Science of the Total Environment xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Temporal variations in arsenic uptake by rice plants in Bangladesh: The role of iron plaque in paddy fields irrigated with groundwater

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ARTICLE INFO

Article history:
Received 22 October 2009
Received in revised form 2 May 2010
Accepted 14 May 2010
Available online xxxx

Keywords:
Arsenic
Rice (*Oryza sativa*)
Paddy soil
Soil water
Iron plaque

ABSTRACT

The transfer of arsenic to rice grains is a human health issue of growing relevance in regions of southern Asia where shallow groundwater used for irrigation of paddy fields is elevated in As. In the present study, As and Fe concentrations in soil water and in the roots of rice plants, primarily the Fe plaque surrounding the roots, were monitored during the 4-month growing season at two sites irrigated with groundwater containing ~130 $\mu g l^{-1}$ As and two control sites irrigated with water containing <15 $\mu g l^{-1}$ As. At both sites irrigated with contaminated water, As concentrations in soil water increased from <10 $\mu g l^{-1}$ to >1000 $\mu g l^{-1}$ during the first five weeks of the growth season and then gradually declined to <10 $\mu g l^{-1}$ during the last five weeks. At the two control sites, concentrations of As in soil water never exceeded 40 $\mu g l^{-1}$. At both contaminated sites, the As content of roots and Fe plaque rose to 1000–1500 $mg kg^{-1}$ towards the middle of the growth season. It then declined to ~300 $mg kg^{-1}$ towards the end, a level still well above As concentration of ~100 $mg kg^{-1}$ in roots and plaque measured throughout the growing season at the two control sites. These time series, combined with simple mass balance considerations, demonstrate that the formation of Fe plaque on the roots of rice plants by micro-aeration significantly limits the uptake of As by rice plants grown in paddy fields. Large variations in the As and Fe content of plant stems at two of the sites irrigated with contaminated water and one of the control sites were also recorded. The origin of these variations, particularly during the last month of the growth season, needs to be better understood because they are likely to influence the uptake of As in rice grains.

1. Introduction

Drinking groundwater supplied by millions of shallow tubewells containing 100–1000 $\mu g l^{-1}$ As is the main origin of chronic exposure to arsenic across the Bengal Basin (Ahmed et al., 2006). As more of the rural population in the region switches to drinking water with <50 $\mu g l^{-1}$ As, rice grown on paddies irrigated with As-contaminated groundwater and containing 0.2–0.4 $mg kg^{-1}$ As could become a comparable source of exposure (Duxbury et al., 2003; Meharg and Rahman, 2003; Williams et al., 2005; Smith et al., 2006; van Geen et al., 2006; Zavala and Duxbury, 2008). Rice could already be the main dietary source of As where drinking water contains <10 $\mu g l^{-1}$ As or rice grains contain >0.4 $mg kg^{-1}$ As because of the large quantities of rice consumed in the region (~500 g/day for adults). Changes in agricultural practices, such as aerobic cultivation (Duxbury and Panaullah, 2007; Xu et al., 2008) or breeding (Meharg and Hartley-Whitaker, 2002) currently explored to lower the As content of rice grown in the region are therefore relevant to human health today and will become increasingly so in the future.

The transfer of As from irrigation water to rice grains involves several steps. The first step, input of As with irrigation water, is difficult to quantify because of a drastic decline of As concentrations in standing water across flooded fields from initial levels as high as 800 $\mu g l^{-1}$ at the irrigation well (Norra et al., 2005; Dittmar et al., 2007; Panaullah et al., 2008). The loss is caused by co-precipitation and/or adsorption of As onto flocs of Fe oxyhydroxide formed upon oxidation of dissolved Fe(II) contained in irrigation water (Roberts et al., 2007). The consequence is that As concentrations in surficial soil can be as high a 70 $mg kg^{-1}$ near the entry point of irrigation water to

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doi:10.1016/j.scitotenv.2010.05.019

a rice field to then decline to essentially background levels <10 mg kg^{-1} As over a distance of a few hundred meters (Dittmar et al., 2007; Panaullah et al., 2008). Arsenic mobilized from the soil during the monsoon contributes to the highly scattered relationship between As levels in irrigation water and surficial soil (Ali et al., 2003; Meharg and Rahman, 2003; Dittmar et al., 2007; Saha and Ali, 2007; Roberts et al., 2010).

Relatively little is known about the next transfer step which is the release of As from soil to soil water when rice paddies are flooded. At an experimental paddy field in Japan irrigated with river water containing little As, Takahashi et al. (2004) observed an increase in soil-water As concentrations from <0.5 μg l^{-1} to ~2 μg l^{-1} at a depth of 20 cm in conjunction with an increase in dissolved Fe from <0.1 to 2 mg l^{-1} during the wet season. Much higher soil-water As concentrations ranging from 150 to 1500 μg l^{-1} over the upper 5 cm of flooded paddy soil were first reported from Bangladesh for a dozen fields irrigated with groundwater containing 80–200 μg l^{-1} As (van Geen et al., 2006). Soil-water As concentrations for a paddy field in the same area of Bangladesh irrigated with groundwater containing <5 μg l^{-1} As did not exceed 30 μg l^{-1}, even in the presence of >1 mg l^{-1} dissolved Fe. Van Geen et al. (2006) and Roberts et al. (2010) showed that soil-water As concentrations are also elevated during the wet season when surface water that is low in As is used for irrigation in those fields irrigated with high-As groundwater during the dry season. A time series of soil-water As in rice paddies of Bangladesh over the entire growing season was first reported by Panaullah et al. (2008). Maximum soil-water As concentrations at a depth of 10 cm were reached ~90 days after transplanting and ranged widely from <100 to 2500 μg l^{-1} as a function of the soil As content (10 to 70 mg kg^{-1}). The few studies of As in soil water conducted to date in Bangladesh therefore indicate that soil processes significantly amplify the As content of irrigation water during at least part of the year, most likely because of the cumulative effect of irrigation during previous years.

The speciation of As in soil water of flooded paddies has been measured in Bangladesh by voltammetry (van Geen et al., 2006), hydride-generation flame atomic-absorption (Panaullah et al., 2008), and ion-exchange separation (Roberts et al., 2010). All three types of measurements indicate a predominance of reduced As(III) in soil water and possibly the presence of As(V), although there is some uncertainty due to analytical and sample preservation issues. The speciation of As in soil water is an important consideration because As(V) uptake by the roots of rice plant occurs via transporters for the nutrient P(V) whereas As(III) shares the same protein transporters as neutral water, urea, and silicic acid molecules (Meharg and Hartley-Whitaker, 2002; Ma et al., 2008). Evidence for speciation-dependent pathways includes suppression of As(V) uptake by plants under aerobic and P-sufficient conditions (Meharg and Macnair, 1992) and the lack of an observable effect of P additions on As(III) uptake in greenhouse studies of flooded rice grown in pots (Abedin et al., 2002).

