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Metal mobility in an anaerobic-digestate-amended soil: the role of two bioenergy crop plants and their metal phytoremediation potential

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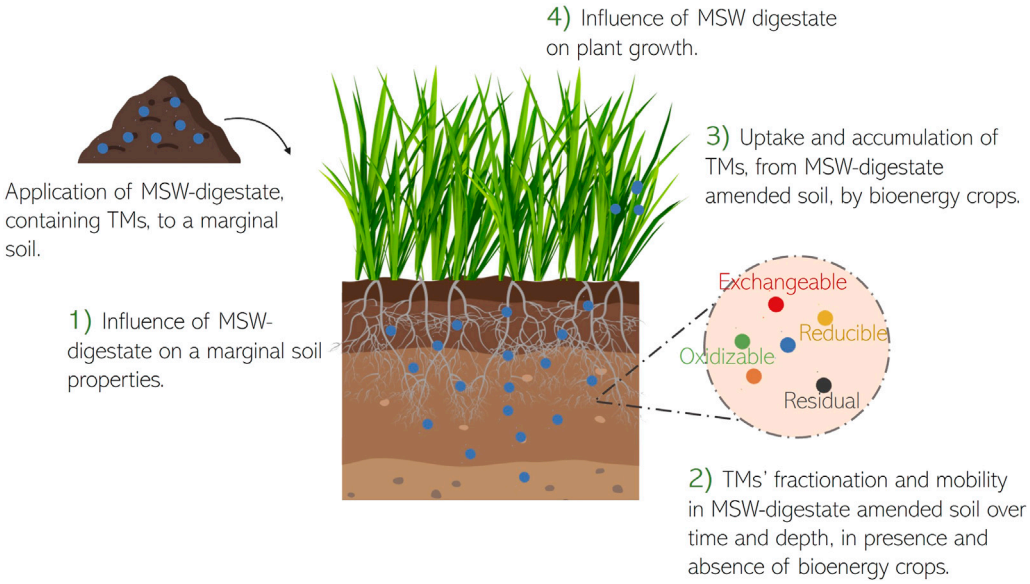
Panicum virgatum and *Pennisetum alopecuroides*, two non-food bioenergy crops, were evaluated for their capacity to phyto-manage trace metals (Pb, Zn, Ni, Fe, Mn, Co, Cr, and Cu) from municipal solid waste digestate after its application to a marginal soil. For that, 90-day vertical soil column mesocosm (columns with 0.6 × 0.2 m) experiments were carried out to assess 1) the impact of digestate application on the health of marginal soil, 2) plant effect on digestate-borne trace metals' mobility along the soil profile (measuring total metal levels and fractionation in different soil layers by atomic absorption spectroscopy, and 3) plant growth performance and trace metal (Pb, Zn, and Cu) uptake capacity. The results showed that trace metals were mostly confined in the 0–0.2 m soil horizon over the course of the experimental period, migrating from the digestate-amended soil layer (0–0.1 m) to the layer underneath (0.1–0.2 m) within the first 21 days and remaining stable afterward. No evidence of the trace metals' mobility to deeper soil layers was detected. Migration of trace metals was reduced in the presence of *P. virgatum* and *P. alopecuroides*, suggesting a phytoremediation (phytostabilization) effect. For both plant species, no trace metal accumulation in the roots was observed (bioconcentration factor <1), although both plants showed a potential for Zn translocation for aboveground tissues (translocation factor >1). The growth of both plants was positively affected by municipal solid waste digestate application, which also improved soil quality (increased concentration of total organic carbon and available phosphorus, as well as cation exchange capacity and water holding capacity).

KEYWORDS

anaerobic digestate, trace metals, phytoremediation, bioenergy crop plants, soil reclamation



GRAPHICAL ABSTRACT

1 Introduction

The world's population is expected to increase by nearly 2 billion people in the next 30 years (UN 2022). This will lead to a consumption of vast amounts of resources, production of billions of tons of waste and wastewater (Kaza et al., 2018), and an unprecedented demand for clean and affordable energy. At the same time, demographic expansion, rising expectations of living standards, and scarcity of natural resources have made soil degradation a major issue now, posing a serious threat to human wellbeing (FAO, 2019).

Anaerobic digestion (AD) has proven to be one of the most economical and effective technologies capable of addressing not only the increasing demand for improved waste treatment practices but also immediate requirements for resource recovery and cleaner energy production (Atelge et al., 2020). This technology is a promising alternative, having the ability of turning a variety of organic waste products, such as organic fraction from municipal solid waste (MSW) into two potentially useful end products: renewable bioenergy in the form of biogas and digested solids, hereafter referred to as "digestate." However, because of its intrinsic benefits, AD is widely applied in Europe (Di Maria et al., 2016) and large amounts of digestate are being produced. Recycling and valorization of digestate through proper digestate management practices is needed.

Digestate is an organic matter and nutrient-rich material that can be applied to soils as fertilizer or soil improver (Alburquerque et al., 2012; ITPS, 2015; M. E. Lee et al., 2021; Monlau et al., 2015; W. Wang and Lee, 2021), which can mitigate the overreliance on environmentally unsustainable chemical fertilizers (Nkoa, 2014). These chemical fertilizers require a high raw material and energy input for their production, and their application can lead to nitrification and loss of soil carbon at higher application rates (Nkoa, 2014; Cheong et al., 2020). In addition, a fraction of the carbon contained in organic amendments, such as digestate, can be sequestered and stabilized in soil. So, land application of digestate could help minimize greenhouse gas emission into the atmosphere (Paustian et al., 2016).

Nonetheless, digestate application is not entirely harmless, as it can contain variable concentrations of harmful chemical pollutants, such as trace metals (TMs), and/or pathogenic microorganisms, especially if it originates from non-separated municipal solid waste. TMs, despite occurring at low concentrations (<1,000 mg kg^{-1}), can have a significant biological effect, either as essential nutrients or as environmental contaminants (Robinson et al., 2009). These TMs may originate from the feedstocks used in AD but can also be added in digesters for optimizing the biogas production yield and rate (Fermoso et al., 2015; Moestedt et al., 2015; Molaey et al., 2018). TMs' total concentrations are always higher in the digestate than in the feedstock, being distributed between the digestate's liquid and solid fractions, but mostly accumulate in the digestate solid fraction together with sulfide and phosphate ions and residual organic fraction (Fermoso et al., 2015). TMs include both elements essential for normal metabolic processes, called micronutrients (e.g., Fe, Mn, Cu, Zn, and Mo), which can become extremely toxic at high concentrations, and elements such as As, Hg, Pb, or Cd, which when present at low concentrations are very noxious to humans and animals, while affecting plant growth and development to a lesser extent.

Hence, one of the main obstacles in applying MSW digestate on soils is related with the possibility of introducing excessive amounts of TMs into the soil ecosystem (Jacobs, 1981; Adriano and Adriano, 2001; Kabata-Pendias and Pendias, 2001). In fact, there are strict legislative norms regarding the maximum amounts of TMs that could be incorporated into arable soil (Mininni et al., 2015; European Commission, 2019). Consequently, for the MSW digestate to be safely classified as a usable "product" rather than

a hazardous "waste," strategies to remove excessive TM concentrations are needed.

The remediation of matrices contaminated with TMs is a challenge because of their non-degradability (Kabata-Pendias and Pendias, 2001; Kabata-Pendias, 2011). Conventional remediation options commonly involve excavation, physicochemical treatments (such as stabilization, soil washing, and chemical reduction/oxidation), and off-site disposal to "secured" landfills. Such remedial options are generally expensive, may produce adverse effects on ecosystems, and often require appropriate methods for waste disposal (Liu et al., 2018).

Phytoremediation is widely viewed as a green, cost-effective, and ecologically responsible alternative to the environmentally destructive physical–chemical remediation methods currently practiced. Phytoremediation can be an *in situ* option to remove excessive TM concentrations and lower TM bioavailability in soil amended with MSW digestate (Tangahu et al., 2011; Awa and Hadibarata, 2020). Phytoremediation of TM-contaminated matrices is based on immobilization of TMs in the rhizosphere soil and roots (phytostabilization) and on the mobilization, uptake, and transfer of TMs into the aboveground plant organs (phytoextraction) (Wei et al., 2021; Bhat et al., 2022). The phytoremediation efficiency depends on multiple factors such as TM speciation and bioavailability, solid matrix properties, and plant species (Terry and Banuelos, 2000; Sayen et al., 2019; Qin et al., 2022). Moreover, plants' presence can further increase the organic matter content in the soil. This organic matter can form water-soluble and/or water-insoluble TM complexes (Zeng et al., 2011). Through the formation of these complexes, organic matter can dissolve, mobilize, and transport TMs in soils and/or accumulate them in certain soil horizons. This can contribute to a reduction of TM mobility toward other reservoirs, namely, living organisms or groundwater.

Among phytoremediation mechanisms, phytoextraction is of special interest due to the possibility of TM recovery. The phytoextraction potential of any plant species is primarily driven by two essential key factors: 1) shoot biomass and 2) total TM concentration accumulated in shoot biomass (Hernández-Allica et al., 2008). Hence, a crucial aspect in phytoremediation trials is the choice of the most appropriate plant species to the conditions and matrices to be remediated. Energy crops could be a suitable option. In fact, these plants are typically grown because of their high-rate biomass production, which can later be used to produce biofuels or combusted to generate heat or electricity. Most of the dedicated energy crops, besides offering the dual benefits of phytoremediation and bioenergy production, easily adapt to unfavorable conditions and are TM accumulators, sequestering exceptionally high amounts of the absorbed TMs into their biomass (Tripathi et al., 2016). These plants could be used for direct TM uptake from soils amended with MSW digestate, while profiting from the fertilizing properties of the digestate itself (Antonkiewicz et al., 2017; M.-S. Lee et al., 2021; Mucha et al., 2019; Seleiman et al., 2012). Eventually, plants' biomass could afterward be used as a feedstock to re-introduce the TMs in the AD process (Garuti et al., 2018), thus allowing it to close the material loop. This is still a scarcely explored subject, and more research to understand the phytoremediation mechanisms in these plants under such conditions is needed. Furthermore, this would allow further expansion of the quadruple task of waste management, soil reclamation, resource recovery, and bioenergy production.

When applying *in situ* TM phytoremediation techniques to the MSW digestate-amended soil, it is essential to follow changes in the TM behavior in the presence of plants. To our knowledge, there is a lack of studies focusing on the effects of MSW digestate contaminated with TMs on the soil, namely, on TM mobility through the soil profile and on the plants' influence on their behavior. Therefore, the present work aimed to evaluate the potential of two dedicated perennial bioenergy crops for the phytomanagement of MSW digestate, contaminated with TMs, after its application to a poor soil, the plants' influence on TM mobility, and how the soil and plants could benefit from the amendment properties of MSW digestate. The selected plant species, switchgrass (*Panicum virgatum*) and fountain grass (*Pennisetum alopecuroides*), have been identified as promising species for TM phytoremediation and bioenergy production. Previous studies have reported the capacity of *P. virgatum* to phytoremediate Cd, Cr, Zn, and Pb from contaminated soils (Chen et al., 2012; Guo et al., 2019), whereas *P. alopecuroides* has been recently identified as an effective Cr phytoremediator for the first time (Jia et al., 2022). Both plant species are potential bioenergy feedstocks (McLaughlin and Adams Kszos, 2005; Fairley, 2011; Tripathi et al., 2016; Zhang et al., 2016).

