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ORIGINAL RESEARCH ARTICLE

Fractionation of heavy metals in soils cultivated with tobacco in Pinar del Río, Cuba

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ABSTRACT

Knowledge of the presence of heavy metals in soils of agricultural areas is important to prevent their accumulation in cultivated plants. The objective of the present investigation was to evaluate the total concentrations and fractions of heavy metals Cd, Pb, Zn, Fe, Mn, Ni, Cu, Cr and Co in the tobacco-growing area of Pinar del Río, Cuba and their relationship with the physicochemical properties of soil. For the study, 59 samples of three types of soils were collected at 20 cm depth. The pseudo-total concentrations of metals in the soils are low and lower than the prevention values registered for Cuban soils. In general, the heavy metals studied present a high affinity for the most stable fractions of the soil, which means a low risk of transfer to the tobacco crop or accumulation in groundwater. The pseudo-total concentrations of heavy metals were low, below the alert values established for soils in the region. The heavy metals studied were mainly associated with the residual fraction, the second fraction with the highest association with metals was that linked to manganese and iron oxides. The principal component analysis showed that their main source is pedogenetic and that these elements are closely related to cation exchange capacity and calcium content.

Keywords: Availability; Fractionation; Heavy Metals; Soil; Tobacco

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1. Introduction

Heavy metals (HMs) are chemical elements harmful to health that can enter the food chain with the capacity to accumulate in living organisms[1]. According to Kabata-Pendias[2] soils are the main source of HMs entering plants, both from natural and anthropogenic causes. Another source of accumulation of HMs are agrochemicals applied in soils[3], whose availability to plants is determined by the way it is found in the environmental reservoir[4].

In Cuba, there are studies on the presence of HMs in soils and their chemical fractionation[5]; however, the soils of the Southern Plain of Pinar del Río province, destined to tobacco (*Nicotiana tabacum* L.) cultivation, have been little studied. The present work aimed to evaluate the pseudo-total concentrations and geochemical fractionation of the HMs: Cd, Pb, Zn, Fe, Mn, Ni, Cu Cr and Co in this area and their relationship with the physicochemical properties of the soil cover.

2. Materials and methods

2.1 Study area and soil sampling

The research was carried out in tobacco growing areas in nine representative agricultural entities, located in the municipalities of Consolación del Sur, Pinar del Río, and San Juan and Martínez, belonging to the southern plain of Pinar del Río province (**Figure 1**), covering an area of 550 km^2.

A total of 59 samples were collected in an equal number of sites (**Table 1**) in Yellowish Ferrallitic Leached (FRAL), Alitic Low Clayey Activity Allitic Marillary (ABA-RA) and Arenosol soils; according to the classification of Hernandez *et al*.[6]; Acrisol Chromic Ferric, Nitisol Rhodic and Arenosol respectively, according to the IUSS[7].

Each sub-sample was taken randomly between 0 and 20 cm depth in the soil. The final sample consisted of 1 kg of soil from 20 sub-samples that were air-dried, crushed and sieved to a diameter of 2 mm.

2.2 Chemical analysis

The methodology proposed by Embrapa[8] was used for this analysis. The pH was determined with a potentiometer in a soil: water ratio (1:2.5). Organic matter (OM) was determined by wet oxidation (Walkley Black method).

Exchangeable Ca and Mg cations were determined with a 1M KCl extractant solution and analysis by flame atomic absorption spectrophotometry (FAS). Assimilable and exchangeable P was extracted by the Mehlich-I method with a solution of HCl (0.05 N) and H_2SO_4 (0.025 N). Assimilable P was measured with a UV-VIS spectrophotometer, while K was determined with a Digimed flame spectrophotometer.

