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POTENTIALLY TOXIC ELEMENTS IN SOILS AND CACAO BEANS IN AGROFORESTRY SYSTEMS OF BAHIA, BRAZIL

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Cacao farming has made many advances in technology and production systems with the goal of increasing final productivity. However, in some cases, the indiscriminate and/or non-technical use of certain agricultural inputs may compromise the quality of the cacao products, like the presence of heavy metals, or potentially toxic elements, such as cadmium (Cd), barium (Ba), copper (Cu) and lead (Pb), compounds of high toxicity and mostly mutagenic and carcinogenic to humans. This work intends to analyze the influence of local lithology and components of agricultural management on the levels of heavy metals in the soil and cacao beans, looking ahead for future evaluations on the consequences of cacao consumption and on the quality demands of the market. The evaluations were made in 15 cacao cropping systems in Southeast Bahia, Brazil. In these cropping systems, cacao was grown under an agroforestry system locally called 'cabruca' or under the shade of rubber or erythrina trees. The results suggest that agricultural practices tend to increase the presence of the heavy metals Cu and Ba in the surface soil layers, reflecting the influence of agricultural inputs, and that the higher content of Cd and Pb in subsurface soil layers reflects the influence of lithology processes. Young soils tend to have higher contents of Cu, Cd and Pb than older soil types. Cacao clone PH-16 accumulated higher Ba than beans of Comum 'forastero' cacao; soil Ba had direct correlation with Ba in cacao beans and inverse correlation with copper. Soil cadmium tended to have a direct correlation with barium in cacao beans.

Key words: Heavy metals, tropical soils, *Theobroma cacao,* cacao bean quality, toxic elements.

Elementos potencialmente tóxicos em solos e amêndoas de cacau em sistemas agroflorestais da Bahia, Brasil. O cultivo do cacau tem passado por muitos avanços em tecnologia e sistemas de produção com objetivo de aumentar a produtividade final. Contudo, em alguns casos, o uso indiscriminado de certos produtos agrícolas pode comprometer a qualidade dos produtos de cacau, como a presença de metais pesados, ou elementos potencialmente tóxicos, como o cádmio (Cd), bário (Ba), cobre (Cu) e chumbo (Pb), compostos de alta toxicidade e em sua maioria mutagênicos e carcinogênicos em seres humanos. Este trabalho pretende analisar a influência da litologia local e de componentes do manejo agrícola nos níveis de metais pesados em solos e amêndoas de cacau, fazendo estimativas para futuras avaliações das consequências do consumo de cacau e das demandas do mercado por qualidade. As avaliações foram realizadas em 15 cultivos de cacau no Sudeste da Bahia, Brasil. Nestes cultivos, cacau foi cultivado em um sistema agroflorestal localmente chamado 'cabruca' ou sob a sombra de seringueira ou eritrina. Os resultados sugerem que as práticas agrícolas tendem a aumentar a presença dos metais pesados Cu e Ba nas camadas superficiais do solo, refletindo a influência dos produtos agrícolas, e dos metais Cd e Pb em subsuperfície, refletindo a influência dos processos litológicos. Solos mais jovens tendem a ter maiores teores de Cu, Cd e Pb do que solos mais velhos. O clone de cacau PH-16 acumulou mais Ba em amêndoas do que o cacau comum; teores de Ba no solo tiveram relação direta com o teor de Ba nas amêndoas e relação inversa com cobre. O teor de cádmium no solo apresentou relação direta com o teor de bário em amêndoas.

Palavras-chave: Metais pesados, solos tropicais, *Theobroma cacao,* qualidade de amêndoas de cacau, elementos tóxicos.

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Introduction

The definitions of "heavy metals" have been modified by several authors (Duffus, 2002). Denominations such as "toxic metals" are also used, although this is still not a precise terminology, since it is the excess that generates toxicity, and some of them, like Cu, Fe, and Zn, are highly dense, can cause toxicity, but are nutrients for humans and plants. Thus, heavy metals can be better described as metal and metalloids associated with environmental pollution, toxicity and adverse effects on biota (Ali and Khan, 2018). However, as this term is considered as part of the language of science (Hubner, Astin and Herbert, 2010), it will be still used in this article.

The metals present in the soil can be classified according to their origin: i) lithogenic: metals originating from a source material; ii) pedogenic: lithogenic metals that have undergone pedogenetic processes; iii) anthropogenic: metals introduced in the soil composition by means of anthropic actions (Kabata-Pendias and Mukherjee, 2007).

The type of rock on which the soil was developed and, mainly, the mineral constituents of its source material have a direct influence on the background levels of metals in the soil, this explains the metal contents found in deeper soil layers. Industrial emissions, effluents, sewage sludge and soil conditioners also contribute to the increase of potentially toxic elements in the soil (Nicholson et al., 2003), this justifies the values found in the top layer of the soil.

Heavy metals can be absorbed by plant roots through specific and/or non-specific transport proteins and transported in the transpiration stream in the xylem from the roots to transpiring shoot parts (Page et al., 2006). After ascension if there is no further redistribution, the heavy metals accumulate primarily in photosynthetically active (transpiring) leaves and can induce oxidative stress, reduce chlorophyll content, and slow down photosynthesis rate (Mourato et al., 2015). The symplastic transport via phloem allows redistribution of absorbed essential and non-essential heavy metals within the plants, depends on the actual source/sink network and on the mobility of each heavy metal.

Cacao bean is the raw material of chocolate, one of the most desired foods in the world. The manufacturing of chocolate involves established strict quality standards, resulting in a strong pressure on the cacao production chain. In the cacao cultivated areas, therefore, an antagonism arises: while there is a challenge of increasing the productivity to fulfil the beans demand, there is a challenge of adding values to cacao production. As the quality of agricultural products is influenced by the naturally occurring contaminants (Edelstein and Ben-Hur, 2018), as well as by contaminants from agricultural inputs (Barañski et al., 2014), the pressure on the quality of the cultivated land with cacao trees can be one way of controlling the quality of cocoa beans (Araujo et al., 2014).

