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Assessment of the Levels of Some Essential Metals in Selected Vegetables Grown on Contaminated Soil Using AAS and UV-Spectrophotometry

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Abstract

Excessive nutrient supply or deficiency in plants poses a major challenge to physiological and metabolic development, both of which are governed by rates of essential metal uptake. This study was conducted to determine the level of uptake of different essential metals from contaminated soils by selected vegetables such as tomato, pepper, garlic, carrot and spinach: *Amaranthus hybridus* and *Celosia argentea*. The amounts of essential metals (Zn, Mg, Mn and Mo) absorbed in the various parts of the vegetables were determined using Atomic Absorption Spectrophotometry after dry ashing at 550 °C. Molybdenum determination was carried out using UV-Spectrophotometry after ashing. From the results obtained, it was observed that the rate of metal uptake varied across and within plant parts. *Celosia argentea* roots, carrot leaves, tomato leaves, and garlic roots displayed the highest level of Mn (96.67 ± 0.03), Zn (67.11 ± 0.16), Mg (188.32 ± 0.22), and Mo (3.74 ± 0.001) uptake respectively. In contrast, the lowest level of metal uptake was recorded in *Celosia argentea* leaves (Mo: 0.71 ± 0.01), tomato roots (Zn: 5.67 ± 0.04), garlic leaves (Mg: 13.81 ± 0.04), and garlic roots (Mn: 0.94 ± 0.004). Our findings are that *Celosia argentea* and carrot plants respectively displayed the highest and lowest essential metals uptake. The difference in essential metal uptake by respective plants may be attributed to the increasing level of soil contamination, as well as the antagonistic and

synergistic effects of metals. From this study, results indicate that metal bioavailability varies widely from element to element and according to element type and plant part.

Keywords: Vegetables, Essential Metals, Contaminated Soil, Atomic Absorption Spectrophotometry, Bioaccumulation.

Introduction

Vegetables are an important component of human diets, providing essential nutrients and trace elements (Singh, 2016). Soils vary in the amounts and composition of mineral nutrients (macro- and micro-elements) as well as in the degree of the uptake availability by the roots. Nutrient storage capacity and accessibility are influenced by soil texture, root depth, and organic matter content; however, the availability is modified by soil moisture and pH (Keller, 2005; Zeng et al., 2011; Neina, 2019). Parameters mentioned above can affect several chemical and biochemical processes occurring in the soil: precipitation-dissolution, adsorption-desorption, complexation-dissociation, and oxidation-reduction, which in essence control the mobility and availability of nutrients to plants (He et al., 2005). Plant-available nutrients are taken up as ions, dissolved in the soil solution, and their uptake depends also on the water flow through the soil-root-shoot pathway (Keller, 2005). Magnesium (Mg) accounts for the highest chemical activity between divalent cations in the cytoplasm, and contributes to the neutralization of sugar compounds, organic acids and amino acids (Etchebarne et al., 2009). Interaction between these two elements is one of the widely known antagonisms in grapevine, and often leads to Mg deficiency (Capps and Wolf, 2000; Haefs et al., 2002). Iron (Fe) and zinc (Zn) are also known to be important mineral elements for grapevine nutrition. Iron deficiency is recognized as one of the main abiotic stresses affecting fruit tree crops growing in calcareous soils in the Mediterranean area (Tagliavini and Rombolà, 2006). The limited availability of Fe in the soil for root absorption leads to reduced yield and quality losses (Bertamini et al., 2002). In addition, Zn deficiency may limit plant growth, since the element plays multiple important roles in various physiological and metabolic processes of the plant (Tsonev and Lidon, 2012). Nutrient availability can be improved by soil or foliar application of a needed element. Soil fertilization is the most established conventional practice, but foliar fertilization, which has been developed in the last 60 years, may be more effective in enhancing nutrient uptake when compared with soil application, particularly for nutrients that can be absorbed on the soil minerals (Tejada and Gonzales, 2004; Kannan, 2010). However, the uptake of one mineral does not depend only on the nutrient availability in the soil, since interactions among elements—synergism and/or antagonism—can influence plant nutrient uptake (Brataševec et al., 2015; White and Brown, 2010). On the other hand, repeated use of metal-enriched chemicals such as fungicides and pesticides, farm

manures, and chemical fertilizers can contribute variable amounts of trace elements, especially heavy metals (e.g., Cu, Mn, Mo, Zn, Cd) (He et al., 2005; Hou et al., 2020).

