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Cd and Pb reduction in cocoa (*Theobroma cacao*) nib using two organic amendments

Reducción de Cd y Pb en la almendra de cacao (*Theobroma cacao*) utilizando dos enmiendas orgánicas

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Abstract In Peru, cocoa (*Theobroma cacao*) cadmium (Cd) and lead (Pb) content may exceed the established limits for international trade and human consumption. Most farmers in the Peruvian jungle generally do not fertilise their cocoa crop parcels with organic amendments (OA). Therefore, the objective of this study was to evaluate the application of compost and poultry manure in the doses of 30, 60 and 90 MT ha^{-1} in cocoa plantations to reduce Cd and Pb content in the cocoa nib. The study was conducted from February to November 2015, in Huánuco, Peru. The textural class of soil was loose silty clay, so it did not change after 9 months of applying the OA. The application of OA decreased available Cd and Pb content in soil over 75% and 98%, respectively. Likewise, the total content of Cd and Pb in cocoa nib decreased more than 95% and 67%, respectively ($P < 0.05$). Therefore, the OA could be a good alternative to reduce Cd and Pb in the cocoa nib.

Key words: *cocoa; cadmium; lead; compost; poultry manure.*

Resumen En Perú, el contenido de cadmio (Cd) y plomo (Pb) en cacao (*Theobroma cacao*) puede exceder los límites establecidos para el comercio internacional y el consumo humano. La mayoría de los agricultores en la selva peruana generalmente no fertilizan sus parcelas de cultivo de cacao con enmiendas orgánicas (EO). Por lo tanto, el objetivo de este estudio fue evaluar la aplicación de compost y estiércol de aves de corral en las dosis de 30, 60 y 90 MT ha^{-1} en las plantaciones de cacao, para reducir el contenido de Cd y Pb en la almendra. El estudio se realizó de febrero a noviembre de 2015, en Huánuco, Perú. La clase de textura del suelo era arcilla limosa suelta, sin cambios después de 9 meses de aplicación de EO. La aplicación de EO disminuyó el contenido de Cd y Pb disponible en el suelo en más del 75% y 98%, respectivamente. Asimismo, el contenido total de Cd y Pb en la almendra de cacao disminuyó más de 95% y 67%, respectivamente ($P < 0.05$). Por lo tanto, la EO podría ser una buena alternativa para reducir Cd y Pb en la almendra de cacao.

Palabras clave: *cacao; cadmio; plomo; compostaje; estiercol de aves.*

Introduction

Cocoa (*Theobroma cacao*) is the main ingredient for chocolate elaboration, Peru is one of the main suppliers of fine flavour cocoa and the world second producer of organic cocoa with markets in Indonesia, Holland, Italy and Spain (MINAGRI, 2019). In Peru, cocoa crop is a sustainable alternative in regions such as Amazonas, Cajamarca, San Martin and Huánuco, where it represents 56% of the Peruvian cocoa production (estimated at 71,838 MT) (OEEE-MINAG, 2013).

Toxic elements like cadmium (Cd) and lead (Pb) are found in Cocoa derived products (Abt et al., 2018). The European Union (EU) has regulated heavy metals content in food and established a maximum limit of 0.1- 0.8 mg kg^{-1} of Cd in chocolate (depending on the cocoa content). Such regulation came into forces in January 2019 (EU Regulation N° 488/2014). Likewise, European importers of dry cocoa nibs have considered a Cd maximum content of 0.5 mg kg^{-1} (CBI, 2018). While no PB limits have been established in cocoa products, its control is becoming more important since origin could proceed from i) contaminated soils or ii) dust during cocoa beans drying on unprotected fields (Abt et al., 2018).

Exposure to Cd and Pb affect human health for their carcinogenic and genotoxic effects. Cd content in cocoa nib from some areas in Peru exceeded the 0.5 mg kg^{-1} limit stablished by the EU cocoa (CBI, 2018; Arévalo-Gardini et al., 2017; Huamaní-Yupanqui et al., 2012).

