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In Vitro Bioaccessibility of Metals in Soils from a Superfund Site in Puerto Rico

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For over 30 years the Municipality of Vega Baja-Puerto Rico (Figure 1) used a 19-acre parcel of land as a domestic, commercial and industrial landfill site where garbage was also cremated, resulting in approximately 8.5 x 10^5 m^3 of disposed waste, a practice which ended by 1979 (USEPA, 2002). During the 1970's people started constructing houses forming a community named "Brisas del Rosario" on this landfill-crematory site, with approximately 200 houses located on 11 acres. The United States Environmental Protection Agency (USEPA), and other federal and local health agencies became concerned with the health risk faced by this community located on a former industrial and domestic landfill. Past soil sampling conducted by the Puerto Rico Environmental Quality Board and the USEPA revealed significant levels of toxic chemicals such as Pb achieving concentrations as high as 26,000 μg/g (ATSDR, 1998; USEPA, 2002). The health risk faced by this community, especially children, living on a hazardous waste site contaminated with high Pb soil concentrations, prompted the USEPA to declared it a Superfund Site (USEPA, 2002). According to the ATSDR (1998) and USEPA (2002), a major route of exposure to Pb for children living in "Brisas del Rosario" is contaminated soil. However, blood screening conducted only for Pb by the Puerto Rico Health Department (PRHD) in 1996 and 1998 in children aged 1-6 years old from "Brisas del Rosario" found blood Pb levels below 10μg/dL which did not reflect high soil Pb concentrations (ATSDR, 1998). Although the PRHD did not provide information on the discrepancy between blood and soil Pb concentrations, this could be partly explained by low bioaccessibility from soils due to high Pb-soil binding capacity impeding its dissociation into the stomach fluid and resulting in lesser amounts available for absorption.

The objective of this study was to determine the bioaccessibility of Pb and other metals (e.g., arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn)) in contaminated soils from "Brisas del Rosario" using synthetic gastric fluid. In addition, a sediment sample from a metal-contaminated estuary, San José Lagoon (SJL), and a standard reference soil (SRM 2710-Montana soil) from the National Institute of Standards and Technology (Gaithersburg, MD) were included for comparisons.

MATERIALS AND METHODS

The top 2.54 cm of soil was collected with 1.9 cm Oakfield tubes (Forestry Suppliers, Inc., Jackson, MS) from yards of three houses identified as B1, B2, and B3 from "Brisas del Rosario", and placed in Whirl-Pak sterile polyethylene plastic bags. The USEPA (2002) ordered the remediation of soils from houses B1 and

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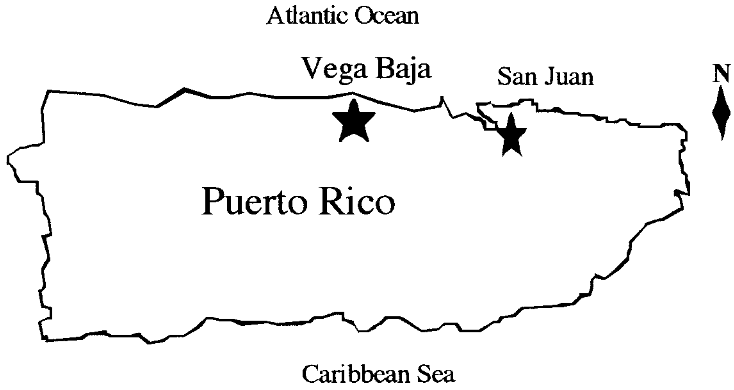

Figure 1 . Map of Puerto Rico showing the Municipality of Vega Baja-Puerto Rico where the impacted community of "Brisas del Rosario" is located.

B3. Soil samples from each house were combined and sieved (<250 μm) into a single composite, providing one composite soil sample per house, transferred to a clean plastic bag and stored at −17°C. The fine soil fraction (<250 μm) is considered the soil particle size that readily adhere to hands and toys, and that is potentially available for ingestion by children (Lanphear et al., 1996; Hwang et al., 1997). Soil and sediment physico-chemical characteristics were determined by the Soil and Plant Analysis Lab (Madison, WI).