Nutrient deficiencies and/or toxicities are widespread and have been documented for various soils all over the world. Nutrient deficiencies occur when the plant cannot acquire sufficient amounts of nutrients for its internal needs, whereas an excessive supply of elements, especially trace metals (e.g., Zn, Cu, Mn), results in toxicity to the plant (He et al., 2005). Specific deficiency symptoms appear on all plant parts, but discoloration (chlorosis) of leaves is most commonly observed. Deficiency symptoms of low mobile nutrients (like Fe and Zn) appear initially and primarily on upper leaves or leaf tips, while deficiency symptoms of mobile nutrients (like Mg) appear primarily on lower fully expanded leaves (Tsonev and Lidon, 2012). Several studies have reported on metal uptake in plants. For instance, Intawongse and Dean (2006) conducted research on uptake of heavy metals by vegetable plants grown on contaminated soil. Similarly, Arora et al. (2008) reported on heavy metals accumulation in vegetables irrigated with water from different sources. Sharma and Nagpal (2020) recently reviewed heavy metal contamination in vegetables globally and its implications for food security. Gupta et al. (2022) investigated heavy metal accumulation in vegetables and associated health risks to humans from their consumption. This study assesses the uptake of essential metals by selected vegetables such as tomato, pepper, garlic, carrot and spinach: *Amaranthus hybridus* and *Celosia argentea* planted on contaminated soil using mineral salts of $ZnSO_4 \cdot H_2O$, $MgSO_4 \cdot H_2O$, and $MnSO_4 \cdot 4H_2O$ as contaminants.

Materials and Methods

Contamination Control

All plastic wares and containers used for the experiment were washed with detergent, rinsed with deionized water and soaked in 10% HNO_3 for 24 hours. They were finally rinsed thoroughly with distilled water followed by deionized water and dried at low temperature in an oven.

Physicochemical Analysis of the Soil Samples

Pre-plant soil samples were collected and the following physicochemical analyses were carried out: Soil pH (ICARDA, 2013), Organic Carbon/matter (Udo et al., 2009), Electrical Conductivity (ICARDA, 2013), and Calcium Carbonate Equivalent (Udo et al., 2009).

Pot Experiment

The experiment consisted of 54 treatments, corresponding to triplicates of (0, 15 and 30 mg/kg) of $ZnSO_4 \cdot H_2O$, $MgSO_4 \cdot H_2O$, $MnSO_4 \cdot 4H_2O$, and Na_2MoO_4 soil application in each pot respectively. Seeds of vegetable plants of tomatoes, pepper, garlic, carrot, spinach: *Amaranthus hybridus* and *Celosia argentea* were obtained from the local market in Bata market, Kano State. These were air dried for 24 hours before planting. Planting was done two weeks after soil contamination. Seeds were sown in each pot and later thinned down to three

seedlings per stand after two weeks of germination. Adequate watering and weeding were observed throughout the growing period.

Digestion of Plant Material

Dried and ground plant material (leaves, root/bulb and fruits) of 1.0 g was weighed in a 30–50 cm^3 porcelain crucible. The porcelain crucible was then placed into a cool muffle furnace, and the temperature was gradually increased to 550 °C. The ashing was continued for 5 hours after attaining 550 °C. The muffle furnace was then shut off and the door was opened cautiously for rapid cooling. After cooling, the porcelain crucible was then taken out carefully. The cooled ash was dissolved in 5 cm^3 portions of 2M HCl and mixed with a plastic rod. After mixing thoroughly, it was allowed to stand for about 30 minutes, and filtered through Whatman No. 42 filter paper, discarding the first portions of the filtrates; the volume was made up to 50 cm^3 with deionized water. The aliquot was then analyzed for Zn, Mn, and Mg (ICARDA, 2013).

