Effects of high concentrations of soil arsenic on the growth of winter wheat (*Triticum aestivum* L) and rape (*Brassica napus*)

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ABSTRACT

Soil arsenic (As) levels are particularly high in parts of China, where wheat and rape are widely grown. Understanding the effects of As concentration on the growth of these two major crops is of significance for food production and security in China. A pot experiment was carried out to study the uptake of As and phosphorus (P), and the soil As bioavailability at different growth stages of wheat and rape. The results indicated that winter wheat was much more sensitive to As stress than rape. Wheat yields were elevated at low rates of As addition (< 60 mg/kg) but reduced at high rates of As concentrations (80–100 mg/kg); while the growth of rape hadn't showed significant responses to As addition. Phosphorus concentrations in wheat at jointing and ear sprouting stages increased with increasing soil As concentrations, and these increases were assumed to contribute a lot to enhanced growth of wheat at low As treatments. Arsenic did not significantly affect P concentrations in rape either. The highest As concentrations in wheat shoot and rape leaf were 8.31 and 3.63 mg/kg, respectively. Arsenic concentrations in wheat and rape grains did not exceed the maximum permissible limit for food stuffs of 1.0 mg/kg. When soil As concentration was less than 60 mg/kg, both wheat and rape could grow satisfactorily without adverse effects; when soil As concentration was 80–100 mg/kg, rape was more suitable to be planted than wheat.

Keywords: arsenic; stimulation; phytotoxicity; phosphorus

Arsenic is a toxic element widely encountered in the environment and in organisms. Arsenic can enter terrestrial and aquatic environments through both natural formation and anthropogenic activities (Tu and Ma 2003).

Persistence of arsenic within soil and its toxicity to plants and animals is of concern. Long-term exposure to low concentrations of As can lead to skin, bladder, lung, and prostate cancer. Non-cancer effects of ingesting As at low levels include cardiovascular disease, diabetes, and anemia (Zhang et al. 2002). There are a number of ways by which people can become exposed to As. The most important one is probably through ingestion of As in drinking water or food (Le et al. 2000). In some areas of Hubei, Shanxi, Yunnan, and Hunan provinces of China, soil As concentrations were much higher compared with other provinces because of coal fuels and metal smelters. For example, fourty hectares of agricultural soil was polluted in Hunan province due to irrigation of As-contaminated water by local farmers (Liao et al. 2004). In 1995, Chinese government constituted the environmental quality standard for soils (GB15618-1995). When soil arsenic concentrations exceed the limitation of 40 mg/kg, the soil is prohibited to plant crops. In 2005, Chinese government constituted the maximum of contaminants in foods (GB2762-2005), and the limitations of inorganic arsenic in some crop products were less than 0.2 mg/kg.

However, in some regions, although soil heavy metals concentrations exceed the limitations, people plant the certain crops, because the produces As concentrations do not exceed the limitations.

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In China both winter wheat and rape live through the winter and they are the main crops in the abovementioned areas. High As concentrations in soil or water may lead to elevated As accumulation in the grains of these two crops or the straw which is used as cattle feed.

Arsenic and P are analogues, which exhibit similar physicochemical behavior in soils and competed directly for the same sorption sites on soil particle surfaces (Hingston et al. 1971). Some researches reported that As sensitivity was intimately linked to P nutrition in plants, and applying P fertilizers could increase As availability in soils and enhanc plant uptake of As (Geng et al. 2006, Wang and Duan 2009, Pigna 2010). In the other way, different soil As concentrations can also affect soil P availability. It is of great significance to investigate the effects of different As concentrations in the soil on the growth of plants when P is applied at a normal rate. Winter wheat and rape were planted in As polluted soil, then As would affect the yield and P accumulations of wheat and rape directly. However, the effects of As on P uptake, accumulation and growth of wheat and rape were not clear.

Consumption of grains grown near these provinces would result in an increased intake of inorganic As. A provisional tolerance weekly intake for inorganic As has been established at 15 μg per kg body mass from all food sources including water (WHO 1989). Therefore the evaluation of the possible health risk to humans or animals through consuming crops cultivated in As contaminated soils is urgently called for. However, less attention has been paid to As accumulation in wheat and rape at different growth stages, and the tolerance ability of wheat and rape to As stress is not clear.

