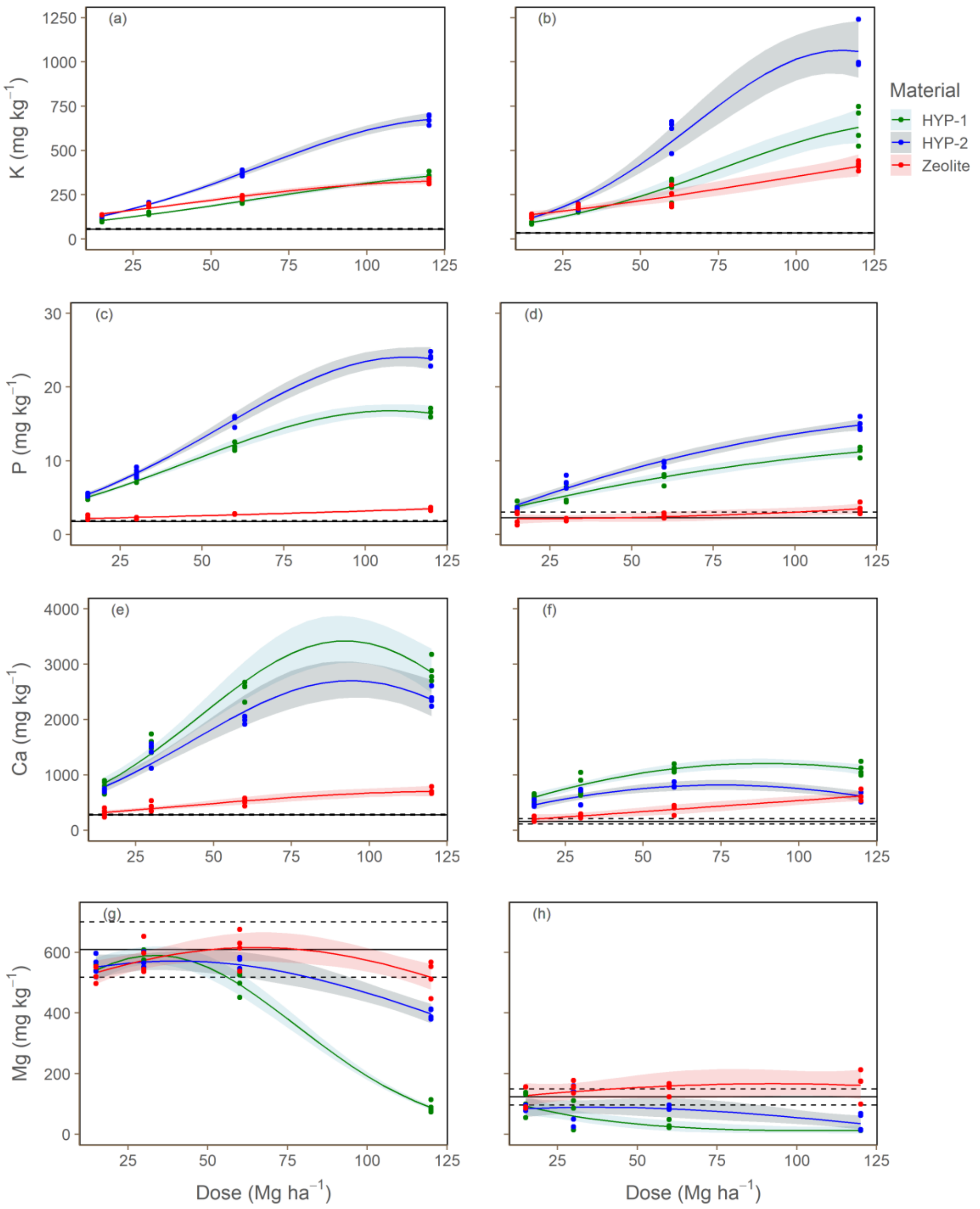


Fig. 1. Concentrations of extractable P (a-Technosol, b-Oxisol), K (c-Technosol, d-Oxisol), Ca (e-Technosol, f-Oxisol), and Mg (g-Technosol, h-Oxisol) after application of increasing doses of HYP-1, HYP-2, and zeolite. The fitted values for each model (solid lines) and their respective 95% confidence interval (shaded areas) are shown. The control means without amendment addition (solid black line) and its 95% confidence interval (black dashed lines) are represented. Non-overlapping confidence intervals indicate significant differences between treatments.

3.2 Microbiological attributes

The Oxisol treated with the highest dose of HYP-1 had significantly higher MBC than the control and soils treated with other rates and other soil amendments ($p<0.05$) (Fig. 2). In the Technosol, the MBC was higher with HYP-1 application at 60 Mg ha^{-1} rate, and in the last dose, both HYPs promote higher MBC than control.

Although MBC increased in the Technosol following the application of increasing doses of HYP-1, in this soil changes of MBR were not significant ($p<0.05$) (Fig. 2b). Nevertheless, in the Oxisol, different products increased MBR compared with the control in each dose tested. In addition to MBR, no clear patterns of response regarding products and doses applied were observed for qCO_2 (microbial metabolic quotient) (Fig. S3).

Concerning fluorescein diacetate hydrolysis (FDA), for the last dose, HYP-1 was superior to HYP-2 and zeolite in this order, in the Technosol. In the Oxisol, the FDA increased with HYPs addition from dose 30 Mg ha^{-1}.

Positive effects of HYP-1 and HYP-2 on MBR and FDA were greater in the Oxisol than in the Technosol. Conversely, positive effects of both HYP-1 and HYP-2 were only observed in the Technosol for MBC. No harmful effects were observed for any product.

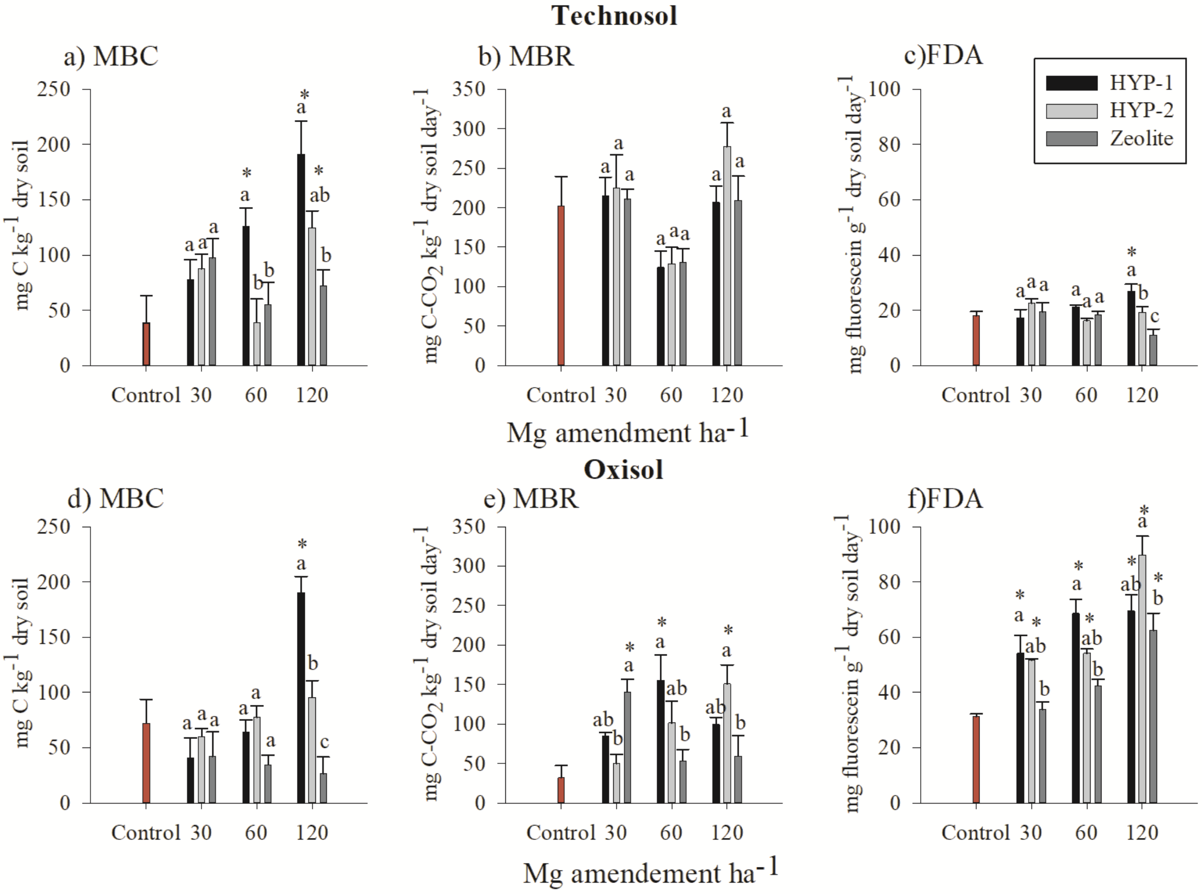


Fig. 2. MBC: microbial biomass carbon (a-Technosol, d-Oxisol); MBR: microbial basal respiration (b-Technosol, e-Oxisol) and FDA: fluorescein diacetate hydrolysis (c-Technosol, f-Oxisol). Lowercase letters compare products with the same dose (Tukey test $p<0.05$). Vertical bars represent standard error (n=4). Asterisks indicate significant differences compared with the control (Dunnett's test $p<0.05$).

3.3 Sequential cultivation

None of the plants tested (*A. gayanus*, *E. grandis,* and *H. vitalbae*) grew/survived in the control and zeolite treatments in both studied soils. In the Technosol, plants of the three species tested started growing with the application of HYPs from the dose of 60 Mg ha^{-1} (Fig. 3a, b, and c). Shoot biomass was 3-fold greater in treatments receiving HYP-1 than in HYP-2 for the rate of 60 Mg ha^{-1}, but no differences between these products were observed in the last dose. In the Oxisol, the three species started growing at the first dose of HYPs tested. No differences between HYP-1 and HYP-2 products were observed for *E. grandis* and *H. vitalbae* shoot biomass production, yet for *A. gayanus*, HYP-1 and HYP-2 promote higher biomass in doses 60 and 120 Mg ha^{-1}, respectively (Fig 3).

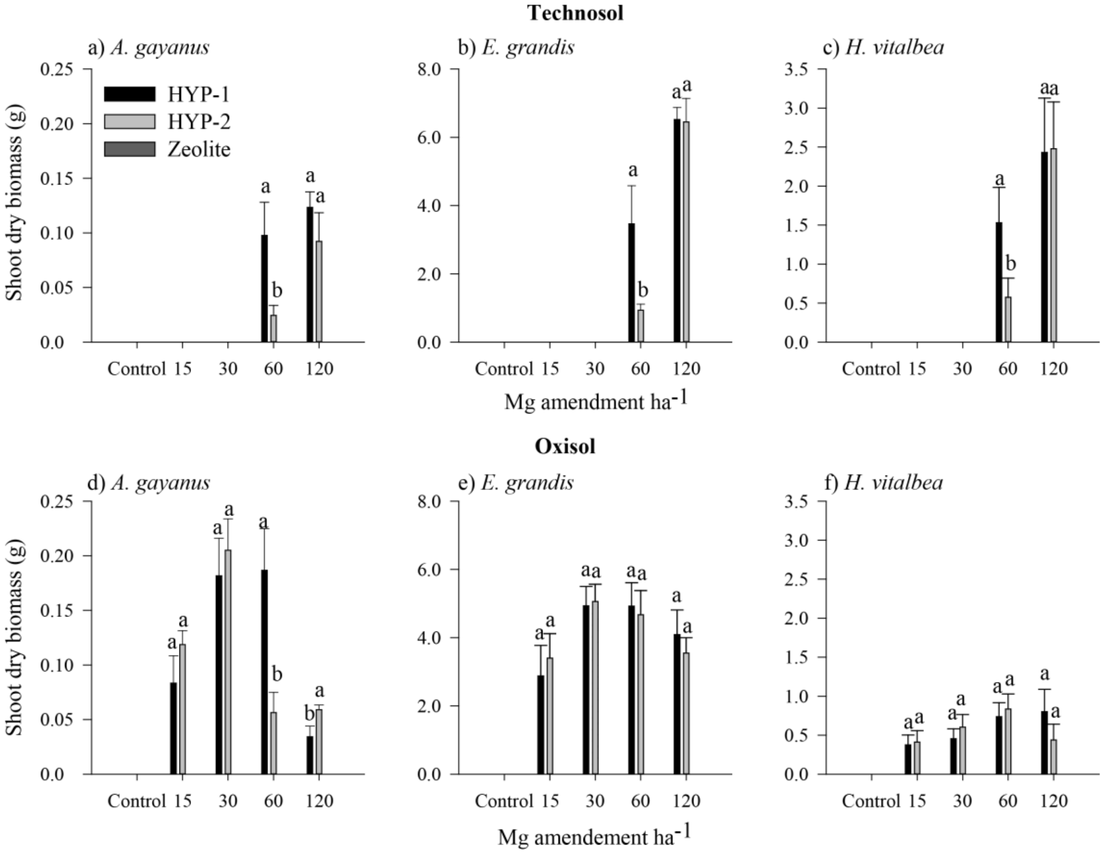


Fig. 3. Shoot dry weight of *Andropogon gayanus* (a-Technosol, d-Oxisol), *Eucalyptus grandis* (b-Technosol, e-Oxisol) and *Heterocondylus vitalbae* (c-Technosol, f-Oxisol). Vertical bars represent standard error (n=4). Lowercase letters compare products with the same dose (Tukey $p<0.05$). Mean values of zeolite and control treatments are missing because plants did not grow or survive in these treatments.

Principal component analyses (PCA) helped explain the results by analyzing the main physico-chemical attributes of amended soils influencing plant growth. PCA explained 77.8%

of the data's total variability in the first two components (Fig. 4a) for Technosol. The PC1, in particular, explained 65.5% of this variation and had a high discriminatory power of different products. The most relevant variables in this discrimination were P, Ca, and pH. These variables exhibited a positive charge on PC1, were positively and significantly related to each other, and were more associated with the HYPs treatments. The variables Cd and Al were also relevant in the discrimination of treatments. They were mainly associated with zeolite and control treatments and evidenced the dose-effect of HYPs.