We hypothesize that the MSW digestate will improve a marginal soil's health and that any TM contamination from the MSW digestate will be tackled by plants through phytoremediation processes, preventing soil contamination and giving a new value to the TM-contaminated MSW digestate. For that, a series of 90-day vertical soil column mesocosm (0.6 × 0.2 m) experiments were performed to assess the effects of the application of the MSW digestate on the quality of a marginal soil and evaluate phytoremediation processes of *P. virgatum* and *P. alopecuroides* over time in terms of 1) plant effects on TM mobility along the soil profile (through the assessment of total metal concentration and BCR sequential extraction at different soil layers), 2) plant growth performance, and 3) plants' TM uptake capacity.

2 Materials and methods

2.1 Soil, digestate, and plants

Soil samples were collected from the vicinity of a construction field in Ermesinde in the municipality of Valongo, North Portugal (41°12′25.7″ N, 8°32′26.7″ W). The soil was identified through the European Soil Database (Hiederer, 2013). Data were complemented with INFOSOLO, the Portuguese online database for soil profile data (Ramos et al., 2017), following the procedure described by Baldasso et al. (2023). The soil was classified as an Entisol deriving from schist, a medium-grade metamorphic rock, with a soil texture ranging from loamy to clayey.

This soil was selected as a good candidate for soil reclamation as it displays poor agronomic qualities (low OM, available nutrients, and water holding capacity (WHC) (Supplementary Table S1)) and low TM content (see Results), ideal to follow the mobility of TMs initially present in the contaminated digestate.

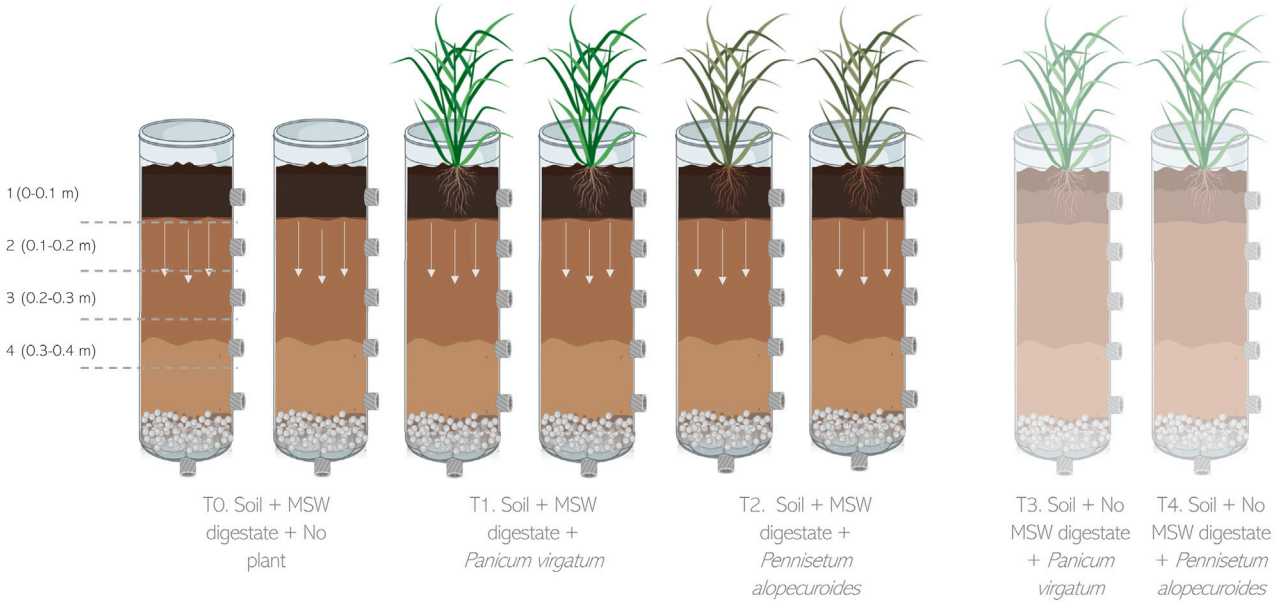


FIGURE 1
Schematic of phytoremediation experiments in soil column mesocosms. T3 and T4 are control columns with no digestate amendment. Figure created using BioRender.com. License agreement number: VT254U6HT3.

The solid fraction of MSW digestate used in these experiments was collected from the mesophilic full-scale anaerobic digestion plant of Tratolixo, a Portuguese company located in the municipality of Mafra, Portugal, that treats the non-source separated organic fraction of MSW at the end of the solid–liquid separation process, specifically after centrifugation.

The physical and chemical characterization of the soil, MSW digestate, and MSW digestate-amended soil is included in Supplementary Material (Supplementary Table S1).

Two energy crops, *P. virgatum* and *P. alopecuroides*, were selected for this study. Plants were selected according to pre-established criteria, considering the following standards: metal (Cr, Mn, Fe, Co, Cd, Ni, Cu, Zn, and Pb) uptake capacity; ability to produce a large volume of biomass; high energy potential; ability to be grown in marginal soils; non-edible; non-invasive behavior and of low-cost maintenance (Chen et al., 2012; Jia et al., 2022; McLaughlin and Adams Kszos, 2005; Shrestha et al., 2019; M. Zhang et al., 2016). One-month-old plants were obtained from a greenhouse located in Vigo, Galicia, Spain, where they were germinated and grown in a commercial substrate at 22$\underline{o}$C ± 2°C.

2.1.1 Initial sample analysis

Elemental composition (Ca, K, Mg, and P) analysis was performed using XRF on the ground sample using ED-XRF SPECTRO XEPOS, XEP05 (SPECTRO Analytical Instruments, AMETEK, France).

The carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content in soil was determined using a 2400 CHNS Organic Elemental Analyzer 100 V (PerkinElmer, MA, USA). Total carbon (TC) content was also obtained through the CHNS analysis.

Dry weight (DW) and water content (WC) were measured according to the oven drying method (ISO: 2720, 1973). The volatile dry weight and volatile organic matter content were determined using the loss on ignition (LOI) approach (Nelson and Sommers, 1996).

The cation exchange capacity (CEC) was determined using the cobalt hexamine method in triplicate for each sample (Ciesielski et al., 1997). The pH value was obtained following the normalized ISO 10390:2005 method, in a 1:5 soil/H_2O solution.

The water holding capacity was determined in accordance with the modified funnel method provided by Bernard (1963).

The particle density was measured using the pycnometer method according to Blake and Hartge (1986). The wet bulk density was determined following the volumetric cylinder standard method (Blake and Hartge, 1986). Porosity was calculated from particle and bulk densities as described in Flint and Flint (2002).

X-ray diffraction (XRD) patterns of the initial soil were recorded. An analysis was conducted using a Bruker D8 ADVANCE diffractometer (Bruker, MA, USA) at the Cu–Kα wavelength (λKα = 0.1541 nm), between 2° and 60° 2θ, with a 0.02° 2θ step and a counting time of 1 s/step and rotating sample holder (15 rpm). Prior to XRD, the sample was gently ground using a non-amorphized ball mill in zirconium oxide (Pulverisette 23, Fritsch). The obtained XRD patterns were processed using EVA software (Bruker). Fourier-transform infrared spectroscopy (FTIR)-ATR (attenuated total reflectance) measurements were performed using an FTIR spectrometer (PerkinElmer, MA, USA) operating in the attenuated total reflectance mode in the middle infrared (MIR) region (4,000–600 cm^{-1}) at a 2 cm^{-1} resolution for 15 scans.

2.2 Experimental design

Experiments were performed in vertical soil columns. Three treatment conditions, each with two replicates, were assembled: T0

(soil + MSW digestate), T1 (soil + MSW digestate + *P. virgatum*), and T2 (soil + MSW digestate + *P. alopecuroides*) (Figure 1). One control column for each plant species, with the soil but no MSW digestate, was also assembled to evaluate both the plant growth and plant natural TM content in the absence of the MSW digestate. A total of eight columns were assembled.

Soil columns consisted of rigid PVC columns (0.2 m diameter × 0.6 m height) with five sampling ports including a hole at the bottom, with a tap, for leachate collection, whenever needed (Figure 1). The lower 0.02 m of each column was filled with inert pebbles (to avoid soil loss and allow water drainage), followed by addition of the homogenized marginal land soil (9.5 L), previously sieved to <2 mm. To avoid positive preferential flows through the soil, the columns were filled without air gaps, enabling an equal level of homogeneity. Finally, the soil was slightly compacted. A thoroughly manually mixed MSW digestate–soil mixture (proportion 1: 1 v/v and 2 L each, to simulate digestate incorporation in the agriculture soil) was placed at the top of the uncontaminated soil in a 0.15-m layer, which is approximately equal to the depth of digestate incorporation on agricultural fields (0.1–0.2 m) (Alburquerque et al., 2012).

Next, the soil present in the columns was saturated by adding a total of 5 L of deionized H_2O. During the experiment (90 days), the soil columns were regularly irrigated to maintain 80% of the water holding capacity, which required adding 200 mL of deionized H_2O weekly. The columns were fully covered with aluminum foil to avoid sunlight interferences.

After 1 week of acclimation (day 7), ten 1-month-old seedlings of *P. virgatum* and *P. alopecuroides*, with similar sizes, were transplanted into the respective column and irrigated with deionized water according to plant requirement, always maintaining a selected WHC. The plants were grown for 83 days, with the total time of the experiment being 90 days.

The experiments were carried out in a semi-open environment, inside of the building of the Faculty of Sciences of the University of Porto, Portugal, with a natural day/night regime and at room temperature (21°C ± 1°C).

Soil samples from T0, T1, and T2 columns were collected on days 0, 7, 21, 35, and 90 at the following depths: 0–0.1 m; 0.1–0.2 m; 0.2–0.3 m; and 0.3–0.4 m using the sampling ports (at each sampling time, one sample at each depth per duplicate columns) (Figure 1). Soil-column leachates were also collected (when available). All the samples were stored at 4°C in polyethylene tubes for subsequent analyses of total metal contents and metal fractionation.

At the end of the experiment, plants from T1, T2 (three plant replicates per duplicate column from each treatment), T3, and T4 (three plant replicates per single column from each treatment) were harvested to assess plant growth and metal content in plant tissues and to estimate the plant metal uptake.

2.3 Metal analysis for total content and fractionation

The metal (Zn, Cu, Pb, Cr, Ni, Mn, Co, Cd, and Fe) content was determined in initial samples (soil, MSW digestate, and MSW digestate-amended soil, each in triplicates), in the soil layer samples collected over time (in duplicates considering the two columns for each treatment), in leachates (when available), and in different plant tissues, with the plants being collected at the end of the experimental period. For each plant tissue, triplicate samples from each column were prepared and analyzed, after homogenizing the total amount of plant tissue from the respective column.

