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1   **Soil attribute regulates assimilation of roxarsone metabolites by rice (*Oryza***
2   ***sativa* L.)**

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ABSTRACT

Roxarsone (ROX), an organoarsenic feed additive, and its metabolites, can be present in animal manure used to fertilize rice. Rice is prone to absorb arsenic, and is subject to straighthead disorder, which reduces rice yield and is linked with organic arsenic compounds. This study aims to elucidate how soil property affect arsenic accumulation in rice plants fertilized with chicken manure containing ROX metabolites. Manures of chickens fed without or with ROX, designated as control manure and ROX treated manure (ROXCM), respectively, were applied in eight paddy soils of different origins, to investigate the assimilation of arsenic species in rice plants. The results show that inorganic arsenic (arsenate and arsenite), monomethylarsonic acid and dimethylarsinic acid (DMA) were detected in all brown rice and husk, trace tetramethylarsonium and trimethylarsine oxide were occasionally found in these both parts, whereas all these arsenic species were determined in straw, irrespective of manure type. ROXCM application specifically and significantly increased brown rice DMA ($P=0.002$), which remarkably enhanced the risk of straighthead disease in rice. Although soil total As impacted grain biomass, soil free-iron oxides and pH dominated arsenic accumulation by rice plants. The significantly increased grain DMA suggests manure bearing ROX metabolites is not suitable to be used in soils with abundant free-iron oxides and/or high pH, if straighthead disorder is to be avoided in rice.

37   *Keywords:* Roxarsone; Animal manure; Rice; Arsenic species; Paddy soil; Soil
38   property

1. Introduction

Roxarsone (4-hydroxy-3-nitro-phenylarsonic acid, ROX), an organoarsenic feed additive in animal production, occurs as itself and its metabolites in animal manure, which is commonly land applied as fertilizer in crop production. ROX metabolites in the manure include inorganic arsenic [iAs, the sum of As(V) and As(III)], monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), 3-amino-4-hydroxyphenylarsonic acid (3-AHPA), 3-acetamido-4-hydroxy-phenylarsonic acid (3-AAHPA) and so on, with iAs dominating (Fisher et al., 2015; Yang et al., 2016; Yao et al., 2016).

Arsenic phytoavailability is closely related to soil properties (Datta et al., 2006; Fu et al., 2011). Soil exchangeable calcium promotes iAs uptake in garland chrysanthemum fertilized from chicken manure contaminated with ROX metabolites (Yao et al., 2017). As(III) and DMA are the primary arsenic species in vegetables grown in aerobic medias amended with chicken manure containing ROX metabolites (Huang et al., 2014; Yao et al., 2009).

Rice, the staple food for about half of the world population (Zhu et al., 2008), is prone to absorb arsenic than other staple food due to its soil anaerobic growth environment, and to highly efficient arsenic transport mechanisms (Su et al., 2010; Williams et al., 2007). Rice consumption is the largest contributor of total iAs intake in Southeast Asia and China (Li et al., 2011; Meharg et al., 2009; Mondal and Polya, 2008), consequently, arsenic accumulation in rice is of global concern. It is reported

that rice grain arsenic is significantly enhanced by addition of ROX to paddy soils (Liu et al., 2009; Wang et al., 2006). However, it is ROX metabolites, rather than ROX itself, that are inevitably introduced into soils as animal manure is land applied as fertilizer. Rice is commonly grown in flooded soils, where the bioavailability of ROX metabolites might be different from that in the aerobic media as irrigation management markedly affects the uptake of arsenic species in rice plant (Carrijo et al., 2019).

The transfer of ROX from chicken diet to rice plant has been documented (Yao et al., 2016). In the present study, we further compare the uptake of arsenic species by rice plants in eight typical paddy soils from China, where two chicken manures, without or with ROX metabolites, were applied, with the aim to identify how soil attributes govern the phytoavailability of ROX metabolites to rice plants, focusing on the edible grain.

