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Effects of Arsenic on Nutrients Uptake of Wheat (*Triticum aestivum* L) at Different Growth Stages

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Arsenic (As) levels in soils have been elevated, especially in some wheat-producing areas of China. Understanding the effects of arsenic pollution on wheat (*Triticum aestivum* L) nutrient elements is of major significance for food production and security. A soil pot experiment was carried out to study the uptake of nutrient elements and arsenic at different growth stages. Arsenic concentrations in wheat shoots is ear sprouting stage > jointing stage > tillering stage. Ear sprouting stage was the main stage that arsenic could affect the nutrient elements uptake. When arsenic concentration in soil was ≤ 60 mg/kg, arsenic increased the straw P, N and K concentrations at the jointing and ear sprouting stages and enhanced the biomass as well as other agronomic parameters such as grain yield. The increase in wheat biomass and yield probably resulted from P, N, K abundance. However, when soil arsenic was ≥ 80 mg/kg, arsenic significantly decreased macroelement (P, K and N) concentrations in the shoots. The reduction in wheat biomass and yield at high arsenic levels probably resulted from both arsenic phytotoxicity and nutrient elements lack. The relationship between shoots arsenic and shoots N, P, K concentrations was quadratic equations. The Cu, Fe, Zn concentrations in wheat shoots significantly were decreased and they were negatively linearly correlated with arsenic concentrations in wheat shoots (R^2 = 0.7064, 0.8265 and 0.8302, respectively). The highest arsenic concentration in wheat grains was 0.39 mg/kg and did not exceed the maximum permissible limit for food stuffs of 0.7 mg/kg.

Key Words: Arsenic, Weat, Nutrient elements, Growth stage.

INTRODUCTION

Arsenic is a ubiquitous natural element which can be detected in almost all soils. The arsenic content of soils is related to the geological substratum and a rather wide range of arsenic levels have been found in soils around the world[1]. Arsenic, however, may accumulate in soils due to human activities such as waste discharges of metal processing plants, burning of fossil fuels, mining of arsenic containing ores and use of arsenical pesticides[2-4].

Areas of the arsenic problem include wheat-growing regions[5]. In China, some areas of Hubei, Shanxi, Yunnan and Hunan provinces have higher concentrations of soil arsenic than the other regions. Soil arsenic concentration was reported to range form 92-840 mg/kg in Changde in Hunan province[6]. High soil arsenic concentration significantly affects the growth and production of wheat as one of the main crops in China. It had reported that arsenic could decrease wheat growth and nutrients uptake[7,8] found arsenic significantly decreased N, P, K, Fe, Cu and Zn uptake of wheat in hydroponic conditions. However, there was little research on nutrients uptake of wheat at different stages. Until now, we do not know which growth stage is the most serious time that arsenic affects wheat nutrient uptake. It is needed to evaluate the effects of arsenic on production and nutrients uptake of wheat and the possible health risk to humans consuming crops cultivated in arsenic-contaminated soil. The objectives of this paper are: (1) to investigate the effects of arsenic on the growth of winter wheat; (2) to examine the uptake of arsenic and nutrient elements at different growth stages of wheat and the transfer of arsenic to human or animals *via* the food chain.

EXPERIMENTAL

Greenhouse: A yellow-brown soil was collected from campus of Huazhong Agricultural University. The pH (5.9) was measured using a 1:1 soil to water ratio; organic matter content was 2.1 %. The background arsenic and phosphorus 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 and KCl, respectively, all of analytically pure grade. Each pot contained 6.5 kg soil. After 10 days, the plants seeds

E-18, each pot of 9 plants, were irrigated with distilled water and a movable waterproof shed was used during the whole crop-growing season. Each treatment was replicated four times. During tillering, jointing and sprouting stage, 1 plant in each pot was harvested and the plants were rinsed thoroughly with deionized water and oven-dried at 65 ºC for 72 h for elemental analysis. Finally, after measuring plants height and tiller numbers, whole wheat 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[9]. The digests were filtered and diluted with deionized water. Total concentrations of potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) were analyzed by ICP-AES (Varain-ICP 700). Total phosphorus (P) and nitrogen (N) were determined by FIC-5000. Total arsenic was determined by HG-AAS (Varain-SpectrAA-220). Acid blanks were analyzed and the recovery of standard was 95 %.

Statistical analysis: The data were average of four replications. All statistically significant differences were tested at $p < 0.05$ by LSD.