All solid samples mentioned previously were initially dried (at room temperature until constant weight). Leachates were acidified with HNO_3 (at 1%) and stored at 4°C until analysis.

TM levels were measured by atomic absorption spectroscopy (AAS), either with flame (PerkinElmer, AAnalyst 200) or electrothermal atomization (PerkinElmer, PinAAcle 900Z, coupled to an AS 900 autosampler), depending on metal levels, after high-pressure digestion in a microwave apparatus (ETHOS 1, Milestone Inc. (Shelton, CT, USA)) following the US EPA 3052 protocol with concentrated HNO_3 (69%) and H_2O_2 (30%), except the leachate samples that were analyzed directly. To quantify TMs in different plant structures, ca. 0.5 g of plant tissues was weighed in a microwave Teflon vial and 1 mL of HNO_3 (69%) and 5 mL of H_2O_2 (30%) were added. The same procedure was used for the MSW digestate and samples collected from the 0–0.1 m layer over time from MSW-digestate-amended columns (T0, T1, and T2) with ca. 0.5 g of each sample being placed in microwave Teflon vials. For the initial soil sample and samples from 0.1–0.4 m layers from the MSW-digestate-amended columns (T0, T1, and T2), only 5 mL of concentrated HNO_3 for ca. 0.25 g of the sample was used. The total running time for the microwave digestion was 25 min: 5 min at 250 W, 5 min at 400 W and 5 min at 500 W, and 10 min at 0 W. The vessels were then allowed to cool at room temperature, and the samples were transferred to 50-mL tubes with the addition of deionized H_2O (up to 20 mL). The solutions were stored at 4°C until AAS analysis. The aforementioned procedures were previously validated in the laboratory (Almeida et al., 2004). Metal quantification was carried out by external calibration with aqueous metal standard solutions. For that, working metal standard solutions were prepared by appropriate dilutions of stock standard solutions with deionized water. Triplicate samples were run to ensure the precision of quantitative results.

Metal fractionation in initial samples (soil, MSW digestate, and MSW digestate-amended soil, each in triplicate) and soil layer samples collected over time from soil columns (in duplicates considering the two columns for each treatment) was estimated by sequential extraction according to the BCR procedure described by Rauret et al. (1999), including minor modifications, as previously reported (Almeida et al., 2004). Three fractions were prepared: 1) exchangeable and bound to carbonates (more bioavailable); 2) reducible and bound to Fe and Mn (hydro) oxides; 3) oxidizable and bound to the organic material and sulfide plus the residual (the less bioavailable). For this purpose, the samples (ca. 0.5 g) were extracted successively with 20 mL of a 0.11 M CH_3COOH solution (exchangeable fraction) and 20 mL of a 0.5 M $NH_2OH.HCl$ solution (fraction bound to Fe and Mn oxyhydroxides). The extractions were carried out using an end-over-end shaker (Unitronic Reciprocating Shaking Bath, JP Selecta) at room temperature. TM concentrations in these solutions were measured by AAS, as described previously. The metal in the third fraction was calculated based on the difference

between the total metal content and the sum of the metal content in the two extraction solutions mentioned previously.

For the initial soil, digestate, and digestate-amended soil samples, fractionation was carried out for Fe, Mn, Zn, Cu, Pb, and Cr. For the samples collected during the soil column experiment, fractionation analysis was carried out only for Zn, Cu, Pb, and Cr.

All the material used in sample preparation was washed with deionized water, placed in a nitric acid solution (20%, v/v) overnight, and washed thoroughly again with deionized water.

2.4 Plant biomass yield and morphological traits

Three young leaves (approximately 7 cm in length) from each column were selected at the beginning of the phytoremediation experiment (day 7) and their height was measured over time (day 21, 35, and 90) to assess the growth dynamics until the end of the experiment, an average was calculated for each treatment and for each time point. At the end of the experiment plants were harvested separately from each column and homogenized, three replicates of each plant tissue (leaves and roots) were taken separately from each column homogenized samples. Both roots and leaves were dried at room temperature (25 ± 2$\underline{o}$C), until a constant weight was reached, to obtain dry weight value, an average for each treatment was calculated.

2.5 Metal accumulation in plants

To evaluate metal accumulation in plants, the bioaccumulation coefficient factor (BCF) and translocation factor (TF) were calculated with concentrations determined in soils and plants at the end of the experiment. The BCF is the ratio of the metal concentration in the root to that in the soil, and the TF is the ratio of the metal concentration in the shoot to that in the root. The BCF and TF were calculated with the formula mentioned as follows, previously reported by Yoon et al. (2006):

- BCF = Croot/Csoil
- TF = Cshoot/Croot

Here, Croot is the metal concentration in the plant root (mg kg^{-1}), Csoil is the metal concentration in the soil (mg kg^{-1}), and Cshoot is the metal concentration in the plant shoot (mg kg^{-1}).

Csoil was calculated as the average of the metal concentration in the two initial soil layers, the 0–0.1 m layer (the MSW digestate-amended soil) and the 0.1–0.2 m layer (the soil layer beneath the MSW digestate-amended soil), as at the end of the experiment (90 days), the plant roots were in contact with both soil layers.

2.6 Statistical analysis

The samples' mean, standard deviation, and maximum and minimum values were analyzed using Microsoft Excel 2019. Statistical tests were performed using SPSS Statistics software (version 26.0).

The Shapiro–Wilk test was used to test if the distribution of the datasets deviates from a comparable normal distribution. The null hypothesis was rejected if p <0.05.

Significant differences among soil column samples collected over time and depth were evaluated through a parametric one-way analysis of variance (ANOVA). The same procedure was followed to evaluate significant differences between metal concentrations in different plant tissues. Two-way ANOVA was used to evaluate the differences in the total metal and metal fraction concentrations between different conditions. The dependent variable was metal concentration in all cases. The variables were defined as continuous.

Significant differences (p <0.05) were detected by a Tukey's Honestly Significant Difference (HSD) *post hoc* test.

Principal component analysis (PCA) and Pearson's correlation were used to identify the potential interrelation among total TM concentrations, and with depth, pH, and presence/absence of the plants, in T0, T1, and T2 columns. Pearson's correlation was tested at 99% confidence level. For the PCA, data were log-transformed and eigenvalues >1 were extracted through a varimax rotation. The analysis were performed using SPSS Statistics (version 26.0) and RStudio (version 4.3.0).

3 Results and discussion

3.1 Initial soil, MSW digestate, and MSW digestate-amended soil characterization

Soil, MSW digestate, and MSW digestate-amended soil (1:1 v/v soil + MSW digestate) were initially characterized. The results are presented in Supplementary Material Supplementary Figure S2, Supplementary Figure S3, and Supplementary Table S1.

X-ray diffraction and attenuated total reflection–Fourier-transform infrared spectroscopy analysis of the soil–solid phase revealed a clay mineral composition of mainly quartz, associated to phyllosilicates such as kaolinite and muscovite (Supplementary Figure S2). Traces of carbonates (<0.5%) were inferred from CHNS analysis before and after acid treatment, and the results are in accordance with weak carbonate band observed on ATR spectra.

The loss on ignition of soil was 2.4% ± 0.1% (Supplementary Table S1), hence considered a poor clayey soil (Brady et al., 2008). This was expected as this was a marginal soil originating from a construction site. The application of MSW digestate, which had an LOI of 22.0% ± 0.8%, substantially increased (p <0.05) the soil LOI to 6.5% ± 0.1%. This is in accordance with what has been previously reported regarding biosolids', such as anaerobic digestate or sewage sludge, ability to increase the soil organic matter content (Gerzabek et al., 2001; Parat et al., 2005; Carabassa et al., 2020). The application of MSW digestate to the soil also increased the soil organic carbon (SOC), the major component of soil organic matter content, from 0.20% ± 0.05% to 4.4% ± 0.7%. Similarly, Tambone et al. (2009) reported a high concentration of organic carbon in the organic municipal solid waste (OFMSW) digestate.

Initial soil pH agrees with the soil mineralogical composition, that is, slightly basic, due to traces of carbonates (Supplementary Table S1). Addition of MSW digestate slightly enhanced the soil pH, from 7.9 ± 0.2 to

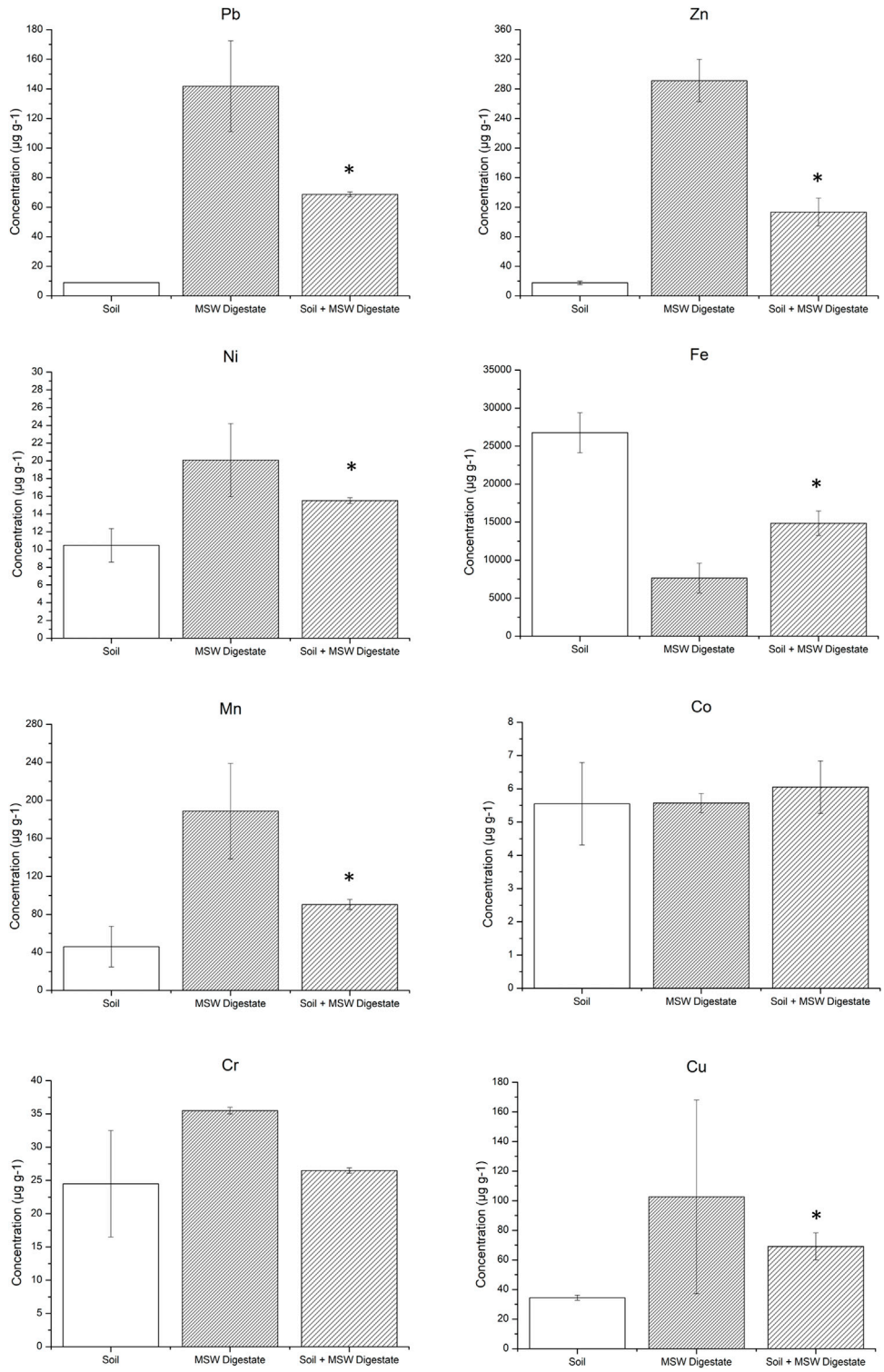


FIGURE 2
Total metal (Pb, Zn, Ni, Fe, Mn, Co, Cr, and Cu) concentration (µg g^{-1}) (+SD) of the initial samples (soil, MSW digestate, and MSW digestate-amended soil). Error bars indicate the standard error of the mean of three measurements (n = 3). * Values in the MSW digestate-amended soil are significantly different than that in the unamended soil at the 0.05 level of significance assessed by Tukey's multiple comparison test.