2. Material and methods

2.1 Chicken manure

Manures were produced from a chicken farm located in Huizhou city, Guangdong province, South China. Two groups of chickens (1320 chickens in each group) of the same breed and age (45 d), were simultaneously fed with ROX contrasting diets. One diet was a typical maize – soybean meal feed without ROX, and the other one was the same formula added with ROX at the recommended dose of 50 mg kg⁻¹. The ROX

(98.0%) was purchased from Zhejiang Rongyao Biotech Co., Ltd, China. Two manures excreted by these two groups of chickens were separately collected, composted for 20 d and then air dried, ground and 2 mm-sieved for use, respectively. The control manure without ROX was designated as CM and the ROX-treated manure as ROXCM. Sieved manure samples were collected for total arsenic and arsenic speciation. Some selected attributes of these both manures are listed in [Table 1](#).

Table 1

Selected properties of two chicken manures without (CM) or bearing roxarsone metabolites (ROMCM).

Item	CM	ROXCM
pH	7.04	7.07
OM (g kg ⁻¹)	502.9±6.6	510.6±17.6
Total N (g kg ⁻¹)	73.2±1.8	72.4±1.0
Total arsenic (mg kg ⁻¹)	1.4±0.1	33.2±0.9
Roxarsone (mg kg ⁻¹)	nd	0.36±0.06
3-AHPA (mg kg ⁻¹)	nd	4.82±0.28
3-AAHPA (mg kg ⁻¹)	nd	0.79±0.02
MMA (mg kg ⁻¹)	nd	0.11±0.02
DMA (mg kg ⁻¹)	0.40±0.0	0.76±0.05
iAs (mg kg ⁻¹)	1.18±0.37	25.9±0.4

2.2 Soil type

Eight typical paddy soils were collected from the main production areas of rice in China. All the soils were collected at 0-25 cm depth, air-dried and then 2 mm-sieved and well mixed. The basic location information of soils is present in [Table S1 \(supporting material\)](#).

2.3 Rice

A conventional indica long grain paddy rice variety “Meixiangzhan”, widely planted in South China, was used. The rice seed was supplied by the Rice Research Institute, Guangdong Academy of Agricultural Sciences. The sterilized seeds were sown into a plastic plate filled with growth substrate (coconut husk:perlite, 3:1), and irrigated with Hoaglands nutrient solution. After being grown for 20 d, the seedlings were transplanted for culture experiment.

2.4 Rice culture experiment

An experiment using the two manures $\times$ eight soils, with four replicates, was conducted in a randomized pot experiment in the green house. In each PVC pot, 100 g (DW) of each manure and 4 kg of each soil were mixed thoroughly. No other fertilizers were added. The soils were flooded for 1 week by adding tap water, and then two 20-day-old seedlings were transplanted into each pot. Rice plants were flooded with 2-3 cm deep water till grain filling, followed by intermittent irrigation till one week before harvest when the soils were drained.

2.5 Sample collection, preparation and chemical analysis

Rice grains and straws were harvested at 106 d after transplanting. The air-dried grain weight was recorded before being dehusked to produce brown rice and husk, and the fresh weight of rice straw was also recorded. Then, all the brown rice, husk and straw samples were lyophilized (Alpha 1-4/LD-plus, Christ) and pulverized by a ball mill (Retsch PM 100) to fine powder for total arsenic and arsenic speciation analysis.

Total arsenic in plant, manure and soil samples was extracted by 1:1 mixture of concentrated HNO_3 and H_2O_2 , and then microwave-digested using appropriate digestion programs, respectively. The internal standard rhodium (Fluka Analytical ICP-MS standard) was used at the concentration of $10 \mu\text{g kg}^{-1}$ to assure the detection quality. The final digestate was detected using ICP-MS (Thermo Scientific iCap Q) for total arsenic. Arsenic species in plant tissues were extracted with 1% concentrated HNO_3 and microwave-digested. 1 mL of the digested plant solution was transferred to the vial, and 10 μL of 30% H_2O_2 was added into the vial to oxidize As(III) to As(V), and the final solution was subjected to IC-ICP-MS for arsenic speciation using the same methods and instruments as described by Signes-Pastor et al. (2017). Arsenic species in manure and soils were extracted with mixture of 65% 50 mM $(\text{NH}_4)_2\text{HPO}_4$ + 35% methanol ($\text{pH} = 6.5$) for 10 h at 45°C , and the extracts were purified by solid-phase cartridges (500 mg 6 mL min^{-1} , Nu Analytical Technology, USA), followed by 0.22 μm films. The filtrates were collected for arsenic speciation as well. The