To determine total concentrations of As, Cd, Cu, Pb, Hg, and Zn, soil samples were digested using USEPA methods 3050B and 7471A (only for Hg) (USEPA, 1996). USEPA method 3050B consisted of digesting 1.1 to 1.2 g dried weight equivalent (dw) of soil, and 0.26 to 0.27 g of the SRM 2710-Montana soil with 5 mL each of distilled deionized water (ddw) and concentrated HNO_3. Soil samples were heated at 95 ± 5°C on hot plates for 15 min in watch glass-covered 250-mL Pyrex glass beakers. An additional 5 mL of HNO_3 was added, covered, and heated for another 30 min. Aliquots of 2-3 mL of 30% H_2O_2 were added to soil samples and re-heated for 10 additional min. Cooled digested soil samples were filtered using a Whatman filter paper #41, diluted to 100 mL with ddw, and transferred to 125-mL Nalgene plastic bottles. Briefly, the digestion for Hg (USEPA method 7471A) in solid materials included concentrated sulfuric acid, and nitric acid in 125-mL glass bottles incubated in a water bath at 95 °C for 2 min, followed by the addition of potassium permanganate to the cooled solution, and re-heated with potassium persulfate. The Hg digestion was terminated with hydroxylamine. Total metal concentrations from the SJL sediment were obtained from Acevedo et al. (2000) that used USEPA methods 3051 and 7471A (USEPA, 1996).

The gastric solution was prepared according to the United States Pharmacopeia methodology (Hamel et al., 1998). A soil to liquid ratio of 1:1000 was used as a fasting reference value, while 2 hrs incubation represented the average time that HCl comes in contact with solids in the stomach at a body temperature of 37°C (Hamel et al., 1998; Rodríguez et al., 1999). The synthetic gastric fluid consisted of a solution of 1.5L ddw, 12g NaCl, 42 mL concentrated HCl, and 19.2g Sigma pepsin (9001-75-61) from porcine stomach mucosa (1,920,000 units/L) in a 18.925-L Nalgene plastic bottle (Hamel et al., 1998). The solution pH was 1.2. Aliquots of 250 mL of gastric fluid were added to 250-mL Nalgene bottles with 0.22 to 0.27 g dw of soil and sediment sample, and incubated in a waterbath at 37°C at 90 cycles/min for 2 hrs. After 2 hrs incubation, a 30 mL aliquot was transferred into a 50-mL Falcon tube and centrifuged at 3110 RPM for 13 min, and the supernatant decanted into a 60-mL Nalgene bottle. Samples were then ready for atomic absorption spectrophotometry (AAS) analysis. The bioaccessibility of metals was determined by dividing the mass of metal from gastric fluid extraction over the mass of the metal obtained using USEPA method 3050B and 3051 (only for sediment) (USEPA, 1996), and values reported after correcting for percent recovery of the SRM 2710.

Except for Hg, metals were analyzed by graphite furnace and direct aspiration modes using a Perkin Elmer (PE) AAS Model 1100B with a deuterium lamp as a background correction (USEPA, 1996; Pérez et al., 2001). Hg was analyzed using a PE MHS-10 flow-injection mercury/hydride generation system coupled to a AAS-1100B (Perkin Elmer, 1987).

RESULTS AND DISCUSSION

Among soil samples of "Brisas del Rosario", physico-chemical properties of B2 were significantly different from B1 and B3 in terms of soil texture with higher sand content (76%) and $CaCO_3$ (23%), suggesting variability in the nature and properties of soils from "Brisas del Rosario" (Table 1). The sediment sample from SJL was very distinct from soils of "Brisas del Rosario" showing generally higher physico-chemical values such as for silt, clay, organic matter, CEC, and $CaCO_3$. The pH for all samples was relatively alkaline (Table 1). Percent recoveries of metals ranged from 79% to 95% for SRM 2710, and 95% to 108% for spiked blanks (Table 2). These recoveries showed that the digestion method was appropriate to determine total metal concentrations from soils of "Brisas del Rosario".

