**Food Chemistry 2019, 282, 1-8**

# Spent coffee grounds improve the nutritional value in minerals of lettuce (*Lactuca sativa L.*) and are an ecological alternative to inorganic fertilizers

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## Abstract

The concentrations of several essential and toxic mineral elements of five groups of lettuces (non-cultivated, cultivated with low or high percentages of spent coffee grounds-SCG, without SCG and with NPK) were compared. The influence of adding increasing amounts of SCG in an *in vitro* assay with a crop soil from Vega of Granada (Spain) was studied. Lettuces with SCG had a higher level of most essential elements (Fe, Co, V, Mn and Zn) as well as some toxic elements (As, Pb and Al), without exceeding the limits established for these elements. Furthermore, blocking of Cd absorption from soil was observed. The direct relations between nutrient absorption and toxic elements and the presence of SCG could be related with minerals chelation by some SCG components, such as melanoidins. In conclusion, the addition of SCG produces lettuces with a higher nutritional value.

**Keywords**: spent coffee grounds; organic amendment; lettuce; mineral nutrient; toxic element; biofortification.

## 1. **Introduction**

Spent coffee grounds (SCG) are a bioresidue obtained during the production of coffee brew (from canteens and restaurants) or from the production of instant coffee powder by treatment of raw coffee powder with hot water or steam under pressure (Mussatto, Machado, Martins & Teixeira, 2011). SCG represent an annual global production of around 6 million tons (Tokimoto, Kawasaki, Nakamura, Akutagawa & Tanada, 2005). Moreover, SCG are the by-product of the coffee industry that involves the highest environmental damage due to its high humidity, acidic pH, polyphenolic compounds and caffeine (Leifa, Pandey & Soccol, 2000). Therefore, an alternative of reuse is necessary for its sustainable management.

Recently, different studies about the reuse of SCG as organic amendment to improve the mineral nutrition of food plants (whether fresh, previously composted or by direct composting on the soil) have been carried out (Cruz, Morais, Mendes, Pereira, Baptista & Casal, 2014; Cruz et al., 2015). The addition of fresh SCG on cultivation substrates decreases Mg, P, Ca, Na Fe, Mn, Zn and Cu, which is attributed by the authors to mineral retention by the coffee matrix through the presence of potentially metal-chelating substances or by the presence of caffeine. However, under the same conditions, composted SCG in low doses improved the status of Mg, Mn, K and Na, due to a better phytoavailability of such elements for plant uptake during composting, as well as to caffeine degradation (Cruz et al., 2014). When these authors composted SCG directly in the soil for 4 months, the 20% and 30% SCG groups showed higher levels of total minerals in comparison to lower SCG percentage groups (Cruz et al., 2015). Other researchers also found an increase in mineral content (Fe, Zn and Mn) in brown rice, after the application of Fe- and Zn-enriched SCG by means of top-dressing application (Morikawa & Saigusa, 2011). Kasongo, Verdoodt, Kanyankogote, Baert & Van Ranst

(2013) also investigated the addition of other types of coffee residues (coffee husk and coffee pulp) in the mineral nutrition of plants and found that the use of this type of waste stimulated the uptake of Ca, Mg, K, N and P, while decreasing the amount of Cu, Zn, Mn and Fe. However, all the previously cited authors did not use regular agricultural soils, but added SCG to very sandy soils, contaminated or to growing substrates, such as peat moss. This is an aspect that must be underlined since the nutritional characteristics of a plant will depend on the chemical, physical and physicochemical properties of the soil type or growing medium employed (Ceglie, Bustamante, Amara, Ben & Tittarelli, 2015; Pinto, Almeida, Aguiar & Ferreira, 2014).

A large number of investigations with SCG have been carried out with lettuce as an edible plant. This is related with fact that lettuce consumption is very high worldwide and has increased in recent decades (Heimler, Vignolini, Arfaioli, Isolani & Romani, 2012). The content of mineral elements in lettuce are a relevant aspect from the human nutrition point of view since lettuce contains essential elements for the human body, such as K, Na, Mg, S and P, which can be involved in reactions of the organism and act as cofactors of vitamins and enzymes (Sofo et al., 2016). In addition, in recent years different studies have been carried out on the health-promoting compounds of lettuce as well as its nutraceutical compounds (Heimler et al., 2012; Pepe et al., 2015).

Finally, the use of SCG as organic amendment could be considered as a multiple solution for the agricultural and environmental problems existing today. This practice facilitates the reuse of such bio-waste, increases the soil organic carbon and decreases $CO_2$ emissions into the atmosphere. In addition the use of SCG increase soil chemical fertility as an alternative to inorganic fertilizers (Cervera-Mata, Pastoriza, Rufián-Henares, Párraga, Martín-García & Delgado, 2018), could in some cases improve the quality of food (Cruz, Baptista, Cunha, Pereira & Casal, 2012) and also could be used to deal with contamination by heavy metals (Kim et al., 2014) and pesticides in the soil (Sánchez-Hernández & Domínguez, 2017).

Taking all these considerations into account, the aim of this work was to determine in an *in vitro* assay, the influence of the addition of increasing amounts of SCG (0, 1, 2, 2.5, 5, 7.5, 10, 12.5 and 15%) to a crop soil from Vega of Granada (Spain), on concentrations of several essential (Mg, Si, P, K, Ca, V, Mn, Fe, Co, Cu, Zn, Se, Mo) and toxic (As, Pb, Cd and Al) minerals of *Lactuca sativa var. longifolia.* These concentrations were also compared with those obtained using commercial inorganic fertilizer (NPK, 15:15:15). Therefore, we tried to determine the possible effect of SCG as organic amendments on lettuces cultivated in a crop soil and so, in an indirect way, to value its role as facilitating or blocking the absorption of essential minerals, as well as mobilizer/immobilizer of toxic elements in soil. The final aim was to evaluate the amelioration of the nutritional value of present minerals in lettuce, as vegetable of usual consumption in the human nutrition.