Concerning the Oxisol, PC1 and PC2 explained 68.4% of the total variability of the data (Fig. 4b). In general, the separation of doses was quite similar to that observed in the PCA with the Technosol (Fig. 4a).

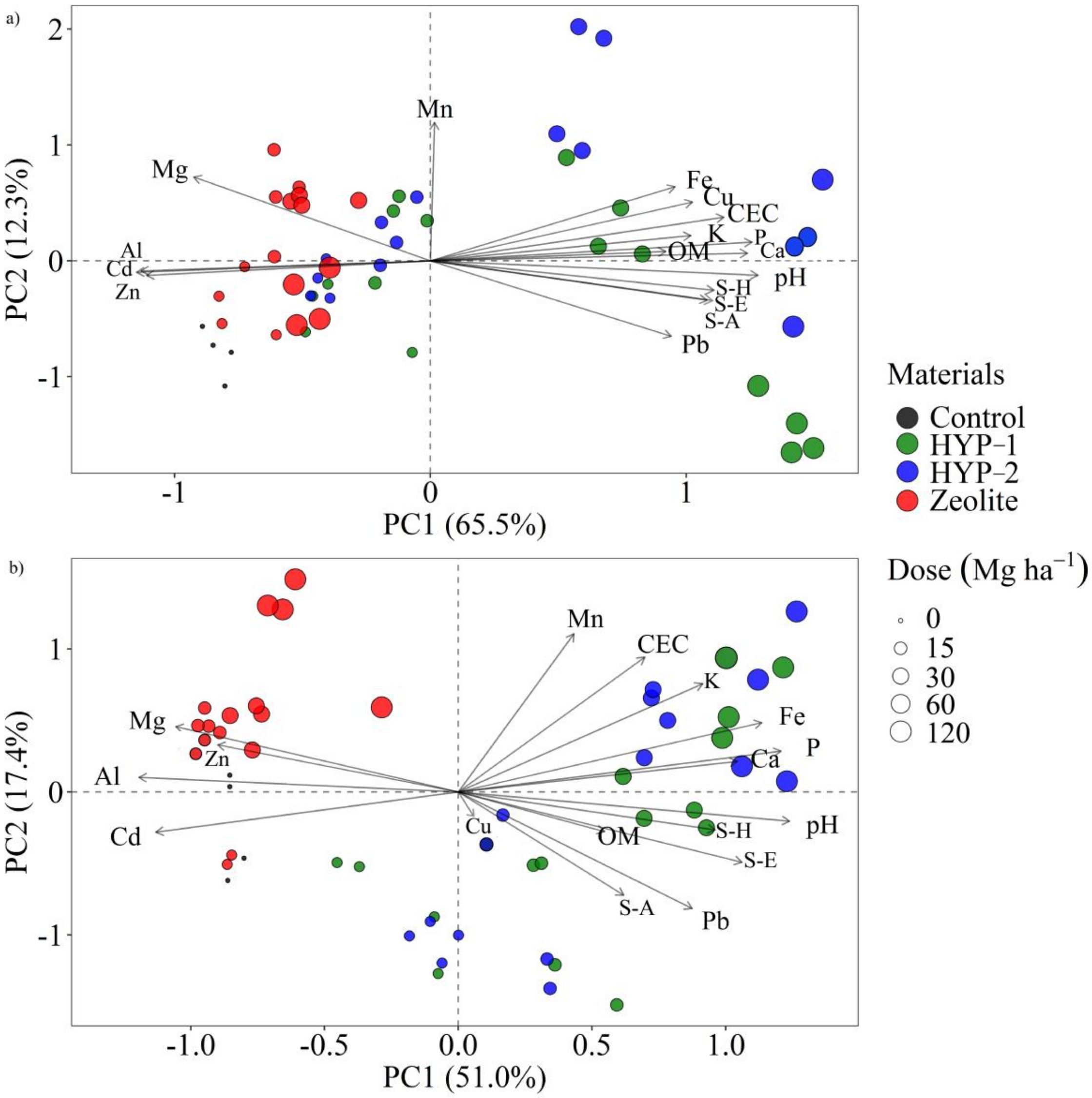


Fig. 4. Principal component analysis (PCA) showing relationships between soil fertility attributes, extractable metal content, and plant shoot dry biomass in (a) Technosol and (b) Oxisol. S-A, S-E, and S-H are *Andropogon gayanus*, *Eucalyptus grandis* and *Heterocondylus vitalbae* shoot dry biomass, respectively; CEC, cation exchange capacity, and OM, organic matter.

3.4 Heavy metal and nutrient concentrations in plant shoot

The application of HYPs increased Pb content in the soil but did not affect the absorption of this element by plants ($p<0.05$) (Table 3). Applying these materials reduced 73 and 93% of

Zn and Cd absorption, respectively, by *E. grandis* in the Oxisol, comparing the first (15 Mg ha^{-1}) and the last dose applied (120 Mg ha^{-1}) (Table 3). For both plants, in the Technosol, the highest reduction of Zn (88.2 - 89.9%) and Cd (83.3 - 85.5%) absorption was observed with HYP-2 and HYP-1 application, respectively. The decrease of metals absorption followed the reduction of these elements in the soil. Likewise, decreasing DTPA-extractable heavy metal content in the soil led to reduced bioaccumulation of these elements (Table S3).

Increasing HYP-1 doses increased Ca content (up to 64.6%) in shoots of *E. grandis* and *H. vitalbae* cultivated in the Technosol (Table 3). In both soils, an increase of K absorption was also observed when applying HYP-2 in *H. vitalbae* (up to 63.8%) and *E. grandis* (up to 73.2%).

Table 3. Elemental analyses in shoot tissues of *Eucaliptus grandis*, and *Heterocondylus vitalbae* cultivated in smelting-affected soils with different doses of HydroPotash. Mean values and standard errors (n = 4) in parentheses.

Technosol													
Plant	**Material**[a]	**Dose**	**Ca***	**K**	**Mg**	**P**	**S**	**Cu**	**Fe**	**Mn**	**Zn**	**Cd**	**Pb**
		Mg ha^{-1}	**---g kg^{-1}---**					**--- mg kg^{-1}---**					
E. grandis	HYP-1	60	10.5 (0.82)a	13.5 (1.01)a	9.75 (0.89)b	2.82 (0.14)a	5.19 (0.28)b	7.20 (0.75)b	37.3 (2.00)a	2,131 (210)b	908 (139)b	4.75 (0.89)b	1.41 (0.19)a
		120	17.4 (2.13)b	19.2 (1.43)b	4.97 (0.88)b	2.46 (0.34)a	2.28 (0.13)a	6.70 (1.25)b	60.7 (5.14)b	617 (59.2)a	195 (22.2)a	0.79 (0.20)a	1.56 (0.19)a
	HYP-2	60	7.12 (1.17)a	16.7 (1.53)a	8.07 (0.65)a	3.09 (0.25)a	6.19 (0.53)c	9.68 (0.31)c	34.8 (2.16)a	1,995 (252)b	1596 (155)c	4.44 (0.16)b	1.16 (0.20)a
		120	9.13 (0.91)a	20.5 (1.78)b	6.11 (0.54)a	2.33 (0.12)a	2.60 (0.14)a	4.11 (0.29)a	44.5 (2.92)a	738 (90.6)a	162 (11.7)a	0.82 (0.17)a	1.11 (0.13)a
H. vitalbae	HYP-1	60	5.15 (0.28)a	33.6 (0.89)b	8.42 (0.18)c	1.75 (0.08)c	5.77 (0.53)c	2.13 (0.08)b	106 (9.37)a	1,083 (163)c	508 (86.7)b	51.81 (6.33)b	1.77 (0.10)a
		120	6.66 (0.74)b	29.8 (2.40)b	2.44 (0.21)a	1.45 (0.12)b	2.86 (0.25)a	1.32 (0.35)a	95.8 (10.9)a	164 (9.71)a	77.5 (9.17)a	7.49 (0.87)a	1.62 (0.13)a
	HYP-2	60	5.54 (0.21)a	21.4 (0.74)a	6.29 (0.78)b	0.91 (0.03)a	4.45 (0.40)b	1.86 (0.16)b	73.3 (7.29)a	517 (43.5)b	738 (53.8)c	13.93 (1.26)a	1.76 (0.10)a
		120	4.70 (0.24)a	35.0 (2.66)b	3.46 (0.29)a	1.87 (0.11)c	3.75 (0.22)b	0.84 (0.34)a	98.9 (19.0)a	186 (14.6)a	87.3 (6.94)a	6.70 (0.27)a	1.37 (0.08)a
Oxisol													
E. grandis	HYP-1	15	13.1(0.87)a	16.4 (0.44)a	7.88 (0.78)b	2.74 (0.19)b	4.18 (0.20)a	11.4 (1.55)a	33.6 (1.38)a	81.9 (19.8)a	439 (89.7)b	14.62 (3.65)a	1.30 (0.04)a
		30	18.7 (2.45)b	21.8 (1.06)a	4.85 (0.34)a	2.28 (0.11)a	5.35 (0.76)b	21.4 (3.74)b	38.5 (2.70)a	73.2 (12.5)a	122 (19.1)a	3.87 (1.17)b	1.53 (0.15)a
		60	18.6 (2.38)b	21.3 (1.21)a	4.19 (0.54)a	2.08 (0.18)a	4.25 (0.18)a	21.5 (1.84)b	44.9 (2.27)b	156 (40.6)b	146 (22.3)a	4.04 (1.12)b	1.51 (0.15)a
		120	20.0(3.02)b	25.6 (2.76)b	3.76 (0.54)a	1.78 (0.19)a	2.71 (0.11)a	28.5 (6.67)b	57.3 (4.20)b	193 (25.7)b	136 (15.4)a	1.07 (0.08)b	1.71 (0.13)a
	HYP-2	15	15.4 (1.55)a	18.9 (1.56)a	7.10 (0.43)b	2.99 (0.25)b	4.27 (0.59)a	10.5 (2.49)a	38.1 (5.07)a	61.9 (11.0)a	445 (64.6)b	16.39 (3.66)a	1.48 (0.17)a
		30	21.4 (2.98)b	24.1 (2.36)b	7.24 (1.42)b	2.59 (0.13)b	6.11 (0.54)b	28.9 (3.53)b	41.1 (3.49)a	80.3 (22.5)a	271 (78.4)a	4.62 (1.03)b	1.76 (0.25)a
		60	22.0 (2.81)b	25.8(1.29)b	5.48 (0.56)a	2.45 (0.33)b	4.29 (0.46)a	28.9 (2.92)b	50.5 (4.87)b	168 (15.0)b	127 (9.46)a	1.69 (0.41)b	1.71 (0.16)a

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		120	13.4 (1.20)a	32.8 (1.97)b	3.26 (0.44)a	2.04 (0.22)a	3.96 (0.60)a	29.3 (3.37)b	49.1 (2.51)b	144 (13.3)b	119 (9.85)a	1.14 (0.33)b	1.54 (0.10)a
H. vitalbae	HYP-1	15	10.8 (0.02)d	29.1 (0.87)a	6.09 (0.26)b	1.40 (0.05)b	3.69 (0.08)b	5.27 (0.50)a	206 (7.91)b	24.5 (0.48)a	381 (15.5)b	74.5 (2.08)e	2.26 (0.15)a
		30	11.3(0.39)d	37.1 (0.53)c	4.71 (0.62)b	1.41 (0.02)b	2.92 (0.05)a	4.90 (0.70)a	116 (8.68)a	29.4 (4.05)a	142 (6.93)b	75.8 (1.01)e	2.68 (0.21)a
		60	9.39 (0.29)c	38.4 (0.70)c	3.20 (0.049)a	1.36 (0.10)b	3.06 (0.14)a	5.12 (0.38)a	179 (26.9)b	33.8 (1.01)a	87.6 (3.89)a	51.6 (1.06)c	2.24 (0.13)a
		120	7.56 (0.29)b	40.4 (0.22)d	2.28 (0.073)a	1.31 (0.06)b	3.56 (0.14)b	6.95 (0.64)b	221 (41.4)b	71.9 (5.10)c	67.4 (4.77)a	35.9 (2.51)b	1.93 (0.15)a
	HYP-2	15	10.0 (0.13)c	31.7 (0.83)b	5.15 (0.18)b	1.59 (0.04)c	3.42 (0.13)b	3.77 (0.16)a	124 (22.6)a	24.1 (1.97)a	270 (7.72)b	68.5 (1.05)d	2.31 (0.09)a
		30	9.34 (0.39)c	38.7 (1.13)c	4.43 (0.55)b	1.55 (0.08)c	3.64 (0.24)b	5.54 (0.38)a	120 (22.2)a	26.3 (0.63)a	154 (25.7)b	68.8 (3.29)d	2.33 (0.08)a
		60	7.63 (0.34)b	38.7 (0.54)c	3.04 (0.18)a	1.28 (0.13)b	3.07 (0.24)a	5.26 (0.29)a	131 (26.0)a	46.8 (6.70)b	78.5 (7.32)a	45.1 (4.45)c	1.96 (0.11)a
		120	6.61(0.18)a	41.8 (1.36)d	2.32 (0.096)a	0.99 (0.03)a	3.09 (0.05)a	6.90 (0.21)b	187 (5.73)b	38.2 (1.58)b	60.9 (0.51)a	19.5 (0.76)a	(0.09)a

[a] Zeolite and control treatments are omitted because none of the plants tested in these treatments grew/survived.

*Means of treatments in each plant growth with same lowercase letters in the vertical columns do not differ by Scott-Knott's test ($p<0.05$).

4. Discussion

The main reason for decreasing the concentrations of extractable Cd and Zn in both studied soils could be related to the increase of soil pH following the application of HYPs, as such products have high pH and high buffering capacity (Table 2) (Ribeiro et al., 2021). Increasing soil pH directly contributes to promoting precipitation of these metals (Kabata-Pendias, 2011), whereas indirectly it increases adsorption sites (variable charges) due to an increase in CEC. The soil pH elevation by HYPs application also led the concentration of exchangeable Al to nearly zero (Table 2). As the soil pH increases, within certain limits, the Al phytotoxic species gradually change to less toxic insoluble species, which mitigated this plant stress factor on the studied soils.

