

Efecto de la biofortificación con hierro y zinc en la producción, acumulación de metales pesados y biomoléculas en lechuga

Effect of biofortification with iron and zinc on production, accumulation of heavy metals and biomolecules in lettuce

Oscar Silva-Marrufo

Tecnológico Nacional de México, Instituto Tecnológico del Valle del Guadiana, Departamento de Ingeniería. Durango, México.

<https://orcid.org/0000-0003-2064-5298>

Angie Tatiana Ortega-Ramirez

Universidad de América, Facultad de Ingenierías, Grupo de Investigación de Procesos Sostenibles (GPS). Bogotá D. C., Colombia.

<https://orcid.org/0000-0002-6364-8432>

Oscar Sariñana-Aldaco

Universidad Autónoma Agraria Antonio Narro, Programa posdoctorado-CONAHCYT. Saltillo, Coahuila, México.

<https://orcid.org/0000-0002-9189-1931>

Rubén Iván Marín-Tinoco*

Universidad Tecnológica del Rodeo, Departamento de Química Área Tecnología Ambiental. Rodeo, Durango. Instituto Mexicano del Seguro Social (IMSS), México.

<https://orcid.org/0000-0003-4885-223X>

Revista de la Facultad de Agronomía

Universidad Nacional de La Plata, Argentina

ISSN: 1669-9513

Periodicidad: Continua

vol. 124, 2025

redaccion.revista@agro.unlp.edu.ar

Recepción: 19 de febrero 2024

Aprobación: 26 de septiembre 2024

Publicación: octubre 2025

URL: <http://portal.amelica>

DOI: <https://doi.org/10.24215/16699513e153>

*Autor de correspondencia: ruben.marin@utrodeo.edu.mx

Resumen

La lechuga (*Lactuca sativa* L.) de la variedad Parris Island, es un cultivo de hoja nativo del sur de Europa y oeste de Asia. Evaluar el rendimiento, calidad nutracéutica y acumulación de plomo en la producción de lechuga biofortificada con hierro y zinc en combinación establecida por NFT (Nutrient Film Technique). El experimento consistió en cinco tratamientos mixtos a diferentes concentraciones de hierro+zinc de 0, 25, 50, 100 y 200 $\mu\text{mol L}^{-1}$. Las variables analizadas en el rendimiento fueron: Peso fresco total, peso fresco de raíz, peso fresco de tallo, peso fresco de hoja, número de hojas, altura de raíz, altura de planta y altura de hoja. Sólo se observan diferencias estadísticas en los tratamientos con 50 $\mu\text{mol L}^{-1}$ y punto de control. Las variables nutracéuticas que analizamos fueron: clorofilas a y b, el mayor contenido fue la concentración de 50 $\mu\text{mol L}^{-1}$, carotenoides en los cuatro tratamientos destacaron a diferencia del testigo, hierro total, la concentración de 200 $\mu\text{mol L}^{-1}$ absorbió 1.2 mg L^{-1} y zinc total la concentración de 50 $\mu\text{mol L}^{-1}$ con un contenido de 2.52 mg L^{-1} , capacidad antioxidante la concentración de 25 $\mu\text{mol L}^{-1}$ fue de 1.895 mg EAA g^{-1} FW. La acumulación de metales pesados en hojas no mostró toxicidad en 100 g de muestra fresca, la calidad nutracéutica a concentraciones de 25 y 100 $\mu\text{mol L}^{-1}$ fueron las que sobresalieron, además de los niveles de clorofilas 50 y 100 $\mu\text{mol L}^{-1}$, la presencia de plomo y arsénico en hojas de lechuga, al aumentar las concentraciones disminuye el contenido de cadmio y se estabiliza en 1.2%. La mejor concentración de hierro+zinc fue de 50 y 100 $\mu\text{mol L}^{-1}$ mejorando hasta un 50% en mayoría de variables estudiadas.

Palabras claves: Biofortificación, calidad nutracéutica, lechuga, hierro, zinc

Abstract

Lettuce (*Lactuca sativa* L.) of the Parris Island variety is a leafy crop native to southern Europe and western Asia. To evaluate the yield, nutraceutical quality and lead accumulation in the production of lettuce biofortified with iron and zinc in combination established by NFT (Nutrient Film Technique). The experiment consisted of five mixed treatments at different iron+zinc concentrations of 0, 25, 50, 100 and 200 $\mu\text{mol L}^{-1}$. The variables analyzed for yield were total fresh weight, root fresh weight, stem fresh weight, leaf fresh weight, number of leaves, root height, plant height and leaf height. Statistical differences were only observed in the treatments with 50 $\mu\text{mol L}^{-1}$ and control point. The nutraceutical variables that we analyzed were: chlorophylls a and b, the highest content was the concentration of 50 $\mu\text{mol L}^{-1}$, carotenoids in the four treatments stood out unlike the control, total iron, the concentration of 200 $\mu\text{mol L}^{-1}$ absorbed 1.2 mg L^{-1} and total zinc the concentration of 50 $\mu\text{mol L}^{-1}$ with a content of 2.52 mg L^{-1} , antioxidant capacity the concentration of 25 $\mu\text{mol L}^{-1}$ was 1.895 mg EAA g^{-1} FW. The accumulation of heavy metals in leaves showed no toxicity in 100 g of fresh sample, the nutraceutical quality at concentrations of 25 and 100 $\mu\text{mol L}^{-1}$ were those that stood out, in addition to the levels of chlorophylls 50 and 100 $\mu\text{mol L}^{-1}$, the presence of lead and arsenic in lettuce leaves, with increasing concentrations the cadmium content decreases and stabilizes at 1.2%. The best concentration of iron+zinc was 50 and 100 $\mu\text{mol L}^{-1}$ improving up to 50% in most of the variables studied.

