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Zinc fertilization as an alternative to increase the concentration of micronutrients in edible parts of vegetables

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ABSTRACT

Because nutrients consumed by human beings are derived from the soil-plant system, biofortification of the edible parts of horticultural crops can be a very useful technique for countering human malnutrition. The objective of this study is to evaluate the transfer of Zn from the soil to the edible parts of carrots, kale and okra in latosol treated with increasing doses of Zn. The experiment was conducted in a randomized block design with a 4 x 3 x 4 factorial scheme, consisting of four Zn doses (0, 50, 150, and 300 mg kg^{-1} of soil), three plant species (carrot, kale and okra) and four parts of the plants (root, stem, leaf and edible parts), with four repetitions. It was found that with an increase in the content of Zn in the soil, the plant species, in general, presented a higher concentration of this element, showing that fertilization practices can increase the availability of Zn to plants. The kale plants exhibited a potential to concentrate Zn in their edible parts, as opposed tocarrot and okra plants. For this reason, these are suitable for use in Zn biofortification programs Zn.

Key words: biofortification, carrot, human malnutrition, kale, okra

Adubação com Zinco como alternativa para aumentar a concentração de micronutrientes em partes comestíveis de hortaliças

RESUMO

Considerando que os nutrientes consumidos pelos seres humanos são provenientes do sistema solo-planta, a biofortificação das partes comestíveis de hortaliças pode ser uma técnica muito útil para combater a desnutrição humana. O objetivo deste estudo é avaliar a transferência de Zn do solo para as partes comestíveis de cenoura, couve e quiabo em latossolo tratado com doses crescentes de Zn. O experimento foi conduzido em um delineamento em blocos casualizados com esquema fatorial 3 x 4 x 4, constituído de quatro doses de Zn (0, 50, 150 e 300 mg kg^{-1} de solo), três espécies vegetais (cenoura, couve e quiabo) e quatro partes de plantas (raiz, caule, folhas e partes comestíveis), com quatro repetições. Verificou-se que com o aumento no teor de Zn no solo, as espécies de plantas, em geral, apresentam maior concentração desse elemento, demonstrando que a prática de fertilização pode aumentar a disponibilidade de Zn para as plantas. Entretanto, as plantas de couve exibiram maior potencial para concentrar Zn nas suas partes comestíveis, em comparação com as plantas de cenoura e quiabo e, por esta razão, são adequados para utilização em programas de biofortificação com Zn.

Palavras-chave: biofortificação, cenoura, desnutrição humana, quiabo, couve-manteiga

Introduction

Micronutrient deficiency is very common and affects more than half the world's population (Mayer et al., 2008). A large part of the populations of Asia, Africa and Latin America continue to base their diets on cereals, which have low micronutrient contents, especially Fe and Zn (Alloway, 2009). Zn deficiency affects more than 25% of global population and is fifth among the most important risk factors for human health in developing countries (Maret & Sandstead, 2006).

Zn is essential for human beings (Alloway, 2009; Broadley et al., 2007) as it is active in more than two hundred enzymes, acting in the synthesis of genetic material, in cellular division, and in the synthesis and degradation of carbohydrates, lipids and proteins (EVM, 2003). Zn deficiency in humans results in mental retardation, damage to the reproductive system, hair loss, diarrhea, loss of appetite, anemia and delayed wound healing (EVM, 2003).

Numerous attempts to solve nutritional deficiencies in human beings have been promoted, principally through the supplementation of food products enriched with micronutrients. However, this strategy has not been adequate due to its elevated cost and low coverage (Yang et al., 2007). In Brazil, the policies for combating malnutrition are characterized by fragility in the coordination of the programs and by difficulties in distributing them to the poorest regions of the country, where the most vulnerable populations are found (Coutinho et al., 2008).

Zn deficiency in human nutrition is a consequence of its scarcity in food (Wu et al., 2007), which is associated with its low availability in a large part of cultivated soils (Cakmak, 2008; Fraige et al., 2007). Even though Zn is the twenty-third most abundant chemical element on the earth (Broadley et al., 2007), more than 90% of this element present in the soil is in an insoluble form. Therefore, it is unavailable to plants, which is the principal reason for its generalized deficiency in crops (Cakmak, 2008). In Brazil, deficiency of Zn in the soil is the most common among the micronutrients, occurring principally in the sandy soils and latosols of the cerrado region (Fraige et al., 2007). Liming practices, which aim to correct the natural acidity of these soils, are frequent and further reduce the availability of Zn to plants, possibly by increasing the affinity of this element to the specific adsorption sites in the soil (Cunha et al., 2008).