## 2. Materials and methods

### *2.1. Spent coffee grounds, soil material and lettuce plants*

SCG were sourced from the preparation of espresso coffee from the canteen of the Faculty of Pharmacy of the University of Granada, Spain. SCG have an acid reaction in water (pH = 5.82), a mineral content of 3426 ppm potassium and 404 ppm phosphorous, and an organic matter content of 46.02% organic carbon and 2.24% nitrogen.

The top soil (arable layer, 0-20cm) of one soil developed under a Mediterranean climate (Andalusia, Southern Spain): 'Vega soil' (37° 14′7.1″N, 3° 45′40.7″W) a brownish-grey (Munsell 10YR 5.5/2) Cambic Calcisol (Aric, Ochric) (IUSS Working Group WRB, 2014), was used for the study. The soil samples were air-dried and sieved

(< 5 mm). Vega soil is clayey (58% clay), with low contents of organic carbon (1.36%) and nitrogen (0.11%), pH moderately alkaline (8.2), electrical conductivity measured at 25°C of 1.3 dS/m and strongly calcareous (39% $CaCO_3$ equivalent) (Cervera-Mata et al., 2018).

The assay was conducted with *Lactuca sativa* var. *longifolia*, ‘Little Duende’. *Lactuca sativa* seeds were germinated and grown for 30 days in cell flats (cell size, in cm=3×3×10) filled with peat and perlite mixture. The flats were placed on benches in an experimental greenhouse in Southern Spain (Saliplant S.L., Granada). Lettuce ‘Little Duende’ is one of the small varieties, and so it is well suited for *in vitro* experiments (Cervera-Mata et al., 2018). In this work, these lettuces not previously grown in soil are called “baby lettuces”.

### *2.2. Experimental design*

The assay was performed with eight replicates and eight SCG doses (1, 2, 2.5, 5, 7.5, 10, 12.5 and 15%). In addition, eight control samples of 0% SCG dose were used. To compare with the conventional fertilization, eight microcosms without SCG were used, but enriched with a triple-15 fertilizer (15% N as N, 15% P as $P_2O_5$ and 15% K as $K_2O$); the concentration added to the soil was 1.75g/Kg. When SCG were added, the soil sample (<5 mm) was mixed with the different amounts of SCG to obtain a total of 400 g of the soil-SCG mixture. The soil-SCG mixtures were transferred to PVC pots of 300 mL capacity closed with a mesh of fiberglass at the base to avoid the loss of fine particles and transplanted with 30-days-old baby lettuces. Then the samples were incubated in a growth chamber under controlled conditions with a relative humidity of 50–60%, temperature of 22/18°C (day/night) and 12/12-h photoperiod at a photosynthetic photon flux density (PPFD) of 350 $\mu mol/m^2/s$ measured at the top of the plant with a 190 SB quantum sensor (LI-COR Inc., Lincoln, NE, USA). To prevent leaching and water stress, the moisture of the pots was maintained between field capacity and permanent wilting point. The irrigation requirements were calculated by weighting, as according to Esmaeelnejad, Shorafa, Gorji & Hosseini (2016). The pots were irrigated every three days with distilled water. A final group consisting of eight samples of baby lettuces was included to compare the effect of SCG on the initial state of the lettuces.

After 40 days of cultivation, lettuce plants were extracted from the pots, washed with distilled water and dried at 65°C (Cervera-Mata et al., 2018). Finally, lettuce samples were homogenized and frozen at -80°C until mineral analysis.

### *2.3. Mineral analysis in lettuce samples*

Samples for quantitative determination of metals were first lyophilized (Telstar, Madrid, Spain). Then, 0.2 g of lyophilized sample was mineralized by attack with $HNO_3$ and $H_2O_2$ of supra-pure quality (Merck, Darmstadt, Germany), in a microwave digester (Milestone, Sorisole, Italy) following a previously optimized protocol (Giampieri et al., 2018). The digest was diluted to 10 mL with Milli-Q water, in order to obtain the analytical dissolution. The determination of total content of essential (Mg, Si, P, K, Ca, V, Mn, Fe, Co, Cu, Zn, Se, Mo) and toxic (As, Pb, Cd and Al) elements in lettuce samples was performed by an ICP-MS instrument (Agilent 7500, Agilent Technologies, Tokyo, Japan) fitted with a Meinhard type nebulizer (Glass Expansion, Romainmotier, Switzerland) and equipped with a He collision cell.

Calibration curves were previously prepared using Ga as an internal standard and by the dilution of stock solutions of 1,000 mg/L in 1% $HNO_3$ for the analyzed elements

(Merck, Darmstadt, Germany). Specifically standard solutions of 100 µg/L of elements in 1% (v/v) $HNO_3$ were prepared from the 1.000 mg/l stock standard solutions (Merck) and used for daily optimizing of the ICP parameters.

The accuracy and precision of Mg, Si, P, K, Ca, V, Mn, Fe, Co, Cu, Zn, Se, Mo, As, Pb and Al measurement procedures were verified by testing the certified reference standard Bovine muscle powder of the National Institute for Standards and Technology (NIST) 8414 (Gaithersburg, MD, USA). No significant differences were found between the mean element concentrations determined in these material and the certified concentrations (Table 1). Additionally, the accuracy of the methods was also tested on the basis of recovery experiments, after complete digestion of spiked lettuce samples with different amounts of elements from the standard solutions (Quintana, Olalla-Herrera, Ruiz-López, Moreno-Montoro & Navarro-Alarcón, 2015). The calculated recoveries for each element were between 95% and 105% in all cases. The concentration (µg/g or ng/g) in samples was obtained by linear calibration. Every element was analyzed in triplicate in each one of the lettuce samples. The concentration of these elements is expressed in fresh weight, which is the usual form of consumption.

### *2.4. Statistical analysis*

The homogeneity of variance was assessed using the Levene test and the normal distribution of the samples with the Shapiro-Wilk test. The Student’s t-test was used to analyze parametric data and the Kruskal-Wallis test to analyze non-parametric data. Regression analysis among elements measured in lettuce samples was evaluated by computing the correlation coefficient by Pearson (for normally distributed data) or Spearman linear correlation (for non-normally distributed data, as in the case of Cd).