This study, therefore, was designed to investigate (1) the effects of As on the growth of both wheat and rape; (2) As and P accumulation by wheat and rape at different growth stages; (3) to recommend the most adaptable plants in agricultural soils with various As contamination levels.

MATERIAL AND METHODS

A yellow-brown soil was collected from the agricultural areas in the campus of Huazhong Agricultural University. The pH (5.9) was measured using a 1:1 soil to water ratio; organic matter content (2.1%) was measured by the Walkley Black method (Nelson et al. 1982). The background As and P concentration in the soil were 24.8 mg/kg and 0.68 g/kg, respectively. After air-drying, the soil was passed through a 1 cm sieve. Then the soil was spiked with Na_2HAsO_4 at rates of 20, 40, 60, 80, and 100 mg/kg. Soil was fertilized with (g/kg soil): N 0.25, P_2O_5 0.15, and K_2O 0.20 supplied as $(NH_4)_2SO_4$, KH_2PO_4, KCl respectively, with all being of analytical grade. Each pot contained 6.5 kg soil. After 10 days, the wheat seeds E-18 (each pot of 9 plants) and rape seeds Zhongyou 821 (5 plants per pot), were irrigated with distilled water and a movable waterproof shed was used during the whole crop-growing season. Each treatment was replicated four times. One plant was harvested from each pot at the tillering, jointing, and sprouting stage of wheat, repectively. and at the seedling, jointing and flowering stage for the rape. Then the harvested plants were rinsed thoroughly with deionized water, and oven-dried at 65°C for 72 h for elemental analysis. Finally, after measuring plants height, whole wheat and rape plants were harvested by cutting at 2 cm above the soil. The grain yield and straw biomass were recorded after drying.

Chemical analysis. Dry plant samples were digested following a HNO_3/H_2O_2 microwave wet-ashing procedure (He et al. 2002). The digests were filtered and diluted with deionized water.

Table 1. Effect of arsenic on biomass and height of wheat and rape

As treatments (mg/kg)	Rape		Wheat	
	biomass (g/pot)	height (cm)	biomass (g/pot)	height (cm)
0	24.9 ± 0.5[a]	130.4 ± 5.5[a]	68.7 ± 2.6[c]	74.2 ± 0.9[b]
20	24.2 ± 1.5[a]	126.5 ± 7.8[a]	81.6 ± 2.1[ab]	79.7 ± 1.2[a]
40	22.9 ± 2.1[a]	121.4 ± 9.1[a]	82.8 ± 4.1[ab]	80.0 ± 2.3[a]
60	23.8 ± 4.1[a]	124.8 ± 9.0[a]	88.6 ± 7.8[a]	80.0 ± 0.8[a]
80	20.7 ± 1.3[a]	122.3 ± 6.5[a]	65.6 ± 7.3[c]	76.0 ± 1.1[b]
100	23.5 ± 4.2[a]	127.5 ± 5.0[a]	60.8 ± 4.4[d]	76.3 ± 1.5[b]

Significant differences ($P < 0.05$) as assessed by the *LSD*-test

Table 2. Effects of arsenic on the agronomic parameters of rape

Treatments As (mg/kg)	Yield (g/pot)	Weight of 1000 grains (g)	Number of ramus (1/plant)	Number of fruitage (1/plant)
0	15.7 ± 0.7[a]	3.86 ± 0.1[a]	7.5 ± 0.5[a]	159 ± 18[a]
20	16.2 ± 2.0[a]	3.84 ± 0.12[a]	7.3 ± 0.9[a]	196 ± 46[a]
40	14.1 ± 2.5[a]	3.65 ± 0.09[a]	7.6 ± 0.6[a]	147 ± 26[a]
60	15.8 ± 0.76[a]	3.71 ± 0.1[a]	7.7 ± 0.5[a]	137 ± 34[a]
80	15.1 ± 2.5[a]	3.77 ± 0.13[a]	7.3 ± 0.9[a]	175 ± 40[a]
100	14.3 ± 2.2[a]	3.65 ± 0.11[a]	6.7 ± 1.4[a]	182 ± 47[a]

Significant differences ($P < 0.05$) as assessed by *LSD*-test

Phosphorus (P) was determined by FIAstar-5000 (Foss, DK-3400, Hillerod, Denmark). Total As was determined by HG-AAs (Varian-SpectrAA-220, Palo Alto, USA). Acid blanks were analyzed and the recovery of standard was > 95%. Total P contents in both wheat and rape shoots were calculated: Total P = biomass × P concentrations

Statistical analysis. Analysis was performed with SPSS. The data were presented as the means of four replicates. All statistically significant differences were tested at $P < 0.05$ by *LSD*.

