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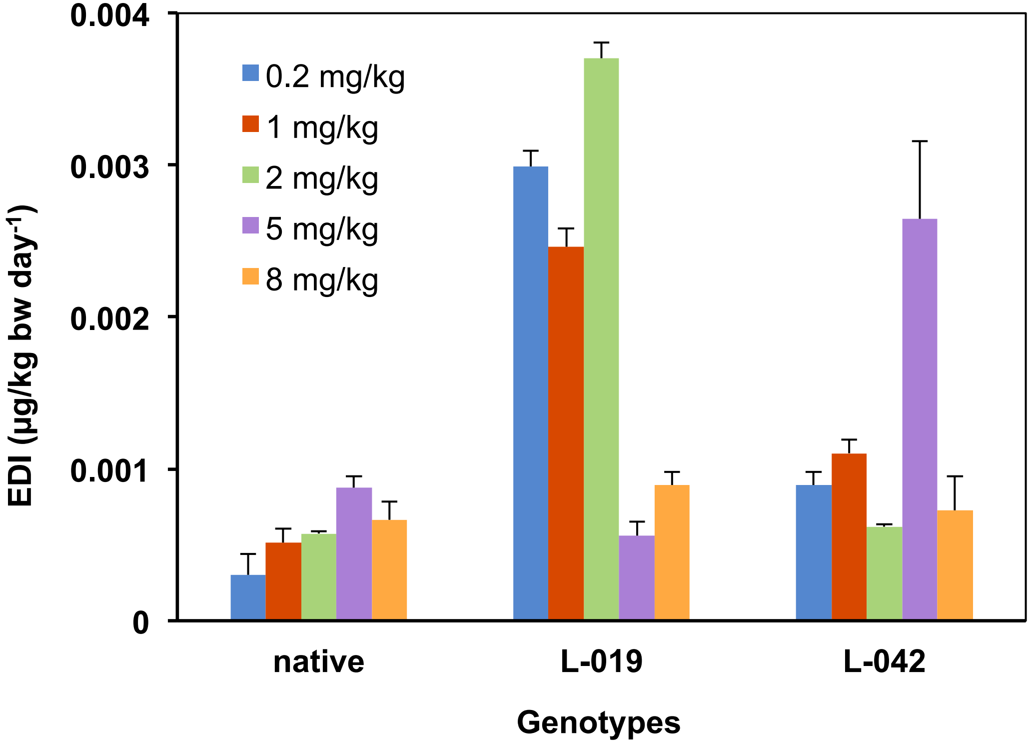
Cowpea

EDI <<< Limit FAO/WHO: 0.57 μg Hg/kg bw day^{-1}



Transfer and bioaccumulation of mercury from soil in cowpea in gold mining sites

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Abstract

In this study, we evaluated the phytoremediation ability of three different genotypes of cowpea grown on mercury-contaminated soils from gold mining areas. In particular we compared a native genotype with two commercial lines L-019 and L-042. The plants were cultivated in soils amended at different concentrations of Hg (i.e. 0.2, 1, 2, 5 and 8 mg kg^{-1}). After three months exposure, we determined plant growth, seed production, and Hg accumulation in different plant tissues (root, leaf, seed and stem). Indices of soil-plant metal transfer such as translocation, bioconcentration and bioaccumulation factors were calculated. Results showed that the native variety presented the highest seed production (3.8 g), however the highest plant biomass (7.9 g) was observed in line L-019, both on Hg-contaminated soil of 1 mg kg^{-1}. The different plant tissues differed in terms of Hg concentration (root > leaf > stem). In the highest treated soil, the line L-042 accumulates higher Hg in both roots and leaves, while line L-019 accumulates more metal in stems. In line L-019, Hg concentrations in the fruit showed significant differences being higher in the valves than in the seeds. The transfer factors were generally lower than 1 and indicates the low accumulation of Hg by cowpeas. The estimated daily Hg intake through cowpea consumption showed values far below the threshold of 0.57 μg/kg dw day^{-1} recommended by the World Health Organization. Our results show cowpea *V. unguiculata* as a good protein-rich food substitute of Hg-contaminated fish for populations living near gold mining sites.

Keywords: Cowpea; Phytoextraction; Mercury; health risk; Vigna unguiculata L. Walp; healthy food

1. Introduction

The growth of the world population and the continuous expansion of industrial production, agriculture, mining and industrial activities have generated serious problems of contamination in the environment (Wang et al., 2017). In soils, particularly in agricultural ones, the presence of considerable amounts of metals such as mercury (Hg), poses a significant threat to human health (Tóth et al., 2016; Sierra et al., 2017). Thus, there is an increasing interest in the transfer of metals from soils to terrestrial food webs (Gall et al., 2015), because the intake of plants (e.g. vegetable) can be a direct route for human exposure to metals (Song et al., 2009).

The specie *V.unguiculata L.* Walp grows in tropical areas characterized by high temperatures and drought periods and is well known for making symbiotic associations to fix nitrogen (Nonnoi et al., 2012). This legume represents one of the first colonizers of poor or degraded soils and thus has been successfully used for restoration of arid and degraded ecosystems (Forti et al., 2006; de Andres et al., 2017). This specie is also known by its use for the phytoremediation of metal contaminated soils (Bezerril et al., 2017; Kopittke et al., 2007a; Kopitthe et al., 2007b), and for the restoration of ecosystems degraded by mining activities (Dary et al., 2010, Moreno-Jiménez et al., 2011). In Colombia, it is primarily grown in the Caribbean region by small producers with economic and technological limitations, because cowpea has the ability to adapt very easily to the different production systems of the region (Araméndiz-Tatis et al., 2011). Some of these soils are highly impacted by artisanal and small-scale gold mining (ASGM) (Marrugo-

Negrete et al., 2017), which is the largest anthropogenic source of Hg contamination worldwide (Salazar-Camacho et al., 2017).

The objective of this study was to evaluate the phytoremediation ability of cowpea (*V. unguiculata L.* Walp) to accumulate Hg from soils. To this end, three genotypes grown in soils amended with different Hg concentrations were analysed in order to simulate soils from ASGM. We determined bulk Hg content in soils, roots, stems and leaves, and the amount of bioavailable Hg in soils. Moreover, we evaluated different indices of soil-plant metal transfer translocation (TF) and bioconcentration factors (BCF) in the plants. Because its low Hg accumulation in plants, this study explored the possibility to use cowpea as a safe protein-rich food alternative to reduce the intake of Hg-contaminated fish in populations living near gold mining sites. Therefore, we calculated the estimated daily intake (EDI) in order to assess the risk to human health posed by Hg exposure due to the consumption of cowpea. The results of this study could be a pioneer contribution determining the risk of human exposure to Hg through cowpeas consumption and unveiling the important role of this plant in contaminated soils near areas impacted by ASGM.

2. Materials and methods

2.1 Soil collection

Soil samples (0-30 cm depth) were collected from agricultural areas located in the Mojana region (8°33'49.6'' N, 75°05'55.4'' W) (Smol inska and Cedzynska, 2007). The samples were packed in plastic bags for their transport to the laboratory. Once in the laboratory, the soil samples were dried at room temperature to constant weight. Then, the soil aggregates were crushed, homogenized and sieved through 2 mm mesh size device (Reis et al., 2010).

2.2 Soil Analysis

The soil chemical characterization was carried out in the soil laboratory of the University of Córdoba, according to the Colombian technical standard (NTC) and the methodologies from IGAC (IGAC, 2006). The pH was characterized through the potentiometric method (NTC 5264, 2008) based on ISO 10390:2005. Organic matter (OM) was characterized using Walkey-Black method (NTC 5403) (Walkey and Black, 1934) and minor elements Cu, Fe, Zn, Mn with modified Olsen method. Available boron was determined through HCl 0.05M (Li and Gupta, 1991), available sulfur was determined through calcium monophosphate 0.008M (Hermida et al, 2013), and available phosphorus through Bray II (Yan et al, 1995). For the characterization of major elements, such as Ca, Mg, K, Na, we used 1.0 M ammonium acetate at pH 7, and the effective cation exchange capacity (CICe) was determined following Reeuwij (2002).

