Mineral enrichment in carrot with different sources and doses of zinc[1]

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ABSTRACT

Zinc (Zn) deficiency in soil and plants and its low nutritional status in the population encourage studies on enrichment of agricultural products. Carrot has potential to enrichment because it is a commonly consumed vegetable. The objective of this study was to evaluate Zn sources (ZnO, $ZnSO_4$, or ZnEDTA) and doses (0, 5, 10, 20, and 30 mg dm^{-3}) applied to the soil to increase the concentration of this mineral in the carrot edible part. Zinc sulfate was the most suitable source for this role because it had a higher accumulation with the increase of treatment dose. The best dose was 19.45 mg dm^{-3} of $ZnSO_4$, which resulted the highest content of Zn in carrots. Moreover, this treatment increased Zn in the root by seven times compared with the control. The low production cost of Zn enrichment of carrots makes them a potential vegetable for the deficiency reduction of this micronutrient in the population nutrition.

Keywords: *Daucus carota L.*; enrichment; nutrient deficiency; micronutrient.

RESUMO

Enriquecimento mineral de cenouras com diferentes fontes e doses de zinco

A deficiência de zinco (Zn) no solo e plantas e seu baixo estado nutricional na população estimulam estudos sobre enriquecimento de produtos agrícolas. A cenoura tem potencial para o enriquecimento, pois é um vegetal comumente consumido. O objetivo deste estudo foi avaliar as fontes de Zn (ZnO, $ZnSO_4$ ou ZnEDTA) e a dose (0, 5, 10, 20 and 30 mg dm^{-3}) aplicadas no solo a fim de aumentar a concentração do mineral na parte comestível da cenoura. $ZnSO_4$ foi mais adequado para esse papel, porque apresentou maior acúmulo com o aumento da dose de tratamento. A melhor dose foi 19,45 mg dm^{-3} de $ZnSO_{4,}$ a qual apresentou maior teor de Zn nas cenouras. Além disso, este tratamento aumentou o teor de Zn na raiz em 7 vezes comparado com o controle. O baixo custo da produção de cenouras enriquecidas com Zn as torna uma hortaliça potencial para a redução da deficiência deste micronutriente na nutrição populacional.

Palavras-chave: *Daucus carota L.*; enriquecimento; deficiência nutricional; micronutriente.

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INTRODUCTION

Zinc (Zn) is a trace element that plays an essential role in several organisms, being essential for human and plant growth (Prado, 2008). Its deficiency in soils is a serious global problem; it is estimated that approximately 60% of the world soil is unsuitable for agriculture due to stress caused by mineral deficiency, unavailability, or toxicity of some nutrients (Pathak *et al*., 2012). In Brazil, Zn deficiency in soil is the most common among the micronutrients, occurring mainly in sandy soils and Latosols in the Cerrado (Brazilian Savannah) region (Fraige *et al*., 2007). Liming, a practice to correct the natural acidity of these soils, is often used and further reduces the availability of Zn to plants, possibly increasing the affinity of this element to specific adsorption sites in the soil (Cunha *et al*., 2008). Low availability of this metal results in significant reductions in productivity and nutritional quality of food (Henriques *et al*., 2012).

Tropical soils have high phosphorus (P) fixation, resulting in the need to use corrective phosphate fertilizer, which can induce Zn deficiency (Alloway, 2009). Regions with soil Zn deficiency are deeply correlated with those that have a high incidence of Zn deficiency in the population (Henriques *et al.*, 2012). When crop cultures are the main alimentary sources for the local human and animal population, Zn deficiency in the diet is unavoidable (Erenoglu *et al*., 2010).

Another important issue is that Zn is a wide-distribution trace element in the human body, necessary for the activity of more than 200 enzymes involved in the maintenance of important metabolic pathways of the organism (Gibson *et al*., 2008). It is estimated that Zn deficiency affects about one third of the world's population (Macêdo *et al*., 2010); it can be potentially identified as a public health problem in many developing countries. In Brazil, studies from different indicators point to low Zn levels in children (Silva *et al*., 2006). To control this deficiency, different methods of food fortification can be adopted to ensure availability of essential nutrients, including Zn, commonly used in widely consumed food.

