Pol. J. Environ. Stud. Vol. 24, No. 2 (2015), 863-870

Original Research

Effects of Long-Term Fertilization on Bioavailability of Heavy Metals in Shajiang Black Soil

Peng-fei Zhu, Fei Liu*

School of Environmental Sciences, Huaibei Normal University,
Huaibei, Anhui 235000, China

Received: 27 September 2013
Accepted: 1 October 2014

Abstract

Though fertilizers can improve grain yield, the trace heavy metals in fertilizers will also enter soil and migrate through food chains, which harbor ecological and health risks. This study was based on more than 30 years of location experiments. The objective was to investigate the effects of different fertilizer treatments on the bioavailability of heavy metals in Shajiang black soil. Total and available contents of Cu, Zn, Pb, and Cd in soil samples were determined by atomic absorption spectrophotometry (AAS), and were compared between different treatments by using single factor analysis of variance and least significant difference. Finally the bioavailability indices (BAIs) of heavy metals were calculated. The results show that after treatments of fertilizers, total contents of Cd, Cu, Pb, and Zn increased, but were all lower than China's Soil Environment Quality Standards (SEQS, level II). Available Cu content was the highest after M treatment; available Zn contents were relatively low after all treatments; available Pb and available Cd contents were the highest after NP treatment. Under different treatments, the BAIs of each heavy metal were largely different. Bioavailability of a heavy metal was affected by soil properties and by its adsorption, desorption, complexation, precipitation, and dissolution in soil. The mean BAI of each metal ranked in the order of Cd>Pb>Cu>Zn; the available Cd and Pb were highly migratory, and thus can be absorbed and enriched by plants, and may finally threaten human health through food chains.

Keywords: heavy metals, extraction, bioavailable, long-term experiment, Shajiang Black Soil

Introduction

The application of chemical fertilizers has been increased to improve grain yield. Chemical fertilizers and organic manure are important measures to improve soil productivity and ensure high grain yield [1]. However, the high-content heavy metals in fertilizers will cause soil quality degradation [2] and environmental pollution [3], which will bring safety hazards to agricultural production. Reportedly, one major cause to heavy metal pollution [4], land pollution [5], and the greenhouse effect that result in global ecological deterioration is the overuse of chemical fertilizers in farmlands, which exceeds the nutrition levels demanded by crops [6, 7]. Other researchers believe that long-term fertilization will not change the levels of heavy metals in soil, either total amount or exchangeable contents [8]. Anyway, it is necessary and urgent to reduce the negative impacts of fertilizers on the environment [9].

As the global population and grain demands are still on the rise, all countries are concerned with how to coordinate the relationship between grain production and environmental protection. One serious concern is soil heavy metal pollution, which is a long-term, complex, hidden,

---

*e-mail: liufei888@163.com

accumulative, and irreversible process. A part of heavy metals enters soil through fertilization. Even though they are necessary trace elements for animals and plants, heavy metals will enrich in soil after long-term fertilization, and at a certain level will impact human health through food chains. Non-essential heavy metals for animals and plants will also be absorbed through the roots and distributed in different organs in plants, causing risks of soil heavy metal pollution [10]. The total amount of heavy metals indicates the risks of soil heavy metal pollution, but whether a heavy metal can be absorbed by plants is dependent on its available state. Due to the complicated dynamic interaction between metal-soil-organism, heavy metals can be only partially absorbed and utilized by organisms [11]. The existing risk assessments for soil heavy metal pollution are mostly based on the assumption that the total amount of a heavy metal can be absorbed and utilized by organisms. Apparently, such assessments are inaccurate because they ignore the available state of a heavy metal, which provides information about its mobility, toxicity and bioavailability [12] and thus is an important means for risk assessments.

Previous location experiments [13, 14] focus on the evolutionary laws of soil fertility, crop yield changes, and crop quality. Yangliu Experiment Base in Suixi County of Anhui Province was built in 1981 to improve Shajiang black soil quality and crop yield. This region is representative of Shajiang black soil in China, so this field base is suitable for studies on Shajiang black soil. However, the accumulative properties of heavy metals in Shajiang black soil have not been reported from the perspective of soil environmental quality. In this paper we discussed the enrichment and cumulative effects of total and available heavy metals in Shajiang black soil. Soils were sampled from areas after different treatments. The physicochemical properties were analyzed. The total contents and available contents of heavy metals were measured, and the long-term effects of different treatments were investigated. This paper provides a scientific foundation for improvement of Shajiang black soil.

Materials and Methods

The Study Area

Shajiang black soil is a major low-yield soil type in Huanghe-Huaihe-Haihe Plain of China, and also the most pervasive low-yield soil type in Huaibei Plain. Shajiang black soil is widely distributed in Anhui, Henan, Shandong, and Jiangsu provinces. Due to many reasons, production in Shajiang black soil is low and unstable, which seriously hinders agricultural development there. The study area with a temperate monsoon climate has an accumulated temperature (≥10ºC) of 4,600~4,800ºC, and 200~220 frost-free days, which are beneficial for two-crop production a year. The annual rainfall is 800 mm, but precipitation is unevenly distributed throughout the year because heavy rains with severe floods are concentrated in July. The recent annual rain pH in the study area was pH=7.2 (2011), pH=7.3 (2012), and pH=7.1 (2013). Shajiang black soil is rich in calcium carbonate; its parent material is sediments of loessial limnetic facies. Shajiang black soil with a heavy texture is mainly composed of montmorillonite (Mg, Ca) $A1_2Si_5O_{16}$. The topsoil is composed of medium loam-heavy loam, and the portion below the topsoil is composed of heavy loam-light clay (about 30% clay). Agriculture there is based mainly on grain production, and the main grain crops are wheat. The study area was far from the downtown, and there are no landfills or steel mills. Therefore, the pollution source of heavy metals there may be fertilization. The study area is shown in Fig. 1.

