Indian Journal of Agricultural Sciences **84** (7): 824–7, July 2014/Article

Genetic Assessment of the suitability of selected methods for species level determination of arsenic in some contaminated inceptisols of West Bengal

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Received: 29 September 2010; Revised accepted: 6 May 2014

ABSTRACT

Elevated level of arsenic in agricultural soils cause potential toxicity to human health, plants and microbes through food-chain, the magnitude being the highest in Bangladesh, followed by West Bengal, India. Since the toxicity and bioavailability varies with arsenic species, it is essential to develop and standardize suitable methodology to estimate arsenic species separately in different matrices. In this study, an attempt has been made to assess the suitabilities of three different extractants, namely orthophosphoric acid (1M H_3PO_4, pH 1.39), ammonium dihydrogen orthophosphate (1M $NH_4H_2PO_4$, pH 4.14) and di ammonium hydrogen orthophosphate [1M $(NH_4)_2HPO_4$, pH 8.26], in determining the arsenic species in selected contaminated inceptisols of West Bengal. The results showed that the extraction efficiency varies with the composition of the extraction solution. The lowest recovery (3.74-17.61%) were obtained with the 1M $(NH_4)_2HPO_4$, pH 8.26, the highest recovery (34.89-73.29%) obtained with the 1M H_3PO_4, pH 1.39 and 4.04-52.30% recovery was obtained using 1M $NH_4H_2PO_4$, pH 4.14.

Key words: Arsenic, Extractant, Soil

Speciation of arsenic has received significant attention over the past 20 years in both mechanistic and exposure assessment research. This is due to species-dependent toxicity of arsenic. The inorganic arsenic species arsenate (As V) and arsenite (As III) have been classified as carcinogenic, and the methylated forms, i.e. monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA), have recently been identified as cancer promoters. Acute toxicities (LD50) of the organic species monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMA) range from 700 to 2 600 mg/kg while inorganic arsenate (AsV) and arsenite (AsIII) are as low as 15 to 20 mg/kg. Therefore the actual health concerns over arsenic exposure are highly related to inorganic species (James *et al.* 2008).

A wide range of analytical methods and techniques have been used to identify and determine As species in soil and sediment samples. High-performance liquid chromatography (HPLC) coupled to an ICP-MS has become an invaluable analytical tool for the determinations of trace levels of individual arsenic compounds (speciation). The extraction procedure is important when determining the speciation of arsenic in soil because different soil type exhibit different extraction efficiencies (Garcia-Manyes *et al.* 2002), which in turn are a reflection of how strongly the As is bound to various mineral phases in the samples. Various extraction procedures have been explored for their efficiency in extracting arsenic species from soil samples.

However, the efficiency of extracting As species from soil samples depends on both the extraction system and the extraction solvent used (Pizarro *et al.* 2003). For example, mild methods, such as methanol/water, extract only a small percentage (<5%) of the arsenic present in soil and sediment samples (Francesconi and Kuehnelt 2004). In contrast various other extractants, such as 0.1M hydroxyl ammonium hydrochloride, 0.2M ammonium oxalate and 0.3M orthophosphoric acid, give a much higher efficiency (10-94%) but exhibit large differences between soil, sediment and sludge (Montperrus *et al.* 2002). Microwave assisted extraction is an option for extracting As species from soil because its automated approach that applies pressure and temperature during extraction and is faster and less labour intensive than traditional extraction method.

We report here the outcome of a study of efficiencies of selected chemical extractants to separate and quantify individual arsenic species from some inceptisols of West Bengal, India, contaminated with geogenic arsenic contamination through prolonged irrigation with arsenic contaminated underground water. In a process to address such objective an attempt has been made to develop a microwave assisted extraction procedure for arsenic species in soils which achieves a good recovery. The determination of arsenic species in soil extracts was performed using LC-

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ICP-MS because of its ability to separate the signals of interfering species from the peaks of interest, its multi element capability and its high detection power.

MATERIALS AND METHODS

All of the solutions were prepared with Mili-Q (Millipore, Bedford, MA, USA) water. For the speciation studies, standard solution (100 mg/l) of As compounds were prepared as follows: i) arsenite ($NaAsO_2$, Perkin Elmer, USA), (ii) arsenate (Na_2HAsO_4, $7H_2O$, Perkin Elmer, USA), (iii) monomethyl arsonate($CH_3AsNa_2O_3$, Sigma-Aldrich Corp St. Louis, MO USA), iv) dimethyl arsenate $(CH_3)_2AsO(OH)$, Sigma) arsenobetaine (As B; Sigma) were dissolved in water.

