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Recycling Electric Arc Furnace Slag into Fertilizer: Effects of "Waste Product" on Growth and Physiology of the Common Bean (*Phaseolus vulgaris* L.)

Sandra Radić [1], Dubravka Sandev [1], Krešimir Maldini [2], Valerija Vujčić Bok [1], Hrvoje Lepeduš [3,4] and Ana-Marija Domijan [5,*]

[1] Faculty of Science, Department of Biology, University of Zagreb, HR-10000 Zagreb, Croatia
[2] Croatian Waters, Main Water Management Laboratory, HR-10000 Zagreb, Croatia
[3] Faculty of Humanities and Social Sciences, Josip Juraj Strossmayer University of Osijek, HR-31000 Osijek, Croatia
[4] Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, HR-31000 Osijek, Croatia
[5] Faculty of Pharmacy and Biochemistry, University of Zagreb, HR-10000 Zagreb, Croatia
* Correspondence: adomijan@pharma.hr; Tel.: +385-1481-8288

Abstract: The aim of this study was to investigate if electric arc furnace (EAF) slag generated during steel production could have an application as a soil enhancer in agriculture. For that purpose, a greenhouse experiment was conducted on common beans (*Phaseolus vulgaris*) cultivated in soil enriched with EAF slag (at 1% and 2% level), synthetic fertilizer (NPK), combined EAF slag and synthetic fertilizer, or in control (untreated) soil. The beans were exposed to test soils until maturity (for 8 weeks). Following that period, physico-chemical properties of the soils, as well as nutrient status, growth, photosynthetic and oxidative stress parameters of bean plants were determined. EAF slag improved the mineral status of the soil and significantly increased Fe, Mg, N, P and K in different bean plant organs. EAF slag and/or NPK increased plant height. EAF slag, especially at lower levels, positively affected dry weight of leaf and seed. Soil supplementation with a lower level of EAF slag, as well as with a combination of EAF slag and NPK, led to significant improvement in gas exchange parameters (net photosynthetic rate, intercellular CO_2 concentration and stomatal conductance) and nitrate reductase activity, indicating a positive influence on bean plants. Potential phytotoxicity of EAF slag was not detected, as evidenced by the oxidative stress parameters. Thus, EAF slag applied at a low level shows promising potential as an efficient soil enhancer, and as a valuable source of nutrients essential to plants, with an equal or even better performance compared to synthetic fertilizer.

Keywords: oxidative stress; photosynthesis; phytotoxicity; soil enhancer; steel production waste

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1. Introduction

During the manufacture of 1.65 billion tons of iron and steel, more than 567 million tons of steel slag is generated globally [1]. Due to growing awareness of environmental protection and economic benefits, steel slag is more and more considered as a potential resource rather than a waste, by which the steel industry contributes to a circular economy [2]. Beside its predominant application in the construction industry (cement production, road base material, etc.), steel slag can also be effectively utilized in agriculture. Today, the production of steel in electric arc furnaces (EAF) holds a relatively high share in total amount of worldwide manufacture of steel, and thus EAF slag has become readily available as a valuable resource [3,4]. The composition of EAF slag varies depending largely on the iron source material. The slag used in this research originated from EAF scrap-based smelting, during which oxidized elements react with added lime to form slag. Typically, EAF slag is a mixture of iron, calcium, magnesium, aluminum, silicon and manganese

oxides associated with the complex compounds of calcium silicates, aluminosilicates and aluminoferrites. The level of a particular oxide depends on the quality of the produced steel, i.e., the quality and composition of the steel scrap used as a source material, the type and proportion of the batch of non-metallic additives, and the type and amount of ferroalloys used, as well as other technological parameters [5]. Consequently, the content of CaO, FeO, Fe_2O_3, SiO_2, MgO, Al_2O_3, MnO, Na_2O, K_2O and P_2O_5 in EAF slag is in the 18–60%, 2.5–41%, 1–31%, 6.5–35%, 1–31%, 1–13.5%, 0.5–12%, 0.06–0.5%, 0.02–0.2% and 0.01–1.8% range, respectively [5]. EAF slag also contains trace amounts of Cu, Zn, Mo, Cr, Pb, Cd, V, As and Hg. However, analysis showed that heavy metal leaching from the soils supplemented with EAF slag is irrelevant in terms of environmental pollution, which classifies EAF slag as non-hazardous waste with possible uses as a soil enhancer or construction material [5,6]. Several studies reported that concentrations of potentially toxic metals leached from fresh and aged steel slag (including EAF slag) to soil are lower than or close to the detection limit [6–9].

The agronomic value of steel slag as a fertilizer or as a liming material has been evaluated previously [10–12]. The studies demonstrated a positive impact of different steel slag on crop yield, however, the impact depended on plant species, type of soil or climate [12–18]. Long-term experiments (over 40 years) carried out on different types of soils, and with different plants utilizing iron and steel slags, revealed that the yields of experimental crops improved significantly without phytotoxic effects [12].

In the mentioned studies, other types of steel slags were utilized as fertilizer or liming material, while there are scant data on the EAF slag either as a lime or as a soil fertilizer. Therefore, the aim of this study was to explore the potential use of EAF slag as a soil enhancer by investigating its effect on soil and plant nutrient status, as well as plant yield and physiological processes (growth, gas exchange, chlorophyll *a* fluorescence kinetics and nitrate reductase (NR) activity) in order to verify its potential use in agronomy. In the study, the common bean (*Phaseolus vulgaris*, bush-type variety) was used as the model plant, since the bean is an important grain legume consumed worldwide as a source of micronutrients and proteins, and has a short generation time. Preliminary studies in which EAF slag was used as a fertilizer at levels up to 8% revealed that optimal growth of bean plants was achieved at relatively low levels of EAF slag. Thus, in the study, EAF slag was used at the levels of 1 and 2%, and its performance was compared to that of NPK fertilizer or a combination of EAF slag and NPK fertilizer. As EAF slag contains trace amounts of heavy metals, the potential phytotoxic effect of EAF slag was determined by measuring indicators of oxidative stress (lipid peroxidation, protein carbonylation and antioxidative enzyme activities) in the model plant.