RESULTS AND DISCUSSION

Growth and biomass of wheat in response to arsenic addition: Compared with the control, the biomass and height of wheat plants in the treatment of 60 mg arsenic/kg soil increased by 24.1 and 7.8 %, respectively. When exposed to higher levels of arsenic at 100 mg/kg, the biomass of plants decreased (Fig. 1).

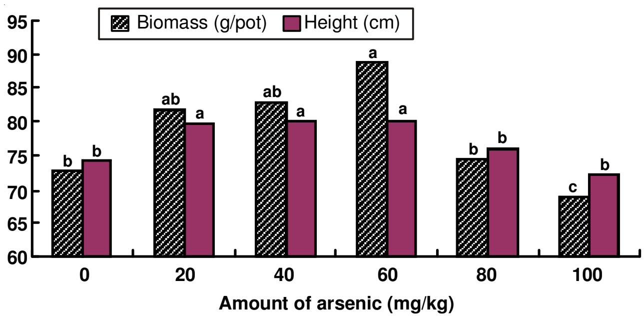

Fig. 1. Effects of arsenic on the biomass and height of wheat at maturity. Significant differences ($p < 0.05$) as assessed by LSD-test

The highest wheat yield of 42.65 g/pot was observed in 60 mg/kg arsenic treatment which was 20.1 % higher than that of control. When arsenic concentration in soil was ≥ 80 mg/kg, wheat yield significantly decreased. The lowest wheat yield of 22.9 g/pot was observed in 100 mg/kg arsenic treatment, which was 35.5 and 46.2 % lower than that in the control and in the 60 mg/kg arsenic treatment, respectively. And the same tendency occurred with weight of 1000 grains. Arsenic did not significantly affect the wheat number of tiller in each pot and the length of spike except in 100 mg/kg arsenic treatment (Table-1).

Uptake and accumulation of arsenic at different growth stage: Wheat accumulated different concentrations of arsenic at different growth stages (Table-2). At the tillering stage, wheat accumulated almost the same concentration of arsenic in arsenic-added treatments, while the plants in the control accumulated less arsenic. At the jointing stage, wheat accumulated more arsenic in all treatments compared with tillering stage, but wheat arsenic concentration did not significantly increase with the addition rate increasing from 40-100 mg/kg. At the ear sprouting stage, arsenic concentration in the wheat shoots was linearly correlated with arsenic addition rates (R^2 = 0.94). When soil arsenic was 100 mg/kg, the wheat shoot arsenic concentration was increased more than 3 times compared with control. The arsenic concentration in grain at the control treatment was only 0.14 mg/kg but increased to 0.39 mg/kg arsenic in the 100 mg/kg arsenic treatment.

Uptake and accumulation of nutrient elements under arsenic stress at different growth stages: At the tillering and jointing stages, arsenic did not significantly affect the concentrations of macroelements (Ca, Mg, K and P) in wheat shoots. At the ear sprouting stage, arsenic significantly decreased the calcium concentration with soil arsenic addition increased. However, when soil arsenic concentration was ≤ 60 mg/kg, arsenic increased the phosphorus concentration at 40 mg/kg arsenic treatment. When soil arsenic concentration was ≥ 80 mg/kg, arsenic reduced P, K and Mg concentrations in shoots compared with the control (Table-3).

At the tillering stage, arsenic did not significantly affect the concentrations of Mn, Fe and Cu in the wheat shoots but decreased the Zn concentrations. At the jointing stage, arsenic decreased the concentrations of Cu and Fe, but the concentrations of Mn and Zn were comparable to those in the control treatment when soil added arsenic was 60 mg/kg. At the sprouting stage, the concentrations of Fe, Cu, Zn and Mn significantly decreased with soil arsenic concentrations increasing (Table-4).

Arsenic is toxic to plant, especially to plant roots. High arsenic concentration in root could restrain the ability of absorbing nutrients and water and decrease the growth of frond. At tiller stage, winter wheat did not need much nutrients and

TABLE-1
EFFECTS OF ARSENIC ON THE AGRONOMIC PARAMETERS OF WHEAT

Soil arsenic (mg/kg)	Yield (g/pot)	Number of tiller (/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	9.40 ± 0.29c	41.62 ± 1.28c

Significant differences ($p < 0.05$) as assessed by LSD-test.