Figure 2 shows that soils from "Brisas del Rosario" contained substantial concentrations of Cu, Cd, Pb, and Zn when compared to the SRM 2710-Montana soil, indicating that people from this community are being exposed to high levels of metals. SRM 2710 is considered a highly elevated metals-contaminated standard reference soil obtained near the former Anaconda smelter plant in Montana (NIST, 2002). Average concentrations of toxic Pb and As in soils of "Brisas de Rosario" were above USEPA soil screening values of 400 μg/g and 22 μg/g, respectively, which are considered a human health threat (Calabrese et al., 1997; Jones and Lawton, 2000). Levels of metals from "Brisas del Rosario" were higher than metal concentrations from a metal-contaminated sediment of a estuarine lagoon (SJL), with the exception of Hg. Total Hg concentrations from soils of "Brisas del Rosario" were low, a reason not to determine bioaccessibility (Figure 2).

The bioaccessible fraction of metals was different within and between solid materials, fluctuating from 4.5% for As to 91% for Cd (Figure 3). Bioaccessibility for Pb for all five samples ranged from 51% to 80%. Higher Pb and Cu bioaccessibilities were obtained for the SRM 2710-Montana soil with 80% and 73%, respectively. Soils from "Brisas del Rosario" showed between 51% to 65% bioaccessibility for Pb, while a large bioaccessible fraction was observed for Cd with 91 % for B3. Zn values ranged from 30% (SRM 2710) to 61% (B2). Using

Table 1. Properties of soils and sediment.

Sample	Texture	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	pH	CEC meq/100g	$CaCO_3$ (%)
B1	sandy loam	69	26	5	7.1	8.0	22.3	13.5
B2	loamy sand	76	19	5	7.1	7.6	19.9	23.0
B3	sandy loam	62	34	4	9.3	7.9	23.3	14.0
SJL	loam	48	44	8	11.6	7.4	32.0	28.0

B1-3 are soil samples from "Brisas del Rosario". SJL is a sediment sample.

Table 2. Percent recoveries from SRM and spiked blanks by the USEPA method 3050B.

Element	Initial concentration		Final concentration		% recovery	
	SRM 2710 ($\mu g/g$) n=3	Spiked blank (mg/L) n=2	SRM 2710 ($\mu g/g$)	Spiked blk (mg/L)	SRM 2710	Spiked blank
As	626	0.02	560 (63)[a]	0.0196 (0.99)	89.5	98
Cd	21.8	0.5	20.7 (0.4)	0.51 (0.00)	95	101
Cu	2950	1.0	2793 (54)	0.97 (0.01)	94.7	97
Hg	32.6	0.005[b]	25.6 (1.3)	0.0045	78.5	90
Pb	5532	1.0	5091 (24)	0.95 (0.01)	92	95
Zn	6952	0.5	6341 (251)	0.54 (0.01)	91.2	108

[a]Number in parenthesis is standard deviation. [b]Represents one replicate.

the SRM 2710-Montana soil for comparison, Hamel et al. (1998) obtained lower average bioaccessibility values for Cd (49.5%), and Pb (34%) than our reported concentrations, while As levels (48%) were similar. Discrepancies in percent bioaccessibility values for Cd and Pb may be due to modifications on digestion and analysis methods. The estuarine sediment from SJL obtained the highest value of bioaccessibility for As (55%), while significantly lower bioaccessibility levels (<10%) were noted with soils from "Brisas del Rosario". The variability in bioaccessibility of metals in different solid materials suggests that the solubility of each metal under synthetic gastric fluid depends on its chemical speciation and binding capacity to different soil and sediment materials (Elkhatib et al., 1991; Hamel et al.,1998; Ruby et al.,1999). An average daily intake of 13 to 2652 μg of Pb per child was calculated using the upper average "Brisas del Rosario" soil Pb concentration of 2,493 $\mu g/g$. This estimate assumed average soil ingestion rates ranging from 9 to 1834 mg/day (Binder et al., 1986; Calabrese et al. 1989; Davis et

