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Research article

Soybean seeds treated with zinc evaluated by X-ray micro-fluorescence spectroscopy

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ABSTRACT: Zinc (Zn) is a micronutrient considered essential to plants which can be supplied through seed treatment. The treatment of soybean [*Glycine max* (L.) Merr.] seeds with Zn, however, is still not well known as regards the uptake and mobilization dynamics of the nutrient during the germination process. This study aimed to evaluate the uptake and distribution dynamics of Zn applied to soybean seeds at two levels of vigor during germination using X-ray micro-fluorescence spectroscopy (μ-XRF). Zinc treatments corresponded to 0, 2, 4 and 8 g of Zn per kg of seeds. High and low vigor seeds that had been treated were located appropriately so as to promote germination. Zn intensity measurements with μ-XRF were taken in different parts ("regions") of the seeds (seed coat, cotyledon, and embryonic axis) after 8, 16 and 24 h of imbibition and seedlings (primary root, hypocotyl, plumule, cotyledon, and seed coat) after 48, 72 and 96 h of germination. High vigor seeds showed higher Zn intensity in the embryonic axis in the first 16 h, while low vigor seeds showed higher intensity after 24 h. After 48, 72 and 96 h of germination low vigor seedlings showed higher Zn intensity than high vigor seedlings in the primary root. It was concluded that μ-XRF is an efficient technique for identifying variances in the dynamics of Zn uptake and mobilization during the germination of soybean seeds with different vigor levels.

Keywords: *Glycine max* (L.) Merr., μ-XRF, seed coating, seed physiology, seed vigor

Introduction

Zinc (Zn) is essential to all organisms (Broadley et al., 2007) and plays a crucial role in plant metabolism. It is an enzyme cofactor and activator which helps maintain the integrity of cellular membranes and gene expression. It is active in the production of auxin (Hafeez et al., 2013) and may be required for chlorophyll biosynthesis in certain plants (Broadley et al., 2007). Thus, the treatment of soybean [*Glycine max* (L.) Merr.] seeds with Zn is a possible alternative supply source to plants. For this reason, the complex dynamics of absorption and distribution by seeds and seedlings is an issue that should be studied.

X-ray micro-fluorescence spectroscopy (μ-XRF) is a well-established analytical tool used in multielemental qualitative and quantitative analysis (Margui and van Grieken, 2013) of a myriad of sample matrices (Montanha et al., 2020a). In this regard, the μ-XRF has proven helpful in exploring the basic distribution pattern in the seeds of several species of economic importance, such as common bean (*Phaseolus vulgaris* L.) (Savassa et al., 2021), rice (*Oryza sativa* L.) (Reis et al., 2020), wheat (*Triticum aestivum* L.) (Singh et al., 2013), and soybean (Montanha et al., 2020b; Romeu et al., 2021).

The efficiency of the μ-XRF was also seen when evaluating the nickel (Ni) uptake/translocation by roots and seeds in soybean (Oliveira et al., 2022); mapping manganese (Mn), iron (Fe) and Zn, located at the radicle tip, and phosphorus (P), sulfate (S), potassium (K) and calcium (Ca), distributed throughout the soybean seeds during germination (Romeu et al., 2021). Additionally, it has demonstrated its effectiveness in soybean and *Zea mays* (L.) treated with copper (Cu), Zn and molybdenum (Mo), whose μ-XRF technique showed a higher concentration of these elements in the tegument or pericarp tissues compared to the roots and seedlings formed (Montanha et al., 2021).

Although agricultural research with μ-XRF has expanded, studies on soybean seeds are still scarce, and this technique could prove promising in the study of nutrient uptake in treated seeds and distribution throughout the germination process. Thus, the purpose of this study was to evaluate the dynamics of Zn absorption and distribution during the germination of soybean treated seeds using μ-XRF. The specific objectives were (i) to investigate the response of high and low vigor soybean seeds to Zn, and (ii) to evaluate Zn mobilization during seed imbibition and seedling development.

Materials and Methods

Seed material and experimental design

Two commercial seed lots from the soybean cultivar M5917 IPRO were used. For this study, the seed lots had different vigor levels (Lot 1 and Lot 2). The seeds had a moisture content of approximately 7 % (wet basis), determined by the oven method at 105 °C for 24 h (MAPA, 2009).

A randomized block design with two factors and, depending on the test, four or five replications were used. The main factor was the seed lot: high vigor or low vigor. The second factor was doses of Zn: 0, 2, 4, and 8 g kg^{-1} of seed.

Imbibition curve, seed germination and vigor tests

The imbibition curve was obtained from two hundred seeds from each lot. The seeds were divided into four subsamples of 50 seeds each, which were weighed on an analytical balance with 0.001 g precision to determine the initial mass (im). The subsamples were rolled in moistened paper towel and kept in a germinator at 25 °C. At two-hour intervals for a period of 26 h, the samples were taken from the germinator, dried to remove water excess, and then weighed to determine the final mass (fm). The results were expressed in percentage of water content (WC) and were calculated as $\%WC = \frac{(fm - im)}{im} \times 100$. The results were used to draw the imbibition curve of each lot.

Seed germination (G) was carried out using five replications of 50 seeds for each seed lot, using paper towel rolls moistened with the quantity of water weighing 2.5-fold the dry weight of the substrate at 25 °C. Next, G was obtained on the fourth (first count of germination - FC) and eighth day after sowing. The test was interpreted according to the Rules for Seed Testing (MAPA, 2009).

For vigor tests, evaluations of accelerated aging after 24 h (AA 24 h), accelerated aging after 48 h (AA 48 h), seedling emergence in sand (SE), emergence speed index (ESI), electrical conductivity (EC), vigor index (VI), uniformity of seedling development (UNF), hypocotyl length (HL), and root length (RL) were conducted.

The AA test using a plastic box (11 cm × 11 cm × 3.5 cm) had the seed samples from each lot distributed in a single layer on a wire mesh above the liquid containing 40 mL distilled water, as described by Dutra and Vieira (2004). The plastic boxes were held in a Bio-Oxygen Demand chamber at 41 °C for 24 h and 48 h, and thereafter we evaluated the percentage of germination with five replications of 50 seeds for each lot. Results were expressed in percentages of normal seedlings for each lot.

