# Experimental study on the uptake and effects of arsenic originated from poultry litter on the growth of *Brassica napus* in greenhouse pot cultivation and health risk assessment

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**Abstract:** Organoarsenics are widely used as growth promoters in poultry industry, resulting in arsenic (As) accumulation in poultry litter. A greenhouse pot study was implemented to investigate the fate of arsenic originated from poultry litter and their effects on the growth of *Brassica napus* (oilseed rape), and assess their potential health risks. Five poultry litter application rates (0, 5%, 10%, 20% and 40%) were used, dividing into two groups: one for soil incubation (SI) and the other for plant cultivation (PC). Experimental results indicated that the total arsenic for composted poultry litter was (10.94±0.23) mg/kg, As(V) and As(III) decreased while methylated arsenic increased after 21 d in SI and PC treatments. Seed germination rates were negatively correlated with monomethylarsenic acid (MMA, $R^2$=0.63, $p$<0.05). The length and biomass of roots and shoots were significantly inhibited by poultry litter, but plant length of 5% treatments was slightly stimulated. Within an average weekly intake of 0.5 kg *Brassica napus*s, the risk quotient (RQ) values induced from roots nearly all surpassed the acceptable limit (1), were two orders magnitude higher than shoots. According to the potential risk to order, child exhibited the highest risk, adolescent ranked secondly, and adult exhibited the lowest risk. Hence, people should better avoid intake *Brassica napus* roots to reduce arsenic potential risk.

**Keywords:** arsenic uptake, poultry litter, arsenic speciation, pot cultivation experiment, *Brassica napus*, health risk assessment
**DOI:** 10.3965/j.ijabe.20160903.2251

**Citation:** Xie H Y, Han D M, Cheng J P, Wang L, Zhou P, Wang W H. Experimental study on the uptake and effects of arsenic originated from poultry litter on the growth of *Brassica napus* in greenhouse pot cultivation and health risk assessment. Int J Agric & Biol Eng, 2016; 9(3): 186－193.

## 1 Introduction

Roxarsone (an organoarsenical compound) is an excellent feed additive to improve poultry feed efficiency and increase egg production[1,2], but this compound is largely excreted and accumulated in poultry litter[1,3]. Local farmers always dispose poultry litter to agricultural soil for fully using their nutrition[4]. Ultimately, the results of repeated and intense localized disposal of poultry litter to farmland soil directly introduced a substantial amount of arsenic to soil[5].

Numerous researchers found that roxarsone can be biotransformed to more leachable forms including arseniteAs(III), arsenateAs(V), monomethylarsenic acid (MMA), dimethylarsenic acid (DMA) and other unidentified species upon composting process[6-8]. Microbes can methylate and demethylate arsenic species in soil, transform organic arsenic to inorganic arsenic and

**Received date:** 2015-12-01 **Accepted date:** 2016-04-13
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vice versa[9]. In addition, As(V) is an analogue of phosphate, it can compete sorption sites[10] and uptake carrier[11] with phosphate. Our previous investigations revealed that under anaerobic circumstance, As(V) and As(III) decreased in treatments applied 60% and 10% rates within initial seven days, subsequently methylated arsenic displayed increasing, suggesting biotic activity transformed inorganoarsenical to methylated arsenic species[8]. In contrast, As(V) dropped in the first seven days but increased thereafter under aerobic circumstances, with methylated arsenic increasing, implying abiotc and biotic activities enhanced arsenic speciation.

The phytoavailability and phytotoxicity of arsenic are primarily determined by its speciation and concentration in the medium[12]. In general, the mobility and availability of arsenic in soil were affected by biotic and abiotic conditions, such as redox, pH and TOC. Inorganic arsenic is the dominant forms of arsenic phytoavailability, and generally exist higher phytotoxicity. As(III) is highly toxic to plants, since As(III) can react with sulfhydryl group(-SH) of enzymes and tissues proteins, leading the emergence of ROS (reactive oxygen species) which would pose inhibition to cellular function, showing phytotoxcity to plant[13]. Crops exposure to arsenic contaminated environment would be associated with serial toxic symptoms, such as smaller germination rates, roots and shoots growth reduction[9]. Arsenic transfer and accumulate into vegetable crops raising the potential risk of an increased arsenic uptake by consumers, increasing human potential health risks.