All of the standard solutions were standardized with respect to As and kept at 4^0C at dark until use. Dilutions of these standard solutions were prepared daily for analysis. Phosphate buffer (30 mmol/l, pH 5.6) was prepared from ammonium dihydrogen orthophosphate (Merck Chemical, 85% purity) and the pH was adjusted by adding ammonium hydroxide. Concentrated nitric acid and concentrated hydrochloride acid (Supra pure; Merck KGaA, Darmstadt, Germany) were used for the aqua regia digestion method. Orthophosphoric acid (Merck Chemical, 85% purity), ammonium hydrogen orthophosphate and ammonium hydrogen orthophosphate were used for microwave extraction.

A microwave digestion system (Multiwave 3000, Anton Par) with a rotor of 48 Teflon digestion vessels was used for sample digestion and extraction. We collected five agricultural soil samples from different location of arsenic affected area of West Bengal, India that have been exposed to contaminated irrigation water.

The EPA 3051 method was followed for sample digestion (method 3051, USEPA 1997). For determining total arsenic in soil samples 0.5 g dried finely powered soil sample was weighed into a dry, clean teflon digestion vessel and 5 ml of aqua regia was added. The vessel was then closed, placed into the rotor. The loaded rotor was then placed into the microwave oven.

The microwave conditions for digestion were

Stages	Condition
Stage1	microwave power 1200W, 300PSI and ramp for 2 minute
Stage2	microwave power 1200W, 300PSI and ramp for 3 minutes
Stage3	5 minute hold and cooling for 30 minutes

The resultant digest was transferred to a 50 ml volumetric flask and analyzed for total arsenic through a Perkin Elmer ELAN DRCe 6100 ICP-MS. The 6100 was equipped with nickel cones, and the DRC was equipped with platinum cones. Sample introduction system components were similar for both instruments: a cyclonic spray chamber (GlassExpansion, Inc., West Melbourne, Australia) and a Meinhard® type A nebulizer.

For speciation analysis selected soil sample and three different extractant solutions namely orthophosphoric acid (1M H_3PO_4, pH 1.39), ammonium dihydrogen orthophosphate (1M $NH_4H_2PO_4$, pH 4.14) and diammonium hydrogen orthophosphate [1M $(NH_4)_2HPO_4$, pH 8.26] were taken to extract different species of arsenic from soil. About 0.5 g of dried finely powered soil sample and 10ml of each extractant solutions were placed in a microwave teflon vessel and the mixture was maintained at 60W for 10 minutes. Then the mixture was transferred to a 50 ml volumetric flask for analysis in a HPLC hyphenated Perkin Elmer ELAN DRCe 6100 ICP-MS the LC-ICP-MS.

For the isocratic method, a Perkin Elmer Series 200 Micro Pump was used instead of the quaternary pump. The isocratic mobile phase was 30 mM $NH_4H_2PO_4$ at pH 5.6. The flow rate was 1.0 mL/min with 100 µL sample injections. The effluent from the LC column was directly connected to the nebulizer with PEEK tubing (1.59 mm o.d.) and a low dead volume PEEK connector (Part No.:WE024375).

RESULTS AND DISCUSSION

Total arsenic content of soil samples was determined through microwave assisted aqua regia digestion in a Perkin Elmer Elan DRC_e II ICP-MS. Results obtained (Table 1) showed that arsenic content of the selected samples ranged from (7900.9-19400.9 µg/kg). The soil samples selected for the study had a neutral soil pH (7.02-7.76). Soils are mostly silty clay to silty clay loam in texture, low to medium in organic matter (oxidizable organic C ranges from 0.30-0.56%), low in available nitrogen (126.0-220.0 kg/ha), medium in available P_2O_5 (44.0-57.0 kg/ha) and moderate in available K_2O (142.0-190.0 kg/ha) content.

Table 1 Physico-chemical properties of experimental soils

Properties	Ghentu-gachhi	Malda	Mitrapur	Gotera	Nona-ghata
pH	7.51	7.2	7.76	7.40	7.02
Organic C (%)	0.56	0.30	0.44	0.51	0.45
Textural class	Silty clay	Sandy loam	Silty clay	Silty clay	Silty clay-loam
%Sand	3.5	54.6	8.0	9.0	16.8
% Silt	46.7	29.9	44.6	46.9	52.2
% Clay	49.8	15.5	47.4	44.1	31.0
CEC c mol (p+)/kg	24.1	30.2	22.1	17.1	24.1
Available nitrogen (kg/ha)	220.0	200.0	178.0	126.0	126.0
Available phosphorus (kg/ha)	57.0	44.0	46.0	54.0	54.0
Available potassium (kg/ha)	190.0	156.0	162.0	142.0	142.0
Total arsenic (µg/kg)	19400.9	7900.9	15300.3	14000.4	11800.2

