Mobilisation of arsenic in soils and in rice (*Oryza sativa* L.) plants affected by organic matter and zinc application in irrigation water contaminated with arsenic

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ABSTRACT

The experiments were conducted to study the effects of arsenic-contaminated irrigation water, zinc and organic matter on the mobilization of arsenic in an Aeric Endoaquept in relation to rice (cv. IET 4786). The results show that the amount of extractable arsenic increased with the progress of submergence decreased with zinc application. The magnitude of such decrease varied with the Zn amount, being greater (0.70 to 1.08 mg/kg) in the treatment where zinc was applied at the rate of 20 mg/kg. With regards to organic matter application, the arsenic content in soil markedly decreased, especially with farmyard manure application. The results of the greenhouse experiment with exposure of graded doses of arsenic to rice suggest that the upper toxic limit of arsenic in soil was 10 mg/kg for rice. The results of the field experiment show that the grain yield of continuous flooding (4.84 t/ha) and intermittent flooding up to 40 days after transplanting followed by continuous flooding (4.83 t/ha) with the application of $ZnSO_4$ at the rate of 25 kg/ha did not vary significantly. The lowest grain yield (3.65 t/ha) was recorded in the treatment where the intermittent flooding was maintained throughout the growth period without the application of zinc. The amount of arsenic was, however, much lower in the treatment where intermittent flooding was maintained throughout the growing period combined with zinc sulphate application.

Keywords: arsenic; interaction; organics; rice; upper toxic limit; zinc

In Asian countries, there is the highest number of people consuming and using arsenic-contaminated drinking water and ground water for irrigation purposes, who consequently suffer from arsenicosis (Chakravarty and Das 1997). Out of nineteen districts in West Bengal, nine districts, where cropping intensities are very high, are arsenic-affected. A huge amount of ground water loaded with arsenic is used for irrigating agricultural crops, particularly for production of boro (summer) rice during the lean period (March to May). 8–9 million people in West Bengal are thus affected by arsenic toxicities as they consume such arsenic-contaminated ground water for drinking purpose (Mandal 1998). Mandal et al. (1996) also reported that the average arsenic contamination in the drinking water is about 0.20 mg/l with a maximum concentration of 3.7 mg/l. According to the World Health Organization (WHO) specification, i.e. 10 μg/l, about 45 percent of the tested drinking water is toxic. Use of such water for irrigation purposes results in an increase of arsenic concentration in soil and it subsequently enters into different parts of crops; Arsenic thus ultimately takes its way to the human and animal body causing various anomalies and carcinogeneses (Chakravarty and Das 1997). Groundwater rich in arsenic mostly occur in the Bengal Delta Plain (BDP), covering the state of West Bengal, the adjoining country of Bangladesh, extending to Bihar, Jharkhand, Uttar Pradesh, Punjab, Chattishgarh and the neighbouring country of Nepal (Bhattacharya et al. 2003). Keeping this in view, the present study was undertaken with the main objective to study mobilization vis-à-vis retention of arsenic in submerged rice (*Oryza sativa* L.) soils as affected by zinc, organic matter and arsenic-contaminated irrigation water.

MATERIAL AND METHODS

The present investigation consisted of three parts: (*i*) laboratory experiment, (*ii*) greenhouse experiment, (*iii*) field experiment.

Laboratory experiment

Set I. Effect of zinc on retention of arsenic in soils. In this set of experiments, soil was collected from the cultivated rice field having following properties: pH 7.6, 0.22 mg/kg of 0.5M $NaHCO_3$ extractable arsenic (Johnston and Barnard 1979), and 0.32 mg/kg of 0.005M DTPA extractable zinc (Lindsay and Norvell 1978). The zinc at the rates of 0, 10 and 20, and arsenic at the rates of 0, 5 and 10 mg/kg were applied to 100 g soil contained in 250-ml polyethylene beaker after combining the respective treatment materials into all possible treatment combinations, i.e. 9. Each treatment combination was replicated thrice in a completely randomized design and was kept for incubation in the laboratory under submerged condition. After lapse of the appropriate period, 0.5M $NaHCO_3$ extractable arsenic and 0.005M DTPA extractable zinc were determined as described below.