The increased DTPA-extractable Pb concentration is possibly due to a dissolution of Pb-organo complex as well as the oxidation of PbS promoted by high soil pH (Bade et al., 2012). This explanation is supported by the high S content found in the Technosol (598 mg kg^{-1}), which probably originated from the ore concentrates processed in this industrial unit (Lopes et al., 2021). It is noteworthy that the total Pb concentration in both soils is lower than the prevention value (i.e. threshold value for soil quality) of this element set by the Brazilian legislation - 72 mg kg^{-1} (CONAMA, 2009).In addition, the highest Pb DTPA-extractable content found after the application of the products (6.79 mg dm^{-3}) (Table 2) is still 90.56% lower.

The increased Ca and K extractable content found in HYPs treatments is mainly related to the composition of these materials (Ribeiro et al., 2021). Since Si is the main component of these products (Table S3), addition of Si may affect bioavailability of other nutrients including P. Thus, the increasing levels of P in soils was probably induced by the displacement of P by Si in the soil adsorption sites (Valle et al., 2016). This especially relevant because it has been demonstrated that Si solubility increases with pH (Schaller et al., 2021), which was observed in HYPs' treatments. Also, increasing pH increases negative charges in soil colloids, thus favoring P availability (Sato and Comerford, 2005).

The Mg reduction in soils receiving HYPs is probably related to pH increase and Al complexation (Miyazawa et al., 2001). At pH > 7.5, Mg may undergo isomorphic coprecipitation with Al species such as $Al(OH)^{-}_{4}$, $Al(OH)^{2-}_{5}$, and $Al(OH)^{3-}_{6}$ (Miyazawa et al., 2001). Moreover, above a pH of 8.4 and 8.5, Mg precipitates as MgAl(OH)n (Miyazawa et al., 2001) and as $MgCO_3$ (Lindsay, 1979), respectively. This pH range was achieved in HYPs´ treatments in the Oxisol. The precipitation also occurs with Ca at pH 7.3 (Miyazawa et al., 2001), but the exchangeable content of this element in the soils was probably not affected because the materials are a source of Ca.

The increased MBC found in HYPs' treatments could be explained by increasing soil pH (Jesus et al., 2009; Jones et al., 2019) as well as the decrease of metal toxicity (Santos et al., 2016) and exchangeable Al content (Jesus et al., 2009; Jones et al., 2019).

The MBR did not followed the MBC trending in the Technosol. This could indicate that a minimum activity and energy produced was used for the maintenance of the microbial cells. On the other hand, the FDA, which indicates the general soil microbial activity (Green et al., 2006; Schnürer and Rosswall, 1982) was stimulated. Increased microbial activity may help the restoration of contaminated environments because microorganisms play key roles in cycling and the availability of nutrients (Santos et al., 2016). In addition, microbial biomass itself plays important role in the immobilization of heavy metals as it produces organic compounds capable of complexing these elements (Ramasamy et al., 2007). In our study, the tested products, especially HYPs, increased MBC and FDA, which may have positively impacted plant growth.

Besides increasing microbial activity, the HYPs, by increasing metals (Cd, Zn and Pb) immobilization and improving chemical properties, probably favored root development and plant establishment. On the other hand, the high levels of heavy metals may have inhibited *A.*

gayanus seed germination and affected the survival of *E. grandis* and *H. vitalbae* seedlings in the control and zeolite treatments. These heavy metals can affect nutrient absorption and promote oxidative stress (Berni et al., 2019; Singh et al., 2020; Wierzbicka and Obidzińska, 1998). Although we did not analyze these endpoints, these effects are commonly associated with heavy metals toxicity. In addition to the chemical restrictions shown in both soils, the Technosol also has physical limitations, which was visually evidenced by soil-crusted layer formation during the plant growth test, mainly in the control treatment. This superficial soil crusting was probably formed due to the low organic matter and high silt content of this soil (Armenise et al., 2018; Zambon et al., 2021).

Principal component analyses (PCA) showed that Cd, Zn, and Al had a negative impact in plant growth, differently of pH, CEC, and K and Ca extractable contents. Decreasing Al soil levels may help plant establishment as this element directly impair the root system and consequently the whole plant (Panda et al., 2009). Also, besides the precipitation effect keeping metals immobilized in soils, increasing nutrients content and their subsequent absorption by plants could have minimized heavy metals accumulation (Huang et al., 2017; Ruthrof et al., 2018). For example, K can alleviate Cd toxicity by a dilution effect (increasing plant biomass) as well as by enhancing the defense system and suppressing Cd uptake by its antagonist absorption effect (Ruthrof et al., 2018). This dilution effect, combined with the low content and low mobility of Pb in the soil (Alloway, 1995) led to the absence of increment of Pb in plant biomass, despite the increased levels of extractable Pb after the addition of HYP. Concerning Ca, research has shown that this nutrient may protect plants against Cd toxicity by, among other factors, improving photosynthesis, reducing Cd uptake, and mitigating oxidative stress (Huang et al., 2017). Also, Ca is reported to decrease the absorption and ameliorate the toxicity of Zn (Baker, 1978; Saleh et al., 1999).

Differences between both soils can be observed by analyzing the distribution of the variable along the PC2. These differences are also observed in plant growth, which occurred starting from dose 15 Mg ha^{-1} in the Oxisol, and from 60 Mg ha^{-1} in the Technosol. This is probably related to soil pH and the increased immobilization of metals. As reported in our previous study, HYPs immobilized almost 100% of soluble Cd, Zn, and Pb in the first dose in the Oxisol and the same effect was observed in the dose of 60 Mg ha^{-1} in the Technosol (Ribeiro et al., 2021). Other factors influencing this dose-effect are the differences in ionic strength, clay content, and ΔpH, by directly affecting colloidal surface charges and ion competition in these soils (Ribeiro et al., 2021).

Although HYPs had similar behavior, HYP-1 promoted higher plant growth compared with HYP-2 with the dose of 60 Mg ha^{-1}, especially in the Technosol. Since HYP-2 has superior or equal improvements in soil chemical properties compared with HYP-1, a possible explanation is the increased MBC by this product in this dose. The positive effect was observed in the Technosol probably because the Oxisol has a higher buffer capacity. In addition, HYP-1 was more effective in reducing Cd, Zn, and Pb pore water concentrations with the dose of 60 Mg ha^{-1} in the Technosol than HYP-2 (Ribeiro et al., 2021).

In our previous study, we sampled soil solution and analyzed concentrations of metals in a period of 84 days (seven pore water extractions) (Ribeiro et al., 2021). The results showed the consistent effect of HYPs on metal immobilization during that period. The current study with sequential cropping systems further proved the capability of metal sorption by HYP. The beneficial and dose effects of HYPs application appeared for all tested plants and indicates that the metal immobilization effect of HYP occurs not only by precipitation, but also by adsorption (Ribeiro et al., 2021), which probably lasts for a long term (e.g., 229 days of study), i.e., a distinctive aging effect

HydroPotash application decreased heavy metals concentration in plant tissues and with few exceptions, all the bioaccumulation indices for the three elements were below 1. This indicates that minimal amounts of metals present in soil accumulated in plant shoot tissue (Buscaroli, 2017). In addition, a trend of decreasing bioaccumulation index with the HYPs doses tested was observed. This suggests that, except for the particular plant defense mechanisms, HYPs helped to keep heavy metals in the soil, which is desirable and recommended for phytostabilization programs (Cundy et al., 2016).

In general, for revegetating contaminated sites, fast-growing plants with large biomass production and metal tolerance are recommended, as these species may control erosion, thus avoiding contamination in areas nearby (Peco et al., 2021). This implies that, except for the highest decreases of metal accumulation, the ideal HYP doses in this study - higher plant biomass and less product application (economically viable) - are 30 Mg ha^{-1} and 60 Mg ha^{-1} for the Oxisol and the Technosol, respectively.

Compared with other common amendments, HYP has advantages since it is a natural material with free biological and metal contaminations, besides its value as a fertilizer. Its sorption capacity has been suggested as long last (Ribeiro et al., 2021). In addition, HydroPotash is an environmentally friendly technology with no waste derived from its production process (Ciceri et al., 2017a). Further studies with field applications are expected to prove beneficial effects found in this study. In other contaminated environments, attention should be devoted to monitoring the levels/bioavailability of micronutrients in soils, since increasing pH may promote their precipitation, hence leading to plant deficiencies.

5. Conclusion

The application of HydroPotash (HYP-1 and HYP-2) in heavy-metal-contaminated soils decreased DTPA-extractable Cd and Zn, exchangeable Al and increased plant-extractable nutrients (K and Ca). The products were not toxic and each had positive effects on soil microbiota. HydroPotashs promote plant growth, whereas in control and zeolite treatments plants did not grow/survive.

Plant shoot biomass was negatively correlated with extractable Cd and Zn and positively with pH, CEC, and Ca content. This work proposed an innovative use of HydroPotash in green remediation projects as an amendment of metal toxicities in soils from a zinc-smelting site. The addition of HYPs as amendments in contaminated soils, besides decreasing heavy metal availability, also has an added advantage due to their value as fertilizer, hence improving soil conditions for plant growth. Moreover, each of the products showed distinct characteristics demonstrating that HydroPotash processing technology can generate amendments with different chemical properties and reactions in soils affecting plant nutrients release and metal immobilization.

Declaration of competing interest

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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Supplementary Material

Hydrothermally-altered feldspar reduces metal toxicity and promotes plant growth in highly metal-contaminated soils

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Samples were mounted on double-sided carbon tape strips and placed in a vacuum chamber. They were then transferred to a carbon evaporator and analyzed by Scanning Electron Microscopy with Energy-Dispersive X-Ray Spectroscopy.

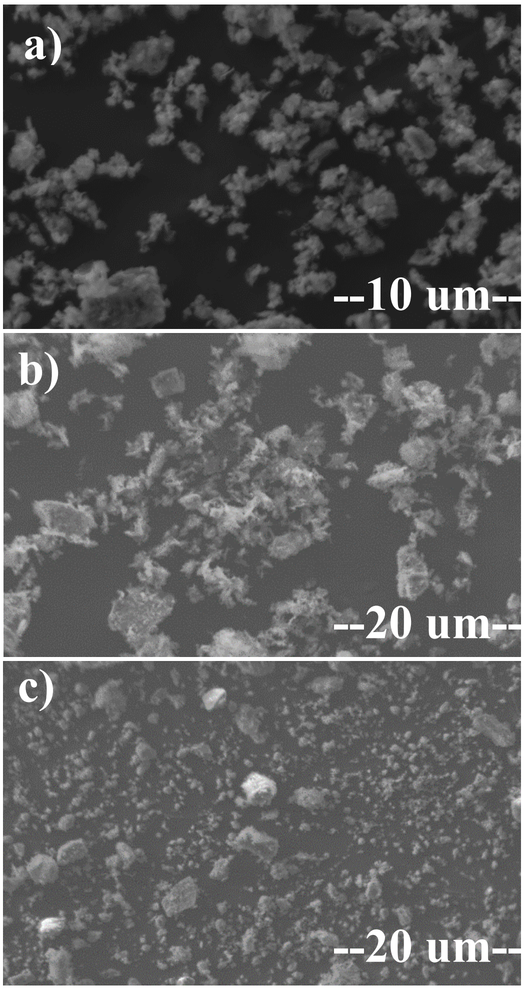

Fig. S1. Electron microscopy images of the materials: (a) HYP-1, (b) HYP-2 and (c) zeolite.

Table S1. Chemical characterization of products using the WDXRF technique

Elements	HYP-1	HYP-2	Zeolite
SiO_2 (%)	34.1	34.6	62.8
CaO (%)	21.9	19.0	3.07
Al_2O_3 (%)	9.20	8.94	10.36
K_2O (%)	8.53	8.80	3.58
Fe_2O_3 (%)	2.81	3.03	1.60
Na_2O (%)	0.69	0.80	0.19
BaO (%)	0.56	0.56	0.11
MgO (%)	0.36	0.37	0.67
TiO_2 (%)	0.25	0.29	0.18
P_2O_5 (%)	0.12	0.12	0.03
MnO (%)	0.06	0.06	0.02
SO_3 (%)	0.02	0.02	0.01

Adapted from Ribeiro et al. (2021).

Ribeiro, P.G., Souza, J.M.P., Rodrigues, M., Ribeiro, I.C.A., de Carvalho, T.S., Lopes, G., Li, Y.C., Guilherme, L.R.G, 2021. Hydrothermally-altered feldspar as an environmentally-friendly technology to promote heavy metals immobilization: batch studies and application in smelting-affected soils. J. Environ. Manage. 291, 112711. https://doi.org/10.1016/j.jenvman.2021.112711.

Table S2. Description of chemical analyses performed in the experiments

Analysis	Description	Reference
Total heavy metals (Cd, Zn, Pb) determination in soils	The 3051A method was used. In brief, air-dried soil samples were ground in a porcelain mortar and passed through a 150-µm nylon sieve. Then, 0.5 g of soil samples were put in a digestion tube with 5 mL of 65% HNO_3. After an overnight period (16 h), digestion tubes were placed in a microwave oven (Mars 5, CEM Corporation, Matthews, NC, USA) and heated at 180°C, with a pressure of 65 psi for 10 min. When cooled, the supernatant was filtered in a filter paper (Whatman No.42), diluted to 10 mL with distilled water, and transferred to acid-washed polypropylene vessels. Heavy metals were quantified using optical emission spectrometry with inductively coupled plasma (ICP-OES, Spectro-model Blue, Germany). For quality control, certified reference material SRM 2711a and SRM 2710a,as well as blank samples were used.	(U.S. EPA., 2007)
Soil pH	The soil pH (1:2.5 soil: water ratio) was determined in a pH meter after mixed for 1 minute and rest for 1 hour.	(Teixeira et al., 2017)
Exchangeable Ca, Mg, and Al	Extracted using 1 mol L^{-1} KCl and quantified by ICP-OES.	(McLean et al., 1958)
Potential acidity	Indirectly determined using a Shomacker–Mclean–Pratt (SMP) solution	(Shoemaker et al., 1961)
Available P and K	Extracted using a Mehlich-1 solution (0.05 mol L^{-1} HCl, 0.0125 mol L^{-1} H_2SO_4). Phosphorus was determined by colorimetry and K, by flame photometry.	(Mehlich, 1953)

Available metal contents - Cd, Zn, and Pb	Extracted using a DTPA solution pH 7.3 (0.005 mol L^{-1} of DTPA + 0.1 mol L^{-1} of triethanolamine + 0.01 mol L^{-1} of $CaCl_2$) in a soil:solution ratio (1:2) and shaking time of 2 h. Metals (Cd, Zn, and Pb) were quantified using ICP-OES.	(Lindsay and Norvell, 1978)

Lindsay, W.L., Norvell, W.A., 1978. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. Soil Sci. Soc. Am. J. 42, 421–428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

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Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na and NH_4. North Carolina Soil Testing Division, Raleigh.