For several decades, organic residues have been employed as amendments for soil fertilization to nourish plant and improve agriculture productivity. Organic amendments (OA) from poultry, swine, sheep and cattle farms have been used to immobilize heavy metals effectively on contaminated soils by means of having them less available for plant absorption (Liu et al, 2009). However, most cocoa farmers in Peru, generally do not fertilise their parcels with OA, unlike Andean farmers who use OA, mainly livestock manure as fertilizer. In that sense, the presence, absorption and accumulation of heavy metals by cocoa plants may be related to soil characteristics such as pH, soil organic matter (OM) content, soil cation exchange capacity (CEC), among others (Chavez et al., 2016; Huamaní-Yupanqui et al., 2012), and these characteristics can be modified by OA application.

OA consist of humic substances (humina, humic and fulvic acids) and non-humic substances (cellulose, lignin and proteins), that can retain the heavy metals in soil (Chavez et al., 2016; Liu et al., 2009) and decrease their bioavailability for cocoa crops. OA application to cocoa trees could be an alternative to reduce Cd and Pb bioavailability from soil and uptake, lowering their content in cocoa nibs.

Therefore, the objective of this research was to evaluate the application of two OA (compost and poultry manure) in cocoa crops to reduce Cd and Pb content in cocoa (*Theobroma cacao*) nib.

Material and methods

Site description

The research was conducted in a private cocoa parcel, located near Tingo María city (latitude: -9.2953196 and longitude: -75.9957428), Huánuco-Peru, at 740 m a.s.l., with 8 year- old CCN-51 cocoa tree clones which were obtained by grafting the CCN-51 clone on the stock of another CCN-51 tree. This agricultural zone has a slope of 2%. The study was conducted from February to November 2015, with meteorological monthly means of 219 mm for precipitation, 25.5 °C for temperature, 83.4% for relative humidity and 158 sunshine hours.

Organic amendments

As an alternative to reduce Cd and Pb content in cocoa nib, soil application of two OA types during the cocoa cropping period (9 months) was tested: i) compost, a dry product from local plant residues; and ii) poultry manure (PM), a wet product from bird droppings from a nearby poultry centre. Both OA are usually found and elaborated in the experimental area and their chemical composition was analysed in duplicate in the Universidad Nacional Agraria de la Selva (UNAS). Analyses made were: i) dry matter content by drying in an oven at 101 °C); ii) ash, by calcination in muffle at 550 °C; iii) OM, obtained by difference of moisture content and ash percentage; iv) carbon (C), obtained as a ratio of OM and 1.724 (conversion factor according to Jackson, 1964); v) nitrogen (N), by acid digestion in Kjehdal equipment; vi) phosphorus pentoxide (P_2O_5) (Olsen et al., 1954); and vii) K, Cd and Pb were quantified in total form by acid digestion, quantified by atomic absorption spectrophotometry (FAO, 1996).

Experimental design

The field experimental area (5,040 m²) was divided in 28 sublots of 180 m² each (15x12 m) with 20 cocoa trees (3 m among trees) (Figure 1). OA was applied using a randomized complete block design with 7 treatments (two OA with three doses each and one control), every treatment had four repetitions (Table 1). No OA was applied to Control (T_1). Due to the low nitrogen content in compost and low C:N rate for PM and knowing also the poor practice of farmers to apply OA in the study area and in general in the jungle, it was decided to test high OA doses as 30, 60 and 90 MT ha⁻¹, similar to some doses employed by Melero et al. (2007).

Table 1. Applied organic amendment dose

Treatment	Organic amendment	Dose	
		Kg tree⁻¹	MT ha⁻¹
T_1	Control	0	0
T_2	Compost	27	30
T_3	Compost	54	60
T_4	Compost	81	90
T_5	Poultry manure	27	30
T_6	Poultry manure	54	60
T_7	Poultry manure	81	90

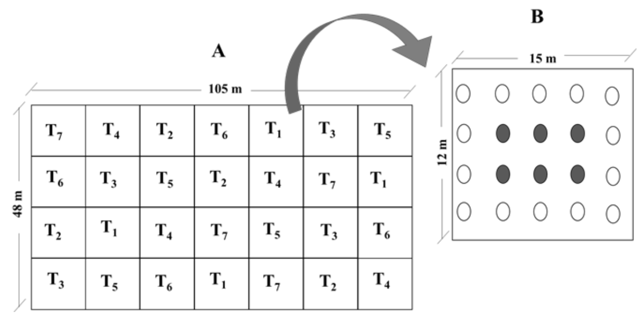

Figure 1. Cocoa growing area. A) Distribution of seven treatments (four replicates for each treatment); B) Distribution of cocoa trees for each replicate

