Bioaccumulation of heavy metals and As in maize (*Zea mays L*) grown close to mine tailings strongly impacts plant development

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Abstract

Potentially toxic metals and metalloids present in mining residues can affect ecosystems, particularly plant growth and development. In this study we evaluated As and heavy metal (Fe, Zn, Cu, Cd, Pb) contents in maize (*Zea mays* L) plants grown in soils collected near (40 m), at intermediate (400 m) and remote (3000 m) distances from mine tailings near Taxco City, Mexico. Soils sampled near and at intermediate sites from the tailings contained high levels of heavy metals which were 3- to 55-fold higher compared to the control samples. The heavy metal and As content in plants reflected the soil contamination being the greatest for most studied elements in root samples followed by stems, leaves, and kernels. Though plants were capable of completing their life cycle and producing the seeds, high bioaccumulation levels had a strong impact on plant development. Abnormalities in the organs like malformations in reproductive structures (tassel and ear), reduction in the phytomer number and the plant height were present. Microscopic studies and morphometric analyses suggest that strongly affected plant growth result from negative and synergistic action of heavy metals and As in soils on cell growth and cell production. This study showed that maize grown near mine tailings accumulates high levels of heavy metals and As which decrease significantly plant yield and could be dangerous if consumed by animals and humans.

Introduction

Through decades, heavy metal and metalloid contamination caused by industrial activities has raised concerns over environmental quality and public health (Wang et al. 2016; Bostick et al. 2018; Prommer et al. 2018; Chen et al. 2019; Alharbi et al. 2020; Hu et al. 2020; Liu et al. 2020a; Wang et al. 2020a, 2020b, 2020c). Heavy metal pollution is a serious environmental problem because metals are non-biodegradable and consequently accumulate in the environment (Ali et al. 2013; Hu and Cheng 2013). Biogeochemical processes can promote the release of potentially toxic elements and their spread through different pathways to the environment. A wide range of environmental media near mining or smelting areas has been contaminated by heavy metals and metalloids, which threat the health of the ecosystem and human beings (Lin et al. 2012; Wang et al. 2017; Liu et al. 2019a, 2019b, 2020b; Yin et al. 2019; Beiyuan et al. 2020; Li et al. 2020; Zhong et al. 2020), especially by the inappropriate disposal of mine tailings, providing negative effects on the exposed organisms (Tovar-Sánchez et al. 2012; Mussali-Galante et al. 2013). Mining has been one of the main economic activities in Mexico since the XVI Century. At present, it provides the raw material for numerous industries. However, exploitation and processing of ore have produced great amounts of residues that constitute a potential source of heavy metals pollution of the environment (Dudka and Adriano 1997; Armienta Hernández et al. 2001; Armienta et al. 2003). Several environmental threats have been produced by ore extraction and processing in the historical mining zone of Taxco, Guerrero state of Mexico (Armienta Hernández et al. 2000, 2001; Armienta et al. 2003; Romero et al. 2007; Ruiz Huerta and Armienta Hernández 2012; Gómez-Bernal et al. 2014; Tovar-Sánchez et al. 2018). Plant cultivation close to these zones represents a public health problem due to the accumulation

of heavy metals in the plant organs and their translocation to humans and animals through food chains. Therefore, these topics become important for public health.

Although physicochemical properties of mining wastes vary according to the ore source and processing characteristics, in general, they contain high concentrations of heavy metals such as Pb, Ni, Cd, Cu, Mn, and Zn together with low concentrations of essential nutrients like P, N, and organic matter (Bradshaw et al. 1978; Alloway 1995; Walder and Chavez 1995; Boulet and Larocque 1998). High levels of heavy metals contamination of soils by mining residues have been reported elsewhere (Álvarez et al. 2003; Clemente et al. 2006; Vázquez et al. 2006; Wei et al. 2020).

Heavy metal pollution is a serious environmental problem. Potentially toxic elements (Cd, Cu, As, or Zn) settled in the soil may be transferred to the liquid phase depending on physicochemical conditions and become available to plants (Norland and Veith 1995; Wong et al. 1998; De Jesús García et al. 2020). Also, mining wastes produce unfavorable health conditions to native vegetation among other factors through substrate acidification (Wong et al. 1998).

Heavy metals may be toxic to wild vegetation even if their concentration does not reach critical levels. Due to the translocation of metals from root to the aerial parts, they may enter the food chains (Mertens et al. 2004; Unterbrunner et al. 2007; Sharma et al. 2018). Many angiosperm plant species possess adaptations allowing survival and reproduction in Zn, Cu, Pb, Cd, Ni, and As highly polluted soils (Dahmani-Muller et al. 2000; Pulford and Watson 2003).

In this study, the impact generated by mine tailings on maize (*Zea mays*) growth was studied. This species was selected as a test plant because it is one of the most common and important cereal crops in Mexico and worldwide. Also, it is considered as a biological, agricultural, cultural and economic patrimony (CONABIO 2006). Maize is an essential component of the Mexican diet and its harvest is an important economic activity for the peasant families. Frequently maize cultivation is located close to mine tailings, but the impact of contaminated soils on plant development is rarely studied even though it represents an important environmental issue with implications on human health along with social consequences (Tovar-Sánchez et al. 2018).

Various experiments have shown heavy metals absorption and accumulation (mainly Zn, Cd, Pb, Hg, and Cu) in maize plants (Wójcik and Tukendorf 1999; Kabata-Pendias 2000; Ali et al. 2002; Wenger et al. 2003; Seregin et al. 2004; Seregin and Kozhevnikova 2005; Souza et al. 2005; Wójcik and Tukiendorf 2005; Pál et al. 2006; Shen et al. 2006; Maksimović et al. 2007, Tovar-Sánchez et al. 2018). Uptake and accumulation of high concentrations of heavy metals by a plant not only produce toxic symptoms and affect the metabolic processes, they also causes physiological, developmental, structural, and ultrastructural changes. High concentrations of heavy metals other than Mn reduce the mesophyll cell size (Zhao et al. 2000), Cd negatively affects chloroplast formation in stem (Barceló et al. 1988), Pb causes a reduction in the number of stomata and trichomes on the abaxial leaf surface caused (Weryszko-Chmielewska and Chwil 2005). Heavy metals induce many other structural and ultrastructural

changes and among others, they exert genotoxic and cytotoxic effects in plant cells, resulting in cellular, physiological, and morphological alterations (Nagajyoti et al. 2010).