The SE test was carried out using four replications of 50 seeds for each treatment for both lots. Seeds were sown in plastic boxes (32.0 × 28.0 × 10.0 cm) containing sand moistened with 60 % of water retention capacity, under 2-cm deep lines, and without complementary fertilization. We counted the number of seedlings that emerged in each repetition daily until the tenth day after sowing. Next, results were averaged to obtain mean values per lot. ESI was determined according to the formula proposed by Maguire (1962).

EC was evaluated according to AOSA Vigor Committee (Baalbaki et al., 2009). Four replications of 50 seeds for each seed lot were used. The seeds were weighed on an analytical balance with 0.001 g precision, immersed in 75 mL of deionized water in plastic cups (200 mL) and held in a germination chamber at 25 °C for 24 h. After that, the electrical conductivity of the soaking solutions was determined using a conductivity meter. The results were expressed in μS cm^{-1} g^{-1} of seeds.

The VI, UNF, HL and RL were evaluated using the Automated Seed Vigor Analysis System - Vigor-S (Rodrigues et al., 2020). Five replications of 20 seeds per lot were used, distributed in two rows of ten seeds in the upper third of two sheets of paper towel covered with a third sheet. The substrate had been moistened previously with a quantity of water weighing 2.5 times the dry weight of the substrate. The seeds in the rolls were germinated at 25 ± 1 °C for three days. Next, the seedlings of each replication were transferred to a sheet of navy-blue ethylene-vinyl acetate (EVA) and digitalized on a scanner fixed upside-down inside an aluminium box (60 cm × 50 cm × 12 cm), at 300 dpi. The images were processed using the Vigor-S software program and the VI and UNF indexes (values from 0 to 1000) and HL and RL (cm) for each lot obtained.

Seed treatment

The seed treatment was applied to each lot at doses of 2, 4 and 8 g of Zn per kg of seed. The control treatment did not receive any treatment (0 g of Zn per kg of seeds). The commercial product used was Maxi Zinc, a product based on Zn oxide. Five hundred grams of seeds were placed in a plastic bag with a capacity of 1 kg. Next, the respective dose was added to the bag using a syringe graduated in millimetres. The bags were shaken manually and steadily for 5 min. Following this, the seeds were spread in plastic trays and kept at room temperature until they dried, and were stored in kraft paper bags. The germination and vigor tests described above evaluated the effect of Zn doses on the physiological potential of seeds from each lot.

Zinc uptake and distribution during germination

The uptake and distribution of Zn during the seed imbibition and seedling development was carried out on a benchtop μ-XRF system, furnished with an Rh X-ray tube operated at 45 kV and 500 μA, using a 25 μm Ni filter without vacuum. The seeds and seedlings were investigated using a 30 μm poly-capillary X-ray beam focused through a polycapillary optical element. The X-ray spectra were acquired in 20 s per point in seeds and 30 s per point in seedlings, using a 30 mm^2 silicon drift detector (SDD), with a dead time of less than 10 %. The Zn intensity results were expressed in count per second (cps). Zn-Kα intensity was normalized in seedlings by their respective Rh-Kα Compton ROI (region of interest) to correct thickness variations between regions. The data was processed by the OriginLab® graphical software tool.

Seed evaluation by X-ray micro-fluorescence

The seeds were germinated as described for the germination test. After 8, 16 and 24 h of germination, the seeds were evaluated. The seeds from each lot were

cross-sectioned using a stainless-steel razor blade to obtain a flat surface. Subsequently, they were placed in a sample holder and assembled using a 6 μm thin polypropylene film. Different regions of the seeds were evaluated using a linescan in which 32 consecutive points were analysed along the seed coat (opposite to hilum), cotyledon and hilum. The embryonic axis was evaluated by measuring four points: one in the root meristem region, one in the region of the plumule meristem and two in the hypocotyl region. Five replicates (seeds) per treatment were measured (Figure 1A and B).

Seedling evaluation by X-ray micro-fluorescence

The seeds were positioned to facilitate the germinating process as described in the germination test. For seedling analysis, a sample holder was developed to achieve the ideal positioning of the seedling in the equipment. The sample holder was built by a 3D printing, printed in ABS polymer (Figure 2A, B and C), using the Tinkercad® for 3D modelling online software program and the Simplify3D® software tool (www.simplify3d.com) to generate the slicing file.

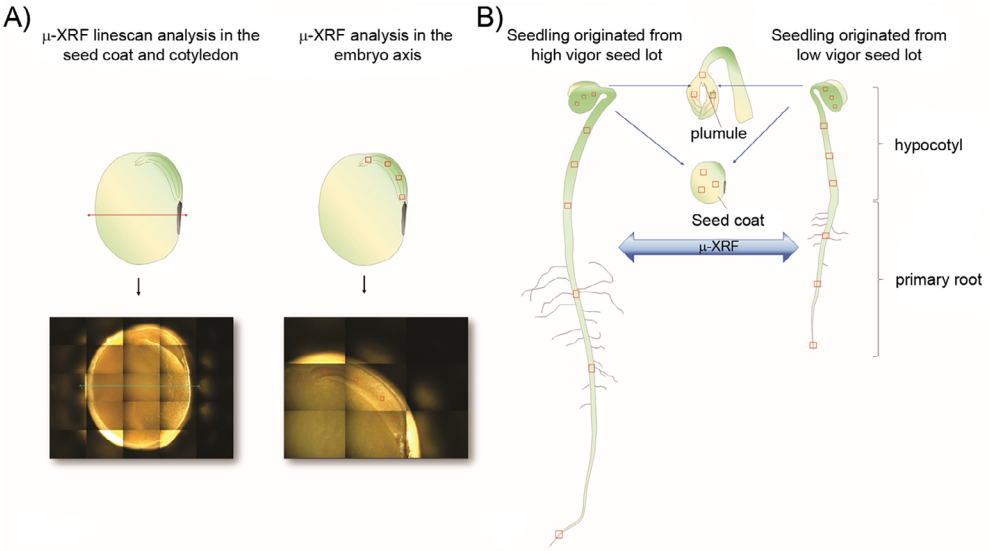

Figure 1 – Representation of μ-XRF analysis of soybean seeds and details of the sampling point (small red dot) in each of the regions assessed (A). Representation of the μ-XRF analysis in different regions of soybean seedlings originating from seeds of both high and low vigor (B). Source: Gomes-Junior and Rohr, 2021.

