Physiological, biochemical and nutritional changes in soybean in response to application of steel slag

Alessandra Vieira da Silva[1*], Angélica Cristina Fernandes Deus[1], Mara Lúcia Cruz de Souza[2], Fernando Broetto[2], Leonardo Theodoro Büll[1]

[1]Department of Soil and Environmental Resources, College of Agronomic Sciences, São Paulo State University (Unesp), Botucatu, São Paulo State, Brazil
[2]Rural Engineering Department, College of Agronomic Sciences, São Paulo State University (Unesp), Botucatu, São Paulo State, Brazil

*Corresponding author: alessandrav.silvaa@gmail.com

Abstract

Steel slag is a powdery industrial residue that has CaO, MgO and SiO_2 in its composition, which enables its use in agriculture for soil acidity correction. Most studies involving this residue have focused on understanding its effects on the soil, not always considering its action on the plant metabolism. Thus, the present study aimed to evaluate physiological, biochemical and nutritional changes in soybean plants due to application of lime and steel slag on the soil surface or by incorporation. The experiment was carried out in the field, with six soil acidity corrective materials: stainless steel slag, steel slag, ladle slag, wollastonite rock, dolomitic lime and calcined dolomite, plus a negative control, which did not receive correctives. Two application methods were adopted: soil surface application or incorporation. Sixty days after application, the soybean crop was established. Soybean response changed with the application method, since incorporation of corrective materials provided greater production of fresh and dry leaf mass and stem fresh mass, increased chlorophyll b and leaf K levels, and contributed to a greater number of plants per hectare and pods per plant, compared to surface application. Besides such benefits, there was no difference between the effect of application methods on soybean yield, indicating that both incorporation and surface application are efficient in increasing soybean grain yield. The correctives steel slag, wollastonite and calcined dolomite provided significant increases in soybean yield.

Keywords: Soil acidity. Liming. Slags. Silicate. *Glycine max.*
Abbreviations: CEC_ Cation Exchange Capacity; ROS_ Reactive Oxygen Species; Fe_ Iron; Mn_ Manganese; Al_ Aluminum; DSW_ Dry Stem Weight; SS_ Steel Slag; W_ Wollastonite; C_Control; LS_ Ladle Slag; SSS_ Stainless Steel Slag; CDL_ Calcined Dolomitic Lime and DL_ Dolomitic Lime. S_ Superficial Application; I_ Incorporation. BS_ Base Saturation. K_ Potassium; N_ Nitrogen; P_ Phosphorus; Ca_ Calcium; Mg_ Magnesium; S_ Sulfur; TSP_ Total Soluble Proteins; POD_ Peroxidase; CAT_ Catalase; SOD_ Superoxide Dismutase; SRA_ Antioxidative System; LA_ Leaf Area; FLW_ Fresh Leaf Weight; DLW_ Dry Leaf Weight; FSW_ Fresh Stem Weight; DSW_ Dry Stem Weight; Si_ Silicon.

Introduction

Soybean (*Glycine max*) is the crop of highest productive expression in Brazil. In the 2019 harvest, the area and volume of soybean production corresponded to approximately 48% of the 240.65 million tons of grains produced in the country (Conab, 2019). Most soybean cultivation involves no-till system (Pauletti et al, 2014) and liming (Lopes et al, 1991)

Brazilian soils have several limiting attributes for the crop yield, including acidity, toxic elements (Al and Mn), and low cation exchange capacity (CEC) (Gama et al., 2007). Liming is frequently used to neutralize the soil acidity, reduce the acidity effect on plant development, improve the microorganism activity, reduce the abiotic stress, and increase the nutrient availability (Martins et al, 2017). Lime is the most used corrective material; however, residues from the metallurgical industries such as steel slags can also be successfully employed in agricultural activity (Deus et al., 2019).

