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Characterization of a *Triticum aestivum* L. Experimental Field to Implement an Agronomic Biofortification Workflow †

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Abstract: Soils provide plants both with a physical home and all the essential nutrients and support they crave to thrive. Such circumstances pave the way for a close analysis of the level of viability of different types of soils, and hence the need to assess the suitability of the experimental field in which to implement an agronomic biofortification itinerary. Thus, soil samples were collected from different sites of a wheat field. A rectangular grid was applied. Afterwards, pH and electrical conductivity were determined with a potentiometer; the mineral quantification was measured using an XRF analyzer and color analysis were performed with a Minolta CR 400 colorimeter. Moisture and organic matter content analyses were also carried out. No significant differences were found when considering the moisture content, pH, electrical conductivity, and the mineral values of Fe and Mn. As opposed to this, slight differences were observed in organic matter content, color parameters, and in Ca, K, S, Cu, and Zn. Concerning the macroelements, the most prevalent mineral was Ca, followed by K and S. As for the microelements, Zn was the least dominant mineral, as opposed to Cu, Mn, and Fe. Data showed that this experimental field has proven to be eligible to implement an agronomic biofortification workflow due to the slightly acid pH and the lower amount of organic matter content.

Keywords: color analyses; mineral quantification; organic matter; soil analyses

1. Introduction

The world population, in 2019, reached around 7.7 billion, and is estimated to grow to about 9 billion in 2050 and to surpass 10 billion people in the year of 2100 [1]. By this means, it is essential to foster new strategies likely to enhance food production within a certain quality standard such as agronomic biofortification of staple crops [2]. The staple crop *Triticum aestivum* L. is considered to be one of the most produced cereals in the world and is forecasted to have a world production of about 770 million tons by 2021/2022 [3]. Soils supply plants with a physical home as well as all the essential nutrients and the support that enable them to prosper [4]. Such a circumstance facilitates a close analysis of the viability degree of different types of soils. Therefore, this work aims to assess the suitability of the experimental field in which to implement an agronomic biofortification itinerary. Hence the need to perform a study on the mineral quantification of the macroelements sulfur (S), potassium (K), and calcium (Ca) and the microelements manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn), in addition to studying the determination of the color parameters (L*, a* and b*), pH, electrical conductivity, moisture, and organic matter contents of soil samples.

2. Materials and Methods

2.1. Experimental Field

On 14 December 2018, soil samples were collected (approximately between 600 and 1000 g), at a depth of about 0–30 cm, from 13 sampling points (in the end we grouped into 4 samples) following a sampling rectangular grid of 23 × 22 m. The experimental field is located in Beja, Portugal (37°57′09.68″ N; 7°30′26.82″ W) and is intended for the cultivation of *Triticum aestivum* L. (cv. Paiva and Roxo) biofortified in different Zn fertilizers.

2.2. Soil Analyses

The soil samples were processed and the determination of moisture content, organic matter content, pH, and electrical conductivity were conducted according to [5] with the minor change that implied using a rectangular grid of 23 × 22 m. An XRF analyzer (model XL3t 950 32 He GOLDD +) was used to measure the mineral content of soil samples under helium atmosphere [6]. The colorimetric parameters (L*—lightness of each sample varying between dark (0) and light (100); a*—color variations between green (−60) and red (+60); and b*—color variations between blue (−60) and yellow (+60)) of the soil samples were analyzed (in triplicate) using a Minolta CR 400 colorimeter (Minolta Corp., Ramsey, NJ, USA) according to [7].

2.3. Statistical Analyses

Data was statistically analyzed using software R (version 3.6.3) to estimate the correlation matrix of the Pearson and Spearman coefficients of the different analyses and a one-way ANOVA ($p \leq 0.05$) was used to assess significant differences. Based on the results, a Tukey's test for mean comparison was performed, considering a 95% confidence level.