Due to the fact that the nutrients consumed by human beings are derived from the soil-plant system, a new approach to solving the problem of micronutrient deficiency in the diet could be biofortification, with an emphasis on the selection of vegetable species with a higher potential to absorb nutrients in the soil and to increase the density and bioavailability of micronutrients in the edible parts (Alloway, 2009; Yang et al., 2007).

In this sense, evaluation of the ability of horticultural plants to transfer Zn from the soil to their edible parts, in regards to the wide natural genetic variation among these plants and the important contribution of these plants to the diet of the Brazilian population (Carvalho et al., 2006), is an essential step for biofortification programs. Therefore, the objective of this work is to evaluate Zn transfer from the soil to the edible parts of carrots, kale and okra in latosol treated with doses of Zn.

Material and Methods

The experiments were performed in a greenhouse. Samples of a typical dystrophic Yellow Latosol were collected at a depth of 0 to 20 cm, then air-dried and sieved through a 2 mm mesh screen for later chemical and physical analysis (Table 1). Determination of available Zn content in the soil was performed by DTPA extraction (Lindsay & Norvell, 1978).

The three vegetables selected for the present study are among the most important in the Brazilian diet and represent three plant groups with different edible parts (tuber, leaf and fruit). The carrot (*Daucus carota*), kale (*Brassica oleracea* var. *Acephala*), and okra (*Abelmoschus esculentus*) seedlings were produced in a greenhouse, in styrofoam trays, using vermicompost as a substrate. The soil samples were fertilized as follows: 250, 240, 150, and 100 mg kg-1 of N, P, K, and S, respectively, added as NH_4SO_2, $NH_4H_2PO_4$, and KNO_3 (Nascimento et al., 2006). For the installation of the experiment, 6.0 kg of previously fertilized soil was transferred to plastic pots. The seedlings were transferred to other pots 20 days after germination and the pots were thinned out after 15 days, leaving only four plants per pot. During the experiment, the soils were kept at 80% of their maximum water retention capacity, mediated by daily weighing and watering to replace water lost by evaporation.

The plants were collected 45 days after seedlings transplanting. Roots, stems, leaves and edible parts were separated. Next, they were washed thoroughly with tap water and then in distilled water, to remove soil particles and impurities present on the surface of the plants. The collected parts were weighed, gathered in paper bags and placed in a stove to dry at 65 -70 ºC during 72h. The dry samples were weighed again, ground in a Wiley mill and then subjected to digestion with a mixture of nitric and perchoric acids to determine the contents of Zn, Fe, Mn and Cu by atomic absorption spectrophotometry.

The experiments were conducted in a randomized block design in a 6 x 3 x 4 factorial scheme. The treatments consisted of six doses of Zn (0, 50, 150, 300, 450 and 550 mg kg^{-1} of soil), three plant species (carrot, kale and okra) and four

Table 1. Chemical and textural attributes of soil used in the experiment for the cultivation of vegetable crops

Attributes	Value
pH (water – 1:2.5)	6.26
P (mg dm^{-3})	19.0
Na^+ (cmol$_c$ dm^{-3})	0.32
K^+ (cmol$_c$ dm^{-3})	0.11
Ca^{+2}+Mg^{+2} (cmol$_c$ dm^{-3})	1.80
Mg^{+2} (cmol$_c$ dm^{-3})	1.20
Al^{+3} (cmol$_c$ dm^{-3})	0.0
H+Al (cmol$_c$ dm^{-3})	3.51
Organic carbon (g kg^{-1})	8.52
Organic matter (g kg^{-1})	14.65
Zn (mg kg^{-1})	2.8
Sand (g kg^{-1})	869.0
Lime (g kg^{-1})	13.0
Clay (g kg^{-1})	118.0

parts of plants (root, stem, leaf and edible parts), with four repetitions. The obtained data were subjected to analysis of variance (ANOVA), and regression equations were fit between the doses of Zn applied to the soil and the micronutrient content in the plants. The average contents of Zn, Fe, Mn and Cu observed in diverse organs of the plants were compared by the Scott-Knott test at 5% significance.

