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Trace Element Composition in Forage Samples from a Military Target Range, Three Agricultural Areas, and One Natural Area in Puerto Rico

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ABSTRACT—Information about trace element composition in vegetation from tropical environments is scarce. Trace elements were evaluated on vegetation samples collected at the Atlantic Fleet Weapons Training Facilities (AFWTF) in Vieques, agricultural areas in Vieques and mainland Puerto Rico, and at the Guánica State Forest. Analyses were conducted using atomic absorption after dry-ashing of samples and extraction with acid. Crops in Vieques contained more lead ($p < 0.05$) and cadmium ($p < 0.10$) than in mainland Puerto Rico. Similar results were observed for lead and cobalt in *Syringodium filiforme*, *Calotropis procera*, and *Sporobolus virginicus* from the AFWTF. The presence of similar trace element profiles in distant terrestrial and marine plant species suggests the presence of different environmental conditions.

INTRODUCTION

Human activities can irreversibly damage natural resources. Agriculture, urban development, industrialism, and military exercises exert obvious pressures on the quality and quantity of natural resources. Among the many environmental pollutants, trace elements such as lead and cadmium have considerable ecological, biological, and public health significance (Ikeda et al., 2000; Lanphear, 1998; Waalkes, 2000).

Although trace elements occur naturally in soil, anthropogenic activities can produce undesirably higher concentrations that may be dispersed through multiple biotic and abiotic processes. The transport, residence time, and fate of pollutants in an ecosystem are serious social concerns. Since the behavior of trace elements in an ecosystem is highly complex, they are usually studied separately for air, water, soil, and biota (Kabata-Pendias, 2001).

Plants are good soil quality indicators and respond directly to air quality. Since plants can naturally uptake pollutants from their local environment, their chemical composition can indicate degree of disturbance when assessed against background values obtained from unpolluted vegetation. The significant role of plants in spreading toxic elements has been well illustrated for several ecosystems (e.g., Lokobauer et al., 1993; Pascoa et al., 1996; Sastre et al., 1999). Plants adapt to great variability of chemical properties in their environment and are intermediate reservoirs through which trace elements in soil, water, or air move to animals and humans (Kabata-Pendias, 2001).

Heavy metals can elicit a variety of adaptive responses in plants, such as detoxification by phytochelating agents (Cobbett, 2000; Jema et al., 1998; Marschner, 1995) that bind metals and protect cells from metal toxicity. Thus, plants have been used as indicators of heavy metal contamination and in the development of environmental restoration technologies such as phytoremediation (e.g., Meagher, 2000; Medina et al., 1998; Raskin et al., 1997). We evaluated the use of plants as natural indicators to measure the flux of specific trace elements in the environment. The composition of trace elements from forage samples was surveyed for various environments in Puerto Rico, including an active military zone, agricultural lands, and a pristine forest reserve.

MATERIALS AND METHODS

Study sites

Vegetation samples were obtained in 2000 and 2001 at four localities in the island of Vieques, Puerto Rico: the Atlantic Fleet Weapons Training Facility (AFWTF, 18°08.32N, 65°18.10W) and three cultivated areas (Monte Carmelo, 18°08.22N, 65°25.61W; Barrio Monte Santo, 18°07.78N, 65°27.44W; and Villa Borinquen, 18°09.12N, 65°26.04W) (Fig. 1). Samples were also taken at the Guánica Forest Reserve (17°57.213N, 66°50.971W) and agricultural areas in Mayagüez (18°10.92N, 67°06.70W) and Las Marías (18°12.43N, 67°00.18W). Reference sampling sites for Vieques were chosen based on comparable geoclimatic conditions or land uses. The analyses were done on *Calotropis procera* (giant milkweed), *Panicum maximum* (guinea grass), *Syringodium filiforme* (Manatee grass), *Sporobolus virginicus*, *Cajanus cajan* (pigeon peas) and *Cucurbita moschata* (squash).

Sampling protocol

Samples were collected manually and identified by botanists at the Mayagüez campus of the University of Puerto Rico. To obtain a representative cross section of each study site, selection and design of sampling protocols were based on Higgins et al. (1996). Each station was randomly sampled in a 2-5 m^2 circular plot. Sampling sites were established independently for each species. Samples were composed of over 40 leaves picked alternately from upper, middle, and the lower sections from 5-10 plants of each species. Fruits of *C. cajan* were sampled in August 18, 2001. Samples were rinsed with distilled water, placed in large plastic bags, stored immediately on an ice cooler, and transported to the laboratory. Samples were handled only with plastic, glass, or porcelain objects. Samples were refrigerated at about 4 °C before the analyses.