Most studies investigated the distribution and accumulation of arsenic in plants mainly on hyperaccumulation plant (like Chinese brake fern)[14,15], cereal crops (like rice)[16] and beet[17], a significant gap of information exists on common vegetables such as *Brassica napus* (oilseed rape). Furthermore, although a number of researches have focused on arsenic speciation under different arsenic concentration, to our knowledge, few studies have investigated the effects of plant cultivation on arsenic transformation in soil amended with poultry litter. The objectives of this study were: (1) to determine arsenic speciation after soil incubation and plant cultivation; (2) examine the arsenic uptake by *Brassica napus* and their effects on *Brassica napus*; (3) perform potential health risk assessment induced from daily intake of edible part of plant.

## 2 Materials and methods

### 2.1 Soil and poultry litter materials

Poultry litter samples were collected from one of the biggest egg poultry farms in Shanghai, where 80 000 to 120 000 feathers of brown laying chickens were fed. Fresh poultry litter were collected with feed debris, roost rubbish and straw powder, then were composted under 50%-60% moisture content for 45 days. After the composting period, the poultry litter was air dried, grounded and passed through a 2 mm sieve for use. To fully mimic the real field circumstances, soil samples (0-20 cm) were collected from an agricultural area near this poultry farm. Soil was air-dried, sieved to pass through a 2 mm sieve and well mixed for experiment.

### 2.2 Soil incubation and plant germination, cultivation

The plant we used in this study was restricted to a popular vegetable of *Brassica napus* (oilseed rape), which were one ordinary food and widely cultivated in China. For germination experiment, the *Brassica napus* seeds were purchased from Shanghai Academy of Agriculture Science.

Five rates (0%, 5%, 10%, 20% and 40%, dry weight) of composted poultry litter were applied to soil samples, and the 0% treatment was taken as control. Treatments with or without poultry litter were further divided into two groups, one group for soil incubation (SI) and another for plant seed germination and cultivation (PC). One kilogram of soil was mixed thoroughly and placed in a plastic pot (13 cm in height and 15 cm in diameter) for each treatment, kept at approximately 70% field water holding capacity by adding deionized water (Mili Q system, Barnstead, USA) prior to soil incubation or plant seed germination and cultivation. The incubation and cultivation experiment both lasted for a period of 21 d in July 2014.

All the treatments were placed in the greenhouse, with conditions follows: light intensity was kept at 5000 lux, with a duration of 16:8 (light: dark) photoperiod cycle and a temperature of (15±0.5) ℃ (dark) and (25±0.5)℃ (light) (SPX-250B-G, Shanghai Boxun Instrument Co.). Plant seed germination and cultivation experiment in this study was performed on the basis of OECD Guidline 208 (Terrestrial Plant Test: Seedling Emergence and Seedling Growth)[14]. *Brassica napus* seeds were socked overnight in distilled water, washed and then treated with Captan to deter fungal growth[13]. Then every 20 *Brassica napus* seeds were placed on the PC treatments soil. Therefore, we got five independent groups of treatments, each group of treatment then split into three replicates. After a period of 3 days, the germination experiment was finished. For the cultivation experiment, only three seedlings were kept in each treatment with three replications to leave enough space for each plant growing. Moisture of the whole treatments were maintained at 70% by periodically weighing these vessels and bottles, adding DI water to compensate for any weight loss throughout the experiment. Pots were randomized on the greenhouse bench and their positions were changed every day.

### 2.3 Sample collection and preparation

At the day of 21, the soil from SI treatments and soil around the *Brassica napus* root in PC treatments were sampled, using a 10 mL polypropylene syringe synchronously. Soil samples were lyophilized and grinded to powder for analysis of total As and arsenic species. The plants in PC treatments were harvested from the pot cautiously, being maintained intact of the plant, then soil around the roots was carefully collected. *Brassica napus* plants were separated into two parts: root (below ground) and shoot (above ground). The roots and shoots were washed with deionized water to eliminate soil particles adsorbed on the plant tissue surfaces. The biomass of roots and shoots were recorded for each pot in fresh weight, and then lyophilized to a constant weight (dry weight) for total As content analysis.

### 2.4 Chemical analysis

Soil samples were digested with concentrated $HNO_3$+$HClO_4$, and total arsenic were determined by using a HG-AFS (AFS930, Beijing Jitian instruments, Beijing, China)[18]. *Brassica napus* samples were digested with concentrated $HNO_3$+$H_2SO_4$ according to Chinese national standard on determination of total arsenic in food. Two Chinese reference materials GBW07408 for soil and GBW07602 for bush plant (Chinese National Reference Materials Center, Beijing) were used to control the analysis quality of the total arsenic in these samples, and the recoveries ranged from 89% to 107%.