Determination of Molybdenum using UV-Visible Spectrophotometer

Digestion of Plant Sample

Oven-dried plant samples (leaves, root/bulb and fruits) of 0.5 g were transferred into a silica crucible; mineralization was carried out by heating slowly in muffle furnace to 550 °C for 2 hours and was maintained at this temperature for a further 2 hours. The ash was moistened with (1–2) cm^3 of water, followed by the addition of 1 cm^3 of concentrated hydrochloric acid. The mixture was heated on a hot plate to near dryness. The residue was dissolved in 10 cm^3 water and filtered through a filter paper, and the insoluble part was washed with hot water twice. The filtrate and washing were collected in a 50 cm^3 volumetric flask. Then, 0.1 cm^3 of standard molybdenum solution (100 mg/dm^3) was placed into a 50 cm^3 volumetric flask, and 3 cm^3 of 2 mol/dm^3 thiourea solution, 3 cm^3 of 3 mol/dm^3 potassium thiocyanate solution and about 0.05 g of copper(I) chloride were added. Then 2 cm^3 of concentrated hydrochloric acid was added, and the solution was diluted to 50 cm^3 with water and left at room temperature for about 5 minutes to allow complete formation of the Mo(V)-SCN complex. The resulting solution was then eluted with 2.5 cm^3 of acetone at a flow rate of 1 ml/minute. The eluents were used directly for the spectrophotometric determination of molybdenum as the thiocyanate complex at 461.0 nm with respect to acetone as blank (Adalet and Rehber, 2003).

Preparation of Soil Sample for Molybdenum Determination

Water volume of 20 cm^3 was added to about 0.2 g of dried soil sample and heated for 2 hours at about 60 °C. The obtained solution was filtered through a filter paper. Then, 0.1 cm^3 of standard molybdenum solution (100 mg/cm^3) was placed into a 50 cm^3 volumetric flask, and 3 cm^3 of 2 mol/cm^3 thiourea solution, 3 ml of 3 mol/cm^3 potassium thiocyanate solution and a piece of copper(I) chloride (about 0.050 g) were added. Then 2 cm^3 of concentrated hydrochloric acid was added, and the solution was diluted to 50 cm^3 with water and left at

room temperature for about 5 minutes to allow complete formation of the Mo(V)-SCN complex. The resulting solution was then eluted with 2.5 cm^3 of acetone at a flow rate of 1 cm^3/minute. The eluents were used directly for the spectrophotometric determination of molybdenum as the thiocyanate complex at 461.0 nm with respect to acetone as blank (Adalet and Rehber, 2003).

Chemicals and Reagents

In the preparation of reagents, chemicals of analytical grade purity and distilled water were used. All the glass and plastic wares were washed with detergent and rinsed with distilled water before drying in an oven. All the weighing was done on a Toledo AB54 Model analytical weighing balance. Zn, Mg, and Mn were determined by Atomic Absorption Spectrophotometer (Agilent 240 AA Model), while molybdenum was determined using UV-Visible spectrophotometer. The samples were collected in triplicates.

Soil and Vegetable Sample Collection and Preparation

The soil samples were collected with a soil auger from the botanical garden, Department of Biological Sciences, Bayero University Kano at 0–20 cm depth. The samples were air dried, sieved with a 2 mm sieve, after which sub-samples of 2 kg each were weighed into 2 L capacity plastic buckets perforated at the bottom to allow for air and water movement. The vegetables were grown in the greenhouse of the Department of Biological Sciences, Bayero University Kano. Contamination of the soil in each pot consisted of the same amount of Zn, Mg, Mn and Mo in the range zero (control), 15 mg/kg and 30 mg/kg of $ZnSO_4 \cdot H_2O$, $MgSO_4 \cdot H_2O$, $MnSO_4 \cdot 4H_2O$ and Na_2MoO_4 respectively. At the stage of harvest, plant organs, including roots, stems, leaves and fruits, were respectively harvested. The collected samples were cleaned with deionized water and prepared for analysis.

One-way analysis of Variance (ANOVA) and least-significance-difference test were performed to evaluate statistical significance using IBM SPSS software. Statistical significance was considered at $p < 0.05$. The complementary part of this section can be found in the electronic supporting information (SI 1.0–1.5).