Keywords: Biofortification, nutraceutical quality, lettuce, iron, zinc

INTRODUCTION

Lettuce is an important crop for small and medium producers, which have low availability of nutrients, moisture and mainly organic matter if they do not give the proper agronomic management (Yu et al., 2022). According to Kim et al. (2016) internationally, lettuce is rich in fiber, fatty acids, amino acids, vitamins A, C, E, B₁, B₂ y B₃, proteins and minerals (Cu, Al, Na, Mg, K y Ca), the leaves are fully edible. Biofortification is currently the most widely used technique to increase the nutraceutical quality of crops. It consists of increasing the nutritional quality of micronutrients, especially in leaves, grains, and seeds, roots and tubers, allowing to improve the nutritional conditions of the producer's family nucleus thanks to self-consumption (Tofiño et al., 2015). Currently, water recirculation systems (hydroponics) are in high demand for the addition and formulation of nutrients called nutrient solution (INTAGRI, 2017).

Nutrients can be used through a nutrient solution source to supply them through foliar sprays at different concentrations, the plant receives them in an optimal and balanced way, thus ensuring a good yield, nutritional quality and low content of heavy metals (Sida-Arreola et al., 2015). Production does not create a serious problem; if not, society in general demands high quality food products, however, it is necessary to reduce hunger and above all to satisfy the needs that contribute to human nutrition. Usually about 22 minerals are needed for proper human development within the system (Ríos-Ruíz et al., 2013).

Iron and zinc in plants are of utmost importance in the productive performance in humans, also the contributions of zinc in leaves, can favor in terms of absorption, however, the above shows that all contributions with these microelements will raise the quality and will be feasible in any crop (Cakmak y Kutma, 2018). Lucena y Hernandez (2017), It has been shown that iron is the fourth element available in the soil, and that the soil generally contains between 1 and 5% of total iron. This information will contribute to mitigate nutritional imbalances, proposing diet diversification programs, high mineral intake, fortification of processed foods (food fortification) and, as we have seen, crop biofortification programs that will enrich our food diet (Unicef Tacro, 2008).

Zinc promotes photosynthetic quality in plants by improving the synthesis of photosynthetic pigments, it is also a micronutrient capable of reducing the absorption and accumulation of heavy metals in plants (Cd, Cr and As), which allows reaching average standards of enzyme activity and improves growth and yield under stress (Hassan et al., 2022). The previous work refers to a tool and key piece to improve their nutritional requirements in order that our crops obtain an adequate development and useful life, so that they can be used for human consumption without the fear of being able to ingest them in their diets (Funes et al., 2015). Therefore, the objective of the present research was to evaluate iron and zinc biofortification in combination on yield, nutraceutical quality and arsenic, lead and cadmium accumulation in parris island lettuce plants, under a hydroponic system, to prove that iron+zinc foliar sprays do not result in high heavy metal contents in the experiment.

METHODOLOGY

The present investigation was developed in the municipality of Rodeo, Durango Mexico. The municipality has an altitude of 1,341 (m.a.s.l.) meters above sea level, as well as an area of 1.854,90 km² (Equivalent to 1,854.90 hectares), with a semi-arid climate. The town has a population of 13,554 inhabitants (Instituto Nacional de Estadística, 2010). In geographic coordinates 25.1705 °N, 104.5364 °W.

AGRONOMIC MANAGEMENT OF PLANT MATERIAL ON THE EXPERIMENTAL AREA

Lettuce seeds of the parris island variety were purchased. Harris Moran Seed Company Certified®. The seeds were planted in polyethylene containers with 200 cavities, which were disinfected with 10% hydrogen peroxide. For sowing, a mixture of peat moss and perlite (1:1 v/v) was used. The experiment was established within the facilities of the Technological University of Rodeo, Rodeo, Durango, in the greenhouse area, the temperature was controlled from 28 to 46 °C with a relative humidity of 60%.

ENABLING AND CONDITIONING OF THE NFT SYSTEM

The present seedlings, once the period of 30 days in the polyethylene containers had been completed, were established in plastic baskets and PVC tubes previously disinfected with 10% hydrogen peroxide, an Evans® submersible pump model AQUA was installed. 60W to generate oxygenation in water and that the plant can be adapted to the production system, in addition a timer was installed to adjust the execution times of the pump. Steiner Nutrient Solution (SN) was developed

according to the water analysis. It was supplied at a concentration of 50% in a 50 L container of stock solution, the application was for one week. The solution was increased to 75% by applying it for 2 weeks, at the end of the crop life cycle stage and reduced to 25% (Steiner, 1984).

FORMULATION OF THE STEINER NUTRIENT SOLUTION FOR PLANT NUTRITION IN LETTUCE PLANTS

The nutrient solution was applied once a week, the first percentage of nutrient solution at 50% was applied 60 days after the transplant and was subsequently applied at 75% at 85 days, at the end of the productive cycle of the crop, the NS (Nutritive solutions) was reduced to 25% (Steiner, 1961), before harvesting it to prevent the lettuce from being bitter, the fertilizers used were: HNO₃ (Nitric acid), H₃PO₄ (Phosphoric acid), CaNO₃ (Calcium nitrate), KNO₃ (Potassium nitrate), K₂SO₄ (Potassium sulfate), MgSO₄·7H₂O (Magnesium Sulfate), Micronutrients and the addition of iron (Fe) and zinc (Zn) in the form of sulfates (Table 1). The pH and electrical conductivity were maintained in ranges between 5.5 dS m⁻¹ and 2.0 dS m⁻¹, (Table 1).

TABLE 1
Amount of fertilizers applied for the 25%, 50% and 75% Steiner solution in 50 L of water.