Leaf epidermis (Chardonnens et al. 1998), trichomes on the leaf surface (Salt et al. 1995; Choi et al. 2001; Ager et al. 2002) or epidermal glands (Lavid et al. 2001a, 2001b) are able to detoxify more Cd than the mesophyll cells. Leaf rib and petiole might also be involved in the storage of Cd, as found in knotweed stems (Shinmachi et al. 2003). Shi et al. (2016) found that plants simultaneously exposed to Pb and Cd suffer morphological and physiological abnormalities in absorption and transport of essential elements, chlorophyll synthesis, growth, and reproduction.

Therefore, this study aimed to determine the impact of mine tailings on soil contamination by heavy metals and As and subsequently on maize growth and development. To this end, concentrations of heavy metals and As were determined in plants and soils which were at different distances from mine source, and morpho-anatomical characteristics were evaluated in maize plants grown in these soils under greenhouse conditions. This work will provide an effective evaluation of the impacts from mine tailings exposed to agricultural soils and maize. We present the evidence that in a mining zone even at a substantial distance from a mine site, both substrate and plants grown in it contain high levels of heavy metals and As that affect significantly plant growth and yield.

Materials And Methods

Sampling location

Sampling was carried out in the mining zone of Taxco, northern Guerrero State, Mexico. Located between 18° 21' and 18° 41' N and 99° 25' and 99° 47' W. This zone has a tropical sub-humid climate and an average temperature of 28 °C. Historical precipitation record indicates a yearly average of 1000 mm (INEGI, 1999). According to FAO/UNESCO (FAO 1977; INEGI 2005), soils are classified as Haplic Phaeozem (IMMSA 1973; COREMI 2013).

Soil sampling and seed material

Soil samples were collected in Santa Rosa municipality in Guerrero State (Figure 1). Soils were sampled at various distances from the border of mine tailings: agricultural non-polluted soil at about 3000 m from mine tailings that corresponds to Taxco sample1 (STAX1), agricultural soil at about 400 m from mine tailings that belongs to Taxco sample2 (STAX2), and soil at about 40 m from mine tailings that corresponds to Taxco sample3 (STAX3) (Figure 1). Sites STAX1 and STAX2 correspond to agricultural soils used for maize cultivation. At each site, three soil samples of 0-20 cm soil depth were taken to make a compound sample. The seeds of H-515 *Z. mays* variety were obtained from plants grown in the region and provided by dwellers. All samples were transported in a container to the Analytical Chemistry Laboratory at the National Autonomous University of Mexico, Mexico City campus.

Greenhouse experimental design

The seeds were washed with water and surface-sterilized by dipping in 2.5% NaOCl for 5 min, and washed 3 times with double-distilled water to remove any traces of chemicals that could interfere with seed germination. The pot experiment was set up in a greenhouse. The pots were 28.3 cm high with the top diameter of 31 cm and a bottom diameter of 22 cm (15 L). Each pot was equipped with an outlet to collect leachates. The pots were arranged in three quintuplet rows in a randomized block design in three tables. Altogether, fifteen pots were filled with soil from each site (STAX1, STAX2, and STAX3), a total of 45 pots, and 5 seeds per pot were sown. The pots were planted/set in January 2009. Maize was grown during 22 weeks in a greenhouse under semi-controlled conditions, where temperature and relative humidity were monitored. Plants grew under natural light and day-night photoperiod and were watered every three days with deionized water avoiding leaching out of the pots. Deionized water was used to avoid possible variability in mineral content of sink water and avoid analytical errors.

Growth conditions in a greenhouse

The plant height was measured in maize plants weekly for 22 weeks, starting from the second week after the sowing. The maximum temperatures during day hours ranged from 25.6 to 41.5 °C, and the minimum temperature during the night hours ranged from 12.9 to 15.6 °C. The average relative humidity was 63.37 % (34.25 % to 84.75 %), and the average of solar radiation was 28078 W/m² (0.6 W/m² to 273.1 W/m²) (Appendix A of Supplementary data). Harvest time was determined based on the end of the life cycle of maize plants when plants stopped their growth and started to dry.

Morphological characteristics

Corresponding morphological characteristics were measured in 180 fresh samples (60 per replicate), taken out from plants grown in 45 pots. To obtain the dry weight of each sampled organ, they were dried at 75 °C until constant weight. The following parameters were collected: fresh and dry weight of aerial parts and the roots, biomass (%), the longest leaf length (cm), number of leafs. For female reproductive structures, length and dry weight of ears, and dry weight of kernels were recorded as well as percentage of their normal or abnormal appearance. For male reproductive structures, tassel length and number of tassel branches were recorded as well as percentage of their normal or abnormal appearance.

Physicochemical soil analysis

The soils samples were collected for analysis after the end of the greenhouse experiment. The roots were carefully extracted from each soil sample (STAX1, STAX2, and STAX3). The soil samples were air dried at room temperature and crushed over plastic trays and were sieved through a sieve of 2 mm mesh size and stored separately for further chemical analyses. Physicochemical parameters: pH, total carbon (%C), organic matter (OM), total nitrogen (N), available phosphorus (P), electrical conductivity (EC), and cationic exchange capacity (CEC) of soil samples were measured in triplicate.

Carbon and nitrogen analyses were performed with an CHNS Perkin Elmer 2400- II element analyzer (Perkin Elmer, Waltham, MA, USA). Measurement of pH was performed with a potentiometer 3301 set

WTW in a 1:2.5 soil: liquid ratio (water and KCl solution/concentration) after 18 h of agitation. Available P was determined with a spectrophotometer Genesys 20. Exchangeable bases were analyzed with an AA Perkin-Elmer 3110 after extraction with ammonium acetate at pH =7.0.