2. Materials and Methods

2.1. Soil Preparation and Experimental Design

The EAF slag sample for this study originated from the Steel Factory, Sisak (Croatia). Previous analysis by Rastovčan-Mioč et al. [6] and Sofilić et al. [5] revealed that EAF slag from the Sisak Steel Factory has a high level of Fe (Fe_2O_3–29.7%) and Ca (CaO–33.2%, $CaCO_3$–8.3%) compounds, followed by SiO_2 (10.8%), Mg (MgO–13.1%, $MgCO_3$–2.5%) compounds, Al_2O_3 (1.7%), MnO (6.2%), K_2O (0.06%), Na_2O (0.02%) and P_2O_5 (0.03%), and the pH-value of the EAF slag is 11.97. The levels of potentially toxic metals in the EAF slag are below the maximum levels allowed by the Croatian Regulation on the protection of agricultural soil from pollution by harmful substances (Zn 82 mg/kg, Cu 59 mg/kg, Cr 10 mg/kg, Pb 25 mg/kg, As 1.2 mg/kg, Mo 1.1 mg/kg, Cd 0.75 mg/kg, Hg < 0.1 mg/kg). Toxicity characterization of the EAF slag eluate (Zn < 1 mg/kg, Cu < 1 mg/kg, Cr < 0.5 mg/kg, Pb < 0.05 mg/kg, As < 0.01 mg/kg, Mo < 0.05 mg/kg, Cd < 0.1 mg/kg, Hg < 0.01 mg/kg) showed that the EAF slag satisfies the prescribed requirements for permanent waste disposal [5].

For this study, the EAF slag was ground, sieved and a fraction of 0–2 mm was used in the experiments. The soil used in the experiments was obtained from the Botanical Garden

of the Faculty of Science, University of Zagreb. Prior to the experiments, the soil was air-dried, ground, sieved to 2 < mm, and stored in polyethylene bags at room temperature. A commercially obtained synthetic mineral fertilizer, Fertilizer (Unichem d.o.o., Vrhnika, Slovenia; NPK 6-3-6 + micronutrients), was used in accordance with the manufacturer's recommendations (100 kg/ha).

A pot experiment with the bush-type variety of bean (*Phaseolus vulgaris* L., Borlotto Lingua di Fuoco, Franchi seeds of Italy) was conducted in a greenhouse in spring 2018. Preliminary experiments showed that this bean variety has the best growth performance in silica sand enriched with either a combination of 2% EAF slag and NPK fertilizer, or only with 2% of EAF slag. Based on those observations, five types of test soils were prepared: control (C, 0% of EAF slag or NPK fertilizer), soil F (F, soil enriched with NPK fertilizer), soil FS2 (FS2, soil enriched with a combination of 2% EAF slag and NPK fertilizer), soil S1, (S1, soil enriched with EAF slag at 1% level), soil S2 (S2, soil enriched with EAF slag at 2% level). Three bean seeds were planted into plastic pots containing 2 kg of either test soil. To achieve 1 and 2% of EAF slag for test soils S1 and S2, 20 g (125 kg/ha) and 40 g (250 kg/ha) of EAF slag was added per pot. Pots were arranged in a randomized block design and irrigated with distilled water (deH_2O) when needed. A week after germination, plants were thinned to one per pot. For each treatment, nine pots were used. The plants were cultivated until maturity in a greenhouse under long day conditions with an average photosynthetically active radiation (PAR) of 1000 µmol/m^2/s, an average daily temperature of 25 ± 2 °C, and night temperature of 16 ± 2 °C, and 60–80% humidity. The experiment lasted eight weeks. All analyses were conducted at the end of the experiment. First, growth parameters (number of leaves and husks, plant height), gas exchange and chlorophyll *a* fluorescence were assessed. Plant material (leaves, husks and seeds) was then collected, washed with deH_2O and prepared according to the relevant protocol or stored at −20 °C until analysis. At that time, soil samples were also collected and kept in polyethylene bags at −20 °C until analysis.

2.2. Physico-Chemical Analysis of Soil Samples and Plant Material

The pH and electrical conductivity of soil samples was determined in a suspension of soil samples prepared in a ratio of 1:5 (soil:deH_2O) [19].

In the soil samples, the content of the elements Mg, K, Ca, Mn, Fe, Si, Cd, Cr, Pb, Zn, Co, Cu and V was determined by using inductively coupled plasma-mass spectrometry (ICP-MS, Elan 9000, Perkin Elmer, Waltham, MA, USA) according to HRN EN ISO 17294-2:2008 norm, and solutions of 20 µg/L Ge, Rh, In and Re were used as internal standards. The calculated relative standard deviation was within 15% for each measured element. The calibration curve of each element, as well as of the internal standards, was made by using Perkin Elmer multi-element standard solutions. The accuracy of the ICP-MS method was verified by measuring the certified reference material (TM-RAIN04, Council Canada) at the beginning and at the end of each batch of samples with a good agreement, within 15%, between the obtained and certified values for each element.

The listed elements were also quantified in the plant material using ICP-MS. The harvested plant material (leaves, husks and seeds) after rinsing with deH_2O was oven dried at 70 °C for 48 h to obtain constant dry weight. Afterwards, approximately 1 g of dry plant material was digested with aqua regia (2.5 mL of Suprapur nitric acid and 7.5 mL of hydrochloric acid) and heated in a Multiwave 3000 (Anton Paar, Graz, Austria) for 30 min at 1000 W, and then quantitatively transferred to a volumetric flask and diluted to 50 mL with deH_2O. The element quantification by ICP-MS was performed as described for the soil samples.

The contents of plant-available N (paN) and P (paP) in the soil samples were determined according to Allen et al. [20] and Temminghoff and Houba [21], respectively. The contents of total N and P in the soil samples and plant material were determined according to ISO/TR 11,905 [22] and ISO 6878 [23].