Because of global problems in human health through food contamination, the issue of heavy metals in cacao beans is taken very seriously. The Agency for Toxic Substances and Disease Registry (ATSDR) labels the chemical elements barium (Ba), cadmium (Cd), copper (Cu) and lead (Pb) as elements that pose a risk to human health, since they are potentially toxic, and humans are frequently exposed to them (ATSDR, 2019). Understanding factors that affect the presence of heavy metals in cacao production systems can support the development of strategies to mitigate the occurrence of these elements in cacao-based foods.

The objective of this study was to evaluate the presence of potentially toxic elements in soils of 15 cacao agroforestry plantations in the Southeast of Bahia state, Brazil, and further assess the interrelationships between heavy metals in soil and their accumulation in cacao beans.

Materials and Methods

Evaluated cacao cropping systems (sites)

From Southeast Bahia major cacao growing region, 15 diversified agroforestry-based cacao farming systems were selected for this study (Table 1 and 2). These field sites cover most representative environments suitable for non-irrigated cacao cropping and include some of the most productive land areas of Brazil.

Table 1. Cacao cropping systems: location, tree composition and soil type of the studied sites (based on Araujo et al., 2013)

Site	Coordinates	Soil Taxonomy	Cropping system	Shade tree/hectare (1)
1	13º 51’ 08" S39º 17’ 54" W	Typic Hapludox	Cacao x Rubber tree	400 (rb)
2	13º 46' 07.0" S39º 17' 52.0" W	Typic Hapludox	Cacao x Rubber tree	350 (rb)
3	13º 40’ 30" S39º 14’ 27" W	Hapludox	Cacao x Rubber tree	150 (rb)
4	13º 45’ 21" S39º 20’ 25" W	Hapludult	Cabruca	60
5	13º 44’ 38" S39º 30’ 10" W	Hapludult	Cacao x Erythrina	60 (er)
6	16º 29’ 02" S39º 23’ 56" W	Hapludult	Cacao x Rubber tree	400 (rb)
7	15º 23’ 15.1" S39º 25’ 48.6" W	Hapludult	Cabruca	35
8	15º 23’ 08" S39º 26’ 04" W	Hapludult	Cabruca	35
9	15º 17’ 04" S39º 28’ 43" W	Hapludult	Cabruca	35
10	14º 31’ 14" S39º 15’ 45" W	Hapludalf	Cabruca	50
11	14º 51’ 36" S39º 14’ 42" W	Dystropept	Cabruca	35
12	14º 42’ 40.9" S39º 20’ 13.2" W	Hapludult	Cabruca	88
13	14º 51’ 47" S39º 06’ 47" W	Hapludox	Cabruca	70
14	14º 51’ 47" S39º 06’ 47" W	Hapludox	Cabruca	70
15	14º 46’ 08" S39º 13’ 26" W	Hapludalf	Cabruca	60

(1) (rb) = Rubber tree (*Hevea* sp); (er) = Erythrina (*Erythrina* sp); Others, on cabruca = native tropical trees.

Table 2 - Summary of the cropping systems methods and lithology of the studied sites

Site	Liming (1)	Fertilization (2)	Cropping Aspects			Lithology (*)	Main Minerals (*)
			Disease control	Pest control	Weed Control		
1	Y	M	Copper Oxide®	Malathion®	Glifosate®	Enderbite; Metatrondhjemite Metasienite; Metamonzonite; Metamonzodiorite; Metagabronorite.Metasienite; Metamonzonite; Metamonzodiorite; Metagabronorite.	Quartz, Plagioclase, Hypertsenium, Calcium pyroxenium, Potassium feldspar, Biotite, Hornblende Labradorite, Anorthite, Andesine, microcline,Potassium feldspar, hornblende, biotite, Hipertsenium, Quartz, Augite Labradorite, Anorthite, Andesine, microcline,Potassium feldspar, hornblende, biotite, Hipertsenio, Quartz, Augite
2	Y	M and O	-	Thiodan®, Decis® and Tamaron®	Roundaup®	Enderbite; Metatrondhjemite Enderbite; Charnockite	Quartz, Plagioclase, Hipertsenium, Potassium feldspar, Biotite, HornblendeQuartz, Microcline, Hipertsenium, Oligoclase, Augite, Magnetite, Biotite, Hornblende, Plagioclase, Calcium pyroxene, Potassium feldaspar
3	Y	M and O	-	Thiodan®, Decis® and Tamaron®	Roundaup®	Conglomeratic Sandstone;Sandy Claystone;Metagabronorite; Ortogranulite.	Aluminosilicates, Quartz, FeldsparPotassium feldspar, Plagioclase, Quartz, Augite, Hipertsenium
4	Y	M and O	-	Thiodan®, Deci®s and Tamaron®	Roundaup®	Metagabronorite; Ortogranulite.Quartz Syenite; Syenite.	Potassium feldspar, Plagioclase, Quartz, Augite, Hipertsenium Potassium feldspar, Quartz, Albite, Biotite, Oligoclase, Hornblende, Orthoclasium, Microcline, Augite
5	-	M	-	Roundaup®	-	Enderbite; Metabasalt; Metadiorite; Metagabbro; Metanorite.Enderbite; Metabasalt; Metadiorite; Metagabbro; Metanorite.	Quartz, Hipertsenio, Potassium feldspar, Biotite, Hornblende, Augite, Labradorite, BytowniteQuartz, Hipertsenio, Potassium feldspar, Biotite, Hornblende, Augite, Labradorite, Bytownite

Continuation Table 2.

