

Trace Elements Analysis in Forage Samples from a US Navy Bombing Range (Vieques, Puerto Rico)

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Abstract: Plants are good environmental sensors of the soil conditions in which they are growing. They also respond directly to the state of air. The tops of plants are collectors of air pollutants, and their chemical composition may be a good indicator for contaminated-areas when it is assessed against background values obtained for unpolluted vegetation. Both, aquatic and terrestrial plants are known to bioaccumulate heavy metals and therefore represent a potential source of these contaminants to the human food chain. An evaluation of heavy metals was conducted from vegetation samples collected at the Atlantic Fleet Weapons Training Facilities (AFWTF) in Vieques, Puerto Rico. In order to understand the potential risks associated to heavy metal mobilization through biological systems, it is first necessary to establish background values obtained from reference locations. This information allows a better interpretation of the significance of anthropogenic factors in changing trace elements status in soil and plants. Since Guánica State Forest is located at a similar geoclimatic zone as the AFWTF, samples at this site were used as a standard reference material and as experimental controls. Both sampling and analysis were conducted as previously described in standardized protocols using acid digestion of dry ashes. Then, levels of heavy metals were obtained by air-acetylene flame detection in an atomic absorption spectrophotometer. Our results from the samples taken at the AFWTF indicate mobilization of undesirable trace elements through the marine and terrestrial food web. Since plants naturally remove heavy metals from soils, they could be employed for the restoration of this and similarly contaminated sites.

Keywords: Vieques, trace elements, military wastes, phytoremediation, tropics, bioindicators.

Introduction

It is estimated that explosives and heavy metals usage have contaminated over a million cubic meters of soil at thousands of former military installations throughout the United States [14]. Major contaminants often include toxic elements such as lead, mercury, uranium and cadmium. Although trace elements occur naturally in soil, pollution with metals in bombing ranges is inevitable. Once in open environments, undesirable concentrations of pollutants could be dispersed through multiple biotic and abiotic processes. Either natural or anthropogenic in origin, soil fragmentation due to explosives will increase the surface/volume ratios of soil and thus geoavailability of metals, dust formation and erosion. The transport, residence time, and fate of pollutants in an ecosystem are serious social concerns.

Plants are good environmental quality indicators and respond directly to air, soil or water quality [2, 3]. 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. Due to the high degree of plant endemism, the Caribbean has been included as one of the ‘hotspots’ for biodiversity [5]. Over 7,000 species of endemic plants have been described in this region alone, accounting for 2.3% of the total number of plant species on earth.

A historically contaminated site with explosive and heavy metal is located at the eastern part of Vieques, Puerto Rico. Various types of explosive and non-explosive ordnance were used at the Atlantic Fleet Weapons Training Facility (AFWTF) until May of 2003 [12, 15]. This 900-acre zone was intensively used for aerial and naval bombardment. After forty years of bombing activity, this location harbors unique diversity of plants specially adapted to tolerate and perhaps attenuate this pollution. This region of Puerto Rico thus provides an excellent location for assessing plant diversity involved in heavy metal restoration.

We evaluated the use of plants as natural indicators of the flux of specific trace elements in the marine and terrestrial environment. This knowledge might lead to the identification of critical pathways of pollutant's transport in the ecosystem as well as the development of more cost effective means for the restoration of this or similarly contaminated sites.

Materials and Methods

Study Sites

Vegetation samples were obtained at the former Atlantic Fleet Weapons Training Facility (AFWTF, 18°08.32N, 65°18.10W) in the island of Vieques, Puerto Rico (Fig. 1). Samples were also taken at Guánica State Forest (17°57.213N, 66°50.971W), a Biosphere Reserve designated by UNESCO in 1981. The analyses were done on *Calotropis procera* (giant milkweed), *Panicum maximum* (guinea grass), *Ipomoea violacea*, *Acacia farnesiana*, *Sporobolus virginicus*, *S. pyramidatus* and *Syringodium filiforme* (Manatee grass). In order to evaluate temporal variation, samples of *S. filiforme* were collected in 2001, 2003 and 2004.

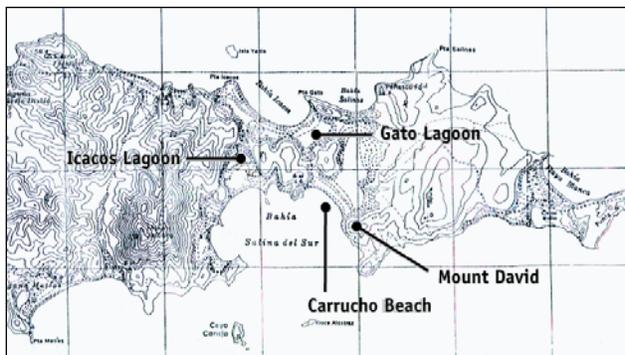


Figure 1: Sampling locations at the former Atlantic Fleet Weapons Training Facility (Vieques, PR).