Steel slags are Ca and Mg silicates containing CaO, MgO, SiO_2, P_2O_5, FeO and MnO in their composition. Their chemical and physical constitution may vary due to the different processes and different raw materials used in metallurgical industries (Deus et al., 2019). Steel slags can be adopted to correct soil acidity; increase pH levels, exchangeable calcium, exchangeable magnesium, CEC, and soil base saturation; improve phosphorus and silicon availability, and reduce Fe, Mn and Al toxicity (Pulz et al., 2008). It must also be emphasized that the use of steel slag in agriculture is a viable alternative for the final disposal of this such material since its waste is inappropriately discarded in landfills and improper places.

Some studies have shown that steel slags efficiently replace lime in soil acidity correction since they dissolve and make elements available to the plants more rapidly and at greater depths (Corrêa et al., 2009; DEUS et al., 2019).

Another advantage of slag application is Si supply to the crop. Silicon has been evidenced to have an indirect effect on the productivity of soybean crops by improving the plant

architecture and the leaf arrangement, which makes the plants stronger and more resistant to lodging (Agarie et al., 1998).

Plants may alter their metabolism when exposed to inadequate environmental conditions and abiotic stress. Excess elements in the soil, such as manganese, may induce abnormal metabolic events that can lead to the production of reactive oxygen species (ROS) (Amaro et al., 2018). Unless ROSs are neutralized, they may affect the cell structure and cause cellular death. Oxidative damage to the plant cell structures creates an imbalance between antioxidant activity and ROS production, which activates enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Qi et al, 2018).

Therefore, better understanding how different types of slag can be used to correct the soil acidity and improve the plant development is important. Steel slag is suggested to alter the capacity of plants to respond to metabolic changes caused by biotic and abiotic factors. Thus, the aim of the present study was to evaluate physiological, biochemical and nutritional changes in soybean plants due to surface application or incorporation of lime and steel slags.

Results

Soybean physiological and nutritional characteristics

The application method influenced Fresh Leaf Weight (FLW), Fresh Stem Weight (FSW), Electrolyte Loss (EL) and Chlorophyll b (Table 3). Incorporation of acidity correctives led to greater FSW and FLW than surface application. Similarly, the highest EL and chlorophyll *b* levels were found when the correctives were incorporated into the soil.

The tested corrective materials had an effect on Dry Leaf Weight (DLW) and Chlorophyll b (Table 3). Stainless steel slag significantly influenced DLW, compared to control, while the remaining treatments had no significant differences. The lowest Dry Stem Weight (DSW) was obtained with the control treatment. Chlorophyll *b* levels ranged from 16.0 μg cm^{-2} for the control to 23.8 μg cm^{-2} for wollastonite; however, there was no significant difference between the studied correctives.

The nutrients K and B were also influenced by the application method, since K level was higher with the incorporation of correctives while B level was higher with surface application. Considering the soil acidity correctives, there was also a significant effect on the leaf levels of Ca, Mg, Fe, Mn, and Zn (Table 4).

Higher levels of the nutrient Ca were provided by the correctives SS, W and SSS, which were similar to each other, whereas limestones did not differ from control (Table 4). Mg levels obtained with DL were higher than those found with SS and W and similar to those resulting from control, LS, SSS and CDL application.

Fe level obtained with the control treatment was superior to that found with LS and W and similar to the levels resulting from SS, SSS, CDL and DL, while for Mn levels, the control was superior to all other treatments. Regarding Zn levels, control treatment was superior to SSS and DL and similar to SS, W, LS and CDL (Table 4).

Biochemical characteristics and soybean productivity

The analyzed biochemical parameters indicated interactions between the application method and the acidity correctives considering the enzyme SOD, which is part of the plant's antioxidative response system (Tables 5 and 6). When incorporation was adopted, SOD activity was the same, regardless of the corrective material. However, for surface application, W and the control were similar to each other in showing higher SOD activity, compared to the other correctives.

Considering the factors separately, there was a difference between the application methods since incorporation resulted in higher number of plants per hectare and higher number of pods per plant, compared to surface application.

The biochemical parameter Total Soluble Proteins (TSP) was influenced by the corrective materials, ranging from 17.7200 mg g^{-1} for the control to 25.9400 mg g^{-1} for steel slag, which showed no difference from the other treatments.

