Arsenic accumulation by rice grown in soil treated with roxarsone

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Abstract

Poultry litter is widely used as a fertilizer for lowland rice in Taiwan and China. However, the organic-arsenic compound roxarsone (additive of poultry feed) in poultry litter can be absorbed by the plants and the resulting arsenic (As) contamination may pose a serious threat to human health. This study used various amounts of poultry litter contaminated with roxarsone in pot experiments to evaluate the effect of roxarsone on rice agronomic parameters and the bioaccumulation of total and inorganic As in rice-plant tissues. Rice-grain yield decreased significantly with increasing As content of the soil, and the critical threshold that killed rice was 200 mg roxarsone (kg soil)$^{-1}$. The As concentrations in root, straw, leaf, husk, and grain increased with increasing soil As ($p < 1\%$). At 100 mg roxarsone per kg of soil, the As concentration in the rice grain exceeded the statutory permissible limit of 1.0 mg As (kg dry weight)$^{-1}$ and at 25 mg roxarsone (kg soil)$^{-1}$, the inorganic As concentrations in grains exceeded the statutory limit of 0.15 mg of inorganic As kg^{-1} in China. For all treatments, the As concentrations in various plant tissues at maturity follow the order: root > stem > leaf > husk > grain. Arsenite was the predominant species in root, straw, and grain, while arsenate was the predominant species in leaf and husk. No significant difference existed between the amounts of arsenite and arsenate when various amounts of poultry litter were applied. This result illustrates that large amounts of added roxarsone are not only toxic to rice but also accumulate in grains in the inorganic As forms, potentially posing a threat to human health *via* the food chain.

Key words: arsenate / arsenite / *Oryza sativa* L. / poultry manure / Taiwan

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1 Introduction

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) is an organic-arsenic compound, which has been used over several decades as an additive to poultry feed, not only to control coccidial intestinal parasites but also to improve feed efficiency and to increase growth. *Mellon* et al. (2001) indicated that roxarsone is frequently added to poultry feed in concentrations of 23–45 g t^{-1}. While small amounts of roxarsone may be retained in the meat of chicken or pork, most ingested roxarsone is excreted in unchanged form from animal bodies (*Moore* et al., 1998). Therefore, poultry litter generally contains some levels of arsenic (As) and the total As concentrations in fresh poultry manure can reach up to 27 mg kg^{-1} (*Hancock* et al., 2002). Several studies could show that roxarsone is stable in dry litter (*Rutherford* et al., 2003) and that 70%–75% of the total As in poultry litter is water-soluble (*Jackson* and *Miller,* 1999). Thus, roxarsone can be easily leached during composting (*Jackson* and *Bertsch*, 2001) and degradation rates of roxarsone increase with the amount of water added. At high-moisture and high-temperature conditions, most roxarsone is transformed into arsenate after 30 d of incubation (*Brown* et al., 2005).

Poultry litter that consists of the manure and bedding material (such as sawdust or wood chips) has a high nutrient content and is used commonly as a fertilizer on cropland and pastures to meet the N requirements of crops. Excrements are generally disposed of by spreading them onto agricultural fields near the poultry houses (*Edwards* et al., 1992). Dry poultry manure with roxarsone is applied to corn fields of Virginia in the USA at an annual rate of about 4.5 t ha^{-1} (*Hyer* et al., 2001), resulting in gradually increasing concentrations of As in water and soil. Soil samples collected in field sites fertilized with roxarsone-rich poultry litter for many years have higher As concentrations than similar soils without poultry litter amendments (*Brown* et al., 2005).

Poultry litter is used in organic agriculture, and in the southern provinces of China most arsenic-bearing wastes (roxarsone or arsanilic acid) are applied into paddy fields as a fertilizer (*Wang* et al., 2006). Wherever poultry litter is used as a fertilizer in paddy fields, this practice is causing As to be released into soil environments with low redox potential (Eh), facilitating its uptake by plants and increasing the risk to human health.

