Developments in Environmental Science, Volume 5
D. Sarkar, R. Datta and R. Hannigan (Editors)
© 2007 Published by Elsevier Ltd.
ISSN: 1474-8177/DOI:10.1016/S1474-8177(07)05015-2

Chapter 15

Effects of incubation time and arsenic load on arsenic bioaccessibility in three Florida soils amended with sodium arsenate

Rupali Datta, Konstantinos C. Makris and Dibyendu Sarkar

Abstract

The potential for human exposure to arsenic (As), a group A carcinogen, has increased tremendously due to the encroachment of suburban areas into former agricultural lands, where arsenical pesticides were used extensively prior to the 1990s. Soil ingestion is the no. 1 route of As exposure, attributable to incidental hand-to-mouth activities by children in playgrounds or house yards having As-contaminated soil. Previous studies have shown that As bioaccessibility in soils is mostly a function of their physicochemical properties. We selected three Florida soils covering a wide range in chemical properties, such as, Fe/Al hydroxides, organic matter, and Ca/Mg content, which are most likely to influence As bioavailability. The soils were amended with sodium arsenate pesticide at loads ranging from 45 to 450 mg As per kg, and subsequently incubated up to 12 months. The overall objectives of this study were to evaluate the effects of incubation time and As load on soil speciation, hence bioaccessibility of As. Results showed a reduction in the water-soluble, and plant-available (water + NH_4Cl-extractable) As fractions of the three soils after four months of incubation, which remained unchanged up to 12 months. This reduction with time was accompanied by a concurrent increase in the NaOH-extractable As fraction, suggesting As sorption by amorphous Fe/Al hydroxides could decrease As bioaccessibility. The effect was most pronounced for the Pahokee Muck soil, which had the greatest amount of amorphous Fe/Al hydroxides (2000 mg kg^{-1}) of all soils. Arsenic associated with the water- and plant-available As fractions is most bioaccessible as indicated by the significant ($\alpha = 0.05$) correlation between the water-soluble, plant-available, and the in-vitro

bioaccessible As fraction in the soils. The NaOH-extractable As fraction was negatively correlated with the in-vitro bioaccessible As fraction, suggesting that the presence of Fe/Al hydroxides could decrease As lability in soils.

15.1. Introduction

Arsenic is a human health concern because it can contribute to skin, bladder, and other cancers (National Research Council-NRC, 1999). Arsenic can also be toxic to plants; an average toxicity threshold of $40\,mg\,kg^{-1}$ soil was established for several crop plants (Sheppard, 1992). Inorganic arsenicals are classified as the number one toxin in the U.S. Environmental Protection Agency (USEPA) list of prioritized pollutants. Arsenic can be found in surface and subsurface water bodies, soils and in many foods; the risk of exposure being the greatest in drinking water (Brown and Ross, 2002). Recommendations by the NRC to lower the current maximum contaminant level (MCL) allowed for As in drinking water from 50 to $10\,\mu g\,l^{-1}$ have been accepted by the USEPA.

While As occurs naturally in the geosphere, there are several anthropogenic sources of As in the environment. Naturally occurring As can be found in soils as the result of the weathering of primary and secondary As-bearing minerals. Sixty percent of the anthropogenic As input comes from only two sources: Cu-smelting and coal combustion (Matschullat, 2000). Other anthropogenic sources of As include herbicide, pesticide, and rodenticide use, as well as waste incineration, mining, batteries, semiconductor industries, steel/glass production, and pressurized wood production (Matschullat, 2000). All of the above listed As sources have significantly increased As levels in soils (total soil As range $7–970\,mg\,kg^{-1}$, Matschullat, 2000).

Arsenic exists in soils mostly in the $+5$ or $+3$ oxidation states. Arsenate (As(V)) is the oxidized form and occurs in well-aerated soils, whereas in chemically –reduced soil environments, arsenite (As(III)) is the prevalent As form. Although arsenite is more toxic than arsenate, arsenate can also have deleterious effects on animals, plants, and microorganisms. Sodium arsenate (SA) was extensively used as a pesticide in the past in agricultural land, elevating soil As concentrations above background levels. The risk of human contact with soil–As has greatly increased in the last two decades as residential areas expanded into former agricultural land. Elevated As concentrations were found in soils used for residential developments located on former apple orchards that had been amended with arsenical pesticides for years (Murphy and Aucott, 1998).

Soil ingestion is the no. 1 route of exposure to As from incidental hand-to-mouth activity of children playing in playgrounds or back yards with As-contaminated soils (Cohen et al., 1998). Bioavailability of As depends upon soil speciation of As, which in turn is a function of the physico-chemical properties of the soil. However, current practices utilize the conservative estimate of 100% As bioavailability in all soil types, ignoring differences in physicochemical properties between soil types. Several in-vivo animal studies have shown that bioavailability of As in soils is significantly lower. Since calculated health risk is a direct function of the input value for chemical dose, the assumption of using an input value of 100% bioavailability for exposure to As-enriched soils potentially overestimates the actual risk, thereby elevating the expenses associated with potential site-cleanup. Hence, there is an increasing need for more studies on the bioavailability of As in contaminated soils of varying physicochemical properties.