Heavy metal and As analysis of soil and plant samples

For the analysis of total heavy metals and As, soil samples were air dried, homogenized, and sieved through 0.63 mm mesh size and ground. 1 g of finely ground soil sample was digested slowly with 20 mL of aqua regia (1:3, v/v: conc. HNO₃ and conc. HCl) and digested for 30 min at 170 °C using a Microwave Accelerated Reaction System (CEM® MARSXpress). The samples were solubilized and dissolved in deionized water and filtered through Whatman 40; this solution was diluted to a final volume of 100 mL until analysis. Analysis of Fe, Zn, Cd, Cu, and Pb was performed by flame atomic absorption spectrometry, and As by hydride generation with a Perkin-Elmer AAnalyst 100. The instruments were calibrated with standard solutions containing known concentrations of each element. High purity Standard solutions were used for calibration curves. Six replicates were used of each soil sample, accuracy was evaluated with the reference soil NIST Montana soil 2711, obtaining from 83 to 100% recovery.

Plants were harvested 22 weeks after germination. In total, nine samples (three plants per pot) were analyzed for metal bioaccumulation. All samples were divided into roots, stems, leaves, and ears (cobs) and vigorously washed with deionized water and soaked in ultrasonic bath machine for 20 minutes. Next, plants were dried at 60 C° for 4 days until constant weight; each organ sample was cut into small pieces and 0.5 g were weighed for the extraction of heavy metals and As. These samples were subjected to open acid digestion with 10 ml of acid concentrated mixture (HNO₃ and HClO₄, 4:1) on a hot plate in a fume hood until appearance of the white fumes indicating complete digestion of plant samples. Then they were cooled, properly filtered, and diluted to a final volume of 50 ml using deionized water and stored in a glass container for further analysis. Thereafter, heavy metal concentrations were measured with an atomic absorption spectrophotometer (Model Perkin-Elmer AAnalyst 100, USA), with flame (Fe, Zn, Cd, and Cu), graphite furnace (Pb), and FIAS-HG (As) mode. The detection limits for all the elements are shown in Table 2. For each measurement, the average values of the five replicates were recorded. All metal concentration values are reported as mg/kg of dry weight.

Scanning electron microscopy (SEM) analysis

From each substrate, ten plants were chosen for SEM analyses. Samples from shoot-borne brace roots, stem, and leaves were taken at the same position within each plant. One-centimeter width stem samples were taken from an internode located between phytomers seven and eight. Adaxial and abaxial leaf samples of 1 cm² were collected from the distal half of leaf seven. For root samples, 1.5-2 cm long portions of adventitious live and turgescient roots near their base were used. Samples were processed using the conventional SEM protocols (Bozzola and Russell 1999) drying at the critical point with a Samdri 780B dryer. Dry samples were attached to aluminum supports with carbon tape (NEM TAPE

8mm) and covered with charcoal and gold in a Bal-Tec SCD 050 sputter coater. Covered samples were observed with a Zeiss JEOL JSM-35CF microscope (applying 15 to 18 kV) at the Chemistry Faculty, UNAM. Measurements were performed from SEM microphotographs. The computer software package Image Tool V.3 was used for image analysis. On transverse sections of roots and shoots, the diameters of roots and vascular bundles were measured in two directions: the longest (major) diameter and the shortest (minor) diameter.

Statistical analysis

Statistical analysis was performed on data using SPSS Version 18.0 (SPSS Inc., Chicago, USA). Descriptive analysis was performed for physicochemical parameters and heavy metal and As contents in soil and plant samples studied (in triplicates). For analysis of the morphological characteristics and morpho-structural data, basic statistics, Pearson's correlations and main components were applied.

Results And Discussion

In this study we analyzed the impact of mine tailings on substrate contamination and plant growth in a range of distances from the mine activity sites. We first analyzed soil samples obtained from about 3000 m from mine tailings (STAX1), agricultural soil at about 400 m from mine tailings (STAX2), and soil at about 40 m from mine tailings (STAX3); the locations are illustrated in Figure 1. After that we addressed accumulation of heavy metals and As in *Z. mays* plants, and finally evaluated impact of contaminated soils on different aspects of plant growth and development.

Soil physicochemical analyses

Near neutral pH values (6.9 to 7.5) were found in soils STAX1 and STAX2 while the STAX3 sample was acid (pH=4.2). pH ranges in soil were extremely high and exceeded the soil background according to the criteria proposed by Castellanos et al. (2000) and Mexican accepted regulations, NOM-021-SEMARNAT-2000 (SEMARNAT 2000). The soils STAX1 followed by STAX2 were the best suited for plant development; as expected, soil samples STAX3 which were closest to tailings, were least appropriate for plant development. Electric conductivity at STAX1 and 2 showed negligible salinity, whereas STAX3 was moderately saline according to the criteria proposed by NOM-021-SEMARNAT-2000 (SEMARNAT 2000). Organic matter was very high at STAX1, high at STAX2, and medium at STAX3 according to the criteria proposed by NOM-021-SEMARNAT-2000 (SEMARNAT 2000). The level of P was very high at STAX1, low at STAX2 and very low at STAX3, according to the criteria proposed by NOM-021-SEMARNAT-2000 (SEMARNAT 2000); Ca was high at the three sample sites; Mg was medium at STAX1 and STAX2 and very low at STAX3; K was very high at STAX1 and medium at STAX2 and 3 according to the criteria proposed by NOM-021-SEMARNAT-2000 (SEMARNAT 2000). Particularly, the lowest levels of total C and N and available P were found in STAX3 soils (see Appendix A of Supplementary data). The most limiting element at this location was P; its level was 62-fold lower compared to STAX1, whereas total C and N levels were lower only 6-and 3-fold, respectively (See Appendix A of Supplementary data). By applying the

correlation matrix analysis, significantly negative correlations in physicochemical parameters in soils were found among the used variables (Appendix B of Supplementary data).