TABLE-2
ARSENIC CONCENTRATION IN THE SHOOTS OF WHEAT AT DIFFERENT GROWTH STAGES

Treatments	Arsenic conc. (mg/kg) in wheat shoots			
Arsenic (mg/kg)	Tillering	Jointing	Ear sprouting	Grain
0	1.69 ± 0.31b	2.33 ± 0.05b	2.84 ± 0.44d	0.14d
20	2.11 ± 0.21ab	2.58 ± 0.16b	3.79 ± 0.06d	0.24c
40	2.52 ± 0.17a	4.03 ± 0.28a	6.29 ± 0.31c	0.23cd
60	2.23 ± 0.35a	4.75 ± 0.28a	6.89 ± 0.75bc	0.43a
80	2.29 ± 0.21a	4.02 ± 0.51a	7.75 ± 1.27ab	0.33bc
100	2.32 ± 0.1a	3.94 ± 0.27a	8.31 ± 0.87a	0.39ab

Significant differences ($p < 0.05$) as assessed by LSD-test.

TABLE-3
EFFECTS OF ARSENIC ON THE SHOOT MACROELEMENTS CONCENTRATIONS OF WHEAT AT DIFFERENT GROWTH STAGES

Arsenic treatment (mg/kg)		0	20	40	60	80	100
Ca (g/kg)	Ear sprouting	2.57a	2.10b	2.04b	1.89b	1.83b	1.81b
	Jointing	2.44a	2.51a	2.45ab	2.28ab	2.21ab	1.93b
	Tillering	4.45a	3.85ab	4.02ab	3.85ab	4.45a	3.81b
Mg (g/kg)	Ear sprouting	1.17a	0.96bc	1.02ab	0.98bc	0.81bc	0.87c
	Jointing	1.37a	1.32ab	1.22ab	1.26ab	1.12b	1.12b
	Tillering	2.03a	1.91a	1.83a	1.58a	1.76a	1.70a
K (g/kg)	Ear sprouting	21.6ab	21.3ab	22.4ab	21.4ab	20.3b	18.2b
	Jointing	23.9a	26.3a	25.1a	25.7a	25.8a	23.5a
	Tillering	42.8a	41.6a	40.5a	38.5a	41.8a	39.0a
P (g/kg)	Ear sprouting	2.31b	2.26bc	2.52a	2.35ab	1.98bc	1.93c
	Jointing	2.84b	2.81b	3.21a	3.12a	3.22a	2.92b
	Tillering	3.86a	3.85a	3.91a	3.95a	4.03a	3.71a
N (g/kg)	Ear sprouting	6.71a	6.58ab	6.82a	6..91a	6.21b	5.68b
	Jointing	14.8a	13.9a	14.4a	15.6a	14.1a	13.6ab
	Tillering	21.2a	20.7a	22.5a	22.1a	20.5a	20.1a

Significant differences ($p < 0.05$) as assessed by LSD-test.

TABLE-4
EFFECTS OF ARSENIC ON THE SHOOT MICRO-ELEMENT CONCENTRATIONS OF WHEAT AT DIFFERENT GROWTH STAGES

Arsenic treatment (mg/kg)		0	20	40	60	80	100
Cu (mg/kg)	Ear sprouting	9.79a	7.10b	5.34bc	3.34d	5.74bc	4.76cd
	Jointing	8.17b	7.57bc	6.67bc	11.53a	6.59bc	6.32c
	Tillering	9.95a	9.79a	8.49a	8.29a	10.49a	8.57a
Fe (mg/kg)	Ear sprouting	711.6a	433.5b	305.8bc	320.3bc	255.9c	254.6c
	Jointing	253.1a	205.7ab	149.7b	197.3ab	164.b	187.8ab
	Tillering	232.4a	200.7a	137.7a	119.4a	142.8a	133.3a
Mn (mg/kg)	Ear sprouting	144.2a	134.2a	138.9a	133.3a	100.5b	119.5ab
	Jointing	138.4a	138.1a	134.2a	138.1a	138.2a	110.6b
	Tillering	195.6b	204.3b	215.9ab	253.2a	238.2ab	234.6ab
Zn (mg/kg)	Ear sprouting	25.4a	21.6b	21.2b	17.4c	16.6c	17.6c
	Jointing	44.4a	44.8a	41.0a	40.9a	38.7a	43.5a
	Tillering	59.4a	46.9ab	42.7b	33.8b	46.2ab	43.9b

Significant differences ($p < 0.05$) as assessed by LSD-test.

water for growing slowly under low temperature. Arsenic did not significantly affect the nutrients uptake because its concentrations in wheat tissue were relatively low. At jointing and ear sprouting stages, wheat grew very fast and needed more nutrient elements. Lots of arsenic was accumulated in wheat tissue with much nutrient elements having been absorbed by wheat. Arsenic concentration in wheat tissue at differential growing stages is ear sprouting > jointing > tillering. It was thus assumed that the ear sprouting stage could be the main stage that arsenic affected nutrients uptake in wheat tissue.