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In the soil of paddy fields, arsenate is readily converted to arsenite because of anaerobic conditions (*Abedin* et al., 2002c). The uptake of arsenite by rice is higher than that of other As species, and high As concentrations in rice are frequently present in highly As-polluted soil or irrigation water, affecting the safe consumption of rice by humans and animals (*Krishnamurti* and *Naidu*, 2002). Different As species have different toxicities to organisms. Typically, inorganic As is more toxic than organic As. Few studies focused on different As species in rice. The aim of this work was to evaluate the effect of roxarsone on rice agronomic parameters and the bioaccumulation of total As and As species in various tissues of rice plants.

2 Materials and methods

2.1 Reagents

Deionized water (electrical conductivity: 0.056 dS m^{-1}) was used for the preparation of reagents and standards. All glassware was soaked in 10% nitric acid for 24 h, then washed with tap water and rinsed three times with deionized water before use. The stock standard solutions of As, monomethyl-arsonic acid (MMA) and dimethyl-arsinic acid (DMA), were prepared by dissolving commercially available As salts in water: arsenite (sodium arsenite; Chem Service), MMA (monosodium methanearsonate, 97.5%, $CH_4AsNaO_3 \cdot 1.5H_2O$; Chem Service), and DMA (dimethylarsenic acid sodium salt, 97%, $C_2H_6AsNaO_2 \cdot 3H_2O$; Merck). The arsenate solution (1000 mg L^{-1}) was obtained from Merck.

2.2 Soil, plant, and poultry-litter material

A pot experiment was conducted in a glasshouse of the National Taiwan University for 130 d from transplanting to the harvest of rice. The temperatures in glasshouse ranged from 21°C to 34°C, and the relative humidity was 60%–70%. The soil (depth of 0–15 cm) and poultry litter used in this study were collected from central Taiwan rice farms, where about 61% of paddy rice of Taiwan is produced. Soil and poultry litter were sun-dried for 10 d. Large soil aggregates were broken by crushing with a hammer and passed through a 2 mm sieve.

Soil pH was measured using a glass electrode and a 1:1 soil- or litter-to-water suspension. Ammonium acetate, adjusted to pH 7.0 (*Page* et al., 1982), was used as extractant to determine soil exchangeable bases and CEC using the leaching method. The samples were digested (at 120°C for 3 h) using HNO_3–H_2O_2 (*Tang* and *Miler,* 1991) to determine total As contents. Available P was determined colorimetrically using 0.5 N $NaHCO_3$ as extractant. Total C and N were determined using a CNHS element analyzer (Perkin Elmer 2400 USA). Soil particle size was determined using the pipette method. Selected physico-chemical parameters of soil and poultry litter are listed in Tab. 1. The pH of soil and poultry litter was 6.9 and 9.2, respectively. Total As contents in soil and poultry litter were 6.8 mg kg^{-1} and 4.7 mg kg^{-1}, respectively. The poultry litter contained 4.23% N.

Table 1: Physico-chemical properties of soil and poultry litter. Data represent means ± SE of three replicates.

Parameters	Soil	Poultry litter
pH	6.9 ± 0.2	9.2 ± 0.3
Moisture (%)	19.2 ± 1.4	12.6 ± 1.2
Sand (%)	34.4 ± 2.2	–
Silt (%)	57.2 ± 3.6	–
Clay (%)	8.4 ± 0.1	–
Organic carbon (%)	5.1 ± 0.5	47.6 ± 2.9
Total nitrogen (%)	0.4 ± 0.1	4.2 ± 0.1
Available phosphorus (%)	0.9 ± 0.1	4.6 ± 0.9
Available potassium (%)	11.1 ± 0.2	2.4 ± 0.2
Total arsenic (mg kg^{-1})	6.8 ± 0.9	4.7 ± 0.9

Pots of 25 cm diameter and 30 cm height were filled with 3 kg soil, amended with poultry litter at rates of 4 and 8 t ha^{-1}, and five levels of roxarsone (0, 25, 50, 100, and 200 mg roxarsone [kg soil]$^{-1}$) were adjusted by addition of 4-hydroxy-3-nitrobenzenearsonic acid. Five 30 day–old seedlings of the widely used lowland rice (*Oryza sativa* L.) cultivar Taiken 8 were transplanted, and the pots were watered daily with tap water to maintain flooded conditions (3–4 cm of floodwater). The experiment was arranged in a factorial completely randomized design with three replications (total of 30 pots).

