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Abstract

Manganese oxides are naturally occurring powerful oxidants and scavenger which can control the mobility and bioavailability of arsenic (As). However, the effect of synthetic nanostructured manganese oxides on the mobilization and transportation of As at actual paddy soils are poorly understood, especially in the low or medium background Mn concentration soil. In the present study, a novel Nano manganese oxide with higher reactivity and surface area has been synthesized. A 90-d soil incubation experiment combined with pot and field rice cultivation trials were designed to evaluate the effectiveness of exogenous α-MnO_2 nanorods on the mobilization and transportation of As in soil-rice systems. Our results proved that the addition of α-MnO_2 nanorods can effectively control the soil-to-solution partitioning of As under anaerobic conditions. After treatment with different amounts of α-MnO_2 nanorods, the content of effective As decreased with the increasing of residual As and insoluble binding As (Ca-As and Fe-As). Besides, the enhanced oxidation of As (III) into As(V) by α-MnO_2 nanorods increased the adsorption of As onto indigenous iron(hydr) oxides which greatly reduced the soil porewater As content. Additionally, pot experiment and filed applications are further proved that the influx of As into aerial parts of rice plants (stems, husk and leaves) was strictly prohibited after treatments with different amount of α-MnO_2 nanorods; more interestingly, significantly negative correlations have been observed between As and Mn in rice, which indicated that as Mn is increased in soil, As in brown rice decreases. Our results demonstrated that the use of α-MnO_2 nanorods in As polluted paddy soil containing low levels of background Mn oxides can be a promising remediation strategy.

Keywords: Arsenic, Nanostructured-MnO_2, Rice, Accumulation, Paddy soil

1. Introduction

Hunan province is world-renowned for its luxuriant deposit of non-ferrous metal ores (tungsten, bismuth, realgar) (Lei et al., 2015; Okkenhaug et al., 2012; Williams et al., 2009). In the past several decades, intensive mineral exploitation, ore extraction and refining activities have caused a large amount of toxic trace elements (Cd, Hg, Pb and As) to be discharged into farmland which has greatly affected the local soil and water environment(Li et al., 2017; Zhao et al., 2015). Among them, arsenic is a ubiquitous and highly toxic metalloid element that has caused severe contamination in Hunan province(Lei et al., 2013; Lei et al., 2015). It is reported that the arsenic-contaminated farmland in Hunan province has already seriously impaired the development of agriculture and posed a serious threat to the health of local residents, because rice is a dominant staple food (Li et al., 2016; Liao et al., 2005).

The existence forms (speciation) of arsenic may be more important than the total arsenic in the soil, which determine its effectiveness and toxicity to organisms. It is generally established that trivalent As species are more toxic than their pentavalent counterparts because they binds to sulfhydryl groups(–SH), impairing the function of many proteins (Fu et al., 2016; Liao et al., 2005; Liu, 2005). In paddy soils, arsenic is predominantly present as the inorganic species arsenate and arsenite (Takahashi et al., 2004). The extent of As mobility and bioavailability in paddy soil is, in part, regulated by the type of minerals exist in the soil system and the oxidation state of As (Fendorf and Kocar, 2009; Ying et al., 2012). Generally, As(III) are far more mobile than As(V); and the relative content of arsenate and arsenite in paddy soil are primarily depending on the redox status of soil (Yamaguchi et al., 2011). Arsenate often exists in anionic forms (e.g., $H_2AsO_4^-$, $HAsO_4^{2-}$) under aerobic conditions with the content can account for 65-98% of total arsenic (Ohtsuka et al., 2013). On the contrary, arsenite takes an electrically uncharged molecule form(H_3AsO_3) under anaerobic reducing conditions (Eh<100mV; pH<9) (Han et al., 2011). The amount of Fe oxides in the soil plays an important role in controlling the concentration of As species in the soil solution; typically, As(V) is strongly adsorbed with metal-(oxyhydr) oxides, whereas As(III) is poorly associated with soil minerals owing to its feature of charge-neutral (Chen et al., 2006; Ehlert et al., 2014); and thus rendering it comparatively effective towards to plants uptake than the As(V) (Xu et al., 2017). However, it is well recognized that during the drastically aerobic-anaerobic transition within paddy fields, the absorbed As will released into soil porewater (Ohtsuka et al., 2013; Xu et al., 2017). The mobilization of As in flooded paddy fields is because of two main processes. Firstly, the reductive dissolution of iron(oxyhydr) oxides have been triggered by soil flooding which caused the sorbed solid phase arsenic releasing into the liquid phase (Lemonte et al., 2017; Weber et al., 2010; Yamaguchi et al., 2011). Secondly, the adsorbed As(V) is reduced to As(III) under the reductive conditions and the latter has a greater tendency to partitioning into the liquid phase than As(V) (Liu et al., 2015; Takahashi et al., 2004). Compared with other terrestrial plant, rice (*Oryza sativa L.*) is efficient in As uptake and translocation, because of the flooded conditions and highly expressed arsenic transporter (Si transporter, aquaporins and phosphate transporters) (Ma et al., 2008; Meharg, 2004; Meharg and Jardine, 2003). Thus, effective measures must be taken to reduce the bio-availability and mobility of As(III) in paddy soil during rice cultivation.

In current literature, several measures have been proposed for reducing the bio-availability of As in soils, such as amendments stabilization (biochar, natural minerals, etc.) (Kumpiene et al., 2008; Li et al., 2018), electro-kinetics (Balasubramanian et al., 2009), acid flushing (Beiyuan et al., 2017; Tokunaga and Hakuta, 2002), phytoremediation (Gilloaiza et al., 2016; Jankong et al., 2007) and agronomic mitigation strategies (Limmer et al., 2018; Seyfferth et al., 2018). However, those methods are hard to meet the actual demand of paddy fields remediation. Due to either their high-cost (Liu et al., 2018), vast energy requirements (Villen-Guzman et al., 2017), or long treatment times (Wan et al., 2016); above all, high cost (or unsustainability) hinder the application of many technologies in polluted farmland (Bontempi, 2017). Chemical stabilization methods, in particular, have been widely accepted in the remediation of As-contaminated soils because they are relatively cost effective (sustainability) and easy to operate and management. Recently, engineered nanoparticles stabilizer such as zero valent iron (Gil-Díaz et al., 2017; Gil-Díaz et al., 2016) and iron phosphate (vivianite) nanoparticles (Liu and Zhao, 2007) has been proved to be an advanced environmental remediation technologies, which could provide cost-effective solutions to some of the most intractable environmental restore problems due to their large surface areas and high surface reactivity (Zhang, 2003). For the variable valence elements (As), by consideration of regulatory measures (in situ oxidation by chemical amendments) to induction the transformation of As(III) to As(V) is considered to be a promising approach which can alleviate the associated environmental risks of As in paddy soil (Lin et al., 2017; Suda and Makino, 2016; Xu et al., 2017). However, to our knowledge, there are few related studies focused on the induction of As to be transformed into low-effective and low-toxicity forms using oxidants in paddy soil.

Manganese oxides are naturally occurring powerful oxidants that can effectively catalyze the oxidation of As(III) to As(V) under natural circumstances (Bruce A. Manning et al., 2002; Ehlert et al., 2014; Han et al., 2011; Lafferty et al., 2010). The As(III) oxidation by manganese oxides can occur across a wide pH ranged from 4.0~8.2, however, the oxidation rates are deeply associated with their structure, surface charge properties, mineral crystallinity and abundance (Oscarson et al., 1983; Scott and Morgan, 1995).The study conducted by (Bruce A. Manning et al., 2002) showed that As(III) can be quickly oxidized into As(V) in the presence of MnO_2 with only about 10% of As(III) was remined after 10 hours reaction. Besides, another profound and detailed researches conducted by Scott and Morgan (Scott and Morgan, 1995) who found that birnessite (δ-MnO_2) can quickly oxidize As(III) to As(V), about 80% of the reaction can be accomplished within 1h and this process was accompanied by the release of Mn^{2+}. Apart from the oxidation ability towards to As(III) by manganese oxides, it was also reported that As(III), after being oxidized by Mn-oxides, can subsequently be adsorbed onto the surfaces of MnOOH (oxidation intermediates) and ferric-(oxyhydr) oxide (Ehlert et al., 2014; Nesbitt et al., 1998); thus the partitioning of As into solution was restrained. Although there are many studies (BA et al., 2002; Ehlert et al., 2014) have been conducted on the oxidation of As(III) by manganese oxides, however, most of these studies are concentrated on pure minerals in aqueous solution.