2.3 Experimental design

Based in previous studies on stability and adaptability in different agronomic tropical environments influenced by the drastic climatic changes (Araméndiz-Tatis et al., 2011; 2016; 2017), three types cowpea beans were selected: the native (genotype V1) and commercial genotypes L-019 (genotype V2) and L-042 (genotype V3) of *V. unguiculata L.* Walp, that were acquired in the Germplasm Bank of the University of Córdoba. Some of their good agronomic attributes are described in Table S1 (reported as Supplementary Information), whereas their nutritional content and quality could be found in previous studies (Aramendiz-Tatis et al., 2016). Each genotype was cultivated in soil amended with $Hg(NO_3)_2$ solution of 50 g L^{-1} to give four different Hg soil concentrations: C1 = 1.0 ± 0.18 mg kg^{-1}, C2 = 2.0 ± 0.29 mg kg^{-1}, C3 = 5.0 ± 0.35 mg kg^{-1} and C4 = 8.0 mg kg^{-1} and were incubated for a period of 3 months. Amendments were successfully maintained (Fig. S1) (reported as Supplementary Information). The soils background level of Hg (C_0) was 0.2 ± 0.08 mg kg^{-1}. Each treatment was evaluated in triplicate, the culture was carried out in plastic containers of 2L capacity, for a total of 45 experimental units.

2.4 Implementation and monitoring of the crop

Prior to planting the seeds in the soil, a disinfection process was performed. The seeds were rinsed with 10% of sodium hypochlorite, and then washed with distilled water according to Finten et al. (2017). A single plant was manually planted at the different containers holding altered soil concentration. In order to maintain soil moisture at field capacity, the greenhouse conditions were 28ºC and 6 hours average of solar brightness (Marrugo-Negrete et al., 2015). Samples were collected after 90 days when 95% of the dried pods showed a cream colour appearance (TJAI, 2010). Once the seeds were sown, the germination time was monitored every 6 hours during a week. Periodic water irrigation was carried out attending to the water needs of the crop. Accordingly and attending the growth rate of the plant and the environmental temperature conditions, the volume and frequency of irrigation was adapted, keeping the soil at three quarters of its field capacity (Marrugo-Negrete et al., 2015). During the development of the experiment the presence of chlorosis and necrosis in leaves was monitored. At the end of the experiment, the plants were harvested, later divided into roots, stems, leaves, valves and seeds. The different parts of the plant were dried in a Binder stove at 40℃ for 72 hours, and afterward the weight was determined using an analytical balance OHAUS Corp. Adventure, model AP2140. Therefore, values are presented as dry weight (dw).

2.5 Determination of Hg in plants and soils

Total mercury (THg) in roots, stems, leafs, valves, seeds and soil was determined using a Direct Mercury Analyzer® (DMA-80 TRICELL; Milestone Inc., Italy) through the EPA 7473 method (EPA, 1998). The quality control in the determination of THg content in the samples was carried out with the certified reference material CRM 1573a "tomato leaves" of 34 ± 4 ng g^{-1} of the NIST, and "Loam Soil" of 0.083±0.017 mg kg^{-1} of ERM®- CC141; the triplicate analysis showed recovery percentages for NIST tomato and ERM soil of 98.3±0.4% and 96.4±0.4%, respectively. The detection limit for THg was 0.1 µg g^{-1} dw in both plants and soil, calculated as three times the standard deviation of the blanks (n=10). The bioavailable Hg in the soil samples was determined in triplicate according to Bloom et al. 2003, and it is equivalent to that extracted in Step 1 of the BCR procedure (Rauret et al., 2000).

2.6 Indices of soil-plant metal transfer

The translocation factor (TF) was calculated by the ratio of the metal concentration in the aerial parts of the plants (shoots) and the concentration of metal in the roots

(Chandra et al., 2017). The bioconcentration factor (BCF) was determined as the ratio of the metal concentration in the roots and the metal concentration in the soil (Chandra et al., 2017). Finally, the bioaccumulation factor (BAF) was calculated as the ratio of the metal concentration in the harvestable part of the plant (i.e. shoots) and the concentration of the metal in the soil (Marrugo-Negrete et al., 2016b).

2.7 Human health risk assessment

The determination of the estimated daily intake (EDI) of THg through the cowpea consumption was calculated using the following equation: $EDI=[THg]xR/Bw$, where THg is the total mercury concentration in the seed; R is the consumption rate of cowpea, and Bw is the adult average body weight (Jia et al., 2017). The average consumption of cowpea in Colombia is 3.86 kg/person/year (10.6 g/person/day) (Tofiño et al. 2011). A value of 70 kg was used as the average adults body weight (WHO, 1993).

2.8 Statistic analysis

The results are expressed as the mean ± the standard deviation of the triplicate determinations. A factorial ANOVA was performed to compare the means between the different treatments; multiple comparisons were made using Fisher's Least Significant Difference (LSD) procedure, a $p < 0.05$ was considered significant. The analysis was carried out with the statistical package Statgraphics Centurion XVI.

3. Results

3.1 Physicochemical characteristics of the soil

Table 1 shows the physicochemical characteristics of the studied soils. The soils have a silty loamy texture and an abundant content of elements needed for crop groth, except for K. Although, the OM showed a slight deficiency and the pH was moderately acidic. The CEC is well above the range of 8-10 cmol+ kg^{-1}, which is generally considered acceptable minimum values to obtain a satisfactory performance if the rest of the parameters are adequate (Sierra et al., 2008). According to Araméndiz-Tatis et al., (2016), we can conclude that the studied soils were suitable for agricultural use in general, and in particular, for the cultivation of *V. unguiculata*.

3.2 Effects of Hg amendments on growth

The plants germinated entirely between the third and fourth day after the sowings. The cowpea plants showed a normal development during their growth in treatments C_0, C1, and C2; while in treatments C3 and C4, moderate and high symptoms of chlorosis and necrosis in the leaves were clearly evidenced. In addition, convex epinasty was presented on the leaves, indicating that at concentrations equal to or greater than 5 mg kg^{-1} of Hg in the soil, symptoms of visible toxicity appear in the foliar tissues.

We saw significant differences ($p<0.05$) in biomass growth (Fig. 1a) and seed production (Fig. 1b) between the different amendments and genotypes. While the biomass of the native genotype (V1) at C1 increased, it progressively decreased above a soils Hg concentration of 2 mg kg^{-1}. The same pattern was observed for the commercial genotype V2 and V3, although the response was more pronounced in genotype V2. Thus, the maximum values of biomass were recorded at concentrations of 1 mg kg^{-1} (C1) in all the evaluated genotypes, with V2 being the highest content (7.9 ± 1.0 g). Noteworthy to mention a 50% decrease of the plant biomass between plants grown in soils amended with 1 mg kg^{-1} (C1) vs. 8 mg kg^{-1} (C4).

Regarding the seed production, the native genotype (V1) stood out as the best one with 3.8 ± 0.1 g/plant at C1. It should be noted that the genotype V3 exhibited a similar behavior than the native one, and surprisingly the genotype V2 exhibited a much lower production. However, the best performance for genotype V2 was obtained at the control (Co). The order of seed production was higher for native cowpea > V3 > V2, showing significant differences among the evaluated genotypes ($p < 0.05$). Therefore our results suggest that these cowpea species can tolerate and adapt to soils moderately contaminated with Hg.

3.3 Concentrations of mercury in plants

Figure 2 shows the accumulation of THg in the tissues of the plant with the order: root > leaf > stem, showing highly significant differences among treatments ($p < 0.05$). For all the evaluated varieties of cowpea the levels increased as Hg soil concentrations increased, however it is emphasized that the commercial genotypes V2 and V3 showed higher concentrations than the native genotype V1. Easily bioavailable Hg concentrations in the evaluated soils remained at very low levels with values of 0.016 ± 0.1 mg kg^{-1}, indicating that in these samples, Hg was mainly in a very stable mineral form.

