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## Innovative use of mine tailings as a soil amendment for growing *Pisum sativum* L.

MOHAMMED KHARBOUCHE<sup>1\*</sup>, KHALID EL KHALIDI<sup>1</sup>, REDOUANE MGHAIOUNI<sup>2</sup>, AHMED AAJJANE<sup>1</sup>, BENDAHHOU ZOURARAH<sup>1</sup>

<sup>1</sup>Department of Geology (Laboratory of Marine Geosciences and Soil Sciences),  
Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco

<sup>2</sup>Advanced Systems Engineering Laboratory, National School of Applied Sciences,  
Ibn Tofail University, Kenitra, Morocco

\*Corresponding author: [kharbouche1969@gmail.com](mailto:kharbouche1969@gmail.com)

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**Abstract:** This study investigates the benefits of using mine tailings (MT) to improve pea (*Pisum sativum* L.) growth and productivity on degraded agricultural soils in semi-arid environments. The research aims to evaluate the use of MT as an innovative soil amendment and to determine the optimal dose required to enhance the micronutrient availability of Zn, Mn, Cu and Fe without affecting soil quality. The experiment was conducted in greenhouse pots with three different soil types amended with different MT doses (control and four doses). Soil samples were collected from the Doukkala region, one of the main agricultural areas in Morocco. Pea was grown in pots and monitored for 87 days until maturity. After harvest, soil and plant samples were weighed, measured and analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The experiment found that moderate doses (0.2 g/kg to 1 g/kg) applied to all soil types promoted optimal pea growth by improving plant height, root and above-ground biomass and pod number. Thus, MT can act as a biostimulant. However, nutrient antagonism negatively affected growth at the highest dose (4 g/kg). Bioconcentration and translocation factors indicated efficient micronutrient uptake and biofortification, while heavy metals remained immobilised in roots, effectively eliminating toxicity risks.

**Keywords:** plant nutrition; agronomic biofortification; soil protection; phytoremediation; circular economy applications

Agricultural soils play an essential role in global food production. Faced with growing demand for food, they are under strong pressure to increase productivity (Kopittke et al. 2019). However, their management is confronted with fertility loss, soil degradation and erosion caused by human activities. Intensive agriculture has led to progressive soil degradation worldwide, threatening food security and ecosystem sustainability (Penuelas et al. 2023). In the African semi-arid zones, particularly in Morocco, the irrational use of agricultural fertilisers represents a serious risk for soil health. The predominant use of macronutrients such as N, P and K, combined with inappropriate farming practices, has aggra-

vated deficiencies in essential micronutrients (Kihara et al. 2020). This situation has reduced not only productivity, but also the nutritional quality of the crops. These agronomic deficiencies, accentuated by recurrent droughts, pose serious problems for food security and agricultural biodiversity (Semba et al. 2021). Micronutrient-deficient diets have contributed to the emergence of hidden hunger. Moreover, the limited technical knowledge of farmers and the high cost of micronutrient supplements have restricted their widespread use in soil improvement.

According to Kirkby (2023), micronutrients include B, Zn, Fe, Cu, Mn, Mo, Ni and Cl. Although micronutrients play an essential role in plant development

and growth, they are only required in small quantities. Their deficiency directly affects both the agronomic performance and nutritional value of crops, while also contributing to public health problems such as stunted growth and immunodeficiency, which mainly affect the vulnerable communities of poor countries (Rodríguez-Espinosa et al. 2024). Biofortification – whether agronomic, genetic or biotechnological thus represents a promising strategy for enriching food crops with essential micronutrients (Stangoulis and Knez 2022). B and Zn deficiencies are common and significant worldwide, but Mn and Fe deficiencies are linked to calcareous and alkaline soils (Dhaliwal et al. 2022). However, toxicity problems are observed in very acidic soils.

Faced with this situation, there is a pressing need to amend agricultural soils using accessible and cost-effective sources of micronutrients (Aparicio et al. 2022). Mine tailings (MT), traditionally considered as environmental waste, offers an innovative opportunity for reuse and valorisation through recycling (Araujo et al. 2022). Due to their high concentrations of essential nutrients, including Zn, Fe, Mn and Cu, they could improve soil fertility and agricultural production. In Morocco, the Kettara mine near Marrakech illustrates a local resource that could be utilised as a soil amendment (Benidire et al. 2022, Kharbouche et al. 2024). According to Singhal et al. (2023), as well as providing essential nutrients, MT could serve as biostimulants, enhancing root development, improving crop resistance to drought and increasing the bioaccumulation of micronutrients.

As a main crop, peas are particularly affected by this problem of micronutrient deficiency because they are grown on depleted soils. The food pea (*Pisum sativum* L.), a species of the Fabaceae family, is a nutritionally important crop for humans and animals. It has grown in 99 countries on six continents. World production of this crop is around 12.4 million tonnes and occupies 121 million hectares (FAOSTAT 2023). It is mainly produced in Europe, followed by North America, Asia and Africa. The pea plays a key role in farming systems, particularly in semi-arid zones, where it is integrated into crop rotations to improve soil sustainability (Chibarabada et al. 2017, Moussadek 2024).

In Morocco, pea cultivation is concentrated between November and June, often alternating with wheat. However, persistent challenges, such as soil depletion and the effects of climate change, compromise its productivity.

To develop sustainable solutions to improve peas' productivity and nutritional quality, it is essential to implement appropriate practices. These approaches will enhance food security and help preserve and effectively manage soils in arid and semi-arid regions. In this context, this study aims to evaluate MT's beneficial effect on peas' growth and biomass, while analysing bioconcentration and translocation factors of micronutrients in the different parts of the plant.

## MATERIAL AND METHODS

**Study areas.** The experiments were conducted in a plastic greenhouse in the Faculty of Science at the Chouaib Doukkali University, El Jadida, Morocco. The materials studied included three agricultural soil types, representative of the Doukkala region (agricultural zone), collected for the experiment. These soils (S1, S2, S3) were classified according to the FAO classification. In addition, the MT was taken from the abandoned Kettara mine, located 35 km north-west of Marrakech.

**Conception of the experimental pot.** The experimental protocol adopted was based on a split-plot arrangement with two factors. Soil type was the main factor, with three levels (S1, S2 and S3) representing the three soil types. MT dose was the secondary factor, with five levels: control 0, 0.2, 1, 2 and 4 g/kg. The experiment was subdivided into three separate blocks to ensure repeatability and minimise random variation. Soil types were classified by textural characteristics and randomly distributed across the three blocks. Firstly, the five doses of TM were randomly and independently distributed in each soil-block combination. Each block contained 15 pots (5-L pots) to ensure a statistically valid assessment of the effects of soil, amendment dose, and effects on the parameters studied.