Soil extraction methods

Arsenic. Five grams of soil sample was taken in a 100-ml conical flask and 50 ml of 0.5M $NaHCO_3$ solution was added. Then the whole material was shaken for 1 h in a "to and fro" horizontal shaker and after completion of shaking, the suspension was filtered through Whatman filter paper No. 42. The filtrate was collected for arsenic analysis with atomic absorption spectrometer (Perkin Elmer model AAnalyst 100) coupled with hydride generator after reducing, with 2 ml 10% KI solution and 2 ml 35% HCl, $NaBH_4$ solution and 4M HCl solution separately from three containers allowed passing to a mixing manifold by a peristaltic pump. From the mixing manifold argon (inert gas) AsH_3 (arsine) generated in the reaction loop. The arsenic was then atomized in a flame of air-acetylene and the direct arsenic concentration in the sample was measured (Johnston and Barnard 1979).

Zinc. Ten grams of soil sample was taken in a conical flask and then 20 ml of 0.005M DTPA solution (pH adjusted to 7.3) was added and the whole material was shaken for 2 h in a "to and fro" horizontal mechanical shaker. After lapse of the shaking period, the soil suspension was filtered through Whatman filter paper No. 42 and the filtrate was ready for Zn analysis with an atomic absorption spectrophotometer (Lindsay and Norvell 1978).

Set II. Effect of organic matter on retention of arsenic in soils. In this set of experiments, the soil was collected from the arsenic contaminated field of Gotera village in the district of Nadia, West Bengal, India, where the subsequent field experiment was conducted. The initial arsenic content of the soil was 1.48 mg/kg. In this experiment, two sources of organic matter, namely well rotten farmyard manure (FYM) and vermicompost (V) were applied. The effect of each source of organic matter was studied separately divided into two series. In both series, three levels each of organic matter (0, 1, and 2% weight of soil) and arsenic (0, 5 and 10 mg/kg) were used. In series I, three levels each of FYM and arsenic were used and in series II, three levels each of vermicompost and arsenic were used separately and kept in the laboratory for incubation under submerged conditions after combining the treatment materials into all possible treatment combinations and replicated thrice in a completely randomized design. After lapse of the appropriate period, arsenic content in soil was extracted with 0.5M $NaHCO_3$ (pH 8.5) as described earlier and determined with an atomic absorption spectrophotometer using hydride generation method.

Greenhouse experiment

Eight kg of soil (non-contaminated with arsenic) was taken in several 10-liter polyethylene pots. In the pots filled with soil, graded doses of soluble arsenic (0, 10, 30, 50, 70, 90, 120, 150, 200 and 300 mg/kg) were added and the soils of each pot were puddled followed by submergence. Then 2–3 thirty-day-old rice seedlings cv. IET 4786 were transplanted in each pot and allowed to grow till the harvest. Periodic monitoring was performed to assess visual symptoms; the soils and plants from each pot were collected regularly and at harvest, and analysed for arsenic content (after digesting the samples with ternary acid mixture, $HClO_4$:HNO_3:H_2SO_4 10:4:1) following the method described by Jackson (1973).

