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Proceeding Paper

Mineral Quantification of *Triticum aestivum* L. Enriched in Zinc—Correlation between Minerals in Soils and Whole Wheat Flours †

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Abstract: *Triticum aestivum* L. is one of the most produced staple crops worldwide in which its zinc biofortification is of the utmost importance to diminish malnutrition. In addition, the pronounced increase of human population demands a higher food production within quality standards. Zinc plays an important role not only in promoting the maintenance of human health, but it is also linked with plant growth. Under this framework, a zinc agronomic biofortification of *Triticum aestivum* L. was implemented in an experimental field with two varieties (Paiva and Roxo) in Beja, Portugal. This itinerary comprised two $ZnSO_4$ foliar spraying along the plant cycle with three different concentrations (control—0; 8.1 and 18.2 kg ha^{-1}). Soil analyses (moisture, organic matter, pH, electrochemical conductivity and mineral quantification) and atomic absorption with the mineral quantification (Ca, K, Mg, P, Fe, Cu and Zn) of whole wheat flours were carried out. Zinc foliar spraying enhanced Zinc content in both varieties in the flours in which was not observed significant differences between $ZnSO_4$ treatments. P and K presented higher values in the flours contrasting with Ca and Mg. In general, there was no significant differences between the soil samples in the respective analyses. It was concluded that wheat flour biofortified in zinc can be a product to help overcome malnutrition.

Keywords: agronomic biofortification; mineral interactions; soil analyses; *Triticum aestivum* L; zinc foliar spraying

1. Introduction

The world's population is expected to increase to more than 8 billion by 2030 [1]. Thereby, according to [2] the food production will have to increase approximately 60% by 2050, in a sustainable way and keeping quality standards. *Triticum aestivum* L. is one of the most produced staple crops worldwide. Thus, it is estimated to reach a production of 776.7 million tons by 2021/2022 [3]. Zinc (Zn) plays an important role (at the function, structure and regulation level) not only in promoting the maintenance of human health, but it is also linked with plant growth [4,5]. Biofortification is likely to diminish malnutrition figures, provided that an essential nutrient in the edible part of staple crops is enhanced and becomes bioavailable [6,7]. This paper aims to analyze the correlations between the minerals present in the sample soils collected from the experimental field which was subjected to a Zn biofortification workflow. A further study was also conducted as a means of investigating the interactions between the minerals present in the whole bread wheat flours of two varieties of *Triticum aestivum* L.

2. Materials and Methods

2.1. Experimental Field

Triticum aestivum L. Roxo and Paiva varieties were cultivated in an experimental field at 37°57′09.68″ N; 7°30′26.82″ W, in Beja (Portugal). The last days of December 2018, brought the sowing of the bread wheat field; whereas the harvest season fell by the end of June 2019. The sowing was conducted in a randomized block design with four repetitions. This field has been divided into 24 plots, each one with an area of 12 m^2 (10 m × 1.2 m), comprising 3 m between repetitions and 0.4 m between plots. A NPK fertilization and 50 kg $Zn{\cdot}ha^{-1}$ were applied in the field beforehand. The Zn biofortification comprised $ZnSO_4$ foliar spraying at booting and heading stages, in late April 2019, with three different concentrations applied (0—control (T0), 8.1 (T1) and 18.2 (T2) $kg{\cdot}ha^{-1}$) and 46% of urea. The total rainfall accumulation was about 5.43 mm, with a daily maximum of 1.85 mm, during the plant life cycle.

2.2. Soil Analyses of the Experimental Field

The soil samples were processed and the determination of moisture content, organic matter content, pH and electrical conductivity were conducted according to [8] with the minor change of using a rectangular grid of 23 × 22 m. An XRF analyzer (model XL3t 950 He GOLDD+) was used to measure the mineral content of soil samples, under helium atmosphere [9].