A sizable and relatively labile reservoir of As in flooded soils is the Fe plaque formed on the roots of aquatic plants by micro-aeration and oxidation of Fe(II) contained in soil water (Otte et al., 1995; Hansel et al., 2002; Norra et al., 2005; Voegelin et al., 2007). Interactions between soil water and Fe plaque could potentially affect both the concentration and the redox state of As in soil water, and therefore also the uptake of As by rice plants. Study of the effect of Fe plaque on the uptake of As by rice plants has been limited largely to hydroponic or greenhouse studies in pots rather than plant material collected from actual paddy fields (Liu et al., 2004a,b; Chen et al., 2005; Xu et al., 2008; Bravin et al., 2008).

A unique feature of the present study is that soil water and rice plant material, including roots and their Fe plaque, were collected repeatedly over the course of an entire growing season from several paddy fields cultivated by farmers in Bangladesh. These fields were irrigated with water spanning a wide range of As concentrations. Our observations differ in significant ways from previous studies that have relied primarily on potted plants grown in a greenhouse. To our knowledge for the first time, we report marked variations in the As content of the roots and stems of rice plants indicating that the last month of the growing season may be particularly important for As translocation to the rice grain. Further study is needed to better understand the underlying processes and contribute to on-going efforts to modify agricultural practices in a way that reduces the uptake of As by rice grains.

2. Methods

Two paddy fields irrigated with groundwater elevated in As (A1, A2) and two control sites (C1, C2) irrigated with low-As water from a deep well and a pond, respectively, were studied in Araihazar upazila, 30 km northeast of Dhaka. Although this could not be confirmed independently, we assume that the history of cultivation and source of winter irrigation water at each of the sites has not changed within the past of few years. We also have no reason to believe that the quantity of fertilizers used (Tri Super Phosphate, KCl, urea, and Zn) differed drastically between sites. In all cases, the paddy field was the first of a sequence of fields irrigated with groundwater from the same source. Soil and rice plants were sampled within a few meters of the inlet of groundwater to the field from January to April, i.e. during the dry season as opposed to the wet season for growing rice which lasts from May to November.

2.1. Field measurements

In situ variations of redox conditions in the first 20 centimetres of the soil at site A1 were recorded in 2006 between days 19 and 91 of the growth season using eight Pt microelectrodes that remained in position throughout the growth season and a Ag/AgCl electrode planted in the soil before taking a sequence of readings. The Pt microelectrodes were embedded within paddy soil and placed horizontally within the cavities of a Teflon plat that was inserted vertically into the soil between two rows of rice plants. Readings were recorded after 3 min of equilibration; redox potentials are expressed relative to a H_2 reference electrode by adding 198.7 mV to the measured values.

2.2. Sampling

Irrigation water was collected at the outlet of the pump and acidified to 1% high purity HCl (Optima) after filtration through a 0.2 μm syringe filter. Soil cores 14–16 cm in length were collected between rows with a stack of 2-cm wide stainless steel rings held together with electrical tape (van Geen et al., 2006). Between 2 and 10 ml of soil water was extracted from the contents of each ring with a stainless steel sediment squeezer (Manheim, 1966) placed in a large clamp. Extraction of pore water by squeezing is a method that is widely used in studies of marine sediments. Soil water coming out of the device was filtered directly through a 0.2 μm syringe filter into scintillation vials with a Poly-Seal cap, and subsequently acidified to 1% high purity HCl (Optima).

Soil-water profiles were obtained 7 to 13 times over the duration of the growth season at sites A1 and C1 in 2005 and at sites A1, A2, and C2 in 2006. One rice plant (*Oryza sativa* L. boro varieties BRRI dhan 28 and 29; specific varieties could not be linked to the different sites) including its roots was collected 3 to 8 times from each of the monitored rice paddies. The timing of soil water and plant sampling is indicated hereon by the number of days elapsed after a particular field was first flooded, which precedes the time seedlings were transplanted by a few days at most. The rice grain analyses reported here are for grains collected from each site near the entry point of irrigation water just before harvesting.

2.3. Analysis of soil water and irrigation water

Irrigation water and soil water collected in 2005 was analyzed for dissolved As, Fe, and P by high-resolution inductively coupled plasma mass-spectrometry (HR ICP-MS) on a VG Axiom magnetic sector

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instrument (Cheng et al., 2004). This method eliminates isobaric interference from ArCl and corrects for potential matrix effects on nebulization efficiency by normalizing counts to that of a Ge spike added at a concentration several orders of magnitude higher than in samples. Irrigation water and soil water collected in 2006 was instead analyzed for As and Fe (and not P) using an Elan DRC II quadrupole ICP-MS (Perkin Elmer). Potential isobaric interference by ArCl was evaluated and determined not to be significant for As. The analytical detection limit of both methods for dissolved As is ~0.1 μg l^{-1} and the precision on the order of 2% for both As and Fe.

Concentrations of As(III) in irrigation water and soil-water samples collected during the first 25 days of the growing season in 2006 at sites A1 and A2 irrigated with contaminated water were analyzed on the day of collection by differential pulse cathodic stripping voltammetry (He et al., 2004). This method has a detection limit of 0.1 μg l^{-1} and a typical reproducibility of 10%. These determinations were combined with total dissolved As measurements by ICP-MS to infer the speciation of As.

2.4. Processing and analysis of roots

Soil was detached from the roots of the rice plants by gently agitating them in several ~10 l volumes of well water, until the water remained clear. The total amount of As contained in the well water that was used is several order of magnitudes lower than in the plant material and therefore should not have affected the measurements. Root samples were air-dried locally, then homogenized and dried at 70 °C before digestion in the laboratory. The concentration of As an Fe in roots and Fe plaque combined was determined after digestion for 5 min in concentrated HNO_3/HCl at 180 °C followed by an additional 10 min in a microwave oven (Ethos Touch Control, Milestone). Root digests were analyzed for As and Fe by inductively coupled plasma atomic emission spectrometry (ICP-AES, Ultima C, Jobin Yvon Horiba). The estimated detection limit and precision for a typical sample of ground root material were 1 mg kg^{-1} and 10%, respectively. To determine reproducibility, these measurements were repeated 2–3 times on certain root sections from the same plants collected at sites A1 and A2 in 2006.

Separation of As contained in Fe plaque from As contained within the bare roots was also attempted at sites A1 and A2 using a combination of dithionite, citrate, and bicarbonate (DCB; Taylor and Crowder, 1983). We report the results of ICP-AES analysis of these digests as well as the residual As and Fe content of roots and plaque released by a final microwave digestion in concentrated HNO_3/HCl following a second DCB extraction.

2.5. Analysis of rice grains

Rice grains collected from a plant from the three sites irrigated with contaminated water and the two control sites, without their husk, were dried overnight in an oven at 70 °C. Ten to twelve rice grains were weighed and digested using a HNO_3/H_2O_2 procedure (van Geen et al., 2006). A reagent blank and an amount of rice flour containing 0.29 ± 0.03 mg kg^{-1} As (Standard Reference Material 1568a from the National Institute of Standards and Technology) equivalent to that of ten to twelve grains were included in the analysis by HR ICP-MS.