Results and Discussion

Soil Analysis

The results indicated that the contaminated soil used for the pot experiment was a silty loam calcareous soil with a slightly alkaline average pH ca. 7.70 ± 0.19, higher than that (pH = 6.67 ± 0.03) found in the background (control) soil, which is within the allowable limits (pH = 6–9) for appropriate growth and efficient uptake of nutrient materials from the soil (Jadia and Fulekar, 2008). The Electrical Conductivity (EC) and Organic Matter (OM) content were lower in the contaminated soil compared to the control soil. The EC for the contaminated soil was 0.4 ± 0.03 dS/m, while the control soil was 1.12 ± 0.27 dS/m. The OM for the contaminated soil was 4%, while the control soil was 6%. Calcium carbonate equivalent

(CCE) for the contaminated soil was 50%, while the control soil was 65%. The concentration of Diethylenetriaminepentaacetic acid (DTPA) extractable metals in the contaminated soil were 68.43 ± 0.101 mg/kg Fe, 196.3 ± 1.52 mg/kg Mn, 50.7 ± 0.42 mg/kg Zn and 31.4 ± 8.72 mg/kg Mo, while 81.00 ± 0.99 mg/kg Fe, 166.3 ± 0.07 mg/kg Mn, 20.7 ± 0.12 mg/kg Zn and 1.4 ± 0.36 mg/kg Mo concentrations were observed in the control soil. All the extractable metals analysed in the soil were below the World Health Organisation permissible limits (FAO/WHO, 2007).

Assessment of Essential Metals Concentrations

Elemental variation in the control and contaminated soil samples can be found in Figure 1– Figure 7 placed in the appendix section. The detailed analyses of the concentrations of the analyzed essential metals are presented in Table 1.0 (in the electronic support information). The level of essential elements present in the leaves of vegetable plants in the control soil samples is shown in Figure 1 placed in the appendix section. Except for carrot plant, the metals with the highest and lowest uptake level in all vegetable plants investigated were Mg and Mo, respectively. The concentrations of Mg in *Celosia argentea* plant leaves, *Amaranthus hybridus* leaves, garlic plant leaves, leaves of tomato plant, leaves of pepper plant were 27.87 ± 0.22, 73.92 ± 0.41, 104.72 ± 0.04, 86.16 ± 0.69, 50.94 ± 0.0, respectively. Low concentration values of Mo recorded in *Celosia argentea* plant leaves, *Amaranthus hybridus* leaves, garlic plant leaves, leaves of tomato plant, leaves of pepper plant were 0.71 ± 0.0, 1.36 ± 0.01, 1.55 ± 0.003, 1.42 ± 0.04, 1.67 ± 0.18, respectively. In carrot plant, metals with highest and lowest mean level of uptake were observed to be Zn (49.93 ± 0.09) and Mg (1.50 ± 0.0), respectively. The concentration of metals in parts of vegetables varies from metal to metal. Magnesium had the highest content in spinach sample and most other vegetable samples analyzed. Excess of magnesium is unlikely to inhibit crop growth though it causes deficiency of calcium and potassium which are essential nutrients of plants (Abbott and Hasnip, 1997).

The concentration of essential metals was investigated in soil application sizes of 15 mg/kg and 30 mg/kg, shown in Figure 2 and Figure 3 placed in the appendix section, respectively. For 15 mg/kg soil application, Mg and Mo respectively displayed the highest and lowest mean metal uptake levels in *Celosia argentea* plant leaves, *Amaranthus hybridus* plant leaves, tomato plant, and pepper plant. In contrast, the highest and lowest metal uptake in garlic plant leaves and carrot plants were Zn and Mg, respectively. Upon increase in the soil application to 30 mg/kg, a similar trend in the level of essential uptake as in 15 mg/kg soil application was observed, although with a variation in the amount of mean concentration in various vegetable plants investigated.

Mean concentration uptake of metals in soil application of 15 mg/kg of the metal salts was higher than that of 30 mg/kg soil application. This diminution could be due to the process of active adsorption of other metals in the soil. Application of higher levels of other metals in growth solution may adversely affect translocation of metals in different plants and parts of the plant. This result is in agreement with Weng et al. (2009), with the assertion that an

ascending trend in the accumulation of Mo content in round leaf cassia plants was seen with increased Mo application up to 2 mg/kg; when higher amounts of Mo were applied in the soil (e.g., 4 mg/kg), lower levels of Mo were observed in the plants. Application of higher levels of Mo in growth solution adversely affects translocation of Fe from red clover roots to shoots (Hangar, 1965).

