Journal of Agricultural Chemistry and Environment, 2022, 11, 209-221
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Effect of Steel Slag on Soil Fertility and Plant Growth

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How to cite this paper: Islam, Z., Tran, Q.T., Koizumi, S., Kato, F., Ito, K., Araki, K.S. and Kubo, M. (2022) Effect of Steel Slag on Soil Fertility and Plant Growth. *Journal of Agricultural Chemistry and Environment*, **11**, 209-221.
https://doi.org/10.4236/jacen.2022.113014

Received: July 5, 2022
Accepted: August 13, 2022
Published: August 16, 2022

Abstract

The effective utilization of steel slag, a byproduct produced in large quantities from the steel refining process, is an important issue. Because steel slag contains abundant mineral components, the effects of steel slag on soil bacterial biomass and plant mineral uptake were analyzed in this study. The soil pH increased in proportion to the amount of steel slag added. A lower concentration (0.2% to 1%) of steel slag addition did not change the bacterial biomass. However, a higher concentration of steel slag (above 1%) had a negative effect on bacterial biomass. A lower amount of steel slag (0.2% to 1%) addition in soil leads to increased mineral (Ca, Mg, and Fe) uptake and plant growth in *Brassica rapa* var. *periviridis* and *Spinacia oleracea* L. However, mineral uptake by the plants decreased when a large amount of steel slag (above 1%) was added to the soil. Low concentrations of steel slag (0.2% to 1%) in soil had positive effects on plant growth, mineral uptake of plants, and bacterial biomass.

Keywords

Bacterial Biomass, Mineral Uptake, Nitrogen Circulation, Phosphorus Circulation, Plant Growth, Steel Slag

1. Introduction

Over the last century, chemical fertilizers have been developed to enhance agricultural activities, and crop and vegetable yields have been substantially en-

hanced [1]. Chemical fertilizers contain mineralized compounds, such as ammonium sulfate, phosphoric acid, and potassium, therefore, plants easily take up these elements [2]. Since chemical fertilizers contain fewer minerals than organic fertilizer, micronutrients in soil have been reduced gradually in this 100-year period [3].

In recent years, organic agriculture has been promoted from the viewpoint of environmental issues and health consciousness [4] [5]. Manure is mainly used as an organic fertilizer, and unfermented materials such as fish meal and bone meal are added as supplements of mineral components [6]. However, the mineral components contained in these materials are unstable and expensive, therefore, the search for new alternative materials is important.

The steel industry discharges a large volume of waste materials, and steel slag is a byproduct of the steel refining process [7] [8] [9]. Since steel slag contains high levels of minerals (Ca, Mg, Fe, Mn, Zn, and Si) and acceptable low ranges of heavy metals (Pb and Cd), steel slag is used for agriculture [10]. Besga *et al.*, [11] reported that the addition of steel slag to soil promotes exchangeable Ca and Mg in the soil. In addition, steel slag addition to soil can be used to increase pH of acidic soils [12] [13]. These studies reported that addition of steel slag enhances mineral uptake by plants. However, the effects of steel slag in organic cultivation system on bacterial biomass and nutrient circulation need to be investigated.

In this study, nutrient concentration and application of steel slag were analyzed for organic agriculture. Plants growth and mineral uptake by plant from steel slag were also evaluated. Furthermore, the effects of steel slag on the soil bacterial biomass and nutrient circulation in the organic soil environment were also investigated.

2. Materials and Methods

2.1. Soil and Slag Use

Previously constructed woodchip-based organic soil was used for this experiment [14] Base soil was constructed using wood chips, mountain soil, black soil, and peat moss at a ratio of 5:3:1:1 (v/v). Organic fertilizers (cow manure 5%, oil cake 0.25%, soybean 0.25%, and bone meal 0.05%, w/w) were mixed with the base soil. **Table 1** shows the properties of the soil. A specimen of steel slag was provided by a steel company (Nippon Steel Corporation, Japan). Total carbon (TC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) and minerals e.g., calcium (Ca), magnesium (Mg), Iron (Fe), and manganese (Mn) of slag were analyzed.

2.2. Plant Cultivation

Brassica rapa var. *periviridis* and *Spinacia oleracea* L. seeds were purchased from Takii & Co., Ltd. (Kyoto, Japan). The pot experiments were carried out in a plant incubation room located at Ritsumeikan University, Shiga, Japan (34˚58'58.0"N 135˚57'49.2"E). The temperature was constant at 23˚C, and the interval of

Table 1. Properties of soil at 30% water content after 1 week incubation.