3. Results

3.1. Groundwater and soil characteristics

The average As content of irrigation water at sites A1 and A2 of ~130 μg l^{-1} is an order of magnitude higher than at the two control sites C1 and C2 (Table 1). The Fe (3–4 mg l^{-1}) and P (~0.8 mg l^{-1}) content of irrigation water was also considerably higher at the contaminated sites compared to the control sites, at least initially. Between 2004 (before the present study started) and 2005, however, Fe and P levels in the irrigation water at C1 increased significantly, accompanied by a more modest increase in As (Table 1). By 2006, the irrigation well at C1 had been abandoned. We speculate that the change in groundwater composition between 2004 and 2005 was due to cracks in the PVC pipe used to construct the irrigation well. This could cause pumping of irrigation water from a different depth or several depths in 2005–2006 containing higher As and Fe concentrations until the well collapsed altogether and could no longer be used.

Table 1
Groundwater and soil characteristics of the five study sites.

Site	A1	A2	C1	C2
Irrigation water (*n*)	16	3	3	1
Year of sampling	2005–2006	2006	2005	2009
As (μg l^{-1})	129 ± 32	136 ± 5	12 ± 3[a]	3.3
Fe (mg l^{-1})	4.4 ± 1.8	3.2 ± 0.2	2.2 ± 0.2[a]	0.2
P (mg l^{-1})	0.8 ± 0.4	0.7 ± 0.04	n.a.	0.02
Soil samples (*n*)	3	1	2	1
Year of sampling	2005–2006	2006	2005	2006
Depth range (cm)	0–15	0–15	0–15	0–10
As (mg kg^{-1})	17.0 ± 2	10.4	4.6 ± 2	5
Fe_2O_3 (%)	5.4 ± 0.1	5.6	4.2 ± 01	4.2
SiO_2 (%)	63.5 ± 2	64.1	66.6 ± 0.01	65.6
Al_2O_3 (%)	14.7 ± 0.2	14.6	12.7 ± 0.1	14.1
Organic matter (%)	2.02 ± 0.05	0.76	3.2 ± 0.05	1.46
P. Olsen (mg kg^{-1})	46.8 ± 6	15.2	5.1 ± 0.5	5.5

n.a.: not available.
[a] In 2004, concentrations of As (3 μg l^{-1}), Fe (0.1 mg l^{-1}), and P (0.04 mg l^{-1}) in groundwater used to irrigate control site C1 were considerably lower than in 2005 (van Geen et al., 2006).

The As content of paddy soil between 0 and 15 cm depth at the sites irrigated with contaminated water is also higher than at the control sites, even if the difference is not as pronounced (10–17 mg kg^{-1} vs. ~5 mg kg^{-1}). With the exception of the Olsen P content of paddy soil at A1 and A2 which is 3–9 times higher than at C1 and C2, the available data show no systematic differences in the basic characteristics of the different sites. Whereas we consider the data collected from all sites averaged over depth in subsequent figures, detailed time series of variations in soil-water profiles are shown in detail only for site A1 in 2006 and site C1 for which the depth and temporal resolution are the highest. The sequence of soil-water profiles for A1 in 2005 and A2 was similar to that of A1 in 2006 (Fig. S1).

3.2. Evolution of Eh in soil water

The Pt electrode data are used here merely as a qualitative indication of changes in redox conditions in the paddy fields, without any claim to a thermodynamic interpretation. Eh data were not collected before the onset irrigation. We therefore use as reference the profile collected on day 91 at the end of the growth season, when surface soils were almost dry and probably aerated. The profile collected 19 days after the onset of irrigation indicates Eh readings that are already significantly lower than at the end of the growth season (Fig. 1a and c). By day 23, lower Eh values indicative of reducing conditions were reached within most of the upper 20 cm of paddy soil and maintained through day 63. The only exception is the 7 cm depth interval where less reducing conditions were apparently maintained through day 31, possibly because of micro-aeration by plant roots at that depth. From days 38 through 63, Eh readings remained uniformly low (Fig. 1b). Starting on day 77, Eh rose first in the shallower intervals in response to decreased flooding. Eh readings then increased to uniformly high values, even in the deepest 20 cm interval, between days 81 and 91.

3.3. As and Fe in soil water

At the beginning and at the very end of the growth season approximately 100 days later, concentrations of Fe in soil water were

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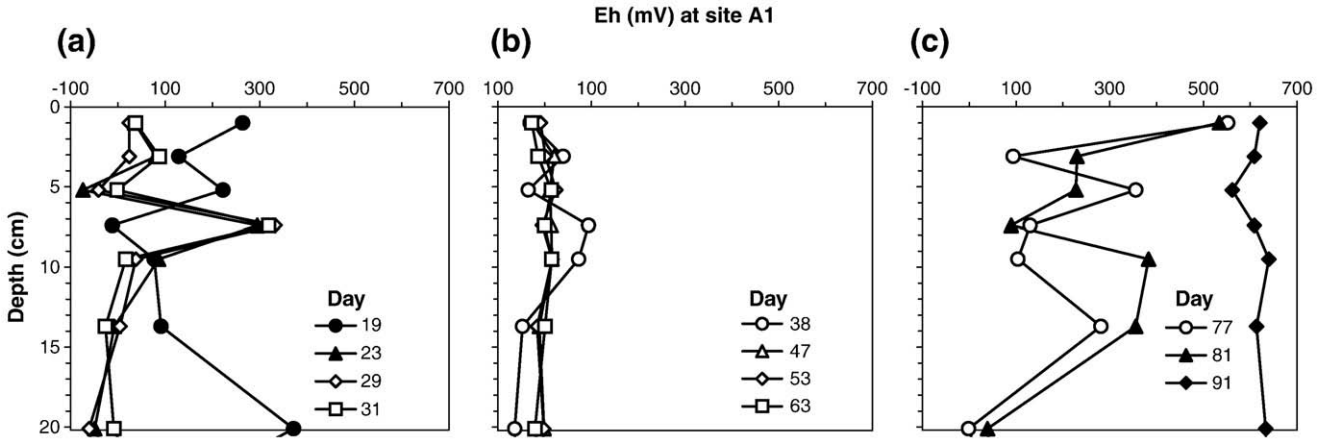

Fig. 1. Temporal evolution of the soil Eh profile measured at site A1 during the winter 2006 growing season. The subset of profiles selected for display corresponds within a few days to when a core was collected from the same paddy for soil-water extraction (Fig. 2). For clarity, the times series is divided in three panels corresponding to days (a) 19–38, (b) 38–63, and (c) 77–91. The electrodes appeared to be insufficiently equilibrated with the soil environment before day 19.

