Density-dependent Accumulation of Heavy Metals in Spring Wheat (*Triticum Aestivum*) and the Risk Assessment from Weak Alkaline Soils, Northwest of China

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Abstract

In order to investigate the effects of planting density on accumulation of heavy metals in spring wheat, a weak alkaline agricultural district mainly contaminated by heavy metals Cu, Zn, Pb and Cd, was chosen in 2014. Five planting densities (114×10^4, 174×10^4, 249×10^4, 369×10^4 and 519×10^4 plants $hectare^{-1}$, respectively) were designed and the level of heavy metals in soil and in various parts (root, stem, leaf, husk and grain) of spring wheat were detected, and then the health risk to human health was assessed via hazard quotient. The results indicate that concentrations of Pb and Cd in each part of spring wheat plant are negatively correlated with planting density, similar but weaker trends are observed for those of Cu and Zn. The hazard quotient of heavy metals through food chain is markedly reduced with increasing planting density, especially for Pb and Cd at higher planting densities (369×10^4 and 519×10^4 plants $hectare^{-1}$). Conclusively, the present study shows that suitable planting density could be a feasible cultivation method for ensuring the safety of wheat products and human health on slightly polluted alkaline soils.

Keywords

Planting Density; Heavy Metals; Spring Wheat; Health Risk; Weak Alkaline Soil

Introduction

Heavy metals pollution in soil has been a worldwide environmental problem because of human activities [17]. Owing to their non-biodegradable, non-thermal degradable and persistant toxic characters, heavy metals are easy to stay in soil and may enter into ground water [6, 12], be taken up by plants and accumulate in human body through food chain, then posing threats to human health [2, 11]. As the second largest crop in the world, wheat as the main food resource for locals is widely grown in northern China [1]. However, wheat grain is no longer safe for consumption because of high soil heavy metals [5]. Although measures have been taken by governments at all levels for protecting the grain crop security, the responses from local farmers are not positive enough for the consideration of yield and economic benefits. So it is urgent to take action to explore a relatively safer planting pattern on spring wheat, especially in slightly polluted and low yield farmland regions.

Moreover, the characteristics of the plant itself to enrich heavy metals have been extensively studied, confirming that the ability essentially depends on plant species. About more than 450 plant species are found to accumulate extraordinarily high amounts of heavy metals, which are termed hyperaccumulators and have the potential to be used for phytoremediation [14]. Wheat belongs to non-hyperaccumulators, but still accumulates high amount of heavy metals, especially in grains [10]. Besides, plant genotypes differ in their uptake, translocation, accumulation of trace metals [4], and similar phenomenon was observed in wheat plants and asparagus bean cultivars [9, 22]. Recently, Hansi et al. found, in lab, that the concentration of Cu in barley shoot would reduce with increasing planting density [8]. All these researches provide important information for breeding crop cultivar that is of low ability to accumulate heavy metals under certain conditions. Nevertheless, it can be different even for the same species to accumulate heavy metals in various types of soil.

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Therefore, the present study aims to (1) make clear about the accumulation ability of spring wheat on heavy metals under different planting densities; (2) clarify whether changing planting densities can be a useful cultivation method for safe production on slightly polluted alkaline soil.

Materials and Methods

Study Area and Planting Density Design

The present study was carried out in Baiyin (103°54′-104°24′E, 36°14′-36°47′N), China. The district of Baiyin is a non-ferrous metal mining and smelting base where the agricultural population is about 1.3 million. Due to the great population and low annual rainfall (about 180 - 450 mm), sewage water was once used for field irrigation, which was the main reason leading to accumulation of heavy metals Cu, Zn, Pb and Cd in local agricultural soil.

Spring wheat (*Triticum aestivum*) is the main crop for local inhabitants, and the variety of Longchun 26 was chosen for sowing. Planting density of local fields was 300 - 400×10^4 plants hectare^{-1}. So, the ideal densities of 150×10^4 (D1), 200×10^4 (D2), 300×10^4 (D3), 400×10^4 (D4) and 550×10^4 (D5) plants hectare^{-1} were designed with each designing plot as 3 m^2 (1.5 m × 2 m) and three replicates. However, the practical planting densities were investigated to be 114×10^4 (D1), 174×10^4 (D2), 249×10^4 (D3), 369×10^4 (D4) and 519×10^4 (D5) plants hectare^{-1}. The field was homogenized before seeding. In order to be realistic, the designed plots were handled in the same way as local people did.