2.3 Sample collection and preservation

The pH and Eh of the soil and the As concentration in the soil solution were analyzed 7, 14, 21, 28, and 35 d after rice transplanting. Soil-solution samples were collected from bottom pipes. The water samples were stored in polyethylene containers, cleaned with 10% nitric acid and then rinsed with deionized water. The water samples were filtered through 0.45 μm filters before analyses. The pH and redox potential (Eh) were directly measured by pH and Eh probes in the soil, reading data after 10 min.

Rice plant–sample collection and preservation were done according to *Abedin* et al. (2002b). Rice plants were harvested by cutting at 4 cm above the soil to avoid basal tissues to be contaminated by the applied roxarsone. Rice spikelets were separated from the panicles by hand, and empty and filled spikelet numbers were recorded. Rice grains were separated from their husks using a mortar and pestle and ground using a stainless-steel grinder (Braun KSM2 Coffer Grinder, Germany). The straw biomass was recorded after oven-drying at 50°C for 48 h. After harvest, the soil was oven-dried at 50°C. Roots were separated from soil during disaggregation and sieving to <2 mm rinsed in deionized water and dried at 50°C for 48 h. Plant samples (root, straw, leaf, grain, and husk) were ground to pass a 1 mm sieve, and the dried samples were stored in a desiccator until chemical analysis.

2.4 Analysis of arsenic in rice

Straw, root, leaf, grain (brown rice), husk, and soil samples were analyzed for total As concentrations. Plant material was digested with concentrated HNO_3 and 30% H_2O_2 at 120°C (*Tang* and *Miller*, 1991; *Abedin* et al., 2002b). Five milliliters of concentrated HNO_3 were added to about 200 mg of dry plant material weighed into a 75 mL digestion tube and allowed to sit overnight. The following day, the digestion tubes were placed on a heating block, and the temperature was raised to 60°C. Three 1 mL aliquots of 30% H_2O_2 were added to each tube, and the temperature was gradually raised to 120°C. The samples were allowed to digest for 3 h, after which the volume was reduced to 3–4 mL. The digests were cooled, diluted to 50 mL with deionized water, and filtered through Whatman No. 42 filter paper into plastic bottles. All glassware and plastic bottles were first acid-washed in 2% HNO_3, rinsed in deionized water, and dried.

The acid digests of soils, roots, straws, leaf, grain, and husks were analyzed for total arsenic by HG/AAS, an atomic-absorption spectrometer (AAS) (AA100 Perkin-Elmer Shelton, USA) and a hydride generator (HG) (FIAS 400 Perkin-Elmer Shelton, USA). To reduce the arsenic to arsine, 0.5% $NaBH_4$ in 0.25% NaOH and 1 N HCl were added into 200 μL of a digested sample. The accuracy of the analysis was checked by the certified standard reference material SRM1568a rice flour from the National Institute of Standards and Technology (NIST). The arsenic concentration in certified reference material was 0.29 ± 0.03 mg kg^{-1} while the measured arsenic was 0.28 ± 0.02 mg kg^{-1}.

2.5 Analysis of arsenic species

Trifluoroacetic acid (TFA) extraction of As species from plant material was conducted according to the method described by *Abedin* et al. (2002b). Portions of 0.25 g ground plant material were weighed into 100 mL glass digestion tubes to which 2 mL of 2 M TFA were added. The digestion tube was then placed on a digestion block and heated at 100°C for 6 h. The digest was rotavapored to dryness, and the residue redissolved with deionized water, filtered through 0.22 μm filters, and made up to a 25 mL volume with deionized water. The extracts were stored at –4°C prior to analysis by HPLC-HG-AAS (*Huang* et al., 2003). A high-performance liquid chromatograph, HPLC (Hitachi 7110, Naka, Japan), equipped with an anion column (Machey-Nagel Nucleosil, 10 μm, 250 × 4.6 mm) and connected to HG/AAS, was used to separate As(III), As(V), DMA, and MMA. All glassware and plasticware were first acid-washed in 2% HNO_3, rinsed in deionized water, and dried. The laboratory procedure recovery rates of As (III), As (V), MMA, and DMA were 101.2%, 97.6%, and 98.1%, respectively. The coefficient of variation (CV) was always below 5%.