Soil and cocoa nib before applying the OA (conditions at initiation of the study), was considered as "T_0". Two months before applying OA in the study area, all cocoa pods were harvested. Then, in a single day, each doses of OA (T_2 – T_7) was applied manually below the top of each tree. Fertilization with macronutrients was also applied (except T_1), consisting of seabird manure and Sulpomag K-Mag® to reach the fertilization formula 60:70:60. Likewise, Borosoil Ulexita® (20 kg ha⁻¹), zinc sulphate, iron sulphate and copper sulphate (5 kg ha⁻¹ each) were used as micronutrients. The soil surface for all treatments was covered with dry leaves and each treatment area was labelled with different coloured ribbons. During the 9-month cultivation period, time necessary for the growth and maturation of cocoa pods, the removal of herbs, pruning, the collection of diseased fruits, among others, was carried out.

Soil analysis

Before applying OA (T_0), six samples of 800 g each of soil were taken from the total study area (5,040 m²) at a depth of 20 cm. Nine months after applying OA (T_2-T_7) with the control (T_1), and after harvesting the cocoa pods, a soil sample of 800 g was collected at 20 cm deep from each repetition (four repetitions per treatment) of each of the 7 treatments. An internal control sample (10 kg) for available Cd and Pb of soil was prepared with the field soil before OA application. Soil control sample was thoroughly mixed and quantified for available Cd and Pb, containing 0.48 mg kg⁻¹ and 0.13 mg kg⁻¹, respectively. This control sample was assayed every 10 samples. All soil samples were kept isolated and analysed separately.

Extraction of soil samples and exploration of the study site was done in zigzag. Dried leaves were removed previously to soil sampling. All soil samples were dried under shade, packed and sent to UNAS laboratory, taking care not to contaminate the samples and using clean materials always. Briefly, samples were ground and sieved in a 2 mm mesh screen, then, the following analyses were carried out: i) soil texture by the Bouyoucos hydrometer method (1962); ii) pH, measured with a potentiometer in a soil suspension and water (1:1); iii) OM by wet oxidation with potassium dichromate in acid medium (Walkley and Black, 1934); iv) cation exchange capacity (CEC) by the ammonium acetate method 1 N, pH: 7.0 (for soils with pH > 5.5) and effective cation exchange capacity (eCEC) by KCl 1 N method (for soils pH < 5.5) (Anderson and Ingram, 1993); v) available Cd and Pb, according to González (1986), treating the soil sample (5 g) with 20 mL EDTA (0.05 M; pH 7), diluting with HCl (0.1 N) before reading absorbance by atomic absorption spectrophotometry (Agilent®, 55B AA model). All samples were analysed by duplicate. Quality assurance of the results were checked by establishing: i) difference among results of two determinations carried out by the same worker, must be less than 0.05 mg kg^{-1} and 0.08 mg kg^{-1} for Cd and Pb assays, respectively; ii) quantification limit for each method was 0.01 mg kg^{-1} and 0.07 mg kg^{-1}, for Cd and Pb assays, respectively; iii) recovery percentage of the addition method (where a known concentration is added to sample) was set from 80% to 110% for both, Cd and Pb.

Pods physical composition

Physical composition of cocoa pods was obtained from 20 fresh mature pods collected randomly from the study area (5,040 m^2) and were sent to ITP (Instituto Tecnológico de la Producción). Each pod was weighed individually (total weight), cut and separated into skin, mucilage, testa, placenta and nib (without testa); subsequently, each part was weighed separately and calculated as percentage of the total weight. Likewise, based on the methodology described in FAO (1996) the following assays were also determined in each part of each cocoa pod: i) moisture content by drying at 101 °C, ii) fat by extraction with diethyl ether in Soxhlet equipment and iii) ash by calcination in a muffle at 550 °C.