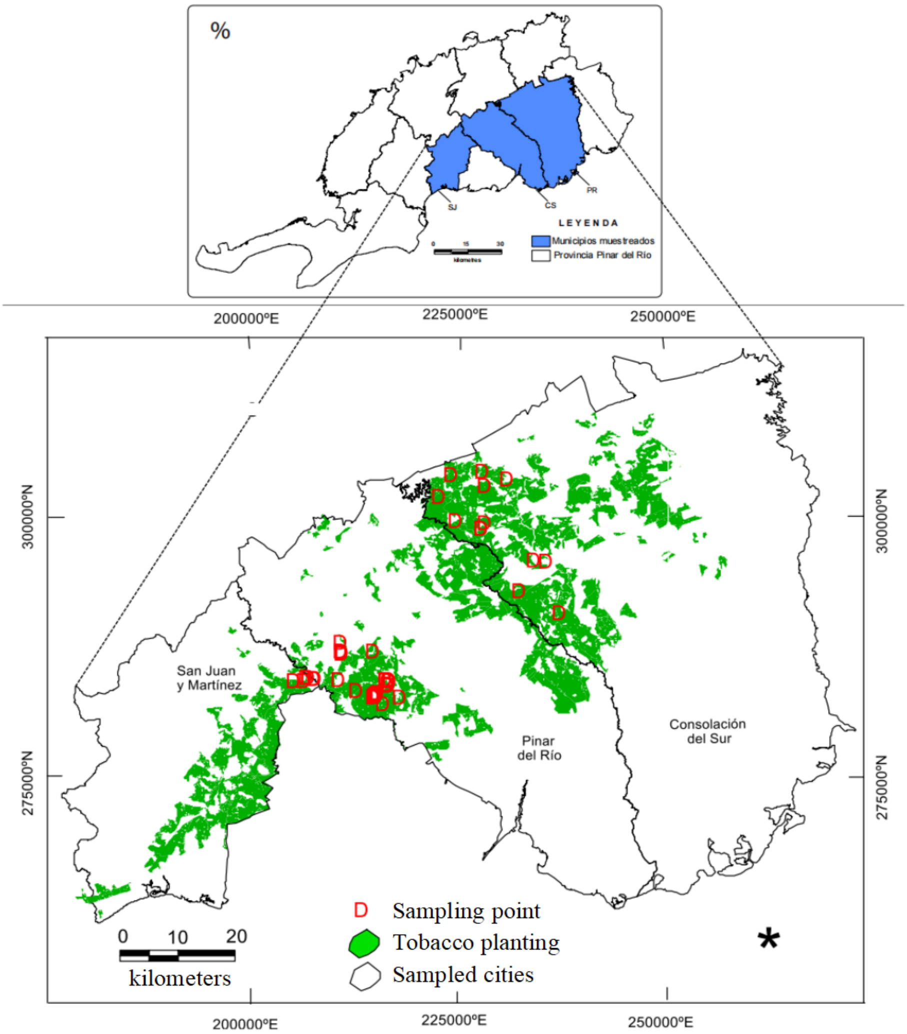

Figure 1. Geographic location of the study area.

Table 1. Distribution of sampling sites by soil type, Pinar del Río province (Cuba)

Municipality	Soil type*	Sampling points Depth (0–20 cm)
South Consolidation	FRAL	12
	ABA-RA	2
	Arenosol	3
Pinar del Río	FRAL	27
	Arenosol	3
San Juan and Martinez	FRAL	5
	ABA-RA	7
Total	-	59

*FRAL: Yellowish Ferrallitic Leached, ABA-RA: Alitic Low Activity Yellowish Red Clay.

Table 2. Scheme used for the sequential extraction of HMs, according to the BCR method

Fractions	Reagents/concentration/pH
F1: Water soluble	H_2O (1 hour)
F2: Soluble acid	CH_3COOH 0.11 M
F3: Bound to iron and manganese oxides	$NH_2OH.HC_l$ 0.1 M pH = 2
F4: Associated with the M.O.	H_2O_2 8.8 M (pH = 2, T=85 °C, 2 h) + CH_3COONH_4 1 M (pH=2)
F5: Residual or total amount*	Aqua regia (inverted) 9 mL HNO_3 + 3 mL HCl

*F5-Residual was obtained by subtracting the contents of HMs from the previous four + Previous stages.

