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# VARIATION IN MINERAL CONTENTS IN THE EDIBLE LEAVES AND STEMS OF *Amaranthus hybridus*, *A. hypochondriacus* AND *A. cruentus* AS A RESULT OF DIFFERENT LOCATIONS OF CULTIVATION AND FERTILIZER APPLICATION

KARINA CANTÚ-LÓPEZ, ENRIQUE ORTIZ-TORRES,  
ARACELI MINERVA VERA-GUZMÁN, JOSÉ LUIS CHÁVEZ-SERVIA,  
PEDRO ANTONIO LÓPEZ AND ADRIÁN ARGUMEDO-MACÍAS

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## SUMMARY

*Amaranth* (*Amaranthus* spp.) is a quelite (edible leaf and stem vegetable) with a long history of use in Mexican cuisine. The objective of this study was to evaluate the mineral content in the edible stems and leaves of three *Amaranthus* species cultivated under different environmental conditions and fertilizer treatments. Using inductively coupled plasma-optical emission spectrometry (ICP-OES), the concentrations of macro- and microelements were determined in 16 genotypes and accessions of *Amaranthus hypochondriacus* L., *A. hybridus* L., and *A. cruentus* L., grown in Huaquechula (Puebla) and Tepetitla (Tlaxcala), Mexico, under fertilized and non-fertilized conditions following a factorial arrangement in a randomized block design. Significant differences ( $p < 0.05$ ,  $0.01$ ) were found in the macro- and microelement con-

centrations in the stems and leaves depending on cultivation site and fertilization. Tepetitla, Tlaxcala, presented more favorable growth conditions in terms of soil moisture and nutrient availability, resulting in higher concentrations of K, Mg, P, S, Na, and Zn, and lower accumulation of Ca, Fe, and Mn. In particular, Fe and Zn concentrations were not influenced by fertilizer application. For Cu, Mn, Na, Ca, K, and S, the decreasing pattern among species was *A. cruentus* > *A. hypochondriacus* > *A. hybridus*; whereas for Fe and Zn, the trend was *A. hybridus* > *A. hypochondriacus* > *A. cruentus*. The genotypes evaluated showed high concentrations of Ca, K, Fe, and Zn. These findings indicate that the consumption of amaranth quelites can contribute substantially to dietary mineral intake and promote consumer health.

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## Introduction

 In all biocultural regions of the world, plants with edible leaves and stems are both gathered and cultivated, forming part of traditional diets. The consumption of

these plants has been documented across diverse cultures, including pre-Columbian societies throughout North and South America. A review by Penafiel *et al.* (2011) underscored the significant contribution of wild or weedy edible plants to local food systems, which also sustain local economies. In most cases, the

collection, consumption, and sale of edible plants occur within local or regional markets and are not reflected in national food production statistics.

However, despite the existence of such potential food sources, approximately 820 million people worldwide suffer from hunger, and in Africa,

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**Karina Cantú-López.** Master of Science in Strategies for Regional Agricultural Development. Colegio de Posgraduados, Mexico. Independent researcher.

**Araceli Minerva Vera-Guzmán** (Corresponding author). Ph.D. in Science, Mexico. Professor-Researcher, CIIDIR-Oaxaca, Instituto Politécnico Nacional. Address: Hornos 1003, Santa Cruz Xoxocotlán, 71230, Oaxaca, Mexico. e-mail: avera@ipn.mx.

**Enrique Ortiz-Torres.** Ph.D. in Sustainable Agriculture, USA. Professor-Researcher, Colegio de Posgraduados, Puebla Campus, Mexico.

**José Luis Chávez-Servia.** Ph.D. in Genetics, Mexico. Professor-Researcher, CIIDIR-Oaxaca, Instituto Politécnico Nacional, Mexico.

**Pedro Antonio López.** Philosophy Doctor in Plant Breeding, USA. Professor-Researcher, Colegio de Posgraduados campus Puebla, Mexico.

**Adrián Argumedo-Macías.** Ph.D. in Planning and Management of Sustainable Rural Development Projects, Spain. Professor-Researcher, Colegio de Posgraduados, Puebla Campus, Mexico.

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Southeast Asia, and Latin America, 26.4% of cases are classified as moderate to severe, including mineral deficiencies and anemia in women of reproductive age (FAO *et al.*, 2019).

In recent decades, interest in amaranth (*Amaranthus* spp.) has re-emerged due to its remarkable contribution as a grain to the family diet, providing minerals, proteins, carbohydrates, and functional compounds with antioxidant, nutritional, and nutraceutical properties. Experimental evidence indicates that amaranth exhibits anti-inflammatory, anticancer, cardioprotective, hepatoprotective, antiviral, antimicrobial, gastroprotective, and antidiabetic activities (Venskutonis and Kraujalis, 2013; Peter and Gandhi, 2017). Consequently, amaranth is recognized as an important source of minerals for human nutrition. For example, *Amaranthus tricolor* has high levels of Ca, Fe, and Zn (Shukla *et al.*, 2006); *A. caudatus*, *A. cruentus*, *A. hybridus* and *A. hypochondriacus* are rich in K, Ca, Mg, and P (Förster *et al.*, 2023); *A. lividus* contains K, Ca, Mg, Fe, Mn, Cu, and Zn; *A. acanthochiton* presents high concentrations of Ca, Mg, and Zn; and *A. deflexus* and *A. viridis* show notable Fe concentrations (Jiménez-Aguilar and Grusak, 2017). Although most species of the genus *Amaranthus* are of American origin, currently cultivated forms have a global distribution, except for some wild species (Das, 2012).

In Mexico and Central America, indigenous communities traditionally consume both wild and cultivated species of amaranth as vegetables (leaves and young stems), which are incorporated into numerous traditional dishes (Espitia-Rangel *et al.*, 2010). The most representative species include *A. cruentus*, *A. hybridus*, *A. hypochondriacus*, *A. powellii*, *A. retroflexus*, *A. palmeri* and *A. spinosus* (Ruiz-Hernández *et al.*, 2018). Román-Cortés *et al.* (2018) evaluated the nutritional and nutraceutical composition of the edible vegetative fraction of *A. hypochondriacus* (known as “quintonil” in Mexico), along with other traditional Mexican vegetables, and found that it constitutes an important source of minerals, proteins, and phenolic compounds with strong antioxidant activity. The young leaves and stems of amaranth are notable dietary sources of K, Ca, Mg, P, S, Fe, Mn, Cu, Zn, and Na (Förster *et al.*, 2023; Sarker and Oba, 2019; Sarker *et al.*, 2020). The leaves of *A. cruentus*, *A. hybridus*, and *A. hypochondriacus* contain 541.8–716.8mg K, 150–497mg Ca, 131.1–230.4mg Mg, 53–139mg P, 0.84–1.31mg Fe, 0.4–0.86mg Zn, 0.1–0.11mg Cu, and 0.76–0.78mg Mn per 100g of fresh weight

(Förster *et al.*, 2023; Jiménez-Aguilar and Grusak, 2017).

The nutritional and chemical composition of edible amaranth stems and leaves depends on multiple factors associated with plant growth, including agroecological conditions, water availability, biotic stress, fertilizer use, species, genotypes, and genotype–environment interactions (Sarker *et al.*, 2014, 2015). Sarker and Oba (2018a) reported high variability among *A. tricolor* genotypes in mineral content, proximate composition, carotenoids, phenolic compounds, vitamin C, and antioxidant capacity under drought stress conditions. Sarker and Oba (2018b) observed that, in addition to genotypic variation, differences in soil moisture levels lead to distinct responses among varieties and genotypes of *A. tricolor*. Jiménez-Aguilar and Grusak (2017) also found interspecific variation in mineral content among amaranth species, with *A. acanthochiton* showing the most pronounced differences. Likewise, Sefasi *et al.* (2025) demonstrated that the contents of proteins, Ca, K, Zn, and Fe are determined by genotype–environment interactions.

Given the dual challenge of malnutrition and obesity faced by many populations in Latin America (Rivera *et al.*, 2014) and the limited access to nutrient-rich foods, amaranth—a native vegetable traditionally consumed by indigenous groups—has been proposed as an important dietary source of essential minerals. However, it remains necessary to clarify how environmental factors, cultivation location, management practices, species, and genotypes influence the nutritional composition of its edible leaves and stems. Therefore, this study aimed to evaluate the mineral content of edible stems and leaves of three amaranth species and to determine the effects of cultivation site and fertilizer application on their composition.

## Materials and Methods

### Experimental biological material

In this study, twelve accessions and varieties belonging to three amaranth species were evaluated, using an improved variety as the control. Among the *Amaranthus hypochondriacus* varieties and accessions, AV25, AV28, AV7, AV3, CP30, Areli, Laura, and Nutrisol were included. Among the *A. hybridus* genotypes, AV17, AV8, AV29, and AV19 were considered; these two species originated from Zapotitlán de Méndez, Puebla. Additionally, the *A. cruentus* varieties AV17, AV8, AV29, and AV19 were

included, originating from Tochimilco, Puebla, and Huazulco, Morelos, Mexico. For further details on the description of the species and genotypes, see Ortiz-Torres *et al.* (2018).