RESULTS AND DISCUSSION

Effects of arsenic on growth and agronomic parameters. Comparing with the control, As addition did not significantly affect both height and biomass of rape (Table 1). However, the biomass and height of wheat, increased by 24.1 and 7.8% separately at 60 mg As/kg soil. When exposed to high levels of As (≥80 mg/kg), the wheat height and biomass decreased significantly (Table 1). The similar results were observed for agronomic parameters: As addition did not significantly affect the agronomic parameters of rape compared with the control (Table 2). But As had increased wheat yield significantly with 20.1% higher than the control when As addition was 60 mg/kg. When soil As concentration was ≥ 80 mg/kg, the wheat yield significantly decreased. And the same tendency occurred with the weight of 1000 grains (Table 3).

Arsenic is not an essential element for plants, but small amounts of arsenic can stimulate plant growth and increase plant biomass (Onken and Hossner 1995). Yield increases due to small additions of arsenic have been observed from corn, potatoes, rye, and wheat (Carbonell et al. 1998, Gulz and Gupta 2000, Gulz et al. 2005). Similar increases in dry matter weight were also observed in tomato plants by Burló et al. (1999). However, As becomes toxic for all plants, causing chlorosis, inhibition of growth and finally death, when soil As concentration is high (Gulz et al. 2005). In the experiment, the stimulating effect of low As concentrations was the reason for wheat growth and yield increasing, and the inhibition of wheat growth and yield rested with As toxicity, respectively. But rape had high tolerance to As and could grow normally even though soil As concentrations was very high (100 mg/kg).

Uptake and accumulation of P at different growth stage. Arsenic addition did not significantly affect rape P concentration at different growth stages (Tables 4 and 5). Total P content of rape in each pot did not significantly change with soil As

Table 3. Effects of arsenic on the agronomic parameters of wheat

Treatments As (mg/kg)	Yield (g/pot)	Number of tiller (1/pot)	Length of spike (cm)	Weight of 1000 grains (g)
0	35.52 ± 4.43b	26 ± 0.96a	10.38 ± 0.05ab	48.27 ± 1.13b
20	37.49 ± 8.72b	27 ± 1.50a	10.83 ± 0.51a	51.91 ± 1.11ab
40	36.22 ± 5.12b	25 ± 3.09a	10.75 ± 0.47a	50.78 ± 0.70a
60	42.65 ± 3.29a	25 ± 1.15a	10.78 ± 0.29a	53.92 ± 2.03a
80	29.02 ± 7.66c	26 ± 1.41a	10.18 ± 0.39b	39.80 ± 3.25c
100	22.97 ± 7.43d	24 ± 2.36b	9.4 ± 0.29c	41.62 ± 1.28c

Significant differences ($P < 0.05$) as assessed by *LSD*-test

Table 4. Uptake of rape P at different growth stage under arsenic stress

Treatments As (mg/kg)	P concentration (g/kg) in rape shoots			
	seeding	jointing	flowering stage	total P (mg/pot)
0	4.36 ± 0.56[a]	3.71 ± 0.17[ab]	3.73 ± 0.29[a]	92.8[a]
20	4.3 ± 0.22[a]	4.36 ± 0.84[a]	3.82 ± 0.31[a]	92.4[a]
40	4.56 ± 0.48[a]	4.41 ± 0.95[a]	3.83 ± 0.67[a]	87.7[a]
60	4.33 ± 0.35[a]	3.91 ± 0.69[a]	3.84 ± 0.26[a]	91.3[a]
80	4.24 ± 0.47[a]	4.54 ± 1.19[a]	4.07 ± 0.32[a]	85.2[ab]
100	4.75 ± 0.82[a]	4.19 ± 0.45[a]	3.87 ± 0.45[a]	90.9[a]

Significant differences ($P < 0.05$) as assessed by *LSD*-test

concentration. However, wheat P concentration increased at jointing stage when soil As ranged from 40 to 80 mg/kg, compared with the control ($P < 0.05$). And soil As significantly decreased wheat P concentration when added arsenic ≥ 80 mg/kg at ear sprouting stage. Total P content in wheat shoots increased when soil added As concentrations < 60 mg/kg, but decreased when added arsenic concentration > 80 mg/kg ($P < 0.05$).