extraction efficiencies of arsenic species were $77.2 \pm 2.1\%$ for As(V), $76.1 \pm 3.4\%$ for As(III), $94.0 \pm 4.0\%$ for MMA and $102.2 \pm 2.6\%$ for DMA in soil, and those were $90.3 \pm 5.3\%$ for ROX, $87.3 \pm 8.9\%$ for 3-AHPA, $97.2 \pm 4.5\%$ for 3-AAHPA, $100.1 \pm 6.0\%$ for As(V), $115.0 \pm 2.3\%$ for As(III), $79.4 \pm 2.5\%$ for MMA and $114.4 \pm 3.2\%$ for DMA in manure.

Soil texture was measured by Bouyocos hydrometer, and soil pH was determined in the suspension of 1:2.5 (soil:water, w/w). Soil organic matter (OM) was quantified using $K_2Cr_2O_7$ oxidation in oil bath method. Soil cation exchangeable capacity (CEC) was evaluated with titrimetric analysis using 1 M CH_3COONH_4 saturation. The soil's free-iron oxides were extracted by mixture of 0.3 M $Na_3C_6H_5O_7$ + 1 M $NaHCO_3$ + 0.5 g $Na_2S_2O_4$ and then measured by phenanthroline colorimetry. Soil exchangeable Ca and Mg were extracted with 1 M CH_3COONH_4 and then detected by atomic adsorption spectrometry, respectively. Soil Olsen-P was extracted using 0.5 M $NaHCO_3$ and determined by Mo-Sb colorimetric method. Analysis methods of the above soil properties are depicted by Lu (2000). Soil total Fe and Mn were measured with the same method as total arsenic using ICP-MS.

2.6 Standards and reagents

Standard reference materials of rice flour (1568b, NIST, USA) and soil (ISE 921) containing certified levels of total arsenic and several arsenic species were used to guarantee the analysis quality of total arsenic and arsenic speciation during the

experiment. The recoveries of total arsenic, iAs, MMA and DMA were $77.4 \pm 3.0\%$, $99.3 \pm 4.6\%$, $97.3 \pm 3.1\%$ and $102.0 \pm 3.2\%$, respectively. Reference materials or pure compounds of ROX (Sigma-Merck, Vertanal™), 3-AHPA (Pfaltz & Bauer), 3-AAHPA (Pfaltz & Bauer), tetramethylarsonium (Tetra, Laboratory synthesized, University of Aberdeen), trimethylarsine oxide (TMAO, Santa Cruz Biotechnology, Inc.), arsenobetaine (AB, BCR Reference material No 626, EC Community Bureau of Reference) and iAs (Inorganic Ventures, certified standard) were used in this study. The detection limits of all arsenic species were $\leq 2.0 \mu\text{g kg}^{-1}$. Multi-Element 2 (Spex Clms-2 Multi-Element Solution 2, matrix: 5% HNO_3) and Multi-Element 4 (Spex Clms-4 Multi-Element Solution 4, matrix: water/Tr-HF) were used to make up all standard solutions in the ranges of 0, 0.1, 0.3, 1, 3, 10, 30 and $100 \mu\text{g kg}^{-1}$ for total arsenic analysis. Ultrapure water was prepared with Millipore Milli-Q Academic.

2.7 Data statistics

All the data were the means of four replicates, and expressed as mean $\pm$ standard deviation. The significance of the effect of manure type and soil type was tested by two-way analysis of variance (ANOVA), followed by comparisons of means using Duncan's. Stepwise linear regression analysis were performed using SAS/STAT (9.2) software. The symbols *, ** and *** show statistical significance at 0.05, 0.01 and 0.001 level, respectively. All variables left in the stepwise regression models are significant at 0.05 level.