To investigate the fates of arsenic speciation in soil after incubation or plant cultivation, different species of arsenic in soil were analyzed. The soil samples were extracted by adding reagent grade phosphoric acid and deionized water, centrifuged on a refrigerated centrifuge, and then passing through C18 cartridge. The concentrations of different species of arsenic in poultry litter were analyzed by a high performance liquid chromatography hydride generation atomic fluorescence spectrometry (HPLC-HG-AFS, Beijing Jitian instruments, Beijing, China), and were equipped with an Hamilton PRP-X100 anion-exchange column (250 mm×4.1 mm, i.d. 10 $\mu$m), using a method adopted from [19]. The detailed description of this method can be found in previous study[8]. And the spiked recoveries for As(III), As(V), MMA, DMA were 98.2%±4.6%, 88.7%±12.9%, 84.1%±8.5% and 93.9%±5.7%, respectively.

### 2.5 Health risk

Potential risk from exposure to arsenic was evaluated for adults, adolescents and children through intake arsenic contaminated *Brassica napus*. The risk quotient (RQ) values were calculated according to following equations[9]:

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \tag{1}$$

$$RQ = ADD / RfD \tag{2}$$

where, *ADD* is average daily dose through food chain, mg/(kg·d); *C* is the concentration of edible part of the plant, mg/kg (fresh weight); *IR* is ingestion rate, kg/d; *EF* is exposure frequency, 209 d/a; *ED* is exposure duration, 50 a for adults,14 a for adolescents and 7 a for children; *BW* is body weight, 70 kg, 54 kg and 15 kg for adult, adolescent and child respectively; *AT* is average life time, equal to 365×*ED*; *RfD* is arsenic toxicity reference dose, 0.0003 mg/(kg·d). According to previous researches[9], $RQ \geq 1$ meaning that health risk arose from exposure to arsenic exceeds the acceptable benchmark, which may pose adverse health effect on people. While if $RQ$<1, no non-carcinogenic harm was expected.

### 2.6 Data statistics

Least significant difference and Pearson correlation coefficients were performed by SPSS17 (SPSS Inc, USA). All data were the mean of three replications, and all the figures were drew by Origin Pro8 (Origin Lab Cor.). The bioaccumulation factor (*BCF*) is a measure assessing their ability to assimilate arsenic from environment. It was computed as Equation (3). The translocation factor (*TF*) is a method to assess ability of plant to translocate arsenic from root to shoot, which was calculated as Equation (4)[13].

$$BCF = \frac{\text{plant concent}}{\text{medium concen}} \quad (3)$$

$$TF = \frac{\text{shoot concen}}{\text{root concent}} \quad (4)$$

## 3 Results and discussion

### 3.1 Fate of TOC, pH and arsenic species

The total arsenic and TOC in agricultural soil and poultry litter were 6.42 g/kg and 245.2 g/kg, (10.27±0.76) mg/kg and (10.94±0.23) mg/kg. As(V) occupied 86.65% total arsenic in soil, however occupied 41.12% in the composted poultry litter. The fate of TOC, pH and different species of arsenic in soil with or without poultry litter was presented in Figure 1. For the original day group of treatments (recorded as OD), applying different rates of poultry litter to soil brought about no discernible changes in pH, As(III) and As(V). However, concentrations of TOC, MMA and DMA showed apparent increase with the poultry litter rates grows, this may be due to TOC and organoarsenic were major in poultry litter.