2.6 Transfer factor

The transfer factor (*TF*), relating the concentration of As in basal to that in upper tissue, was used to estimate the propensity of arsenic accumulation in various plant parts:

$$TF = \frac{C_{ut}}{C_{bt}} \quad (1)$$

where C_{ut} is the As concentration in upper tissue and C_{bt} is the As concentration in basal tissue.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) was adopted to examine the statistical differences among various treatments of effective tiller numbers, grain yield, 1000-grain weight, and As concentrations in various plant parts. The SAS package was used for statistical analyses at a 95% level of probability.

3 Results

3.1 Soil pH, Eh, and As in soil solution

With 4 t ha^{-1} of added poultry litter, soil pH ranged from 6.8 to 7.2 and Eh ranged from –231 to –398 mV. With 8 t ha^{-1} of poultry litter, soil pH was 6.8 to 7.1 and Eh was –160 to –398 mV. Figure 1 shows the temporal dynamics of As species in soil solution. Arsenite was the predominant species throughout the experiment due to the flooded and reduced conditions in soil solution. The As speciation over 35 d indicated that As(III), As(V), MMA, and DMA made up 56%, 35%, 6%, and 2%, respectively, of the total As with 8 t ha^{-1} and 63%, 31%, 4%, and 2%, respectively, with 4 t ha^{-1} of added poultry litter.

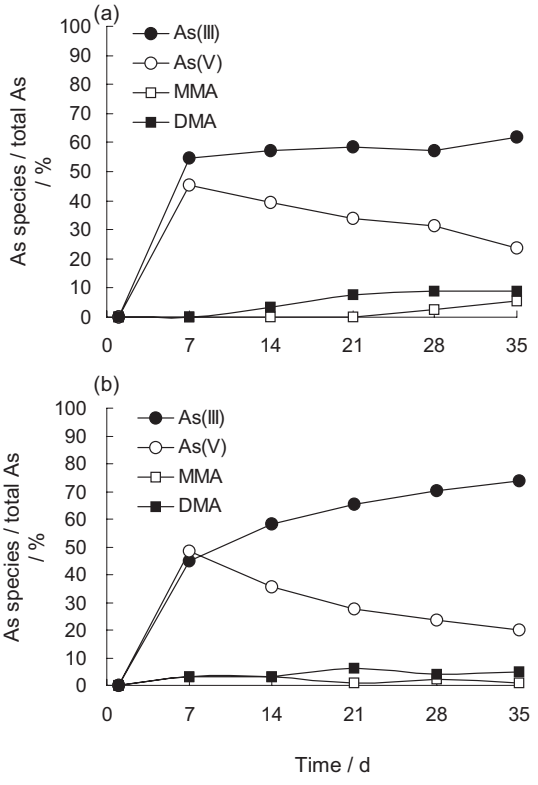

Figure 1: Dynamics of arsenic species in soil solution for flooded–paddy field conditions (*i.e.*, saturation to permanent immersion of the soil up to 3–4 cm of solution), soil amended with dry poultry litter of (a) 8 t ha^{-1} and (b) 4 t ha^{-1}. As(III): Arsenite, As(V): Arsenate, DMA: Dimethyl-arsinic acid, MMA: Monomethyl-arsonic acid.

3.2 Rice performance and arsenic concentrations

Rice plants withered after 56 d in the treatment with 200 mg roxarsone (kg soil)$^{-1}$. Yield, tiller numbers, and 1000-grain weight of rice grown in 0, 25, 50, and 100 mg roxarsone (kg soil)$^{-1}$ are presented in Fig. 2. The amount of poultry litter added did not significantly affect the plant parameters, except for the straw weight in the 25 mg– and 50 mg–roxarsone treatments. However, the amount of roxarsone added significantly reduced tiller number, 1000-grain weight, and grain yield (p < 5%). This As-induced reduction was most pronounced in tiller number and grain yield (Fig. 2).