2.3. Mineral Quantification of Whole Bread Wheat Flours through Atomic Absorption Spectrometry

Whole bread wheat flour samples were analyzed according to the method of [10] using the Perkin Elmer Instruments AAnalyst 200 Atomic Absorption Spectrophotometer, with AA WinLAb software. Initially, about 1 g of each sample was weighed, placed in a 50 mL Erlenmeyer flask and 10 mL of nitric acid was added (acid digestion). Then, it was heated between 100 and 150 °C until total evaporation occurred and a solution of HNO_3:$HClO_4$ (2:3 mL) was added. Afterwards, the whole procedure was repeated, and the precipitate was dissolved in a 2% HCl solution, being filtered with Whatman paper nº 4 into a 50 mL volumetric flask. A standard solution of 2% HCl was prepared and the absorbances of the flour samples were measured in the spectrophotometer.

2.4. Statistical Analyses

Software R (version 3.6.3) was used to statistically analyze data. Such analyzes comprised One-Way ANOVA ($p \leq 0.05$) to evaluate the differences between the samples of different varieties and treatments. Considering a 95% confidence level, a Tukey's test for mean comparison was performed. Furthermore, this software permitted the obtainment of the correlation matrix of the Spearman and Pearson coefficients for the minerals present in the soils and in the whole bread wheat flours.

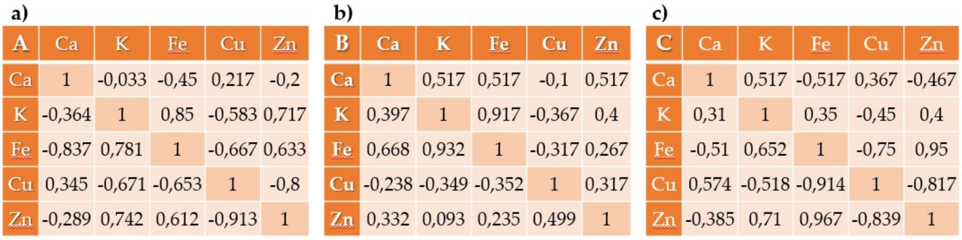
3. Results

Soil analyzes pH, electrical conductivity, organic matter and moisture contents and the values of calcium (Ca), iron (Fe) and Zn did not show significant differences among the different soil samples (Table 1). The values of pH were slightly above 7 and the electrical conductivity varied between 412 and 568 μS.cm^{-1}. Potassium (K) showed lower values when compared to Ca (almost twice the values of K). The minerals quantification demonstrated higher levels of Fe, followed by Zn and copper (Cu) (Cu and Zn showed similar values). The minerals Mg and P presented values lower than 1500 and 200 mg.kg^{-1}, respectively. There was a strong and positive correlation between the minerals: K and Fe for samples A and B; K and Zn for samples A and C; and Fe and Zn for the sample C (Table 2). By contrast, there was a strong and negative correlation between the minerals: Cu and Zn for samples A and C; Fe and Ca for sample A; and Fe and Cu for the sample C.

Table 1. Soil analyses (samples collected at 0–30 cm deep) of *Triticum aestivum* L. experimental field ($n = 3$ for pH, electrical conductivity, organic matter and moisture contents; and $n = 9$ for mineral quantification of K, Ca, Fe, Cu, Zn). 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	pH (H_2O)	Electrical Conductivity	Organic Matter	Moisture	K	Ca	Fe	Cu	Zn	Mg	P
		μS.cm^{-1}		%				mg.kg^{-1}			
A	7.70 ± 0.05 a	543 ± 44 a	6.92 ± 0.02 a	22.52 ± 0.17 a	0.652 ± 0.008 a	1.26 ± 0.11 a	38,136 ± 278 a	43.45 ± 2.31 b	59.11 ± 1.20 a	<1500	<200
B	7.57 ± 0.10 a	412 ± 19 a	6.71 ± 0.07 a	22.62 ± 0.16 a	0.643 ± 0.029 ab	1.01 ± 0.05 a	39,390 ± 257 a	44.56 ± 1.057 ab	60.36 ± 1.00 a		
C	7.73 ± 0.08 a	568 ± 47 a	6.95 ± 0.12 a	22.29 ± 0.38 a	0.572 ± 0.020 b	1.23 ± 0.12 a	38,806 ± 1065 a	52.70 ± 3.591 a	59.96 ± 1.78 a		