Metal analysis in vegetation

Analyses of heavy metals followed Montgomery et al. (1977) and Thompson (1969). Samples were rinsed thoroughly with deionized water, shaken to remove most of the water, allowed to air dry, and ground in a ceramic mortar. Two grams of finely cut material was weighed in a porcelain dish that had been heated at 525 °C for 2 h. Samples were then dried in an oven at 105 °C for 24 h, allowed to cool in a desiccator, weighed, and incinerated in a muffle furnace for 2-3 h at 525 °C. Ashes were dissolved in 5 ml of 20 % HCl and the solution was transferred to a 600 ml beaker. The porcelain dish was rinsed twice with 6-8 ml of 20 % HCl and the solution was added to the same beaker. Samples were covered with a watch glass and heated on a hot plate until boiling. Additional 20 % HCl was added as needed to avoid sample dry-

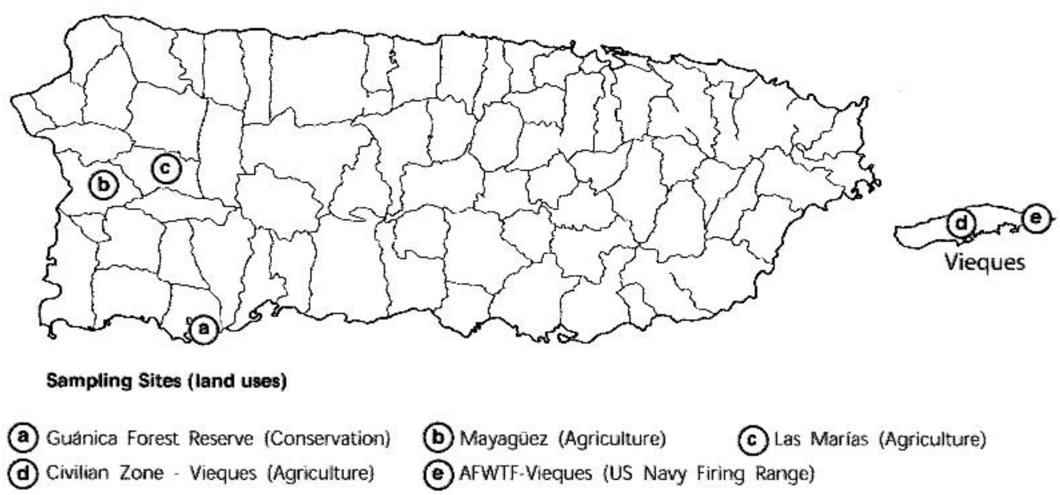

FIG. 1. Location and land uses of sampling sites.

ing. Samples were filtered through Whatman # 40 paper with 10 % HCl. The final volume was adjusted with 10 % HCl. Samples were stored in acid washed bottles and sealed tightly until analysis. The concentration of acid-extractable elements was determined by air-acetylene flame detection in an atomic absorption spectrophotometer (Perkin Elmer Model 2380). Instrument calibration was verified with ICP/ICP-MS grade standard solutions for each metal. Strict controls were used to insure the validity of the data. Only high purity reagents were used (Fischer Sci., ACS grade). Glassware was cleaned with detergent, rinsed with Milli-Q water, cleaned with concentrated HNO_3, and finally rinsed with deionized water. Samples were analyzed at least in duplicate. ANOVA tests were performed using SYSTAT version 9.1.

Results and Discussion

Different species of plants showed different trace element profiles (Tables 1 and 2). Copper and nickel were more abundant in *Sporobolus virginicus* and manganese was more abundant in *Syringodium filiforme* at the Guanica forest reserve. The concentration of many trace elements, particularly lead, was higher in Vieques than in mainland Puerto Rico. Natural lead concentration in plants growing in uncontaminated areas is usually low, ranging from 0.1 to 10 μg/g (dry weight) and averaging 2 μg/g (Kabata-Pendias, 2001).