Field experiment

Field experiment was carried out on arsenic-contaminated Aeric Endoaquept soil of Gotera village in the district of Nadia, with summer rice (cv. IET 4786) as a test crop; the soil had following physico-chemical properties: pH 7.6, cation exchange capacity (CEC) 17.86 cmol (p^+/kg), organic carbon content 7.8 g/kg, DTPA – extractable Zn 0.46 mg/kg, 0.5M $NaHCO_3$ extractable arsenic 1.48 mg/kg. After puddling, the experimental plot (30 × 17.5 m^2) was divided in 12 main plots (6 × 4.5 m^2), with each main plot being further divided in to 2 subplots (3 × 4.5 m^2) in a split plot design replicated four times. Recommended levels of 100, 50 and 50 kg/ha of N, P_2O_5 and K_2O, respectively, were applied to each subplot, where half of the N and full P_2O_5 and K_2O were applied as basal and 1/4 of the N was top dressed at active tillering and panicle initiation stages. 2–3 thirty-day-old rice seedlings were transplanted at the spacing of 15 × 15 cm. The source of irrigation water was a shallow tube well containing 0.26 mg/l arsenic. The main plot treatments consisted of three irrigation methods, namely (i) continuous ponding (I_0), (ii) intermittent ponding (I_1) and (iii) intermittent ponding up to 40 days of crop growth and then continuous ponding till harvest (I_2). The subplot treatment included two levels of Zn, i.e. Zn_0 (no zinc) and Zn_1 (25 kg/ha $ZnSO_4$). Afterwards, the rice plants were allowed to grow till the harvest. Soil samples after 75 days of crop growth (corresponding to grain filling stage) and plant samples at harvest were analysed for arsenic content. Arsenic was determined with an atomic absorption spectrometer after extracting soils with 0.5M $NaHCO_3$ at pH 8.5 (Johnston and Barnard 1979), and plant samples after digesting with ternary acid mixture $HClO_4$:HNO_3:H_2SO_4 (10:4:1) as described earlier.

Statistical analysis. Statistical analyses of the data were made following the method described by Panse and Sukhatme (1989).

RESULTS AND DISCUSSION

Effect of zinc on the mobilization of arsenic in soils

The results (Figure 1) show that the amount of arsenic in native soil slightly increased due to submergence compared to the absolute control (As_0Zn_0); however, it decreased significantly with the application of zinc, irrespective of level. A higher decrease (38.4%) of arsenic in soil was recorded with 10 mg/kg than with 20 mg/kg (35.1%) zinc application after 42 days of submergence. The results also show that the arsenic content consistently increased with the progress of submergence due to application of arsenic. As to the interaction between Zn and arsenic, it was observed that the arsenic content decreased; a greater decrease was recorded with $Zn_{10}As_5$ treatment, where the application of Zn and arsenic at the doses of 10 and 5 mg/kg, respectively, was used. The results further suggest that the arsenic consistently increased up to 42 days of submergence in the As_0Zn_0 treatment; a decrease was recorded when zinc and arsenic were applied in combination. The lowest

