Enseñar la explotación de la tierra, no la del hombre

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Maestría en Ciencias en Recursos Naturales y Medio Ambiente en Zonas Áridas

Evaluación ambiental de la distribución y concentración de metales pesados en agua, suelo y planta (*Sorghum bicolor* L. Moench)

Environmental assessment of the distribution and concentration of heavy metals in water, soil and plant (*Sorghum bicolor* L. Moench)

T HE S I S

Submitted as a partial requirement to obtain the degree of:

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Environmental assessment of the distribution and concentration of heavy metals in water, soil and plant (*Sorghum bicolor* L. Moench)

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Evaluación ambiental de la distribución y concentración de metales pesados en agua, suelo y planta (*Sorghum bicolor* L. Moench)

RESUMEN GENERAL

La contaminación por metales pesados en agroecosistemas áridos y semiáridos es un problema creciente para la seguridad alimentaria y el medio ambiente. La Comarca Lagunera, en el norte de México, es una de las zonas agrícolas más importantes del país. Donde la utilización de agua subterránea permite mantener la producción de forraje a pesar de la escasez de agua. En este estudio se evaluó la acumulación y distribución de elementos esenciales y tóxicos en *Sorghum bicolor* L. Moench. Ubicado en Gómez Palacio, Durango, a lo largo de cuatro etapas fenológicas. En el estudio se analizaron raíces, tallos, hojas, espigas, además de muestras de suelo y agua de riego. Los elementos determinados fueron cobre, zinc, calcio, magnesio, hierro, manganeso, arsénico, plomo y cadmio. Estos fueron medidos usando espectrometría de masas con plasma acoplado inductivamente (ICP-MS). Los resultados mostraron que el cobre y el zinc tendieron a acumularse en hojas y tallos, mientras que calcio y magnesio se concentraron en raíces y hojas. El hierro y manganeso mostraron baja movilidad interna, reteniéndose principalmente en las raíces. En contraste, los metales presentaron una movilidad limitada, donde el arsénico, plomo y cadmio se acumularon sobre todo en raíces y hojas, con baja translocación hacia los órganos reproductivos. En el suelo, la concentración de estos elementos disminuyó con el ciclo del cultivo, a diferencia de los nutrientes esenciales que se mantuvieron estables. En el agua de riego, calcio y magnesio permanecieron constantes, mientras que hierro, manganeso, arsénico y plomo mostraron ligeras reducciones hacia la madurez. La baja translocación de As, Pb y Cd hacia las espigas indica un riesgo reducido para el consumo directo del sorgo. Sin embargo, la acumulación progresiva en suelos y aguas de riego representa un riesgo potencial a largo plazo, lo que subraya la necesidad de implementar estrategias de monitoreo y manejo sostenible en sistemas agrícolas de zonas áridas.

Palabras clave: bioacumulación, movilidad de metales, absorción vegetal

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Environmental assessment of the distribution and concentration of heavy metals in water, soil and plant (*Sorghum bicolor* L. Moench)

GENERAL ABSTRACT

Heavy metal contamination in arid and semi-arid agroecosystems is an increasing concern for food security and environmental sustainability. The Comarca Lagunera region in northern Mexico is one of the country's most important agricultural zones, where the use of groundwater enables forage production despite severe water scarcity. This study evaluated the accumulation and distribution of essential and toxic elements in *Sorghum bicolor* L. Moench grown in Gómez Palacio, Durango, across four phenological stages. Samples of roots, stems, leaves, and spikes were analyzed, along with soil and irrigation water. The elements quantified included copper, zinc, calcium, magnesium, iron, manganese, arsenic, lead, and cadmium, measured using inductively coupled plasma mass spectrometry (ICP-MS). The results showed that copper and zinc tended to accumulate in leaves and stems, whereas calcium and magnesium were concentrated primarily in roots and leaves. Iron and manganese exhibited low internal mobility, remaining mostly in the roots. In contrast, toxic metals displayed limited mobility arsenic, lead, and cadmium accumulated mainly in roots and leaves, with minimal translocation to reproductive organs. In the soil, the concentrations of toxic metals decreased throughout the crop cycle, unlike essential nutrients, which remained stable. In irrigation water, calcium and magnesium remained constant, while iron, manganese, arsenic, and lead showed slight reductions toward plant maturity. These findings highlight the influence of arid conditions on metal dynamics, where soil properties and plant physiology determine the mobility and accumulation of each element. The low translocation of As, Pb, and Cd into the spikes indicates a reduced risk for the direct consumption of sorghum; however, the progressive accumulation of these contaminants in soil and irrigation water represents a potential long-term risk, underscoring the need for monitoring and sustainable management strategies in arid agricultural systems.

Keywords: plant uptake, bioaccumulation, toxic metal mobility

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CHAPTER 1. GENERAL INTRODUCTION

Heavy metals (HMs) in arid and semi-arid agroecosystems represent a significant environmental and production problem. These elements are characterized by their persistence, bioaccumulation, and toxicity. This compromises ecosystem stability, crop productivity, and food security (Navarro-Aviño et al., 2007; Londoño-Franco et al., 2016). Their accumulation in soil, irrigation water, and plant tissues poses risks to plants, livestock, and human populations dependent on contaminated food sources (Londoño-Franco et al., 2016).

Certain metallic elements, such as Cu, Zn, Mn, Fe, Ca, and Mg, are essential micronutrients required by plant metabolism. Nonetheless, the abundance of those elements or under unfavorable environmental conditions, may disrupt physiological processes, reduce biomass accumulation, and compromise the nutritional quality of forage (Méndez et al., 2009; Mishra et al., 2021; Çevik et al., 2025).

These interactions become particularly relevant in arid regions, where prolonged drought and low soil fertility alter metal mobility and bioavailability (Rodríguez-Eugenio et al., 2019; Alsafran et al., 2023). Climatic conditions such as high evapotranspiration, combined with low organic matter content, modify solubility and absorption dynamics (Hossain et al., 2022; Jiang et al., 2021).

Also, intensive agriculture relies on the use of groundwater. This irrigation water can contain elevated levels of metals, due to the natural composition of aquifers or contamination. This is associated with mining and industry (Ponce-Hernández et al., 2025). The interaction of all these factors promotes the gradual accumulation of metals in the soil and crops, increasing the exposure of organisms and the risk of transfer through the food chain.

The Comarca Lagunera region, located in northern Mexico, has reported high concentrations of metals such as arsenic (As), cadmium (Cd), and lead (Pb), which frequently exceed the maximum permissible limits, according to Zamora et al. (2025) and Huizar-Álvarez & Carrillo-Rivera (2021). The result of the combined effects of intensive mining and metallurgy, aquifer overexploitation, and the widespread (Valenzuela-Noriega, 2021).

These conditions have affected not only water and soil quality but also agricultural productivity and food security throughout the region (Azpilcueta-Pérez et al., 2017). Addressing these problems requires a detailed understanding of the dynamics of metals in agricultural systems, including their accumulation in soil and irrigation water, as well as their subsequent absorption and translocation in major crops (Page & Feller2015).

Among forage species, forage sorghum (Sorghum bicolor L. Moench) stands out for its adaptability to arid conditions, high biomass yield, and nutritional importance in animal feed (Rodríguez-Gómez, 2021; Hossain et al., 2022). In 2020, more than 19,000 hectares were dedicated to sorghum cultivation, producing over 85,000 tons and ranking as the third most important forage crop after alfalfa and silage maize (Zavala-Borrego et al., 2021). However, its capacity to absorb and accumulate heavy metals raises food safety and sustainability concerns.

However, this species' capacity to absorb and accumulate heavy metals raises potential food security and long-term sustainability concerns for local agricultural systems. Therefore, it is crucial to understand how these elements are distributed and translocated in different plant organs under arid soil conditions and irrigation with groundwater (Gorelova et al., 2023).

Despite the regional relevance of forage crops, limited information exists on the temporal dynamics of metal accumulation and translocation in *Sorghum bicolor*, particularly across phenological stages and under real field conditions (Mishra et al., 2021). Sorghum has demonstrated a strong capacity to absorb and retain both essential and toxic metals, especially under environmental stress, making it a suitable species for examining contamination pathways in arid systems (Çevik et al., 2025). However, current studies rarely provide holistic analysis integrating soil chemistry, irrigation water quality, and metal distribution in plant organs.

For this reason, the main objective of the present study was to evaluate the accumulation and translocation of both essential elements and metals in *Sorghum bicolor* cultivated in the Comarca Lagunera region. This research aims to generate scientific evidence to support the design of agricultural management, monitoring, and remediation strategies to address heavy metal contamination.

In this way, it seeks to improve the sustainability of arid agroecosystems as well as zones like the Laguna region, in addition to reducing potential risks along the food chain. The findings are also expected to provide a useful reference for evaluating forage crops under comparable environmental conditions. In a broader sense, such knowledge may support more effective environmental management and help strengthen food security in regions affected by limited water availability and heavy metal exposure.

Objective

General Objective

To determine the accumulation and translocation of heavy metals (As, Cd, and Pb) in *Sorghum bicolor* cultivated in the Comarca Lagunera region and the dynamic of those in soil, irrigation water, and plant organs in different phenological stages, to estimate potential risks to food security and forage production sustainability.

Hypothesis

Sorghum bicolor cultivated in the Comarca Lagunera accumulates and translocates heavy metals from soil and irrigation water into plant tissues, with variations determined by phenological stage. Essential nutrients are expected to exhibit stage-dependent changes related to their physiological functions and their competitive interactions with heavy metals, influencing their distribution within the plant.

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CHAPTER 2. LITERATURE REVIEW

2.1 Contamination of heavy metals in agroecosystems

Heavy metal pollution is one of the main environmental challenges for agriculture, human health, and ecosystem stability. Unlike many organic contaminants, heavy persist and accumulate in soil, irrigate water, and plant tissues over time (Alengebawy et al., 2021). This issue is critical in agricultural areas with intensive industrial activity. Frequent fertilizer application, and limited access to clean water, conditions commonly found in arid and semi-arid lands (Rodríguez-Eugenio et al., 2019; Alsafran et al., 2023; Valenzuela-Noriega, 2021).

In recent years, a growing body of research has shown that metal contamination in agroecosystems affects not only soil and water quality but also food security, since toxic elements can move from the environment into crops, livestock, and eventually humans (Collado-López et al., 2022; Shetty et al., 2025). Worldwide, the presence of arsenic (As), lead (Pb), and cadmium (Cd) in agricultural systems has been linked to genotoxic effects, neurological disorders, and kidney damage in humans, as well as to reductions in plant growth and yield (Jomova et al., 2025).

The problem is more severe in semi-arid lands, where dry conditions and soil properties limit the natural leaching of contaminants, promoting their accumulation near the surface (Torres-Martínez et al., 2021; Velázquez-Chávez et al., 2022). The Comarca Lagunera in Mexico illustrates this situation. The overexploited aquifers, intensive livestock activity, and prolonged use of groundwater with elevated levels of HM have maintained a long-term ecological imbalance (Méndez-Gómez et al., 2008; Ponce-Hernández et al., 2025).

In agricultural systems soil enriched with heavy metals tends to lose fertility and disturb the microbial communities responsible for nutrient cycling (Popoola et al. 2024). Metal accumulation often changes the physical arrangement of soils, reducing their porosity and limiting how water and oxygen move through the root zone (Haider et al., 2021). A comprehensive understanding of this problem involves examining soil chemical properties, irrigation water quality, and plant physiological responses as interconnected factors.

Within this context, sorghum (*Sorghum bicolor*) stands out as a species of high scientific and productive interest. Its broad adaptation to water stress conditions, its extensive use as forage, and its ability to tolerate moderate concentrations of heavy metals make it a suitable bioindicator for environmental pollution studies (Mishra et al., 2021). Furthermore, its well-defined phenological cycle allows for a clear assessment of the dynamics of element accumulation and redistribution throughout its development.

2.2 Natural and anthropogenic sources of metallic pollution

Heavy metals reach agricultural systems through both natural and human-related processes. Naturally, they arise from the slow weathering of mineral-rich rocks, wind erosion, volcanic emissions, and the atmospheric movement of fine particles (Acharya, 2024). These processes contribute to the natural metal content of soils, but the resulting concentrations are typically low and rarely threaten plant or human health.

