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Bioavailability of arsenic in rice in arsenic endemic areas of West Bengal, India

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ABSTRACT

Rice is a potentially important route of human exposure to arsenic, especially with rice-based diets. The WHO standard for As in drinking water of 10 µg L^{-1} has been adopted by many countries. Arsenic in water is generally inorganic and can be a mixture of arsenite (As (III)) and arsenate (As V). Arsenic in rice is of special concern because of the much higher levels of As in rice grain compared to other staple cereal crops. An effort has been made, through the present study, to take an account of arsenic speciation in rice in the arsenic affected villages of Chakdaha block, Nadia district, West Bengal, India having an arsenic concentration of irrigation water drifted from the shallow tube wells 0.32 mg l^{-1}. The present study indicated that inorganic arsenic shared maximum arsenic load in rice straw while in grains it is considerably low. As species recovered from rice straw and grain are principally As-III and As-V. Rice grain As has been found to be principally As-III while in straw As-V predominated over As-III. The maximum dietary risk of exposure to inorganic arsenic through transplanted aus paddy in the present investigation was calculated to be almost 700 % of PTWI (Provisional Tolerable Weekly Intake) for an adult of 60 kg bodyweight.

Key words: *arsenic, bioavailability, rice, speciation, risk assessment. West Bengal*

Rice is the most important crop of India and second principal food crop of the world. In India, rice is predominantly grown in the Indo-Gangetic plains, on 13.5 million ha or 85 percent of the cultivated land area with ground water as a principal source of irrigation (Samra *et al.*, 2004). Most of the shallow groundwater in southern Bangladesh and eastern part of West Bengal, India, is geogenicaly contaminated with arsenic (As), exposing more than 40 million people at risk of As in drinking water (World Bank, 2005). Arsenic contamination of water and soil can also adversely affect food safety. A global normal range of 0.08 to 0.2 mg As kg^{-1} has been suggested for rice (Zavala and Duxbury, 2008), but values as high as 0.25 mg As kg.$^{-1}$ have been found in rice (Mandal *et al.*, 2007). The average daily intake of As from rice for an Indian adult is approximately 100 mg As (NNMB, 2002) (400 g dry wt x 0.25 mg As kg^{-1}), which is 5 times the 20 mg As intake from consumption of 2 L of water as the WHO limit of 10 ug l^{-1} (WHO, 1993).

Arsenic contamination in groundwater in the state of West Bengal has assumed the proportion of 12 endemic districts, 111 endemic blocks and above 50 million people exposed to threats of arsenic related health hazard (School of Environmental Science, J.U, 2006). It is only the agricultural sector which enjoys the major share (> 90%) of such contaminated groundwater as source of irrigation and received attention for quantifying the influence of arsenic in soil-plant system (Abedin *et al.*, 2002, Mukhopadhyay and Sanyal, 2004). Mondal and Polya (2008) reported that the contribution of rice to the total arsenic intake in some parts of India is as high as that of arsenic contaminated drinking water, indicating that As-tainted rice can be a significant source of arsenic.

In this context, an experiment has been conducted in the arsenic endemic area of West Bengal to explore the behavior of arsenic in soil, water and principal crops, quantifying the net toxicities and bioavailabilities of arsenic in soil-water-plant with regard to species level information of the toxic metalloid, assessing risks of dietary exposures and exploring for possible mitigation options.

MATERIALS AND METHODS

The experiment was conducted at farmer's field in the village Ghentughachi (block Chakdaha, district Nadia, West Bengal, India for two years (2008 and 2009) during May to September. The autumn rice crop, variety *GS 3* which is widely grown in the arsenic affected area of West Bengal was selected for the study. The crop was sown during first week of May. Seed rate was 100 kg ha^{-1} and spacing maintained at 30X10 cm. Weeding was done twice at 20 and 40 days after sowing (DAS). Rice fields were irrigated both from shallow tube well water (STW- As concentration @ 0.32 mg l^{-1}) and pond water (PW - As concentration @ 0.03 mg l^{-1}).