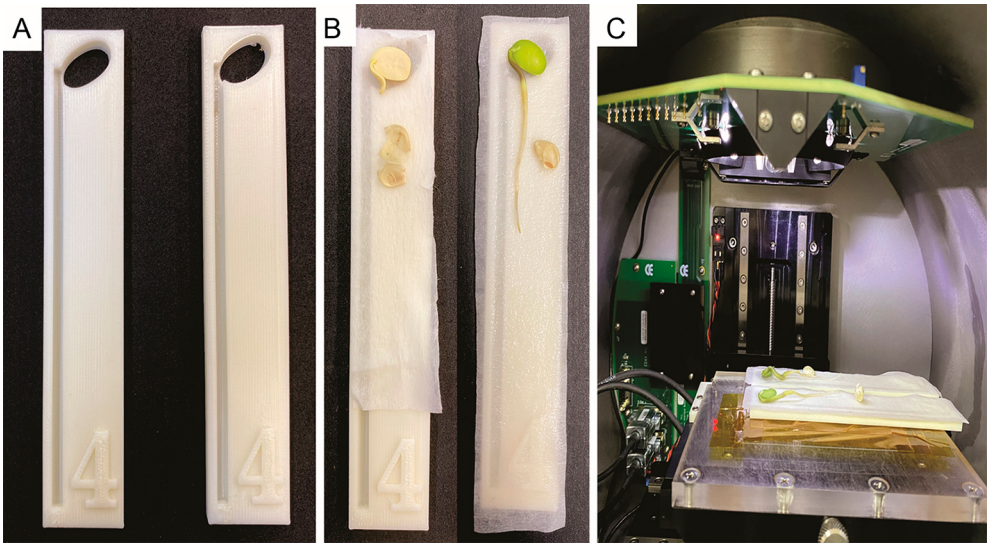

Figure 2 – Sample holder for seedling analysis (A), positioning of seedlings on the sample holder (B) and positioning of the sample holder in the equipment (C). Source: Rohr (2021).

Sample preparation consisted of removing one cotyledon of the seedling using a stainless-steel razor blade to expose the plumule for evaluation. The sample holder was covered with a moistened paper towel to prevent the seedling from dehydrating during evaluation. The evaluations were carried out at 48, 72 and 96 h after germination at doses of 0, 2 and 8 g kg^{-1}. As there were no discrepancies or differences in the behavior of the seeds at either the 2 g kg^{-1} or the 4 g kg^{-1}dose, the 4 g kg^{-1} dose was not evaluated. Three points in the regions of the plumule, cotyledon (internal region), seed coat, primary root meristem and hypocotyl were made with five replicates (seedlings) per treatment.

Statistical analysis

The data obtained were submitted to presuppositions based on normality and homogeneity of residual variances. The Shapiro-Wilk normality test showed that data were normally distributed. The analysis of variance was formulated by the F test ($p < 0.05$). Where significant variance was observed in the analysis, the complementary Tukey test ($p < 0.05$) was applied. These statistical analyses were conducted using the PROC GLM procedure in SAS (Statistical Analysis System, v. 9.4).

Results and Discussion

The seed lots showed similar WC, with a variation of 0.3 percentage points (Table 1). A variation below two percentage points was deemed acceptable for validating a comparison between the results of germination and vigor (Marcos-Filho, 2015). Both high and low vigor seeds exceeded the minimum percentage of germination standards established by law for the commercialization of soybean seeds in Brazil (G ≥ 80 %).

The results of germination and vigor tests showed differences ($p < 0.05$) between high and low vigor seeds, supporting our early characterization of high vigor (L1) and low vigor (L2) groups.

Zn at 8 g per kg of seed was phytotoxic to both lots; the effects were more drastic on the low vigor lot (Table 2). Lourenço et al. (2020) observed that Zn coating in rice (*Oryza sativa* L.) influenced seed vigor. The authors compared two sources of Zn (ZnO and $ZnSO_4$). Although the sources presented no difference in germination, ZnO presented better results in seed vigor tests. Moreover, the increase in ZnO doses increased the values of normal seedling germination in the cold test with a maximum value of 107.5 g kg^{-1} of Zn in seeds.

The 8 g kg^{-1} dosage negatively affected FC, SE, VI, UNF, HL and RL in the high vigor seeds, while all tests except RL were also negatively affected

Table 1 – Physiological characterization of high and low vigor soybean seed.

Vigor	WC+	G	FC	AA 24 h	AA 48 h	SE	EC	ESI	VI	UNF	HL	RL
	%						μS cm^{-1} g^{-1}	index			cm	
High	7.3	96	96	94	93	100	113.7	11.6	782	807	2.98	5.42
Low	7.0	86	85	83	52	91	159.7	8.5	427	714	1.82	2.00
F test		44.31*	607.54*	98.97*	217.81*	762.85*	50.54*	426.84*	918.03*	38.50*	257.23*	572.52*
CV (%)		4.52	1.56	4.16	11.86	0.98	14.95	4.64	6.15	6.21	9.55	12.19

WC+ = Water content; G = germination rate; FC = first count of germination; AA 24 h = accelerated aging after 24 h; AA 48 h = accelerated aging after 48 h; SE = seedling emergence in sand; EC = electrical conductivity ; ESI = emergence speed index; VI = vigor index; UNF = uniformity of seedling development ; HL = hypocotyl length; RL = root length; *Significant at 5 % of probability.

