Bull. Environ. Contam. Toxicol. (2004) 72:240–247

DOI: 10.1007/s00128-003-9031-6

Environmental Contamination and Toxicology

Arsenic Fate and Bioavailability in Two Soils Contaminated with Sodium Arsenate Pesticide: An Incubation Study

D. Sarkar, R. Datta

Department of Earth and Environmental Science, University of Texas at San Antonio, 6900 North Loop 1604 West, San Antonio, TX 78249-0663, USA

Received: 9 May 2003/Accepted: 27 November 2003

The United States Environmental Protection Agency (USEPA) has classified arsenic (As) as a Group-A human carcinogen (Southworth, 1995) and banned the usage of inorganic As-based pesticides in the late eighties. However, long-time application of pesticides in agricultural lands had already resulted in high levels of As residues in certain soils (Murphy and Aucott, 1998). An elevated health risk is associated with long-term human exposure to As in such soils. During the last two decades, rapid encroachment of suburban development on lands previously used for agriculture in fast expanding metropolitan areas has tremendously increased the potential for human contact. Soil ingestion from incidental hand-to-mouth activity by children is a very important issue in assessing human health risk associated with exposure to arsenical pesticide-applied former agricultural soils, which are now being used for residential purposes. A critical parameter that allows for more realistic health risk assessment in As-containing soils is an estimate of "bioavailable" As, i.e., the extent of absorption of a chemical into the bloodstream from the gastrointestinal tract, lungs, or skin (Halmes and Roberts, 1997). Many baseline risk assessments of Superfund sites have used the conservative estimate that all (100%) As present in soil is bioavailable by equating As bioavailability in water with that in soils. However, several *in-vivo* animal studies have shown that bioavailability of As in soils may be significantly less than that in water (Rodriguez et al., 1999). The assumption of an input value of 100% bioavailability potentially overestimates the actual risk, thereby elevating the expenses associated with site-cleanup. Moreover, As exists in many geochemical and soil mineralogical forms, many of which are geochemically stable and/or insoluble in human gastric/intestinal juices and hence are not likely to be available for systemic absorption.

The major reason why As bioavailability estimate is generally taken as 100% is the tremendous cost associated with performing site-specific animal trials (*in-vivo* bioavailability studies). Hence, several *in-vitro* methods to estimate bioavailable As have been developed in recent years that have simulated *in-vivo* data with varying degree of success (Davis et al., 1992; Rodriguez et al., 1999). However, all the *in-vitro* methods developed for As deal with severely contaminated waste disposal sites, and not pesticide-applied soils with marginally higher than

---

Correspondence to: D. Sarkar

background As concentrations. Dose-response curves are often not linear at lower doses, as most toxins have a hormetic effect (Teaf, 1999). Moreover, these studies did not take into account the issue of variability in soil properties and its effect on bioavailable As concentration. Adriano (2001) summarized several soil properties that are most likely to influence soil availability of As, namely, pH, clay content, amorphous Fe-Al oxides, organic matter content, S, P concentrations and soil redox conditions. Consequently, the rather simplistic "one size fits all" approach used so far in assessing As bioavailability needs to be re-evaluated.

The current study aimed at addressing the issue of soil variability on As speciation and bioavailability. The primary goal of this study was to establish the relationship between geochemical fate of arsenic as a function of soil properties, using pesticide-contaminated soils. In this report, we present preliminary data obtained from two pesticide-contaminated soils, one with minimum As retention capacity and the other with relatively high As retention potential. The effect of soil properties on As speciation and bioavailability is discussed.