**Pea cultivation.** *Pisum sativum* L. (a local cultivar) was selected for this study because of its nutritional importance and particular sensitivity to micronutrients. The seeds were disinfected and sown in 5-L pots containing 3 kg of soil and MT mix. These pots were perforated to ensure optimum drainage, and leaching liquids were collected and reintroduced to maintain a continuous supply of nutrients. The plant pots were arranged in a greenhouse maintained under normal conditions. The temperature varied between 7 °C and 21 °C. The photoperiod varied from 10 h to 12 h of natural light, and relative humidity fluctuated around 70–90%.

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The monitored plants were continuously measured from 20/01/2023 for 87 days. At the start of the experiment, 10 seeds were sown. The plants were then irrigated and fertilised with an NPK nutrient programme adapted to their growth stage. Weeds were removed manually to maintain favourable conditions for plant development.

**Determination of germination rates and growth measurements.** After 10 days, the germination percentage was determined as the ratio of the number of successfully germinated seeds to the total number of seeds originally planted in each pot. Finally, 5 plants per pot were monitored throughout the experiment.

Plant growth was monitored over 87 days, with weekly measurements of the main development and growth parameters. Plant height was measured using a graduated ruler, while biomass was determined after drying the samples at 72 °C to a constant weight. At the end of the experiment, the number of pods and the total dry weight of seeds per plant were determined.

**Physicochemical analyses of soils and MT.** Soil and MT samples were analysed using standardised protocols. Robinson et al. (1997) used the method to determine granulometry. The pH (1:2.5) was measured. Walkley and Black's method (1934) was used to evaluate organic carbon. Carbonate content was measured using a Bernard calcimeter. Electrical conductivity (1:5 solution) was determined. Assimilable (K and P) content was determined using the method of Olsen (1954). The extractable cations (Mg, Na, K and Ca) were also analysed. CEC (cation exchange capacity) was determined by the Metson (1957) method. Total nitrogen was analysed using the method of Bremner and Mulvaney (1982), while nitric and ammoniacal nitrogen contents were determined using the methods of Sims and Jackson (1971) and Dorich and Nelson (1983). Finally, the micronutrient content was analysed using Lindsay and Norvell's method (1978).

Based on the FAO classification, three initial soils were analysed and classified (Table 1). S1 has a Vertisol, generally characterised by high water and nutrient retention, but can suffer from low drainage and compaction

Table 1. Physico-chemical properties of the three soil types and the mining tailings used in the experiment

Parameter	S1	S2	S3	Mine tailing
Clay	43.30	26.12	17.87	11.3
Silt	15.45	35.76	8.72	5.3
Sand	42.25	38.12	73.41	83.4
Soil organic carbon (%)	1.83	1.38	1.29	1.17
CaCO <sub>3</sub>	1.35	1.08	1.23	–
CaO	–	–	–	1.05
pH	8.15	7.72	7.82	2.3
Electrical conductivity (mS/cm)	0.46	0.31	0.54	7.67
Cation exchange capacity (mmol <sub>+</sub> /kg)	304.1	288.2	176.1	66.9
P	39.3	18.8	35.3	1.27
Ca	4 783	2 606	3 318	1 451
K	240	169	162	50
Mg	1 138	751	521	1 891
Na	631	560	347	245
N-NO <sub>3</sub> (mg/kg)	35.62	27.78	19.24	–
N-NH <sub>4</sub>	3.72	4.13	6.59	–
Zn	0.91	0.42	0.94	185
Mn	21.64	24.38	21.52	27.4
Fe	13.55	12.93	9.81	12 728
Cu	0.69	0.87	0.59	1 336

Parameters include pH, electrical conductivity, organic carbon content, cation exchange capacity, particle size distribution (sand, silt, and clay), and total concentrations of key micronutrients (Cu, Fe, Mn, and Zn). S1 – Vertisol; S2 – Luvisol; S3 – Arenosol

problems. S2 is Luvisol, balancing water retention and drainage, which is beneficial for plant growth due to the increased availability of nutrients. S3 is Arenosol, with excellent drainage, but subject to leaching and reduced fertility due to low water and nutrient retention. Soil texture determines water retention, drainage, nutrient availability and vulnerability to erosion.

**Analysis of micronutrients in plant tissues and grains of pea.** The pea plants were harvested after 87 days. Root, shoot and leaf tissues were washed, desiccated at 70 °C for 3 days and weighed. Seeds, roots and aerial parts were ground and calcined for 4 h at 450 °C. The incineration ash was digested with *aqua regia* (HNO<sub>3</sub>/HClO<sub>4</sub>), filtered and analysed by plasma atomic emission spectrometry (ICP-AES, PerkinElmer Optima 8000, Waltham, USA) for Zn, Mn, Cu and Fe contents.

The focus on four micronutrients (Zn, Mn, Cu and Fe) was chosen because of their dual relevance: they are essential for plant growth and human nutrition, and they are present in significant concentrations in the tailings studied. However, mine tailings can also contain potentially toxic elements such as cadmium, lead, arsenic and chromium. Although these elements are beyond the scope of this study, it is important to monitor them to ensure the environmental safety of TM reuse.

**Determination of bioconcentration factor and translocation factor.**

Eq. (1) is used to calculate bioconcentration factor (BCF) as follows:

$$BCF = \frac{\text{micronutrient}_{\text{grains concentration (mg/kg)}}}{\text{micronutrient}_{\text{soil concentration (mg/kg)}}} \quad (1)$$

Eq. (2) used to calculate the translocation factor (FT) is as follows:

$$FT = \frac{\text{micronutrient}_{\text{aboveground concentration (mg/kg)}}}{\text{micronutrient}_{\text{root concentration (mg/kg)}}} \quad (2)$$

**Statistical analysis.** Analysis of variance (ANOVA 2) was used to determine significant differences between experimental groups using SPSS version 20 software (New York, USA). For multiple comparisons of means, the *LSD* (least significant difference) test was used with a significance level of  $P < 0.05$ . Furthermore, the regression method was used to establish the relationship between growth parameters and pea biomass with MT doses applied by soil type.

## RESULTS AND DISCUSSION

**Soil and mine tailing chemical properties.** Table 1 presents the chemical characteristics of the soil and MT samples. The data indicates that all three soil

types (S1, S2 and S3) are alkaline, with pH values of 8.15, 7.82 and an intermediate value for S2 (Table 1). This alkalinity may reduce the availability of micronutrients (Cu, Zn, Fe, Mn) to plants. Organic carbon (OC) content is low in all soils, with S3 being the most vulnerable to nutrient leaching due to its Arenosol texture.

CEC is highest in soil S1 (304.1 mmol<sub>+</sub>/kg) due to its clay content, followed by soil S2 (288.2 mmol<sub>+</sub>/kg), while soil S3 has the lowest capacity (176.1 mmol<sub>+</sub>/kg), increasing the risk of nutrient loss.

