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Evaluation of slag fertilizer potential in *Capsicum annuum* L. cultivation and production

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Abstract: The search for new approaches for sustainable and economic agricultural fertilization is becoming of great interest worldwide. The potential use of steel slag as a soil improver has been evaluated in this study to derive various benefits for agricultural cropping systems. Scanning electron microscopy (SEM), and inductively coupled plasma atomic emission spectroscopy (ICP-AES) confirmed their richness in minerals (Ca, Fe, Si, Mg, Mn, S, and P) beneficial for plant growth. In this regard, the objective of this study was to evaluate the effect of steel slag on the growth of *Capsicum annuum* L. plants. Three concentrations of slag (5, 20, and 30 g kg^{-1}) were applied. Chlorophylls, carotenoids, mineral elements, and proteins content were then evaluated. The application of slag at 20 g kg^{-1} generated significant results on the growth parameters, namely, length of shoot (40.16 cm), root (22.16 cm), number of leaves (14 per plant), internodes (15 per plant), flowers and dry weights. Thus, this treatment allows the plants to reach fructification and form fruits of good nutritional quality as compared to the negative and positive (NPK fertilizer) control treatments. The application of the studied steel slag specifically at the concentration of 20 g kg^{-1} induced an increase in photosynthetic pigments (35.32 mg g^{-1} FW of total chlorophyll and 7.26 mg g^{-1} FW of carotenoids), essential elements (N, P, K and Ca) and a high level of proteins compared to the controls. The slag showed an improvement on *C. annuum* development and could be proposed as potential plant fertilizer to enhance crop productivity. Overall, the study confirms the importance of adopting appropriate sustainable practices when spreading slag on agricultural soils and monitoring its potential impact on the environment, particularly the risk of long-term metal release.

Keywords: Steel slag, Growth, Capsicum annuum L, Photosynthetic pigments, Protein, Nutritive elements.

Introduction

Since the green revolution in the global agro-ecosystem, the application of chemical fertilizers in agricultural production is particularly important contributing to sustained economic growth and food production (Adeniyan and Ojeniyi, 2005; Akanni, 2011). However, these chemical fertilizers are often applied in excessive quantities, which can lead to different problems such as altering the physical and chemical soil properties, including erosion and increased soil porosity, and consequently surface water pollution, and soil acidification and compaction (Pagliai et al., 2004; Yan et al., 2018). In addition, it may lead to modifications of the biological soil properties caused by the deterioration of soil organisms playing key roles in soil fertility (Pahalvi et al., 2021), ultimately altering the quality of vegetables and fruits, and increasing susceptibility to diseases and pests (Pahalvi et al., 2021). These undesirable effects of fertilizers, together with their high production and environmental costs make them an impractical strategy. For this reason, alternative research has evaluated other products with fertilization potential and multiple benefits to increase the nutritional value of plants and respect the environment. In traditional agriculture, organic wastes are widely known to have beneficial effects. Manure is among the most widely used, and non-fermented materials such as fishmeal and bone meal added as additional mineral components. Nevertheless, these materials are expensive and unstable, and may be a source of soil and water contamination if not properly degraded. Hence the importance of the search for new alternative materials (Westerman and Bicudo, 2005).

The steel industry produces a large volume of waste, especially steel slag, which is a by-product of the iron and steel making process. Typically, it contains Lime (CaO), Silica (SiO_2), Ferric Oxide (Fe_2O_3), Magnesia (MgO), Manganese (MnO) and Phosphorus (P_2O_5) (Kimio, 2015). Currently, large-scale recycling of slag is now a fundamental solution to the environmental problems caused by theirs dump, which has attracted the attention of many scientists (Das, et al., 2020a; Islam et al., 2022). Slag can be used as a sintering material to replace commercial lime, applied for carbon dioxide capture and flue gas desulphurization, and used for fabrication of other products such as building materials, ceramics production, colorful pavers and tiles, cement and concrete (Branca and Colla, 2012; Gencel et al., 2021; Yi et al., 2012). Therefore, its application in the treatment of industrial wastewater has been the subject of several studies dealing with the adsorption capacity of mercury, calcium carbonate, nitrogen, phosphorus, and organic impurities (Oh et al., 2012).

The physical and chemical properties of slag, as well as its mineralogical richness in calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe) and phosphorus (P) (Chand et al., 2015; Meddich et al., 2023), suggest their use in agriculture as an alternative to chemical fertilizers (Das et al., 2020a; Islam et al., 2022; Meddich et al., 2023; Radić et al., 2022; Wang and Cai, 2006). Although slag may contain traces of heavy metals, these are generally below the toxicity thresholds established by the United States (Das et al., 2020a; Guo et al., 2018; Gwon et al., 2018), encouraging its beneficial fertilization potential in agricultural practices. Neverthless, the possible long-term release of heavy metals into the soil is the main problem posed by the use of slag in agriculture. Thus, to avoid any risk linked to their potential toxicity, it is necessary to evaluate the recommended levels and adopt appropriate ecological and sustainable methodologies to maximize the multiple benefits associated with their use in agriculture.

The present study was designed to effectively reuse slag, not only to reduce pollution causing landfill sites, but also to harness its benefits for sustainable agricultural crops, as well as to develop a new strategy of recycling and destocking of slag to contribute to the "zero waste" objective. For that, *Capsicum annuum* L. was supplied with steelmaking as fertilizer. Growth, physiological and biochemical parameters were evaluated. *Capsicum annuum* is one of the main vegetables grown in Morocco, especially in the Tadla region, which accounts for more than 80% of the national production and has excellent nutritional values (Ahmad et al., 2022; Zaki et al., 2013a; Zaki et al., 2013b).

Material and methods

Steel Slag analysis

Steel slag provided by Concamine Company located in Berrchid city, Morocco, was used as amendment. This waste was ground into a fine powder using a Pulverisette 1-Classic line Jaw crusher, and the obtained crushed material was then fed to a Pulverisette 9 Vibrating Cup mill to obtain a finer material. A manual sieving was then conducted, and the particles with a size below 2 mm were kept for further use.