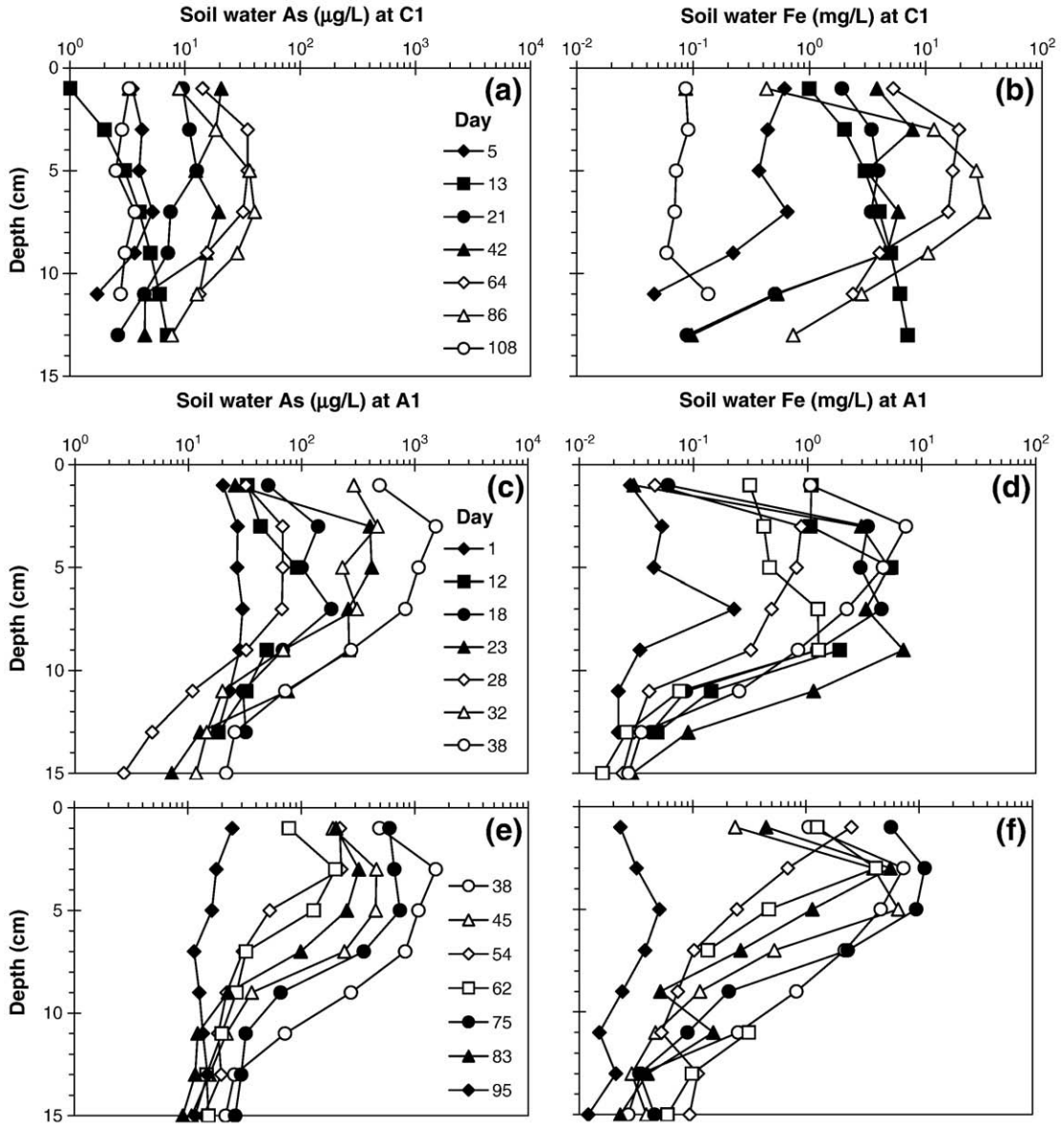

Fig. 2. Vertical profiles of As and Fe concentrations in soil water obtained over the course of a growing season cycle at control site C1 (a–b) during winter 2005 and at contaminated site A1 during winter 2006 divided in two panels corresponding to days (a, d) 1–38 and days (e, f) 38–95. Note that concentrations are shown on a log scale.

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<0.1 mg l^{-1} at both site A1 irrigated with contaminated water and control site C1 (Fig. 2b, d and f). During the intervening period, soil-water Fe concentrations generally exceeded 1 mg l^{-1} between 1 and 9 cm depth and occasionally rose to 10 mg l^{-1}. Below 9 cm depth, soil-water Fe levels rarely exceeded 1 mg l^{-1} throughout the growth season. Within a general pattern of Fe release to soil water during the first half of the growth season followed by loss from soil water during the second, the data show considerable variability over time and from site to site, even after the Fe content of soil water is averaged over the upper 9 cm (Fig. 3c).

Unlike Fe, there is a striking contrast between As concentrations reached in soil water at the sites irrigated with contaminated water and the control sites. The highest levels reached in several depth intervals containing >10 mg l^{-1} Fe on days 64 and 86 at control site C1 remained <40 μg l^{-1} As (Fig. 2a). At A1 instead, soil-water As concentrations in the upper 9 cm typically exceeded 100 μg l^{-1} and occasionally 1000 μg l^{-1} between days 18 and 83 (Fig. 2c and e). Similarly to Fe, the release of As to soil water below 9 cm depth was muted and concentrations rarely exceeded 100 μg l^{-1}. With a few exceptions, the average As content of soil water in the upper 9 cm at sites A1 and A2 averaged approximately around 500 μg l^{-1} between day 30 and day 80 of the growth season (Fig. 3a). This is the depth range where the highest abundance of plant roots was maintained throughout the growth season, even if an occasional root would extend to greater depth.

The speciation data obtained in 2006 indicates that 85 ± 13% (n = 20) of total As in soil water in the 50–300 μg l^{-1} range was present in the reduced As(III) form (Fig. S2). The proportion of As(III) was lower and more variable at As in soil-water concentrations <50 μg l^{-1} (60 ± 27%; n = 25).

3.4. Variations in the As and Fe content of roots and Fe plaque

The composition of roots and Fe plaque determined by microwave digestion evolved more gradually than As and Fe concentrations in soil water at all sites. The Fe content of root and plaque rose from an initial ~10 g kg^{-1} to a fairly steady level of 50 g kg^{-1} around day 80 of the growth season at contaminated site A1 and control site C1 (Fig. 3d). Data from the other contaminated and control sites (A2 and C2) indicate a higher initial Fe content of root and plaque followed by a steady decline between days 40 and 100. Comparison with measurements targeting Fe oxyhydroxides indicates that at least 50%, and typically more, of the Fe contained in the roots and plaque combined was liberated by the DCB extraction at A1 and A2 (Fig. 4c and d). The extraction data also show that little residual Fe remained in the roots after a second DCB extraction.

Concentrations of As in combined root and plaque measured by microwave digestion at the two sites irrigated with contaminated water ranged from 50 to 350 mg kg^{-1} during the first two weeks of the growth season to then gradually (with the exception of one outlier) reach a plateau of ~800 mg kg^{-1} between days 70 and 80 (Fig. 3b). At the end of this period, the As content of root and plaque at the sites irrigated with contaminated water returned precipitously to lower levels that are only a factor of two higher than the average As contents of roots and plaque at the two control sites. Similarly to Fe, in the case of Fe, DCB extractions liberated at last half, and typically most, of the As contained in roots and plaque combined and very little As remained in the roots after two DCB extractions (Fig. 4a and b). The implication is that most of the Fe and As liberated by microwave digestion reflects the composition of the Fe plaque rather than the contents of the root itself.