2.3. Analysis of Physiological and Oxidative Stress Parameters in Bean Plants

2.3.1. Growth Parameters

The plant growth and yield were estimated at the end of the experiment (after eight weeks) by assessing the height of the plants (cm) and the total number of leaves and husks, as well as the dry weight of leaves, husks and seeds.

2.3.2. Gas Exchange Measurements

Net photosynthetic rate (PS; μmol $CO_2/m^2/s$), transpiration rate (T; μmol $H_2O/m^2/s$), stomatal conductance (gs; μmol $H_2O/m^2/s$), intercellular CO_2 concentration (C*i*; μmol $CO_2/m^2/s^1$) and incident irradiance at leaf surface (PAR; μmol$/m^2/s$) were measured at the end of the experiment using an LCpro portable photosynthesis system (ADC, Bio Scientific Ltd., Hoddesdon, UK) connected to a broadleaf chamber. Measurements were performed on the main leaflet of the topmost, fully expanded trifoliate leaf, on nine plants per treatment. Average values per plant were calculated from three recorded measurements per leaf. Temperature and incident irradiance in the leaf chamber were adjusted each time (records under artificial conditions set by analysis system). Measurements were carried out from 10:00 to 12:00 h a.m., at 380 ± 5 μmol/mol CO_2 concentration.

2.3.3. Chlorophyll a Fluorescence and Level of Photosynthetic Pigments

The minimal minimum (F_0) and maximum (F_m) chlorophyll *a* fluorescence yields were measured using a Handy-PEA fluorimeter (Hansatech Instruments Ltd., Norfolk, UK). The leaves were adapted to the dark for 30 min, and then the F_0 was recorded at 50 μs, followed by a pulse of saturating red light (32,000 μmol$/m^2/s$, peak at 650 nm) during 1 s in order to induce F_m. The maximum quantum yield of photosystem II (F_v/F_m) was calculated according to Strasser et al. [24]. The plant material extracts were prepared in acetone 80% (*v*/*v*) and centrifuged. In the supernatants the content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total carotenoids (Car) was determined at 470, 661.6 and 644.8 nm and calculated in mg/g fresh weight according to Lichtenthaler [25].

2.3.4. In Vivo Nitrate Reductase (NR) Assay

The first and rate-limiting step of nitrate assimilation in plants is catalyzed by molybdenum-containing NAD(P)H:nitrate reductase (NR; EC 1.7.1.1-3). The in vivo assay of NR activity in the leaves was carried out according to Randall [26] with slight modifications. Fresh leaves were cut into slices and placed in ice-cold 100 mM potassium phosphate buffer (pH 7.4) containing 30 mM KNO_3 and 5% propanol (*v*/*v*). The tubes were incubated in a water bath at 30 °C for 30 min under dark conditions. At the end of the incubation period, tubes were transferred to a boiling water bath for 5 min to stop the enzyme activity and then allowed to cool to room temperature. Next, 1% sulphanilamide in 3 M HCl was added to each tube, mixed for 15 s, followed by an addition of 0.02% N-(1-Naphthyl)ethylenediamine, after which the tubes were mixed again. The pink color due to diazotization was allowed to develop for 20 min. The activity was measured at 540 nm. NR activity was expressed as μmol $NO_2/h/g$ fresh weight.

2.3.5. Oxidative Stress Parameters

Lipid peroxidation, protein carbonylation, and soluble proteins, as well as the activities of superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), and peroxidase (POX; EC 1.11.1.7) were determined in the plant leaves as reported previously [27].

An indicator of lipid peroxidation, malondialdehyde (MDA), was evaluated using thiobarbituric acid (TBA). The MDA-TBA complex was measured at 532 and 600 nm and its concentration was estimated based on an absorption coefficient of 155/mM/cm. Protein carbonyls were assessed in reaction with 2,4-dinitrophenol hydrazine (DNPH) at 370 nm using an absorption coefficient of 22/mM/cm. For antioxidant enzyme activities, the bean leaves were ground in liquid nitrogen and then homogenized in 50 mM potassium

phosphate buffer (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (Sigma-Aldrich, St. Louis, MO, USA) and polyvinylpolypyrrolidone (Sigma-Aldrich, St. Louis, MO, USA). The homogenates were centrifuged (3K18 Centrifuge, Sigma, Osterode am Harz, Germany) at 25,000× *g* for 30 min at 4 °C, and the supernatants were used for enzyme activity and protein content assays. Total soluble protein contents in the supernatants were estimated using bovine albumin serum (Sigma-Aldrich, St. Louis, MO, USA) as standard. SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Sigma-Aldrich, St. Louis, MO, USA) at 560 nm. One unit of SOD was taken as the activity of the enzyme extract producing 50% inhibition of nitroblue tetrazolium reduction. Activity of APX was determined by monitoring the oxidation of ascorbate at 290 nm and using its absorption coefficient (ε = 2.8/mM/cm). One enzyme unit was defined as µmol oxidized ascorbate/g fresh weight/min. For the determination of POX, the formation of tetraguaiacol was followed at 470 nm and was quantified by taking its absorption coefficient (ε = 26.6/mM/cm) into account. One enzyme unit was defined as µmol produced tetraguaiacol/g fresh weight/min. Specific enzyme activity for all enzymes was expressed as units/mg protein. All absorbance measurements were performed on a spectrophotometer Specord 40 (Analytik Jena, Jena, Germany).

2.3.6. Statistical Analysis

Analysis was carried out using STATISTICA 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). Normality of the data was tested by Shapiro–Wilk's W test. Homogeneity of variance for each dependent variable was evaluated by Levene's test. The possible difference among the treatments was assessed by one-way ANOVA, followed by Duncan's post hoc comparison test. In all of the statistical tests the significance level was set to ($p < 0.05$). Between different parameters Pearson's correlation coefficients (r) were calculated.