To the best of our knowledges, there are very few related studies on the oxidation of As(III) by synthetic nanostructured-MnO_2 in actual paddy soils; especially in the low or medium background Mn concentration soil. In China, it has been reported that the content of Mn in soil was varied between 10-5532 $mg{\cdot}kg^{-1}$ with an average amount of 710 $mg{\cdot}kg^{-1}$(Liu et al., 1983). The Mn concentrations which below the average value of 710mg kg^{-1} can be classified as low manganese soil. Recently, a soil incubation experiment conducted by Xu et al (Xu et al., 2017) showed that additions of synthetic Mn oxide (hausmannite) in low background Mn content paddy soils can effectively control the partitioning of As from solid phase to liquid phase due to the oxidation of As(III) However, they only have considered the efficiency of micrometer scale Mn oxides under the laboratory conditions but did not test it under complex field trials. Therefore, we have proposed a hypothesis that the endogenous iron oxides in paddy soil can be used to retain the oxidized As(V) after incorporation with nanostructured manganese oxides under flooded conditions, thereby reducing the bioavailability of As towards to rice. The major objectives of this present study are therefore to (i) investigated the potential of synthetic α-MnO_2 nanorods on the control of solid to solution distribution of As under flooded conditions; (ii) determined whether synthetic α-MnO_2 nanorods could reduce As uptake into rice grow in paddy soil with low endogenous Mn concentration at fields scales; and (iii) elucidated the associated mechanisms regarding to the reduced of As uptake by rice.

2. Material and methods

2.1 Chemicals and reagents

All chemicals, including manganese sulfate ($MnSO_4{\cdot}H_2O$), potassium persulfate ($K_2S_2O_8$), ammonium phosphate ($(NH_4)_3PO_4{\cdot}3H_2O$), urea ($CO(NH_2)_2$), potassium carbonate (K_2CO_3), hydrochloric acid (HCl), nitric acid (HNO_3) and sulfuric acid (H_2SO_4) used in this study were of analytical grade without any further purification. The α-MnO_2 nanorods was synthesized following the protocol previously outlined by(Yu et al., 2013) with minor modification; Briefly, the α-MnO_2 nanorods were hydrothermally synthesized using a solution containing a certain amount of $MnSO_4{\cdot}H_2O$ (0.3415 g, 2 mmol) and $K_2S_2O_8$ (0.5434 g, 2 mmol), the detailed procedures can be found in supplementary material. The obtained α-MnO_2 nano materials are a very stable black powdery solid. The powder X-ray diffraction (XRD) patterns of the obtained materials were recorded on a Bruker D8 Advance XRD diffractometer with Cu Kα radiation (Voltage: 40 kV; Current: 40 mA; Scanning rate: 10°/min). The morphologies of the samples were observed by emission scanning electron microscopy (SEM, Quanta F250, FEI, USA) Ultrapure water (18.2 MΩ cm) was used in all experiments, unless otherwise stated. All experimental containers were soaked with 10% HNO_3 overnight and rinsed several times with deionized water before use.

2.2 Site characterization and soil sampling

Chenzhou City lies between 24°53′ and 26°50′ latitudes and between 112°13′ and 114°14′ longitudes(Lei et al., 2015). The research paddy field is located in Dengjiatang (25°36'N, 113°00'E) village, Su Xian district, Chenzhou City. An As-product factory was located at Dengjiatang in 1992, but it has been out of production since 1999(Lei et al., 2015; Liao et al., 2004; Liao et al., 2005).

Bulk arsenic contaminated soil samples were collected from the plow layers (0-20cm). The soil samples were air-dried at room temperature, ground, and passed through a 5-mm nylon sieve. The elementary physicochemical properties were analyzed, and the results were shown in Table S1 in supplementary material.

2.3 Soil incubation and As sequential extraction experiment

Soil incubation experiments were designed to explore the effect of α-MnO_2 nanorods on the variations of As fractionation in flooded paddy soil. To prepare the *α*-MnO_2 treated samples, 15 kg sieved soil was weighted carefully and packed into a polyethylene pot (50 cm×22 cm). Subsequently, the soil was amended with *α*-MnO_2 nanorods to maintain the rates of 0.2%, 0.5%, 1.0% and 2.0% of soil weight. The pot was first pre-incubated for 24 h in the dark with soil moisture content being maintained at 70% field water holding capacity. After that, the pots were incubated at 25°C in the growth chamber, with daily additions of ultra-pure water to maintain the water level of 3cm above the soil surface.

After 90 days, the soil samples were collected from the surface (0-20 cm depth) of the soil profile. After being air-dried at ambient temperature, obtained soil will first ground to pass through 1mm screen, and then ground again using agate mortar and passed through 0.15 mm screen prior to analysis. A sequential As fractionation schemes was employed to determine the operationally defined As fractionation(Van et al., 2003; Wu et al., 2006). The detail operation procedure was described in supplementary material. (Table S2). All the treatments and extractions procedures were run in triplicates unless stated otherwise. The extraction efficiency of arsenic fractionation was presented in Table S3.

2.4 Pot experiment designs

The pot experiment was carried out in a greenhouse of Hunan agricultural university. Firstly, 15.0 kg homogenized arsenic contaminated soil was packed in each polyethylene pot with a height of 50 cm and a diameter of 22 cm. Ammonium phosphate ($(NH_4)_3PO_4 \cdot 3H_2O$), urea ($CO(NH_2)_2$), potassium carbonate (K_2CO_3), were added to each pot as basal fertilizers at dosage of 4.29, 2.93, and 3.30 g for N, P, and K supply, respectively. Then the soil was amended with α-MnO_2 nanorods at rates of 0.2%, 0.5%, 1.0% and 2.0% of soil weight. Each pot was then saturated with distilled water and drained down to an equilibrium state for 7 days under natural conditions. All the treatments were triplicated and randomly arrangement and three blank controls (without α-MnO_2 nanorods addition) were provided.

The rice seeds (*O. sativa L* Yuzhenxiang, obtained from Hunan Rice Research Institute) were disinfected in 30% H_2O_2 solution for 10 minutes, followed by thorough washing with deionized water and soaking in deionized water for 24 h. Rice seeds were germinated in moist vermiculite trays until the three-leaf stage and then transplanted into pots. During the whole growth period, all pots were irrigated with distilled water daily to maintain the water level of 3 cm above the soil surface. Porewater samples were collected at a 15-days interval after tillering stage (after 45 days) using a porous fiber tube. The pH, Mn and As concentrations in soil solutions were analyzed. Rice was harvested at the 105th day. At each growth stage (tillering, heading and maturing stages) rice plants were collected, digested, and then the concentrations of Mn and As in the organs (roots, stems, leaves, husk and grains) of rice plants were analyzed.

2.5 Field application experiment designs

2.5.1 Experimental design

The field experiment has carried out in May 2013 at a paddy field in Dengjiatang, Chenzhou City, Hunan Province, (25°36'N, 113°00'E). The paddy fields were divided into 1.5 m ×1.5 m sub-plots with a 40-cm buffer zone between each. Before rice planting, the top soil (0–20cm depth) was subject to manual plowing. α-MnO_2 nanorods was then added into the soil at a rate of 0.2%, 0.5%, 1.0% and 2.0% of soil weight (0-20 cm), respectively, and thoroughly mixed with top soil. All treatment was conducted in triplicate with a completely randomized factorial design, and three blank controls (without α-MnO_2 nanorods addition) were provided. Each plot was then let to equilibrium for 7 days under natural conditions. Rice cultivars used in the pot experiment (Yuzhenxiang) were also used in the field experiment. Rice seedlings were transplanted after germination for 30 days (on June 6, 2013) and harvested on September 30, 2013. To facilitate in situ sampling of pore water, during the growth of rice, ‘Rhizon’ soil solution samplers (Rhizon Research Products, Wageningen, The Netherlands) were buried in each plot while the rice was transplanted. During the whole growth period, water layer of about 3.0 cm above the topsoil of the paddy field was maintained. The other cultivation methods were the same as the local paddy cultivation methods until the rice has matured.