Micronutrient analysis indicates that S1 is potentially deficient in Cu (0.69 mg/kg), while S2 has the highest Cu content (0.87 mg/kg) and S3 the lowest (0.59 mg/kg). Fe is sufficient in S1 and S2, but its availability is limited by soil alkalinity. S3 also has the lowest Fe content. Mn is present in sufficient quantities in all soils, although pH can affect uptake. Soil S1 contains adequate Zn levels, but its high pH may limit Zn availability. At the same time, soil S2 has a high risk of Zn deficiency; soil S3, although with a slightly higher Zn content than soil S1, remains at risk.

Finally, the analysis of MT reveals that they are rich in Fe (12 728 mg/kg), Cu (1 336 mg/kg) and Zn (185 mg/kg). Therefore, using these MT as an amendment for agricultural soils is important.

**Physicochemical analysis of soil after application of MT.** The use of MT in the soil resulted in major physico-chemical changes, including a significant increase in Cu and Fe concentrations, as shown in Figure 1. The values measured after treatment exceed those of the initial control soil, indicating a significant effect of incorporating MT.

This enrichment can be attributed to the high initial content of Fe and Cu in the MT material, which was gradually released into the soil matrix. The observed trends suggest that even at low application doses (0.2 and 1 g/kg DM (dry matter)), there is a significant improvement in micronutrient availability. However, the increase becomes more pronounced at higher doses (2 and 4 g/kg DM), especially for Vertisol (S1), which generally has a higher CEC and can retain added metals more effectively. However, the Mn and Zn concentration variations were less consistent across treatments and soil types.

These results highlight the potential of MT as a micronutrient-rich soil amendment capable of enhancing soil fertility and contributing to agronomic biofortification. However, monitoring and optimisation of MT application doses are essential to balance nutrient enrichment with environmental safety.

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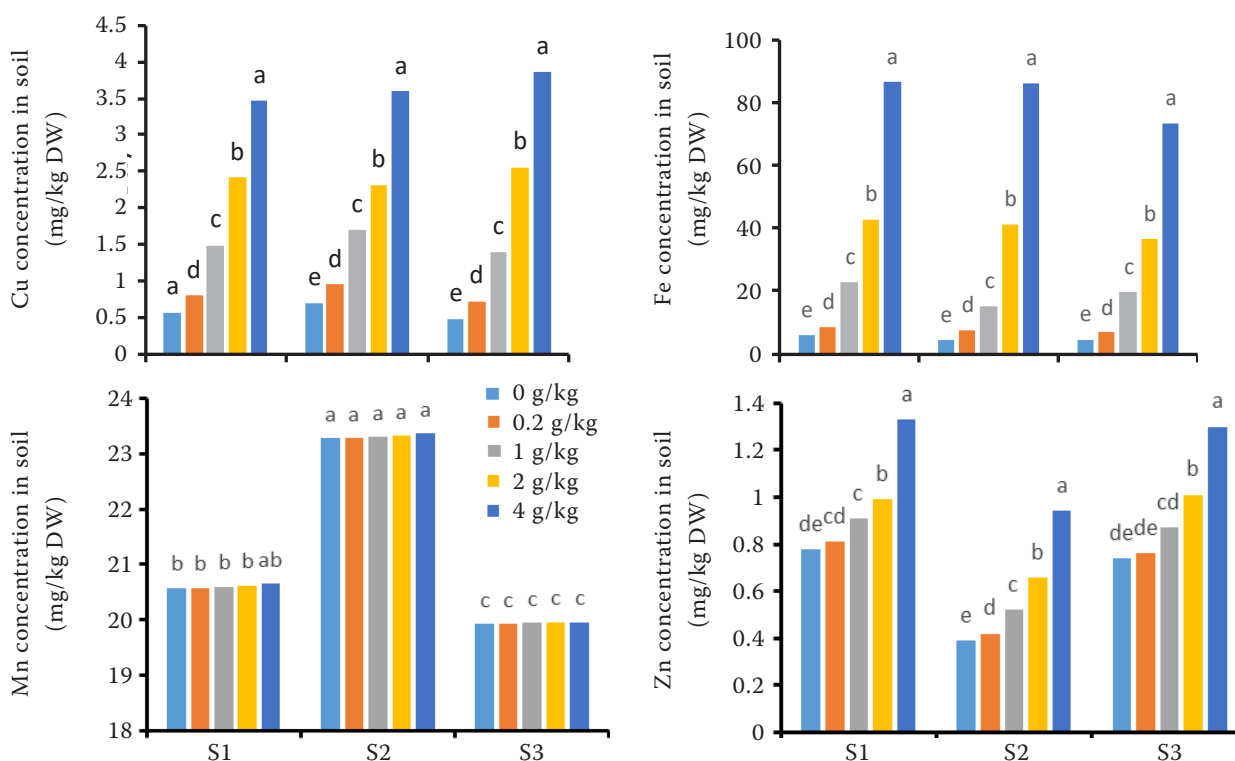


Figure 1. Concentrations of Cu, Fe, Mn, and Zn in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol – after the application of different doses of mining residues (0, 0.2, 1, 2, and 4 g/kg of soil dry matter), measured 87 days after sowing at pea harvest. Vertical bars represent the standard error of the mean ( $\pm$  SE), calculated from three replicates. Different letters indicate statistically significant differences at  $P < 0.05$  within the same soil type. DW – dry weight

**Effects of MT on percentage germination, growth and morphology of pea plants.** In this experiment, at doses lower than 1 g/kg, the results indicated that the application of MT had no significant effect ( $P < 0.05$ ) on the germination percentage of pea. As shown in Figure 2, the germination percentages observed for the treated soils were comparable to those for the control. This result is in concordance with the results of previous research (Mondal and Bose 2019), which generally indicate improved germination and crop performance when essential micronutrients are available at optimal levels. Figure 2 shows that the incorporation of MT has a significant effect ( $P < 0.05$ ) on the growth and morphology of *Pisum sativum* L., with the effects depending on the doses applied. The maximum height (51 cm) was obtained at a level of 1 g/kg, while a minimum height (33 cm) was observed at 4 g/kg. This reduction at higher doses is attributed to the antagonism of the micronutrients, which limits their availability to the plants (Rietra et al. 2017).

The essential micronutrients (Cu, Fe, Mn, Zn) present in MT facilitate processes such as chlorophyll synthesis, biological nitrogen fixation and

growth hormone regulation (Nazir et al. 2019). The maximum number of pods (2.67) was found at 1 g/kg in the Luvisol soil. This increase was attributed to improved stem structure and increased pod fertility.

Roots are observed to be longer and more vigorous at moderate doses (0.2–1 g/kg), particularly in Arenosol soils, improving nutrient uptake and biomass yield. However, high doses  $\geq 2$  g/kg have a negative effect on root growth (Saquee et al. 2023).