### Experimental design and planting

A factorial arrangement of 16 genotypes or accessions, with and without fertilization, was established under a randomized block design with two replicates at two locations. The experiment was conducted in plots consisting of two rows, each 5m long, with 0.8m between rows, in heterogeneous parcels managed by collaborating farmers, which restricted the use of additional replications and more complex experimental designs.

One planting was carried out in Huaquechula, Puebla (18°45′58.3″ N, 98°33′25.14″ W; 1577 m a.s.l.) on September 8, 2018, and another in Tepetitla, Tlaxcala, Mexico (19°16′31.31″ N, 98°23′20.07″ W; 2228 m a.s.l.) on August 24, 2018.

Sixteen amaranth accessions and varieties were used, and the fertilization factor comprised two levels (with and without), following the 18N–07P–09K formula, using urea (46N–0P–0K), diammonium phosphate (18N–46P–0K), and potassium chloride (0N–0P–60K), which were incorporated at the time of sowing.

Sampling of leaves and young stems (shoots) was performed 40 days after sowing, after which the plant material was shade-dried for 15 days. In addition, at each site, temperature and precipitation were recorded, and a soil analysis (0–30cm depth) was conducted prior to sowing, following the information previously reported by Cantú-López *et al.* (2022).

### Preparation of samples for analysis

The dried samples of young leaves and stems were subjected to a second drying process in a forced-air oven (Thermo Scientific) for 48h at 48°C under constant temperature and humidity conditions. The samples were then placed in flasks and stored in a cool, dry place until analysis. Approximately 100g of each field sample was ground using a Krups® coffee grinder (model GX4100, Mexico). Once pulverized, the samples were preserved in amber bottles sealed with Parafilm® and kept at –20°C until analysis.

### Determination of minerals

A 2g portion of the pulverized sample was oven-dried at 100°C

(Barnstead/Thermolyne Oven series 9000, USA) until a constant weight was reached, following AACC Method 44-15. The material was subsequently incinerated at 57°C in a muffle furnace (Barnstead/Thermolyne 1400, USA) until reaching a constant weight (AACC Method 08-01.01; AACC, 1976). The obtained ashes were dissolved with 3ml of concentrated hydrochloric acid (JT Baker®) and diluted with deionized water to a final volume of 50ml. The solution was then filtered through fine-pore filter paper.

Additionally, a blank was prepared without sample, following the same procedure to rule out possible contamination from reagents.

The quantification of micro- and macroelements (Cu, Fe, Mn, Zn, Ca, K, Mg, Na, P, and S) was performed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6500 DUO, England) with radial and axial configurations and an autosampler (CETAC ASX-520, USA), using argon as the auxiliary gas. The analysis was conducted under the following conditions: auxiliary gas flow 0.4l·min<sup>-1</sup>, RF power 1200W, peristaltic pump stabilization time 10s, and analysis speed 50rpm.

Quantification was based on multielement certified reference standards (≥ 99.9% purity; High Purity Standards®, USA), within the following concentration ranges: 1–100 mg·l<sup>-1</sup> for P, Mg, K, Ca, Fe, and Na, and 0.2–5 µg·ml<sup>-1</sup> for Cu, S, Mn, and Zn.

The lower detection limits were 0.00006, 0.0001, 0.0002, 0.002, 0.003, and 0.005 mg L<sup>-1</sup> for Mn, Cu, Zn, Mg, Na, and Fe, respectively, and 0.01 mg L<sup>-1</sup> for P, K, and Ca (Martínez-Martínez *et al.*, 2019).

Recovery rates for Cu, Fe, Mn, Zn, Ca, K, Mg, Na, P, and S were 100.5, 104.6, 101.6, 102.6, 102.6, 101.6, 105.4, 105.1, 102.5, and 99.4%, respectively. All analyses were performed in triplicate and results were expressed as g·kg<sup>-1</sup> for macroelements and mg·100g<sup>-1</sup> for microelements.

#### Statistical analysis

A database was constructed from the laboratory results, and based on the field experimental design, combined analyses of variance (ANOVA) were carried out using a linear model of a randomized complete block design, with nesting of replicates within locations and nesting of accessions or varieties within species.

As a result, the partitioning of the sums of squares and variances

for each mineral was obtained to compare the magnitude of variability and determine statistical significance relative to the error variance. Multiple comparisons of means were performed using Tukey's test ( $p < 0.05$ ) for each factor and interaction. All statistical analyses were performed using the SAS software package (SAS Institute, 2006).

#### Results

In the combined analysis of variance, significant differences ( $p \leq 0.05$ , 0.01) were detected for the main effects (locations, fertilization, species, and genotypes) and their interactions for all minerals evaluated. Exceptions were observed in the following cases: for Cu, no significant differences were found between locations or in the interactions location × species, species × fertilization, fertilization × genotypes, location × fertilization × species, and location × fertilization × genotypes; for Na, no significant effect of fertilization was detected; for Zn, the interaction location × fertilization × species was not significant; and for S, the interaction location × fertilization × species was also not significant. The coefficients of variation ranged from 0.4% to 22.5% (Table I). These findings indicate that most of the factors and their interactions influenced the mineral content of leaves and edible stems of amaranth.

The mineral content of amaranth leaves and stems varied between growing locations. Higher concentrations of K, Mg, P, S, Na, and Zn were detected in plants grown in Tepetitla, Tlaxcala, compared with those grown in Huaquechula, Puebla, whereas the opposite trend was observed for Ca, Fe, and Mn, indicating that agroecological conditions influence mineral composition. Regarding the effect of fertilizer application, mineral contents were generally higher in plots with fertilization than in those without fertilization; for example, Ca, K, Mg, P, Cu, Mn, and Na concentrations were higher, but S, Fe, and Zn were not affected. Consequently, both location and fertilization significantly affected most minerals evaluated. In Huaquechula, fertilized plants showed higher contents of Ca, Cu, and Mn, whereas in Tepetitla, fertilized plants accumulated higher Mg levels. However, S and Zn contents were higher in non-fertilized plants at both locations. In contrast, K, P, and Na concentrations did not show consistent variation with fertilization in either location. Overall, a general trend of higher mineral accumulation under fertilizer application was observed in both localities (Table II).

The mineral contents differed significantly among the three amaranth species (*A. hypochondriacus*, *A. hybridus*, and *A. cruentus*). *A. cruentus* exhibited significantly higher concentrations of Cu, Mn, Na, Ca, K, and S, whereas *A. hybridus* showed the highest contents of Fe, Zn, Mg, and P. The general decreasing trend for Cu, Mn, Na, Ca, K, and S among species was: *A. cruentus* > *A. hypochondriacus* > *A. hybridus*, whereas for Fe and Zn the trend was: *A. hybridus* > *A. hypochondriacus* > *A. cruentus*.

Significant differences were also detected among genotypes within each species. Several accessions exceeded the commercial control variety in more than two macro- and microelements. In *A. hypochondriacus*, genotypes AV3, AV7, AV25, and AV28 had higher mineral contents; in *A. hybridus*, genotypes AV8, AV17, and AV19 stood out; and in *A. cruentus*, genotypes CP15, CP38, and CP39 performed similarly to the commercial variety Benito. The range of variation among genotypes was wide; for example, Fe content ranged from 31.9 to 66.3mg·100 g<sup>-1</sup>, Zn ranged from 4.59 to 5.69mg·100 g<sup>-1</sup>, and Ca ranged from 21.1 to 29.9g·kg<sup>-1</sup> (Table III).

In the location × genotype and species interactions, significant differences were detected for all minerals evaluated, showing differential trends depending on the element analyzed. The microelement concentrations differed among amaranth genotypes across growing locations. Higher Fe and Mn concentrations were recorded in plants grown in Huaquechula, Puebla, than in those grown in Tepetitla, Tlaxcala, whereas the opposite trend was observed for Na, with greater Na contents in plants from Tepetitla. For Cu and Zn, no marked differences were observed between locations, as similar concentrations were detected at both sites (Table IV).