3. Results and Discussion

3.1. Effect of EAF Slag on Nutrient Status in Soil and Bean Plant Organs

The soil enriched with the EAF slag had higher pH-value and conductivity in comparison to the control soil (Table 1). The effect of the EAF slag on the soil pH-value and conductivity can be ascribed to its high content of oxides, primarily free calcium oxide. These compounds can be dissolved in the aqueous solution of the soil, resulting in the release of hydroxide ions and thus higher alkalinity [16,18,28,29].

The contents of the assessed nutrients elevated in the soils supplemented with the EAF slag (Table 1). Several studies have reported that concentrations and availability of Mg, Ca and K cations rise with time in soil enriched with steel slag [15,18,28,29]. Khan et al. [29] concluded that increase of soil pH-value due to the addition of EAF slag improves the ratio of Ca:Mg ions and thus ensures the greater availability of Ca ions that are generally unavailable in acid soils. In this study, elevated concentrations of Mg, Ca and K in the soils enriched with the EAF slag, and even in the soil enriched with combined 2% EAF slag and NPK, were detected. Interestingly, the contents of Mg and K in the soil increased with an increase of the level of EAF slag, while the content of Ca was the highest in the soil enriched with 1% EAF slag.

The significant uptake of Mg and K by plant organs coincided with increased concentrations of those nutrients in the slag enriched soil (Table 2). The EAF slag supplementation markedly increased leaf and seed Mg content, showing even better performance than NPK fertilizer (Table 2). The lower level of the EAF slag significantly increased the leaf and husk K content. Enhanced nutrient uptake by plants in the EAF slag enriched soils might be connected with the beneficial effects of slag on soil bacterial communities, which in turn increased nutrient availability [17,18].

Table 1. Physico-chemical analysis of: control soil (C–without EAF slag or NPK fertilizer), soil enriched with synthetic NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2), and soil enriched with EAF slag (S1–at 1%, S2–at 2% level).

Parameter	C	F	FS2	S1	S2
pH	7.71	7.42	8.47	8.53	8.77
EC (µS/cm)	398	494	595	473	464
N (g/kg)	7.01	7.92	8.05	7.84	7.57
paN (g/kg)	3.04	3.69	3.96	3.46	3.37
P (g/kg)	6.22	6.97	7.13	7.36	6.87
paP (g/kg)	3.86	4.01	4.17	4.30	4.00
Mg (g/kg)	6.86	6.61	8.32	8.70	9.25
K (g/kg)	5.56	5.61	5.85	6.07	6.11
Ca (g/kg)	36.5	41.5	46.3	56.9	50.8
Fe (g/kg)	4.77	5.25	5.34	5.49	5.20
Si (g/kg)	1.20	1.42	1.56	3.38	1.78
Mn (mg/kg)	128	139	153	201	158
Cd (mg/kg)	0.534	0.551	0.562	0.568	0.584
Cr (mg/kg)	25.0	23.5	34.6	31.3	35.5
Pb (mg/kg)	52.5	51.6	49.9	54.0	59.6
Zn (mg/kg)	106	117	123	107	120
Co (mg/kg)	8.07	8.56	9.19	8.55	9.81
Cu (mg/kg)	46.0	42.8	44.4	46.8	50.8
V (mg/kg)	173	178	182	194	220

Numbers present an average of the two replicates. paN—plant available N, paP—plant available P.

Table 2. Macro- and microelements in leaf, husk and seed of bean plants cultivated in: control soil (C—without EAF slag or NPK fertilizer), soil enriched with synthetic NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2), and soil enriched with EAF slag (S1—at 1%, S2—at 2% level).

Parameter	C	F	FS2	S1	S2
			Leaf		
Mn (mg/kg)	35.00 a	38.57 a	35.03 a	36.78 a	35.78 a
Fe (mg/kg)	78.43 c	155.88 a	105.05 b	115.72 b	109.72 b
Mg (g/kg)	3.49 c	4.27 b	4.38 b	4.47 b	5.04 a
N (g/kg)	21.28 c	29.67 a	29.52 a	25.68 b	23.21 c
P (g/kg)	1.96 c	2.00 c	2.05 c	2.93 a	2.61 b
K (g/kg)	15.57 c	20.63 a	17.99 b	18.19 b	15.92 c
			Husk		
Mn (mg/kg)	35.01 d	40.26 cd	44.25 bc	56.69 a	51.65 ab
Fe (mg/kg)	37.59 c	44.86 bc	57.02 a	48.96 ab	53.14 a
Mg (g/kg)	3.55 a	3.79 a	3.85 a	3.69 a	3.53 a
N (g/kg)	17.47 b	20.80 a	21.20 a	20.98 a	20.96 a
P (g/kg)	4.31 a	4.21 a	4.72 a	4.59 a	4.33 a
K (g/kg)	30.71 b	35.40 ab	33.21 ab	37.58 a	35.22 ab
			Seed		
Mn (mg/kg)	30.03 a	22.92 b	29.10 a	31.32 a	31.36 a
Fe (mg/kg)	82.17 a	84.14 a	88.47 a	90.22 a	88.93 a
Mg (g/kg)	1.90 b	1.64 c	1.94 b	2.19 a	2.11 a
N (g/kg)	30.14 c	32.94 bc	38.63 a	37.17 a	35.93 ab
P (g/kg)	5.41 b	5.49 b	5.77 ab	6.48 a	5.53 b
K (g/kg)	18.51 a	18.29 a	19.19 a	20.40 a	19.77 a

Numbers present an average of the four replicates. Standard deviations were less than 10%. Different letters within each row indicate significant difference at $p < 0.05$.