2.5.2 Sampling and, analyses

Extractions of pore water were conducted in the tillering stage (45 days after germination) and were extracted every 15 days during the last period (days 45-105). The soil solution pH was recorded at the same time intervals as for pore water sampling. The concentrations of Mn in pore water were measured in acidified subsamples by inductively coupled Plasma optical emission spectrometer (ICP-OES, PerkinElmer Optima 8300, USA). As concentrations in soil pore water was determined by atomic fluorescence spectrometer (AFS-920, Beijing Titan Instruments Co., Ltd.). At each specifically growth period, the plants sample was collected at the tillering, heading and maturing stages. Rice plant samples were separated into roots, stems, leaves and grain. And washed three times using distilled water, the cleaned plant samples were placed in an oven at 105°C for 2 h and dried at 70°C for 3 days to constant weight, and then ground to pass a 100-mesh sieve with a micro plant grinding machine.

The method for total As digestion and determination in rice plants was conducted following the protocol of GB/T 5009.11-2003 which issued by Ministry of Environmental Protection (MEP) of China (Geng et al., 2017). Typically, 1.0 g of dry rice sample was digested using a mixture of acids (4:1 HNO_3: $HClO_4$, v/v) at 180°C on a graphite digestion furnace. After digestion, the solution will let to cool down to ambient temperature and made to 25 mL using UP water, each digested solution will store in 50mL polyethylene bottle at 4°C before analysis. As concentrations in plants tissues will determined by atomic fluorescence spectrometer (AFS-920, Beijing Titan Instruments Co., Ltd.), Mn concentrations will measure by ICP-OES (Optima8300 PerkinElmer). For quality assurance and quality control purposes, blanks and standard plant reference material (shrub branches and leaves GBW07603 (GSV-2), rice material (GBW10010 (GSB-1) were obtained from China Standard Materials Research Center, Beijing, P.R. China and digested along with the unknown samples and used for the QA/QC program. All the glassware was washed with detergent firstly, soaked with 20% HNO_3 solution for 24 h, and then rinsed with UP water for three times before use.

2.6 Statistical analyses

All statistical analyses were performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Differences between the control and treatment samples were determined using ANOVA and Tukey multiple comparisons analysis with p<0.05

indicating statistical significance. Correlations were obtained by Pearson correlation coefficient in bivariate correlations.

3. Results

3.1 Characterization of synthesized α-MnO_2 nanorods

The x-ray diffraction (XRD) pattern and scanning transmission electron microscopy (SEM) images of the synthesized α-MnO_2 nanorods are shown in Fig.S1, Fig.S2, respectively. It can be seen that manganese dioxide is clustered; the structure is uniformly well dispersed spherical agglomerate particles. High-magnification SEM images showed that the α-MnO_2 is an urchin-like spherical with a diameter of 1-1.5 μm, which consists of several straight and radially grown nanorods with uniform diameter around 30-40nm (Fig.S2a, b). The synthesized α-MnO_2 nanorods has an obvious characteristic diffraction peak in the XRD pattern (Fig.S1), which indicated that the high crystallization degree of nanostructured manganese dioxide, and the characteristic peak was also in agreement with the standard data given in its JCPDS card (24-0072).

3.2 Effects of different amount of α-MnO_2 nanorods on distribution of arsenic fractionation

The As sequential extraction procedure is widely used to evaluate As distribution within soil fractionation, which can help us to understand the mobility and bioavailability of As in soil(Jin et al., 2011; Wenzel et al., 2001; Zhang et al., 2017).The effect of different dosages of α-MnO_2 nanorods on the distribution and percentages of Arsenic fractionation in paddy soil are presented in Fig.1.

In general, the application of α-MnO_2 amendment increased the residual fractionation and reduced the effective forms of As to some extent, indicating that α-MnO_2 can effectively control the bioavailability of As in soil. As shown in Fig.1, in the treated and untreated soil, loosely bound As comprised the smallest proportions of all the As fractionation (<3%); what's more, the content of loosely bound As in the soil with 1.0% MnO_2 treatment was significantly lower than that in other treatment. However, residual fractionation contained the most proportions of As (about 50%), whether or not to add α-MnO_2 into soil. Besides, proportions of residual As in the 1.0% MnO_2 treated soil was increased to 57.08% which indicated that the addition of 1.0% MnO_2 increased the residual As and reduced the other forms of As in the soil.

For binding state-As (Al-As, Ca-As and Fe-As), there was no significant difference in the content of Al-As in the soil after treatment with different amounts of α-MnO_2, whereas Ca-As decreased to 10.23% after treatment with 2% α-MnO_2; and with the increase of concentration, Ca-As content showed an obviously decreasing trend, which indicated that the addition of α-MnO_2 in paddy soil has a certain inhibitory effect on the binding of As and Ca.

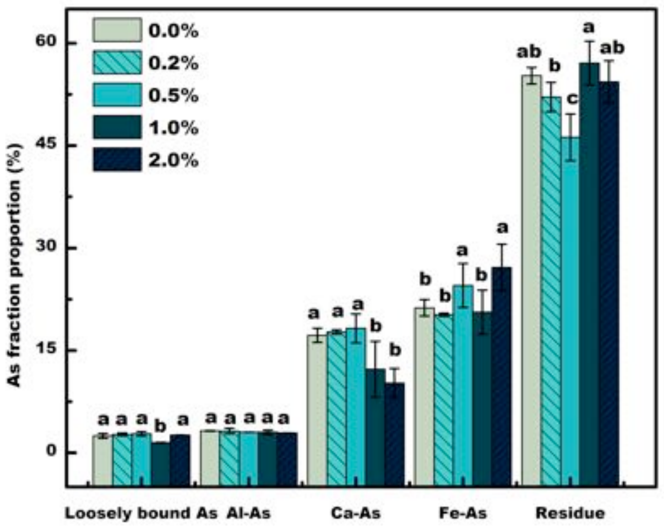


Fig.1. Fraction distributions of arsenic in paddy soil amended with different amounts of α-MnO_2 ;different letters indicate significant difference between different treatments (*P* < 0.05), arsenic fraction tested was separately from each other.

For Fe-bound As, there was no regular change of Fe-As in the soil after the addition of α-MnO_2. However, it can be seen that the content of Fe-As in soils increased to 24.53% and 27.16% at rates of 0.5% and 2.0%, respectively. However, at dosages of 0.2% and 1%, the amount of Fe-As in the soil has reduced, and the addition of 1% was more obvious, which was 20.62%.

3.3 Pot experiments

3.3.1 Effect of α-MnO_2 on the dynamic pH variations of soil solution

The dynamics variations of pH in the pore water during growth of rice under treatments of different amounts of α-MnO_2 were presented in Fig.S3. In general, during the whole growth stage (45-105 days), the soil pore water pH either increased to or remained stable in the near-neutral range after amended with different amounts of α-MnO_2. The pH range of the soil solution was around 7.36-7.55 for the 45th day, which was higher than the pH (7.25) of CK (Control treatments). The highest pH value of the pore water was recorded at the 45th day (7.25-7.55). Afterwards, the pH of the pore water decreased with the rice growth and reached the lowest value (6.28-6.76) at the 105th days. Compared to the control, α-MnO_2 treatments slightly reduced the pH of the paddy soil under flooded condition, the pH was reduced by 0.58-1.06 unit after supplementation with α-MnO_2.