Sampling Protocol

Samples were collected manually and identified by botanists at the Mayagüez Campus of the University of Puerto Rico. Each location was randomly sampled in a 2-5 m² 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 foliar sections from 5-10 plants of each species. When possible, samples of root material, stems and fruits were collected. After collection, samples were placed in large plastic bags and immediately transported to the laboratory. Samples were handled only with plastic, glass, or porcelain objects and 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 grounded in a

ceramic mortar. Approximately three grams of finely cut material was weighed in a porcelain dish that had been heated at 600°C for 2h. Samples were then dried in an oven at 65°C for 24h, allowed to cool in a desiccator, weighed, and incinerated in a muffle furnace for 2-3 h at 575°C. Ashes were dissolved in 5ml of 20% HCl and filtered. The concentration of acid-extractable elements was determined by air-acetylene flame detection in an atomic absorption spectrophotometer (Perkin Elmer Model AA100). Results were evaluated by the t-test using SYSTAT version 11.

Results and Discussion

The elemental composition of vegetative samples obtained at the Vieques's bombing range is presented in table 1. In general, distinctive profiles are observed within the studied species thus reflecting differences in their physiological properties. Copper and nickel were most abundant in *Sporobolus virginicus* and manganese was more abundant in *Syringodium filiforme* at the Guánica State Forest. In contrast, higher levels of lead were detected at the AFWTF than control populations collected from mainland Puerto Rico (Figure 2). The content of lead in pasture grass (*P. maximum*) from Vieques was as high as 13 g/g (dry weight), which is above safety guidelines [1]. Lead is used in ammunition, metal products (targets), batteries, and paints. The vegetation therefore, could be an intermediate reservoir through which trace elements in soil, water, or air move to animals and humans [2, 3]. From 1984 to 2000, the US Navy allowed local farmers to graze cows in the eastern part of Vieques including at the AFWTF. The potential for direct exposure and the impact on human health is exemplified by this pathway. Trace element analysis conducted on the island residents has demonstrated significantly higher concentration of various elements including mercury, lead and cadmium [6, 7].

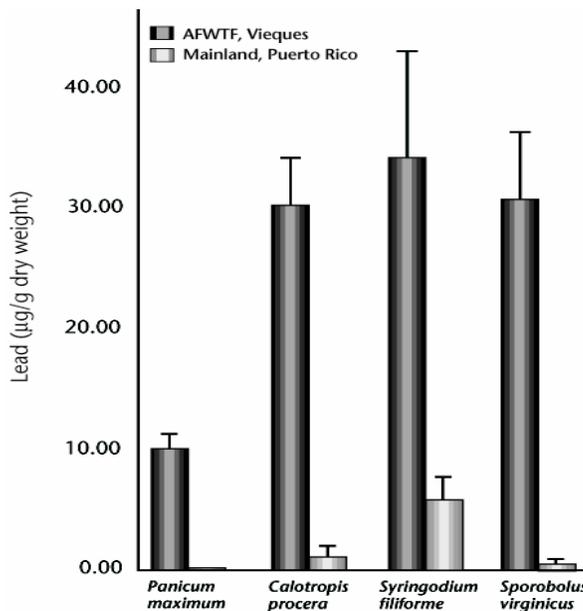


Figure 2: Average content of lead (Pb) in various plant species from the AFWTF (Vieques) and reference location (Mainland, Puerto Rico).

Table 1: Elemental analysis of terrestrial plant material collected at the AFWTF, Vieques (Puerto Rico).

	¹ Chemical Element (g/g dry weight)						
	Pb	Co	Ni	Mn	Cr	Cd	Cu
Mount David							
<i>Panicum maximum</i> (roots)	12.85 (2.10)	63.66 (0.86)	14.19 (4.72)	115.4 (47.1)	21.04 (8.56)	2.89 (0.43)	17.10 (8.11)
<i>P. maximum</i> (leaves)	10.25 (1.27)	46.65 (8.91)	5.08 (3.25)	135.0 (31.7)	5.99 (0.58)	1.57 (0.14)	4.03 (3.34)
<i>Calotropis procera</i> (leaves)	30.05 (3.63)	68.40 (9.08)	18.08 (2.50)	287.9 (3.4)	12.68 (2.82)	3.11 (2.09)	4.63 (4.25)
<i>Acacia farnesiana</i> (stems)	8.13 (1.74)	10.15 (1.08)	5.33 (1.29)	18.3 (0.1)	2.86 (0.92)	nd	4.04 (1.31)
Icacos Lagoon							
<i>Aquatic plant</i> (leaves)	2.69 (1.07)	36.31 (26.66)	29.31 (2.45)	1,740.4 (145.5)	42.14 (3.52)	4.63 (1.68)	12.93 (3.66)
Gato Lagoon							
<i>Ipomoea violacea</i> (fruits)	32.61 (9.17)	25.75 (3.89)	7.64 (4.31)	40.3 (0.1)	1.17 (0.03)	nd	9.39 (3.52)
<i>Sporobolus virginicus</i> (roots)	17.17 (1.81)	68.61 (5.59)	78.26 (12.81)	882.4 (99.8)	182.62 (9.59)	1.18 (0.07)	10.36 (11.44)
<i>S. virginicus</i> (leaves)	30.45 (5.36)	34.81 (1.80)	nd	670.6 (267.9)	5.32 (0.99)	nd	nd
<i>S. pyramidatus</i> (roots)	18.77 (5.27)	22.91 (2.61)	7.14 (2.67)	156.2 (1.8)	15.77 (2.09)	2.25 (1.07)	9.02 (2.14)
<i>S. pyramidatus</i> (leaves)	12.19 (2.71)	2.73 (0.13)	1.25 (0.52)	31.8 (3.0)	3.03 (0.29)	nd	nd