Agrochemicals	Nutrition solution to 25%	Nutrition solution to 50%	Nutrition solution to 75%
Nitric acid (HNO ₃)	0.5 mL	1.5 mL	2.2 mL
Phosphoric acid (H ₃ PO ₄)	2.2 mL	4.5 mL	6.15 mL
Calcium nitrate (CaNO ₃)	8 g	16 g	22 g
Potassium nitrate (KNO ₃)	4.5 g	9 g	13.65 g
Potassium sulfate (K ₂ SO ₄)	3.5 g	7.2 g	10 g
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	5.2 g	10.5 g	17.2 g
Element micro	0.5 g	1.5 g	2 g

At the end of the activities, application doses were prepared in the form of plant assimilation in mg, which were weighed on a Scientific® brand digital scale, after that, using 1 L of water and 1 mL of Agri Best® brand dispersant. In five sprinklers marked with the treatment number, the minerals were diluted, three applications were carried out in a period of 15 days, after the seedling was established in the recirculating water system (hydroponics). It is worth mentioning that the foliar application doses of the two elements were established based on the form of assimilation of the plant (Iron and zinc), mixed in a single preprinted micro sprinkler with trigger (TOLCO, Mexico) (10 mL) with a volume and frequency of 0.2 mL plants⁻¹ (Table 2).

TABLE 2
Formulation of iron+zinc concentrations applied by foliar spray in lettuce.

Treatments of iron sulfate (μmol L ⁻¹)	Iron sulfate (g L ⁻¹)	Treatments of zinc sulfate (μmol L ⁻¹)	Zinc sulfate (g L ⁻¹)
0	0	0	0
25	0.0037977	25	0.00404
50	0.0075955	50	0.00807
100	0.0151910	100	0.01615
200	0.0303820	200	0.03229

EXPERIMENTAL DESIGN

The present data collected adhered to a completely randomized design; since given the circumstances they were submitted in 25 experimental units, that each plant is an experimental unit by means of a comparison of means with the Tukey test at 0.05%.

VARIABLES EVALUATED

Analytical Assessments

The variables evaluated were yield (root height, root weight, and leaf height, per plant), product quality parameters (total weight, number of leaves, and plant height), as well as antioxidant capacity, accumulation of total iron and zinc and accumulation of heavy metals (Lead, arsenic and cadmium) due to the effect of foliar spraying in combination of iron and zinc in parris island type lettuce plants (Figure 1).



FIGURE 1

Harvest of parris island type lettuce in NFT system with 5 treatments and 5 repetitions respectively, where: a). Control, b). $25 \mu\text{mol L}^{-1}$ iron+zinc, c). $50 \mu\text{mol L}^{-1}$ iron+zinc, d). $100 \mu\text{mol L}^{-1}$ iron+zinc and e). $200 \mu\text{mol L}^{-1}$ iron+zinc.

Extraction and quantification of photosynthetic pigments in plant sample

The quantification of photosynthetic pigments was developed following the technique of Wellburn (1994), 60 mg of fresh plant sample (leaves) were weighed using a portable analytical balance Scout® Pro SP202 (Ohaus, Parsippany-Troy Hills, USA), They were then placed in a test tube with a lid and 10 mL of methanol were added and incubated for 24 h. The readings and absorbance were obtained using a Genesys30sV UV spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA), at a wavelength of 666, 653 and 470 nm, and the data were processed using the following formulas (1-4), the Data were expressed in milligrams per gram of fresh weight (mg g^{-1} FW).

$$\text{Chl } a = [15.65 (A_{666}) - 7.34 (A_{653})] \quad (1)$$

$$\text{Chl } b = [27.05 (A_{653}) - 11.21 (A_{666})] \quad (2)$$

$$\text{Chl totals} = [(\text{Chl } a) + (\text{Chl } b)] \quad (3)$$

$$\text{Carotenoids} = \frac{[1000 (A_{470}) - 2.86 (\text{Chl } a) - 129.2 (\text{Chl } b)]}{221} \quad (4)$$

Total quantification of antioxidant capacity under the ABTS+ method

The absorptions by the ABTS reaction at 7 mM of the radical, were obtained by means of potassium persulfate at 2.45 mM (1:1) (v/v), in the dark over a period of 16 hours, after which a solution was made with 20% methanol, with a 0.1 M phosphate buffer (pH 7-7.2), was homogenized with the help of a vortex (Maxi Mix II Thermo Scientific®) for 10 s, until obtaining an absorbance of 0.7 ± 0.01 at a measurement of 754 nm (Re et al., 1999). The absorbance was measured using a UV spectrophotometer Genesys30sV (Thermo Scientific, Waltham, Massachusetts, USA) at 754 nm. The final results were represented as equivalent milligrams of ascorbic acid per gram of fresh weight (mg AAE g⁻¹ FW).

Quantification of iron and total zinc in lettuce leaves

The quantification of total iron and zinc was performed using EPA techniques (1978a; 1978b; 1978c), with slight modifications the samples were calcined in an oven for a period of 48 h, the standard solutions of Fe and Zn were prepared by successive dilution from standard Fe and Zn solutions of 1000 µg/mL in 5% v/v CH₃OH, in 1% v/v HNO₃ in 2.5% m/v NaCl, 0.5% m/v MgCl₂ and 0.8% m/v CaCl₂. For the calibration of the equipment, a blank sample composed of Milli Q water, 5% v/v CH₃OH, 1% v/v HNO₃ and 2.5% m/v NaCl, 0.5% m/v MgCl₂ was prepared, and CaCl₂ at 0.8% m/v. Subsequently crushed using a ceramic mortar and subjected to an atomic absorption spectrophotometer (Analyst 300, PerkinElmer, Germany). Identifying total iron and zinc in each plant sample evaluated and for each treatment, the data were reported in mg/L.

Accumulation of heavy metals due to foliar spraying of iron+zinc in lettuce

The quantification of heavy metals in the plant material of the lettuce, was a total of four samples with their respective witness (checkpoint), it was necessary for each sample of at least 20 g for each treatment. The green plant samples were placed in virgin polyethylene bags, sealed, coded and taken to the laboratory where quartering and sub-sampling (100-200 g) were carried out on the same day (to avoid dehydration of the leaves). Under the methodology and methods proposed by EPA (1978a; 1978b; 1978c).