In Huaquechula, Puebla, a locality characterized by low precipitation and high temperatures, the concentrations of P, Mg, K, and S were lower than those found in plants grown in Tepetitla, Tlaxcala. However, Ca content showed the reverse pattern. Genotypes exhibited contrasting responses within each species and mineral evaluated; for example, genotypes AV17, Benito, CP38, AV3, AV7, and AV25 (4.50–5.07g·kg<sup>-1</sup>) had the highest P values in Huaquechula, but in Tepetitla, only genotype AV3 maintained similar P levels. Genotypes AV7 and AV25 of *A. hypochondriacus* showed P contents (9.95–11.10 g·kg<sup>-1</sup>) that were almost double in Huaquechula compared with Tepetitla, a trend also observed for Mg, K, and S. In contrast, Ca content followed

TABLE I  
SIGNIFICANCE OF SQUARE MEANS IN MINERAL CONTENT IN EDIBLE LEAVES AND STEMS OF AMARANTH,  
CULTIVATED IN TLAXCALA AND PUEBLA, MEXICO

| Sources of variation         | Microelements      |                   |                    |                    |                    |
|------------------------------|--------------------|-------------------|--------------------|--------------------|--------------------|
|                              | Cu                 | Fe                | Mn                 | Na                 | Zn                 |
| Locations (L)                | 0.11 <sup>ns</sup> | 46921**           | 3823.2**           | 36388**            | 27.8**             |
| Fertilization (F)            | 0.31*              | 250**             | 214.2**            | 0.11 <sup>ns</sup> | 7.8**              |
| Species (E)                  | 0.19*              | 3129**            | 58.5**             | 184*               | 2.9**              |
| Genotypes (G)/E <sup>1</sup> | 0.15**             | 658**             | 9.6**              | 4387**             | 1.1**              |
| L x F                        | 0.32**             | 665**             | 125.2**            | 649**              | 2.4**              |
| L x E                        | 0.08 <sup>ns</sup> | 2052**            | 42.1**             | 1010**             | 4.0**              |
| F x E                        | 0.10 <sup>ns</sup> | 227**             | 0.5**              | 554**              | 1.8**              |
| L x G/E                      | 0.12**             | 455**             | 11.5**             | 287**              | 1.4**              |
| F x G/E                      | 0.06 <sup>ns</sup> | 371**             | 8.3**              | 382**              | 0.8**              |
| L x F x E                    | 0.02 <sup>ns</sup> | 299**             | 12.2**             | 716**              | 0.01 <sup>ns</sup> |
| L x F x G/E                  | 0.03 <sup>ns</sup> | 236**             | 4.7**              | 347**              | 0.92**             |
| Repeat/L <sup>1</sup>        | 0.03 <sup>ns</sup> | 1.1 <sup>ns</sup> | 0.03 <sup>ns</sup> | 76 <sup>ns</sup>   | 0.01 <sup>ns</sup> |
| Error                        | 0.04               | 1.2               | 0.02               | 47                 | 0.01               |
| Coeff. of variation (%)      | 22.5               | 2.6               | 2.1                | 12.8               | 1.8                |

| Sources of variation         | Macroelements      |                    |                    |                    |                    |
|------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                              | Ca                 | K                  | Mg                 | P                  | S                  |
| Locations (L)                | 1027.77**          | 39170.8**          | 4293.97**          | 3449.6**           | 56.43**            |
| Fertilization (F)            | 83.33**            | 30.2**             | 22.45**            | 18.9**             | 27.84**            |
| Species (E)                  | 60.06**            | 1002.3**           | 155.55**           | 44.8**             | 10.26**            |
| Genotypes (G)/E <sup>1</sup> | 65.12**            | 153.9**            | 21.86**            | 11.7**             | 1.84**             |
| L x F                        | 4.11**             | 12.3**             | 0.24**             | 13.9**             | 4.90**             |
| L x E                        | 387.66**           | 83.6**             | 78.93**            | 35.4**             | 2.89**             |
| F x E                        | 9.57**             | 4.7**              | 0.84**             | 1.6**              | 0.26*              |
| L x G/E                      | 88.12**            | 61.1**             | 12.66**            | 5.9**              | 0.99**             |
| F x G/E                      | 44.30**            | 40.7**             | 10.85**            | 2.5**              | 0.32**             |
| L x F x E                    | 28.53**            | 46.2**             | 3.04**             | 8.0**              | 0.18 <sup>ns</sup> |
| L x F x G/E                  | 29.22**            | 57.0**             | 11.72**            | 3.4**              | 1.23**             |
| Repeat/L <sup>1</sup>        | 0.09 <sup>ns</sup> | 1.10 <sup>ns</sup> | 0.02 <sup>ns</sup> | 0.04 <sup>ns</sup> | 0.11 <sup>ns</sup> |
| Error                        | 0.127              | 0.77               | 0.024              | 0.06               | 0.061              |
| Coeff. of variation (%)      | 0.72               | 1.0                | 0.4                | 1.0                | 2.5                |

<sup>ns</sup> Not significant ( $p > 0.05$ ); \* Significant at  $p \leq 0.05$ ; \*\* Significant at  $p \leq 0.01$ . <sup>1</sup> Indicates nesting of genotypes in species and nesting of repetitions in locations.

this pattern only in genotype CP39, which showed a higher Ca concentration in Huaquechula (34.3g·kgv<sup>-1</sup>) than in Tepetitla (25.2g·kg<sup>-1</sup>) (Table IV).

The fertilization × species and fertilization × genotype interactions had significant effects on all minerals evaluated, with the exception of Cu, for which no significant interaction was detected. With fertilizer application, Fe content among genotypes ranged from 9.6 to 15.7mg·100g<sup>-1</sup>, while without fertilizer, Fe values ranged from 10.7 to 15.6mg·100g<sup>-1</sup>, indicating a slight increase in Fe concentration in non-fertilized plants of certain genotypes (AV19, CP15, Areli, AV3, AV7, and CP30), although in others

the opposite effect was observed. This interaction pattern was similar for Mn, Na, and Zn, confirming a differential genotype response to fertilization.

These results indicate the feasibility of discriminating genotypes based on microelement response to fertilizer application, with genotypes AV19, CP15, Areli, CP30, and Laura exhibiting variable mineral responses depending on fertilization. In the case of macroelements, Ca variation followed a similar pattern to Fe, although different genotypes were involved. The improved varieties Areli, Laura, and Nutrisol showed a reduction in P content in the absence of fertilizer. For Mg, a slight increase in concentration was

observed under fertilization. In contrast, genotypes Benito and AV exhibited similar mineral compositions without fertilization, while genotypes AV29, CP15, AV25, AV28, and CP30 showed higher K contents in non-fertilized plants. Differentiation based on macroelement content between fertilized and non-fertilized plants is useful for selecting genotypes that perform well under low-input conditions (Table V).

In the three-way interaction among locations, fertilization, and genotypes, no significant differences were detected for Cu content, although its concentrations ranged from 0.7 to 1.7mg·100 g<sup>-1</sup>. A significant interaction



TABLE II  
MINERAL CONTENT IN LEAVES AND STEMS OF AMARANTH CULTIVATED IN PUEBLA AND TLAXCALA, MEXICO,  
WITH AND WITHOUT FERTILIZER

| Minerals                                | Locations <sup>1</sup> |                     | Fertilization <sup>1</sup> |                        | Locations-fertilization interaction <sup>1</sup> |         |                  |          |
|-----------------------------------------|------------------------|---------------------|----------------------------|------------------------|--------------------------------------------------|---------|------------------|----------|
|                                         | Huaquechula,<br>Pue.   | Tepetitla,<br>Tlax. | With fert.<br>(C/F)        | Without<br>fert. (S/F) | Huaquechula, Pue.                                |         | Tepetitla, Tlax. |          |
|                                         |                        |                     |                            |                        | C/F                                              | S/F     | C/F              | S/F      |
| Macroelements (g·kg <sup>-1</sup> )     |                        |                     |                            |                        |                                                  |         |                  |          |
| Ca                                      | 27.10 a                | 22.57 b             | 24.48 a                    | 24.20 b                | 27.87 a                                          | 26.25 b | 23.00 c          | 22.18 d  |
| K                                       | 53.13 b                | 103.31 a            | 78.78 a                    | 77.66 b                | 54.06 b                                          | 52.15 c | 103.25 a         | 103.03 a |
| Mg                                      | 11.59 b                | 18.94 a             | 15.51 a                    | 15.01 b                | 11.82 c                                          | 11.33 d | 19.19 a          | 18.70 b  |
| P                                       | 4.42 b                 | 8.73 a              | 6.72 a                     | 6.43 b                 | 4.66 b                                           | 4.18 c  | 8.75 a           | 8.68 a   |
| S                                       | 0.89 b                 | 1.12 a              | 0.93 b                     | 1.08 a                 | 0.79 d                                           | 1.00 c  | 1.07 b           | 1.16 a   |
| Microelements (mg 100·g <sup>-1</sup> ) |                        |                     |                            |                        |                                                  |         |                  |          |
| Cu                                      | 0.98 a                 | 0.93 a              | 1.00 a                     | 0.91 b                 | 1.07 a                                           | 0.89 b  | 0.93 b           | 0.93 b   |
| Fe                                      | 58.12 a                | 27.08 b             | 41.85 b                    | 43.35 a                | 55.55 b                                          | 60.31 a | 27.76 c          | 26.51 d  |
| Mn                                      | 12.35 a                | 3.19 b              | 8.81 a                     | 6.69 b                 | 14.14 a                                          | 10.53 b | 3.54 c           | 3.28 d   |
| Na                                      | 38.0 b                 | 67.0 a              | 57.0 a                     | 52.0 b                 | 36.22 c                                          | 40.57 b | 69.76 a          | 66.69 a  |
| Zn                                      | 4.56 b                 | 5.45 a              | 4.76 b                     | 5.25 a                 | 4.19 d                                           | 4.93 c  | 5.33 b           | 5.58 a   |

<sup>1</sup> In the rows of localities, fertilization and location–fertilization interactions, means with the same letter are not significantly different (Tukey's test,  $p \leq 0.05$ ).