6	-	M and O	-	Decis®	Roundaup®	Monzodiorite; Monzonite; SyeniteMetadiorite; Granitic Gneiss; Tonalitic Orthogneiss.	Biotite, Hornblende, Augite, Quartz, Hipertsenium, Andean, Microcline, Albite, OligoclaseHornblende, Biotite, Augite, Microcline, Albite, Oligoclase, Andean, Quartz
7	Y	M	Dithane®, Carbomax® Endosulfan®	Thamaron®, Malathion® Parathion®	Roundaup®	Metadiorite; Granitic Gneiss; Tonalitic Orthogneiss. Monzodiorite; Monzonite; Syenite	Hornblende, Biotite, Augite, Microcline, Albite, Oligoclase, Andean, Quartz Biotite, Hornblende, Augite, Quartz, Hipertsenium, Andean, Microcline, Albite, Oligoclase
8	-	M	Dithane®, Carbomax® Endosulfan®	Thamaron®, Malathion® and Parathion®	Roundaup®	Enderbite; Metatrondhjemite Metasienite; Metamonzonite; Metamonzodiorite; Metagabronorite.	Quartz, Plagioclase, Hypertsenium, Calcium pyroxenium, Potassium feldspar, Biotite, Hornblende Labradorite, Anorthite, Andesine, microcline,Potassium feldspar, hornblende, biotite, Hipertsenium, Quartz, Augite
9	-	M	-	Thiodan®, Decis® Dipterex®	Glifosate®	Metasienite; Metamonzonite; Metamonzodiorite; Metagabronorite. Enderbite; Metatrondhjemite	Labradorite, Anorthite, Andesine, microcline,Potassium feldspar, hornblende, biotite, Hipertsenio, Quartz, Augite Quartz, Plagioclase, Hipertsenium, Potassium feldspar, Biotite, Hornblende
10	Y	M	-	Decis®	Roundaup®	Enderbite; CharnockiteConglomeratic Sandstone;Sandy Claystone.	Quartz, Microcline, Hipertsenium, Oligoclase, Augite, Magnetite, Biotite, Hornblende, Plagioclase, Calcium pyroxene, Potassium feldasparAluminosilicates, Quartz, Feldspar
11	Y	O	Sucrose and Fermented soup	Manipueira (Cassava)	-	Metagabronorite; Ortogranulite. Metagabronorite; Ortogranulite.	Potassium feldspar, Plagioclase, Quartz, Augite, Hipertsenium Potassium feldspar, Plagioclase, Quartz, Augite, Hipertsenium
12	-	M	Chemical	Cevin 500®, Stron®	Roundaup®	Quartz Syenite; Syenite.	Potassium feldspar, Quartz, Albite, Biotite, Oligoclase, Hornblende, Orthoclasium, Microcline, Augite
13	Y	M	-	Decis®	-	Enderbite; Metabasalt; Metadiorite; Metagabbro; Metanorite.Enderbite; Metabasalt; Metadiorite; Metagabbro; Metanorite.	Quartz, Hipertsenio, Potassium feldspar, Biotite, Hornblende, Augite, Labradorite, BytowniteQuartz, Hipertsenio, Potassium feldspar, Biotite, Hornblende, Augite, Labradorite, Bytownite
14	Y	M	-	Decis®	-	Monzodiorite; Monzonite; SyeniteMetadiorite; Granitic Gneiss; Tonalitic Orthogneiss.	Biotite, Hornblende, Augite, Quartz, Hipertsenium, Andean, Microcline, Albite, OligoclaseHornblende, Biotite, Augite, Microcline, Albite, Oligoclase, Andean, Quartz
15	-	-	-	-	-	Metadiorite; Granitic Gneiss; Tonalitic Orthogneiss.	Hornblende, Biotite, Augite, Microcline, Albite, Oligoclase, Andean, Quartz

(1) Y = yes; (2) M = mineral; O = organic; (*) Source: Geological map of Brazil to the millionth, sheet Sd-24 (CPRM, 2004). Multimedia Encyclopedia of Minerals and Atlas of Rocks (Machado et al., 2003).

Cropping systems and genetic material

Cacao cropping systems selected for this study encompass: 'cabruca' (cacao trees under shade of thinned heterogeneous native trees), agroforestry system (cacao inter cropped with Rubber trees) and conventional (cacao under homogeneous shade, mainly of *Erythrina glauca* trees).

The cacao accession in the majority of selected cacao cropping systems was the genotype PH-16, a hybrid resulting from the crossing between the mother genotype PA-150 which belongs to the Marañón cluster, Parinari IV subcluster, also classified as the Peruvian Upper Amazon Forastero genetic group, and the father genotype ICS 1 which belongs to the Trinitario group, a selection performed by the Imperial College of Tropical Agriculture in Trinidad and Tobago on hybrids resulted from a crossing between the Criollo "native" of Trinidad and Tobago and Forastero genotypes brought from Venezuela (Motamayor et al., 2008; Motilal and Sreenivasan, 2012; Yang et al., 2013; Turnbull and Hadley, 2020). However, two cropping systems (14 and 15) were planted with the Forastero type, Comum cacao, a typical cacao genotype cultivated in Southeast of Bahia state, Brazil for over 200 years, which was used to start plantations in African, South Asian and Oceanian countries (Santos et al., 2015).

Soil Sampling and Method of Analysis

In each study site approximately 1 to 2 hectares of area was selected and subdivided into three equal collection areas, which were characterized by similar soil types and with designated cropping system. From each collection area, 10 discrete samples were collected at random and thoroughly mixed to obtain one uniform homogeneous composite sample. At each of the cropping systems, 3 composite soil samples were collected from 0-15 cm (D1) and 35-50 cm (D2) depths.