The high concentration of lead in *S. filiforme* (manatee grass) from Carrucho Beach, located at the southern section of the AFWTF, indicates the potential for dispersion and dangerous bioaccumulation along the marine food chain (Table 1). Fishes, crustaceans, and manatees directly or indirectly consume this marine plant. The US Fish and Wildlife Service has reported manatees feeding in Vieques. The high level of lead in *S. filiforme* cannot be explained by natural processes. The oceanic pH (approximately 8.0 ± 0.5) limits the solubility of many metals, including lead, and metals must be dissolved in order to be available for marine plants to accumulate in their tissues. At the AFWTF, the US EPA (1984-1999) Discharge Monitoring Reports identified excessive concentrations of lead with occasional average levels of up to 5 mg/L, as well as fluctuations in pH. These parameters could enhance metal bioavailability, thus increasing uptake of metals by aquatic life. Heavy metals are undetectable in seawater when military practices are not taking place (URS Greiner Woodward Clyde, 2000).

The establishment of a typical value is not a simple task, but background concentrations can be established from populations grown under various conditions. *Cajanus cajan* (pigeon peas) and *Cucurbita*

TABLE 1. Elemental analysis of leaf samples collected at two locations in Puerto Rico.

	Chemical element (μg/g dry weight)[1]						
	Pb	Co	Ni	Mn	Cr	Cd	Cu
Guánica, State Forest							
Calotropis procera	1.29 (1.10)	2.59 (0.00)	5.17 (1.46)	17.85 (1.10)	9.31 (0.73)	1.03 (0.00)	5.69 (1.46)
Syringodium filiforme	5.57 (1.90)	4.19 (0.06)	14.64 (4.74)	251.44 (1.39)	27.93 (1.60)	2.79 (0.04)	15.39 (4.16)
Sporobolus virginicus	0.60 (0.32)	3.11 (2.06)	5.36 (0.59)	7.54 (0.44)	7.35 (0.71)	0.79 (0.05)	2.76 (0.40)
Panicum maximum	nd	4.69 (0.16)	10.50 (2.95)	35.19 (1.24)	12.24 (3.71)	0.60 (0.85)	12.93 (2.11)
Vieques, AFWTF							
Calotropis procera	30.05 (3.63)	68.40 (9.08)	18.08 (2.50)	287.94 (3.39)	12.68 (2.82)	3.11 (2.09)	4.63 (4.25)
Syringodium filiforme	33.32 (10.77)	29.60 (5.51)	28.66 (1.58)	58.23 (1.86)	2.78 (1.33)	2.78 (1.33)	30.48 (3.63)
Sporobolus virginicus	30.45 (5.36)	34.81 (1.80)	nd	670.57 (267.85)	5.32 (0.99)	nd	nd
Panicum maximum	10.25 (1.27)	46.65 (8.91)	5.08 (3.25)	135.02 (31.72)	5.99 (0.58)	1.57 (0.14)	4.03 (3.34)

[1]Average (standard deviation; n = 2); nd = not-detectable.

TABLE 2. Trace element composition in forage from the civilian area of Vieques and mainland Puerto Rico.

	Chemical element (μg/g dry weight)[1,2]					
	Pb	Co	Ni	Mn	Cd	Cu
Mainland, Puerto Rico[3]						
Cajanus cajan	4.93 (0.28)	4.31 (0.30)	7.44 (0.56)	137.43 (6.68)	0.90 (0.13)	11.04 (0.41)
Cucurbita moschata	4.51 (1.54)	– (–)	17.43 (0.23)	113.03 (55.73)	0.65 (0.32)	21.16 (3.71)
Vieques, Puerto Rico[4]						
Cajanus cajan	29.14* (17.61)	10.42** (5.15)	7.38 (3.05)	126.32 (54.85)	1.79** (0.78)	20.58* (5.53)
Cucurbita moschata	33.08* (19.06)	7.95 (2.44)	10.32 (4.27)	92.54 (20.92)	2.34** (1.44)	29.76* (5.25)

[1]Average (standard deviation; n = 4 to 6); Dash = not available.
[2]Significant differences $*p < 0.05$; $**p < 0.10$.
[3]Mainland, includes samples from Mayagüez and Las Marías.
[4]Vieques, includes samples from Monte Carmelo, Villa Borinquen and Monte Santo.