TABLE III  
EFFECT OF SPECIES AND GENOTYPES ON THE CONTENT OF MINERALS IN LEAVES AND STEMS OF AMARANTH,  
CULTIVATED IN TLAXCALA AND PUEBLA, MEXICO

| Genotypes                 | Cu <sup>1</sup>                                | Fe     | Mn      | Na     | Zn     | Ca                                        | K      | Mg     | P     | S     |
|---------------------------|------------------------------------------------|--------|---------|--------|--------|-------------------------------------------|--------|--------|-------|-------|
|                           | ----- mg ·100 g <sup>-1</sup> dry weight ----- |        |         |        |        | ----- g·kg <sup>-1</sup> dry weight ----- |        |        |       |       |
| <i>A. hypochondriacus</i> |                                                |        |         |        |        |                                           |        |        |       |       |
| AV7                       | 0.96 a                                         | 39.8 b | 7.21 b  | 55.0 a | 4.79 b | 23.7 b                                    | 84.6 a | 14.6 b | 7.5 a | 1.1 a |
| AV25                      | 0.92 a                                         | 42.5 b | 6.97 b  | 52.0 a | 4.62 c | 22.8 c                                    | 86.3 a | 14.4 d | 7.5 a | 1.1 a |
| AV28                      | 0.98 a                                         | 45.8 b | 8.13 a  | 59.0 a | 4.94 a | 24.8 a                                    | 88.6 a | 15.2 b | 7.0 a | 1.1 a |
| CP30                      | 0.83 b                                         | 34.8 c | 6.27 b  | 50.0 a | 4.88 a | 25.3 a                                    | 75.0 c | 13.8 e | 6.3 d | 0.1 c |
| Areli                     | 0.90 b                                         | 32.2 c | 6.02 c  | 49.0 a | 5.12 a | 23.5 b                                    | 74.4 c | 12.9 f | 6.0 e | 0.9 e |
| Laura                     | 1.04 ab                                        | 35.8 c | 6.91 b  | 38.0 b | 5.42 a | 23.9 b                                    | 71.4 e | 13.6 e | 6.8 b | 1.1 a |
| Nutrisol                  | 0.75 b                                         | 52.7 a | 7.92 a  | 54.0 a | 5.69 a | 25.7 a                                    | 67.1 e | 14.6 b | 6.7 b | 0.9 b |
| Average                   | 0.93 B                                         | 41.0 B | 7.13 C  | 52.0 B | 5.09 B | 24.8 B                                    | 77.9 B | 14.5 C | 6.0 C | 1.0 B |
| <i>A. hybridus</i>        |                                                |        |         |        |        |                                           |        |        |       |       |
| AV8                       | 1.01 a                                         | 52.8 a | 8.58 a  | 49.0 a | 5.28 a | 27.2 a                                    | 64.7 f | 18.8 a | 5.8 f | 0.9 d |
| AV17                      | 0.86 b                                         | 66.3 a | 7.37 b  | 61.0 a | 5.53 a | 23.5 b                                    | 66.2 e | 16.7 a | 6.4 d | 0.8 g |
| AV19                      | 1.07 a                                         | 47.3 b | 6.81 b  | 44.0 a | 4.89 a | 22.6 b                                    | 74.1 c | 16.5 b | 6.5 c | 1.0 b |
| AV29                      | 0.81 b                                         | 41.5 b | 8.41 a  | 54.0 a | 4.84 b | 21.1 c                                    | 76.4 b | 16.2 b | 5.4 g | 0.9 f |
| Average                   | 0.94 B                                         | 52.0 A | 7.79 B  | 52.0 B | 5.14 A | 23.6 C                                    | 70.3 C | 17.0 A | 6.9 A | 0.9 C |
| <i>A. cruentus</i>        |                                                |        |         |        |        |                                           |        |        |       |       |
| CP15                      | 0.98 a                                         | 41.0 b | 10.06 a | 55.0 a | 4.78 b | 26.4 a                                    | 85.3 a | 15.3 b | 6.0 d | 1.0 b |
| CP38                      | 0.94 a                                         | 33.7 c | 7.73 a  | 56.0 a | 4.69 c | 23.6 b                                    | 89.0 a | 13.9 d | 6.5 d | 1.0 a |
| CP39                      | 1.24 a                                         | 39.5 b | 10.09 a | 56.0 a | 4.76 b | 29.9 a                                    | 83.2 a | 16.1 b | 6.2 d | 1.2 a |
| Benito                    | 0.97 a                                         | 31.9 d | 8.27 a  | 54.0 a | 4.59 d | 24.7 a                                    | 89.4 a | 15.0 b | 6.7 b | 1.1 a |
| Average                   | 1.03 A                                         | 36.5 C | 9.04 A  | 55.0A  | 4.71 C | 26.1 A                                    | 86.7 A | 15.1 B | 6.4 B | 1.1 A |

<sup>1</sup> In a column, the means of genotypes or species with the same letter do not differ significantly (Tukey's test,  $p \leq 0.05$ ).

TABLE IV  
RESPONSES TO INTERACTION OF LOCALITIES-SPECIES AND GENOTYPES OF AMARANTH IN MINERAL  
CONTENT IN EDIBLE LEAVES AND STEMS

| Genotypes                               | Fe <sup>1</sup> | Mn   | Cu <sup>1</sup> | Na <sup>2</sup> | Zn <sup>1</sup> | P <sup>2</sup> | Mg <sup>2</sup> | Ca <sup>2</sup> | K <sup>2</sup> | S <sup>2</sup> |
|-----------------------------------------|-----------------|------|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|----------------|
| Location 1: Huaquechula, Puebla, Mexico |                 |      |                 |                 |                 |                |                 |                 |                |                |
| <i>Amaranthus hybridus</i>              |                 |      |                 |                 |                 |                |                 |                 |                |                |
| AV8                                     | 82.5            | 13.2 | 1.07            | 0.42            | 5.13            | 4.14           | 12.6            | 23.7            | 49.7           | 0.89           |
| AV17                                    | 97.4            | 10.8 | 0.74            | 0.56            | 4.80            | 4.78           | 11.5            | 22.7            | 43.4           | 0.73           |
| AV19                                    | 64.4            | 9.8  | 1.09            | 0.30            | 4.17            | 4.31           | 11.9            | 23.1            | 50.2           | 0.87           |
| AV29                                    | 58.1            | 12.6 | 0.75            | 0.42            | 4.91            | 3.70           | 12.2            | 25.3            | 45.4           | 0.85           |
| <i>Amaranthus cruentus</i>              |                 |      |                 |                 |                 |                |                 |                 |                |                |
| Benito                                  | 37.3            | 13.3 | 0.96            | 0.35            | 4.37            | 4.81           | 11.6            | 29.5            | 61.8           | 0.88           |
| CP15                                    | 61.6            | 16.7 | 1.06            | 0.37            | 5.10            | 4.30           | 13.3            | 34.4            | 54.0           | 0.89           |
| CP38                                    | 44.3            | 12.2 | 0.94            | 0.41            | 4.11            | 4.82           | 11.0            | 28.9            | 57.8           | 0.94           |
| CP39                                    | 52.4            | 16.5 | 1.41            | 0.31            | 4.68            | 4.32           | 12.9            | 34.3            | 56.7           | 0.91           |
| <i>Amaranthus hypochondriacus</i>       |                 |      |                 |                 |                 |                |                 |                 |                |                |
| Arelí                                   | 45.3            | 9.2  | 0.95            | 0.37            | 4.72            | 4.36           | 9.7             | 23.1            | 48.8           | 0.84           |
| AV3                                     | 46.9            | 11.2 | 0.90            | 0.30            | 4.22            | 4.50           | 12.0            | 28.6            | 57.8           | 0.98           |
| AV7                                     | 49.3            | 10.6 | 0.87            | 0.39            | 3.93            | 4.91           | 10.6            | 24.5            | 60.5           | 1.03           |
| AV25                                    | 60.8            | 11.1 | 0.90            | 0.37            | 3.84            | 5.07           | 11.4            | 27.4            | 61.4           | 1.03           |
| AV28                                    | 60.6            | 13.3 | 1.05            | 0.43            | 3.98            | 4.02           | 13.0            | 31.9            | 57.0           | 1.01           |
| CP30                                    | 48.1            | 11.0 | 0.88            | 0.31            | 4.54            | 4.43           | 10.1            | 24.9            | 49.3           | 0.84           |
| Laura                                   | 49.7            | 11.5 | 1.29            | 0.33            | 5.06            | 4.07           | 11.5            | 25.2            | 48.3           | 0.81           |
| Nutrisol                                | 68.7            | 13.9 | 0.76            | 0.42            | 5.33            | 4.08           | 10.3            | 25.2            | 45.8           | 0.85           |
| Location 2: Tepetitla, Tlaxcala, Mexico |                 |      |                 |                 |                 |                |                 |                 |                |                |
| <i>Amaranthus hybridus</i>              |                 |      |                 |                 |                 |                |                 |                 |                |                |
| AV8                                     | 23.1            | 4.04 | 0.96            | 0.56            | 5.43            | 7.32           | 25.1            | 30.8            | 79.5           | 0.97           |
| AV17                                    | 35.5            | 4.03 | 0.99            | 0.70            | 6.19            | 8.01           | 22.1            | 24.5            | 86.3           | 0.88           |
| AV19                                    | 30.2            | 3.81 | 1.05            | 0.58            | 5.62            | 8.72           | 21.3            | 22.1            | 98.0           | 1.10           |
| AV29                                    | 24.9            | 3.77 | 0.83            | 0.62            | 4.78            | 7.11           | 20.1            | 16.5            | 107.0          | 0.90           |
| <i>Amaranthus cruentus</i>              |                 |      |                 |                 |                 |                |                 |                 |                |                |
| Benito                                  | 27.5            | 3.28 | 1.01            | 0.79            | 4.80            | 8.56           | 18.4            | 19.9            | 117.0          | 1.30           |
| CP15                                    | 20.0            | 3.33 | 0.89            | 0.73            | 4.46            | 7.76           | 17.3            | 18.5            | 117.0          | 1.11           |
| CP38                                    | 23.0            | 3.23 | 0.94            | 0.72            | 5.28            | 8.18           | 16.9            | 18.2            | 120.0          | 1.13           |
| CP39                                    | 25.8            | 3.62 | 1.05            | 0.79            | 4.83            | 8.11           | 19.2            | 25.2            | 109.0          | 1.44           |
| <i>Amaranthus hypochondriacus</i>       |                 |      |                 |                 |                 |                |                 |                 |                |                |
| Arelí                                   | 19.0            | 2.86 | 0.84            | 0.60            | 5.51            | 7.69           | 16.2            | 24.0            | 100.0          | 0.95           |
| AV3                                     | 41.2            | 3.87 | 1.13            | 0.86            | 6.28            | 11.10          | 21.2            | 28.9            | 94.1           | 1.19           |
| AV7                                     | 30.8            | 3.81 | 1.05            | 0.72            | 5.68            | 10.10          | 18.6            | 22.8            | 108.0          | 1.16           |
| AV25                                    | 24.2            | 2.89 | 0.95            | 0.68            | 5.41            | 9.95           | 17.5            | 18.1            | 111.0          | 1.13           |
| AV28                                    | 34.1            | 4.70 | 0.89            | 0.69            | 5.74            | 9.17           | 16.6            | 19.1            | 107.0          | 1.14           |
| CP30                                    | 21.4            | 1.51 | 0.79            | 0.70            | 5.22            | 8.21           | 17.5            | 25.6            | 101.0          | 1.03           |
| Laura                                   | 22.1            | 2.33 | 0.80            | 0.44            | 5.77            | 9.47           | 15.7            | 22.6            | 94.5           | 1.31           |
| Nutrisol                                | 35.1            | 1.81 | 0.73            | 0.62            | 6.05            | 9.21           | 18.8            | 26.1            | 88.0           | 1.03           |
| DSH-Tukey <sup>3</sup>                  | 2.94            | 0.48 | 0.48            | 0.16            | 0.20            | 0.30           | 0.26            | 8.25            | 3.62           | 0.11           |