THg levels in the fruit of *V. unguiculata* was discriminated in seed and valves (Fig 3). For all treatments there were significant differences ($p < 0.05$) among the Hg content in the different parts of the fruit (Fig. 3). The results show that V1 reach the lowest THg concentration in seed for almost all the treatments, being lower than the recommended by the Chinese national food standards agency 10 µg kg^{-1} for foods of vegetable origin (Jia et al., 2017). While the commercial genotypes (V2 and V3) showed higher concentrations, with the genotype V2 being a more enriched for Hg in the seeds than V3 in all the treatments (except C3); thus, reaching maximum difference in the soils ≥ 2 mg kg^{-1} of Hg. Similar to the seed results, Hg concentrations in the valves showed statistically significant differences among treatments ($p < 0.05$). Concentrations of Hg in the valves tend to be lower for the native genotype than in commercial ones.

3.4 Translocation, bioconcentration and bioaccumulation factors

The results of the translocation, bioconcentration and bioaccumulation factors are shown in Table 2. In general, the increase of the soil Hg concentration decreases the TF. However, at the lowest Hg soils concentration (Co), the commercial genotype V2 stands out. Similarly, the TF leaf/root, with values greater than 1, was high for the commercial genotypes V2 and V3 in Co. This demonstrates the higher transfer of Hg from soils to leaves of cowpea in commercial genotypes with respect to native. The results of the BCF root/soil showed the commercial genotypes V2 and V3 as the ones that are closer to the unit in soil of 5 mg Hg kg^{-1} (see bold numbers in Table 2). Maximum BCF values for the seed/soil ratio correspond to genotype V2 in Co, however these values are low (Table 2). The highest BAF for the leaf/soil (0.374) was observed in genotype V3 at the lowest dose of Hg. In contrast, very low BAF for stem/soil values were observed in any amendment.

3.5 Determination of estimated daily intake

The estimated daily intake (EDI) of Hg through cowpea consumption (Fig. 4) did not exceed the maximum limit established by the WHO/FAO Expert Committee on Food Additives that established a provisional tolerable daily intake (PTDI) of 0.57 µg/kg.bw.day^{-1} (JECFA, 2010). In particular the native genotype V1 presented the lowest EDI values (average 0.0006 µg/kg.bw.day^{-1}). The commercial genotypes show similar average EDI values between them (V1: 0.0017 µg/kg.bw.day^{-1} vs. V2: 0.0010 µg/kg.bw.day^{-1}), with the highest value of 0.004 µg/kg.bw.day^{-1} for V2 genotype at C2.

4. Discussion

4.1 Soil characteristics: an important regulator of Hg transfer to cowpea

As mention above, the soil characterization (Table 1) showed that the studied soils were suitable for the cultivation of cowpea (Aramendiz-Tatis et al. 2016). Low OM and moderately acidic pH facilitate metals solubility, mobility and thus its bioavailability (Olaniran et al., 2013; Sandrin and Hoffman, 2007), which might therefore enhance metal plant absorption. In particular, it has been shown that the maximum absorption of Hg^{2+} occurs in pH between 4 and 5 (McLaughlin et al., 1999), which is similar to measured values in the studied soils. However, the observed low transfer and absorption of soil Hg to the roots and aerial parts (Fig. 2) suggest an underlying mechanism of the plant to protect against metals (Patra and Sarma, 2000, Moreno-Jiménez et al., 2006).

4.2 Effects of Hg on growth and seed production

The observed adverse effects on the development of the studied plants, especially for higher Hg concentrations (5 and 8 mg kg^{-1}) in soils, are in agreement with previous work of Cho and Park (2000), and confirm a reduction on plant biomass and chlorophyll content in the leaves (Azevedo and Rodriguez, 2012). Also, Shiyab et al. (2009) observed a reduction in the biomass and in the relative content of water in leaves, as well as densely stained areas that surround the vascular bundles of the leaves, in *Brassica juncea* crops exposed to Hg. The same behavior was also evidenced in the present study for *V. unguiculata* at Hg concentrations higher than 5 mg kg^{-1}. Moreover, other studies conducted with metals in *V. unguiculata* cowpea reported that even low concentrations of Pb are highly toxic to plants and can reduce their development by more than 10% to 0.4 mg L^{-1} (Kopittke et al., 2007). It is noteworthy that inhibition of growth and reduction of biomass production are frequent phenomena observed in plants exposed to toxic levels of Hg and other metals (Patra and Sharma, 2000).

The production of seeds was also reduced at concentrations higher than 5 mg kg^{-1} (Fig. 1). At lower concentrations, the average production remains around 300 kg ha^{-1} (Table 3) for all the varieties, which is lower than the average yield of 521 kg ha^{-1} estimated by the FAO (2013) for standard conditions worldwide. Cowpea seed production in Colombia is centralized basically in the Caribbean region and it is slightly higher (600 kg ha^{-1}) than the global average (Araméndiz-Tatis et al., 2011).

In the present study, the native variety stands out presenting an average yield of 667 kg ha^{-1} at low Hg soil concentration (e.g. 1 mg kg^{-1}) showing that this variety maintains a good production in soils moderately contaminated.

4.3 Mercury concentrations in soils and tissues

The three evaluated genotypes showed higher concentrations of Hg in the roots followed by the leaves and stems, increasing as the contaminant increases in the amendments (Fig. 2). These results are similar to those obtained under field conditions with native plants such as *Jatropha curcas* (Marrugo et al., 2016b). Several authors have reported that most plants tend to accumulate Hg in the roots, followed by the aerial part of the plant (Moreno-Jiménez et al., 2006; Cargnelutti et al., 2006, Azevedo & Rodriguez, 2012, Marrugo-Negrete et al., 2015, Marrugo-Negrete et al., 2016b). This is due to the absorption of nutrients and dissolved salts by the root, and by being in direct contact with the contaminated medium, as well as acting as a barrier to prevent the metal from reaching the aerial parts of the plant (Patra and Sarma, 2000, Moreno-Jiménez et al., 2006). It should be noted that at lower concentrations of Hg (e.g. Co) the improved varieties accumulated more Hg in the leaves than in the other tissues, this may be due to the influence of the volatilized Hg^0 in the air that can be captured by the stomata of the leaves (Frescholtz et al. 2003, Marrugo-Negrete et al., 2016b).

Accumulation of Hg in seeds of commercial plants has been studied by Kabata-Pendias (2000), who revealed some ranges of the Hg content in different species of plants of economic interest such as wheat seeds *T. aestivum* (0.2 – 33.0 μg kg^{-1}), barley *H. vulgare* (5.0 – 82.0 μg kg^{-1}), oats *A. sativa* (<4.0 – 45.0 μg kg^{-1}), rye *S.*

cereale (3.0 -18.0 μg kg^{-1}), corn *Z. mays* (3.0 – 4.6 μg kg^{-1}), and beans *P. vulgaris* (3.0 – 11.0 μg kg^{-1}). All those, presented higher THg concentrations than those found in the present study (Fig. 3) for the native cowpea. In contrast, the studied commercial genotypes V2 and V3 showed a higher Hg concentration range. Our results thus suggest that the studied native cowpea has more efficient mechanisms to restrict the translocation of contaminants from the soils to the seeds in comparison with the modified varieties (Fig. 3a).

4.4 Accumulation factors for mercury

In the native cowpea, TF, BCF and BAF factors values were less than 1, indicating that *V. unguiculata* is not a good bioaccumulator of Hg (Raskin and Ensley, 2000, Tu et. al., 2003). Some plants have developed strategies to restrict the entry of metal into the root and its transport to the shoots (Sun et al., 2016). Few examples with other metals are *T. mongolicum* for Zn, *C. communis* for Cd, *O. biennis* for Cd and Cu, and *R. chinensis* for Cu (Wei et al., 2015). In contrast, genotypes V2 and V3 showed transfer values higher than or equal to 1 but only at the lowest concentrations (Co), indicating that commercial genotypes presented a stronger capacity to store Hg in their aerial parts, even at the lowest tested concentrations. Moreover, these genotypes are not capable of preserving the incorporation of toxic Hg to fruits (Andres et al., 2010). BAF and BCF were less than 1, which indicates that none of the tested genotypes of *V. unguiculata* evaluated in the study could not be used for the phytoextraction of Hg (Chandra et al., 2017).