Sample Preparation and Chemical Analysis

Spring wheat was sowed on March 22, 2014 and harvested on June 30, 2014. The whole plant was collected and taken to lab with polyethylene bags. After that, samples were rinsed with tab water and washed three times with deionized water. Then wheat plants were divided into root, stem, leaf, husk and grain. Finally, various parts were dried at 70°C in a constant tempreture oven for 48 h and dry matters were determined. Meanwhile, corresponding topsoils (0~20 cm) were sampled when sampling the plant, dried at room temperature and ground to pass through a 0.15 mm nylon sieve. At least 6 plants were collected from each plot and mixed into a composite sample.

Soil pH was measured in H_2O (1:2.5, soil:water ratio, w/v). Plant sample about 0.5 g was added to 50 ml teflon crucible, digested with HNO_3-$HClO_4$ till the solution became transparent. The solution obtained was cooled and filtered through Whatman neutral filter paper. The filtered solution was then diluted to 25 ml and kept at ambient temperature for further analysis. For soil sample, 0.5 g dry matter was used for digestion with 10 ml HCl, 10 ml HNO_3, 5 ml $HClO_4$ and 3 ml HF. When the mixture became transparent, it was cooled, filtered with Whatman neutral paper and diluted to 25 ml with deionized water.

Determination of the heavy metals in solution was performed on atomic absorption spectrophotometer (AAS, ZEEnit 700P, Analytik Jena, Germany). To ensure the reliability of the detecting results, reagent blanks were used to correct the instrument readings. Quality controls similar to the digestion procedure of plant sample and soil were carried out in digestion of Certified Reference Materials for the Chemical Composition of Biological Sample (GBW 10052, GSB - 30) and Soil Sample (GBW 07402, GSS - 2). The relatively standard deviation (% RSD) was < 5% and the recovery rate was > 95%.

Health Risk Assessment

The noncarcinogenic health risk from consumption of wheat grain is expressed in hazard quotient (HQ) by Eq.(1) [5]. If HQ < 1, the population unlikely to suffer adverse effects. The higher the HQ, the more attention should be paid to.

$$HQ = \frac{DIM}{RfD} \tag{1}$$

Where, RfD is the reference dose of individual metal and is defined as the maximum permissible level of metal that is unlikely to pose deleterious effects on human health. RfD are based on 0.04, 0.3, 0.004 and 0.001 mg/kg/day for Cu, Zn, Pb and Cd, respectively [23]; DIM is the daily intake of individual metal and is expressed in Eq.(2).

$$DIM = \frac{C_{metal} \times D_{intake}}{BW} \tag{2}$$

Where, C_{metal} represents the concentration of individual metal in wheat grain, D_{intake} represents the daily intake rate of grain (0.47 kg /person/ day) and BW represents the average body weight of 65 kg for adults.

Bioconcentration factor (BCF) is an index of accumulation degree of metal in plants, and is calculated by Eq.(3).

$$BCF = \frac{C_{mt}}{C_{ms}} \tag{3}$$

Where, C_{mt} represents the concentration of individual metal in whole aerial part (shoot) or any organ or tissue; C_{ms} is the concentration of individual metal in soil.

Statistical Analysis

All data were computed with statistical package SPSS 19.0 (IBM, Amonk, NY, USA). One-way ANOVA test (LSD) was performed to assess the influence of different planting densities on metal concentrations in wheat plant and soil ($p<0.05$). The histograms were generated by OriginLab pro 9.0 (OriginLab. Inc., USA).

Results and Discussion

Heavy Metal Concentration in Soil

From Table 1, it can be seen that the soil is slightly alkaline, and the total concentrations of the four selected elements are lower than the environmental quality standard for soils of China (GB15618-1995), except for Cd, and the concentrations of metals are also lower than that from the previous work done in this region (60.05 mg/kg, 230.66 mg/kg, 100.7 mg/kg, 5.7 mg/kg for Cu, Zn, Pb and Cd, respectively) [5]. However, concentrations of all the four elements exceeded their background values, indicating that over the past few decades the environment-unfriendly development of industry and agriculture might cause the local soil polluted. The reduced content of heavy metals in this region respects to that of the last ten years may be due to annually crop plant uptake, runoff wash out and leaching [18].