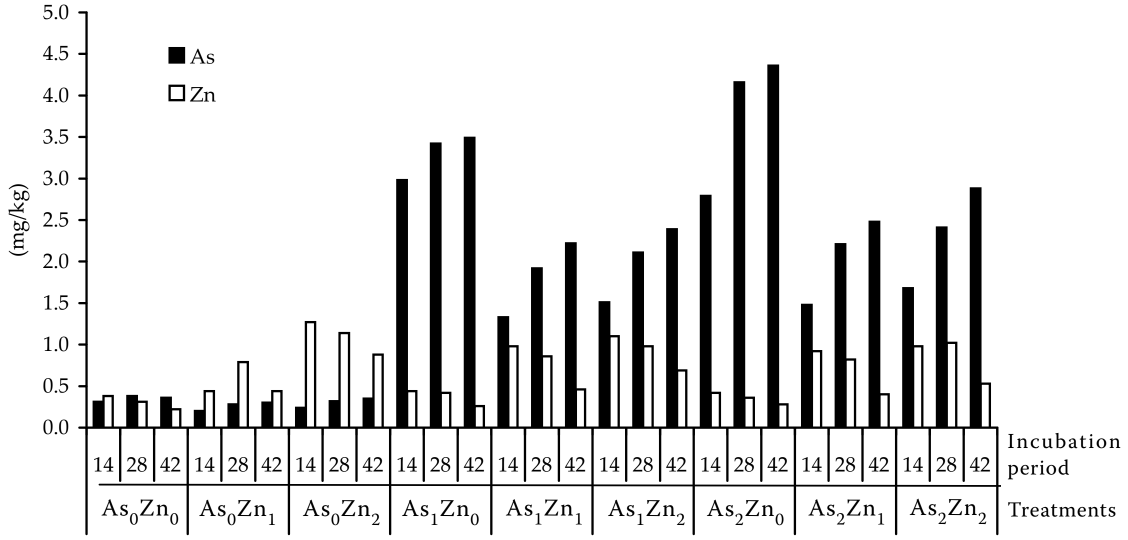

Figure 1. Effect of Zn on retention of arsenic in soil

Table 1. Effect of organic matter (farmyard manure) on retention of arsenic in soil

Treatments	Arsenic concentration (mg/kg)											
	10 days				25 days				50 days			
	As_0	As_1	As_2	mean	As_0	As_1	As_2	mean	As_0	As_1	As_2	mean
O_0	2.05	2.58	3.73	2.78	2.89	3.32	4.14	3.45	2.63	3.13	3.89	3.10
O_1	2.09	2.50	3.74	2.77	2.73	3.15	3.87	3.25	2.43	3.87	3.78	3.03
O_2	2.15	2.43	3.66	2.74	2.67	3.23	3.78	3.22	2.12	2.63	3.53	2.76
Mean	2.09	2.50	3.71		2.76	3.23	3.90		2.39	2.88	3.73	

arsenic content was recorded in As_0Zn_1 treatment compared to the other combinations.

The results (Figure 1) also show that the amount of DTPA-extractable Zn content was found to increase due to Zn application, being higher with higher level of Zn application. Nevertheless, this increase was found to gradually diminish with the progress of submergence, irrespective of Zn levels. As to the effect of arsenic application, it was observed that the amount of Zn consistently decreased up to 42 days of submergence with the application of arsenic; a greater decrease was recorded with higher levels of arsenic. As regards the interaction effect between arsenic and zinc, the Zn content decreased markedly in the As_2Zn_1 treatment where arsenic and zinc were both applied at the dose of 10 mg/kg at 42 days of submergence. It may be concluded from the results that the arsenic content decreased with the application of zinc, being greater with 10 mg/kg Zn compared to 20 mg/kg Zn.

Effect of organic matter on mobilisation of arsenic in soils

The results (Tables 1 and 2) show that the arsenic content decreased with the application of organic matter irrespective of sources. The source and amount of organic matter however influenced the extent of such decrease; a greater decrease (18.30%) was recorded in the case of application of higher levels of vermicompost (2% of weight of soil) compared to corresponding level of well rotten FYM (14.01%) after 50 days of submergence. The same trend was observed for the changes of arsenic concentration in soil due to application of arsenic; the concentration increased up to 25 days of submergence and thereafter, it decreased at 50 days of submergence. Considering the interaction effect between vermicompost and arsenic, the amount of arsenic decreased irrespective of treatment combinations; the highest decrease was recorded in As_0V_1 and As_0V_2 treatments where no arsenic was applied. A decrease was recorded also in As_1V_2 combination where arsenic was applied at the dose of 5 mg/kg combined with vermicompost (2% of the weight of soil), which might be caused by formation of insoluble arseno-organic complexes and their adsorption on to organic colloids. From the results, it may be concluded that the mobilization of applied arsenic was found to decrease with the application of farmyard manure (FYM) and vermicompost; a greater decrease was reported for combined applications of 5 mg/kg arsenic and vermicompost (2% of the weight of soil).