4.5 Risk assessment

Figure 4 shows the EDI values of Hg for the consumption of the studied cowpea seeds by adults. The average EDI (0.001 μg/kg.bw.day^{-1}) considering all the genotypes was over 57 times the PTDI threshold limit of 0.57 μg/kg.bw.day^{-1} considered safe by the JECFA. However, it should be noted that in risk assessment this threshold limit is considered for a whole diet, not only restricted to some specific food items. Thus, in our discussion it is actually mentioned that cowpea is a good food alternative that can replace the consumption of fish. In any case, for practical purposes, it is hopeless to consider the possibility of completely replacing fish from the diet of these populations. Therefore, more detailed studies on risk should be carried out considering the consumption of both cowpea and fish. Considering previous argument, several studies in the Caribbean (Marrugo-Negrete et al., 2013, Fuentes-Gandara et al., 2018, Carranza-López et al., 2019) indicate that according to Hg in fish and consumption rate, the EDI values in these populations are very much higher than PTDI. In fact, EDI values based in fish consumption of contaminated species in the Caribbean region are 1-3 orders of magnitude higher than those EDI values in cowpea.

4.6. Nutritional facts and diet custom of local people

From a nutritional point of view, cowpea is a particular food that provides protein, calories, fiber, minerals and vitamins (Kabas et al., 2007). Indeed, cowpea is a good source of iron, zinc, calories, fiber, vitamins, protein (24%), carbohydrates (53%) and fats (2%) (De-Paula et al., 2018; FAO, 2016). A study has revealed that the genotypes evaluated here have a high content of protein, vitamins and minerals as (Araméndiz-Tatis, 2016). Therefore, these genotypic lines have significant economic, social, and cultural importance for low-income people in the Caribbean region (Cardona-Ayala et al., 2013; Araméndiz et al., 2011). On the other hand, if we pretend to include this food in the diet of local people theis dietary customs should also be considered. In fact, the dietary habits of people living near ASGM areas have a marked preference of fish consumption, and thus, fish is their main source of animal protein. Unfortunately, fish caught near ASGM is usually Hg-contaminated and its high consumption may become a serious health problem for consumers. Therefore, cowpea may represent a good substitute to replace protein-rich foods as fish, but with lower levels of Hg. In this sense, since no regulation on Hg content in foods (except for fish) exists in Colombia, it should be interesting to compare cowpea Hg contents with other worldwide regulations. The native cowpea genotype transfers the contaminant to the seed in Hg concentrations lower than the reference value of 10 μg kg^{-1} established by China for foods of plant origin (Jia et al., 2017), and also it is lower than reference value of 100 μg kg^{-1} stated by Europe for food (EU, 2015).

5. Conclusions

The evaluated cowpea plants showed differences in the accumulation of Hg in their tissues. It should be highlighted that of the three varieties evaluated, the native cowpea adapted very well in soils with Hg levels of 2 μg kg^{-1} leading to a production of biomass and seeds that is within the normal limits for cultivation in tropical areas. However, in all the evaluated varieties subjected to soils with Hg concentrations higher than 2 μg kg^{-1}, both the biomass and the production decreased more than the 50%. The native variety showed resistance to translocate

Hg to seed, demonstrating that it can be grown in soils with high metal content. This is of paramount importance in populations with high fish consumption in their dietary habits and inhabiting areas close to ASGM. Cowpea is considered a protein-rich food and our study can ensure good yield and harmlessness food in terms of Hg concentrations, and it can thus replace several fishmeals. Cowpea could be a substitute of fish without losing nutritional properties and decreasing the amount of Hg in their body burden. We conclude that it should be considered as an economic and agri-food alternative to fish in ASGM areas.

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References

Albán M. 2012. Manual del cultivo de frijol caupí. Editorial: Asociación de Productores Agropecuarios del Distrito de Morropón/ Proyecto Norte Emprendedor Swisscontact Fundación Suiza para la Cooperación del Desarrollo Técnico/ Dirección Regional Agraria Piura, Primera edición Piura, Perú, Pp 28.

Andres A., Millán R., Esteban E. 2010. Evaluación del Uso Agrícola de Vicia sativa L. en Suelos Contaminados con Mercurio. UAM. Departamento de Química Agrícola. CIEMAT. Departamento de Medio Ambiente. España. P. 61.

Araméndiz Tatis, H., & Espitia Camacho, M. 2011. Comportamiento agronómico de líneas promisorias de fríjol caupí Vigna unguiculata L. Walp en el Valle del Sinú. Revista Temas Agrarios 16(2) .

Araméndiz-Tatis H., Cardona-Ayala C.E., Combatt-Caballero E. M. 2016. Contenido Nutricional de Líneas de Fríjol Caupí (*Vigna unguiculata* L. Walp.) Seleccionadas de una Población Criolla. Información Tecnológica. Vol. 27(2), 53-60.

Araméndiz-Tatis, H. A., Camacho, M. E., & Ayala, C. C. 2017. Adaptabilidad y estabilidad fenotípica en cultivares de fríjol Caupí en el caribe húmedo Colombiano. Biotecnología en el sector agropecuario y agroindustrial, 15(2), 14-22.

Azevedo R. & Rodriguez E. 2012. Phytotoxicity of Mercury in Plants: A Review. J. Bot. Vol. 6.

Bezerril M. N., Oliveira M., Gomes-Rochette N., de Menezes A., Gonçalves A., Barreto F., Costa J., Sá Borges S., Ferreira R., Fernandes D. 2017. Effect of lead on physiological and antioxidant responses in two *Vigna unguiculata* cultivars differing in Pb-accumulation. Chemosphere (176) 397-404.

Cargnelutti, D., Tabaldi, L.A., Spanevello, R.M., Jucoski, G.O., Battisti, V., Redin, M.,Linares, C.E.B., Dressler, V.L., Flores, E.M.M., Nicoloso, F.T., Morsch, V.M., Schetinger, M.R.C., 2006. Mercury toxicity induces oxidative stress in growing cucumber seedlings. Chemosphere 65, 999–1006.

Cardona-Ayala, C., Araméndiz-Tatis, H., Jarma-Orozco, A. 2013. Variabilidad genética en líneas de fríjol caupí (Vigna unguiculata L. Walp). Revista Agronomía. 21(2):7-18.

Carranza-Lopez, L., Caballero-Gallardo, K., Cervantes-Ceballos, L., Turizo-Tapia, A., Olivero-Verbel, J. 2019. Multicompartment Mercury Contamination in Major Gold Mining Districts at the Department of Bolivar, Colombia. Archives of Environmental Contamination and Toxicology. 76:640–649.

Chandra R., Yadav S., Yadav S. 2017. Phytoextraction potential of heavy metals by native wetland plantsgrowing on chlorolignin containing sludge of pulp and paper industry. Ecol. Eng. 98 134–145.

Cho U. H. and Park J. O. 2000. Mercury-induced oxidative stress in tomato seedlings. Plant Sci. 156 1–9.

Cordy, P., Veiga, M., Salih, I., Al-Saadi, S., Console, S., García, O., Mesa, L., Velásquez, P., Roeser, M., 2011. Mercury contamination from artisanal gold mining in Antioquia, Colombia: the world's highest per capita mercury pollution. Sci. Total Environ. 410, 154–160.

Dary M., Chamber-Pérez M.A., Palomares A.J., Pajuelo E. 2010. “In situ” phytostabilisation of heavy metal pullited soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J. Hazard. Mater. 177, 323-330.

de Andres, F., Walter, I., Tenorio, J.L. 2007. Revegetation of abandoned agricultural land amended with biosolids. Sci. Total Environ. 378, 31-83.

De Gouveia M., Bolívar A., López M., Salih A., Pérez H. 2005. Participación de agricultores en la selección de materiales genéticos de fríjol (*Vigna unguiculata*) evaluados en suelos ácidos de la Parroquia Espino estado Guárico (Venezuela). Cuadernos de Desarrollo Rural (54), 2005 - pp 113-129.

De Paula, C. D., Jarma, S., & Aramendiz, H. (2016). Caracterización nutricional y sensorial de frijol caupí (Vigna unguiculata (L.) Walp.). Agronomía Colombiana, 34(1Supl), S1131-S1134.