The significance level was set at 5% ($p < 0.05$) in all tests. SPSS 22.0 for Windows (IBM SPSS Inc., New York, USA) was used for data analyses. Principal component analysis (PCA) was used for clustering samples and their relationships to mineral elements. This statistical treatment was performed in the statistical software in Excel (XLSTAT).

## 3. Results and discussion

Table 2 shows the levels of essential (Mg, Si, P, K, Ca, V, Mn, Fe, Co, Cu, Zn, Se and Mo) and toxic (As, Pb, Cd and Al) minerals in baby lettuces, lettuce cultivated in soils added with SCG (low percentages of SCG: 1%, 2%, 2.5% and 5% and high percentages of SCG: 7.5%, 10%, 12.5% and 15%), lettuce grown without SCG (0% SCG) and lettuces grown with NPK fertilizer. To understand the results included in this table, a comparative analysis between the results for the five groups of lettuces was carried out.

### *3.1. Mineralt levels in cultivated lettuces on soils added with low (1-5%) and high (7.5-15%) SCG percentages vs. non-cultivated lettuces (baby lettuces).*

When the levels of mineral elements of baby lettuces and lettuces cultivated during 40 days in soils added with SCG were compared, an increase in the levels of most of the elements analyzed was observed (Fig. 1a). These increases were only significant ($p < 0.05$) for Ca, Mn, Co and V (high and low percentages of SCG added) and for Fe (high percentages of SCG added). Conversely, the levels of P, Cu and Mo decreased when low and high percentages were added. In relation to toxic elements, the addition of low and high percentages of SCG increased significantly ($p < 0.05$) the levels of As, Pb, Cd and Al. These results show the potential use of SCG as soil organic amendment, since this agricultural practice facilitates the biofortification of lettuce, increasing many essential elements and therefore, improving their nutritional value. However, in a counterproductive way, the use of SCG increases the levels of toxic metals, although these levels are very low and would not suppose problems for human health, as will be discussed later.

These results are opposite to those found by Pinto et al. (2014), who stated that the nutritional value was higher in young lettuces, after monitoring at 5 stages of lettuce growth and mineral content at three experimental agricultural greenhouse fields. These authors only found an increase in K levels along the study period in lettuces grown in the three experimental fields. They also concluded that the lettuce nutritional value was also strongly dependent on the soil. These differences can be attributed to the fact that these authors used a substrate rich in organic carbon (50% approx) mixed with a sandy material (more than 80% of sand), with a neutral pH (6.8). In our study, Vega soil is a crop soil, clayey, with a low organic carbon content, rich in carbonates and its pH is moderately alkaline (8.2). According to Ceglie et al. (2015), the plant response is related to the physical, chemical and physicochemical characteristics of the growing medium used. In this sense, the substrates rich in organic matter have a high capacity for retention and blocking of nutrients (Ociepa, Ociepa-Kubicka, Okoniewska & Lach, 2013). Therefore, the elements could be blocked in the matrix of SCG (very organic material) and could not be made available to the plant, which would also explain the results of Cruz et al. (2012).

### *3.2. Mineral levels in lettuces cultivated on soils added with low (1-5%) and high (7.5-15%) SCG percentages vs. those cultivated without SCG (0% SCG)*

The comparison of the mineral levels in lettuces cultivated during 40 days in soils added with SCG (1-5% SCG and 7.5-15% SCG) and lettuces cultivated without SCG showed an increase in the content of most of the elements analyzed (Fig. 1b). However, when low percentages of SCG were added, the amounts of Mn and K decreased. Significant increases in Fe, Co and V levels ($p < 0.05$) were found in low and high percentages of SCG treatments, while Mn level was significantly higher only in the high percentage of SCG treatment, and Zn concentration only in the low percentage of SCG. In relation to toxic metals, the addition of high and low percentages of SCG increased the levels of As, Pb and Al, while decreased the levels of Cd.

Cruz et al. (2014) found the same effect (an increase of nutrient elements) in lettuce cultivated with composted SCG or cultivated in the substrate with SCG that have been transformed directly in the soil. However, these authors found the opposite effect when fresh SCG were added. The results of this study could be different to Cruz et al. (2014) since these authors cultivated lettuce in organic substrates, which have very different characteristics to the soil used in this study, in terms of pH, moisture, bulk density, N, etc. (Cruz et al., 2012). The differences in the plant nutrition response to the type of soil are also indicated by Pinto et al. (2014), and Ceglie et al. (2015), as we pointed out in the previous section. Additionally, the use of organic substrates in research related with plant nutrition could have the disadvantage of obtaining results not extrapolated to the agricultural reality. So, during the last few years, concern about the environmental impact associated with peat moss extraction has increased (Bullock, Collier& Convery, 2012; Holmes, 2009). This substrate has been used as standard media used in conventional and organic seedling production and has currently been considered a non-renewable resource (Ceglie et al., 2015).

### *3.3. Mineral levels in lettuces cultivated on soils added with low (1-5%) and high (7.5-15%) SCG percentages vs. those cultivated with NPK fertilizer*

When mineral levels of lettuces cultivated during 40 days in soils added with SCG (1-5% SCG and 7.5-15% SCG) and lettuces cultivated with NPK were compared, an increase in the levels of most of the elements analyzed was observed (Fig. 1c). Significant increases in P, Ca, V, Mn, Co, Fe, Cu and Zn levels ($p < 0.05$) were found in low and high percentages of SCG treatments. In relation to toxic metals, the addition of high and low percentages of SCG increased significantly ($p < 0.05$) the levels of Pd and Al, as well as the level of Cd (at low SCG percentages) and As (only at high SCG percentage). These results are of great interest since they compare a conventional agricultural practice (such as adding NPK inorganic fertilizer) with the addition of a bio-waste (SCG) as organic amendment. As stated above, the addition of SCG could be an effective method of biofortification of essential minerals (P, Ca, Fe, Cu, Zn, Mn, Co, V, Si and Mg) in lettuces, which supposes an increase of the nutritional value of the lettuce. A negative aspect of the addition of SCG was the increase in the levels of toxic elements such as As, Pb, Cd and Al, although the slight nutritional implications of these increase will also be discussed later. Furthermore, these results are different from those found by Herencia, García-Galavis, Ruiz Dorado & Maqueda (2011), who concluded that it is not possible to assert a higher nutritional quality of organic crops according only to the criteria of fertilizer type, when comparing the nutritional quality of the crops grown in an organic and conventional fertilized soil.