The major determinants in N availability in a soil solution are the content of the soil organic matter and conditions for mineralization (moisture, temperature, aeration), while the soil pH has minimal effect on the turnover of N in alkaline soils [30]. Higher plant-available N was recorded in the soils supplemented either by the EAF slag or NPK fertilizer compared to the control soil (Table 1). This might be related to the higher degree of nitrification in soils at a pH greater than 6.0, since the optimal pH for nitrification is established to be around 8.5 [31]. Interestingly, the increase of leaf and seed N content was higher in plants cultivated in soils supplemented by a lower level of the EAF slag than in those supplemented by a higher level of it. A moderate-to-strong positive relationship was found between N and K content in different plant organs (r from 0.68 to 0.84), suggesting the possible effect of K on the N level (Table 3). This could be the case in our study, since it was demonstrated that a higher amount of K in the slag, when released into the soil, can increase the availability of NH_4^+ ions in the process of cation exchange [32,33]. In this study, a significant induction of leaf NR activity was observed in all amended soils (Table 4), which corroborates the assumption of a higher nitrification rate in tested soils. In addition, NR activity and content of leaf N were closely related, as evidenced by a very strong positive correlation (r = 0.89; Table 3). In a study by Das et al. [17], it was determined that steel slag amendment improved the N uptake of rice straw. The authors suggested that increased N uptake could be connected with the stimulation of nitrogen fixation in soil amended with steel slag.

Both total and plant available P was higher in the soils supplemented either by the EAF slag or NPK fertilizer than in the control soil (Table 1). Available P in alkaline soils is generally low; it may, however, increase depending on the amount of soluble organic matter, as P tends to be less stable at higher pH [34]. Kristen and Erstad [35] found that the increase of P in soil could be related to the presence of Si in slag. Specifically, Si can be replaced with plant-available soil P. In this study, the increase of plant-available P coincided with the increase of Si in the EAF slag amended soils, especially in those amended with 1% EAF slag (Table 1). Accordingly, the leaf and seed P content significantly increased in plants cultivated in slag supplemented soils (Table 2).

Fe solubility is low in calcareous soils. At pH between 7 and 8.5, this microelement is mainly present in the soil solution in the form of $Fe(OH)_2^+$, $Fe(OH)_3$ and $Fe(OH)_4^-$ [30]. Despite high soil pH in the slag amended soils, the Fe content was higher in those soils compared to the control soil (Table 1). This result was expected, as the EAF slag contains a considerable amount of Fe oxides. The Fe uptake in leaves and husks of plants increased either by the EAF slag or NPK fertilizer supplementation compared to the control (Table 2). These results could be explained by the better solubility of $Fe(OH)_4^-$ at pH higher than 8.5 [36]. It is interesting that a positive moderate-to-strong correlation was established among Fe, Mg, N, P and K contents in different plant organs, indicating their mutual dependency (Table 3). Similarly, Torkashvand [16] found that application of steel converter slag at 0.5 and 1% levels significantly increased extractable Fe in calcareous soil, and uptake of Fe, Mn, K, and P in maize shoots, after a two-month growth period. Steel slag also stimulated accumulation of Fe and other nutrients (N, P, K, Mn and Zn) in maize [14] and radish plants, especially when organic matter was added to calcareous soil [37]. Additionally, Islam et al. [18] detected increased uptake of Fe, Mg and Ca in turnips and spinach cultivated in soils amended with steel slag.

Several investigations found a negative correlation between Fe and Mn uptake in shoots, suggesting an antagonistic effect between these micronutrients [38,39], however, that was not the case in our study. Here, the EAF slag application significantly increased Mn content in the soil and in the husk of bean plants, while the leaf and seed Mn content was similar to that in the control soil.

Table 3. Pearson's coefficient of correlation between parameters with corresponding r values.

	Chl a + b	Height	No. Husk	DW Leaf	DW Seed	DW Husk	PS	NR	Fe Leaf	Mg Leaf	N Leaf	P Leaf	K Leaf	Fe Husk	Mg Husk	N Husk	P Husk	K Husk	Fe Seed	Mg Seed	N Seed	P Seed	K Seed	MDA
Height	0.16																							
No. husk	−0.19	0.42																						
DW leaf	0.14	−0.17	0.28																					
DW seed	0.07	−0.04	−0.17	0.09																				
DW husk	−0.22	−0.17	−0.07	−0.06	−0.16																			
PS	−0.06	0.18	0.31	0.00	0.14	−0.05																		
NR	0.00	0.36	0.10	−0.42	−0.24	0.31	0.64																	
Fe leaf	−0.28	0.35	0.28	−0.32	−0.04	0.54	0.03	0.42																
Mg leaf	0.11	0.36	0.49	0.33	0.39	0.01	0.35	0.20	0.34															
N leaf	−0.16	0.37	0.33	−0.46	−0.40	0.41	0.56	0.89	0.59	0.22														
P leaf	0.21	0.27	0.61	0.65	0.23	−0.12	0.08	−0.16	0.06	0.56	−0.14													
K leaf	−0.13	0.42	0.22	0.55	−0.25	0.55	0.21	0.75	0.81	0.15	0.84	−0.04												
Fe husk	0.12	0.32	0.43	0.26	0.12	−0.10	0.78	0.58	0.07	0.72	0.48	0.33	0.13											
Mg husk	0.00	0.28	0.22	0.53	0.14	0.30	0.47	0.55	0.31	0.24	0.61	−0.18	0.53	0.28										
N husk	−0.04	0.45	0.54	0.07	0.18	0.19	0.68	0.65	0.56	0.80	0.66	0.43	0.54	0.83	0.43									
P husk	0.08	0.35	0.09	−0.27	0.37	−0.43	0.53	0.21	−0.24	0.26	0.18	0.01	−0.08	0.40	0.54	0.26								
K husk	0.02	0.56	0.63	0.20	0.17	0.17	0.22	0.27	0.64	0.65	0.37	0.76	0.52	0.42	0.14	0.75	0.05							
Fe seed	0.17	0.48	0.64	0.49	0.21	−0.21	0.60	0.29	0.05	0.72	0.24	0.77	0.06	0.82	0.09	0.76	0.36	0.71						
Mg seed	0.45	0.07	0.33	0.71	0.37	−0.32	0.24	−0.28	−0.39	0.36	−0.34	0.77	−0.45	0.40	−0.21	0.21	0.23	0.37	0.71					
N seed	0.14	0.47	0.47	0.23	0.16	−0.18	0.80	0.59	0.04	0.65	0.47	0.48	0.19	0.94	0.28	0.82	0.51	0.55	0.91	0.49				
P seed	0.02	0.46	0.62	0.20	0.15	−0.04	0.40	0.19	0.07	0.30	0.23	0.73	0.26	0.31	0.28	0.45	0.38	0.67	0.71	0.55	0.59			
K seed	0.15	0.39	0.60	0.59	0.42	−0.22	0.37	−0.06	−0.10	0.58	−0.10	0.87	−0.13	0.52	0.01	0.50	0.29	0.64	0.88	0.82	0.68	0.80		
MDA	0.08	0.27	−0.13	−0.43	−0.22	−0.12	0.28	0.65	0.03	−0.03	0.47	−0.27	0.40	0.32	0.08	0.23	0.05	−0.06	0.10	−0.31	0.34	−0.04	−0.13	
APX	−0.07	0.19	−0.19	−0.44	−0.40	0.10	0.11	0.58	0.24	−0.06	0.57	−0.34	0.40	0.25	0.08	0.22	0.16	0.04	−0.02	−0.35	0.21	−0.21	−0.40	0.45

