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Arsenic uptake by two vegetables grown in two soils amended with As-bearing animal manures

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ABSTRACT

Organoarsenicals are widely used as growth promoters in animal feed, resulting in unabsorbed arsenic (As) left in animal manures. A pot experiment was conducted to investigate the growth and As uptake of amaranth (*Amaranthus tricolor Linn*, a crop with an axial root system) and water spinach (*Ipomoea aquatica Forsk*, a crop with a fibrous root system) grown in a paddy soil (PS) and a lateritic red soil (LRS) amended with 2% and 4% (w/w) As-bearing chicken manure and pig manure, respectively. Soils without any fertilizers were the controls. The biomass, As contents and total As uptake of the shoots, As transfer factors (TFs) from roots to shoots and the root/shoot (R/S) ratios of water spinach were significantly higher than those of amaranth ($p < 0.0015$). The biomass, total As uptake and R/S ratios showed significant difference for soil types ($p < 0.0031$). Manure amendments increased the biomass of both vegetables, reduced the As contents in amaranth but increased those in water spinach. The As contents were negatively correlated with the biomass in amaranth, but positive correlation was observed for water spinach. The total As uptake by amaranth was decreased in PS and insignificantly affected in LRS by manure application, but that by water spinach was significantly increased in both soils. We suggest that the higher As uptake by water spinach might be related to its root structure and R/S ratio. Heavy application of As-bearing animal manures should be avoided in water spinach.

1. Introduction

Organoarsenical compounds have been used to promote growth and suppress bacterial and parasitic diseases in intensive animal production since the early 20th century [1]. Roxarsone (3-nitro-4-hydroxylphenylarsonic acid) and arsanilic acid (4-aminophenylarsonic acid) are two widely used organoarsenicals. Their ordinary rates in diets range 25–100 mg kg^{-1} [2,3]. However, organoarsenicals are excreted almost unchanged in feces and urine [4,5]. Arsenic (As) contents in animal manures range from not detectable to 315.1 mg kg^{-1}, as reported in literatures [6–12]. An investigation showed that 13.5% of the chicken manure samples and 52.9% of the pig manures exceeded the As limit of 50 mg kg^{-1} specified in the Chinese National Organic–Inorganic Compound Criteria (GB18877-2002) [12], which was ascribed to over-use of organoarsenicals by some farmers in China [13]. Morrison [14] showed that the As contents in two feed crops (alfalfa and clover) were insignificantly affected by application of As-containing chicken litter (As 15–30 mg kg^{-1}) at 4–6 tons/acre for 20 years. However, Jackson et al. [8] and Rutherford et al. [15] reported that 70%–90% of the total As in animal manures is water-soluble. It is the high water-solubility of As in manures that make us interested in the bioavailability of As to crops rather than the two feed crops documented by Morrison [14].

China is a major meat producer having chickens and pigs numbers in stocks accounting for 26.0 and 50.9% of the world's stocks in 2004, respectively [16]. A huge amount of animal manure is produced. Animal manures are commonly applied to land as fertilizer. Rutherford et al. [15] reported As build-up in soil after one application at 6 tons/acre and after two applications at both 3 and 6 tons/acre. Wang et al. [17] demonstrated that the As contents in different parts of rice plants increase by increased rate of roxarsone and arsanilic acid. Consequently, the potential risk of As contamination of crops in soil amended with As-bearing manures is not clear but urgently needs investigation. The experiment reported here aims to reveal the growth of vegetables and As uptake in soils on

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Table 1
Selected properties of paddy soil and lateritic red soil used

	Paddy soil	Lateritic red soil
pH[a]	6.77	6.44
OM ($g\,kg^{-1}$)	23.9	20.2
Sand ($g\,kg^{-1}$)	326	726
Silt ($g\,kg^{-1}$)	380	220
Clay ($g\,kg^{-1}$)	294	54
CEC ($cmol\,kg^{-1}$)	13.71	8.42
Total soluble salts ($g\,kg^{-1}$)[b]	1.2	0.9
Alkalized N ($mg\,kg^{-1}$)	74.2	67.2
Available P ($mg\,kg^{-1}$)	63.2	41.3
Available K ($mg\,kg^{-1}$)	161.7	113.3
Total As ($mg\,kg^{-1}$)	33.1	23.9
Water-soluble As ($mg\,kg^{-1}$)[c]	0.607	0.547

[a] Measured in a 1:2.5 soil to water (w/v) suspension.
[b] Measured in a 1:5 soil to water (w/v) suspension.
[c] Measured in a 1:10 soil to water (w/v) suspension.

which As-containing animal manures were applied, as influenced by vegetable species and soil types, and to provide information for making policy decisions on animal manures.