These results are interesting from the point of view of organic farming and obtaining food with the Integrated Production identification label. The addition of SCG bio-waste *versus* the traditional NPK inorganic fertilizer, in addition to increase the nutritional value of lettuces, has an additional positive effect on the environment, ensuring the implementation of a good agricultural practice and sustainable development, due to the consequent decrease in the use of conventional fertilizers (NPK) in crops. This point was previously stated by Cervera-Mata et al. (2018), who reported that SCG addition provides nutrients (N, P and K) thus reducing the need of inorganic fertilizers. On the other hand, the same authors stated that the incorporation of one ton of SCG would suppose C sequestration, with the consequent reduction of 506 kg of $CO_2$ emitted into the atmosphere, what is an important fact from the environmental point of view.

In addition, as stated by Ciesielczuk & Rosik-Dulewska (2015), SCG would also behave like slow action fertilizers, decreasing nutrient loss by leaching. The increase in nutritional value due to the increase in the content of multiple essential minerals would also justify the higher price of organic and integrated production food. Taking the previous results into account, in future studies it would be necessary to determine if this increase in the nutritive value in reference to several essential minerals is kept when lettuces are cultivated directly in greenhouse experimental fields, as well as how that nutritional value would evolve in the following cultivation seasons. Therefore, the agriculture based on organic amendments ensures greater sustainability of food production in the medium/long term, and foods of greater nutritional value. Organic agriculture is more profitable and environmentally friendly, and deliver equally or more nutritious foods that contain less (or no) pesticide residues, compared with conventional farming (Reganold &Watchter, 2016).

### *3.4. Mineral levels in lettuces cultivated on soils without the SCG (0% SCG) vs. those cultivated with NPK fertilizer*

All the minerals analyzed had a higher level in lettuces cultivated without SCG than those cultivated with NPK, except for As (Table 2). Significant ($p < 0.05$) increases in Cu, Mo and Cd levels were found. This result and those stated previously (related to the comparison between SCG and NPK treatments) indicate that inorganic fertilization does not improve the level of nutrients in lettuce. An opposite result occurred when SCG were added. Conventional agriculture not only reduces the nutritional quality of crops (as stated previously), but also increases pesticide residues, promote the loss of biodiversity and ecosystem services and reduces soil quality (Reganold & Watchter, 2016).

### *3.5. Influence of the increase of SCG percentages (1, 2, 2.5, 5, 7.5, 10, 12.5, 15%) on mineral levels in lettuces*

In this section, the influence of the progressive increase of SCG percentages added to the soil on the final level of the elements analyzed in lettuces was also evaluated (Supplementary Table 1). When the amount of SCG added was increased, an enriching trend in most of the minerals studied was observed. In most cases, these trends were not linear, except for Mn (Fig. 2a). In this case, a progressive increase of Mn levels by SCG addition was observed. The increase in the levels of nutrient elements by SCG and other coffee wastes addition has been related to the chelating effect of some of its components such as melanoidins, polyphenols and carbohydrates (Morikawa & Saigusa, 2008; Kasongo, Verdoodt, Kanyankogote, Baert & Van Ranst, 2011; Liu et al., 2015). In this sense, Rufián Henares & de la Cueva (2009) determined the chelating capacity of melanoidins extracted from coffee against Fe and observed how these compounds decreased free Fe while increasing chelated Fe. Specifically, fifty percent of the iron was chelated at a coffee melanoidin concentration of 2.5 mg/mL.

These chelates formed by melanoidins and other chelating molecules would be released little by little, while SCG are degraded in the soil. In this way, SCG could become slow action fertilizers (Ciesielczuk & Rosik-Dulewska, 2015). Therefore, in future studies it would be necessary to determine which of the referred components (melanoidins, polyphenols and/or carbohydrates) are responsible for increasing the concentrations of different minerals in lettuces after SCG addition. This activity could be considered as a biofortifying effect.

In relation to toxic metals, its behavior was similar to that of essential minerals (Supplementary Table 1), except for Cd (Fig. 2b). When the amount of SCG added was increased, a decreasing trend in the Cd levels was observed. This effect is opposed to the behavior of Mn. Probably certain compounds present in SCG would form insoluble complexes with soil Cd, limiting its absorption by the plant. In recent years it has been indicated that organic fertilizers (such as SCG), are effective in immobilizing heavy metals in contaminated farmlands (Wu, Wu, Liu, Chen, Wu & Yu, 2017). In our study the results obtained are also very interesting in the case of crop soils that contain high levels of Cd, since the addition of SCG as organic amendments could limit the availability of Cd and therefore the final content of this toxic element in plants. In this way, the addition of SCG to crop soils could be an effective method to remedy soil contamination, since SCG contain very small amounts of heavy metals and can retain them by chelation (Kim et al., 2014). These researchers stated that the bioavailable heavy metals content (Cd, Cu, Pb and Zn) decrease in soils treated with SCG or its biochar, similar finding than that reported in our case for Cd.