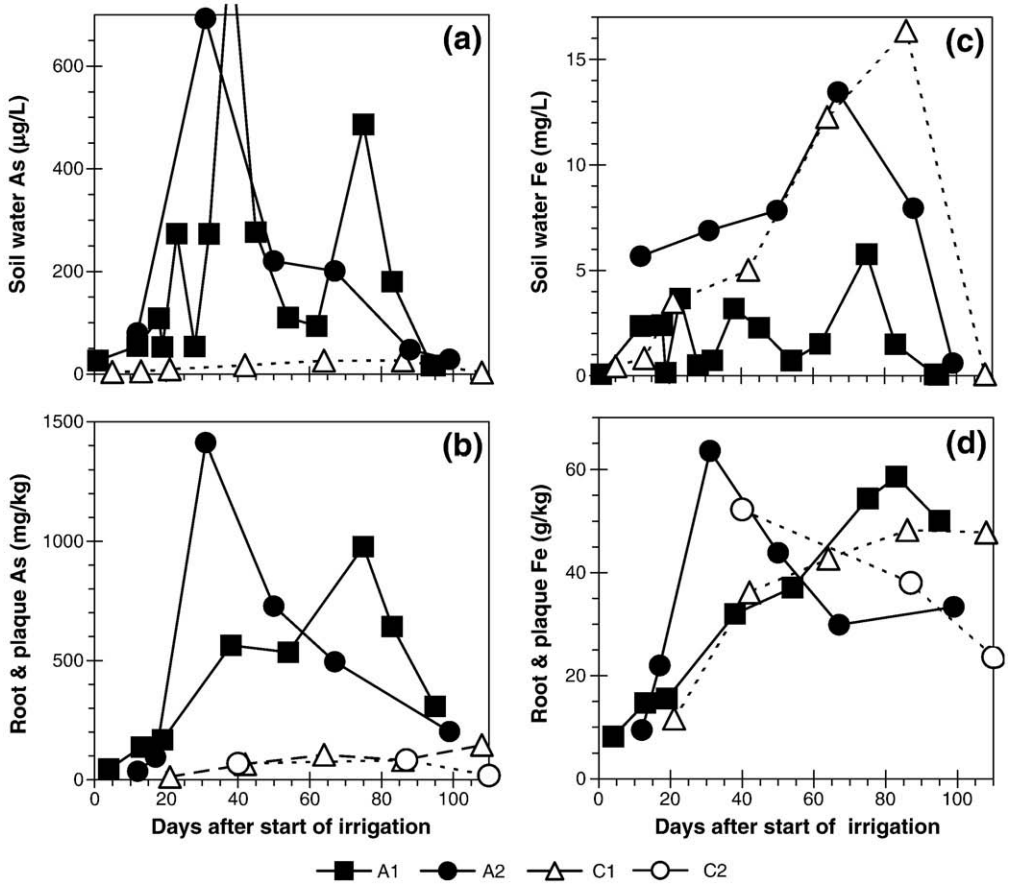

Fig. 3. Variations in the (a) As and (c) Fe content of soil water averaged over the upper 9 cm of the paddy. Also shown are variations in average (b) As and (d) Fe content of combined root and plaque determined by microwave digestion. Filled symbols corresponded to contaminated sites and open symbols to control sites.

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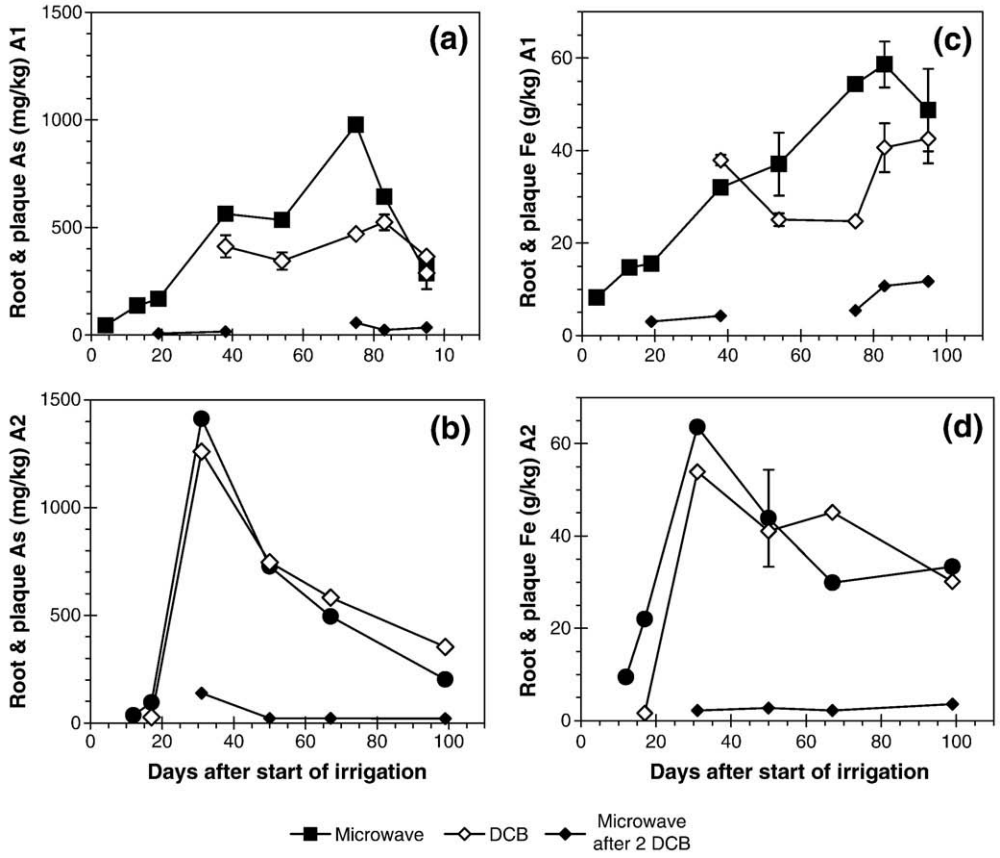

Fig. 4. Variations in the As and Fe content root and plaque over the course of the growth season determined by total microwave digestion and DCB extraction. Error bars indicate the standard deviation of 2–3 measurements on different root sections from the same plant on days 38, 54, 83, and 95 at site A1 '06 and days 50 and 67 at site A2. In some cases, the error bars are smaller than the size of the symbol. Two duplicate measurements on roots obtained from two different plants are combined on day 95 at site A1 '06. Also shown are single measurements of the residual As and Fe content of roots after two sequential DCB extractions.

3.5. Concentrations of As and Fe in plant stems

The limited data available from sites A1 and A2 irrigated with contaminated water indicate significant variations in the Fe and As composition of plant stems over the course of the growth season (Table 2). The concentration of Fe in stems gradually declined at both sites from ~1.5 g kg^{-1} after day 20 to <0.3 g kg^{-1} around on day 70. In parallel, the As content of stems declined over the same period at site A1 from 12 mg kg^{-1} on day 13 to a minimum of 3.7 g kg^{-1} on day 75, and at site A2 from 17 mg kg^{-1} on 12 to a minimum of 4 mg kg^{-1} on day 67. The As content of stems notably rose back to 5–10 mg kg^{-1} at sites A1 and A2 during the last month of the growth season. Plant stems were analyzed only on days 40 and 120 at control site C2 and contained low levels of Fe (0.2–0.4 g kg^{-1}) and As (0.4–0.5 mg kg^{-1}) (Table 2).