2. Materials and methods

2.1. Soils, animal manures and vegetable species

Two soils were used in this experiment. One was a paddy soil (PS) collected from a rice–vegetable–rice rotation production system in the Panyu district (22°51′32″N, 113°27′34″E) of Guangzhou, South China. The other was a lateritic red soil (LRS) collected from the Crop Experiment Station of the Guangdong Academy of Agricultural Sciences (23°8′43″N, 113°20′50″E) located in Guangzhou. Prior to use, soils were air-dried and ground to pass through a 5.0-mm sieve. Selected properties of the two soils are shown in Table 1.

A chicken manure (CM) and a pig manure (PM) were separately gathered at a chicken farm and a pig farm where organoarsenicals were fed in the animal diets in Huizhou city, Guangdong province. Manures were air-dried, feathers removed and ground to pass through a 2-mm sieve. Basic properties of the two manures are presented in Table 2.

Two popular leafy vegetables grown in China, amaranth (*Amaranthus tricolor Linn*) and water spinach (*Ipomoea aquatica Forsk*) were used. The seeds were purchased from the Guangzhou Vegetable Institute, Guangzhou.

Table 2
Selected properties of the chicken manure (CM) and pig manure (PM) used

	Chicken manure	Pig manure
pH[a]	6.17	6.23
OM ($g\,kg^{-1}$)	636	593
Total N ($g\,kg^{-1}$)	19.0	24.0
Total P ($g\,kg^{-1}$)	5.9	5.9
Total K ($g\,kg^{-1}$)	8.1	8.5
Total soluble salts ($g\,kg^{-1}$)[b]	36.5	22.1
Total As ($mg\,kg^{-1}$)	32.0	31.8
Water-soluble As ($mg\,kg^{-1}$)[c]	10.3	10.5
Total Cu ($mg\,kg^{-1}$)	146.4	126.2
Total Zn ($mg\,kg^{-1}$)	171.1	163.0
Acheomycin ($mg\,kg^{-1}$)	nd[d]	nd
Terramycin ($mg\,kg^{-1}$)	nd	nd
Aureomycin ($mg\,kg^{-1}$)	nd	nd
Olaquindox ($mg\,kg^{-1}$)	nd	nd

[a] Measured in a 1:5 manure to water (w/v) suspension.
[b] Measured in a 1:25 manure to water (w/v) suspension.
[c] Measured in a 1:10 manure to water (w/v) suspension.
[d] Not detected.

2.2. Pot experiment

A pot experiment with five treatments and four replications was conducted in a green house. The two manures were added at rates of 2 or 4% (w/w): CM (2%CM and 4%CM) and PM (2%PM and 4%PM). Soils without any fertilizer were the controls (CK). 7 kg soil was mixed with manure thoroughly and put into a plastic pot. Soil moisture was kept at 75% field water holding capacity by adding de-ionized water for 10 d, and then seeds were sown with 7 seedlings kept in each pot.

2.3. Sample collection

Vegetables were harvested at 48 d after seeding. The shoots and roots were gathered separately, then washed with tap water and rinsed with de-ionized water, with the fresh weight recorded. The plants were oven-dried at 65 °C to a constant weight, and the dry weight recorded. Plant tissues were finely powered with a stainless steel miller for analysis.