moschata (squash) are fast growing plants consumed throughout Puerto Rico. Table 2 presents the trace element concentration in samples of these species collected in Vieques and in mainland Puerto Rico. Vieques plants contained up to 10 times more lead and 3 times more cadmium than samples from mainland Puerto Rico. Differences were significant for lead ($p < 0.05$), copper ($p < 0.05$), and cadmium ($p < 0.10$). Average concentrations for cadmium and lead in Vieques exceeded safety standards (CEU, 1999) (Fig. 2). In contrast, cadmium and lead concentrations were below levels of concern (Council of European Union, 1999) in the forage samples collected in Las Marías and Mayagüez, even though these metals are naturally more common in the volcanic soils of those areas. According to the Council of the European Union for Health and Food Safety Affairs, excessive values for cadmium and lead are 1.2 and 11.2 μg/g dry weight, respectively. These rigorous levels of toxicity, as opposed to those suggested by Smith and Huyck (1999), correspond to plants used for animal or human consumption. Significant differences in concentration were also found for manganese and nickel.

Forage samples of *C. cajan* were sampled again in Monte Carmelo (Vieques) on August 18, 2001. The samples included all the individuals sampled a year earlier. Results (Table 3) confirm the metal concentrations detected in 2000 in Vieques. Lead concentration in leaves of *C. cajan* was higher than the preceding year (Fig. 2). Metal concentration was similar or slightly higher in

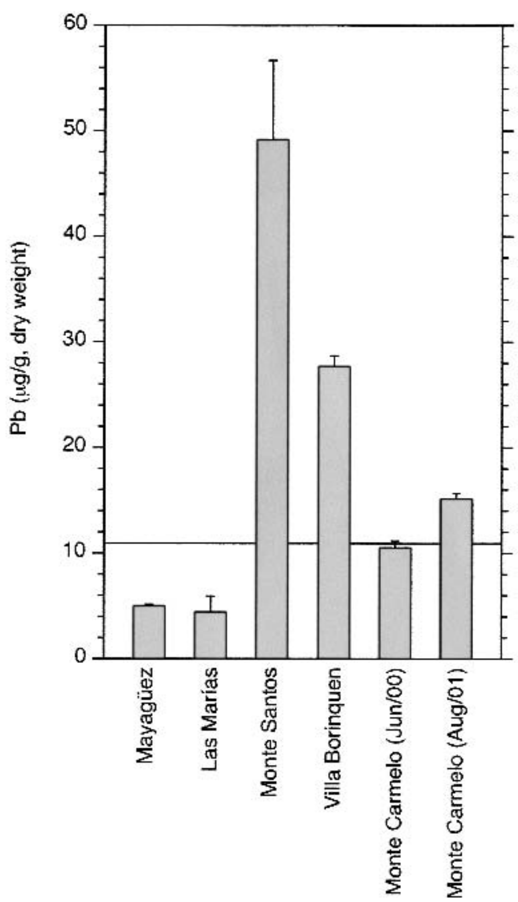

FIG. 2. Lead (Pb) content in *Cajanus cajan* grown in Vieques and elsewhere. Levels below the solid line are considered safe for human consumption (CEU, 1999).

TABLE 3. Chemical elements in leaves and fruits of *Cajanus cajans* collected at [1]Monte Carmelo (Vieques, Puerto Rico) [μg/g dry weight].

	Cu	Zn	Ni	Pb	Cd	Co
Leaves	10.72 (6.06)	32.12 (9.95)	2.26 (0.28)	15.12 (0.59)	2.42 (0.23)	17.46 (0.95)
Fruits	8.22 (1.69)	34.00 (1.86)	4.80 (0.75)	6.35 (2.06)	3.17 (1.36)	25.75 (3.48)
Fruits/leaves[2]	0.8	1.1	2.1	0.4	1.3	1.5

[1]Samples were collected from all available plants (aprox. 20 individuals) on August 18, 2001.
[2]Fruits/leaves = 1.0, equal metal concentration in both plant tissue samples. Fruits/leaves > 1.0, higher metal concentration in the fruits. Fruits/leaves < 1.0, lower metal concentration in fruits.

fruits than in leaves, with the exception of lead concentration, which was below levels of concern in fruits.

Soil contaminated with heavy metals can produce apparently normal crops that may be unsafe for consumption. Uptake of metals from air pollution has also been documented (Hughes et al., 1980). The excessive levels of trace elements found in edible plants cultivated in Vieques may be due to local anthropogenic impacts, including military exercises conducted in the nearby Atlantic Fleet Weapons Training Facility.

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