Table 2 – Physiological response of high and low vigor soybean seed to Zn.

Zn dose	G+	FC	AA 24 h	AA 48 h	SE	ESI	VI	UNF	HL	RL
g kg^{-1}	%					index			cm	
High vigor										
0	96 a[1]	96 a	94 a	95 a	100 a	11.7 a	797 ab	859 a	3.26 a	6.09 a
2	96 a	96 a	94 a	94 a	99 a	11.7 a	774 ab	790 ab	3.18 ab	5.47 ab
4	98 a	98 a	96 a	94 a	99 a	11.5 a	820 a	822 ab	2.96 ab	5.43 ab
8	93 a	91 b	91 a	91 a	96 b	11.3 a	738 b	755 b	2.52 b	4.70 b
CV (%)	3.71	1.04	3.51	2.44	0.72	2.82	5.75	6.15	9.69	9.27
Low vigor										
0	86 ab	85 b	86 a	38 b	92 a	9.2 a	461 a	766 a	1.84 a	2.03 a
2	90 a	88 a	86 a	59 a	92 a	8.8 ab	430 ab	712 ab	1.85 a	2.03 a
4	88 ab	87 a	82 ab	64 a	91 a	8.1 b	428 ab	721 ab	2.01 a	2.19 a
8	80 b	78 c	78 b	47 b	88 b	7.9 b	386 b	658 b	1.57 b	1.74 a
CV (%)	5.25	0.84	2.88	11.07	0.88	6.06	6.22	6.80	6.33	15.45

G+ = germination rate; FC = first count of germination; AA 24 h = accelerated aging after 24 h; AA 48 h = accelerated aging after 48 h; SE = seedling emergence in sand; ESI = emergence speed index; VI = vigor index; UNF = uniformity of seedling development; HL = hypocotyl length; RL = root length; [1]Mean comparison within each column for each lot (Tukey test, $p < 0.05$).

in the low vigor seeds. Results showed that seed quality decreased as the Zn dosage increased. Lemes et al. (2017) also determined and verified the effects of seed treatment with Zn in soybeans. The authors identified that treatments at doses between 2 to 6 g kg^{-1} of seed yielded the best results for maintaining the physiological potential of seeds.

The seed imbibition curve, from 0 to 26 h for both lots presented a similar pattern (Figure 3). However, the high vigor seeds presented a lower water uptake rate at the beginning of the process (first minutes of imbibition) and, shortly after that, higher water content was observed, up to 24 h of imbibitions, compared to the seeds of the low vigor lot. Conversely, in the period of 24 to 26 h of imbibition, the water content of the seeds in the low vigor lot continued to increase differently compared with the high vigor lot. This behavior matches that Marcos-Filho (2015) described, in which the seeds in a more advanced stage of deterioration, such as low vigor seeds, generally present faster initial water imbibitions. However, during the process, the seeds with higher physiological potential require more significant quantities of water for the continuity of the metabolism.

The process of water uptake by the seeds follows a three-phase pattern. The first phase, in general, lasts from 8 to 16 h, but the duration of the second is variable depending on the species, and for soybeans, this period can last up to eight to ten times longer than the first phase, and the beginning of the third phase is identified by the protrusion of the primary root (Bewley and Black, 1978). As seen in the dotted red lines indicated on the imbibition curve of the two seed lots, Zn uptake assessments were carried out during the first and second phases of the process.

Even without Zn treatment (dose of 0 g kg^{-1}) and regardless of the imbibition time, the seed coat and cotyledon of both high and low vigor soybean seeds had low Zn intensity (around 10 cps), (Figure 4). On the other hand, the highest Zn intensity for treated seeds was observed in the seed coat and the hilum region. Our findings corroborated the study by Montanha et al. (2020a), who also used μ-XRF for tracing Zn distribution in soybean seeds, in which Zn presented the maximum count rate on the seed coat, and then sharply decreased on the seed coat-cotyledon interface.

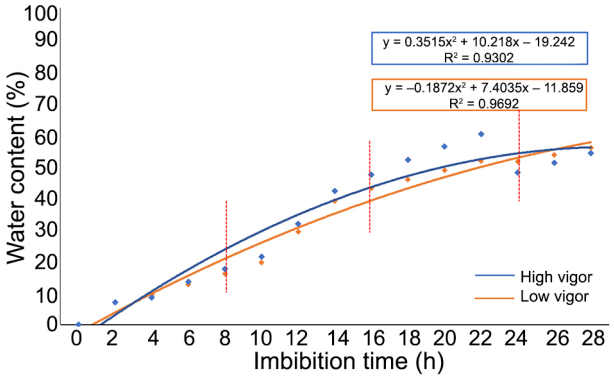

Figure 3 – Water uptake by soybean seeds during the first 28 h of germination, from both high and low vigor seed lots. The vertical dotted red lines correspond to the times when μ-XRF were evaluated.

For the 2 and 4 g kg^{-1} doses Zn intensity in the hilum region was approximately 100 times higher than that found in the central region of the cotyledon, while at the 8 g kg^{-1} dose it was almost 1,000 times higher. In general, as the dose and the imbibition time increased, the greater the Zn intensity in the seed coat, cotyledon and hilum, and as regards the seed vigor levels, a similar Zn absorption pattern was observed in the cotyledon region between the seeds of the high and low vigor lots, regardless of the dose used in the seed treatment.

These results can be explained by the seed hydration process observed by Pietrzak et al. (2002). Using nuclear magnetic resonance imaging, the authors observed that water uptake is a multistage process. It begins to enter the seed through the micropyle and hilum, and when inside the seed, it first fills the voids between cotyledons and between cotyledons and seed coat. Next, it enters the embryonic axis, and from there, it is distributed into cotyledons. Thus, as water acts as a solvent for Zn, the behavior observed in this research study indicates that the dynamics of Zn uptake were a response to the dynamics of water absorption.