2.4. Chemical analysis

Soil and manure pH were determined by a 1:2.5 (soil:water, w/v) and a 1:5 (manure:water, w/v) suspension, respectively, soil OM by the method of $K_2Cr_2O_7$–H_2SO_4 oxidation using oil bath heating, soil CEC by extracting with 1.0 mol L^{-1} NH_4OAc solution at pH 7.0, soil alkalized N by the titration method with 2% H_3BO_3, soil available P by the method of Olsen-P, soil available K by the extraction method with 0.1 mol L^{-1} NH_4OAC using flame photometry. Total soluble salts (TSSs) in soil and manure samples were extracted by 1:5 (soil:water, w/v) and 1:25 (manure:water, w/v) suspensions, respectively, and measured as the mass of the residues after the evaporation of solutions. Manure samples were digested with concentrated $H_2SO_4 + H_2O_2$, and determined the total N, P and K by Kjeldahl method, vanadium–ammonium molybdate colorimetry and flame photometry, respectively.

Soil and manure samples were digested with concentrated $HNO_3 + HClO_4$ and the total As was determined by HG-AFS (AFS930, Jitian, Beijing, China). Manure samples were digested with concentrated $HNO_3 + HClO_4$ (5:1) and the total Cu and Zn contents were measured with HG-AAS (Hitachi Z-5000). Water-soluble As in manures and soils was extracted in a 1:10 (soil:water, w/v) suspension [18] and determined by HG-AFS. Plant tissue samples were digested with concentrated $HNO_3 + H_2SO_4$, then the total As was measured by HG-AFS using the Chinese standard method for food (GB/T 5009.11-2003). Two Chinese standard materials, GBW07602 and GBW07408, were used to control the analysis quality of total As of plants, soils and manures, respectively, with recoveries of 92–101%.

Four antibiotics including acheomycin, terramycin, aureomycin and olaquindox in the two manures were also determined. Acheomycin, terramycin and aureomycin were extracted with 5% $HClO_4$, and the centrifuged suspension was measured by HPLC (Agilent 1100, USA) using the Chinese standard methods (GB/T 5009.116-2003). Olaquindox was extracted with 40% methanol and determined by HPLC using the standard method issued by the Ministry of Agriculture of China. Antibiotic detection was conducted by the Guangzhou Agricultural Standards and Supervisory Center. The detection limits for acheomycin, terramycin, aureomycin and olaquindox were 0.25, 0.15, 0.65 and 0.88 mg kg^{-1}, respectively.

2.5. Statistical analysis

All data were the mean of four replications. The As transfer factors were computed as the ratios of total As contents (fresh weight) in vegetable shoots/total As contents (fresh weight) in the roots. The

Table 3
Biomass of the shoots of amaranth and water spinach as affected by vegetable species, soil types and soil amendments

Variables	Biomass of the shoot (g pot^{-1})	ANOVA			
		Main effects	DF	*F*	*p*-Value
Vegetable species			1	86.89	0.0001
Amaranth	71.40				
Water spinach	90.85				
Soil types			1	9.56	0.0031
Paddy soil	84.36				
Lateritic red soil	77.92				
Soil amendments			4	107.27	0.0001
2%CM	92.73				
4%CM	63.95				
2%PM	88.16				
4%PM	110.84				
CK	50.01				

root/shoot ratios were calculated as the ratios of dry weight of the vegetable roots/dry weight of the shoots. ANOVA was performed using SAS/STAT software (1989–1996, SAS Institute Inc., Cary, NC, USA), with LSD ($p < 0.05$). Pearson correlation coefficients for the linear relationship between the As contents and the biomass of vegetables were calculated by SPSS software (1989–1999, SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Growth of amaranth and water spinach