Elemental analysis in the soil samples were done by extracting soils with aqua regia (3:1 HCl/HNO_3) using the method described by Mcgrath and Cunliffe (1985), and elements were quantified by inductively coupled plasma spectrometry.

Pod Sampling, bean fermentation and bean analysis

Pods were collected, during the spring of 2008, within 50 m from the soil identifying points, in the three collection areas. Thus, the origin of each sample of pods and beans corresponds to a properly identified and classified soil in each study site. Each sample (three for each study site) corresponds to 50 mature cacao pods. Considering all orchards and replicates, 45 composite samples of fruits were obtained for fermentation.

Styrofoam boxes (30 x 20 x 30 cm) with a capacity of approximately 8 kg were used and cacao beans were fermented with mucilage which corresponded to 50 cacao pods. The fermentation process of cacao bens occurred for 168 hours (7 days) with peak temperature of 51°C, occurred on the 3rd day. During the fermentation process, after 48 hours, for oxygenation beans were mixed on daily basis. After being fermented, cacao beans were continuously dried in forced ventilation oven with temperatures ranging between 35 and 45°C for 192 hours (8 days). After drying, the cacao beans contained approximately 7% of moisture. The dry cacao beans were manually peeled with tweezers for a complete separation between the seed coat and endosperm, and only the endosperm (cotyledons and embryo) was milled for chemical analysis. In this study the term dry cacao beans refer to the endosperm of dry cacao beans.

Beans were ground in Wiley mill and passed through 2 mm mesh sieve. Bean samples were digested in nitric-perchloric acid and the elements in extractants were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) based on the method EPA-3050B (USEPA, 1996).

Statistical analysis

The experiment was designed in a Completely Randomized Bock Design (CRBD). The data was submitted to analysis of variance using SAS. The means were compared using the Scheffé test ($p<0.05$) using R.

Results and Discussion

In different cropping systems, at both soil depths heavy metal contents varied significantly (Table 3). The soil Cu content was higher at D1 than in D2 in 4 out of 15 cropping systems, in only one cropping system was recorded Cu content higher in D2, and in the remaining 10 sites there were no statistically significant differences between the studied soil layers (Table 3).

Table 3. Average content of the metals Cu, Cd, Ba, and Pb (mg kg^{-1}) in soil, at two depths in 15 sites in Southeast Bahia, Brazil

Site	Cu		Cd		Ba		Pb	
	D1*	D2*	D1	D2	D1	D2	D1	D2
1	35.95 BC a	21.35 B b	2.10 AB a	1.35 CD b	26.10 C a	19.40 C a	108.20 A a	103.70 AB b
2	10.45 DE a	11.30 B a	0.95 AB a	1.35 CD a	37.85 BC b	41.90 C a	60.90 BCDE b	93.20 ABCD a
3	32.20 C a	28.05 B b	1.35 AB a	1.40 BCD a	5.15 C a	5.40 C a	93.05 AB a	98.00 ABC a
4	25.40 CD a	18.65 B a	1.1 AB a	0.35 D a	23.65 C a	13.70 C a	48.45 BCDE a	52.00 DE a
5	8.35 DE a	9.30 B a	0.6 B b	1.45 BCD a	99.70 BC a	131.65 BC a	24.05 DE b	69.80 BCDE a
6	3.10 E a	2.80 B a	0.6 B b	1.00 CD a	9.00 C a	9.55 C a	23.00 E b	57.70 CDE a
7	22.65 CD a	19.50 B a	1.55 AB a	1.25 CD a	41.20 BC a	30.40 C b	50.80 BCDE a	51.60 DE a
8	95.60 A a	21.50 B b	1.75 AB a	1.55 BC a	218.55 B a	91.35 BC b	70.85 ABCD a	71.95 BCDE a
9	35.70 BC a	14.50 B b	2.00 AB a	1.55 BC a	28.15 BC a	18.40 C a	81.10 AB a	82.05 ABCDE a
10	20.75 CDE a	20.95 B a	1.75 AB a	1.85 BC a	128.55 BC a	101.70 BC b	58.55 BCDE b	94.05 ABCD a
11	54.35 B b	115.40 A a	2.35 AB b	3.85 A a	58.25 BC a	57.35 C a	70.60 ABCD b	125.50 A a
12	32.85 C a	25.65 B a	2.95 A a	2.55 B a	628.85 A a	358.80 AB b	77.05 ABC a	78.35 BCDE a
13	6.75 DE a	8.60 B a	0.6 B a	0.75 CD a	9.50 C a	6.80 C b	30.10 CDE a	36.45 E a
14	6.75 DE a	8.60 B a	0.6 B a	0.75 CD a	9.50 C a	6.80 C b	30.10 CDE a	36.45 E a
15	20.40 CDE a	15.10 B a	1.40 AB a	1.75 BC a	524.60 A a	569.10 A a	46.30 BCDE b	73.10 BCDE a

* D1 = 0-15 cm; D2 = 35-50 cm. Means with the same letter are not different by Scheffé Test 5%, capital letters for means among lines; small letters for means between columns for each studied element.

Such higher Cu accumulation in D1 layer is possibly related to the adopted cropping method (Table 2). The soil Cu content at Site 8 was nearly four times higher in D1 compared to D2, suggesting high effect of anthropogenic actions in this site (Table 2 and 3). This corroborates with the findings of Reboredo et al. (2018), who stated that the high use of fertilizers, fungicides, herbicides, and insecticides can increase the soil metal contents. In addition, the high affinity of Cu by organic matter (Lockwood et al., 2015), a soil fraction usually more present in the surface layer of the soil, can explain the high accumulation of this element in D1. In organic matter, Cu is adsorbed by humic and fulvic acids forming stable complexes (Medina et al., 2017).

