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Relationships between Cadmium in Tissues of Cacao Trees and Soils in Plantations of Trinidad and Tobago

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Abstract

The primary source of cadmium in cocoa beans has been linked to its direct uptake by the cacao plant from cadmium contaminated soils. This research was conducted to evaluate and interpret significant relationships between cadmium levels in tissues of the cacao plant and soils from cocoa-growing areas in Trinidad and Tobago. Total (HNO_3-extractable) concentrations of cadmium in both tissues and soils were determined. The levels of cadmium measured varied in the order: leaves > pods > shells > nibs > soil. Cadmium levels in all the cacao tissues analyzed were significantly ($p < 0.05$), positively and strongly correlated with each other. Additionally, significant ($p < 0.05$) positive relationships were also identified between Cd in cacao tissues and corresponding total HNO_3-extractable Cd levels in soils. These findings suggest that they can possibly be used as predictive tools for assessing Cd levels in cacao.

Keywords

Cadmium, Cacao, Soils, Relationships

1. Introduction

Contamination of soils with heavy metals is an increasing problem globally and a threat to environmental biota,

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as these metals accumulate in soils and plants [1] [2]. In plants, some metals, including copper, zinc and nickel, are considered essential micronutrients [3]; however, when present in excess, these essential and non-essential heavy metals, such as cadmium (Cd), can accumulate to toxic levels in the tissues of plants used for human or animal nutrition [4].

Generally, the intake of Cd in very small amounts by humans is unavoidable, with agricultural produce being a major source [5]. However, its toxicity can present significant health risks if Cd-contaminated foodstuffs are consumed [6] [7]. Health effects include abdominal cramps, headaches, vomiting and diarrhea [8]. With long-term exposure, Cd causes renal damage [9], severe loss of bone minerals and painful fractures [10].

Over the years, foods such as vegetables [11] [12] rice [10] and other cereals [13] [14] and shellfish [15] have been monitored for Cd contamination. Also of great public significance are the levels of Cd in cocoa beans, cocoa powders and cocoa liquors used for chocolate production [1] [16]-[19]. The Cd content in chocolates is a function of the cocoa bean nib content and diminishes in the order: bitter chocolate (used for baking), dark chocolate (bittersweet and semi-sweet), milk chocolate and white chocolate [18]. It is thus not surprising that food safety concerns have been generated over the presence and levels of Cd in cocoa, as many consume these products daily. As a result, international legislative bodies, as well as chocolate manufacturing countries, have introduced new regulations for the protection of the health of their consumers [20]-[22] in addition to pending stricter EU standards [23].

It is claimed that Cd levels in cocoa beans are due to their uptake by cacao plants from soils [17]. Some soils, especially those of volcanic origins, can contain high levels of Cd, which can be taken up by the cacao plant and concentrated in the beans [24]. Cadmium is not reported to be essential for any biological process in plants [25], but is still absorbed by both root and leaf systems from soils [26]-[29]. Since Cd in cacao is absorbed from soils and subsequently distributed among its various tissues [16] [17], including leaves, pods and beans, the reduction of Cd uptake from soils is a major strategy for its reduction in beans, to allow producers to meet increasingly stringent food safety standards for cocoa products. However, it is first necessary to determine any correlations in Cd levels among the various cacao tissues and soils of cacao estates to better understand the mechanisms of uptake and distribution in cacao tissues as well as to determine the effectiveness of soil treatments to reduce Cd uptake.

In this study, Cd levels among cacao leaves, pods, bean nibs, testae/shells and soils, on which cacao trees were grown, were determined and compared, to identify whether it was possible to relate soil Cd with cacao tissues and especially cacao beans (nibs) from which edible products were derived. Such correlations can be used to assess the effectiveness of soil treatments to reduce Cd uptake and redistribution in cacao tissues, especially the nibs. The potential to estimate cadmium levels in cacao beans via correlations with other cacao tissues was also investigated, to provide a basis for subsequent studies in Cd absorption and distribution in cacao trees. A recent study of cadmium in cacao tissues and soils in Ecuador [30] focused largely on cadmium correlations between beans and soils and possible origins of contamination, but not between cacao tissues, as done in this study.

2. Materials and Methods

All water used for sample preparation and cleaning of glassware in this study was glass-distilled and deionized. To avoid trace metal contamination, all laboratory glassware and utensils used were washed with a commercial detergent and tap water, immersed in a 2 M nitric acid HNO_3 (J.T. Baker, USA) bath for at least 24 h, then rinsed with distilled deionized water before drying in an oven at 50˚C. All reagents used for sample preparation and analyses in this study were of analytical grade and were tested in blank analyses, to correct for Cd background levels.

2.1. Collection and Preparation of Samples

Cacao samples were collected from 45 plantations throughout different cacao-growing locations on all major soil types (I-VII) in Trinidad and Tobago (**Table 1**). At each sampling site, 8 - 12 cacao trees were randomly selected and soil cores (0 - 30 cm depth) taken with an auger around the drip zone of each tree, since cacao trees have extensive feeding root systems close to the surface [31]. From each tree sampled, cacao tissues, namely mature and sound leaves and ripe pods, were randomly taken and placed in polyethylene bags, tied at the mouths, immediately labeled for identification and transported to the laboratory for processing.