2.3 Pseudo-total concentrations and heavy metal fractionation

To determine the pseudo-total concentrations of HMs, 1 g of the sieved soil sample was taken, digested by heating with the MARS Xpress® Digester, USEPA Method 3051A using inverted aqua regia[9]. The resulting extracts were analyzed by EAA in a VARIAN-55B equipment where the metallic elements Cd, Pb, Zn, Fe, Mn, Ni, Cu, Cr and Co were quantified.

Subsequently, 1 g of soil was taken and sequential extraction was performed according to the BCR method described by Ure *et al.*[10], adding the water-soluble fraction. The blank samples (control) without soil addition were analyzed using the above procedure for each stage of analysis in three replicates. The procedure used, the reagents and the five fractions obtained are included in **Table 2**.

The contents of HMs in the extracts were quantified by Plasma Emission Spectrometer (ICP-OES). The limit of detection (LOD) of the method was calculated by the mean of the blank values plus three times the standard deviation of the blank of all analyses (10 replicates). Finally, the factor mobility (FM) was determined by the difference of the labile or mobile fractions of the metal and the result of the complete extraction scheme. Equation proposed by Iwegbue[11] was used for this purpose:

$$\boldsymbol{FM} = \frac{\boldsymbol{F1} + \boldsymbol{F2}}{\boldsymbol{F1} + \boldsymbol{F2} + \boldsymbol{F3} + \boldsymbol{F4} + \boldsymbol{F5}} * \mathbf{100}$$

Where, *FM* is the mobility factor of the fractions; *F1–Fn* are the geochemical fractions of the metal.

2.4 Statistical analysis

The data were analyzed by the descriptive method and multivariate principal component analysis (PCA)[12]. Statistical processing was performed using SPSS v.22 for Windows.

3. Results and discussion

3.1 Soil properties and heavy metal concentration

The soils are characterized by low fertility and clay content, slightly acid pH (5.5–6.5)[13] (**Table 3**), M.O content ≤1% due to intensive tillage which has contributed to degradation[14]. The exchangeable base content and cation exchange capacity are low, which is common in soils cultivated with tobacco in Pinar del Río province[15]. Soluble P values are ≥45 mg/dm^3, as a result of fertilizer application and the tendency to accumulate this element in the soil[16]. The pseudo-total concentrations of the metals studied in the soil are lower than those established as prevention values for Cuban soils[17] (**Table 4**).

With the exception of the Co content in the ABA-RA soil (23 mg/kg), in the others this metal presented values different from the prevention value (25 mg/kg). Cd is the metal with the lowest concentration in the soil with values <1 mg/kg. In this soil, higher values were presented in all cases, which may be due to the fact that despite having a similar texture to the others, it has a higher clay content. In addition, it exhibits slightly higher values of Fe and Mn. According to Amaral-Sobrinho *et al.*[3], Fe and

Mn oxides have a significant effect on the absorption of HMs.

In the analysis of the pseudo-total concentrations of HMs, it was generally found that these elements are associated with the parent material, pedogenetic processes, and the geomorphological and climatic conditions of each region. In studies similar to the present research, Fãssler *et al*.[18] and Bashir[19] found low concentrations of HMs in soils intended for tobacco cultivation, with values lower than the prevention values established by the standards of several countries. Taking as a reference the total concentrations of these HMs reported by several authors in soils used for tobacco cultivation, it was found that, despite intensive use, this crop has not caused significant increases in HM concentrations.