3. Results

Soil analyses of pH, electrical conductivity, and moisture content did not yield significant differences among the four different soil samples (Table 1). It was verified that the sample S1 stands out from samples S2, S3, and S4, presenting the lowest values concerning electrical conductivity and moisture content as opposed to the highest values for pH and organic matter. The opposite was confirmed for samples S2 and S3 (except moisture content). The values of pH were all approximately 7, in which they presented as slightly acidic. Electrical conductivity varied between 271 and 361 $\mu S \cdot cm^{-1}$ and moisture content presented an interval of values from 11.5 to 17.3%. The sample S1 showed higher values when compared to the samples S2 and S3 (almost half the values of S1).

Table 1. Soil analyses (samples collected at depth of 0–30 cm) of *Triticum aestivum* L. experimental field's pH, electrical conductivity, organic matter, and moisture contents ($n = 3$). Letters a, b indicate significant differences of each parameter, considering different samples (statistical analyses using the single-factor ANOVA test, $p < 0.05$).

Samples	pH (H_2O)	Electrical Conductivity	Moisture Content	Organic Matter Content
		$\mu S \cdot cm^{-1}$	%	
S1	7.06 ± 0.188 a	271 ± 25.7 a	11.5 ± 2.61 a	7.11 ± 0.646 a
S2	6.77 ± 0.213 a	361 ± 15.2 a	15.3 ± 1.69 a	4.44 ± 0.473 b
S3	6.76 ± 0.0613 a	358 ± 44.3 a	16 ± 0.445 a	4.64 ± 0.136 b
S4	6.83 ± 0.0921 a	313 ± 39.4 a	17.3 ± 1.15 a	5.31 ± 0.0463 b

The minerals S, K, Ca (except for in samples S1 and S3), Cu (apart from in samples S1, S2 and S3), and Zn showed significant differences among the different soil samples, whereas Mn and Fe did not (Table 2). Concerning the macroelements, the most prevalent mineral was Ca, followed by K and S. As for the microelements, Zn was the least dominant mineral, as opposed to Cu, Mn, and Fe. The sample S4 revealed the highest values for all the microelements. Moreover, S3 was the top sample for S and Ca (S1 was the highest for K). As for the macroelements K and Ca, although S4 presented the lowest values, S2 revealed the lowest values for S. Regarding S1 and S2, these samples showed lower values for Cu and Zn, and for Mn and Fe, respectively. The minerals Mg and P presented values lower than 1500 and 200 $mg \cdot kg^{-1}$, respectively. In general, there was a strong and positive correlation between the minerals relating to the Spearman correlation: Ca–K for samples S1, S3, and S4; Zn–Cu for samples S1 and S3; Zn–Fe for samples S1, S2, and S3; Zn–Mn for samples S1 and S2; Cu–Mn for samples S1 and S3; and Fe–Mn (Table 3). In addition, there was a strong and positive correlation between the minerals regarding the Pearson correlation: Cu–Zn for samples S1, S2, and S3; Fe–Zn for samples S1, S2, and S3; Fe–Cu for samples S1, S2, and S3; Mn–Zn for samples S1, S2, and S3; Mn–Cu for samples S1, S2, and S3; and Mn–Fe. By contrast, for both Spearman and Pearson correlations, there was a strong and negative correlation between the minerals for the samples: S1 (the mineral S with the minerals Zn, Cu, Fe, and Mn); S2 (the mineral Ca with the minerals Zn, Fe, and Mn; and the mineral S with K); S3 (the mineral K with the minerals S, Zn, and Cu only for the Pearson correlation); and S4 (the mineral Cu with the minerals Ca, K, Fe, and Mn).

Table 2. Soil analyses (samples collected at depth of 0–30 cm) of *Triticum aestivum* L. experimental field's mineral quantification of S, K, Ca, Mn, Fe, Cu, Zn ($n = 9$). Letters a, b indicate significant differences of each parameter, considering different samples (statistical analysis using the single-factor ANOVA test, $p < 0.05$). Mg and P presented values lower than the detection limit of the equipment.