In-vivo bioavailability studies using animal models are expensive and time-consuming. The elevated cost associated with performing in-vivo bioavailability studies has encouraged the use of in-vitro methods to estimate bioavailable- or bioaccessible-As (Davis et al., 1992; Sheppard et al., 1995; Rodriguez et al., 1999). We chose to utilize the recently developed term *bioaccessibility* to better define the ambiguous use of *bioavailability* that has been used for both in-vivo biological and in-vitro chemical extraction assays. According to Semple et al. (2004), the *bioaccessible* compound is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical. However, the chemical may be either physically removed from the organism or only bioavailable after a period of time (Semple et al., 2004). A *bioavailable* compound is defined as the compound which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time (Semple et al., 2004).

Most of the in-vitro methods to estimate bioaccessibility developed for As have dealt with severely contaminated waste disposal sites, but were not applied to arsenical pesticide-treated soils, which usually contain marginally higher than background As concentrations. A few recent studies have utilized soils contaminated with lower levels of As ($\sim$45 mg kg^{-1}) to evaluate the effect of different soil types on the magnitude of bioaccessible As, where dimethylarsinic acid (DMA) (Sarkar et al., 2004) and sodium arsenite (Datta and Sarkar, 2004) were used as the As pesticide source. Sarkar et al. (2004) emphasized the influence of soil type on the geochemical fractionation and bioaccessibility of As; soils with high concentrations of amorphous Fe/Al hydroxides retained more As–DMA, rendering As less bioaccessible.

The current study aims at addressing the effect of soil physicochemical properties on As fractionation and bioaccessibility in three soils incubated with sodium arsenate. We hypothesized that poorly As sorbing soils (low in amorphous Fe/Al hydroxides) would exhibit high degree of As bioaccessibility, whereas soils with higher amorphous Fe/Al content would show increased As retention. The main objectives of this study were: (i) to evaluate the effects of incubation time and As load on As geochemical fractionation and bioaccessibility in three FL soils and (ii) to determine the degree of correlation between various soil–As forms, as obtained by sequential extraction and different measures of As bioaccessibility obtained by in-vitro tests.

15.2. Materials and methods

Soil sampling and characterization. Three surface (0–12 cm depth) FL soils were collected for this study: soil samples from the Immokalee series were collected from Southwest Florida Research and Education Center, Immokalee, Florida; Millhopper soil samples were collected from the University of Florida campus at Gainesville, Florida, and the Pahokee Muck soil was collected from Everglades Research and Education Center at Belle Glade, Florida. The soils were selected based on their presumed As retention capacities. Immokalee soil is a sand (93%) with minimum As retention capacity, Millhopper soil is a sandy loam with relatively high concentration of Fe/Al hydroxides, thus, higher As sorption capacity than Immokalee soil. The third soil used was a Pahokee Muck soil series, which is expected to show the highest affinity for As, since it is characterized by large amounts of Fe/Al and Ca/Mg contents, as well as, increased organic matter content.

Soils were air-dried, passed through a 2-mm sieve, and analyzed for pH, electrical conductivity, particle size, and water content using standard protocols. Organic matter content was determined using the loss-on-ignition method (Klute, 1996). Exchangeable cations were extracted in 1 M ammonium acetate (pH 7.0) and cation exchange capacity was determined with the ammonium acetate method (Rhoades, 1982). Plant-available Ca, Mg, and P were determined using the Mehlich III soil test (Mehlich, 1984). Oxalate-extractable Fe and Al concentrations of the soils were determined using Tamm's reagent (Klute, 1996). Total-recoverable Ca, Mg, Fe, Al, P, and As concentrations were measured in acid digests according to USEPA method 3050B (US EPA, 2000).

Phosphorus was colorimetrically measured with a UV/vis spectrophotometer, using the molybdate–ascorbic acid method (Watanabe and

Olsen, 1965). Soluble Fe, Al, Ca, and Mg were analyzed using flame atomic absorption spectrometry (FAAS). Total soluble As was analyzed using graphite furnace atomic absorption spectrometry (GFAAS).

Soil amendments. Two hundred grams of the Immokalee, Millhopper, and Pahokee soils were amended with low ($45\,mg\,kg^{-1}$), medium ($225\,mg\,kg^{-1}$), and high ($450\,mg\,kg^{-1}$) As loads as sodium arsenate (SA). These As loads represent average As concentrations in soils treated with arsenical pesticides ($45\,mg\,kg^{-1}$), or loads that may be encountered in soils with a prolonged history of arsenical pesticide applications ($450\,mg\,kg^{-1}$ load) (Chisholm et al., 1955). Water content was maintained at 70% of the water holding capacity of the soils. Samples were thoroughly mixed and were stored in tightly sealed bags at room temperature. Soils were aerated regularly and constant water content (70% of the water holding capacity) was maintained biweekly. Extractable As was quantified in soil sub-samples taken at time zero (immediately after sodium arsenate application), 4 and 12 months of incubation, using sequential extractions and in-vitro procedures as described below.

Sequential As extractions. Sequential extraction of As was performed using the method of Chunguo and Zihui (1988) with a few modifications (Datta and Sarkar, 2004) for the following operationally defined As 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 sum of water- and NH_4Cl-extractable As pools is designated as plant-available pool, based on earlier phosphorus (P) fractionation experiments (Hedley et al., 1982). Extracts were filtered and analyzed for total soluble As using GFAAS.

In-vitro test. Bioaccessible As was determined following the method of Rodriguez et al. (1999) who developed the "physiologically based extraction test" (PBET) to predict metal bioavailability in human gastric juices (stomach phase).