Although the Cu content was higher in D1 in many of the cropping systems, at site 11 the Cu content was more than twice the level in D2 (Table 3 and Figure 1). Site 11 has a young soil (Dystropept), a soil with primary minerals and rocks usually less than two meters from the surface, suggesting the effect of lithology. In addition, site 11 is organically farmed and has no Cu inputs received through conventional agricultural practices, that usually leads to accumulation of Cu at the surface soil layer. However, to state that anthropogenic activities promote higher Cu content in D1 than in D2 in cacao agroforestry systems, further studies including more organic cropping systems are needed.

Especially in a forest system, it is expected that the soil heavy metal content in D2 will highly correlate with the content in D1, due to the process called biological uplift, where plant available metals are absorbed in deeper soil layers and deposited on the soil surface through litterfall (Blaser et al., 2000; Starr et al., 2003; Bern, Townsend and Farmer, 2005; Kraepiel et al., 2015). However, biological lift is reported as being variable among heavy metals in the forest environment (Kraepiel et al., 2015) and is affected by the differential effect of the biosphere on the heavy metals biogeochemical cycling (Reimann, Fabian and Flem, 2019). A low positive correlation was recorded between soil Cu content in D1 and D2 (Table 4), which suggests the effect of unnatural factors sourcing Cu, that occurred differentially among the sites (Table 2), and differentially affect Cu content in D1 and D2, resulting in reduced correlation. Cu is the only plant nutrient among the evaluated elements in the current study, it is accumulated in cacao beans more than Cd, Ba and Pb (Table 5) and it is highly exported through cacao harvesting which possibly interfere in the edaphic system equilibrium, besides the effect of other anthropogenic activities.

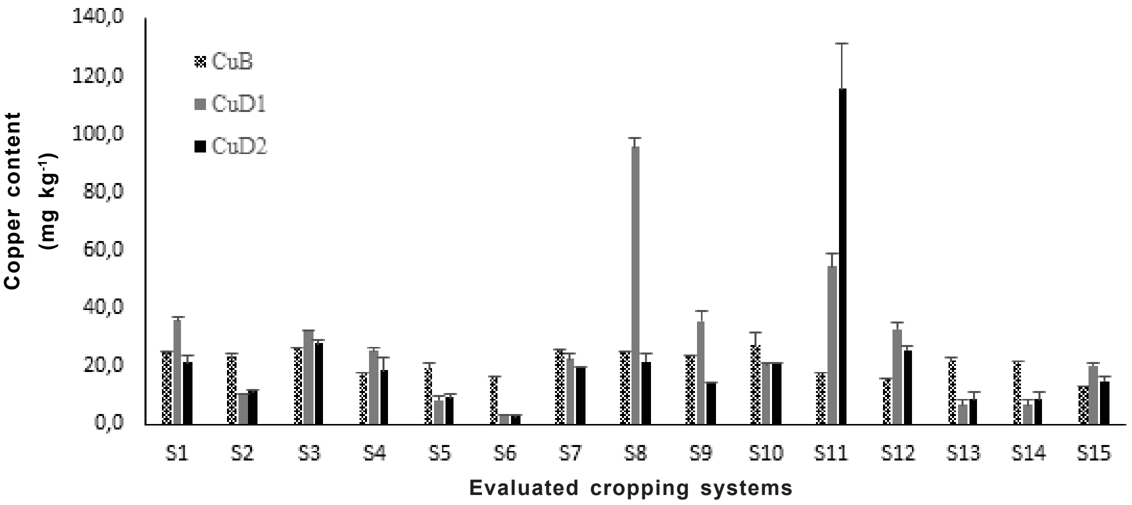

Figure 1. Content of copper (mg kg^{-1}) in the cacao beans (CuB) and in two soil depths, CuD1 (0-15 cm), and CuD2 (35-50 cm) in 15 cacao cropping systems in Southeast Bahia, Brazil.

Table 4. Linear correlation between the metal contents in two soil depths, D1 (0-15 cm) and D2 (35-50 cm), from 15 sites in Southeast Bahia, Brazil

	CuD1	CdD1	BaD1	PbD1	CuD2	CdD2	BaD2	PbD2
CuD1	1	0.60*	0.19 ^{ns}	0.57 *	0.45 ^{ns}	0.43 ^{ns}	0.03 ^{ns}	0.41 ^{ns}
CdD1		1	0.51 *	0.75 *	0.52 *	0.71 *	0.31 ^{ns}	0.61 *
BaD1			1	0.09 ^{ns}	0.01 ^{ns}	0.39 ^{ns}	0.92 *	0.07 ^{ns}
PbD1				1	0.34 ^{ns}	0.37 ^{ns}	-0.04 ^{ns}	0.71 *
CuD2					1	0.83 *	-0.02 ^{ns}	0.65 *
CdD2						1	0.33 ^{ns}	0.74 *
BaD2							1	0.07 ^{ns}
PbD2								1

* significant ^{ns} not significant. Values equal or above 0.5 are considered significant correlation.

Table 5. Metal content in cacao beans (mg kg^{-1}) in 15 sites in Southeast Bahia, Brazil

Site	Cu	Cd	Ba	Pb*
1	24.60 AB	0.45 DEF	4.20 CDE	< 4.3
2	23.10 AB	0.30 EF	3.20 CDE	< 4.3
3	25.90 A	0.25 F	2.30 E	< 4.3
4	17.25 AB	0.70 CDEF	6.45 BCD	< 4.3
5	19.35 AB	0.60 CDEF	5.65 BCDE	< 4.3
6	16.20 AB	0.50 CDEF	2.75 CDE	< 4.3
7	25.25 A	1.35 A	4.65 CDE	< 4.3
8	24.95 AB	0.30 EF	2.65 DE	< 4.3
9	23.50 AB	0.50 DCDEF	3.45 CDE	< 4.3
10	27.10 A	0.30 EF	8.90 AB	< 4.3
11	17.70 AB	0.60 CDEF	11.05 A	< 4.3
12	15.40 AB	0.75 BCDE	11.40 A	< 4.3
13	22.05 AB	0.90 ABCD	6.50 BC	< 4.3
14	21.40 AB	0.95 ABC	4.95 CDE	< 4.3
15	12.97 B	1.20 AB	8.85 AB	< 4.3

Means followed by the same letters in the column are not statistically different by the Scheffé Test 5%.* below the detection content

The soil Cd content was higher at D2 than in D1 in 3 out of 15 cropping systems, in only one site was Cd content higher in D1, in the remaining 11 sites there were no statistically significant differences between the studied soil layers (Table 3).