Samples	S	K	Ca	Mn	Fe	Cu	Zn	Mg	P
		%				$mg \cdot kg^{-1}$			
S1	0.0195 ± 0.0005 ab	0.0899 ± 0.004 a	1.182 ± 0.053 a	495 ± 59 a	21759 ± 1895 a	79.9 ± 1.9 b	20.9 ± 1.3 b	<1500	<200
S2	0.0191 ± 0.001 b	0.0841 ± 0.002 ab	1.042 ± 0.063 ab	446 ± 37 a	21296 ± 1572 a	91.2 ± 4.71 b	22.1 ± 0.939 ab		
S3	0.0218 ± 0.0005 a	0.0835 ± 0.001 ab	1.183 ± 0.053 a	480 ± 42 a	22408 ± 1424 a	79.9 ± 4.81 b	22.2 ± 1.12 ab		
S4	0.0209 ± 0.0009 ab	0.0755 ± 0.002 b	0.9787 ± 0.026 b	619 ± 59 a	24311 ± 1010 a	116 ± 1.88 a	26.2 ± 0.885 a		

Table 3. Correlation matrices of Spearman (the top of the diagonal) and Pearson (the bottom of the diagonal) coefficients of mineral quantification of soils (Ca, K, S, Zn, Cu, Fe, and Mn) for soil samples S1 (**a**), S2 (**b**), S3 (**c**), and S4 (**d**).

(**a**)

S1	**Ca**	**K**	**S**	**Zn**	**Cu**	**Fe**	**Mn**
Ca	1	1	0.5	−0.5	−0.5	−0.5	−0.5
K	0.93	1	0.5	−0.5	−0.5	−0.5	−0.5
S	0.35	−0.015	1	−1	−1	−1	−1
Zn	−0.69	−0.38	−0.92	1	1	1	1
Cu	−0.89	−0.67	−0.73	0.94	1	1	1
Fe	−0.6	−0.27	−0.96	0.99	0.89	1	1
Mn	−0.78	−0.5	−0.86	0.99	0.98	0.97	1

(**b**)

S2	**Ca**	**K**	**S**	**Zn**	**Cu**	**Fe**	**Mn**
Ca	1	0.5	−0.5	−1	−0.5	−1	−1
K	−0.14	1	−1	−0.5	0.5	−0.5	−0.5
S	0.2	−1	1	0.5	−0.5	0.5	0.5
Zn	−0.93	−0.23	0.17	1	0.5	1	1
Cu	−1	0.21	−0.27	0.91	1	0.5	0.5
Fe	−0.95	−0.17	0.1	1	0.93	1	1
Mn	−1	0.11	−0.18	0.94	1	0.96	1

(**c**)

S3	**Ca**	**K**	**S**	**Zn**	**Cu**	**Fe**	**Mn**
Ca	1	1	−0.33	−0.32	−0.32	0.32	0.32
K	0.61	1	−0.33	−0.32	−0.32	0.32	0.32
S	−0.19	−0.89	1	0.95	0.95	0.74	0.74
Zn	−0.003	−0.74	0.91	1	0.8	0.8	0.6
Cu	−0.19	−0.89	1	0.91	1	0.6	0.8
Fe	0.53	−0.35	0.73	0.79	0.73	1	0.8
Mn	0.56	−0.31	0.71	0.74	0.71	1	1

(**d**)

S4	**Ca**	**K**	**S**	**Zn**	**Cu**	**Fe**	**Mn**
Ca	1	1	−0.5	−0.5	−1	1	1
K	0.82	1	−0.5	−0.5	−1	1	1
S	0.021	−0.55	1	−0.5	0.5	−0.5	−0.5
Zn	−0.42	0.17	−0.92	1	0.5	−0.5	−0.5
Cu	−0.96	−0.95	0.27	0.14	1	−1	−1
Fe	0.85	1	−0.51	0.11	−0.97	1	1
Mn	1	0.86	−0.054	−0.35	−0.98	0.89	1