The steel slag was examined by scanning electron microscopy (SEM) to characterize its configuration, angles, surface texture and to reveal information about the crystallographic structure. In order to assess the slag mineralogical composition, the macronutrient and micronutrient levels (P, K, Ca, Mg, Fe, Si, Mn, S, and Zn) were determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES-PerkinElmer-Version 5.5).

Experimental Design

Seedling preparation

The study was conducted using *Capsicum annuum* (Solanaceae) plants cultivated in a growth chamber (Light/dark cycle of 13h/11 h, light intensity of 200 µmol. m^{-2}. s^{-1}, temperature of 26 ± 2 °C and Relative Humidity (RH) of 70-80%) in the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco, from February to May 2022 (Counting from sowing to transplanting and harvesting). Seeds were surface sterilized with a 12 % sodium hypochlorite solution for 15 minutes, rinsed thoroughly with sterile distilled water, and then placed on the surface of sterile Whatman paper in Petri dishes for germination in the dark and at 25°C. After 7 days, the germinated seeds were selected to be sown in peat-filled trays (at the same culture conditions listed above) until the first leaves appearence.

Culture conditions

Capsicum annuum seedlings were then transplanted into 1.5 L plastic pots containing 1 kg of sand per pot in a homogeneous mixture with different concentrations (5, 20, and 30 $g.kg^{-1}$) of steel slag powder. The experiment was set up in a completely randomized design with 10 replicates per treatment (two plants per pot). A group of plants grown with the recommended dose of NPK chemical fertilizer (15% of nitrogen, 15% of phosphorus and 15% of potassium) applied two weeks after transplantation, served as a positive control, while another group of plants without any fertilizers served as a negative control. During the experiment, each pot was irrigated with Hoagland's nutrient solution (Hoagland et al., 1950) once a week (Isah et al., 2014; Sarwar et al., 2018). Plants from different treatments were grown under the same water regime (70% of field capacity). The watering scheme was one irrigation per 48 hours. The experiment lasted until the fruiting stage, and then plants were harvested for the determination of morphological and biochemical parameters.

Growth parameters

After harvest, the root system was separated from the shoot. The following biometrical data were assessed: shoot and root length (cm), number of leaves, flowers, and internodes per plant. Then the shoot, root, and fruit were dried separately at 75 °C for 48 h to record their dry weights (mg/plant).

Physiological analysis

Pigment determination

Pigment measurements were carried out according to the method described by Lichtenthaler (1987) and Shabala *et al.* (1998). Briefly, 50 mg of leaves were suspended in 95.5% acetone and incubated at 4 °C for 48 h in the dark. Thereafter, centrifugation for 5 min at 5000 rpm was performed. The optical density (OD) of the supernatant was read at different absorption wavelengths (470, 644, and 662 nm). Chlorophylls and carotenoids contents were determined in ten independent aliquots and expressed as mg. g^{-1} of fresh weight (mg. g^{-1} FW) using the following formula:

Chlorophyll a=(9.784 × OD at 662 nm)-(0.99 × OD at 644 nm)
Chlorophyll b=(21.42 × OD at 644 nm)-(4.65 × OD at 662 nm)
Total Chlorophyll=Chl a+Chl b
Carotenoids=((1000 ×OD at 470 nm)-(1.9×Chl a)-(63.14×Chl b))/214

Nitrogen determination

Nitrogen content in plant parts was determined using the Kjeldahl method as previously described by Lahrouni *et al*. (2012). Briefly, 0.5 g of dry plant material was digested with 10 mL of sulfuric acid (98%) and 1 g of a mixture of Kjeldahl catalysts until boiling at 420 °C for 90 min. After complete mineralization, the samples were recovered in 100 mL of distilled water; 40 mL of these solutions were transferred to Kjeldahl flasks containing a few drops of 1 % phenolphthalein and NaOH (8N). The resulting distillate was collected over boric acid and titrated with 0.05N sulfuric acid.

Minerals determination

Different samples of the harvested plants (aerial, root and fruit parts) corresponding to different treatments were separately crushed in a mill to determine their mineral constituents (P, K, Ca, S, Fe, Si, Zn, Cu, As, Pb, Y, Th, Zr, Se) using XRF fluorescence X spectrometer (Handheld X-ray Fluorescence- DELTA PREMIUM-Olympus NDT, INC. Waltham, MA USA).

Protein determination

The protein content was quantified according to the Bradford method (Bradford, 1976). Shortly, 250 mg of different plant parts (leaves, roots, and fruits) were homogenized with 2 mL of phosphate buffer (0.05M, pH 6), and the homogenates were centrifuged at 12,500 × *g* for 20 min. The extraction was carried out in ten replicates. Thereafter, 2 mL of Bradford's reagent and 100 µL of distilled water were added to 100 µL of each supernatant. After shaking and incubation at room temperature, the OD was measured at 595 nm and the protein contents were expressed as mg. g^{-1} FW (in comparison to a calibration curve of bovine serum albumin (BSA; lyophilized powder with purity ≥96% and purchased from Sigma-USA) as standard solution.

Statistical analysis

Data were analyzed statistically for standard deviation using SPSS 21.0 statistical software (IBM Corp (2012). IBM SPSS Statistics (version 21.0). IBM Corporation). All measurements were performed in ten replicates. The values were presented as mean ± standard error (SE). Means of different treatments were assessed using One-way ANOVA and compared using Tukey's test. Significant differences were considered at $p < 0.05$ and were indicated by different letters. The Kolmogorov-Smirnov test was used beforehand to evaluate the normality.