In contrast, most of the contamination in farming soils comes from human activity (Acharya, 2024). The main sources of contamination include industrial discharges, mining operations, and the frequent use of fertilizers, pesticides, and sewage sludge that contain trace metals (Alengebawy et al., 2021). In water-scarce regions, irrigation often depends on groundwater or wastewater sources, a condition that may gradually increase the accumulation of metals in agricultural soils (Torres-Martínez et al., 2021).

Hydrogeochemical analyses in the Comarca Lagunera have identified both natural and anthropogenic influences on groundwater composition (Dorjderem et al., 2020). Arsenic, lead, and cadmium originate from the dissolution of arsenic-bearing minerals and from infiltration linked to industrial and livestock activities (Méndez-Gómez et al., 2008). As a result, metal concentrations frequently exceed the limits established by NOM-127-SSA1-1994 and by the U.S. EPA, affecting the suitability of water for irrigation as well as for human consumption.

The semi-arid climate intensifies this problem because limited rainfall and strong evaporation promote the buildup of salts and metals near the surface of soil (Etikala et al., 2021). Over time, these conditions cause soils to act as long-term reservoirs of pollution, slowly releasing metals into the soil solution and increasing their availability for plant uptake (Velázquez-Chávez et al., 2022).

The movement and bioavailability of metals depend on both their total concentration and environmental physicochemical properties (Rahman et al., 2022). Factors such as pH, electrical conductivity, organic matter, and soil texture are involved (Abbas et al., 2018). In the Comarca Lagunera, predominantly alkaline soils with low organic matter affect metal retention, promoting fixation of some elements while increasing arsenic mobility and plant uptake (Martínez-Castillo et al., 2022).

2.3 Toxicity and physiological effects of heavy metals on plants

The toxicity of these elements becomes evident from the earliest stages of plant development. Seed germination may decline as a result of disrupted water uptake and reduced activity of enzymes involved in reserve mobilization. Root elongation is often inhibited, and overall growth is diminished due to structural damage to cell membranes and tissues (Nogueira et al., 2021; Mohamed et al., 2025) Additionally, In addition, photosynthetic performance is adversely affected, since alterations in chlorophyll biosynthesis, damage to chloroplast structures, and decreased CO_2 fixation collectively restrict biomass accumulation (Mohamed et al., 2025).

Likewise, heavy metals disrupt cellular homeostasis and nutritional balance. Cadmium, for example, can compete with calcium, magnesium, and iron in membrane transport systems, leading to metabolic imbalances. These alterations affect processes such as cellular respiration and nitrogen assimilation (Ali, et al., 2020). Visibly, symptoms such as chlorosis and foliar necrosis may appear, reflecting a general physiological deterioration (Mohamed et al., 2025).

Despite these negative effects, plants have developed tolerance and detoxification mechanisms that help them cope with metal stress. A key strategy is the synthesis of phytochelatins and metallothioneins, which bind metal ions and promote their sequestration in vacuoles (Hassan et al., 2025; Mir & Hayat, 2021). However, the effectiveness of these mechanisms depends on the plant species, metal concentration, and environmental conditions.

2.4 Behavior of heavy metals in the soil-water-plant system

The movement of heavy metals in the agricultural environment responds to a complex dynamic determined by physicochemical, biological, and climatic factors. In this sense, the soil-water-plant system constitutes an interdependent network in which the mobility and bioavailability of elements depend on soil properties, irrigation water characteristics, and crop physiology (Abbas et al., 2018; Alengebawy et al., 2021).

Agricultural soils function as a primary reservoir of metals. How metal ions attach to or detach from soil particles depends mainly on pH, the amount of organic matter, and the texture of the soil. In alkaline environments, such as those prevalent in the Comarca Lagunera region, some metals like lead and copper tend to precipitate or remain in poorly soluble fractions; however, arsenic, especially in its arsenate form, remains more mobile and bioavailable (Trentin et al., 2022).

Irrigation water is a key pathway for metal entry into agricultural soils. As groundwater flows through mineral-rich geological layers, it can dissolve metals and transport them to farming areas (Economou-Eliopoulos & Megremi, 2021). Repeated irrigation promotes accumulation in surface soils, especially where leaching is limited, as in semi-arid regions (Torres-Martínez et al., 2021). In water-scarce areas, reuse of irrigation water and reliance on deeper wells further increase metal concentrations.

Once metals enter the soil, they may be taken up by roots, bound to organic compounds, or released into the soil solution. This last fraction is called bioavailable**,** what plants can absorb (Kumar, 2023). The size of these particles depends on several factors, including the soil's cation exchange, the number of organic ligands, redox conditions, and overall salt content (Abbas et al., 2018). In soils that contain little organic matter and are affected by salinity, cadmium and zinc tend to move more freely, which raises the likelihood of their absorption by plants (Ullah, et al., 2024).

The flow of metals into the plant occurs mainly through passive transport in the rhizosphere, although it can be modulated by the presence of root exudates that act as chelating or mobilizing agents (Haider et al., 2021). Once inside the root, the elements can be stored in cortical tissues, immobilized by binding proteins, or translocated to aerial organs via the xylem. Still, the way this process unfolds depends both on how much metal is present and on the developmental stage of the crop (Mishra et al., 2021).

In summary, the soil-water-plant system acts as a functional unit where the mobility, bioavailability, and accumulation of metals are closely interconnected. Understanding these relationships allows for the development of more effective management and monitoring strategies to reduce environmental and agricultural risks.

2.5 *Sorghum bicolor* L. Moench theoretical foundations and phenology

Sorghum (*Sorghum bicolor*), a member of the Poaceae family, is extensively cultivated in arid and semi-arid environments because of its efficient water use and its capacity to grow in nutrient-poor soils (Msweli et al., 2025). In Mexico, it serves as a strategic forage crop, particularly in the Comarca Lagunera, where livestock production requires substantial amounts of plant biomass (Zavala-Borrego et al., 2021).

From a physiological point of view, sorghum is a C4 plant, and this feature allows it to keep photosynthesis and carbon fixation active even under strong sunlight and high temperatures (Mishra et al., 2021). Because of these adaptations**,** along with its hardiness and fast growth**,** the crop often performs better than others when facing environmental stresses such as drought, salinity, or the presence of heavy metals (Msweli et al., 2025).

Sorghum growth occurs in three main phenological stages the vegetative, flowering, and maturity. During the vegetative phase, active leaf and root development increases nutrient demand and enhances the uptake of metals present in the soil. In the flowering stage, nutrients are redistributed toward reproductive structures, modifying the internal concentration of several elements. As the crop reaches maturity, nutrient translocation slows, and metal accumulation stabilizes or slightly decreases due to dilution within the expanding biomass (Çevik et al., 2025; Mishra et al., 2021).

These physiological changes make it essential to examine each growth stage separately, since metal concentrations often fluctuate across the crop cycle. In sorghum, the way plants respond to metal stress varies according to their genetic background, such as the cultivar or hybrid, and the surrounding environment, particularly soil type and water quality (Gorelova et al., 2023).

2.6 Physiological and molecular mechanisms of absorption and translocation in Sorghum bicolor

Metal absorption occurs primarily in the roots. Metals dissolved in soil water enter the root along with the nutrient solution. However, the root does not allow the free passage of all elements. Internal barriers regulate their entry, and transport proteins control the amount of metal that can cross cell membranes (Kumar et al., 2017). Recent studies show that different sorghum varieties have varying capacities to absorb and retain cadmium, indicating a genetic basis for this process (Sun et al., 2019).

Once inside the root, the metal can be retained or transported to the aerial parts of the plant. Transport to stems and leaves occurs through the xylem, the tissue that also transports water. In many cases, Sorghum bicolor tends to accumulate higher concentrations of metal in the roots than in the leaves, which reduces damage to photosynthetic tissues. This behavior has been confirmed in recent studies conducted in contaminated soils, where low translocation factors were observed (Sagimbayeva et al., 2025).

At the molecular level, specific transport proteins participate in the movement of metals within the plant. Among these, HMA-type ATPases and ABC transporters stand out, which can influence cadmium loading and mobilization. Recent research has identified variations in genes such as SbABCB11, associated with differences in Cd translocation among sorghum genotypes, demonstrating that transport control is also a genetically regulated process (Sagimbayeva et al., 2025).

The presence of heavy metals generates stress in the plant because it increases the production of reactive molecules that can damage cells and membranes. To protect itself, sorghum activates antioxidant enzymes that help neutralize these compounds. This physiological response has been described as one of the main mechanisms of tolerance to metal stress (Mishra et al., 2021).

Furthermore, sorghum can form associations with mycorrhizal fungi and respond to regulatory compounds such as polyamines, which enhances its ability to handle cadmium. Recent studies show that the combination of mycorrhizae and organic compounds can increase metal retention in the roots and decrease its movement to the leaves, reinforcing the plant's tolerance (Hamed et al., 2024; Ofori-Agyemang et al., 2024).

2.7 Interactions between Heavy Metals and Essential Nutrients in Plants

The absorption of heavy metals and essential nutrients shares physiological and molecular pathways, so both groups of elements exhibit competitive, synergistic, or inhibitory interactions in the rhizosphere and within plant tissues (Pasricha et al., 2021; Haider et al., 2021). At the root level, many heavy metals enter using the same transporters as essential nutrients.

Cadmium (Cd^{2+}) competes with iron (Fe^{2+}) and zinc (Zn^{2+}) for ZIP and IRT transporters, which can induce functional iron deficiency even when its total concentration in the soil is adequate (Connorton & Balk, 2017; Kobayashi et al., 2019). Similarly, lead (Pb^{2+}) interferes with the absorption of calcium (Ca^{2+}) and magnesium (Mg^{2+}), affecting membrane stability and apoplastic transport (Demidchik et al., 2018; Rani et al., 2024).

These interactions explain the preferential accumulation of Cd and Pb in roots and their limited translocation to stems and leaves (Qi et al., 2020; Yang et al., 2023). The bioavailability of heavy metals is also modulated by soil nutrient status. High concentrations of Ca^{2+} and Mg^{2+} can reduce Cd^{2+} uptake by competing for binding sites on the plasma membrane, while high phosphorus (P) levels form poorly soluble complexes with Cd and Pb, decreasing their mobility (Chen et al., 2018; Jia et al., 2025).

In alkaline soils with low organic matter, a typical condition of the Comarca Lagunera region, this set of interactions tends to immobilize Pb and maintain As in more mobile forms (Farooq et al., 2019;). Within the plant, translocation to aerial organs depends on the balance between xylem mobility and detoxification mechanisms. Metals such as Cd, Pb, and As are rapidly sequestered in roots by phytochelatins and metallothioneins, limiting their redistribution and favoring their accumulation in basal tissues (Haider et al., 2021; Mondal et al., 2022).

Conversely, micronutrients such as Zn and Cu exhibit high mobility during vegetative stages, which explains their relative increase in leaves and stems even under metal stress conditions (Kirkby & Römheld, 2008; Mir et al., 2021).These interactions show that heavy metal uptake depends not only on environmental availability but also on ionic competition, nutritional homeostasis, and the crop's phenological stage. (Wu et al., 2020; Jomova et al., 2025).

2.8 Phytoremediation and the role of sorghum as an environmental bioindicator

Phytoremediation is an environmental cleanup technique that uses plants to capture, accumulate, or immobilize contaminants, including heavy metals, present in contaminated soils and water. This strategy is part of the so called biological remediation methods because it takes advantage of plants' natural physiological processes to remove or stabilize toxic substances in the contaminated environment (Soudek et al., 2014; Sagimbayeva et al., 2025). Phytoremediation is considered a more environmentally friendly and lower-cost alternative to traditional physicochemical options, as it can be applied directly to the affected soil without the need for deep excavation or transport of contaminated materials.

Heavy metals such as lead (Pb), cadmium (Cd), and arsenic (As) pose a serious challenge to agricultural soils, water bodies, and sediments due to their toxicity, persistence, and ability to bioaccumulate in organisms. These characteristics make heavy metals especially problematic for human health and ecosystems, as they do not degrade easily and can enter the food chain through crops and pastures. For this reason, phytoremediation systems using plant species capable of accumulating metals or reducing their mobility have been the subject of intensive research in recent decades (Soudek et al., 2014).