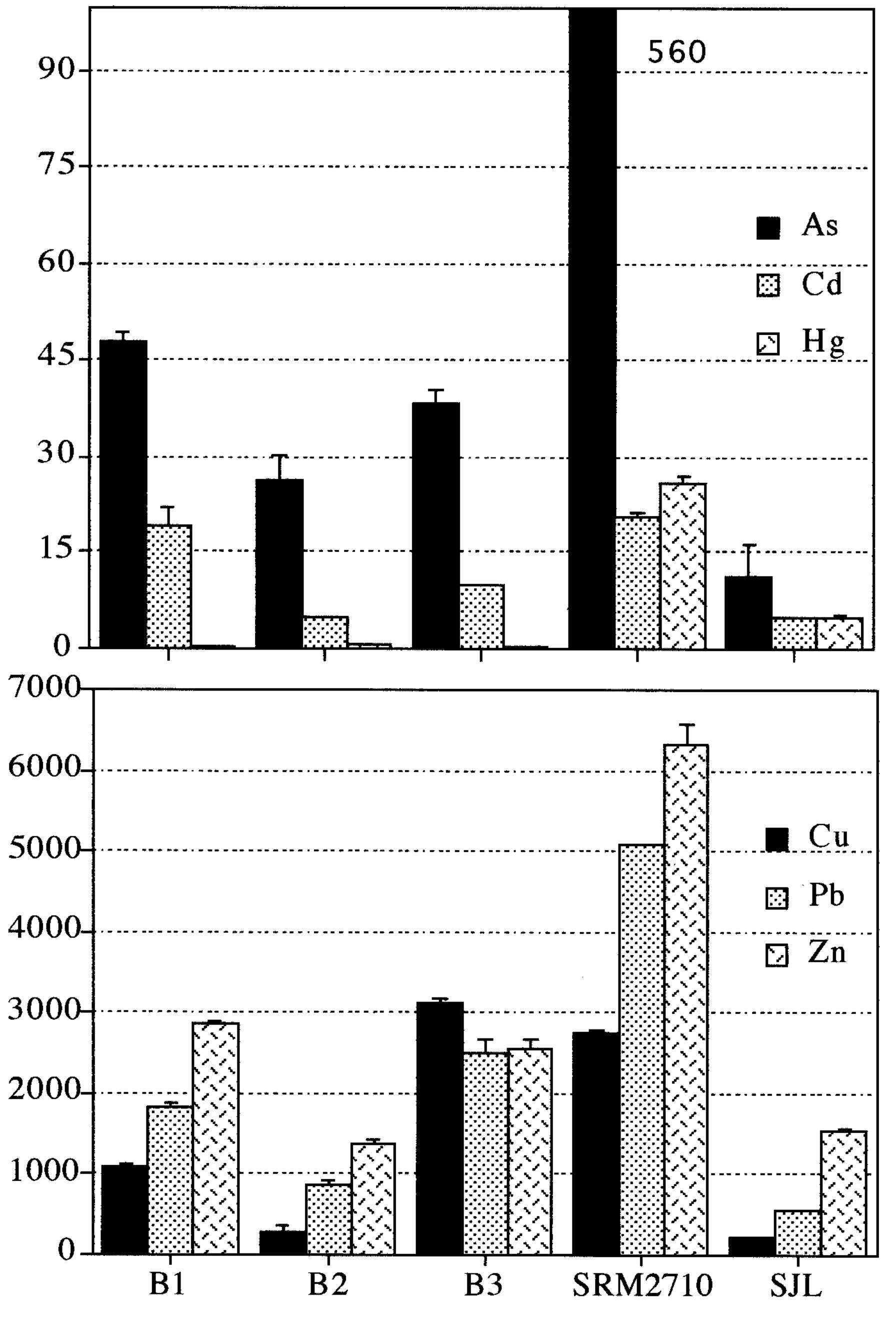

Figure 2. Average total metal concentrations (μg/g dw) in soils from "Brisas del Rosario" (B1 to B3), a sediment sample (SJL), and SRM 2710-Montana soil. Data points represent triplicates(n=3), except for As of B3 in which n=2, ± standard deviation.