Durango-Hernández J., Díaz-Fernández L., Marrugo-Negrete J., Cruz-Esquivel Á., Enamorado-Montes G. 2016. Evaluación de la capacidad fitoestabilzadora de mercurio por parte de la Cebolla (*Allium Cepa* L), en cultivos hidropónicos Revista Alimentos Hoy. Vol 24, No 39.

EPA, 1998. "Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry," Revision 0. Washington, DC.

EU, 2015. Commission Regulation 2015/186, amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, fluorine, lead, mercury, endosulfan and Ambrosia sedes. Wew. Accessed 21 October 2019. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.031.01.0011.01.ENG

FAO, 2013. Food and Agriculture Organization. The State of Food and Agriculture. Roma: FAO. p109. http://www.fao.org/publications/sofa/2013/en/. Accessed 22 October 2019

FAO, 2016. Food and Agriculture Organization. Codex Alimentarius Commission. *Vigna unguiculata*. https://www.google.com/url?client=internal-uds-cse&cx=018170620143701104933:qq82jsfba7w&q=http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/%3Flnk%3D1%26url%3Dhttps%25253A%25252F%25252Fworkspace.fao.org%25252Fsites%25252Fcodex%25252FMeetings%25252FCX-718-48%25252FCRD%25252Fpr48_crd24x.pdf&sa=U&ved=2ahUKEwil4Me0yq_lAhUnzYUKHcF6BHcQFjACegQIABAC&usg=AOvVaw0BrncaHjUIG5beX4VMmWsr Accessed 22 October 2019

Finten G., Agüero M.V., Jagus R.J. 2017. Citric acid as alternative to sodium hypochlorite for washing and disinfection of experimentally-infected spinach leaves. LWT - Food Sci. Technol. 82 318-325.

Forti, M., Lavie, Y., Ben-Dov, Y., Pauker, R. 2006. Long-Term survival and develoment under dryland conditions in an experimental site in the semi-arid Negev of Israel. J. Arid. Environ. 65, 1-28.

Frescholtz, T.F., Gustin, M.S., Schorran, D.E., Fernandez, G.C., 2003. Assessing the source of mercury in foliar tissue of quaking aspen. Environ. Toxicol. Chem. 22, 2114–2119.

Gall, J.E., Boyd ,R.S., Rajakaruna, N. 2015 Transfer of heavy metals through terrestrial food webs: a review. Environ Monit Assess. 187, 201

Hermida, J. J. F., Toro, M. C. H., Guzmán, M., & Cabrera, R. I. (2013). Determining nutrient diagnostic norms for greenhouse roses. *HortScience*, *48*(11), 1403-1410.

IGAC Instituto Geográfico Agustín Codazzi. 2016. Métodos analíticos del laboratorio de suelos. Subdirección de Agrología, 6a Edición, 450-499, Bogotá, Colombia.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2010. Joint FAO/WHO food standards programme, committee of the codex alimentarius commission, thirty third session. http://www.fsis.usda.gov/PDF/2010-CAC/cac33_15e.pdf. Accessed 5 November 2019.

Jia, Q., Zhu, X., Hao, Y., Yang, Z., Wang, Q., Fu, H., and Yu, H. 2017. Mercury in soil, vegetable and human hair in a typicalmining area in China: Implication for human exposure, J. Environ. Sci.

Kabaata-Pendias A. 2000. Trace elements in soils and plants.Third Edition. CRC Press, Boca Raton, usa. 413p.

Kabas, O.; Yilmaz, E.; Ozmerzi, A. e I. Akinci. (2007). Some physical and nutritional properties of cowpea seed (*Vigna sinensis* L.), J. Food Eng. 79, 1405–1409.

Kopittke P. M., Asher C. J., Kopittke R. A., Menzies N. W. 2007a Toxic effects of Pb^{2+} on growth of cowpea (*Vigna unguiculata*) Environ. Pollut. 50 280-287.

Kopittke P.M., Dart P.J., and Menzies N.W. 2007b. Toxic effects of low concentrations of Cu on nodulation of cowpea (*Vigna unguiculata)*. Environ. Pollut. 145, 309-315.

Li, R., and U. C. Gupta. 1991. Extraction of soil B for predicting its availability to plants. Communications in Soil Science and Plant Analysis 22:1003–1012.

Marrugo-Negrete J., Durango-Hernández J., Pinedo-Hernández J., Enamorado-Montes G., S.Díez. 2016a. Mercury uptake and effects on growth in *Jatropha curcas*. J. Environ. Sci. 48. 120-125.

Marrugo-Negrete J., Marrugo-Madrid S., Pinedo-Hernández J., Durango-Hernández J., Díez, S.. 2016b. Screening of native plant species for phytoremediation potential at a Hg-contaminated mining site. Sci. Total Environ. 542 809–816.

Marrugo-Negrete J., Durango-Hernández J., Pinedo-Hernández J., Olivero-Verbel J., Sergi Díez. 2015. Phytoremediation of mercury-contaminated soils by Jatropha curcas. Chemosphere 127 58–63.

Marrugo-Negrete J., Pinedo-Hernández J., Díez S. 2017. Assessment of heavy metal pollution, spatial distribution and origin in agricultural soils along the Sinú River Basin, Colombia. Environ. Res. 154: 380–388.

Marrugo-Negrete J.L., Benitez L.N., Olivero-Verbel J. 2008b. Distribution of mercury in several environmental compartments in an aquatic ecosystem impacted by gold mining in Northern Colombia. Arch. Environ. Contam. Toxicol. 55:305-16. http://dx.doi.org/10.1007/s00244-007-9129-7

Marrugo-Negrete J.L., Olivero-Verbel J., Lans E., Benitez L.N. 2008a**.**Total mercury and methylmercury concentrations in fish from the Mojana region of Colombia. Environ. Geochem. Health. 2008;30:21-30. http://dx.doi.org/10.1007/s10653-007-9104-2

McLaughlin, M.J., D.R. Parker y J.M. Clarke. 1999. Metals and micronutrients – food safety issues. Field Crops Res. 60, 143-163.

Moreno-Jiménez E., Gamarra R., Carpena-Ruiz R.O., Millán R., Penálosa J.M., Esteban E. 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén área. Chemosphere 63 1969–1973.

Moreno-Jiménez E., Vázquez S., Carpena-Ruiz R. O., Esteban E., Peñalosa J. M. 2011. Using Mediterranean shrubs for the phytoremediation of a soil impacted by pyritic wastes in Southern Spain: A field experiment. J. Environ. Manag. 92 1584-1590.

Nonnoi F., Chinnaswamy A., García de la Torre V. S., Coba de la Peña T., Lucas M. M., Pueyo J. 2012. Metal tolerance of rhizobial strains isolated from nodules of herbaceous legumes (*Medicago spp. and Trifolium spp*.) growing in mercury-contaminated soils. Applied Soil Ecol. 61 49– 59.

NTC-Norma Técnica Colombiana - 5264. Bogotá, D. C; Colombia: 2008. Calidad de Suelo, Determinación del pH. https://docplayer.es/57197445-Norma-tecnica-colombiana-5264.html. Accessed 5 November 2019.

NTC-Norma Técnica Colombiana - 5403. Bogotá, D. C; Colombia: 2013. Calidad de Suelo, Determinación del carbono orgánico. https://docplayer.es/53210008-Norma-tecnica-colombiana-5403.htm. Accessed 5 November 2019.

Olaniran, A.O., Balgobind, A., Pillay, B. 2013. Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and posible improvement strategies int. J. Mol. Sci. (14), 10197–10228.

Patra M. and Sharma A. 2000. Mercury toxicity in plants. Bot. Rev. 66. 379–422.

Pinedo-Hernández J., Marrugo-Negrete J., Díez S. 2015. Speciation and bioavailability of mercury in sediments impacted by gold mining in Colombia. Chemosphere 119:1289–1295.

Rauret, G., López-Sánchez, J. F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A. M., Quevauviller, P. (2000). Application of a modified BCR sequential extraction (threestep) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil ref- erence material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. Journal of Environmental Monitoring, 2, 228–233.

Raskin I. and Ensley B.D. 2000. Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley & Sons, New York. 304 pp.

Reeuwij, L.P. (2002). Procedures for soil analysis. Sixth edition. International Soil Reference and Information Centre—Food and Agriculture Organization of the United Nations. Technical Paper 9.