### *3.6. Correlation among mineral levels measured in lettuce cultivated in soils added with SCG as organic amendment*

There were multiple statistically significant positive linear correlations among the elements analyzed in lettuces (Supplementary Table 2). Those relations with a high correlation coefficient ($r \geq 0.800$) have been highlighted: Ca and Mg ($r = 0.941$), Fe and V ($r = 0.949$), K and Mg ($r = 0.851$), Fe and Si ($r = 0.828$), Co and V ($r = 0.827$), Se and V ($r = 0.881$), I and Si ($r = 0.807$), Fe and Co ($r = 0.866$) and Se and Fe ($r = 0.861$). In the case of toxic metals the high correlation coefficients ($r \geq 0.800$) have been set for the relations among Al and V ($r = 0.992$), Al and Fe ($r = 0.962$), Al and Co ($r = 0.852$), and Al and Se ($r = 0.886$). Cd is the only element that presents significant negative relations with P ($r = -0.522$), Cu ($r = -0.461$) and Mn ($r = -0.437$). Some of these relations are logical from the plant nutrition point of view. For example, Mg and P are synergistic elements, since the increase of one assists the assimilation of the other. However, other correlations are opposite to this logic, like the correlations between K-Ca or K-Mg. These elements are antagonistic since the increase of one obstructs the assimilation of the other. The high number of positive correlations, shown by the principal component analysis (Supplementary Fig. 1) demonstrated that all the elements are highly related to the contents of SCG in the soil. Minerals were positively related to each other and to the low- high contents of SCG, as stated previously. Cd was closer to the treatment 0% SCG since its content in these lettuces is higher, while Cu, P and Mo were closer to baby lettuces, for the same reason. NPK, 0% SCG treatments and baby lettuce were all grouped on the opposite side of the graph. This result supports what was previously discussed about the nutrients chelation and toxic elements by some components of SCG (melanoidins, polyphenols, carbohydrates, etc.) and their higher availability by plants. In addition, the nature of the vegetable (in this case lettuce) could force relations between elements. Similar relationships have been described fur lettuce by Nali, Balducci, Frati, Loppi & Lorenzini (2009) who also found good correlations among the crustal element Al with Co, Fe and V.

### *3.7. Nutritional implications of the addition of SCG to the soil*

Taking into consideration the results of Table 2, the amounts of the different minerals provided by a daily lettuce portion (estimated between 150-200g; Carbajal & Sánchez-Muniz, 2003) were calculated (Table 3). The content of nutrients and toxic minerals would increase when lettuces are cultivated with SCG at high doses, low or in both cases, with respect to the cultivation without SCG or with NPK, except for Cd and Si. The mineral increase in the average lettuces grown in high and low doses of SCG (Table 2) compared to lettuces grown without SCG addition (or with NPK) were 32% or 184% for Zn, 161% or 229% for Co, 191% or 285% for the Fe, 56% or 180% for Mn and 306% or 440% for V, respectively.

Table 3 shows the mineral content of lettuces grown without SCG, the mean value for those grown with SCG and those cultivated with NPK. From these results, the percent of dietary reference intakes (DRIs) for healthy adult men and women (Institute of Medicine, 2005) provided by a portion of lettuce (150 g) were calculated (Table 4). As clearly demonstrated in Table 4, SCG addition increases the percent of DRIs covered by a portion of lettuce, particularly important in the case of Mn (18.86% of DRIs in women), and Fe (10.7% of DRIs in men and 4.75% in women). These data are of particular importance due to the prevalence of iron-deficiency anemia (24.8%) in the world population (WHO, 2008). To a lesser extent for Ca, Cu and Mo the increase of the percent of DRIs are highlighted, approximately 8% in all three cases.

In relation to toxic elements, 150 g of lettuce provide an amount of Al that rises by 352% (Table 3) when lettuces are grown in soils added with SCG (average of low and high percentages), compared to those grown in soils not added (0% SCG) and by 560% compared to those grown with NPK. However, this portion provides 965 µg of Al, when the usual daily diet provides an intake between 1600-13000 µg of Al (Aguilar et al., 2008); such intake corresponds to an exposure of approximately 200 to 1500 µg/Kg body weight per week from water and food in a 60 kg adult (Aguilar et al., 2008). Therefore, in relation to Al, the consumption of a lettuce portion grown in soils added with SCG would not suppose a health risk for consumers.

The contents of Cd behave inversely to those of Al; in other words, it decreased when the lettuces are grown with SCG. The maximum levels of Cd reported for lettuce are 0.2 µg/g fresh weight (Commission regulation, 2006). Table 2 shows that the levels in those lettuces cultivated with high SCG percentages were significantly lower (0.015 µg/g fresh weight), while those cultivated in soils with low SCG percentages were 0.133 µg/g fresh weight. However, this level does not exceed the maximum limit defined by the legislation, so this concentration would not cause toxicity problems in human beings. In relation to Pb, its amounts increased when lettuces were cultivated with SCG (0.0131 µg/g, fresh weight; Table 2). However, this concentration does not exceed the maximum limit defined by Commission regulation (2006): 0.1 µg/g fresh weight.

## 4. Conclusions

The use of SCG as organic amendment increases the levels of multiple essential elements such as Fe, Co, V, Mn and Zn, some of them of great nutritional importance, therefore increasing the nutritional value of lettuce. In addition, the levels of these minerals correlate positively with each other, despite some of them are antagonistic elements at the absorption level. This behavior could be related to the formation of soluble chelates with some SCG compounds (such as melanoidins, polyphenols or carbohydrates), which could be liberated into the soil by SCG degradation. SCG (as organic amendment) *versus* NPK (as traditional fertilizer) increases the levels of numerous essential elements (P, Si, Ca, V, Mn, Co, Fe, Cu, Zn, Mg and Mo), which could reduce the use of chemical fertilizers, ensuring an improvement in environmental sustainability. SCG as organic amendment give rise to the production of a food product that could be classified as ecological and, at the same time, improves its nutritional value. The latter could also justify the higher price of the organic/ecological products so far obtained.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

This work was supported by project AGL2014-53895-R from the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund (FEDER). This paper will form part of Ana Cervera-Mata’s doctoral thesis, which is being developed within the context of the “Nutrition and Food Sciences Programme” at the University of Granada.

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## Figure captions

**Fig. 1.** Variation in the content of mineral elements in lettuce grown for 40 days in soils added with SCG (high and low percentages of SCG) compared to other groups of lettuces: a) Non-cultivated lettuce (baby lettuce); b) Lettuce grown for 40 days without SCG; c) Lettuce grown for 40 days with NPK. The units of each element are different so that the data have been multiplied or divided in order to adapt the height of the bars to represent them in the graph.