Among the interventions that are being used to solve Zn deficiency in humans, fortification and supplementation are being widely applied in some countries. However, these approaches have a high cost and are not readily available in developing countries. Biofortification with fertilizer is presented as a quick, cost-effective, and useful way in complementary approach to improve nutrient concentrations (Cakmak, 2008). On the other hand, genetic biofortification can present itself as a viable strategy to fight nutritional deficiencies (Rios *et al*., 2009); however, a multidisciplinary approach is necessary, which includes agronomic knowledge (Moraes *et al*., 2012).

Carrot (*Daucus carota L.*) has great potential for mineral enrichment because is a commonly consumed vegetable by the Brazilian population (Brasil, 2009). Therefore, it is important to verify the best ways to get more nutritious cultivars through agronomic practices. Enrichment using micronutrients in common foods access can be an alternative to reduce the growing nutritional problems in the population.

Considering the impact of the lack of Zn in soil and plants and nutritional status of the population, studies on the agronomic enrichment of food with this element become necessary. In addition, the use of fortified fertilizer with Zn is a recent issue that is rarely studied, mainly in Brazil, despite its importance. Besides, the appropriate use of this micronutrient should be considered because, when applied in high doses, it can cause damage to plants and contamination of soil. Thus, the objective of this study was to evaluate the sources and Zn doses applied to the soil to increase the concentration of this element in the edible part of carrot, without impairing plant growth and production.

MATERIAL AND METHODS

Description of the Experimental Area

The experiment was conducted in the experimental area of the Universidade Federal do Pampa, Campus Itaqui (29º07'31" S; 56º33'11" W; altitude 57 m) in a greenhouse. The climate is CFa (temperate subtropical in Köppen classification) and is characterized by having the temperature of the warmest month above 22 °C and of the coldest month not lower than 3 °C.

Experimental Design and Treatment

The experimental design was a completely randomized design and treatments were five doses of Zn (0, 5, 10, 20, and 30 mg dm^{-3}) and three sources of Zn (zinc sulfate, zinc oxide, and chelated (EDTA) zinc) with four replicates, applied to the soil. We used 52 vessels with a capacity of 5 L, filled with 3 kg of sand and 2 kg vermiculite each. Cultivar Nantes carrots was seeded, with eight seeds distributed in a circular format. After thinning, four plants remained per vessel. The vessels were daily irrigated with deionized water and modified (without zinc) nutrient solution of Hoagland & Arnold (1950); the ionic strength of the solution started by 10%, being increased with the development of treatments up to the total of 100%. The vessels were identified according to the treatment, dose value, and repetition number.

Evaluation

On the 34th day after planting and two days before harvest (day 103) the measurement of foliage height was carried out. At the end of the experimental period, plants were harvested and the leaf area was separated from the root for determination of fresh and dry matter of both parts, in addition to measurement of root length and diameter. Deformities were also evaluated in the roots and classified according to the Programa Brasileiro para Melhoria dos Padrões Comerciais e Embalagens de Hortigranjeiros (Brazilian Program for the Improvement of Commercial Standards and Horticultural Packaging) (Brasil, 2000).

After these evaluations, samples were prepared for Zn and Fe determinations.

Sample Preparation

The plant tissues were washed in deionized water, followed by weighing and drying in an oven with forced-air circulation at 65 °C until constant weight. The plant material was ground in a Wiley mill and passed through a sieve of 20 "mesh" (0.841 mm). Dried samples were decomposed by mixture of HNO_3 and H_2O_2 (3:1) in digester block according to the methodology described by Silva (2009).

Zinc and Iron Determination

Analysis of Zn concentration was performed in an atomic absorption spectrophotometer novAA 300, with acetylene/air flame, according to the methodology indicated by the equipment software.

To check one possible interference of Zn in the iron (Fe) absorption by the plant, the concentrations of Fe in the sample were determined according to NBR 13934 (ABNT, 1997), with adaptations, in UV-Visible (8453 HP-Agilent).

Statistical Analysis

Statistical analysis was carried out with the program Graph Pad PRISM® 5.0 (2007) by one-way ANOVA and Dunnett comparison test when necessary. Results with p values < 0.05 were considered significant.

In order to determine the optimal dose for the accumulation of Zn, the segmented regression by broken-line model was performed (Portz *et al.*, 2000; Robbins *et al.*, 2006). This model is obtained by the method of least squares and aims to determine the break point, i.e., the point representing the lowest sum of squares. These determinations were performed in Proc NLIN of SAS software.