Table 2. Effect of vermicompost on retention of arsenic in soil

Treatments	Arsenic concentration (mg/kg)											
	10 days				25 days				50 days			
	As_0	As_1	As_2	mean	As_0	As_1	As_2	mean	As_0	As_1	As_2	mean
V_0	2.13	2.48	3.89	2.83	2.76	3.51	4.18	3.48	2.59	3.23	3.71	3.17
V_1	2.12	2.67	3.77	2.85	2.54	3.42	3.86	3.27	2.24	2.88	3.43	2.85
V_2	2.16	2.42	3.64	2.74	2.69	3.59	3.71	3.33	2.01	2.65	3.13	2.59
Mean	2.13	2.52	3.76		2.66	3.50	3.92		2.28	2.91	3.42	

Effect of arsenic-contaminated irrigation water and zinc on the accumulation of arsenic in soils in relation to rice

The results (Table 3) show that the amount of arsenic built up in soil until 75 days of submergence (3.98 mg/kg) significantly increased in the treatment where zinc was not applied. With regards to irrigation water management practices, it was reported that the highest amount of arsenic in soil at 75 days of rice growth (3.64 mg/kg) was found in I_0 treatment followed by I_1 (3.58 mg/kg) and I_2 (2.75 mg/kg), irrespective of zinc applications. This arsenic accumulation in soil was however further counteracted by application of $ZnSO_4$ at the dose of 25 kg/ha as evidenced from the interaction effects between Zn and methods of applying irrigation water. Considering the interaction effect between Zn and methods of irrigation, the lowest amount of arsenic in soil (1.98 mg/kg) was recorded in Zn_1I_1 treatment combination where $ZnSO_4$ was applied at the rate of 25 kg/ha followed by intermittent ponding up to 75 days of crop growth.

The highest grain yield of rice (4.98 t/ha) was recorded in Zn_1I_0 treatment, closely followed by 4.83 t/ha in Zn_1I_1 combination. Although the yield slightly decreased (4.19 t/ha) in Zn_1I_2 treatment combination, As deposit in soil was significantly lower and thus, despite the arsenic pollution problems, Zn_1I_1 treatment combination was proved superior because of marginal yield increase and reduction of arsenic concentration both in soil and plants.

The results (Table 4) further show that the accumulation of arsenic in different parts of rice plants varied with treatments in the order:

root > stem > leaf > grain

Such accumulation of arsenic in rice root, stem, leaf and grain was found to decrease significantly with the application of either Zn or management of irrigation water or interaction effect of both treatments; this decrease was significantly lowest in root (7.15 mg/kg), followed by stem (5.14 mg/kg), leaf (5.49 mg/kg) and grain (0.81 mg/kg) with the application of Zn under intermittent ponding throughout the growing period of rice compared to other water management practices. The highest amount of water used in the treatment I_0 (160 cm/ha) followed by I_1 (124 cm/ha) and I_2 (102 cm/ha) for the growth of rice without affecting the yield of rice and without increasing the concentration of arsenic in both soils and its uptake by rice plant. However, the results of the present investigation concluded that the arsenic content in different parts of rice viz. root, straw and grain was moderated with the application of Zn. The arsenic content in root was significantly higher followed by straw, while the amount in grain was the lowest. The upper toxic limit of arsenic in soils for rice is 10 mg/kg; beyond that normal growth of rice was not recorded.

Effects of graded doses of arsenic exposure to rice

The results (Table 5) show that arsenic level of 30 mg/kg affected the growth, namely height and tiller numbers, and caused acute chlorotic symptoms with chaffy grains etc.; plants on soils supplied

Table 3. Effect of arsenic-contaminated irrigation water and Zn fertilizer on the arsenic content in soil after 75 days of crop growth and yield of rice (*Oryza sativa* L.)