Results and Discussion

The Zn applied to the soil affected the concentrations of micronutrients in the evaluated plants (Table 2). The Zn and Cu contents in the carrot roots increased linearly, while a quadratic response was observed in relation to the Fe content, and no significant effect was observed for Mn. The linear increase of Zn and Cu observed in the carrot roots agrees with the observations of Nair et al. (2008), who evaluated the content of these elements in beet roots grown in solutions with different doses of these micronutrients.

Table 2. Regression equations for the levels of micronutrients in plant parts of carrot, kale and okra in response to increasing doses of Zn in soil

Nutrients	Parts of plants	Regression equations	R^2
		Carrot	
Zn	Root	$\hat{y}$ = 707.43 + 24.91***x	0.89
	Leaf	$\hat{y}$ = 76.96 + 18.45***x - 0.04***x²	0.97
	Tuber	$\hat{y}$ = 65,70	
Fe	Root	$\hat{y}$ = 174.17 + 17.11***x - 0.05***x²	0.99
	Leaf	$\hat{y}$ = 194.26	-
	Tuber	$\hat{y}$ = 266.76	-
Mn	Root	$\hat{y}$ = 342.97	-
	Leaf	$\hat{y}$ = 387.09 + 3.95***x - 0.011***x²	0.99
	Tuber	$\hat{y}$ = 80.09 + 0.46*x	0.98
Cu	Root	$\hat{y}$ = 36.16 + 0.04*x	0.88
	Leaf	$\hat{y}$ = 14.45 + 0.032*x	0.94
	Tuber	$\hat{y}$ = 14.57	-
		Kale	
Zn	Root	$\hat{y}$ = 11.36 + 16.84***x	0.99
	Stem	$\hat{y}$ = 17.83 + 2.38*x	0.99
	Leaf	$\hat{y}$ = 95.07 + 5,62***x	0.97
Fe	Root	$\hat{y}$ = 718.89 + 5.12***x – 0.12***x²	0.99
	Stem	$\hat{y}$ = 60.73	-
	Leaf	$\hat{y}$ = 202.57	-
Mn	Root	$\hat{y}$ = 77.16	-
	Stem	$\hat{y}$ = 15.63	-
	Leaf	$\hat{y}$ = 134.74 + 0.41*x	0.95
Cu	Root	$\hat{y}$ = 38.49 + 0.069***x	0.84
	Stem	$\hat{y}$ = 1.23 + 0.048*x	0.85
	Leaf	$\hat{y}$ = 15.40	-
		Okra	
Zn	Root	$\hat{y}$ = 35.47 + 26.33***x – 0.06***x²	0.98
	Stem	$\hat{y}$ = 26.22 + 2.94**x	0.99
	Leaf	$\hat{y}$ = 69.10 + 23.17***x	0.99
	Fruits	$\hat{y}$ = 28.72	
Fe	Root	$\hat{y}$ = 281.36 + 3.84***x – 0.01*x²	0.87
	Stem	$\hat{y}$ = 42.59	-
	Leaf	$\hat{y}$ = 161.61	-
	Fruits	$\hat{y}$ = 239.50	-
Mn	Root	$\hat{y}$ = 35.70	-
	Stem	$\hat{y}$ = 14.81	-
	Leaf	$\hat{y}$ = 280.36 + 1.20***x	0.98
	Fruits	$\hat{y}$ = 51.19	
Cu	Root	$\hat{y}$ = 30.36 + 0.18**x – 0.0006**x²	0.75
	Stem	$\hat{y}$ = 0.76 +0.28***x– 0.0008***x²	0.79
	Leaf	$\hat{y}$ = 14.29	-
	Fruits	$\hat{y}$ = 12.08	-

*, ** e *** significant at 5,1, e 0,1% respectively; NS = not significant

The linear increase in Cu content found in the carrot leaves can be associated with the high Zn content of the soil, which promoted a loss of cellular membrane selectivity (Sarma et al., 2006), resulting in higher absorption of Cu by the plants and a subsequent transfer to the leaves. However, the Zn and Mn content in the carrot leaves exhibited a quadratic response, while the Fe concentration was not significantly affected by the Zn content of the soil. The Mn content evaluated in the carrot tubers increased as a function of adding increasing doses of Zn to the soil, while the Zn, Fe and Cu concentrations were not influenced. The increase in Mn content of the edible part of the carrot (tuber) can make this food more nutritious, in view of the essentiality of this micronutrient for human beings (Kostava et al., 2008).