3. Results and discussion

3.1 Characterization of eight paddy soils

The selected attributes and arsenic species of eight soils are present in Table 2 and 3. These soils contained 103.2~417.6 g kg⁻¹ of clay, 247.3~744.7 g kg⁻¹ of silt and 17.3~646.7 g kg⁻¹ of sand, indicating greatly variable soil texture. Soil pH differed from 5.17 to 8.14, and soil OM was in the range of 18.6~55.3 g kg⁻¹, with the CEC of 6.80~36.30 cmol kg⁻¹. Soil Olsen-P varied from 7.9 to 156.9 mg kg⁻¹. Soil exchangeable Ca and Mg ranged from 645 to 5092 mg kg⁻¹ and from 18 to 320 mg kg⁻¹, respectively. These soils contained 8.4~54.7 mg kg⁻¹ of total Fe, 1.4~30.3 mg kg⁻¹ of free-iron oxides and 59~930 mg kg⁻¹ of total Mn, respectively. Soil total arsenic was in the range of 1.8~25.0 mg kg⁻¹. As(V) was the dominating arsenic species detected in all soils, with the concentrations of 0.57±0.38~9.69±0.19 mg kg⁻¹. As(III) was analyzed at low levels in six soils, with the exception of Soil FJ and Soil SC. DMA was only found in Soil PY, and MMA was observed in Soil GZ and Soil PY. The above indicates that these soils considerably differed in physiochemical properties.

Table 3

Artenic species in eight paddy soils.

Soil	As(V) (mg kg ⁻¹)	As(III) (mg kg ⁻¹)	DMA (mg kg ⁻¹)	MMA (mg kg ⁻¹)
Soil GZ	9.47±0.27	0.32±0.06	<LOD	0.09±0.02
Soil PY	9.69±0.19	0.38±0.01	0.05±0.0	0.07±0.02

Soil GX	1.44±0.08	0.07±0.01	<LOD	<LOD
Soil FJ	0.57±0.38	<LOD	<LOD	<LOD
Soil HB	4.89±0.09	0.06±0.03	<LOD	<LOD
Soil SC	3.16±0.18	<LOD	<LOD	<LOD
Soil SX	6.65±0.28	0.04±0.02	<LOD	<LOD
Soil AH	4.33±0.46	0.40±0.02	<LOD	<LOD

188

189 **Table 2**

190 Selected properties of eight paddy soils.

Soil	Clay (g kg ⁻¹)	Silt (g kg ⁻¹)	Sand (g kg ⁻¹)	pH	Organic matter (g kg ⁻¹)	CEC (cmol (+) kg ⁻¹)	Olsen-P (mg kg ⁻¹)	Exchangeab le Ca (mg kg ⁻¹)	Exchangeab le Mg (mg kg ⁻¹)	Total Fe (mg kg ⁻¹)	Free-iron oxides (g kg ⁻¹)	Total Mn (mg kg ⁻¹)	Total arsenic (mg kg ⁻¹)
Soil GZ	144.0	352.4	503.6	6.15	26.2	11.35	49.0	1259	19	29.3	15.4	281	15.2
Soil PY	417.6	538.9	43.5	5.17	23.7	36.30	156.9	737	206	54.7	30.3	631	25.0
Soil GX	106.0	247.3	646.7	6.31	18.6	6.80	49.1	939	18	12.0	8.0	59	3.3
Soil FJ	103.2	353.8	543	5.54	36.4	12.55	45.4	645	43	8.4	1.4	388	1.8
Soil HB	345.6	543.2	111.2	8.14	31.6	31.20	15.5	3844	320	38.4	13.9	706	9.5
Soil SC	191.2	491.2	317.6	7.44	20.9	19.10	7.9	3601	111	26.0	6.4	697	5.9
Soil SX	257.2	664.4	78.4	7.34	55.3	18.55	70.8	5092	294	38.0	10.8	930	10.3