**Fig. 2.** Influence of the enhancement of SCG percentages on: a) Mn levels b) Cd levels.

**Figure 1**

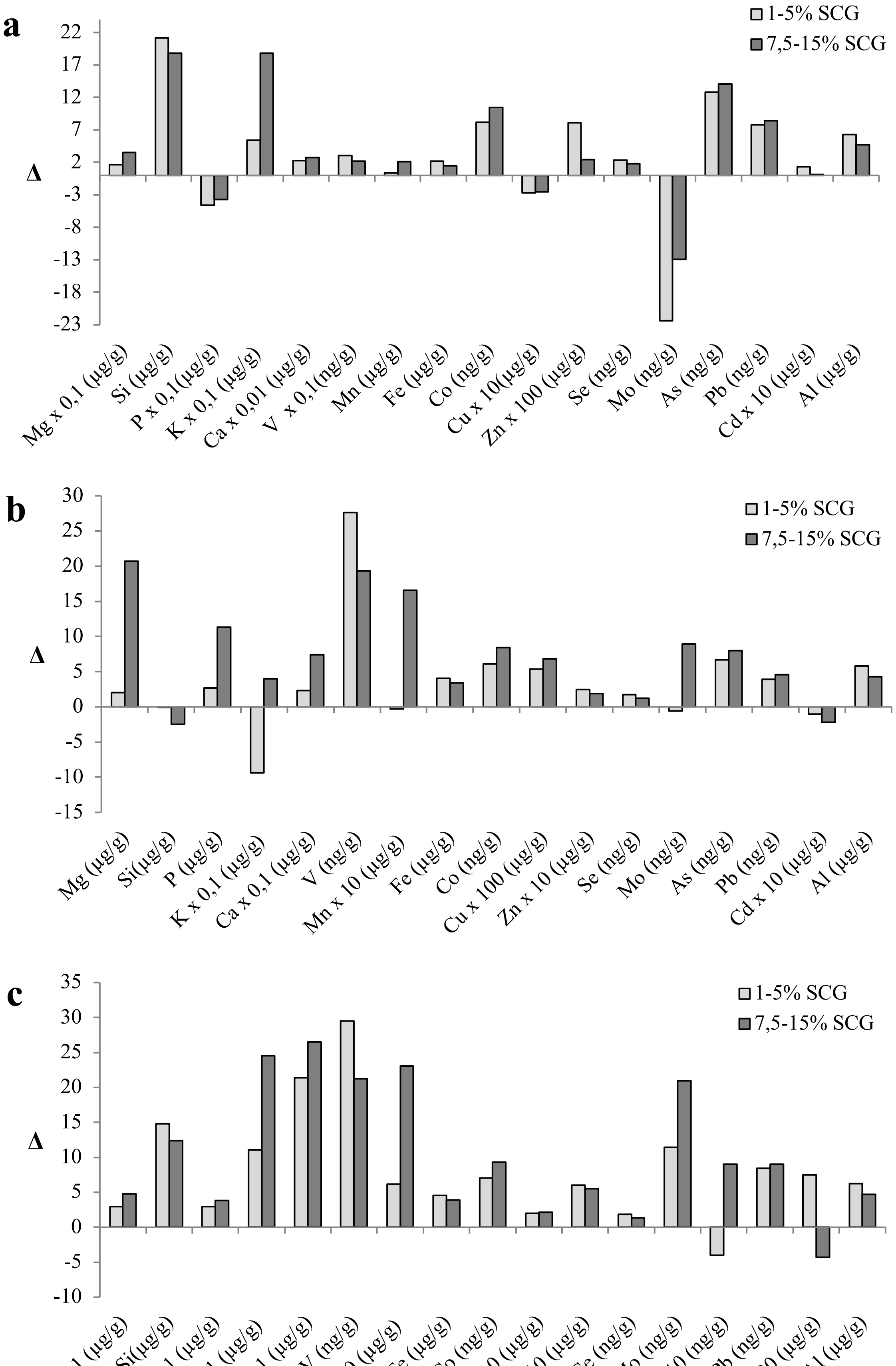
a
1-5% SCG
7,5-15% SCG
Δ
22
17
12
7
2
-3
-8
-13
-18
-23
Mg x 0,1 (µg/g)
Si (µg/g)
P x 0,1(µg/g)
K x 0,1 (µg/g)
Ca x 0,01 (µg/g)
V x 0,1(ng/g)
Mn (µg/g)
Fe (µg/g)
Co (ng/g)
Cu x 10(µg/g)
Zn x 100 (µg/g)
Se (ng/g)
Mo (ng/g)
As (ng/g)
Pb (ng/g)
Cd x 10 (µg/g)
Al (µg/g)
b
1-5% SCG
7,5-15% SCG
Δ
30
25
20
15
10
5
0
-5
-10
-15
Mg (µg/g)
Si(µg/g)
P (µg/g)
K x 0,1 (µg/g)
Ca x 0,1 (µg/g)
V (ng/g)
Mn x 10 (µg/g)
Fe (µg/g)
Co (ng/g)
Cu x 100 (µg/g)
Zn x 10 (µg/g)
Se (ng/g)
Mo (ng/g)
As (ng/g)
Pb (ng/g)
Cd x 10 (µg/g)
Al (µg/g)
c
1-5% SCG
7,5-15% SCG
Δ
35
30
25
20
15
10
5
0
-5
-10
Mg x 0,1 (µg/g)
Si(µg/g)
P x 0,1 (µg/g)
K x 0,1 (µg/g)
Ca x 0,1 (µg/g)
V (ng/g)
Mn x 10 (µg/g)
Fe (µg/g)
Co (ng/g)
Cu x 10 (µg/g)
Zn x 10 (µg/g)
Se (ng/g)
Mo (ng/g)
As x 10 (ng/g)
Pb (ng/g)
Cd x 100 (µg/g)
Al (µg/g)