Root length, shoot height and number of pods per plant increased at moderate doses (0.2–1 g/kg). At higher doses, these parameters' values were reduced, indicating the toxic effects of an excessive micronutrient content. High doses of MT contain elevated concentrations of micronutrients, which can induce toxicity in pea (*Pisum sativum* L.). This toxicity is manifested through various physiological and biochemical disorders, including chlorosis, inhibition of root growth, oxidative stress, reduced photosynthetic activity, metal accumulation in roots, foliar necrosis, and overall impairment of root system development (Shahid et al. 2017).

**Effect of MT on root biomass, shoot biomass and grain biomass.** The results show a significant



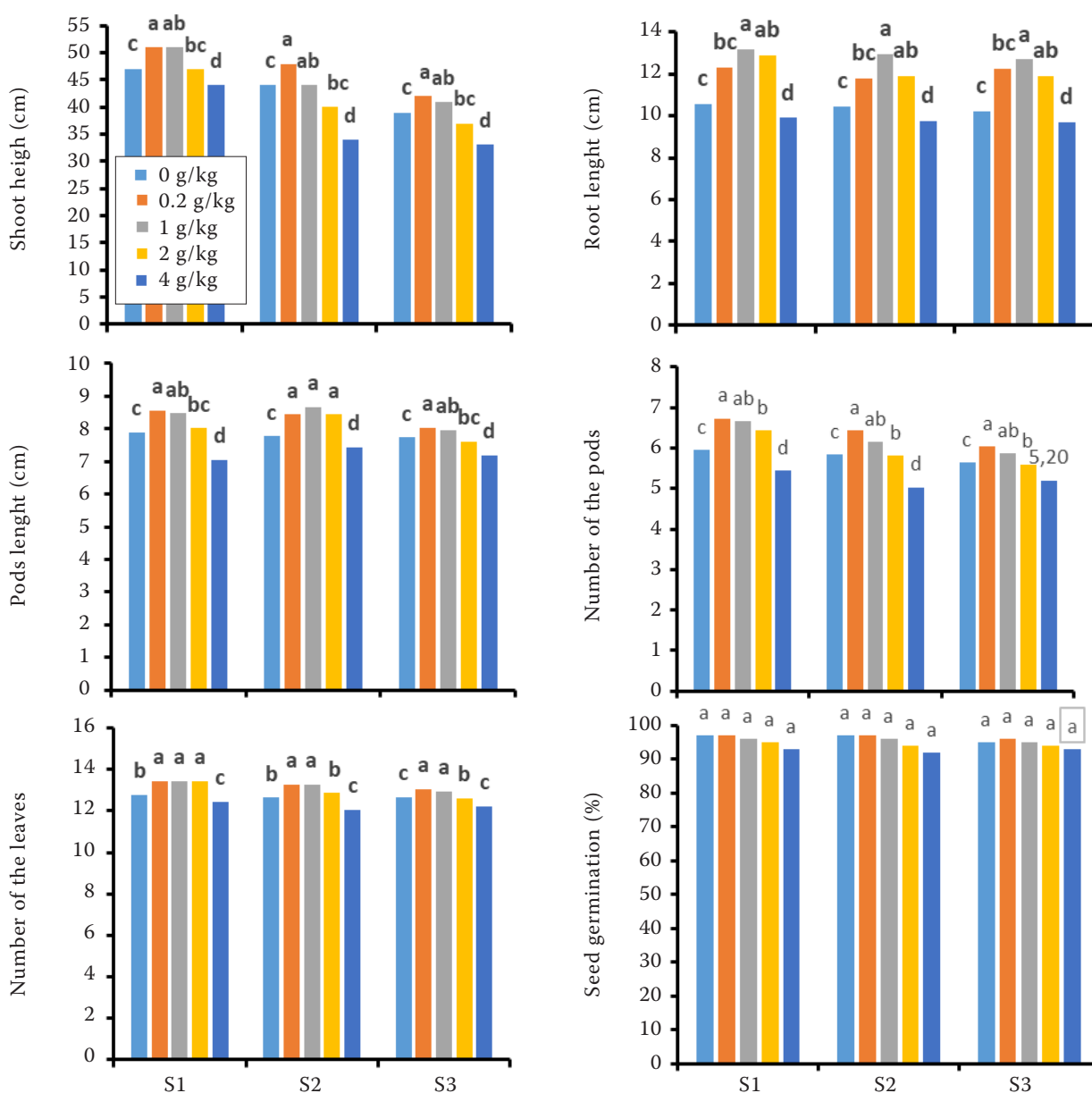


Figure 2. Effects of different treatment levels (0, 0.2, 1, 2, and 4 g/kg soil dry matter) on germination rate, root length, shoot height, number of leaves, number of pods, and pod length of pea (*Pisum sativum* L.) plants after 87 days of growth in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol. Different letters indicate statistically significant differences at  $P < 0.05$  within the same soil type

variation in peas' above-ground and root biomass depending on the doses of MT applied (Figure 3). Low to moderate doses of amendments  $\leq 1$  g/kg stimulated root, shoot and grain biomass thanks to a balanced supply of essential micronutrients, particularly Zn, Mn, Fe, and Cu. These minerals are critical for photosynthesis, enzyme formation and cell growth (Marschner 2012). This trend is coherent with observations on other crops such as triticale (*Triticosecale* Wittm) and maize (*Zea mays* L.),

where optimal micronutrient supply maximises growth and yield (Rashid and Ryan 2008). At high doses ( $> 2$  g/kg), biomass decreased significantly ( $P < 0.05$ ) due to excessive accumulation of micronutrients, leading to toxic effects and nutritional imbalances. Excessive concentrations of Zn or Cu reduce the assimilation of other soil nutrients, such as Fe, leading to secondary deficiencies (Kabata-Pendias 2011). Toxicity is manifested by the excess production of reactive oxygen species (ROS), which deteriorates