Reis A. T., Rodrigues S. M., Davidson C. M., Pereira E., Duarte A. C. 2010. Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated áreas. Chemosphere 81 1369–1377.

Restrepo-Sánchez N.E., Acevedo-Betancourth L., Henao-Murillo B., Peláez-Jaramillo C. 2015. Remediation effect of compost on soluble mercury transfer in a crop of *Phaseolus vulgaris*. J. Environ Sci. 31 61 – 67.

Romero S. E., Marrugo-Negrete J.L., Arias J.E., Hadad H., & Maine M.A. 2011. Hg, Cu, Pb, Cd, and Zn Accumulation in Macrophytes Growing in Tropical Wetlands. Water Air Soil Pollut. 216:361–373.

Salazar-Camacho C., Salas-Moreno M., Marrugo-Madrid S., Marrugo-Negrete J., Díez S. 2017. Dietary human exposure to mercury in two artisanal small-scale gold mining communities of northwestern Colombia. Environ. Int. 107, 47–54.

Sandrin, T.R., Hoffman, D.R., 2007. Bioremediation of organic and metal cocontaminated environments: effects of metal toxicity, speciation, and bioavailability on biodegradation. Environ. Bioremed. Technol., 1–34.

Schuster, E. 1991. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes - A review of the literature. Water Air Soil Pollut. 56:1, 667–680.

Shiyab, S., Chen J., Han, F., Monts, D., Matta, F., Gu. M. and Su, Y. 2009. Phytotoxicity of mercury in Indian mustard (*Brassica juncea* L.). Ecotox Environ Safe. 72, 619-625.

Sierra M.J., Millán R., Esteban E. 2008 Potential use of Solanum melongena in agricultural areas with high mercury background concentrations. Food Chem. Toxicol. 46 2143–2149.

Sierra, M. J., López-Nicolás, R., González-Bermúdez, C., Frontela-Saseta C., and Millán R. 2017. Cultivation of *Solanum tuberosum* in a former mining district for a safe human consumption integrating simulated digestion. J Sci Food Agric. DOI 10.1002/jsfa.8412.

Smolinska, B. and Cedzynska, K. 2007. EDTA and urease effects on Hg accumulation by *Lepidium sativum*. Chemosphere 69, 1388–1395.

Song B., Lei M., Chen T., Zheng Y., Xie Y., LI X., Gao D. 2009. Assessing the health risk of heavy metals in vegetables to the general population in Beijing, China J. Environ. Sci. 21 1702-1709.

Sun Z., Chen J., Wang X., Lv C. 2016. Heavy metal accumulation in native plants at a metallurgy waste sitein rural areas of Northern China. Ecol. Eng. 86 60–68.

TJAI, Thomas Jefferson Agricultural Institute. 2010. Cowpea: a versatile legume for hot, dry conditions. Columbia, MO. http://www.jeffersoninstitute.org/pubs/cowpea.shtml

Tofiño A, Tofiño R, Cabal D, Melo A, Camarillo W, Pachón H. 2011. Evaluación agronómica y sensorial de fríjol (Phaseolus vulgaris L.) mejorado nutricionalmente en el norte del departamento del Cesar, Colombia. Perspect Nutr Humana. 13:161-177.

Tóth G., Hermann T., Da Silva M.R., Montanarella L. 2016. Heavy metals in agricultural soils of the European Union with implicationsfor food safety. Environ. Int. 88 299–309.

Tu C., Ma l.Q. y Bondada B. 2003. Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation. J. Environ. Qual. 31, 1671-1675.

Walkley, A., Black, I.A. 1934. An examination of Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid tritation method. Soil Science 37:29-38.

Wang, L.K., Chen, J.P., Hung, YT, Shammas, N.K. 2017. Heavy Metals in the Environment. 1st Edition. CRC press, Boca Raton.

Wei, S., Zhou, Q., and Wang X. 2005. Identification of weed plants excluding the uptake of heavy metals. Environ. Int. 31 829 – 834.

Wenzel, W., Bunkowski, M., Puschenrerter, M., Horak, O. 2003. Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ. Pollut. 123:131 – 8.

World Health Organization (WHO) 1993. Evaluation of Certain Food Additives and Contaminants. In: Forty-First Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva. Technical Series 837.

Yan, X., Beebe, S. E., & Lynch, J. P. (1995). Genetic variation for phosphorus efficiency of common bean in contrasting soil types: II. Yield response. *Crop Science*, *35*(4), 1094-1099.

Figure 1. a) Biomass of the plant and b) seed production (g) of three different cowpeas genotypes (V1: native genotype; V2: genotype L-019 and V3: genotype L-042) grew at different concentrations of total Hg: Co: 0.2 mg kg^{-1} (control); C1: 1 mg kg^{-1}; C2: 2 mg kg^{-1}; C3: 5 mg kg^{-1}; C4: 8 mg kg^{-1}. Different letters indicate significant statistical differences $p < 0.05$ among treatments.

Figure 2. Concentrations of total mercury (THg in μg kg^{-1}) in a) root, b) stem, and c) leaf of the studied cowpeas genotypes (V1: native genotype; V2: genotype L-019 and V3: genotype L-042) evaluated in the different amended soils (Co: 0.2 mg kg^{-1} (control); C1: 1 mg kg^{-1}; C2: 2 mg kg^{-1}; C3: 5 mg kg^{-1}; C4: 8 mg kg^{-1}). Different letters indicate significant statistical differences $p < 0.05$. Phytotoxic traits observed in leaf, moderate (*), high (**).

Figure 3. Concentration of total mercury (THg in μg kg^{-1}) in seed a) and valves b) of *Vigna unguiculata L* genotypes (V1: native genotype; V2: genotype L-019 and V3: genotype L-042) exposed to different soil THg concentrations (Co:0.2 mg kg^{-1} (control); C1: 1 mg kg^{-1}; C2: 2 mg kg^{-1}; C3: 5 mg kg^{-1}; C4: 8 mg kg^{-1}). The letters mean significant statistical differences ($p < 0.05$).

Figure 4. Provisional Daily Intake (PTDI, μg/kg.bw.day^{-1}) for total mercury according to the consumption of the cowpea seeds (V1: native genotype; V2: genotype L-019 and V3: genotype L-042) evaluated at the different THg soil concentrations (Co:0.2 mg kg^{-1} (control); C1: 1 mg kg^{-1}; C2: 2 mg kg^{-1}; C3: 5 mg kg^{-1}; C4: 8 mg kg^{-1}). Letters mean significant statistical differences ($p < 0.05$).

Table 1. Physicochemical characterization of the soil (n=3)

pH	4.86±0.03
%OM	0.85±0.04
%Clay	20.3
%Silt	73.8
%Sand	5.8
Texture	Silty loam
Elements (mg kg^{-1})	
Cu	37.9±0.1
Fe	56.5±0.9
Mn	109.7±2.5
S	78.5±3.3
P	107.1±6.1
B	0.32±0.01
Zn	24.6±2.0
Total Hg	0.2±0.08
Hg bioavailable	0.016 ± 0.1
CEC (cmol^{+} kg^{-1})	
Na	0.70±0.02
Ca	17.5±1.2
Mg	13.4±0.8
K	0.18±0.02

Table 2. Translocation factor (TF). bioaccumulation factor (BAF) and bioconcentration factor (BCF) for native and commercial lines L-019 and L-042 genotypes.