Parameter	Value
TC (mg/kg)	38,000
TN (mg/kg)	1380
TP (mg/kg)	850
TK (mg/kg)	5100
Bacterial biomass ($\times 10^8$ cells/g-soil)	12.6
Nitrogen circulation activity (point)	32
Phosphorus circulation activity (point)	67
pH	6
EC (ds/cm)	0.7
Water holding capacity (ml/kg)	1500
Bulk density (g/cm^3)	0.55

light and dark periods was 12 h/12 h. Two experiments were conducted in this study.

The first experiment was conducted to cultivate *B. rapa* at different application rates (0%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, 1.2%, and 1.4%) of steel slag. One seedling of *B. rapa* was transplanted into each small pot that contained 200 g of the soil. The water content of the soil was maintained at 30% during the cultivation period. This experiment was carried out in one replicate for each treatment. The bacterial biomass and pH value of soil with each steel slag concentration were analyzed at the start point (0 week) and after plant cultivation (4 weeks). Fresh shoot weight as plant growth and mineral concentrations (Ca, Mg, Fe, and Mn) of dried shoots were measured.

In the second experiment, two different plant species (*B. rapa* and *S. oleracea*) were cultivated in the soil with 0% (T_0), 0.5% ($T_{0.5}$), and 1% (T_1) steel slag in triplicate. A total of 2.5 kg of soil was put in a Wagner pot (1/5000a) and maintained at 30% water content. Three seedlings of *B. rapa* and *S. oleracea* were transplanted into each pot. Bacterial biomass, nitrogen circulation activity, and phosphorus circulation activity in the soil were measured at the initial experiment and after plant harvest. After 4 weeks for *B. rapa* and 6 weeks for *S. oleracea* fresh shoot weight, SPAD value, nitrate, and mineral concentrations (Ca, Mg, Fe, and Mn) of dried shoots were analyzed.

2.3. Analytical Methods

TC was analyzed by using a Total Organic Carbon Analyser (TOC-VCPH, Shimadzu, Kyoto, Japan). TN, TP, and TK were extracted with $CuSO_4 \cdot 5H_2O$, H_2SO_4, and H_2O_2 at 420˚C [15]. Ca, Mg, Fe, and Mn were extracted with HNO_3 and $HClO_4$ at 215˚C. After extraction, TN and TP were determined by the indophenol blue method [16] and molybdenum blue method [17] respectively.

The TK, Ca, Mg, Fe, and Mn concentrations in the extracts were measured by usinga an atomic absorption spectrophotometer (Hitachi Z2300, Tokyo, Japan). Total bacterial biomass was analyzed by quantification of environmental DNA (eDNA) extracted by the slow-stirring method [18].

The evaluation of P circulation activities was carried out by following our previous procedure [19] [20]. A soil sample of 1 g was added to a phytic acid solution (pH 7) containing 3.9 mg of P and incubated for 3 days at 25˚C. Control treatment with only distilled water was also prepared simultaneously. Water-soluble phosphorous (SP) was extracted with 20 mL of distilled water and analyzed by the molybdenum blue method. The increase in SP was defined as the P circulation activity. The following formula was used to calculate P circulation and expressed in points (0 to 100).

$$\text{P circulation activity}\left(\text{point}\right)=\frac{\left(SP\text{ in }\mathrm{P_3}-SP\text{ in }\mathrm{P_0}\right)\left(SP\text{ in }\mathrm{W_3}-SP\text{ in }\mathrm{W_0}\right)}{\text{Total added P}}\times 100$$

where, P_0 and W_0 denote phytic acid and distilled water added tube at day 0, and P_3 and W_3 denote phytic acid and distilled water added tube at day 3.

Nitrogen (N) circulation activity was analyzed based on the bacterial biomass, ammonium oxidation rate and nitrite oxidation rate and expressed in points [21]. The bacterial biomass of 6.0×10^8 cells g^{-1} was defined as 100 points. Soil mixed with ammonium sulfate or sodium nitrite (60 μg N g^{-1} dry soil) was incubated at 25˚C and after 3 days of incubation, the percentage of reduction in the added N was defined as the ammonium or nitrite oxidation rate. Using the scores of bacterial biomasses, ammonium oxidation rate and nitrite oxidation rate, a radar chart was constructed, and the relative area of the inner triangle is expressed as the N circulation activity.