Results

Steel Slag analysis

The morphological characteristics, dimensions, and structure of the slag sample used in this study were revealed by the scanning electron microscope (SEM) shown in Figure 1. Examination unveiled a surface exhibiting a rough texture with the presence of particles resembling the form of sub-rounded to angular grains with an average diameter of a few microns (Figure 1). ICP-AES analysis determined the contents of the mineral components present in the slag used in this study. The tested steel slag primarily contained a relatively higher amount of Ca, Fe, Si, Mg, Mn, S and P (Table 1).

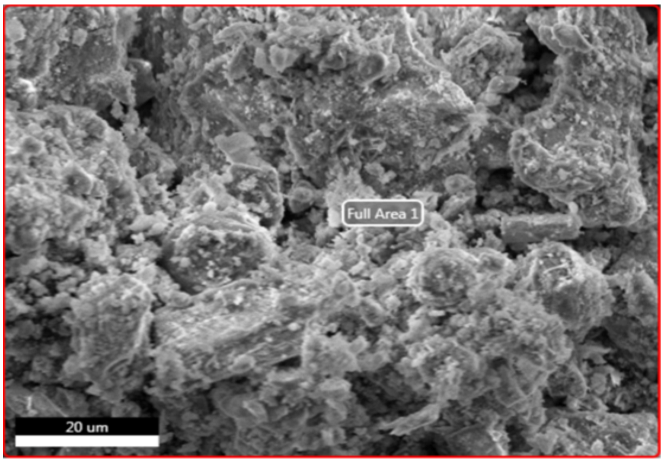

Figure 1 - Scanning electron microscope (SEM) of slag sample.

Table 1 - Steel slag mineral analysis by ICP-AES

Mineral element	Content (%)
P	0.41 ± 0.01
K	0.15 ± 0.09
Ca	46.27 ± 4.34
Mg	6.68 ± 1.30
Fe	27.17 ± 3.49
Si	11.89 ± 0.89
Mn	4.81 ± 0.72
S	0.96 ± 0.25
Zn	0.05 ±0.002

Note. P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; Fe: Iron Si: Silicon; Mn: Manganese; S: Sulfur; Zn: Zinc

Effect of steel slag on C. annuum L. growth

Several biological parameters were evaluated on different plants parts. The results of the length of the shoot and root parts, number of leaves, flowers, internode per plant, also the dry weight of different parts of the plant are summarized in Tables 2 and 3.

The addition of steel slag into *C. annuum* culture displayed a remarkable effect on the different growth parameters. The length of shoots and roots of *C. annuum* showed a variable behavior depending on the slag concentration. Indeed, the treatment with 20 g. kg^{-1} of slag significantly increased the shoot and root lengths (40.16 and 22.16 cm, respectively) in comparison to the other tested concentrations. In fact, at 5 and 30 g. kg^{-1} of slag concentrations, the root elongation was equivalent to the negative control (Table 2). The effect of slag on the number of leaves, flowering, and the number of internodes differed depending on the applied treatment. For the treatment with 20 g. kg^{-1} of slag all the evaluated parameters showed the same pattern as the positive control (NPK Fertilizer). For 30 g. kg^{-1} slag concentration, the plants showed a higher number of leaves and internodes, and the same number of flowers as compared with the negative control (plants without fertilization). The lowest 5 g. kg^{-1} slag concentration induced the lowest flower number, but a higher leaves number than the negative control (Table 2).

Table 2 - Effect of steel slag fertilizer on growth traits of C. annuum. Means of 10 replicates ± SE (standard error). Different letters indicate significant differences based on a comparison of means with ANOVA test ($p < 0.05$) and Tukey test.

	Shoot length (cm)	Root length (cm)	Number of leaves per plant	Number of flowers per plant	Number of internodes per plant
5 g. kg^{-1}	29.33 ± 1.21^{e}	18.5 ± 0.83^{c}	8.5 ±1.04^{b}	0.5 ± 0.81^{c}	9 ± 0.98^{c}
20 g. kg^{-1}	40.16 ± 1.47^{b}	22.16 ± 1.47^{b}	14.16 ± 0.98^{a}	2.33 ± 0.51^{a}	14.83 ± 0.75^{a}
30 g. kg^{-1}	36.00 ± 0.63^{c}	18.83 ± 1.47^{c}	10.83 ± 0.98^{b}	1.16 ± 0.40^{b}	11 ± 0.89^{b}
Positive control	45.50 ± 1.37^{a}	25 ± 1.41^{a}	14 ± 0.63^{a}	2.33 ± 0.51^{a}	15.5 ± 0.83^{a}
Negative control	32.00 ± 1.09^{d}	19.5 ± 0.54^{c}	5.33 ± 0.81^{c}	1 ± 0.63^{b}	7.33 ± 0.98^{c}

On the other hand, the evaluation of plant biomass showed that both shoot and root dry weights (DW) of positive control were higher than all the tested slag concentrations. At 20 g. kg^{-1} both shoots and roots had DW higher than the negative control, while 5 and 30 g. kg^{-1} treatments induced DW closer or lower than the negative control (Table 3). The obtained fruits showed DW significantly higher than the positive control for all slag treatments applied in this study. Plants treated with 20 g. kg^{-1} exhibited the highest fruit DW followed by 5 then 30 g. kg^{-1} slag concentrations. Plants of the negative control did not show fruits (Table 3).

Table 3 - Dry weight of the shoot, root, and fruit part of C. annuum. Means of 10 replicates ± SE (standard error). Different letters indicate Significant differences based on a comparison of means with ANOVA test (p < 0.05) and Tukey test.