Discussion

Application of correctives by incorporation resulted in the highest values of the parameters FLW, FSW, EL, Chlorophyll b, K level, number of plants, and number of pods/plants, compared to surface application. This was possibly due to the improved distribution of correctives (0-0.20 m) provided by incorporation, also favoring soil aeration and, consequently, improving the development of the plant's root system with higher nutrient and water absorption.

Wollastonite application had the greatest effect on Chlorophyll *b*, while the remaining treatments showed no significant differences (Table 4). Chlorophyll *b* is characterized as an accessory pigment, responsible for aiding in light absorption (Silva et al., 2014), maximizing energy absorption that effectively acts in the photochemical reactions (Gill & Tuteja, 2010) and in the photosynthesis maintenance (Imtiaz et al., 2016).

Increased leaf Ca levels provided by the treatment with Wollastonite were due to the Ca percentage present in this corrective material.

Mg concentration in soybean leaves was higher with DL, compared the other treatments. This increase is related to the composition of such material, which has higher MgO values than the other correctives (Table 2), combined with the rain that occurred during the experimental period, leading to good nutrient distribution. The leaf levels of the nutrients Ca, Mg, N and P remained within the adequate range for soybean, except K, which kept below the optimal range, according to Ambrosano et al. (1997).

The higher leaf level of B provided by the surface application of acidity correctives can be explained by the storage of part of the available form of this element in the superficial layer of the soil, together with organic matter (Raij et al. 1997). Leaf Fe level was higher with the control than with the other treatments.

The increased Mn level in soybean leaves was not within the adequate range proposed by Ambrosano for the crop: from 20 to 100 mg kg^{-1}. In this study, Mn values were higher than 100 mg kg^{-1} for all treatments. According to Rosolem et al. (1992), the range from 140 mg kg^{-1} to 300 mg kg^{-1} Mn in mature leaves corresponds to toxic levels for soybean crops. The highest Mn levels (300 mg kg^{-1}) obtained with the control treatment might have contributed to the lower number of pods per plant in the control (Table 4), compared to the other treatments, corroborating the study by Heenan & Campbel (1980).

TSP content is regarded as a reference indicator to determine and quantify several other enzyme activities. SS, either incorporated or applied on the soil surface, was the

Table 1. Chemical characterization of the soil before the installation of the experiment, according to the different treatments, correctives and form of application.

Correctives	Application	pH CaCl$_2$	O.M. g/dm³	P resina mg/dm³	H+Al	K	Ca	Mg	SB	CEC	BS %
					\------------------mmolc dm^{-3}------------						
SS	I	4.4	28	12	65	0.8	23	4	27	90	30
W	I	4.3	27	12	64	0.6	24	4	28	92	31
LS	I	5.0	26	36	38	0.8	42	8	51	89	57
SSS	I	4.5	37	33	53	0.9	32	7	41	94	44
CDL	I	4.5	32	10	52	0.3	29	7	36	88	41
DL	I	4.9	33	31	44	0.9	31	11	43	87	49
SS	S	4.6	34	11	55	1.1	35	5	41	96	43
W	S	4.4	27	14	62	0.5	28	4	32	94	34
LS	S	4.7	32	15	48	0.9	36	7	44	92	48
SSS	S	4.5	33	26	54	1.3	48	12	62	116	53
CDL	S	4.4	32	9	59	1.1	26	7	35	94	37
DL	S	5.2	31	28	35	0.6	42	14	57	91	62
Medium		4.6	31	20	48	0.8	33	8	41	87	44
C	I	3.8	25	58	110	0.9	7	2	10	120	8
C	S	4.2	28	88	62	1.3	22	5	28	90	31