The biomass of the shoots of amaranth and water spinach showed significant differences for vegetable species, soil types and soil amendments ($p < 0.0031$) (Table 3). All manure amended treatments (except the 4%CM treatment for water spinach in LRS) increased the biomass of the two vegetables in either soil, compared to their controls (Fig. 1). Though more nutrients were introduced, the biomass of both vegetables in the 4%CM treatment were similar to or considerably reduced, compared to the 2%CM treatment. This might be ascribed to the complex constituents of animal manures. Animal manures contain not only N, P and K nutrients but also soluble salts. In our previous field plot experiment, the TSSs contents in soils increased by 1.4–2.0 g kg^{-1} when poultry manures (29.7–42.3 TSSs kg^{-1}) were successively amended at 56.25–450 kg N/ha for three crops [19], and resulted in considerable yield reduction of vegetable [20]. However, this experiment was conducted for only for a single crop, and the TSSs in the CM were low in animal manures [12]. Hence, TSSs were unlikely to be the main reason for the biomass reduction of both vegetables. It has been reported that animal manures commonly contain antioxidants, antibiotic residues and organic pollutants [11,21,22], which might be toxic to soil microbes and influence soil quality [21], leading to a harmful influence on plant growth. Four antibiotics – acheomycin, terramycin, aureomycin and olaquindox – could not be detected in either manure; these are commonly found in manures from intensive animal production systems [11]. Hence, the biomass reduction by application of higher CM was attributed to some unknown component(s) in the CM. However, there was no yield reduction with high applications of pig manure. Growth of the two vegetables was improved by higher PM addition. Generally, the growth response to the two manures for the two vegetables in two soils may be attributed to: (1) amaranth has an axial root system with a few adventitious roots, but water spinach develops a strong fibrous root system; (2) PS has higher CEC and OM with the texture of loamy clay, leading to greater buffering capability compared to LRS. Moreover, it also had a higher background nutrient fertility. Consequently, water spinach produced more biomass in PS with higher fertility, amaranth performed better in LRS when PM was applied, which was a sandy loam with better permeability for its root development than in PS. However, amaranth did not show the same rule when CM was used due to its complex constituents.

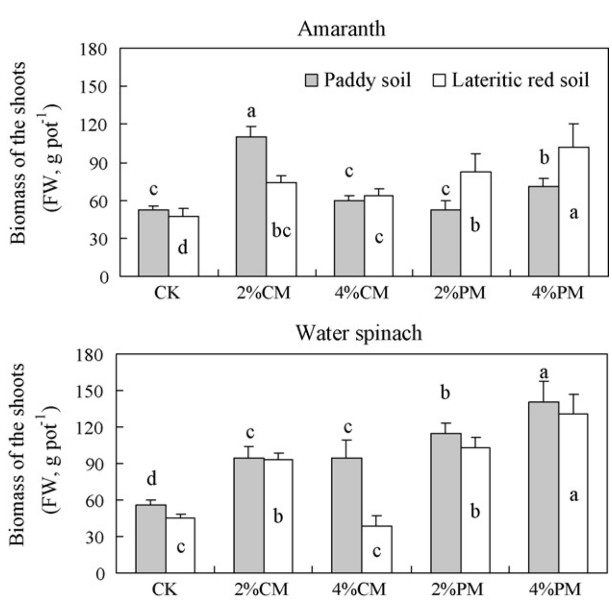

Fig. 1. Biomass of the shoots in amaranth and water spinach in paddy soil and lateritic red soil as affected by application of chicken manure (2%CM and 4%CM) and pig manure (2%PM and 4%PM). Bars with different letters significantly differ at the level of 0.05.

3.2. As contents in the shoots of amaranth and water spinach

Table 4 indicates that water spinach had significantly ($p < 0.0001$) higher As content (0.099 mg kg^{-1}) than amaranth (0.058 mg kg^{-1}). Significant differences were also detected for soil amendments but not for soil types ($p < 0.4638$). The As contents in the two vegetables do not exceed the As limit (0.5 mg kg^{-1} based on the fresh weight) of the Chinese food hygiene standard (GB18406-2001). Manure amendments except the 4%PM treatment in LRS decreased the As contents in amaranth, but both manures increased the As contents in water spinach, compared to their controls (Fig. 2). Generally, higher CM rate decreased the As contents in amaranth but higher PM dose increased them, and higher rates of both CM and PM increased them in water spinach.

Table 4
As contents in the shoots of amaranth and water spinach as affected by vegetable species, soil types and soil amendments

Variables	As content in the shoot (mg kg^{-1})	ANOVA			
		Main effects	DF	*F*	*p*-Value
Vegetable species			1	201.80	0.0001
Amaranth	0.058				
Water spinach	0.099				
Soil types			1	0.54	0.4638
Paddy soil	0.079				
Lateritic red soil	0.077				
Soil amendments			4	5.02	0.0015
2%CM	0.072				
4%CM	0.082				
2%PM	0.077				
4%PM	0.089				
CK	0.071				