Numbers marked with red color are significant at $p < 0.05$.

Table 4. Physiological parameters in bean leaves cultivated in: control soil (C—without EAF slag or NPK fertilizer), soil enriched with synthetic NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2) and soil enriched with EAF slag (S1—at 1% level, S2—at 2% level).

Parameter	C	F	FS2	S1	S2
PAR	1301	1393	1359	1302	1379
C*i*	239 (13.38) b	255 (11.68) b	271 (6.50) a	262 (23.13) ab	249 (24.0) b
T	8.05 (0.30) a	8.06 (0.14) a	8.73 (0.73) a	8.06 (0.77) a	8.28 (0.54) a
gs	0.27 (0.026) b	0.27 (0.013) b	0.32 (0.014) a	0.30 (0.021) ab	0.28 (0.011) b
NR	274.3 (27.9) e	615.0 (85.9) b	720.6 (47.9) a	474.4 (63.1) c	360.0 (32.1) d

Numbers present an average of the nine replicates. Standard deviation (except for PAR) is shown in parenthesis. Different letters within each row indicate significant difference at $p < 0.05$. PAR—photosynthetically active radiation, C*i*—intercellular CO_2 concentration, T—transpiration rate, gs—stomatal conductance, NR—nitrate reductase (μmol NO_2/h/g fresh weight).

The EAF slag supplementation did not cause substantial change in the contents of non-essential, potentially toxic metals (Cd, Pb, Cr) (Table 1). The content of those metals in the soil amended with 2% EAF slag was slightly higher than in the soils amended with 1% EAF slag and the control soil. In this study, the uptake of non-essential metals in plant organs was not determined, but based on the available data, steel slag at levels up to 2% does not significantly affect the content of those metals in several model plants, including the bean [15,40–42].

3.2. Effect of EAF Slag on Growth and NR Activity of Bean Plant

The application of either EAF slag or NPK fertilizer significantly increased plant height compared to the control soil (16–21% increase compared to the control) (Figure 1a). EAF slag increased the leaf dry weight (Figure 1c). However, only amended with 1% EAF slag resulted with a significant increase in the number of husks (Figure 1b) and in seed dry weight (Figure 1c). A marked rise in the number of husks was also seen after application of combined 2% EAF slag and NPK fertilizer (Figure 1c). Such positive impact of the EAF slag on growth parameters may be connected to the increased contents of N, P, K, Mg and Fe in different plant organs (Table 2).

Specifically, a positive correlation between the leaf dry weight and P, K and Mg content in different plant organs was found (r from 0.55 to 0.71) (Table 3). In addition, the number of husks was closely related to the husk-N and -K, and Fe, P and K content in different plant organs (r from 0.54 to 0.64) (Table 3). Previous studies demonstrated that converter steel slag at level 1 and 2% caused an increase of Fe, P and K uptake and concomitant increase of shoot dry matter in maize [13,14]. On the other hand, Negim et al. [15] reported that steel slag-promoted growth of dwarf beans could be related to an increased foliar Ca content. In a study by Chen et al. [42], molybdenum slag at levels up to 5% improved the growth of pak choi seedlings cultivated in calcareous soil by providing nutrients. Interestingly, in comparison to the performance of NPK amendment, remobilization of N from leaf to seed (which is important for the seed-filling period) was much more effective in plants cultivated in the EAF slag-amended soil that resulted in maximum seed dry weight (Table 2). This might be due to disturbed N metabolism and higher oxidative damage in plants cultivated in the soil enriched with NPK fertilizer (Table 5). Indeed, recent research suggests that excessive N application led to considerable changes in N metabolism and to increased lipid peroxidation, which consequently altered grain filling in wheat [43].

Nitrate reductase (NR) is a crucial enzyme for the acquisition of N in plants and it is a reliable indicator of plant-N status in leaves [44]. The activity of NR increased in a following order: C < S2 < S1 < F < FS2 (Table 4). A very strong correlation was found between NR activity and leaf N (r = 0.89), but the activity of that enzyme also correlated with N contents of husk and seed (r from 0.59 to 0.65) (Table 3).