MATERIALS AND METHODS

Surface soil from the Immokalee series was collected from Southwest Florida Research and Education Center, Immokalee, Florida and the Orelia soil series was collected from Corpus Christi, Texas. Orelia series soil is distributed in the Gulf Coast Prairie of southern Texas. Approximately half of Orelia soils are cropped to cotton where arsenical pesticides were widely used. The soils were characterized for pH, electrical conductivity, particle size, Cation exchange capacity, water and organic matter content using standard protocols (Klute, 1996). Plant-available Ca, Mg and P were extracted by Mehlich III solution (Mehlich, 1984). Oxalate-extractable Fe and Al was obtained using Tamm's reagent (Sparks, 1996). Total P was extracted using the ignition method (Sparks, 1996). Total recoverable Ca, Mg, Fe, Al, P, and As was obtained by soil digestion according to USEPA method 3050B (USEPA, 1996). Phosphorus was measured colorimetrically by an UV/Visible light spectrophotometer using the molybdate-ascorbic acid method (Sparks, 1996). Iron was determined colorimetrically according to Olson and Ellis (1982). Calcium, Mg and Al were analyzed using flame atomic absorption spectrometry (FAAS). Arsenic was analyzed using graphite furnace (GFAAS).

Immokalee and Orelia series soils were incubated after spiking with sodium arsenate (the most widely used arsenical pesticide in the cotton fields) for a period of four months (mon). Two hundred grams of soil were spiked with sodium arsenate at the rate of 45, 225 and 450 mg As/kg soil. Water content was maintained at 70% of the water holding capacity of the soils. The pesticide was thoroughly mixed with soils, which were then stored in sealed bags at room temperature. The soils were aerated regularly, and constant water content was maintained. Comparative As speciation and bioavailability studies at 0 time (immediately after spiking the soils with pesticides) and after a 4 mon incubation period are reported.

Sequential extraction of As was performed following the procedure developed by

Table 1. Soil Properties

Properties		Immokalee	Orelia
pH		6.0	8.2
EC[§] (μS/cm)		59	203
CEC[¶] (Cmol/kg)		777	3,810
SOM[¥] (%)		0.84	2.39
As (mg/kg)		15.0	16.5
P (mg/kg)	Mehlich 3	4.0	62
	Total	208	1,688
Ca+Mg (mg/kg)	Mehlich 3	266	3,099
	Total	1,178	13,125
Fe+Al (mg/kg)	Oxalate	66	384
	Total	212	6,060

[§]EC = Electrical Conductivity; [¶]CEC = Cation Exchange Capacity;
[¥]SOM = Soil Organic Matter (n=3).

Chungao and Zihui (1988) for the following operationally-defined forms: 1) Water-soluble phase 2) Exchangeable phase 3) Fe- and Al-bound phase 4) Ca- and Mg-bound phase 5) Organic matter and Sulfide-bound phase and 6) Residual phase. The extracts were filtered and analyzed for As using the GFAAS. Bioavailable As was estimated following the *in-vitro* gastrointestinal method of Rodriguez et al. (1999) with certain modifications (Datta and Sarkar, 2003). All analyses were carried out in triplicate and results are shown as mean values. Replicates had to fall within 95-105% to be considered acceptable. Recoveries of 90-110% of spikes and external standards were considered acceptable.

RESULTS AND DISCUSSION

Characterization data for Immokalee and Orelia series soils are given in Table 1. Immokalee soil is a sandy spodosol with low Fe/Al, Ca/Mg and P contents. Being sandy and lacking in positively charged adsorptive surfaces (e.g. amorphous Fe/Al oxides), the Immokalee soil is likely to have minimal As retention capacity (Pierce and Moore, 1980; Oscarson et al., 1981), thereby potentially increasing the bioavailable fraction of As. This soil was used as a control to study the effects of high CEC and high concentrations of Fe, Al, Ca, Mg, P present in Orelia soil. Orelia is a sandy clay loam with high pH, and differs greatly from Immokalee in salinity (measured as electrical conductivity), CEC, Ca, Mg, Fe and Al content. According to Chen et al. (1999), the major factors controlling trace metal concentrations in soils are the clay content, organic carbon content, pH, CEC, Fe and Al content. Reportedly, As is strongly adsorbed on Fe/Al oxides (Barringer et al., 1998); hence, Orelia soil may have strong As retention capability. However, high pH and high P content of the Orelia soil could result in desorption of retained As. Both soils had similar low native As concentration (Table 1).