Cocoa nib analysis

Pod harvest was done in 2 days, where 16 cocoa pods were collected from 6 trees located in the central part of each repetition of each treatment (Figure 1b). 16 cocoa pods were stored and assayed separately. The cocoa nib (naturally embedded with mucilage) was extracted from pods and dried directly in the sun for 4 days without fermentation. Then, through precise and superficial cut in the dried cocoa beans, testa was removed, to finally obtain the cocoa nib (around 1 kg from 16 cocoa pods) which was assayed in duplicate in UNAS laboratory. Analyses included: i) moisture, determined by oven drying at 101 °C; ii) ash, obtained by calcination in a muffle furnace at 550 °C; iii) OM, collected by difference subtracting the moisture and ash content; iv) nitrogen (N), by acid digestion in Kjeldahl equipment and v) total Cd and Pb content by calcination (10 g cocoa nib), dilution with HCl (0.1 N) and quantification by atomic absorption spectrophotometry according to AOAC 999.11 (2002), where quality assurance of the results was checked as in soil analysis. In addition, for assuring the quality of the results of the cocoa nibs assayed in UNAS, some cocoa nibs samples (with known results) were sent, assayed and verified in ITP physicochemical laboratory, where methods are accredited for total Cd and Pb in bivalves under ISO-IEC 17025: 2005 (Barriga and Aranda, 2018) by atomic absorption spectrophotometry (Perkin Elmer®, Analysis 800, USA).

Data analysis

The statistical program SPSS version 18 (IBM Corp., NY, USA) was used to perform variance analysis (ANOVA) of one factor. The two means of organic amendments chemical composition were compared by least significant difference (LSD) test at a significance level ($P < 0.05$) and means for other analyses were compared by the Duncan test for multiple comparisons at a significance level ($P < 0.05$).

Results

Fresh cocoa pods characterisation

Cocoa pods had an average weight of 704.8 ± 81.0 g (n = 20). Regarding the whole pod of fresh cocoa (not fermented), the skin represents more than 75% (w/w), constituting the main residue of the cocoa activity; while the nib (without testa) is main part that enters in the chocolate production, represents about 11% (w/w) (Table 2). In addition, the mucilage with testa and placenta account for about 10% (w/w). The nib had a lower moisture content and a higher fat content (more than 30%), compared to other parts of the cocoa pod (around 1%) ($P < 0.05$).

Organic amendment

Table 3 shows OA chemical composition. Under the conditions used, the compost presented a higher dry matter, OM and ash content ($P < 0.05$) than PM. N content in the compost (0.71%) was lower ($P < 0.05$) than PM (2.68%), and for this reason the compost has a C:N rate nearly 4 times higher than the PM.

Soil physical composition

Physical composition of soil before (T_0) and 9 months after applying compost and PM (T_1-T_7) in different concentrations in the cocoa farming is presented in Table 4. There was no significant difference ($P > 0.05$) in the physical composition of soil, before and after applying OA; in both cases, the soil presented a textural class of loose silty clay, formed mainly by silt (around 43%), followed by clay and sand.

Chemical analysis of the soil

Soil pH was 5.92 before OA application (T_0); after applying organic amendments and 9 months after the cultivation time, soil pH decreased around 1 unit (with exception of T_3 and T_7), where T_1 had a pH (4.73) lower than other treatments. However, differences recorded for all treatments were no significant ($P > 0.05$) (Table 5).

In relation to the initial soil condition (T_0), soil OM content increased in all the treatments with OA (T_2 to T_7) and also in the control (T_1), but the increase was not significant ($P > 0.05$). Treatments T_2, T_5, and T_6 showed increments of OM higher than other treatments. Before applying OA (T_0), the CEC was 10.03 mEq 100 g soil^{-1}, whereas after applying OA, the eCEC of soil showed highs and lows, where T_2 y T_7 decreased the eCEC, whereas the T_1, T_3, T_4, T_5 and T_6 increased the eCEC, in relation to T_0; however, these slight variations in eCEC were not significant ($P > 0.05$). In short, in spite of applying high doses of OA in the study area

Table 2. Physical composition and moisture, fat and ash content of cocoa pods (n = 20)