The area of the inner triangle in the radar chart is calculated as follows

$$\text{Area}=\frac{\left(a\times b\right)+\left(b\times c\right)+\left(c\times a\right)}{4}\times\frac{\sqrt{3}}{100}$$

where *a*, *b*, and *c* denote scores of bacterial numbers, ammonium oxidation rate and nitrite oxidation rate, respectively. Nitrogen circulation activity was analyzed by calculating the relative area of the inner triangle as follows

$$\text{N circulation activity}\left(\text{point}\right)=\frac{\text{Area of the inner triangle}}{\text{Area of the outer triangle}}\times 100$$

Soil pH was analyzed at a 1:2.5 ratio of soil and distilled water using a pH metre (LAQUA F-72, Horiba, Kyoto, Japan). The chlorophyll content in the leaves was measured by a Soil Plant Analysis Development (SPAD) meter (SPAD-502, Konica Minolta Sensing, Osaka, Japan). The SPAD value was an average value of 10 measurements. For nitrate analysis, fresh plants were mixed at a ratio of 1:5 (w/v) of plant and distilled water by using waring blender for 1 min. Subsequently, the supernatant was collected for nitrate measurement after centrifuging at 14,000 rpm for 5 min. Nitrate was analyzed following the brucine method with slight modification [22] by using spectrophotometer (Hitachi U-1900, Tokyo,

Japan).

2.4. Statistical Analysis

Mean and standard deviation was performed using SPSS 25.0 software (Armond, NY, USA). Significant differences were determined by one-way analysis of variance (ANOVA, $p < 0.05$), followed by Tukey's post-hoc test.

3. Results

3.1. Mineral Concentrations of Steel Slag

To understand the features of steel slag, the chemical composition was analyzed. The steel slag mainly contained Ca (228,000 mg/kg) and Fe (210,000 mg/kg) followed by Mn (40,400 mg/kg) and Mg (35,100 mg/kg) (Table 2). This result indicates that steel slag contains minerals and has the possibility of increasing micronutrients in agricultural soil.

3.2. Effect of Steel Slag Application on Soil Bacterial Biomass and pH

Table 3 shows the effect of steel slag on the soil bacterial biomass and pH value at 0 and 4 weeks. The steel slag additions from 0.2% to 1% did not change bacterial biomass compared to soil without steel slag. However, higher steel slag concentrations (1.2% and 1.4%) decreased bacterial biomass in the soil at weeks 0 and 4. The pH values of the soil gradually increased with the increase in steel slag addition.

3.3. Effect Steel Slag on Plant (*B. rapa*)

The growth and mineral uptake of *B. rapa* were analyzed to determine the suitable concentration of steel slag addition to soil. When 0.2% to 1.4% steel slag were added to the soil, the shoot weight of the plants increased. Steel slag concentrations of 0.4% to 1.4% seem to be suitable for plant growth (Figure 1).

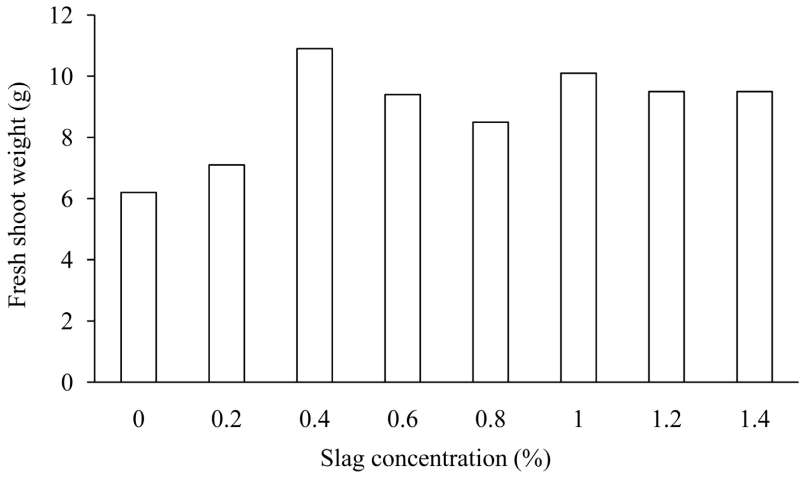

Figure 1. Effect of steel slag amendment on fresh shoot weight of *Brassica rapa*.