8.4 ± 0.1 (p <0.05). This could be explained by the alkaline nature of MSW digestate (8.8 ± 0.4). Furthermore, there could be an increase of negatively charged functional groups in the soil, coming from the MSW digestate-borne organic matter, such as carboxyl (-COOH), phenolic (-OH), and amino (-NH_2) groups (Adusei-Gyamfi et al., 2019).

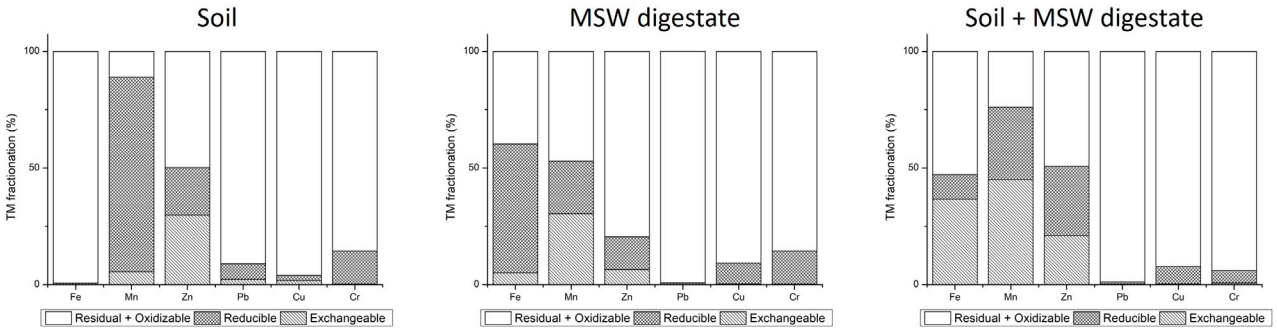


FIGURE 3
Percentage of selected metals (Fe, Mn, Zn, Pb, Cu, and Cr) in different fractions (exchangeable, reducible, and residual + oxidizable) in the initial samples (soil, MSW digestate, and MSW digestate-amended soil). Exchangeable (acid-soluble and bound to carbonates); reducible (bound to Fe and Mn oxides); residual + oxidizable (bound to the soil matrix and organic matter). Error bars are not illustrated in the figure; the standard error of the mean of three measurements (n = 3) are indicated in Supplementary Table S4.

There was also a significant increase (p <0.05) in the cation exchange capacity in the soil amended with MSW digestate (from 4.4 ± 0.2 to 15.2 ± 2 cmol (+)/kg) (Supplementary Table S1), probably induced by the higher amount of negatively charged sites, as previously mentioned. Overall, this would increase the capacity of the soil to hold positively charged ions. The increase of the CEC in organic component-amended soil has also been previously reported (Panuccio et al., 2021). The CEC of MSW digestate was 40 ± 3 cmol (+)/kg, which is similar to the values found in literature (20.3%–53.4%) (Teglia et al., 2011a; 2011b). It is worth mentioning that the increase of the CEC of soils is of particular interest in the context of soil improvement, for example, an increased CEC has been proven to reduce the entry of nutrient loads into groundwater systems (Bargmann et al., 2014).

The application of MSW digestate also increased the levels of available P (P_2O_5) in the soil from 0.1% ± 0.02% to 0.78% ± 0.03% (Supplementary Table S1), similarly to what was observed by Bachmann et al. (2014), Hupfauf et al. (2016), and Tan et al. (2021). These studies showed that the application of the digestate increased the content of plant-available P in the soil to the same extent as highly soluble mineral P fertilizer and undigested dairy slurry, so the digestate could be a suitable substitute for inorganic fertilizers. Ultimately, these changes can promote plant growth and yield. The contents of total nitrogen (TN) in soil also increased after MSW digestate application. These results were consistent with the previous findings, showing that organic matter, N, and P in the digestate improved both plant biomass and chemical properties of the soil (Garg et al., 2005; Arthurson, 2009; Głowacka et al., 2020).

Soil calcium (CaO) and magnesium (MgO) also increased (p <0.05) with MSW digestate amendment, from 1.3% ± 0.6% to 8.0% ± 0.4% and from 0.30 ± 0.08% to 2.6% ± 0.9%, respectively (Supplementary Table S1). High values have been found in digestate-amended soils (Doyeni et al., 2021). CaO and MgO are important adsorbents of TMs in soils. They are binders that can react with TM salts, and hence induce TM precipitation (i.e., insoluble complex compounds or hydroxides) due to their alkaline nature (Spence and Shi, 2004).

Both the bulk and particle density were lower in the soil amended with MSW digestate, decreasing from 2.2 ± 0.3 and 2.54 ± 0.01 g mL^{-1} to 1.3 ± 0.2 (p <0.05) and 1.8 ± 0.9 g mL^{-1} (p >0.05), respectively (Supplementary Table S1). Organic matter found in the digestate can make clay soils less dense and heavy (Pagliai et al., 1981; Pagliai and Antisari, 1993). A field experiment by Garg et al. (2005) showed that the amendment of soils with liquid digestate from agricultural waste reduced the bulk density and increased the saturated hydraulic conductivity and moisture retention capacity of soils.

These findings corroborate the results on the amending properties of typical anaerobic digestate, and they suggest that MSW digestate could be considered an effective organic amendment material. These changes in soil characteristics could influence the TM content, distribution, bioavailability, and, ultimately, toxicity (Adriano and Adriano, 2001; Kabata-Pendias and Pendias, 2001) and influence plants' development and of soil microbial communities. Nevertheless, it is worth noting that these results were obtained after the recent amendment of the soil, and long-term experiments should be conducted to determine the MSW-digestate effects in the soil over an extended timeframe.

3.2 Total and fractionation metal content in the soil, MSW digestate, and MSW digestate-amended soil

The total concentrations of TMs in the soil, MSW digestate, and MSW digestate-amended soil are given in Figure 2. Cd was below the limit of quantification (LOQ) (0.5 μg g^{-1}) in all samples; therefore, this metal was not considered for further analysis. Cr and Ni concentrations in the MSW digestate were below the limit values stated in Portuguese (Decree Law No 73/2011, 2011) and European ((EU) 2019/1,009 (European Commission, 2019)) regulations for Class III (soil for plant crops not intended for human and animal food), whereas Pb, Zn, and Cu exceeded values stipulated in the Portuguese regulation (Supplementary Table S2). No limit values for Mn and Fe were found in the legislations.

Overall, MSW digestate amendment markedly increased (p <0.05) the concentrations of Pb, Zn, Ni, Mn, and Cu in the soil. The increase of total TM concentrations in the top soil after the application of biosolid fertilizers has been extensively reported (Koutroubas et al., 2014; Latare et al., 2014; Lloret et al., 2016;

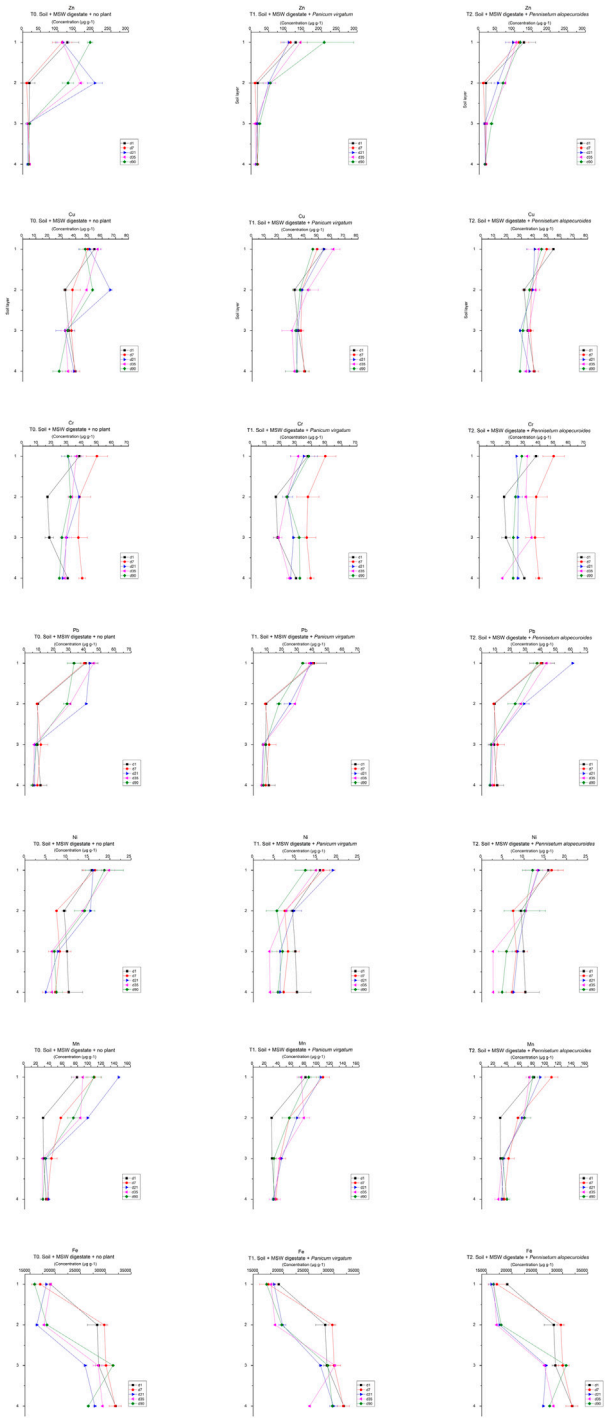


FIGURE 4
Total metal (Zn, Cu, Cr, Pb, Ni, Mn, and Fe) concentration in soil solid phase along soil depth; layers (1, 0–0.1 m; 2, 0.1–0.2 m; 3, 0.2–0.3 m; 4, 0.3–0.4 m) in soil columns with and without plants over time (days 1, 7, 21, 35, and 90). Plants were transplanted into the respective columns at day 7. Error bars indicate the standard error of the mean of the three measurements per each duplicated column (n = 2 × 3).