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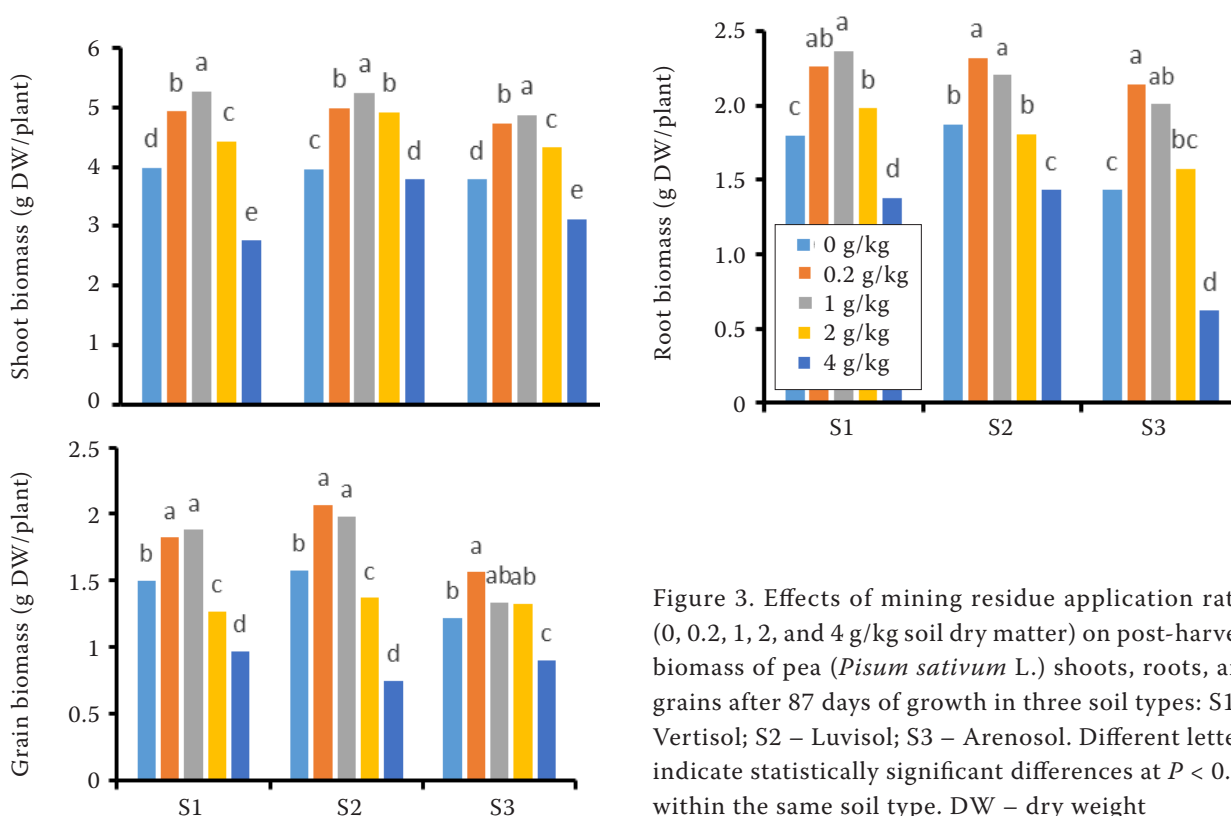


Figure 3. Effects of mining residue application rates (0, 0.2, 1, 2, and 4 g/kg soil dry matter) on post-harvest biomass of pea (*Pisum sativum* L.) shoots, roots, and grains after 87 days of growth in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol. Different letters indicate statistically significant differences at  $P < 0.05$  within the same soil type. DW – dry weight

the proteins and lipids of plant cells and disrupts normal metabolic processes (Sharma et al. 2012).

Although pot trials under controlled conditions have demonstrated the feasibility of biofortification and agronomic restoration using Kettara mine tailings, these results need to be validated in the field. In this respect, the 1 132 m<sup>2</sup> study carried out in China (Liaoning province) showed that an amendment combining mine tailings, plants and bacteria could significantly reduce bulk density, improve soil porosity and promote plant growth (Cui et al. 2021). Similarly, in Finland, the incorporation of biochar and compost on mine tailings optimised plant cover and limited metal bioaccumulation (Hagner et al. 2021). This research confirms that the pot approach is a solid starting point, provided that it is supplemented by trials in real environments.

Moderate doses of mine tailings (0.2–1 g/kg) significantly improved pea growth parameters – germination, shoot height, root length, pod number, and biomass – compared to the control ( $P < 0.05$ ). The highest plant height (51 cm) and biomass were recorded at 1 g/kg, while higher doses ( $\geq 2$  g/kg) caused toxicity symptoms and growth inhibition. Root vigour was particularly enhanced in Arenosol at moderate doses. These results demonstrate a clear

dose-dependent effect, with optimal benefits at 1 g/kg and detrimental impacts at excessive levels.

**Relationships between micronutrient extractable from the soil and their concentrations in pea plants (roots, stems and grains).** The concentrations of Zn, Fe, Mn, and Cu were analysed in the roots, shoots and grains of plants grown on three different soils (S1, S2, and S3) treated with increasing doses of mining residues (0 to 4 g/kg) (Figure 4). The results showed significant variations depending on the dose and plant part. In all three soil types, micronutrient concentrations generally increased for doses below 0.2 g/kg, with less pronounced changes in the shoots. Between 0.2 and 1 g/kg, concentrations remained stable. However, a decline in micronutrient levels was observed at higher doses (1 to 4 g/kg). The roots showed the highest accumulation among plant tissues, followed by the stems and grains.

Cu and Zn concentrations increased with the MT dose, indicating active transfer to the roots, the primary site of bioaccumulation. This aligns with Kabata-Pendias (2011), who observed that roots often concentrate metallic elements in response to soil amendments. In some cases, Cu and Zn concentrations in roots sometimes exceeded the safety limits of 20 mg/kg for Cu and 60 mg/kg for Zn, as