	Shoot dry weight (mg)	Root dry weight (mg)	Fruit dry weight (mg)
5 g. kg^{-1}	786.61 ± 15.55^{c}	367.06 ± 18.05^{cd}	636.47 ± 3.39^{b}
20 g. kg^{-1}	831.61 ± 16.72^{b}	493.00 ± 19.75^{b}	895.35 ± 3.80^{a}
30 g. kg^{-1}	724.10 ± 5.51^{d}	356.22 ± 16.45^{d}	511.7 ± 3.65^{c}
Positive control	924.60 ± 5.04^{a}	622.13 ± 13.32^{a}	137.24 ± 1.00^{d}
Negative control	735.87 ± 5.53^{d}	411.25 ± 15.89^{c}	-

Effect of steel slag on C. annuum physiology

Effects of steel slag on photosynthetic pigments

In order to evaluate the effect of steel slag on the photosynthetic activity in *C. annuum* plants, the determination of pigment contents (chlorophylls and carotenoids) was performed (Figure 2). The treatments with different steel slag concentrations induced a significant variation in the photosynthetic pigments content (Chlorophyll *a*, *b,* and Carotenoids) compared to the controls. According to the obtained results, most of the plants exposed to all steel slag concentrations (5, 20, and 30 g. kg^{-1}) showed higher levels of Chlorophyll *a* (19.63, 20.35, and 20.79 mg. g^{-1} FW, respectively) compared to the negative control (15.09 mg. g^{-1} FW) and closer to those recorded from the positive control plants (22.73 mg. g^{-1} FW) (Figure 2a). Chlorophyll *b* revealed high values, especially in plants treated with 20 g. kg^{-1} of slag (14.49 mg. g^{-1} FW) compared to the negative control (10.54 mg. g^{-1} FW) (Figure 2b), and approaching the chlorophyll *b* content of the positive control (17.57 mg. g^{-1} FW). High content of total chlorophyll was recorded in plants treated with 20 and 30 g. kg^{-1} (35.32, 31.37 mg g^{-1} FW successively) compared to the untreated plants (25.63 mg g^{-1} FW). The values obtained-with 20 g. kg^{-1} were closer to plants treated with NPK fertilizer (40.31 mg g^{-1} FW) (Figure 2c). Moreover, compared to both controls, the carotenoid content was more expressive in plants treated with 20 and 30 g. kg^{-1} of slag, while the values observed in plants treated with 5 g kg^{-1} slag did not showed significant differences with the negative control, approaching those of the positive control (Figure 2d).

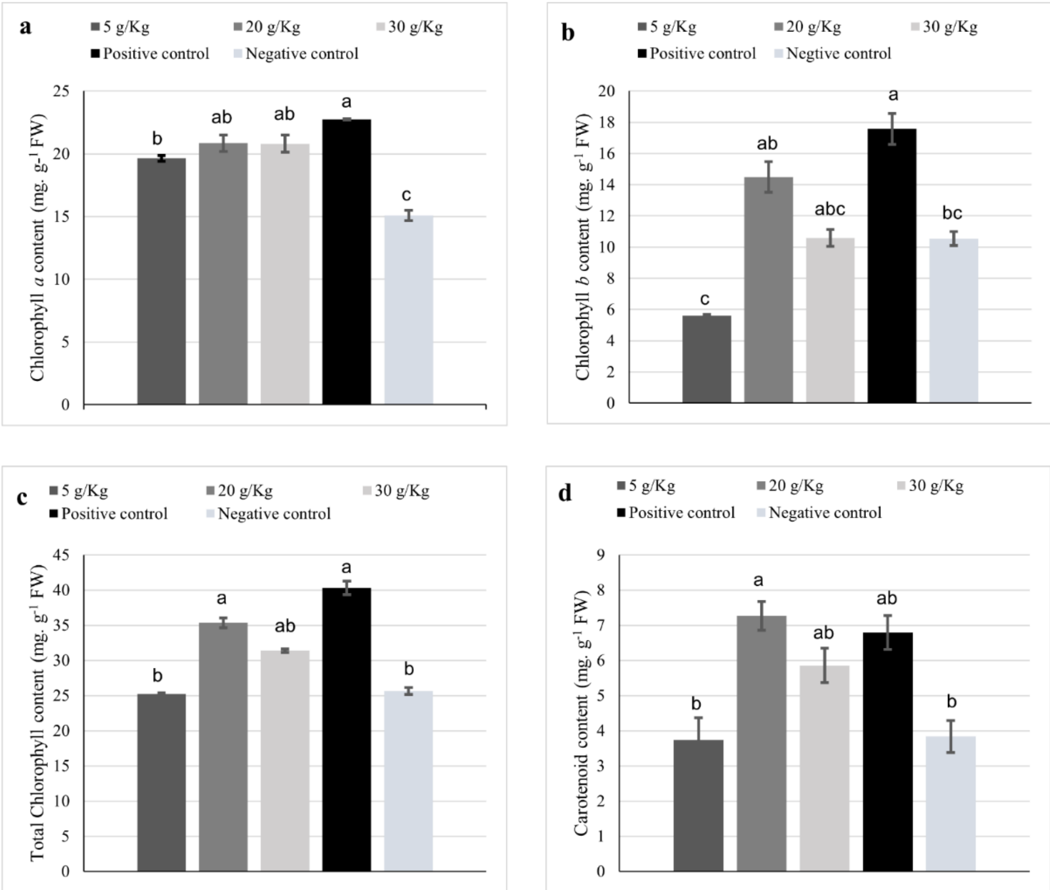

Figure 2 - Variation of chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoids (d) in C. annuum. Means of 10 replicates ± SE (standard error). Different letters indicate significant differences based on a comparison of means with ANOVA test at $p < 0.05$ and Tukey test.