It has been reported that P and As exhibit similar physicochemical behavior in soils and they competed directly for the same sorption sites on soil particle surfaces (Hingston et al. 1971). Therefore, increasing As concentrations are expected to cause release of phosphate and enhance the phosphate bioavailability in soil. According to Gulz et al. (2005), different plant species had different availability to absorb and accumulate phosphate from the same As contaminated soil. They also reported that a better P nutrition status reduced the toxicity of As present in the plants and thus the inhibition of growth. Pigna et al. (2010) reported that As toxicity in crops could be more prevalent in situation where As contamination was found coexisting with low available P. Lu et al. (2010) reported rice grain arsenic was correlated with P concentration and molar ration of P/As in shoot.

In the experiment, As enhanced wheat P accumulation at low soil As concentrations (≤ 60 mg/kg) and decreased wheat P when soil arsenic concentrations were 80–100 mg/kg at the jointing and ear sprouting stage, respectively. That may partly explain the stimulation or inhibition of wheat growth and yield at different soil As concentrations. However, rape P was not affected by As significantly in the whole growth stage. It implied that the rape has high tolerance to As stress and keeps normal P levels in tissue to alleviate As phytotoxicity.

Other researches also reported that not only As concentration in the plants was the factor governing growth, but also plants phosphate statue was another important factor (Gulz et al. 2005, Cozzolino et al. 2010). Wang and Duan (2009) reported that P deficiency increased the sensitivity of rice to arsenate and increased external phosphate supply could alleviate As toxicity. It could be assumed that the effects of soil As on wheat and rape depended on the concentrations of both P and As in the two plants.

Uptake and accumulation of As at different growth stages. With the advance of growth stages, As concentrations significantly increased in wheat shoot and the highest shoot As concentration was

Table 5. Uptake of wheat P at different growth stage under arsenic stress

Treatments As (mg/kg)	P concentration (g/kg) in wheat shoots			
	tillering	jointing	ear sprouting	total P (mg/pot)
0	3.86 ± 0.03[a]	2.84 ± 0.11[b]	2.31 ± 0.33[a]	158.7[c]
20	3.85 ± 0.35[a]	2.81 ± 0.36[b]	2.26 ± 0.17[bc]	184.4[b]
40	3.91 ± 0.25[a]	3.21 ± 0.28[a]	2.52 ± 0.27[a]	208.7[a]
60	3.95 ± 0.15[a]	3.12 ± 0.28[a]	2.36 ± 0.27[a]	208.1[a]
80	4.03 ± 0.26[a]	3.22 ± 0.32[a]	1.98 ± 0.05[bc]	145.6[c]
100	3.71 ± 0.43[a]	2.92 ± 0.13[b]	1.93 ± 0.19[c]	133.6[d]

Significant differences ($P < 0.05$) as assessed by *LSD*-test

Table 6. Arsenic concentrations in the shoots of rape at different growth stages

Treatments As (mg/kg)	As concentration (mg/kg) at different growth stages				
	seeding	jointing	flowering (in stem)	flowering (in leaf)	grain
0	0.14 ± 0.05^{c}	0.8 ± 0.14^{b}	0.77 ± 0.06^{b}	1.42 ± 0.08^{d}	0.021^{c}
20	0.43 ± 0.17^{b}	0.97 ± 0.21^{b}	0.88 ± 0.11^{b}	1.57 ± 0.12^{d}	0.025^{abc}
40	0.56 ± 0.26^{b}	0.77 ± 0.11^{b}	0.93 ± 0.11^{b}	2.31 ± 0.05^{c}	0.023^{bc}
60	0.66 ± 0.1^{ab}	1.07 ± 0.12^{b}	1.02 ± 0.12^{b}	2.75 ± 0.7^{bc}	0.029^{ab}
80	0.72 ± 0.22^{a}	1.63 ± 0.54^{a}	1.34 ± 0.15^{a}	3.11 ± 0.42^{ab}	0.031^{a}
100	0.91 ± 0.11^{a}	2.03 ± 0.37^{a}	1.36 ± 0.45^{a}	3.63 ± 0.44^{a}	0.027^{abc}