Table 2. Correlation matrix of Spearman (the top of the diagonal) and Pearson's (the bottom of the diagonal) and coefficients of the minerals Ca, K, Fe, Cu and Zn of soil samples A (a), B (b) and C (c) for the experimental field.



a)

A	Ca	K	Fe	Cu	Zn
Ca	1	-0,033	-0,45	0,217	-0,2
K	-0,364	1	0,85	-0,583	0,717
Fe	-0,837	0,781	1	-0,667	0,633
Cu	0,345	-0,671	-0,653	1	-0,8
Zn	-0,289	0,742	0,612	-0,913	1

b)

B	Ca	K	Fe	Cu	Zn
Ca	1	0,517	0,517	-0,1	0,517
K	0,397	1	0,917	-0,367	0,4
Fe	0,668	0,932	1	-0,317	0,267
Cu	-0,238	-0,349	-0,352	1	0,317
Zn	0,332	0,093	0,235	0,499	1

c)

C	Ca	K	Fe	Cu	Zn
Ca	1	0,517	-0,517	0,367	-0,467
K	0,31	1	0,35	-0,45	0,4
Fe	-0,51	0,652	1	-0,75	0,95
Cu	0,574	-0,518	-0,914	1	-0,817
Zn	-0,385	0,71	0,967	-0,839	1

No significant differences were observed between $ZnSO_4$ treatments for both varieties in the minerals magnesium (Mg), phosphor (P), Ca and Cu (Table 3). Relatively to the minerals, P and K presented higher values in the flours contrasting with Mg and Ca. While assessing values of the microelements, Cu presented lower values than Zn and Fe. When comparing control samples (T0), it was found that Paiva variety presented higher mineral content for all the minerals. Zinc foliar spraying enhanced Zn content in Paiva and Roxo varieties in the flours. After applying Zn fertilizer in Paiva a decrease of the mineral content for Fe, K, Ca and Mg was observed. In contrast, considering Roxo, an increase of the mineral content for the minerals P, K, Mg, Fe, Zn and Cu regarding control samples, was observed. A strong and positive correlation was presented between the minerals: Zn and Ca, and K and Zn for samples PT0, RT0 and RT1; K and P for samples PT0, PT1 and RT1; K and Mg, Mg and Zn and Mg and Ca for samples PT0, PT1, RT0 and RT1; P and Mg for samples PT0, PT1, PT2 and RT1; K and Ca for samples PT0, PT1, PT2, RT0, RT1 and RT2;

Zn and Cu, K and Cu, and Mg and Cu for samples PT1, RT0, and RT1; Zn and P and Cu and P for samples PT1 and RT1; Fe and Ca and Fe and K for samples PT1 and PT2; Fe and Mg and P and Fe for samples PT1 and RT2; Fe and Cu for sample PT1; Cu and Ca for samples RT0 and RT1; and P and Ca for samples RT1 and RT2 (Table 4). Conversely, there was a strong and negative correlation between the minerals: Fe and Cu for sample PT0; Zn and P for samples PT2, RT0 and RT2; Cu and P for samples RT0 and RT2; K and Mg for sample PT2 and RT2; K and P and P and Ca for samples PT2 and RT0; P and Ca, and P and Mg for sample RT0; P and Fe, Fe and Mg, and Mg and Ca for sample PT2; and Zn and Fe, Cu and Ca, Zn and Ca, and K and Cu for the sample RT2.

Table 3. Mean mineral contents of whole wheat flour of *Triticum aestivum* L. (cvs. Paiva and Roxo) (n = 3) after foliar spraying.