The measurements taken on the embryonic axis showed that in the control treatment, regardless of the imbibition time, Zn intensity was similar to that found in the cotyledon, seed coat and hilum regions, that is to say, values close to 10 cps (Figure 5). However, when the seeds were treated with Zn doses, higher intensity of the element was observed in the region of the embryonic axis when compared to the cotyledon region. The previous study carried out by Romeu et al. (2021) pointed out that the elemental distribution pattern in the embryo axis of pristine soybean seeds diverges from those observed in the imbibed ones. The μ-XRF maps recorded from pristine to imbibition (24 h and 48 h) seeds revealed a possible trend of elemental redistribution during seed germination from cotyledon to embryonic axis.

Water movement is faster in embryonic tissues since these tissues capture water in a more uniformly and continuously on account of the germination process involving both cell division and elongation, which requires a greater volume of water (McDonald et al., 1988b). Therefore, the cotyledons and seed coat tissues follow not only the water absorption pattern to uptake Zn but also the embryonic axis.

As regards the seed vigor levels, differences in Zn intensity was observed in the measurements taken after 8 and 16 h of imbibition for both the 2 and 4 g kg^{-1} doses. Zn intensity in the embryonic axis of the seeds from the high vigor lot was more significant than that in the seeds from the low vigor lot.

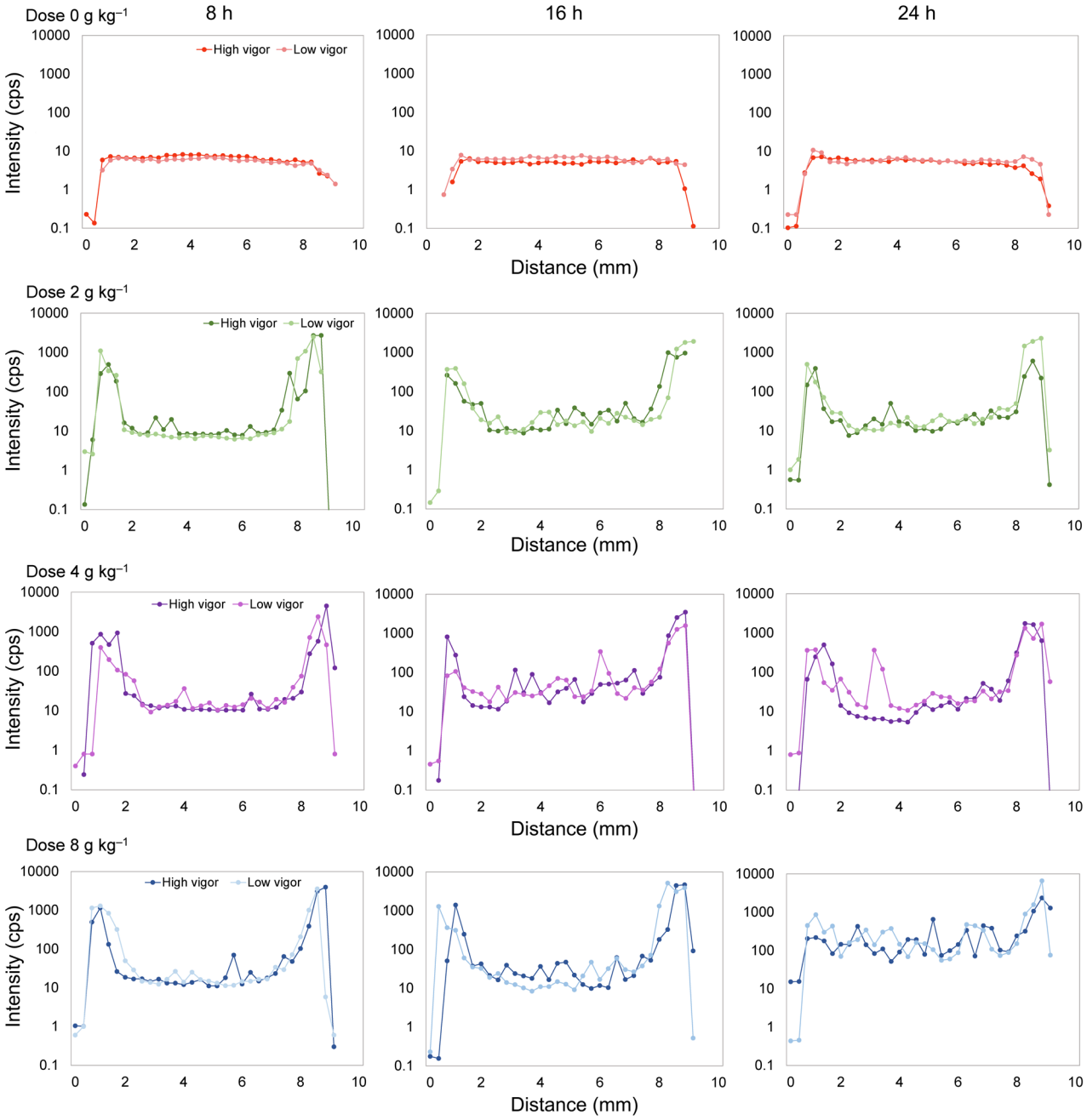

Figure 4 – Zinc intensity (cps – counts per second) measured with μ-XRF after 8 h, 16 h and 24 h of imbibition at the seed coat, cotyledon and hilum of soybean seeds treated with 0, 2, 4 and 8 g of Zn per seed kg^{-1}. Zn intensity corresponding to the seed coat, cotyledon and hilum are interpreted by looking at each linescan graph: from left (0 mm = seed coat) to right (8 mm = hilum), according to representation in Figure 1. The higher the value in the y axis the higher the Zn intensity.

The differences between the seed vigor levels can be associated with the behaviour presented in the imbibition curve, which may be attributable to differences in the metabolism of the seeds from each lot, and, therefore, in the capacity of water absorption by the embryonic axis. Moreover, it is known that seeds from lots with less physiological potential require more time to repair damage accumulated during the deterioration process of the seeds (McDonald, 1999). Thus, the permeability of membranes from less vigorous lots is less efficient when compared to seeds from lots with greater vigor, since the integrity of the tissues of these seeds, especially the seed coat, is affected more.