Table 2
Variations in the As and Fe content of rice plant stems collected over the course of the 2006 growing season from three paddies in Araihazar, Bangladesh.

Site A1			Site A2			Site C2		
Day	As (mg kg^{-1})	Fe (g kg^{-1})	Day	As (mg kg^{-1})	Fe (g kg^{-1})	Day	As (mg kg^{-1})	Fe (g kg^{-1})
13	12.13	0.59	12	3.24	2.25	40	0.58	0.44
19	8.80	1.71	17	16.74	1.48	110	0.41	0.19
38	5.22	1.42	31	18.29	0.87			
54	1.09	0.78	50	7.64	0.35			
75	3.68	0.24	67	4.01	0.28			
82	5.42	1.22	99	9.80	0.24			

3.6. Concentrations of As in rice grains

The concentration of As measured in SRM 1568a rice flour was 0.26 mg $kg^{-1} \pm 0.02$ ($n = 5$), which is slightly lower but not inconsistent with the certified value (Table 3). Rice grains analyzed without their husk from the control sites C1 and C2 contained less As than grains from the sites irrigated with contaminated water (Table 3), but by no more than a factor of three when averaging all measurements from the control site (0.14 ± 0.03 mg kg^{-1} $n = 3$) and sites irrigated with contaminated water (0.38 ± 0.04 mg kg^{-1}, $n = 6$).

4. Discussion

The data from paddy fields cultivated by local farmers in Araihazar confirms that irrigation with groundwater containing >100 μg l^{-1} As dramatically impacts rice paddies at several levels compared to control sites irrigated with low-As groundwater or surface water. The underlying assumption, confirmed by discussions with local villagers

Table 3
As content of rice flour and rice grains.

Sample	As (mg kg^{-1})	N
SRM1568a certified	0.29 ± 0.03	–
SRM1568a measured	0.26 ± 0.02	5
Site A1	0.27 ± 0.03	2
Site A2	0.58 ± 0.05	2
Site C1	0.15 ± 0.03	2
Site C2	0.12	1

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or farmers, is that the source of irrigation water sampled in 2005–2006 was representative of the irrigation history of the sites over at least the past several years. This is consistent with the elevated soil As concentration at sites irrigated with contaminated water relative to the control sites (Table 1). Soil-water As concentrations averaged within the upper 9 cm reached a maximum around the middle of the growth season of about 500 μg l^{-1} at the two sites irrigated with contaminated water that was ten-fold higher than at the control sites (Fig. 3a). This was accompanied by a roughly proportional increase in the As content of roots and plaque of ~800 mg kg^{-1} relative to the As content of roots and plaque at the control sites of <100 mg kg^{-1} (Fig. 3b). The soil-water data reported here are consistent with the limited number of previous measurements from Bangladesh using three different extraction methods (van Geen et al., 2006; Panaullah et al., 2008; Roberts et al., 2010) and, for reasons that are unclear, more than an order of magnitude higher than soil-water As concentrations recorded during a greenhouse study of rice plants in flooded soil amended with 10 mg kg^{-1} As (Xu et al., 2008).

The range of As concentrations measured in roots and plaque as well as stems from Araihazar at the end of the growth season is comparable to the composition of plant material from impacted fields and control sites across the Bengal Basin (Ali et al., 2003; Norra et al., 2005; Huq et al., 2006; Panaullah et al., 2008). The contribution of the present study is the demonstration that Fe plaque, which clearly dominates the combination of root and plaque, as well as stem As concentrations vary considerably over the course of the growth season, both above and below the As content in stems reached at harvest time. Despite much higher soil-water As concentrations, the As content of stems from Araihazar (1–10 mg kg^{-1}) and elsewhere in Bangladesh (3–13 mg kg^{-1}; Panaullah et al., 2008) is significantly lower than the 13–30 mg kg^{-1} range reported for stems at maturity in the aforementioned greenhouse study of rice plants grown in flooded soils, and this for pots with soil both unamended and amended with As (Xu et al., 2008).

Although the number of rice grain analyses reported here is very limited, the results suggest that the order of magnitude higher As concentrations in soil water at the sites irrigated with contaminated water compared to the control sites did not result in a proportional increase in the As content of rice grains. Panaullah et al. (2008) also reported a limited range of 0.3–0.6 mg kg^{-1} for the As content of rice grown in paddies containing 10–70 mg kg^{-1} As in soil and levels as high as 2500 μg l^{-1} As in soil water. In contrast, rice grains from the greenhouse study by Xu et al. (2008) collected from plants grown in flooded pots, both unamended and amended with As, contained much higher levels of As in rice grains (1–2.5 mg kg^{-1}). For reasons that are presently unclear, the greenhouse study of Xu et al. (2008) underestimates how much As is released to soil water in actual paddy fields while at the same time overestimating the amount of As that is transferred to the rice grain (van Geen and Duxbury, 2009; Zhao et al., 2009).

The pattern of rising As concentrations in both soil water and root plaque observed at sites irrigated with contaminated water in Araihazar during the first half of the growth season (Fig. 3a and b), followed by a gradual decline during the second half, suggests some form of exchange between these two reservoirs. Evaluating the nature of this interaction and its consequence for As uptake by the plant requires an estimate of the relative size of the As and Fe pools in soil, soil water, whole roots, as well as plant stems. On the assumption that 1 dm^3 of soil contains about 1 g of plaque and root biomass (Yang et al., 2004), an average concentration of 800 μg l^{-1} As in soil water within the upper 10 cm of paddy soil, and 800 mg As kg^{-1} in whole roots including plaque, the amount of As stored in roots and plaque combined is about three times higher than the amount of As contained in soil water (Table 4). Concentrations of As in soil water, and to a lesser extent roots and plaque combined, should therefore be quite sensitive to transfer between these two pools.

Table 4
Inventory of As and Fe contained in paddy soil, including plant roots and stems. The range of concentrations of As and Fe in various compartments reflects documented variations over the course of the growth cycle and/or differences between impacted and control sites.

	Soil	Soil water	Whole roots	Plant stem
1 dm^3	2 kg	0.4 l	1 g	5 g
Fe conc.	4%	0.1–10 mg l^{-1}	10–50 mg kg^{-1}	0.2–2 mg kg^{-1}
As conc.	2–20 mg kg^{-1}	10–1500 μg l^{-1}	100–800 mg kg^{-1}	1–20 mg kg^{-1}
Fe pool (mg)	80,000	0.04–4	0.01–0.05	0.001–0.01
As pool (μg)	4000–40,000	4–600	100–800	5–100

Similar calculations show that the size of the pools of As in soil water and whole roots combined is dwarfed by a 10 to 100-fold larger reservoir of As in soil (Table 4). The implication is that the release of a very small fraction of As contained in soil is sufficient to dramatically raise As levels in both soil water and roots and plaque combined. The same reasoning should apply to soil As concentrations at the control sites, and yet concentrations of As in both soil water and combined roots and plaque remain much lower than at the contaminated sites (Fig. 3a and c). This suggests that the pool of As added to soil by irrigation is particularly prone to release by reductive dissolution of Fe oxyhydroxides and is cycled between soil water and plaque during each growth season. Soil-water measurements from Bangladesh obtained during the wet season suggest this probably occurs twice a year (van Geen et al., 2006; Roberts et al., 2010).