Soil speciation is the process of identification and quantification of the various

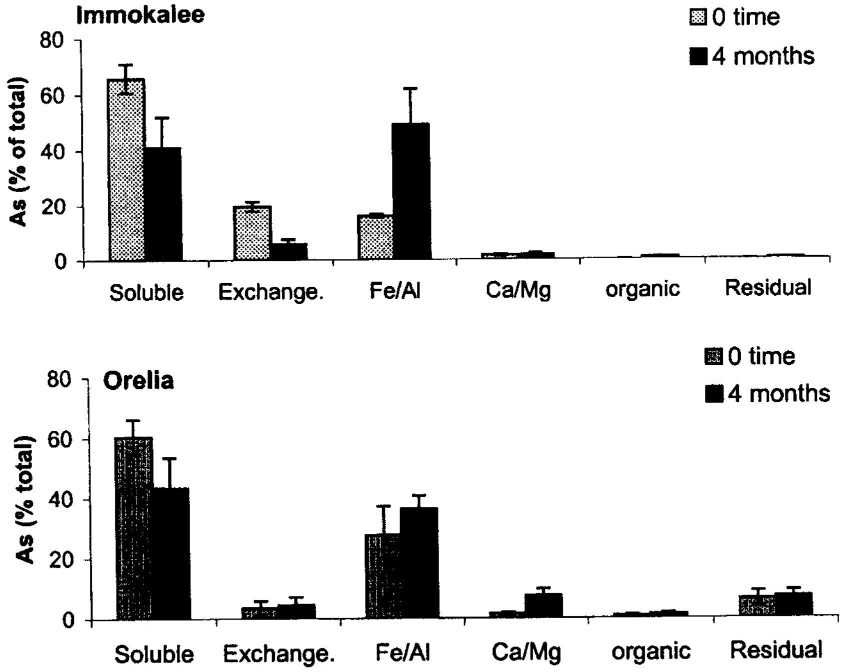

Figure 1. Geochemical speciation of As in Immokalee and Orelia soils (n=3).

"operationally defined" species, forms or phases of chemicals occurring in the soil (Pickering, 1981). Figure 1 shows the percentage of As extracted at each step of the sequential extraction procedure for both Immokalee and Orelia soils contaminated with 45 mg/kg of As. In case of the Immokalee soil, about 65% of total applied As was extracted in the soluble fraction immediately after spiking the soil with pesticides (0 time). Approximately 20% was extracted in the exchangeable fraction and the Fe/Al-bound fraction contributed to about 15% of total As. Very little As was extracted in the remaining fractions; cumulatively, the Ca/Mg-bound, organic-bound, sulfide-bound, and residual As contributed to less than 1% of the total applied As. Since soluble and exchangeable fractions of As are considered both bioavailable and phytoavailable, approximately 85% of the total As would have been available for plant uptake immediately after pesticide application. After a 4 mon incubation period, the amount of As extracted in the soluble fraction decreased to 40% and the amount in the exchangeable fraction decreased to 6%. As a result of soil-pesticide equilibration, a significant fraction of the initially soluble/exchangeable As changed forms and became associated with the Fe/Al fraction. Interestingly, although Immokalee has a relatively low Fe/Al content, a 3-fold increase was observed in the amount of As associated with the Fe/Al fraction after the 4 mon incubation period, indicating a strong tendency of As oxyanions to be adsorbed by Fe/Al oxides. As adsorbed hysteretically to Fe/Al oxides is considered to be unavailable for plant uptake, and is also not likely to be bioavailable to human gastrointestinal system (Rodriguez et al, 1998).