As mentioned previously, the extraction efficiency of arsenic species from soils depends on sample matrix and extraction system. In this study different extractants were tested to obtain optimal recovery. The above mentioned soil samples were selected as the test sample because of its high total arsenic concentration. Different extractant solutions, such as orthophosphoric acid (1M H_3PO_4, pH 1.39), ammonium dihydrogen orthophosphate (1M NH_4H_2 PO_4, pH 4.14) and di ammonium hydrogen orthophosphate *(*1M $(NH_4)_2H$ PO_4*,* pH 8.26*),* were assayed independently. The extraction was performed at 60W microwave power. After extraction the arsenic content in the extract was measured by LC-ICP-MS and the percentage of recoveries of arsenic were calculated by comparing the obtained values with those obtained from aqua regia extraction.

The observations were recorded in Table 2 showed that AsV appeared to be the major species while As (III) was found at a minor concentration. It indicates that Cl^- in sample did not interfere with the determination of As species. No methylated arsenic species were detected in these soils probably due to poor organic fractions and low microbial activity. In temperate regions with organic rich soils however contrasting findings were obtained. Giacomino *et al.* (2010) reported that AsV prevails over AsIII whereas more than 40% of total arsenic has been recovered in organic form.

The results (Table 2) show that the extraction efficiency varies with the composition of the extraction solution. The lowest recovery (3.74-17.61%) were obtained with the 1M $(NH_4)_2H$ PO_4, pH 8.26, the highest recovery (34.89-73.29%) obtained with the 1M H_3PO_4, pH 1.39 and 4.04-52.30% recovery was obtained using 1M NH_4H_2 PO_4, pH 4.14. This is in accordance with the results obtained earlier by Chen *et al.* (2008). Naidu *et al.* (2009) also reported that arsenic speciation in soil is based on extraction with phosphate solutions including orthophosphoric acid, ammonium dihydrogen phosphate, ammoum hydrogen orthophosphate while the highest extraction efficiency was obtained through 1M orthophosphoric acid. Their study revealed that AsV was the predominant species in soil solution. However As III was detected in some soil samples. This may be due to the fact that the pH of the extracting solution influences the speciation of arsenic (AsIII, pka: 9.2 and AsV. Pka_1: 2.8, pka_2: 6.3, pka_3: 11.8) in soil and the phosphate (Pka_1: 2, pka_2: 7, pka_3: 12) in the extractant (Gallardo *et al.* 2001). This in turn impacts on the ion exchange between As species in soil and phosphate in the extractant, leading to different recoveries for arsenic species from different soils. The extraction efficiency of the selected reagents were observed to be substantially low in a number of soils as the majority of As is strongly bonded to Fe, Al and Mn particles of the soil and arsenic adsorption is highly dependent on Fe, Al and Mn concentrations in the soil as well as on the soil pH (Chen *et al.* 2008).

It has also been found that different efficiencies were obtained for As species when extractants with varying ambient pH were used. For example, both AsIII and AsV were released through 1M H_3PO_4, pH 1.39 and 1M NH_4H_2 PO_4, pH 4.14. However, the concentration of AsV (133.70 ìg/kg) using 1M H_3PO_4 at pH 1.39 is higher than that (51.49 ìg/kg) obtained from 1M NH_4H_2 PO_4, pH 4.14. The difference could be the result of ion-exchange between AsV and $H_3PO_4^-$ since As III appears in solution as a neutral

Table 2 Extraction efficiency of different extractant and their recovery percentage

