Arsenic biogeochemistry and human health risk assessment in organo-arsenical pesticide-applied acidic and alkaline soils: An incubation study

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Abstract

Organo-arsenical compounds are considered non-carcinogenic, and hence, are still allowed by the regulatory agencies for use in agriculture as pesticides. Due to rapid encroachment of suburban areas into former agricultural lands, the potential for human exposure to soil-arsenic has increased tremendously in recent years. However, insufficient data is available on the stability of organo-arsenicals in soils; as to whether they remain in an organic form, or are converted over time to potentially carcinogenic inorganic forms. A static incubation study was conducted to estimate soil speciation and in-vitro bioavailability (i.e., bio-accessibility) of arsenic as a function of soil properties. Two chemically variant soil types were chosen, based on their potential differences with respect to arsenic reactivity: an acid sand with minimal arsenic retention capacity and an alkaline clay loam with relatively high concentrations of Fe/Al and Ca/Mg. The soils were amended with dimethylarsenic acid (DMA) at three rates, 45, 225 and 450 mg/kg, and incubated for 1 year. A sequential extraction scheme was employed to identify the geochemical forms of arsenic in soils, which were correlated with the in-vitro bioavailable fractions of arsenic. Human health risk calculated in terms of excess cancer risk (ECR) showed that risk assessment based on bioaccessible arsenic concentrations instead of the traditional total soil arsenic is a more realistic approach. Results showed that soil properties (such as pH, Fe/Al content and soil texture) of the two soils dictated the geochemical speciation, and hence, bioaccessibility of arsenic from DMA, indicating that the use of organic arsenicals as pesticides in mineral soils may not be a safe practice from a human health risk perspective.

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1. Introduction

Biogeochemical cycling of arsenic has been under intensive investigation due to the health risk associated with this group A human carcinogen (Frey and Edwards, 1997; Kim and Nriagu, 2000). Anthropogenic activities are considered to be the major contributor of arsenic found in contaminated sites (Cullen and Reimer, 1989; Nriagu, 1994; Davis et al., 2001). Several authors have addressed the problem of soil and groundwater contamination due to the use of arsenical pesticides (Carolyn et al., 2002; Chun, 2002; Brouwere et al., 2004). In the late eighties and early nineties, the USEPA banned usage of many inorganic arsenic-based pesticides (USEPA, 1992; Southworth, 1995). However, organic forms of
arsenic are considered non-carcinogenic and are still being used on agricultural lands.

Organo-arsenical pesticides, such as monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA), are used mainly as herbicides (Pongratz, 1998). Documented data indicate that transformation of organic and inorganic forms occur in soil systems, mainly through redox reactions and bio-transformation processes (Cullen et al., 1979; Henry et al., 1979), indicating that the transformation of non-carcinogenic organic arsenicals to carcinogenic inorganic species is possible in soil systems (Rodriguez, 1998), which might pose a significant health risk. However, the issue of the influence of soil properties on the transformation of organo-arsenical pesticides to inorganic forms has not been adequately addressed in a comprehensive study involving a variety of pesticide-contaminated soil types.

A critical parameter for realistic health risk assessment in arsenic-enriched soils is an estimate of “bioavailable” arsenic, which is the extent of absorption of a chemical into the bloodstream from the gastrointestinal tract, lungs or skin (Halmes and Roberts, 1997). Bioaccessibility of arsenic in soil types is dependent on several parameters that are controlled by the chemical composition of the soil. Evidence suggests that pH, redox potential, clay content, oxides/hydroxides, carbonates, organic matter and microbial community structure play a key role in speciation, retention and bioaccessibility of arsenic in soils (Woolson, 1977; Polemio et al., 1982; Sadiq, 1997; Adriano, 2001).