3.3.2 Effect of α-MnO_2 on the dynamic variations of As and Mn in pore water

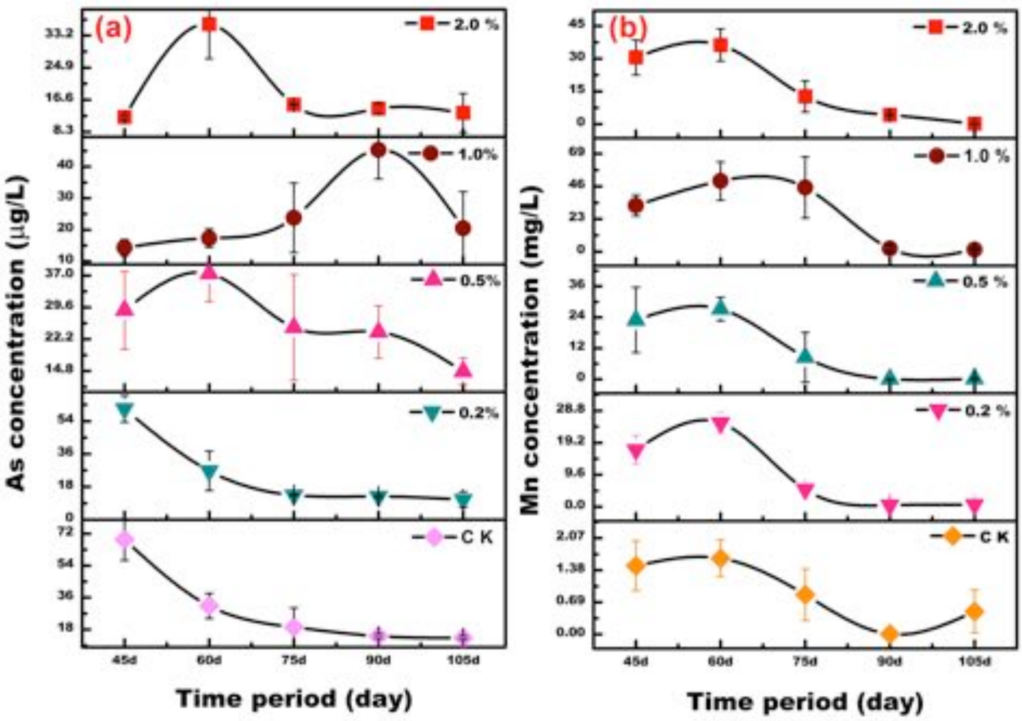


Fig.2. Dynamics variations of pore water As (a) and Mn (b) in the α-MnO_2 treatments throughout the rice cultivation period. Data are means SE (n= 3).

After treatments with different dosage of α-MnO_2 the dynamic variation of arsenic and manganese content in pore water at different growth period of rice is shown in Fig.2. Different reduction patterns of arsenic in soil pore water were observed among with different treatments. Regardless of the addition of α-MnO_2, the arsenic content in soil pore water of CK was decreased from the 45th day to the 105th day, and reaching the minimum value of 13.26±0.94 $\mu g \cdot L^{-1}$ at the 105th day; possibly due to the adsorbed by newly formed endogeneity amorphous iron oxides (Amstaetter et al., 2012; Cismasu et al., 2015). However, compared with CK, the content of arsenic in pore water after adding α-MnO_2 with different dosages decreased markedly at the 45th day. All treatments showed an obvious dosage dependent reduction trend of arsenic in soil pore water. Similarly, the decline trend was observed between CK and 0.2% treatments; however, with the increasing of dosages the content of arsenic began to rebound after 45 days. Arsenic concentrations peaked at 60 days of reaction at 0.5% treatments; 90 day for 1.0% treatments and 60 day of 2.0% treatments. In spite of fluctuation, what worth affirming was that arsenic in soil pore water still decreased to a constant level (10.94-14.69 $\mu g \cdot L^{-1}$) at the end of rice cultivation period (90-105 day), except for 1% treatment, nearly 2-fold (20.49 $\mu g \cdot L^{-1}$) increased in porewater As was observed during 90-105 day.

Unlike As, α-MnO_2 treatments increased porewater Mn levels (Fig.2b), four treatments (0.2%, 0.5% ,1.0% and 2.0%) showed that substantial increased in the porewater Mn concentration at the 45th day, whereas Mn concentration in the CK soil porewater remained low level throughout the whole cultivation period (45-105 days). What's more, Mn concentrations kept increasing after another 15 days; with 17-33 folds augment compared with that of control soil. This is more likely coupled with the enhanced dissolution of manganese resulting from oxidation of As(III) to As(V) by manganese oxide (Xu et al., 2017; Z et al., 2017). Yet, effluent Mn(II) concentrations in soil porewater began to drop at the 60th day, with concentrations declining to a constant level of 0.13-0.60 mg·L^{-1} after 90 days of cultivation. Furthermore, among all the four treatments, 1.0 % retained the highest porewater Mn dissolution rate compared with other treatments during day 45-75 days and reaching a peak of 49.77 mg·L^{-1} on the 60th day. In the meantime, however, the other three treatments have already dropped to low levels (5.2-12.6 mg·L^{-1}).

3.3.3 Effect of α-MnO_2 on the accumulation of As and Mn in rice plants parts during different growth stage

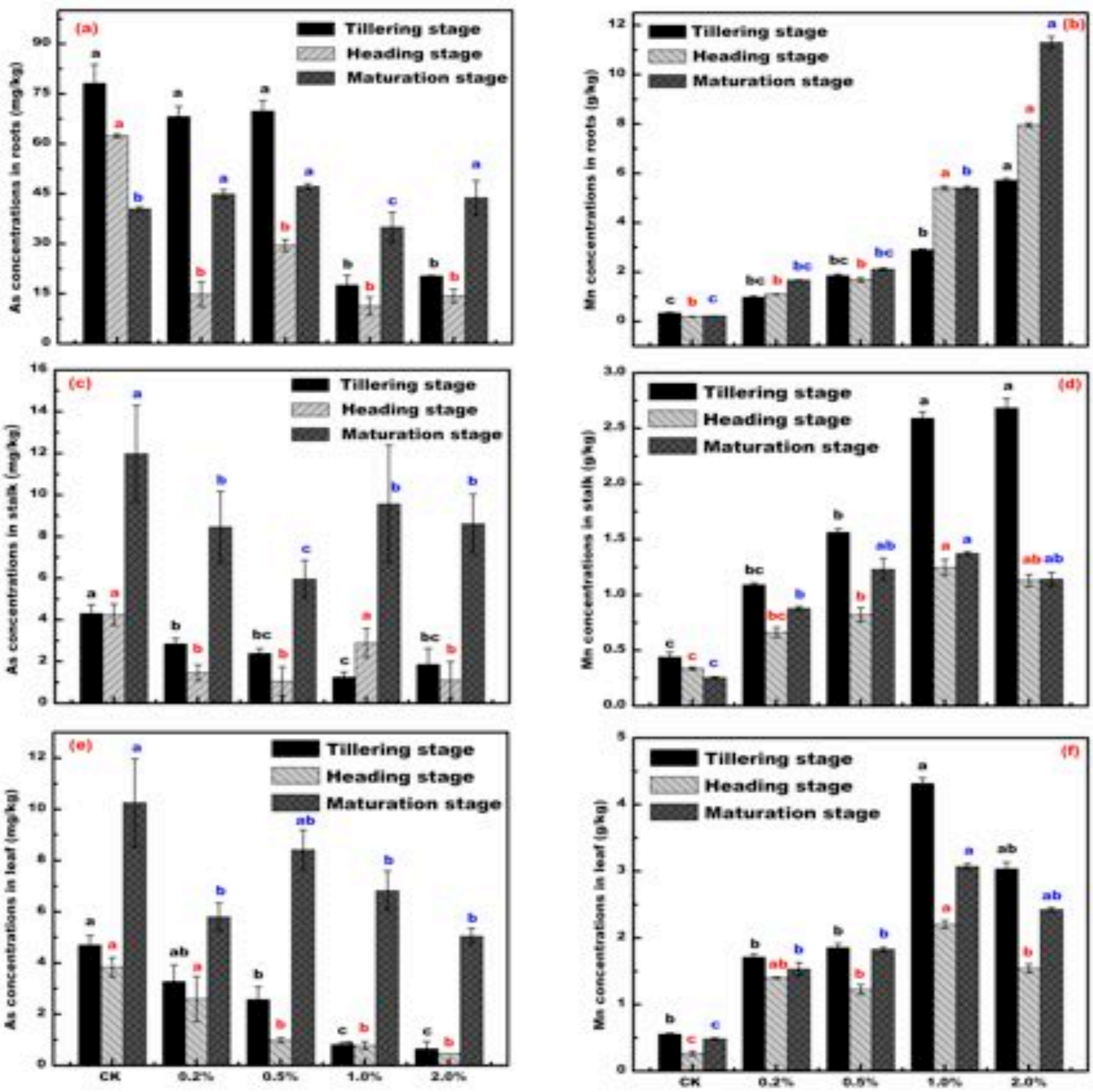


Fig.3. As and Mn concentrations in the root (a, b), straw (c, d), leaf (e, f) in the α-MnO_2 treatments; Data are

means SE (n= 3), different letters indicate significant difference between different treatments (*P* < 0.05), significant tests are separate from each other (black letter indicates tillering stage, red letter indicates heading stage, blue letter indicates maturation stage)