SS: Steel slag; W: Wollastonite; C: Control; LS: Ladle slag; SSS: Stainless steel slag; CDL: Calcined dolomitic lime and DL: Dolomitic lime. S: Superficial and I: Incorporated.
SB: Sum of Bases. BS: Base saturation. CEC: Cation exchange capacity
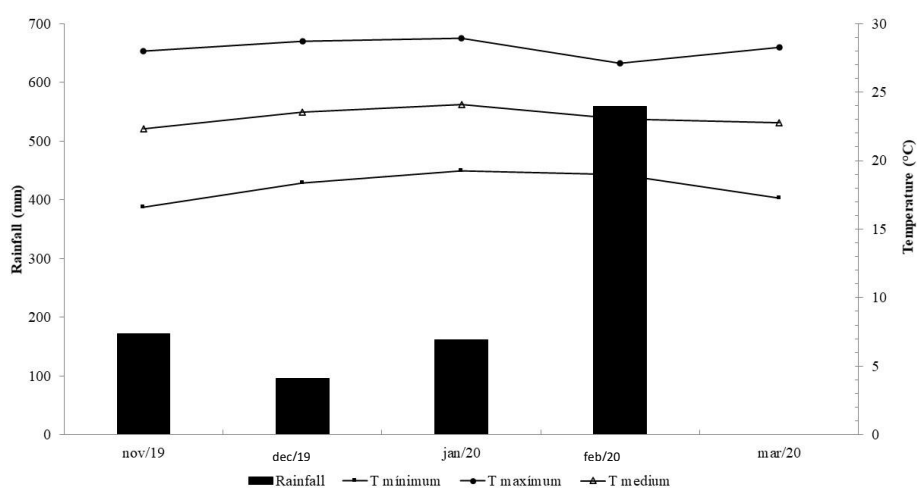
Fig 1. Monthly rainfall and maximum, medium and minimum temperature in Botucatu, State of São Paulo, Brazil during the study period, from November 2019 to March 2020.

Table 2. Chemical and physical characterization of soil acidity correctives.

Correctives	Result in Percent %					
	CaO	MgO	RE	NP	PRNT	Moisture
Stainless steel slag	39.67	11.33	70.97	86	61	2.2
Steel slag	35.30	13.10	73.00	72	53	0.9
Calcined dolomitic lime	52.30	17.90	97.00	139	134	0.2
Dolomitic lime	31.18	16.29	89.36	82	74	1.9
Ladle slag	33.35	6.05	74.11	68	51	1.2
Wollastonite	33.38	4.40	99.64	65	64	0.1

RE= reactivity, expresses the corrective percentage that reacts in three months; NP= neutralizing power, expresses the corrective chemical potential, in CaCO$_3$ equivalent.

Table 3. Leaf area (LA), leaf fresh mass (FLW), leaf dry mass (DLW), stem fresh mass (FSW), stem dry mass (DSW), leaf silicon content (Si), relative water content in leaf tissue (RWC), loss of electrolytes (EL) and pigment content: chlorophyll a (Clo a), chlorophyll b (Clo b) and carotenoids (Car) as a function of the effect of the application mode and correctives of soil acidity on soybean cultivar TMG 7062 IPRO.