Table 2. Properties of slag.

Parameter	Value
TC (mg/kg)	12,130
TN (mg/kg)	80
TP (mg/kg)	20,500
TK (mg/kg)	15,220
Ca (mg/kg)	228,000
Mg (mg/kg)	35,100
Fe (mg/kg)	210,000
Mn (mg/kg)	40,400

Table 3. Bacterial biomass, and pH values after the addition of steel slag to the soil at weeks 0 and 4.

Steel slag concentration (%)	Bacterial biomass ($\times 10^8$ cells/g)		pH	
	Week 0	Week 4	Week 0	Week 4
0	9.0	10.2	5.9	7.1
0.2	10.0	9.8	6.4	7.6
0.4	10.5	9.9	6.7	7.7
0.6	10.5	10.3	6.6	8.1
0.8	10.0	10.2	7.1	8.2
1.0	10.4	9.6	7.2	8.3
1.2	7.9	7.3	7.4	8.5
1.4	7.6	7.4	7.0	8.3

Calcium uptake by *B. rapa* proportionally increased when the steel slag was added at 0.2% to 1%, and the highest calcium uptake was 24,980 mg/kg at 1% steel slag (Table 4). However, the addition of a high concentration of steel slag (above 1%) did not increase the uptake of Ca. A similar tendency was observed in the case of Mg, Fe, and Mn uptake by *B. rapa*.

3.4. Cultivation of Two Different Species of Plants (*B. rapa* and *S. oleracea*) with a Suitable Concentration of Steel Slag

Three different treatments (T_0, $T_{0.5}$, and T_1) were carried out for the evaluation bacterial biomass, N-circulation activity, P-circulation activity, and growth of *B. rapa* and *S. oleracea*. The effects of steel slag on soil fertility are shown on Table 5 and Table 6. The bacterial biomass in the soil was almost similar in all treatments. The addition of steel slag did not change N-circulation and P-circulation significantly at week 0 ($p > 0.05$). Meanwhile, a significant increase in N-circulation activities was observed at weeks 4 and 6 after harvest *B. rapa* and *S. oleracea* respectively. On the other hand, P-circulation activities decreased at weeks 4 and 6 ($p > 0.05$).

Table 4. Mineral concentrations in the dried plant shoot (*Brassica rapa*).

Steel slag concentration (%)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
0	10,780	2580	1040	380
0.2	13,500	2880	1040	430
0.4	16,740	2860	2020	440
0.6	15,000	2860	2300	480
0.8	20,260	3040	2500	480
1.0	24,980	3140	2800	460
1.2	22,800	2900	2700	480
1.4	22,820	2880	2420	540

Table 5. Soil bacterial biomass, N circulation activity, and P circulation activity in three treatments of *Brassica rapa* cultivated soil at 0 and 4 weeks.

Treatment	Bacterial biomass ($\times 10^8$ cells/g)		N-circulation activity (point)		P-circulation activity (point)	
	Week 0	Week 4	Week 0	Week 4	Week 0	Week 4
T_0	9.6 ± 0.2 [a]	8.7 ± 0.2 [a]	37.3 ± 2.5 [a]	49.6 ± 5.3 [c]	53.3 ± 1.7 [a]	19.3 ± 3.3 [a]
$T_{0.5}$	9.3 ± 0.2 [a]	8.4 ± 0.3 [a]	38.3 ± 7.1 [a]	92 ± 3.5 [b]	50.6 ± 6.3 [a]	8.0 ± 2.1 [c]
T_1	9.4 ± 0.1 [a]	8.5 ± 0.5 [a]	37.6 ± 2.6 [a]	95.7 ± 2.3 [a]	50 ± 6.4 [a]	12.6 ± 4.1 [b]

Means followed by different letters within a column are significantly different at $p < 0.05$.

Table 6. Soil bacterial biomass, N circulation activity, and P circulation activity in three treatments of *Spinacia oleracea* cultivated soil at weeks 0 and 6.