Among the species evaluated for this purpose, sorghum (Sorghum bicolor) stands out for several reasons: it is a plant with high biomass production, tolerance to adverse environmental conditions, and an extensive root system, which allows it to interact efficiently with large volumes of contaminated soil (Sagimbayeva et al., 2025). In laboratory and field studies, S. bicolor has demonstrated a considerable capacity to absorb heavy metals such as cadmium and lead from contaminated media, suggesting that certain sorghum varieties can be used to extract metals from the soil or limit their mobility (Soudek et al., 2014).

Research by Sagimbayeva et al. (2025) evaluated the phytostabilization potential of Sorghum bicolor under arid conditions and in soils contaminated with Pb, Cd, and Co. The results showed that most of the metals were retained in the roots, with translocation factors below one (TF < 1), indicating that the crop primarily functioned as a phytostabilizer limiting metal mobility and reducing the risk of dispersal rather than translocating large quantities to the aerial parts, which would facilitate harvesting for extraction (Sagimbayeva et al., 2025). This is significant because retaining metals in the roots can help prevent their entry into other ecosystem components, although it also implies that complete metal removal from the site may require additional strategies.

A complementary study found that Sorghum bicolor can accumulate metals in different organs depending on the experimental conditions, showing that cadmium and zinc generally concentrate in the roots when grown in hydroponic solutions under metal stress, although the presence of antioxidants can modulate this accumulation (Soudek et al., 2014). These behaviors suggest that the accumulation profile (which organ accumulates the most metal) can vary depending on the metal species, environmental conditions, and experimental practices, but in general, sorghum possesses physiological characteristics that make it suitable for monitoring metals in contaminated soils.

Furthermore, studies have demonstrated that sorghum can function as an environmental bioindicator, since the levels of metals accumulated in its tissues reflect the availability of these elements in the soil where it is grown. This allows the use of bicolor sorghum as a biological sensor to assess the presence and degree of contamination in agricultural or industrial environments before the large-scale implementation of remediation strategies (Soudek et al., 2014).

Additional research has explored combinations of sorghum with other agronomic and microbiological practices to improve remediation efficacy. For example, studies have investigated the association of S. bicolor with humic/fulvic acids and mycorrhizal fungi to enhance biomass production and metal uptake in agricultural soils contaminated with Pb, Cd, and Zn (Ofori-Agyemang, 2024). These integrated approaches aim to increase the efficiency of heavy metal accumulation while maintaining plant growth and productivity.

2.9 National and international regulations on metals in soil, water, and plants

The regulation of heavy metals in agricultural and food matrices plays a critical role in ensuring environmental protection and food safety. In Mexico, the main regulatory instruments are NOM-147-SEMARNAT/SSA1-2004 (SEMARNAT, 2007), which defines maximum permissible limits (MPLs) for heavy metals in soils; NOM-021-SEMARNAT-2000 (SEMARNAT, 2002), which establishes procedures for soil classification and analysis; and NOM-127-SSA1-1994 (2021) (Secretaría de Salud, 2021), which regulates water quality for human consumption.

According to NOM-147-SEMARNAT/SSA1-2004 (SEMARNAT, 2007), the maximum permissible levels for contaminated soils are 22 mg kg^{-1} for arsenic (As), 37 mg kg^{-1} for cadmium (Cd), and 400 mg kg^{-1} for lead (Pb). These limits function as technical benchmarks for environmental risk assessment and remediation planning. Their application is particularly relevant in agricultural areas where prolonged irrigation or industrial activities may contribute to metal accumulation in soils.

Regarding water quality, NOM-127-SSA1-1994/2021 establishes that arsenic must remain below 0.025 mg L^{-1}, lead below 0.01 mg L^{-1}, and cadmium below 0.005 mg L^{-1} (SSA, 2021). These thresholds are especially significant in semi-arid regions such as the Comarca Lagunera, where groundwater is the primary source of irrigation. In such contexts, naturally occurring or anthropogenic metal concentrations may exceed recommended values, increasing environmental and agricultural risks.

At the international level, the Codex Alimentarius Commission (Codex Alimentarius, 2019) establishes maximum limits for contaminants in food and animal feed. For livestock feed, the allowable concentrations are 0.5 mg kg^{-1} for arsenic and 0.2 mg kg^{-1} for lead, while sorghum grain and other cereals should remain below 0.1 mg kg^{-1}. These guidelines aim to reduce the transfer of toxic elements through the food chain and protect consumer health.

Despite existing standards, heavy metal accumulation in forage crops continues to raise concerns. Livestock consumes large quantities of plant biomass, which facilitates the bioaccumulation and biomagnification of contaminants. As a result, metals stored in plant tissues may be transferred to animal derived products such as meat and milk. This dynamic represents a potential risk to food quality and public health.

Compliance with national and international MPLs is therefore essential for sustainable agricultural production and food security. However, many regulations do not fully consider variables such as crop growth stage, soil properties, and forage characteristics. Additionally, several Mexican standards have not been updated in over a decade, limiting their alignment with current advances in environmental toxicology and risk assessment (Velázquez-Chávez et al., 2022). Consequently, interpreting metal concentrations requires a broader agroecological perspective.

2.10 Economic and environmental impact of HM pollution.

The presence of heavy metals in agroecosystems has far-reaching economic and environmental effects. Reduced productivity, loss of soil fertility, and declining forage quality translate into lower earnings for producers. Beyond the farm level, governments and communities face high expenses linked to land restoration and public health concerns (Alsafran et al., 2023).

The Comarca Lagunera depends heavily on forage crops and dairy production to support its economy. When heavy metals accumulate in soil or water, the problem goes beyond the environment; it directly threatens food safety and the region's economic balance (Velázquez-Chávez et al., 2022). If exposure continues, both milk and meat output may decline, product quality may fall, and producers can face significant financial losses at home and abroad.

From an environmental perspective, heavy metals disturb the biological balance of soils by damaging beneficial microorganisms and disrupting essential processes such as nutrient cycling and mineralization. Because these elements persist for long periods, they also reduce biodiversity and weaken the resilience of agricultural ecosystems (Haider et al., 2021).

Assessing the economic impact of this contamination requires including its social cost, accounting not only for direct production losses but also for expenses related to healthcare and mitigation. Viewing the issue in this broader sense underscores the importance of studies like the present one, which provide empirical data on pollution levels and their implications for both agricultural productivity and public health.

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CHAPTER 3. ENVIRONMENTAL ASSESSMENT OF THE LEVELS AND DISTRIBUTION OF HEAVY METALS IN WATER, SOIL AND PLANT TISSUES OF *SORGHUM BICOLOR* L. (MOENCH)

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Abstract

Heavy metal contamination in agricultural ecosystems poses significant environmental and food security risks. In the Comarca Lagunera region of Mexico, intensive agricultural activity has increased heavy metal pollution. Moreover, *Sorghum bicolor* L. Moench, a key forage crop, is susceptible to the absorption of heavy metals, which could affect the health of livestock and humans. This study evaluates the levels and distribution of heavy metals in sorghum plants, soil, and irrigation water to assess bioaccumulation patterns. The study was made in Gómez Palacio, Durango, Mexico, located at 25° 41′ 52.71″ N and 103° 22′ 5.31″ WW acrossfour phenological stages of S. bicolor. Water, soil, and plant samples (roots, stems, leaves, and spikes) were analyzed for Cu, Zn, Ca, Mg, Fe, Mn, As, Pb, and Cd using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analyses were performed to identify significant differences in metal concentrations across growth stages. Cu and Zn accumulated mainly in leaves and stems, while Ca and Mg were concentrated in roots and leaves. Fe and Mn were retained in roots, indicating limited translocation. As and Pb accumulated in roots and leaves, suggesting restricted mobility and potential atmospheric deposition. Cd was primarily confined to the roots. In the soil, Pb, As, Cd, and Cu concentrations decreased throughout the crop cycle, whereas Fe, Ca, Zn, and Mg remained stable. Ca and Mg levels were constant in water, while Mn, Fe, As, and Pb slightly declined toward maturity. The study highlights the variable uptake and distribution of heavy metals in *Sorghum bicolor* influenced by soil properties and plant metabolism. While essential metals followed expected physiological pathways. These findings emphasize the need for improved soil and water management to mitigate heavy metal contamination in agroecosystems.

Keywords: Plant metal uptake, Bioaccumulation, Trace metals, Pollution Dynamics, Soil-water-plant interaction, forage crops

Journal: *Case Studies in the Environment* *Status: Under review*

Introduction

Heavy metals (HMs) are a diverse group of elements that play varying roles in environmental and biological systems, depending on their chemical properties and concentrations. They are typically defined as elements with densities greater than 5 g cm^{-3} (Jomova et al., 2024). Some HMs, such as zinc (Zn), copper (Cu), nickel (Ni), and trivalent chromium (Cr^{3+}), are essential trace elements involved in vital biological processes. In contrast, others, like cadmium (Cd), mercury (Hg), lead (Pb), and hexavalent chromium (Cr^{6+}), are non-essential and even toxic at low concentrations (Shetty et al., 2025). These elements occur naturally in ecosystems, with background levels influenced by local geology. However, anthropogenic activities such as mining, smelting, industrial discharge, and intensive agriculture have significantly altered their environmental distribution (Collado-López et al., 2022; Alsafran et al., 2023).

In agroecosystems, the use of fertilizers and pesticides is one of the main sources of heavy metals accumulation in soils (Alengebawy, et al., 2021). Over time, this contamination poses risks to ecosystems and human health, especially in regions with high agricultural activity (Scutaraș & Trincă, et al., 2023). A primary route of humans' exposure to heavy metals is through the consumption of contaminated food. Once absorbed by plants from contaminated soil or irrigation water, these elements can accumulate in edible plant tissues, transfer to livestock through forage, and ultimately enter the human food chain (Jiang et al., 2021; Waqas et al., 2024; Li et al., 2023).

Northern Mexico is characterized by intensive agricultural and livestock production, as well as an important and strong agro-industrial sector (Torres, et al., 2021; Gutiérrez-Núñez, 2023). However, long-term agricultural activities have contributed to increasing environmental pollution, highlighting the need for effective monitoring, prevention, and remediation strategies (Ocampo-López et al., 2024). In the Comarca Lagunera, a key agricultural region, studies have reported elevated levels of heavy metals and dissolved salts in agricultural zones, influenced by seasonal variations and environmental factors (Velázquez-Chávez et al. 2022).

Water quality monitoring has revealed that contaminants such as arsenic, lead, and mercury exceed the maximum permissible limits set by NOM-127-SSA1-1994, the Mexican standard for drinking and agricultural water. Specifically, arsenic surpasses the 0.025 mg L^{-1} limit in 39% of cases, lead exceeds 0.001 mg L^{-1} in 5.3%, and mercury exceeds the 0.006 mg L^{-1} limit in 0.2% (Magadán, 2024). Additionally, the soil in the region contains hazardous levels of lead (Pb), copper (Cu), and zinc (Zn), often surpassing the safety thresholds defined by NOM-147-SEMARNAT/SSA1. In some areas of Coahuila, lead concentrations have been recorded as high as 21,101.64 mg kg-1, far exceeding the 250 mg kg-1 limit, while cadmium levels have reached 760 mg kg-1 (Ponce-Hernández, 2025).

Forage crops play a fundamental role in regional agricultural systems, particularly in the Comarca Lagunera, where they are a crucial component of livestock production. Among them, forage sorghum (*Sorghum bicolor* L. Moench) stands out due to its adaptability to arid or semi-arid regions and its high biomass yield (Hossain et al., 2022). In 2020, the region cultivated 19,564 hectares of forage sorghum, yielding 85,245.9 tons with a commercial value of 32,957,547.87 USD, making it the third most important forage crop in the area (Zavala-Borrego et al., 2021).

However, the potential contamination of forage crops with heavy metals is a growing concern. Since livestock consume large amounts of forage, contaminants accumulated in plants can be transferred to meat and dairy products, impacting food safety. International standards such as the Codex Alimentarius (FAO/WHO, 2024) set strict maximum permissible limits (MPLs) for heavy metals in sorghum grains and animal feed. For example, the arsenic (As) limit is 0.5 mg kg^{-1} in feed and 0.1 mg kg^{-1} in grain, while lead (Pb) cannot exceed 0.2 mg kg^{-1} in feed and 0.1 mg kg^{-1} in grain. Adherence to these limits is essential to ensure food safety in agricultural production.