Effects of steel slag on mineral content

The results of the mineral analysis of the various plant parts are listed in Table 4. Steel slag treatments induced an improvement in the levels of the main inorganic nutrients essential for the growth of *C. annuum*. In particular, the most significant improvement was observed with a dosage of 20 g. kg^{-1} steel slag (Table 4). The application of steel slag resulted in a moderate increase in nitrogen (N) levels in plant shoots and roots with the application of 20 g. kg^{-1} compared to the negative control (Table 4a and b). A greater concentration of this nutrient was also noticed in the fruits of plants treated with 20 g. kg^{-1} compared to the other tested steel slag concentrations (5 and 30 g. kg^{-1}) (Tale 4c). In addition, in the aerial part of the plants, the application of steel scraps, especially 20 g. kg^{-1}, resulted in a slight increase in the phosphorus content (P) compared to the negative control. In contrast, at 20 g. kg^{-1} the potassium (K) content was very high compared to the negative control and comparable to that of the positive control. The levels of calcium (Ca) and iron (Fe) were higher at 20 g. kg^{-1} than those observed in the negative and positive controls (plants fertilized with NPK). Similarly, the application of 5 g. kg^{-1} produced results similar of sulfur (S) content to that of positive control. However, the content of other elements (silicon (Si) and zinc (Zn) showed no significant change. (Table 4a). Particularly high concentrations of P, Ca, and Fe were detected in the roots, inversely to the potassium K, mainly when 20 g. kg^{-1} of steel slag was applied (Table 4b). The contents of S and Zn in the roots were higher in plants treated with 30 g.kg^{-1} of steel slag when compared to the negative control and close to the positive controls. The steel stag studied at all these levels (5, 20 and 30 g.kg^{-1}) promoted increases in the root Si content when compared to both two controls (Table 4b). It is worth highlighting that the plants treated with 20 g. kg^{-1} of slag notably exhibited similar or higher mineral element contents (P, K, Ca, S, and Fe) in their fruits compared to the positive control (plants with NPK fertilizer) (Table 4c). With regard to the contents of Si in fruits from plants treated with 5 and 30 g. kg^{-1} of steel slag, more similar results to the positive control were recorded, while Zn showed no significant evolution following the application of the slag (Table 4c). The nutrient contents (P, K, Ca, S, and Fe) were significantly higher with the application of 20 g. kg^{-1}.

On the other hand, the elements like copper (Cu), arsenic (As), lead (Pb), yttrium (Y), thorium (Th), zirconium (Zr), and selenium (Se) were not detected in *C. annuum* shoots, roots, and fruits.

Evaluation of the influence of slag on the total protein content

The total protein contents are summarized in Figure 3. In comparison to the negative control, the total protein content in shoots was slightly increased (0.81; 0.81, and 0.78 mg g^{-1} FW) respectively in plants supplemented with 5, 20, and 30 g. kg^{-1} slag, However, the highest value of protein was recorded in plants treated with NPK fertilizer (0.88 mg g^{-1} FW) (Figure 3a). In the roots, plants treated with 20 g. kg^{-1} showed a significant increase in protein content compared to the negative control and more similar values to positive control (Figure 3b), although the 5 and 30 g. kg^{-1} treatments showed no significant effect (Figure 3b). The evaluation of the protein content in the fruits showed a significant increase reaching 0.10 and 0.09 mg g^{-1} FW successively at 20 and 30 g. kg^{-1} treatments compared to the treatment of 5 g.kg^{-1}, whereas the fruits obtained by the treatment with fertilizer NPK presented on average the highest value of 0.14 mg g^{-1} FW compared to fruits from treated plants (Figure 3c).

Table 4 - Effects of steel slag fertilizer on the mineral content of C. annuum plant; Shoot (a), Root (b), Fruit (c). Means of 10 replicates ± SE (standard error). Different letters indicate significant differences based on a comparison of means with ANOVA test ($p < 0.05$) and Tukey test. N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; S: Sulfur; Fe: Iron; Si: Silicon; Zn: Zinc

a

Minerals	5 g kg^{-1}	20 g kg^{-1}	30 g kg^{-1}	Positive control	Negative control
N (%)	3.02 ± 2.29^{b}	3.15 ± 0.30^{b}	2.64 ± 0.48^{b}	6.24 ± 0.59^{a}	1.49 ± 0.16^{c}
P (g. kg $^{-1}$)	1.17 ± 0.05^{c}	1.34 ± 0.05^{ab}	1.28 ± 0.05^{b}	1.40 ± 0.05^{a}	1.29 ± 0.05^{b}
K (g. kg $^{-1}$)	14.70 ± 0.1^{c}	16.20 ± 0.1^{a}	15.40 ± 0.1^{b}	16.20 ± 0.1^{a}	15.40 ± 0.1^{b}
Ca (g. kg $^{-1}$)	16.90 ± 0.1^{b}	19.50 ± 0.1^{a}	19.40 ± 0.1^{a}	15.40 ± 0.1^{c}	13.50 ± 0.09^{d}
S (g. kg $^{-1}$)	0.60 ± 0.03^{a}	0.54 ± 0.02^{b}	0.54 ± 0.02^{b}	0.58 ± 0.0^{a}	0.55 ± 0.02^{b}
Fe (g. kg $^{-1}$)	0.27 ± 0.01^{c}	0.62 ± 0.02^{a}	0.29 ± 0.02^{c}	0.35 ± 0.0^{b}	0.30 ± 0.02^{c}
Si (g. kg $^{-1}$)	4.36 ± 0.12^{a}	4.26 ± 0.11^{a}	4.30 ± 0.12^{a}	4.40 ± 0.12^{a}	4.45 ± 0.12^{a}
Zn (mg. kg $^{-1}$)	36 ± 3.25^{ab}	30 ± 3.94^{b}	33 ± 3.39^{ab}	38 ± 2.83^{a}	34 ± 2.81^{ab}