Sample id	Extractant	Arsenic species					Sum of species (ppb)	Total As after aqua regia extraction (ppb)	Recovery (%)
		As B (ppb)	As III (ppb)	DMA (ppb)	MMA (ppb)	As V (ppb)			
Ghentugachhi soil	1M H_3PO_4	nd	900.15± 14.17	nd	nd	13300.70±122.75	14200.85±136.91	19400.9±205.53	73.29±1.48
	1M NH_4H_2 PO_4	nd	200.91± 41.57	nd	nd	5100.49±171.53	5400.40±128.46		27.91±1.36
	1M $(NH_4)_2H$ PO_4	nd	nd	nd	nd	n.d.	0.00		0.00
Mitrapur soil	1M H_3PO_4	nd	nd	nd	nd	7500.89±145.18	7500.89±148.59	15300.3±163.66	49.50±0.51
	1M NH_4H_2 PO_4	nd	nd	nd	nd	4000.74±164.76	4000.74±95.66		26.58±1.22
	1M $(NH_4)_2H$ PO_4	nd	nd	nd	nd	2700.00±128.44	2700.00±110.88		17.61±1.31
Malda soil	1M H_3PO_4	nd	200.53± 54.62	nd	nd	2500.35±123.55	2700.88±126.24	7900.9±81.65	34.89±1.33
	1M NH_4H_2 PO_4	nd	nd	nd	nd	300.23±82.57	300.23±67.89		4.04±0.77
	1M $(NH_4)_2H$ PO_4	nd	nd	nd	nd	n.d.	0.00		0.00
Gotera soil	1M H_3PO_4	nd	nd	nd	nd	6200.73±245.79	6200.73±129.64	14000.4±140.67	44.68±1.37
	1M NH_4H_2 PO_4	nd	nd	nd	nd	3700.66±132.44	3700.66±88.36		26.82±1.76
	1M $(NH_4)_2H$ PO_4	nd	nd	nd	nd	500.25±45.72	500.25±59.60		3.74±0.88
Nonaghata soil	1M H_3PO_4	nd	300.37±64.50	nd	nd	7900.29±187.94	8200.66±222.32	11800.2±133.73	69.93±1.49
	1M NH_4H_2 PO_4	nd	nd	nd	nd	6100.82±135.32	6100.82±179.62		52.30±1.24
	1M $(NH_4)_2H$ PO_4	nd	nd	nd	nd	500.08±48.95	500.08±59.08		4.30±1.11

nd - not detectable

species at both pH values. But when 1M $(NH_4)_2H\ PO_4$, pH 8.26 was used as an extractant, only AsV was found in some soils but the efficiency was too low. The results indicated that both As III and As V were extracted efficiently in an acidic medium.

Through the present investigation, 1M H_3PO_4 @ pH 1.39 has emerged as the best chemical extractant for determination of arsenic species in some contaminated Inceptisols (gangetic alluvium) of West Bengal. The microwave conditions were optimized at 60W microwave power with 10 ml extractant for 10 minutes extraction. Predominant arsenic species detected from experimental soils was AsV (arsenate).

ACKNOWLEDGEMENTS

This work was supported by the ICAR, NAIP (Component-4), Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India.

REFERENCES

Chen Z, Akter K F, Rahman M M and Naidu R. 2008. The separation of arsenic species in soils and plant tissues by anion-exchange chromatography with inductively coupled mass spectrometry using various mobile phases. *Microchemical Journal* **89** (1): 20–8.

Francesconi K A and Kuehnelt D. 2004. Determination of arsenic species: A critical review of methods and applications. *Analyst* **129**: 373–95.

Gallardo MV, Bohari Y, Astruc A, Potin-Gautier M and Astruc M. 2001. Speciation analysis of arsenic in environmental samples by high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry following orthophosphoric acid extraction. *Analytica Chimica Acta* **441**: 257–68.

Garcia-Manyes S, Jimenez G, Padro A, Rubio R and Rauret G. 2002. Arsenic speciation in contaminated soils. *Talanta* **58**: 97–09.

Giacomino A, Malandrino M, Abollino O, Velayutham M, Chinnathangavel T and Mentasti E. 2010. An approach for arsenic in a contaminated soil: Speciation, fractionation, extraction and effluent decontamination. *Environment Pollution* **2**: 416–23.

James WD, Raghvan T, Gentry T J, Shan G and Loeppert R H. 2008. Arsenic speciation: HPLC followed by ICP- MS or INAA. *Journal of Radioanalytical and Nuclear Chemistry* **278** (2): 267–70.

Montperrus M, Bohari Y, Bueno M, Astruc A and Astruc M. 2002. Comparison of extraction procedures for arsenic speciation in environmental solid reference materials by high-performance liquid chromatography hydride generation-atomic fluorescence spectrometry. *Applied Organometallic Chemistry* **16**: 347–54.

Pizarro I, Gomez M, Camara C and Palacios M A. 2003. Arsenic speciation in environmental and biological samples extraction and stability studies. *Analytica Chimica Acta* **495**: 85–98.

Rahman M M, Chen Z and Naidu R. 2009. Extraction of arsenic species in soils using microwave-assisted extraction detected by ion chromatography coupled to inductively coupled plasma mass spectrometry. *Environmental Geochemistry and Health* **31**: 93–102.

USEPA. 1997. Method 3051 A. Microwave assisted acid digestion of sediments, sludge's, soils and oils. United States Environmental Protection Agency (USEPA),Washington, DC.