The experiment has been laid out in a 2 factor randomized block design with three replications. Factorial experimental treatments were two levels of irrigation (irrigation through shallow tube well water and irrigation through surface water) and four levels of organic manures namely FYM@10t.ha^{-1}, vermicompost @ 3 t.ha^{-1}, municipal sludge@10 t.ha^{-1}and mustard cake@1.0 t.ha^{-1}. The soils were amended with well decomposed FYM, vermicompost, municipal sludge and mustard cake in respective treated plots followed by a couple of ploughing operations 25 days before sowing. The recommended doses of N, P, K fertilizers (N: P-$_2$O$_{5:}$ K$_2$O:: 100 : 50 : 50) kg. ha^{-1} were applied to the soils irrespective of treatments. The entire P and K fertilizers were applied basally while N fertilizer has been applied in three splits (50% as basal and rest 50% top dressed at 30 DAS and 45 DAS). The initial and post-harvest soil samples were collected through soil auger at a depth of 15 cm. At least 10 sub (core) samples were collected to have the composite sample from one replication. Plant samples (whole plant) were collected at different growth stages i.e. at 30, 60 and 90 DAS.

Soils samples were collected, tagged and packed in brown polythene packets and taken to the laboratory. The soil samples were air-dried, ground and sieved through 2 mm sieve and packed in air tight polythene containers. The plant samples were oven dried for 24 hours at 105°C, ground and packed in air tight polythene container. Soil samples were analyzed for detailed characterization with respect to the important physico-chemical properties (pH, organic carbon, available N, P_2O_5 & K_2O, total and extractable arsenic) following the standard methods (Page, 1982).

Available N content of soil was determined by the Kjeldahl method (Subbiah and Asija, 1956), available P by 0.5 M $NaHCO_3$ (pH 8.5) (Olsen and Sommers,1982) exchangeable K by 1M NH_4OAc (pH 7.0) (Knudsen *et al*., 1982), oxydizable organic C (Walkley and Black, 1934), texture (Dewis and Freitas, 1984), Olsen extractable As by 0.5 M $NaHCO_3$, pH 8.5 (Olsen and Sommers, 1982) and total As by tri-acid digestion (Sparks, 2006). Plant samples were digested with a mixture of acids *i.e.* HNO_3, $HClO_4$ and H_2SO_4 in a proportion of 10:4:1 (v/v) for total As measurement. Olsen extractable P was analyzed colorimetrically, ammonium acetate extractable K was analyzed by flame photometry. Sodium bicarbonate extractable As, total soil As and plant As were determined through atomic absorption spectrophotometer (PerkinElmer AAnalyst 200) coupled with flow injection system (FIAS-400).

The humic acid (HA) and fulvic acid (FA) fractions were extracted from the manures used with 0.5 M Na_2CO_3, followed by their fractionation into humic and fulvic acid constituents and the complexation equilibria between arsenic and the humic/fulvic substances were examined following the standard method (Schnitzer and Skinner, 1966) and the stability constants (Log k) of the arsenic-humic/fulvic complexes formed were recorded.

About 0.2 g of rice grain or straw sample were weighed into a microwave Teflon vessel and 7 ml of concentrated nitric acid was added to it and left to stand overnight at room temperature. Samples were then digested in a microwave maintained at 200 ^{0}C for 20 minutes. Samples were then cooled and transferred to a 50 ml volumetric flask for total arsenic analysis through Perkin Elmer ELAN DRC$_e$ 6000 ICP-MS.

For speciation analysis about 0.2 g of rice grain or straw sample were weighed into a microwave Teflon vessel and 2 ml of 2.0 M TFA was added to it. Samples were then digested in a microwave maintained at 90^{0}C for 20minutes. Samples were then cooled and transferred to a 50 ml volumetric flask for speciation analysis (Abedin *et al.*, 2002). Attempts here have been made to assess the toxicity level in grain and straw. Few selected samples, precisely those who responded better against the interventions employed in terms of total arsenic accumulation, accumulation of arsenic species have been determined by TFA (@pH 6.0) extraction followed by detection and quantification through a Perkin-Elmer ELAN DRC$_e$ HPLC-ICP-MS and the outcome has been recorded

RESULTS AND DISCUSSION

The results indicated that the agricultural soil of the study area has become highly contaminated with arsenic (19.17 mg.kg^{-1}) due to the excessive use of arsenic rich groundwater (0.32 mg.l^{-1}) for irrigation (Table 1). Long term use of arsenic contaminated groundwater for irrigation may result in the further increase of arsenic concentration in the agricultural soil and eventually hyper-accumulation in rice plants.