The absence of an increase in the Zn content in the carrot tubers indicates a low capacity to allocate this element in this organ (Yang et al., 2007) and, consequently, its low potential for use in biofortification programs aimed at Zn supplementation in the diet of a given population. The maximum Zn content in the carrot leaves was observed at a dose of 230 mg kg^{-1} of Zn in the soil. This possibly indicates that, beginning at this concentration in the soil, a phytotoxic effect occurred with the consequent development of disturbances in the plant (Bosiacki and Tyksiñski, 2009), inhibiting its absorption and allocation to the leaves.

With the increase of Zn content in the soil, it was observed that all parts of the kale plants exhibited increased concentrations of this element. This indicates that this species absorbs and distributes Zn to all of its organs (Yang et al., 2007), indicating a high capacity for translocation of this element. A similar behavior was also verified in relation to Cu content in the kale roots and stems and for Mn in the kale leaves. In contrast, the Mn (root and stem), Fe (stem and leaf) and Cu (leaf) contents in the kale plants were found to be unaffected by the application of increasing doses of Zn in the soil.

The Zn content encountered in the roots, stems and leaves of okra was significantly affected by the increasing doses of this element applied to the soil. A linear increase of Zn was found in the stems and leaves, whereas in the roots the content of this element presented a quadratic response with a maximum value of 2537.77 mg kg^{-1} of dry matter, observed at a dose of 150 mg kg^{-1} of Zn in the soil. A quadratic response was also observed for Fe and Cu content in okra roots. The reduction in concentration of these elements upon reaching the point of maximum concentration in the roots may be due to phytotoxicity caused by the high Zn content present in the soil (Alloway, 2009).

The Zn, Fe, Mn and Cu contents observed in the okra fruits were not affected by the use of increased doses of Zn in the soil. This demonstrates that Zn is not easily translocated from other organs to the fruit, suggesting that biofortification of okra fruits with Zn is not viable.

In general, with the increase of Zn content in the soil, it was found that the plant species exhibited a higher concentration of this element, showing that fertilization practices can increase its availability in plants (Table 3). However, independently of the Zn dose applied to the soil, higher concentrations were observed preferentially in the roots, with low translocation

Table 3. Concentration of micronutrients in plants, carrots, kale and okra grown in soil with increasing doses of Zn

Culture	Parts of plants	Dose of Zn (mg kg^{-1}) 0	50	150	300
		Zn (mg kg^{-1})			
Carrot	Root	420.60 a	2419.22 a	4600.40 a	8024.37 a
	Leaf	44.42 b	523.62 b	1879.82 b	1703.90 b
	Tuber	69.72 b	72.85 c	105.30 c	113.40 c
Kale	Root	77.87 a	725.60 a	2610.65 a	5059.20 a
	Stem	26.18 a	149.87 b	335.52 c	747.90 c
	Leaf	62.60 a	553.72 a	1174.17 b	1801.05 b
Okra	Root	55.40 a	954.55 a	2537.77 a	1975.87 a
	Stem	14.41 a	188.67 b	466.85 b	908.75 b
	Leaf	39.95 a	1147.75 a	2323.67 a	2258.12 a
	Fruits	28.72 a	28.92 c	33.00 c	48.22 c
CV (%)		16.58			
		Fe (mg kg^{-1})			
Carrot	Root	198.60 a	859.35 a	1626.97 a	748.80 a
	Leaf	192.37 a	211.17 b	227.82 b	250.32 b
	Tuber	283.57 a	237.97 b	274.40 b	204.36 b
Kale	Root	713.67 a	949.95 a	1172.02 a	1016.70 a
	Stem	63.36 c	47.95 c	44.67 c	39.17 c
	Leaf	220.05 b	124.62 b	119.65 b	115.55 b
Okra	Root	235.10 a	533.40 a	599.85 a	595.47 a
	Stem	41.48 c	51.37 c	53.47 c	41.75 c
	Leaf	160.40 b	157.72 b	145.65 b	142.80 b
	Fruits	258.52 a	158.72 b	154.75 b	135.60 b
CV (%)		17.46			
		Mn (mg kg^{-1})			
Carrot	Root	349.05 a	349.15 b	351.42 b	388.52 b
	Leaf	381.42 a	566.82 a	722.27 a	566.72 a
	Tuber	72.87 b	114.70 c	143.70 c	218.06 c
Kale	Root	82.50 a	91.97 a	139.40 a	140.75 b
	Stem	15.25 b	16.90 b	17.82 b	28.37 c
	Leaf	140.72 a	158.52 a	179.55 a	266.77 a
Okra	Root	30.60 b	43.20 b	59.55 b	64.70 c
	Stem	15.02 b	19.45 b	28.70 b	37.92 c
	Leaf	300.92 a	311.47 a	468.75 a	643.20 a
	Fruits	57.13 b	60.00 b	75.95 b	121.83 b
CV (%)		18.03			
		Cu (mg kg^{-1})			
Carrot	Root	36.75 a	39.80 a	39.82 a	51.62 a
	Leaf	14.20 b	17.10 b	17.90 b	24.40 b
	Tuber	13.85 b	16.57 b	16.47 b	19.50 b
Kale	Root	37.77 a	39.35 a	54.28 a	56.38 a
	Stem	2.78 c	4.10 c	4.52 c	17.47 b
	Leaf	15.22 b	16.35 b	17.40 b	20.60 b
Okra	Root	27.37 a	43.40 a	40.90 a	29.68 a
	Stem	2.81 c	4.42 c	25.10 b	5.42 c
	Leaf	13.25 b	16.20 b	16.62 b	18.47 b
	Fruits	11.07 b	13.60 b	13.15 b	13.87 b
CV (%)		14.86			