Table 3. Physico-chemical properties of soils[a] in tobacco growing areas

Properties **FRAL (n = 132)**		**Type of soil[b]**		
		ABA-RA (n = 27)	**Arenosol (n = 18)**	
pH (H_2O)		6.1 ± 0.80a*	6.4 ± 0.20a	5.5 ± 0.20b
MO (%)		0.9 ± 0.30a	1 ± 0.20a	0.7 ± 0.10b
Ca	cmol·dm^{-3}	3.23 ± 1.57b	7.5 ± 0.55a	2.49 ± 0.79b
Mg^{2+}		1.43 ± 0.80a	1.66 ± 0.39a	0.6 ± 0.26b
K^{+}		0.38 ± 0.24a	0.22 ± 0.09b	0.23 ± 0.09b
Al^{3+}		0.42 ± 1.42	ND	0.20 ± 0.30
CIC pH 7.0		11.29 ± 1.72b	14.95 ± 1.105a	9.74 ± 1.18c
P	mg·dm^{-3}	177.57 ± 133.80a	82.24 ± 7.56b	47.09 ± 22.15b
Clay	%	8.56 ± 1.57a	9.20 ± 2.67a	6.94 ± 1.46b
Limo		30.43 ± 9.18a	30.16 ± 5.24a	28.59 ± 7.80a
Sand		61.02 ± 8.88b	60.66 ± 5.84b	64.46 ± 8.76a
Textural class		Sandy loam	Sandy loam	Clayey sand

[a]Average values ± standard deviation.

***** Different letters in the row indicate significant differences *($P \leq 0.05$)*.

Table 4. Pseudo-total HM concentrations in soils used for tobacco cultivation in Pinar del Río (Cuba)

FRAL MPs (n = 132)		**Type of soil**			**Prevention value[1]**
		ABA-RA (n = 27)	**Arenosol (n = 18)**		
Cu	mg/kg	11.23 ± 4.8	15.56 ± 1.24	6.42 ± 1.20	150
Zn		33.27 ± 15.83	49.72 ±5.56	16.2 ± 4.80	300
Cd		0.07 ± 0.09	0.33 ± 0.4	ND	2
Pb		13.97 ± 9.04	28.17 ± 2.94	10.52 ± 4.83	72
Fe	g/kg	11.38	19	13.76	-
Mn	mg/kg	335.6 ± 232.43	1401.37 ± 315.03	89.82 ± 49.03	-
Ni		12.4 ± 6.24	37.88 ± 5.18	8.9 ± 1.50	300
Cr		21.58 ± 12.26	33.31 ± 4.24	26.4 ± 7.61	300
Co		5.77 ± 3.17	22.99 ± 4.86	3.33 ± 0.90	25

[1]Proposed prevention value for Cuban soils.

Geochemical fractionation provided insight into the main associations of HMs in the soil (**Figure 2**). The Cu distribution pattern indicated a greater association with the residual fraction, with values > 60%, followed by the fraction bound to the OM. These metals can be found adsorbed on the surface of kaolinite-type clays, in oxides, or bound to the OM forming chelates[20]. Only 10% of this element was found in the reducible fraction bound to oxides in these soils; on the other hand, very small portions were found in the soluble and exchangeable fractions, indicating a low risk of contamination, if the same agricultural practices were maintained.