Treatments	Arsenic content (mg/kg) in soil after 75 days of growth			Grain yield (t/ha)		
	Zn_0	Zn_1	mean	Zn_0	Zn_1	mean
I_0	4.48*	2.81*	3.64*	4.01	4.84	4.42*
I_1	3.95*	3.21	3.58*	3.80	4.83	4.31*
I_2	3.53*	1.98*	2.75*	3.65	4.19	3.92*
Mean	3.98*	2.66*		3.82*	4.62*	
CD (P = 0.05)						
I		0.66			0.29	
Zn		0.82			0.42	
I × Zn		1.42			0.73	

*significant at 5% level

Table 4. Effect of arsenic-contaminated irrigation water and Zn fertilizer on the arsenic content in rice (*Oryza sativa* L.)

Treatments	Arsenic content in rice (mg/kg)											
	root			stem			leaf			grain		
	Zn_0	Zn_1	mean	Zn_0	Zn_1	mean	Zn_0	Zn_1	mean	Zn_0	Zn_1	mean
I_0	8.93*	8.87*	8.90*	6.97*	6.87*	6.92*	6.98*	6.86*	6.92*	1.03	0.94	0.98
I_1	8.31*	8.01*	8.16*	6.19*	5.98*	6.08*	6.24*	6.10*	6.17*	0.98	0.88	0.93
I_2	7.71*	7.15*	7.43*	6.35*	5.14*	5.24*	5.78*	5.49*	5.63*	0.96	0.81	0.88
Mean	8.31*	8.01*		6.17	5.99		6.33	6.15		0.99	0.87	
CD (P = 0.05)												
I		0.29			0.37			0.35			0.29	
Zn		0.19			0.28			0.31			0.23	
I × Zn		0.37			0.48			0.53			0.39	

*significant at 5% level

with 50, 70, 90, 120, 150, 200 and 300 mg/kg of arsenic showed visual symptoms of drying up from the tip portion of older leaves after exhibiting initial symptoms of leaf sheath and then the whole plants gradually dried up at harvest time. However, the rice plants supplied with 10 mg/kg arsenic showed no remarkable changes in terms of growth, height and tiller numbers. The residual amount of arsenic in soil increased compared to arsenic uptake by rice plant indicating lower mobility of arsenic within the plant. The results suggest that soils containing 10 mg/kg arsenic do not affect normal growth of rice plants; hence, 10 mg/kg arsenic content in soil might be considered as upper toxic limit for rice. The transfer coefficient of arsenic in soil was calculated based on the arsenic concentration in the aboveground parts of rice plants divided by the residual arsenic concentration in the soil. The obtained transfer coefficient of arsenic varied from 0.08 to 0.29 at 0 and 300 mg/kg arsenic application, respectively. The transfer coefficient of arsenic in soil was 0.12 in the case of 10 mg/kg arsenic applied into soil, which exhibited almost normal growth of the rice plant. Moreover, the results of our investigation proved that the amount of arsenic decreased with the application of zinc, this decrease was more significant with 10 mg/kg Zn compared to 20 mg/kg Zn. The arsenic content in different parts of rice viz. root, straw and grain was found to be moderated with the application of Zn as well; it was significantly higher in roots followed by straw and the lowest in grain. It can be concluded that the upper toxic limit of arsenic in soils for rice might be 10 mg/kg.

The arsenic contamination in soils may be reduced by applying sulphates of zinc, iron and aluminium to the soil (Brady 1974, Garai et al. 2000). Craw and Chappell (2000) explained that such decrease in the concentration of arsenic due to Zn application might be caused by the precipitation/fixation of arsenic as Zn-arsenate unavailable to plants. The following mechanism may be responsible for it:

$$\text{arsenate} + ZnSO_4 \rightarrow \text{Zn-arsenate}$$
$$\text{(insoluble and retained in the soil)}$$

Bhattacharya et al. (1997, 2004) and Heikens et al. (2007) studied the relation with the secondary Fe, Al and Mn phase and concluded that the surface reactivity of Fe and Al phases play an important

Table 5. Effect of graded levels of arsenic application on its uptake (mg/kg) by rice and residual arsenic content (mg/kg) in soil