Heavy metal and As content in substrates at different distances from mine tailings

It is important to point out that soils were heavily contaminated by the mining activities and the heavy metal and As contamination showed a decreasing trend with increased distance from mine tailings. As shown in Table 1, the average heavy metal and As contents in soil were distributed as follows Fe> Zn> Pb> Cu> As> Cd. All the studied metals increased in the following way STAX3>STAX1>STAX2 as shown in Table 1. The highest concentrations of contaminant elements were detected in the closest soil to mine tailings (STAX3) and were 3- (Fe), 24- (Zn), 6- (Cu), 8- (Cd), 55- (Pb), and 4 (As) and 9-fold higher at STAX3 compared to STAX2 (Table 1). Concentrations of all the studied elements at STAX3 were above the limits established by US EPA (1992), IHOBE, (2003), and Mexican NOM-147-SEMARNAT/SSA1-2004, and at STAX1 were higher for Cu. Alloway (1995) proposed a limit of 60-125 mg/kg of Cu for soils which was exceeded at STAX1 and STAX3 only. European Union's regulatory commission (EU) (2006) sets a limit of 100 mg/kg of Cu in soils which was exceeded at STAX1 and STAX3 only. Despite its proximity to mine tailings, metal concentrations were lower in arable land (STAX2) compared to the control site (STAX1), except for Fe and As ($P>0.05$, Table 1). Various authors found heavy metal concentrations in soils and maize plants were lower at other sites than those measured in this study in the following way: STAX3>STAX1>STAX2 as shown in Table 2 (Oladipo et al. 2016; Bai et al. 2016).

Concentrations of potentially toxic elements at 40 m from mine tailings (STAX3) detected in our study were within the range of the contents of the same elements reported earlier for Taxco mine tailings, which were for As = 190 - 800 mg/kg, Pb = 378 - 4932 mg/kg, Zn = 58 - 2949 mg/kg and Cu = 25 - 122 mg/kg (Romero et al. 2008) (compare with data shown in Table 1), suggesting the fast rate of migration of these elements in the soils.

The significantly positive correlation in soil heavy metal contents was found as follows Fe-S with Cd-Ps, Zn-Pr, Zn-Pl, Cd-Pr, Zn-Ps, and Cd-Pl (.999, $P\leq 0.05$); Zn-S with Cd-S, As-S, and Cu-Ps (1, $P\leq 0.01$); Cd-S with As-S (1, $P\leq 0.01$), and Cu-Ps (.999, $P\leq 0.05$); Pb-S with Cd-Ps, Zn-Pr, Zn-Pl, Cd-Pr, Zn-Ps, and Cd-Pl (.999, $P\leq 0.05$) and As-S with Cu-Ps (.999, $P\leq 0.05$) (Appendix B of Supplementary data). The evidently positive correlations between heavy metals and As in the soils indicated that these elements were probably originated from the same sources and shared the same pathways (Zhang et al. 2018b; Liu et al. 2019d; Zeng et al. 2020).

Heavy Metal and As accumulation in *Z. mays*

Application of Cd and As alone or in combination substantially reduces the plant growth (plant height, number of leaves per plant, leaf area, stem diameter, and shoot fresh and dry weight) (Anjum et al. 2016). Du et al. (2017) and Liu et al. (2009) found that maize plant can be more tolerant under As stress opposite to our study, which shows that maize is not enough tolerant under As stress. Content of As and analyzed metals was highest in roots compared to the aerial parts (stem, leaves, and kernels) (Table 2).

Higher heavy metals and As contents were found in plants grown in STAX3 substrate compared to more distant sites ($P < 0.05$), while plants grown in STAX1 and STAX2 had values of a similar range ($P > 0.05$)

Another research found that maize grown in a greenhouse under semi-controlled conditions showed greater accumulation of Cd and Pb in the roots than in the shoots (Figlioli et al. 2019). Besides, Lv et al. (2019), found that maize under moderate toxic metal stress of Pb (29.7 mg/kg in soil) and Cd (0.02 mg/kg in soil) showed the following accumulation trend of roots > stems and leaves > grains.

Principal component analysis (PCA) in the organs of the maize (Figure 2) showed that Cu-R (R, in roots), Pb-S (S, in stem), Cu-L (L, in leaves) Fe-L, and As-R did not have significant relation with the other parameters and Zn-R with Cd-R, Cd-L, Zn-S, Cd-S, and Zn-L; Zn-S with Zn-L, Cd-S, and Cd-L; As-S with Pb-K (K, in kernels); Fe-K with Zn-K, As-K, and Cu-K, hence these had significant relation.

Heavy metal and As bioaccumulation in different plant organs

Roots. Metal and As content was determined only in the adventitious shoot-borne roots developed from the basal stem node; these roots are considered brace roots (Hochholdinger et al. 2004). The highest heavy metal concentrations in roots were found in plants grown in STAX3 soils (Table 2). Interestingly that Zn and Pb concentrations in roots were higher in plants grown in STAX1 than in STAX2 while the metal content in the soil was greater in STAX2 (Table 1). This suggests that the lowest concentration of Zn and Pb in substrate is less toxic and facilitates the uptake of these elements compared to substrates with the higher metal content. However, Fe and As concentrations were higher in roots of STAX2 than STAX1. The observed trend reflects a relationship between the substrate and the amount of metal absorbed by the plants causing inhibition of root growth by heavy metal. These data are in good agreement with barrier function of plant roots (De-Jesús-García et al. 2020). The significant positive correlation of heavy metal content in roots was found as follows Fe-R with Fe-S (.998, $P \leq 0.05$), and As-L (.997, $P \leq 0.05$); Zn-R with Cd-R, Cd-L, Zn-S, Cd-S, and Zn-L (1, $P \leq 0.01$); Cu-R with As-S and Pb-K (-.998, $P \leq 0.01$) (Appendix B of Supplementary data).

Stem. Similarly, to roots, the highest metal content was found in stems of plants grown in STAX3, except for Pb and As (Table 2) The As concentration was the same in plants grown in STAX1 and STAX3 but greater in STAX2. Interestingly, the level of Zn, Cu, and Cd, in stems was found to be the same in plants grown in STAX1 and STAX2, suggesting low root to shoot translocation of these elements. However, in STAX3, the content of Zn in stems increased 28-fold for Zn and only 2- and 7-fold for Cu and Cd, respectively, compared to plants grown in STAX1 (Table 2) The only element whose content was progressively increased with a decreased distance to mine tailings was Fe (Table 2). The Pb content in stem samples was surprisingly similar in plants grown in STAX1 and STAX3. stems. Output of the correlation analysis showed significantly higher positive correlation in content of Fe-S with As-L (1, $P \leq 0.01$); Zn-S with Zn-L, Cd-S, and Cd-L (1, $P \leq 0.01$); Cd-S with Zn-L and Cd-L (1, $P \leq 0.01$); As-S with Pb-K (1, $P \leq 0.01$); Zn-L with Cd-L (1, $P \leq 0.01$) (Appendix B of Supplementary data). This analysis showed that barrier function of plant roots (De-Jesús-García et al. 2020) is efficient to a different degree for different elements and at different soil contamination levels. Close to mine tailings, the plants can contain rather

high levels of Fe and Zn and moderate levels of Cu and Cd, therefore a plant forage could be harmful for animal consumption.