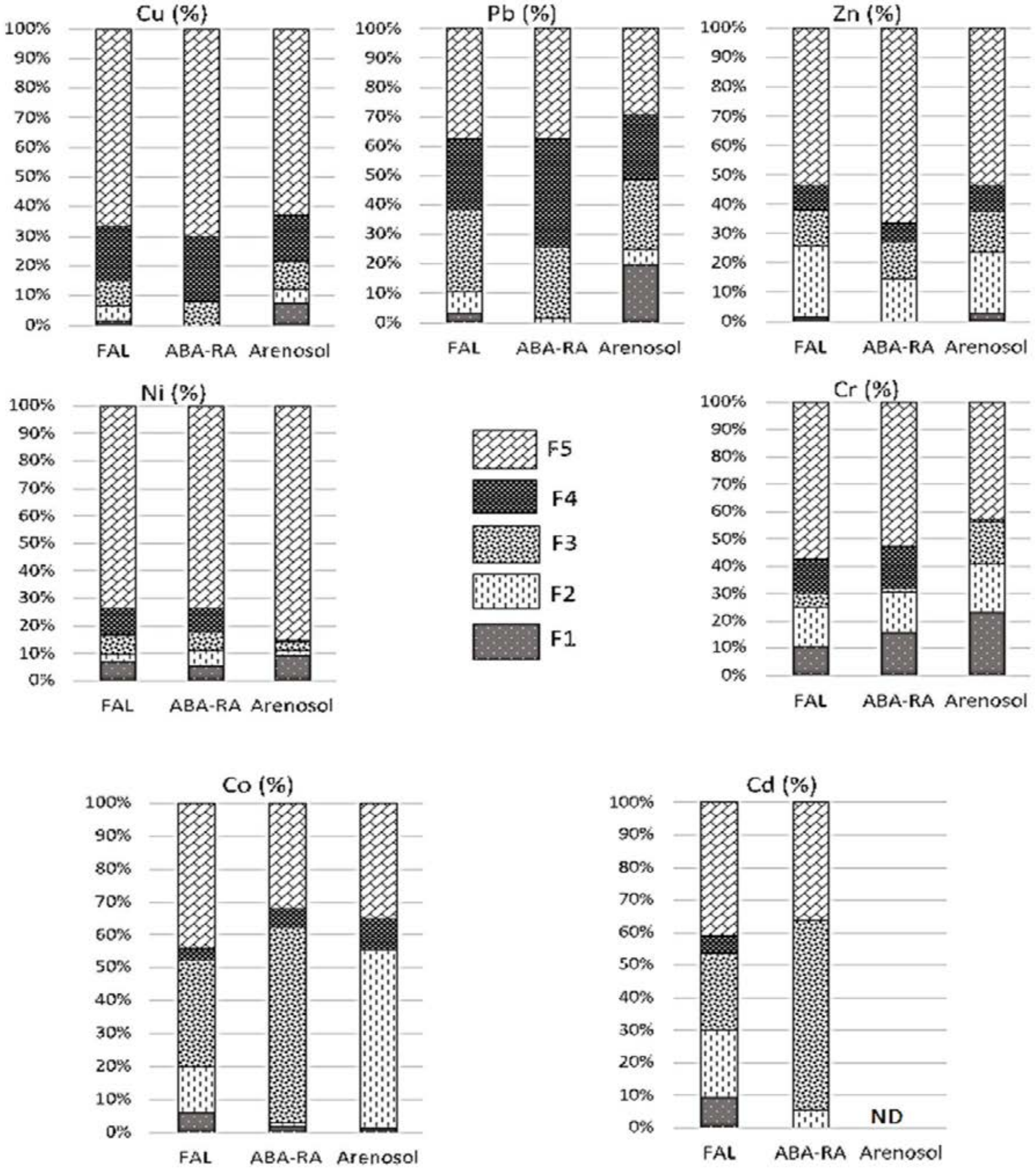

Figure 2. Cu, Pb, Zn, Ni, Cr, Co and Cd metal fractions in the soils.
F1: water soluble fraction, F2: acid soluble fraction, F3: reducible fraction bound to iron and manganese oxides, F4: oxidizable fraction bound to organic matter, F5: residual fraction, ND: not detected.

Pb was found to be mainly associated with the residual fraction (F5) and bound to organic matter (F4). Li *et al*.[21] found low concentrations of this element in the soil, mainly in the residual fraction. As total concentrations increased, they moved to the exchangeable fraction. In the fraction bound to iron and manganese oxides (F3), values between 24 and 28% were found. The HMs present in this fraction can shift to bioavailable forms, if the oxidation-reduction conditions change[22]. In the most labile fractions (water-soluble and acid-soluble), the values found were low and did not exceed 10%, except in the Arenosol, where a value of 20% was recorded; this may be due to the fact that this soil has the lowest pH(5.5). It has been shown that pH is negatively correlated with Pb mobility, since in these conditions this metal appears in the form of free ions, increasing its bioavailability[23–25].