Marguí et al., 2016; Dragicevic et al., 2018). However, for Fe, an opposite trend was observed as the Fe content was much higher in the soil than in MSW digestate-amended soil (p <0.05). No significant differences (p >0.05) were observed in concentrations of Cr and Co between the soil and the MSW digestate-amended soil, although Cr concentration tends to be higher in MSW digestate.

A modified BCR sequential extraction protocol (Rauret et al., 1999) was carried out to evaluate TM availability changes upon soil amendment with the MSW digestate for Fe, Mn, Cu, Zn, Pb, and Cr (Figure 3). For Cd, Co, and Ni, no metal fractionation was carried out, as their levels in the initial matrices were low. The sequential extraction method identifies TMs in the MSW

digestate-amended soil which could be potentially mobilized in the short and long term and ultimately become an environmental hazard or TMs that could be more bioavailable to plant and microbial communities, either potentiating plant uptake or increasing metal toxicity. In the present study, chemical fractionation of TMs in solid matrices consisted in three fractions (exchangeable, reducible, and oxidizable + residual). Preliminary tests indicated that the percentages in the oxidizable fraction were very low (results not shown), so it was decided to join this fraction to the residual one.

Overall, MSW digestate amendment increased the potential mobility and availability of Fe and Mn (p <0.05) (exchangeable fraction) in the soil, while no significant (p >0.05) influence was found in the Pb, Cu, and Cr fraction distribution. In the initial soil, Fe seemed to be mainly present in the residual and oxidizable fraction (least mobile forms). Application of MSW digestate significantly influenced Fe distribution, increasing exchangeable and reducible fractions (p <0.05), and thus its potential availability. Ali et al. (2015) reported that the application of biofertilizers led to a drop in soil pH, which resulted in an increase of available Fe. However in this study, MSW digestate induced a slight increase in the soil pH, which is generally linked to an increase in the cationic metal retention in soil surfaces via sorption, inner-sphere surface complexation, and/or precipitation and multinuclear type reactions (McBride, 1994). Therefore, the increase in the available forms, in this case, could be caused by 1) trace organic acids present in the MSW digestate or 2) recent TM soil enrichment, which was not yet allowed to become sequestered and adsorbed by soil constituents.

Similarly, the application of MSW digestate increased (p <0.05) the exchangeable fraction of Mn, while it decreased the reducible fraction in the soil. Considering the recent application of the MSW digestate into the soil, this result could be attributed to MSW digestate containing Mn in exchangeable form and the subsequent release into the soil after amendment. Similar results were reported by Karimi et al. (2020), who studied Mn availability in the biochar-amended soil. The decrease of the reducible fraction and an increase in the residual + oxidizable fraction could be explained by the transformation of Mn into a more stable fraction through the formation of insoluble complexes with organic matter functional groups (Dhaliwal et al., 2019; Li et al., 2021).

Upon addition of MSW digestate to the soil, the Zn concentration in the reducible fraction increased (p <0.05), whereas that in the exchangeable fraction dropped. However, the total amount of oxidizable + residual fractions was not significantly affected by MSW digestate. This could be linked to the presence of Mn in the MSW digestate. Hydrous Mn oxides, along with organic matter, can decrease the reactivity of metals in the soil through adsorption (Jing et al., 2023).

Similarly, Qiao et al. (2003) reported an increase of Zn concentration in the reducible fraction of paddy soil after biosolid application, as well as Bose et al. (2008), who found similar results on an industrial waste-amended soil. However, other case studies showed an increase in the proportion of Zn bound to the acid-extractable fraction after biosolid amendments (Planquart et al., 1999; Qiao et al., 2003; Yang et al., 2018; Wydro et al., 2021).

Both in the initial soil and MSW digestate-amended soil, the largest portion of Pb, Cr, and Cu was found distributed among the oxidizable + residual fractions (bound to organic matter and sulfides and the remaining non-silicate-bound TMs). It is considered that TMs are dominantly present in the residual fraction, as preliminary results showed low TM amounts in the oxidizable fraction. This indicates that these TMs are less readily bioavailable to plants.

MSW digestate amendment in the soil seemed to increase the bioavailability of Fe and Mn, whereas it maintained low bioavailability for Pb, Cu, and Cr. For Zn, on the other hand, the MSW digestate slightly decreased its bioavailability. Based on the comparison with previously published data, the results also seem to indicate that the origin, type, and properties of the digestate, as well as field conditions, will differently influence TM bioavailability. However, the results in this section reflect the effect of a recent application of MSW digestate to the soil; the following sections will describe and discuss the longer-term effects.

3.3 Total metal concentration along the soil profile through time

Understanding the migration and fractionation of TMs in the soil environment after MSW digestate amendment is of great significance for implementing relevant risk-control strategies (Latosińska et al., 2021).

The total content of Zn, Cu, Cr, Pb, Mn, Ni, and Fe in the different layers of the soil column after 7, 21, 35, and 90 days of MSW digestate application is shown in Figure 4. Levels of Cd and Co were not determined due to their low concentrations, which would prevent observing significant differences over time.

Overall, in a short-term period (90 days), relatively little downward movement of TMs occurred. TMs (Cr, Mn, Ni, Cu, Zn, and Pb) tended to mostly remain in the upper layer (0–0.1 m), with the one with MSW digestate-amended soil, moving slowly from the surface to the second layer, within the top 0.2 m of the soil profile.

After the stabilization period, at day 7 before plant transplantation, concentrations for all TMs were significantly higher (p <0.05) in the 0–0.1 m soil layer (MSW digestate-amended soil) when compared to deeper layers and decreased from the top to bottom, except for Fe, which already presented a higher concentration in the initial soil (p <0.05).

In soil columns with no plant (T0, Figure 4.), as a general trend, at day 21, significant increases (p <0.05) of Zn, Cu, Pb, Cr, Ni, and Mn concentrations were observed in the 0.1–0.2 m layer, the layer below the MSW-amended soil, after which TM concentration did not vary significantly (p >0.05). These results suggest the migration of Zn, Cu, Pb, Cr, Ni, and Mn from the MSW digestate-amended soil to the soil layer beneath (0.1–0.2 m) during the first 21 days after MSW digestate application, which then remained relatively stable until the end of the experimental period (day 90), with no statistically significant differences (p >0.05) among days 21, 35, and 90. No evidence of TM movement deep into the soil profile was detected since the TM concentration in the depths below 0.2 m essentially remained unchanged for all treatments during the whole experimental period. Similar results have been previously reported (Harris and Urie, 1986; Farrell et al., 2010) showing that TM levels in the soil after bio-amendment applications remained essentially constant in the top 0.1 m of soil, while it progressively declined

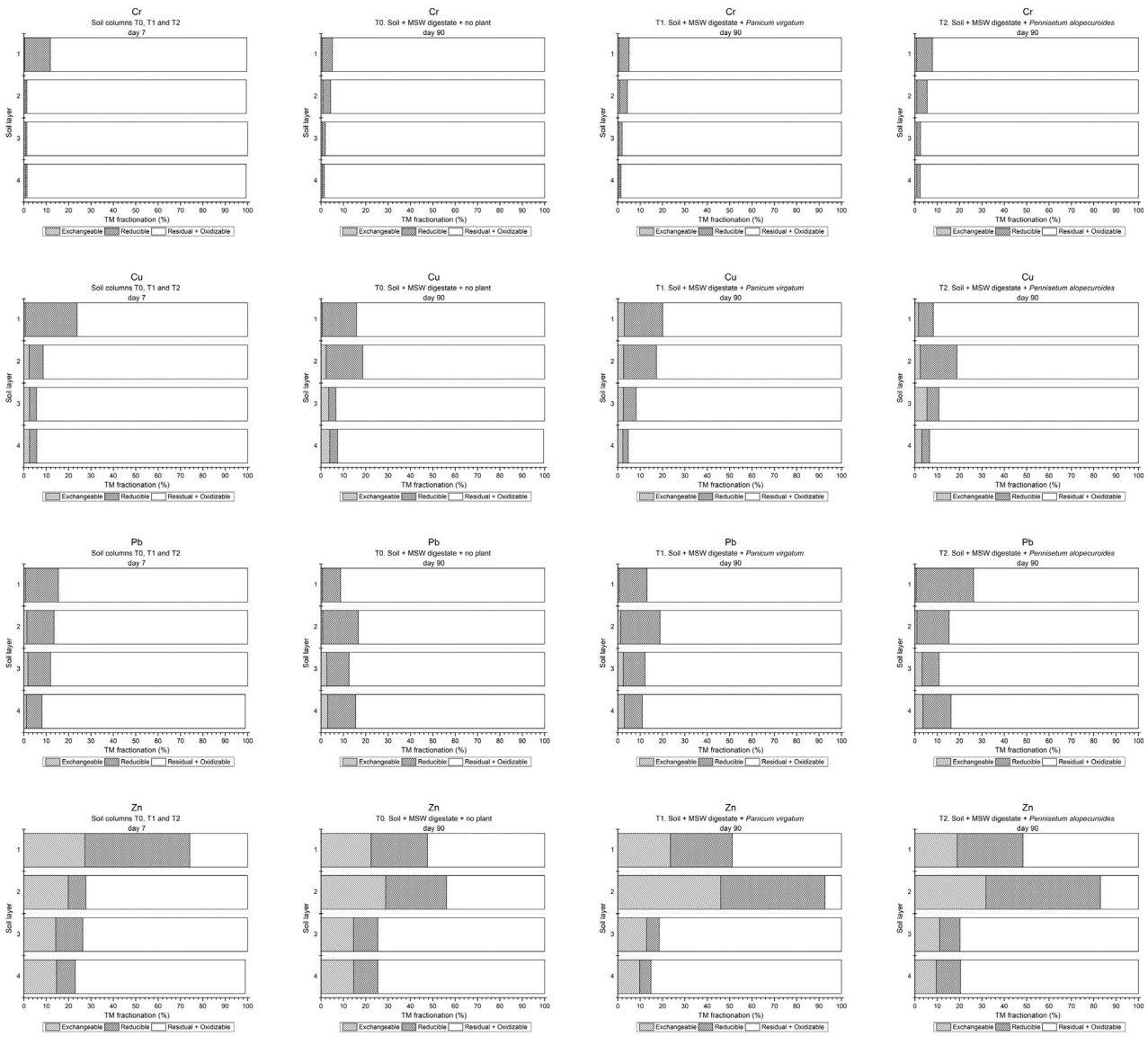


FIGURE 5
Percentage of metal fractions (Cr, Cu, Pb, and Zn) (exchangeable, reducible, and residual + oxidizable) along soil layers (1, 0–0.1 m; 2, 0.1–0.2 m; 3, 0.2–0.3 m; 4, 0.3–0.4 m) in soil columns after the initial stabilization period (day 7) and the end of the experimental period (day 90). Three measurements per each duplicated column (n = 2 × 3) were performed.

in the sub-surface layers after a longer period. Similarly, a recent study conducted by Pikuła and Stępień (2021) showed that the addition of increasing amounts of organic matter in the soil reduced the leaching of TMs deep into the soil profile (especially on light soils that are highly sandy).