TABLE 1. SOIL pH, SOIL HEAVY METAL CONCENTRATIONS (mg/kg, DW; n=5).

	pH	Cu	Zn	Pb	Cd
Data from the present study	7.75	43.86±3.64	147.68±27.24	54.99±4.71	1.23±0.35
Permissible limits of Chinese standards[a]	>7.5	100	300	350	1
Soil element background values[b]	-	24.1	68.5	18.8	0.116

a: Set by Environment Protection Administration of China(GB15618-1995)
b: From Chinese Soil Element Background Values (1990)

Heavy Metal Concentration in Spring Wheat

It can be seen that the concentrations of four heavy metals in any part of spring wheat from five planting densities are all below the thresholds of plant toxicity [3]. Zn has the highest concentration followed by Cu, and they are two or more times higher than Pb and Cd, which may be due to the essentiality of Cu and Zn for plant physiology, while Pb and Cd are the stress factors to photosynthesis, chlorophyll synthesis and antioxidant enzymes metabolism [15, 16]. Grain always has the lowest concentrations of Cu, Pb and Cd in all plant parts from D1 to D5, except for Zn, which is in consistent with Nan et al.'s report about heavy metal accumulation in wheat grain planted on alkaline soil [13].

The heavy metal concentrations varied with the various parts of wheat (Figure 1), for Cu: root > husk > leaf > stem > grain; for Zn: root > grain > leaf > husk > stem; for Cd: root > leaf > stem > husk > grain and for Pb: root > leaf > husk > grain > stem. The present results are partly different with the former reports about Cu [19], Pb and Cd [20]. Reasons for these differences may be the soil properties, plant species or cultivars and ratio of the bioavailable heavy metals in soil [21].