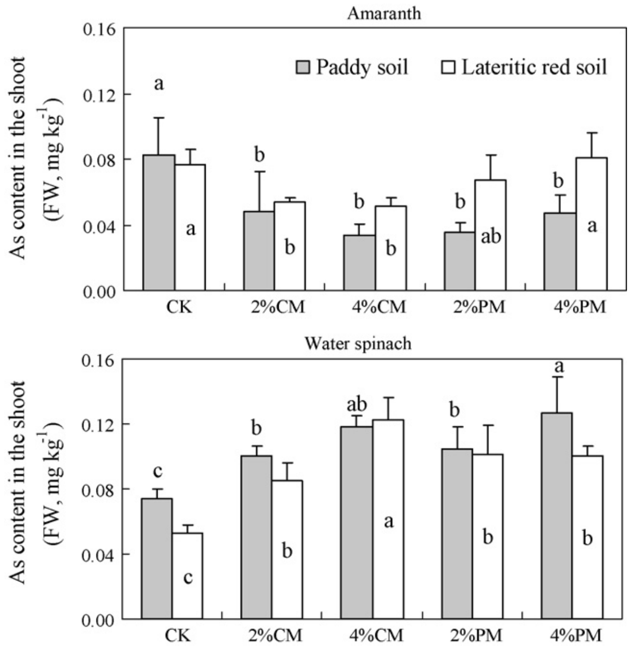

Fig. 2. As contents in the shoots of amaranth and water spinach in paddy soil and lateritic red soil as affected by application of chicken manure (2%CM and 4%CM) and pig manure (2%PM and 4%PM). Bars with different letters significantly differ at the level of 0.05.

3.3. Correlations between As contents and biomass of amaranth and water spinach

The results of correlation analysis imply that the As contents of amaranth are negatively but not significantly ($r = -0.412$, $p < 0.071$) correlated with the biomass in PS (Fig. 3). However, negative and significant ($r = -0.601^{**}$, $p < 0.006$) correlation was observed in LRS. Positive and significant ($r = 0.612^{**}$, $p < 0.004$) correlation was found between the As contents and the biomass of water spinach in PS, but insignificantly positive ($r = 0.060$, $p < 0.806$) correlation was found in LRS. Though 32.2 and 33.0% of the total As in the CM and PM were water-soluble, which were 14-fold higher than those in both soils (1.8% in PS and 2.3% in LRS), the As contents in amaranth were diluted by the biomass. Hence, the nutritional value of the manure to improve the growth of amaranth could not be ignored when evaluating the potential risk of As contamination as affected by As-containing manures. Manure application improved not only the growth of water spinach but also its capability to absorb As, indicating that water spinach is a crop that preferentially accumulates As. Heavy application of As-bearing animal manures should be avoided.

3.4. Total As amount in the shoots of amaranth and water spinach

Water spinach (9.61 μg As pot^{-1}) took up nearly threefold total As than amaranth (3.52 μg As pot^{-1}) (Table 5). The total As uptake by vegetables was significantly higher in PS than in LRS ($p < 0.0006$). Significant differences were also observed for soil amendments ($p < 0.0001$) (Table 5). Manure application except the 2%CM treatment significantly decreased the total As in amaranth in PS compared to the control, and insignificantly affected the total As in LRS except the 2%PM treatment (Fig. 4). Though amaranth accumulated more As in the control in PS than in LRS, higher amount of total As was taken up by manure application in LRS than in PS. The total As in all manure amended treatments for water spinach was twofold to fourfold higher than those in the control plants in both soils. The differences of the total As in water spinach among treatments were similar to those of the biomass (Fig. 1), showing that the total As in water spinach was primarily determined by its biomass. Water spinach accumulated more As in PS than in LRS for all treatments.