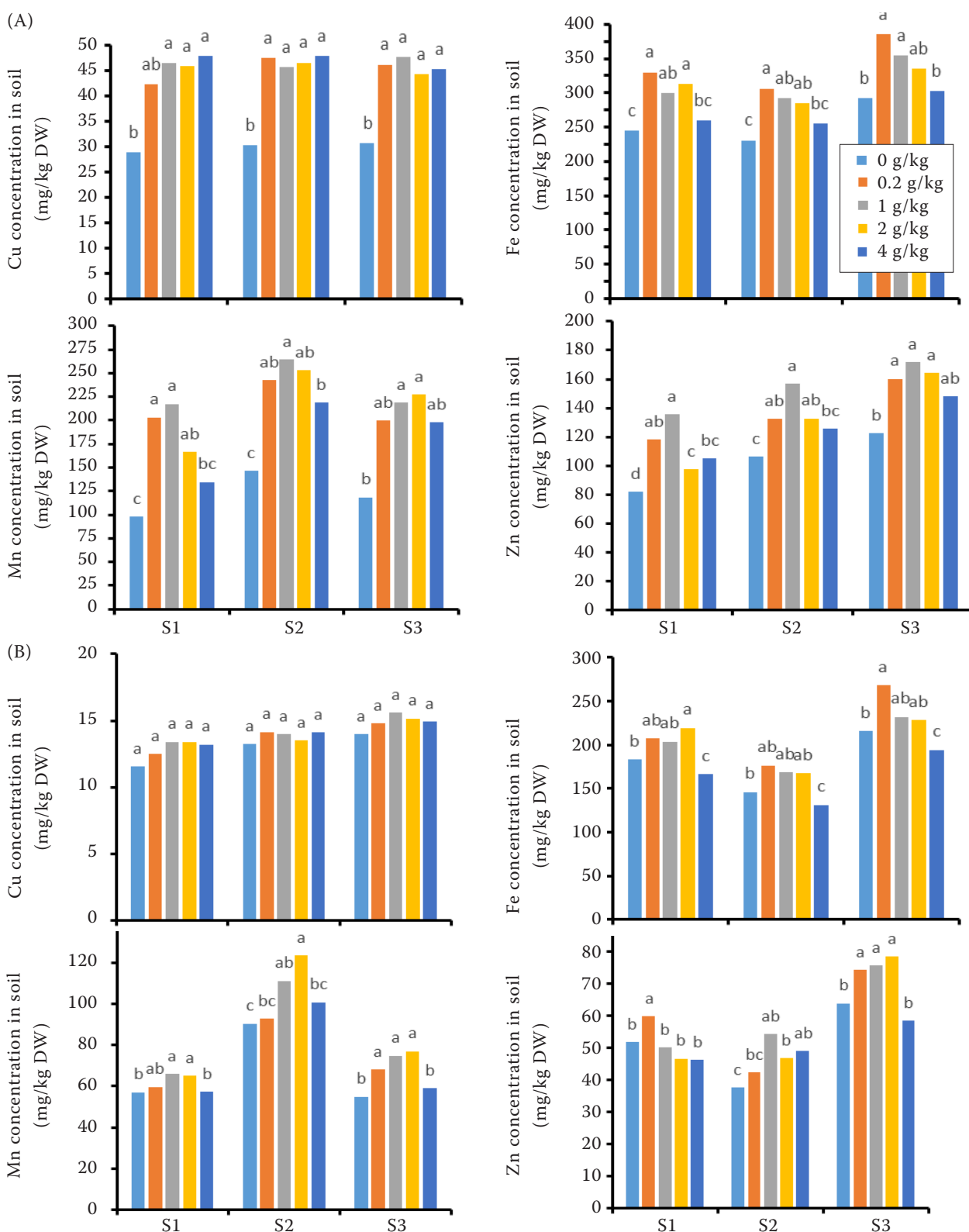
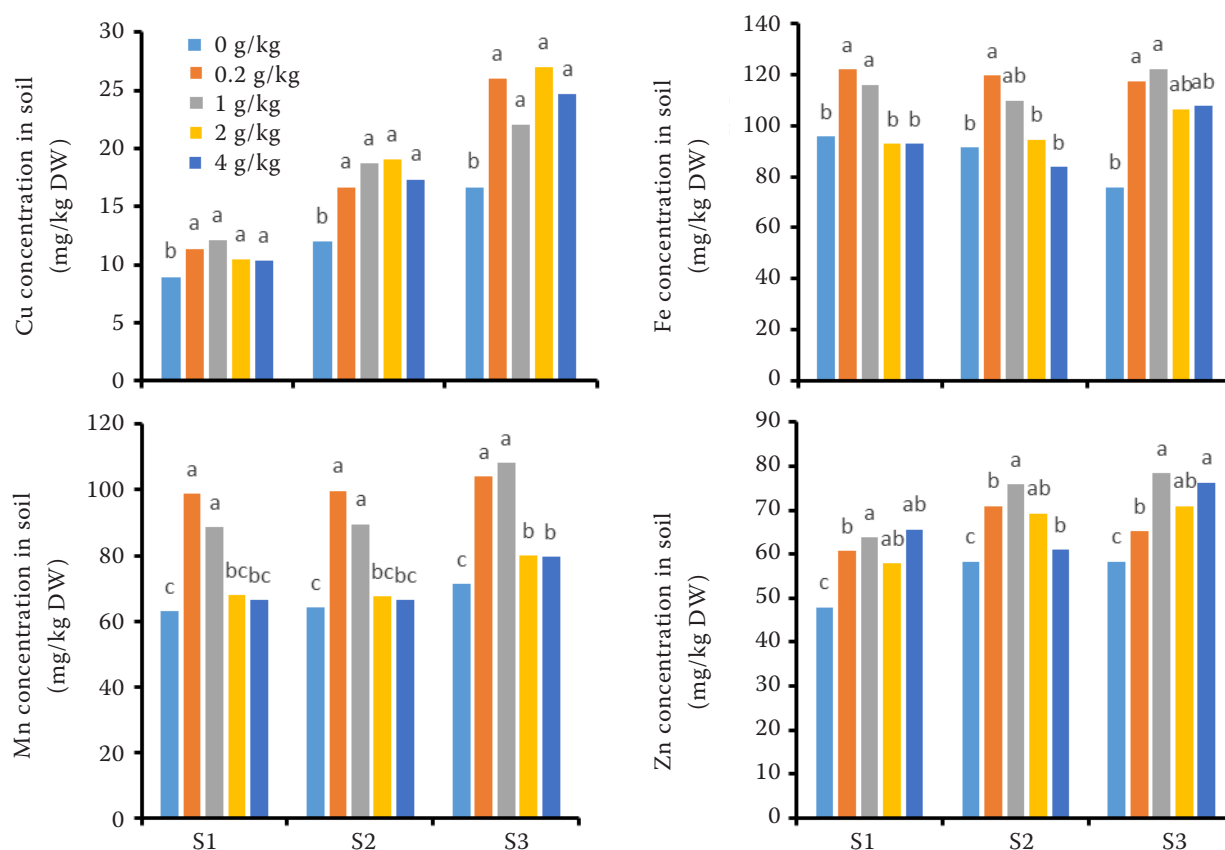


Figure 4. Mean concentrations of micronutrients (Cu, Fe, Mn, and Zn) in (A) the roots and (B) the shoots of pea plants (*Pisum sativum* L.) harvested 87 days after sowing, as affected by different application levels of mining residues (0, 0.2, 1, 2, and 4 g/kg soil dry matter) in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol. Vertical bars represent the standard error of the mean ( $\pm$  SE) calculated from three replicates. Different letters indicate statistically significant differences at  $P < 0.05$  within the same soil type. DW – dry weight



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Continued Figure 4. Mean concentrations of micronutrients (Cu, Fe, Mn, and Zn) in the grains of pea plants (*Pisum sativum* L.) harvested 87 days after sowing, following the application of different doses of mining residue amendments (0, 0.2, 1, 2, and 4 g/kg soil dry matter) in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol. Different letters indicate statistically significant differences at  $P < 0.05$  within the same soil type. DW – dry weight

defined by Joint (2011), but remained below phytotoxic thresholds (100–150 mg/kg for Zn). Fe and Mn concentrations, while relatively high, stayed well below the phytotoxic levels for Fe ( $> 1\,000$  mg/kg) and Mn ( $> 400$  mg/kg), reflecting plant tolerance at the studied doses. These levels were higher than those reported in similar studies (Fageria 2016), suggesting the potential of MT for grain biofortification.

Although this study highlights the potential of mine tailings for agronomic biofortification and soil restoration, it does not include an assessment of the economic feasibility or risks associated with large-scale application. Future research should therefore focus on assessing the cost-effectiveness of processing and transporting mine tailings, as well as identifying potential logistical constraints. In addition, comprehensive risk assessments are needed to understand the long-term implications for the environment and human health, particularly with regard to the potential accumulation of toxic elements. Considering these factors is essential to ensure that the re-use of MT is

in line with the principles of a safe, sustainable and economically viable circular economy in agriculture.