¹Average (standard deviation; n = 2 to 4);
nd, not-detectable.

In addition, lead levels up to 33 g/g (dry weight) were detected in fruit material of *Ipomoea violacea*. Both local and migratory birds such as *Zenaida aurita* (Zenaida Dove) and *Spindalis portoricensis* (Puerto Rican Spindalis) feed on this fruit [11]. Natural lead concentration in plants growing in uncontaminated areas is usually low, ranging from 0.1 to 10 g/g (dry weight) and averaging 2 g/g [3]. Moreover, the concentration of cobalt and manganese found in the vegetation in Vieques was greater than those detected in the control population. Fire, decomposition of dead vegetable tissue, aerial dispersion and consumption by herbivores could all be historic and/or on-going transport pathways out of the former bombing range.

At the marine ecosystem, levels of lead detected in *Syringodium filiforme* from the AFWTF indicate the dispersion of metals throughout the marine food chain (Table 2). In 2001, differences were significantly higher ($p < 0.05$) at the AFWTF for lead, copper, nickel and cobalt. Only aluminum was higher ($p < 0.05$) in plants collected at Guánica. The content of lead in *S. filiforme* cannot be explained solely as a result of 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 Environmental Protection Agency

Discharge Monitoring Reports from 1984-1999 identified excessive concentrations of lead with occasional average levels of up to 5 mg/L, as well as fluctuations in pH [13]. These parameters could enhance metal bioavailability, thus increasing uptake of metals by marine life during military maneuvers. Heavy metals were undetectable in seawater when military practices did not take place [10]. In May 2003, military operations ceased at the AFWTF. Samples obtained in 2004 of *S. filiforme* showed lower concentrations ($p < 0.05$) of cobalt, copper, nickel and lead to those levels observed in 2001. At the AFWTF however, the level of these elements are yet higher ($p < 0.05$) than mainland, Puerto Rico.

Syringodium is commonly found in shallow waters in southern Puerto Rico. Tribble (1981) demonstrated that coral reef fish have a preference for *Syringodium* rather than other marine plants such as *Thalassia*. Their distribution in coral reefs is usually limited by selective grazing activity [9]. The level of lead and other elements in *S. filiforme* demonstrate the potential for dispersion and dangerous bioaccumulation along the marine food chain. Fishes, crustaceans, and manatees directly or indirectly consume this marine plant. The US Fish and Wildlife Service reported manatees feeding in Vieques and most intensively near the former AFWTF. Understanding the dynamics of trace elements and other

Table 2: Elemental analysis of *Syringodium filiforme* collected at the AFWTF and Guánica State Forest

	¹ Chemical Element (g/g dry weight)						
	Pb	Co	Ni	Al	As	Cd	Cu
AFWTF-2001 (Active)	33.32 (10.77)	29.60 (5.51)	28.66 (1.58)	na na	na na	0.28 (0.13)	30.48 (3.63)
AFWTF-2004 (Non-active)	8.14 (3.15)	10.61 (4.21)	3.43 (2.13)	154.3 (67.7)	0.61 (0.46)	0.15 (0.15)	17.42 (1.83)
Guánica-2001	5.58 (1.90)	4.19 (0.06)	14.64 (4.75)	na na	na na	0.28 (0.01)	15.39 (4.16)
Guánica-2003/2004	2.33 (2.24)	1.82 (0.43)	3.75 (1.85)	341.2 (112.9)	1.04 (0.25)	0.28 (0.22)	12.16 (2.83)

¹Average (standard deviation; n = 2 to 10);
na = not available.

pollutants at this location could help establish management practices intended to prevent further exposure to human and endangered species. In turn, mitigation and better restoration mechanisms might be developed.

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