Soil AH	238.0	744.7	17.3	5.62	20.0	19.65	31.4	2101	179	23.3	12.8	239	8.1

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3.2 Plant biomass

There was no significant difference for the biomass of both rice grain and straw produced in soils amended with two manures ($P=0.901$), irrespective of ROX metabolites in the manure or not (Fig. 1). The plant biomass in the present work further confirms our previous observation that ROX addition at allowable dose in chicken diet did not suppress rice growth (Yao et al., 2016), which was ascribed to the nourishment of the manure to rice plant and low concentrations of ROX metabolites in these ROXCM-amended soils. The growth response of rice plant to ROX metabolites in the manure differed from the previous studies (Liu et al., 2009; Wang et al., 2006), where rice biomass was significantly decreased by external ROX addition directly in soils at high levels, e.g., $\geq 10 \text{ mg As kg}^{-1}$ or $\geq 25 \text{ mg ROX kg}^{-1}$. However, both grain and straw biomass was significantly influenced by soil type ($P=0.008$ and 0.004), implying that soil properties dominated the growth of rice plant fertilized with two manures in the present work.

The stepwise analysis further shows that grain biomass was primarily dependent on soil total As since the variance of soil total As explained 90.5% ($P<0.001$) and 73.9% ($P=0.006$) of the variability of grain biomass in CM- and ROXCM-amended soils, respectively. Soil clay had the main effect on straw biomass in CM-amended soil, the difference of which contributed to 70.7% ($P=0.009$) of the variance of straw biomass. However, no dominating soil factor was observed for straw biomass in

ROXCM-amended soil because no variable met the 0.050 significance level for entry into the model between straw biomass and soil attribute. The above indicates that rice grain was more sensitive to soil arsenic than rice straw.

Fig. 1. Biomass of rice plants (a: grain; b: straw) grown in eight soils fertilized with two chicken manures without or bearing roxarsone metabolites (CM and ROXCM), respectively.

ns refers to not significant at 0.05 level.

3.3 Total arsenic in rice plant

ROXCM application led to significantly elevated total arsenic in brown rice ($P<0.001$), but not in husk and straw, as compared to the CM treatment (Fig. 2). Moreover, total arsenic in brown rice, husk and straw significantly differed with soil type ($P<0.001$).

Fig. 2. Total arsenic in rice plants (a: brown rice; b: husk; c: straw) grown in eight soils fertilized with two chicken manures without or bearing roxarsone metabolites (CM and ROXCM), respectively.

ns refers to not significant at 0.05 level.

3.3 Arsenic species in rice plant

Although ROX, 3-AHPA, 3-AAHPA, As(III), As(V), MMA, DMA and some unknown arsenic species were detectable in the ROXCM, only iAs, MMA and DMA were determined in brown rice from all soils fertilized with ROXCM, the same as those from soils amended with the control CM (Fig. 3a). Meanwhile, trace amounts of Tetra were found in brown rice from the Soil PY-CM, Soil AH-CM and Soil AH-ROXCM treatments as well. The occurrence of Tetra in rice grain was first identified by Hansen et al. (2011), and was found in rice grain from Hunan province, China by Jia et al. (2012).

Besides iAs, MMA and DMA, trace Tetra was only observed in the husk from the Soil AH-ROXCM treatment, while TMAO was detected in the husk from both manure treatments in Soil GZ, Soil PY, Soil AH and the Soil HB-ROXCM treatment (Fig. 3b).

All the five arsenic species were observed in the straw from all soils amended with two manures, suggesting that the appearance of Tetra and TMAO in rice plants were not associated with ROX metabolites in the manure (Fig. 3c). It has been documented that TMAO in rice plant is taken up by rice root from soil solution and cannot be converted by other arsenic species in rice plant (Arao et al., 2011; Jia et al., 2012).