Genotypes	**Treatments (mg kg^{-1})**				
	0.2	**1**	**2**	**5**	**8**
			TF (Seed/Root)		
V1:native	0.046	0.012	0.012	0.015	0.002
V2:L-019	**0.832**	0.285	0.051	0.001	0.003
V3: L-042	0.195	0.017	0.008	0.006	0.001
			TF (Leaf/Root)		
V1:native	0.982	0.154	0.183	0.463	0.100
V2:L-019	**1.542**	0.879	0.116	0.174	0.026
V3: L-042	**2.083**	0.091	0.111	0.034	0.217
			BAF (Stem/Soil)		
V1:native	0.047	0.010	0.005	0.005	0.017
V2:L-019	0.041	0.013	0.007	0.005	0.004
V3: L-042	0.039	0.045	0.010	0.008	0.011
			BAF (Leaf/Soil)		
V1:native	0.255	0.050	0.031	0.039	0.032
V2:L-019	0.217	0.057	0.030	0.132	0.008
V3: L-042	**0.374**	0.045	0.033	0.022	0.108
			BCF (Root/Soil)		
V1:native	0.260	0.323	0.170	0.083	0.320
V2:L-019	0.140	0.065	0.261	**0.762**	0.302
V3: L-042	0.179	0.491	0.301	**0.635**	0.495
			BCF (Seed/Soil)		
V1:native	0.012	0.004	0.002	0.001	0.001
V2:L-019	0.117	0.019	0.013	0.001	0.001
V3: L-042	0.035	0.008	0.002	0.004	0.001

Table 3. Seed production and standard deviation (SD) by hectare for each of the amendments (Co: no amendment ~0.2 mg kg^{-1}, C1: 1 mg kg^{-1}; C2: 2 mg kg^{-1}; C3: 5 mg kg^{-1}; C4: 8 mg kg^{-1}, and the studied genotypes (V1: native; V2: L-019; V3: L-042).

Genotype	**Amendments**	**Seed production (kg. ha^{-1})**	**SD**
V1	Co	439	18
	C1	667	18
	C2	219	26
	C3	167	9
	C4	13	4
V2	Co	332	11
	C1	164	27
	C2	89	11
	C3	66	4
	C4	45	6
V3	Co	237	26
	C1	562	70
	C2	263	88
	C3	298	18
	C4	18	4

Figure 1.

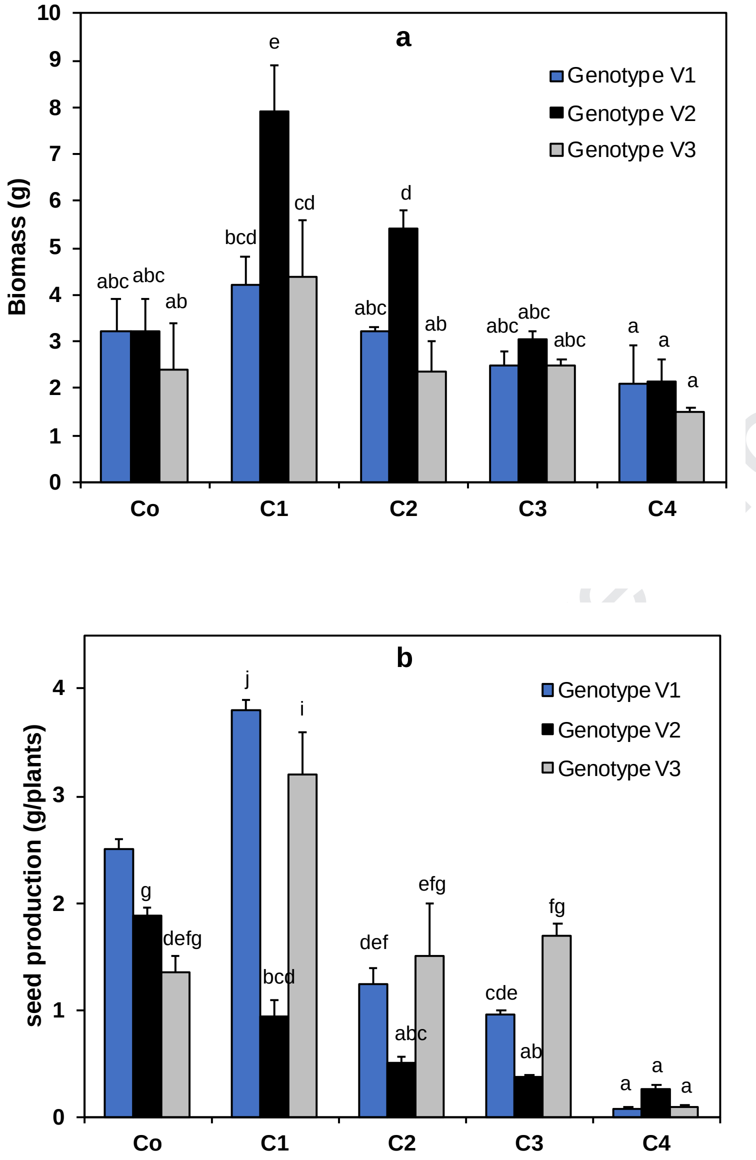

a
Biomass (g)
10
9
8
7
6
5
4
3
2
1
0
Genotype V1
Genotype V2
Genotype V3
abc
abc
ab
bcd
e
cd
abc
d
ab
abc
abc
abc
a
a
a
Co
C1
C2
C3
C4
b
seed production (g/plants)
4
3
2
1
0
Genotype V1
Genotype V2
Genotype V3
g
defg
j
bcd
i
def
abc
efg
cde
ab
fg
a
a
a
Co
C1
C2
C3
C4

Figure 2.

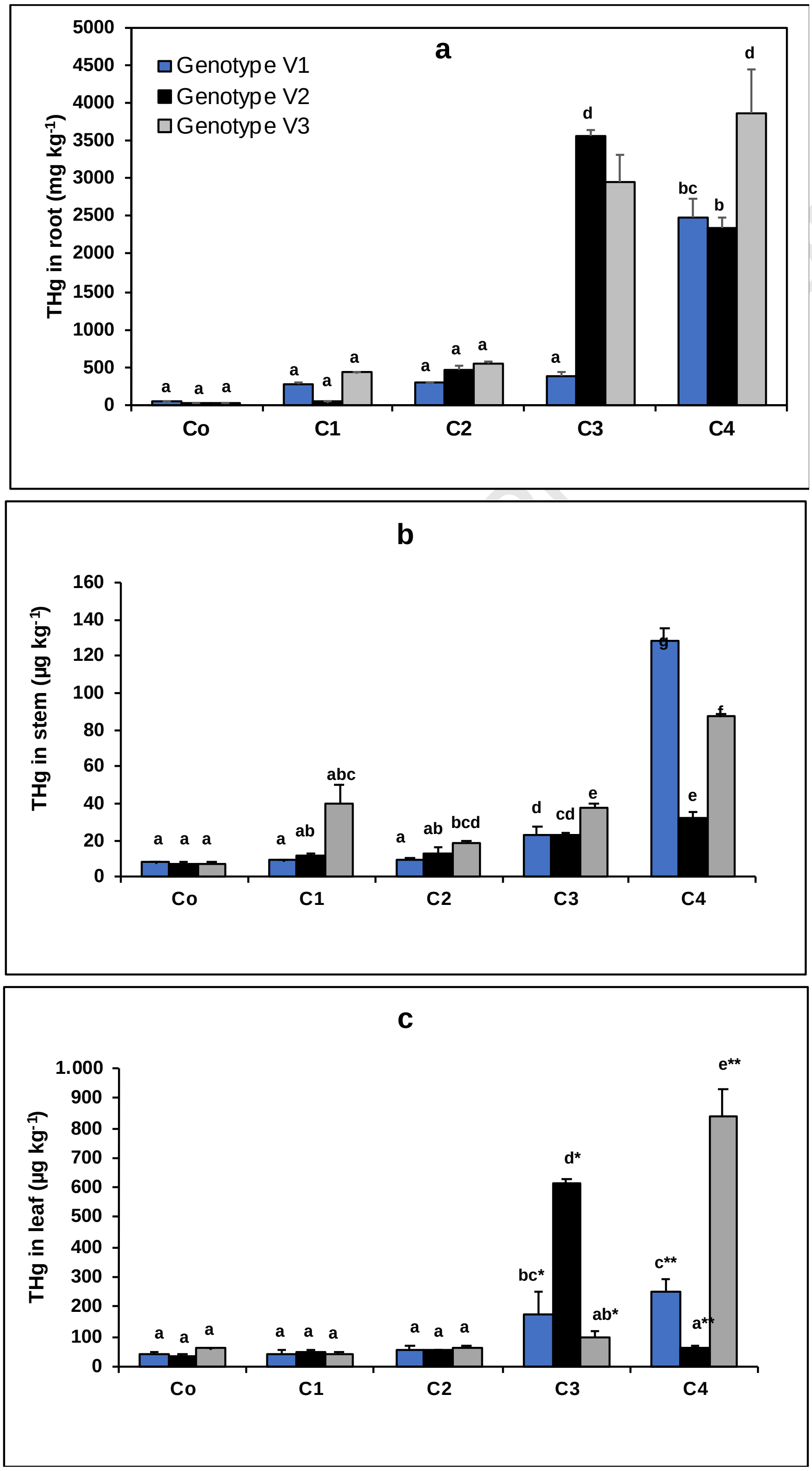

a
THg in root (mg kg^{-1})
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Genotype V1
Genotype V2
Genotype V3
a a a
a a a
a a a
a d
bc b d
Co
C1
C2
C3
C4
b
THg in stem (µg kg^{-1})
160
140
120
100
80
60
40
20
0
a a a
a ab abc
a ab bcd
d cd e
g e f
Co
C1
C2
C3
C4
c
THg in leaf (µg kg^{-1})
1.000
900
800
700
600
500
400
300
200
100
0
a a a
a a a
a a a
bc* d* ab*
c** a** e**
Co
C1
C2
C3
C4

Figure 3.