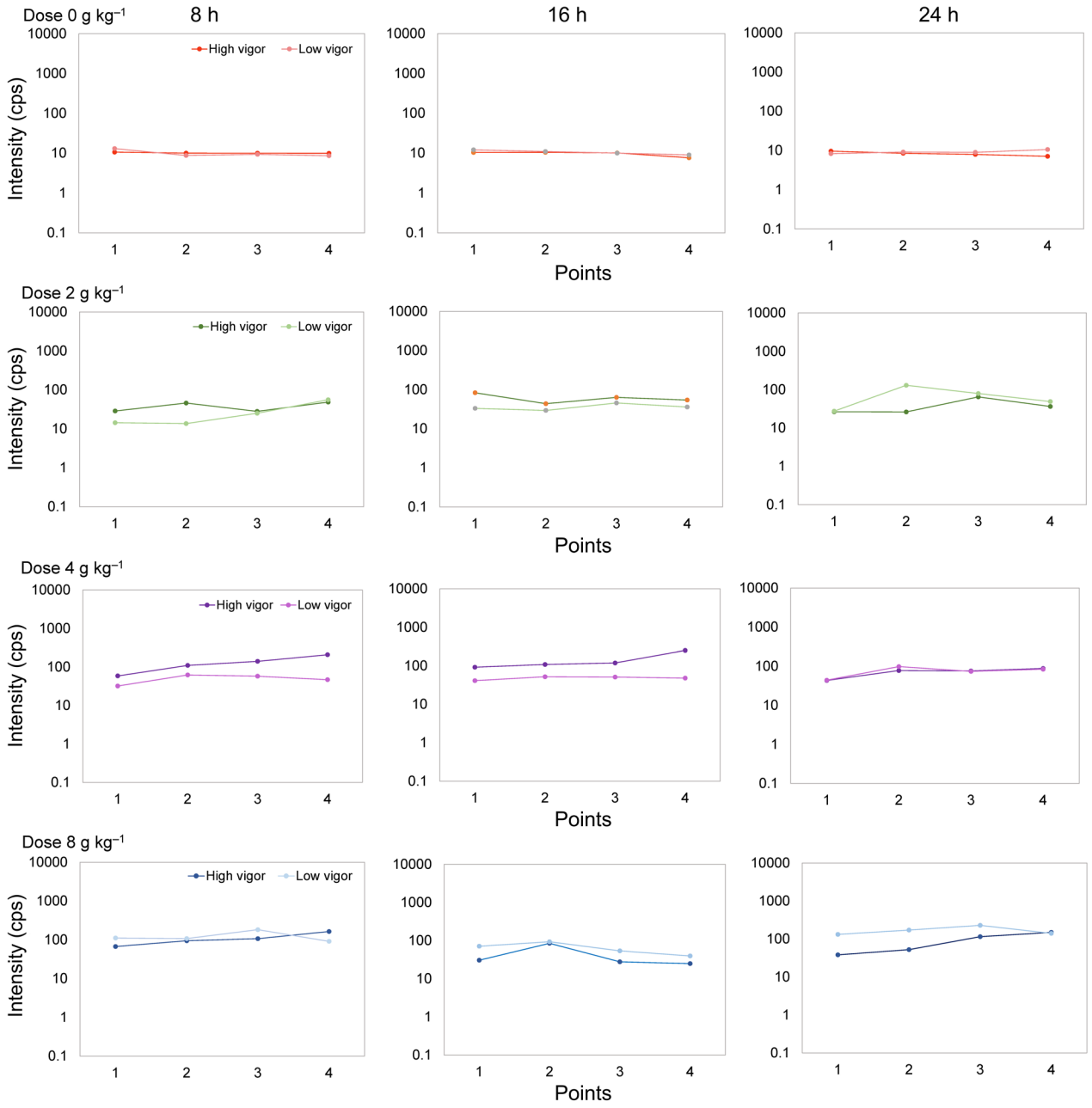

Figure 5 – Zinc intensity (cps – counts per second) measured with μ-XRF after 8 h, 16 h and 24 h of imbibition in the embryonic axis (P1 represents the measure in the region of the root meristem, P2 and P3 are the measures in the region of the hypocotyl, and P4 is the measure in the region of the plumular meristem) of both high and low vigor soybean seeds treated with 0, 2, 4 and 8 g of Zn per kg of seed.

Based on the Zn intensity in count per second (cps) measured in the four regions of the embryonic axis, the average values were calculated for each lot and dose of Zn, within each imbibition time (Table 3). There were no differences ($p > 0.05$) in Zn intensity between the seed lots in any of the evaluation times for the control treatment. However, for the 2 g kg^{-1} dose, after 16 h of imbibition, the high vigor lot had Zn intensity higher ($p < 0.05$) than the low vigor lot. Similar behavior was found with the 4 g kg^{-1} dose, after 8 and 16 h of imbibition, with Zn intensity in the high vigor lot approximately three times higher than the seeds of the low vigor lot. These results indicate that the seed vigor has considerable influence on the mobilization of Zn to the embryonic axis, mainly during the period from 0 to 16 h of imbibition.

The results also show a reduction in Zn intensity in the embryonic axis of seeds from the high vigor lot,

Table 3 – Zinc intensity (cps) determined with μ-XRF during the imbibition in the embryonic axis of soybean seeds treated with Zn.