Figure 4 placed in the appendix section shows the level of essential metals in the roots/bulbs of the vegetable samples planted in control soil application. Unlike in the case of essential metals present in the leaves of vegetables planted in the control soil, metals with the highest and lowest metal uptake levels were Mg and Mo, respectively, in all the vegetable samples without exception. The mean concentrations of Mg in *Celosia argentea* plant roots, *Amaranthus hybridus* roots, garlic plant roots, carrot plant roots, tomato plant roots, pepper plant roots were 97.24 ± 0.09, 92.78 ± 0.14, 83.64 ± 0.18, 36.06 ± 0.17, 59.19 ± 0.15, 65.81 ± 0.07 respectively. In this study, the concentration of metals in plant roots was observed to be higher compared to that of the leaves of vegetable plants. This differs from Intawongse and Dean (2006), who reported that that Mn and Zn were more enriched in the leaves than in the roots. Naz et al. (2015) reported high concentration of metals in roots, which is also in agreement with Palma et al. (2002) indicating the presence of high concentration of metals in *Solanum lycopersicum* (tomato) roots. Recent studies by Gavrilescu (2022) and Hou et al. (2020) have emphasized that the environmental behavior of metals in soils is governed primarily by persistence and bioavailability rather than total concentration alone, with soil pH and organic matter content modulating these processes through complex biogeochemical transformations.

From Figure 5 placed in the appendix section, analyses of the level of essential metal uptake revealed Mg and Mo as the metals with highest and lowest mean concentration, respectively, in all the vegetable samples except carrot plant under 15 mg/kg soil application. For carrot plants, Zn was observed to show the highest level of uptake, while the least amount was similarly noticed to be Mo. Upon increase of the soil application to 30 mg/kg, all vegetables without exception demonstrated a similar trend as the 15 mg/kg soil application with Mg and Mo as metals with the highest and lowest uptake level respectively, as shown in **Figure 6** placed in the appendix section. However, the correlation between soil application and mean metal concentration was not absolutely linear. For instance, the overall mean concentration of essential metal uptake was observed to increase in *Amaranthus hybridus* leaves, carrot plant, and tomato plant upon increase in soil application from 15 mg/kg to 30 mg/kg. On the contrary, lower mean concentration of essential metals uptake levels were noticed in *Celosia argentea* plant leaves, garlic, and pepper roots upon increase in soil application from 15 mg/kg to 30 mg/kg. This non-linear relation between the concentration of essential metals and amount of soil application may be attributed to the accumulation of certain metal ions in plants in an order of magnitude greater than the surrounding medium. Similar to our findings, Weng et al. (2013) reported that uptake capacity differs among vegetable species. However, a deviation to these trends was reported by Weng et al. (2009). White and Brown (2010) highlighted that

micronutrient imbalances can result in physiological disorders affecting plant growth, yield and metabolism, emphasizing the importance of maintaining optimal nutrient ratios in agricultural systems.

Figure 7 placed in the appendix section shows the level of essential metal uptake in tomato fruits and pepper fruits. In the control soil application for both vegetable fruits, the highest and lowest metal uptake was Mg and Mo, respectively. For the tomato fruits, the mean concentration of Mg and Mo was 38.81 ± 0.10 and 1.65 ± 0.06, respectively; while in pepper fruit, Mg and Mo were recorded as 40.55 ± 0.05 and 2.88 ± 0.01, respectively. Similarly, upon increase in the soil application from 15 mg/kg to 30 mg/kg, the trend in the metal uptake level was observed to be the same in the form Mg > Zn > Mn > Mo. To evaluate the health risk related with these metals, comparison of the levels of essential metal in the studied vegetables with FAO and WHO permissible limits was carried out as shown in Figure 8, which indicates that Zn, Mg and Mn are within the permissible limit of the FAO/WHO standards, while Molybdenum was above the permissible limit of FAO/WHO (FAO/WHO, 2001). Variation in essential metal contents across these vegetables is likely attributable to differential absorption from soil, which is influenced by factors including pH of the soil (Mubofu, 2012), the organic matter content (Mubofu, 2012), and the interaction of other metals such as iron in the growing areas. Zeng et al. (2011) demonstrated that soil pH and organic matter content significantly influence heavy metal availability and uptake by rice plants, with alkaline pH and high organic matter reducing metal bioavailability through enhanced adsorption and complexation. The concentration of these metal elements in the soil, the absorption capacity of metal elements by vegetable species may also cause the differences in the contents. Elevated metal levels in some vegetables may also reflect contamination from irrigation water or agricultural soil. Furthermore, inter-species variation in metal transport and accumulation is likely governed by genetic factors. Gupta et al. (2021) emphasized the need for continuous monitoring of soil and vegetables to prohibit excessive heavy metal accumulation in the food chain, particularly in regions with intensive agricultural activity. Agronomic practices such as the mineral fertilizer applied and water managements on growing these vegetables could also affect the uptake of these metals.