Table 1. Distribution of soil physical and chemical properties across the different locations sampled.

Characteristic	Soil type						
	I	II	III	IV	V	VI	VII
Sand (%)	31	64	47	35	33	25	19
Silt (%)	18	7	21	20	17	14	10
Clay (%)	42	25	29	49	32	59	33
pH	4.9	4.5	4.9	4.9	5.3	7.6	6.2
EC (mhos × 10^6)	37	43	138	16	41	99	55
CEC (meq/100g)	11.5	4.7	8.2	8.5	4.4	6.3	34.2
OM (%)	1.55	1.03	2.41	0.86	1.89	3.96	0.52
Base saturation (%)	18	13	49	28	32	100	100
Ca (meq/100g)	1.3	0.5	3.5	1.8	0.8	8.0	15.6
Mg (meq/100g)	7	0.2	0.7	0.9	0.7	0.4	31.1

From each cacao pod, the beans were removed and rinsed in deionized water to remove excess mucilage. The beans were then oven-dried on glass plates at 105˚C for 4 - 5 h, separated into nibs and shells, and each ground separately in ceramic mortars to <1 mm diameter particle size. Pod husks were rinsed with deionized water, grated finely (<1 mm) on a plastic grater and dried on glass plates at 105˚C for 3 h. Leaves were washed with deionized water, chopped to <1 mm in a food processor, then oven-dried on aluminum foil at 105˚C for about 24 h. Soil samples were air-dried for 48 h, ground and sieved through a 2 mm sieve, as recommended for soil analyses [32]. Each dried sample was placed in a clean polyethylene bag, mixed well, labeled and stored until needed for analysis.

2.2. Extraction and Analysis of Samples

2.2.1. Determination of Cd in Cacao Tissues

The method of determination for Cd in cacao tissues was optimized for highest recovered levels, using local samples of cacao leaves, pods, nibs and shells or testae. Sample mass: acid volume, digestion conditions and extraction times were systematically varied, to determine the highest levels of Cd recovered from each sample type. For these analyses, optimal recoveries were obtained for 0.5 g aliquots of nib, shells, pod and leaf samples, to each of which 10 mL concentrated HNO_3 (J.T. Baker, USA) were added and samples allowed to pre-digest at room temperature for 12 h, followed by complete digestion on a heating block at 130˚C for 6 h. Digested extracts were cooled, diluted with 5mL distilled water, filtered through Whatman No. 542 filters and made up to 25 mL, for Cd determination by flame atomic absorption spectrometry (FAAS).

Reference Cocoa Materials certified for Cd were unfortunately unavailable to our laboratory at the time of the study, necessitating the use of NIST SRM 1570a Spinach Leaves, (National Institutes of Standard and Technology, Maryland, USA), to validate the test method. Replicate analyses (n = 10) of this SRM were done. Once validated, this method was used to analyze all cacao tissues samples, with appropriate instrumental and sample quality control measures taken, to maintain the accuracy of analyses. Quality control results shown in **Table 2**.

2.2.2. Determination of Total HNO_3-Extractable Cd in Soil

As for cacao tissues, the method of determination of total Cd in soil was similarly optimized, using a soil sample from a local cocoa farm. For optimal extractions, to triplicate 0.5 g aliquots of soil samples, 10 mL concentrated HNO_3 was added to each and samples pre-digested at room temperature for 12 h, followed by exhaustive digestion on a heating block at 130˚C for 8 h. Digested extracts were cooled, diluted with 5 mL distilled water, filtered through Whatman No. 542 filters and made up to 25 mL, for Cd determinations by FAAS.

A Certified Reference Soil, namely NIST SRM 2710 Montana Soil was analyzed in replicate (n = 10) with this method and % recoveries for Cd determined. Appropriate quality control measures, as done with cacao

Table 2. Mean cadmium concentrations ± Std. deviation and % recoveries from NIST Certified Reference Materials SRM1570a and SRM 2710.

	Certified value (µg/g)	Mean, n = 23 triplicates (µg/g)	% Recovery	CV
SRM 1570a (Spinach leaves)	2.89 ± 0.07	2.69 ± 0.05	95.5	4.71
SRM 2710 (Montana soil)	21.80 ± 0.2	21.58 ± 0.31	99.00	1.43

tissues were also taken to maintain accuracy of analyses. Results of quality control for soil samples are shown in **Table 2**.

3. Statistical Analysis

Correlation analyses used to evaluate and interpret significant relationships between the selected heavy metal concentrations in various cacao sample types were done with Minitab 16 software.

4. Results and Discussion

Data generated from 23 sets of replicate analyses of leaf and soil CRM over the 18-month experimental period, including 10 replicates for method validation, are shown in **Table 2**. The mean CRM recoveries for cadmium for each sample type over this period were consistent with their certified values (**Table 2**). In addition, the RSD values (<10%) for each metal determined demonstrate the high precision and reproducibility of the test method.