Table 2 presents the total As concentrations in rice plants. Increasing the amount of roxarsone significantly increased the As concentrations in root, straw, leaf, husk, and grain. Total As concentrations in rice tissues followed in all treatments the order: root > straw > husk > leaf > grain. Adding more roxarsone to the soil resulted in larger differences between plant organs. However, DMA and MMA were not detected in all rice tissues. Inorganic arsenic in rice tissues also followed the same order: root > straw > husk > leaf > grain. The extraction efficiencies ranged from 73.22% to 97.6%.

Figure 3 shows variations of inorganic As for different treatments. Arsenate and arsenite were present in rice root, straw, and leaf. However, arsenate was not found in grain and arsenite was not found in husk. Arsenite concentrations were higher than arsenate concentrations in root, straw, and grain, while arsenate concentrations were greater in leaf and husk.

Most arsenite and arsenate concentrations did not vary with the amount of poultry litter added (4 or 8 t ha^{-1}), except for arsenite in root and straw with 100 mg of roxarsone and arsenate in the leaf with 50 mg roxarsone added. In the 100 mg roxarsone (kg soil)$^{-1}$ treatment, arsenite in straw and arsenate in leaf increased significantly with the amount of poultry litter added. Generally, increasing the amount of roxarsone significantly increased the inorganic-As concentrations in root, straw, leaf, husk, and grain (p < 1%; Tab. 3).

3.3 Arsenic transfer from soil to shoot and grain

The ratios of arsenic uptake from soil to root of rice ranged from 1.2 to 4.3 (calculated from Tab. 2). However, As-transfer factors for different plant parts in rice were all less than 1.0 (Fig. 4). Across treatments, the average As transfer from root to straw was 0.25, indicating that most As accumulated in the root. Moreover, As-transfer factors from straw to leaves,

Table 2: Arsenic concentrations in different plant parts of rice amended with increasing amounts of roxarsone. Data represent means ± SE of three replicates. Extraction efficiency (%) = sum of As species (TFA extraction) / total As (nitric acid digestion).

Roxarsone treatment	Total As	Inorganic As	extraction efficiency
/ mg (kg soil)$^{-1}$	/ μg g^{-1}	/ μg g^{-1}	/%
	root		
0	8 ± 0.52	6 ± 0.37	73 ± 2
25	56 ± 2.57	45 ± 1.71	79 ± 1
50	134 ± 9.27	103 ± 12.86	76 ± 5
100	395 ± 12.90	334 ± 20.70	84 ± 3
	straw		
0	2.35 ± 0.07	2.02 ± 0.09	85 ± 2
25	17.56 ± 1.77	13.96 ± 0.41	80 ± 6
50	27.26 ± 2.13	22.88 ± 3.30	84 ± 11
100	72.57 ± 6.62	60.02 ± 8.99	82 ± 5
	leaf		
0	1.21 ± 0.07	0.93 ± 0.05	76 ± 4
25	14.96 ± 0.87	14.50 ± 1.00	97 ± 12
50	21.95 ± 0.33	18.06 ± 0.54	82 ± 3
100	33.29 ± 1.92	27.32 ± 1.85	82 ± 4
	husk		
0	0.35 ± 0.03	0.28 ± 0.02	79 ± 2
25	2.27 ± 0.33	1.74 ± 0.36	75 ± 5
50	2.80 ± 0.20	2.05 ± 0.16	73 ± 1
100	3.80 ± 0.40	3.48 ± 0.17	92 ± 6
	grain		
0	0.17 ± 0.01	0.13 ± 0.01	78 ± 4
25	0.31 ± 0.01	0.23 ± 0.04	73 ± 8
50	0.50 ± 0.04	0.42 ± 0.03	84 ± 4
100	1.09 ± 0.05	0.84 ± 0.04	77 ± 3