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Teixeira, P.C., Donagemma, G.K., Fontana, A., Teixeira, W.G. (Eds.), 2017. Manual of Soil Analysis Methods, third ed. Embrapa, Brasília (in Portuguese).

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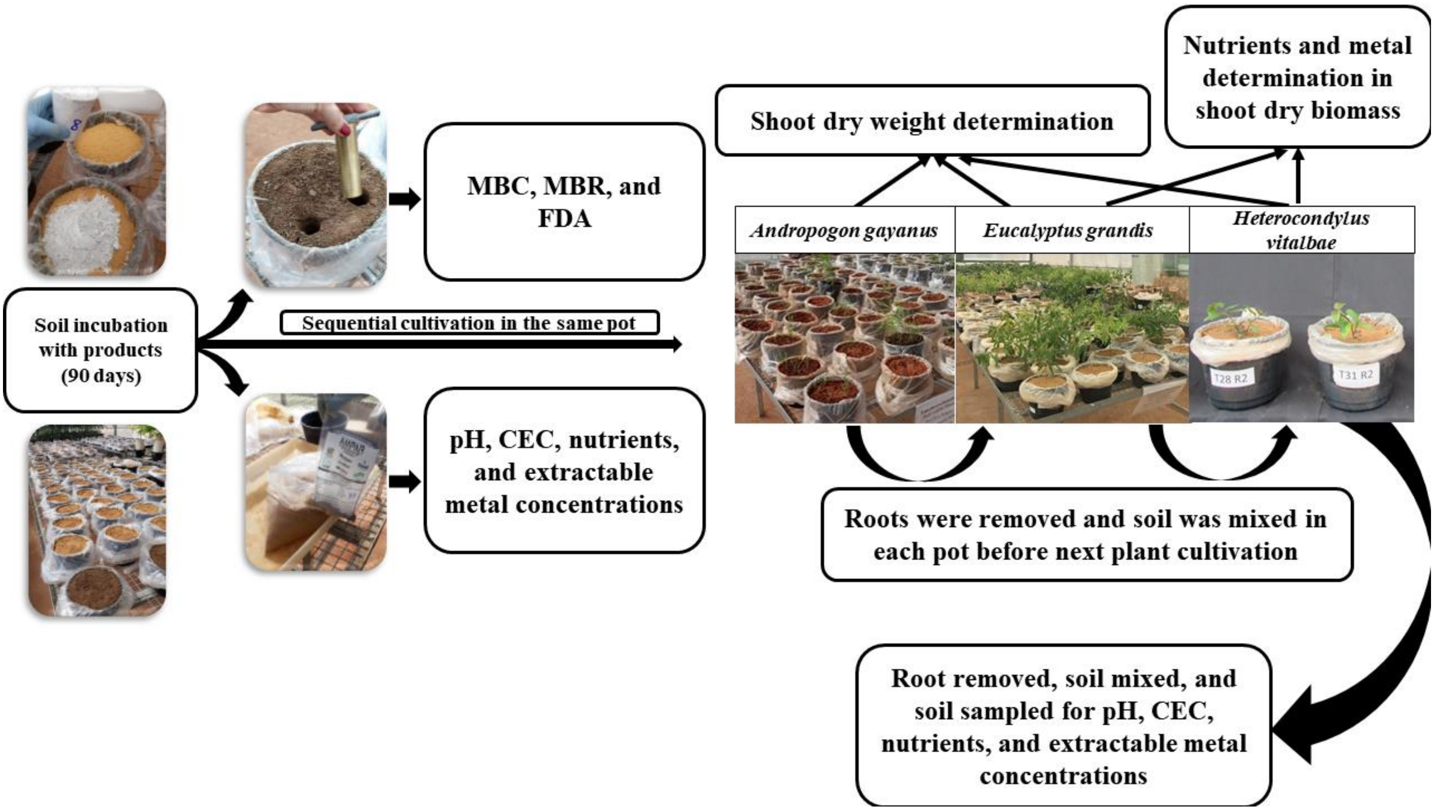


Fig. S2. Organogram of the methodological sequence of procedures performed in the experiments. MBC = microbial biomass carbon; MBR = microbial basal respiration; FDA = fluorescein diacetate hydrolysis; and, CEC = cation exchange capacity.

Table S3. Phytoavailable Cd, Zn, and Pb in zinc-smelting soils treated with the products and cultivated with *Andropogon gayanus, Eucalyptus grandis,* and *Heterocondylus vitalbae*.

Material	Dose Mg ha^{-1}	Cd	Pb	Zn
			mg kg^{-1}	
		Technosol		
Control	0	3.54 (0.32)	0.05 (0.02)	3053 (178)
HYP-1	15	2.97 (0.24)	0.12 (0.11)	1889 (224)
	30	2.58 (0.12)	0.01 (0.00)	1500 (246)
	60	1.72 (0.04)	0.92 (0.05)	877 (28.6)
	120	0.77 (0.21)	0.79 (0.10)	396 (85.1)
HYP-2	15	2.81 (0.30)	0.02 (0.01)	2235 (147)
	30	2.80 (0.04)	0.02 (0.02)	1221 (36.1)
	60	1.36 (0.02)	0.24 (0.02)	970 (23.8)
	120	0.60 (0.01)	0.61 (0.03)	658 (32.5)
Zeolite	15	3.20 (0.52)	0.07 (0.01)	3018 (344)
	30	1.89 (0.25)	0.03 (0.02)	2405 (301)
	60	1.42 (0.33)	0.06 (0.03)	1900 (470)
	120	1.25 (0.07)	0.01 (0.00)	2127 (168)
		Oxisol		
Control	0	27.3 (0.97)	0.38 (0.25)	1065 (59.6)
HYP-1	15	27.2 (1.01)	3.99 (1.41)	906 (27.6)
	30	20.1 (0.81)	7.83 (2.04)	696 (44.5)
	60	13.2 (0.42)	3.76 (0.14)	478 (9.38)
	120	7.77 (0.17)	3.09 (0.04)	404 (23.1)
HYP-2	15	23.6 (0.32)	4.38 (0.10)	637 (25.9)
	30	18.9 (0.45)	6.71 (1.72)	762 (42.8)
	60	9.31 (0.52)	3.29 (0.02)	466 (26.6)
	120	5.56 (0.37)	4.81 (1.18)	521 (38.7)
Zeolite	15	26.8 (0.60)	1.29 (0.87)	972 (89.7)
	30	24.6 (0.77)	0.16 (0.05)	1087 (69.3)
	60	24.2 (0.45)	0.09 (0.01)	1106 (40.4)
	120	25.1 (0.44)	1.29 (1.02)	921 (73.3)

Table S4. Fertility attributes in zinc-smelting soils treated with the products and cultivated with *Andropogon gayanus*, *Eucalyptus grandis*, and *Heterocondylus vitalbae*.

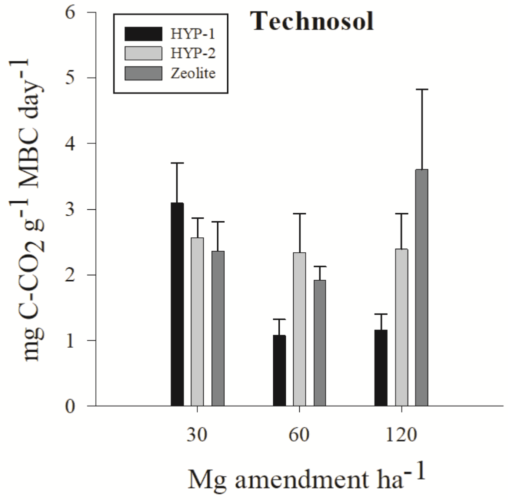
Material	Dose Mg ha^{-1}	pH	K	P	Ca	Mg	Al	CEC	CEC pH$_{7.0}$	S
			----- mg kg^{-1} -----		------------------------------ cmol$_c$ kg^{-1}------------------------------					mg kg^{-1}
Technosol										
Control	0	4.45 (0.03)	193 (2.43)	392 (23.7)	3.53 (0.13)	5.87 (0.22)	0.10 (0.00)	10.0 (0.11)	17.2 (1.44)	1467 (91.8)
HYP-1	15	4.93 (0.09)	273 (18.6)	523 (52.5)	9.15 (0.85)	5.84 (0.43)	0.10 (0.00)	15.8 (0.58)	18.4 (0.12)	1295 (94.7)
	30	5.20 (0.14)	302 (10.9)	574 (20.6)	11.5 (0.97)	4.89 (0.43)	0.13 (0.03)	17.3 (0.75)	21.0 (1.23)	1006 (106)
	60	6.43 (0.12)	438 (9.30)	499 (43.1)	19.4 (0.20)	4.95 (0.18)	0.15 (0.03)	25.6 (0.33)	30.6 (2.79)	705 (42.3)
	120	7.85 (0.25)	801 (25.7)	431 (28.1)	23.4 (0.34)	1.78 (0.04)	0.17 (0.02)	27.4 (0.45)	28.0 (0.43)	289 (10.5)
HYP-2	15	4.85 (0.06)	279 (4.69)	419 (7.79)	7.46 (0.17)	5.41 (0.65)	0.08 (0.03)	13.7 (0.75)	19.3 (1.19)	1201 (65.2)
	30	5.33 (0.02)	428 (11.2)	502 (4.55)	11.6 (0.25)	5.09 (0.15)	0.13 (0.03)	17.9 (0.16)	22.6 (2.44)	1011 (22.8)
	60	5.98 (0.13)	768 (9.05)	519 (27.6)	17.7 (0.30)	5.06 (0.28)	0.18 (0.03)	24.9 (0.52)	25.8 (0.58)	732 (9.71)
	120	8.00 (0.00)	1492 (58.3)	399 (26.6)	19.6 (0.52)	2.16 (0.08)	0.15 (0.03)	25.7 (0.66)	26.3 (0.67)	315 (7.34)
Zeolite	15	4.55 (0.05)	295 (7.44)	372 (19.6)	3.94 (0.20)	5.05 (1.13)	0.10 (0.00)	9.85 (1.30)	14.6 (1.53)	1323 (326)
	30	4.55 (0.03)	400 (4.42)	435 (74.0)	4.62 (0.29)	4.97 (0.56)	0.10 (0.00)	10.7 (0.76)	15.3 (0.19)	591 (332)
	60	4.57 (0.02)	528 (1.80)	526 (78.0)	6.61 (0.40)	5.17 (1.10)	0.10 (0.00)	13.2 (1.50)	17.1 (0.60)	1056 (209)
	120	4.83 (0.05)	834 (4.00)	391 (13.5)	8.22 (0.30)	4.65 (0.63)	0.13 (0.03)	15.1 (0.82)	21.1 (1.21)	1192 (43.8)
Oxisol										
Control	0	5.23 (0.06)	189 (6.31)	336 (37.9)	2.28 (0.21)	1.52 (0.17)	0.03 (0.03)	4.31 (0.40)	9.06 (0.48)	270 (44.5)
HYP-1	15	6.35 (0.21)	274 (8.21)	346 (21.5)	5.51 (0.39)	1.51 (0.20)	0.08 (0.03)	7.81 (0.61)	9.68 (0.68)	250 (40.8)
	30	6.98 (0.18)	347 (7.97)	405 (18.1)	6.09 (0.66)	1.09 (0.23)	0.08 (0.03)	8.15 (0.90)	9.22 (0.900	187 (52.0)
	60	7.53 (0.11)	575 (20.4)	469 (73.9)	7.59 (0.37)	0.78 (0.02)	0.10 (0.00)	9.94 (0.39)	10.8 (0.35)	116 (5.41)
	120	8.13 (0.02)	1017 (9.76)	371 (17.9)	7.48 (0.28)	0.40 (0.02)	0.10 (0.00)	10.6 (0.30)	11.2 (0.310	171 (4.54)
HYP-2	15	5.83 (0.08)	328 (9.02)	340 (18.3)	4.79 (0.13)	1.33 (0.06)	0.08 (0.03)	7.03 (0.17)	8.93 (0.26)	182 (8.42)
	30	6.68 (0.06)	516 (8.91)	371 (16.2)	5.40 (0.32)	1.20 (0.18)	0.10 (0.00)	8.02 (0.47)	9.22 (0.53)	180 (32.2)
	60	7.38 (0.21)	996 (13.3)	531 (77.0)	7.34 (0.12)	1.04 (0.02)	0.10 (0.04)	11.0 (0.14)	11.8 (0.15)	137 (3.74)

	120	8.13 (0.11)	1746 (49.4)	328 (52.7)	5.64 (0.79)	0.51 (0.09)	0.05 (0.03)	10.7 (0.79)	11.2 (0.80)	213 (34.1)
Zeolite	15	5.13 (0.13)	325 (6.54)	336 (13.3)	2.96 (0.18)	1.50 (0.12)	0.05 (0.03)	5.34 (0.31)	9.92 (0.31)	294 (54.7)
	30	4.98 (0.05)	446 (5.86)	348 (21.2)	3.78 (0.04)	1.93 (0.25)	0.03 (0.03)	6.88 (0.31)	11.5 (0.55)	436 (36.0)
	60	4.98 (0.03)	586 (8.26)	353 (26.9)	4.74 (0.23)	1.89 (0.04)	0.10 (0.00)	8.23 (0.23)	12.9 (0.18)	462 (22.0)
	120	5.08 (0.09)	794 (13.1)	248 (20.1)	6.10 (0.28)	1.85 (0.24)	0.10 (0.00)	10.1 (0.50)	14.1 (0.63)	318 (75.1)

The metabolic coefficient was determined by the ratio of microbial biomass carbon and microbial basal respiration (MBR) MBR/MBC (Anderson; Domsch, 1993).