Treatments	LA	FLW	DLW	FSW	DSW	Leaf silicon content (Si)	RWC	EL	Clo a	Clo b	Car
Application (A)	cm² plant^{-1}	------------ g plant $^{-1}$ ------------				mg kg^{-1}	%		μg cm^{-2} of leaf		
Incorporated	4924.9 a	54.0 a	24.3 a	149.7 a	43.35 a	3.05 a	84.8 a	6.3 a	35. 0 a	22.3 a	8.7 a
Superficial	3509.1 a	39.7 b	19.3 a	96.2 b	43.92 a	2.89 a	88.3 a	5.2 b	34.0 a	19.2 b	8.5 a
F	47.82^{ns}	11.59*	3.73^{ns}	20.71*	6.37^{ns}	0.22^{ns}	3.19^{ns}	1.29*	1.16^{ns}	20.71*	6.37^{ns}
Correctives (C)											
SS	4117.4 a	43.8 a	21.9 ab	126.8 a	43.00 ab	2.37 a	88.8 a	6.2 a	35.0 a	20.8 a	8.6 a
W	4244.7 a	46.0 a	22.1 ab	120.5 a	44.50 ab	3.18 a	87.3 a	4.5 a	36.7 a	23.8 a	8.8 a
C	3109.7 a	35.7 a	17.5 b	88.7 a	49.00 a	3.35 a	89.3 a	5.7 a	30.8 a	16.0 b	8.0 a
LS	4537.5 a	47.6 a	22.5 ab	136.1 a	43.00 ab	2.62 a	83.9 a	6.7 a	35.0 a	21.6 a	8.3 a
SSS	5274.4 a	58.2 a	25.6 a	150.1 a	40.62 b	2.75 a	84.2 a	4.8 a	36.9 a	21.9 a	9.1 a
CDL	4313.3 a	47.3 a	21.9 ab	124.0 a	45.12 ab	2.78 a	82.8 a	5.3 a	35.1 a	21.4 a	9.1 a
DL	3922.0 a	49.1 a	21.1 ab	114.7 a	40.25 b	2.73 a	88.4 a	7.2 a	34.2 a	20.1 ab	8.2 a
F	0.28^{ns}	1.24^{ns}	2.12*	1.41^{ns}	2.52*	2.00^{ns}	0.69^{ns}	0.79^{ns}	1.19^{ns}	5.84*	0.74^{ns}
A x C	2.02^{ns}	1.72^{ns}	1.82^{ns}	1.97^{ns}	2.07^{ns}	1.92^{ns}	0.39^{ns}	0.81^{ns}	0.65^{ns}	0.48^{ns}	0.64^{ns}
VC (%) plot	55	33	43	35	46	42	8	61	3	10	4
VC (%) subplot	29	36	21	36	22	21	11	54	15	13	16

Averages followed by different letters in the columns differ among themselves. By the Tukey test at 5% (*) and 1% (**) probability. SS – Steel slag. W – Wollastonite. C – control. LS – Ladle slag. SSS – Stainless steel slag. CDL – Calcined dolomitic lime. DL – Dolomitic lime. ns - not significant.

Table 4. Average leaf contents of macronutrients (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Fe, Mn and Zn) in soybeans, harvest 2019/20 as a function of the incorporated and superficial application of acidity correction materials from soil.

Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Application (A)	------------ g Kg $^{-1}$ ------------						------------ mg Kg $^{-1}$ ------------				
Incorporated	49.85 a	2.95 a	16.96 a	7.5 a	5.02 a	1.90 a	43.96 b	10.75 a	145 a	183 a	43.35 a
Superficial	47.78 a	2.86 a	14.46 b	7.0 a	5.01 a	2.06 a	47.82 a	10.96 a	130 a	150 a	43.92 a
F	3.10^{ns}	0.47^{ns}	12.54*	2.52^{ns}	0.01^{ns}	0.57^{ns}	47.82*	0.68^{ns}	0.36^{ns}	8.68^{ns}	0.05^{ns}
Correctives (C)											
SS	48.75 a	2.88 a	15.12 a	7.8 ab	4.76 b	2.03 a	43.12 a	10.12 a	130 ab	134 b	43.00 ab
W	48.50 a	2.93 a	15.75 a	8.0 a	4.66 b	2.01 a	47.62 a	11.12 a	118 b	103 b	44.50 ab
C	48.87 a	3.01 a	17.50 a	7.0 c	4.87 ab	2.01 a	46.62 a	11.50 a	181 a	312 a	49.00 a
LS	50.50 a	2.92 a	15.25 a	7.5 abc	5.05 ab	2.02 a	47.25 a	11.12 a	120 b	187 b	43.00 ab
SSS	49.12 a	2.97 a	16.00 a	7.7 ab	5.08 ab	1.90 a	46.25 a	10.75 a	131 ab	152 b	40.62 b
CDL	48.50 a	2.91 a	15.87 a	7.2 bc	5.47 ab	1.91 a	46.37 a	11.25 a	133 ab	170 b	45.12 ab
DL	47.50 a	2.72 a	14.50 a	7.2 bc	5.23 ab	1.93 a	44.00 a	10.12 a	150 ab	111 b	40.25 b
F	0.92^{ns}	1.64^{ns}	2.15^{ns}	2.53*	2.53*	0.65^{ns}	0.71^{ns}	0.12^{ns}	3.12*	11.67**	2.53*
A x C	2.31^{ns}	1.21^{ns}	0.79^{ns}	1.77^{ns}	0.32^{ns}	0.57^{ns}	0.84^{ns}	0.17^{ns}	2.20^{ns}	1.00^{ns}	0.32^{ns}
VC (%) plot	9	17	16	8	11	39	9	16	37	24	23
VC (%) subplot	5	6	12	7	8	12	12	10	25	35	12