Treatment	Bacterial biomass ($\times 10^8$ cells/g)		N-circulation activity (point)		P-circulation activity (point)	
	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6
T_0	9.3 ± 0.21 [a]	8.4 ± 0.04 [a]	37.3 ± 2.4 [a]	50.0 ± 5.3 [c]	53.3 ± 1.7 [a]	19.3 ± 3.3 [a]
$T_{0.5}$	9.6 ± 0.34 [a]	8.1 ± 0.41 [a]	38.3 ± 7.1 [a]	93.7 ± 4.0 [b]	50.6 ± 6.3 [a]	8.0 ± 2.2 [c]
T_1	9.5 ± 0.16 [a]	8.2 ± 0.26 [a]	39.6 ± 6.8 [a]	95.7 ± 2.4 [a]	49.6 ± 6.8 [a]	12.7 ± 4.1 [b]

Means followed by different letters within a column are significantly different at $p < 0.05$.

Figure 2 and Figure 3 show the growth of *B. rapa* and *S. oleracea* under the three different treatments. The fresh shoot weight in the T_1 treatment was significantly higher than those in the T_0 and $T_{0.5}$ treatments ($p < 0.05$) (Figure 4(a)). The SPAD values of both plant species were the highest in the T_1 treatment (Figure 4(b)). The addition of the steel slag to the soil did not show any significant differences with nitrate uptake in the two plant species ($p > 0.05$) (Figure 4(c)).

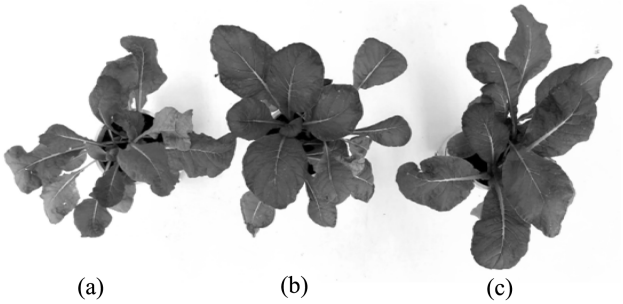

Figure 2. Cultivation of *Brassica rapa* at 0% (a); 0.5% (b); and 1% (c) concentrations of steel slag addition.

Figure 3. Cultivation of *Spinacia oleracea* at 0% (a); 0.5% (b); and 1% (c) concentrations of steel slag addition.