The maximum accumulation of arsenic was observed in root (34.84-75.25 mg.kg^{-1}), followed by leaf (4.56-18.63 mg.kg^{-1}), shoot (2.28-18.00 mg.kg^{-1}) and grain (0.44-1.33 mg.kg^{-1}) (Table 2). Results revealed that the arsenic accumulation in different parts of rice remained in an order of root>leaf>shoot>grain in both the experimental years (2008 and 2009) which has been found to increase with advancement of growth stages (Fig.1). Similar observations were also reported by Abedin *et al.*, (2002). Very little share of the total arsenic accumulation has been found to be translocated to grain (2-4%), although the level is alarming (0.44-1.33 mg. kg^{-1}). Rice grain samples from arsenic-endemic areas in West Bengal, India were also reported to contain high concentrations of As with a mean value of 0.45 mg kg^{-1} (range 0.19–0.78 mg kg^{-1}) for Boro rice and a mean concentration of 0.33 mg kg^{-1} (range 0.06–0.60 mg kg^{-1} for Aman rice (Bhattacharya *et al.*, 2010).

Table 1. Physico-chemical properties of experimental site

Properties	Observation
Soil	
pH	7.51
Organic C (%)	0.56
Textural class	Silty clay
%Sand	3.5
% Silt	46.7
% Clay	49.8
Available nitrogen (kg.ha^{-1})	220.0
Available phosphorus (kg.ha^{-1})	57.0
Available potassium (kg.ha^{-1})	190.0
Total arsenic (kg.ha^{-1})	19.17
Available arsenic (kg.ha^{-1})	5.30
Water	
Arsenic in pond water (ppm)	0.03
Arsenic in shallow water (ppm)	0.32

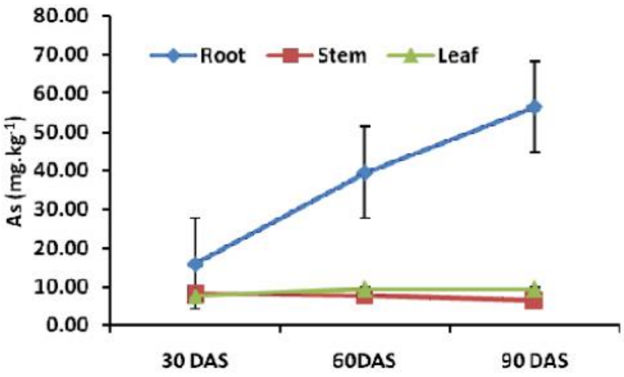

Fig. 1 Progressive changes in arsenic accumulation in different plant parts of *autumn* rice with advancement of growth

Based on a comparative analysis of samples from different origins Shraim (2014) reported that American rice accumulated highest arsenic concentration (Mean 0.25mg kg^{-1}) followed by the Thai rice (mean 0.200 mg kg^{-1}) the Pakishani rice (mean 0.147 mg kg^{-1}), the Indian rice (mean 0.103 mg kg^{-1}).

The results indicated that incorporation of organic manures has marked effect on reduction of arsenic accumulation in different plant parts of wet season rice. It was observed that incorporation of organic manures significantly reduced the arsenic uptake by different plant parts of rice over the control counter part under both the irrigation regimes (STW and PW). Such beneficial role exerted by different organic sources has been found to be most pronounced and consistent with FYM and vermicompost. Das *et al* (2005) also observed that available soil arsenic content decreased with the increase of organic matter application. Such changes in arsenic accumulation in rice manifested either through using surface water as irrigation source or through organic amendments, may be attributed to similar changes in soil available arsenic

Table 2 Arsenic accumulations in different plant parts of rice recorded at different growth stages as affected by intervention of organic manures and source of irrigation