Variety	Treatment	Mg	P	K	Ca	Fe	Cu	Zn
				%			mg.kg^{-1}	
Paiva (P)	T0	8.87 ± 0.03 a	115 ± 3.00 a	65.54 ± 5.32 b	1.87 ± 0.13 a	5.71 ± 0.10 a	0.241 ± 0.002 a	0.653 ± 0.013 b
	T1	8.78 ± 0.30 a	138 ± 6.50 a	63.47 ± 1.39 a	1.04 ± 0.12 a	0.58 ± 0.17 ab	0.276 ± 0.013 a	0.739 ± 0.035 a
	T2	8.32 ± 0.44 a	118 ± 11.6 a	63.89 ± 4.88 b	1.19 ± 0.30 a	1.44 ± 0.99 c	0.239 ± 0.006 a	1.143 ± 0.099 a
Roxo (R)	T0	8.56 ± 0.13 a	98.1 ± 6.20 a	59.41 ± 5.97 b	1.31 ± 0.26 a	3.33 ± 0.06 bc	0.225 ± 0.021 a	0.638 ± 0.145 b
	T1	9.04 ± 0.14 a	111 ± 3.20 a	69.73 ± 1.14 ab	1.58 ± 0.055 a	4.13 ± 0.10 ab	0.263 ± 0.003 a	1.177 ± 0.018 a
	T2	8.92 ± 0.03 a	118 ± 2.80 a	60.92 ± 4.95 b	1.24 ± 0.10 a	3.970 ± 0.10 ab	0.257 ± 0.001 a	1.175 ± 0.011 a

Table 4. Correlation matrix of Spearman (the top of the diagonal) and Pearson (the bottom of the diagonal) and coefficients of the minerals Zn, Cu, Fe, Ca, K, P and Mg of *Triticum aestivum* L. (cvs Roxo and Paiva) whole bread wheat flours for experimental field. With the foliar application of $ZnSO_4$: T0 = control ((a,d)); T1 corresponds to 8.1 ((b,e)) and T2 to 18.2 kg·ha^{-1} ((c,f)).

a)

Paiva T0	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	-0,5	0,5	1	0,5	0,5	0,5
Cu	-0,514	1	-1	-0,5	0,5	0,5	0,5
Fe	0,645	-0,987	1	0,5	-0,5	-0,5	-0,5
Ca	0,982	-0,342	0,487	1	0,5	0,5	0,5
K	0,831	0,05	0,111	0,922	1	1	1
P	0,547	0,436	-0,287	0,697	0,92	1	1
Mg	0,823	0,064	0,096	0,916	1	0,926	1

b)

Paiva T1	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	1	0,5	0,5	0,5	1	0,5
Cu	0,968	1	0,5	0,5	0,5	1	0,5
Fe	0,521	0,719	1	1	1	0,5	1
Ca	0,203	0,443	0,941	1	1	0,5	1
K	0,668	0,834	0,983	0,864	1	0,5	1
P	0,927	0,992	0,803	0,556	0,899	1	0,5
Mg	0,895	0,978	0,847	0,619	0,93	0,997	1

c)

Paiva T2	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	0,5	0,5	0,5	0,5	-0,5	-0,5
Cu	0,343	1	-0,5	-0,5	-0,5	0,5	0,5
Fe	0,486	-0,654	1	1	1	-1	-1
Ca	0,635	-0,508	0,984	1	1	-1	-1
K	0,695	-0,437	0,966	0,997	1	-1	-1
P	-0,721	0,403	-0,956	-0,993	-0,999	1	1
Mg	-0,697	0,434	-0,965	-0,997	-1	0,999	1

d)

Roxo T0	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	0,5	0,5	0,5	1	-1	0,5
Cu	0,971	1	-0,5	1	0,5	-0,5	1
Fe	0,452	0,225	1	-0,5	0,5	-0,5	-0,5
Ca	0,784	0,91	-0,199	1	0,5	-0,5	1
K	1	0,973	0,443	0,79	1	-1	0,5
P	-0,999	-0,96	-0,488	-0,758	-0,999	1	-0,5
Mg	0,8	0,92	-0,174	1	0,806	-0,775	1

e)

Roxo T1	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	0,5	-0,5	0,5	0,5	1	0,5
Cu	0,873	1	0,5	1	1	0,5	1
Fe	-0,462	0,03	1	0,5	0,5	-0,5	0,5
Ca	0,942	0,986	-0,138	1	1	0,5	1
K	0,935	0,989	-0,119	1	1	0,5	1
P	0,995	0,821	-0,547	0,905	0,896	1	0,5
Mg	0,817	0,994	0,135	0,963	0,968	0,756	1

f)