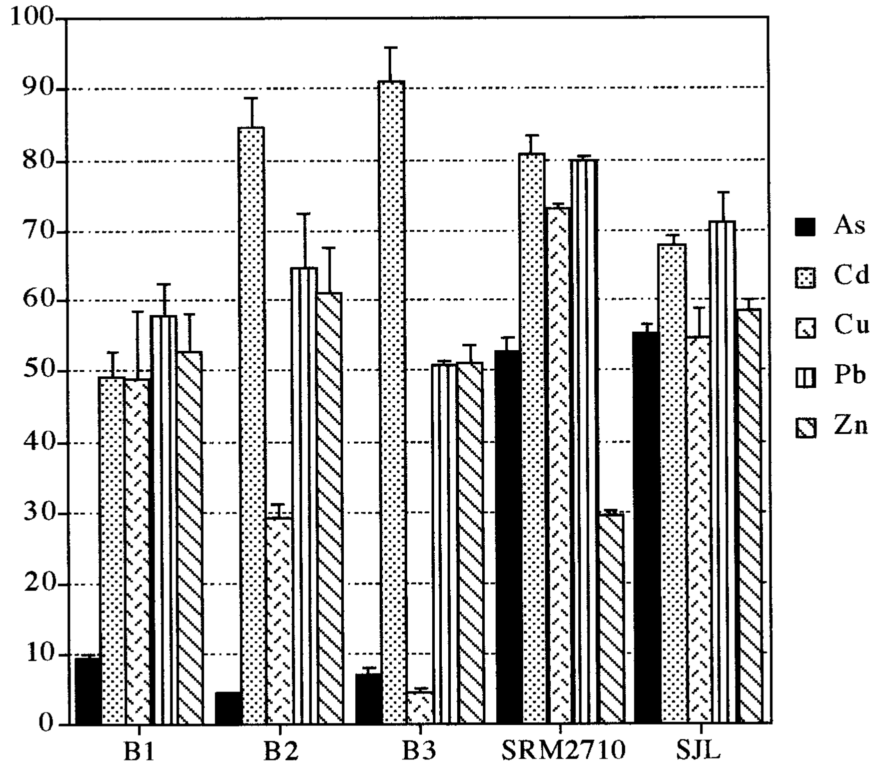

Figure 3. Average percent (%) bioaccessibility of metals in soils from "Brisas del Rosario" (B1 to B3), a sediment sample (SJL), and Montana Soil. Data points represent triplicates (n=3) except for As of SRM-2710 in which n=2, ± standard deviation.

al., 1990; van Wijnen et al., 1990; Hwang et al., 1997; Stanek and Calabrese 2000) after correcting for an average 58% Pb bioaccessibility.

Results showed that metal contaminated soils, in particular Pb, is bioaccessible in synthetic gastric fluid, potentially becoming available for absorption from the stomach compartment into the bloodstream. Therefore, it is unlikely that tightly bound Pb to ingested soil particles is a main reason for the low blood Pb levels found in children from "Brisas del Rosario". This community is located in a region characterized by calcareous soils. Although $CaCO_3$–rich soil is known to show high adsorption capacity for Pb in the form of $PbCO_3$, increased bioaccessibility would be expected under acidic conditions of the stomach (Elkhatib et al., 1991; Ruby et al.,1999). Probably low blood Pb concentrations in children reflected (1) modification in parent and child behavior that minimizes exposure to metal-contaminated soils, and (2) that blood-Pb is a marker of recent exposure returning to normal levels even when exposure is excessive (Needleman et al., 1979). Further studies are needed to determine the association between the bioaccessibility of Pb and other metals in soils, biomarkers of exposure, and health effects, to obtain an accurate health risk assessment of this community.

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