<sup>1</sup> Average in mg·100 g<sup>-1</sup>; <sup>2</sup> Averages in g·kg<sup>-1</sup>; <sup>3</sup> DSH-Tukey: Differences between means with values greater than the honest significant difference (DSH) are statistically significant (Tukey,  $p \leq 0.05$ ).

effect of fertilization  $\times$  genotype  $\times$  location was observed for Fe content. Fe concentration was approximately doubled in most genotypes when fertilizer was applied at both locations (Huaquechula, Puebla, and Tepetitla, Tlaxcala), a trend that was also observed

for Mn content. In contrast, Na and Zn concentrations were generally higher in plants grown without fertilizer. For example, Na ranged from 2.2 to 6.1 mg·100 g<sup>-1</sup> with fertilizer and from 3.4 to 9.2mg·100 g<sup>-1</sup> without fertilizer, whereas Zn ranged from 3.3 to

6.0mg·100 g<sup>-1</sup> with fertilizer and from 4.1 to 7.0mg·100 g<sup>-1</sup> without fertilizer.

Regarding species responses to the interaction with locations and fertilization, higher Fe and Mn concentrations were detected under fertilizer application, while no significant

TABLE V  
EFFECTS OF THE INTERACTIONS BETWEEN FERTILIZER USED AND GENOTYPES OF AMARANTH ON  
MINERAL CONTENT IN LEAVES AND STEMS

| Genotypes                   | Fe <sup>1</sup> | Mn <sup>1</sup> | Cu <sup>1</sup> | Na <sup>2</sup> | Zn <sup>1</sup> | P <sup>2</sup> | Mg <sup>2</sup> | Ca <sup>2</sup> | K <sup>2</sup> | S <sup>2</sup> |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|----------------|
| With fertilizer application |                 |                 |                 |                 |                 |                |                 |                 |                |                |
| AV8                         | 12.8            | 8.20            | 1.01            | 0.54            | 4.66            | 7.36           | 15.1            | 25.0            | 92.1           | 1.04           |
| AV17                        | 13.2            | 6.83            | 0.86            | 0.51            | 5.46            | 5.96           | 13.6            | 26.1            | 71.9           | 0.96           |
| AV19                        | 12.3            | 9.23            | 0.86            | 0.64            | 5.32            | 6.50           | 17.4            | 24.5            | 65.7           | 0.73           |
| AV29                        | 13.2            | 6.24            | 1.00            | 0.43            | 4.79            | 6.25           | 16.2            | 24.1            | 76.5           | 1.08           |
| Benito                      | 11.9            | 8.05            | 0.93            | 0.63            | 5.23            | 6.68           | 15.3            | 24.6            | 85.2           | 1.16           |
| CP15                        | 11.0            | 9.52            | 0.87            | 0.54            | 4.58            | 5.50           | 15.7            | 22.2            | 75.2           | 0.81           |
| CP38                        | 15.7            | 8.47            | 1.01            | 0.65            | 4.95            | 8.07           | 16.2            | 29.1            | 75.5           | 0.95           |
| CP39                        | 14.7            | 6.59            | 1.03            | 0.52            | 5.54            | 7.53           | 17.0            | 28.4            | 76.4           | 1.21           |
| Arelí                       | 11.5            | 5.22            | 0.93            | 0.46            | 4.77            | 6.08           | 12.3            | 21.1            | 76.9           | 0.83           |
| AV3                         | 13.2            | 8.72            | 0.94            | 0.45            | 4.62            | 7.81           | 16.1            | 26.3            | 83.4           | 1.00           |
| AV7                         | 9.7             | 5.28            | 0.91            | 0.60            | 4.62            | 7.21           | 12.7            | 19.3            | 89.1           | 1.16           |
| AV25                        | 12.2            | 5.56            | 0.87            | 0.63            | 5.67            | 6.30           | 16.2            | 22.6            | 66.0           | 0.87           |
| AV28                        | 10.2            | 7.37            | 1.14            | 0.45            | 5.00            | 6.78           | 16.7            | 21.0            | 71.7           | 0.88           |
| CP30                        | 9.6             | 7.28            | 0.73            | 0.50            | 5.08            | 5.38           | 16.6            | 20.0            | 77.2           | 0.92           |
| Laura                       | 14.4            | 7.74            | 1.03            | 0.54            | 4.74            | 7.83           | 15.3            | 25.9            | 77.9           | 1.03           |
| Nutrisol                    | 11.6            | 6.74            | 0.89            | 0.57            | 4.87            | 7.14           | 13.9            | 21.4            | 90.4           | 1.15           |
| No fertilizer application   |                 |                 |                 |                 |                 |                |                 |                 |                |                |
| AV8                         | 12.1            | 8.76            | 0.89            | 0.41            | 4.52            | 6.67           | 14.1            | 24.0            | 91.2           | 0.94           |
| AV17                        | 14.6            | 7.55            | 0.90            | 0.48            | 5.48            | 5.47           | 18.8            | 27.1            | 60.6           | 0.94           |
| AV19                        | 15.0            | 9.88            | 1.06            | 0.53            | 4.54            | 7.25           | 15.9            | 28.4            | 90.1           | 1.09           |
| AV29                        | 14.5            | 8.79            | 0.98            | 0.50            | 4.92            | 6.03           | 14.9            | 27.0            | 86.1           | 1.09           |
| Benito                      | 10.7            | 6.71            | 0.99            | 0.72            | 4.87            | 6.32           | 13.8            | 23.1            | 86.9           | 1.12           |
| CP15                        | 15.0            | 10.8            | 1.14            | 0.50            | 4.79            | 6.26           | 15.8            | 30.3            | 84.4           | 1.10           |
| CP38                        | 13.4            | 8.19            | 1.30            | 0.38            | 4.37            | 6.43           | 14.1            | 24.6            | 73.8           | 0.93           |
| CP39                        | 12.8            | 5.63            | 0.79            | 0.39            | 6.47            | 7.11           | 13.1            | 23.1            | 69.0           | 1.18           |
| Arelí                       | 14.5            | 9.68            | 1.13            | 0.50            | 5.09            | 5.99           | 18.9            | 27.5            | 68.5           | 0.91           |
| AV3                         | 15.0            | 8.70            | 0.93            | 0.51            | 4.22            | 6.03           | 15.0            | 27.3            | 72.6           | 0.85           |
| AV7                         | 11.6            | 3.83            | 0.74            | 0.49            | 5.54            | 6.61           | 12.5            | 23.2            | 77.3           | 1.02           |
| AV25                        | 10.7            | 6.68            | 0.91            | 0.61            | 4.63            | 6.13           | 14.1            | 21.0            | 88.3           | 1.09           |
| AV28                        | 12.5            | 11.3            | 0.97            | 0.60            | 4.64            | 6.03           | 15.7            | 25.9            | 84.7           | 0.90           |
| CP30                        | 15.6            | 9.31            | 1.31            | 0.59            | 4.73            | 6.17           | 16.3            | 29.3            | 81.6           | 1.25           |
| Laura                       | 12.3            | 9.64            | 0.77            | 0.71            | 5.27            | 6.69           | 13.8            | 23.9            | 74.8           | 0.85           |
| Nutrisol                    | 14.8            | 6.09            | 0.72            | 0.34            | 6.11            | 6.61           | 15.3            | 27.4            | 59.0           | 1.03           |
| DSH-Tukey <sup>3</sup>      | 2.93            | 0.4             | NS              | 0.16            | 0.20            | 0.30           | 0.26            | 0.82            | 3.62           | 0.11           |