Irrigation Sources (I)	Organic matters (O)	Arsenic accumulation in mg.kg^{-1}							
		2008				2009			
		Root	Shoot	Leaf	Grain	Root	Shoot	Leaf	Grain
Shallow tube-well water	C	67.67±1.53	18.00±0.19	18.63±0.10	1.33±0.04	75.25±0.25	4.94±0.06	12.15±0.12	0.92±0.08
	O_1	68.33±2.96	13.08±0.29	16.13±0.20	0.76±0.03	54.22±0.47	3.38±0.05	8.77±0.08	0.75±0.06
	O_2	65.75±0.74	8.53±0.17	7.46±0.09	1.08±0.06	42.41±0.17	4.46±0.08	6.13±0.05	0.90±0.05
	O_3	65.50±0.41	7.40±0.09	11.89±0.14	0.60±0.02	38.33±0.43	2.78±0.11	6.41±0.11	0.66±0.07
	O_4	63.92±1.31	9.03±0.19	13.50±0.10	0.67±0.08	49.45±0.13	3.01±0.05	6.75±0.09	0.68±0.04
	Mean	66.23	11.21	13.52	0.89	51.93	3.71	8.04	0.78
Pond water	C	65.33±0.77	13.92±0.21	10.84±0.15	1.17±0.14	69.21±0.33	3.68±0.09	9.77±0.11	0.82±0.06
	O_1	68.58±0.31	9.31±0.14	9.36±0.23	0.64±0.09	49.49±0.20	3.25±0.11	6.23±0.09	0.63±0.03
	O_2	56.33±0.72	7.97±0.11	8.54±0.18	0.48±0.06	37.68±0.22	2.28±0.06	4.56±0.05	0.62±0.05
	O_3	58.17±0.31	5.23±0.18	7.53±0.13	0.44±0.11	34.84±0.47	2.58±0.12	6.84±0.07	0.63±0.04
	O_4	59.75±0.41	6.36±0.08	9.39±0.17	0.51±0.07	41.32±0.79	2.85±0.06	6.28±0.04	0.68±0.03
	Mean	61.63	8.56	9.13	0.65	46.51	2.93	6.74	0.68
CD ($P<0.05$)									
I		1.19	0.15	0.11	0.01	0.34	0.04	0.05	0.02
O		1.87	0.24	0.18	0.02	0.54	0.06	0.08	0.03
I × O		2.65	0.34	0.26	0.03	0.77	0.09	0.12	0.04

C = Control, O_1 = Mustard cake@1t ha^{-1}, O_2 = Farm Yard Manure@10t ha^{-1}, O_3 = Vermicompost @3t ha^{-1} and O_4 = Municipal sludge@10t ha^{-1}.

Table 3. Correlation between available soil arsenic and total uptake of rice at harvest

Irrigation sources(I)	Treatment (T)	2008		2009	
		Available arsenic (kg.ha^{-1})	Total uptake (mg.kg^{-1})	Available arsenic (kg.ha^{-1})	Total uptake (mg.kg^{-1})
Shallow tube well water	C	4.46	105.63	4.32	93.26
	O_1	4.19	98.3	4.14	67.13
	O_2	4.01	82.82	3.87	53.9
	O_3	3.97	85.43	3.49	48.18
	O_4	4.28	87.12	4.13	59.89
	Mean	4.18	91.86	3.99	64.47
Pond water	C	3.93	91.26	4.26	83.48
	O_1	3.66	87.85	3.71	59.6
	O_2	3.03	73.32	2.97	45.14
	O_3	3.31	71.37	3.22	44.87
	O_4	3.51	76.01	3.35	51.14
	Mean	3.49	79.96	3.50	56.85
Correlation		0.8685**		0.8466**	

C = Control, O_1 = Mustard cake @1t ha^{-1}, O_2 = Farm Yard Manure @10 t ha^{-1}, O_3 = Vermicompost @3t ha^{-1} and O_4 = Municipal sludge @10 t ha^{-1}.

under similar situations, as reflected in significant correlation drawn between total arsenic uptake by rice at harvest and available arsenic in post-harvest soil of rice (Table 3). The magnitude of such decreases, however, varied with sources and levels of applied organic matter while such decrease remained most pronounced with vermicompost, which might be due to formation of insoluble arseno-organic complexes and its adsorption on to organic colloids.

Organic amendments such as composts and manures which contain a high amount of humified organic matter can decrease the bioavailability of heavy metals through adsorption and by forming stable complexes with humic substances. (Chen *et al.*, 2000). Jones (2000) reported that the reduced accumulation of arsenic in plants are due to low availability of the toxicant from soil due to amended through compost, manures etc. Rahaman *et* *al.* (2011) showed that combined applications of lathyrus + vermicompost + poultry manure reduced arsenic transport in plant parts (root, straw, husk, whole grains and milled grain). Precipitation and flocculation of humic acids by heavy metals were observed in both acidic and calcareous soils (Clemente and Bernal, 2006). Humic acids have great capacity to retain and bind metals. Their molecular structure is usually larger than the soil pore size resulting in the low mobility and little leaching through soil profile. (Halim *et al.*, 2003).