Conclusion

In conclusion, the selected vegetables (garlic, carrot, tomato, pepper, and spinach) differ in their capacity to accumulate essential metals (Zn, Mn, Mg, and Mo). At 15 mg/kg soil application, the uptake of Zinc and Magnesium are within the permissible levels of FAO/WHO of 2001, while Molybdenum is above the permissible limit *(Figure 8)* placed in the appendix section. The results obtained compare favourably with findings reported elsewhere. The concentration of metals in the edible part of the vegetables varies from metal to metal. The observed reduction in metal uptake may be attributable to antagonistic interactions among metals in the soil or during root absorption. Furthermore, the study confirms that, in addition to soil physicochemical parameters, uptake is also governed by the concentration of individual

elements in the soil. Biofortification programmes have been increasingly recognized as promising solutions for global nutritional deficiencies, with studies by Cakmak (2008) and Alloway (2009) demonstrating successful Zn enrichment in crops through agronomic approaches and targeted fertilization strategies.

Recommendation

Further experiments should be conducted using lower soil contamination levels (5–15 mg/kg) to characterise metal uptake in these vegetables across a finer concentration range. Also, the soil pH, electrical conductivity, organic matter and calcium carbonate equivalence should be considered since soil uptake of elements depends on soil parameters.

The pattern emerging from these results suggests that controlled application of inorganic salts to soil may represent a viable approach to vegetable biofortification. Additional research assessing a broader range of essential metals in vegetables grown on contaminated soils of varying types is recommended.

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Appendix

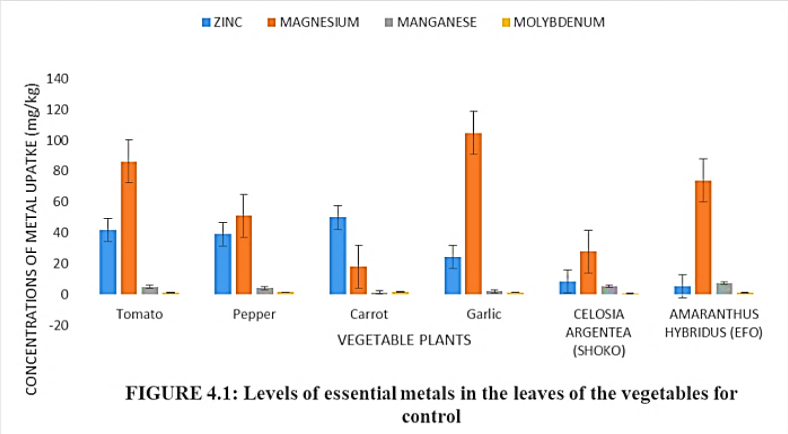


Figure 1. Level of essential metals in the leaves of vegetable plants in control soil application.

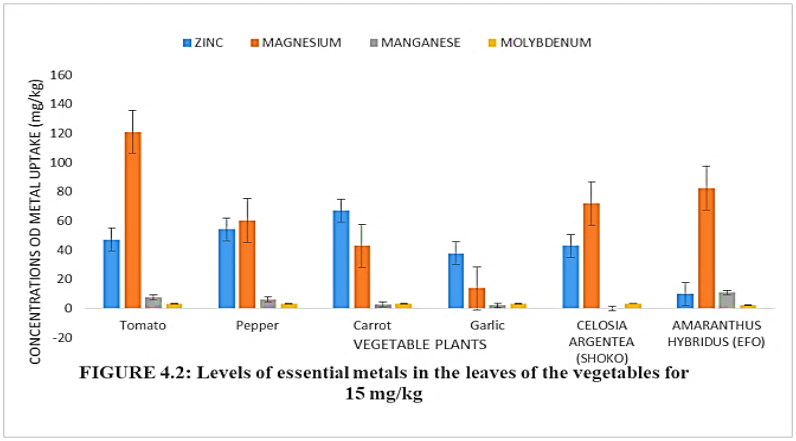


Figure 2. Level of essential metals in the leaves of vegetable plants under 15 mg/kg soil application.