Due to rapid expansion of suburbia in the U.S., pesticide-applied former agricultural soils are now routinely being converted to residential developments. Ingestion of arsenic-contaminated soil due to incidental hand-to-mouth activity by children is now being increasingly taken into account in assessing human health risks associated with exposure to such soils. However, realistic methods to quantify the percentage of bioaccessible arsenic are rarely employed. The majority of the baseline risk assessment studies of Superfund sites have used the extremely conservative estimate that all (100%) arsenic present in soil is bioaccessible by equating arsenic solubility in water with that in soils. However, several in-vivo animal studies have shown that bioavailability of arsenic in soils may be significantly less than that in water. Ng et al. (1993) used rats to obtain a mean arsenic bioavailability value of 69%; Greon et al. (1994) used dogs to obtain an absolute arsenic bioavailability value of 8%; Rodriguez et al. (1999) used immature swine (the sole USEPA-approved model) and estimated relative bioavailable arsenic to vary between 2.7% and 42.8% depending on arsenic concentrations in soil ingestion dose. Because calculated health risk is a direct function of the input value for chemical dose, the assumption of using an input value of 100% bioaccessibility for exposure to arsenic-enriched soils potentially overestimates the actual risk, thereby elevating the expenses associated with site-cleanup. Moreover, arsenic exists in many geochemical/mineralogical forms in soils. Several of these arsenic species are geochemically stable and/or insoluble in human gastric/intestinal juices and, hence, are not likely to be available for systemic absorption. Therefore, an appropriate evaluation of pathway-specific arsenic bioaccessibility requires accurate, case-specific information on geochemical fate of arsenic.

Dimethylarsenic acid is considered to be a less toxic organic form of arsenic, and is commonly used as herbicide (Cai et al., 2002). While the toxicity of DMA has been extensively studied (Kenyon and Hughes, 2001), the environmental fate, stability, distribution and bioaccessibility of DMA and other organo-arsenicals in soils are still rather incompletely understood. Hence, a static incubation study was initiated as the first step toward understanding arsenic geochemistry in soils with varying chemical properties amended with DMA. The major objectives of this study reported here were: (i) to estimate bioaccessibility using an in-vitro technique, (ii) to decipher the relationship between soil-arsenic speciation and bioaccessibility, and (iii) to calculate the human health risk ensuing from exposure to DMA-contaminated soils. A follow-up column study allowing for dynamic interactions between soils, water, DMA and plants is currently in progress in a temperature and humidity controlled greenhouse setup.

2. Materials and methods

2.1. Soils sampling, characterization and laboratory incubation study design

The two types of soils used in this study, the Immokalee series Spodosol and the Tobosa series Vertisol, were collected from the surface horizons of Southwest Florida Research and Education Center, Immokalee, Florida and the Texas Agricultural Experiment Station, San Angelo, Texas, respectively. The soils were selected such that they vary widely in their pH, clay content, Fe/Al and Ca/Mg concentrations, to represent a range of properties that are important for arsenic retention and/or bioaccessibility. Air-dried and sieved (2 mm) soils were used for the determination of pH, electrical conductivity (EC), soil moisture and organic matter content using standard protocols (Sparks, 1996). Exchangeable cations were extracted by 1 M ammonium acetate (pH 7.0) and cation exchange
capacity (CEC) was determined by the removal of ammonium ions (Rhoades, 1982). 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 by the ignition method (Saunders and Williams, 1955). Total recoverable Ca, Mg, Fe, Al, and P was determined by acid digestion according to the USEPA Method 3050B (USEPA, 1996).

The concentrations of P and Fe in the extracts and digests were determined colorimetrically using the molybdate-ascorbic acid method (Watanabe and Olsen, 1965) and 1,10-phenanthroline-reagent method (Olson and Ellis, 1982), respectively by a Varian Cary-50 UV–Visible light spectrometer. The concentrations of Ca, Mg and Al in the extracts/digests were analyzed using a Perkin-Elmer PE 7000 atomic absorption spectrophotometer in the flame mode (FAAS). The digests were analyzed for arsenic using a graphite furnace atomic absorption spectrophotometer (GFAAS).

The laboratory incubation study was conducted for a period of 1 year using Immokalee and Tobosa series soils spiked with DMA (MP Biomedicals Inc.). Two hundred grams of soil each from the Immokalee and Tobosa series were amended with DMA to achieve a final concentration of 45, 225 and 450 mg/kg (mass of arsenic/mass of dry soil), which represent background arsenic concentrations typically found at agricultural soils resulting from 1, 5 and 10 years of continuous pesticide application, respectively (Chisholm et al., 1955). Moisture content was maintained at 70% of the water holding capacity of the soils. The pesticide was thoroughly mixed with the soils by hand, which were then stored in bags at room temperature. Spiked soil was weekly aerated and the soil moisture content was maintained by periodic addition of required water. Soil samples were extracted for arsenic immediately after the amendment (time 0), after 4 months and 12 months of incubation. The soil samples were extracted for arsenic using a customized sequential extraction method and two in-vitro procedures described below. All experiments were carried out in triplicate and mean values are reported.