The colorimetric parameters L* (lightness), a* (red–green transitions), and b* (yellow–blue transitions) showed significant differences among the different soil samples before and after performing organic matter content, except for the parameter a* (Figure 1). The soil samples, before the organic matter content, presented lower values in the three parameters, excluding the S2 (-a and -b) and S3 (-a and -b) samples in the L* parameter. Concerning the samples before the organic matter content, S3a and S4a displayed, respectively, the highest and the lowest values of the three parameters. Furthermore, after the analysis, for the parameters L* and a*, the sample S1b showed the highest values as did the sample S2b in b*. Finally, S4b revealed the lowest values in the parameters L* and b*. Conversely, S3b presented the lowest value in a*. After the analysis was run, samples S1 and S2 revealed a circa elevenfold increase compared to the color before and, approximately, a thirtyfold increase concerning S4. In general, the results of the three parameters indicated a major contribution of the dark, green, and blue colors.

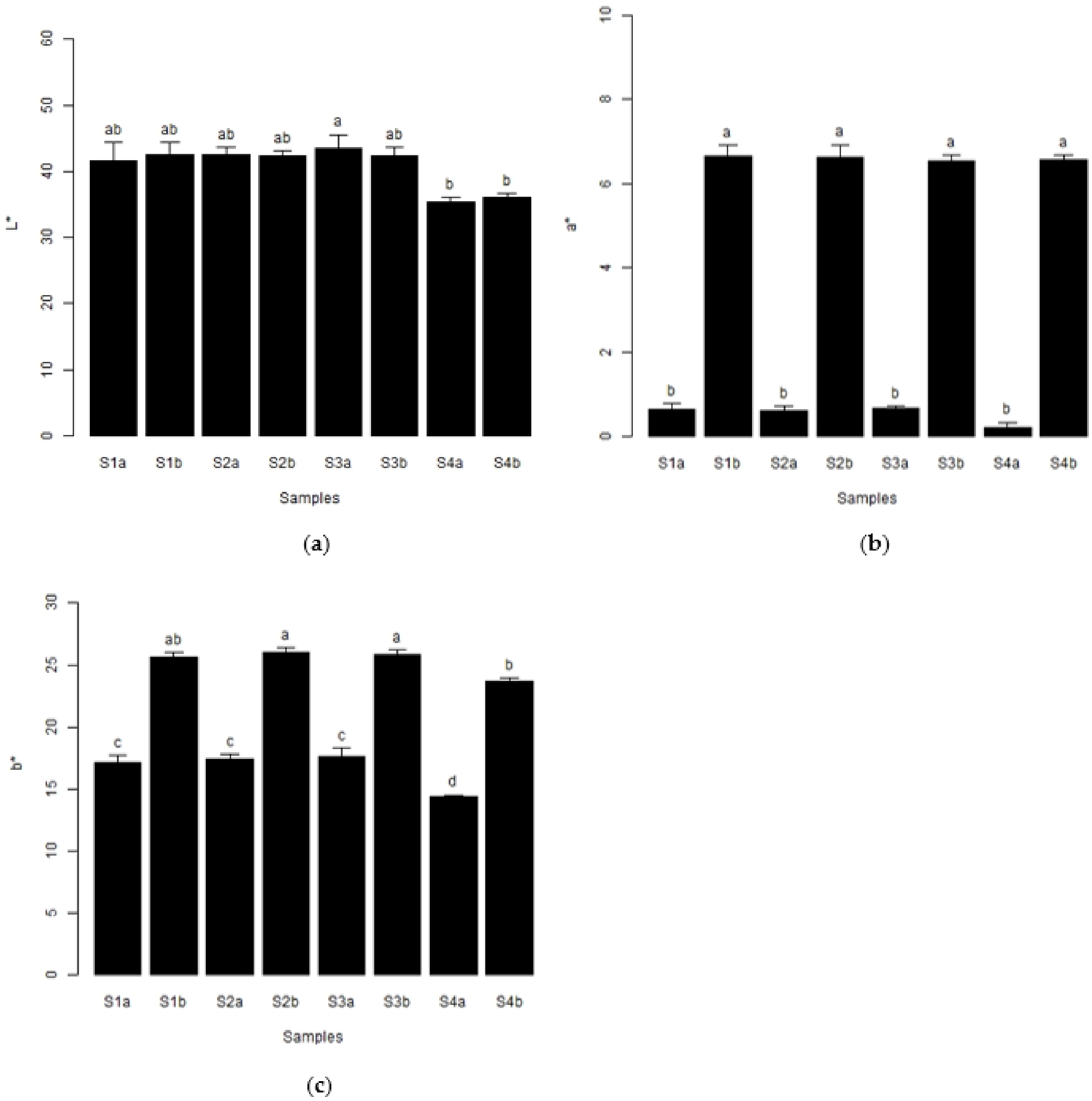

Figure 1. Colorimeter CIELab System with the color parameters (n = 9): (**a**) L* (lightness); (**b**) a* (red–green transitions); and (**c**) b* (yellow–blue transitions) of soil samples before (S1a, S2a, S3a, and S4a) and after (S1b, S2b, S3b, and S4b) performing organic matter content. Letters a, b, c, d indicate significant differences of each parameter, considering different samples (statistical analysis using the single-factor ANOVA test, $p < 0.05$).

4. Discussion

To begin with, as pH, electrical conductivity, and moisture content did not present significant differences, we can presume that this field is not heterogenous. It is verified that for soils with a basic pH, Zn becomes less available in the soil according to [8]. The range of values between 5.5 and 7.0 is considered to be ideal for wheat to thrive [9]. Bearing this in mind, the fact that the values obtained for the pH were within the range of 6.76 to 7.06 might indicate that this field is suitable to implement in the study. Nevertheless, soils with low levels of organic matter content