Roxo T2	Zn	Cu	Fe	Ca	K	P	Mg
Zn	1	0,5	-1	-0,5	0,5	-0,5	-0,5
Cu	0,699	1	-0,5	-1	-0,5	-1	0,5
Fe	-0,835	-0,192	1	0,5	-0,5	0,5	0,5
Ca	-0,761	-0,996	0,279	1	0,5	1	-0,5
K	-0,29	-0,887	-0,283	0,842	1	0,5	-1
P	-0,994	-0,774	0,77	0,828	0,394	1	-0,5
Mg	-0,454	0,319	0,869	-0,232	-0,72	0,353	1

4. Discussion

Since all the soil analyzes, except for the minerals K and Cu, did not present significant differences, we can presume that the experimental field is homogeneous. The mineral K plays an important roll in plants metabolism, for example in the regulation of the opening and closing of stomates, activation of some enzymes and balancing the use of N. This mineral has an antagonist effect on absorb Ca, Mg and P [11]. According to [12,13], K presents a synergetic interaction with Fe. Besides, K moves in soils by diffusion and is mobile in the plant [12,13]. The minerals Mg, Ca, Fe, Cu and Zn move in the soil by mass flow, whereas the minerals P and Fe, move in soil by diffusion [13]. Regarding the mineral's mobility in plant, the minerals are P, mobile and the minerals are Mg (relatively immobile), Ca, Fe, Cu and Zn immobile [12,13].

One of the functions of Ca is to be a cofactor of various enzymes of ATP. The mineral has an antagonist interaction with Cu, Fe and Zn, but also interacts with Cu and Zn in a synergetic way [8,13]. Magnesium is a component of chlorophyll and functions as an enzyme activator in plants. This mineral interacts with Cu, Fe and Zn in an antagonist way, however, presents a synergetic interaction with Zn [12,13]. The mineral P is an important constituent of nucleic acids, proteins, metabolic substrates and coenzymes. This mineral has both antagonist and synergetic interactions with Cu, Fe and Zn [12,13]. Iron plays an important role in plants in chlorophyll synthesis and also in enzyme electron transfer. Iron presents an antagonist interaction with Ca, Mg and P, whereas it only interacts with P in a synergetic way [13]. The mineral Cu is part of a diversity of enzymes and works as a catalyst for respiration. Copper interacts with Ca and P in both antagonist and synergetic ways, and only presents an antagonist interaction with Mg [13]. Zinc has a myriad of functions in plants like being part of enzymes from regulation and has both antagonist and synergetic interactions with Ca, Mg and P [12,13].

Taking everything into account, most of the results obtained were not in line with what was said by the authors [13] has most of the minerals presented strong positive correlations in the whole wheat flours, so the majority of the minerals showed a synergetic interaction.

5. Conclusions

In general, there were no significant differences between the soil samples in the various parameters analyzed. Considering macroelements, Ca presented higher values in the soils. Conversely, Fe was the dominant microelement. In the soil samples, it was observed that only the minerals K, Fe and Zn were strongly and positively correlated, however, the minerals Fe, Cu, Ca (only with Fe) and Zn (only with Cu) had a strong and negative correlation. When compared to Roxo, Paiva variety presented higher mineral content for all the minerals in the flours of the control samples (P0 and R0). When applying Zn fertilizer in Paiva a decrease of the mineral content for Fe, K, Ca and Mg, was observed. Nevertheless, an increase of the mineral content for the minerals P, K, Mg, Fe, Zn and Cu regarding control samples was observed in Roxo. Zn foliar spraying enhanced Zn content in both varieties. Thus, wheat flour biofortified in zinc can be a product to help overcome malnutrition. Regarding whole bread wheat flours, it was observed that, in general, the minerals were strongly and positively correlated, although in some cases the minerals also had a strong and negative correlation.

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Abbreviations

The following abbreviations are used in this manuscript:

P	*Triticum aestivum* L. Paiva variety;
R	*Triticum aestivum* L. Roxo variety;
T0	control;
T1	corresponds to the foliar spray of $ZnSO_4$ with the concentration of 8.1 $kg.ha^{-1}$;
T2	corresponds to the foliar spray of $ZnSO_4$ with the concentration of 18.2 $kg.ha^{-1}$;

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