2.2. Sequential extraction for soil speciation of arsenic

Sequential extraction of arsenic was performed following the procedure reported by Chunguo and Zihui (1988) with a few modifications (Datta and Sarkar, 2004) 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 arsenic using the GFAAS.

2.3. In-vitro extraction method for bioaccessible arsenic

Bioaccessible arsenic was estimated following the in-vitro gastrointestinal method of Rodriguez et al. (1999) with certain modifications made by Sarkar and Datta (2003). Arsenic concentrations in the simulated stomach phase (IVG-S) and the absorbed intestinal phase (IVG-AI) were analyzed using the GFAAS.

2.4. Human health risk calculation using bioaccessibility data

The amount of soil ingested by a child is generally estimated as 100 mg/day (Sarkar and Datta, 2004). Based on this value, chronic daily intake (CDI) was calculated as (Sarkar and Datta, 2004):

\[ CDI = \frac{AsCS \times \% \text{Bioaccessibility}}{0.0001} \]

where CDI=chronic daily intake of As (mg) per day, AsCS=As concentration in soil (mg/kg) and 0.0001=amount of soil (kg) ingested by a child per day.

The potential risk for cancer was calculated as the product of CDI and the appropriate slope factor. This value is defined as excess cancer risk (ECR) (Sarkar and Datta, 2004).

\[ ECR = CDI \times SF \]

where ECR is the estimate of the excess lifetime probability of developing cancer resulting from exposure to As (dimensionless), CDI=chronic daily intake (mg/kg/day) and SF=slope factor of As for carcinogenic effects (mg/kg/day).

Slope factor is defined as a plausible upper-bound estimate of the probability of a response per unit intake of the chemical over a lifetime. For arsenic, the slope factor was assumed to be 1.5 mg/kg/day, obtained from USEPA’s Integrated Risk Information System (IRIS) (USEPA, 2001) and Health Effects Assessment Summary Tables (HEAST) (USEPA, 1997) databases.

3. Results and discussion

3.1. Soil properties as determinants of arsenic fate in soils

Chen et al. (1999) suggested that the retention and bioaccessibility of trace metals in soils are dependent on their pH, CEC, clay content, organic carbon content, and
Fe and Al content. The physico-chemical properties of Immokalee and Tobosa series soils are shown in Table 1. Being sandy and lacking positively charged surfaces (e.g. amorphous Fe/Al oxides), the Immokalee soil is likely to have minimal arsenic retention capacity (Pierce and Moore, 1980; Oscarson et al., 1981), thereby potentially increasing the bioaccessible fraction of arsenic. The Tobosa soil is a vertisol with about 38.6% clay content. In addition to having higher concentrations of Fe, Al, Ca and Mg, it also has much higher pH compared to Immokalee soil (Table 1); and so, Immokalee is classified as an acid soil and Tobosa is alkaline. The pH of the both soil did not change appreciably. The soils also vary widely in their salinity (measured as EC), and the CEC, both likely to affect arsenic geochemistry. Reportedly, arsenic is strongly adsorbed onto Fe and Al oxides (Jacobs et al., 1970; Barringer et al., 1998); hence, Tobosa soil, with higher Fe/Al content is likely to have higher arsenic retention capability compared to Immokalee. Generally, sorption of arsenic decreases with increasing pH (Adriano, 2001). This can be attributed to the negative surface charge on the adsorptive surface at higher pH, given the negative charge of arsenic oxyanions (Wasay et al., 2000). However, the low retention of arsenic in the acid Immokalee soil system may be controlled to its low sorption capacity being predominantly sandy and devoid of appreciable amounts of retainer phases, such as Fe/Al-oxides or clays or organic matter. On the other hand, irrespective of the high soil pH, appreciable amount of arsenic was retained in the Tobosa soil, which may be attributed to its precipitation as Ca/Mg phases. Arsenic bound to the Ca/Mg fraction in Tobosa soil has the potential to solubilize in the highly acidic environment of human stomach, thus becoming bioaccessible. Both the soils studied had similar native arsenic concentration, ranging between 15 mg/kg in Immokalee and 16.5 mg/kg in Tobosa.