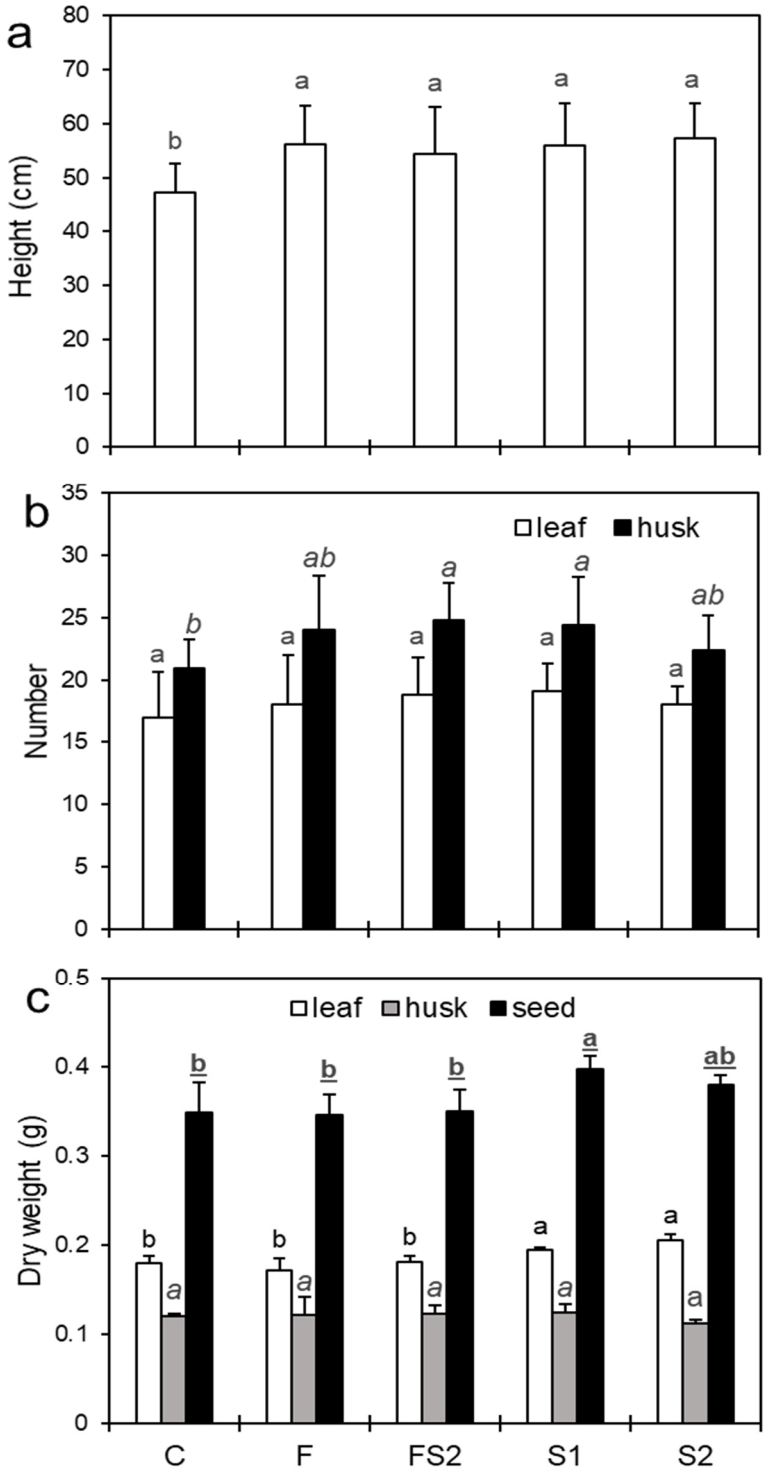

Figure 1. Effect of EAF slag on plant growth. (**a**) Plant height, (**b**) number of leaves and husks, and (**c**) dry weight of leaf, husk and seed in bean plants cultivated in: control soil (C—soil without EAF slag or NPK fertilizer), soil enriched with NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2), and soil enriched with EAF slag (S1—at 1%, S2—at 2% level). Error bars present standard deviations. Different letters indicate significantly different values at $p < 0.05$.

Table 5. Parameters of oxidative stress in bean leaves cultivated in: control soil (C—without EAF slag or NPK fertilizer), soil enriched with synthetic NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2) and with EAF slag (S1—1%, S2—2% level).

Parameter	C	F	FS2	S1	S2
MDA	17.6 (1.91) b	21.8 (2.17) a	20.9 (0.88) a	17.2 (0.86) b	17.4 (0.81) b
C=O	40.8 (4.77) a	43.3 (5.28) a	40.6 (2.55) a	42.1 (3.88) a	40.7 (1.27) a
SOD	10.9 (0.70) a	9.7 (0.21) a	10.4 (0.94) a	11.0 (0.42) a	10.8 (0.83) a
APX	2.67 (0.13) b	3.45 (0.37) a	3.56 (0.57) a	2.79 (0.31) b	2.83 (0.20) b
POX	2.73 (0.10) a	2.56 (0.28) a	2.38 (0.13) a	2.36 (0.23) a	2.41 (0.17) a

Numbers present an average of the six replicates. Standard deviation is shown in parenthesis. Different letters within each row indicate significant difference at $p < 0.05$. MDA—malondialdehyde (nmol/g fresh weight), C=O—carbonyls (nmol/mg protein), SOD—superoxide dismutase (U/mg protein), APX—ascorbate peroxidase (U/mg protein), POX—guaiacol peroxidase (U/mg protein).

3.3. Effect of EAF Slag on Photosynthetic Parameters of Bean Leaves

In this study, the activity of the photosynthetic apparatus was evaluated by estimating maximum quantum yield of PSII (F_v/F_m), net photosynthetic rate of CO_2 assimilation (PS), and content of chlorophylls and carotenoids (Table 6). Soil amendment with either the EAF slag or NPK fertilizer caused no significant change in F_v/F_m values, nor in the contents of chlorophylls and carotenoids and their ratios in comparison to the control. Since these parameters directly describe the regulation of the processes of absorption and trapping energy fluxes, they are extraordinarily important for maintaining effective primary photochemistry of the PSII [45,46]. Based on these data, it can be concluded that PSII was fully functional in all investigated bean plants, which allowed a truthful direct comparison of net photosynthetic rates between investigated plants.

Table 6. Photosynthetic parameters in bean leaves cultivated in: control soil (C—without EAF slag or NPK fertilizer), soil enriched with synthetic NPK fertilizer (F), soil enriched with a combination of 2% EAF slag and NPK fertilizer (FS2), and with EAF slag (S1—1%, S2—2% level).