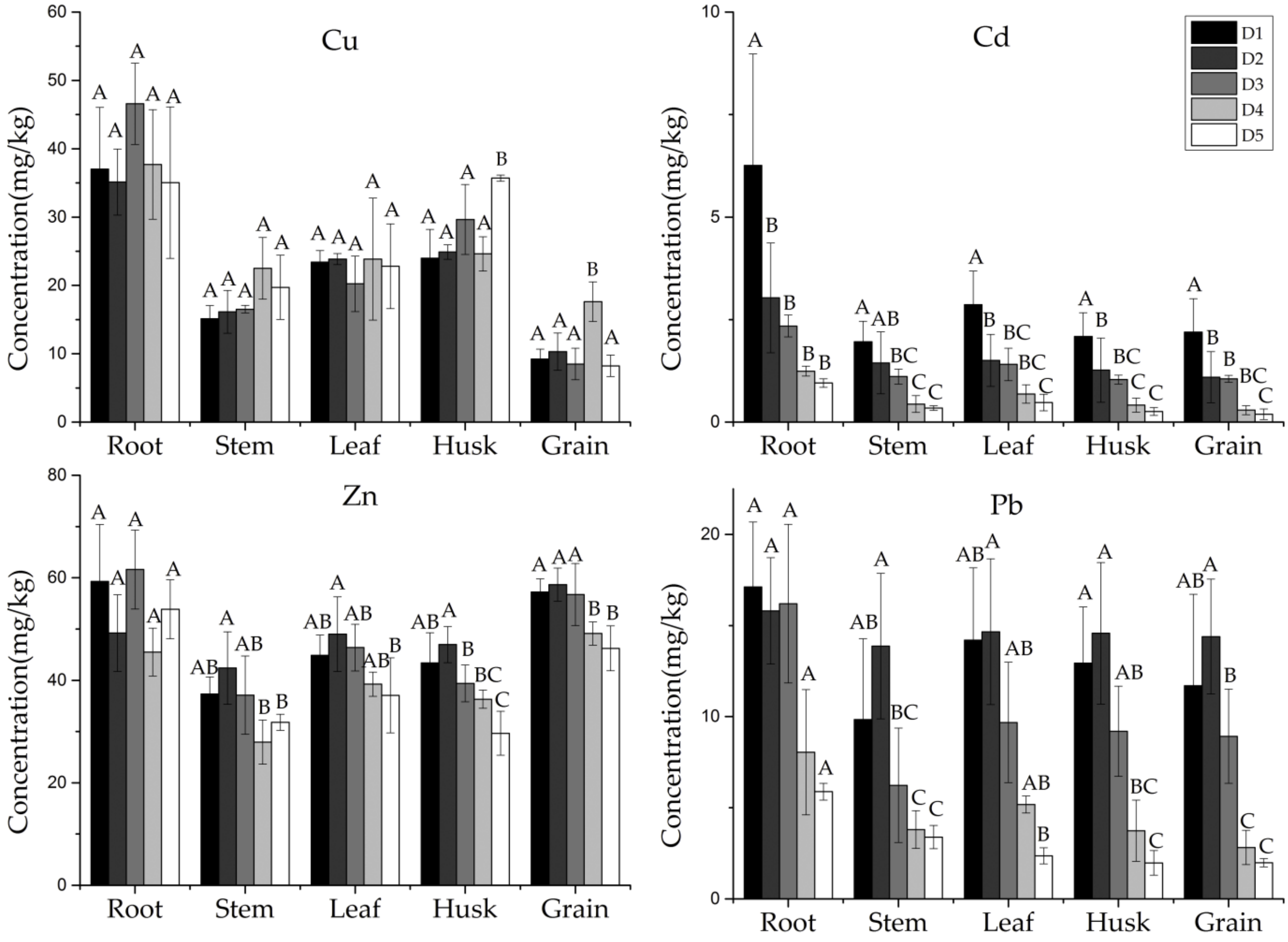

FIGURE 1. HEAVY METAL CONCENTRATION IN EACH PART OF SPRING WHEAT FROM DIFFERENT PLANTING DENSITIES IN BAIYIN DISTRICT. VALUES WITH DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT AT P<0.05(A, B AND C INDICATE DIFFERENCES AMONG DIFFERENT PLANTING DENSITIES).

TABLE 2. PEARSON'S CORRELATION BETWEEN PLANTING DENSITY AND TISSUES OF SPRING WHEAT (n=5).

		Root	Stem	Leaf	Husk	Grain
Cu		-0.650	0.303	-0.300	0.681**	0.090
Zn	Planting density	-0.131	-0.554*	-0.614*	-0.834**	-0.787**
Pb		-0.616*	-0.708*	-0.650**	-0.803**	-0.823**
Cd		-0.731**	-0.824**	-0.790**	-0.808**	-0.784**

* and ** mean difference or significant difference at p<0.05 or p<0.01(2 tailed).

Among the experimental densities of D1-D5, it is showed that the concentrations of heavy metals Pb, Cd and Zn in wheat are negatively related with the wheat planting density (Table 2), which suggests that in the selected five densities the higher the density, the lower the concentration of Pb, Cd and Zn in wheat tissues (except for Zn in root), while there is no distinct regularity of Cu content in wheat.

Feasibility of Reducing Heavy Metal Content in Wheat Grain

Heavy metals in grain of different plant densities are shown in Table 3. In all planting densities, Cu concentration, except for D4, is under the limit of China Standards. Only a significant decrease is observed at D5, though it is slightly lowered compared to that at D1. Meanwhile, Zn concentration at D1, D2 and D3 is beyond the limit (50 mg/kg), then it goes to the safe range at D4 and D5. Similar but much obvious tendency is found in Pb and Cd of grain (Table 3). Although they are still above the limits at the highest density (D5), the concentrations have already decreased about 78% and 75% for Pb and Cd , respectively, compared to previous work done in the same region [5]. Above all, the higher the planting density is, the lower the Pb and Cd concentrations in wheat grain are, which means elevating planting density has a significant effect on reduction of Pb and Cd and a weaker effect on Cu and Zn in wheat grain.

The safety of grain is directly related to the consumers' health. Therefore, the assessment of health risks to inhabitants posed by heavy metals via intake of grain is done (Table 4). HQs of Cu, Zn Pb and Cd are above 1 at all five planting densities, indicating there are potential noncarcinogenic effects to the consumers. Pb has the highest HQ followed by Cd in all densities, while a significant decrease of HQ_{Pb} is found with the increasing of planting density, and the integrative risk also significantly decreased from D3 to D5 respect to the previous work (21.98 of Pb and 6.72 of Cd in local planting density [5]. So planting density could be considered as an alternative cultivation way for safer production on slightly polluted soils.

However, yield is under consideration as well as grain safety. As is known, production would increase with increasing planting density in certain ranges [7]. In present study, the grain production has elevated with planting density, getting to the maximum at D4 and going down at D5 (Table 4), but no significance appears among the five selected densities ($p<0.05$). Meanwhile, the HQs of Pb and Cd at D4 and D5 significantly declined compared to that in D1, D2 and D3, thus, planting density is again confirmed to be feasible for safer production on slightly contaminated soils. In addition, further study is required for the most appropriate density, which will keep health risk and yield in balance.