Cd is easily absorbed and translocated through plant xylem (She et al., 2018), in the biogeochemical cycling it is transported between soil and plant and *vice-versa*. It can be extracted from the system *e.g.* if allocated in harvested plant parts. In the soil it is highly adsorbed to the organic matter and its retention correlates with soil pH, Cation Exchange Capacity (CEC), and specific surface (Tahervand and Jalali, 2016; Yang et al., 2018).

Higher Cd accumulation in D2 layer could be from natural origin rather than any of the anthropogenic activities adopted in the cropping systems. The topsoil layer of site 12 had the highest Cd content (Table 3 and Figure 2) and among the Cd contents at the

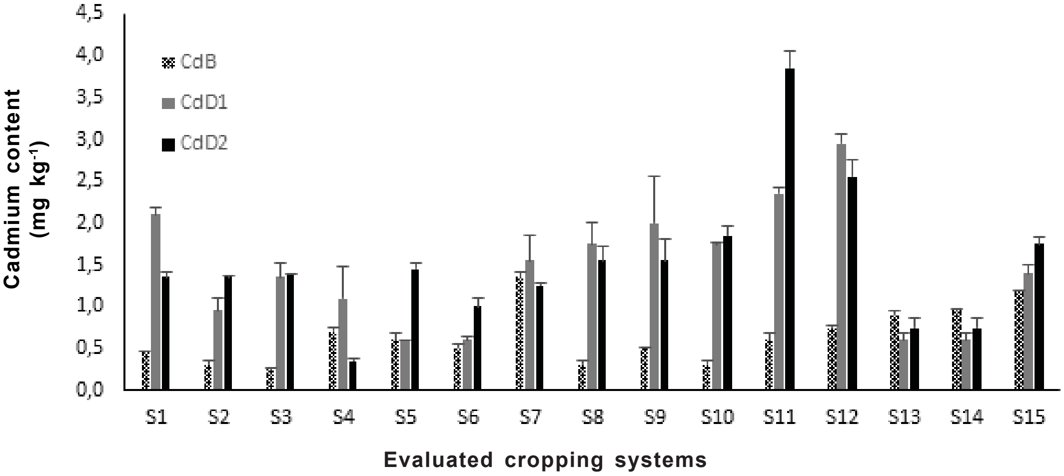

Figure 2. Content of cadmium (mg kg^{-1}) in the cacao beans (CdB) and in two soil depths, CdD1 (0-15 cm), and CdD2 (35-50 cm) in 15 cacao cropping systems in Southeast Bahia, Brazil.

deeper layer the site 11 presented the highest content. Site 11 has a partially weathered soil (Dystropept) suggesting the effect of the local lithology on soil Cd content. This site also presented one of the higher Cd contents in the top layer of the soil (Table 3). This could be caused by the fact that this site was the only one that received phosphorus in the form of ground natural rock phosphate (unpublished data), which is recognized for containing naturally high levels of Cd (Roberts, 2014). Also, litterfall in the biogeochemical cycling may influence the Cd content in the surface soil layer, as shown by the high positive correlation between the Cd content in D1 and D2 (Table 4). Mineral phosphate fertilizers are also recognized as source of Cd (Bizarro, Meurer and Tatsch, 2008) and were used in most of the sites (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14) (Table 2).

The element Ba showed statistically higher content in the top layer of the soil (Table 3 and Figure 3), in six sites (7, 8,10,12,13 and 14). Site 12 had the highest content in the topsoil layer, and site 15 had high content in both soil layers. Ba is usually associated with soil K content due to their similar ionic radii, and thus usually is present in soils with high occurrence with minerals like alkali feldspar and biotite (Madejón, 2013). However, in these cropping systems the detailed information on the nature of mineralogy was not done to confirm such hypothesis. Ba content in D1 highly correlates with its content in D2 (Table 4) suggesting that the occurrence of this heavy metal in D1 is highly dependent on the lithology and the sourcing through litterfall.

Soil contamination with Pb is a cumulative process, usually irreversible, which makes the increase of the content and the persistent availability for plant absorption (Demir, Pamukcu and Shrestha, 2015; Fabian, Reimann and Caritat, 2017). Except for cropping system 1, in all the other cropping systems higher Pb contents was recorded in subsurface soil layer (CdD2) (Table 3 and Figure 4), and statistically higher Pb levels were observed in D2 in the sites 2, 5, 6, 10, 11, 15. Sites 1 and 3 stand out by the high and similar contents in both layers. The sites 1, 2, and 3 deserve a special consideration due to the high soil Pb contents, and to the existing similarities among these cropping systems with regards to nature of shade trees present, lithology and proximity to each other (Table 1, 2 and 3). Pb content in D2 highly correlates with Pb content in D1 (Table 4), suggesting the effect of litterfall sourcing this element to D1 from D2, through biogeochemical cycling. Pb content in D2 also showed high positive correlation with Cd content in D2 and in D1, and Cu content in D2, but does not show high correlation neither positive nor negative with Cu content in D1, and Ba content in D1 and D2 (Table 4). Pb content in D1 does not show high correlation with any of the other potentially toxic elements evaluated in D2 layer, except with itself, however Pb content in D1 showed high positive correlation with Cu content in D1 and Cd content in D1 layer (Table 4).