RESULTS AND DISCUSSION

Plant Growth and Roots Evaluation

The measurement of the plant foliage height was carried out to check if there was interference of each treatment on their growth and development. The results performed on the 34th day after planting are shown in Figure 1. When comparing the groups of plantas treated with different sources and doses of Zn, ZnEDTA group dose 30 mg dm^{-3} showed a lower growth of the foliar part of plant compared with the control group in the initial growth. On the other hand, there was no significant difference between groups (data not shown) in relation to the heights measured on the 103rd day. Silva *et al.* (2014) reported that the source of chelated Zn reduces the absorption of the elements by the plant. Thus, initially, the use of treatment with ZnEDTA at its highest dosage maintained the nutrients adsorbed in the soil, presenting growth deficit in the plant, which was gradually corrected physiologically during the experiment, being adapted to the conditions to which it was submitted. This may be the explanation for lower leaf growth in ZnEDTA treated plants in the early days, which was no longer significant at the end of the experiment.

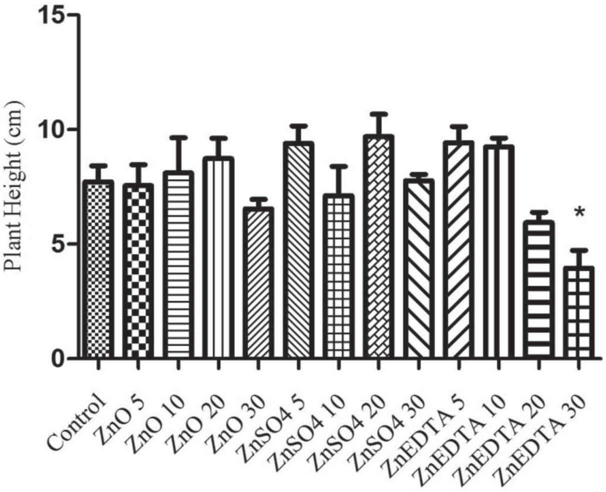
After harvesting, weight, length, Zn content in the edible part, and carrot appearance for purchase intent were evaluated. The replicates that presented carrots with defects are described in Table 1. Only one replicate of 5 mg dm^{-3} ZnEDTA dose presented carrots with defects as well as 30 mg dm^{-3} ZnSO4 and 30 mg dm^{-3} ZnO. On the other hand, for the larger doses of ZnEDTA, more than one vase showed defects, in dose 20 mg dm^{-3}, two replicates, and in 30 mg dm^{-3}, all replicates, sometimes with more than one defect. In Table 1, they are only the treatments that showed defects according to the classification of the Brazilian Program for the Improvement of Standards Commercial and Horticultural Packaging (Brasil, 2000), in which the categorization is done by comparing the product obtained with the established standards. According to the classification norm, the minimum quality standard does not tolerate marketing roots with serious defects, such as deformation, cleavage, rot, wilt, among others. In all treatments in which defects were found, these were classified as 'serious'; the other treatments not presented in Table 1 showed no defects. Therefore, all samples with the dosage 30 mg dm^{-3} of ZnEDTA were considered unmarketable because all the repetitions of this treatment displayed a serious defect. The treatment with EDTA led to a greater number of groups with defects. Moreover, it was observed that the application of a higher dosage, regardless of source, resulted in an increased frequency of defects.

In relation to root weight, the 20 mg dm^{-3} ZnEDTA treatment was the only one that presented lower average weight when compared with the control (Figure 2A). The ZnEDTA treatment also affected root growth, with a length reduction with the use of higher doses (20 and 30 mg dm^{-3}) when compared with the control group (Figure 2B). Thus, this treatment showed some degree of toxicity, reducing the size of produced vegetable. According to Sandall (2015), ZnEDTA is more effective when used with dry fertilizer because of its ability to mobility; when used with liquid fertilizers, it has the same effectiveness of inorganics; and as main disadvantage, ZnEDTA is more expensive and, therefore, require a greater cost to meet the needs of Zn in soil. Thus, when evaluating the disadvantages imposed by the application of the doses 20 and 30 mg dm^{-3} of ZnEDTA source, is possible to conclude that for carrot enrichment, this treatment is not adequate.