Anderson, T.H., Domsch, K.H. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biology and Biochemistry, p. 393-395, 1993.

a) qCO_2



b) qCO_2

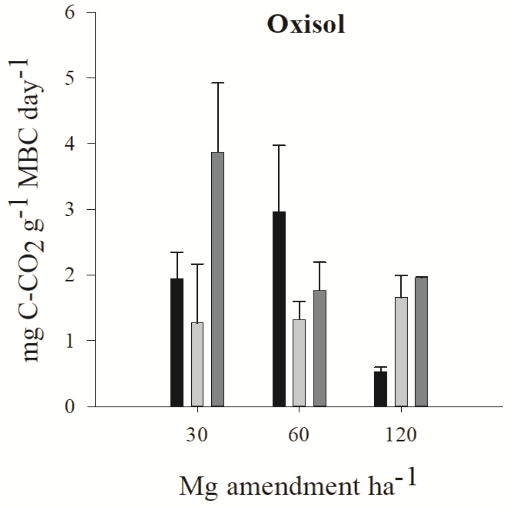


Fig. S3. Metabolic quotient (qCO_2) of (a) Technosol and (b) Oxisol

Bioaccumulation coefficients for shoots - ratio between the concentration of Cd, Zn, and Pb in *E. grandis* and *H. vitalbae* shoots and the concentration of these metals in soil (Buscaroli, 2017).

Buscaroli, A. (2017). An overview of indexes to evaluate terrestrial plants for phytoremediation purposes (Review). Ecological Indicators 82, 367-380.

Table S5. Bioaccumulation of Zn, Cd, and Pb in shoots of *Eucalyptus grandis* and *Heterocondylus vitalbae*.

Plant	Soil	Material	Dose (Mg ha^{-1})	Zn	Cd	Pb
Eucalyptus grandis	Technosol	HYP-1	60	0.14	0.31	0.02
			120	0.03	0.05	0.02
		HYP-2	60	0.24	0.29	0.02
			120	0.02	0.05	0.02
	Oxisol	HYP-1	15	0.19	0.23	0.02
			30	0.05	0.06	0.03
			60	0.06	0.06	0.03
			120	0.06	0.02	0.03
		HYP-2	15	0.20	0.26	0.03
			30	0.12	0.07	0.03
			60	0.06	0.03	0.03
			120	0.05	0.02	0.03
Heterocondylus vitalbae	Technosol	HYP-1	60	0.08	3.43	0.03
			120	0.01	0.50	0.03
		HYP-2	60	0.11	0.92	0.03
			120	0.01	0.44	0.02
	Oxisol	HYP-1	15	0.17	1.20	0.04
			30	0.06	1.22	0.05
			60	0.04	0.83	0.04
			120	0.03	0.58	0.04
		HYP-2	15	0.12	1.10	0.04
			30	0.07	1.10	0.04
			60	0.03	0.72	0.04
			120	0.03	0.31	0.04

MANUSCRIPT 4: Combined effects of hydrothermally-altered feldspar and water regime on decreasing cadmium accumulation in rice[1]

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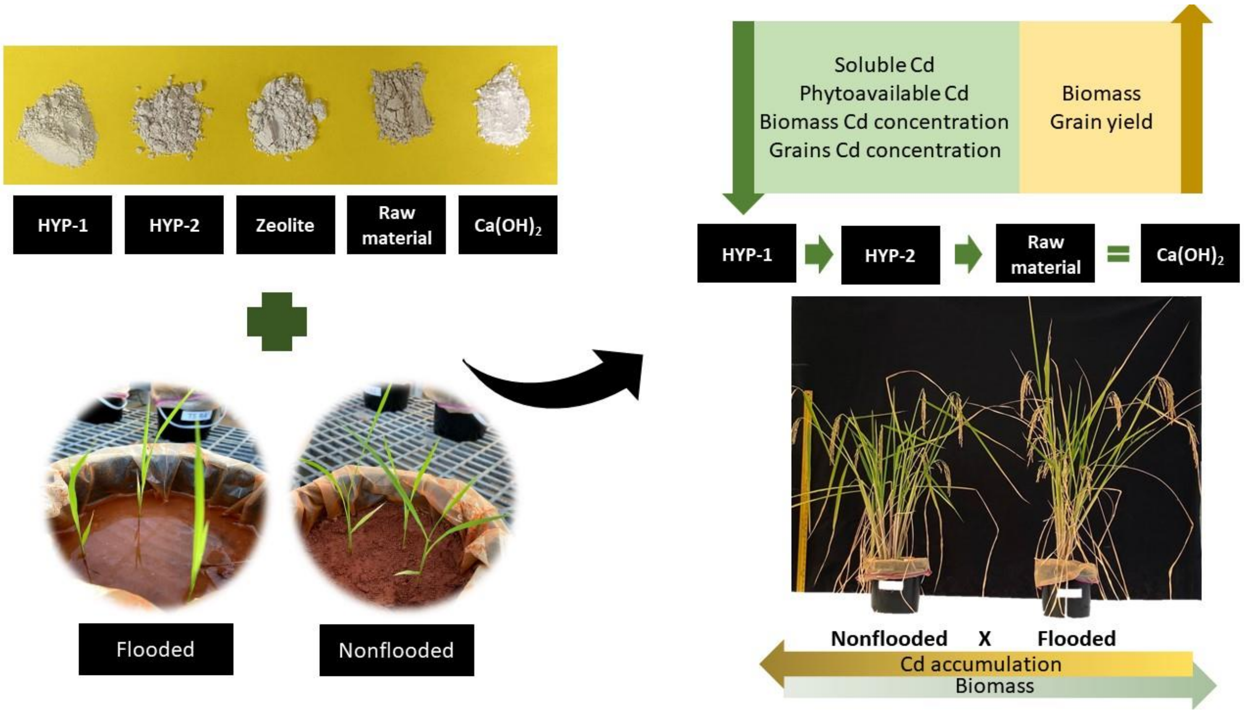
[1] The article was written according to the rules of the scientific journal Chemosphere. This article is a preliminary version and, therefore, changes may appear to adapt it.

Abstract

The accumulation of cadmium (Cd) in grains and edible parts of crops poses a risk to human health. Because rice is the staple food of more than half of the world population, reducing Cd uptake by rice is critical for food safety. HydroPotash (HYP), an innovative potassium fertilizer produced with hydrothermal process, has the characteristics for immobilizing heavy metals and potential use for remediating Cd contaminated soils. The objective of this study was to evaluate the HYP as a soil amendment to immobilize Cd in an acidic soil and to reduce the accumulation of Cd in rice tissues. The experiment was performed in a greenhouse with a Cecil sandy loam soil (pH 5.3 and spiked with 3 mg Cd kg^{-1}) under either flooding conditions (water level at 4 cm above the soil surface) or at field capacity. Two hydrothermal materials (HYP-1 and HYP-2) were compared with zeolite, K-feldspar + $Ca(OH)_2$ (the raw material used for producing HYP), $Ca(OH)_2$, and a control (without amendment). HydroPotashs, the raw material, and $Ca(OH)_2$ increased pH and electrical conductivity of the soil solution collected 30 days after applying treatments. These materials reduced the soluble concentration of Cd (up to 99.7%) compared with the control ($p < 0.05$). Under the flooded regime, irrespectively of the materials applied, plant growth was favored. HydroPotash-1 was more effective for increasing dry biomass compared with other amendments under both water regimes. HydroPotashs reduced phytoavailable Cd in soil, Cd content in plant biomass at tillering and maturing stage, and were efficient in minimizing Cd accumulation in rice grains.

Keywords: food safety; HydroPotash; cadmium fractionation; soil amendment; green technology; irrigation

Graphical abstract



Highlights

HydroPotash decreased Cd accumulation in rice biomass at tillering stage

HydroPotash decreased Cd accumulation in rice biomass at the maturation stage

HydroPotash decreased Cd accumulation in rice grains

The highest decreases of Cd absorption were obtained under the flooded regime

1. Introduction

Much has been discussed about food security worldwide and cadmium (Cd) is a recurring concern. Cadmium contamination in soil occurs due to weathering of rocks, mining activities, and the use of fertilizers, pesticides, and wastewater (Rai et al., 2019; Srivastava et al., 2017; Wuana and Okieimen, 2011). One of the most common pathways for Cd exposure is through ingestion of food produced from Cd contaminated soils (ATSDR, 2012). Chronic oral exposure to high levels of Cd by humans can cause epidermal lesions, kidney damage, and other health problems (ATSDR, 2012).

There are many cases of rice (*Oryza sativa*) contaminated with Cd (Aoshima, 2016; Du et al., 2013; Fangmin et al., 2006). The most famous one is the Cd-related disease of Itai Itai, where an area cultivated with rice was irrigated with Cd-contaminated wastewater from a zinc mining in Japan (Aoshima, 2016; Yoshida et al., 1999). In areas used for food production, decreasing the accumulation of toxic elements in food grains can be achieved through genetic improvement techniques, optimization of water and fertilizer management, by growing cover crops, and thru the application of soil amendments (Sebastian and Prasad, 2014).

Hydrothermally-altered Feldspar (HydroPotash, HYP) Advanced Potash Technologies is a novel multi-nutritional (potassium, silicon, and calcium), chloride-free, environmentally friendly (natural raw materials) fertilizer produced from K-feldspar. HydroPotash can be produced anywhere globally, as K-feldspar-rich rocks are abundant worldwide (Ciceri et al., 2017). This fertilizer is therefore suited for countries that currently depend on the importation of potassium fertilizer and can help to address global food security towards 2050. The production process is sustainable and does not produce any by-products or waste. This fertilizer also possesses the ability to immobilize heavy metals and can be used to remediate Cd-contaminated soils due to its high buffering pH and cation exchange capacity (Ribeiro et al., 2021).

Considering that absorption and accumulation of Cd in grains and edible parts of cultivated plants lead to a risk to human health (Afonne and Ifediba, 2020; Rai et al., 2019) and that rice is the staple food of more than half of the world population, immobilization of Cd in rice-growing Cd contaminated soils is critical for food safety and food quality (Hussain et al., 2021; Sebastian and Prasad, 2014; Seyfferth et al., 2019). We hypothesize that the application of HYP in acidic soils will immobilize Cd, reduce Cd uptake by rice, and improve rice growth and yield.

The main objective of this study was to evaluate the use of HYP as a soil amendment to immobilize Cd in acidic soil and reduce the accumulation of Cd in rice. As rice is produced in different water regimes and this directly influences Cd absorption by plants (Seyfferth et al., 2019), the experiment was carried out under different soil moisture conditions (nonflooded and flooded). The zeolite, $Ca(OH)_2$, a mixture of raw materials used for producing HYP (K-feldspar + $Ca(OH)_2$), and a control (soil without amendment) were also included in the experiment for comparison.

2. Material and Methods

2.1 Soil and amendments characterization

The study was carried out in a greenhouse with an average temperature of 28(± 3) ºC during February and August, in Homestead, FL, USA. The Cecil sandy loam (USDA, 2009) was collected from the Wall Pit Mine in Taylor County, Georgia, USA (Vargas et al., 2017). The soil was sieved through 4 mm for the pot experiment and 2 mm for the chemical analyses. The physical characterization of the soil was performed based on methods described by Vargas et al. (2017). The basic properties of the soil are 56.6% sand, 5.3% silt, 38% clay, 7.78% organic matter, and 0.039 mg kg^{-1} total Cd before spiking with Cd. After air-drying, the soil was spiked with $Cd(NO_3)_2.4H_2O$ (Sigma-Aldrich, St. Louis, MO) to raise Cd concentration to 3 mg kg^{-1} as a Cd-contaminated soil.

Two hydrothermally-altered feldspars (HYP-1 and HYP-2), raw materials of HYP (K-feldspar plus $Ca(OH)_2$), and $Ca(OH)_2$ were provided by Advanced Potash Technologies Ltd. The detailed preparation of HYPs was described by Ciceri et al. (2017). Briefly, feldspars (>80% pure $KAlSi_3O_8$) and $CaOH_2$ were mixed with a hydrothermal process (HYP-1: 200 ºC for 1h and HYP-2: 230 ºC for 2.5 h) (Ciceri et al., 2017, Ribeiro et al., 2021), milled, and dried. A zeolite (hydrated aluminosilicate mineral) was obtained from Celta Brasil (Cotia, Brazil). The above materials were measured for pH in water (1:10 product: water ratio) after 30 minutes of agitation (Seyfferth et al., 2019) using an equipment AR60, Fisher, Fisher Scientific, Fairlawn, NJ and showed pH values of 11.9, 11.6, 7.3, 12.2, and 12.2 for HYP-1, HYP-2, zeolite, the raw material of HYPs, and $Ca(OH)_2$, respectively.

2.2 Experimental design

Forty-eight nursery pots (4 L) were packed with 3.5 kg of air-dried soil and the stock solution of cadmium nitrate uniformly applied on the soil. Then Cd spiked soil was incubated for 30 days with soil moisture at 70% field capacity. After Cd aging in the soil, the amendments were applied (hand-mixing) at the rate of 2% for HYP-1, HYP-2, zeolite, and raw-material [product: soil ratio (w:w)]. The amount of $Ca(OH)_2$ applied was its percentage in feed material (0.187 of 2%). A soil without amendment was used as control. During the experiment, 24 pots were flooded to 4 cm above the soil surface representing the flooded treatment, and the remaining pots were kept under field capacity as a nonflooded treatment. Irrigation was made by hand using tap water (<0.15 µg Cd L^{-1}). The experiment was conducted using a completely randomized design with four replicates of each treatment.

2.3 Soil analyses before cultivation

After 30-days of incubation of soil with amendments, soil solution was collected utilizing Rhizon samplers (Part no. 192101, Eijkelkamp, Agrisearch Equipment, The Netherlands). A portion of the extract was used to measure soil solution pH and EC immediately after extraction using a pH meter (AR60, Fisher, Fisher Scientific, Fairlawn, NJ). The second portion of the extract was analyzed for Cd using inductively coupled plasma mass spectrometry ICP-MS (HP4500 PLUS, Agilent Technologies, Palo Alto, CA, USA) after acidifying samples with HNO_3 - 2% v/v. The concentrations of chloride, nitrite, bromide, nitrate, phosphate, sulfate, sodium, ammonium, potassium, magnesium, and calcium were also determined using ion chromatography (Dionex, Thermo Fisher Scientific, Waltham, MA). The Cd and ions concentrations were entered in MINTEQ 3.1 software to predict Cd speciation in soil solution.