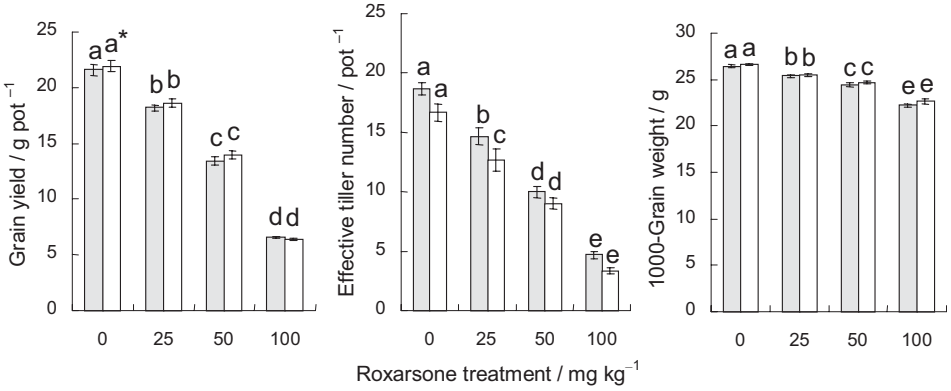

Figure 2: Effect of treating soil with roxarsone on rice-biomass parameters for two amounts of poultry litter added; black: 8 t ha^{-1}, white: 4 t ha^{-1}. Error bars represent ± SE from three replications. *Mean ± SE bars followed by same letters do not differ significantly from each with p = 5%.