Parts	Physical (W/W) (%)	Moisture (%)	Fat (%)	Ash (%)
Skin	76.4^a (3.9)	83.26^a (2.05)	1.40^a (0.35)	1.78^a (0.39)
Mucilage with testa	7.7^b (1.4)	77.6^b (2.45)	0.38^b (0.10)	0.68^b (0.07)
Placenta	1.9^c (0.8)	77.07^b (1.68)	1.15^a (0.14)	1.42^a (0.39)
Nib (without testa)	10.8^b (2.0)	32.82^c (1.95)	33.30^c (1.96)	2.58^c (0.25)

Different superscript letters in the same column indicate statistically significant difference ($P < 0.05$).

Table 3. Chemical composition of Compost and Poultry manure

	Compost	Poultry manure
Dry matter (%)	98.46^a (0.54)	42.20^b (0.98)
Ash (%)	68.72^a (1.46)	19.05^b (0.43)
Organic matter (%)	29.71^a (1.92)	23.63^b (1.87)
C (%)	21.65^a (2.05)	18.80^a (1.13)
Nitrogen (%)	0.71^a (0.06)	2.68^b (0.13)
Relation C/N	30.47^a (0.46)	7.03^b (0.07)
Phosphorus (%)	0.05^a (0.01)	2.42^b (0.16)
Potassium (%)	2.11^a (0.13)	10.65^b (0.35)
Cadmium (mg kg^{-1})	0.01^a (0.00)	0.01^a (0.00)
Lead (mg kg^{-1})	8.45^a (0.64)	5.68^b (0.04)

Different superscript letters in the same row indicate statistically significant difference ($P < 0.05$). Average of 2 results.

Table 4. Physical composition of soil in the study site of cocoa crops

Treatment	Sand (%)	Clay (%)	Silt (%)	Textural class
T_0*	25.33[a] (4.53)	30.67[a] (3.08)	44.00[a] (2.52)	Loose silty clay
T_1	25.50[a] (6.61)	31.50[a] (1.00)	43.00[a] (3.73)	Loose silty clay
T_2	27.50[a] (3.79)	30.00[a] (2.58)	42.50[a] (3.42)	Loose silty clay
T_3	27.00[a] (3.83)	29.50[a] (2.65)	43.50[a] (3.79)	Loose silty clay
T_4	23.00[a] (3.46)	32.50[a] (2.52)	44.50[a] (3.79)	Loose silty clay
T_5	25.00[a] (3.46)	30.50[a] (1.91)	44.50[a] (3.00)	Loose silty clay
T_6	24.50[a] (1.91)	31.00[a] (1.63)	44.50[a] (1.00)	Loose silty clay
T_7	25.50[a] (2.52)	31.00[a] (1.63)	43.50[a] (1.91)	Loose silty clay

Same superscript letters in the same column indicate no statistically significant differences ($P > 0.05$).
(*) Soil before applying organic amendments

Table 5. Chemical analysis of the soil in the study site of cocoa crops

Treatment	pH	OM (%)	eCEC (mEq/100 g)
T_0	5.92[a] (0.48)	2.24[a] (0.67)	10.03[a] (3.38) *
T_1	4.73[a] (0.25)	2.83[a] (0.94)	11.05[a] (4.12)
T_2	4.87[a] (0.78)	3.74[a] (1.84)	8.96[a] (1.69)
T_3	4.97[a] (0.75)	2.44[a] (0.54)	11.73[a] (5.21)
T_4	4.83[a] (0.82)	2.52[a] (1.34)	10.79[a] (3.15)
T_5	4.81[a] (0.76)	3.82[a] (0.77)	12.01[a] (5.86)
T_6	4.79[a] (0.78)	3.44[a] (0.52)	12.91[a] (5.12)
T_7	4.98[a] (0.95)	2.29[a] (0.76)	9.66[a] (0.67)

Same superscript letters in the same column indicate statistically no significant difference ($P > 0.05$). (*) Unique value from CEC, for having a pH > 5.5

(T_2-T_7), this resulted in slight variations in pH, OM and eCEC in each treatment, however, this was not significant ($P > 0.05$) even for the control treatment (T_1).