However, an opposite trend was recorded here for Fe, that presented an upward trend over depth (lower concentration was observed in upper layers) and non-significant differences (p >0.05) over time, except for a big decline observed at the 0.1–0.2 m layer between day 7 and day 21, which after that point remained stable. This could be explained by the fact that the Fe concentration did not originate from the MSW digestate, as observed in the initial samples' characterization, which showed that MSW digestate amendment induced a decrease in the soil's Fe concentration.

The observed Zn, Cd, Pb, Cr, Ni, and Cd behavior in T0 could be explained by the known affinity of TMs for different fractions of soil organic matter (Fujikawa et al., 2000; Fujikawa and Fukui, 2001; Milne et al., 2003; Hartland et al., 2012; Šípková et al., 2013) and larger levels of organic matter in top layers induced by the MSW digestate amendment. Within organic matter, several distinct functional groups (such as hydroxyl, carbonyl, carboxyl, carbohydrate, and phenol) exhibit a strong capacity for forming complex compounds with metals, including insoluble complexes which can ultimately reduce metal mobility and transport (Tang et al., 2014; Borggaard et al., 2019). An additional piece of evidence is the increase of negatively charged sites in the soil (CEC) upon MSW amendment, previously explained (Supplementary Table S1), which was likely because of the increase of organic matter, indicating the

ability of the upper layer of soil to hold positively charged ions (such as TM cations) (Sumner and Miller, 1996).

Another possible explanation is the observed increase in the soil pH upon MSW digestate amendment (from 7.9 to 8.4, Supplementary Table S1), a value that was maintained until the end of the experiment (Supplementary Table S3). The maintained higher pH over time could be explained by various processes, with emphasis on the release of OH^- ions due to the decarboxylation of organic anions during the mineralization of organic carbon and the consumption of H^+ ions through the protonation of organic molecules (Shi et al., 2019; Yan et al., 1996). Many researchers have observed a significant reduction in the leaching of TMs under an increase in soil pH value (Fulekar and Dave, 1991; Wang et al., 2020). This slightly basic pH can induce some TM precipitation under the form of hydroxides and/or carbonates (Ford and Sparks, 2001). Furthermore, this pH value is favorable for the sorption of TMs onto clay minerals, present in the studied soil (Harter, 1983; Lair et al., 2007). Finally, it has been observed in the first section that MSW digestate amendment increased CaO and MgO concentrations in the soil (Supplementary Table S1), which are considered to be major adsorbents of TMs in soils due to their alkaline nature (Spence and Shi, 2004), which could also explain the high retention of TMs in the upper layers. A similar trend of TM migration was found in soil columns with *P. virgatum* and *P. alopecuroides* (treatments T1 and T2), with the TM content also remaining mainly confined in the 0–0.2-m layers over the course of the experiment. However, TM concentrations in the 0.1–0.2 m-layer on days 21, 35, and 90 were significantly (p <0.05) lower when compared to those in soil columns without plants (T0), indicating a potential protective effect of the studied plants, preventing metal mobility through the soil profile. A clear decline in TM concentration over depth can be observed, especially for Zn, Cu, and Pb. As previously mentioned, the TM distribution at depths below 0.2 m remained constant during the experiment for all treatments.

Similarly, *P. virgatum* has been widely reported to reduce Zn concentrations in soil (Aderholt et al., 2017; B.-C; Chen et al., 2012; Kacprzak et al., 2014; Masters et al., 2016), as well as Cr (Li et al., 2011), which was not observed in the current study. Fewer studies focus on *P. alopecuroides* (Chen et al., 2020). However, other species of the same genus have shown the TM uptake capacity, reducing TM concentrations in soils, similar to what was found in this study. For example, Lin et al. (2020) used *Pennisetum hydridum* to treat municipal sewage sludge. In the study of He et al. (2021), digestate application was used to improve the Cd phytoremediation potential of *Pennisetum hydridum*.

Small but not significant differences (p >0.05) were observed between columns with different plant species, except for the Zn concentration in the 0.1–0.2 m layer at day 90, which was lower (p <0.05) in the presence of *P. virgatum* than with *P. alopecuroides*. The reduced migration of TMs observed when plants were present could be explained by the activity in the rhizosphere. Both plants and rhizosphere microorganisms (bacteria, archaea, and fungi) can contribute to lowering TM mobility (Plekhanova et al., 2022). They both can release chelating compounds outside cells, for example, secondary metabolites such as biosurfactants, TM-binding proteins (metallothionein), metallophores, and low molecular weight organic acids and iron-chelating compounds (siderophores) (Tao et al., 2004; Lambers et al., 2009; Seshadri et al., 2015; Fresno et al., 2017; Barra Caracciolo and Terenzi, 2021), which can induce sorption and/or precipitation processes and decrease the mobility and accessibility of TMs significantly. A recent study from Grybos et al. (2022) showed the potential of microorganisms to release chelating agents for Fe and Pb.

Furthermore, these compounds can enhance the plant's and rhizosphere microorganisms' metabolically active uptake of TMs (McGrath et al., 2001). In fact, the Zn and Cu concentration decrease could be explained by plant uptake due to their essential nature for plants, as they were involved in many enzymatic processes necessary, for example, for proper photosynthesis, metabolism of carbohydrates and proteins, oxidation processes, and synthesis of DNA, RNA, and chlorophyll.

It is important to note that in both the absence and presence of plants, Pb, Zn, Ni, Fe, Mn, Cr, and Cu concentrations in column leachates collected over time were below the detection limit (250 μg L^{-1}, 17 μg·L^{-}1, 125 μg L^{-1}, 174 μg·L^{-}1, 66 μg L^{-1}, 0.75 μg L^{-1}, and 100 μg L^{-1}, respectively), indicating no significant leaching of TMs from the soil column.

To better visualize the obtained findings, correlation coefficients were calculated (Pearson's at the 0.05 significance level) and principal component analysis was performed to determine the relationships between total concentrations of TMs in the soil profile and among the pH, depth, and presence/absence of plants in soil columns T0, T1, and T2 (Supplementary Material, Supplementary Table S5, Supplementary Figure S3). As previously observed, Pb, Cr, Zn, Ni, Mn, and Cu were negatively correlated with depth (p <0.05), whereas the opposite was observed for Fe (p <0.05). Concentrations of Pb, Cr, Zn, Ni, Mn, and Cu over time and depth were positively correlated with each other (p <0.05), while they were negatively correlated with Fe (p <0.05). The positively correlated metal concentrations with each other could indicate a synergy among the leaching of these metals through the soil profile (Gräfe et al., 2004; Kumpiene et al., 2008). Regarding plant presence, Mn, Zn, Ni, and Cu total concentrations in the soil profile were negatively correlated with the presence of both *P. alopecuroides* and *P. virgatum*, while Pb was negatively correlated with *P. virgatum*, and Cr was negatively correlated with *P. alopecuroides*, although these correlations were not significant (p >0.05).

3.4 Metal fractionation as a function of time, depth, and presence of plants

To evaluate the evolution of the potential availability of TMs in the soil amended with MSW digestate over time and depth, and possible plant presence effects, metal fractionation was determined in samples collected in soil columns after the stabilization period (7 days) and at the end of the experimental period (90 days) for Cu, Cr, Pb, and Zn. The TMs of concern could cause ecotoxicological effects in the soil ecosystem upon amendment (Figure 5).

At day 7, Cu, Cr, and Pb were found mostly in the oxidizable + residual fraction in all soil layers, indicating that TMs were in their less mobile state, as previously observed for the initial samples. This was followed by the reducible fraction (TMs bound to oxides which

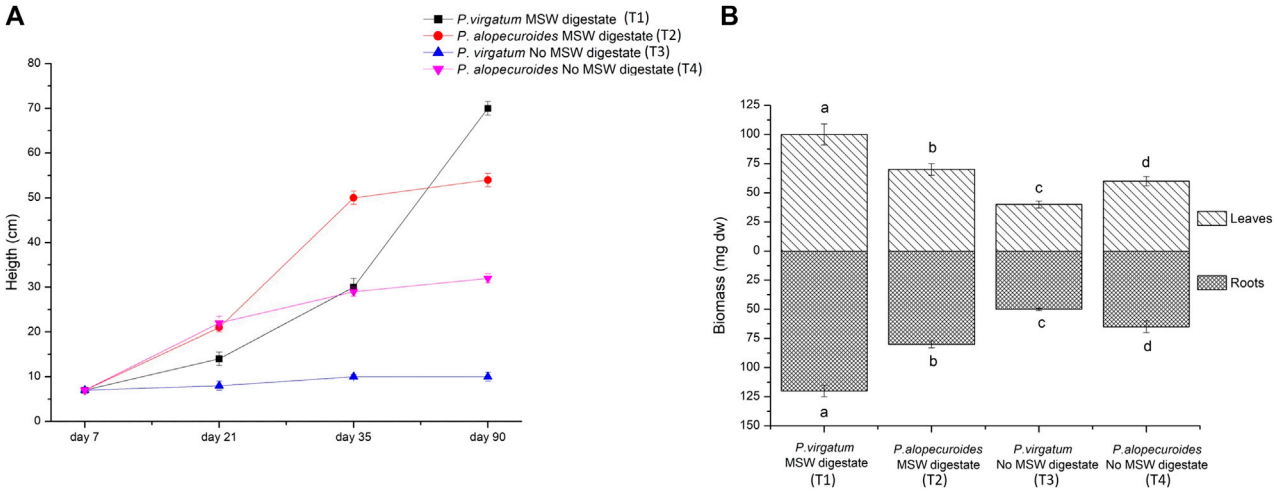


FIGURE 6
(A) Average height (cm) (+SD) over time of *P. virgatum* and *P. alopecuroides* grown in the presence or absence of MSW digestate. **(B)** Average dry mass (mg) (+SD) at the end of the experimental period (day 90) of *P. virgatum* and *P. alopecuroides* grown in the presence or absence of MSW digestate. Error bars indicate the standard error of the mean of three measurements per each duplicated column (n = 2 × 3) in the case of plants from MSW-amended columns (T1 and T2) and three measurement per single column (n = 1 × 3) for plants in the unamended column (T3 and T4). Columns with different letters are significantly different at the 0.05 level of significance by Tukey's multiple comparison test.

are unstable under reducing conditions), while the exchangeable fraction (bound to the soil by weak adsorption in the particles, therefore the most "mobile" fraction) was very low. Zn was found to be the most bioavailable TM; in the top layer (0–0.1 m), approximately 25% of the total concentration of Zn corresponded to the exchangeable fraction, whereas 47% corresponded to the reducible fraction. According to the literature, the mobility of the selected TMs in soils decreases in the following order: Zn >Cu >Pb (Finzgar et al., 2007).