<sup>1</sup> Average in mg·100 g<sup>-1</sup>; <sup>2</sup> Averages in g·kg<sup>-1</sup>; <sup>3</sup> DSH-Tukey: Differences between means with values greater than the honest significant difference (DSH) are statistically significant (Tukey,  $p \leq 0.05$ ). NS: Nonsignificant differences (Tukey,  $p > 0.05$ ).

differences were observed in Na and Zn contents in unfertilized plants. Among species, *A. cruentus* showed the highest mineral concentrations across locations, and in *A. hybridus*, Fe content was higher in Tepetitla under fertilization, whereas Na content was higher in plants from Tepetitla without fertilizer (Figure 1).

The macroelement content of edible leaves and stems was also influenced by the interaction of location  $\times$  fertilization  $\times$  genotype in the three species evaluated. For Ca, higher concentrations were observed with fertilizer application, with genotype values ranging from 19.4 to 34.7g·kg<sup>-1</sup> across both locations;

without fertilizer, Ca concentrations ranged from 14.1 to 31.0g·kg<sup>-1</sup>. This trend was reversed for K, Mg, P, and S, whose highest concentrations were recorded without fertilizer.

A slight increase in K concentration was observed in genotypes cultivated in Tepetitla compared with Huaquechula under non-fertilized conditions, possibly due to higher rainfall in that region. For Mg, genotypes showed values ranging from 8.3 to 13.8g·kg<sup>-1</sup> with fertilizer and from 14.0 to 30.4g·kg<sup>-1</sup> without fertilizer. In some cases, P concentration doubled in the absence of fertilizer, ranging from 3.5 to 5.6g·kg<sup>-1</sup> with

fertilizer and from 6.9 to 11.3g·kg<sup>-1</sup> without fertilizer. Similarly, S concentration was higher in plants grown without fertilizer, and plants cultivated in Tepetitla showed higher mineral contents than those from Huaquechula (Figure 2).

## Discussion

Amaranth (*Amaranthus* spp.), consumed as edible leaves and stems, is an important source of mineral micro- and macroelements, including K, Ca, Mg, Fe, Mn, Cu, and Zn (Sarker and Oba, 2019; Jiménez-Aguilar and Grusak, 2017; Shukla *et al.*, 2006). In Mexico, the

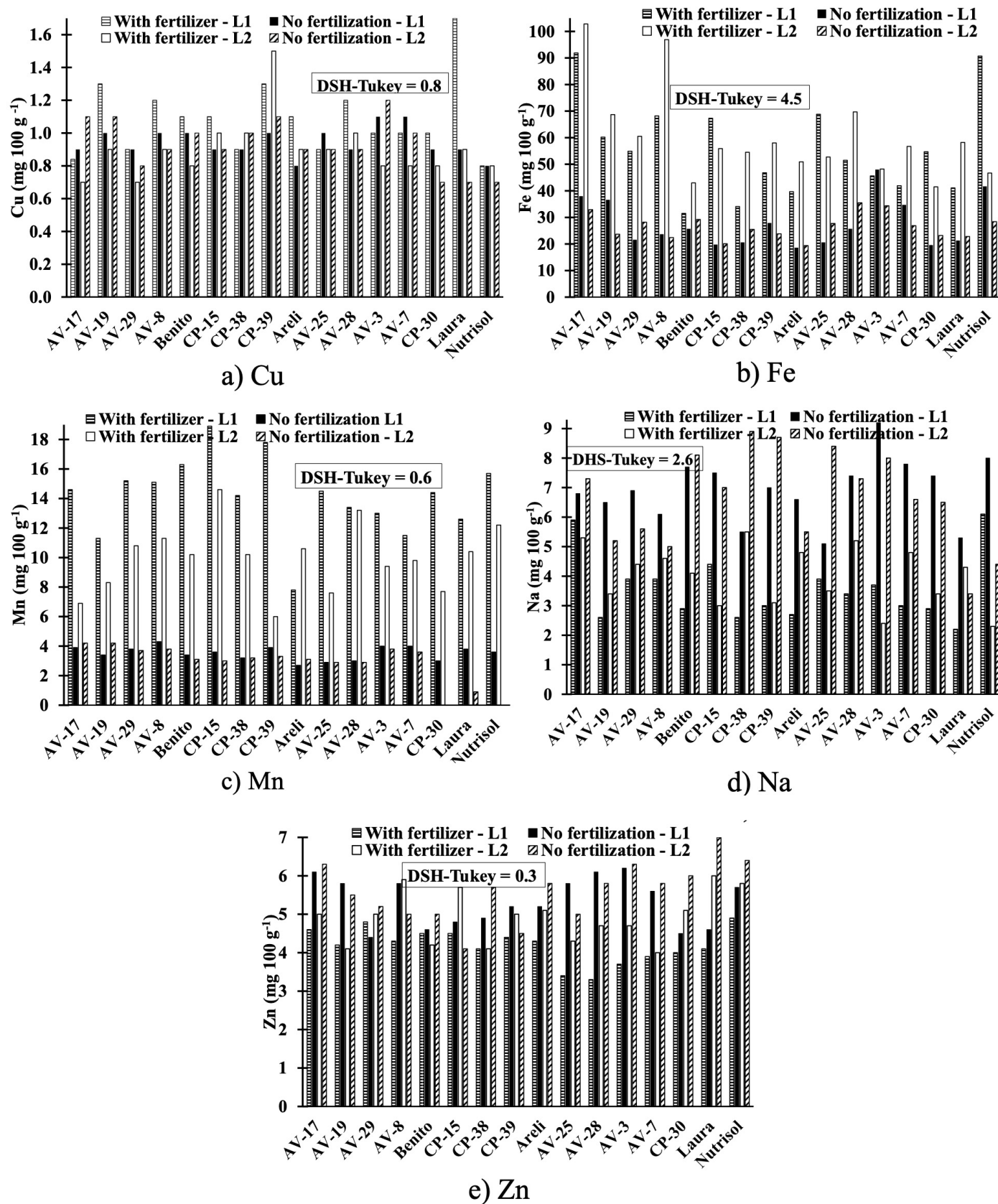


Figure 1. Interaction of locations, fertilization and genotypes of three species of amaranth in microelement content. L1: Huaquechula, Puebla, Mexico; L2; Tepetitla, Tlaxcala, Mexico.