At ear sprouting stage, arsenic concentration in wheat shoots increased significantly with soil arsenic increasing. Arsenic affected the accumulation and distribution of nutrient elements in wheat significantly. The relationship between shoots arsenic and shoots K, P and N concentrations is quadratic equation with correlation coefficients (R^2) being 0.8735, 0.7244 and 0.7925, respectively (Table-5) and relationship between shoots arsenic and shoots Ca, Mg, Cu, Fe and Zn concentrations is simple equation (Table-5).

When soil arsenic concentrations was < 60 mg/kg, N and K concentrations in wheat shoots had tendency to increase at jointing and ear sprouting stages. However, P concentration in shoot increased significantly comparing with control. Phosphate and arsenate are analogues and have similar physical and chemical character[10]. Arsenate added to the soil induced more P uptake as more P was replaced from the soil by arsenate[11-13]. It was assumed that soil arsenic enhanced N, K and P uptake in wheat shoots when soil arsenic concentration was < 60 mg/kg.

TABLE-5
RELATIONSHIP BETWEEN ARSENIC AND MACRO-MICROELEMENTS IN WHEAT SHOOTS AT THE EAR SPROUTING STAGE

Element	Equations	Correlation coefficients (R^2)
Ca	Y = -119.2x + 2748.8	R^2 = 0.8256
Mg	Y = -45.4x + 1238.4	R^2 = 0.6186
K	Y = -369.9x^2 + 3656.1x + 13652	R^2 = 0.8735
P	Y = -54.8x^2 + 549.9x + 1109	R^2 = 0.7244
N	Y = -113.7x^2 + 1129.8x + 4229	R^2 = 0.7925
Cu	Y = -0.8621x + 11.137	R^2 = 0.7064
Fe	Y = -73.411x + 816.71	R^2 = 0.8265
Mn	Y = -5.0768x + 158.61	R^2 = 0.4734
Zn	Y = -1.4229x + 28.426	R^2 = 0.8302

X: Arsenic concentrations in wheat shoots (mg/kg); Y: Macro-microelements concentration in wheat shoots (mg/kg).

This might be one of the reasons why arsenic enhanced the wheat biomass and yield. However, When the spiked arsenic concentrations were > 80 mg/kg, arsenic decreased the macroelements (Ca, Mg, N, P and K) concentrations of wheat. Calcium is of fundamental importance for maintaining membrane permeability and cell integrity[12] and magnesium mainly serves as the central atom of the chlorophyll molecule and a co-factor in many enzymes activating phosphorylation processes. Potassium and nitrogen in plants have close relationships with protein, cytokinin supply and plant growth. The decline of Ca, Mg, N and K in wheat tissue could induce nutrient elements lack and reduce biomass and yield. Phosphate in plants is important for energy transfer and protein metabolism[14]. Phosphate and arsenate are analogues and are taken up by plants through the same transport system[10,15]. It is likely that arsenic will compete with phosphate for uptake, resulting in a lower accumulation of phosphate in wheat shoots. Similar results have been reported by other authors[15]. The reduction in nutrient elements accumulation at high arsenic levels probably resulted from either arsenic phytotoxicity or competitive uptake. This was the reason why arsenic decreased wheat biomass and yield when soli arsenic concentration was 80-100 mg/kg.

The Cu, Fe, Zn concentrations in wheat shoots significantly decreased with the increasing arsenic addition rates at ear sprouting and the correlation coefficients (R^2) was 0.7064, 0.8265 and 0.8302, respectively (Table-5). This indicated that arsenic easily limited wheat microelements uptake when soil arsenic concentration was 20-100 mg/kg. Microelements are associated with various enzymes systems[14] and are generally believed to be intermediate in mobility. Arsenic-induced reduction in micronutrients in wheat shoots was probably due to arsenic phytotoxicity. Fe, Zn and Cu are associated with various enzymes systems and takes part in the function of photosynthesis. This means that activities of various enzymes and photosynthesis were restrained by arsenic.

When soil arsenic concentration increased, wheat grain arsenic concentration increased at the ear sprouting stage (R^2 = 0.93). The grain arsenic concentrations in our experiment increased significantly from 0.14 mg/kg in the control treatment to 0.39 mg/kg in the highest arsenate treatment, but did not exceed the maximum permissible limit for food stuffs of 0.7 mg/kg. Therefore, the grains were safe to be consumed.

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