Given the widespread contamination of water and soil in the region, an integrated assessment is necessary to understand the extent of heavy metal accumulation in agricultural systems (Acharya, 2024). Although previous studies have shown that crops such as *Sorghum bicolor L.* are capable of absorbing and translocating heavy metals under contaminated, given its high biomass production, adaptability to adverse environments and tolerance to the presence of toxic elements such as Cd, Pb and Zn (Gorelova, et al., 2023; Çevık & Ekinci, 2025) most of this research has been conducted under controlled or generalized conditions. Recent reviews also highlight that heavy metal exposure can severely affect sorghum's morpho-physiological traits, enzymatic activity, and yield potential (Mishra et al., 2020). However, there is limited information on how these processes occur under the specific environmental and agricultural conditions of the Comarca Lagunera.

Therefore, this study aims to evaluate the levels and distribution of heavy metals in irrigation water, soil, and tissues of sorghum, to understand their accumulation patterns and potential transfer to the food chain. This research was designed to contribute to the development of monitoring, prevention, and remediation strategies that help mitigate the risks of contamination. This study was designed to investigate the dynamic accumulation of essential nutrients (Cu, Zn, Ca, Mg, Fe, and Mn) and toxic metals (As, Pb, and Cd) in sorghum (*Sorghum bicolor* L.) tissues (roots, stems, leaves, and spikes) under field conditions, considering their interaction with soil and irrigation water across different phenological stages.

We hypothesized that the concentration and distribution of these elements vary significantly among plant organs and developmental phases, reflecting changes in uptake, translocation, and storage mechanisms, and that these patterns are influenced by environmental inputs such as soil composition and water quality. The main objectives were: (1) to quantify the concentrations of selected nutrients and toxic elements in sorghum tissues during vegetative, flowering, and grain-filling stages; (2) to evaluate their corresponding levels in soil and irrigation water; and (3) to assess whether elemental accumulation in edible plant parts remains within safe limits established by international standards for food and feed safety.

Materials and Methods

Study site

This study was conducted in Gómez Palacio, Durango, México. The sampling area is located at 25° 41′ 52.71″ N and 103° 22′ 5.31″ W. Its altitude is 1100 meters above sea level. The region is characterized by a desert climate with annual precipitation ranging from 250 to 400 mm and an average annual evaporation rate of approximately 2,000 mm. Rain is concentrated during the summer and winter months (INEGI, 2021).

The study area is regularly used for forage crop cultivation. For this evaluation, the locally grown crop was *Sorghum bicolor*, widely used for livestock feed in the region due to its high forage yield and drought resistance (Hossain et al., 2022). It is important to mention that agricultural practices, including fertilizing, crop management, and irrigation frequency, remain consistent with local practices throughout the study.

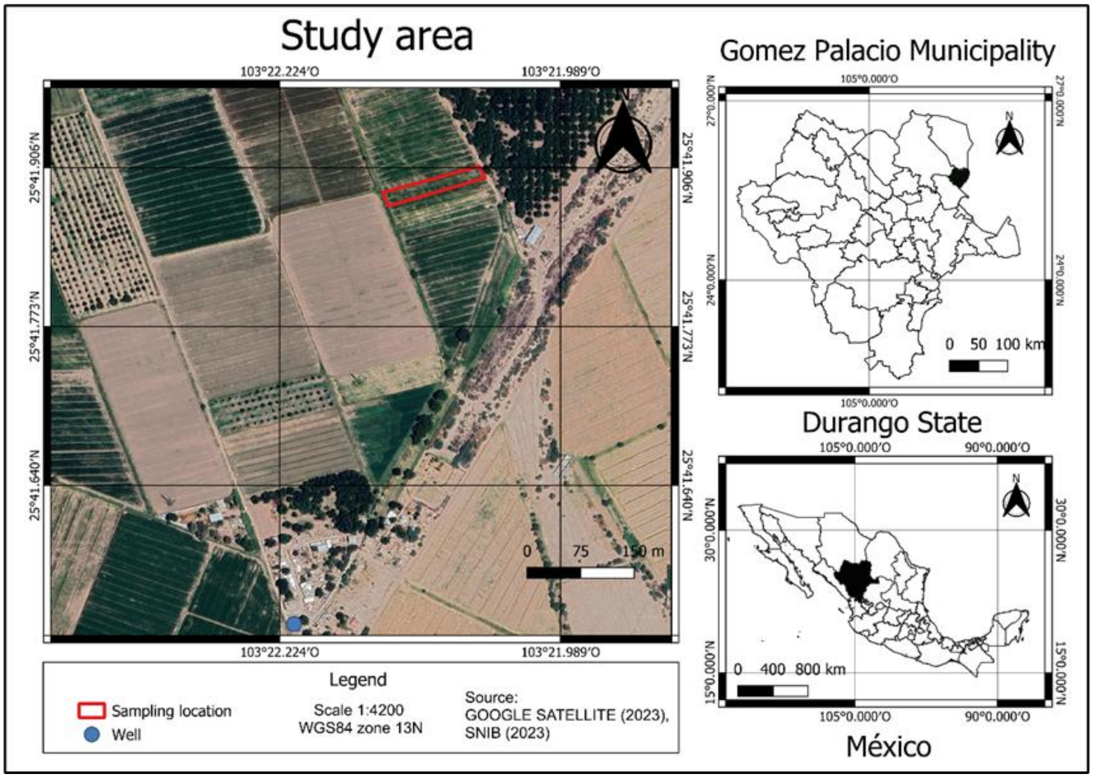

Figure 1. Geographic location of the study area (self-development).

Sampling design and frequency

The study adopted a case study approach with a quantitative and descriptive design. Samples were collected five times over the sorghum growth cycle, at about fifteen-day intervals, beginning in May and finishing in July 2024. Each collection was planned to align with one of the crop's main phenological stages.

Samples were taken from three environmental components irrigation water, soil, and plant tissues (including leaves, stems, roots, and spikes when present). Once collected, all samples were carefully labeled and transported under controlled conditions to preserve their integrity until laboratory analysis.

The collection and preservation of samples followed national standards designed to prevent contamination and reduce degradation. These guidelines include, among other aspects, the use of clean, inert containers and specific storage methods, such as refrigeration or acidification, depending on the type of sample. They also establish maximum holding periods before laboratory analysis. All procedures adhered to the corresponding Mexican Official Standards (Secretaría de Medio Ambiente y Recursos Naturales, 2000; Secretaría de Economía, 2001, 2016).

Water

Irrigation water samples were collected from a local well, representing the primary source of agricultural irrigation, as indicated in the Mexican standard NMX-AA-051-SCFI-2001 (Secretaría de Economía, 2001). To prevent the precipitation of dissolved metals, each sample was acidified in the field with concentrated nitric acid (HNO3). Samples were stored in polyethylene containers and refrigerated at 4°C until the analysis was done.

Soil

Soil samples were collected from three depths: 0-10 cm, 10-20 cm, and 20-30 cm to obtain representative bulk soil. To characterize the general soil conditions of the agricultural system, particularly those influenced by irrigation and environmental factors. An Edelman-type auger was used, which allows efficient extraction of the soil without significantly altering its structure.

After collecting, the samples were left to air-dry and then carefully cleaned by hand to remove roots, stones, or any other debris. Afterward, the soil was sieved through a 0.2 mm mesh so that only the fine, uniform fraction remained for chemical analysis. Each sample was then sealed in a clean, labeled container and kept under proper conditions until testing. The entire procedure followed the methodology described in the Mexican Official Standard NOM-021-SEMARNAT-2000 (Secretaría de Medio Ambiente y Recursos Naturales, 2000).

Plant

Plant material was carefully divided into separate organs, including leaves, stems, roots, and spikes when available. The samples were oven-dried at 45 °C until they reached a constant weight, which allowed for the determination of dry biomass (JeioTech, OF-02G-2C). Once dried, the tissues were finely ground and passed through a 0.2 mm mesh to obtain a homogeneous powder suitable for digestion.

Glassware preparation

Before use, all glassware employed in handling and digestion was immersed for 24 hours in a 10% nitric acid (HNO_3) bath to remove any trace contamination. The material was then thoroughly rinsed with distilled water and left to air-dry. These steps followed the procedure established in the Mexican standard NMX-AA-132-SCFI-2016 (Secretaría de Economía, 2016).

Sample digestion for heavy metal analysis

To extract heavy metals, soil and plant tissue samples were digested using the acid digestion procedure described in EPA Method 3050B (USEPA, 1996). Approximately 0.5 ± 0.001 g of each sample was treated with 10 mL of concentrated nitric acid and heated at 150 °C on a hot plate until the organic matter was fully decomposed. The digested solution was then filtered through Whatman No. 32 filter paper and brought to a final volume of 50 ml with deionized water. All digested samples were stored in acid-washed containers at 4 °C until analysis.

Analysis performance

Heavy metal concentrations in all samples were determined using analyzed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS; NexION 300X

PerkinElmer). Analyses were conducted at the National Center for Disciplinary research on Water-Soil-Plant-Atmosphere Relationships (CENID RASPA), part of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). Calibration was performed using certified multi-element and single-element standard solutions (High-Purity Standards, Charleston, SC, USA), diluted in a 2% acid mixture (HNO3, HCl, HF, and H2SO4). The instrumental operating conditions of the ICP.MS instruments are described in Table 1.

Table 1. Operating Conditions of the PerkinElmer NexION 300X ICP-MS.

ICP-MS instrument	**ICP-MS; NexION 300X PerkinElmer**
Forward power	1.25 kW at 27 MHz
Reflected power	< 10 W
Viewing height	4.0-mm square window centered 14 mm above the load coil
Torch	Fassel ® type
Gas flow-rate	0.5 a 1.2 L/min
Coolant	12.0 1 rain -l
Auxiliary	0.4 1 rain - 1
Injector	1.0 1 rain- t
Spray chamber	Scott ® double-pass type
Nebuliser	Concentric glass (Meinhard ® TR-30-3A)
Solution uptake rate	1.0 ml rain -l (unpumped)
Uptake tube	310 × 0.5-mmi.d. polyethylene
Nebulizer tip wash	0.5 ml of de-ionized water containing 1% v/v Photo-rio ® between sample solutions

Data Analysis

Descriptive and inferential statistical analyses were performed using SPSS software (version 18, SPSS Inc., Chicago IL). A multiple analysis of variance (ANOVA) was conducted to assess differences in heavy metal concentrations across matrices and sampling dates. When significant differences were observed, the meanings were separated using Tukey´s multiple range test at a significance level of $p<0.05$.

Comparison with Regulatory Limits

Sampling was conducted in alignment with the main phenological stages of the crop. Specimens were taken from three environmental matrices: irrigation water, soil, and plant tissues (including leaves, stems, roots, and spikes when available). All collected samples were clearly labeled and transported under controlled conditions to preserve their original characteristics until laboratory analysis. The handling and storage process complied with national standards intended to prevent contamination and degradation. The protocols required the use of clean and chemically inert containers to avoid contamination. Each sample was stored under conditions suitable for its type. For example, refrigeration for biological material or acid preservation for water and soil extracts. The storage time before analysis was kept within the limits established by national regulations. All activities were carried out in compliance with the Mexican Official Standards (Secretaría de Medio Ambiente y Recursos Naturales, 2000; Secretaría de Economía, 2001, 2016). In plant tissues, concentrations were compared with the maximum permissible levels set by the Codex Alimentarius (Codex Alimentarius Commission, 2019) and Directive 2002/32/EC (European Commission, 2002), the latter applicable to animal feed.

Results and Discussion

This study aimed to evaluate the accumulation of heavy metals in the Comarca Lagunera region and understand their behavior within *Sorghum bicolor* L. during its development. To this end, the concentrations of some essential nutrients (Cu, Zn, Ca, Mg, Fe, and Mn) and toxic metals (As, Pb, and Cd) were analyzed in irrigation water, soil, and plant tissues (roots, stems, leaves, and spikes) under realistic field conditions and at different phenological stages. The following results provide insight into the environmental levels of contamination, their relationship with plant development, and the potential risks for entry into the food chain.

Nutrients content

Copper (Cu)

Copper (Cu) is an essential micronutrient for plant development, with optimal concentrations in plant tissues generally ranging from 5 to 30 mg kg^{-1}. However, when these levels are exceeded, copper can become toxic to plants (Kumar et al., 2020).