The complexation studies of arsenic with humic acid and fulvic acid fractions isolated from the selected organic manures used in the present experiment revealed that HA-FA fractions extracted from vermicompost have the capacity of making strongest complexes with soil arsenic, as expressed in the computed log K values (Table 4) which may be attributed to the reduction in available arsenic load in soil-plant system through respective interventions. This is in good agreement with the findings as obtained earlier by Mukhopadhyay and Sanyal (2004) and Sinha and Bhattacharyya (2011) who reported that there was an ability of native or added soil organic fractions to sorb arsenic, thereby moderating its toxicity in soil-plant system. Das (2007) also observed 18.30% and 14.01% decrease in 0.5 M $NaHCO_3$- extractable soil As from the control counterpart when the soil was amended with vermicompost and well-rotten FYM, due to formation of organo-As complexation.

Table 4. Characterization of the selected organic manures

Feature	FYM	Vermicompost	Sludge	Mustard cake
TOC (%)	25.9	25.0	17.0	12.0
N (%)	0.5	0.25	0.5	5.0
P (%)	1.5	1.0	1.5	2.0
K (%)	1.0	1.0	1.0	1.5
Zn (ppm)	52.0	48.0	80.0	39.0
Cu (ppm)	8.0	12.0	40.0	19.0
Fe (ppm)	1500	1025	1838	2705
Mn (ppm)	53.0	56.0	62.0	70.0
C: N	20:1	15:1	18:1	12:1
As (ppm)	3.54	3.02	3.64	0.38
Log k (HA)	4.12	4.86	3.54	2.67
Log k (FA)	8.65	10.27	7.97	4.95

It is now commonly accepted that toxicity and bioavailability varies with arsenic species and assessing toxicity and risk associated with As exposure based on total concentrations only may lead to artifacts. Rice has been shown to accumulate various forms of arsenic like arsenite As (III) arsenate (As V), methylarsonic acid and dimethyl arsinic acid that differ in toxicity to living beings, the first two being more toxic than the other two species (Hughes, 2002). The recovery of arsenic species through TFA extraction remained at quite satisfactory level (63 to 103 % of total arsenic determined through microwave assisted HNO_3 digestion). The As-III and As-V remained the major arsenic species in most of the grain and straw samples analyzed. It is interesting to note that As-III accounted for the major As species recovered from grains of

Table 5. Arsenic speciation of selected straw and grain samples of *aus* paddy by TFA (@ pH 6.2) extraction through HPLC-ICP-MS

Sample	Irrigation	Manure	Arsenic species					Sum of	Total As (ppb)	Per cent
			As B (ppb)	As-III (ppb)	DMA (ppb)	MMA (ppb)	As-V (ppb)	Species	(HNO_3 digestion)	recovery
Grain	PW	C	nd	320.4±22.31	113.4±7.57	nd	251.4±14.38	685.2±29.14	669.0±33.07	102.4±6.29
		VC	nd	284.4±15.65	nd	nd	118.8±12.51	403.2±26.4	390.0±28.83	103.4±5.35
		FYM	nd	288.6±12.84	nd	nd	121.9 ±9.97	410.4±21.9	434.7±23.01	94.4±1.57
	STW	C	nd	328.0 ±25.5	nd	nd	183.3 ±7.13	511.3±22.5	743.7±22.87	68.8±2.98
		VC	nd	307.6±25.69	nd	nd	134.7±10.01	442.3±18.55	557.3±22.79	79.4±1.51
		FYM	nd	314.6±20.98	nd	nd	147.2 ±8.94	461.9±24.1	585.7±19.25	78.9±2.40
Straw	PW	C	nd	369.0±28.74	208.0±9.78	nd	3428.5 ±106	4005.5±75.5	3988.0±88.27	100.4±2.03
		VC	nd	187.6±12.41	nd	nd	2987.4±89.3	3175.0±65.7	3879.0±108	81.9±1.76
		FYM	nd	224.2±20.04	nd	nd	2763.0 ±105	2987.2±78.3	4120.0±96.7	72.5±2.98
	STW	C	nd	387.6±30.76	202.8±13.41	nd	4169.4 ±113	4759.8±69.0	4836.0±109.4	98.4±3.01
		VC	nd	106.8 ±8.61	nd	nd	2691.6±93.6	2798.4±59.5	4398.0±94.6	63.6±3.55
		FYM	nd	328.9±22.88		nd	3578.6±88.9	3907.5±68.2	4587.0±83.9	85.2±4.20