b

Minerals	5 g kg^{-1}	20 g kg^{-1}	30 g kg^{-1}	Positive control	Negative control
N (%)	3.91 ± 0.11^{c}	4.96 ± 0.11^{b}	3.38 ± 0.18^{cd}	7.94 ± 0.29^{a}	2.89 ± 0.26^{d}
P (g. kg $^{-1}$)	1.31 ± 0.05^{bc}	1.44 ± 0.05^{a}	1.38 ± 0.05^{ab}	1.46 ± 0.05^{a}	1.29 ± 0.05^{c}
K (g. kg $^{-1}$)	12.80 ± 0.1^{e}	14.60 ± 0.1^{c}	13.40 ± 0.1^{d}	18.20 ± 0.1^{a}	17.50 ± 0.1^{b}
Ca (g. kg $^{-1}$)	11.20 ± 0.1^{c}	14.30 ± 0.1^{a}	12.20 ± 0.1^{b}	12.20 ± 0.3^{b}	10.50 ± 0.6^{c}
S (g. kg $^{-1}$)	0.58 ± 0.03^{b}	ND	0.62 ± 0.03^{a}	0.61 ± 0.03^{a}	0.58 ± 0.03^{b}
Fe (g. kg $^{-1}$)	1.86 ± 0.03^{e}	2.64 ± 0.04^{b}	2.22 ± 0.04^{c}	2.79 ± 0.04^{a}	2.23 ± 0.04^{c}
Si (g. kg $^{-1}$)	4.81 ± 0.13^{a}	4.76 ± 0.12^{a}	4.67 ± 0.12^{a}	4.39 ± 0.11^{b}	4.34 ± 0.12^{b}
Zn (mg. kg $^{-1}$)	30 ± 3.65^{b}	30 ± 3.20^{b}	40 ± 3.39^{a}	37 ± 3.84^{ab}	31 ± 3.20^{b}

c

Minerals	5 g kg^{-1}	20 g kg^{-1}	30 g kg^{-1}	Positive control	Negative control
N (%)	5.74 ± 0.36^{b}	6.49 ± 0.29^{b}	5.24 ± 0.26^{b}	8.58 ± 0.85^{a}	-
P (g. kg $^{-1}$)	1.06 ± 0.04^{b}	1.29 ± 0.05^{a}	1.27 ± 0.02^{a}	1.30 ± 0.05^{a}	-
K (g. kg $^{-1}$)	9.00 ± 0.1^{d}	12.10 ± 0.1^{a}	9.30 ± 0.05^{c}	11.80 ± 0.1^{b}	-
Ca (g. kg $^{-1}$)	7.50 ± 0.4^{c}	18.80 ± 0.1^{a}	8.50 ± 0.1^{b}	4.6 ± 00.1^{d}	-
S (g. kg $^{-1}$)	0.39 ± 0.02^{b}	0.55 ± 0.02^{a}	0.53 ± 0.1^{a}	0.54 ± 0.03^{a}	-
Fe (g. kg $^{-1}$)	0.22 ± 0.01^{d}	0.41 ± 0.02^{a}	0.35 ± 0.5^{b}	0.30 ± 0.02^{c}	-
Si (g. kg $^{-1}$)	4.34 ± 0.11^{a}	3.75 ± 0.10^{b}	4.58 ± 0.02^{a}	4.43 ± 0.11^{a}	-
Zn (mg. kg $^{-1}$)	27 ± 2.54^{b}	31 ± 3.74^{ab}	29 ± 2.65^{b}	35 ± 2.20^{a}	-

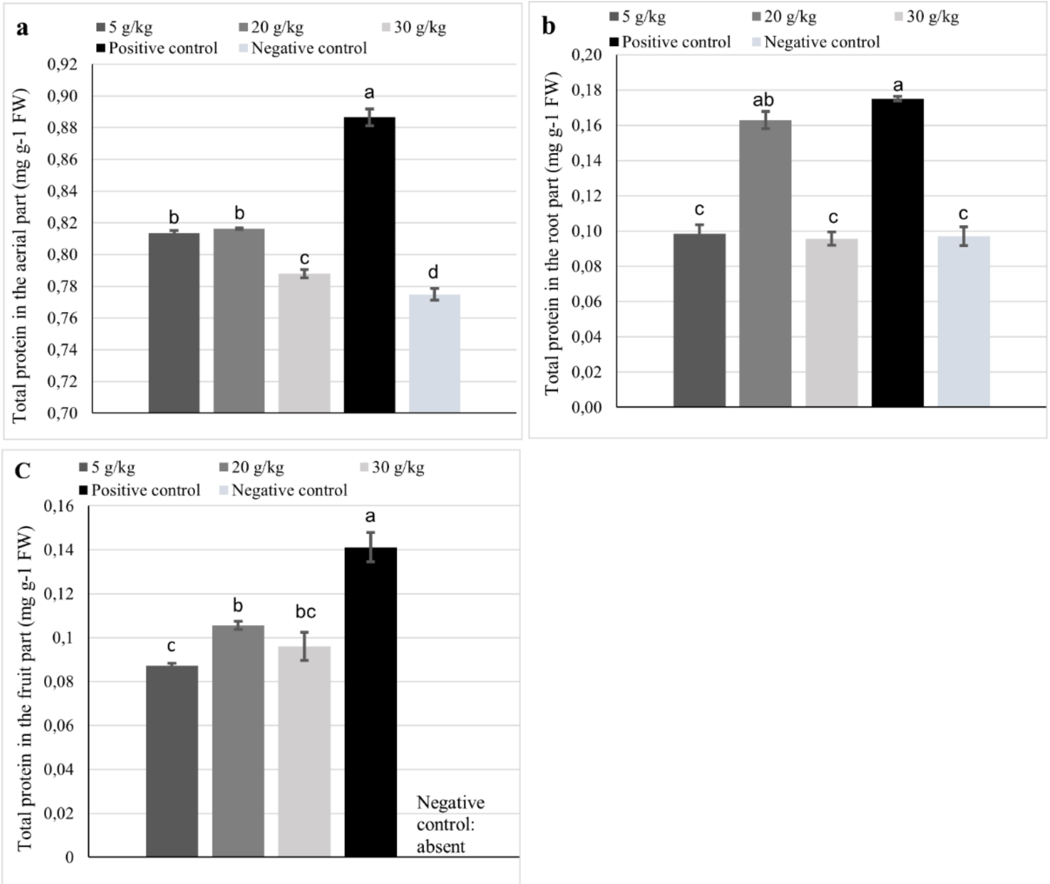

Figure 3 - Total protein content in Capsicum annuum L. a: Aerial part, b: Root part and c: fruit part. Mean of 10 replicates ± SE (standard error of the mean). Different letters indicate significant differences based on a comparison of means with ANOVA test at $p < 0.05$ and Tukey test.