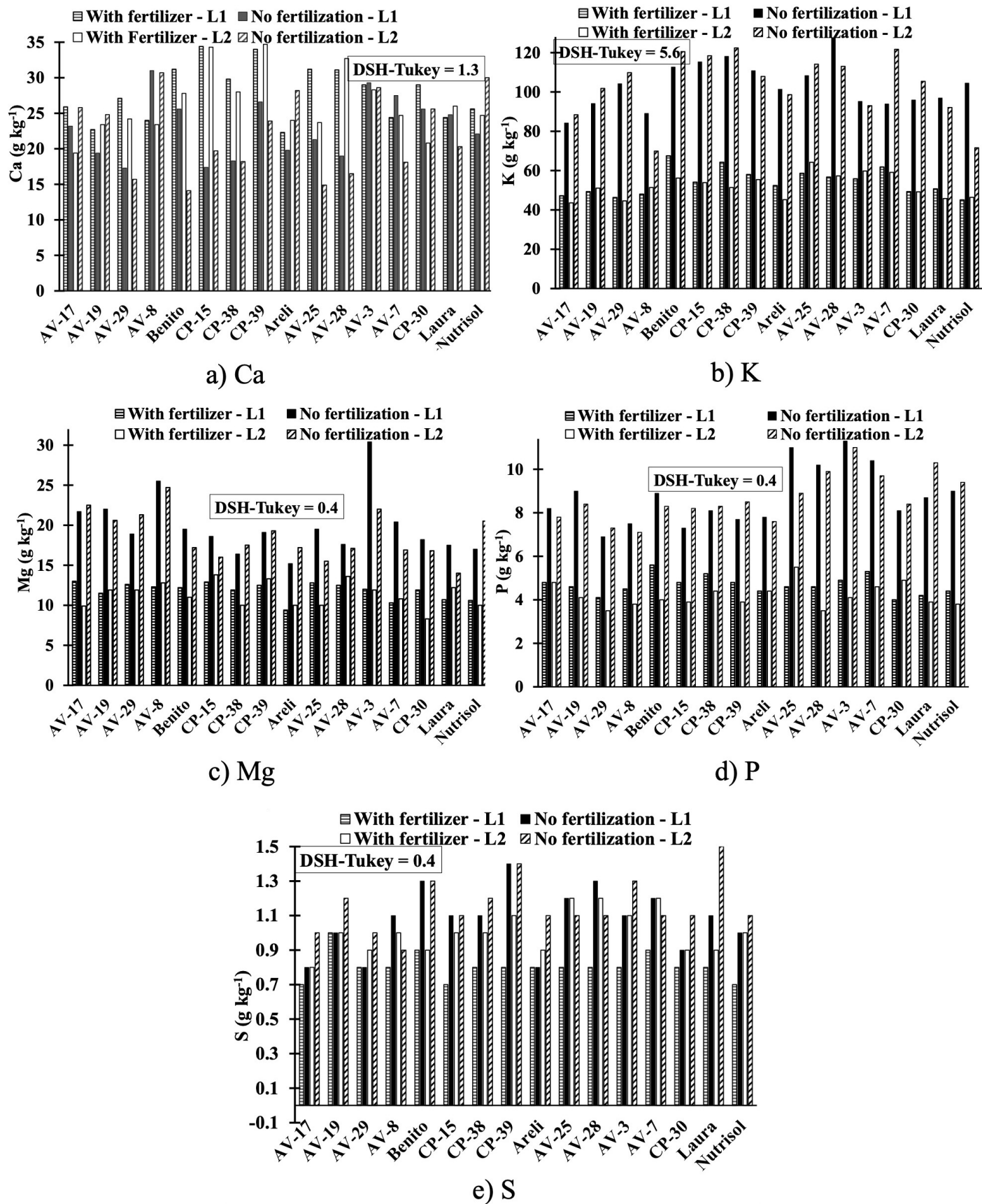


Figure 2. Interaction of locations, fertilization and genotypes of three species of amaranth, in content of macroelements. L1: Huaquechula, Puebla, Mexico; L2: Tepetitla, Tlaxcala, Mexico..

most representative green amaranth species are *A. hybridus*, *A. hypochondriacus*, and *A. cruentus*, which occur both in wild and cultivated forms. These species are tolerant to environmental stress factors such as high temperature and drought and have low water requirements (Barrales *et al.*, 2010; Mukuwapasi *et al.*, 2024).

During cultivation, various agronomic and environmental factors influence the mineral composition of young amaranth leaves and stems, including fertilization, soil type, species, genotype, and agroecological conditions (Ohshiro *et al.*, 2016; Managa and Nematodzi, 2023). The results of this study indicate that the composition of mineral micro- and macroelements in amaranth leaves and stems is significantly affected by cultivation site, fertilization, species, genotype, and the interactions among these factors. Among the main effects, the variance explained by cultivation location was greater than that explained by fertilization, species, genotype, or their interactions.

Amaranth plants grown in Tepetitla exhibited higher concentrations of K, Mg, P, S, Cu, Na, and Zn, whereas plants from Huaquechula showed higher contents of Ca, Cu, Fe, and Mn. This behavior demonstrates that the agroecological conditions of each site directly affect mineral accumulation. Environmental data confirm that Tepetitla, Tlaxcala, presents more favorable growth conditions, with fertile soils and well-distributed rainfall during the rainy season, whereas Huaquechula, Puebla, has less fertile soils and experienced a 16-day drought period (Cantú-López *et al.*, 2022).

From an agronomic perspective, water availability is a determining factor for the translocation and accumulation of minerals. For instance, in *A. tricolor*, water stress increases Ca, Mg, K, S, Mn, Cu, and Na contents while decreasing P, Fe, and Zn concentrations (Sarker and Oba, 2018b), although no consistent pattern was observed among elements. Overall, environmental factors collectively influence mineral accumulation. For example, Managa and Nematodzi (2023) found that in *A. cruentus* and *A. graecizans* grown under field and greenhouse conditions, mineral composition was influenced by the cultivation system. Open-field plants exhibited higher concentrations of Ca, Mg, P, Fe, Zn, Cu, and Mn, whereas greenhouse plants accumulated more K, Na, and S.

Amaranth (*Amaranthus* spp.) requires adequate availability of nitrogen (N), phosphorus (P), potassium (K), and other macroelements to ensure efficient translocation, accumulation, and utilization within plant tissues (Ohshiro *et al.*, 2016). In this study, fertilization with the 18N-07P-09K formula in *A. hybridus*, *A. hypochondriacus*, and *A. cruentus* increased the concentrations of Ca, K, Mg, P, Cu, Mn, and Na, whereas S, Fe, and Zn contents decreased in leaves and young stems. However, the nutritional response to fertilization depends on both dose and fertilizer type; for example, in *A. cruentus* cultivated in Nigeria, the application of organic fertilizer (compost) increased Fe and Zn levels in shoots compared with inorganic fertilizer (Okunlola, 2024). Similarly, Makus and Rettiarachchy (1999) and Makus (2003) reported in *A. tricolor* that the source and concentration of nitrogen influence Ca, K, S, P, Fe, Mn, Cu, and Mg contents, while combined N, P, and K application increases Na, P, Ca, and Mg levels (Ohshiro *et al.*, 2016). Mineral assimilation and accumulation also depend on genotype (homogeneous amaranth lines) and on interactions between available soil nitrogen and applied fertilizer (Akamine *et al.*, 2021). The results of the present study confirmed that cultivation location and fertilization significantly affected micro- and macroelement concentrations in leaves and young stems, generally increasing mineral levels when fertilizer was applied.

The mineral composition of amaranth leaves and young stems is affected by genotype, cultivation practices, and environmental growing conditions. In this study, significant differences and wide variation were observed among *A. hypochondriacus*, *A. hybridus*, and *A. cruentus*, as well as among genotypes within each species. *A. cruentus* showed the highest Ca content (26.14g·kg<sup>-1</sup>), a value similar to that reported by Kachiguma *et al.* (2015) (18.83–22.5g·kg<sup>-1</sup>). Other amaranth species, such as *A. dubius*, also exhibit high Ca values (30.14–31.61g·kg<sup>-1</sup>) (Molina *et al.*, 2011), while *A. tricolor* has shown even greater variation (55–169g·kg<sup>-1</sup>) (Ohshiro *et al.*, 2016). The mean K concentrations recorded in *A. hypochondriacus*, *A. hybridus*, and *A. cruentus* ranged from 70.34 to 86.74g·kg<sup>-1</sup>, which exceed previously reported values of 6.6 to 16.77g·kg<sup>-1</sup> (Kachiguma *et al.*, 2015; Seguin *et al.*, 2013). For Cu, Seguin *et al.* (2013) reported concentrations of 13.8–14.7mg·kg<sup>-1</sup> (1.38–1.47

mg·100g<sup>-1</sup>) in *A. cruentus*, values comparable to those obtained in this study. The Fe contents found in *A. hypochondriacus*, *A. hybridus*, and *A. cruentus* were higher than those reported by Kachiguma *et al.* (2015) but lower than values previously recorded in *A. dubius*, *A. cruentus*, and *A. tricolor* (Molina *et al.*, 2011; Montero-Quintero *et al.*, 2011; Ohshiro *et al.*, 2016). Zn content ranged from 4.71 to 5.14mg·100 g<sup>-1</sup> in the three species evaluated, exceeding values of 1.26–3.46mg·100 g<sup>-1</sup> reported by Kachiguma *et al.* (2015).

The mineral content of amaranth leaves (quelites) from *A. hypochondriacus*, *A. hybridus*, and *A. cruentus* varies according to species, genotype, and fertilization regime. However, their consumption contributes essential micro- and macroelements to the human diet. Elements such as Cu, Fe, Mn, Zn, Ca, Mg, P, and K are fundamental to structural maintenance and metabolic regulation (Awuchi *et al.*, 2020). Cu, Fe, Mn, and Zn function as enzymatic cofactors and play key roles in cellular homeostasis and immune response (Gombart *et al.*, 2020), whereas Ca, Mg, P, and K are associated with a reduced risk of osteoporosis, cardiovascular disease, diabetes, and some types of cancer (Ciosek *et al.*, 2021; Cormick and Belizán, 2019; Hoyt *et al.*, 2022).

Fe and Zn are especially important in vulnerable populations, such as children, pregnant women, and the elderly, and the amaranth species evaluated in this study, when consumed as vegetables, can contribute significantly to meeting daily dietary needs for these minerals. For example, a fresh or cooked portion equivalent to 100g dry weight provides 50–73.42% of the daily Zn requirement, and 16g dry weight meets 100% of daily Fe requirements for children and adults. Likewise, consuming 100g of fresh amaranth leaves per day supplies 4–8% of the Zn requirement, 25–140% of Fe, 11–27% of Cu, 4–90% of Mn, 10–30% of K, 26–95% of Mg, 4–11% of P, and 12–29% of Ca for adults, and 31–76% of Ca for children (Institute of Medicine of the National Academies, 2006).

In general, the mineral composition of leaves and stems in *A. hypochondriacus*, *A. hybridus*, and *A. cruentus* results from the interaction of multiple agronomic and environmental factors, and each factor—alone or combined—affects mineral accumulation differently (Sarker

and Oba, 2018b; Managa and Nemadodzi, 2023; Sefasi *et al.*, 2025). The interaction among location, fertilization, genotype, and species produced complex responses: in some cases, location effects predominated, whereas in others fertilization, species identity, or genotypic variation within species had greater influence.