This element participates in several key physiological processes, such as mitochondrial respiration, electron transport in photosynthesis, oxidative stress regulation, hormone signaling, and cell wall metabolism. Its two oxidation states (Cu^{2+} and Cu^{+}) enable it to perform diverse biochemical roles. In addition, copper serves as a cofactor for various metalloproteins that are fundamental for normal growth and development (Wani et al., 2024).

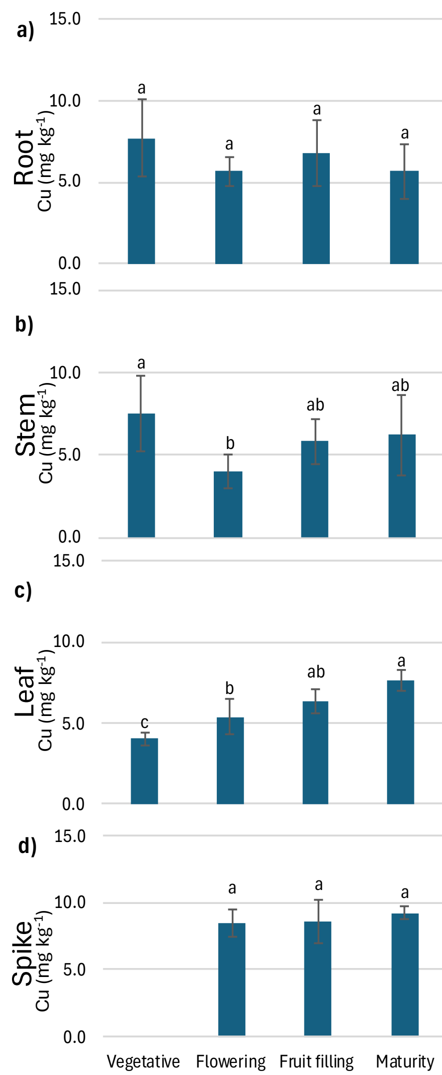

Figure 2. Copper concentrations in *S. bicolor* plants along growth cycle. Values indicate the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

In root tissues showed no significant variation among phenological stages ($F = 1.051$, $p = 0.391$) although slightly higher values were detected during the vegetative phase (Figure 2a). A comparable trend was observed in stems, where Cu levels reached their highest point at the vegetative stage, with significant differences between the vegetative and flowering phases (F =4.431, p=0.015) (Figure 2b). In leaf tissues, Cu content changed significantly over the course of development (F =15.963, p= 0.001) (Figure 2c), reflecting a gradual accumulation as the plant advanced toward maturity. In contrast, spikes maintained nearly uniform Cu concentrations across all stages, with no statistically significant differences (F =0.5, p= 0.615) (Figure 2d). Overall, copper accumulation in the plant displayed a slight upward trend toward maturity, remaining within a relatively narrow range (0.5–10.0 mg kg⁻¹). This behavior suggests efficient uptake and redistribution of Cu during plant growth, especially throughout the flowering and reproductive stages.

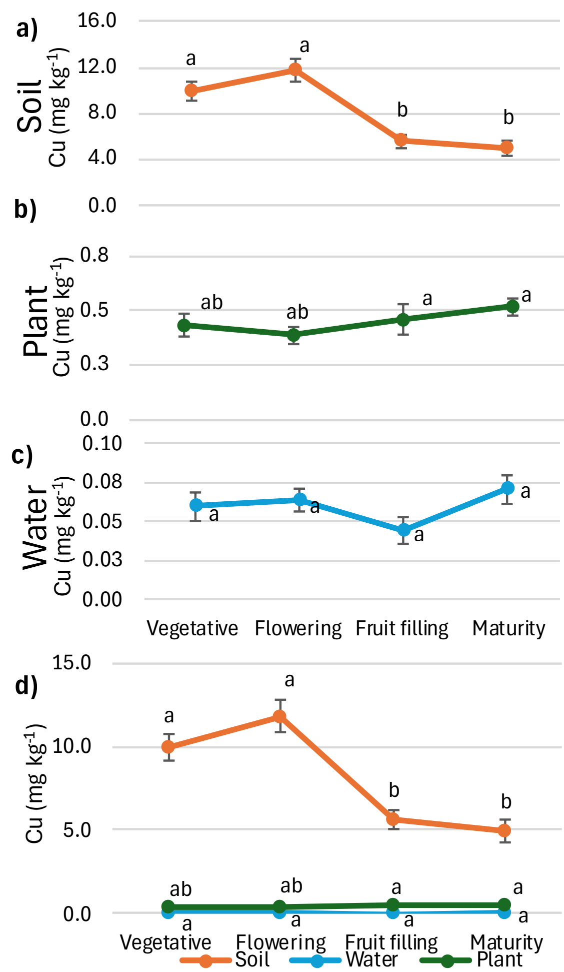

Figure 3. Copper concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, p ≤ 0.05).

In soil, copper concentrations fluctuated significantly (F =8.42, p= 0.001) from 5 to 12.5 mg kg-1. This fluctuation may be attributed to plant uptake dynamics, fertilizer applications, or irrigation inputs during early growth. As well, copper concentrations in irrigated water remain consistently low (0.04 to 0.05 mg kg^{-1}) with no significant changes across sampling periods ($F = 1.32$, $p = 0.32$).

The results indicate that Cu behavior in these concentrations remain well below the maximum limit of 0.2 mg L^{-1} (200 µg L^{-1}) recommended for irrigation water to avoid phytotoxic effects (Espinoza-Rivas et al., 2022). The results suggest that the behavior of Cu in the *Sorghum bicolor* system is mainly influenced by soil levels, particularly during the flowering stage, when translocation toward stems and leaves tends to increase (Wairich et al., 2022). The relatively constant Cu content found in roots points to a buffering function that helps maintain internal homeostasis (Trentin et al., 2022). Moreover, the small variation observed in irrigation water confirms that water was not a significant source of Cu input, underscoring instead the central role of soil composition and management practices in determining Cu dynamics (Mir et al., 2021).

Zn

Zinc (Zn) is another essential micronutrient for crop growth, with typical concentrations ranging from 30 to 200 µg g^{-1} of dry weight (Kaur & Garg, 2021). It plays a crucial part in several physiological processes, including photosynthesis, protein formation, pollen development, and plant defense mechanisms. When zinc is scarce, growth and yield are often reduced, and many metabolic reactions operate less efficiently. On the other hand, excessive Zn can become harmful, disrupting cell division and limiting biomass accumulation (Hassan et al., 2020; Rudani et al., 2018).

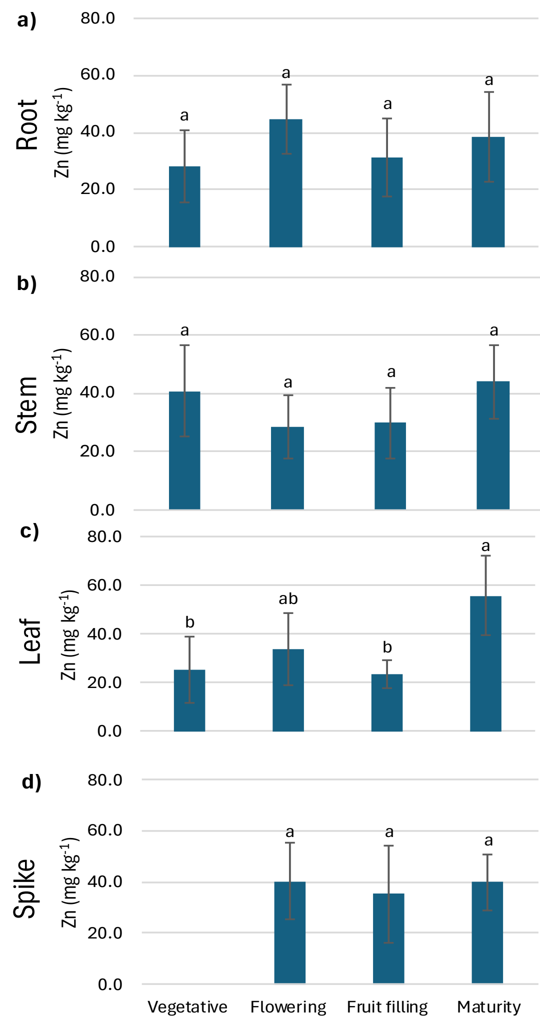

Figure 4. Zinc concentrations in *S. bicolor* plants throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

In this study, zinc concentrations in roots ($F = 1.79$, $p = 0.177$), stems ($F = 1.124$, $p = 0.36$), and spikes ($F = 1.32$, $p = 0.32$) remained relatively stable throughout the different phenological stages, with no statistically significant differences detected ($p > 0.05$) (Figures 4a, 4b, and 4d). However, higher zinc concentrations were found in root tissue during the flowering stage. Conversely, higher zinc concentrations were observed in the leaves and stems during the maturity stage. In the leaves, significant statistical differences were found (F =2.91, p= 0.05) (Figure 4c) among the growth stages of sorghum plants. This pattern may be attributed to zinc´s role in the activity of carbonic anhydrase (CA), a key enzyme involved in photosynthesis and carbohydrate metabolism. Higher concentrations of zinc in the leaves during advanced development stages are likely to the increase activity of CA during these periods (Kirkby & Römheld, 2008). Furthermore, Zn is an essential component of numerous proteins, and its accumulation in the grain may depend on the availability of these proteins for transport and storage (Dionisio et al., 2018; Rehman et al., 2021). According to Kirkby and Römheld (2008), Zn accumulation in leaf tissue could be related to its role in the activity of CA, which regulates CO_2 fixation and carbohydrate metabolism. The high demand for Zn in these processes explains its greater leaf accumulation during the last stages of plant development.

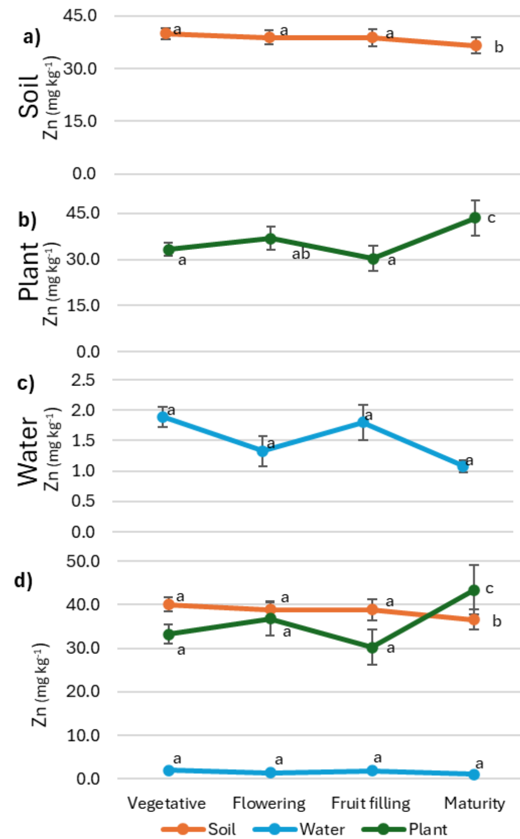

Figure 5. Zinc concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, p ≤ 0.05).

Ca

Calcium (Ca) is one of the three most abundant macronutrients in crops (Wdowiak et al., 2024). It not only provides structural support by reinforcing cell walls and maintaining membrane stability but also acts as a key messenger in numerous physiological processes. Among its many functions are the regulation of root and pollen tube growth and the coordination of plant responses to environmental and biological stress (Thor, 2019). When calcium levels are insufficient, developing tissues are among the first to show damage, which can hinder growth and compromise stress resilience (Wani et al., 2024).