Figure 3 shows the decrease of Cd and Pb available in the soil due to OA application. The available Cd and Pb content in the soil decreased significantly ($P < 0.05$) in all treatments (including T_1) with respect to T_0 (before using the OA) after 9 months of the harvest period. The contents of available forms of Cd and Pb decreased by more than 75% and 98%, respectively, with respect to its initial content (T_0).

Cocoa nib chemical analysis

Table 6 presents coca nibs chemical analysis, before and after OA application, where moisture content, ash, OM and N in all the treatments did not show significant differences ($P > 0.05$) before (T_0) and after (T_1-T_7) OA application. It can be inferred that the macromolecular components such as fat, carbohydrate and proteins, which make up the OM of the cocoa nib, were not affected by the addition of the OA in the soil.

Cocoa nibs Cd and Pb content

Figure 4 shows decrease of total content of Cd and Pb in the nib due to application of OA. After the cultivation period of 9 months, Cd and Pb achieved a significant decrease ($P < 0.05$) in the cocoa nib, with respect to the nib before the study (T_0). The total content of Cd and Pb decreased by approximately 95% and 67%, respectively, in cocoa nib, this indicates that the application of the OA (in the doses indicated in Table 1) allowed a significant decrease in these heavy metals in cocoa nib.

Discussion

Two OA (compost and PM) were applied at open field of cocoa plantations to evaluate its effect in the reduction of Cd and Pb content in cocoa nibs

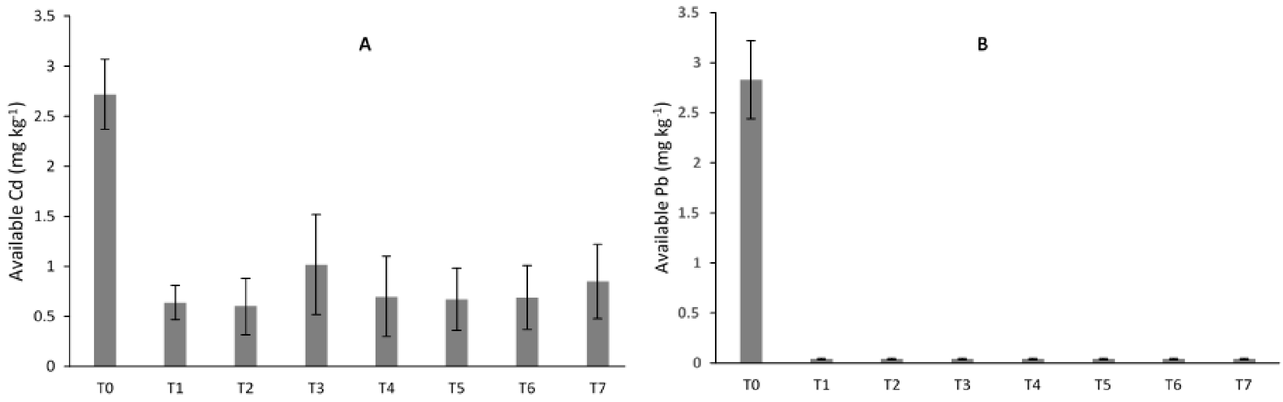

Figure 3. Decrease of Cd (A) and Pb (B) available in the soil

Table 6. Cocoa nibs chemical analysis

Treatment	Moisture (%)	Ash (%)	OM (%)	N (%)
T_0	6.01^{a} (0.93)	3.99^{a} (0.58)	90.23^{a} (1.19)	2.29^{a} (0.51)
T_1	6.51^{a} (1.05)	3.69^{a} (0.37)	90.32^{a} (1.11)	2.09^{a} (0.68)
T_2	6.06^{a} (0.76)	3.29^{a} (1.57)	90.65^{a} (0.98)	2.66^{a} (0.29)
T_3	6.32^{a} (1.16)	4.13^{a} (0.16)	89.86^{a} (1.22)	2.48^{a} (0.07)
T_4	6.10^{a} (1.17)	4.33^{a} (0.78)	89.56^{a} (1.69)	2.38^{a} (0.13)
T_5	5.50^{a} (1.49)	4.23^{a} (0.42)	90.27^{a} (1.10)	2.28^{a} (0.43)
T_6	4.95^{a} (0.83)	4.64^{a} (1.64)	90.13^{a} (1.68)	2.49^{a} (0.17)
T_7	7.23^{a} (2.92)	3.78^{a} (1.13)	88.99^{a} (2.32)	2.41^{a} (0.09)

Same superscript letters in the same column indicate no statistically significant difference ($P > 0.05$).