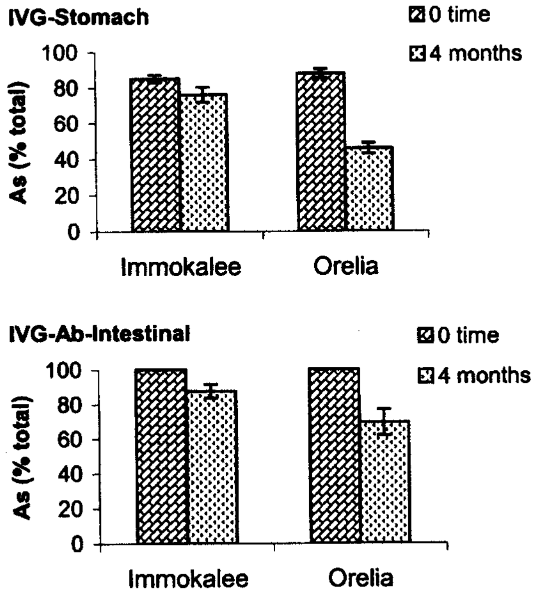

Figure 2. Bioavailable As extracted by the IVG-stomach method and the IVG absorbed-intestinal method (expressed as percentage of total As; n=3).

In Orelia soil, the amount of As extracted in the soluble/exchangeable and Fe/Al-bound fractions was similar to those of Immokalee at 0 time (Figure 1) despite having vastly different soil chemistry (high CEC, high Ca/Mg and Fe/Al contents) indicating inadequate soil-pesticide equilibration. Majority of As was extracted in the soluble/exchangeable fractions; a minor amount was extracted in the Fe/Al fraction with negligible amounts extracted in the other fractions. After 4 months, soluble As decreased by approximately 66% of the original concentrations, whereas Fe/Al-bound As increased by 133%. Furthermore, Orelia soil showed a 5-fold increase in the amount of As associated with the Ca/Mg fraction and a 1.5-fold increase in the amount of As associated with the organic fraction. Apparently, the high concentrations of Fe, Al, Ca, Mg, and CEC in Orelia soil dictated geochemical speciation of As after the soil had time to equilibrate with the pesticide. Similar results were obtained in Immokalee and Orelia soils contaminated with 225 and 450 mg/kg of As (data not shown).

The risk from As exposure is associated only with those forms of As that are potentially extractable by the human gastrointestinal juices. As evidenced earlier, As may exist in several geochemical forms depending on soil chemical properties; these forms may or may not be bioavailable. Two important mechanisms are involved in human digestion of As contaminated soil: solubility of the chemical in the digestive juices, and its absorption across the intestinal membrane (Rodriguez

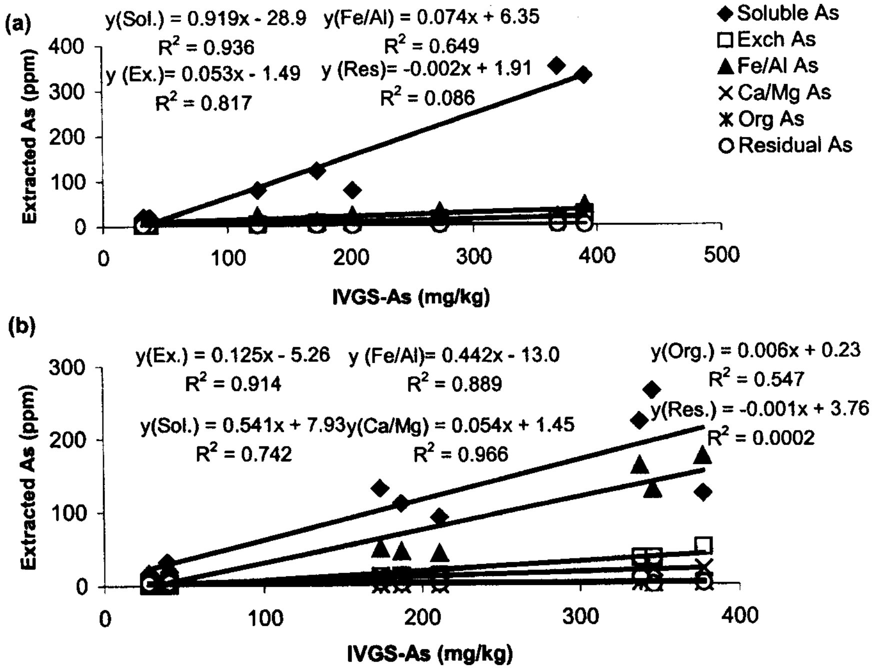

Figure 3. Correlation between bioavailable As obtained by the IVG-stomach phase and various geochemical forms of As (a) 0 time (b) 4 months