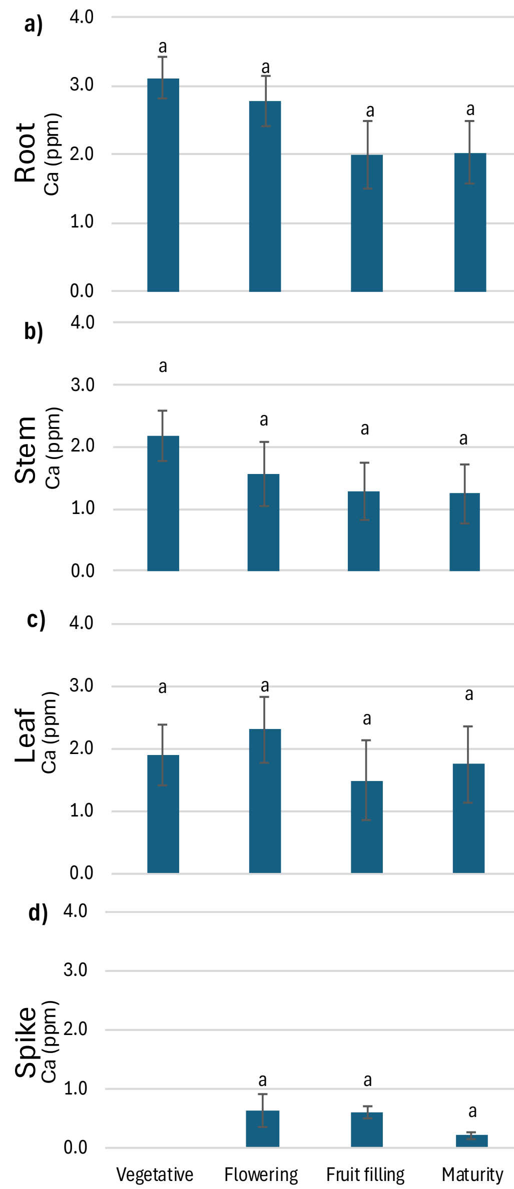

Figure 6. Calcium concentrations in S. bicolor plants throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$)

Throughout the phenological stages evaluated, calcium concentrations in plant tissues did not vary significantly ($p > 0.05$). However, roots consistently showed higher Ca levels than the other plant organs (Figure 6a), suggesting that they play a major role in calcium storage and regulation within the plant system. In the stem, calcium concentration increased during the vegetative stage but showed a slight decrease towards maturity (Figure 6b). On the other hand, leaf calcium was higher during the flowering phase (Figure 6c). In the spike, calcium levels were considerably lower than in other organs (Figure 6d), which may be attributed to calcium´s limited mobility in the phloem and its dependence on xylem transport (Hocking, et al., 2016).

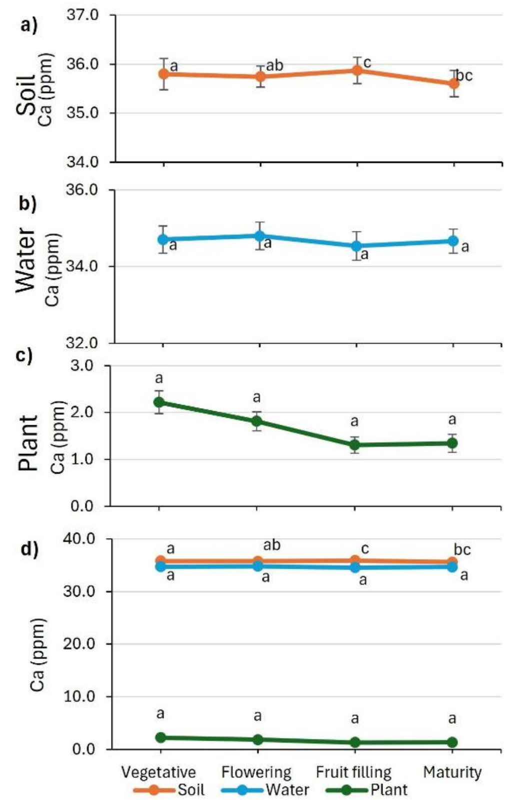

Figure 7. Calcium concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Previous studies have demonstrated that calcium accumulation is restricted in organs with low transpiration rates, such as ripening fruits because transpiration is critical in calcium transport (Demidchik et al., 2018). During the early stages of development, when transpiration rates decline, calcium influx is reduced across different tissues (Hocking et al., 2016). The higher concentrations of calcium in root and leaf tissues suggest that calcium distribution is influenced by water flow dynamics within the plant, with greater accumulation occurring in organs that maintain active transpiration and vascular transport during early growth stages (Kim, et al. 2022; He, et al., 2021).

Mg

Similar to Calcium, magnesium (Mg) is an essential macronutrient. It plays a vital role in photosynthesis, protein synthesis, and enzyme activation. Magnesium deficiency can severely affect crop growth and quality (Chen et al., 2018). The optimal magnesium requirement for plant growth ranges from 1.5 to 3.5 g kg-1 (1500 to 3500 ppm) in vegetative tissues (Guo et al., 2016).

In this study, no statistically significant differences in Mg concentration were detected across phenological stages ($p > 0.05$). A slightly higher mean value was observed during the vegetative stage, particularly in whole-plant samples, suggesting a possible trend toward greater Mg accumulation at early growth (Figure 8d). Magnesium content in plant tissues did not show statistically significant variations across the analyzed phenological stages ($p > 0.05$) (Figures 8a and 8b). In both roots and stems, magnesium concentrations declined slightly as the crop reached maturity, with the highest values recorded during the vegetative stage. In the leaves, magnesium levels rose noticeably during

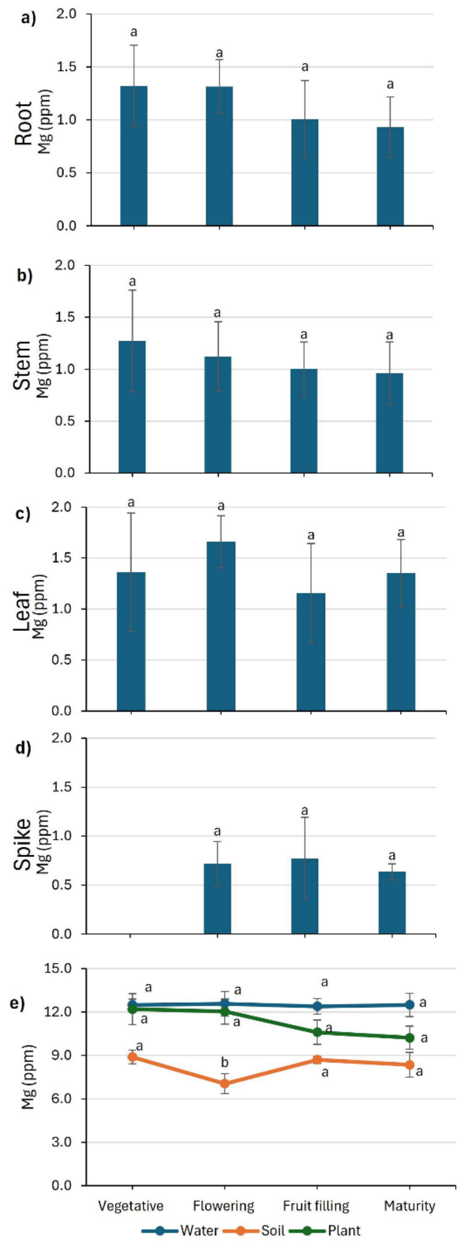

Figure 8.Magnesium concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

the flowering stage (Figure 8c). By contrast, the spikes showed the lowest concentrations, a pattern similar to that observed for calcium (Figure 8d). These observations suggest that magnesium uptake and movement within the plant depend strongly on its availability in both soil and irrigation water. Overall, Mg concentrations changed little across the growth stages, which implies that absorption was relatively stable but limited through the crop cycle. Such consistency likely results from low bioavailability in the soil–water environment, restricting the plant’s ability to accumulate enough magnesium to satisfy its metabolic needs.

Because magnesium is essential for chlorophyll synthesis and ATP production, its presence is critical for sustaining photosynthetic performance (Tanoi & Kobayashi, 2015). The higher Mg concentration found in leaves during the flowering phase may therefore correspond to increased photosynthetic demand and energy production. This interpretation agrees with earlier studies showing that Mg tends to be redistributed toward younger, metabolically active tissues during reproductive growth (Farhat et al., 2016). The gradual decline observed toward maturity may indicate the remobilization of Mg from senescing tissues to developing grains, a process previously reported in other cereal species (Tanoi & Kobayashi, 2015; Farhat et al., 2016).

Fe

ron (Fe) is an essential micronutrient that plays a central role in plant growth, productivity, and crop quality (Berger et al., 2023). It takes part in several physiological processes, such as respiration, chlorophyll formation, and photosynthesis. In addition, Fe serves as a cofactor for enzymes involved in electron and oxygen transport, which are key to maintaining cellular energy flow. Most of the iron absorbed by plants comes from the soil, and its availability is largely determined by pH and redox potential (Connorton et al., 2017).

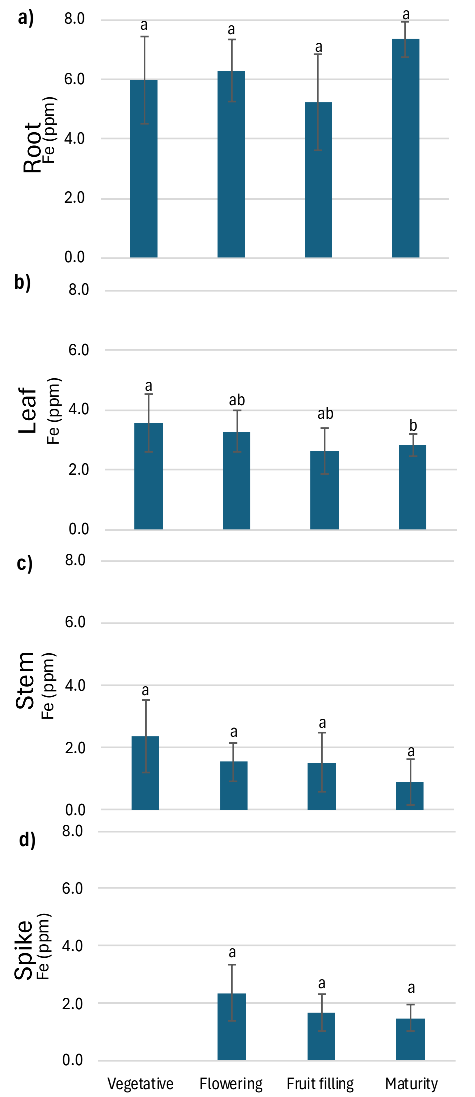

Figure 9. Iron concentrations in *S. bicolor* plants throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Most of the iron measured in the plant was concentrated in the roots, which appear to act as the main reservoir throughout development. In contrast, the stems maintained nearly constant Fe levels F =1.55, p= 0.22) (Figure 9a). The leaves showed a different pattern, with the highest concentrations recorded during the vegetative stage and a gradual decline as plants matured (F =1.38, p= 0.04). In spikes, Fe concentrations were uniformly low and showed no major changes (F =0.84, p= 0.44) (Figure 9b). These results point to the limited mobility of iron once absorbed by the root system.

These results indicate that the roots act as the main reservoir of Fe, most likely because the element is not easily translocated to the aerial parts of the plant. This restricted movement could be related to the limited activity of specific transport proteins or to the chemical form in which iron occurs within the tissues (Zulfiqar et al., 2023). The gradual decline in leaf Fe content as the crop matures suggests an internal redistribution of nutrients, in which younger and actively growing organs are prioritized. Kobayashi et al. (2019) noted that when plants experience iron deficiency, they tend to channel the available Fe toward younger, actively growing tissues. As a result, older leaves show a gradual decline in concentration as development advances. In the surrounding environment, Fe levels in the soil stayed relatively constant throughout all growth stages, with no statistically significant differences detected (F =1.01, p= 0.41) (Figure 10a). Concentrations varied only slightly, from 13.8 to 14.9 ppm, suggesting that iron remained accessible to the plants during the entire cultivation period.