For the different microwave-assisted digestions, it was necessary to use a CEM digester (Mars 6, USA®). Each sample was weighed 0.5 g (dried and sieved) in EasyPrep Plus® reaction vessels and 10.00 mL of diluted acid was added (HNO₃, 4.8 mol L⁻¹). In temperature programming, the following activity was necessary: a). Power 400-1800 W, b). Temperature ramp: 15 min until reaching 200 °C. In an estimated period of 10 min at 200 °C, pressure 500 psi. For all the calibrations it was necessary to add 10 mL of pure methanol into the cuvettes simultaneously with the samples. Once the different digestions were finished, it was quantitatively transferred and gauged to a volume of 20.00 mL with ultrapure water. The main measurement wavelengths used were 283.3 nm (Pb, Cd and Ar). For all samples, graphite tubes made of pyrolytic coating materials (Thermo Scientific®) and 99.99% argon gas were supported were subjected to an atomic absorption spectrophotometer (Analyst 300, PerkinElmer, Germany) (Tissot et al., 2020). The data was reported in mg kg⁻¹.

PLANT SAMPLING

For each response variable evaluated and, in the treatments, they are described below: 0, 25, 50, 100 and 200 µmol L⁻¹ of iron and zinc in combination, each application had five replicates, therefore, 40 experimental units were obtained (each plant was an experimental unit). All the treatments were subjected every 8 days in foliar form with the help of an atomizer with a capacity of 1 L and 1 mL of dispersant, starting two weeks after the transplant, the comparisons were under greenhouse conditions and the comparison was between the treatments (Iron and zinc concentrations), crop yield, nutraceutical quality and the accumulation of three heavy metals.

ANALYSIS OF THE DATA OBTAINED

The data obtained were analyzed by means of a comparison of means between the treatments, the data obtained were submit betweened using a statistical program IBM SPSS® version 2020, the database was set up in Microsoft Excel for Windows 10 (x64), the comparisons between means and a Tukey test at ($p \leq 0.05$) and adhering to a standard deviation between the treatments (Rivadeneira et al., 2020).

RESULTS

YIELD, GROWTH AND BIOMASS ACCUMULATION

The biomass accumulation by foliar application in combination of iron and zinc underwent changes with a concentration of 50 $\mu\text{mol L}^{-1}$, observing 360.6 g of total weight, 54.4 g of root fresh weight, 66.8 g of stem biomass, fresh weight in leaves with 247.6 g, 46 leaves per plant and leaf height 27.1 cm. 6 g, 46 leaves per plant and leaf height 27.1 cm, this concentration excelled in almost all the response variables studied, except for plant height, which was 43.8 cm lower than the control (44.1 cm), indicating that the treatments are similar ($p \leq 0.05$). On the other hand, spraying 100 $\mu\text{mol L}^{-1}$ Fe and Zn decreased yield in the variables studied with 229.8 g, total weight 35.8 g in root fresh weight, 40 g stem fresh weight, 150.6 g fresh weight in leaves, 40 leaves per plant, 17 cm root height, 36.8 cm plant height and 19.4 cm leaf height ($p \leq 0.05$), however, in the case of spraying with 200 $\mu\text{mol L}^{-1}$ Fe and Zn, it caused a stabilization in almost all the variables studied (Table 3).

TABLE 3

Effect of foliar application of iron+zinc on fresh biomass of lettuce seedlings. Different letters within each column indicate significant difference between treatments (Tukey; $p \leq 0.05$), $n=5$; FW: Fresh weight.

Iron+zinc ($\mu\text{mol L}^{-1}$)	Total biomass	Root biomass	Stem biomass	Leaves biomass	Number of sheets	Root height	Plant height	Leaves height
-----g plant ⁻¹ -----					-----cm-----			
0	354.8 ab	53.2 ab	54 ab	239 ab	40.2 ab	18.9 a	44.1 a	25.2 a
25	304.2 ab	41.6 ab	54.4 ab	203.4 ab	43.2 ab	14.7 b	38.7 ab	24 ab
50	360.6 a	54.4 a	66.8 a	247.6 a	46.4 a	18.7 a	43.8 a	27.1 a
100	229.8 b	35.8 b	40 b	150.6 ab	40.4 ab	17.4 ab	36.8 b	19.4 ab
200	258 ab	44.6 ab	42.6 ab	167.6 ab	39.2 b	18.1 ab	41.5 ab	23.4 ab

The present results according to the Tukey $p \leq 0.05$ test, it was observed that the present concentrations of iron (Fe) and zinc (Zn) in combination did not significantly affect the fresh plant material of lettuce (Parris Island), demonstrating the highest lettuce plant material, the present foliar sprays are with 50 $\mu\text{mol L}^{-1}$, in combination with iron+zinc in almost all the elaborated variables, surpassing the rest of the treatments and some studied variables.

QUANTIFICATION OF PHOTOSYNTHETIC PIGMENTS IN LETTUCE LEAVES

Table 4, shows the results for photosynthetic pigments. The different concentrations of iron and zinc sulfates in combination did not cause significant changes ($p \leq 0.05$) in the concentration of pigments in Parris Island lettuce. The concentration of photosynthetic pigments did not increase with the concentrations studied, therefore there is no statistical difference in these compounds.

This concentration increased the content of chlorophylls a, b, total and carotenoids by 0.723, 0.072, 0.795 and 0.722 $\mu\text{g g}^{-1}$ FW, respectively, compared to the high concentration of 200 $\mu\text{mol L}^{-1}$ iron+zinc, which caused the lowest values in these response variables, observing 0.021, 0.002, 0.023 and 0.020 mg g^{-1} FW.

TABLE 4

Average values of the variables chlorophyll a, b, total and carotenoids due to the effect of applying iron+zinc in different concentrations. Different letters within each column indicate significant difference between treatments (Tukey; $p \leq 0.05$), $n=5$; FW: Fresh weight.