The contents of As and Mn in different parts of rice at various growth stages are shown in Fig.3. Three different rice growth stages (Tillering, heading and mature stage) were selected to estimate the efficiency of α-MnO_2 in controlling the accumulation of As and Mn in rice. In general, regardless of the addition of α-MnO_2, the distribution pattern of arsenic in rice roots, stems and leaves follows the order of roots > stems > leaves. However, with the addition of α-MnO_2, the arsenic content in various parts of rice at each growth period was reduced to varying degrees compared with the CK. That is, the addition of α-MnO_2 can effectively impede the migration of arsenic to rice plants. In α-MnO_2 treatments, root As was decreased by 10.63-77.51% at tillering stage (Fig.3a), 52.71-81.94% at heading stage compared to the control. However, the interesting thing is that the reduction rate of arsenic in the mature stage was much lower than that of the other two growth period, which was indicated that the heading and tillering stage may be the key time period to control the transportation and migration of As (Li et al., 2015; Zheng et al., 2011).

On the contrary, the distribution pattern of Mn content in rice roots was clearly different from that of As in rice. Compared with CK, the Mn content in various parts of rice plants has significantly increased ($p<0.05$) at different growth stages after addition of α-MnO_2 and the increasing trend was obviously dosage dependent, which illustrated that the order of Mn in roots, stems and leaves of rice was: 2.0% > 1.0% > 0.5% > 0.2% > CK (Fig.3b). The overall Mn concentration in rice roots was increased by 1.9-15.87 times at tillering stage, 4.58-39.46 times at heading stage and 6.5-49.89

times at the mature stage, respectively.

Although α-MnO_2 treatments were effective in reducing and accumulating of As and Mn in rice roots; their effects, however, on As, Mn transportation in the straw and leaf was less satisfactory. For the re-distribution of As in rice stalk and leaf, regardless of the addition of α-MnO_2 and rice growth period sequences, the higher accumulation efficiency were observed at mature stage for both stalk and leaf; which indicated that mature stage were the crucial period for the translocation and migration of As in rice (Fig.3ce). However, the accumulation of Mn in stalks and leaves showed different patterns (Fig.3df), rice tended to accumulate more Mn during tillering stage, which was probably due to the enhanced dissolution of Mn in soil porewater at early stages (45-60 days) of rice growth after amended with α-MnO_2 nanorods.

In addition, the observed results also implied that the re-distribution of As in rice part did not interfered by the added α-MnO_2 after the tillering stage and this phenomenon was less time-dependent; which also confirmed that the As was sequestered in rice roots and thus the subsequent influx of As into other rice parts was restrained (Fig.3ce).

The content of As and Mn in brown rice and husk can further prove this phenomenon (Fig.4). The addition of α-MnO_2 Nano-rods significantly reduced the total arsenic content in brown rice and husk ($p<0.05$), while the Mn content was significantly increased ($p<0.05$). Compared with CK, the contents of total arsenic in the husks decreased by 36.4%, 24.0%, 12.6% and 15.5%, respectively. Furthermore, the content of total arsenic in brown rice was decreased by 17.8%, 36.4%, 65.4% and

60.7%, respectively, which demonstrated that the addition of α-MnO_2 nanorods can effectively prevent arsenic uptake by brown rice. Meanwhile, Mn concentrations in rice husk and brown rice increased in a dosage dependent way, which demonstrated that the Mn concentrations increased with increasing dosages of α-MnO_2 nanorods. The content of Mn in the husk increased by 55.8%, 79.3%, 102.0% and 133.3%, respectively; and the content of Mn in brown rice increased by 148.7%, 174.6%, 295.5% and 310.4%, respectively (Fig.4).

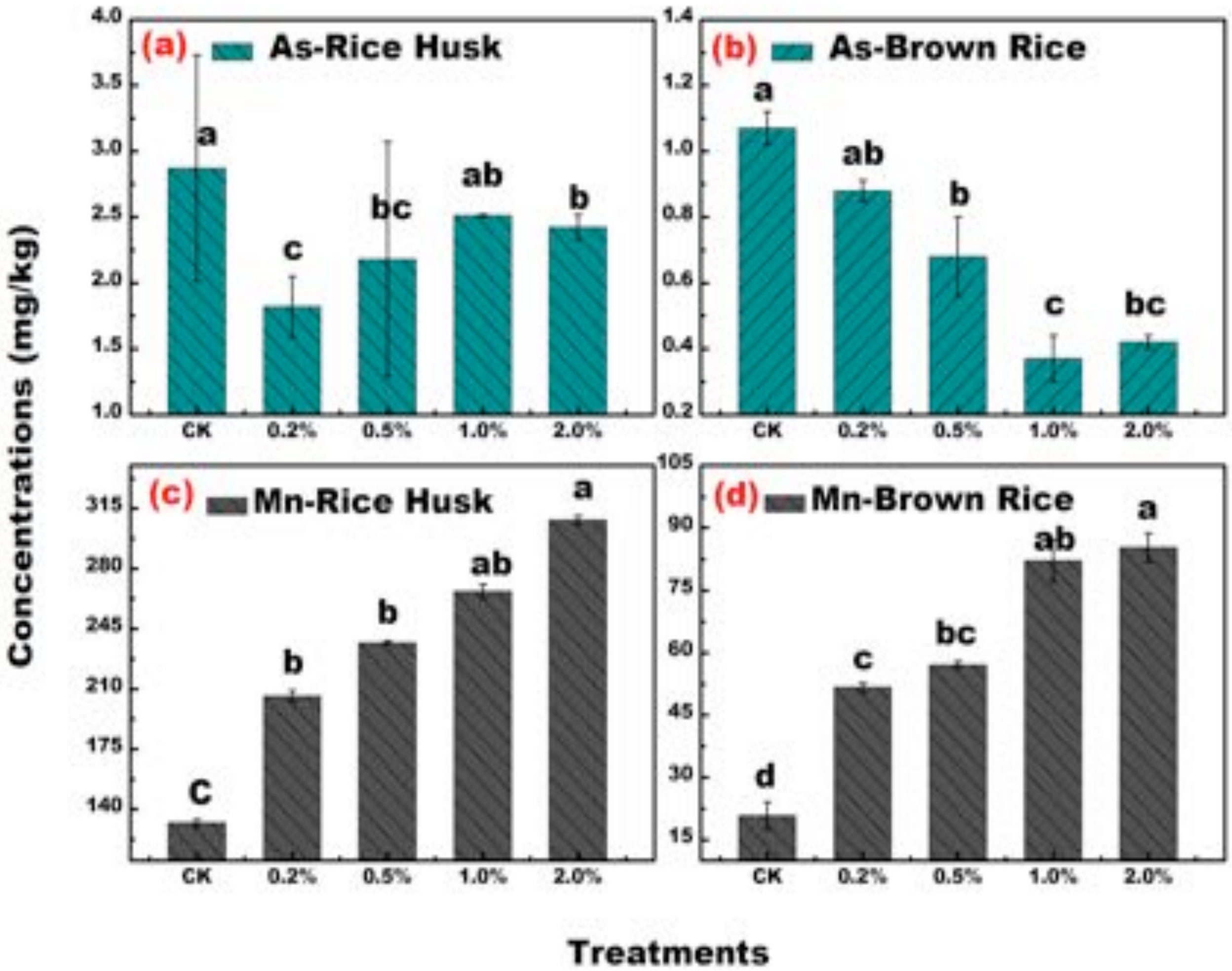


Fig.4 Concentrations of As and Mn in rice husk and brown rice (Pot experiment),(a) As-rice husk, (b) As-brown rice,(c) Mn-rice husk,(d) Mn-brown rice; Data are means SE (n= 3), different letters indicate significant difference between different treatments (*P* < 0.05).