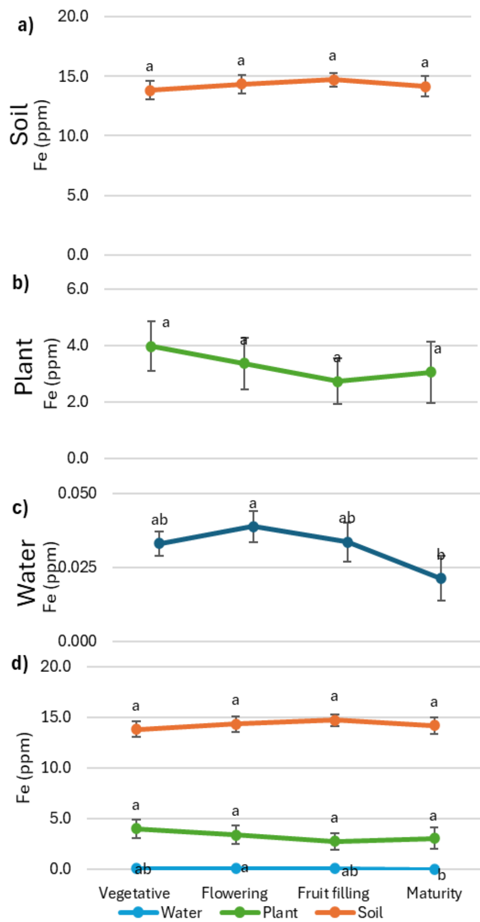

Figure 10. Iron concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

In contrast, the Fe content in irrigation water was much lower, between 0.02 and 0.04 ppm, and did vary significantly among stages ($p \leq 0.05$). The highest concentration was recorded during flowering, whereas the lowest occurred at maturity ($F = 0.43$, $p = 0.03$). Although these values are minor compared with those in the soil, water irrigation may still provide a small but useful contribution of Fe during the early stages of plant development.

Mn

Manganese (Mn) is another essential micronutrient with important roles in photosynthesis, iron reduction, cellular respiration, reactive oxygen species (ROS) scavenging, hormone regulation, and stress responses (Vatansever et al., 2017).

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Manganese levels in roots and stems were highest during the vegetative phase, followed by a slight decline as the plants reached maturity. In contrast, leaf Mn concentrations stayed relatively constant, with no significant variation among the different growth stages. The spike

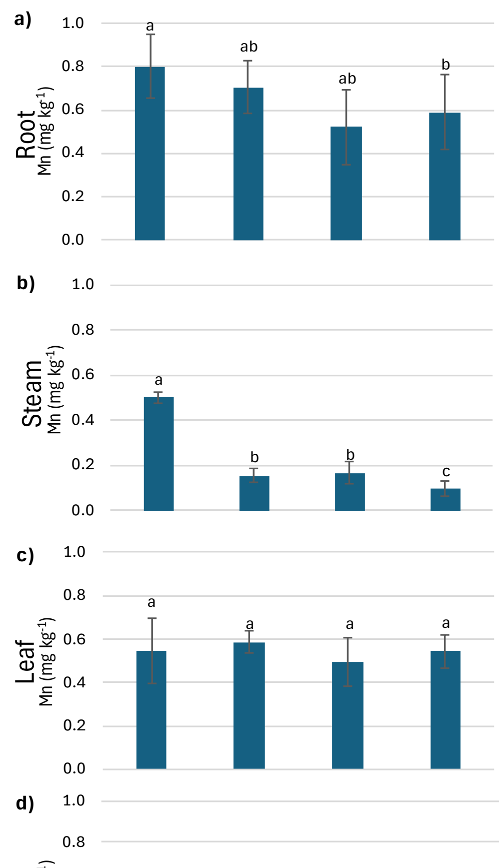

Figure 11.Manganese concentrations in *S. bicolor* plants throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$)

remained constant during the flowering, fruit filling, and maturity stages. These results imply that Mn accumulates primarily in roots, which may serve as a Mn reservoir. The observed decrease in stem Mn content and its stability in the leaves. This could be related to the low phloem mobility of this element, a phenomenon previously reported by Millaleo et al. (2010). Leaf Mn homeostasis might be maintained by regulatory mechanisms that ensure sufficient supply for photosynthesis and enzymatic activity, especially given its role in the oxygen-evolving complex of photosystem II.

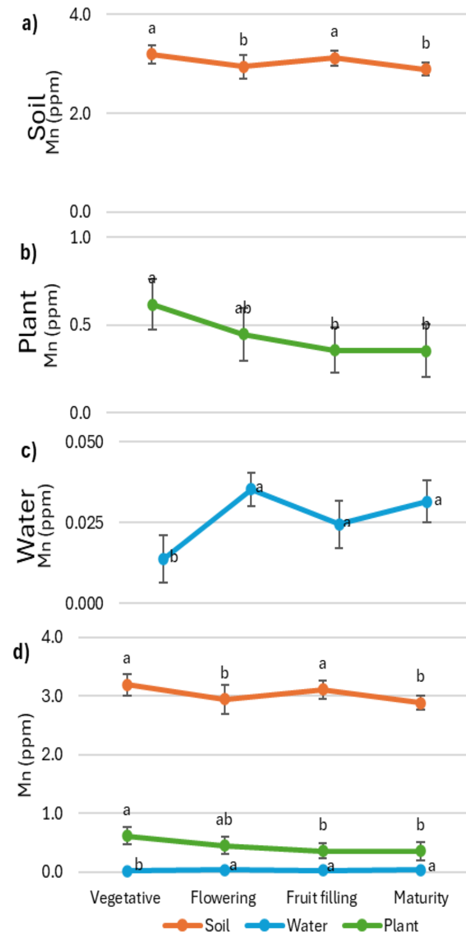

Figure 12. Manganese concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, p ≤ 0.05*)*

Across the growing stages, Mn levels in the soil stayed within a narrow range, averaging between 2.8 and 3.2 ppm. Although the differences were statistically significant, they were relatively small, with a slight decline observed as plants moved from the vegetative stage to flowering and maturity (Figure 11a). The pattern probably reflects the combined effects of plant uptake and variations in Mn bioavailability.

Mn in irrigation water was detected at considerably lower concentrations (0.015–0.035 ppm), although significant differences were also observed between stages (Figure 11c). The highest content was recorded during flowering, while the lowest levels were observed in the vegetative stage. Although these amounts are minimal compared to those in the soil, water could contribute small amounts of Mn, particularly at times when the soil has lower bioavailability.

Heavy metal content

As

Arsenic (As) does not play any beneficial role in plant nutrition, but it is widely distributed in soils and waters around the world. Even small amounts can disturb vital plant functions, leading to biochemical and structural damage (Martínez-Castillo et al., 2022; Sinha et al., 2023). This metal interferes with core metabolic processes, promotes the generation of reactive oxygen species (ROS), and weakens both photosynthetic and protein metabolism (Abbas et al., 2018; Hassan et al., 2025).

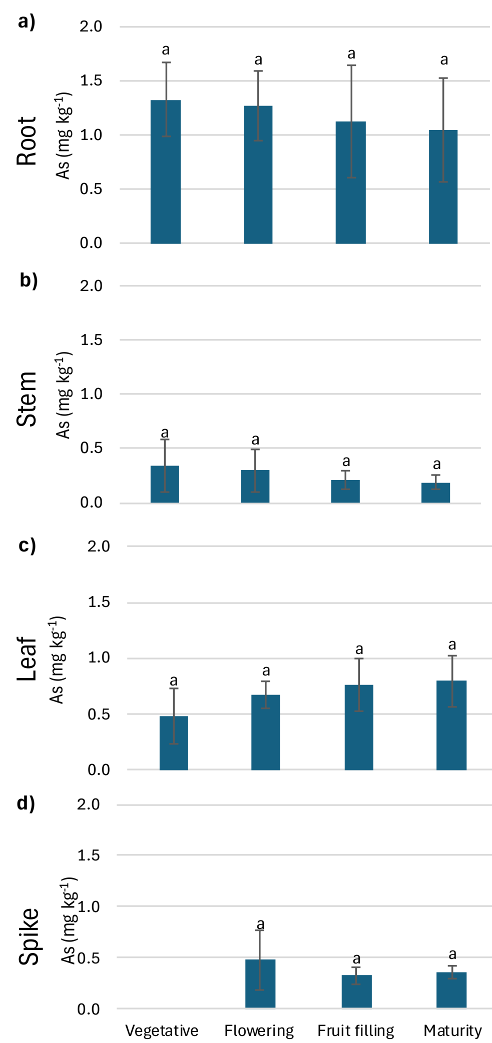

Figure 13. Arsenic concentrations in *S. bicolor* plants throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Arsenic content in plant tissues did not show statistically significant differences among growth stages evaluated. However, higher As levels were recorded in roots during the vegetative and flowering stages (Figure 13a). In the stem, arsenic concentrations decreased over time. While in leaves, a gradual increase was observed toward maturity. Finally, in spikes, As content decreased across stages (Figure 13d).

These may be explained by the ability of plants to limit arsenic translocation through exclusion processes and regulate arsenic uptake and translocation through detoxification mechanisms. The reduction of arsenate (As^{5+}) to arsenite (As^{3+}) allows the latter to be sequestered inside vacuoles through its binding to thiol-containing peptides (Mondal et al., 2022). In parallel, the activation of antioxidant enzymes and the accumulation of osmoprotectants—such as proline and glycine betaine—help the plant to counteract oxidative damage caused by arsenic stress. Together, these protective responses, along with the gradual decrease in arsenic availability in the soil as the crop develops, could explain the relatively steady concentrations of this element found in plant tissues. Wheat (Triticum aestivum) has demonstrated a notable ability to tolerate and accumulate arsenic through a range of physiological and microbiological adaptations.

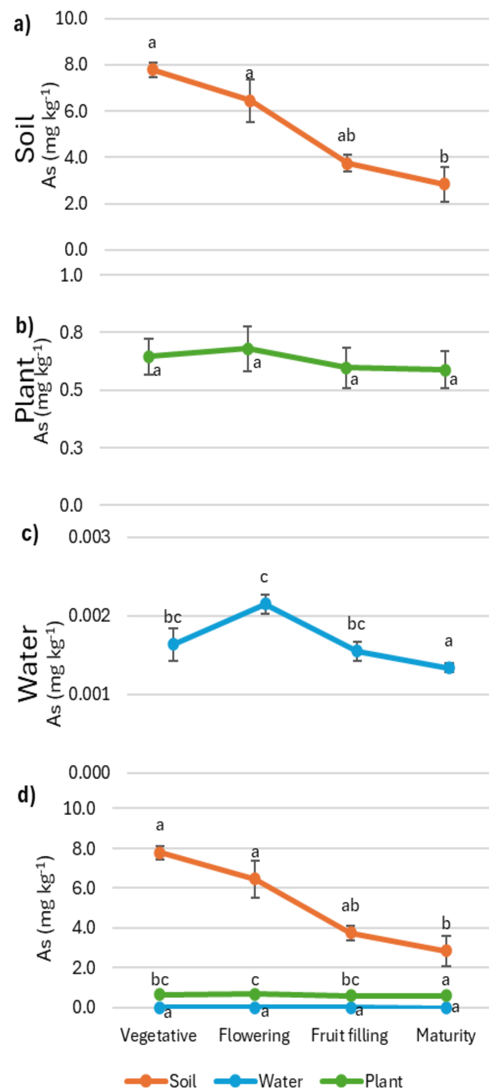

Figure 14. Arsenic concentrations *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Tolerance involves enhanced antioxidant activity and close interaction with metal-tolerant rhizobacteria that promote detoxification and stress resistance (Kwok & Chan, 2022).

In rice systems, the way water is managed has a decisive influence on how arsenic behaves in the soil. Farooq et al. (2019) observed that during periods of flooding caused by rainfall, arsenic dissolution reached about 1,086 μg kg^{-1}, notably higher than the 309 μg kg^{-1} measured under groundwater irrigation. The results show that too much water can push arsenic downward through the soil, increasing its availability to plants.

Pb

Lead (Pb) is a highly toxic heavy metal with no biological function in plants. It causes severe physiological and morphological impairments and poses major health risks when present in agricultural systems. In the Comarca Lagunera region, factors such as mining, metallurgical activities, deep well drilling, and excessive groundwater extraction have exacerbated Pb contamination (Méndez-Gómez et al., 2008; Revelo, et al., 2024; Valenzuela-Noriega, 2021).

Pb accumulation reached its maximum in roots and stems at the vegetative stage and declined progressively as growth advanced (Figures 15a and 15b). Leaf concentrations differed significantly among stages F =7.42, p= 0.002), with the highest values recorded during vegetative growth (Figure 15c). In contrast, Pb content in the spikes remained low and fairly stable throughout the crop cycle (Figure 15d).