After incubation, soil samples were also collected, dried, and sieved through 2mm, and extracted with DTPA-TEA (pH= 7.3) (Lindsay and Norvell, 1978) for analysis of extractable Cd and other elements with ICP-MS (HP4500 PLUS, Agilent Technologies, Palo Alto, CA, USA).

2.4 Rice cultivation and analyses

The seeds of a long grain rice cultivar (XL753) were soaked in distilled water for 24 h before planted in containers filled with Cecil soil (no Cd spiked). At 21 days after germination, two seedlings with two fully expanded leaves were transplanted to pots with both Cd spiked control soil and amended soil treatments. The same amounts of the regular fertilizers with N, P, and K were applied for all pots following the description of Zhang et al. (2019). In sum, N was applied as urea at rates of 200 mg kg^{-1} as basal fertilization, 120 mg kg^{-1} at the tillering stage, and 80 mg kg^{-1} at the panicle stage. Monocalcium phosphate was applied at a rate of 200 mg kg^{-1}, and muriate of potash was applied at rates of 112 mg kg^{-1} as basal fertilization, and 48 mg kg $^{-1}$ at the panicle stage, except for HYP-1 and HYP-2 that had only received 48 mg kg^{-1} of K with consideration of K in these products. Sulfur was applied as magnesium sulfate at a 50 mg kg^{-1} rate. After some plants showing micronutrient deficiencies at the tillering stage, a mix of micronutrients consisted of 0.2% boric acid, 0.3% manganese sulfate, 0.5% zinc sulfate, 0.1% ammonium molybdate, and 20% iron as iron sulfate was applied twice with 15 days apart via foliar spray. Copper sulfate was applied in the soil at a rate of 1.5 mg kg^{-1}.

At 30 days after transplanting, one plant was collected from each pot, oven-dried, and the dry shoot mass was recorded. The Cd contents in removed plants were determined using ICP-MS (HP4500 PLUS, Agilent Technologies, Palo Alto, CA, USA) after the digestion with nitric acid + hydrogen peroxide (Modified EPA 3051a method).

Rice plants were harvested 145 days after transplanting and grains were separated from plants. The biomasses of plants were washed in running tap water following by distilled water. Both biomass and grains were dried in an oven at 70ºC for 72 h. After dried, grains were separated from husks. Grains and shoots were weighted for dry weight and grounded in a blender before total Cd determination (same methodology described before).

2.5 Soil analyses after cultivation

Soil DTPA-extractable Cd was determined as described above. The fractionation of Cd forms was assessed with the method of Tessier sequential extraction (Tessier et al., 1979). Briefly, one g dried soil was extracted with five extractants [1 M $MgCl_2$; 1 M CH_3COONa (pH = 5); 0.04 M $NH_2OH.HCl$; 0.02 M HNO_3 + 30% H_2O_2 and 3.2 M NH_4OAc in 20% HNO_3) for four Cd factions [exchangeable (Cd-EXC), carbonate bounded (Cd-CAR), iron and manganese oxides bounded (Cd-OXI), organic (Cd-OM)]. The residual Cd (Cd-RES) was obtained by subtracting these fractions from the total Cd (Chavez et al., 2016) which was determined by microwave digestion with HCl. After each extraction, the samples were washed with 8 mL of distilled water and centrifuged again (12,000 x g) before adding the next solution (Tessier, 1979).

2.6 Statistical analysis

After analyses of variance, Dunnett's test was used to compared products' effect with the control treatment ($p<0.05$). Tukey's test compared the effect of water regime and various products in the same water regime. The analyses were made in the R Environment (R Core Team, 2017).

3. Results and discussion

3.1 Effects of soil amendments on Cd chemistry of soil solution and bioavailability of Cd in the soil after incubation for 30 days

The addition of HYPs, $Ca(OH)_2$, and raw material of HYP increased pH of soil solution significantly for both flooded and nonflooded regimes, and consequently, Cd concentrations in soil solutions collected from these treatments were dramatically decreased (up to 99.8%) compared with the control and zeolite treatments ($p<0.05$) (Table 1). These amendments play the role of lime materials because of their high pH (11.6-12.2).

Effects of soil amendments on EC of soil solutions were similar to those on pH of soil solutions (Table 1). All treatments except flooded and nonflooded zeolite increased EC of soil solutions compared with the control without any amendment. The EC values increased in soil solution were due to the dissolution of the materials applied. As expected, flooding reduced the EC of soil solutions for all treatments except zeolite.

According to the calculations of a chemical equilibrium model (MINTEQ 3.1), the free form (Cd^{2+}) was the dominant species of Cd (42.5 – 82.8%) in soil solutions from all treatments (Table S1). This form of Cd was much higher in the control (78.3 – 80.9%) and the treatment with zeolite (78.5-82.8%) in both water regimes than in treatments with other products (42.5-67.2%). The addition of alkaline materials decreased free forms of Cd in soil solution, following the results of pH and soluble Cd in solution (Table 1). The highest decreases in Cd^{2+} form compared with the control were obtained with the application of HYP-2 (29.1%) and $Ca(OH)_2$ (47.5%) at nonflooded and flooded regimes, respectively.

Table 1. Soil solution characterization after 30 days of product application. Means and standard errors (n=4)

Water regime	**Material**	**pH^{1}***	**EC^{1} (μS cm^{-1})**	**Cd (μg L^{-1})**
Nonflooded	Control	5.7 (0.07)	359 (18.2)	72.9 (20.3)
	Zeolite	5.5 (0.16)	287 (42.8)	96.1 (7.6)
	HYP-1	7.9 (0.03)*	1,410 (74.3)*	0.29 (0.03)*
	HYP-2	8.0 (0.02) *	1,890 (193)*	0.36 (0.02)*
	Raw material	8.0 (0.11)*	1,018 (36.1)*	0.60 (0.09)*
	$Ca(OH)_2$	8.3 (0.25)*	1,270 (114)*	0.29 (0.05)*
Flooded	Control	6.8 (0.02)	328 (14.1)	94.6 (11.9)

Zeolite	6.8 (0.14)	413 (31.04)	72.01 (20.0)
HYP-1	7.9 (0.05)*	1,195 (43.3)*	0.37 (0.04)*
HYP-2	8.0 (0.03)*	1,243 (32.0)*	0.25 (0.03)*
Raw material	8.0 (0.29)*	612 (228)*	0.97 (0.69)*
$Ca(OH)_2$	10 (0.29)*	834 (31.7)*	0.20 (0.06)*

* Different to control group at $p<0.05$ according to Dunnett´s test

Redox potential varies with soil water content and directly influences Cd mobility (Seyfferth et al., 2019). When the soil is flooded, the potential redox decreases, and the reductive condition directly increases the mobility of Cd because the reduction of Fe and Mn oxides (Suda and Makino, 2016) and dissolution of organic matter (Ponting et al., 2021) releasing Cd previously adsorbed. Contrarily, Cd solubility may decrease in reductive environments due to Cd sulfide formation and the transformation of the Cd soluble fraction into other forms due to an increase in the soil pH (Ponting et al., 2021; Sumi et al., 2014). It is well documented that due to reduction flooded soils undergo an increase in soil pH. In this study, the soil solution pH increased 1.1 units (5.7 - 6.8) in the control group at the flooded regime compared with nonflooded (Table 1).

Besides the water regimes, it is clear that the application of alkaline materials contributed to lower Cd concentration in soil solution. Also, phytoavailable Cd in soils amended with these products decreased after 30 days of incubation (Table 2). In fact, HYPs reduced 32.6% and 21.8% of the phytoavailable Cd under nonflooded and flooded regimes, respectively. The soil pH is the main factor controlling Cd availability (Yu et al., 2016) because it may directly promote metal precipitation (Kabata-Pendias, 2011) or increase soil CEC in soil with variable charges. Under the nonflooded regime, HYPs were the most effective products reducing Cd phytoavailability, better than the raw material and $Ca(OH)_2$. It indicates that besides precipitation, adsorption may play the role of immobilization of Cd in soils amended with these products (Ribeiro et al., 2021). The water regime affected Cd phytoavailability only with $Ca(OH)_2$, control, and zeolite treatments ($p<0.05$).

As a potash fertilizer, HYPs increased K phytoavailable content in the soil, which was 12 and 36 fold-greater with HYP-2 treatments compared with the control at nonflooded and flooded regimes, respectively. It is interesting to notice the role of hydrothermal alteration in the K availability. HydroPotash-1 and HYP-2 increased up to 1390% the K availability compared with the raw material after 30 days of soil incubation (Table 2). The hydrolytic dissolution of the feldspar structure coupled with the incorporation of Ca^{2+} instead of K^+ may be responsible for this increased availability of K (Ciceri et al., 2017).

Except for zeolite, all products increased about 50% of phytoavailable Ca under both water regimes compared with the control (Table 2). The application of alkaline materials decreased phytoavailable Mg in soils as observed in a previous experiment (Ribeiro et al., 2021 submitted). This is probably related to the pH increase causing Mg precipitation and isomorphic coprecipitation with Al species (Miyazawa et al., 2001). Except for Mn, all other micronutrient content in soil increased with HYPs application.

Table 2 – Phytoavailable Cd and other nutrients in soil without amendment and amended with products and incubated for 30 days under flooded and nonflooded water regimes. Mean values and standard error (n=4).

System	Material	Cd*	Zn	Cu	Ca	Mg	Al	Fe	Mn	K
Nonflooded	----------	----------	----------	----------	---------- mg kg^{-1} ----------	----------	----------	----------	----------	----------
	Control	0.95 (0.02)Aab	0.93 (0.28)	0.11 (0.01)	341.15 (4.19)	37.90 (0.50)	4.23 (0.51)	5.06 (0.38)	1.71 (0.09)	4.91 (0.15)
	Zeolite	0.98 (0.02)Aa	0.74 (0.13)	0.09 (0.01)	361.11 (4.15)	33.39 (1.09)	4.37 (1.15)	6.10 (0.65)	1.88 (0.08)	36.74 (1.30)
	HYP-1	0.69 (0.06)Ad	1.78 (0.14)	0.20 (0.02)	676.02 (69.90)	8.00 (0.85)	1.73 (0.54)	6.98 (0.76)	0.85 (0.08)	31.38 (3.47)
	HYP-2	0.64 (0.01)Ad	1.77 (0.15)	0.18 (0.01)	683.77 (47.01)	5.18 (0.38)	0.83 (0.27)	6.24 (0.40)	0.73 (0.05)	58.73 (4.31)
	Raw material	0.85 (0.01)Abc	2.31 (0.44)	0.22 (0.03)	675.93 (9.37)	7.82 (0.37)	5.37 (2.23)	8.60 (0.76)	1.07 (0.03)	13.96 (0.11)
	$Ca(OH)_2$	0.82 (0.01)Ac	1.72 (0.39)	0.25 (0.06)	687.02 (6.48)	7.04 (0.26)	3.33 (0.76)	6.86 (0.09)	0.99 (0.03)	7.67 (0.21)
Flooded	Control	0.87 (0.02)Ba	0.70 (0.12)	0.08 (0.01)	353.26 (10.45)	37.58 (1.12)	4.50 (0.97)	4.81 (0.32)	1.34 (0.09)	4.79 (0.20)
	Zeolite	0.83 (0.01)Ba	0.80 (0.23)	0.13 (0.06)	368.36 (6.70)	32.84 (1.16)	2.78 (0.31)	4.64 (0.47)	1.62 (0.05)	35.96 (1.47)
	HYP-1	0.72 (0.04)Abc	1.06 (0.27)	0.20 (0.03)	663.02 (24.66)	6.36 (0.32)	3.89 (0.62)	9.52 (1.56)	1.17 (0.07)	86.17 (5.23)

HYP-2	0.68 (0.01)Ac	0.63 (0.07)	0.30 (0.10)	656.13 (18.65)	6.15 (0.31)	9.24 (1.79)	7.44 (0.41)	1.14 (0.06)	172.10 (3.27)
Raw material	0.80 (0.03)Aab	0.58 (0.04)	0.16 (0.04)	634.95 (97.00)	6.80 (0.10)	3.21 (0.25)	6.48 (0.98)	1.26 (0.03)	11.55 (2.39)
$Ca(OH)_2$	0.71 (0.02)Bbc	0.51 (0.06)	0.17 (0.01)	700.61 (26.36)	5.28 (0.28)	4.16 (1.31)	7.43 (0.40)	1.00 (0.02)	7.36 (0.06)

*Lower case letters compare products at the same water regime and capital letters compare the effect of water regime in each product.

3.2 Rice cultivation and analyses

3.2.1 Biomass and Cd content at the tillering stage

Rice dry biomass at the tillering stage was superior in flooded compared with nonflooded water regime ($p < 0.05$) (Fig 1a). No interactions between water regime and materials were observed. Considering both systems, HYP-1 was superior to the control and the raw material ($p < 0.05$). Due to the small amount of material, the Cd concentration reported in Fig.1b is the concentration found in a composite sample of four repetitions (no statistical analysis was applied). The application of all tested materials reduced Cd concentration in plant tissues compared with the control.

At the tillering stage, plants growing in control treatments at nonflooded conditions were affected by Cd toxicity. The decreased shoot growth and chlorosis could be the direct effect of this element in photosynthesis and respiration suppression, oxidative stress, and altered enzyme activity and nutrient absorption (Kabata-Pendias, 2011).