The ANOVA (Table 2) shows that soil types, applied treatments, and their interaction significantly influence trace element concentrations in grain ( $P < 0.05$ ). These complex interactions highlight the central role of soil conditions in nutrient bioavailability and transfer to plant tissues. These findings are consistent with studies by Martinka et al. (2014), which indicate that micronutrient enrichment varies according to plant organs, growing conditions and amendments used. He highlights the importance of using MT as amendments, offering a sustainable solution for their valorisation and contributing to food security.

Cu, Zn, Fe, and Mn concentrations in pea roots, shoots, and grains varied significantly with mine tailing doses and soil types. A notable increase occurred at 0.2 g/kg, especially for Cu and Zn in roots, followed by stabilisation and a decline at higher doses. Roots accumulated significantly more micronutrients than

Table 2. Effects of mining tailings amendment at different doses (0, 0.2, 1, 2, and 4 g/kg soil dry matter) on the concentrations of heavy metals (Cu, Fe, Mn, and Zn; mg/kg) in the grains of pea (*Pisum sativum* L.) harvested after 87 days

Source	df	Cu		Fe		Mn		Zn	
		F-value	P	F-value	P	F-value	P	F-value	P
Rep (block)	2	0.008	0.992	4.000	0.032	1.893	0.172	4.332	0.025
Main (soil type)	2	83.535	0.000	0.684	0.514	141.913	0.000	10.612	0.000
Main × rep	4	1.547	0.220	8.334	0.000	9.819	0.000	5.165	0.004
Sub (treatment)	4	7.816	0.000	8.265	0.000	15.989	0.000	8.903	0.000
Main × sub	8	1.973	0.095	1.080	0.410	1.198	0.341	1.438	0.232

Data are presented as mean values from three replicates per treatment. *F*-values represent results from a two-way ANOVA with degrees of freedom (*df*) = 2 × 4. Means were compared using the least significant difference (*LSD*) test, and differences are considered statistically significant at *P* < 0.05

shoots and grains. ANOVA confirmed significant effects of soil and treatment (*P* < 0.05), highlighting the potential of MT for targeted biofortification.

#### Micronutrient bioaccumulation factors in grain.

Figure 5 presents the results of micronutrient biocon-

centration (Zn, Mn, Fe, and Cu) in pea grains according to the MT dose applied per soil type (S1, S2 and S3). The data reveal significant variation depending on the elements studied and the experimental conditions. The bioaccumulation factor (BF) is below 1

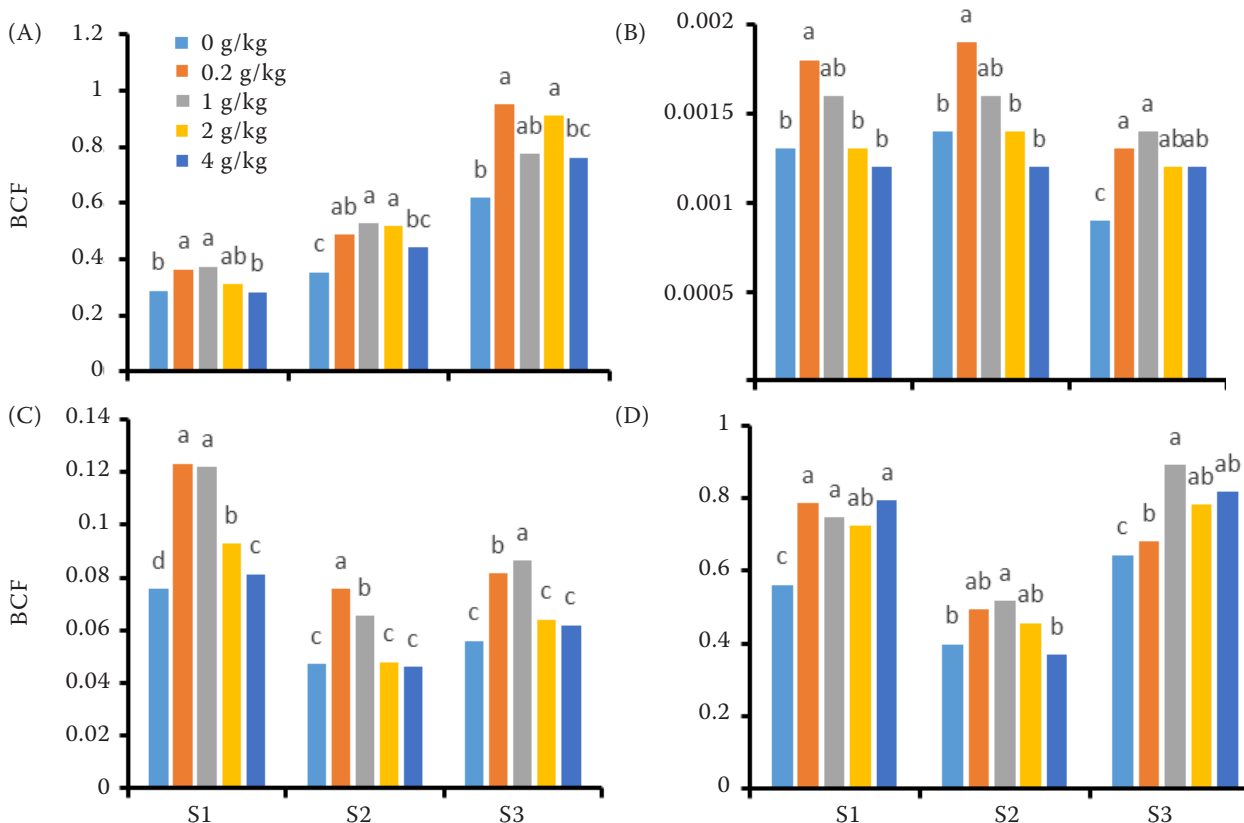


Figure 5. Average bioconcentration factor (BCF) of micronutrients (A) Cu; (B) Fe; (C) Mn, and (D) Zn from soil to grain in pea (*Pisum sativum* L.) cultivated in three soil types: S1 – Vertisol; S2 – Luvisol; S3 – Arenosol – amended with different doses of mining tailings (0, 0.2, 1, 2, and 4 g/kg soil dry matter). Measurements were taken 87 days after sowing. The bioconcentration factor was calculated as the ratio of micronutrient concentration in the grain to that in the corresponding soil. Different letters indicate statistically significant differences at *P* < 0.05 within the same soil type