Leaves. Results showed that Fe, Zn, Cd, and As concentrations in leaves were higher in STAX3 samples than STAX1 and STAX2, except for Cu and Pb presenting a higher or the same content in STAX1, respectively (Table 2). Interestingly, for Zn and Cd the content in leaves was the same in the samples of STAX1 and STAX2. However, overall analysis showed that accumulation was at maximum for Zn, and Cd in STAX3 their content was 6- and 2-fold greater than in STAX1 samples, respectively (Table 2). Some authors reported that heavy metal concentrations affect the structure of the leaves in different ways. Navarro-Pedreño et al. (2003) found that heavy metal content affects preferentially younger leaves than mature leaves. Also, Sharma et al. 2003 found that toxic elements in *Z. mays* causes visible lesions such as chlorosis, vein clearing in young leaves, curling of leaf margin, and yellowing.

Kernels. The content of heavy metals and As in maize kernels ranged from 26.2 to 30.5 mg/kg for Fe, from 29.0 to 41.9 mg/kg for Zn, from 2.8 to 5.2 mg/kg for Cu, from 0.5 to 1.4 mg/kg for Cd, from 0.5 to 0.6 mg/kg for Pb, and 0.0 to 0.2 mg/kg for As and no higher level of either metals or As in STAX3 samples compared to STAX 1 was found (Table 2). Unexpectedly, the highest metal content was detected in kernels of plants grown in soils from STAX1 and it was 18, 30, and 56% higher for Zn, Cu, and Cd compared to STAX3 samples (Table 2). These results further confirmed that maize grains at sampling sites were heavily contaminated and these contents may cause health risks to consumers. Barać et al. (2016) found that maize kernel heavy metal concentrations vary depending on growth location (Nigeria, USA, Sweden, and Kosovo). However, the reported metal contents are lower than in this study.

Importantly, As and some metal contents detected at different sites in our study were within the range detected in sweet maize kernels produced by plants grown in natural non-contaminated soils (Kabata-Pendias 2000), whose concentration ranges for Cu were from 1.4 to 2.1 mg/kg, Zn from 25 to 36 mg/kg, As from 0.03 to 0.4 mg/kg, and Pb from 0.3 to 3.0 mg/kg (Table 3). Our study thus demonstrates that despite seriously contaminated soils and a high level of accumulation of heavy metals and As in vegetative organs, kernels accumulated the contaminants to a very limited extent even in the most contaminated soil samples of STAX3.

Alamgir et al. (2016) found that in the aerial part of maize plant the maximum content of Cu was 0.29 mg/kg of dry weight, and of Pb was 5.8 mg/kg. These values were similar to those obtained in our study; for Cu; it was on average 0.21 mg/kg, and for Pb 5.34 mg/kg. In this study, highly significant and positive relationships were observed between Fe-K with Zn-K, As-K, and Cu-K (1, $P \leq 0.01$), and Cd-K (.999 $P \leq 0.01$); Zn-K with Cu-K, As-K (1, $P \leq 0.01$) and Cd-K (.998, $P \leq 0.05$); Cu-K with As-K (1, $P \leq 0.01$), and Cd-K (.998, $P \leq 0.01$); Cd-K with As-K (.998, $P \leq 0.01$) (Appendix B of Supplementary data). Overall, analysis of heavy metals and As contents in plants grown in contaminated soils showed the highest level of elements in roots is followed, for the majority of elements, by those in leaves, stems and kernels. These results clearly indicate that consumption of maize plants as forage by animals even at sites as far as 3 km from a

mining site can represent a danger and the elements consumed could be transferred to the food chain to humans.

Plant Growth and Development

To better understand plant growth and developmental abnormalities, some growth parameters and developmental aspects of plants grown in the three soil samples were analyzed. The timing of seed germination in soils was recorded. The seed germination started at 3 or 4 days after the sowing and was completed by 15 days. The germination time differences could be related to slight differences in soil depth where the seeds were imbibed. For a total of 225 maize seeds (5 seeds per pot) planted, a 95% germination was observed irrespectively of soil types. After germination of most plants and seedlings emerged, 30 plants were extracted, 10 from each substrate, to determine wet and dry weights and size.

In plants of 20 days of growth no differences were found among plants grown in different substrates. The average length of the aerial part ranged from 8 to 12 cm, fresh weight was 1 g for seedlings grown in all substrates, and dry weight was from 0.28 to 0.32 g. All plants (from STAX1, STAX2, and STAX3) by this time developed 3 leaves. The 20-day seedlings did not show any morphological signs of abnormal development. However, after 20 days of growth, maize plantlets were more vigorous when grown in the STAX1 substrates, the most remote of the tailings, followed by STAX2 and STAX3. Plants grown in STAX3 soil showed visual symptoms of toxicity such as retarded growth, thin leaves with necrosis, chlorosis and brown hues. These effects were among other factors due to the high concentrations of heavy metals (Table 1) causing phytotoxic effects and were similar to the reported cases (Lagriffoul et al. 1998; Souza and Rauser 2003; Souza et al. 2005).

Maize plants grown at STAX1 soils at about 22 weeks reached a height of an average of 132 cm and were healthy, while those grown in substrate STAX2 were 86 cm and in STAX3 64 cm of height (Figure 3). Also, a certain degree of plant stress was observed as plant leaves were yellowish and reddish. The observed plant height trend was STAX1>STAX2>STAX3. This corresponded well with decreased distance-dependent negative impact of contamination from mine tailing soils. As by 20 days there were no differences in the shoot length among plants grown in different substrates, these differences at the end of experiment also reflected changes in relative growth rate under these conditions.