Regarding soil columns without plants (treatment T0), in general, at the end of the experimental period, Zn, Cu, Cr, and Pb were mostly found in the oxidizable and residual fractions. As previously detailed, these TMs are probably non-mobile, not available, being bound to stable, high-molecular weight substances (Kazi et al., 2005; Van Poucke et al., 2020a; Van Poucke et al., 2020b), being probably sequestered by organic matter in the soil matrix. Overall, the reducible fraction of TMs in the first soil layer (0–0.1 m) decreased over time when compared to that on day 7, increasing in the second soil layer (0.1 m–0.2 m), accordingly to the migration of TMs observed for the total TM concentration. Layers below 0.2 m remained practically unchanged. Concerning Zn, both exchangeable and reducible fractions decreased in the 0–0.1 m layer, while both fractions increased in the 0.1–0.2 m layer. However, no significant changes were observed in TM fractions between day 7 and day 90.

Previous studies reported that there is a risk in the application of bio-amendments from municipal waste or urban sources, such as digestate or sewage sludge, in soil since both quantity and bioavailability of TMs may increase in the soil (Cambier et al., 2019). However, in the present study, a low metal mobility was observed without a significant increase in metal bioavailability. This was even more noticeable when the plants were present.

No significant changes between presence (T1 and T2) and absence of plants (T0) were observed in Cu and Cr fractionation. However, for Pb, while its fractionation in columns with *P. virgatum* (T1) was similar to that found in soil columns without plant (T0), *P. alopecuroides's* presence (T2) seemed to slightly increase the Pb reducible fraction. These results are in accordance with the reported high solubility of Pb at low pH (Sauvé et al., 1997). For Zn, however, both plants clearly changed Zn fractionation and availability, increasing it in the second soil layer (0.1–0.2 m). This could be explained by the rhizosphere activities, discussed in the previous section, which can in turn induce a decrease in pH (Supplementary Table S3).

The 0–0.2 m (rhizosphere) layer of both soil columns with the plants (*P. virgatum* and *P. alopecuroides*) showed a one-unit pH decrease compared to the 0.2–0.4 m layer, 7.1 vs. 8.2. This pH decrease could be explained by a root-mediated pH shift, which could be attributed to a combination of mechanisms, including 1) the cation–anion exchange balance; 2) organic acid release; 3) root exudation and respiration, 4) redox-coupled processes; 5) release of H^+ ions by the roots in their immediate vicinity (Seitz Valerie A. et al., 2022). Soil pH decrease can increase TM mobility and availability since it plays a major role in the sorption of metals as it directly controls the solubility of metal hydroxides, as well as of metal carbonates and phosphates (Sauvé et al., 2000; Michaud et al., 2007; Blossfeld et al., 2010; Seshadri et al., 2015; Antoniadis et al., 2017). Soil pH can also affect metal hydrolysis, ion-pair formation, and organic matter solubility.

Nonetheless, despite some changes in metal availability in upper layers, the combined study of total TM concentration and fractionation indicated that the presence of plants reduced metal transport through the soil profile in the short term (90 days).

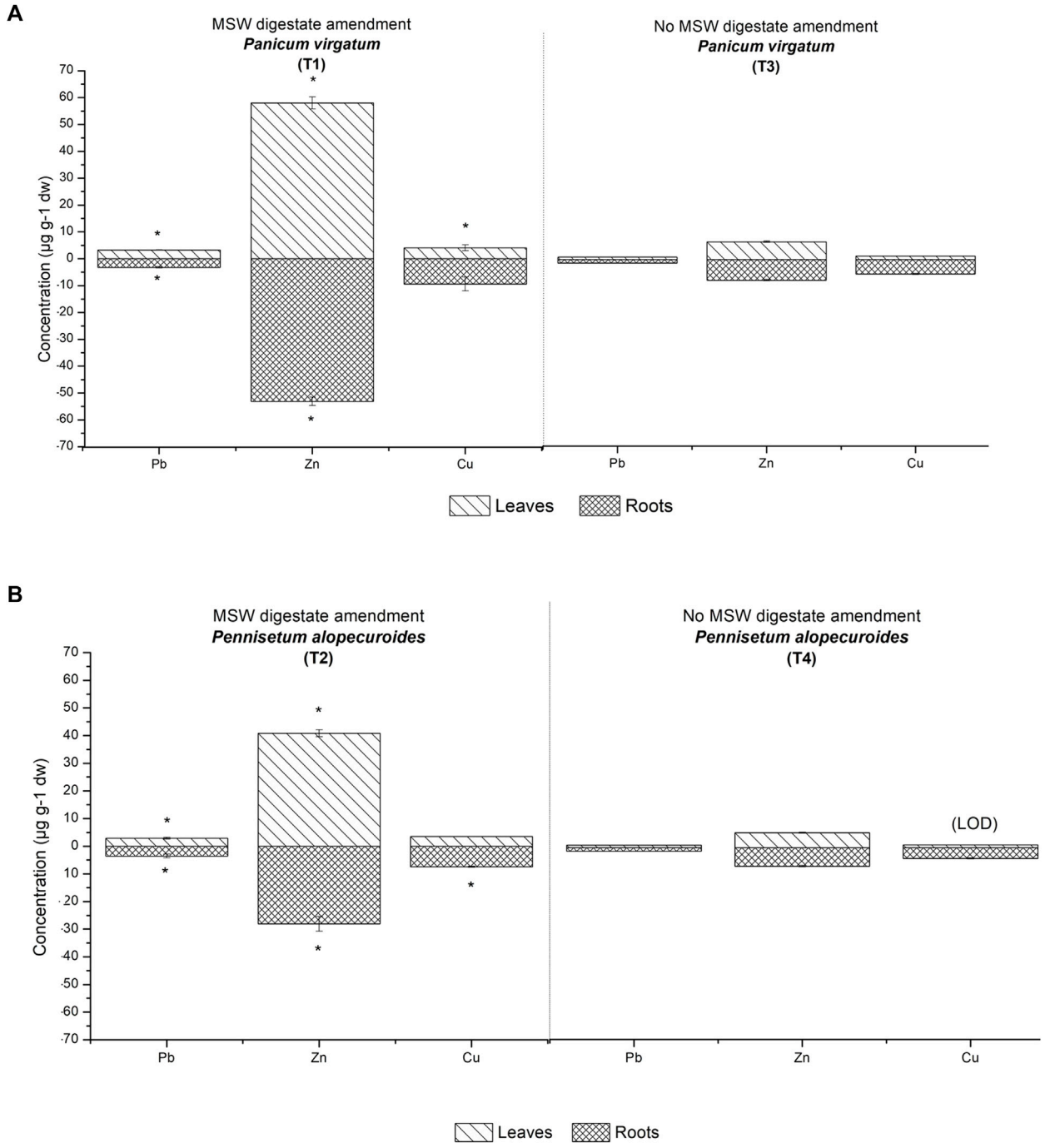


FIGURE 7
Concentration (µg g^{-1}) on dry weight basis (+SD) of Pb, Zn, and Cu at the end of the experimental period (day 90) in roots and leaves of *P. virgatum* and *P. alopecuroides* grown in the presence or absence of MSW digestate. **(A)** *P. virgatum* grown in the presence and absence of MSW digestate, **(B)** *P. alopecuroides* grown in the presence and absence of MSW digestate. (*) indicates the significant differences of each metal concentration between the presence and absence of the MSW amendment at the 0.05 level of significance by Tukey's multiple comparison test. Error bars indicate the standard error of the mean of the three measurements from two columns (n = 2 × 3) for T1 and T2, and the mean of three measurements from a single column (n = 1 × 3) for T3 and T4. The values were below the limit of detection (LOD) for Cu (1.5 µg g^{-1}).

3.5 Effects of MSW digestate on plant growth

Positive significant effects (p <0.05) of MSW digestate amendment were observed on the growth of both plants (Figure 6). At the end of the experimental period, both plants presented a significantly higher (p <0.05) dry matter biomass in MSW digestate-amended soil conditions when compared to the unamended soil (Figure 6). *P. virgatum* in the MSW digestate-amended soil presented a greater (p <0.05) total biomass than *P. alopecuroides* under the same

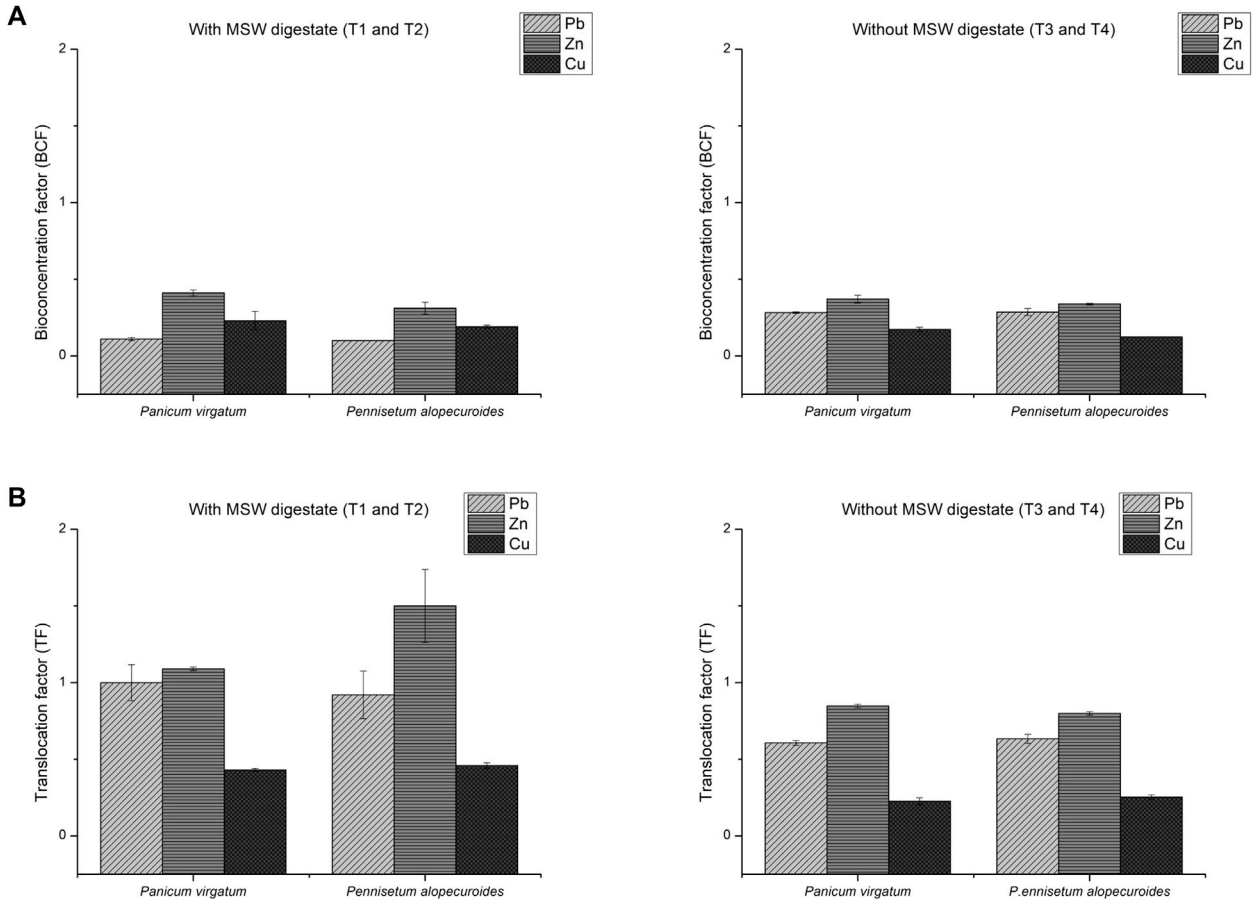


FIGURE 8
Root bioconcentration factor (BCF) and translocation factor (TF) (+SD) of Pb, Zn, and Cu for *P. virgatum* and *P. alopecuroides* grown in the **(A)** MSW digestate amended soil and **(B)** unamended soil, at the end of the experimental period (day 90). Error bars indicate the standard error of the mean of the three measurements from two columns (n = 2 × 3) for T1 and T2 and the mean of three measurements from a single column (n = 1 × 3) for T3 and T4.

conditions. Furthermore, plants' height evolution over time was increased with MSW digestate amendment (Figure 6).