Zinc Root Concentration

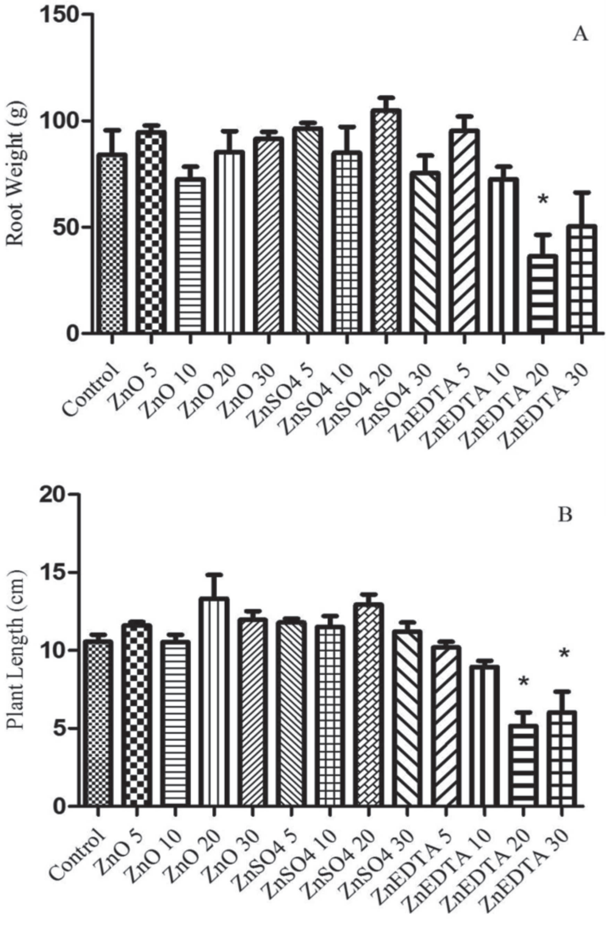
As shown in Figure 3, the carrots treated with ZnO kept increasing Zn absorption with the increase of metal dose, and up to the maximum dose, it was not possible to observe accumulation stability. In the ZnSO$_4$ treatment, it was observed an absorption peak at 20 mg dm^{-3} dosage, and afterwards, there was a small drop, which may indicate a certain toxicity at the highest dose. According to Alloway (2009), the reduction in concentration of these elements to reach the point of maximum concentration in the roots may be due to phytotoxicity caused by the high Zn content in the soil. Chelated Zinc treatment had no increase of Zn accumulation in the root with increment of treatment dosage.

When analyzing the results of the Zn content in mg kg^{-1} (Table 2), we verified that the highest contents were found in treatments ZnO and 30 mg kg^{-1} ZnSO$_4$; however, these have a high standard deviation evidencing a non-uniformity



*Different from Control (one-way ANOVA; Dunnett test; $p < 0.05$).

Figure 1: Average of plant foliage with different sources and doses of zinc on the 34th day after planting.



* Different from Control (one-way ANOVA; Dunnett test; $p < 0.05$).

Figure 2: Root weight *in natura* (A) and root length (B) with different sources and doses of zinc.

Table 1: Defect type and classification according to the Brazilian Program for the Improvement of Standards Commercial and Horticultural Packaging of roots evaluated. Only treatments with defective carrots are presented

Treatment	Defect	Classification
ZnEDTA 5* R1**	Deformation	Serious defect
ZnEDTA 30* R1**	Crack	Serious defect
ZnEDTA 30* R2**	Deformation and Crack	Serious defect
ZnEDTA 30* R3**	Deformation and Crack	Serious defect
ZnEDTA 30* R4**	Rootless	Serious defect
ZnEDTA 20* R1**	Crack	Serious defect
ZnEDTA 20* R3**	Crack	Serious defect
ZnSO$_4$ 30* R3**	Crack	Serious defect
ZnO 30* R4**	Deformation	Serious defect

* Dosage.