**Figure 2**

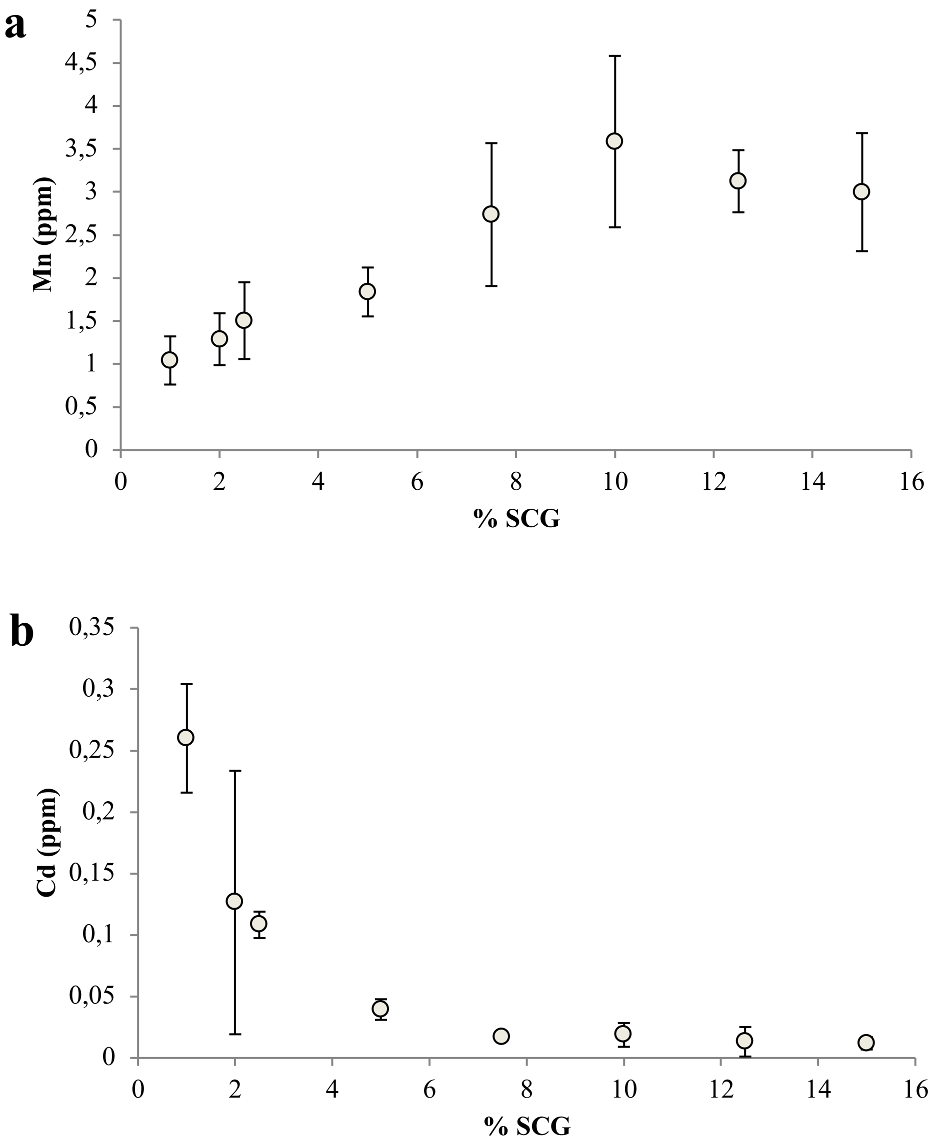
a
Mn (ppm)
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0
2
4
6
8
10
12
14
16
% SCG
b
Cd (ppm)
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0
2
4
6
8
10
12
14
16
% SCG

**Table 1.** Checking of accuracy and precision of the method for measurement of different minerals, employing the standard reference material Bovine muscle powder 8414 of the National Institute for Standards and Technology (NIST).

| Element | (mean SD, µg/g) | |
|---|---|---|
| | Found level | Certified level |
| Mg | 1006 ± 143 | 960 ± 95 |
| Al | 1.60 ± 0.08 | 1.70 ± 1.40 |
| P | 8646 ± 445 | 8360 ± 450 |
| Ca | 167 ± 16.4 | 145 ± 20.0 |
| Mn | 0.270 ± 0.056 | 0.370 ± 0.090 |
| Fe | 84.53 ± 1.89 | 71.2 ± 9.2 |
| V | - | 0.005* |
| K | - | 15170 ± 370 |
| Cu | 3.47 ± 0.08 | 2.84 ± 0.45 |
| Zn | 164.48 ± 3.39 | 142 ± 14 |
| As | 0.0055 ± 0.0045 | 0.0090 ± 0.003 |
| Se | 0.093 ± 0.046 | 0.076 ± 0.010 |
| Mo | 0.036 ± 0.019 | 0.080 ± 0.060 |
| Cd | 0.0049 ± 0.0035 | 0.013 ± 0.011 |
| Pb | 0.496 ± 0.122 | 0.380 ± 0.240 |

*Concentration estimated for V provided as information value by the NIST.