Dose (g kg^{-1})	Imbibition time (h)					
	8		16		24	
	HV	LV	HV	LV	HV	LV
0	10.10 Ad[1]	9.87 Ad	9.63 Ad	10.51 Ac	8.25 Ac	9.20 Ac
2	37.74 Ac	27.19 Ac	61.11 Ab	36.00 Bb	38.48 Ab	71.56 Ab
4	147.20 Aa	49.74 Bb	155.30 Aa	48.10 Bab	78.35 Aa	75.08 Ab
8	125.90 Ab	122.80 Aa	31.48 Bc	64.32 Aa	117.20 Ba	167.40 Aa
CV (%)	9.53		11.61		7.96	

HV = high vigor; LV = low vigor; cps = counts per second; [1]Means followed by different capital letters on the line and within each imbibition time and means followed by different lower-case letters within each column differ by the Tukey test at 5 % probability.

comparing the imbibition times of 16 and 24 h for doses of 2 (61.11 to 38.48 cps) and 4 (155.30 to 78.35 cps) g kg^{-1}. On the other hand, for seeds with low vigor, the behavior was the opposite; that is, there was an increase in Zn intensity in the embryonic axis with the application of the 2 (36.00 to 71.56 cps) and 4 (48.10 to 75.08 cps) g kg^{-1} doses. These results highlight differences in Zn absorption and the influence of assimilation metabolism of seed vigor.

The periods between 16 and 24 h are related to the second phase of the three-phase water absorption pattern described by Bewley and Black (1978). According to Marcos-Filho (2015), during the hydration process in the second phase, several events occur, such as organelle and membrane reorganization, increases in respiratory activity, synthesis and consumption of ATP, synthesis of proteins and mRNA and activation of enzymes. This results in the initiation of the mobilization of reserves which promotes the accumulation of solutes and the subsequent entry of water into the cells, whose expansion culminates in the elongation of the embryonic axis.

Possibly, the reduction in the intensity of Zn in the seeds with greater vigor with the advance in soaking time is associated with a more intense metabolism which promotes greater elongation of the embryonic axis and mobilization of Zn into the tissues. Consequently, this results in a lower concentration of the element per unit area compared to the embryonic axis of seeds with less vigor, which has a less developed embryonic axis. Thus, the Zn intensity in the embryonic axis of seeds with low vigor was higher after 24 h of imbibition on account of the higher tissue density.

As regards the seed treatment with the dose of 8 g kg^{-1} dose, the results of both lots were inconsistent with higher Zn intensity in the embryonic axis after 8 and 24 h of imbibition and lower at 16 h. This reveals the harmful effect of this dose on seed metabolism, as observed in the results of the physiological potential assessments (Table 2).

The water absorption capacity of each part of the seed is also related to the difference in the chemical composition of each structure. The studies developed by McDonald et al., (1988a, b) revealed that the embryonic axis of soybean has a greater capacity for water absorption and retention than the cotyledon and the seed coat. The authors attribute this capacity to differences in the chemical composition of each structure. Our results suggest that the mobilization of Zn, whether it is the Zn naturally present in seeds, from a high or low vigor lot, or the Zn applied through seed treatment, has the embryonic axis as its destination, that is to say, it moves to the region with the highest seed metabolism. This assumption may also find support in the results obtained by Ozturk et al. (2006) in wheat, Takahashi et al. (2009) in rice, and Romeu et al. (2021) in soybean. Based on μ-XRF measurements taken during the first 24 h of imbibition of rice seeds, Takahashi et al. (2009) observed that Zn was most abundant in the embryo and Zn in the endosperm decreased compared to Zn in the embryo. Furthermore, high levels of Zn in the embryo accumulated in the radicle and leaf primordium. Ozturk et al. (2006) also studied the concentration and localization of Zn during seed development and germination in wheat. They found high Zn concentrations in newly developed radicle and coleoptile over 36 h of seed germination, which expresses a critical physiological role for Zn during early seedling development.

Among the events that occur in the first two stages of seed imbibition are mRNA synthesis, the beginning of mRNA transcription, protein synthesis activation, DNA repair, "*de novo*" mRNA transcription and "*de novo*" enzyme synthesis (Obroucheva, 2010; Bewley et al., 2013). In connection with this, Zn is an element that is essential to making this happen. According to Vallee and Auld (1990), Zn plays a functional (catalytic) and structural role in enzyme reactions, and these metabolic functions are established based on its tendency to structure tetrahedral complexes with N-, O- and S-ligands. In addition, Broadley et al. (2007) maintained that among transition metals, it is the second most abundant in organisms and the only one detected in all enzyme classes, which are oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Sites of Zn binding are also found in a wide range of proteins, membrane lipids and DNA / RNA molecules. Thus, the differences in the metabolism of high and low vigor seeds can be associated with the speed of biochemical reactions and, as mentioned before, the capability

of cell membranes to reorganize themselves quickly (membrane permeability).

Measurements in seedlings of both lots revealed that Zn intensity increased in all regions of the seedlings (primary root, hypocotyl, plumule, cotyledon and seed coat) according to the dose applied (Table 4). In the control treatment, Zn intensity in the seed coat of the high vigor lot was higher than that of the seeds of the low vigor lot. However, these differences were not found when the seeds were treated with doses of 2 and 8 g kg^{-1}.

Evaluation of the cotyledon after 48, 72 and 96 h of germination showed no differences ($p > 0.05$) between lots when doses of 2 and 8 g kg^{-1} were applied. Similar results were observed when the evaluations were carried out in the hypocotyl, with differences only in seedlings with 48 h of germination for doses of 0 and 2 g kg^{-1}, when the values of Zn intensity for the low vigor lot were higher. On the other hand, when the primary root and the plumule were evaluated, the differences between the seed lots were more regular. In the control treatment (0 g kg^{-1}) Zn intensity in the primary root at 48 h of germination was similar between seedlings originating from seeds of both high and low vigor. However, with the advance of the germination time (72 and 96 h) Zn intensity in the primary root of the seedlings from the low vigor lot decreased and was lower ($p < 0.05$) than the intensity observed for the high vigor lot. Our results corroborate the findings of Takahashi et al. (2009) in rice seedlings after 36 h of germination in which Zn stored in the endosperm was transported to the scutellum and collected in the vascular bundle of the scutellum and then transported to the seminal root and leaf primordium.

When the seeds were treated with 2 g kg^{-1}, the results revealed greater Zn intensity in the primary root of seedlings from the low vigor seed lot for all three evaluation periods. Similar results were observed when the plumule was evaluated with higher Zn intensity values for seedlings from low vigor seeds. As regards the 8 g kg^{-1} dose, except for the higher Zn intensity in the plumule of the low vigor lot, all other treatments revealed no differences between seed lots.