Means followed by the same letters in the columns do not differ significantly by Scott & Knott test at 5% significance. Small letters compare the contents of the elements between the parts of each plant, by column.

to the shoots. Zn is minimally translocated to the aerial part of the plants (Kabata-Pendias & Mukherjee, 2007) due to a natural impediment present in plant roots (Andrade et al., 2008). Retention of Zn in the root may be the result of the thickening of the endodermis and the casparian strips, reducing translocation of this element, possibly to preserve the physiological processes in the aerial parts of the plants (Sharma & Dubey, 2005).

Increased Zn content in the kale plants was observed in the roots, while the leaves concentrated intermediate values between 62.72 and 1801.05 mg kg^{-1} of Zn in the dry matter, as a function of the Zn doses. The kale presented great potential for transferring Zn from the soil to the leaves (edible part), demonstrating that its utilization in biofortification programs is feasible. However, biofortification programs should evaluate whether the Zn content allocated to the edible part of the plant adequately guarantees food safety, as ingestion of excessive quantities of Zn results in serious health problems (Scherz & Kirchhof, 2006). In biofortification programs, it is also necessary to evaluate the presence of "antinutrient" substances in the plants (Campos-Bowers & Wittenmyer, 2007). Since these substances inhibit the absorption of Zn by the organism, an increase of Zn concentration in the edible part may not be effective in increasing the bioavailable content in food.

The low allocation of Zn in the edible parts of the carrot (tuber) and okra (fruit) plants indicates that these species are not appropriate for use in biofortification programs with this particular element by means of agronomic practices through soil fertilization management (Alloway, 2009)..

The distribution of Fe in carrot plants grown in soil with a natural concentration of Zn (dose of 0) did not significantly differ among the organs. However, it was observed that the plants grown in soil with the addition of Zn (50 to 300 mg kg^{-1}) presented higher relative contents of Fe in the roots, supporting the natural variation of plants in relation to the preferential allocation of micronutrients in the roots (Smical et al., 2008).

In general, it was observed for all the vegetables that the Mn content is higher in the leaves. According to Chandilyan et al. (2006), plants exhibit a higher concentration of Mn in the leaves than in the other organs. This may be due to its relative importance in photosynthesis and its high natural mobility in plants. The application of increasing doses of Zn to the soil resulted in an increase in the concentration of Mn in the carrot leaves, although it promoted a decrease in the content of this element in the edible part (tuber). Interference of Zn in the Mn content in the plant organs seems not to be a rule, and it seems to depend on the plant species, since the concentration of this element in the kale and okra plants was not affected.

The presence of Zn in the soil, independent of the applied dose, did not affect the Cu content in any of the evaluated plants. This demonstrates that the content of this element in the plants has little relation with the Zn content present in the soil.

As a consequence of the increasing doses of Zn in the soil, it was found that the largest percentage of this element was transferred to the leaves of kale, while the concentrations in the leaves of carrot and okra were not affected (Figure 1). These leafy vegetables presented higher potential for transferring Zn from the soil to their edible parts compared with other horticultural plants (Bosiacki & Tyksiñski, 2009). These results demonstrate the differences among species in relation to the capacity to absorb and concentrate Zn in tissues (Yang et al., 2007). The low Zn content in the edible parts of the carrot (tuber root) indicates a reduced capacity of this species to accumulate Zn from the soil in this organ (Yang et al., 2007).