Digestates can enhance plant growth directly through physiological and nutritional effects. Some substances found in digestates can function as natural plant hormones (auxins and gibberellins) and can improve seed germination, root initiation, and uptake of plant nutrients. Digestates can also serve as sources of biologically available N, P, and K, which can contribute to plant growth (Liang et al., 1996; Tambone et al., 2010). For instance, in this study, it has been shown that MSW digestate application increased P_2O_5 concentration in soil (Supplementary Table S1). Indirectly, digestates may affect plant growth through modifications of the physical, chemical, and biological properties of the soil, for example, enhanced soil water holding capacity and CEC and improved soil structure (Stevenson, 1994). In addition, recent studies have identified beneficial microbes in digestates, such as plant growth promoting bacteria (PGPB) (Qi et al., 2017).

In fact, it has been demonstrated that the application of different digestates from different sources improved crop yields (Zheng et al., 2019; Jimenez et al., 2020; Jamison et al., 2021; Panuccio et al., 2021), as observed in the current study. Tan et al. (2021) showed that the bioenergy crop hybrid giant Napier grass increased the total yield when digestate was applied. In addition, Walsh et al. (2012) found that grasses amended with liquid digestate gave similar or better yields than those receiving either N or NPK inorganic fertilizers. Alburquerque et al. (2012) found that the addition of the digestate increased the soil microbial biomass and activities, which provided a greater amount of organic carbon to the soil, ultimately causing a positive effect on crop yields. Lopushniak et al. (2021) performed prognostic models of *P. virgatum L.*, and according to their results, the use of sewage sludge provided a higher dry biomass yield than unamended soil. Rodgers & Anderson (1989) already demonstrated the benefits of sludge amendment in *P. virgatum* and other grass crop species. Yue et al. (2017) reported how municipal sewage sludge biochar amendment induced an improvement of poor urban soil fertility and turf grass nutrition and growth.

Therefore, the amendment with the MSW digestate was beneficial to plants. Higher plant growth can lead to higher exudation of compounds which can influence TM mobility and availability and could influence the TM behaviors as discussed in the previous sections, contributing for a higher retention of TMs in the layer of the soil amended with the MSW digestate, reducing TM mobility in the layer of the soil beneath it. A higher plant growth and an increase in plant biomass can also lead to a high amount of TMs being removed from contaminated matrices due to a higher metal

uptake, which will also reduce the possibility of TM mobility through the soil profile.

3.6 Plant metal uptake

After harvesting the plants at the end of the experiment, the total concentration of the two essential TMs (Zn and Cu) and one non-essential TM (Pb) in plant tissues was determined to evaluate whether the plants were adopting a phytoextraction strategy.

When analyzing phytoextraction, different factors should be taken into consideration: 1) the plant's potential to accumulate the TM and 2) the plant's ability to transfer the accumulated contaminants from belowground to the aboveground tissues. Therefore, the bioconcentration factor and translocation factor were calculated to evaluate TM uptake, mobilization into the plant tissues, and storage in the shoot parts. The BCF is described as the ability of plants for elemental accumulation from the substrate (Radziemska, 2018), being used to measure the TM accumulation efficiency in plants; BCF values >1 indicate a potential TM hyperaccumulator species (Zhang et al., 2002). The TF explains the ability of a plant to translocate the TM from the roots through the shoots and leaves, mediated by the xylem and/or phloem cells (Rascio and Navari-Izzo, 2011), being used to evaluate plants' capacity for phytoextraction.

Pb, Zn, and Cu concentrations in plant tissues grown in the MSW digestate-amended soil was significantly higher than in plants grown in the unamended soil (p <0.05) (Figure 7), being the most noticeable for Zn. In general, Zn levels were statistically higher in *P. virgatum*. In both plant species, the concentration of TMs in the leaves and roots followed the order Zn >Cu >Pb; both in the MSW digestate-amended and -unamended soil, however, the differences between Cu and Pb concentrations were not statistically significant. This was in accordance with the metal fractionation results where Zn was found mostly in exchangeable and reducible fractions, considered to be the most bioavailable TM in the MSW digestate-amended soil and so more easily taken up. In addition, according to the total TM concentration, Zn was the most abundant TM.

Both in the unamended and MSW digestate-amended soil, the BCF <1 was observed for both plants for all the TMs. BCF values were slightly higher for *P. virgatum* (Figure 8). Even though Zn and Cu are essential elements for all living organisms (Bowen, 1966), no evidence of bioconcentration was found. In addition, despite Zn being the most available TM, no evidence of hyperaccumulation in plants was found.

Regarding TM translocation abilities, for Zn a TF >1 was observed for both plant species. As mentioned previously, a TF value higher than 1 indicates that the TM is stored mainly in the aboveground part of the plant, which is primarily responsible for phytoextraction (Nirola et al., 2015). The observed results suggest a potential for both plants to relocate Zn to the aboveground biomass. The highest accumulation of the contaminants in the aerial part is interesting from the phytoextraction point of view. Once the contaminants are in the aerial part, the biomass harvest will contain the metals removed from the MSW digestate-amended soil.

Previous studies have shown that *P. virgatum* can uptake TMs such as Cd, Cr, and Zn from contaminated soils (Cui et al., 2011; Aderholt et al., 2017; Afzal et al., 2017; Guo et al., 2019). Mei et al. (2020) also showed *P. alopecuroides* had good Cu, Pb, Zn, and Cd uptake abilities when exposed to synthetic stormwater in bioretention plants, as also observed in this study, although without the bioconcentration capacity.

Differences in TM accumulation between the two plant species could be attributed to different plant uptake capacities; variations in the plant biomass, as *P. virgatum* presented a higher biomass; or the differences in the rhizosphere microbial community structure (not determined), rather than metal availability differences, which were not significant, as discussed previously. On the other hand, differences of the TF between the MSW digestate-amended and unamended soil could be explained by the differences in TM bioavailability and the plant biomass between the different conditions, as well as the differences in rhizosphere microbial diversity. The TM type, sources, physical and chemical behavior, environmental factors (Usman et al., 2020), plant biomass, and rhizosphere microbial diversity (Wood et al., 2016) can all play a role in TM extraction and accumulation by plants.

Therefore, the decrease in Zn, Cu, and Pb in the MSW digestate-amended soil layer and reduced migration into the soil layer beneath it when plants were present could be more closely associated with the physical and chemical stabilization of TMs in the soil through phytostabilization processes rather than direct root metal accumulation by the plants. However, the plants showed a capacity to translocate and accumulate Zn in aboveground tissues. The results suggest that different phytoremediation processes may be occurring, which combined, can help immobilize TMs in the rhizosphere and plant tissues (Kidd et al., 2009; Alkorta et al., 2010) and protect deeper layers of the soil from TM migration. Further research would help identify the phytoremediation mechanisms adopted by the plants, which in turn would help optimize the process.

4 Conclusion

The results showed that MSW digestate application increased the organic matter content and the macro- and micronutrients in the marginal soil, indicating a potentially suitable use in soil reclamation. Furthermore, MSW digestate exhibited a positive impact on the growth rate and biomass yield of *P. virgatum* and *P. alopecuroides*, with the latter showing a slightly less biomass growth.

Overall, total TM concentrations increased in the 0.1–0.2 m soil layer over time, the soil layer beneath the digestate-amended soil, suggesting a downward migration of TMs. Nevertheless, total TM concentrations tended to be confined in the upper layers (0–0.2 m) of the soil profile, indicating no metal migration to the deep soil layers and no evidence of risk, for instance, of groundwater contamination. The highest concentrations in bioavailable metal fractions were found in the first layers, the 0–0.1-m MSW digestate-amended soil and 0.1–0.2 m, especially for Zn. The other TMs, namely, Cu, Cr, Fe, Mn, and Pb, were mostly found in oxidizable–residual fractions in all layers of the soil profile, showing a low availability. *P. virgatum* and *P. alopecuroides* reduced TM migration to the 0.1–0.2 m layer, in particular that of Zn, Cu, and Pb, suggesting a protective effect. Moreover, in general, no significant differences were observed among TM fractionation between the presence and absence of plants. In fact, only an increase in Zn availability in the 0.1–0.2 m

layer was observed when *P. virgatum* was present. Within the studied conditions, higher concentrations of TMs were found in both plants' tissues when exposed to MSW digestate-amended soils relatively to unamended soils. However, no bioconcentration capacity was observed for the two plants.

Although the current findings cannot be extended to all soils, for the bio-amendment types and field situations, it is expected that the current research will add to the body of knowledge regarding the potential benefit of the MSW digestate, along with its impact on soil attributes and the use of phytoremediation technologies as an economically green alternative to increase the safety of such practices. Further experiments are needed to analyze the long-term effects and large-scale implementation.

Ultimately, this approach may help achieve the Mission Board's proposal to the European Commission that aims to ensure 75% of healthy soils by 2030 for food, people, nature, and climate (EC, 2020). Furthermore, it may also help address the United Nations Sustainable Development Goals (SDGs) 7 ("Ensure access to affordable, reliable, sustainable and modern energy for all"), 13 ("Climate action"), and 15 ("Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss") (United Nations, 2016).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

NB-G: conceptualization, data curation, formal analysis, investigation, methodology, visualization, and writing–original draft. VB: methodology and writing–review and editing. VR: methodology, validation, visualization, and writing–review and editing. CG: resources and writing–review and editing. GG: funding acquisition and writing–review and editing. MA: resources and writing–review and editing. RC: resources and writing–review and editing. AM: funding acquisition, supervision, and writing–review and editing. MA: funding acquisition, supervision, validation, visualization, and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1267463/full#supplementary-material

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