Extension of this calculation shows that the pool of As contained in plant stems is about an order of magnitude smaller than the amount of As in soil water and in Fe plaque at the impacted sites as well as at the control sites (Table 4). Intriguingly, it is roughly mid-way through the growing season, when As levels in soil water and in Fe plaque are particularly high, that As concentrations in the stems of rice plants are at a minimum (Table 2). One possible explanation is that Fe plaque is particularly effective in trapping As and inhibiting uptake via roots towards the middle of the growth season while rapid growth of the rice plant (e.g. Ziska et al., 1997) above ground dilutes the As that was taken up during the first few weeks of the growth season. To our knowledge, there is no comparable published time series of plant stem composition from actual paddy fields other than the study of Fageria (2004), which reported a decline in the Fe content of the stems of rice plants during the growth season.

Unfortunately, it is precisely around the time of panicle initiation between days 60 and 70 that As concentrations in the stem of rice plants start to rise again (Table 2). The process underlying this increase is of considerable interest because it could be an important factor controlling the ultimate As content of rice plants and rice grains. Consideration of variations in As/Fe ratios in the various relevant pools provides some additional clues. During the first month of the growth season, the time series indicate a parallel increase in soil-water As/Fe and root and plaque As/Fe (Fig. 5b and c). Then, after Eh values stabilize at uniformly low levels (Figs. 1b and 5a) and until about the time of panicle initiation, As/Fe ratios in roots and plaque are relatively constant (Fig. 5c). In soil water instead, As/Fe ratios actually decrease by about an order of magnitude during this period (Fig. 5b). If the uptake of As by plant roots competes with As incorporation in root plaque, the resulting drop in soil-water As/Fe could be an indication that As concentrations in plant stems decline over this time span because the continuous formation of fresh Fe plaque constitutes an active sink for As.

The decline in As/Fe ratios in combined roots and Fe plaque while As/Fe ratios in soil water start to rise again during the last few weeks of the growth season is probably not a coincidence and deserves further study because it follows panicle initiation (Fig. 5b and c). The data show that whereas both As and Fe concentrations in soil water decline following panicle initiation, the drop in dissolved Fe levels is

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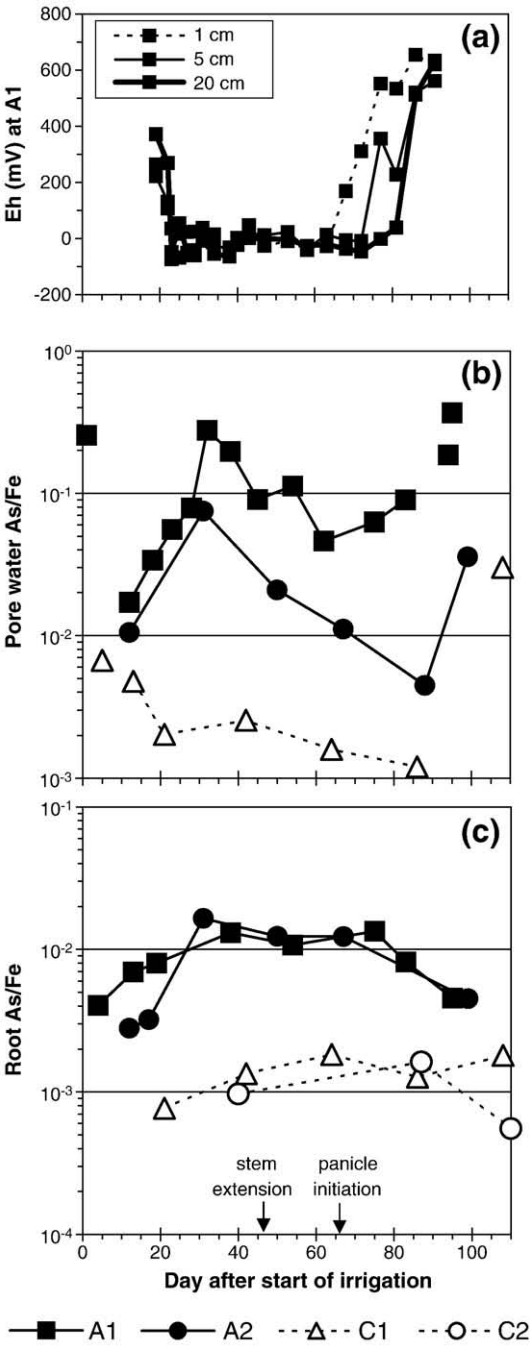

Fig. 5. Temporal variations in the properties of soil water and combined root at contaminated and control sites. (a) Variations in Eh at site A1 for three soil depths during winter 2006 are shown as an indication of the evolution of the redox state of the soil. Molar As/Fe ratios are shown on a log scale for (b) soil water averaged over the upper 9 cm and (c) combined root and plaque determined by microwave digestion. Filled symbols corresponded to contaminated sites and open symbols to control sites. Symbols are not connected with a line for soil-water samples containing <0.5 mg l^{-1} Fe averaged over the upper 9 cm.

more precipitous. The result may be a drastic decline in the rate of fresh Fe plaque formation and therefore diminished As removal from soil water. The rise in stem As during the last month of the growth season suggests this was paired with greater uptake of As from soil water by the rice plant (Table 2).

Why might the continuous formation of fresh Fe plaque be needed to inhibit the uptake of As by rice plants? One possible explanation is based on laboratory studies showing that crystallization of amorphous Fe oxyhydroxides, or just their aging, reduces their capacity for incorporating and adsorbing As(V), the likely redox state of As in Fe plaque (Hansel et al., 2002; Fuller et al., 1993). As soil-water Fe levels decline during the last third of the growth season, the release of As accompanying recrystallization and/or aging may therefore insufficiently be compensated by the formation of fresh Fe plaque. Elevated soil-water P concentrations (data not shown) indicate that competitive effects may also reduce the capacity of Fe plaque to incorporate As at the contaminated sites. Further study is clearly required to unravel the processes underlying the rise in plant stem As during the last month of the growth season. Such understanding may help further reduce the transfer of As to the rice plant and rice grain by aerobic cultivation or planting on raised beds (Xu et al., 2008; Panaullah et al. 2008). An added benefit of reducing the As content of rice stems is that it would reduce the exposure of cattle eating rice straw and therefore, if this route is significant, reducing propagation of As originating from irrigation water through the food chain (Abedin et al., 2002). Of greater consequence for human health, however, may be the recently unveiled exposure by inhalation of women using rice straw elevated in As as fuel for cooking (Pal et al., 2007).