et al., 1999). While majority of the studies so far have estimated the amount of chemicals solubilized under gastrointestinal conditions (Ruby et al., 1996), only one has taken the intestinal absorption of chemicals into account while estimating bioavailability. Rodriquez et al. (1999) used iron hydroxide gel in the *in-vitro* gastrointestinal method to simulate intestinal absorption of As. In the current study, we used iron-oxide coated filter papers inserted in nylon bags as a proxy for the intestinal lining. The results of the gastrointestinal bioavailability studies in Immokalee and Orelia soils spiked with 45 mg/kg of As are shown in Figure 2. In the gastric (stomach) phase, approximately 85% of the total As was bioavailable at 0 time in the Immokalee soil, whereas about of 88% of As was bioavailable in Orelia. After 4 mon equilibration, percent bioavailability dropped to about 76% in the Immokalee soil. However, the number was significantly lower in case of Orelia; only 46%. of total As applied was bioavailable after the pesticide was allowed to equilibrate with the soil. Similar trends were noted in the intestinal phase, although it generally extracted more As than the stomach phase. At 0 time, 100% of applied As was bioavailable, which decreased to 88% in Immokalee and to 69% in Orelia. Apparently, As was much more strongly retained in Orelia soil compared to Immokalee. The potentially irreversibly adsorbed As fraction rendered a significant portion of the total soil As unavailable to human GI system in Orelia. Similar trends were observed in the Immokalee and Orelia series soils contaminated with 225 and 450 mg/kg of As (data not shown). Figure 3 shows the correlation between As extracted by *in-vitro* gastrointestinal-

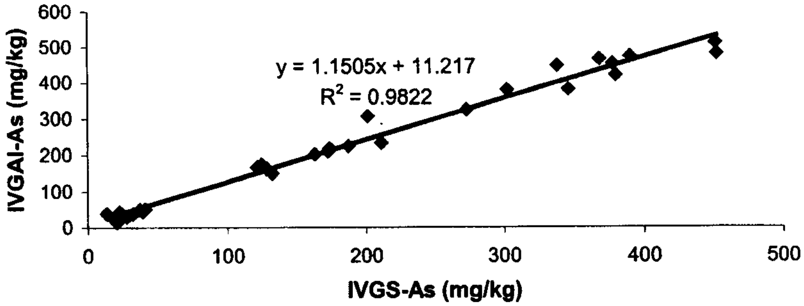

Figure 4. Comparison of bioavailable As obtained by the IVG-stomach and IVG-absorbed intestinal phases in Immokalee and Orelia soils. Data from 0 time and 4 months at 3 application rates have been pooled.

stomach (IVGS) method with As extracted by the different steps of the sequential extraction method for Orelia soil spiked with 45, 225 and 450 mg/kg of As. Immediately after spiking the soil with pesticide, bioavailable As was found to correlate highly with the soluble and the exchangeable fractions (93% and 81%, respectively) and to a certain extent with the Fe/Al-bound fraction (65%). After the 4 mon incubation period, the soluble and exchangeable fractions of As still correlated very highly with *in-vitro* bioavailable As (74% and 91%, respectively). Correlation with the Fe/Al-bound fraction increased to 89%; this is somewhat surprising because Fe/Al oxides are reported to adsorb As hysteritically, thereby decreasing potentially bioavailable As (Chen, 1999). The correlation between bioavailable As and the Ca/Mg-bound fraction increased to 97% after 4 months. Arsenic possibly precipitated as Ca/Mg compounds, which re-dissolved in the low pH gastric system, making the reversibly retained As soluble and hence, available for human absorption. However, this fraction is not likely to be available for plant uptake since the extractant pH has to be low enough to dissolve the precipitates. Bioavailable As also correlated significantly with organic-bound As (54%); SOM possibly solubilized a portion of soil As, thereby making it bioavailable (Gough et al., 1996). Similar results were observed when As obtained by the different steps of sequential extraction was correlated with bioavailable As obtained by the IVG absorbed-intestinal method (data not shown). Figure 4 shows the correlation between As extracted by the two IVG methods. A regression coefficient of 0.98 was obtained, indicating that results from both methods were statistically similar, and that both *in-vitro* methods were extracting As from similar soil As pools.