Statistical analyses. Data were analyzed for the main and interaction effects of the incubation time and As load treatments in a 3×3 completely randomized design using the Design-Expert software (Design-Expert, 2001). Potential outliers were identified and were eliminated from statistical analyses. Pearson correlation coefficients were computed using the Statistix 7 software package. Data are reported as the means of triplicate measurements $\pm$ one standard deviation.

15.3. Results and discussion

Soil properties. The three surface FL soils were acidic, with electrical conductivities and cation-exchange capacities in the order of

Table 15.1. General chemical properties of the three FL soils

		Immokalee	Millhopper	Pahokee
pH		6.0	6.4	5.9
EC ($\mu S\,cm^{-1}$)		59	145	558
CEC ($cmol\,kg^{-1}$)		777	2356	18,908
Soil organic matter ($g\,kg^{-1}$)		8.4	43.8	854
Total-recoverable As ($mg\,kg^{-1}$)		0.8	1.8	1.9
P ($mg\,kg^{-1}$)	Mehlich 3	4.0	134	36.0
	Total	210	4900	6800
Ca + Mg ($mg\,kg^{-1}$)	Mehlich 3	270	900	10,000
	Total	1200	3200	41,000
Fe + Al ($mg\,kg^{-1}$)	Oxalate	66	704	2000
	Total	212	4800	6000

EC, Electrical conductivity; CEC, cation-exchange capacity.

Immokalee < Millhopper < Pahokee (Table 15.1). Soil organic matter content was least for the Immokalee (0.84%), higher in Millhopper (4.3%), and extremely high (85%) for the Pahokee Muck soil. Total As concentrations of the three soils were $< 2\,mg\,kg^{-1}$, and within the range of background soil As concentrations (0.1–40 $mg\,kg^{-1}$ soil) (Kabata-Pendias and Pendias, 1992). Total-recoverable P, Ca + Mg, and Fe + Al concentrations increased in the Immokalee < Millhopper < Pahokee order (Table 15.1). Oxalate-extractable Fe + Al concentrations for all three soils were < 40% of the total concentrations, suggesting the presence of Fe/Al hydroxides and potential for oxyanion (As or P) retention.

Sequential As extraction. Soil sub-samples taken at 0, 4, and 12 months were fractionated into different operationally defined As pools. Fractionation data for the unamended (no As added) soils showed As concentrations in the order of $< 0.5\,mg\,kg^{-1}$ in each fraction, and the sum of all As fractions was $\sim 2\,mg\,kg^{-1}$ for all three soils, being consistent with total-recoverable As concentrations measured independently via acid digestion (data not shown). Right after pesticide amendment to the soils (time zero), most of the added As was found in the water-soluble phase, representing > 60% of the total As for all As loads of the Immokalee and Pahokee soils (Fig. 15.1). Immokalee soil had negligible As retention capacity due to the absence of As-adsorbents such as Fe/Al oxides, or As-precipitators, such as Ca and Mg. The amount of water-soluble As was high in Pahokee Muck soil despite the high oxalate-extractable Fe/Al and Ca + Mg concentrations (Table 15.1). The large amount of organic matter (85%) in this soil probably covered the mineral surfaces and retarded As sorption by stabilizing As into solution. The relatively high amorphous Fe/Al content ($700\,mg\,kg^{-1}$) of the Millhopper soil could