3.2. Geochemical forms of arsenic

Chemical speciation of arsenic was performed via identification and quantification of the various “operationally defined” species, forms or phases occurring in the soil. Operationally defined species of arsenic in soils is characterized by the procedure of isolation of arsenic, depending on its physical (solubility) or chemical (bonding or reactivity) properties (Van Herreweghe et al., 2003). Analysis of such geochemical forms is important because it can provide crucial information on reactivity, bioaccessibility and toxicity of arsenic. The distribution of DMA in different chemical phases within Immokalee and Tobosa soils was investigated at three time points (0 time, 4 months and 1 year) at 45 mg/kg of arsenic loading rate (Figs. 1 and 2). The speciation of arsenic in Immokalee soil shows that nearly 89% of total soil arsenic was present in the water soluble fraction, and 8% of arsenic was present in the exchangeable fraction immediately after spiking the soil with DMA pesticides (0 time). Only 3% of arsenic was associated with Fe/Al oxide fraction, while other species including the organic phase constituted less than 1% of the total arsenic amended (Fig. 1). After a 12-month incubation period, the amount of arsenic extracted in the soluble fraction decreased to 63% with an increase in Fe/Al-bound fraction to 21%. Small increases were also observed in the Ca/Mg-bound and organic-bound forms of arsenic. These results are in agreement with the data reported by Woolson and Kearney (1973).

In the Tobosa soil, the amount of arsenic extracted in the soluble fraction (85%) was similar to that in Immokalee at 0 time indicating lack of soil pesticide equilibration (Fig. 2). While 4% of the arsenic was extracted from the Fe/Al-bound fraction, approximately 8% of arsenic was extracted as Ca/Mg phase, owing to the large amounts of Ca/Mg present in Tobosa soil. Other fractions showed negligible retention of arsenic. While after 4 months of incubation, the soluble arsenic fraction declined to about 42%, the Fe/Al-bound fraction increased almost eight-fold to 34%. This trend continued up to the 12-month incubation period, when the soluble fraction of arsenic decreased further to 26%. Also, 23% of the arsenic was retained by the Ca/Mg fraction and 8% by the residual fraction (Fig. 2). Decrease in arsenic concentration in the soluble fraction coupled with the corresponding increase in the Fe/Al and Ca/Mg-bound

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Table 1
Selective properties of the soils

<table>
<thead>
<tr>
<th>Properties</th>
<th>Immokalee</th>
<th>Tobosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.0</td>
<td>8.05</td>
</tr>
<tr>
<td>ECa (μS/cm)</td>
<td>59</td>
<td>136.5</td>
</tr>
<tr>
<td>CECb (Cmol/kg)</td>
<td>777</td>
<td>4770</td>
</tr>
<tr>
<td>SOMc (%)</td>
<td>0.84</td>
<td>4.49</td>
</tr>
<tr>
<td>Soil</td>
<td>Sand</td>
<td>98.7</td>
</tr>
<tr>
<td>Texture (%)</td>
<td>Silt</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>0.3</td>
</tr>
<tr>
<td>As (mg/kg)</td>
<td>15.0</td>
<td>16.5</td>
</tr>
<tr>
<td>P (mg/kg)</td>
<td>Mehlich 3</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>208</td>
</tr>
<tr>
<td>Ca+Mg (mg/kg)</td>
<td>Mehlich 3</td>
<td>266</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1178</td>
</tr>
<tr>
<td>Fe+Al (mg/kg)</td>
<td>Oxalate</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>212</td>
</tr>
</tbody>
</table>

*a*EC=electrical conductivity; *b*CEC=cation exchange capacity; *c*SOM=soil organic matter. Data represent mean of three replicates. The relative percentage error between the replicates was less than 10%.
fraction indicates the effect of soil properties and equi-
libration time on soil speciation of arsenic.