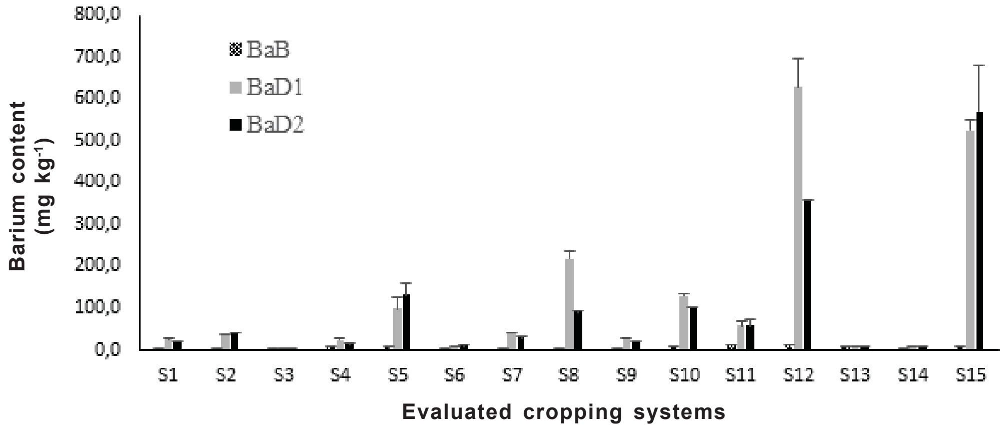

Figure 3. Content of barium (mg kg^{-1}) in the cacao beans (BaB) and in two soil depths, BaD1 (0-15 cm), and BaD2 (35-50 cm) in 15 cacao cropping systems in Southeast Bahia, Brazil.

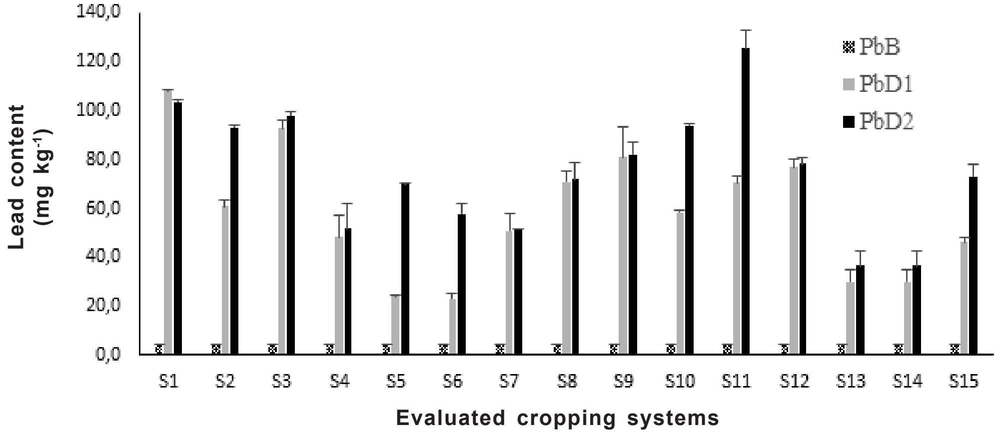

Figure 4. Content of lead (mg kg^{-1}) in the cacao beans (PbB) and in two soil depths, PbD1 (0-15 cm), and PbD2 (35-50 cm) in 15 cacao cropping systems in Southeast Bahia, Brazil.

In general, based on the higher soil heavy metals contents, special attention should be considered with the sites: 1 ,2, 3 and 9 (Pb), 11 (Cu, Cd, Pb), 12 (Cd, Ba) and 15 (Ba). Sites 11 and 15, both organically farmed, were situated on the youngest soils and derived from lithology that might be sourcing many mineral elements (desirable or undesirable), and the site 12 had possible effects of high inputs used in adopted conventional agricultural production system (Table 1 and 2).

Most plant species grown in soils contaminated with heavy metals can only limit partially the uptake and translocation of these elements from the root system towards the shoot (Baker, 1981). Cacao tree can absorb heavy metals from soil and accumulate them in seeds (Arévalo-Gardini et al., 2017; Lewis et al., 2018), producing risks to the human consumption of chocolate and other derived products due to the close relation between chemical composition of the beans and quality of the derived products (Bertoldi et al., 2016).

The site 10 presented the highest content of Cu in the beans (27.1 mg kg^{-1}) (Table 5), statistically equal to the sites 7 (25.2 mg kg^{-1}) and 3 (25.9 mg kg^{-1}). The smallest Cu content in beans was observed at site 15 (12.9 mg kg^{-1}). There was no significant correlation observed between the soil Cu content and the Cu content in the beans (Table 6).

The highest content of Cd in beans was found at the site 7 (1.35 mg kg^{-1}), within the range reported by Chavez et al. (2015) in cacao beans from Equador; Ramtahal et al. (2016) in nibs from orchards in Trinidad and Tobago; Gramlich et al. (2018) in cacao beans from Honduras; Arévalo-Gardini et al. (2017) in cacao

Table 6. Linear correlation between the metal contents in cacao beans (B) and in soil (S) in two soil depths (D) in 15 sites in Southeast Bahia, Brazil