Parameter	C	F	FS2	S1	S2
PS	11.96 (1.66) c	13.77 (0.95) bc	19.68 (1.75) a	16.05 (1.49) b	14.21 (1.55) bc
F_v/F_m	0.846 (0.010) a	0.848 (0.008) a	0.843 (0.009) a	0.848 (0.006) a	0.843 (0.011) a
Chl *a*	0.832 (0.028) a	0.839 (0.047) a	0.848 (0.045) a	0.863 (0.045) a	0.831 (0.069) a
Chl *b*	0.337 (0.020) a	0.337 (0.040) a	0.350 (0.056) a	0.364 (0.030) a	0.339 (0.042) a
Chl *a* + *b*	1.169 (0.034) a	1.176 (0.087) a	1.198 (0.099) a	1.227 (0.009) a	1.169 (0.109) a
Car	0.346 (0.015) a	0.332 (0.021) a	0.346 (0.025) a	0.355 (0.009) a	0.340 (0.024) a
Chl *a*/*b*	2.479 (0.165) a	2.51 (0.167) a	2.457 (0.239) a	2.375 (0.087) a	2.462 (0.151) a
Chl *a* + *b*/Car	3.382 (0.111) a	3.537 (0.079) a	3.460 (0.103) a	3.459 (0.125) a	3.436 (0.139) a

Numbers present an average of the nine replicates. Standard deviation is shown in parenthesis. Different letters within each row indicate significant difference at $p < 0.05$. PS—net photosynthetic rate, Fv/Fm—maximum quantum yield of PSII, contents of Chl *a*—chlorophyll *a* (mg/g fresh weight), Chl *b*—chlorophyll *b* (mg/g fresh weight), Chl *a* + *b*—total chlorophylls (mg/g fresh weight), Car—carotenoids (mg/g fresh weight), Chl *a*/*b*—ratio of chlorophyll *a* to chlorophyll *b*, and Chl *a* + *b*/Car—chlorophyll *a* + *b* to carotenoid ratio.

Gas exchange measurements provide a direct measure of the net rate of photosynthetic carbon assimilation [47]. Photosynthetic parameters obtained by those measurements showed that the EAF slag boosted photosynthesis, as evidenced by increased net photosynthetic rate PS (Table 6). The highest gain of PS (65% compared to the control) was noted after application of combined 2% EAF slag and NPK fertilizer, whereas a significant increase of PS (35% compared to control) was also observed on application of 1% EAF slag. It seems that the photosynthetic rate is affected by leaf N and NR activity, as the correlation between those parameters was significant and ranged from 0.56 to 0.64 (Table 3). The relation between PS and leaf-N content could be explained, at least to some extent, by the relatively high investment of N in the proteins of the Calvin cycle and thylakoids, in particular RuBisCO in C3 plants [48–50].

Other physiological parameters, such as intercellular CO_2 concentration and stomatal conductance, were improved in plants cultivated in the soil enriched with 1% EAF slag

and in the soil enriched with combined 2% EAF slag and NPK fertilizer (Table 4). On the other hand, the transpiration rate was not affected by either the EAF slag or NPK fertilizer supplementation, in comparison to the control.

3.4. Effect of EAF Slag on Oxidative Stress Parameters of Bean Leaves

Oxidative stress arising due to the imbalance between the surplus production of ROS and immediate inefficiency in their neutralization often occurs in response to a variety of natural and anthropogenic factors. Stress-induced accumulated ROS can harm vital biomolecules, causing protein cross-linking, inhibition of enzyme activity, alterations in membrane fluidity and solute transport, and other detrimental processes, which eventually lead to cell death [51]. Since a higher oxidation rate occurs in plant leaves, in this study, oxidative stress parameters were assessed only in bean leaves. As markers of oxidative damage to membrane lipids and proteins in the bean leaves, MDA and carbonyl group contents were evaluated. Carbonylation of leaf proteins was not affected by either the EAF slag or NPK fertilizer application, implying that there was no direct oxidation of proteins by ROS (Table 5). However, the level of MDA significantly increased on application of NPK fertilizer compared to the control. Simultaneously, a significant rise in activity of APX, one of the H_2O_2 detoxifying enzymes, was determined on application of NPK fertilizer. On the other hand, the POX enzyme was obviously not induced in the degradation of the H_2O_2, as evidenced by unchanged POX activity in the plants cultivated in the amended soils. Since a positive correlation (Table 3) was established between NR activity and MDA ($r = 0.65$), a higher peroxidation of membrane lipids might be related to a faster rate of N assimilation and higher ROS formation [43,52]. Moreover, APX activity correlated with NR activity ($r = 0.58$) and leaf N content ($r = 0.57$), corroborating the connection between N metabolism and ROS accumulation. Activity of SOD, one of the major antioxidative enzymes, was unchanged in the plants cultivated in the soils enriched with either the EAF slag and/or NPK fertilizer, which points to another source of H_2O_2 formation (Table 5). The uncharged and freely diffusible oxygen species can be generated via several enzymatic and non-enzymatic reactions such as the oxidation of glycolate during photorespiration, amine oxidase, xanthine oxidase, NADPH oxidase and so forth [53].

4. Conclusions

This investigation demonstrated that EAF slag, characterized as non-hazardous waste from steelmaking, displays promising potential in agricultural practice as a soil enhancer and a fertilizing material without any phytotoxic effects, at least at the applied levels. The effects of EAF slag on the mineral status of soil, bean growth and nutrition was comparable to the performance of the synthetic NPK fertilizer. The study also revealed that a combination of EAF slag and an NPK fertilizer proved satisfactory in bean cultivation, yet not superior compared to EAF slag (in particular at 1% level). Overall, the recycling and utilization of EAF slag in crop cultivation provides additional advantages in the management of the steel industry by-product—reducing the volume of the slag that otherwise would occupy a large area in landfills and reducing the amount of synthetic fertilizers used in agriculture. However, further investigation is needed to evaluate the efficiency of EAF slag on crop yield under field conditions, with special consideration given to monitoring heavy metals mobility in soil and crops.

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