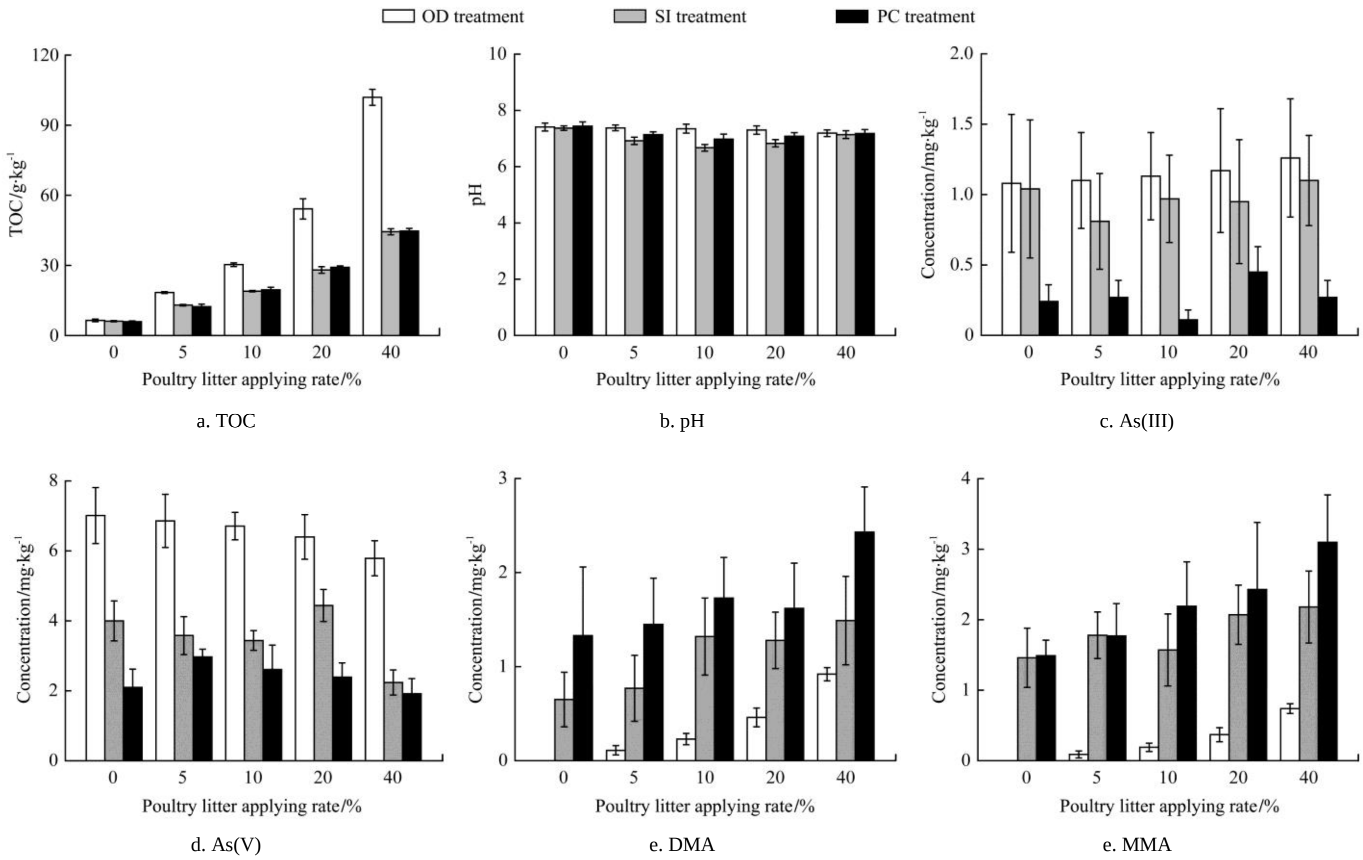


Figure1 TOC, pH and arsenic speciation in soil (bars represent three replicates)

Comparison of SI treatments with OD treatments shows that TOC, pH and different species of arsenic experienced different fates after 21 days' period of incubation. With the increasing of poultry litter applied to soil, TOC in SI treatments decreased more apparently (Figure 1a), decreasing ranging from 29.43% to 56.45% of OD concentrations, this probably because TOC provided as nutrition for microbial activities in soil. As for pH, there were no apparent variations in each treatment. Inorganic arsenic species displayed different levels decreasing: As(III) decreased slightly (Figure 1c), while As(V) decreased from about 7 mg/kg to approximately 4 mg/kg, the decreasing rates ranged from 30.62% to 61.31% (Figure 1d). Similarly, Cortinas et al.[20] found that As(V) and As(III) decreased in the initial 24 days during incubation. Contrast to inorganic arsenic species, MMA and DMA concentrations increase rather than drop (Figures 1e and 1f). Numerous researches[20,21] reported that inorganic arsenic could be methylated by microorganisms, generating organoarsenical compounds, such as MMA and DMA. Applying poultry litter to soil, nutrient such as TOC (organic matters), $NO_3$ and $PO_4$ would be increasing, leading microbes biotransform inorganic arsenic to organic arsenic species be enhanced[22]. Furthermore, the 40% SI treatments showed the biggest TOC and As(V) drop, but the highest increase of MMA and DMA, indirectly supports our deduction that biotic activities caused arsenic speciation, since the availability of nutrient is the key factor influencing microbe activity[15].