Metal accumulation varies depending on the plant developmental stage as shown for 30 and 70 day maize plants (Ruiz and Armienta 2012). It is known that heavy metals affect significantly plant growth in maize, including roots, leaves, cobs, and stems (Wei et al. 2020; Anjhum et al. 2016; Tovar et al. 2018). Additionally, Ruiz and Armienta, (2012) and Drlickova et al. (2013) found that As is also harmful for maize growth. This indicates that the effects on the maize plant are manifested at an early stage, however, it is important to highlight the ability of these species to withstand high concentrations of potentially toxic metals, complete their physiological cycle and reach the reproductive developmental stage and form ears and tassels.

Morphological and physiological characteristics of *Z. mays* grown in contaminated soils

After 22 days of growth, plants grown in STAX2 and STAX3 soils showed the greatest structural and morphological visible changes. As shown in Figure 4, differences in leaf maximum length were found in plants grown in the studied soil substrates (73 cm, 42 cm, and 25 cm, for STAX1, STAX2, and STAX3, respectively, Table 3).

Plant growth was significantly affected in plants grown in STAX2 and STAX3 substrates compared to those in STAX1. The fresh weight of aerial part of plants grown in STAX2 and STAX3 was 5- and 12-fold reduced compared to that in STAX1 ($P < 0.05$, Table 3). Plants grown in STAX3 soils presented the greatest developmental problems, while plants grown in STAX1 soil, far from the mining wastes, were healthy and had developed on average 13 phytomers (Table 3). Average number of phytomers developed by the end of experiment was also progressively diminished (10 at STAX2, and 8 at STAX3, $P < 0.05$) (Table 3), suggesting that the activity of shoot apical meristem was severely compromised when plants grew in presence of heavy metal and As contaminated soils. Similarly, a progressive decrease in root fresh weight was found in increasingly contaminated soils (Table 3).

Despite severe growth abnormalities, maize plants grown in contaminated substrates reached the reproductive stage and formed male (spikes) and female (ears) inflorescences, however, clear differences in their development and size were observed (Figure 5, Table 4). Ear weight was following the trend STAX1>STAX2>STAX3. Abnormalities in the reproductive organ development in plants grown in soils STAX2 and STAX3 were detected in 54 and 72 % (ear), and 48 and 92% (tassel) of plants, respectively. Despite this abnormal development, in plants grown in the soil closest to mine tailings (STAX3), ears were only 20% shorter than in plants grown in STAX1 and STAX2 soils ($P < 0.05$, Table 3). The ear dry weight was progressively diminished in plants grown in STAX2 and STAX3 compared to those grown in STAX1 (Table 3). Differences in the general plant and ear appearance, including stem pigmentation, in 22-week plants grown in the soils tested are shown in Figure 4.

Kernel formation was also affected in maize plants grown in contaminated soils. Kernels harvested from STAX1 plants weighed 0.18 g, from STAX2, 0.11 g, and STAX3, 0.06 g and no differences were found among STAX2 and STAX3 samples (Table 3). This suggests that even intermediate level of contamination has a strong effect on ear and kernel development.

Tassel development, particularly their growth in length, was progressively and significantly diminished with the following trend STAX1>STAX2>STAX3 (Table 3). Similarly, their branching was significantly affected: in STAX3 plants no branching was present while in plants grown in STAX1 soils 5 branches on average were formed (Table 3). Furthermore, a fusion of the reproductive structures (tassel joint with ear) in STAX3 were also found and reflected developmental alterations in maize plants grown in this substrate (Figure 4). These results clearly indicate that heavy metal content influenced the morphogenesis and development of reproductive organs. The detected abnormalities can be used as easily detectable symptoms for the assessment of the negative impact of mining wastes in maize plants grown nearby and are related to a high level of heavy metals and As in mining-impacted soils.

In this study, it was evidenced that maize plants are capable to complete all development stages, including generative development and seed formation. Our results are also consistent with other studies of maize influenced by heavy metals and As where the authors refer to developmental changes in maize plant. For example, Tovar-Sánchez et al. (2018) found altered micromorphology in younger leaves of *Z. mays* samples from polluted sites; Anjum et al. (2017) found that application of Cd and As was detrimental for morpho-physiological features and yield performance of maize cultivars; Ruiz-Huerta et al. (2017) reported that the bioaccumulation of As in maize is determined by the ratio of As in roots over the total As in soil. Therefore, our results show that *Z. mays* is a species with a great capacity and resistance to grow in hostile environments, particularly in substrates that contain high heavy metals and As contents.

Application of heavy metals and As in combination substantially reduced maize growth (plant height, number of leaves per plant, leaf area, stem diameter, and shoot fresh and dry weight). These results are consistent with those reported earlier (Nagajyoti et al. 2010; Xu and Shi 2000). Significant morphological and physiological differences were observed among plants grown in soils with different As and heavy metal contents. Although studies have been developed on metal absorption by maize, few ones have been performed with adult plants (Shi et al. 2016). Results showed high contaminants absorption and a significant decrease in physiologic and metabolic rate, decreasing the biomass and producing abnormalities in the fertilization or reproduction stage. Reported morphological changes in maize plants include chlorosis, necrosis in young leaves, decrease in the number of leaves, and roots and brown appearance in roots (Kabata-Pendias 2000). Plant height and leaf area are reduced when plants are exposed to Pb and Cd in many plant species (Gopal and Rizvi 2008).

Significant morphological and physiological differences were observed among plants grown in soils with different As and heavy metal contents. Wei et al. (2020) found that most of the metals, such as Pb, Zn, Cr, and Mn, were mainly accumulated in the roots. Results show that in general, individuals exposed to multimetal contaminated soils were smaller, had altered leaf shape structures, and altered micromorphological characters (Ruiz-Huerta et al. 2017; Tovar-Sánchez et al. 2018; Kabata-Pendias 2000). All these data are consistent with the results obtained in this study. In addition, our study suggests that the combined effect of simultaneous exposure to different heavy metals and As has a strong synergic effect, as the developmental and morphological alteration in plant development seem to be more severe than those reported in the literature when treated with individual contaminated agents.