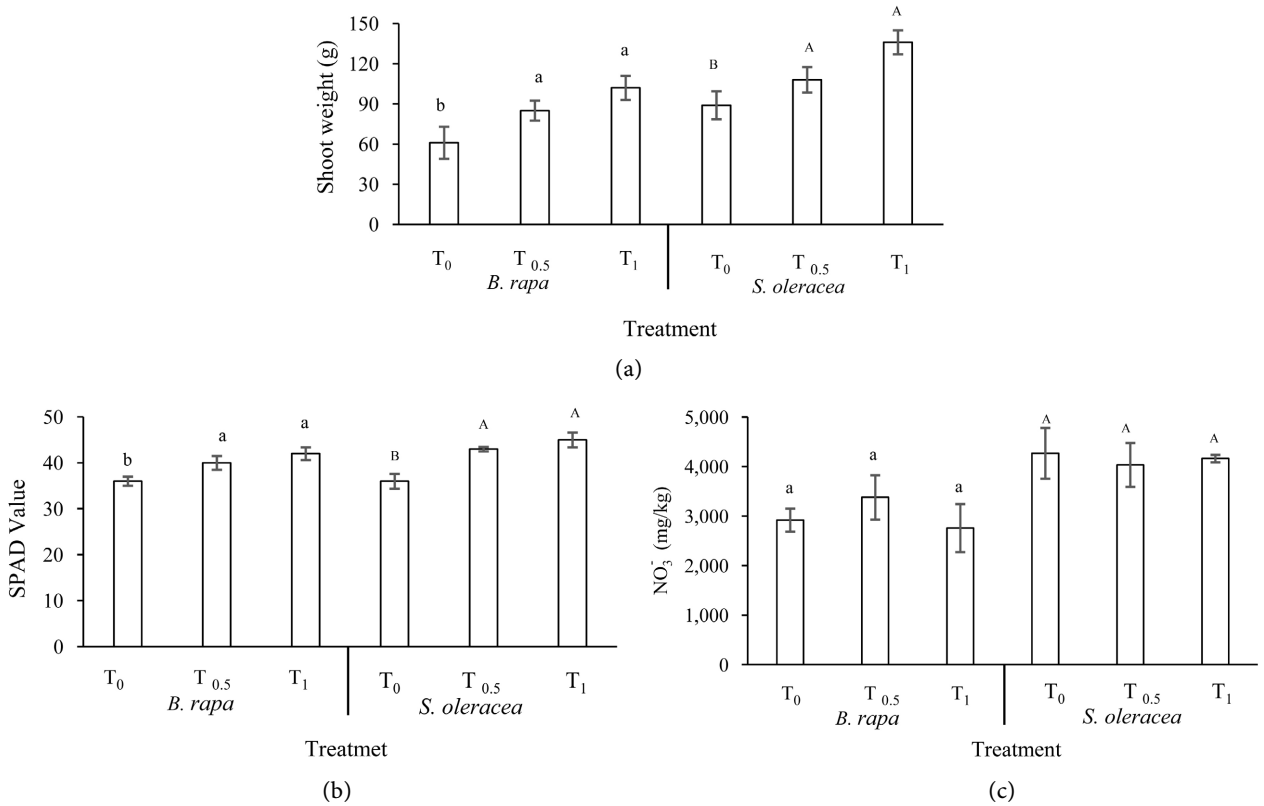

Figure 4. Shoot weight (a); SPAD value (b); and nitrate uptake (c) of *Brassica rapa* and *Spinacia oleracea* under different treatments. Means with different letters indicate significant differences at $p < 0.05$.

The uptake of Ca, Mg, and Fe increased in *B. rapa* and *S. oleracea* with increasing steel slag concentration in the soil. The highest contents of Ca (16,333 mg/kg), Mg (4166 mg/kg), and Fe (1460 mg/kg) in *B. rapa* were observed in the T_1 treatment (Figures 5(a)-(c)). Similarly, the same tendency was found in the case of *S. oleracea*. These results suggest that 0.5% to 1.0% steel slag addition seems to be a suitable condition not only plant growth but also for nutrient circulation in soil.

4. Discussion

Organic agriculture has been promoted, however the mineral components contained in the main organic fertilizers such as manure are limited. In this research, the fertilization possibility of steel slag, which is byproduct from the steel industry, for organic agriculture was investigated.