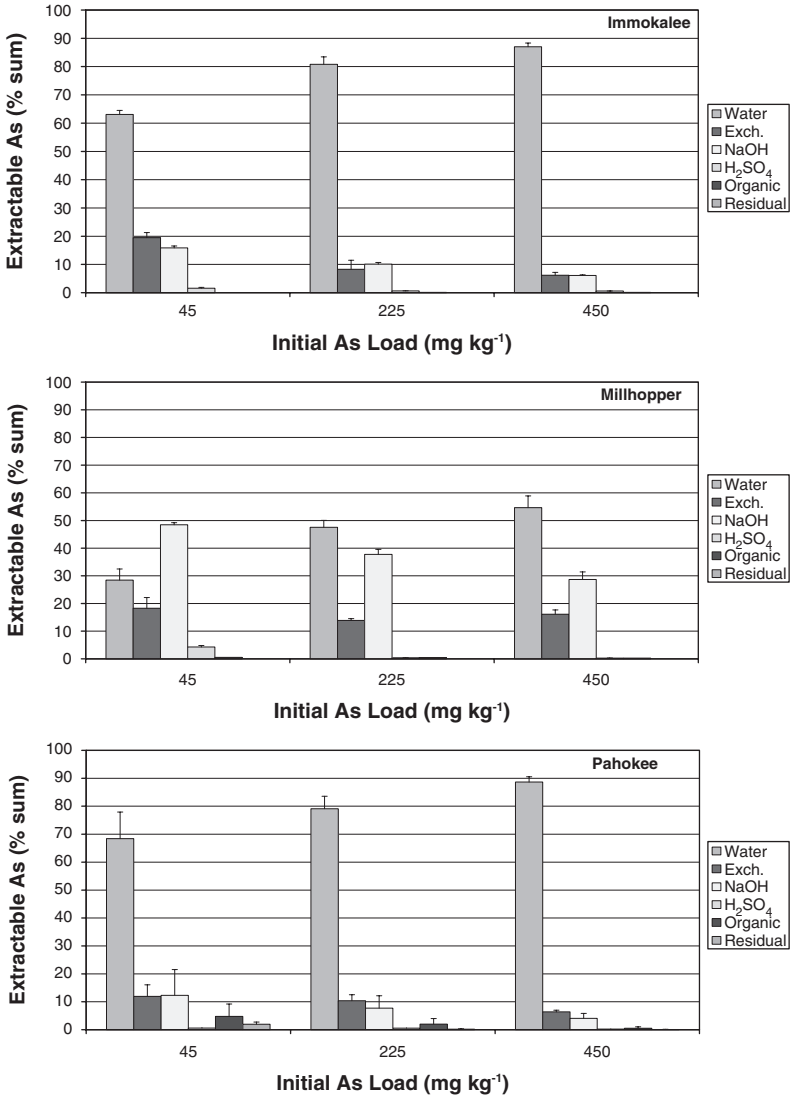

Figure 15.1. Sequential As fractionation data as a function of As load for the three soils at time zero. Data are the average of three replicates $\pm$ one standard deviation.

serve as a sink for As and resulted in low amounts of As in the water-soluble phase at time zero (30–50%, all As loads) (Fig. 15.1). Increasing As loads from 45 to 450 mg kg^{-1} resulted in greater amount of As extracted in the water-soluble phase for all soils, suggesting greater risk

of As mobility (Fig. 15.1). Concurrently, As in the exchangeable and NaOH-extractable phase decreased with increasing As load.

Sub-samples were removed after 4- and 12-month incubation, and subjected to the sequential As fractionation scheme (Fig. 15.2). Water-soluble As in the Immokalee and Pahokee soils decreased after 4- and 12-month incubation, paralleling increases in the NaOH-extractable As fraction. Results for the Millhopper soil were less clear, but within the trends observed with the other two soils (Fig. 15.2). Reductions in water-soluble As fractions of the soils with incubation time suggest that soluble As was allowed to react and sorb onto Fe/Al- or Ca/Mg-bearing soil phases. Analysis of variance showed that there was a significant ($\alpha = 0.05$) interaction between incubation time and As load, rendering impossible to draw conclusions from the significant ($\alpha = 0.05$) main effects of time and As load as shown in Figs. 15.1 and 15.2.

The sum of water-soluble and exchangeable-As is frequently cited as the plant-available As fraction. Incubation time and As load showed an interactive effect on the plant-available As fraction of the Immokalee soil; plant-available As concentrations decreased with incubation time only at the lowest As load ($45\,mg\,kg^{-1}$) (Fig. 15.3). At 225 and $450\,mg\,kg^{-1}$, plant-available As remained unchanged with time, suggesting that the minimum As sorption capacity of the Immokalee soil was unable to retain As added at high loads (225 and 450 mg As per kg). No significant interaction between As load and incubation time was observed for the plant-available fraction of the Pahokee soil (Fig. 15.3); plant-available As concentrations significantly ($\alpha = 0.05$) increased with As load at all incubation times (Fig. 15.3). Plant-available As of the Pahokee soil was significantly ($\alpha = 0.05$) reduced within four months of incubation from ~83% to 53%, and remained unchanged thereafter (Fig. 15.3). Data for the Millhopper soil were similar and are not shown. Incubation experiments with the Immokalee and Millhopper soils amended with dimethylarsinic acid(V) (DMA) showed a similar decrease in the water-soluble As fraction with incubation time (Sarkar et al., 2004). Despite the obvious decrease in plant-available As concentrations with incubation time, a significant amount of As (~50% of the total As added) remains in solution, and it is accessible to plants and microorganisms, posing a serious health risk for living organisms.

The observed decreases in plant-available As concentrations with incubation time could be explained on the basis of a concurrent increase in the NaOH-extractable As fraction with incubation time (Fig. 15.4). In the