Making a comparison of PC with OD treatments, pH held nearly unchangeable regardless of cultivating plants or not, but arsenic experienced different fates. As(V) showed sharp decreases in each treatment in PC, and the decreasing concentrations ranged from 3.89 mg/kg to 4.91 mg/kg. It was well established that As(V) and phosphate have similar characterization, both transport cross plasma membrane into roots sharing the same carrier[11]. As(III) in PC treatments decreased to 22.50%, 24.67%, 9.44%, 38.18% and 21.43% of the corresponding OD treatments, decreased more than that in SI treatments, the enhanced reduction most probably was absorbed by plant[23]. The enhanced As(V) reduction was in accord with previous studies[13] that plants prefer to uptake As(V) than As(III). MMA and DMA showed sharper increases than SI treatments, indicating cultivating plant creating better circumstance that enhanced organoarsenical compounds shaping.

### 3.2 Brassica *napus* germination

Significant inhibitory efforts of poultry litter were observed, and exhibited a dose-dependent response on *Brassica napus* germination (Table 1). For control treatments (0%), the average germination rates were 71%±8%, which was 1.6, 2.7, 3.7 and 3.9 times higher than 5%, 10%, 20% and 40% rates treatments, respectively. Li et al.[24] found that wheat germination percentage displayed decreasing trend with the increase of arsenic concentrations. The decreasing germination may be because phytotoxicity induced from different arsenic species inhibited seeds germination. In addition, adding higher rate poultry litter to soil more nutrition would be for microorganism[25], enhancing shaping anaerobic soil circumstance, ultimately resulting in hostile environment for seeds germination.

**Table 1 *Brassica napus* germination and uptake data in both roots and shoots**

| Treatment | Germination/% | Concentration/mg $kg^{-1}$ (dry weight) | |
|---|---|---|---|
| | | root | shoot |
| 0% | 71±8 | 6.36±3.65 | 0.17±0.02 |
| 5% | 44±32 | 8.19±2.59 | 0.29±0.04 |
| 10% | 26±10 | 12.10±6.91 | 0.47±0.07 |
| 20% | 19±6 | 10.81±1.47 | 0.58±0.16 |
| 40% | 18±7 | 13.30±2.17 | 0.54±0.36 |

### 3.3 Brassica *napus* growth

In roots, the uptake of total arsenic was significantly higher than in shoots (Table 1). For roots, the mean uptake by *Brassica napus* in control treatments was (6.36±3.65) mg/kg (dry weight), while in 5%, 10%, 20% and 40% treatments were (8.19±2.59) mg/kg, (12.10±6.91) mg/kg, (10.81±1.47) mg/kg and (13.30±2.17) mg/kg, respectively. The uptake of total arsenic by shoots was all smaller than 1 mg/kg, which were far lower than roots. High retention of arsenic in roots were also reported in other plants, like tomato[26] and rice[27]. It could be plausibly explained that upon absorption arsenic, plants usually accumulate arsenic in roots for limiting toxic arsenic translocation to shoots[9], also was seen as plant detoxification process[11].

*Brassica napus* root length and shoot length were negatively affected by poultry litter in the whole (Figure 2a). The roots and shoots in treatments with 5% poultry litter were (5.67±0.04) cm and (4.68±0.08) cm respectively, which were stimulated compared with the control. With increasing poultry litter rates, the lengths of *Brassica napus* roots and shoots were inhibited, and were significantly negative with MMA and DMA (Table 2). Reduced roots and shoot growth in response to arsenic exposure were reported by a number of investigators in other plants[9,16]. Inorganic arsenic exist high phytotoxicity, As(III) can react with sulfhydryl group(-SH) of enzymes and tissues proteins, leading to inhibition to cellular function[11]. Also, As(V) would reduce to As(III) in the plant, enzymatic antioxidants and nonenzymatic antioxidants would be synthesized during the arsenic reduction process[14], posing inhibition to cellular function[13].