4.1. Cadmium Concentrations in Cacao Tissues and Soils Sampled

More than 50% of the locations sampled contained detectable (>0.3 µg/g) Cd in the cacao tissues sampled. Generally, Cd levels measured in the tissues of the cacao plants varied in the order: leaves (0.54 - 5.21 µg/g) > pods (0.53 - 4.49 µg/g) > shells (0.44 - 4.41 µg/g) > nibs (0.35 - 3.82 µg/g). This trend is consistent with some previous reports of differing Cd levels among organs or tissues in the same plant [16] [17] [33], but differs from those reported for Ecuadorean cacao [30], possibly because of differences in germplasm [34]. Its distribution in the cacao tree may be as a result of the mobilization of protective mechanisms in plants, which inhibit the transport of metals to other tissues and organs [35].

Conversely, the total HNO_3-extractable concentrations of Cd in soils sampled across cacao plantations ranged from 0.3 - 1.7 µg/g. These levels were generally lower than those of cacao tissues of trees grown on the soils, consistent with previous reports [16]. Recycling of underlying organic materials such as leaf and pod materials on soils of plantations can contribute to uptake of Cd by cacao plants [16] [17]. It may also be due to the volcanic nature of soils in a few areas sampled, bearing in mind that volcanic soils can contain high levels of Cd [24]. In addition, it was noted that some of these areas were either prone to flooding during the wet season, with the possibility of Cd contamination from industrial sources [10] [36], or in some cases had a long history of fertilizer applications. Phosphate fertilizers are known to be contaminated with Cd [37] and have contributed to elevated levels of Cd in soil in cacao plantations in Malaysia [38].

4.2. Correlation Analysis of Cd in Cacao Tissues and Soils

Correlation analyses were used to evaluate and interpret significant relationships for Cd concentrations in the various cacao tissues (**Table 3**).

Cadmium levels in all the cacao tissues analyzed were significantly ($p < 0.05$), positively and strongly correlated with each other: (Nib/Shell) $r = 0.86$; (Nib/Leaf) $r = 0.79$; (Nib/Pod) $r = 0.76$; (Shell/Leaf) $r = 0.83$; (Shell/Pod) $r = 0.68$); and (Leaf/Pod) $r = 0.80$. This indicates that the concentrations of Cd concentrations in cacao nibs, shells, leaves and pods vary proportionately with each other. It also suggests that the Cd distribution and concentration ratios within tissues are related. Such strong correlations suggest that cadmium levels in cacao beans can be estimated through its other tissues, when pods are not available. This may offer a means of early screening of cacao cultivars for cadmium accumulation, for selecting low-cadmium varieties, to allow cacao growers to meet the proposed European Union standard [23] for cadmium in beans.

Table 3. Pearson correlation coefficients (r) between Cd levels in cacao tissues and soil.

	Cd (Nib)	Cd (Shell)	Cd (Leaf)	Cd (Pod)
Cd (Shell)	**0.86**			
Cd (Leaf)	**0.79**	**0.83**		
Cd (Pod)	**0.76**	**0.68**	**0.80**	
Cd (Soil)	**0.35**	**0.43**	**0.34**	**0.50**

Bold values are significant at $p < 0.05$.

Significant ($p < 0.05$) relationships were also identified between Cd in cacao tissues and corresponding total HNO_3-extractable Cd levels in soils: (Nib/Soil) r = 0.35; (Shell/Soil) r = 0.43; (Leaf/Soil) r = 0.34; and (Pod/Soil) r = 0.50. It is notable that such correlations were obtained for a wide variety of cacao cultivars and soil types and should be applicable to other cacao-growing countries.

It is well known that most plants which accumulate heavy metals in their aerial tissues primarily reflect the concentrations of their respective metal in the soil [39]. However, although significant ($p < 0.05$), the correlations obtained with local soils were also weak, suggesting that the measurement of total Cd may not be appropriate for estimating metal availability to plants. The bioavailability of metals in soils for uptake by plants is often not the same as their total metal concentrations, since only a fraction may be available for absorption [40]. The study on Ecuadorean cacao tissues and soils [30] demonstrated that selective extraction methods for soil cadmium provide stronger correlations between cacao beans and soils. Subsequent to the present study, it was shown that bioavailable soil Cd determined using single-extractant protocols such as DTPA, AB-DTPA and EDTA are strongly correlated with tissues of cacao plants grown on Cd-contaminated soils [41]. Future studies on the uptake and distribution of Cd from soils to cacao tissues should therefore use bio-available instead of total extractable Cd.

5. Conclusion

This study demonstrated that strong and significant correlations exist between Cd levels in cacao tissues over a wide range of cacao cultivars. These findings suggest that it may be possible to predict Cd levels in cocoa beans from those in mature leaves and pods. This can be especially useful in assessing cacao trees, growing areas and soil types for possible elevated Cd levels in beans, even when pods are unavailable. Such an approach can be used by cacao farmers and purchasing agents of cocoa beans to estimate whether beans from any farm or area are likely to meet specific Food Safety Standards for Cd. Similarly, the effectiveness of soil treatments to minimize Cd uptake from soils by cacao plants can be monitored through tissue Cd levels before and following remedial soil treatments.

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