Iron+zinc ($\mu\text{mol L}^{-1}$)	Clorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Total carotenoids (mg g^{-1} FW)
			Sheet	
0	0.552 ab	0.052 ab	0.604 ab	0.551 ab
25	0.367 b	0.036 ab	0.403 b	0.366 a
50	0.723 a	0.072 a	0.795 a	0.722 a
100	0.687 a	0.068 a	0.755 a	0.686 ab
200	0.021 b	0.002 ab	0.023 b	0.020 b

Quantification of total iron and zinc in parris island lettuce leaves

The effects of applying zinc in the form of sulfate in lettuce leaves, it was evidenced that biofortified lettuce with application mediation showed better values, also its nutritional value increased. In the case of zinc sulfate, the increase in zinc is not relevant compared to iron, it is the case of the concentration of $50 \mu\text{mol L}^{-1}$ of iron and zinc in combination the composition of iron in the leaf was of $1.32 \pm 0.200 \text{ mg L}^{-1}$ and $0.524 \pm 0.100 \text{ mg L}^{-1}$ ($p \leq 0.05$), respectively, the composition of zinc in the leaf at that same concentration was $2.52 \pm 0.400 \text{ mg L}^{-1}$, respectively; which is explained due to the fact that the plant naturally has a greater absorption of iron than of zinc, which is also attributed to the applications of nutrient solutions at different application percentages.

The concentration of $200 \mu\text{mol L}^{-1}$ was the one that stood out among the other foliar treatments of iron and zinc in sulfate form with an absorption of $1.320 \pm 0.200 \text{ mg L}^{-1}$ of iron, while the accumulation of zinc was the concentration with $50 \mu\text{mol L}^{-1}$ in sulfate form, an absorption of $2.52 \pm 0.400 \text{ mg L}^{-1}$ was observed, the analysis of the standard deviation evidenced that these two foliar sprays are the ones with the highest Fe and Zn abundance ($p \leq 0.05$) (Table 5).

TABLE 5

Average content of iron and zinc micronutrients in parris island lettuce leaf tissue, due to the effect of applying iron+zinc in different concentrations. Different letters within each column indicate significant difference between treatments (Tukey; $p \leq 0.05$), $n=5$; \pm SD. FW: Fresh weight.

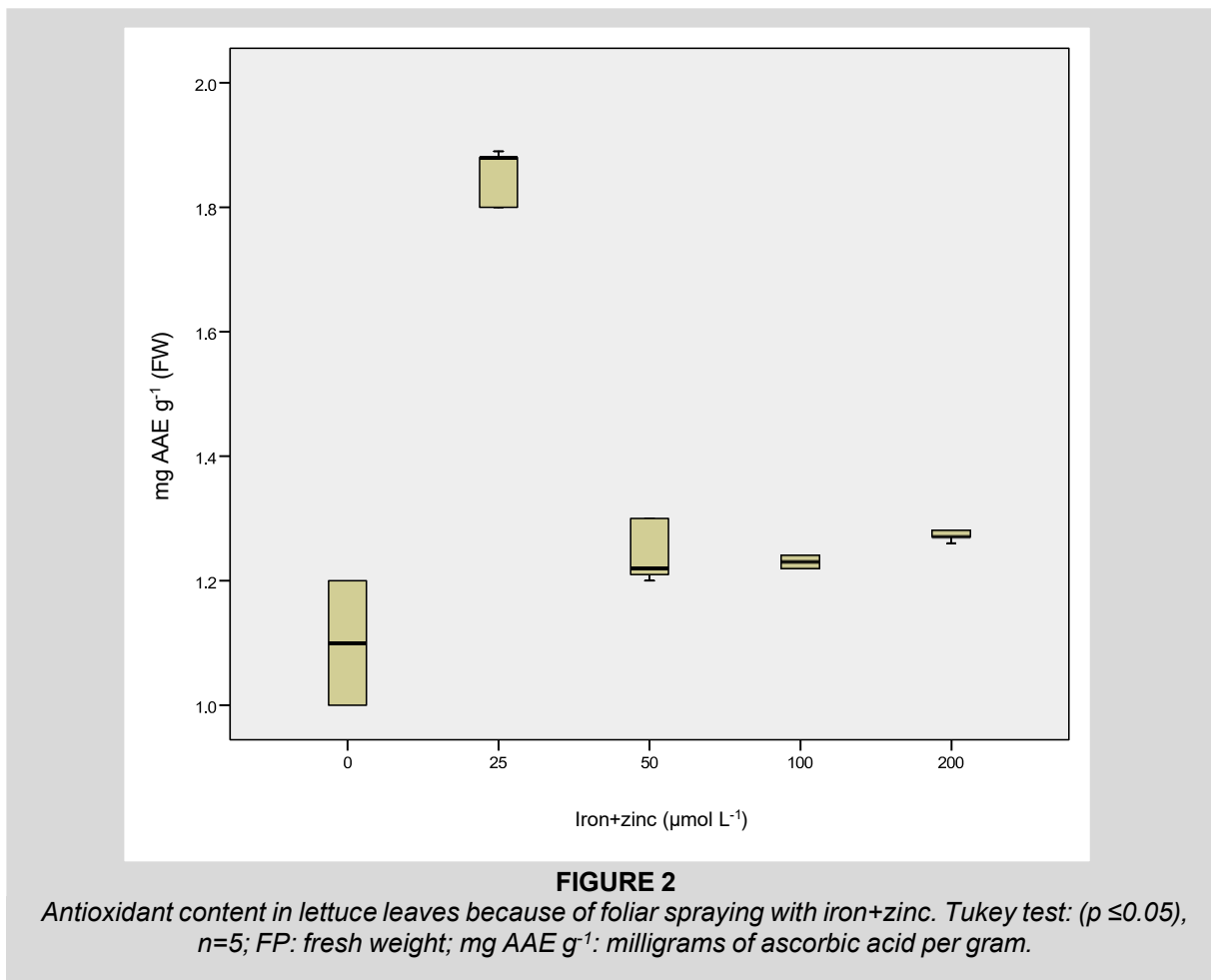
Iron + zinc ($\mu\text{mol L}^{-1}$)	Iron		Zinc
	Leaf mg L^{-1}		
0	0.839 ± 0.100 ab		1.99 ± 0.300 ab
25	0.712 ± 0.100 ab		1.95 ± 0.300 ab
50	0.923 ± 0.100 ab		2.52 ± 0.400 a
100	0.524 ± 0.100 ab		1.13 ± 0.200 b
200	1.320 ± 0.200 a		1.51 ± 0.300 b