These results indicate potential mineralization of
DMA into more stable inorganic arsenic in soil systems. Woolson (1983, 1989) suggested that two types of oxi-
dation reactions are generally responsible for the trans-
formation of organo-arsenicals to inorganic arsenic oxyanions: in one type, the carbon-arsenic bond is de-
stroyed primarily due to microbial activity, and in the
other type, the change in oxidation state of arsenic is due
to its interaction with soil mineral phases. In the reported
system, either or both of these oxidation mechanisms
may have played a role in transforming organo-arsenical
species into more stable inorganic arsenic oxyanions,
which were then strongly retained by the Fe/Al oxides
(via adsorption) and Ca/Mg (via precipitation) in the
Tobosa soil. However, specific information on the
mechanisms of arsenic retention could be only speculated
at this time; further studies involving advanced spectro-
scopic techniques, such as XAFS, XANES, etc., are
required to directly identify solid phase arsenic specia-
tion. Arsenic adsorbed irreversibly to Fe/Al oxides
is considered to be unavailable for plant uptake and is also
relatively not available to the human gastrointestinal
systems (Rodriguez, 1998). However, Ca/Mg-bound
forms are likely to dissolve and become bioaccessible
in the strongly acidic environment of the stomach.

Similar results were obtained in soils contaminated
with 225 and 450 mg/kg of arsenic in the form of DMA
(data not shown). These findings suggest that arsenic

Fig. 1. Geochemical speciation of arsenic in Immokalee soil amended with DMA (45 mg/kg As) at time zero, and after 4-month and 12-month equilibration.

Fig. 2. Geochemical speciation of arsenic in Tobosa soil amended with DMA (45 mg/kg As) at time zero, and after 4-month and 12-month equilibration.
from DMA application has tremendous potential to become mobile in sandy soils, particularly those with low amorphous Fe/Al oxide contents. Even in soils with high Fe/Al, arsenic from DMA may become available for leaching through the soil profile immediately after pesticide application. Pattern of distribution of applied DMA in soils used in our study suggests that magnitude of speciation of arsenic is a function of active retention sites in the soil and equilibration time. It is interesting to note here that Tobosa soil with clayey texture and some organic matter (4.49%) is likely to be more microbially active, resulting in higher amounts of DMA being converted into inorganic form of arsenic, which could subsequently speciate to Fe/Al- and Ca/Mg-bound fractions. On the contrary, Immokalee, a sandy soil low in organic carbon, is likely to be less biologically active.

3.3. Estimation of bioaccessible arsenic

The risk from arsenic exposure is associated only with those forms of arsenic that are potentially available to the human gastrointestinal system. It has been increasingly accepted that certain geochemical forms of arsenic are not bioaccessible. Although in-vivo studies using animal models are the most appropriate methods for determining bioavailability of arsenic in soils, the time required for the animal experiments and the high expense associated with them prohibit the use of animal models for routine bioavailability assessments. As a consequence, in-vitro chemical methods (“beaker” models) simulating gastrointestinal conditions in the human gastrointestinal system have been developed in recent years. Two important mechanisms involved in determining bioaccessibility of arsenic in contaminated soils are: (1) solubility of arsenic in the digestive juices and (2) absorption of arsenic across the intestinal membrane (Rodriguez et al., 1999). While the majority of the studies reported so far estimated the amount of metals solubilized under gastrointestinal conditions, very few reported studies have taken the intestinal absorption of metals into account while estimating soil-arsenic bioaccessibility (Rodriguez et al., 1999; Sarkar and Datta, 2003).

Data obtained from the in-vitro gastrointestinal bioaccessibility studies are presented in Figs. 3 and 4. The IVG-stomach method extracted 100% of the arsenic in Immokalee soil, which decreased to 69% after 12 months. In contrast, in Tobosa soil, while 100% of the arsenic was bioaccessible at 0 time, bioaccessibility decreased to 43% after 4-month and 33% after 12-month incubation period (Fig. 3). The IVG-adsorbed intestinal method extracted higher amounts of arsenic from both the soils. While 100% of the arsenic was bioaccessible immediately after amendment in both soils studied, bioaccessibility decreased to 79% in Immokalee and 51% in Tobosa soil after 12 months of incubation (Fig. 4).

Similar trends were observed in soils contaminated with 225 and 450 mg/kg of arsenic in the form of DMA (data not shown). Decreased bioaccessibility of arsenic in both soils after 12-month incubation presumably depended upon the extent of transformation of DMA to inorganic arsenic species with lower solubility and greater retention potential. Arsenic was more strongly retained by the abundant Fe/Al phases in the Tobosa soil.