	$Cu_{(S)}$	$Cd_{(S)}$	$Ba_{(S)}$	$Cu_{(B)}$	$Cd_{(B)}$	$Ba_{(B)}$
D_1 (0-15 cm)						
$Cu_{(S)}$	1	0.56 *	0.16 ^{ns}	0.23 ^{ns}	-0.27 ^{ns}	-0.03 ^{ns}
$Cd_{(S)}$		1	0.48 ^{ns}	0.01 ^{ns}	-0.32 ^{ns}	0.49 ^{ns}
$Ba_{(S)}$			1	-0.54 *	0.32 ^{ns}	0.59 *
D_2 (35-50 cm)						
	$Cu_{(S)}$	**$Cd_{(S)}$**	**$Ba_{(S)}$**	**$Cu_{(B)}$**	**$Cd_{(B)}$**	**$Ba_{(B)}$**
$Cu_{(S)}$	1	0.83 *	-0.05 ^{ns}	-0.12 ^{ns}	-0.05 ^{ns}	0.51 ^{ns}
$Cd_{(S)}$		1	0.31 ^{ns}	-0.14 ^{ns}	-0.06 ^{ns}	0.63 *
$Ba_{(S)}$			1	-0.61 *	0.44 ^{ns}	0.56 *

* significant ^{ns} not significant.

samples from Peru; and Zarcinas et al. (2004) in cacao beans produced in Malaysia, while the smallest bean Cd content was found in the site 3. As with Cu, there was no significant correlation observed between the soil Cd content and the Cd content in the beans (Table 6). In a study performed in Trinidad and Tobago, a significant ($P < 0.05$) correlation was found between leaf Cd content and soil Cd content, for both soil Cd extractants, DTPA ($r = 0.66$) or Mehlich 3 ($r = 0.66$), but the relationship was not significant when the soil extractant was aqua regia, like the one that was used in the current study, or nitric acid (Lewis et al., 2021). In the Trinidad and Tobago study, DTPA or Mehlich 3 extracted significantly ($P < 0.05$) lower amounts of Cd from the soil compared to nitric acid or aqua regia, suggesting that these last two extractors extract Cd fractions from soil that are not available for absorption by cacao plants (Lewis et al., 2021).

The higher contents of Ba in the beans were found at the sites 12 (11.40 $mg\ kg^{-1}$) and 11 (11.05 $mg\ kg^{-1}$) similar to those values reported by Bertoldi et al. (2016). The smallest Ba content was found at the site 3 (2.30 $mg\ kg^{-1}$) and was well below to the values verified by Bertoldi et al. (2016) in cacao beans. The Ba values found in the current study were much lower than the values reported in Brazil nuts (96 to 1990 $mg\ kg^{-1}$) (Parekh et al., 2008), and similar to the values reported for cereals (6 $mg\ kg^{-1}$) (Kabata-Pendias and Pendias, 2001). Positive correlation was observed between Ba content of soil and beans, and a slightly high significant negative correlation was observed between Ba content in soil and Cu content in beans in both soil depths. Also, the content of Ba in beans presented a positive correlation with the content of Cd in soil but only in the deeper evaluated soil layer (Table 6).

By the adopted analytical method, it was not possible to identify Pb presence in cacao beans, as levels of Pb were lower than detectable levels by the ICP instrument used.

Considering the sites 14 and 15, which were planted with same cacao type-Comum (Forastero) and with similar cropping aspects, but these sites differed in soil types, Hapludox versus Hapludalf, a further comparison was done (Table 7). The cacao beans of Forastero trees grown in a Hapludox (site 14) showed higher Cu contents than those from trees grown in a Hapludalf soil (site 15) (Table 7), possibly since the cacao trees in site 14 were cropped by a conventional method while the trees grown in site 15 were organically farmed. On the other hand, beans from site 15 showed higher content of Cd and Ba, suggesting the effect of the soil age, or the presence of source material close to the surface, on the availability of minerals like Ba, as identified in this study (Table 1, 2 and 3).

In another comparison (Table 8), cacao trees of PH-16 were grown in site 13 and Forastero grown on site 14, both sites had Hapludox soil and Cabruca

Table 7. Content of metals ($mg\ kg^{-1}$) in Comum cacao beans in two sites in Southeast Bahia, Brazil

Site	Soil	Cu	Cd	Ba	Pb*
14	Hapludox	21.40 A	0.96 B	4.96 B	< 4.3
15	Hapludalf	12.96 B	1.20 A	8.86 A	< 4.3
C.V.%		5.7	3.7	5.6	-

Means followed by the same letters in the column are not statistically different by the Scheffé Test 5%. C.V. = coefficient of variation. * below the detection content.

Table 8. Content of metals ($mg\ kg^{-1}$) in cacao beans of different genetic material at the same soil type (Hapludox) in Southeast Bahia, Brazil

Site	Genetic Material	Cu	Cd	Ba	Pb*
13	PH-16	22.05 A	0.90 A	6.50 A	< 4.3
14	Comum cacao	21.40 A	0.95 A	4.95 B	< 4.3
C.V.		7.28	8.74	9.55	-

Means followed by the same letters in the column are not statistically different by the Scheffé Test 5%. C.V. = coefficient of variation. * below the detection content.

cultivation system (Table 1 and 2). The Ba content of beans of PH-16 were higher than beans of Forastero while Cu and Cd contents were not statistically different. This shows the possible existence of cacao varietal differences in accumulation of heavy metals.

The results showed in this study are from a broad and first evaluation that deserves further and deeper analysis of differences imposed by soil types, cacao varieties and adapted management systems on accumulations of heavy metals by cacao beans.

Conclusion

The conventional cacao cropping methods usually increase the heavy metal (such as Cu and Ba) content in the top layer of the soil, this could be the result of adapting chemical sprays to control disease, insect and weeds and applications of inorganic fertilizers which contain these heavy elements, and the possible excess will end up on top of the soil surface.

The heavy metal Pb and Cd are usually influenced by the lithology, showing generally higher content in the deeper layer of the soil, however some of the results of this study suggest the effect of anthropogenic action and higher accumulation of these elements in the top layer of the soil. Young soils have higher content of Cu, Cd and Pb through their profile. The cacao clone PH-16 accumulated more Ba in the beans than the beans of Comum 'forastero' cacao.

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