Contaminated soils impact in *Z. mays* development at the structural level

Morphometric and qualitative changes in roots

Adventitious roots were analyzed near their connection with the stem. At this level, roots had epidermal layer followed by 5-7 cortical cell layers and by endodermis. Inside to the endodermis, a central (vascular) cylinder is recognized as it is shown in figure 5 (1a-1c). Plants grown in STAX3 had the thinnest roots and the root cells were of the smallest diameter compared to those in plants grown in STAX1 and STAX2. Besides, STAX3 roots showed other alterations, a decrease in the central cylinder diameter and the

thickness of cortex (Figure 5 and Table 4). All the measured root parameters: root diameter, central cylinder diameter and the number of metaxylem sectors followed the same pattern and the values were significantly and progressively diminished from STAX1 to STAX2 to STAX3 samples (Table 4). It is known that in field-grown maize, lateral root diameter at the base positively correlates with total root length (Wu et al. 2016). It seems that the same may hold for the studied here adventitious roots. In agreement with this a clear correlation is found between a significantly diminished root biomass (Table 3) and a decreased root diameter and the number of metaxylem sectors (Table 4) in plants grown in STAX2 and STAX3 soils compared to STAX1.

Morphometric and qualitative changes in stems

Similarly to roots, stem diameter was progressively diminished from STAX to STAX3 samples. Deformation of the stem due to the pressure of developing wrapping leaves was also observed (Figure 5). In plant grown in STAX2 and STAX3 soils, the number of vascular bundles and average parenchyma stem cell diameter was significantly diminished and was 63 and 45% of STAX1 (for bundles) and 73 and 33% of STAX1 (for cell diameter). Despite that cell diameter in STAX3 stems decreased 3-fold compared to STAX1, cell density (number of cells per stem area) increased in STAX3 only 2.1-fold (Table 4), suggesting that cell production by the shoot apical meristem was significantly affected in the plants grown in contaminated soils.

Morphometric and qualitative changes in leaves

Analyzing abaxial and adaxial leaf surface in plants grown in STAX2 and STAX3 soils, no clear differences were found compared to STAX1 samples (Figure 5). Maize leaves in plants grown in STAX1 and STAX2 soils, had normal appearance of macro-trichomes, micro-trichomes, glandular trichomes, stomata on their epidermis. In samples growing in STAX3 soils cutin plugs (CP) were present. This corresponds to the decreased leaf length in STAX3 samples compared to the other samples (Table 4).

Analysis of leaf surface showed no decrease in the number of stomata in plants grown in contaminated soils and only epidermis pavement cell length was affected in STAX3 samples which were 77% of that in STAX1 (Table 4). Considering that leaf length in STAX3 decreased 2.9-fold in STAX3 compared to STAX 1 (Table 3) but cell length was diminished less than 2-fold, the data suggest that the number of cells and thus cell proliferation during leaf development was strongly affected in plants grown in contaminated soil. Overall this analysis shows that both vegetative and generative maize organs are negatively affected by mining impacted soils through a number of developmental abnormalities and negative effects on cell production and cell growth.

Conclusions

In this study we showed that the mining activity strongly affects the level of contamination of nearby soils and the highest contamination levels were found at STAX3 site, except for As which was the greatest in STAX2 soil. This contamination affected strongly both vegetative and generative organ

development which was a consequence of bioaccumulation of metals and As in plant organs, which were in most cases the greatest in plants grown at the closes to mine tailings. Plants grown in STAX2 and STAX3 were negatively affected compared to those in STAX3 in a number of developmental aspects, most significantly their height, phytomer number, reproductive structures (tassel and ear), leaf and root growth, stem diamantes and biomass.

Maize may tolerate and grow in soils with high-heavy metals concentrations without interrupting its biological life cycle and reach the reproductive stage. This is consistent with the fact that in the maize genome a number of metal-detoxifying genes have been identified (Vielle-Calzada et al., 2009). However, development of various organs in plants grown close to mining-site-impacted soils was affected, as data suggest, mainly through inhibition of cell production and cell growth resulted in developmental and growth abnormalities. Morphological characteristics of reproductive structures (tassel, ear, and kernel) may be important factors for visual identification of damage from mining influence. Morphometric analysis showed the soil contamination affects plant development depending on their closeness to the mining residues. These effects are reflected in the size and density of some structures in tissues, as well as cell morphology such as the number of metaxylem sectors by area, the abundance of the vascular bundle and others. Morphological alterations are clear to the naked eye and they may be used as a marker of plant damage from the stress caused by mining-impacted soils.

Declarations

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Tables

Table 1. Heavy metals and As average concentrations in soils (mg/kg dry weight).

Site	Fe %	Zn	Cu	Cd	Pb	As
STAX1	2.9 (0.5)a	687.4 (198.2)b	447.1 (107.4)b	6.9 (2.4)b	229.6 (50.6)b	46.8 (9.5)a
STAX2	3.2 (0.1)a	147.2 (17.3) a	62.4 (3.5)a	3.5 (0.6)a	59.9 (0.1)a	22.3 (0.9)a
STAX3	9.6 (0.8)b	3494.0 (352.4)c	388.1 (32.4)b	26.7 (1.9)c	3269.7 (53.7)c	192.7 (44.8)b
Limit value of NOM-147-SEMARNAT-2004				37	400	22
Limit value of WHO 1996 soils		50	36	0.8	85	
Limit value of EPA 2002		24.7	16.4	0.48	3.9	
STD	2.4(4.7)	304.0(3.7)	105.2(7.0)	37.9(0.0)	1100.0(2.7)	107.2(2.5)
Mon 2711**	2.89	350.4	114	41.7	1162	105
Recovery (%)	83	87	92	91	95	100

Different letters in a column correspond to statistically different values ($P<0.05$). Numbers in parenthesis correspond to standard deviation values ($n=5$). **Montana (Mon) soil 2711 NIST reported concentration. Detection limits (LD), mgkg^{-1} : Fe= 10, Zn=5, Cu= 5, Cd=2, Pb=20, and As= 5.

Table 2. Concentrations of metals and As (mg/kg of dry weight) in maize organs (root, stem, leaf, and kernel) sampled at 22 weeks of growth.