It was also confirmed that, as a function of the increasing doses of Zn applied to the soil, there was a reduction in the Fe content in the edible parts of carrot and okra. This is a consequence of competition among these nutrients (Aloway, 2008). In contrast, Zn increased the concentration of Mn in the edible parts of carrot and kale. This synergistic effect of Zn on Mn concentration in plants was also observed by Montezano

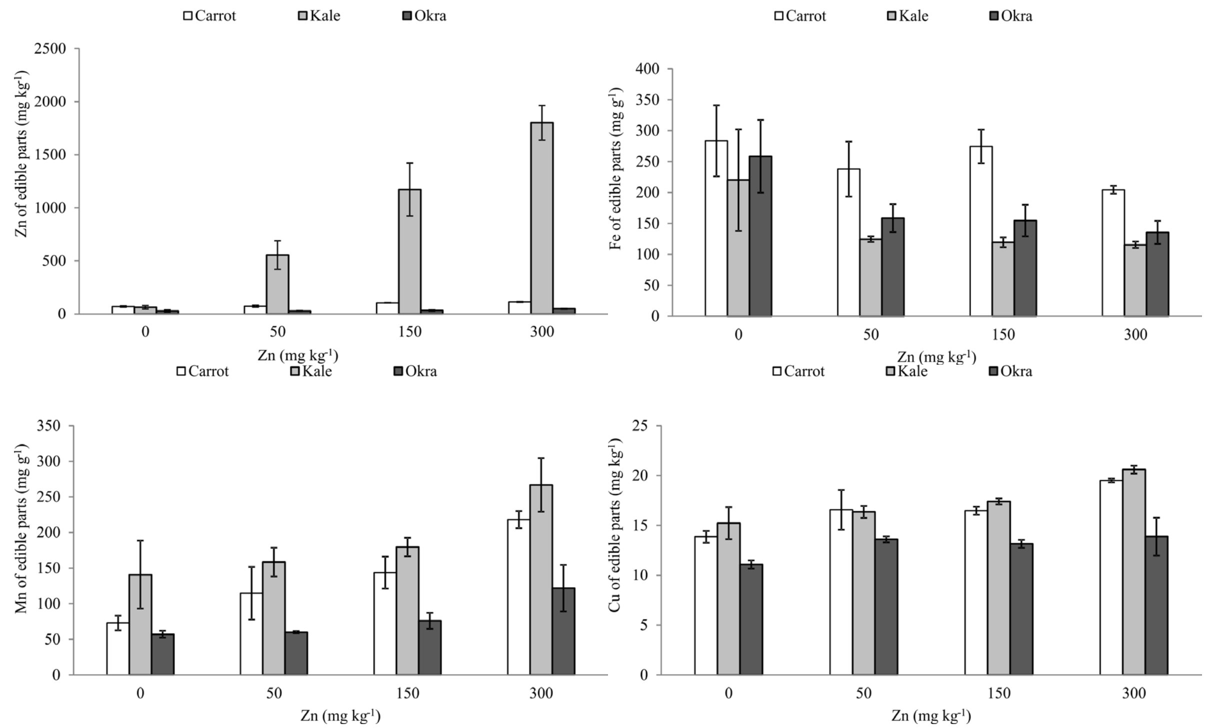

Figure 1. Concentration of Zn, Fe, Mn and Cu in plants of carrot (tuber), cabbage (leaf) and okra (fruit) depending on the doses of Zn in the soil

et al., (2008) and may contribute to increasing the nutritive potential of plants by means of biofortification with Mn, another important micronutrient in the human diet.

Zn did not exert a significant effect on Cu concentrations observed in the edible parts of the plants, although Alloway (2009) alleged that high Zn concentrations in the soil can reduce Cu availability due to competition for absorption sites on the roots, affecting absorption by the plant.

Conclusions

The kale plants exhibited the potential to concentrate Zn in their edible parts, indicating their usefulness for biofortification programs.

As a result of Zn application to the soil, an increase in Mn content was found in carrot tubers and there was a reduction of Fe in the okra fruits, affecting the nutritional balance of plant.

The carrot and okra plants did not transfer the Zn applied to the soil to their edible parts. For this reason, they are not appropriate for use in biofortification programs with Zn.

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