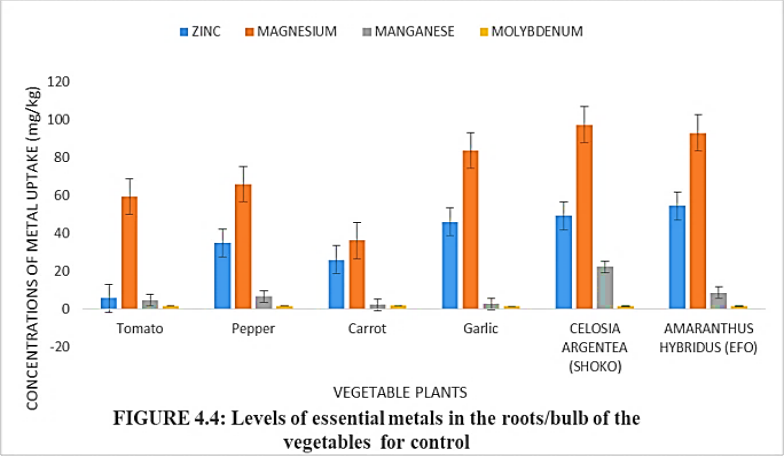


FIGURE 4.4: Levels of essential metals in the roots/bulb of the vegetables for control

Figure 3. Level of essential metals in the leaves of vegetable plants under 30 mg/kg soil application.

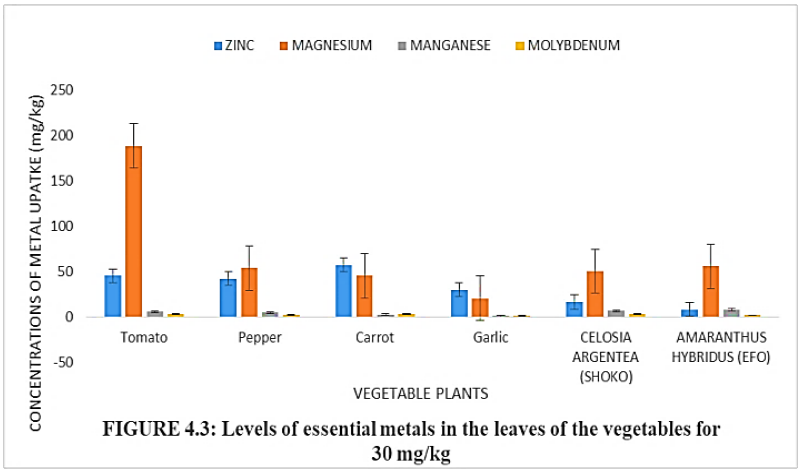


FIGURE 4.3: Levels of essential metals in the leaves of the vegetables for 30 mg/kg

Figure 4. Level of essential metals in the roots/bulbs of vegetable samples planted in control soil application.

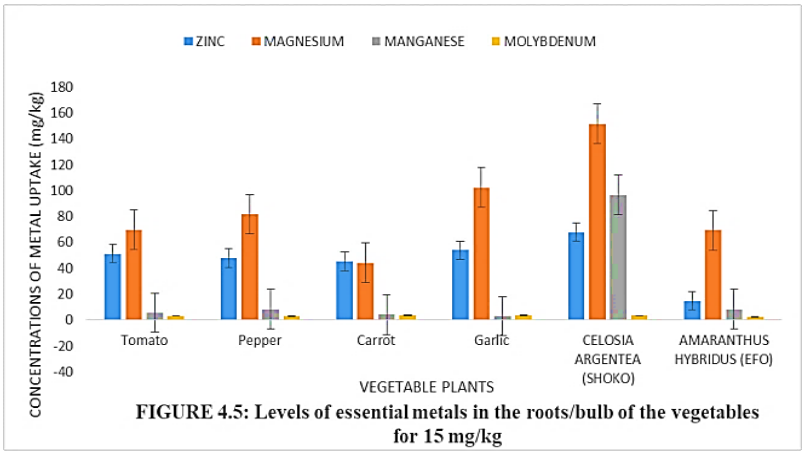


FIGURE 4.5: Levels of essential metals in the roots/bulb of the vegetables for 15 mg/kg

Figure 5. Level of essential metals in the roots/bulbs of vegetable samples under 15 mg/kg soil application.