The study demonstrated that soil properties have a marked impact on geochemical speciation of As. Soil chemistry dictated the operationally defined forms of As. Arsenic bioavailability was mostly a function of geochemical speciation.

Acknowledgments. We thank the Texas Higher Education Coordinating Board Advanced Research Program for funding this study. The Center for Water Research, UTSA is acknowledged for providing the support for a technical assistant and the administrative assistance of Ms. Hermina Simpson. Thanks to Drs. T. Obreza and J. Matocha for providing the soils.

REFERENCES

Adriano DC (2001) Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals. Springer, New York, NY

Barringer JL, Szabo Z, Barringer, TH (1998) USGS WRI Rep 98-4016. US Geological Survey, West Trenton, NJ

Chen M, Ma LQ, Harris WG (1999) Baseline concentrations of 15 trace elements in Florida surface soils. J Environ Qual 28:1173-1181

Chunguo C, Zihui L (1988) Chemical speciation of As in water, suspended solids and sediment of Xiangjiang river. China Sci Total Environ 77:69-82

Datta R, Sarkar, D (2003) National Association of Environmental Professionals, 28th Annual Conference Proc, June 22-25, San Antonio, TX.

Davis A, Ruby MV, Bergstrom PD (1992) Bioavailability of arsenic and lead in soils from the Butte mining district. Environ Sci Technol 26:461-468

Gough LP, Kotra RK, Holmes CW, Briggs PH, Crock JG, Fey DL, Hageman PL, Meter AL (1996) USGS Open File Report 96-091, Washingtion, DC

Halmes NCH, Roberts SM (1997) Arsenic bioavailability: A review of the literature. Technical Report 97-02, CEHT, University of Florida

Klute A (1996) Methods of Soil Analysis: Part 1: Physical and Mineralogical Methods. Soil Sci Soc Am Publications, Madison, WI

Mehlich A (1984) Mehlich No 3 soil test extractant: A modification of Mehlich No 2 extractant. Commun Soil Sci Plant Anal 15:1409-1416

Murphy E, Aucott M (1998) An assessment of the amounts of arsenical pesticide used historically in a geographic area. Sci Total Environ 218:89-101

Olson RV, Ellis R (1982) Methods of Soil Analysis Part 2. In: Agron Monogr 9 Am Soc Agron and Soil Sci Soc Am, Madison, WI. pp 301-312

Oscarson DW, Huang PM, Defosse C, Herbillon A (1981) Oxidative power of Mn(IV) and Fe(III) oxides with respect to As(III) in terrestrial and aquatic environments. Nature 291:50-51

Pickering WF (1981) Selective chemical extraction of soil components and bound metal species. Critical Rev Anal Chem 12:233-266

Pierce ML, Moore CB (1980) Adsorption of arsenite on amorphous iron hydroxide from dilute aqueous solution. Environ Sci Technol 14: 214-216

Rodriguez RR, Basta NT, Casteel SW Pace LW (1999) An *in-vitro* gastro-intestinal method to estimate bioavailable arsenic in contaminated soil and solid media. Environ Sci Technol 33:642-649

Ruby MV, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB, Bergstrom P (1993) Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ Sci Technol 27:2870-2877

Sparks DL (1996) Methods of soil analysis: Chemical methods. Soil Sci Soc Am Book Series (Part 3). Soil Sci Soc Am, Inc, Madison, WI

Southworth RM (1995) Land application pollutant limit for arsenic. Part 503 USEPA, Washington, DC

Teaf CM (1999) Can regulatory agency actions accommodate hormesis, adaptation and mechanisms of toxicity? In: Belle Newsletter 8 Northeast Regional Environmental Public Health Center, Amherst, MA. pp 1-5

USEPA (1996) Test methods for evaluating solid waste SW 846 3rd ed. Office of solid waste and emergency response, Washington, DC