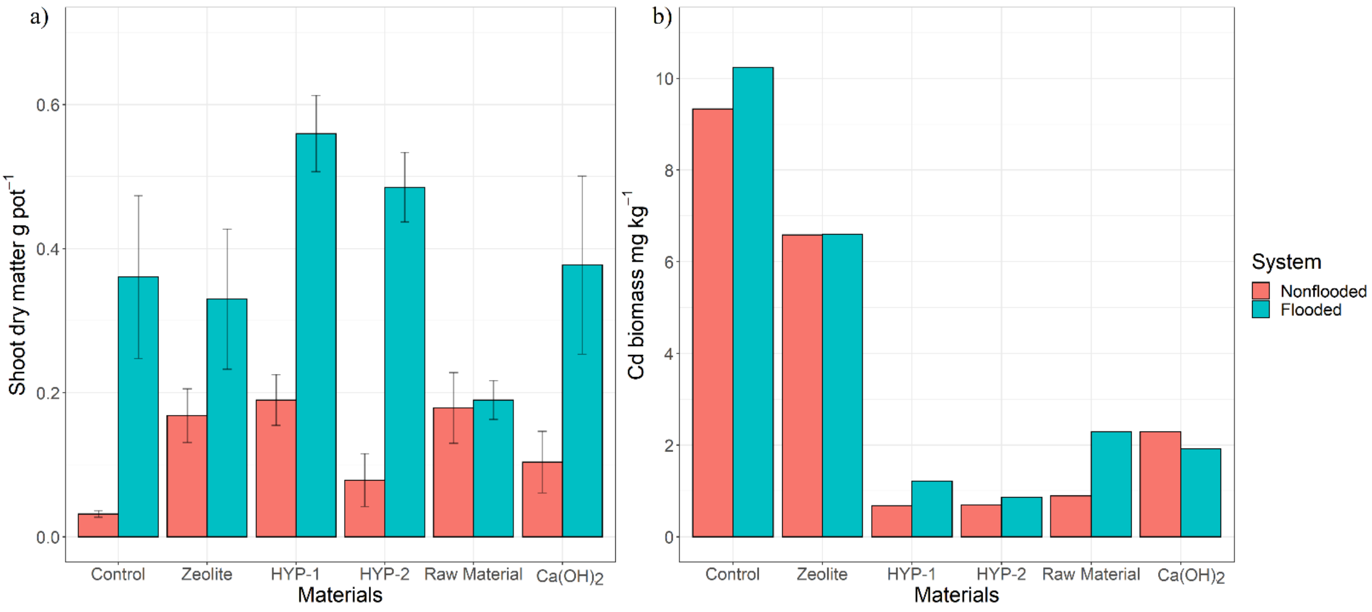


Fig. 1. Dry shoot mass (g) (a) and metal content (mg kg^{-1}) (b) in rice plant at tillering stage. Vertical bars represent standard errors (n=4). Due to the limited amount of dry matter for chemical analysis, concentrations of Cd reported in (Fig.1b) were based on a composite sample of four replicates.

3.2.2 Biomass and Cd content at harvest

No interactions between water regime and materials were observed for biomass production at maturing stage ($p < 0.05$) (Fig 2a). Shoot dry biomass at harvest showed that rice grew better under the flooded than the nonflooded water regime (Fig. 2a). Considering both water regimes, HYP-1 and zeolite produced higher biomass than the raw material and $Ca(OH)_2$ ($p < 0.05$).

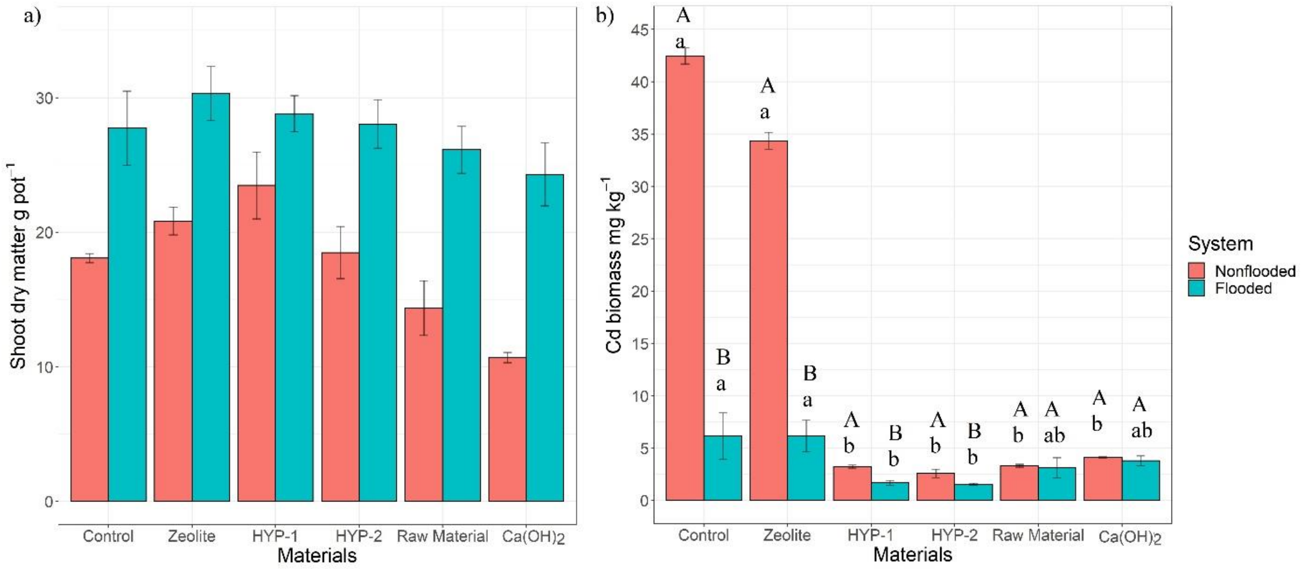


Fig. 2. Rice dry mass production (g pot^{-1}) (a) and Cd concentration in rice biomass (mg kg^{-1}) (b) at harvest. Lower case letters compare products at the same water regime, and capital letters compare the effect of the water regime on each product. Vertical bars represent standard errors (n=4).

Cadmium concentrations in rice biomass under flooded regime decreased drastically for control and zeolite treatments when compared with nonflooded regime (Fig.2b). The water regime also affected Cd uptake by rice treated with HYPs, and HYP-2 reduced up to 94 and 75% of Cd concentration in rice biomass at nonflooded and flooded regimes, respectively, when compared with the control. Flooding decreased 86% of Cd accumulation in rice biomass compared with the control in both water regimes. Alkaline materials (HYP-1, HYP-2, raw material, and $Ca(OH)_2$) also reduced Cd absorption even at the flooded regime. It is worth mentioning that although not statistically different, HYP-1 and HYP-2 had additional effects in Cd minimization. These products reduced up to 50.6 and 59% of Cd concentration in rice biomass when compared with the raw material and $Ca(OH)_2$, respectively.

3.2.3 Grain production and Cd in grain

Production of rice grain was much higher under flooded regime than under nonflooded regime. The product application did not affect grain production under the flooded regime ($p<0.05$) (Fig 3a), probably because flooding already reduced Cd availability and consequently reduced its impact on plant production. It is worth mentioning that although not statistically different, rice production was 23.3% higher under HYP-1 treatment compared with the control under the flooded regime. Although the effect of decreasing Cd accumulation in grains, the application of the raw material and $Ca(OH)_2$ decreased rice grain production at nonflooded conditions (Fig. 3b). On the other hand, the application of HYP-1 did not reduce rice production and reduce this element content in rice grains by 77.5% compared with the control.

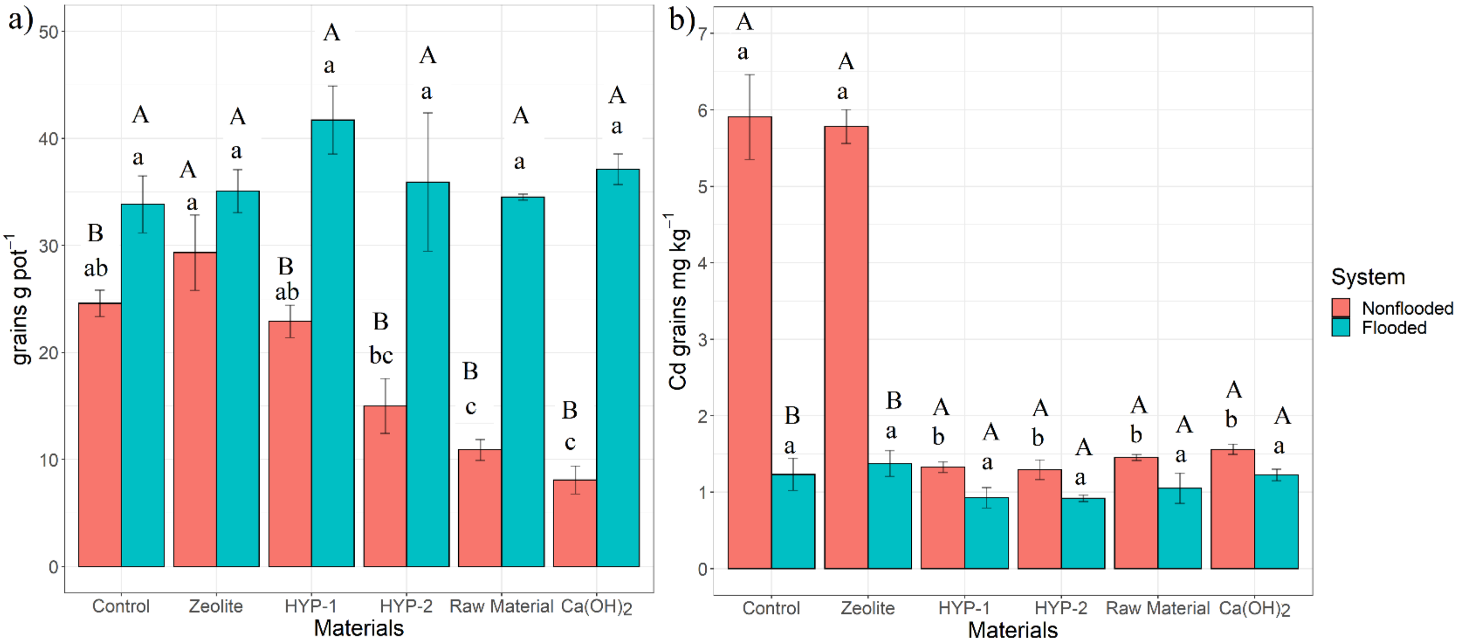


Fig. 3. Grains production (g)(a) and Cd concentration in rice grains (mg kg^{-1}) (b). Lower case letters compare products at the same water regime, and capital letters compare the effect of the water regime on each product. Vertical bars represent standard errors ($n=4$).

The nutrient content in plant biomass and grains are shown in Supplementary Materials (Table S2 and S3).

3.3 Analyses of soil collected after harvest

The DTPA analyses after cultivation are shown in the Supplementary Material (Table S4). In general, the control and zeolite treatments presented lower phytoavailable Cd contents at this time. This can be easily explained by the high accumulation of this element in biomass and rice grains (Fig. 1, 2, and 3), decreasing Cd content in the soil after cultivation. It is interesting to notice that Cd phytoavailable content was lower at the flooded regime for all treatments. Before cultivation, this only occurred in zeolite, the control, and $Ca(OH)_2$ treatments (Table 2).

Sequential extraction was performed further to investigate the impact of products on soil Cd phytoavailability. In general, alkaline materials decreased exchangeable Cd compared with the control at nonflooded conditions (Fig 4). However, products had an opposite effect on the soil carbonate-Cd, which increased in both water regimes. Organic matter-Cd and residual had the smallest percentages in studied soils. With few exceptions, the Cd dominant fraction for all treatments was bounded to Fe and Mn oxides (Fig. 4).

According to the standard protocol used in this study (i.e., the Tessier methodology), the soil should be dried for analysis of sequential metal extraction. Although the samples were dried and consequently the Cd may have remobilized to other fractions, the sequential fractionation analysis results indicate differences between fractions in soils under flooded and nonflooded conditions. Under flooded conditions, the exchangeable forms decreased compared with non-flooded, and the oxides form increased. Although we did not analyze amorphous and crystalline iron forms, the higher oxide-Cd fraction could have occurred due to the remobilization of exchangeable-Cd into amorphous Fe oxide bounded form (higher adsorption capacity than crystalline Fe). In a soil previously flooded, drying can change its fractions, decreasing oxidizable and residual fractions and increasing Cd reducible fraction (Qi et al., 2014). Also Fe oxidation have been described in reductive environments due to rice plants aerenchyma and roots (Hu et al., 2015; Yu et al., 2016).

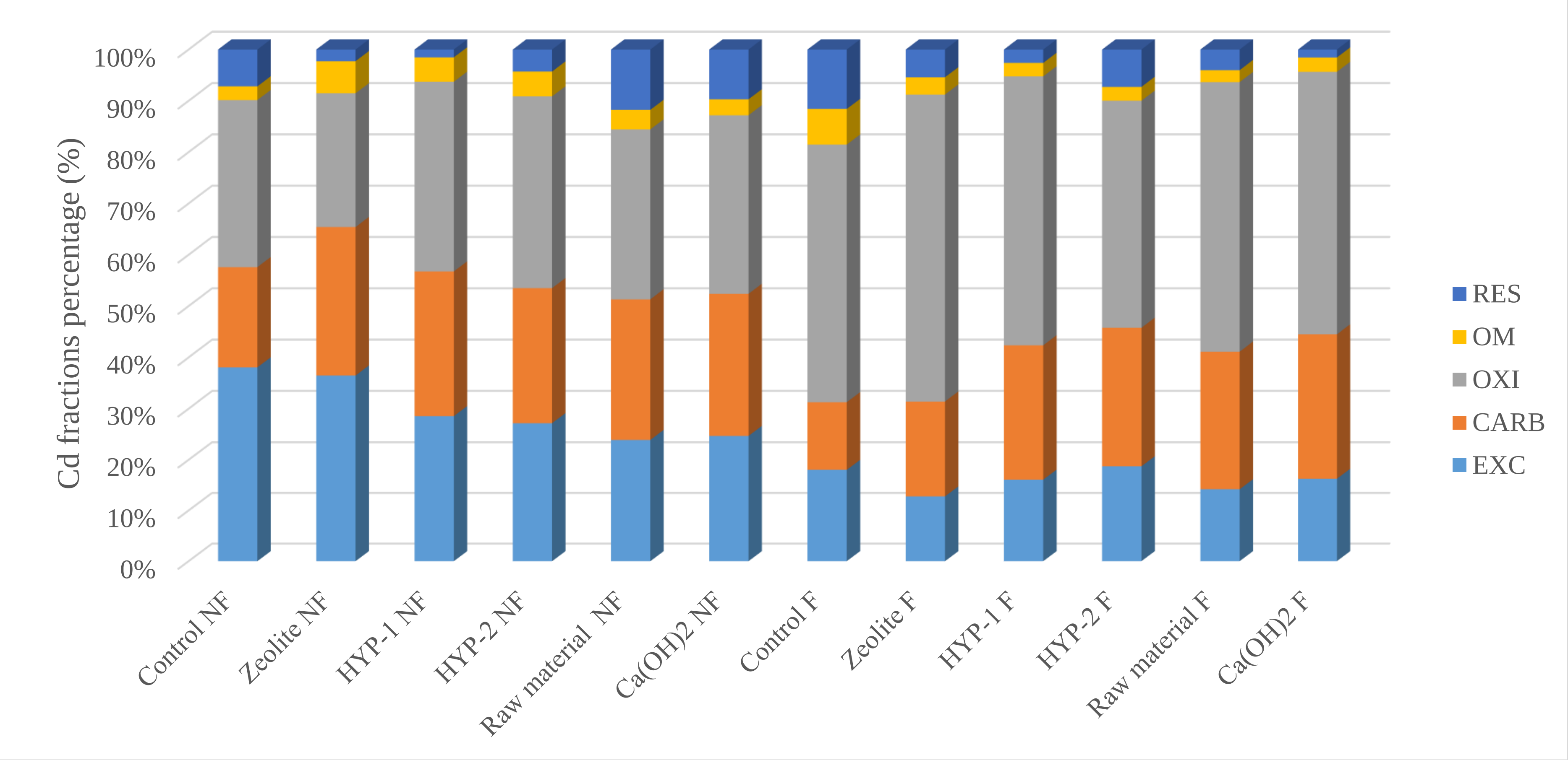


Fig. 4. Percentage of different Cd fractions in soil with different products application and water regimes. NF – nonflooded, F – flooded, Cd-EXC – exchangeable, Cd-CARB – carbonate bounded, Cd-OXI – iron and manganese oxides bounded, Cd-OM – organic matter bounded, Cd-RES – residual Cd.