Essentially, after seed treatment with Zn, evaluations after 48 h indicated that the low vigor lot has higher Zn intensity in the regions with a higher metabolism (primary root and plumule). These results may also be related to the fact that the speed

Table 4 – Normalized Zn intensity (cps) determined with μ-XRF during the germination in different regions of soybean seedlings originated from seeds treated with Zn.

Primary root Dose (g kg^{-1})	Germination time (h)					
	48		72		96	
	HV	LV	HV	LV	HV	LV
0	0.0504 Ab[1]	0.0552 Ab	0.0522 Ab	0.0334 Bb	0.0664 Ab	0.0409 Bb
2	0.0795 Bb	0.2249 Aa	0.1131 Ba	0.2369 Aa	0.0512 Bb	0.1525 Aa
8	0.5805 Aa	0.5184 Aa	0.1900 Aa	2.2060 Aa	0.1858 Aa	0.1015 Aa
CV (%)	26.87		20.33		22.34	
Hypocotyl						
0	0.0264 Bc	0.0404 Ab	0.0269 Ab	0.0244 Ac	0.0185 Ab	0.0218 Ac
2	0.0525 Bb	0.1386 Aa	0.0628 Aa	0.0554 Ab	0.0427 Aa	0.0523 Ab
8	0.1683 Aa	0.2035 Aa	0.0911 Aa	0.1426 Aa	0.0696 Aa	0.1098 Aa
CV (%)	14.03		12.67		9.66	
Plumule						
0	0.0356 Ab	0.0371 Ab	0.0334 Ab	0.0328 Ab	0.0331 Ab	0.0325 Ab
2	0.1212 Aa	0.1804 Aa	0.0661Bab	0.1864 Aa	0.0761 Ab	0.1105 Aa
8	0.2015 Aa	0.1226 Aa	0.1099 Ba	0.1734 Aa	0.2155 Aa	0.1841 Aa
CV (%)	19.01		17.33		18.96	
Cotyledon						
0	0.0429 Ab	0.0268 Bb	0.0461 Ab	0.0294 Ab	0.0341 Ab	0.0322 Ab
2	0.1262 Aa	0.1045 Aa	0.1217 Aa	0.1558 Aa	0.1121Aab	0.1265 Aa
8	0.1909 Aa	0.1653 Aa	0.1626 Aa	0.1787 Aa	0.2349 Aa	0.2395 Aa
CV (%)	14.51		14.32		20.07	
Seed coat						
0	0.0541 Ab	0.0236 Bb	0.0541 Ab	0.0255 Bc	0.0461 Ab	0.0249 Bc
2	3.5734 Ab	2.3880 Aa	5.8128 Ab	2.9982 Ab	5.4626 Ab	5.1402 Ab
8	12.527 Aa	10.075 Aa	34.648 Aa	25.269 Aa	14.171 Aa	24.238 Aa
CV (%)	25.94		27.20		21.49	

HV = high vigor; LV = low vigor; cps = counts per second; [1]Means followed by different capital letters in the row and within each germination time and means followed by different lower-case letters within each column for each seedling region differ from each other by the Tukey test at 5 % probability.

of biochemical reactions was different between the two lots. A hypothesis that may justify this behavior is that the low vigor lot requires more energy to generate biochemical reactions at this stage of germination than the high vigor lot, which generates greater demand for the Zn as a result of the seed treatment. Moreover, the higher Zn intensity found in the seeds of the low vigor lot may be due to the higher concentration of the element per unit area. It should be noted that the seedlings originating from the low vigor lot were smaller, as presented in HL and RL results (Table 2), than seedlings originating from the high vigor lot. Thus, Zn intensity in the low vigor lot is more significant due to the higher density of the tissues.

The uptake of various trace elements in wheat, buckwheat (*Fagopyrum esculentum* Möench L.) and quinoa (*Chenopodium quinoa* Willd.) were studied by Lintschinger et al. (1997). The total germination period was 96 h for wheat and quinoa and 72 h for buckwheat. The authors observed similar uptake behavior for Zn and other elements, which was characterized by relatively high uptake of the element during soaking, a subsequent decrease on the first day of germination, and an increase on the second and third days. As regards the metabolism of reserves in soybean seeds, Henning et al. (2010) concluded that highly vigorous seeds have higher levels of soluble proteins, starch and soluble sugars, and greater capacity to mobilize reserves in germination. This may support the results found in our study related to Zn uptake and distribution in seeds of both low and high vigor. Several research studies have shown that μ-XRF can be widely applied to the examination of seed germination in different species (Takahashi et al., 2009; Singh et al., 2013; Reis et al., 2020; Montanha et al., 2020b; Savassa et al., 2021; Romeu et al., 2021). However, to our knowledge, no other studies have used μ-XRF to investigate the uptake and mobilization of Zn during the germination process after seed treatment, mainly when using seed lots with different levels of vigor.

Conclusions

The results of this study provided evidence of the efficiency of the μ-XRF in identifying variances in the dynamics of Zn uptake and mobilization during the germination of soybean seeds. The evidence found suggests no differences in Zn uptake and distribution dynamics during the germination of soybean seeds with both low and high vigor when μ-XRF measurements are taken of the cotyledon. For both levels of seed vigor Zn intensity rises with increases in the dose applied via seed treatment and the period of imbibition. However, when measurements were taken of the region of the embryonic axis, identifiable differences emerged in the dynamics of Zn uptake between high and low vigor seeds. High vigor seeds had higher Zn intensity in the embryonic axis at the beginning of the water uptake process (first 16 h), while low vigor seeds presented higher Zn intensity in the embryonic axis in the last stages of water uptake (after 24 h) before seed germination. Moreover, there is higher Zn intensity in the primary root of 3, 4 and 5-day-old seedlings in the low vigor lot than in high vigor lot seedlings which is probably attributable to the higher concentration of the element per unit area as the root is less developed.

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Authors' Contributions

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