Fig. 3. Arsenic bioaccessibility in DMA-applied soils (45 mg/kg As) using the IVG-stomach in-vitro methods at time zero, and after 4-month and 12-month equilibration.

Fig. 4. Arsenic bioaccessibility in DMA-applied soils (45 mg/kg As) using the IVG-absorbed intestinal in-vitro methods at time zero, and after 4-month and 12-month equilibration.
and was relatively more stable (hence, less bioaccessible) compared to the Immokalee soil. Tobosa soil also has higher clay content compared to Immokalee soil, which has been reported to decrease bioaccessibility of metals (Baath, 1989). This study clearly indicates that soil properties have a major impact on the bioaccessibility of arsenic in soils. However, till date, total arsenic, and not bioaccessible fraction of arsenic, is considered for human health risk assessment of contaminated soils (Khan and Scullion, 2000; Chew et al., 2001). As a consequence, the human health risk of contaminated sites may be overestimated leading to higher estimated clean-up costs.

3.4. Correlation between soil speciation and bioaccessibility of arsenic

Correlation between arsenic extracted by in-vitro gastrointestinal-stomach method with arsenic extracted by different steps of sequential extraction scheme for the Immokalee and Tobosa soils amended with 45, 225 and 450 mg/kg arsenic at 0 time and after 4 and 12 months of incubation are shown in Tables 2 and 3, respectively. Bioaccessible arsenic in Immokalee soil was significantly correlated with the soluble fractions at 0 time ($R^2=0.66$, $p<0.1$) as well as after the 12-month ($R^2=0.98$, $p<0.1$) incubation period (Fig. 5). With increasing incubation time, correlation ($R^2$) between bioaccessible arsenic and the exchangeable fraction of sequentially extracted arsenic increased from 0.88 to 0.95 (Table 2) after 1 year. In case of Tobosa soil, the correlation with soluble arsenic remained high throughout the study period (Table 3). The correlation ($R^2$) with Fe/Al and Ca/Mg-bound fractions increased to 0.93 ($p<0.1$) and 0.86 ($p<0.1$), respectively, after 12 months of soil ageing, suggesting re-dissolution of arsenic-bearing Fe/Al or Ca/Mg phases in the harsh chemical environment of the simulated gastrointestinal system. In addition, bioaccessible arsenic also correlated with the organic-bound form of arsenic in Tobosa soil. These results indicate that DMA, which is considered to be a non-carcinogenic, relatively less toxic organo-arsenical species, undergoes potential chemical transformation to the more toxic and carcinogenic inorganic arsenicals when exposed to a strongly reducing environment, such as the human gastrointestinal system, thereby questioning the validity of the current USEPA policy that still allows usage of organo-arsenical pesticides in soils. Similar results were obtained when sequentially extracted arsenic was correlated with bioaccessible arsenic obtained by the in-vitro gastrointestinal absorbed intestinal method (data not shown).

3.5. Correlation between arsenic extracted by the IVG-S and IVG-AI methods

Data obtained from two IVG methods were correlated for the Immokalee and Tobosa soils contaminated with 45, 225 and 450 mg/kg arsenic (Fig. 5). A regression coefficient of 0.90 ($p<0.1$) was obtained, indicating that results from both methods were statistically similar, and that both in-vitro methods were extracting arsenic from

<table>
<thead>
<tr>
<th>Soil As species</th>
<th>$R^2$</th>
<th>0 time</th>
<th>4 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble As</td>
<td>0.55</td>
<td>0.86</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Exchangeable As</td>
<td>0.88</td>
<td>0.76</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Fe/Al-bound As</td>
<td>0.54</td>
<td>0.78</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Ca/Mg-bound As</td>
<td>0.55</td>
<td>–</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Organic-bound As</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Residual As</td>
<td>–</td>
<td>0.59</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

The $R^2$ values reported here are significant at $p<0.1$. Non-significant correlations are not shown.
similar soil arsenic pools. However, the IVG-AI method proved to be a bit more conservative and extracted more arsenic when compared to the IVG-S method, as evident from the slope factor greater than 1 (Fig. 5).