4. Conclusion

HYPs reduced soluble Cd in soil solution, phytoavailable Cd in soil, and Cd contents in the plant at the tillering stage. These products also decreased Cd accumulation in rice biomass at the maturing stage and in rice grains.

By itself, a flooding condition can reduce Cd in rice biomass. However, HYP can decrease Cd uptake at a similar range in a nonflooded regime if flooding is not possible. Contrarily to $Ca(OH)_2$ and the raw material, HYP-1 combined positive effects on rice production and Cd minimization in grains.

Declaration of competing interest

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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Supplementary Material

Combined effects of hydrothermally-altered feldspar and water regime on decreasing cadmium accumulation in rice

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Table S1. Speciation of Cd predicted with a chemical equilibrium model (MINTEQ 3.1) in soil solution collected after incubating 30 days of soils without amendment (control) and with amendments (HYP-1, HYP-2, raw material, and $Ca(OH)_2$)

Water regime	Nonflooded						Flooded					
(%)	Control	Zeolite	HYP-1	HYP-2	Raw material	$Ca(OH)_2$	Control	Zeolite	HYP-1	HYP-2	Raw material	$Ca(OH)_2$
Cd^{+2}	78.3	82.8	59.8	55.5	64.9	59.6	80.9	78.5	62.6	65.4	67.2	42.5
$CdOH^+$	-	-	0.24	0.27	0.35	0.60	0.03	0.03	0.26	0.36	0.38	23.7
$Cd(OH)_2$ (aq)	-	-	-	-	-	-	-	-	-	-	-	13.3
$Cd(OH)_3^-$	-	-	-	-	-	-	-	-	-	-	-	0.02
$CdCl^+$	19.2	15.9	13.6	15.5	12.8	17.7	15.7	17.2	12.8	12.6	15.2	9.72
$CdCl_2$ (aq)	0.24	0.16	0.18	0.26	0.14	0.31	0.15	0.19	0.15	0.14	0.19	0.12
$CdBr^+$	0.03	0.03	0.04	0.03	0.05	0.06	0.02	0.03	0.04	0.03	0.04	0.03
$CdSO_4$ (aq)	2.11	0.94	23.0	24.4	19.4	20.0	3.10	4.02	21.7	20.4	14.0	9.36
$Cd(SO_4)_2^{-2}$	-	-	1.66	2.29	0.98	1.31	0.01	0.02	1.29	1.02	0.42	0.31
$CdNH_3^{+2}$	-	-	0.09	-	0.06	0.05	-	-	-	-	-	0.81
$CdNO_2^+$	0.12	0.12	0.04	0.04	0.09	0.02	-	-	-	-	0.11	0.07
$CdNO_3^+$	-	-	1.31	1.71	0.04	0.18	0.01	-	-	-	-	
$CdHPO_4$ (aq)	-	-	-	-	1.20	0.21	-	-	1.21	-	2.46	0.02

Table S2 – Elemental analyses in shoot tissues of rice cultivated in artificially Cd contaminated soil with different products application. Mean values and standard error (n=4).

System	Materials	Ca	Mg	K	Fe	Cu	Zn	Mn
		--------------------- g kg^{-1}---------------------			------------------------------------- mg kg^{-1}------------------------------------			
Nonflooded	HYP-1	2.69 (0.45)	5.08 (0.15)	18.88 (1.46)	115.7 (10.99)	8.20 (1.04)	5.97 (2.10)	150.2 (28.59)
	HYP-2	2.61 (0.47)	4.22 (0.17)	23.39 (1.06)	120.4 (19.24)	6.56 (0.67)	3.39 (0.74)	141.5 (11.37)
	Zeolite	3.22 (0.16)	5.68 (0.29)	25.58 (1.42)	92.34 (5.60)	3.86 (0.52)	21.30 (2.85)	1371 (53.9)
	Raw Material	6.26 (0.10)	8.73 (1.36)	19.32 (1.30)	113.5 (36.40)	4.95 (0.79)	21.96 (6.22)	238.5 (19.35)
	$Ca(OH)_2$	5.11 (0.78)	7.67 (0.74)	20.96 (0.56)	136.6 (18.37)	10.75 (4.61)	79.75 (43.59)	256.2 (23.62)
	Control	3.12 (0.23)	11.31 (0.58)	15.69 (0.25)	115.9 (18.27)	4.58 (0.61)	101.4 (25.66)	1281 (32.46)
Flooded	HYP1	1.60 (0.35)	5.45 (0.62)	14.49 (0.58)	88.65 (6.80)	3.14 (1.06)	55.84 (20.96)	99.37 (7.38)
	HYP2	1.64 (0.25)	4.88 (0.37)	17.79 (0.69)	94.81 (11.87)	4.14 (0.86)	69.08 (23.08)	101.34 (10.34)
	Zeolite	1.70 (0.21)	4.51 (0.67)	20.59 (2.25)	107.9 (6.99)	0.70 (0.55)	42.05 (6.82)	194.34 (49.75)
	Raw Material	2.82 (0.48)	7.09 (0.36)	14.20 (0.78)	148.9 (14.50)	1.51 (0.38)	40.60 (2.72)	219.5 (33.00)
	$Ca(OH)_2$	3.33 (0.47)	10.8 (0.73)	14.80 (0.75)	156.4 (15.43)	6.63 (3.03)	40.39 (17.26)	197.1 (28.57)
	Control	2.42 (0.37)	7.71 (1.46)	12.10 (1.22)	151.0 (8.30)	6.29 (5.26)	48.08 (7.19)	188.4 (52.40)

Table S3 – Elemental analyses in grains of rice cultivated in artificially Cd contaminated soil with different products application; Mean values and standard error (n=4).

System	Material	Ca	Mg	K	Fe	Cu	Zn	Cd	Al	Mn
		-- mg kg^{-1} --	---------- g kg^{-1} ---------		-- mg kg^{-1} --					
Nonflooded	HYP-1	72.74 (7.37)	1.82 (0.12)	2.08 (0.12)	12.46 (1.80)	4.99 (0.23)	18.80 (6.79)	1.33 (0.07)	14.12 (6.17)	12.49 (0.63)
	HYP-2	80.18 (38.59)	1.71 (0.22)	2.25 (0.24)	24.18 (3.71)	4.63 (0.64)	4.87 (1.70)	1.30 (0.15)	15.12 (3.14)	12.33 (0.84)
	Zeolite	42.37 (5.84)	2.02 (0.10)	2.54 (0.12)	30.03 (4.99)	4.32 (0.34)	16.05 (1.51)	5.78 (0.22)	11.37 (1.22)	18.17 (0.61)
	Raw Material	98.50 (16.77)	1.89 (0.09)	2.42 (0.06)	28.15 (4.20)	6.44 (0.83)	16.76 (2.58)	1.45 (0.04)	15.99 (3.01)	16.09 (0.11)
	$Ca(OH)_2$	90.79 (4.97)	1.90 (0.14)	2.40 (0.18)	30.80 (1.47)	4.37 (0.24)	12.20 (1.64)	1.56 (0.08)	16.06 (2.52)	15.87 (1.54)
	Control	44.30 (10.98)	2.26 (0.04)	2.63 (0.16)	33.48 (1.79)	5.08 (0.50)	23.18 (7.15)	5.91 (0.64)	25.00 (5.85)	18.79 (1.26)
Flooded	HYP-1	49.87 (28.36)	2.51 (0.07)	2.41 (0.07)	49.14 (8.16)	4.16 (0.34)	18.32 (3.31)	0.93 (0.13)	12.67 (1.02)	16.63 (0.67)
	HYP-2	111.1 (34.04)	2.31 (0.21)	2.28 (0.07)	35.76 (0.77)	4.75 (0.15)	21.37 (9.15)	0.92 (0.04)	14.91 (1.93)	15.03 (0.67)
	Zeolite	81.30 (17.50)	2.63 (0.10)	2.66 (0.06)	50.88 (2.52)	3.50 (0.53)	21.12 (1.04)	1.37 (0.17)	17.28 (4.08)	21.47 (1.08)
	Raw Material	86.32 (9.79)	2.62 (0.11)	2.48 (0.06)	57.44 (6.69)	4.83 (0.56)	18.15 (2.57)	1.05 (0.20)	33.22 (7.70)	22.69 (3.60)
	$Ca(OH)_2$	101.5 (16.65)	2.56 (0.14)	2.38 (0.09)	48.81 (7.39)	5.36 (0.39)	14.52 (1.64)	1.23 (0.08)	18.46 (5.48)	18.53 (0.37)
	Control	60.55 (7.02)	2.70 (0.08)	2.67 (0.04)	57.70 (11.23)	4.20 (0.74)	22.87 (3.02)	1.23 (0.21)	19.93 (7.58)	20.88 (0.90)

Table S4– DTPA extractable Cd and nutrients in soil after amended with products and cultivated with rice. Mean values and standard error (n=4).

System	Material	Cd	Zn	Cu	Ca	Mg	Al	Fe	Pb	Ni	Mn	K
		mg kg^{-1}										
Nonflooded	HYP-1	0.74 (0.02)Aa	1.52 (0.17)	0.50 (0.09)	779.23 (36.30)	36.42 (4.20)	6.68 (2.91)	9.29 (0.71)	0.24 (0.01)	0.18 (0.03)	1.71 (0.18)	16.04 (3.19)
	HYP-2	0.68 (0.01)Aa	2.20 (0.30)	0.57 (0.08)	768.92 (38.79)	41.78 (3.01)	5.90 (0.85)	8.89 (0.44)	0.24 (0.02)	0.20 (0.03)	1.75 (0.15)	89.62 (9.34)
	Zeolite	0.59 (0.03)Aa	2.48 (0.20)	0.46 (0.06)	513.63 (6.68)	62.06 (2.84)	4.16 (0.29)	6.56 (0.20)	0.22 (0.003)	0.15 (0.02)	1.05 (0.20)	10.24 (1.68)
	Raw Material	0.72 (0.03)Aa	2.23 (0.15)	0.41 (0.03)	836.28 (38.97)	37.44 (3.91)	4.70 (0.40)	8.76 (0.41)	0.23 (0.01)	0.17 (0.01)	1.75 (0.14)	10.04 (0.81)
	Ca(OH)$_2$	0.71 (0.04)Aa	1.80 (0.19)	0.48 (0.09)	916.06 (45.64)	44.71 (5.61)	4.15 (0.42)	8.10 (0.37)	0.21 (0.01)	0.15 (0.01)	1.89 (0.18)	20.54 (1.53)
	Control	0.67 (0.10)Aa	1.73 (0.19)	0.44 (0.04)	495.31 (17.21)	64.19 (15.54)	5.97 (1.03)	6.84 (0.19)	0.23 (0.02)	0.12 (0.003)	1.76 (0.61)	1.65 (0.03)
Flooded	HYP-1	0.47 (0.01)Ba	1.09 (0.11)	0.62 (0.04)	656.16 (8.91)	40.20 (2.36)	7.25 (2.90)	46.22 (1.81)	0.36 (0.01)	0.20 (0.02)	3.15 (0.09)	25.35 (2.10)
	HYP-2	0.54 (0.04)Ba	1.79 (0.17)	0.61 (0.06)	664.22 (14.50)	43.33 (2.41)	4.30 (0.76)	31.52 (2.69)	0.32 (0.02)	0.23 (0.03)	3.35 (0.09)	85.98 (3.06)
	Zeolite	0.19 (0.02)Bb	1.65 (0.22)	0.47 (0.04)	504.74 (10.69)	60.11 (3.90)	5.42 (1.56)	52.50 (6.49)	0.30 (0.01)	0.12 (0.01)	3.21 (0.24)	12.83 (0.92)
	Raw Material	0.37 (0.06)Bab	1.92 (0.31)	0.54 (0.06)	647.16 (53.87)	41.58 (2.15)	8.02 (1.62)	36.65 (2.05)	0.29 (0.01)	0.15 (0.01)	2.72 (0.14)	7.24 (0.52)
	Ca(OH)$_2$	0.46 (0.02)Ba	1.59 (0.19)	0.60 (0.08)	731.73 (12.29)	26.28 (2.40)	6.63 (1.02)	30.26 (2.91)	0.30 (0.02)	0.17 (0.02)	2.34 (0.14)	4.77 (0.85)
	Control	0.24 (0.02)Bb	1.73 (0.25)	0.50 (0.05)	507.75 (20.46)	56.59 (5.95)	7.77 (1.02)	55.14 (10.02)	0.27 (0.02)	0.16 (0.05)	2.77 (0.29)	1.83 (0.16)

*Lower case letters compare products at the same water regime and capital letters compare the effect of water regime in each product