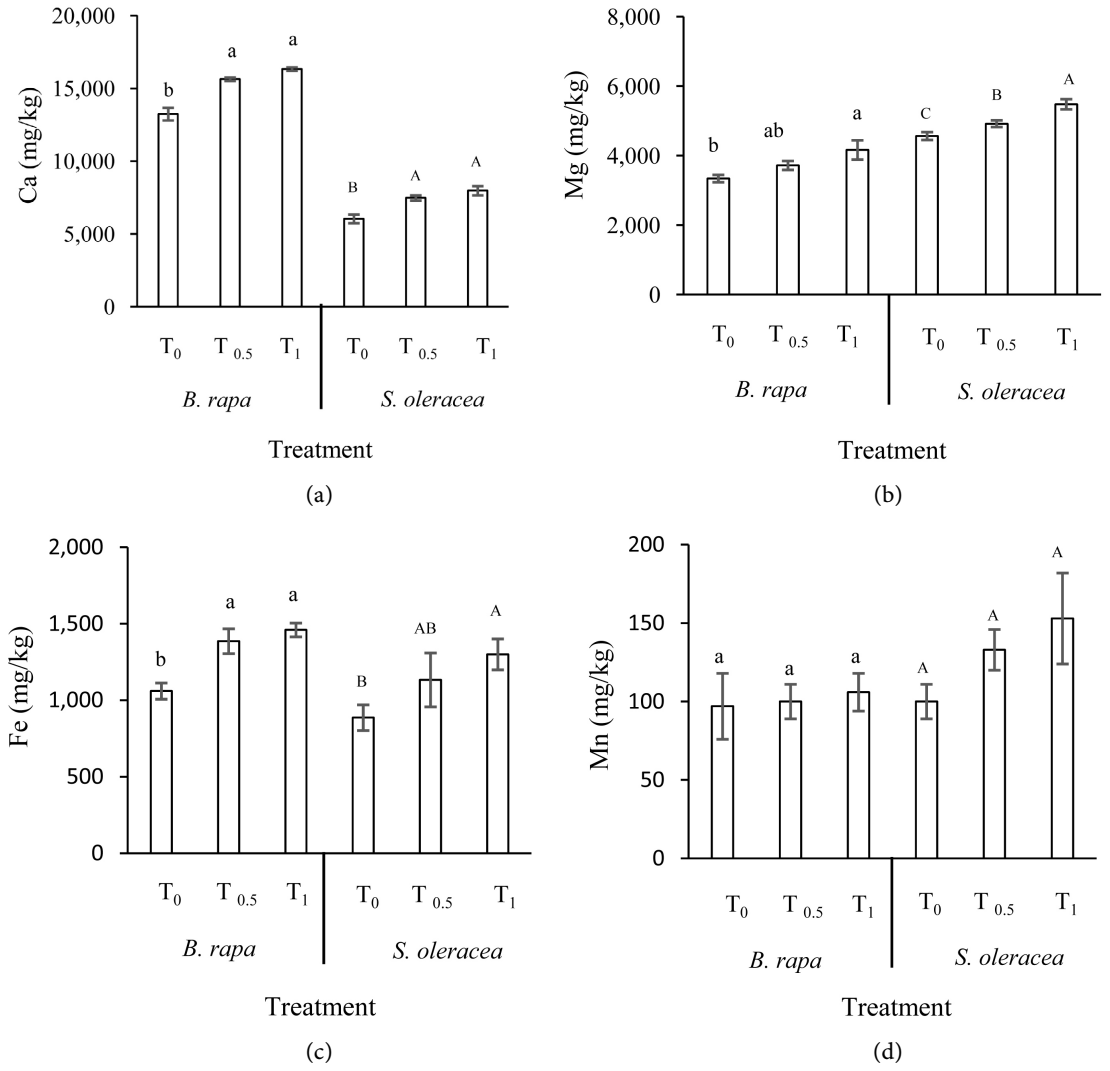

Figure 5. Ca concentration (a); Mg concentration (b); Fe concentration (c); and Mn concentration (d) in dried shoot weight in *Brassica rapa* and *Spinacia oleracea* under different treatments. Means with different letters indicate significant differences at $p < 0.05$.

Low concentrations of steel slag addition did not have a negative effect on soil bacterial biomass. Previous studies reported that slag addition in soil increases total bacterial biomass, but decreases the relative abundance of Genus *Acidobacteria*, *Bacteriodates*, *Nitrospirae*, *and Chloroflexi* [23] [24]. The interaction of steel slag and microorganisms in soil is still unclear, and more investigation is needed to determine the relationship between soil microbes and slag addition.

The addition of steel slag leads to an increase in soil pH, and the pH values of the soil gradually increased with increasing steel slag addition. When slag is added to soil, CaO and MgO dissolve with water and release OH^- which increases the pH values of the soil [25]. The minerals in steel slag seem to elute slowly in the soil environment and the effect upon 1% addition continues for approximately 1 year.

On the other hand, addition of steel slag into soil negatively affects P-circulation activity because increased calcium ions precipitate with phosphate in soil [26]. However, a positive effect on N-circulation activity was observed. This effect may be caused by the activation of ammonia and nitrite oxidizing bacteria in soil [24]. The effective addition of steel slag should consider the balance between P-circulation and N-circulation activities.

Plant growth and mineral uptake significantly increased when steel slag was added to the soil. This result supports previous studies [27] [28] [29]. Enhanced N circulation activity among treatments facilitated N uptake by plant which could be a reason for plant growth. The increase in mineral uptake by plants suggests that steel slag contains exchangeable mineral or could be mineralized by microorganisms.

Ionization of steel slag is promoted by a large number of soil microorganisms, therefore, the effect of steel slag may be more effective in organic agricultural fields [30]. Since, the bacterial biomass is lower in the agricultural field cultivated with chemical fertilizers, so the effect of slag may be lower compared to the organic cultivation system. Further investigation is progressing to determine the effect of slag in conventional cutivation system.

5. Conclusion

Mineral uptake by the plants decreased when a large amount of steel slag (above 1%) was added to the soil, however, a lower amount of steel slag (0.2% to 1%) addition to the soil led to increased mineral (Ca, Mg, and Fe) uptake and plant growth. Low concentrations of steel slag (0.5% to 1%) in soil had positive effects on plant growth, mineral uptake of plants, and bacterial biomass during short-term cultivation practices. Further studies are suggested on the long-term cultivation and repetitive application of slag.

Funding

This work was supported by Nippon Steel Corporation, Japan.

Conflicts of Interest

The authors declare that they have no known competing interests.

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