3.3.4 Relationships between As and Mn concentration in rice plants parts under different growth stages

To further identify the relationship between the concentrations of As and Mn in rice (Fig.S4) and different growth stages (Fig.5), we performed a correlation analysis; and the results were presented in Fig.S4 and Fig.5 The concentration of As showed an obviously significant negative correlation (p<0.05) with the amount of Mn in the rice, indicating that the As content of rice plants decreased significantly with the increase of Mn concentrations. And this also indicated that the application of α-MnO_2 nanorods was extremely effective at decreasing the available As in the soil

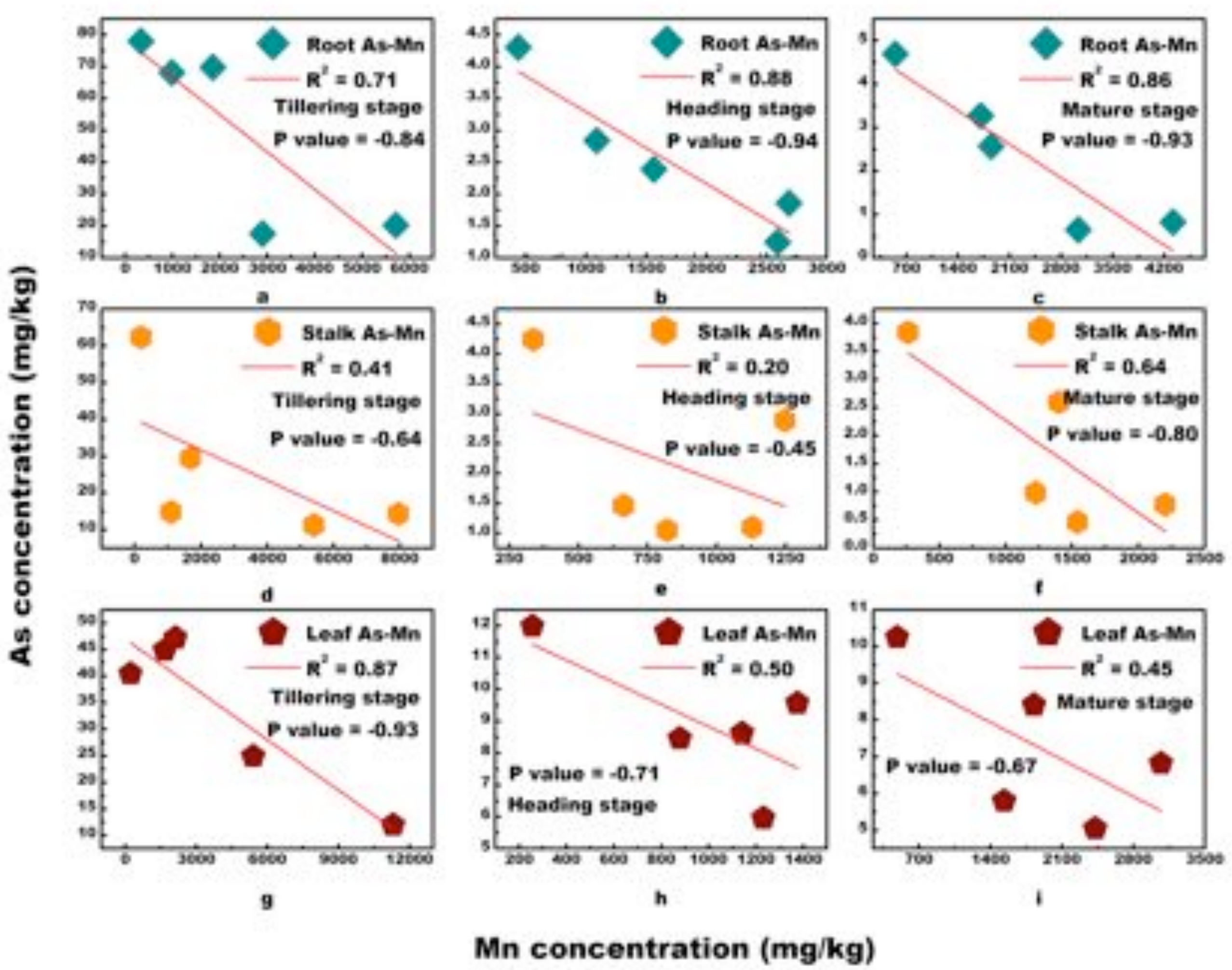


Fig.5. Relationships between As and Mn concentration in rice plants during different growth stage (a, b, c,

root-As-Mn), (d, e, f, stalk -As-Mn) and (g, h, I, leaf-As-Mn); Data are means SE (n= 3).

In the meantime, the relationships between As and Mn in different rice parts at different growth stages were also analyzed, and the correlations were presented in Fig.5, as can be seen from the above picture, overall As and Mn correlations in various parts showed obviously negative correlations. Especially for root As and Mn, their correlation showed higher R^2 value compared with other two parts, regardless of the growth stages (R^2=0.71-0.88, p<0.05; Fig.5abc). In contrast, although at certain growth stages, the As and Mn in rice parts showed extremely high significance; their overall relationships in stalks (R^2=0.20-0.63; Fig.5def) and leaves (R^2=0.44-0.87; Fig.5ghi), however, were poor than roots As-Mn.