Consequently, iron presents low accumulations in the leaf at a concentration of $100 \mu\text{mol L}^{-1}$, as does zinc at the same concentration, this indicates that the higher the combined concentration of iron and zinc sulfates, the greater the presence of iron. On the other hand, the data obtained do not show a significant difference ($p \leq 0.05$), the concentrations with the control, observing $1.320 \pm 0.200 \text{ mg L}^{-1}$ Fe ($200 \text{ iron+zinc } \mu\text{mol L}^{-1}$) and Zn $2.52 \pm 0.400 \text{ mg L}^{-1}$ ($50 \text{ Iron+zinc } \mu\text{mol L}^{-1}$) according to the standard deviation. The presence of zinc in lettuce leaves may be sufficient evidence to enrich different types of lettuce and strengthen their nutritional quality. However, it is evident that high concentrations of iron+zinc (100 and $200 \mu\text{mol L}^{-1}$) via the foliar route in hydroponic lettuces in the absorption of total zinc was not significant ($p \leq 0.05$).

QUANTIFICATION OF THE ANTIOXIDANT ACTIVITY WITH THE ABTS+ METHOD

In Figure 2, it demonstrates in the micro assays under the ABTS+ method, values of 1.895 mg AAE g⁻¹ FW are exposed, with low enrichment in the combination of iron and zinc of 25 μmol L⁻¹, the present concentration exceeded between the others and the minimum was enriched with 100 μmol L⁻¹ of iron and zinc with 1.240 mg AAE g⁻¹ FW and the other combinations of 50 and 200 μmol L⁻¹ were stabilized, as was the control (in the absence of iron and zinc). There was no significant increase ($p \leq 0.05$).

For both trace elements, it was observed that the iron composition was higher since, by foliar spraying from the beginning, it shows a higher antioxidant activity where it is protected in the edible parts, by obtaining a higher interaction with the free radical under the ABTS+ method (Figure 2).



QUANTIFICATION OF ARSENIC, LEAD AND CADMIUM BY FOLIAR SPRAYING WITH IRON+ZINC

It represents an importance; since it indicates the degree of toxicity generated during biofortification with iron+zinc at different supplies, in the case of the heavy metal lead (Pb), in the control a total of 1.988±0.825 mg kg⁻¹ was identified as the point of control, however, it was identified that high concentrations applied by iron+zinc foliar spraying do not generate a large accumulation of lead in the edible parts of the lettuce, along with the heavy metal arsenic, the greatest presence was the control with 0.466±0.083 mg kg⁻¹, compared to cadmium ($p \leq 0.05$), which obtained 0.194±0.39 mg kg⁻¹; as previously mentioned by the permissible limits of heavy metals according to EU (2006) (Table 6). However, the effects of Ar and Pb reduction by the application of 100 μmol L⁻¹ iron+zinc was 0.269±0.002, 1.017±0.010 mg kg⁻¹ and Cd at the concentration of 200 μmol L⁻¹ was 0.194±0.39 mg kg⁻¹.

TABLE 6

Quantification of heavy metals (mg kg^{-1}) by foliar spraying of iron+zinc combination in parris island lettuce leaves. Different letters within the same column mean significant differences according to Tukey's test; $p \leq 0.05$, $n=5$; \pm SD. FW: Fresh weight.

Iron+zinc ($\mu\text{mol L}^{-1}$)	Arsenic (Ar)	Lead (Pb) Leaf mg kg^{-1}	Cadmium (Cd)
0	0.466 \pm 0.083 a	1.988 \pm 0.825 a	0.114 \pm 0.02 b
25	0.269 \pm 0.002 b	1.917 \pm 0.464 ab	0.100 \pm 0.01 b
50	0.287 \pm 0.003 ab	1.254 \pm 0.220 ab	0.139 \pm 0.36 ab
100	0.269 \pm 0.002 b	1.017 \pm 0.010 b	0.124 \pm 0.34 ab
200	0.323 \pm 0.061 a	1.088 \pm 0.020 b	0.194 \pm 0.39 a

DISCUSSION

The data submitted made it clear that despite the fact that Fe is an important element for yield and growth, so to speak in many plants, just like Zn, it also participates in the Fenton reaction, as stated by Martí-Echeverría et al. (2017), that all plant productions need the formation of reactive oxygen species (ROS), with the support of foliar sprays of trace elements, such as iron and zinc, may cause cell wall toxicity, for the reason that trace elements play an important role in the reaction with polyunsaturated fatty acids, proteins and nucleic acids (Briat et al., 2015).

Following the same tonic citing Giordano et al. (2019), reported increases on the fresh weight of *Lactuca sativa* L. This research was submitted by foliar spraying with Fe, compared to untreated plants, the response variables studied were plant height, number of leaves, as well as fresh weight of the plant decreased as iron concentrations increased (Wang et al., 2022). In the present study similar values were obtained with 2.52 \pm 4 mg kg^{-1} of zinc, for the concentration of 50 $\mu\text{mol L}^{-1}$ In combination with iron+zinc, no great reduction is observed with increasing concentrations in combination of iron and zinc.