3.6. Human health risk calculations on the basis of bioaccessible arsenic in soils

Arsenic biogeochemistry has been under intensive investigation owing to its relation to human health risk. Because toxicological effects of arsenic in soils are a function of its bioaccessibility, such effects depend mainly on the association of arsenic with various soil components rather than to its total soil concentration. However, risk calculations are generally performed using total arsenic data without considering speciation, mobility and bioaccessibility of different geochemical forms of arsenic (Alexander, 2000). As demonstrated earlier, speciation of arsenic in soils determines its bioaccessibility, which in turn has a major implication on human health risk. Remediation of contaminated sites is usually based on risk calculations, following identification of unsafe levels of exposure. In accordance with USEPA regulations, risk to human health is calculated in terms of excess cancer risk, which is the additional risk above the background risk of developing cancer as a result of a specific exposure. Using the data obtained from in-vitro bioaccessibility studies, the reduction in potential cancer risk was calculated using soil-specific bioaccessibility data as opposed to total arsenic data (Table 4). The ECR values were substantially reduced in both soils when bioavailability values were used instead of total arsenic values. The ECR values were also observed to decrease with increasing incubation period as arsenic became less bioaccessible with soil ageing. This trend was more evident in Tobosa soil compared to Immokalee soil. In Tobosa soil the ECR values (after 12 months of incubation) were 0.23, 1.59 and 3.78 as opposed to the values of 0.32, 2.33 and 4.35 in Immokalee soil amended with 45, 225 and 450 mg/kg of arsenic, respectively. These values are substantially lower than the ECR values expected if total arsenic values were used for risk calculations instead of bioaccessible arsenic.

4. Summary and conclusions

Despite extensive research on soils contaminated with arsenical pesticides, reports on long-term evaluation of environmental fate and bioaccessibility of organo-arsenicals in relation to human health risk are rare. Hence, the reported study examined the pattern of speciation and bioaccessibility in two soils with varying chemical properties amended with DMA. The study demonstrated that soil properties have a marked impact on geochemical speciation of arsenic. After equilibration, soil chemistry dictated geochemical speciation of arsenic, and arsenic bioaccessibility was primarily a function of its soil chemical forms. In the Tobosa series soil, which has high amounts of Fe/Al, Ca/Mg and higher clay content, arsenic bioaccessibility decreased markedly after 12-month incubation, compared to the Immokalee series soil, with low Fe/Al and Ca/Mg content. Bioaccessible arsenic exhibited high correlation with soluble and exchangeable arsenic in both Immokalee and Tobosa soils. In the Tobosa soil, correlation between Fe/Al- and Ca./Mg-bound arsenic and bioaccessible arsenic increased significantly with equilibration time. Other soil arsenic phases, such as the organically complexed arsenicals also showed a noticeable increase in their correlation with bioaccessible arsenic with increasing equilibration time. Results indicate that DMA, which is considered to be a relatively less toxic organo-arsenical species, undergoes chemical transformation to potentially carcinogenic inorganic arsenicals when exposed to a strongly reducing environment of the human gastrointestinal system, thereby questioning the validity of the current USEPA policy that still allows the use of

<table>
<thead>
<tr>
<th>Soil series</th>
<th>AsCS$^a$ (mg/kg)</th>
<th>Bioaccessibility (%)</th>
<th>CDI$^b$ (mg/kg/day)</th>
<th>ECR$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 months</td>
<td>12 months</td>
<td>Total</td>
<td>4 months</td>
</tr>
<tr>
<td>Immokalee</td>
<td>45</td>
<td>80.7</td>
<td>46.1</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>225</td>
<td>94.3</td>
<td>68.8</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>75.8</td>
<td>64.4</td>
<td>4.50</td>
</tr>
<tr>
<td>Tobosa</td>
<td>45</td>
<td>42.9</td>
<td>32.5</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>225</td>
<td>58.4</td>
<td>46.9</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>63.4</td>
<td>55.9</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Data represent mean of three replicates.

$^a$AsCS—As concentration in soil; $^b$CDI—chronic daily intake; $^c$ECR—excess cancer risk.
organic arsenic as pesticides. Health risk evaluation in terms of excess cancer risk index showed that human health risk assessments based on bioaccessible arsenic instead of the traditional total soil arsenic is a more realistic approach, which prevents overestimation of human health risk, and hence, site clean-up costs. Results indicate that soil properties need to be taken into consideration while evaluating the human health risk from exposure to arsenic-contaminated soils.

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