3.4 Field applications

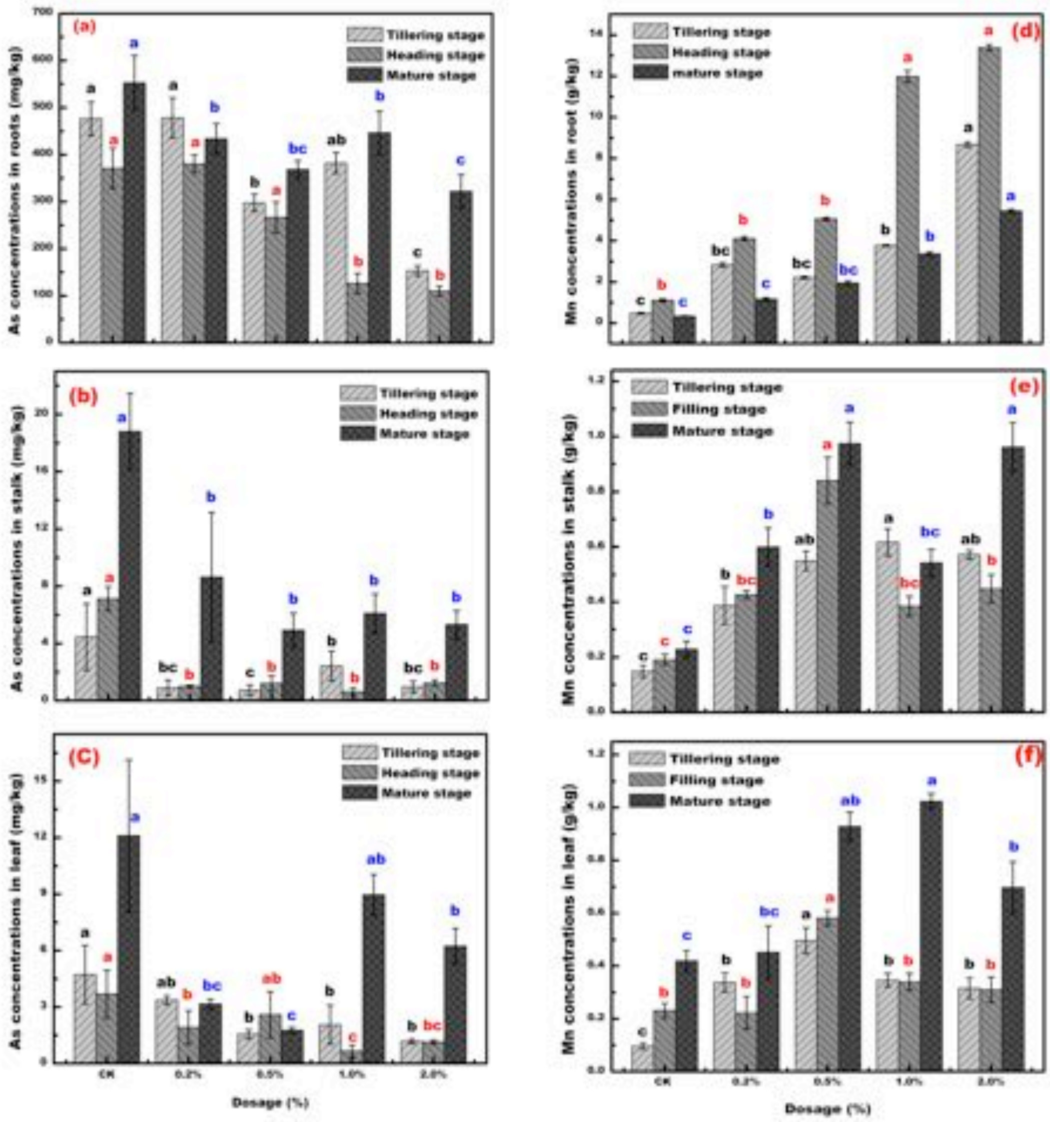
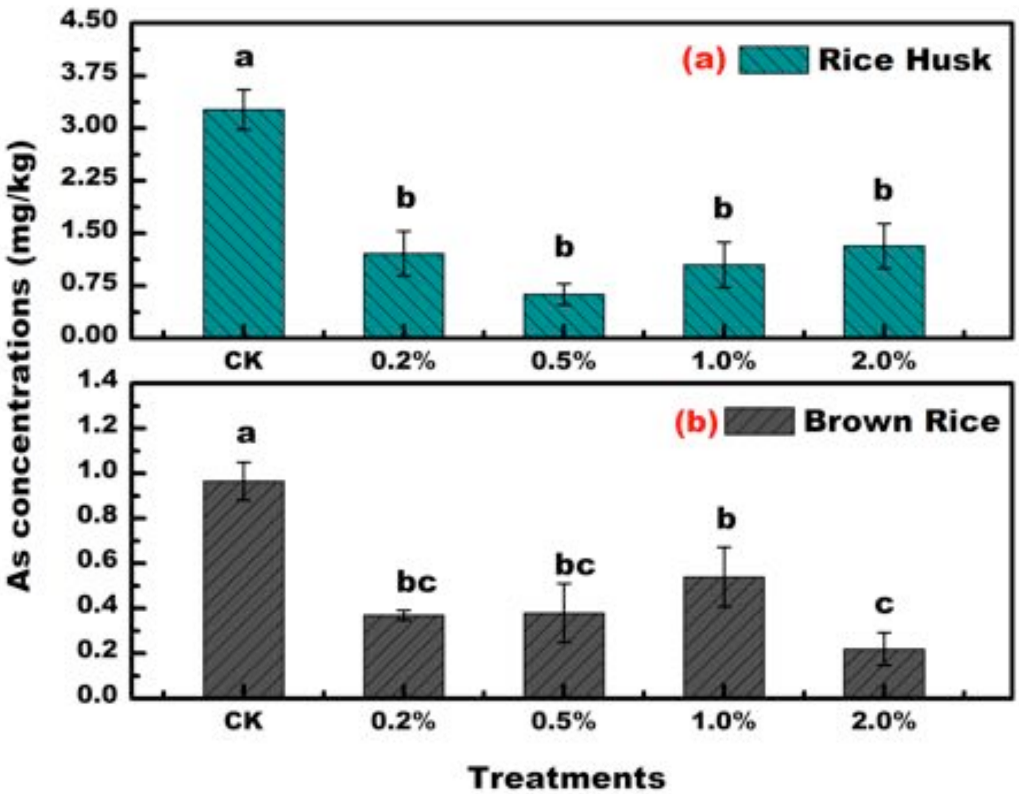


Fig.6. As and Mn concentrations in the root (a, d), straw (b, e), and leaf (c, f) of rice in the α-MnO_2 treatments (Field application); Data are means SE (n= 3), different letters indicate significant difference between different treatments (*P* < 0.05), significant tests are separate from each other (black letter indicates tillering stage, red letter indicates heading stage, blue letter indicates maturation stage)

Previous studies have shown that the addition of α-MnO_2 nanorods in arsenic-contaminated soil can effectively control the transport of arsenic from soil to rice in pot experiments. This result provides a theoretical basis for the application of α-MnO_2 nanorods in field trials. With this in mind, field trials were designed to further evaluate the effectiveness of α-MnO_2 nanorods in controlling the mobility and bio-availability of arsenic in soil-rice interfaces under complex natural systems. The content of arsenic and manganese in different parts of rice at various stages in the field experiment was shown in Fig.6. With the addition of α-MnO_2 nanorods, the arsenic content in various parts of rice at each period was reduced to varying degrees compared with the CK group (Fig.6b). It was also can be seen that the arsenic concentration in rice roots was much higher than that in the stalks and leaves. Furthermore, the similar results have been observed in stalks and leaves compared with pot experiment, in which rice tended to accumulate more As at mature stage. And the observed results also indicated that most of the As was sequestered in rice roots (Fig.6bc). Meanwhile, after treatments with different amounts of α-MnO_2 nanorods, the content of Mn in various parts of rice plants increased significantly (p<0.05) at different growth periods compared with the CK. And this result was consistent with the results obtained in the pot experiment, which also proved that the α-MnO_2 nanorods can indeed reduce the bio-availability of arsenic in soil. After treatment with α-MnO_2 nanorods, total As content in brown rice and husk was significantly reduced (Fig.7; p<0.05). Arsenic content in the husk of CK was 3.06±0.41mg·kg^{-1} and 0.96±0.08mg·kg^{-1} in brown rice. Compared with CK, the contents of total As in husks decreased by 60.5%, 79.6%, 65.7%, and 56.9%, respectively; and the content of total As in brown rice was decreased by 61.5%, 60.4%, 43.4%, and 77.1%, respectively. Among them, the lowest content



(0.22mg·kg^{-1}) of As in brown rice was obtained under the condition of 2.0% treatments.

Fig.7. Concentrations of As in rice husk and brown rice (Field application), (a) rice husk, (b) brown rice; Data are means SE (n= 3), different letters indicate significant difference between different treatments (*P* < 0.05).

4. Discussion

4.1 Effects of different dosages of α-MnO_2 nanorods on the solid-liquid partitioning of As in paddy soil

Since long is it established that the toxicity, activity and bioavailability of As in paddy soil are closely related to its presence in the soil (Yamaguchi et al., 2011). The hazards of As in soil are not only related to its content, but also related to its effectiveness in the soil and its associated binding forms (classification). In the present study, a 90d soil incubation experiment combined with sequential extraction procedure, therefore, was conducted to study the arsenic fractionation transformed in soil under flooded conditions after amended with α-MnO_2 nanorods; because it can furnish us an indication for the mobility and bioavailability of As in paddy soil after amended with α-MnO_2 nanorods. As can be draw from the above results (Fig.1), after treatment with different amounts of α-MnO_2 nanorods the content of effective As decreased with the increasing of residual As and insoluble binding As (Ca-As and Fe-As), indicating that supplementation of α-MnO_2 nanorods can effectively control the bio-availability of As in soil and reduced the associated influx of As into rice plants. Generally, loosely bound As (water soluble and exchangeable As) are highly bio-availability, and they are easily absorbed by organisms, resulting in greater toxicity. The Fe-As and other encapsulated state As are not easily absorbed by organisms and enter the water body, their harmfulness is relatively low. Fe-As, Al-As bind closely to soil, their toxicity to organisms are generally less than Ca-As.