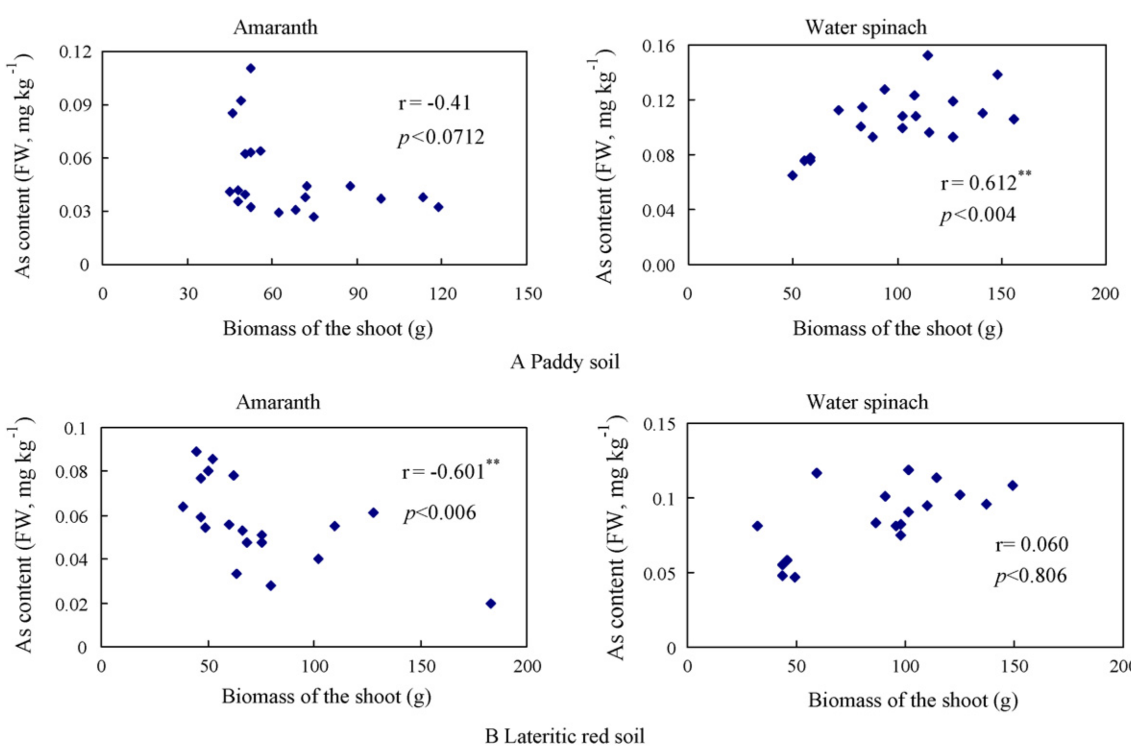

Fig. 3. Correlations between As contents and biomass of the shoots of amaranth and water spinach in paddy soil (A) and lateritic red soil (B). Correlation coefficients were -0.412 ($p < 0.071$) and -0.601^{**} ($p < 0.006$) for amaranth and 0.612^{**} ($p < 0.004$) and 0.060 ($p < 0.806$) for water spinach in paddy soil and lateritic red soil, respectively.

Table 5
Total As uptake in the shoots of amaranth and water spinach as affected by vegetable species, soil types and soil amendments

Variables	Total As in the shoot ($\mu g\ pot^{-1}$)	ANOVA			
		Main effects	DF	*F*	*p*-Value
Vegetable species			1	306.03	0.0001
Amaranth	3.52				
Water spinach	9.61				
Soil types			1	13.32	0.0006
Paddy soil	7.20				
Lateritic red soil	5.93				
Soil amendments			4	35.87	0.0001
2%CM	6.33				
4%CM	5.60				
2%PM	7.35				
4%PM	9.94				
CK	3.60				

Table 6
As transfer factors from roots to shoots in amaranth and water spinach as affected by vegetable species, soil types and soil amendments

Variables	As transfer factor	ANOVA			
		Main effects	DF	*F*	*p*-Value
Vegetable species			1	56.86	0.0001
Amaranth	0.10				
Water spinach	0.26				
Soil types			1	0.01	0.9347
Paddy soil	0.18				
Lateritic red soil	0.18				
Soil amendments			4	5.31	0.0010
2%CM	0.14				
4%CM	0.15				
2%PM	0.15				
4%PM	0.19				
CK	0.28				

3.5. As translocation in amaranth and water spinach

The As transfer factors (TFs) were used to evaluate the translocation of As from roots to shoots in plants. The As TFs showed significant differences for both vegetable species ($p < 0.0001$) and soil amendments ($p < 0.0010$), but not for soil types ($p < 0.9347$), as presented in Table 6. Water spinach had significantly ($p < 0.0001$) stronger capability to transport As than amaranth. The As TFs in amaranth for manure amended treatments (except the 4%PM treatment in LRS) in both soils were more than fivefold and twofold lower than the control values (Fig. 5). Manure amendments decreased the As TFs in water spinach, with values of 0.197–0.295 in PS, compared with the As TF of 0.339 for the control. The As TFs (0.229–0.388) with manure treatments were similar to those in the LRS control (0.267) or even significantly larger. The higher manure doses enhanced the As translocation for water spinach in both soils.