** Repetition number.

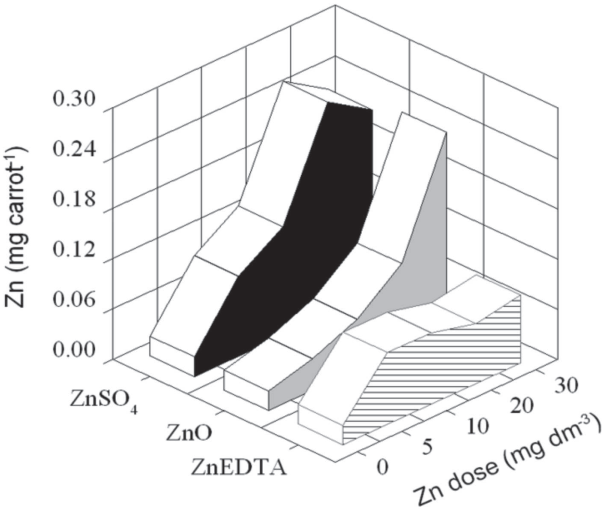


Figure 3: Root Zn accumulation in relation to dose in treatments, with different sources and doses of zinc.

between the concentrations found in different replicates. The treatment 20 mg kg^{-1} ZnSO$_4$ does not result in a higher concentration of Zn but presents a low deviation between the replicates and resulted in a greater accumulation by carrot because they exhibited larger size and better appearance in relation to the other treatments.

About the accumulation of Zn in the edible part of carrot, it was found that, due to increase in the Zn content in the soil, the plants generally have higher concentrations of this element, demonstrating that fertilization practice can increase the availability of Zn to plants, which is potentially absorbed. According to Kabata-Pendias & Mukherjee (2007), regardless of Zn dose applied in the soil, the highest concentrations are observed mainly in roots, which have low translocation to the shoot. Zinc is minimally translocated to the shoot due to a natural impediment present in its roots (Andrade *et al*., 2008), so the carrot has a high potential for enrichment with this element in the edible part, justifying the considerable metal accumulation found in this work.

These results demonstrate that the best treatment for Zn fortification in carrots is the zinc sulfate source at a dose of 20 mg dm^{-3} because no deformity was observed in this treatment, and it presented the highest root weight and element accumulation. Zinc oxide also presents an increasing accumulation, but would require a larger dose, which is a disadvantage to this source because it is little soluble. Sandall (2015) mentioned that the ZnSO$_4$ is the Zn source most used as fertilizer, which, being an inorganic compound relatively soluble in soluble and effective in granular form, should be applied in the areas of soil with low levels of this mineral.

The segmented regression by the broken-line model resulted in break point in dose 19.48 mg dm^{-3} (Figure 4), indicating that from this, there is no significant difference in Zn accumulate per carrot and confirming that the dose near 20 mg dm^{-3} is the ideal.

The Fe concentration found in the samples was not affected. Table 2 shows that there was no significant difference in Fe content from different sources and doses of zinc, which is presented as a positive feature. Since carrot is a food source of Fe, it becomes important to check any reduction of its content. In the study by Lima *et al.* (2015), Zn levels found in carrot roots increased linearly, whereas a quadratic response was observed in relation to the Fe content in these plants; thus, it was also confirmed that, with application of increasing doses of Zn in the soil, there was a reduction of Fe content in the edible parts of carrot. Smical *et al*. (2008) found that lettuce grown in soil with the addition of Zn (50 to 300 mg kg^{-1}) had higher relative contents of Fe in the roots, supporting the natural variation of the plant in relation to preferential assignment

Table 2: Average (± s.d.) Fe and Zn content found in the roots with different sources and doses of zinc (mg dm^{-3}) (n = 4)

Treatment	Zn mg kg^{-1}	Fe mg kg^{-1}
Control	10.26±1.74	64.83±20.61
ZnO 5	11.79±1.55	44.02±7.91
ZnO 10	17.32±1.71*	66.65±18.60
ZnO 20	35.81±12.08*	65.45±6.68
ZnO 30	79.09±21.62*	62.05±17.34
ZnSO$_4$ 5	30.47±2.69*	73.01±25.11
ZnSO$_4$ 10	46.21±7.60*	70.16±21.34
ZnSO$_4$ 20	72.31±8.45*	64.26±14.88
ZnSO$_4$ 30	88.74±19.20*	60.35±4.06
ZnEDTA 5	26.30±2.09*	66.92±6.87
ZnEDTA 10	34.66±6.39*	52.73±6.52
ZnEDTA 20	48.34±17.93*	61.72±15.79
ZnEDTA 30	56.68±14.19*	69.15±7.20