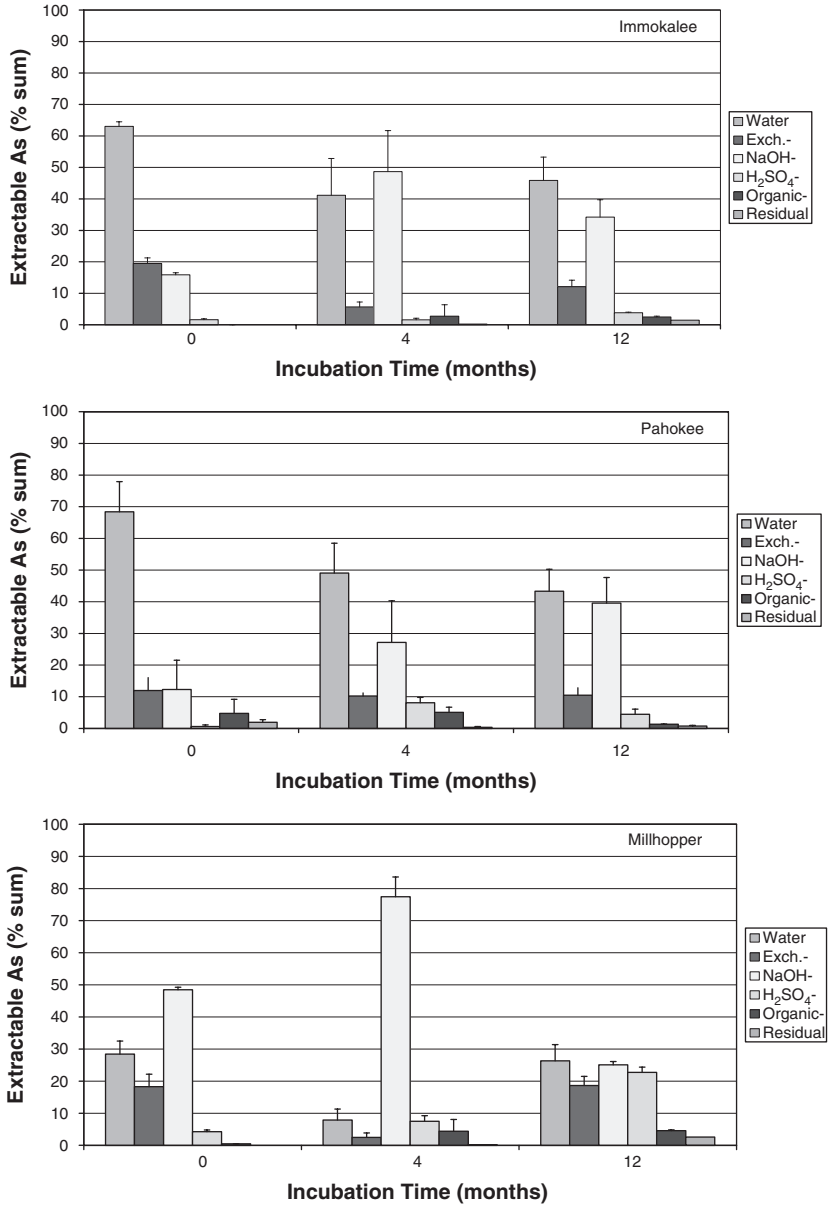

Figure 15.2. Effect of incubation time on the different As fractions for the three soils amended with 45 mg As per kg. Data are the average of three replicates ± one standard deviation.

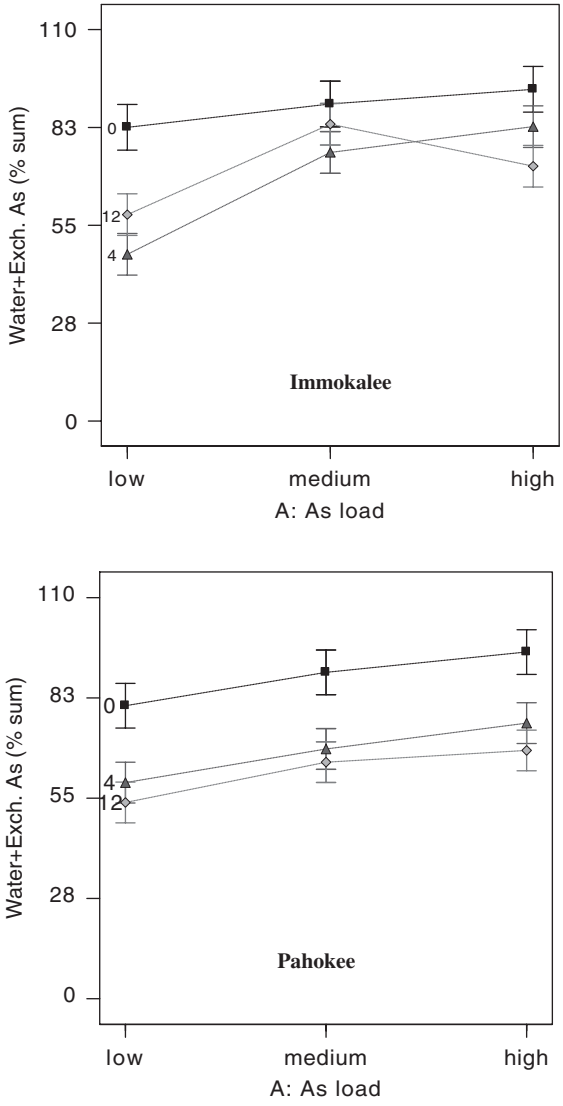

Figure 15.3. Effects of sodium arsenate application rate (low = 45, medium = 225, and high = 450 mg kg^{-1}) and incubation time (0, 4, and 12 months after amendment application) on the plant-available As (water + NH_4Cl – extractable As) fractions of the Immokalee and Pahokee soils (expressed as percent of the sum of all As fractions). The error bars represent the least significant difference (LSD) at the 95% confidence level. Data are the mean of three replicates. Data for the Millhopper were similar (not shown).