For instance, in Huaquechula, Puebla (1577 m a.s.l.), where rainfall is low and temperatures are high, plants accumulated higher concentrations of Fe, Mn, and Ca than those cultivated in Tepetitla, Tlaxcala (2228 m a.s.l.), a locality with higher precipitation and lower temperatures. Species–environment interactions were also evident; for example, warm or sub-warm climates favor higher Fe concentrations in *A. hybridus* genotypes (Kachiguma *et al.*, 2015), as well as in *A. hybridus* var. *cruentus*, *A. hypochondriacus*, *A. tricolor*, and *A. thunbergii* (Modi, 2007). In the present study, genotypes AV8, CP15, and AV28 recorded higher mineral contents in Huaquechula, whereas AV17, CP39, and AV3 were superior in Tepetitla, corresponding to *A. hybridus*, *A. cruentus*, and *A. hypochondriacus*, respectively. These findings confirm a strong interaction between genotype and cultivation environment.

In the location  $\times$  genotype/species interaction, plants grown in Huaquechula, Puebla generally exhibited lower concentrations of P, Mg, K, and S, whereas higher Ca concentrations were recorded in plants cultivated in Tepetitla, Tlaxcala. However, the response of each genotype varied according to species and mineral. For example, genotypes AV3, AV7, and AV25 of *A. hypochondriacus* accumulated higher levels of P, Mg, K, and S in Huaquechula, and their concentrations nearly doubled in Tepetitla, indicating an environmental effect associated with precipitation and temperature differences between sites. In contrast, genotype CP39 of *A. cruentus* showed higher Ca accumulation in Huaquechula than in Tepetitla, suggesting that low humidity or drought conditions may favor Ca accumulation in young leaves and stems.

Although the response to drought is species— and genotype—dependent, previous research in *A. cruentus* reported a reduction in K, P, and Mg contents and an increase in Ca under water deficit conditions (Tetyannikov *et al.*, 2022), whereas in *A. tricolor*, drought stress increased Ca, Mg, K, S, Mn, Cu, and Na concentrations (Sarker and Oba, 2018b). Similarly, Modi (2007) found

that Ca concentration increases in amaranth leaves grown under warm conditions. These findings support the existence of differentiated response patterns caused by interaction effects among environmental factors, fertilization, species, and genotype.

The fertilization  $\times$  genotype/species interaction significantly affected all mineral concentrations except Cu. Genotypes AV19 and AV29 (*A. hybridus*), CP15 (*A. cruentus*), and CP30 (*A. hypochondriacus*) accumulated higher Fe, Mn, P, Ca, K, and S contents in the absence of fertilizer, indicating their potential for cultivation in low-input systems or nutrient-deficient soils, which is especially relevant for smallholder farmers.

In the three-way interaction among location  $\times$  fertilization  $\times$  genotype, Fe and Mn contents increased markedly with fertilization, while Na, K, Mg, P, and S showed higher values without fertilization in both Huaquechula and Tepetitla. Ca content was highest in fertilized plants of *A. cruentus*, K content was greater without fertilization, Mg content was greater in *A. hybridus*, and P and S contents were higher in *A. hypochondriacus* regardless of location.

## Conclusions

The evaluation of mineral contents in the edible leaves and stems of 16 genotypes from three green amaranth species revealed that the effect of cultivation location was greater than the effects of fertilization, species, and genotype within species, with the exception of Cu, and that all main factors showed significant interactions. The concentrations of most macroelements increased under favorable soil moisture and nutrient conditions, in contrast to the behavior observed for most microelements. S, Fe, and Zn contents in young leaves and stems did not respond positively to fertilizer application.

For both micro- and macroelements, significant effects were detected for location  $\times$  fertilization and location  $\times$  species interactions. The general trend in mineral concentration among species followed the order: *A. cruentus* > *A. hypochondriacus* > *A. hybridus* for Cu, Mn, Na, Ca, K, and S, whereas for Fe and Zn the pattern was: *A. hybridus* > *A. hypochondriacus* > *A. cruentus*.

Within each species, Ca, K, Fe, and Zn contents varied significantly among genotypes, indicating a strong

influence of genetic factors on mineral accumulation. Based on these findings, amaranth consumed as a leafy vegetable can be considered a valuable dietary source of essential minerals and may contribute substantially to human nutrition.

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## VARIACIÓN EN EL CONTENIDO DE MINERALES EN HOJAS Y TALLOS COMESTIBLES DE *Amaranthus hybridus*, *A. hypochondriacus* Y *A. cruentus* COMO RESULTADO DE DIFERENTES LUGARES DE CULTIVO Y APLICACIÓN DE FERTILIZANTE

Karina Cantú-López, Enrique Ortiz-Torres, Araceli Minerva Vera-Guzmán, José Luis Chávez-Servia, Pedro Antonio López y Adrián Argumedo-Macias

### RESUMEN

El amaranto (*Amaranthus* spp.) es un quelite (hortaliza de hojas y tallos comestibles) con una larga historia de uso en la gastronomía mexicana. El objetivo de este estudio fue evaluar el contenido mineral en los tallos y hojas comestibles de tres especies de *Amaranthus* cultivadas bajo diferentes condiciones ambientales y tratamientos de fertilización. Mediante espectrometría de emisión óptica con plasma acoplado inductivamente (ICP-OES), se determinaron las concentraciones de macro y microelementos en 16 genotipos y accesiones de *Amaranthus hypochondriacus* L., *A. hybridus* L. y *A. cruentus* L., cultivados en Huaquechula (Puebla) y Tepetitla (Tlaxcala), México, bajo condiciones con y sin fertilización, siguiendo un arreglo factorial en un diseño de bloques al azar. Se encontraron diferencias significativas ( $p < 0.05$ ,  $0.01$ ) en las concentraciones de macro y microelementos en tallos y

hojas, según el sitio de cultivo y la fertilización aplicada. Tepetitla, Tlaxcala, presentó condiciones de crecimiento más favorables en términos de humedad del suelo y disponibilidad de nutrientes, lo que resultó en mayores concentraciones de K, Mg, P, S, Na y Zn, y menor acumulación de Ca, Fe y Mn. En particular, las concentraciones de Fe y Zn no fueron influenciadas por la aplicación de fertilizante. Para Cu, Mn, Na, Ca, K y S, el patrón decreciente entre especies fue *A. cruentus* > *A. hypochondriacus* > *A. hybridus*, mientras que para Fe y Zn la tendencia fue *A. hybridus* > *A. hypochondriacus* > *A. cruentus*. Los genotipos evaluados mostraron altas concentraciones de Ca, K, Fe y Zn. Estos resultados indican que el consumo de quelites de amaranto puede contribuir de manera importante a la ingesta dietética de minerales y favorecer la salud de los consumidores.

## VARIAÇÃO NOS TEORES MINERAIS NAS FOLHAS E CAULES COMESTÍVEIS DE *Amaranthus hybridus*, *A. hypochondriacus* E *A. cruentus* COMO RESULTADO DE DIFERENTES LOCAIS DE CULTIVO E APLICAÇÃO DE FERTILIZANTE

Karina Cantú-López, Enrique Ortiz-Torres, Araceli Minerva Vera-Guzmán, José Luis Chávez-Servia, Pedro Antonio López e Adrián Argumedo-Macias

### RESUMO

O amaranto (*Amaranthus* spp.) é um quelite (hortaliça de folhas e caules comestíveis) com uma longa história de uso na culinária mexicana. O objetivo deste estudo foi avaliar o teor mineral nos caules e folhas comestíveis de três espécies de *Amaranthus* cultivadas sob diferentes condições ambientais e tratamentos de fertilização. Por meio da espectrometria de emissão óptica com plasma indutivamente acoplado (ICP-OES), foram determinadas as concentrações de macro e microelementos em 16 genótipos e acessos de *Amaranthus hypochondriacus* L., *A. hybridus* L. e *A. cruentus* L., cultivados em Huaquechula (Puebla) e Tepetitla (Tlaxcala), México, com e sem fertilização, seguindo um arranjo fatorial em delineamento de blocos ao acaso. Foram observadas diferenças significativas ( $p < 0.05$ ,  $0.01$ ) nas concentrações de macro e microelementos em caules e fo-

lhas, de acordo com o local de cultivo e a fertilização aplicada. Tepetitla, Tlaxcala, apresentou condições de crescimento mais favoráveis em termos de umidade do solo e disponibilidade de nutrientes, resultando em maiores concentrações de K, Mg, P, S, Na e Zn, e menor acúmulo de Ca, Fe e Mn. Em particular, as concentrações de Fe e Zn não foram influenciadas pela aplicação de fertilizante. Para Cu, Mn, Na, Ca, K e S, o padrão decrescente entre espécies foi *A. cruentus* > *A. hypochondriacus* > *A. hybridus*, enquanto para Fe e Zn a tendência foi *A. hybridus* > *A. hypochondriacus* > *A. cruentus*. Os genótipos avaliados apresentaram altas concentrações de Ca, K, Fe e Zn. Esses resultados indicam que o consumo de quelites de amaranto pode contribuir de maneira significativa para a ingestão dietética de minerais e promover a saúde dos consumidores.