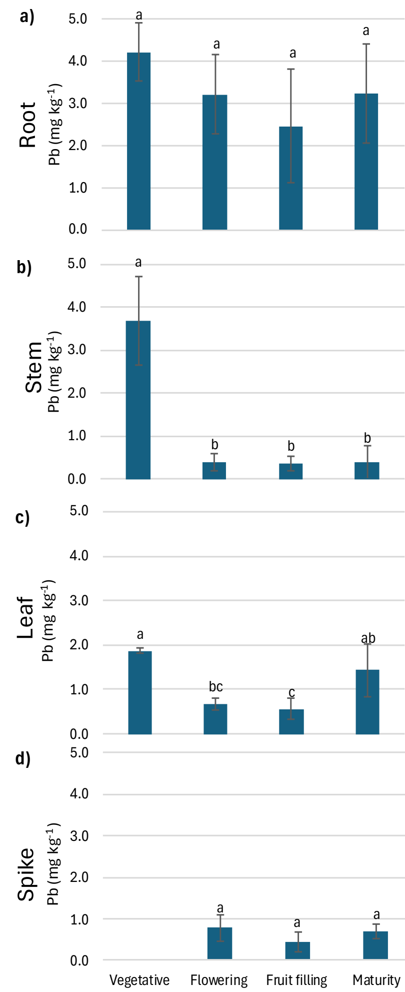

Figure 15. Lead concentrations in *S. bicolor plants* throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, p ≤ 0.05).

The comparison between soil and plant samples revealed a Pb distribution similar to that of arsenic. Lead accumulated mainly in the roots, acting as the first barrier to metal uptake and translocation (Rani et al., 2024). The steady concentrations observed across developmental stages confirm this defensive role. Still, some of the metal can reach the leaves through direct contact with airborne dust, a process that helps explain the higher Pb values found during the initial stages of growth. Pb´s interference with photosynthetic processes, especially inhibition of the Hill reaction and photophosphorylation, has been documented in chloroplasts (Romanowska et al., 2008). Furthermore, Pb can alter mitochondria function by increasing the oxidation rate of key substrates, impacting overall energy metabolism (glycine, succinate, and malate at higher rates than untreated plants (Jagota et al., 2024).

As the crop matures, the leaves hold less Pb, most likely because the plant locks the metal away inside vacuoles, reducing its impact on the cells responsible for photosynthesis (Rani et al., 2024; Romanowska et al., 2008). Roots also play a role by blocking Pb movement toward the shoots (Jagota et al., 2024). Thanks to these strategies, the plant can continue photosynthesizing efficiently even after being exposed to high metal levels.

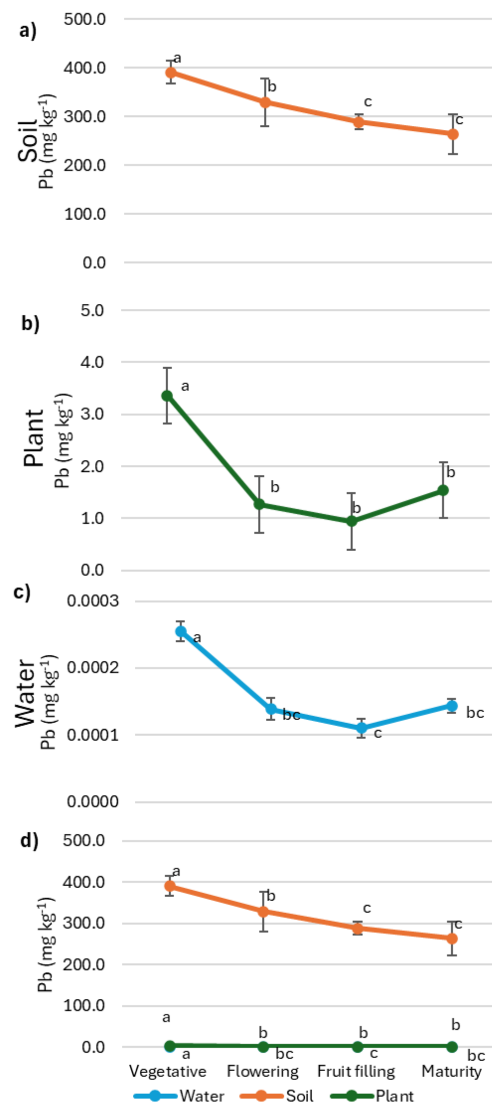

Figure 16. Lead concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent the mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Cd

Cadmium (Cd) occurs naturally in the environment, mainly in rocks, soil, and water, but it is not an element required for plant growth. Once absorbed, it becomes highly toxic, triggering oxidative stress, disturbing water and nutrient uptake, and altering key physiological functions (Wdowiak et al., 2024; Haider et al., 2021).

During the experiment, Cd levels in roots, stems, and leaves increased slightly with time, though no significant variation was detected between growth stages. Notably, the highest Cd concentrations were found in all plant tissues at maturity, with the lowest levels recorded in spikes throughout development (Figures 17a, 17b, 17c, and 17d). In contrast to the other contaminants, Pb and As, the concentration of this element in plant tissues is higher than that found in the soil. Plants

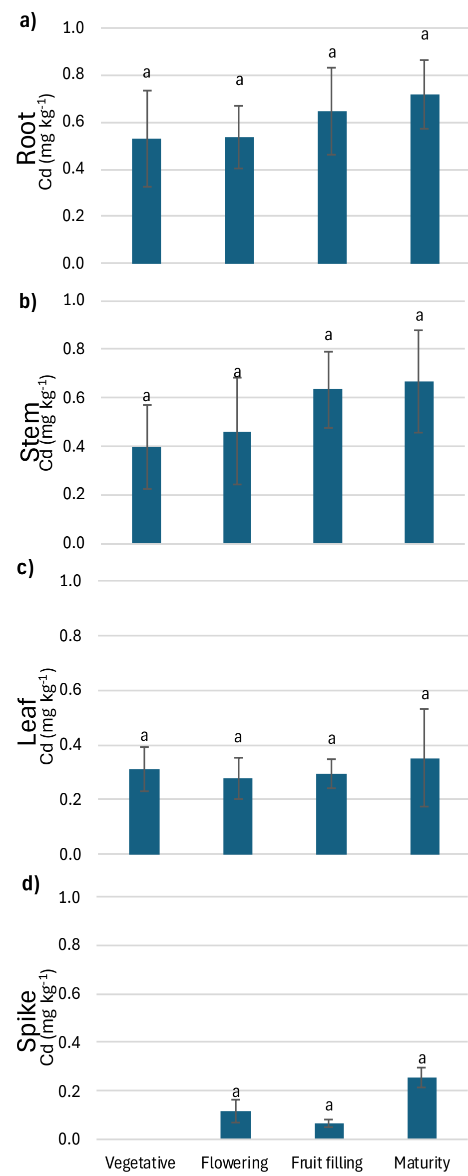

Figure 17. Cadmium concentrations in *S. bicolor* plants throughout the growth cycle. Values represent mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

showed a higher tendency to absorb and accumulate cadmium compared with other analyzed elements. The accumulation pattern appears to result from apoplastic barriers within the roots that restrict Cd movement to aerial organs, promoting its retention in root cell walls (Qi et al., 2020). The elevated Cd values found during maturity, particularly in spikes, imply that translocation to reproductive organs occurs gradually toward the end of the cycle. This may be due to nutrient redistribution during late growth stages (Li et al., 2023). Soil and water analysis indicate that soil is the main source of Cd contamination, and while plants absorb Cd, their internal barriers effectively limit its movement to protect vital tissues, particularly reproductive structures.

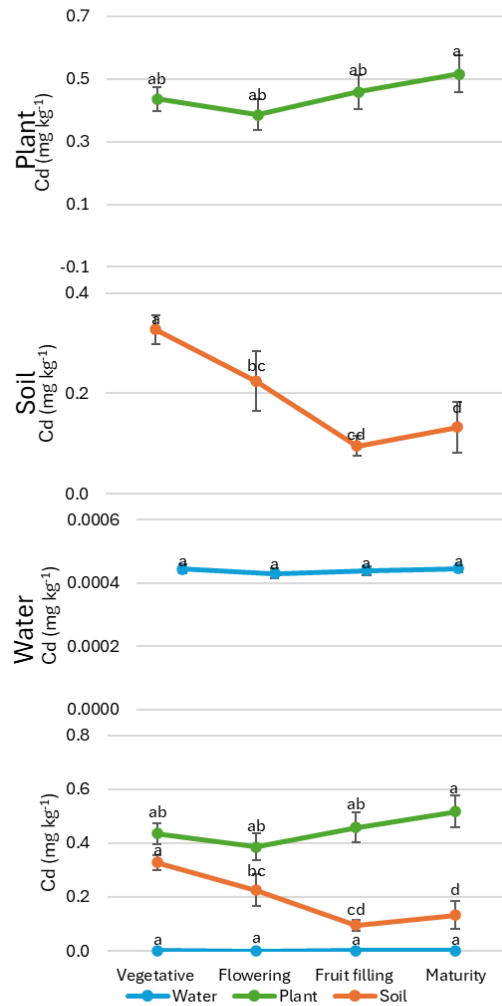

Figure 18. Cadmium concentrations in *S. bicolor* plants, soil and water throughout the growth cycle. Values represent mean ± standard deviation. Common lowercase letters within each row (i.e., across phenological stages) are not significantly different (Tukey HSD, $p \leq 0.05$).

Adherence to permissible metal and essential element levels

The concentrations of metals in the water are within the permissible limits established by NOM-127-SSA1-1994, the EPA, and Directive 98/83/EC. Both essential elements, such as Cu, Zn, Fe, and Mn, and potentially toxic elements like As, Pb, and Cd, remained within safe ranges throughout all crop development stages. Overall, the water met the quality requirements for agricultural use and did not show signs of contamination that could endanger the crops.

In the soil, the concentrations of Fe, Cu, Zn, and Mn were found to be within the ranges established as suitable by NOM-021-SEMARNAT-2000, while Ca and Mg presented high values. As for the soil, the concentration of micronutrients such as Fe, Cu, Zn, and Mn stayed within the suitable range described in NOM-021-SEMARNAT-2000, while Ca and Mg appeared in relatively high amounts. In all crop stages As, Cd, and Pb were within the limits set by NOM-147-SEMARNAT/SSA1-2004. Lead levels were a bit higher than the limits set by the European Directive 86/278/EEC. They could represent a slight environmental risk. However, Pb levels surpassed those permitted by the European Directive 86/278/EEC, suggesting that under European standards, potential risks may exist. In contrast, arsenic and cadmium remain within acceptable limits under both Mexican and European frameworks.

In plant tissues, the concentrations of arsenic As, Pb, and Cd exceeded the maximum permissible levels set by the Codex Alimentarius at all development stages. However, these values remained within the limits established by Directive 2002/32/EC, which applies to animal feed. This suggests a potential health risk if the plants are intended for direct human consumption under Codex standards.

Conclusions

This study examined the biodynamic distribution of heavy metals (Cu, Zn, Ca, Mg, Fe, Mn, As, Pb, Cd) in *Sorghum bicolor*, soil, and irrigation water across different phenological stages. Results showed that Cu and Zn accumulation patterns depend on soil interactions and plant development, with Zn reaching up to 58.3 mg/kg in leaves during grain filling. Ca distribution was limited in spikes due to its xylem-dependent transport, while low Mg availability (<40 mg/kg in soil) restricted uptake. Fe and Mn are predominantly accumulated in roots, supporting their roles in early growth and enzymatic functions.

Toxic elements showed concerning trends: As concentrations in leaves increased to 0.28 mg/kg at maturity, Pb peaked at 0.34 mg/kg during early development, and Cd remained root-bound, with values up to 0.09 mg/kg. Although soil and water met Mexican and international standards (NOM-127-SSA1-1994; EPA; Directive 98/83/EC), Pb in soil exceeded EU thresholds (>70 mg/kg). In plant tissues, As, Pb, and Cd surpassed Codex Alimentarius limits for human consumption, but remained within acceptable levels for animal feed (Directive 2002/32/EC).

This integrative approach highlights the need for tailored soil and water management strategies to mitigate metal toxicity and ensure safe agricultural practices in contaminated environments. Considering that *Sorghum bicolor* is primarily used as livestock forage in the region, the findings of this study raise important concerns regarding food safety. Although animal-derived products were not directly assessed, the detection of toxic metals in plant tissues indicates the potential for these contaminants to be transferred through the food chain into products such as meat or milk. This could pose a risk to public health, particularly in agricultural systems exposed to heavy metal contamination. Therefore, future research should focus on examining this possible transfer to better understand its implications for human health and to develop appropriate mitigation strategies.

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