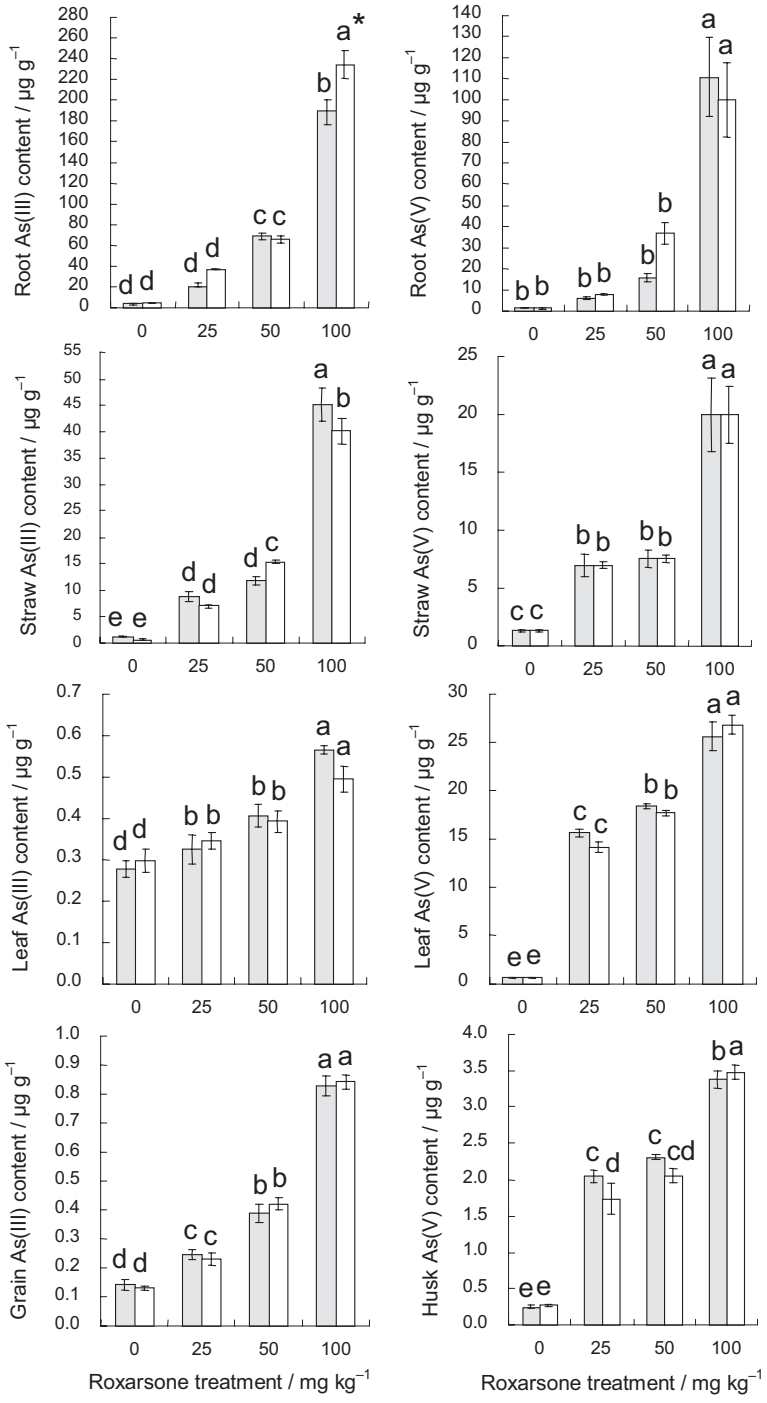

Figure 3: Arsenite and arsenate concentrations in rice plant parts for two amounts of poultry litter added; black: 8 t ha^{-1}, white: 4 t ha^{-1}. Error bars represent ± SE from three replications. *Means ± SE bars followed by same letters differ significantly from each other with p = 5%.

husk, and grain were 0.66, 0.11, and 0.03, respectively, indicating the largest amounts of As being transferred from straw into leaf and only little into grain.

4 Discussion

4.1 Effect of arsenic on rice growth

The experiment showed that when added in excess of 200 mg kg^{-1}, roxarsone was toxic to paddy rice. Arsenic has been associated with metabolic processes and found to inhibit plant growth, sometimes leading to death (*Marin* et al., 1993). Our work demonstrated that rice-straw weights decreased significantly (p < 5%) as the soil As contents increased. Similar findings were reported by *Abedin* et al. (2002a, b) and by *Wang* et al. (2006). Exposure to As reportedly also reduced shoot biomass and growth of rice (*Tang* and *Miller,* 1991). This study showed a significant decrease in rice-grain yield as the soil As content increased. The toxicity of As is usually manifested as the "straighthead disease" as observed in the USA (*Meharg* and *Hartley-Whitaker*, 2002).

Table 3: Correlation between the amount of applied roxarsone and the arsenic concentrations in soil and various plant parts of rice.

	Roxarsone	Soil total As	Root inorg. As	Straw inorg. As	Leaf inorg. As	Husk inorg. As	Grain inorg. As
Roxarsone	1	0.99*	0.96	0.92	0.91	0.87	0.98
Soil total As		1	0.96	0.97	0.90	0.87	0.98
Root inorg. As			1	0.95	0.82	0.75	0.93
Straw inorg. As				1	0.82	0.85	0.94
Leaf inorg. As					1	0.86	0.87
Husk inorg. As						1	0.87
Grain inorg. As							1

*All correlation values with $p < 1\%$

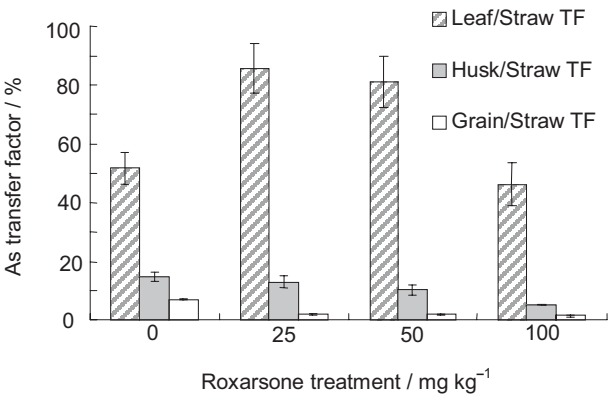

Figure 4: Arsenic transfer factors (TF) between various plant parts of rice. Means ± SE of three replicates.