	Fe	Zn	Cu	Cd	Pb	As
Brace roots						
STAX1	820.6(34.3)a	73.4(6.4)b	78.1(16.8)b	1.1(32.6)a	14.6(31.7)b	0.2(68.0)a
STAX2	2651.5(23.9)b	45.3(8.5)a	17.6(21.2)b	1.0(0.7)a	7.9(23.3)a	0.7(49.5)b
STAX3	4040.6(34.4)c	2302.7(7.6)c	82.2(22.0)b	63.2(49.1)b	23.8 c	1.6(6.1)c
Stem						
STAX1	13.3(25.6)a	53.8(71.2)a	2.6(62.1)a	0.5(2.7)a	3.1(15.2)b	0.1(29.1)a
STAX2	33.5(27.7)b	52.2(0.7)a	2.0(34.7)a	0.5(0.7)a	1.5(1.5)a	0.3(30.4)b
STAX3	52.8(56.9)c	1521.5(3.8)b	5.3(26.4)b	3.6(10.1)b	3.8(10.8)b	0.1(37.5)a
Leaf						
STAX1	145.1(5.7)a	47.3(0.8)a	10.8(52.0)b	0.7(46.5)a	5.2(38.8)ab	0.2(14.5)a
STAX2	177.6(76.9)b	47.4(50.5)a	6.2(16.3)a	0.7(46.5)a	4.0(36.9)a	0.3(7.7)ab
STAX3	186.9(16.5)b	287.4(31.8)b	6.8(10.1)a	1.7(60.6)b	6.4(3.8)b	0.4(1.7)b
Kernel						
STAX1	30.5(10.0)a	41.9(21.8)b	5.2(32.9)b	1.4(47.6)b	0.5(15.0)a	0.2(59.1)b
STAX2	26.2(20.9)a	29.0(29.7)a	2.8 (18.7)a	0.5(55.3)a	0.6(3.2)a	0.0(76.8)a
STAX3	28.3(1.3)a	35.4(69.8)a	4.0(41.0)a	0.9(48.0)a	0.5(18.7)a	0.1(10.1)b

Different letters in a column correspond to statistically different values ($P<0.05$). Numbers inside parenthesis correspond to standard deviation values ($n=3$). Detection limits (LD), mgkg^{-1} : $Fe= 2.5$, $Zn=2.5$, $Cu=2.5$, $Cd= 0.05$, $Pb=0.05$, and $As= 0.015$.

Table 3. Morphological characteristics of vegetative and generative organs in plants grown for 22 weeks in STAX1, STAX2, and STAX3 soils.

Parameters	Sites		
Morphology and biomass	STAX1 n=45	STAX2 n=45	STAX3 n=45
Total fresh weight –aerial part (g)	52(25)c	11(3)b	4(2) a
Total dry weight- aerial part (g)	21(10)c	4 (2)b	2(1)a
Fresh weight- root (g)	17(7)c	4 (2)b	2(0.4)a
Dry weight-root (g)	5(3)c	1(0.4)b	1(0.3)a
Biomass* (%)	41(15)a	38(18)b	41(22)a
Number of phytomers	13(6)c	10(6)b	8(3)a
Longest leaf length (cm)	73(30)c	42(23)b	25(12)a
Ears	STAX1 n= 76	STAX2 n= 76	STAX3 n= 32
Length (cm)	10(3) ^b	8 (2) ^a	8(2) ^a
Dry weight of ears (g)	3 (2) ^c	0.5 (0.4) ^b	0.2 (0.1) ^a
Normal appearance (%)	78(12) ^c	46 (15) ^b	28 (9) ^a
Dry weight of kernels (g)	0.18 (0.01) ^b	0.11 (0.02) ^a	0.06(0.002) ^a
Tassel	STAX1 n=64	STAX2 n=46	STAX3 n=13
Length (cm)	15(5)c	7(4)b	4 (3)a
Number of branches	5(3)b	2(1)a	1(0.3)a
Normal appearance (%)	84(12)c	52 (14)b	8 (4)a

Different letters in a row correspond to statistically different values ($P<0.05$). Numbers inside parenthesis indicate standard deviation values. *Biomass (%) = (Fresh weight–dry weight) in percentage¹

Table 4. Morphometric analysis of roots and stems on transverse sections and of leaf surface in plants grown for 22 weeks in STAX1, STAX2, and STAX3 soils.

Morphometric parameters	Sites		
Shoot-borne brace roots	STAX1	STAX2	STAX3
Major root diameter (µm)	1580(533) ^c	1287(313) ^b	1200(177) ^a
Minor root diameter (µm)	1450(425) ^c	1285(386) ^b	1163(185) ^a
Shortest (minor) vascular bundle diameter (µm)	833(365) ^c	699(176) ^b	640(68) ^a
Longest (major) vascular bundle diameter (µm)	908(478) ^c	763(245) ^b	655(94) ^a
Number of metaxylem sectors per section	25(2) ^b	15(3) ^a	12(2) ^a
Stem	STAX1	STAX2	STAX3
Diameter (mm)	4.5(0.8) ^c	2.6(0.6) ^b	1.6(0.4) ^a
Number of vascular bundles	11(3) ^c	7(4) ^b	5(2) ^a
Parenchyma cell diameter (µm)	106(63) ^c	77(24) ^b	35(12) ^a
Leaf	STAX1	STAX2	STAX3
Stomata number (adaxial)	5(1) ^a	6(2) ^a	5(2) ^a
Stomata number (abaxial)	9(2) ^a	9(1) ^a	11(3) ^b
Epidermal cell length (µm).	88(13) ^b	86(18) ^b	68(14) ^a

Different letters in rows correspond to statistical different values ($P < 0.05$). Numbers inside parenthesis correspond to standard deviation values $n=20$.

Supplementary

Appendices A and B are not available with this version.

Figures

Figure 1

Location of sampled soils in Taxco, Guerrero, México

Figure 2

The principal component analysis of heavy metal and As content in maize organs. abbreviations: _K is Kernel; _L is Leaves; _R is Roots; _S is Soils

Figure 3

Changes in height of maize plants during 22 weeks. Different letters indicate statistical difference ($P < 0.05$), $n = 60$, SE.

Figure 4

Maize plants grown during 22 weeks in the studied soils: STAX1, STAX2, and STAX3 showing visible morphological differences. A) plant appearance. B) ear morphology. C) tassel morphology.

Figure 5

Scanning electron microscopy analysis of plants grown in different soils samples. A) STAX1; B) STAX2, C) STAX3, grown in the three substrates. Microphotographs. 1) Transverse sections of roots (55x); 2) stem, (43x); 3) abaxial leaf surface and 4)