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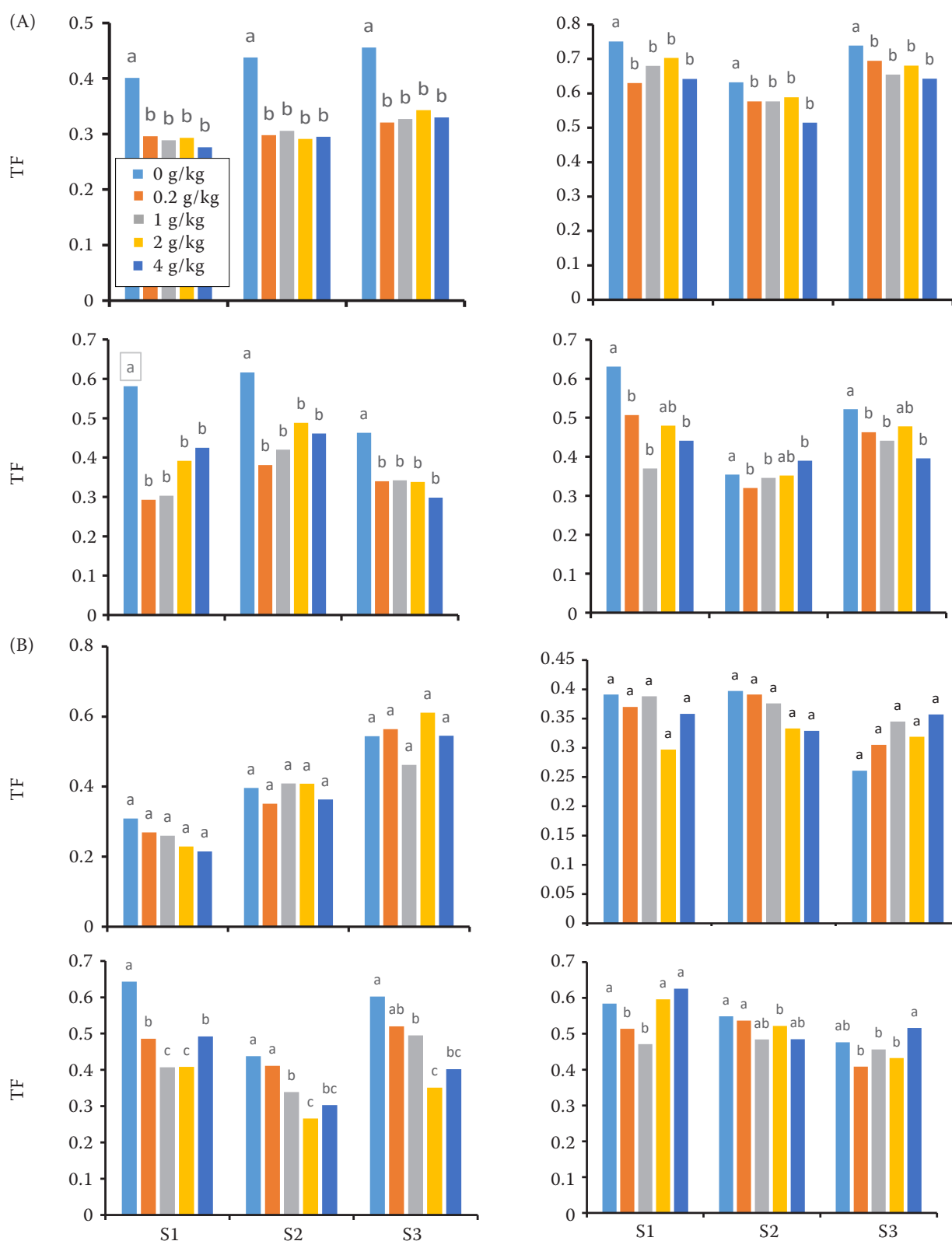


Figure 6. Average translocation factor (TF) of micronutrients (Cu, Fe, Mn, and Zn) from (A) root to shoot and (B) root to grain in pea (*Pisum sativum* L.) cultivated in three soil types – S1 – Vertisol; S2 – Luvisol; S3 – Arenosol – amended with different doses of mining tailings (0, 0.2, 1, 2, and 4 g/kg soil dry matter). Plants were harvested 87 days after sowing. The translocation factor was calculated as the ratio of micronutrient concentration in the shoot to that in the root

for most micronutrients (Figure 5). In general, Cu had the highest average total BF, followed by Zn, Mn and Fe. The highest BF were detected in pea grains between 0.2 and 2 g/kg, although the lowest values were recorded in control soils.

These results highlight the capacity of pea grains to accumulate essential micronutrients beneficial for both human and animal nutrition (Demirezen and Temizgül 2014).

The differences between soils are due to their physicochemical properties. Soil texture, OC content and pH have a significant effect on micronutrient availability. S3 presents generally higher bioconcentration factors, especially for Cu and Zn, indicating better availability or absorption in this soil type. At higher doses (2 and 4 g/kg), a decreasing trend is observed, particularly marked in S3. These variations could result from competition with other nutrient ions in the soil, as in the case of antagonism between Zn and Fe, or toxicity at high doses, as has been reported by Yang et al. (2022).

Bioaccumulation factors (BFs) for Cu, Zn, Mn, and Fe in pea grains varied significantly with MT dose and soil type, with Cu showing the highest overall BF. The highest BFs occurred at moderate doses (0.2–2 g/kg), especially in Arenosol (S3), while higher doses ( $\geq 2$  g/kg) caused a decline. These differences reflect soil properties and nutrient interactions, highlighting optimal biofortification potential at moderate MT levels.

**Translocation factors.** Figure 6 illustrates the changes in TF for different pea tissues, micronutrient and MT application doses. For all micronutrients, TF were inferior to 1.0. The FT values for Cu and Fe were lower in grains compared to stems. The sequence of FTs from roots to grains was as follows: Cu > Mn > Zn, while the order from roots to stems was Mn > Zn > Cu > Fe. The highest FT values for Cu, Fe and Mn were observed in pea tissue treated at 0.2 g/kg and in the untreated soil, whereas the lowest were found with treatment at 4 g/kg. However, the highest FT values for Zn were found in pea tissue treated with 4 g/kg, with the lowest FT values obtained in untreated soil. Root-shoot translocation factors show a general decrease with increasing tailings doses, which could be attributed to preferential accumulation in roots due to toxicity.

Micronutrient translocation in pea is influenced by both biological tolerance mechanisms and environmental factors. According to Cui et al. (2004), plants tend to restrict the upward movement of

potentially toxic elements by retaining them in the roots, thereby minimising damage to aerial tissues. In addition, variation in soil physicochemical properties influences element mobility (Yang et al. 2022). A competitive interaction between a certain type of nutrient, such as Zn and Fe, restricts their transfer to grains at high concentrations (Yang et al. 2022). The results show that micronutrient translocation strongly depends on tailings doses and soil properties. While low doses promote transfer to grains, high doses reduce this translocation, due to toxic effects or saturation of transport pathways.

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