4.2 Arsenic in different parts of rice plants

The results show that total As in rice plant parts significantly increased with soil As content. The As concentrations in different plant tissues in all treatments followed the order: root > straw > leaf > husk > grain. Arsenic reportedly accumulated more in roots than in aboveground plant parts (*Chaturvedi,* 2006). Also in our study, the root stored the largest amount of As. However, As may be adsorbed in large amounts by the iron plaque on the root surface (*Liu* et al., 2006), but iron plaque was not analyzed in our study.

The As speciation indicated that arsenite is the dominant species in root, straw, and grain, while arsenate is the dominant species in leaf and husk. However, this work does not detect the presence of MMA and DMA. A possible reason may be the reportedly low uptake of organic As compared to that of inorganic As species (*Odanaka* et al., 1987). Particularly DMA and MMA are taken up by rice roots at a slow rate (*Abedin* et al., 2002c). On average, ratios of inorganic As to organic As in soil solution are 93 to 7 and the ratios of arsenite to arsenate are 60 to 33.

Several factors affect the As speciation in a plant. Arsenite is the dominant species in submerged soils (*Marin* et al., 1993). Arsenate and arsenite are the inorganic and phytoavailable forms of As in soil, being available to rice and following the order: DMA < As(V) < MMA < As(III) (*Marin* et al., 1992). Plants take up arsenate, the predominant form of As in aerobic soils, through phosphate transporters (*Meharg* and *Hartley-Whitaker*, 2002). However, because paddy soils are flooded during the rice-growing season, arsenite becomes the predominant chemical species of As and is absorbed *via* aquaporins into the rice root (*Meharg* and *Rahman*, 2003).

In the reduced environment of a flooded soil (*Marin* et al., 1993), As is present predominantly in the inorganic form with arsenite concentrations exceeding those of arsenate. Thus, arsenite is the dominant species in root and straw of rice. Additionally in this work, the arsenite exceeded arsenate concentrations in straw, but the arsenate exceeded the arsenite in leaf and husk, indicating a possible oxidation of arsenite in the plants as suggested by *Schmidt* et al. (2004).

4.3 Potential health hazards of arsenic in rice grain and straw

The shoots and grains of rice grown in As-contaminated soil can accumulate high levels of As (*Abedin* et al., 2002a, b). Therefore, the uptake of As by rice plants plays an important role in the transfer of this toxic element into food chains, resulting in potential threats to human health (*Meharg* and *Rahman*, 2003). This study indicates that the total As and inorganic As concentrations in rice grains are 1.14 mg kg^{-1} and 0.89 mg kg^{-1} after application of 100 mg roxarsone (kg soil)$^{-1}$. The total As concentration in rice exceeded the statutory limit of 1.0 mg As (kg dry weight)$^{-1}$ in rice grain in Australia (*National Food Authority*, 1993) and the inorganic As exceeded the limit of 0.15 mg kg^{-1} in China (GB2762–2005 China National Standard).

The assessment of health risks due to As in rice has largely been based on the inorganic-As concentration because inorganic species are considered to be more toxic than the organic As. Also in our research, inorganic As makes up nearly 80% of the total As in grain, which is similar to the 86% reported by *Schoof* et al. (1998). Assuming an average inorganic-As content of 75% of the total As, rice grain with 1 mg kg^{-1} of total As contains 0.75 mg kg^{-1} of inorganic As, which exceeds by far the health standard of China (0.15 mg kg^{-1}). Additionally, this work shows that the As concentration in rice straw may reach 60 mg (kg dry weight)$^{-1}$

with arsenite being the predominant As species. Rice straw is widely used as cattle feed (*Abedin* et al., 2002a), and feeding As-contaminated straw to cattle may result in elevated As contents in meat and milk. Thus, a high As concentration in the straw may not only pose a direct threat to animal health but also an indirect threat to human health.

5 Conclusion

The study reveals that roxarsone added to paddy fields is transformed predominantly into arsenite and to a lesser extent to arsenate. Di- and mono-methylarsinic acid only occur in trace levels. The average As contents in grain in this study exceeded the statutory limits for both total and inorganic As in plants. We conclude that high application rates of roxarsone to rice fields result in As toxicity in rice and potentially threaten human health *via* food chain exposure.

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