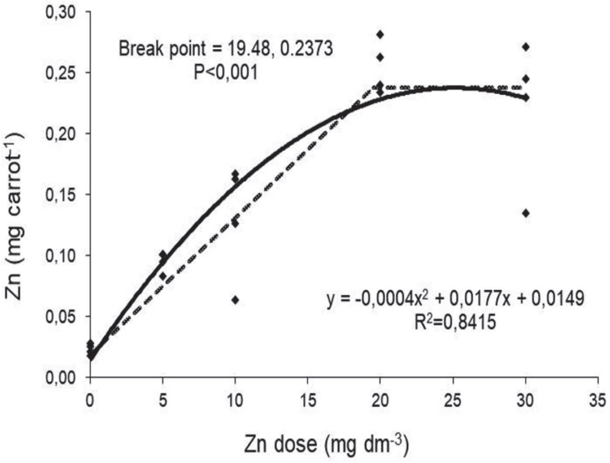
* Different from control (one-way ANOVA; Dunnett test; $p < 0.05$).

of micronutrients to the roots, differing from that found in this study with carrots.

Zinc and Fe content found in carrot samples of this study showed higher values when compared with the levels of the same vegetable expressed in the Brazilian Table of Food Composition (TACO) (2 mg kg^{-1} of Zn and 1 mg kg^{-1} of Fe) (NEPA, 2011). This difference can be explained by several factors such as the variety used is not specified in TACO Table, besides the experimental design. According to Andrade (2010) and Zapelini & Zapelini (2007), the trials in which the researcher maintains the phenomena in control conditions and reduces external influences, as occurs in greenhouse and in experiments in vessels, in scientific terms, is the most accurate design. While the field research is subjected to the influence of several variables, since it is applied in natural settings, research in the greenhouse, the control of variables allows the achievement of results under the influence of certain variable analyzed separately by controlled conditions (Couto *et al.*, 2011) so the trend of nutrient accumulation in greenhouse samples is more comprehensible.

Comparing the treatments studied, $ZnSO_4$ has been proved to be the most suitable for enrichment for having a greater accumulation of Zn with increasing doses. The best dose was 19.45 mg dm^{-3} of $ZnSO_4$ as it demonstrated higher content depending on the carrot size. Silva *et al.* (2014) verified in the foliar analysis that the absorption by the plants was differentiated and, also observed that the sulfated source obtained better availability of the nutrient for the plants than that chelated in the bean culture.

Providing Zn for plant through the application of fortified fertilizer in the soil and/or foliar application seems important to ensure the concentration of this element (Macêdo *et al.*, 2010). The application of fertilizers with Zn has shown promising results, Pascoalino (2014) described an average increase of Zn concentration in wheat grains of 1.06 times compared with the control. Therefore, carrot has more potential for Zn biofortification compared with wheat, as this mineral remains more concentrated in the root and has greater difficulty in translocation to aerial parts (Lima *et al.*, 2015).



Segmented regression by the broken-line model (——); tendency line for non-linear regression.

Figure 4: Application of the segmented regression and linear quadratic models to the results of $ZnSO_4$ treatment, fitted to the root Zn accumulation in per vase response.

The introduction of biofortified crops with a higher content of minerals and vitamins complements the existing nutrition interventions and provides a sustainable and inexpensive manner to reach populations with limited access to formal market systems and health (Nutti *et al.*, 2005). In recent years, evaluating among the studied nutrients, the best responses have been obtained with the application of Zn because it is possible to select efficient plants for greater element accumulation and, also responsive to the addition of this micronutrient by the fertilization (Moraes *et al*., 2009; White & Bloadley, 2005). Yilmaz *et al.* (1997) and Cakmak *et al.* (2010) evaluated Zn application methods and demonstrated that the application of Zn doses in the soil improved productivity and Zn content in wheat grain.

With enrichment using the treatment with best results for plant development (20 mg dm^{-3} $ZnSO_4$), the Zn concentration in the root increased by seven times compared with the control (Table 2). According to Institute of Medicine (2001), Zn recommendation for healthy adults is 8 mg day^{-1} for women and 11 mg day^{-1} for men. Thus, the consumption of fortified 100 g Carrot (72.31 mg kg^{-1}) would easily reach this recommendation and reduce considerably the potential for this micronutrient deficiency in the population since it corresponds to substantially all the Zn required in daily intake.

CONCLUSION

In this study, it was possible to identify the source that seems promising for Zn enrichment in carrots.

Comparing the sulfate, oxide, and complex (EDTA) sources, the 19.45 mg dm^{-3} zinc sulfate dose increased vegetable growth and no defect that impairs marketing was detected.

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