(Herreweghe et al., 2003)found that water-soluble As and exchangeable As are soluble As in the soil or As adsorbed on the surface of soil particles, which accounts for less than 3% of total As. Similar results have been observed in our experiment, the proportion of loosely bound As in the soil was less than 3% regardless of the addition of α-MnO_2 nanorods, which indicated that the mobilization pool of As in paddy soil depends on other forms. Thus, the content of binding As (such as Fe-As, Al-As) may be the most contributing component in soil. Under aerobic conditions, As(V) is strongly adsorbed on most mineral constituents, including Fe and Al(hydr)oxides (Goldberg, 2002) whereas, arsenite is more mobile because of its poor affinity for mineral surfaces (Han et al., 2011). Manganese oxide has long be regarded as natural occurring powerful oxidants (Han et al., 2011; Ren et al., 2013), which can rapidly convert As(III) to As(V) over the pH range of 4~8.2 under natural conditions (Bruce A. Manning et al., 2002). In the present study, in order to simulate an authentic rice growing environment, the soil have been flooded with water along with the whole incubation period. It is well accepted that incubation under flooded conditions can cause solid As to be distributed to liquid phase(Lemonte et al., 2017; Xu et al., 2017), in which the dominating As species was arsenite ; so As can be re-mobilized into soil when As(V) is reduced to As(III). In spite of this circumstance, as can be seen from the Fig.1, the content of Fe-As still increased after treatment with 0.5% and 2.0% of α-MnO_2 nanorods, which proved that the increased binding state As was more likely due to the oxidation of As(III) into As(V) by α-MnO_2 nanorods and subsequently resulted to the enhanced adsorption onto ferric(hydr)oxides and Al mineral. The subsequently re-allocation of As into the soil solution was, thus effectively reduced. Besides, the content of Ca-As was decreased with an increase in the associated residual As at the dosage of 1%, which indicated that the other encapsulated As fractionation was also transformed to less effective forms.

Combined the obtained results in soil incubation experiments, which can provide us with a reliable theoretical basis for our subsequent experiment. The hypothesis of the present study was proposed that in the rice rhizosphere micro-environment, addition of α-MnO_2 nanorods can affect the chemical speciation of As in the soil solution through in situ oxidation which in turn affect the bio-availability and mobility of As for rice uptake. As already discussed in soil incubation experiment, the partitioning of As into the soil solution was significantly controlled by As fractionation transformed. This part of observed results can be further proved by the dynamic monitoring of As variations in soil solution (Fig.2ab). As can be seen from Fig.2a, the content of porewater As was significantly decreased as a dosage dependent way at the 45th day with the increasing content of α-MnO_2 nanorods. And during the whole growth stages (45-105 days), in spite of slightly fluctuation, the overall concentrations of As was decreased. Several studies have been deciphered that the effluent of Mn is attributed to the process of arsenite oxidation by manganese oxides (Lafferty et al., 2010; Oscarson et al., 1983; Tournassat et al., 2002); and it is widely accepted that the oxidation of As(III) to As(V) involves in a two-step process, including the release of Mn(II) and sorption of Mn(II) on oxidation intermediate (Nesbitt et al., 1998).

Our results are extremely consistent with those previous studies. As can be drawn from the above picture (Fig.2b) the content of Mn has been increased to an extraordinary magnitude (17-33 folds) compared with the untreated soil. Nevertheless, unfortunately the persistence increasing of Fe(II) and Mn(II) in soil porewater under reduced conditions at early growth stages (45-60 days) can inhibited the abiotic oxidation of As(III) by α-MnO_2 nanorods (Ehlert et al., 2014). Another study reported by Chen et al. (2006) showed that the oxidation rates could be also impaired by soil organic matter(Chen et al., 2006). Those reported studies explained that why the Mn concentrations in the soil porewater tend to decrease with the rice growth time prolonged (60-105days) (Fig.2b).

4.2 Enhanced impeded of As accumulation in rice by α-MnO_2 nanorods.

Combined the results which obtained from soil incubation experiments, we can draw a conclusion that the partitioning of As into soil porewater has been rigorous hindered (Fig.2a). Moreover, the impeded As elution has also in turn affected the accumulation of As into rice. But the interplay between the addition of α-MnO_2 nanorods and As in plant cultivation systems was far more complexity than the pure soil incubation. Pot and field rice cultivation together with soil incubation experiments were therefore combined to clarify the effects of different amounts of α-MnO_2 nanorods on the accumulation and translocation of As into rice (Fig.3-7). On the basis of the present study, we believe that there are at least two factors appear to play crucial roles in controlling the As influx into rice plants.

Firstly, in situ oxidation of As(III) to As(V) in the presence of α-MnO_2 nanorods leads to the subsequently enhanced adsorption of As onto endogenous iron(oxyhydr) oxides; greatly reduced the re-allocation of As into soil porewater, and thus directly cut down the total As transportation into rice parts. As can be seen from the above picture (Fig.3 and Fig.6) The addition of Mn oxides significantly impeded the accumulation of As into subterranean parts (roots p<0.05); and the associated influx of As into aerial parts (stems, leaves, husk and brown rice) has been also obstructed (Fig.3 and Fig.6; p<0.05). Correlations analysis (Fig.S4 and Fig.5) between As and Mn in rice parts can also prove that the transportation of As has been greatly limited.

Secondly, apart from those existing fact, As bioavailability could be also mediated through iron and manganese plaque formation on the rice roots and so influence As uptake by rice plants (Liu, 2005; Liu et al., 2005). Therefore, enhanced sequestrate of As by rice roots were probably due to the iron and manganese plaque formation of the rice roots (Fig.3a and Fig.5a). Besides, the addition of manganese oxides can also promote Fe(II) oxidation (Postma and Appelo, 2000); Ehlert et al. (Ehlert et al., 2014) reported that birnessite additions can promote Fe(II) oxidation to Fe(III), thereby creating newly-formed Fe(III) hydroxides which could serve as efficient sorbents for As(III).

Hence, by combination of the obtained facts, we can draw a conclusion that the addition of α-MnO_2 nanorods served as a multifunctional role on the As mobilization and transportation in paddy fields. Firstly, the effects on the As fractionation in paddy soil leads to the less dissolution of As in soil porewater. Secondly, As(III) oxidation trigged by α-MnO_2 nanorods resulted to the adsorption of water soluble As(V) onto iron oxides. Lastly, the enhanced dissolution of Mn(II) and Fe(III) can lead to the more iron and manganese plaque formation, which can sequester more As on the surface of rice roots.

5 Conclusions

In the present study, soil incubation experiments which combined with pot and field rice cultivation trials were designed to evaluate the effectiveness of exogenous α-MnO_2 nanorods on the mobilization and transportation of As in soil-rice systems. Our results proved that the addition of α-MnO_2 nanorods can effectively control the soil-to-solution partitioning of As under anaerobic conditions. The As fractionation can be transformed from effective forms into less effective forms impeded the re-allocation of As into soil porewater, and thus reduced the total amounts of As influx into rice. Besides, the enhanced oxidation of As(III) into As(V) by α-MnO_2 nanorods greatly increased the adsorption of As onto indigenous iron(hydr) oxides, thus, reduced the soil porewater As. Furthermore, with the simultaneous co-occurrences of the oxidation intermediates of Mn(II) and Fe(III), which can probably lead to the enhanced formation of iron and manganese plaque on the surface of rice roots. Combined, the influx of As into aerial parts of rice plants (stems, husk and leaves) was strictly prohibited by rice roots. Nevertheless, it should be noted that the abiotic oxidation of As by α-MnO_2 nanorods are greatly impaired by various environmental factors (such as DOM, microbial activities and ligands), thus, further work is still needed to verify these results under fields or laboratory scales.

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