Zn fractionation indicated that this metal is more than 50% bound to the residual fraction. There is a tendency of clay minerals to absorb Zn irreversibly by penetration into the crystal lattice[4], which is why the ABA-RA soil has the highest percentage of this element. In the fraction bound to M.O and oxides, values of approximately 7% and 11%, respectively, were observed. The soluble acid fraction (F2) showed values higher than 15%. These values agree with the findings of Kennou *et al.*[26] under conditions similar to those of this study. The water soluble fraction was low with values < 3%. Low values in this fraction are common because, in uncontaminated mineral soils Zn is mainly associated with the reducible fraction bound to Fe, Mn and Al oxides and its mobile forms are less frequent[4,11]. However, this result may vary if soil properties, especially change of pH value[27,28].

Ni presented the highest affinity for the residual fraction, with high values above 70% in the soils studied. In uncontaminated soils this metal is mainly found in this fraction, indicating its lithogenic origin. However, the presence of this element in the most labile and bioavailable fractions (F1 + F2), with values close to 10%, indicated that this metal presents risks of accumulation in plant-available forms. In Pinar del Río (Cuba), most of Ni was found in forms not assimilable by plants. However, sandy-textured soils, due to their chemical and physical characteristics, maintain a higher proportion of Ni in plant-available form than in other types of soils[29].

Cr also showed greater affinity for the residual fraction. This element was found in low pseudo-total concentrations (**Table 4**) and its content is mainly due to the source material. Some affinity was also observed with the M.O. bound fraction, being the FRAL soil the one with the highest affinity, with a value of 20%. The soils did not show affinity with the fraction bound to oxides. The sum of the mobile fractions (F1 + F2) showed values close to or greater than 25% however, the bioavailability of this element is generally low, presenting values that do not exceed 3% of the total in the soil[22].

Co presented a different trend from the other HMs. The association with the residual fraction was slightly lower than the values recorded for the rest of the metals, with values between 32 and 44%. The fraction bound to OM was low in all three soil types with values <10%. The fraction bound to oxides presented high values in FRAL (33%) and ABA-RA (59%) soils; however, in the case of Arenosol no association with this fraction was observed. The fractions that determine the mobility of the metal (F1 + F2), presented discrepant values among the main soil types in comparison with other metals. In the Arenosol, Co showed the highest mobility, with 55% in both fractions, followed by the FRAL soil with 20%. These results allow inferring that under these conditions this metal may represent transfer risks to the tobacco crop or to other cultivated species.

Cd showed high affinity for the residual and reducible fractions bound to oxides. However, this element also showed a high association with the most mobile fractions (F1 + F2), with a value of approximately 30% in FRAL soil. In soils cultivated with tobacco, Ortega *et al.*[30] found that the Cd fraction was the second highest in concentration and bioavailability, followed by the fraction bound to carbonates; in Arenosols this metal was not detected. Cd under natural conditions is mainly associated with carbonates and the organic fraction. The high sand content in these soils determines the low reserve and buffer capacity to retain cations mainly Cd[31].

From the analysis of the data obtained, differences in the mobility factor (MF) of the PMs under study were recorded, according to the following order: Cd > Cr = Cr = Zn = Zn = Co > Co > Pb = Ni > Cu (**Table 5**). The FM was high for Cd, Cr, Zn and Co with values above 10% indicating that these metals can potentially be extracted and accumulated by the tobacco plant or leached into groundwater as possible routes of contamination.