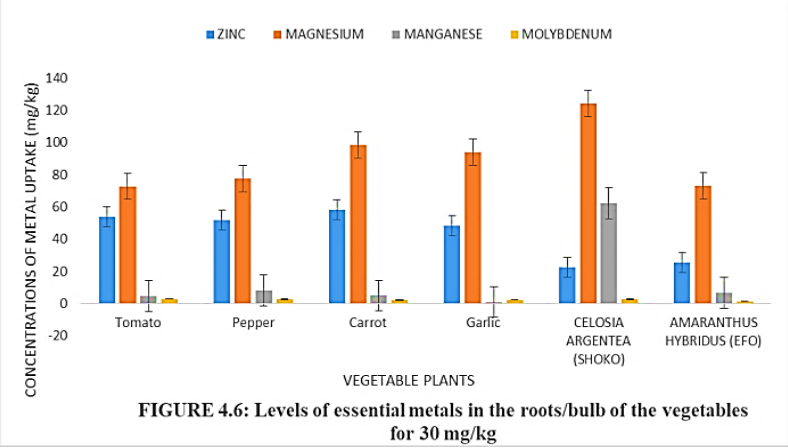


FIGURE 4.6: Levels of essential metals in the roots/bulb of the vegetables for 30 mg/kg

Figure 6. Level of essential metals in the roots/bulbs of vegetable samples under 30 mg/kg soil application.

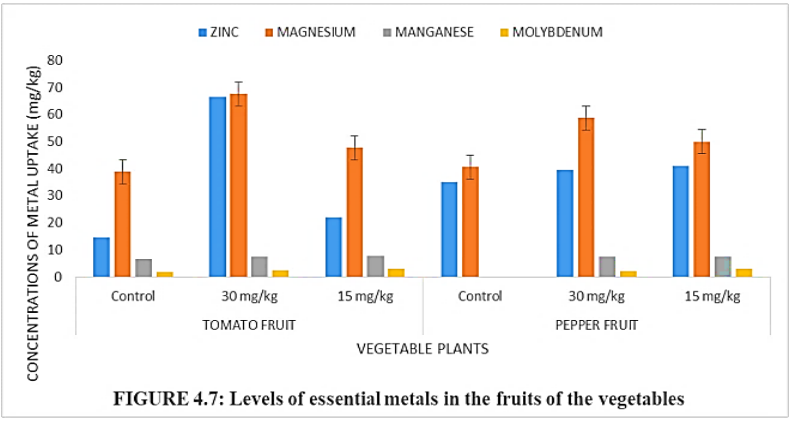


FIGURE 4.7: Levels of essential metals in the fruits of the vegetables

Figure 7. Level of essential metal uptake in tomato fruits and pepper fruits.

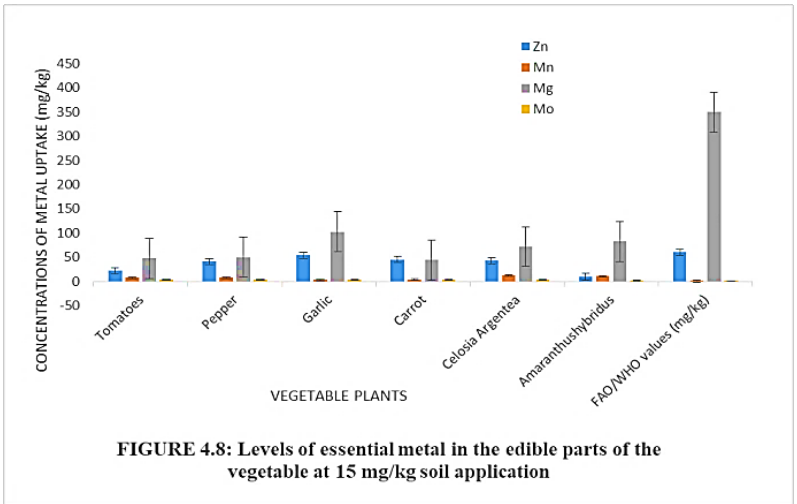


FIGURE 4.8: Levels of essential metal in the edible parts of the vegetable at 15 mg/kg soil application

Figure 8. Comparison of essential metal levels in studied vegetables with FAO/WHO permissible limits.