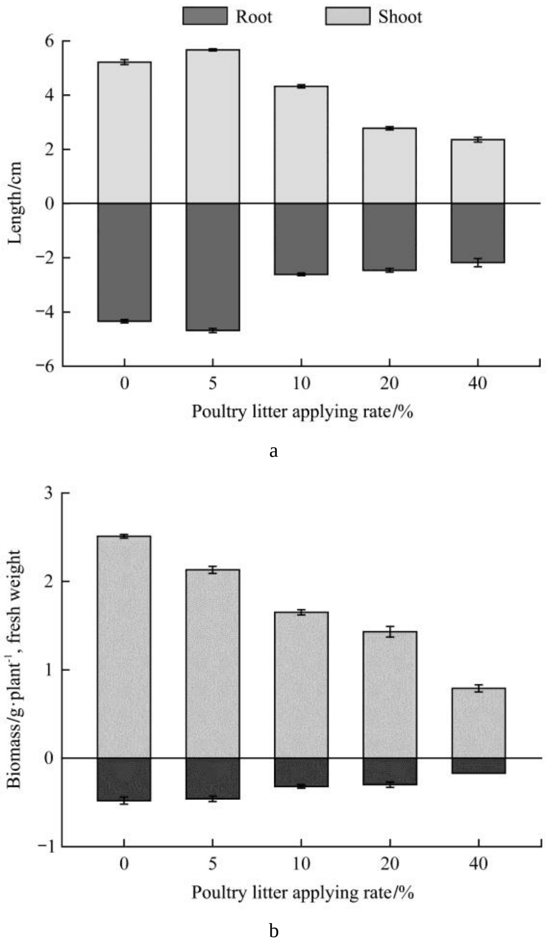


Figure 2 Length (a) and biomass (b) of *Brassica napus* roots and shoots after 21 days' cultivation (bars represent three replicates)

As shown in Figure 2b, *Brassica napus* roots and shoots biomass displayed apparent inhibitory effects of arsenic toxicity across the range of treatments. The shoot and root biomass in control treatments were (2.51±0.02) g/plant (fresh weight) and (0.48±0.04) g/plant respectively. Relative to the controls, the maximum reduction in shoots and roots biomass was observed in 40% treatments, which were about one third and one quarter of the controls for shoots and roots, respectively. This result was in accord with the research[25] that arsenic had inhibition on tomato shoots and roots biomass which were cultivated in arsenic contaminated area. The biomass reduction was more pronounced for shoots, such reduction could be excepted that the arsenic absorbed by plant usually trends to retained and accumulated in plant roots, and with smaller concentration in parts above the ground[12]. In addition, germination, length and biomass of roots and shoots were always positively correlated with each other; indirectly proving similar factor affected their inhibitory behaviors.

**Table 2 Pearson's correlation coefficient of As species with plant germination and growth (*n*=15)**

| | DMA | Germination | Root length | Shoot length | Root biomass | Shoot biomass |
|---|---|---|---|---|---|---|
| MMA | 0.67 | −0.63* | −0.62* | −0.67** | −0.67** | −0.73** |
| DMA | | −0.63 | −0.57 | −0.52* | −0.70** | −0.58* |
| Germination | | | 0.71** | 0.70** | 0.71** | 0.77** |
| Root length | | | | 0.92** | 0.90** | 0.88** |
| Shoot length | | | | | 0.91** | 0.91** |
| Root biomass | | | | | | 0.97** |

Note: *: at significant level at $p$<0.05; **: at significant level at $p$<0.01.

### 3.4 Arsenic soil-plant bioaccumulation and translocation factor

As shown in Table 3, the mean calculated BCF for roots in the control and 5% rate treatments were (0.61±0.34) and (0.79±0.24), respectively. Treatments of 10% and 20% shared similar BCF values, and 40% exhibited the highest BCF with value of (1.26±0.19). However, arsenic BCF of shoots were smaller than 0.1 regardless poultry litter applying quantity. This is consistent with the observation of beets[18] which with BCF values ranged from 0.027 to 0.055. The transfer coefficients of roots were 24-37 times bigger than shoots, indicated a higher proportion of arsenic accumulated into roots of *Brassica napus.*

**Table 3 BCF and TF values of *Brassica napus***

| Treatment | BCF (roots) | BCF (shoots) | TF |
|---|---|---|---|
| 0% | 0.61±0.34 | 0.02±0 | 0.04±0.02 |
| 5% | 0.79±0.24 | 0.03±0 | 0.04±0.01 |
| 10% | 1.17±0.66 | 0.05±0.01 | 0.04±0.02 |
| 20% | 1.04±0.14 | 0.06±0.02 | 0.05±0.01 |
| 40% | 1.26±0.19 | 0.05±0.03 | 0.04±0.03 |