Averages followed by different letters in the columns differ among themselves. By the Tukey test at 5% (*) and 1% (**) probability. SS – Steel slag. W – Wollastonite. C – control. LS – Ladle slag. SSS – Stainless steel slag. CDL – Calcined dolomitic lime. DL – Dolomitic lime. ns - not significant.

only treatment that had an effect on soluble proteins, probably because it provided greater protease activity, which decreased manganese toxicity for SOD in soybean leaves. Plants are capable of developing biochemical responses to survive stress; such responses are generated by an antioxidative system (SRA) and have been evaluated as stress indicators (Souza et al, 2020). In the present study, there was an increase in the activity of the enzyme SOD in response to the surface application of acidity correctives. The greater SOD activity obtained with the control treatment can be related to the excessive leaf level of manganese present in the treatment, compared to the incorporated correctives, which showed low SOD activity.

Thus, activity of other enzymes (CAT and POD) was not observed, i.e., only the enzyme that is considered the first line of the plant defense against stress was activated: SOD. However, this evidences that the plant has adapted to the environment and has not activated the other enzymes (CAT and POD) through its defense mechanism.

Critical Mn^{+2} levels in the leaves (below or above the adequate range) cause oxidative stress due to an increase in the concentration of hydrogen peroxide and malondialdehyde, an indicator of lipid peroxidation (Amaro et al., 2018). Such oxidative stress was evidenced by the enzyme SOD in soybean leaves. In addition, in the present study, even though Mn^{+2} was above the optimal level for

Table 5. Total soluble protein content (PTS), activity of the enzymes Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT), N°. of plants per hectare, N°. of pod / plant, N°. of grains / plant and productivity depending on the application and the corrective effect of soil acidity on soybean cultivar TMG 7062 IPRO.

Treatments	TSP	SOD	POD	CAT	Number of plants per hectare	Number of pods/plant	Number of grains/plant	Silicon content in the grain	Yield (t ha^{-1})
	(mg g^{-1})	(UI µg proteín^{-1})	µKat µg proteín-1					mg kg^{-1}	
Application (A)									
Incorporated	24.3 a	0.98 a	2.7 a	370.4 a	550594 a	78 a	2.0 a	1.05 a	6.46 a
Superficial	20.8 a	1.03 a	2.8 a	362.5 a	444346 b	53 b	2.0 a	1.04 a	5.30 a
F	37.42^{ns}	1.01^{ns}	0,57^{ns}	0,05^{ns}	46,15**	17,81*	1,00^{ns}	0,79^{ns}	3,88^{ns}
Correctives (C)									
SS	25.9 a	0.86 b	2.7 a	291.9 a	463194 a	75 a	2.0 a	1.09 a	7.18 a
W	22.2 ab	1.10 ab	2.7 a	426.9 a	515277 a	53 c	2.0 a	1.21 a	6.25 ab
C	17.7 b	1.16 a	2.8 a	345.7 a	519443 a	55 bc	2.0 a	1.01 a	4.32 c
LS	23.8 ab	0.94 ab	2.8 a	389.4 a	513548 a	64 abc	2.0 a	0.86 a	5.98 abc
SSS	21.6 ab	1.02 ab	2.7 a	419.8 a	503471 a	75 a	2.0 a	1.16 a	5.22 bc
CDL	22.4 ab	1.09 ab	2.8 a	383.5 a	474999 a	68 ab	2.0 a	1.03 a	6.40 ab
DL	24.2 ab	0.87 ab	2.8 a	307.9 a	492360 a	65 abc	2.0 a	0.94 a	5.80 abc
F	2.49*	3.08*	1.62^{ns}	1.67^{ns}	0.43^{ns}	2.87*	0.64^{ns}	0.64^{n}	4.86**
A x C	0.66^{ns}	2.76*	0.80^{ns}	1.91^{ns}	0.43^{ns}	1.88^{ns}	1.53^{ns}	0.33^{ns}	1.00^{ns}
VC (%) plot	21	18	6	34	12	34	7	26	37
VC (%) subplot	20	19	4	31	19	23	15	29	20