The present investigation was carried out under protected systems and the experimental samples were subjected to temperatures between 38 and 42 °C where chlorophyll a denoted large absorption with 0.723 mg g^{-1} FW, chlorophyll b with 0.072 mg g^{-1} FW and carotenoids of 0.336 mg g^{-1} FW, respectively. Comparing the present data with Acuña et al. (2017), reported different interactions between different factors, demonstrated that chlorophyll a, b and carotenoids maintained greater stability with alkaline solutions and under conditions of temperatures close to 5 °C (Ambra et al., 2023). The present results showed values of 0.357 \pm 0.02 $\mu\text{g mL}^{-1}$ in lettuce culture applying iron and zinc in combination developed in a NFT (Nutrient Film Technique) system, the last treatment, remained almost constant except for the second treatment that was at a concentration of 25 $\mu\text{mol L}^{-1}$ of the additive, these results are similar to the present work with the addition of 25 $\mu\text{mol L}^{-1}$ iron+zinc; since it implies that the rest of the treatments are statistically equal, so there were no significant differences between treatments according to the Tukey test ($p \leq 0.05$). It should be noted that, for both scenarios, the results are obtained after 90 days.

It was demonstrated in the present study that minimal zinc uptakes are experienced in the leaves, to iron, without zinc the iron content increased, and manganese 36.8, 12.6 and 26.5%, especially in the application of zinc sulfate in the leaves., in the present study the dose with 25 $\mu\text{mol L}^{-1}$ in combination of iron and zinc, iron and zinc increased on a small scale, the above was compared, but in cowpea bean cultivation (Guillén-Molina et al., 2016; Lopez-Morales et al., 2020). The results reported by Legesse et al. (2017), regarding zinc, they applied around four times to chickpea and Mastewal plants at different concentrations, the different concentrations were compared with the yield and zinc content, the concentration of zinc stored in the grain found a significant difference between the varieties, the observed results they range from 34 mg kg^{-1} for Mastewal to 42 mg kg^{-1} for chickpea cultivation.

The applications of zinc in the form of sulfate do not represent an advantage to the plant as a fertilizer through foliar sprays, the sprays were in the form of chelate, according to Sida-Arreola et al. (2017), demonstrated that at concentrations of 25 $\mu\text{mol L}^{-1}$ of zinc in its chelated form with zinc sulfate improved the nutraceutical quality in bean crops and, therefore, the health of the consumer, in the present study the concentration with 50 $\mu\text{mol L}^{-1}$ demonstrated high concentrations of zinc and concentrations of 200 $\mu\text{mol L}^{-1}$ improved the absorption of iron. Secondly, Balandrán-Valladares et al. (2021), reported a concentration of zinc₂₊ between treatments that obtained values over 17.21 \pm 0.30

and $42.00 \pm 2.84 \text{ mg kg}^{-1}$, the highest value was observed with the concentration of $200 \mu\text{mol L}^{-1}$ zinc²⁺ by foliar spray.

One of the works carried out by Rivera-Marchant et al. (2016), reported and exposed the lettuce leaves cvs. 'Kristine RZ' and 'Versai RZ' with UV-B radiation, in both crops, the interaction factor was significant between both factors, the above was with the intention that the different crops obtained average values of $44.6 \mu\text{g ET g}^{-1}$, in this study they did not present significant differences in antioxidant capacity, but in the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method compared on UV-B radiation intensities in the first harvest (Hao et al., 2022).

Compared to the study of Lin et al. (2016), reported greater effects of the inhibition of the radical, the sample exerted it with $25 \mu\text{mol L}^{-1}$ the other enrichment concentrations, the absorption of the ABTS radical was stabilized, where it is eliminated with a wide range of compounds, mainly phenolic acids and flavonoids. It was noted that the experiments performed by Vázquez-Vázquez et al. (2020), underwent treatments with application of nutrient solution and with organic fertilizer, in corn cultivation, showed that the application of earthworm leaching obtained high yields, greater antioxidant capacity, phenolic contents among all the nutrient solutions (Giordano et al., 2019). In the case of sulfur, the variables stabilized in response, the present investigation increased the antioxidant capacity in the concentration of $25 \mu\text{mol L}^{-1}$, compared to the authors.

On the other hand, previous research by Preciado-Rangel et al. (2022), applied iron by foliar spraying on lettuce, which did not observe symptoms of toxicity in leaves; since toxicity may vary between species and type of dose to plants, improved lettuce yields and increased bioactive compounds the present data shown show that the concentrations implemented in this research represent adequate ranges and did not show low yield in fresh weight, along with the combination with Fe and Zn, no great toxicity was generated according to the quantification of heavy metals in the leaves; since the witness treatment (checkpoint), showed an accumulation of $1.988 \text{ mg kg}^{-1} \pm 0.825$ according to the standard deviation. A pointed out by Sariñana-Aldaco et al. (2023), in their study they applied high concentrations of indole 3 acetic acid melon crops and did not generate toxicity in seedlings. However, there was a 26.38% increase in proteins.

Guzmán-Morales et al. (2021), identified accumulation of seven heavy metals (Fe, Co, Zn, Mn, Cu, Ni and Pb) at high levels in the aerial part was the highest accumulation, which represents a risk for local food security in lettuce leaves; but against discussion the iron and zinc. The present data quantified arsenic and cadmium that did not present high concentrations of toxicity, however, lead minimally exceeded the permissible limits in concentrations of $25 \mu\text{mol L}^{-1}$, iron+zinc and checkpoint, which generates those high foliar contributions of iron and zinc in combination decrease the presence of lead and does not significantly affect the lettuce plant, by means of the variables studied.