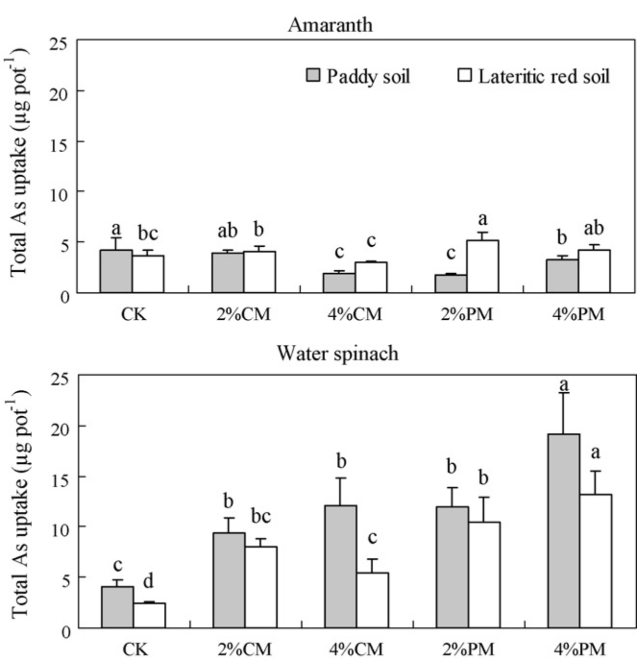

Fig. 4. Total As uptake in the shoots of amaranth and water spinach in paddy soil and lateritic red soil as affected by application of chicken manure (2%CM and 4%CM) and pig manure (2%PM and 4%PM). Bars with different letters significantly differ at the level of 0.05.

3.6. Root/shoot (R/S) ratios for amaranth and water spinach

As previously noted, amaranth and water spinach are crops with different root structures. Vegetable species, soil types and soil amendments significantly affected the R/S ratios of these vegetables at the levels of 0.0001 (Table 7). Water spinach had a higher R/S ratio than amaranth, and the two vegetables developed stronger roots in LRS with the texture of sandy loam than in PS with the texture of loamy clay. Manure additions significantly decreased the R/S ratios of amaranth in both soils (Fig. 6), which was attributed to growth priority of roots over shoots when a plant is subjected to nutrient stress [23,24]. The R/S ratios of water spinach for manure amended treatments were insignificantly decreased or significantly increased, compared to the control values, except for significant reduction in both CM treatments in PS. It was probably the differences between root structures and R/S ratios for the two vegetables that leaded to the discrepancies between their growth and capability to absorb and transfer As when manures were amended. More work need to be done to confirm this. However, crop species must be considered when As-bearing manures are applied.

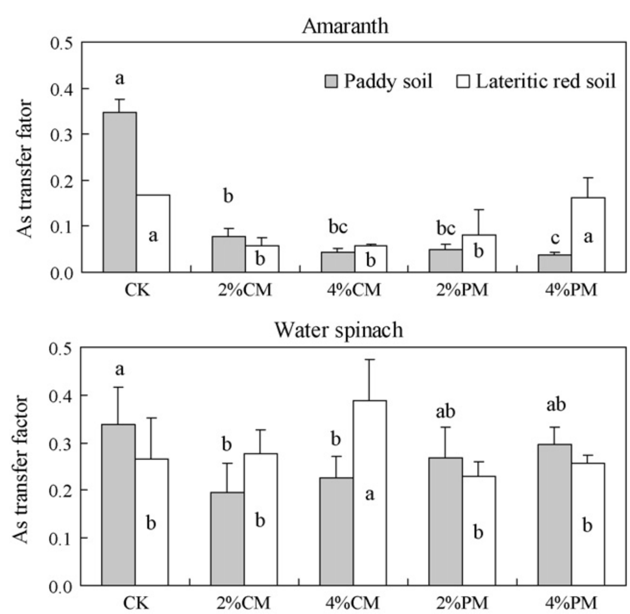

Fig. 5. As transfer factors from roots to shoots in amaranth and water spinach in paddy soil and lateritic red soil as affected by application of chicken manure (2%CM and 4%CM) and pig manure (2%PM and 4%PM). Bars with different letters significantly differ at the level of 0.05.