C-Control, VC- Vermicompost, FYM-Farm yard manure transplanted aus paddy while As-V predominates As recoveries from rice straw (Table 5). Meharg *et al.*, 2002 also observed that arsenic species in rice straw extracted with TFA are arsenate, arsenite and DMA. The proportion of arsenate, arsenite and DMA were 72-84%, 15-26% and 1-4%, respectively.

Meharg *et al.*, 2008 showed that rice grain arsenic speciation is dominated by inorganic arsenic and DMA. DMA has been recovered from few grain and straw samples where interventions through organic manures have not been made. The inorganic arsenic of grain has been found to increase with increasing levels of total grain arsenic (Fig. 2).

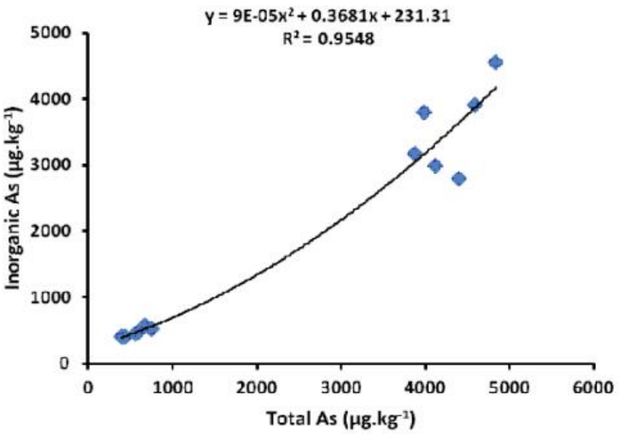

Fig. 2 Changes in inorganic arsenic in grains of transplanted autumn rice with changes in total arsenic thereof

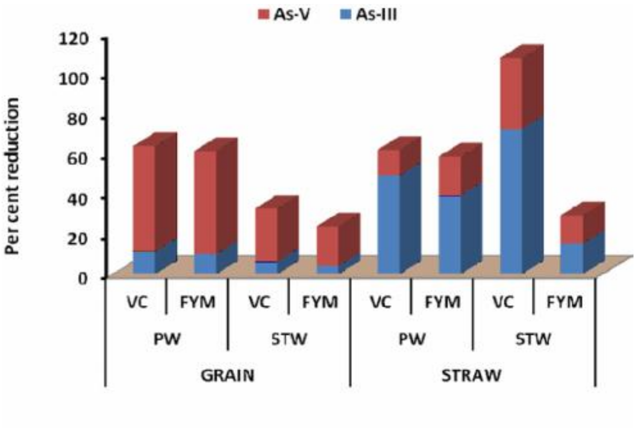

Fig. 3 Per cent reduction in inorganic arsenic species accumulation in grain and straw of transplanted aus paddy through organic intervention and changing irrigation source

VC – vermicompost, FYM- Farm yard manure, Pw – Pond water, STW – Shallow tubewell water

Soil amendment through organic intervention (Vermicompost > FYM) reduced arsenic accumulation in rice grain and straw which has been principally manifested through reduction of As-V in grain and As-III in straw (Fig.3). The assessment of risks for dietary exposure to food items (rice grain) is quite imperative since the proportions of arsenic toxicity contributed through As-III remained quite significant (44 to 73% of total As recovered through HNO_3 digest) as reflected in the present study . The maximum dietary risk of exposure to inorganic arsenic through autumn paddy in the present experiment was calculated to almost 700 % of PTWI (Provisional Tolerable Weekly Intake) for an adult of 60 kg bodyweight.

From the present investigation it can be concluded that the As-III and As-V remained the major arsenic species in most of the grain and straw samples of *autumn* rice analyzed. As-III accounted for the major As species recovered from the grains, while As-V predominated As recoveries from rice straw. Soil amendment through organic intervention reduced arsenic accumulation in rice grain and straw which has been principally manifested through reduction of inorganic As.

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