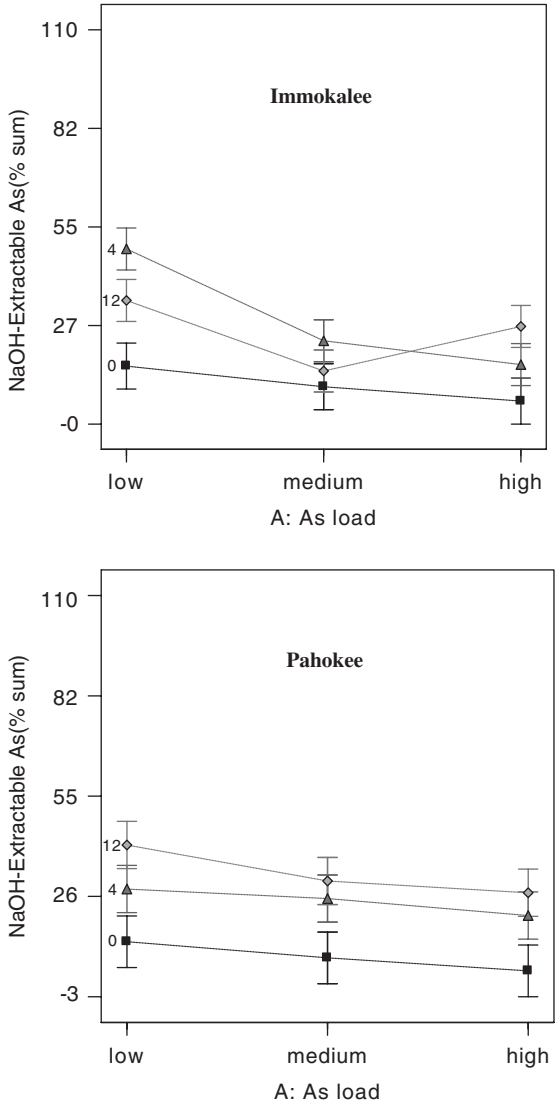

Figure 15.4. Effects of sodium arsenate application rate (low = 45, medium = 225, and high = 450 mg kg^{-1}) and incubation time (0, 4, and 12 months after amendment application) on the NaOH-extractable As fractions of the Immokalee and Pahokee soils (expressed as percent of the sum of all As fractions). The error bars represent the least significant difference (LSD) at the 95% confidence level. Data are the mean of three replicates. Data for the Millhopper were similar (not shown).

case of Immokalee soil, there was a significant ($\alpha = 0.05$) increase in NaOH-extractable As concentration with time, but only in the 45 mg kg^{-1} As load, consistent with the observed temporal decreases in plant-available As concentrations (Fig. 15.4). A positive incubation time effect on the NaOH-extractable As concentration in the Pahokee soil was observed for all As loads, which is also consistent with the temporal trends of the plant-available As (Fig. 15.4). The Millhopper soil showed similar trends (data not shown). Regardless of the initial As load (by mass), the relatively high amorphous Fe/Al content of the Pahokee soil (2000 mg kg^{-1}) was responsible for decreasing labile As concentrations, rendering sorbed As less bioaccessible. Arsenic adsorption is positively correlated with the Fe and Al hydroxide content of soils (Goldberg, 2002). Similar to the NaOH-extractable As, the H_2SO_4-extractable concentrations increased with time for all three soils, suggesting that As was transferred toward less-labile pools, such as Ca/Mg compounds (data not shown).

The two mineral soils (Immokalee and Millhopper) showed no significant effect of incubation time or As load on the organically bound As fraction (data not shown). Interestingly, the soil with the highest organic matter content (Pahokee Muck) also showed no effect of incubation time, or As load on the magnitude of the organic-bound As fraction, indicating that the major As sorbents in the three FL soils, are mostly inorganic (Fe/Al and Ca/Mg pools).

In-vitro bioaccessibility of As. Sub-samples of the three FL soils were subjected to the in-vitro stomach phase to simulate soil–As particle bioaccessibility when present in human gastric juices. For the stomach phase of the Immokalee soil, there was a significant ($\alpha = 0.05$) increase in bioaccessible As concentrations with increasing As load, and a concurrent decrease in As bioaccessibility with incubation time (Fig. 15.5). Despite the significance of the decrease in bioaccessible As with time, remaining levels (~55% of total As added) were still high, being consistent with the large water-soluble and plant-available As concentrations that remained even after 12 months of incubation (Figs. 15.1–15.3). Similar results were obtained with the Pahokee Muck and Millhopper soils; there was a significant reduction in bioaccessible As of the stomach phase with time (Fig. 15.5), but large concentrations of bioaccessible As remained, which could pose a serious threat to human health. It is noteworthy that the remaining bioaccessible As levels were approximately equal for all three soils (50–60%). These data mandate the need for extensive research to develop novel and cost-effective methods to lower As bioaccessibility and, thus, bioavailability in As-contaminated soils.