Translocation of arsenic from roots to shoots of the *Brassica napus* was rather constant, with TF values ranged from 0.02 to 0.07. Previous research[17] reported that TF values of beets grew in different arsenic concentrations were with the range of 0.96-1.76, implying

*Brassica napus* trend to accumulate arsenic in root, detoxify below the ground. The low translocation coefficients implying *Brassica napus* translocation process was a active transport mechanism that involved proteins, enzymes and water transportation system[9].

### 3.5 Health risk assessment

The risk quotient (RQ) values for average weekly intake of 0.5 kg *Brassica napus*s across the range of treatments were calculated, the results were shown in Table 4. In general, RQ values induced from ingestion *Brassica napus* roots were two orders magnitude higher than induced from shoots. Apparent increasing RQ values were observed while the poultry litter applying rate growing, paralleling with a fluctuation of 20% treatments. For adults, average RQ values originated from uptake roots were 0.94±0.46, 1.60±0.54, 2.19±1.43, 1.85±0.22 and 2.23±0.43 for the control, 5%, 10%, 20% and 40% treatments, respectively. This risk values were a bit bigger than others' results of 0.6 to 1.0 for tomato[26]. Comparing with the criteria 1, except the control was close to this threshold, other treatments were unacceptable level, implying potential noncarcinogenic healthy risk for adults through uptake arsenic contaminated roots. As sensitive people groups, adolescents and children were 1.2 and 4.7 times higher than adults respectively, all surpassed the acceptable criteria. According to the potential risk to order, child exhibited the highest risk, adolescent ranked secondly, and adult exhibited the lowest risk. As for shoots, three groups of people suffered low risk, suggesting uptakes of shoots would not generate apparent risk. These results suggest that strategies to reduce potential health risk by avoiding uptake *Brassica napus* roots would be very efficient.

**Table 4 Risk quotient(RQ) for human health risk**

| Treatment | Roots | | | Shoots | | |
|---|---|---|---|---|---|---|
| | adult | adolescent | child | adult | adolescent | child |
| 0% | 0.94±0.46 | 1.16±0.56 | 4.39±2.13 | 0.03 | 0.03±0.01 | 0.12±0.02 |
| 5% | 1.60±0.54 | 1.97±0.66 | 7.48±2.51 | 0.04±0.01 | 0.05±0.01 | 0.20±0.03 |
| 10% | 2.19±1.43 | 2.69±1.76 | 10.22±6.68 | 0.07±0.01 | 0.08±0.01 | 0.32±0.04 |
| 20% | 1.85±0.22 | 2.27±0.27 | 8.63±1.02 | 0.08±0.02 | 0.10±0.03 | 0.39±0.11 |
| 40% | 2.23±0.43 | 2.74±0.53 | 10.43±2.01 | 0.08±0.05 | 0.10±0.07 | 0.38±0.25 |

## 4 Conclusions

The greenhouse pot study revealed that roxarsone degraded to inorganic arsenic and methylated arsenic after composting. During 21 d soil incubation, As(V) and As(III) were transformed to MMA and DMA through microbial methylation. Since plant uptake, As(V) and As(III) experienced enhanced decreases in PC treatments than in SI. *Brassica napus* seeds germination rate displayed decreasing trend with the increase of poultry litter. *Brassica napus* root uptake more than 10 times bigger arsenic than shoot, which was seen as plant detoxification process. Moreover, root exhibited pronounced elongation and biomass inhibitory by applying poultry litter than shoot. The BCF values for roots ranged around 1, which were 24-37 times bigger than shoots, indicating arsenic translocation process of *Brassica napus* was an active transport mechanism. The mean healthy risks induced from intake roots for adults were with range of 0.94-2.23, all surpassed the criterion 1, but for shoot within 0.1. The risks rising from roots were two orders magnitude higher than that from shoots. Furthermore, child exhibited the highest risk and adult exhibited the lowest risk in general. Avoiding intake *Brassica napus* roots would be efficient for reducing potential health risk.

## Acknowledgment

This study was financially supported by National Natural Science Foundation of China (No. 21177087) and the National High Technology Research and Development Program of China (2012AA101405).

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