Though, being observed in the ROXCM, ROX was not detectable in all the rice plants, which implies that rice could not take up ROX or ROX had completely disappeared before rice root could absorb it. In our previous study, ROX and its preliminary ROX metabolites such as 3-AHPA and 3-AAHPA completely degraded while rice plant was harvested in soils applied with CM bearing ROX metabolites (Yao et al., 2016). Since Tetra and TMAO in rice grain is not frequently found in previous investigations, it is uncertain whether manure application is beneficial to form these both arsenic compounds in flooded soil.

Additionally, DMA is the primary arsenic species in brown rice in this work since methylated arsenic species such as DMA and MMA are easily translocated to the above ground tissues (Zhao et al., 2010), which is different from the observation that

arenite is the predominant species in brown rice grown in soils directly spiked with external ROX (Liu et al., 2009). Hence, if ROX metabolites in the manure were neglected, the potential direct human health risk caused by rice consumption associated with ROX utilization in animal production would be exaggerated.

Fig. 3. Arsenic species in rice plants (a: brown rice; b: husk; c: straw) grown in eight soils fertilized with two chicken manures without or bearing roxarsone metabolites (CM and ROXCM), respectively.

ns refers to not significant at 0.05 level.

The concentrations of all arsenic species in rice plants were not influenced by manure type, with the exception of significantly increased DMA in brown rice fertilized with ROXCM as compared to that with CM ($P=0.002$). The elevated brown rice DMA can be explained by the dual effect of DMA supply in the ROXCM per se, and the promotion of organic matter from the manure (Yao et al., 2016). It indicates that though ROX and its metabolites occurred in the ROXCM, ROXCM application solely and significantly increased DMA in brown rice, which contributes to the significantly increased total arsenic in brown rice. However, soil type significantly

affected the concentrations of all the detectable arsenic species in rice plants
($P < 0.001$).

3.5 Effect of soil properties on arsenic uptake by rice plant

Stepwise regression analysis shows that total arsenic in both brown rice and husk from CM- or ROXCM-amended soils were solely dependent on soil free-iron oxides, with the variance of soil free-iron oxides accounting for approximately 93.7%, 88.6%, 92.5% and 89.4% of the difference of total arsenic in brown rice from both manure-amended soils at the level of < 0.001 (Table 4), respectively. However, straw total arsenic from both manure-amended soils was primarily determined by soil pH. The variation of soil pH explained approximately 66.9% and 74.9% of the variability of straw total arsenic at the level of ≤ 0.007 , respectively.

Although brown rice iAs in CM-amended soil was jointly influenced by soil pH, soil total arsenic and total Mn, soil pH dominated the discrepancy of iAs among soils with the variance accounting for 89.7% of the variability of iAs, and soil total arsenic and total Mn had minor effect. In ROXCM-amended soil, brown rice iAs was solely associated with soil pH. Brown rice DMA and MMA were dependent on soil free-iron oxides. The variation of soil free-iron oxides contributed to 83.6~94.9% of the total variance of brown rice DMA and MMA in both manure-amended soils at the level of < 0.001 , with the exception that soil pH explained 4.8% of the total variance of brown rice MMA in CM-amended soil. Though soil silt, soil total Mn or total Fe might be

affecting attributes, generally, arsenic species (iAs, DMA and MMA) in husk from both manure-applied soils were primarily dependent on soil free-iron oxides. For the five detectable arsenic species in straw from both manure-applied soils (with the exception of straw MMA in CM-amended soil), soil pH was the only significant variable determining arsenic accumulation in the straw ($P \leq 0.016$), with the variance of soil pH accounting for 64.2~89.0% of the total variability of arsenic species.