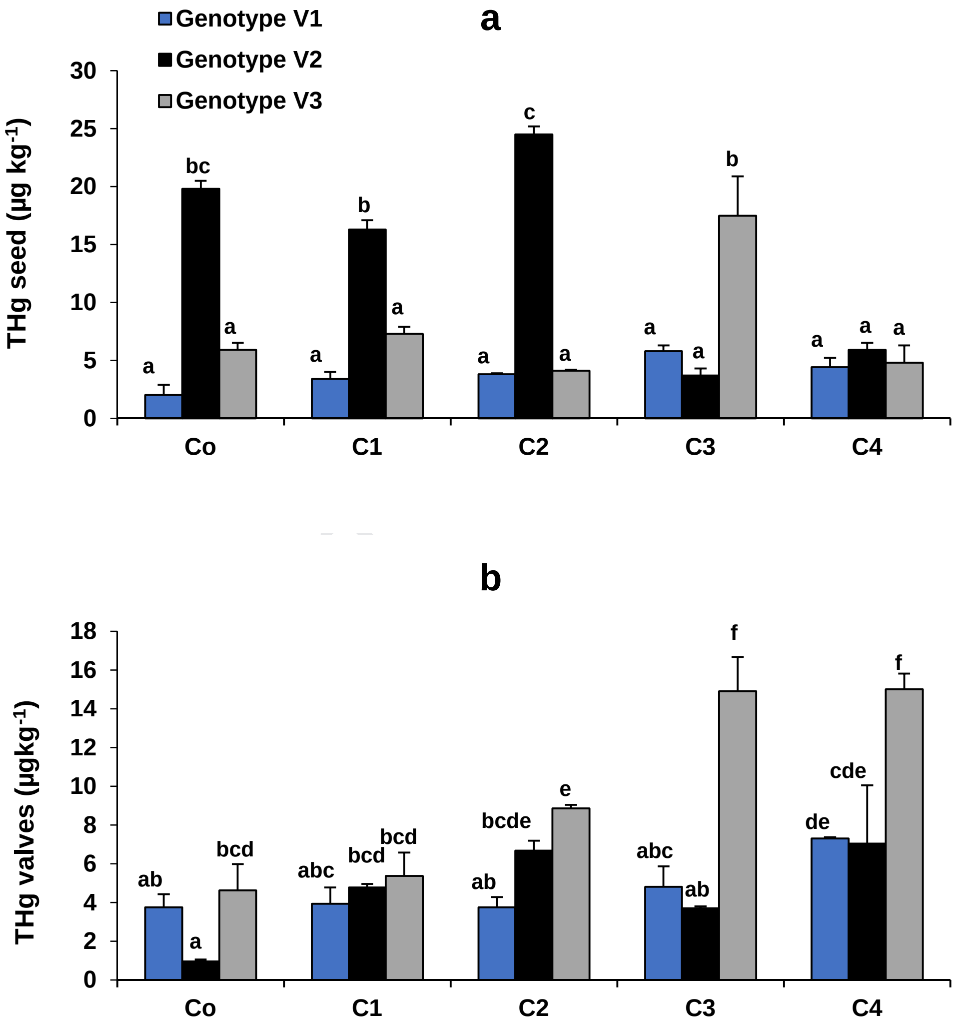
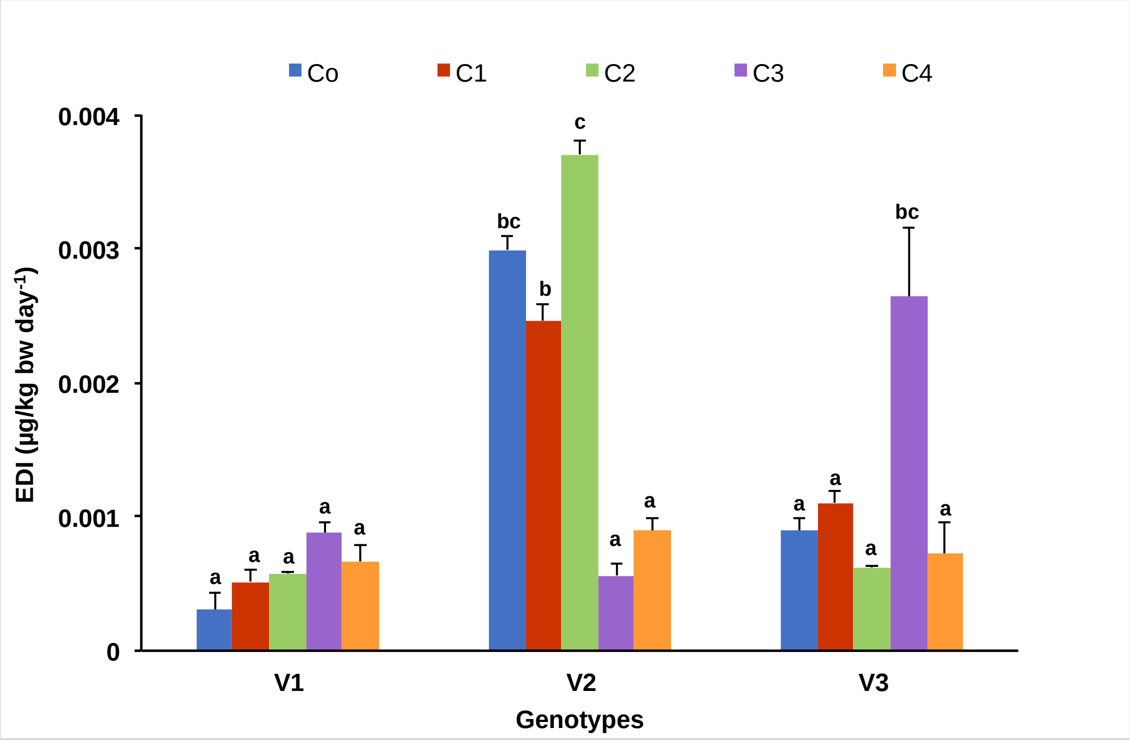

■ Genotype V1
■ Genotype V2
■ Genotype V3
a
THg seed (µg kg-1)
30
25
20
15
10
5
0
a
bc
a
a
b
a
a
c
a
a
a
b
a
a
a
Co
C1
C2
C3
C4
b
THg valves (µgkg-1)
18
16
14
12
10
8
6
4
2
0
ab
a
bcd
abc
bcd
bcd
ab
bcde
e
abc
ab
f
de
cde
f
Co
C1
C2
C3
C4

Figure 4.


Co
C1
C2
C3
C4
0.004
0.003
0.002
0.001
0
EDI (µg/kg bw day^{-1})
a
a
a
a
a
bc
b
c
a
a
a
a
a
bc
a
V1
V2
V3
Genotypes

Highlights

- Mercury uptake in cowpea was determined in three genotypes using polluted soil
- In all the lines, Hg levels in plant tissues followed this order root> leaf> stem
- Native variety showed resistance to translocate Hg to the fruit vs commercial lines
- The daily Hg intake through cowpea are far below the health risk according to WHO
- Cowpea is a protein-rich food that can reduce the high intake of contaminated fish