Parameters	Application of graded levels of arsenic (mg/kg)									
	0	10	30	50	70	90	120	150	200	300
Arsenic uptake	0.33	3.25	7.89	9.98	12.67	15.73	20.19	28.37	49.33	60.14
Residual arsenic	0.12	1.93	5.89	12.13	18.19	25.28	35.16	44.93	60.57	93.87

role in adsorbing the bulk of As in the sedimentary aquifers in the Bengal basin; this mechanism may be applied also for the present study since it was carried out under submerged conditions. A consistent increase in the concentration of arsenic due to submergence might result from varying intensity of reduction of arsenate to arsenite. Retention or mobility of As under varying redox conditions is based on the interaction of the aqueous phase with different mineral phases in the soil sediments (Bhattacharya et al. 2002, Roberts et al. 2007).

The application of organic matter to the soil very frequently interacts with different inorganic pollutants including applied arsenic rendering the latter unavailable to plants. Mukhopadhyay et al. (2002) showed that extractable arsenic from soil was higher at longer incubation period than at shorter period, and also in presence of added arsenic. They also reported that the interaction effect between applied organic matter and arsenic helps to moderate the concentration of arsenic, which might be related to binding of arsenic to the soil matrix. Thanabalasingam and Pickering (1986) reported that the humic acids help for the adsorption of As (III) and As (V) where the humic acid acts as an anion exchanger in which the basic amino functional groups seem to be involved in retaining the acidic groups. Lund and Fobian (1991) reported arsenic to be retained by soils containing high amount of organic matter. Our study confirms these findings. The accumulation of arsenic in the edible parts of most plants is usually low (O'Neill 1995). However, the uptake of arsenic by plants occurs primarily through the root system and the highest arsenic concentrations were recorded in plant roots and tubers (Marin et al. 1993). Sheppard (1992) reported that the type of soil is the only significant variable when considering the phytotoxicity of arsenic. He also reported that inorganic arsenic was five times more toxic to plants in sandy soils (40 mg/kg) than in clayey soils containing 200 mg As/kg. The phytotoxicity of arsenic is expected to be greater in sandy soils than in other types of soil, since sandy soil usually contain low amount of Fe and Al oxides and clays. Irrigation water, soils and plants from adjacent rice and wheat fields were analysed for arsenic and other elements and showed that rice and wheat grains were not contaminated by arsenic, but concentrations in rice roots exceeded 160 mg/kg due to an Fe-rich plaque around rice roots (Das et al. 2005, Norra et al. 2005).

Meharg and his co-workers recently carried out a series of experiments to elucidate As uptake mechanisms by rice plants (Abedin et al. 2002, Meharg and Jardine 2003) and were the first to show that arsenite is actively translocated across plant plasma membranes. However, for paddy rice, one of the most common aquatic crop plants, iron plaque is commonly formed on root surfaces and may subsequently affect As dynamics in the rhizosphere and As accumulation by rice plants (Liu et al. 2004).

A continuous application of arsenic-loaded irrigation water to the soil ultimately leads to an increase of soil arsenic level, which might be reduced with the application of $ZnSO_4$ at the rate of 25 kg/ha (Garai et al. 2000). The highest accumulation of arsenic was recorded in roots compared to stems, leaves and grains; it might be caused by As mobility within the plant, which would support the results reported by Liu et al. (1985) who showed the distribution of arsenic in plants in the descending order (root > stem > leaf > edible part). Das et al. (2005) reported that the accumulation of arsenic in rice root, stem, leaves and grains might be significantly decreased with the application of Zn, by management of irrigation water, or both. The values recorded in individual plant parts with the application of Zn under intermittent ponding throughout the growth period of rice compared to other water management practices were as follows: roots (7.15 mg/kg), stem (5.14 mg/kg), leaf (5.49 mg/kg) and grain (0.81 mg/kg).

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