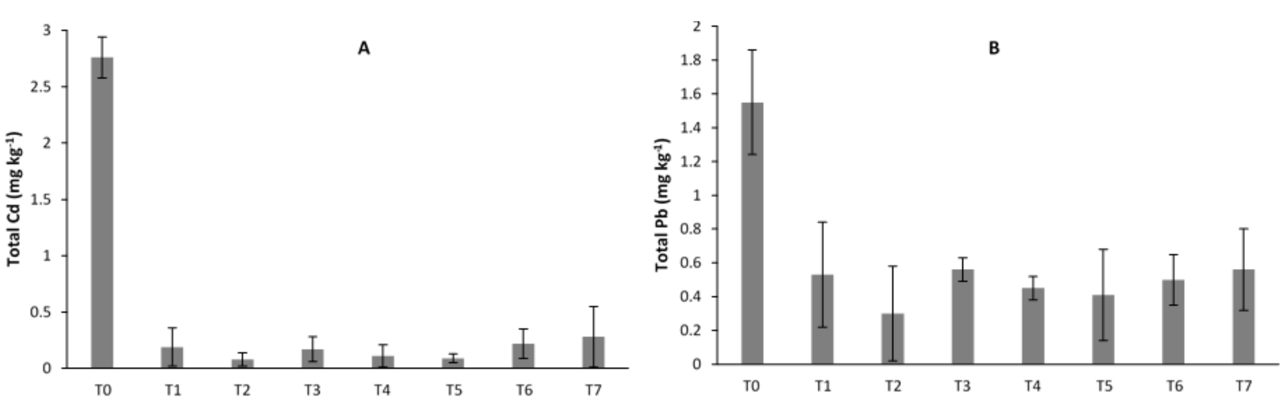

Figure 4. Decrease of total Cd (A) and Pb (B) in cocoa nibs

after 9 months of the crop period. C:N relation for compost was 30, having a higher degree of humification and resistance to microbial degradation (Bernal et al, 1998) than PM with a C:N relation of 7, similar to broiler poultry manure reported by Qian and Schoenau (2002) (C:N 7.6). N content for PM was 4 times higher than compost and this could be contributing with mineral N to the soil system, favouring net mineralization (Jansson and Persson, 1982). Both OA are important nutrient source for cocoa plantation soil. As there are no strict criteria to assess a fertilizer or OA for crops that demand months or years, Flavel and Murphy (2006) suggested the use of C:N rate as an adequate predictor to assess the impact of amendments on the N cycle.

Due to the high humification degree of OM, compost is more resistant to microbial degradation than manure (Walker et al, 2003), this could indicate that the light soil pH decrease observed was due to the high microbial charge developed basically by PM since fetid odours were detected 5 days after its application. Tejada et al. (2006) reported that the soil microbial biomass in PM-amended soils was 37% higher than in compost-amended soils. The production of organic acids (aminoacids, glycine, cysteine and humic acids) during mineralization (amminization and ammonification) of organic materials by heterotrophs and nitrification by autotrophs could also explain the decrease in soil pH (Angelova *et al*, 2013). However, Liu et al. (2009) detected soil pH increase in 0.5 to 1.2 units after 4 months of wheat cultivation using chicken manure compost. The pH during the 9 months cultivation of the present study was not measured. According to the classification of Garrido (1993), in the present study, the pH of the soil (T_0) was acidic (pH of 5.5-6.5), whereas 9 months after applying the OA, the pH of all treated soils had a very acid grade (pH less than 5.0). Liu et al. (2009) reported a significant increase in soil OM after applying chicken manure compost in wheat (4 months) cultivated in greenhouse where rain could not remove or lixiviate the OA, these are very different conditions from this study in open field.