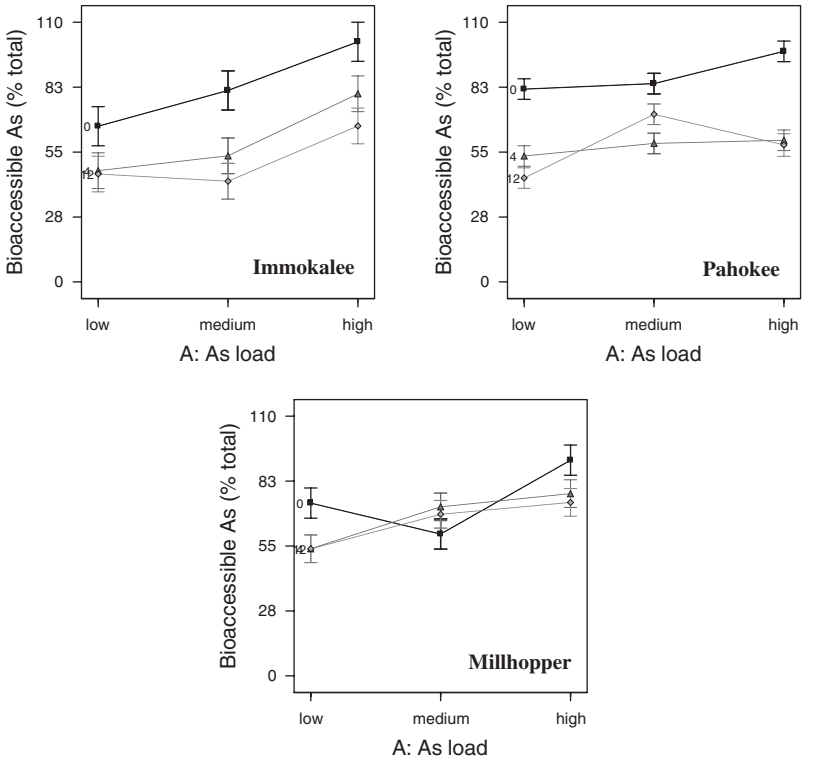

Figure 15.5. Effects of sodium arsenate application rate (low = 45, medium = 225, and high = 450 mg kg^{-1}) and incubation time (0, 4, and 12 months after amendment application) on the bioaccessible As fractions as measured by the in-vitro gastric phase of the three soils (expressed as percent of the sum of all As fractions). The error bars represent the least significant difference (LSD) at the 95% confidence level. Data are the mean of three replicates.

Correlations. We attempted to linearly correlate the different As fractions of the sequential fractionation scheme with the in-vitro As bioavailable fraction, i.e. the stomach-As phase, utilizing all incubation times and As load data. For the Immokalee soil, the only As fraction that was significantly ($\alpha = 0.05$) correlated with the in-vitro stomach method was the water-soluble ($r^2 = 0.56$) and the (water + NH_4Cl)-extractable As fractions ($r^2 = 0.53$) (Table 15.2). The NaOH-extractable As fraction of the Immokalee soil was negatively correlated with the stomach method ($r^2 = 0.54$), suggesting resistance of the Fe/Al-bound As to dissolution, when present in the acidic content of the in-vitro gastric test.

Table 15.2. Pearson correlation coefficients for different measures of As bioaccessibility in the soils

Extractable As	Water	NH_4Cl^-	Plant-available	$NaOH^-$	$H_2SO_4^-$	$H_2O_2^-$	HNO_3^-	Stomach phase
Immokalee								
NH_4Cl^-	−0.26	1.00						
Plant-available[@]	0.94**	0.09	1.00					
$NaOH^-$	−0.93**	0.10	−0.99**	1.00				
$H_2SO_4^-$	−0.61*	0.31	−0.52*	0.43	1.00			
$H_2O_2^-$	−0.52*	−0.18	−0.60*	0.49	0.42	1.00		
HNO_3^-	0.05	0.18	0.40	0.38	0.86**	0.46*	1.00	
Stomach phase	0.56*	−0.15	0.53*	−0.55*	−0.17	−0.22	0.09	1.00
Pahokee Muck								
NH_4Cl^-	−0.39	1.00						
Plant-Available[@]	0.97**	−0.17	1.00					
$NaOH^-$	−0.95**	0.20	−0.96**	1.00				
H_2SO_4	−0.57*	0.03	−0.61*	0.43	1.00			
$H_2O_2^-$	−0.11	−0.05	−0.13	0.08	0.28	1.00		
HNO_3^-	0.05	0.04	0.04	0.02	0.12	0.15	1.00	
Stomach phase	0.77**	−0.11	0.79**	−0.72**	−0.71**	−0.10	0.08	1.00
Millhopper								
NH_4Cl^-	0.91**	1.00						
Plant-Available[@]	0.99**	0.95**	1.00					
$NaOH^-$	0.56*	0.51*	0.56*	1.00				
$H_2SO_4^-$	0.45	0.68*	0.52*	0.43	1.00			
$H_2O_2^-$	0.11	0.11	0.13	0.24	0.0.36	1.00		
HNO_3^-	0.11	0.23	0.15	−0.22	0.59*	0.16	1.00	
Stomach phase	0.70**	−0.61*	0.70**	0.47*	0.29		0.09	1.00

*Significant at the $\alpha = 0.05$ probability.
**Significant at the $\alpha = 0.01$ probability.

For the Pahokee soil, positive linear correlations were observed between the water-soluble ($r^2 = 0.77$) and (water + NH_4Cl)-extractable ($r^2 = 0.79$) As fractions with the bioaccessible-As of the stomach phase (Table 15.2). Similar positive correlations were observed for different batches of the Immokalee and Millhoper soils amended with DMA (Sarkar et al., 2004). Similar to Immokalee, a negative linear correlation was observed for the NaOH-extractable As fraction with bioavailable As in the gastric phase of the Pahokee and Millhopper soils. The H_2SO_4-extractable As fraction was also correlated with bioaccessible As obtained from the in-vitro test in the Pahokee Muck soil, consistent with the results of Sarkar et al. (2004). It seems that Fe/Al, and to a lesser extent, Ca/Mg solid phases control As bioaccessibility in Pahokee Muck soil. This may be the result of the greater amorphous Fe/Al content of the Pahokee soil (oxalate-extractable Fe + Al = 2000 mg kg^{-1}) when compared to 704 and 66 mg kg^{-1} in the Millhopper and Immokalee soils, respectively.

15.4. Conclusions

This study demonstrated that soil physicochemical properties impact the relative As distribution between labile and non-labile soil phases, and thus, influence the magnitude of bioaccessible As, as estimated by the in-vitro test. Soil solid phases such as amorphous Fe/Al hydroxides as well as Ca/Mg compounds (to a lesser extent) may be the predominant sorbents for As in soils. Incubating the soils with different As loads up to 12 months showed that there was a significant reduction in the water-soluble and the plant-available As fractions with time, but a significant bioaccessible pool of As remained in the soils. Observed decreases in the plant-available As fraction with incubation time were paralleled by significant temporal increases in the NaOH-extractable As fraction of all three soils, which represents the amorphous Fe/Al-bound As fraction. Another As fraction that increased with time was the H_2SO_4-extractable As fraction, which represents As bound to Ca/Mg compounds. This effect was seen in the Millhopper and the Pahokee soils that had large amounts of total Ca/Mg concentrations, but not in the Immokalee soil. There was no effect of time or As load on the organic-bound As fraction, suggesting that mineral phases control As availability in the soils studied.