TABLE 3. CONCENTRATIONS OF HEAVY METALS IN WHEAT GRAIN. (mg/kg, DW; n=3)

Densities	Cu	Zn	Pb	Cd
	Mean±StDev	Mean±StDev	Mean±StDev	Mean±StDev
D1	9.24±1.43^{a}	57.25±2.55^{a}	11.70±0.81^{ab}	2.19±0.81^{a}
D2	10.31±2.71^{a}	58.66±3.25^{a}	14.39±3.15^{a}	1.09±0.62^{b}
D3	8.50±2.31^{a}	56.75±6.06^{a}	8.92±2.58^{b}	1.06±0.08^{b}
D4	17.62±8.89^{b}	49.12±2.26^{b}	2.82±0.94^{c}	0.29±0.11^{bc}
D5	8.26±1.56^{b}	46.23±4.37^{b}	1.99±1.22^{c}	0.19±0.13^{c}
Limits	10^{m}	50^{n}	0.2^{x}	0.1^{x}

$^{a, b, c}$ mean significant differences among different densities ($p<0.05$).
$^{m, n, x}$ represent the permissible limits of China Standards GB15199-94, 13106-91 and 2762-2012, respectively.

TABLE 4. HQ OF HEAVY METALS IN GRAIN TO INHABITANTS AND YIELDS(KG/HA) OF WHEAT GRAIN IN DIFFERENT DENSITIES

Densities	Yields(n=3)	HQ			
		Cu	Zn	Pb	Cd
D1	4153.8±745.8^{a}	1.67^{a}	1.37^{a}	21.2^{ab}	15.8^{a}
D2	2918.6±571.9^{a}	1.86^{a}	1.41^{a}	26.0^{a}	7.90^{b}
D3	5477.2±1082.2^{a}	1.54^{a}	1.36^{a}	16.13^{b}	7.64^{b}
D4	5541.2±802.6^{a}	3.18^{a}	1.18^{b}	5.10^{c}	2.09^{bc}
D5	5330.1±2325.6^{a}	1.48^{a}	1.11^{b}	3.59^{c}	1.37^{c}

$^{a, b, c}$ mean significant differences among different densities ($p<0.05$).

Effect of Planting Density on BCFs

BCFs of the four metals in grain followed the sequence of Cd > Zn > Cu > Pb (Table 5), which is different from Dai et al's result of Zn > Cd > Cu > Pb [5]. The difference of BCFs may be due to cultivars, which could cause variety of ability to accumulate heavy metals. The BCFs vary in different parts of spring wheat, but only the BCFs of Cd are relatively higher, especially in root at all densities (except Cu in root at D3) are > 1 (values in bold, Table 5), suggesting that Longchun 26 could be an ideal excluder for Cd. Thus, planting density negatively affect the bioaccumulating ability of wheat for heavy metals, meanwhile, can ensure the safety of the edible part.

TABLE 5. THE BCFS OF WHEAT TISSUES

Elements	Densities	BCFs				
		Root	Stem	Leaf	Husk	Grain
Cu	D1	0.93	0.38	0.59	0.6	0.23
	D2	0.76	0.35	0.52	0.54	0.22
	D3	**1.02**	0.36	0.45	0.66	0.19
	D4	0.9	0.54	0.57	0.59	0.42
	D5	0.95	0.54	0.62	0.97	0.22
Zn	D1	0.35	0.22	0.27	0.26	0.34
	D2	0.31	0.27	0.31	0.29	0.37
	D3	0.41	0.25	0.31	0.26	0.38
	D4	0.34	0.21	0.29	0.27	0.37
	D5	0.33	0.21	0.23	0.19	0.3
Pb	D1	0.25	0.14	0.20	0.19	0.17
	D2	0.25	0.21	0.23	0.23	0.22
	D3	0.29	0.11	0.17	0.16	0.16
	D4	0.15	0.07	0.1	0.07	0.05
	D5	0.12	0.07	0.05	0.04	0.04
Cd	D1	**3.3**	0.98	**1.44**	**1.05**	**1.09**
	D2	**1.9**	0.91	0.95	0.8	0.69
	D3	**1.8**	0.83	**1.04**	0.78	0.81
	D4	**1.3**	0.47	0.70	0.42	0.30
	D5	**1.2**	0.43	0.62	0.32	0.25

Conclusions

The present study aims to make sure the effect of different planting densities on the accumulation of heavy metals in spring wheat. The results have shown that with the increasing of planting density, the concentrations of Pb and Cd in spring wheat have decreased significantly, while that of Zn especially Cu are barely affected, and heavy metals in aerial parts, especially in grain could be lowered, which lead to a reduction of health risk. In summary, changing planting density of spring wheat could be a feasible method for safer production on the slightly polluted alkaline soil.

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