Totally, there was no significant difference for arsenic uptake by rice plants between CM and ROXCM, with the exception of significant enhanced brown rice DMA in the ROXCM treatment. This indicates that whether ROX metabolites were present in the CM, or not, they did not alter the role of soil property on arsenic uptake by rice plant. Moreover, soil factors governing the accumulation of total arsenic or arsenic species in rice plant varied with plant tissue and arsenic species. The assimilation of arsenic species in rice plants was enhanced predominantly by soil iron oxides and pH in the present study, which is explained by the following mechanisms. Firstly, iron oxides are the predominant arsenic adsorbents in hydromorphic soils/paddy soils (Vithanage et al., 2013; Zhu et al., 2019). The two manures used were abundant in organic matter, generating dissolved organic carbon into the soils, which desorbs more arsenic in soils rich in indigenous iron oxides binding arsenic and increases mobile arsenic in soil solutions for plant uptake (Syu et al., 2019; Williams et al., 2011). Also, soils with high pH are commonly low in arsenic retention

325 capability ([Syu et al., 2019](#); [Wang et al., 2015](#)), which facilitates arsenic assimilation
326 in rice plants.

327 **Table 4**

328 Regression models for arsenic in rice plant (Y) and soil property (variable) in soils fertilized with two chicken manures without or bearing

329 roxarsone metabolites (CM and ROXCM), respectively.

Y	Model	P value of variable	Partial R ² of variable	P value of model
Brown rice total arsenic in CM-amended soil	Y=0.0392Soil free-iron oxides	Soil free-iron oxide: <0.001	0.937	<0.001
Brown rice total arsenic in ROXCM-amended soil	Y=0.4312Soil free-iron oxides	Soil free-iron oxides: <0.001	0.886	<0.001
Husk total arsenic in CM-amended soil	Y=0.0609Soil free-iron oxides	Soil free-iron oxides: <0.001	0.925	<0.001
Husk total arsenic in ROXCM-amended soil	Y=0.0681Soil free-iron oxides	Soil free-iron oxides: <0.001	0.894	<0.001
Straw total arsenic in CM-amended soil	Y=0.6755Soil pH	Soil pH: 0.007	0.669	0.007
Straw total arsenic in ROXCM-amended soil	Y=0.6791Soil pH	Soil pH: 0.003	0.749	0.003
Brown rice iAs in CM-amended soil	Y=0.0322Soil pH + 0.0010Soil total arsenic - 0.0002Soil total Mn	Soil pH: 0.001	0.897	<0.001
		Soil total arsenic: 0.004	0.064	
		Soil total Mn: 0.047	0.022	
Brown rice iAs in ROXCM-amended soil	Y=0.0338Soil pH	Soil pH: <0.001	0.941	<0.001
Brown rice DMA in CM-amended soil	Y=0.0380Soil free-iron oxides	Soil free-iron oxides: <0.001	0.876	<0.001
Brown rice DMA in ROXCM-amended soil	Y=0.0458Soil free-iron oxides	Soil free-iron oxides: <0.001	0.836	<0.001
Brown rice MMA in CM-amended soil	Y= -0.0026Soil pH + 0.0039Soil free-iron oxides	Soil pH: 0.018	0.048	<0.001
		Soil free-iron oxides: <0.001	0.924	