5. Conclusion

Time series of detailed soil-water profiles from a suite of paddy fields in Bangladesh demonstrate a dramatic impact of irrigation with groundwater elevated in As within the depth range tapped by the roots of rice plants. The range of As concentrations observed in soil water supports the notion that greenhouse studies of potted plants fail to capture the magnitude of As released in flooded soils. A sizeable proportion of the As released to soil water by reductive dissolution of Fe oxyhydroxides is subsequently trapped, however, on the Fe plaque precipitated on the roots of rice plants as a result of micro-aeration. A minimum in soil-water As/Fe ratios is reached around the time of panicle initiation. This coincides with a minimum in the As content of plant stems and suggests that the formation of fresh Fe plaque inhibits the uptake of As by rice plants. The new field data suggest that more attention should be paid in future studies to processes regulating the transfer of As from soil water to the rice plant during the last month of the growth season. This period is likely to have a disproportionate effect on the As content of rice straw and rice grains.

Acknowledgments

This research was funded by the ACI “Ecodyn” from the French Ministry of Research and NIEHS Superfund Basic Research Program grant NIH 1 P42 ES10349.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2010.05.019.

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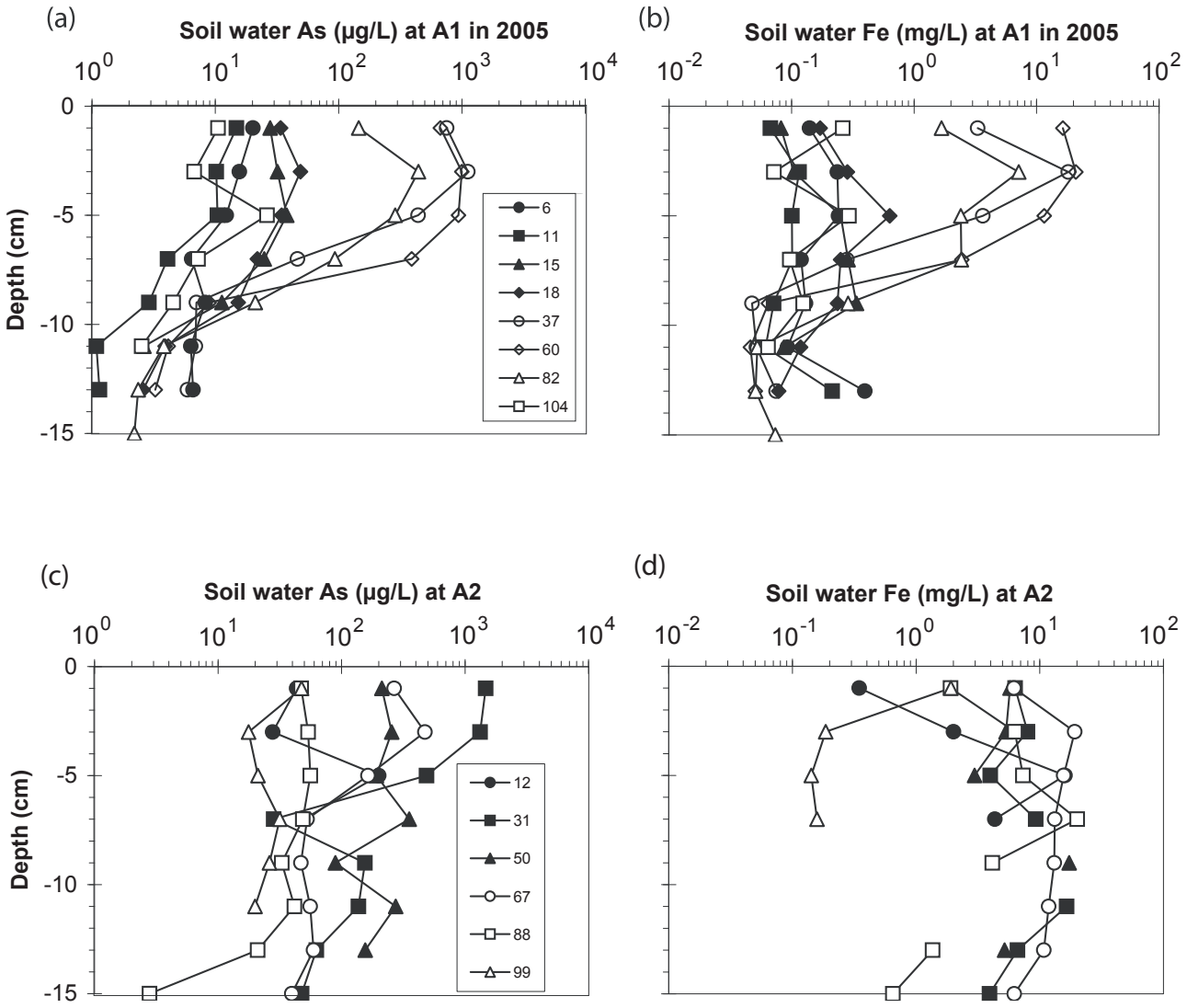
(a)
Soil water As (µg/L) at A1 in 2005
10^0 10^1 10^2 10^3 10^4
0
-5
-10
-15
Depth (cm)
6
11
15
18
37
60
82
104
(b)
Soil water Fe (mg/L) at A1 in 2005
10^{-2} 10^{-1} 10^0 10^1 10^2
(c)
Soil water As (µg/L) at A2
10^0 10^1 10^2 10^3 10^4
0
-5
-10
-15
Depth (cm)
12
31
50
67
88
99
(d)
Soil water Fe (mg/L) at A2
10^{-2} 10^{-1} 10^0 10^1 10^2

Figure S1

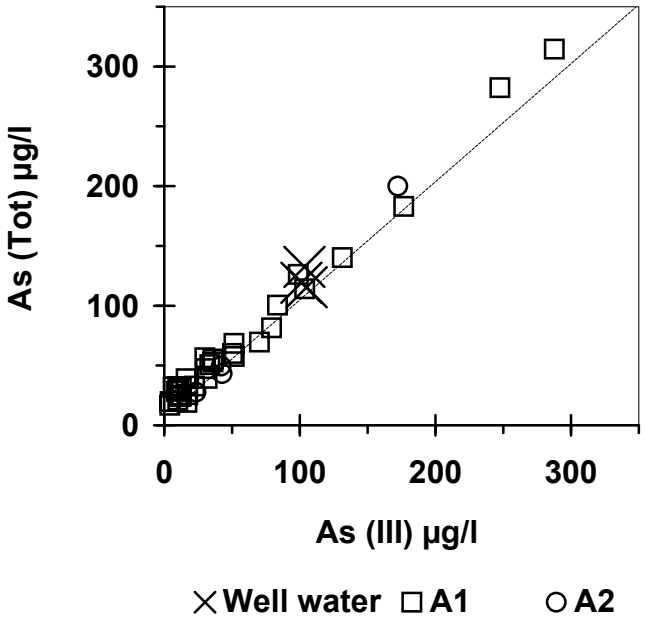

Figure S2