Discussion

Steel slag was proposed and tested in this study as a fertilizer for agricultural applications. This choice is based on their nutritional benefits for plants, their ease of application, and their cost-effectiveness, as well as their economic advantages for agriculture (Branca and Colla, 2012). At the same time, their reuse and recycling reduce their impact on the environment and contributes to the "zero waste" objective of the Agenda 2030 and waste management legislation (Branca et al., 2020). In this context, the effect of different concentrations (5, 20, and 30 g. kg^{-1}) of slag on vegetative growth, and physiological and biochemical parameters of *Capsicum annum* was evaluated in the present study.

The results of the mineralogical characterization obtained in this study confirmed that the slag used could be considered a valuable resource for plant improvement due to its high content of mineral elements (Ca, Fe, Si, Mg, Mn, S, and P). The mineralogical richness of the slag used in this study correlates with several other research that have characterized the mineralogical composition and abandonment of slag in different countries (Japan, Brazil, Sweden, France, and China) and reported that slag accumulates essential mineral elements on its surface, with proportions ranging from 52% for Calcium, 15% Silicon, 15% Magnesium, 35% Iron, 4% Manganese and 3% Phosphorus (Guo et al., 2018; Yi et al., 2012a; Yildirim and Prezzi, 2011). To this interest, the chemical composition of this steel slag is close to that of mineral fertilizers, necessary for plant growth, and it might be possible to recycle it for smart-agriculture practices (Council, 2019). The mineral profile of slag offers fertilizing effects (Ahmad et al., 2022; Ali and Shahram, 2007; Branca and Colla, 2012; Meddich et al., 2023) involved in several plant physiological processes such as, Ca that can strengthen the root system and promote the absorption of K; Fe that can help the conversion of hydrogen sulfide present in the soil into iron sulfide, making it harmless and reducing the problem of Fe chlorosis (Wang and Cai, 2006); Mn, an important element for chlorophyll, which can promote photosynthesis (Kimio, 2015); Si that increases plant resistance to biotic and abiotic stresses such as Fusariosis, water stress, and salinity (Bouzoubaâ et al., 2009; Yavaş and Ünay, 2017). The latter, when released during steelmaking processes is more soluble and easily consumed by plants (Pereira et al., 2004), and therefore encouraged the manufacture of slag-based silicate fertilizers, commercialized in Japan since 2001. The increased availability of mineral elements in the soil and their uptake by plants result from the effect of improving the pH of acidic soils by amending them with slag (Ali and Shahram, 2007; Gao et al., 2020). Consequently, due to its composition, this by-product has already been recycled for the production of silica, phosphorus, and trace element fertilizers in Germany, the United States, France, and Japan (Chand et al., 2015; Yi et al., 2012).

The richness of the slag applied in this study in terms of mineral elements beneficial to crops has enabled us to obtain encouraging results on the improvement of *C. annuum*, contributing to the valorization of Moroccan slag for agricultural applications by implementing sustainable and ecological approaches to integrate it into the agricultural sector. In the present study, a significant improvement in the biological and physiological condition of *C. annuum* plants was observed with the addition of slag, which is consistent with several previous studies showing that slag increases crop yield (Das et al., 2020b; Islam et al., 2022; Meddich et al., 2023; Radić et al., 2022; Wang and Cai, 2006). The results showed that the application of 20 g of slag per Kg of sand presented a significant improvement in the morphological parameters of *C. annuum* such as shoot and root length, number of leaves, flowers, and internodes, and plant dry weight, and a remarkable effectiveness in improving fruit nutritional quality. This is in agreement with several studies, such as the work of Wang and Cai (2006), which reported that the addition of 20 g of slag to 1 kg of sandy loam soil resulted in a significant improvement in maize dry matter as well as Fe, P and K uptake by the plants. Negim et al. (2010) reported also that the application of 1% and 2% of slag treatments in 1 kg of soil gave greater increases in shoot yield of *Phaseolus vulgaris* L. In addition, the increase of the pigment's contents (Chlorophyll *a, b*, total, and carotenoids) in *C. annuum* amended with 20 g. kg^{-1} of slag, revealed the positive impact of the tested input on the photosynthetic activity. Our results are in accordance with those carried out by Das et al. (2020b), showing that slag application into the soil induced high photosynthetic rate. The increase in chlorophyll and carotenoids was related to magnesium, manganese and iron contained in the applied slag and are the main elements of chlorophylls promoting photosynthesis (Kimio, 2015; Radić et al., 2022). The increase in protein content of *C. annuum* plants after slag application (mainly at 20 g. kg^{-1}), could be related to the increase in nitrogen previously reported to be related to organic matter production and amino acid biosynthesis (Anas et al., 2020). This is confirmed by Errouh et al. (2023) and Meddich et al. (2023) evaluating the same slag studied in our work originating from the same company, which reported an increase in protein content in *Triticum durum* Desf regardless of the presence or absence of salt and water stress. Similarly, Radić et al. (2022) reported a significant increase in protein content in *P. vulgaris* leaves grown under slag treatment.