Significant differences ($P < 0.05$) as assessed by *LSD*-test

Table 7. Arsenic concentration in the shoots of wheat at different growth stages

Treatments As (mg/kg)	As concentration (mg/kg) at different growth stage			
	tillering	jointing	ear sprouting	grain
0	1.69 ± 0.31^{b}	2.33 ± 0.05^{b}	2.84 ± 0.44^{d}	0.014^{d}
20	2.11 ± 0.21^{ab}	2.58 ± 0.16^{b}	3.79 ± 0.06^{d}	0.024^{c}
40	2.52 ± 0.17^{a}	4.03 ± 0.28^{a}	6.29 ± 0.31^{c}	0.023^{cd}
60	2.23 ± 0.35^{a}	4.75 ± 0.28^{a}	6.89 ± 0.75^{bc}	0.043^{a}
80	2.29 ± 0.21^{a}	4.02 ± 0.51^{a}	7.75 ± 1.27^{ab}	0.033^{bc}
100	2.32 ± 0.1^{a}	3.94 ± 0.27^{a}	8.31 ± 0.87^{a}	0.039^{ab}

Significant differences ($P < 0.05$) as assessed by *LSD*-test

observed at ear sprouting stage. Arsenic concentration in the wheat shoots was 8.31 mg/kg when soil added arsenic was 100 mg/kg (Table 7). However, arsenic concentration in rape shoot did not change significantly at different growth stages. Rape shoot accumulated much less arsenic than wheat shoot at different arsenic treatments (Table 6). Arsenic concentrations were about 0.01–0.05 mg/kg in the grains of both wheat and rape.

Arsenic concentration in the rape stem or leaf was much less than in the wheat shoot. It can be assumed that rape is less sensitive to arsenic than wheat. Some authors report that plants belonging to the order Cruciferae have high tolerance to As, such as Indian mustard varieties, rape etc.. (Chaturvedi 2006, Zhong et al. 2011). The grain As concentrations of wheat and rape increased with arsenic added in soil but did not exceed the maximum permissible limit for food stuffs of 1.0 mg/kg (National Food Authority 1993). A provisional tolerance weekly intake (PTWT) for inorganic As has been established at 15 µg/kg body mass from all food sources including water (WHO 1989). Assuming a body weight of 60 kg, then the PTWI is 900 µg. If each person consuming 1 kg of grains per day would induce exposure of 70–350 µg As per week, which did not exceed the PTWI limit of 900 µg. Therefore, the grains of wheat and rape are safe to be consumed.

Arsenic accumulation in the straw of up to about 8.3 mg/kg in the highest arsenate treatment showed that the wheat straw has the potential to accumulate high level of arsenic. Straw in the UK fed to cattle contained arsenic less than 0.20 mg/kg (Nicholson et al. 1999). Sheep fed with organoarsenicas showed a significantly increased arsenic concentration in tissue and milk (Shariatpanahi and Anderson 1984). Therefore, it is possible that cattle fed with arsenic contaminated straw may show elevated arsenic in the meat and/or milk. Cattle fed with high arsenic contaminated straw could be a direct threat to their health and to human health via ingesting arsenic contaminated meat and drinking milk with elevated arsenic concentration (Abedin et al. 2002). However, arsenic concentrations of rape stem and leaf is of 0.77–3.63 mg/kg which is much less than that in wheat shoots. It can be assumed that rape could transfer less arsenic to the food chain and thus pose lower risk to human or animal health than wheat.

Therefore, when soil arsenic concentrations is 0–60 mg/kg, both wheat and rape are suitable to be planted; when soil arsenic concentrations is 80–100 mg/kg, rape is more suitable to be planted because of rape's higher tolerance to arsenic and considerable yield.

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