Results from the in-vitro test confirmed the trends observed in the sequential fractionation experiments. Bioaccessible As measured in the stomach phase decreased with incubation time at all As loads for the three soils, but not to levels that would minimize the risk associated with exposure to soil–As particles. Arsenic associated with the water- and plant-available As fractions is most bioaccessible as indicated by the significant ($\alpha = 0.05$) correlation between the water-soluble, plant-available As concentrations, and the in-vitro bioaccessible As fraction in the soils. The NaOH-extractable As fraction was negatively correlated with the in-vitro bioaccessible As fraction, suggesting that the presence of Fe/Al hydroxides could decrease As lability in soils.

The results obtained in this study provide an useful estimation of As bioavailability in soils varying in physicochemical properties. Despite the decrease in bioaccessible As concentrations with time for all soils, a large amount of bioaccessible As remained in the soils even after 12-month incubation, which could potentially pose a serious health hazard for humans. This information can be used for As risk assessment studies in FL soils. However, since the data were generated in a static incubation study, caution should be exercised in extrapolating the data to the field scale. A greenhouse study is currently being conducted to evaluating As bioaccessibility using the same soils to validate the observed trends in the incubation experiments.

ACKNOWLEDGMENTS

We acknowledge the USEPA-STAR Program for funding this study. We would like to thank Drs. E. Hanlon and S. Daroub, Mr. Schwandes and Mr. Taylor of the University of Florida for providing the soils used in this study.

REFERENCES

Brown, K.G., Ross, G.L., 2002. Arsenic, drinking water, and health: A position paper of the American Council on Science and Health. Regul. Toxicol. Pharm. 36, 162–174.

Chisholm, D., MacPhee, W., MacEachern, C.R., 1955. Effect of repeated applications of pesticides to soil. Can. J. Agr. Sci. 35, 433–439.

Chunguo, C., Zihui, L., 1988. Speciation of arsenic in water, suspended solids and sediment of Xiangjiang river. China Sci. Total Environ. 77, 69–82.

Cohen, J.T., Beck, B.D., Bowers, T.S., Bornschein, R.L., Calabrese, E.J., 1998. An arsenic exposure model: Probabilistic validation using empirical data. Hum. Ecol. Risk Assess. 4, 341–377.

Datta, R., Sarkar, D., 2004. Arsenic geochemistry in three soils contaminated with sodium arsenite pesticide: An incubation study. Environ. Geosci. 11, 87–97.

Davis, A., Ruby, M.V., Bergstrom, P.D., 1992. Bioavailability of arsenic and lead in soils from the Butte, Montana, mining district. Environ. Sci. Technol. 26, 461–468.

Design-Expert, 2001. Software for design of experiments, version 6.0.5, Stat-Ease Inc. Minneapolis, MN.

Goldberg, S., 2002. Competitive adsorption of arsenate and arsenite on oxides and clay minerals. Soil Sci. Soc. Am. J. 66, 413–421.

Hedley, M.J., Stewart, J.E.B., Chauhan, B.S., 1982. Changes in phosphorus fractions in soils under intensive plant growth. Soil Sci. Soc. Am. J. 46, 970–976.

Kabata-Pendias, A., Pendias, H., 1992. Trace Elements in Soils and Plants, second ed.. CRC Press, Boca Raton, FL.

Klute, A., 1996. Methods of Soil Analysis: Part 1: Physical and Mineralogical Methods. SSSA Publications, Madison, WI.

Matschullat, J., 2000. Arsenic in the geosphere—A review. Sci. Total Environ. 249, 297–312.

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.A., Aucott, M., 1998. An assessment of the amounts of arsenical pesticide used historically in a geographic area. Sci. Total Environ. 218, 89–101.

National Research Council, 1999. Arsenic in Drinking Water. National Academy Press, Washington, DC, p. 273.

Rhoades, J.D., 1982. Methods of Soil Analysis. Part 2. In: Agron. Monogr 9. ASA and SSSA, Madison, WI, pp. 149–158.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., Pace, L.W., 1999. An in-vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ. Sci. Technol. 33, 642–649.

Sarkar, D., Datta, R., Sharma, S., 2004. Fate and bioavailability of arsenic in organo-arsenical pesticide-applied soils. Part-I: Incubation study. Chemosphere 60, 188–195.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ. Sci. Technol. 38, 228A–231A.

Sheppard, S.C., 1992. Summary of phytotoxic levels of soil arsenic. Water Air Soil Pollut. 64, 539–550.

Sheppard, S.C., Evenden, W.G., Schwartz, W.J., 1995. Ingested soil: Bioavailability of sorbed lead, cadmium, cesium, iodine, and mercury. J. Environ. Qual. 24, 498–505.

US EPA, 2000. Test methods for evaluating solid waste, Physical/Chemical Methods, USEPA-65 FR 70678, Draft Update IVB SW-846. U.S. Governmental Printing Office, Washington, DC.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29, 677–678.