These positive effects of steel slag treatments on the biomass of *C. annuum* plants could be due to rapid assimilations of minerals by the plants which promotes significant growth (Radić et al., 2022). The improvement of nitrogen contents in plants treated with slag at 20 g. kg^{-1} is consistent with the results of previous reports (Wang et al., 2018; Das et al., 2020b), which showed that there is a strong impact of steel slag fertilization on availability of soil N, well-expressed in terms of crop yield and plant biomass of rice. These results could be mainly explained by the effect of silicate ions released through the slags that contribute to NO_3 and NH_4 availability for plants (Wang et al., 2018). The silicate ions contained in slag are also linked to the improvement of P status in soils and thus to the increase of their availability for plants (Lee et al., 2004). In order to strengthen the resistance of steel slag to low temperatures, the manufacture of slag goes through a process of dephosphorization to release phosphorus (Branca and Colla, 2012). The recovery of the latter provides raw material for the production of cheaper phosphate fertilizers, which will produce substantial agricultural and economic benefits (Gao et al., 2020). An increase in Ca levels in *C. annuum* plants treated with 20 g kg^{-1} of slag, might allow the plants to absorb more K which is an essential element for plant growth, having a positive impact on plant response to abiotic stress such as drought (Yang et al., 2019; Radić et al., 2022). Overall, mineral accumulation in *C. annuum* plants supplied with 20 g. kg^{-1} of slag, is in line with previous studies in maize plants, indicating accumulation of Fe, Mn, Mg, K, and P after slag application (Radić et al., 2022).

In the present work, the application of 20 g. kg^{-1} of slag showed a remarkable improvement in the different growth parameters of the vegetative biomass. On the other hand, the application of 30 g. kg^{-1} of slag showed a decrease in growth parameters compared to the positive control (NPK fertilizer). This could be due to the accumulation of excessive amounts of mineral elements at the concentration of 30 g kg^{-1} of slag which causes toxicity in *C. annuum* plants. This is in agreement with several other studies. Cai et al. (2022) and Islam et al. (2022) reported that a moderate rate of added slag is beneficial for height, biomass, root growth of plants, and an increase in mineral uptake (Ca, Mg, Fe) as well as an improvement in the process of chlorophyll synthesis, while the high rate of slag has a contradictory effect which induces a reduction in plant growth.

Our study shows the possibility of alternating the application of NPK fertilizer with steel slag as an inexpensive and cost-effective fertilizer. The set of results obtained in this study are in close conformity with the study conducted by Radlc et al. (2013), which shows that steel slag is comparable and can replace the NPK fertilizer in maize, and by Radić et al. (2022) revealing the effectiveness of slag on soil nutritional improvement with a similar performance compared to synthetic fertilizers.

On the other hand, studies showing the ability of slag to reduce the impact of abiotic stresses such as drought and salinity noted that the same slag used in this study when applied at 10 g. kg^{-1} of soil significantly improved several growth parameters of *T. durum* (height of spikes, fresh weight of shoots, fresh weight of the spikes, dry weight of shoots and roots) (Negim et al., 2010; Errouh et al., 2023; Meddich et al., 2023). These studies showed that this dose of slag allowed the plant protection against salt stress by an increase in stomatal conductance, and fluorescence of chlorophyll with an accumulation of soluble sugars and proteins. In addition, the improvement in the pH of agricultural soils amended by slag is accompanied by the remediation of heavy metals in the soil and a reduction in their adsorption by plants (Ghisman et al., 2022), such as the detoxification of Cd, Cu, Zn, and As, in rice tissues amended with slag (Gwon et al., 2018; Ning et al., 2016).

However, slag may contain metallic contaminants (V, Cr, As, Pb, Cd, Co, Ba, Hg, Se, Sb, Ag, Zn, and Ni), the concentration of which depends specifically on the metallurgical processes adopted, (Gwon et al., 2018; Piatak et al., 2019; Ilyushechkin et al., 2012). Nevertheless several studies have shown that the heavy metals contained in slag are, in most cases, below the toxicity potential set by the United States (Das et al., 2020a; Guo et al., 2018). In this study, the levels toxic heavy metals in *C. annuum* plants were below the detection limit, presumably, because these elements are present at very low levels or even absent. The obtained results in this study correlate with several other researches, which showed that there is no significant increase in heavy metal content in plants when the steel slag is applied as fertilizer, which means that heavy metals tend to bind to the slag matrix and are therefore not available to plants, or are released in non-toxic forms to plants (Hiltunen and Hiltunen, 2004; Gutierrez et al., 2010; Negim et al., 2010; Branca and Colla, 2012; Schlögl et al., 2023; Pietrini et al., 2017; Wang et al., 2015). However, the long-term implications of this type of amendment require further research into the behavior of heavy metals release and their immobilization in the soil to achieve a more efficient and sustainable economic use of slag in agriculture.

Overall, our results suggest that the enriching composition of the slag used in this study enhanced the development of *C. annuum* plants treated with 20 g. kg^{-1}. This is shown by the reported improvement of photosynthesis activity, mineral elements, and protein content at the plant level, as well as the production of fruits of good nutritional quality. In summary, compared to chemical fertilizers, the slag used in this research seems to be suitable for *C. annuum* production, both in terms of costs and agronomic efficiency. This study encourages the careful and controlled application of slag to soils to improve their fertility and provide beneficial nutrients for sustainable and environmentally-friendly agricultural production, but also to reduce the slag landfill sites.

Conclusion

The addition of slag-based fertilizers at moderate amendment levels (20 g. kg^{-1}) promotes the growth of *Capsicum annuum* biomass by increasing the content of photosynthetic pigments, mineral nutrients (N, P, K, Ca, Fe), and proteins, to levels similar to fertilization with chemical fertilizers (NPK). The steel slag used has a promising potential in crop improvement and could be applied on a large scale to improve crop production. Overall, the results of this study showed, on the one hand, the potential fertilizing effect of steel slag for crop improvement and, on the other hand, the discovery of an ecological strategy adopted for the recycling of these by-products, in terms of environmental preservation. This research opens up innovative prospects for sustainable agriculture, aims to make slag suitable for harmonious integration into farming practices, and involves careful assessment of environmental implications to avoid any risk of long-term heavy metal contamination.

Author contributions

O.O.; Conducting experiments; O.O., F.K. and B.O. Methodology, analysis, and article writing. Y.E.; Methodology and analysis. H.K and H.C.; Slag samples provision from The Concamine company; A.M.; Revision and correction of the manuscript; F.K. and B.O.; Planning the work and Article revisions and corrections. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interests.

Data Availability

The data generated and/or analysed during the current study are contained in this manuscript and presented in tables and figures.

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