Respect to CEC in the soil, according to the classification of Garrido (1993), T_2 and T_7 were less than 10 mEq/100 g considered as very low level of eCEC, while T_1, T_3, T_4, T_5 and T_6 were from 10 to 20 mEq 100 g^{-1} considered as low level of eCEC (Table 5).

According to Walker et al. (2003) manure was more effective than compost with respect to decreasing heavy metal bioavailability in the highly Murcia contaminated soil. In this study, we have observed a significant decrease ($P < 0.05$) in the soil bioavailability of heavy metals, however we can no state that OA (compost or PM) was more effective in this action since there may be a mix of OA due to the lixiviation caused by the rain in the whole experimental field, even in the case of control treatment (T_1).

Respect to the non-significant difference obtained in soil chemical analysis (Table 5), this could be explained due to i) some result variability found in each treatment (seven treatments) reflected by the standard deviation (shown in all results) due to natural fluctuations registered in each repetition (four repetitions for every treatment) because this study was done on an open field of 5,040 m^2; and ii) the strong rains during the 9-month period that normally occurs in this zone and in general in all the Peruvian jungle, since the rain may have lixiviated or carried OA to other treated areas, even affecting the Control treatment (T_1). These facts may not lead to observe significant difference due to the OA effect. Respect to soil pH, our results differ from those reported by Liu et al. (2015) who mentions that in order to reduce the Cd absorption by plants, the soil pH need to increase with the increase of OM in the soil. Chavez et al (2016) reported the soil pH increase up to 60 days after application of vermicompost and zeolite on cocoa plantation soil, but on day 75 the soil pH decreased. In our study, we registered a slight soil pH change after 9 months of applying the organic amendments (compost and poultry manure). It is possible that after the application of the OAs an increase of pH the soil pH reduced with the increase of

OM in the soil. In general, we consider that OA decrease the bioavailability of Cd and Pb and its transfer to plants; however, this could not happen in all cases, because different types of soil respond differently, for example, Pinto et al. (2004) reported an increase in translocation of Cd from the root to the plant of sorghum with the application of OM, suggesting a larger Cd solubility inside the plant; in another study, a 3-fold increase in Cd concentration was observed in the sorghum bud with the use of OM (Pinto et al., 2005).

The composition of organic matter and the mineral phase of the soil specifically influence Cd speciation; for example, soils with high contents of organic matter or iron oxides adsorbed more Cd than those with significant amounts of 2:1 clay type, although they have high CEC (Reyes and Barreto, 2011). Pérez (2006) has studied the effect of adsorption on Cd, Cu, Pb and Zn simultaneously by organic matter in soils, suggesting that a higher content of organic matter increases the retention of Cd on Zn. This explains that the high applied dose of organic amendments (compost and poultry manure) in the cocoa crop causes the retention of heavy metals such as cadmium and lead, making them less absorbable by the cocoa tree.

Soil type also influences the effect of OM from amendments in the mobility and bioavailability of Cd and Pb. This is possibly due to the presence of microorganisms that develop during fertilisation and capture these metals, making them less available for absorption (Liu et al., 2009) by cocoa trees. Compost, which has a high content of humified organic matter, also could decrease the bioavailability of heavy metals by means of absorption and forming humic substances in soil (Shuman, 1999), lowering the heavy metal absorption by cocoa trees. Liu et al (2009) observed a significant decrease in soluble Cd in soil and in total Cd in wheat after 4 months of application of chicken manure compost on soil contaminated with Cd, where they argued that soluble/exchangeable Cd was converted to organic-bound and inorganic precipitates forms.

In summary, this study evidenced the Cd and Pb content of cocoa nibs decreased in all the treatments containing OA (T_2 – T_7) and in the control (T_1) after 9 months. Likewise, this study was performed in a real open field of cocoa plantation, consisted of 5,040 m^2 of unprotected field and without control of temperature, in a very humid tropical forest climate, where the rain promoted the mixing and lixiviation of the treatments in the experimental field.

Conclusion

Application of OA, such as compost and PM and after the cultivation period (9 months), allowed the significant decrease in the Cd and Pb content in cocoa nib. Organic amendments (compost and PM) applied at the indicated doses in cocoa tree plantations can be an effective alternative to decrease Cd and Pb content in cocoa nibs.

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