Therefore, Reyes et al. (2016), obtained concentration levels of 0.3 mg kg^{-1} of lead in leafy vegetables, it was identified that the presence of heavy metals in food is using water sources of mining origin. These sources of water resources with the presence of lead are used to irrigate crops (Scutarasu y Trinca, 2023). The lettuces of the present work under greenhouse conditions, the content of Pb (lead), Ar (arsenic) and Cd (cadmium) are low compared to the control (control) observing 0.825 mg kg^{-1} , the high doses applied reduced the presence of lead, arsenic and cadmium in leaves, which does not significantly affect the yield, nutritional quality and antioxidant capacity. In this way, in the so-called Green Revolution, where the massive use of chemical fertilizers and pesticides was promoted as a solution to increase agricultural and food production, it brought negative effects both on the environment and on human health (Goltz et al., 2020).

Regarding the environment, chemical fertilizers and pesticides have a significant impact on soil and water quality. Fertilizers, when applied in large quantities, cause loss of soil fertility and contamination of groundwater by leaching. On the other hand, pesticides are toxic to insects, birds and other animals that are important to biodiversity and the food chain (Peña-Arrancibia et al., 2021).

Regarding human health, the excessive use of chemical pesticides leads to the exposure of agricultural workers and consumers to toxic substances. This causes health problems such as cancer, respiratory and neurological diseases, among others. In addition, exposure to these chemicals also has a negative impact on leafy crops. (Dubé et al., 2010; De-Assis et al., 2021).

For this reason, it is important to look for more sustainable and healthy alternatives for food production, such as biofortification and other sustainable agricultural practices that minimize the use of chemical products and promote environmental conservation and human health (Sperotto et al., 2018). The production of lettuce biofortified with iron and zinc improves the nutritional quality of the food, which is important in terms of food security, especially in regions where nutrition is precarious. In addition, biofortification is presented as a cheaper and more sustainable alternative to improve nutrition, compared to micronutrient supplementation, which is expensive and difficult to implement in vulnerable populations. Therefore, rigorous studies must be carried out to monitor the accumulation of

heavy metals in biofortified crops and ensure that food is safe for human consumption (Yadav et al., 2020).

In the same way, biofortification contributes to the reduction of soil and water contamination. Instead of adding chemical fertilizers and pesticides to the soil, iron and zinc biofortification uses biological amendments, which improves soil health and reduces the leaching of nutrients and contaminants into groundwater (Cakmakc et al., 2023). The results obtained represent a valuable contribution to the fight against micronutrient deficiency and environmental pollution, and could contribute to creating a safer, healthier and more sustainable world.

It should be noted that it is important to consider the possible side effects of biofortification, such as the accumulation of heavy metals in plants. Lettuce is a plant that is widely cultivated and commonly consumed. Our research found that biofortification with iron and zinc in lettuce had a positive effect on the production and accumulation of essential nutrients, but we also observed an accumulation of heavy metals in lettuce leaves in lesser proportion, which is why the present minerals are feasible in the production of our studied crop. The accumulation of heavy metals in plants is a public health problem, especially for people who regularly consume foods that contain high concentrations of these metals. Prolonged exposure to heavy metals has negative health effects, including nervous system damage, reproductive problems, and increased risk of cancer (Pipoyan et al., 2023).

Finally, it is suggested to address in other investigations the accumulation of heavy metals in plants, especially in staple food crops such as lettuce. More research needs to be done to better understand the effects of biofortification on the accumulation of heavy metals in different types of plants and in different environmental conditions, before being brought to human consumption. In addition, strategies must be developed to minimize heavy metal accumulation in biofortified plants, including selecting plant varieties that are resistant to heavy metal accumulation and implementing more sustainable and healthy agricultural practices, this will be reflected in the increase of our crop yields. (Rashid et al., 2023; Ortega-Ramirez et al., 2023; Guerrero-Martin et al., 2023a; 2023b; Ortega-Ramirez et al., 2021; Ramirez et al., 2019).

CONCLUSION

The results indicated Biofortification is a viable alternative for the yield of leafy crops, such as lettuce, once the combined addition of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), as a solution and by foliar route does not represent the level of toxicity of the three heavy metals mentioned above. The yield in the present study the effect of the concentration with $50 \mu\text{mol L}^{-1}$ with iron and zinc by foliar spray on parris island lettuce leaves, was the one that showed good increases in almost all the response variables, which is suitable for the lettuce production as opposed to the variable root height with 18.9 cm.

The high concentrations of iron+zinc at concentrations of $200 \mu\text{mol L}^{-1}$ showed an absorption of lead of $1.088 \pm 0.020 \text{ mg kg}^{-1}$ in 100 g of sample, on the other hand, a low concentration of all the metals studied (arsenic, lead and cadmium) was observed, which presents data below the previously permissible limits. The biofortified lettuce in combination with iron+zinc did not show toxicity and is suitable for human consumption.

This study highlights the importance of biofortification in the production of more nutritious foods enriched with micronutrients such as iron and zinc. The need to monitor the levels of heavy metals in food and the importance of evaluating the content of biomolecules in food are also highlighted. These findings contribute to research on food safety and provide valuable information for the development of more sustainable and healthier agricultural practices. Nonetheless, biofortification can be a valuable tool in addressing dietary nutrient deficiency, but it is important to be aware of potential side effects and take steps to minimize them.

Acknowledgements

This work was supported for publication by Universidad Tecnológica de Rodeo (Durango-México) for providing high core laboratories and Fundación Universidad de América (Bogotá-Colombia) for the constant support through its professors-researchers to produce high level human capital.

Author Contributions

References. 1-Project administration; 2-Funding acquisition; 3-Formal analysis; 4- Conceptualization; 5-Data curation; 6-Writing, review & editing; 7-Investigation; 8-Methodology; 9-Resources; 10-Writing, original draft; 11-Software; 12-Supervision; 13-Validation; 14-Visualization.

Author Name	Academic Collaboration													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Oscar Silva Marufo	X		X					X				X		
Angie Tatiana Ortega Ramirez						X						X	X	X
Oscar Sarinana Aldaco							X		X	X	X			
Rubén Iván Marín Tinoco		X			X								X	X

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