Among the HMs studied, the highest FM (>50%) was observed in Cd, which indicated that this metal, despite low pseudo-total contents in soils, accumulates in plant-available forms and may constitute a risk to the tobacco crop. Cd is known to be a more mobile and soluble element than many other

Table 5. Mobility factors of the heavy metals included in the study

	Cd	Pb	Cu	Zn	Ni	Cr	Co
	%						
Median	60a*	8.7c	2.6d	21.8b	8.5c	26.8b	20.6b
RIC[a]	85.7	17.2	6.4	19.0	14.8	38.0	36.4

Soils cultivated with tobacco, Pinar del Río (Cuba) (n = 177). [a]RIC: Interquartile range. *Values followed by different letters in the same row differ from each other ($P < 0.05$).

PMs in soil. High FM of this metal is common in sandy soils, where pH fluctuations predominate, being a key factor in controlling its availability[32]. The high FM of other elements, such as Cr, is not common, being this one of the most stable metallic elements in Cuban soils. The results of Cr content in the most labile fractions in uncultivated soils of Cuba do not exceed 3% of total Cr[17].

Zn is also highly mobile and is one of the elements that commonly present alterations in its biogeochemical cycle in the soil, due to anthropogenic causes[33]. However, it is essential for plant development and is not toxic at low concentrations. Co is an element that appears occluded in secondary minerals in the form of co-precipitate generally with manganese oxides. The high FM observed in this study may be due to transformations in soil conditions, such as pH changes, which cause the solubility of these precipitates. The ionic adsorption properties of Fe and Mn oxides depend, to a large extent, on pH, which is why in the Arenosol (pH 5.5) the highest amount of Co (55%) was recorded in the F1 and F2 fractions.

However, the high mobility of the aforementioned HMs, due to their low concentrations in the soil, have a low bioavailability for the tobacco plant. The rest of the HMs presented low mobility, being Cu the element with the highest stability in the soil. The low mobility of this element is common in mineral soils with low organic matter content.

The Principal Component Analysis (PCA) allowed us to identify three components, which accounted for 78.7% of the total variance of the original data (**Table 6**). The first one explained 47.5% of the total variance. The variables that appear in this analysis with the highest loadings are CEC and calcium content, in addition to all the PMs under study, the latter with a loading >0.7, except Cu and Cr. The fundamental source of these variables in this component may be due to natural causes. HMs can be adsorbed on the surface of clays, or on iron and manganese oxyhydroxides and can also be present in the crystal lattice of primary and secondary minerals such as carbonates, sulfates and oxides.

Table 6. Matrix of rotated components and total variance explained of the variables under study

	Components		
	1	2	3
M.O	0.167	0.081	0.908
P	-0.237	0.793	0.354
pH	0.400	0.712	-0.310
$\text{CIC}_{\text{pH=7}}$	0.735	0.153	0.565
Ca	0.853	0.238	0.184
Total (Ni)	0.943	0.033	0.111
Total (Co)	0.913	-0.035	-0.001
Total (Cd)	0.740	0.354	0.046
Total (Cu)	0.647	0.603	0.257
Total (Zn)	0.716	0.592	0.089
Total (Pb)	0.742	0.379	0.194
Total (Cr)	0.663	0.033	0.051

Total variance explained, sum of the squared saturations of the rotation of each component:

Total	5.705	2.209	1.529
Percentage of variance	47.540	18.407	12.741
Cumulative percentage	47.540	65.947	78.688

4. Conclusions

The pseudo-total concentrations of Cu, Zn, Cd, Pb, Fe, Mn, Ni, Cr and Co metals in soils cultivated with tobacco in Cuba are low and lower than the prevention values recorded for these soils.

In general, the heavy metals studied have a high affinity for the most stable fractions of the soil (F3, F4 and F5), which represents a low risk of transfer to the tobacco crop or accumulation in groundwater.

The association of all heavy metals in principal component 1 is evidence that their main source of origin is pedogenetic and that these elements are closely related to cation exchange capacity and calcium content.

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Conflict of interest

The authors declared no conflict of interest.

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