Averages followed by different letters in the columns differ among themselves. By the Tukey test at 5% (*) and 1% (**) probability. SS – Steel slag. W – Wollastonite. C – control. LS – Ladle slag. SSS – Stainless steel slag. CDL – Calcined dolomitic lime. DL – Dolomitic lime. ns - not significant.

Table 6. Deployment of the interaction x corrective for the enzyme Superoxide dismutase (SOD) (UI µg proteín^{-1}).

Correctives	Forms of application	
	Incorporated	Superficial
SS	0.90 aA	0.81 aC
W	0.97 aA	1.24 aB
C	0.99 bA	1.33 aA
LS	0.98 aA	0.91 aC
SSS	0.90 aA	1.13 aC
CDL	1.23 aA	0.95 aC
DL	0.89 aA	0.85 aC

SS – Steel slag. W – Wollastonite. C – control. LS – Ladle slag. SSS – Stainless steel slag. CDL – Calcined dolomitic lime. DL – Dolomitic lime. ns - not significant. Capital letters correspond to the comparisons between the soil acidity correctives within the same mode of application. Lower case letters correspond to comparisons between the mode of application for the same soil acidity correction by the Tukey test at a level of 5% (*) and 1% (**) probability.

soybean leaves, it did not inhibit the availability of Ca^{+2}, Mg^{+2} and Fe^{+2} in the leaves. The number of plants per linear meter increased with the incorporation of soil acidity correctives in a no-till system (Rheinheimer et al, 2000). The number of pods per plant varied from 53 for incorporation to 78 for surface application (Table 4). This might have occurred because larger soybean plant populations are capable of producing more pods due to the greater number of ramifications, which determines higher knot production potential and, consequently, higher number of pods per plant (Mauad et al., 2010). Thus, this positively influenced the number of pods per plant considering the two analyzed factors.

There were satisfactory differences in productivity (t ha^{-1}) with the application of the soil acidity correctives; steel slag led to the highest productivity, showing an average value of 7.18 t ha^{-1}, while control had an average value of 4.32 t ha^{-1}. Thus, the increased productivity observed in the present study is due to the residual effect of using steel slags as soil acidity corrective over time in the same area, which acted as a nutrient supplier to the soil and had a positive effect on productivity. It must be highlighted that productivity is also favored by other factors like genotypes of high productive

potential and some environmental factors (Prado et al., 2001).

Material and Methods

Description of the experimental site and soil
The experiment was conducted between July 2019 and February 2020, under field conditions, at the College of Agronomic Sciences – São Paulo State University (Unesp), Botucatu, São Paulo State, Brazil (22°50'19" S and 48°25'54" W, at 738m above sea level). The soil in that area was classified as Rhodic Hapludox (Soil Survey Staff, 2014), a dystrophic Red Latosol, according to the classification of Embrapa (2018).

The experiment was established in 2010 with the surface application and incorporation of the soil acidity corrective materials described on item 1.4. Still in 2010, before application of the corrective materials, the plots designated to receive treatments by incorporation were prepared by plowing with a reversible disc plough and harrowing with leveling disc harrow. Then, the corrective materials were manually applied on the soil surface and mechanically incorporated to 0–20 m. After application, the no-tillage

system was followed (Deus et al., 2020) for all treatments until 2013.

The liming was reapplied in 2013 and 2017, considering the soil chemical analysis for those years and the chemical characteristics of each corrective material; the dose was calculated to increase base saturation to 70%. The corrective materials were applied on the soil surface and incorporated to 0-20 cm depth, following the proposed treatments. After each reapplication, the crops were conducted in a no-tillage system for all treatments.