**Table 2.** Contents of essential and toxic* mineral elements in lettuce samples. Data are presented as mean ± SD.

| Mineral (unit) | 0% SCG | 1-5% SCG | 7,5-15% SCG | NPK | Baby lettuce |
|---|---|---|---|---|---|
| Mg (µg/g) | $109.5 \pm 22.7$ | $111.5 \pm 25.7$ | $130.2 \pm 34.4^{a}$ | $82.1 \pm 7.16^{a}$ | $95.11 \pm 9.11$ |
| Si (µg/g) | $21.3 \pm 10.4$ | $21.2 \pm 9.93^{a}$ | $18.8 \pm 10.65$ | $6.39 \pm 3.51^{a}$ | Not detected |
| P (µg/g) | $94.2 \pm 15,3^{a,b}$ | $96.9 \pm 14,7^{c,d}$ | $105.5 \pm 21.4^{e,f}$ | $67.1 \pm 6.74^{a,c,d,e,g}$ | $142.8 \pm 14.6^{b,d,f,g}$ |
| K (µg/g) | $1377 \pm 141.3$ | $1283 \pm 201,2$ | $1417 \pm 232.4$ | $1172 \pm 89,8$ | $1229\pm 127.5$ |
| Ca (µg/g) | $489.0 \pm 107.7$ | $512.3 \pm 112.2^{a,b}$ | $563.4 \pm 130.3^{c,d}$ | $298.1 \pm 11.2^{a.c}$ | $286.8 \pm 24.4^{b,d}$ |
| V (ng/g) | $7.67 \pm 5.97^{a,b}$ | $35.3 \pm 17.7^{a,c,d}$ | $27.0 \pm 13.7^{b,e,f}$ | $5.76 \pm 4,13^{c,e}$ | $4.84 \pm 0.571^{d,f}$ |
| Mn (µg/g) | $1.45 \pm 0.365^{a}$ | $1.42 \pm 0,418^{b,c,d}$ | $3.11 \pm 0,719^{a,b,e,f}$ | $0.806 \pm 0,170^{c,e}$ | $1.03 \pm 0.046^{d,\cancel{e,}f}$ |
| Fe (µg/g) | $1.96 \pm 0.983^{a,b}$ | $6.03 \pm 2.57^{a,c}$ | $5.38 \pm 2.32^{b,d,e}$ | $1.48 \pm 0.442^{c,d}$ | $3.86 \pm 0.246^{e}$ |
| Co (µg/g) | $4.50 \pm 1.47^{a,b}$ | $10.6 \pm 4,35^{a,c,d}$ | $12.9 \pm 3.94^{b,e,f}$ | $3.57 \pm 0.988^{c,e}$ | $2.45 \pm 0.020^{d,f}$ |
| Cu (µg/g) | $0.404 \pm 0.015^{a,b}$ | $0.458 \pm 0.071^{c,d}$ | $0.472 \pm 0.120^{e,f}$ | $0.257 \pm 0.044^{a,c,e,g}$ | $0.726 \pm 0.081^{b,d,f,g}$ |
| Zn (µg/g) | $0.675 \pm 0.076^{a}$ | $0.918 \pm 0.192^{a,b}$ | $0.861 \pm 0.201^{c}$ | $0.313 \pm 0.136^{b,c}$ | $0.837 \pm 0.098$ |
| Se (ng/g) | $1.96 \pm 1.08$ | $3.70 \pm 2.14$ | $3.18 \pm 2.06$ | $1.83 \pm 1.32$ | $1.37 \pm 0.070$ |
| Mo (ng/g) | $19.8 \pm 5.99^{a,b}$ | $19.2 \pm 5.73^{c,d}$ | $28.7 \pm 21.6$ | $7.77 \pm 1.85^{c,a,e}$ | $41.6 \pm 5.24^{d,b,e}$ |
| *As (ng/g) | $13.5 \pm 5.23$ | $20.2 \pm 5.10^{a}$ | $21.5 \pm 5.28^{b}$ | $20.6 \pm 2.96^{c}$ | $7.41 \pm 0.848^{a,b,c}$ |
| *Pb (ng/g) | $8.86 \pm 4.66$ | $12.8 \pm 5.00^{a,b}$ | $13.4 \pm 9.41$ | $4.36 \pm 1.47^{a}$ | $4.98 \pm 0.081^{b}$ |
| *Cd (µg/g) | $0.237 \pm 0.073^{a,b,c}$ | $0.133 \pm 0.097^{d,e,f}$ | $0.015 \pm 0.008^{a,d,g,h}$ | $0.058 \pm 0.008^{b,e,g,i}$ | $0.003 \pm 0.001^{c,f,,g,h,i}$ |
| *Al (µg/g) | $1.39 \pm 1.35^{a,b}$ | $7.19 \pm 3.83^{a,c.d}$ | $5.67 \pm 2.93^{b,e,f}$ | $0.975 \pm 0.692^{c,e}$ | $0.921 \pm 0.075^{d,f}$ |

$^{a,b,c,d}$ The same letters in the same line, indicate statistically significant differences ($p$ <0.05) in the levels of this mineral in the lettuce samples between treatments.

**Table 3**. Amount of the different minerals provided by a portion of lettuce (150 g) grown with the different treatments tested.

| Mineral (unit) | 0% SCG | SCG (mean)** | NPK |
|---|---|---|---|
| Fe (mg) | 0.294 | 0.856 | 0.222 |
| Co (µg) | 0.675 | 1.763 | 0.535 |
| V (µg) | 1.150 | 4.673 | 0.864 |
| Mn (mg) | 0.217 | 0.340 | 0.121 |
| Zn (mg) | 0.101 | 0.134 | 0.047 |
| Mg (mg) | 16.42 | 18.13 | 12.31 |
| Si (mg) | 3.195 | 3.000 | 0.958 |
| P (mg) | 14.13 | 15.18 | 10.06 |
| K (mg) | 206.6 | 202.5 | 175.8 |
| Ca (mg) | 73.30 | 80.65 | 44.70 |
| Se (µg) | 0.294 | 0.516 | 0.274 |
| Cu (µg) | 60.60 | 69.75 | 38.50 |
| Mo (µg) | 2.970 | 3.593 | 1.166 |
| *As (µg) | 2.025 | 3.128 | 3.090 |
| *Pb (µg) | 1.329 | 1.965 | 0.654 |
| *Cd (µg) | 35.55 | 11.08 | 8.700 |
| *Al (µg) | 208.5 | 964.5 | 146.2 |

*Toxic metals; **Average between low and high percentages of SCG.

**Table 4**. Mineral dietary reference intakes (DRIs) and percent of DRIs, for healthy adult men and women (Institute of Medicine, 2005) provided by a portion of lettuce (150 g) grown with SCG (average between low and high SCG values)

| Mineral | DRIs (mg) | | %DRIS | |
|---|---|---|---|---|
| | | | SCG (mean)* | |
| | Women | Men | Women | Men |
| Fe | 18 | 8 | 4.75 | 10.69 |
| Mn | 1.8 | 2.3 | 18.86 | 14.76 |
| Zn | 8 | 11 | 1.67 | 1.21 |
| Mg | 320 | 420 | 5.66 | 4.32 |
| P | 700 | 700 | 2.17 | 2.17 |
| K | 4700 | 4700 | 4.31 | 4.31 |
| Ca | 1000 | 1000 | 8.07 | 8.07 |
| Se | 0.055 | 0.055 | 0.94 | 0.94 |
| Cu | 0.9 | 0.9 | 7.75 | 7.75 |
| Mo | 0.045 | 0.045 | 7.98 | 7.98 |

*Average between low and high percentages of SCG