Brown rice MMA in ROXCM-amended soil	Y=0.0029	Soil free-iron oxides	Soil free-iron oxides: <0.001	0.949	<0.001
Husk iAs CM-amended soil	Y=0.0008	Soil silt + 0.0244	Soil silt: <0.001	0.070	<0.001
		Soil free-iron oxides - 0.001			
		Soil Mg - 0.0012			
		Olsen-P			
			Soil free-iron oxides: <0.001	0.901	<0.001
			Soil Mg: 0.002	0.023	
			Soil Olsen-P: 0.035	0.005	
Husk iAs ROXCM-amended soil	Y=0.0169	Soil total Fe	Soil total Fe: <0.001	0.926	<0.001
Husk DMA in CM-amended soil	Y=0.0209	Soil free-iron oxides	Soil free-iron oxides: <0.001	0.854	<0.001
Husk DMA in ROXCM-amended soil	Y=0.0013	Soil silt - 0.0075	Soil silt: <0.001	0.710	<0.001
		Soil total Mn	Soil total Mn: 0.005	0.221	
Husk MMA in CM-amended soil	Y=0.0045	Soil free-iron oxides	Soil free-iron oxides: <0.001	0.951	<0.001
Husk MMA in ROXCM-amended soil	Y=0.0051	Soil free-iron oxides	Soil free-iron oxides: <0.001	0.952	<0.001
Straw iAs in CM-amended soil	Y=0.6938	Soil pH	Soil pH: 0.009	0.642	0.009
Straw iAs in ROXCM-amended soil	Y=0.6930	Soil pH	Soil pH: 0.002	0.770	0.002
Straw DMA in CM-amended soil	Y=0.0215	Soil pH	Soil pH: 0.009	0.642	0.009
Straw DMA in ROXCM-amended soil	Y=0.0233	Soil pH	Soil pH: 0.005	0.690	0.005
Straw MMA in CM-amended soil	Y=0.0006	Soil sand	Soil sand: 0.016	0.585	0.016
Straw MMA in ROXCM-amended soil	Y=0.0343	Soil pH	Soil pH: 0.007	0.670	0.007
Straw Tetra in CM-amended soil	Y=0.0012	Soil pH	Soil pH: 0.006	0.684	0.006
Straw Tetra in ROXCM-amended soil	Y=0.0012	Soil pH	Soil pH: 0.003	0.748	0.003
Straw TMAO in CM-amended soil	Y=0.0044	Soil pH	Soil pH: <0.001	0.822	<0.001
Straw TMAO in ROXCM-amended soil	Y=0.0047	Soil pH	Soil pH: <0.001	0.890	<0.001

It is noticeable that the increased DMA in brown rice caused by ROXCM application might not pose serious arsenic health impact to human due to its low toxicity to organisms (Zhao et al., 2010), however, it might lead to “straighthead” symptom, a physical disorder linked with arsenic (DMA in particular) in rice plant (Agrama and Yan, 2009; Limmer et al., 2018; Rahman et al., 2008; Yan et al., 2005). “Straighthead” is characterized by sterile florets, poorly developed panicles and yield loss, and reported in the U.S.A, Thailand, Japan, Australia, Argentina and elsewhere (Belefant-Miller and Beaty, 2007; Dunn et al., 2006; Takeoka et al., 1990; Weerapat, 1979; Yan et al., 2010). Incorporation of monosodium methylarsenate (Belefant-Miller, 2012; Yan et al., 2008), organic matter such as barley straw and stubble residues of rice or winter cereal (Williams, 2005) and external DMA (Limmer et al., 2018) easily induces or intensifies straighthead.

Though the biomass of rice plants, grain yield in particular in all soils were not inhibited by ROX metabolites in the present investigation, we are not sure whether ROXCM application facilitates the growth environment suitable for straighthead occurrence because cultivar difference of resistance to straighthead is indeed found in rice (Li et al., 2017; Yan et al., 2005) and the susceptibility of the used cultivar “Meixiangzhan” to straighthead was not be examined. It is noticeable that only nineteen cultivars, 18 indica and 1 japonica, among a total of 124 Chinese cultivars including 109 indica and 15 japonica, have been identified as straighthead resistant (Yan et al., 2005). Natural occurred straighthead disorder has not yet be documented

in China, but the possibility of straighthead disorder caused by application of animal manure containing ROX metabolites is worthy to be further investigated because crop losses range from 10% to 30% in medium grains and up to 90% in short and long grains in other countries ([Takeoka et al., 1990](#); [Williams, 2005](#)).

4. Conclusion

The growth of rice plant was not influenced by ROX metabolites in the manure, but significantly varied with soil type. Arsenic species detectable in rice plant were unrelated to ROX metabolites. ROX metabolites did not increase total arsenic or arsenic species in straw and husk, except that total arsenic in brown rice was significantly enhanced by ROX metabolites, most of which generated from significantly increased DMA. The accumulation of total arsenic and arsenic species in rice plant was governed by soil free-iron oxides or pH. The results indicate that animal manure bearing ROX metabolites should not be applied in soils rich in free-iron and/or with high pH to minimum the occurrence of straighthead disorder in rice.

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Declarations of interest

None.

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