In July 2019, chemical analysis was performed to characterize the soil at the 0-20cm layer, following the methodology proposed by Raij et al. (2001).

Treatments, experimental design and study development

Treatments consisted of two application methods (surface application and incorporation) of six soil acidity corrective materials, namely, steel slag (SS), ladle slag (LS), stainless-steel slag (SSS), Wollastonite (W), Dolomitic lime (DL) and calcined dolomitic lime (CDL), in addition to one control treatment (C), to which no corrective material was applied.

Experimental design was in randomized blocks with $42m^2$ (6m×7m) subplots and four replicates. The major plots were compared for the application method, surface application or incorporation, while the sub-plots were compared for the applied corrective materials.

The plots that received treatments by incorporation were prepared with a grid of disc levelers; then, in October 2019, the correctives were manually applied on the soil surface and mechanically incorporated to 0–20 m with an intermediate degree grid. For the plots receiving surface treatments, the corrective materials were manually applied on the soil surface.

The used dose of each corrective was calculated to increase base saturation to 70%, considering the result of the soil chemical analysis (Table 1) and the chemical composition of each corrective (Table 2). The chemical and physical characterization of corrective materials was carried out according to the Brazilian Legislation for Limestone (Alcarde, 2009).

After 60 days of application of the acidity correctives, soybeans of the cultivar 'TMG 7062 IPRO' were sown. The seeds were previously treated with the fungicide carboxin (carboxanilide) + thiram (dimethyldithiocarbamate) (9.6 g a.i. per 40 kg seeds) and the insecticide thiamethoxam (34 g a.i. per 40 kg seeds) and inoculated with liquid inoculum (5 x 10^9 CFU *Bradyrhizobium japonicum* mL^{-1}).

Sowing was performed in November 2019, adopting 0.45 cm row spacing and 20 seeds m^{-1} sowing rate. Fertilization was conducted based on the recommendations for soybean by Raij et al. (1996) and on the chemical characteristics obtained in the soil analysis (Table 1), considering the average of all treatments, except the control treatment. Thus, 300 kg ha-^{1} of the formula 02-20-20 (N-P_2O_5-K_2O) were applied.

Plant Measurements

During full blooming (70 days after germination), analyses included leaf area (cm^2) measurement with a leaf area meter model LICOR LI-3000; fresh and dry masses of leaves and stems; relative water content (RWC) (Barr et al. 1962); electrolyte loss (EL) (Lafuente et al., 1991); pigment content (Hiscox & Israelstam, 1979); leaf levels of macro and micronutrients (Malavolta et al., 1997), Total Soluble Proteins (TSP) (Bradford, 1976) and silicon, and activity of the enzymes SOD (Giannopolitis and Ries, 1977), POD and CAT (Lock, 1963). For both variables, 2 to 5 leaves were collected from the middle third of the plant to perform all analyses.

At the end of the crop cycle, evaluations included number of pods per plant, number of grains per pod, silicon content in the grain, mass of 100 grains, and yield. Soybean harvest was manually performed 115 days after seedling emergence and consisted of collecting 2 lines of 2 meters per subplot; then, humidity was corrected to 13% and the yield was calculated as t^{-1} ha.

Statistical analysis

Data were subjected to Shapiro-Wilk test for normality. Results underwent analysis of variance and, when significant, were compared according to Tukey's test at 5% probability. Statistical analysis was performed with the statistical software AgroEstat (Barbosa; Maldonado, 2015).

Conclusions

Application of correctives by incorporation favored soil aeration, which increased leaf biomass, stem weight, number of plants per linear meter, pigment content and, consequently, number of pods per plant.

The levels of micronutrients (B, Cu, Fe and Zn) were within the adequate range for the crop, except manganese, which was toxic, triggering SOD activity (stress indicator) and promoting, especially for the control treatment, a decrease in the number of pods per plant and consequently lower soybean productivity.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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