tend to be deficient in Zn [9], whereas the results of our study revealed values between 4.44 and 7.11%. According to [10], the minerals K, Fe, and Mn move in the soil by diffusion, whereas Zn and Mn move by root interception and, finally, S, Ca, Fe, Cu, and Zn move by mass flow. There are studies that reveal that the uptake of Zn by wheat is inhibited in the presence of K and Ca, as observed in our work [8]. Our data implied a synergistic interaction between Zn and Fe which is corroborated by [11], whereas the antagonistic relationships between S and Fe, and between Ca and the minerals Zn, Fe, Cu, and Mn are in line with [10].

5. Conclusions

Soil analyses of moisture content, electrical conductivity, and pH did not show significant differences among the different soil samples; nevertheless, it was verified that the sample S1 stood out, presenting the lowest values concerning moisture content and electrical conductivity, and the highest values for organic matter and pH. Regarding the macroelements, the most predominant was Ca, followed by K and S, whereas for the microelements, Zn was the least dominant, as opposed to Cu, Mn, and Fe (in which S4 showed the highest values for all the microelements). The color of the soil samples, before the organic matter content was analyzed, presented lower values in the three parameters. After the analysis, samples S1 and S2 revealed a circa elevenfold increase compared to the color before and, approximately, a thirtyfold increase concerning S4. In general, the results of the three parameters indicated a major contribution of the dark, green, and blue colors. To sum up, this experimental field is proven to be eligible to implement an agronomic biofortification workflow.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IOCAG2022-12304/s1.

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Abbreviations

The following abbreviations are used in this manuscript:

S (1, 2, 3, and 4)	Soil samples collected from different places in the experimental field.
S (1, 2, 3, and 4) a	Soil samples before the analysis of organic matter content.
S (1, 2, 3, and 4) b	Soil samples after the analysis of organic matter content.

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