Table 7
Root/shoot ratios for amaranth and water spinach as affected by vegetable species, soil types and soil amendments

Variables	Root/shoot ratio	ANOVA			
		Main effects	DF	*F*	*p*-Value
Vegetable species			1	261.15	0.0001
Amaranth	0.18				
Water spinach	0.30				
Soil types			1	45.23	0.0001
Paddy soil	0.21				
Lateritic red soil	0.27				
Soil amendments			4	19.38	0.0001
2%CM	0.22				
4%CM	0.19				
2%PM	0.27				
4%PM	0.25				
CK	0.28				

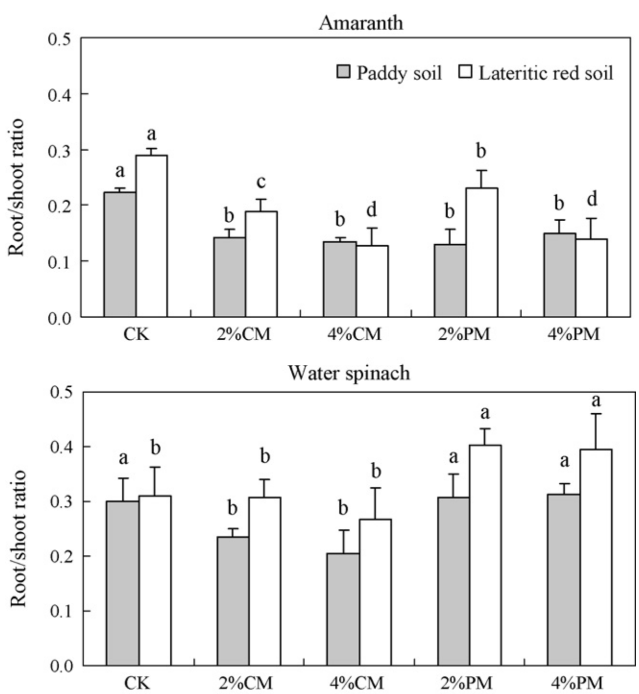

Fig. 6. Root/shoot ratios for amaranth and water spinach in paddy soil and lateritic red soil as affected by application of chicken manure (2%CM and 4%CM) and pig manure (2%PM and 4%PM). Bars with different letters significantly differ at the level of 0.05.

4. Conclusions

In our experiment, vegetable species and soil amendments significantly affected the biomass, As contents, total As uptake of the shoots, As TFs and the R/S ratios of amaranth and water spinach. The biomass, the total As uptake and R/S ratios showed significant difference for soil types.

Manure application increased the biomass of both vegetables, reduced the As contents in amaranth, but increased those in water spinach. Generally, the higher manure doses increased the As contents more than lower ones. The As contents were negatively correlated with the biomass in amaranth, positively correlated in water spinach. Significant correlations were observed for amaranth in LRS and for water spinach in PS. The total As uptake by amaranth was decreased in PS and insignificantly affected in LRS by manure application, that by water spinach was significantly increased in both soils. In general, the capability of transporting As in amaranth was significantly decreased, and that in water spinach was insignificantly decreased by manure amendments. Manure amendments significantly lowered the R/S ratios for amaranth, and insignificantly decreased those for water spinach except the two CM treatments in PS. We assume the root structures and R/S ratios may result in the discrepancies between As uptake by the two vegetables. Water spinach showed higher potential risk of As contamination while higher rate of As-bearing animal manures were used in PS.

The results showed that crop species and soil type should be considered while As-containing animal manures were used. And, As speciation needs to be investigated to further elucidate the phytoavailability of As in manures resulted from organoarsenical additives.

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