Design of Experiment

This experiment began in September 1981 in the Yangliu Experiment Base in Suixi County of Anhui, and the tested soil was Shajiang black soil.

Before the experiment, the basic conditions of the soil layer (0~20 cm) were: organic matter 10.22 $g\cdot kg^{-1}$; total nitrogen 0.78 $g\cdot kg^{-1}$; total phosphorus 0.47 $g\cdot kg^{-1}$; alkali-hydrolyzable nitrogen 64.1 $mg\cdot kg^{-1}$; available phosphorus 2.5 $mg\cdot kg^{-1}$; and pH 7.6. Five treatments were used: (1) only chemical fertilizers (named NP, where N is urea and P is calcium superphosphate) containing 300 $kg\cdot hm^{-2}$ N, and 98 $kg\cdot hm^{-2}$ P_2O_5; (2) only organic manure (M, farmyard manure) containing 300 kg kg hm^{-2} N, but the P_2O_5 content in organic manure changed year by year; (3) organic manure + chemical fertilizers with equal amounts of N (MNP, half the amount of NP + M); (4) organic manure + chemical fertilizers with high N (HMNP, 80% of the amount of NP + M); (5) no fertilization (CK). Nitrogen was fixed during fertilization. Each experiment with an initial area of 180 m^2 (40 m×4.5 m) was conducted in duplicate. Local farmyard manure, urea, and calcium superphosphate were used as organic manure, nitrogen fertilizer, and phosphate fertilizer, respectively.

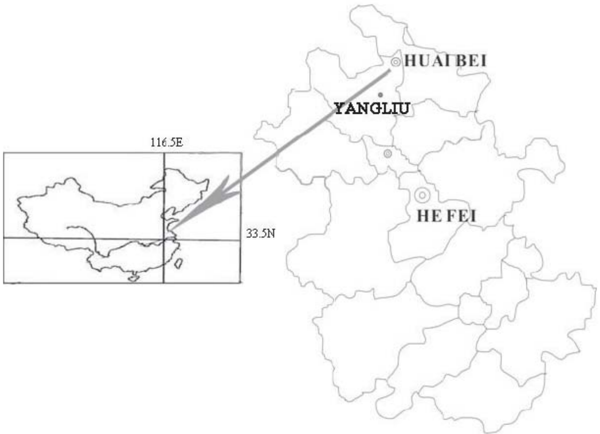

Fig. 1. Location of the study area.

Table 1. Effect of fertilizer treatments on chemical and physical characteristics of soil.

Treatments	pH (H_2O)	Organic carbon ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Available N ($mg \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Total N ($mg \cdot kg^{-1}$)	Available K ($mg \cdot kg^{-1}$)
NP	6.24	11.62	0.95	103.5	0.57	39.3	381.7	94.7
M	7.52	17.85	1.39	134.8	0.97	69	591.3	149
MNP	7.17	13.6	1.08	116.6	0.81	57.6	492.2	119
HMNP	7.46	15.5	1.32	142.7	1.03	66.5	496.8	125.5
CK	7.03	10.43	0.67	38.6	0.33	2.6	457.1	114.2
base data	7.6	10.2	0.78	64.1	0.4	2.5	/	/

Sampling and Analysis

In each treatment area, eight representative sampling points distributed in a snake-shape were selected in October 2011. At each point, 40 dots of soil in the mixed topsoil (0~20 cm) were collected perpendicular to ridges using a soil auger. The 40 dots of topsoil were fully mixed. About 1 kg of soil was kept using a quartering method, and stored in a valve bag. Also, sectional soil was collected by selecting 10 sampling dots and storing them until use.

Then in a dry room, the wet samples were poured onto white enamel discs or plastic films, spread into a 20-cm thick layer, and crushed and flipped using a glass stick to dry them evenly. During drying, impurities such as gravel, sand, and plant residues were picked out. Then each dried sample (100-200 g) was placed onto an organic glass board and was crushed again using a wooden stick. After repeated treatment, each sample could fully pass a 10-mesh nylon sieve (aperture = 2 mm), and was stored in a wide-mouth bottle. The samples for measurements of total contents and available contents of heavy metals passed a 100-mesh sieve and a 20-mesh sieve, respectively. Methods of measurements were listed as follows: Total nitrogen was measured using semimicro Kjeldahl determination; alkali-hydrolyzable nitrogen was measured using diffusion method; total phosphorus was measured using NaOH liquate-colorimetry; available P was measured using $NaHCO_3$ leaching-spectrophotometry; pH: electric potential method (soil:water = 2.5:1); organic matter: potassium bichromate and heat capacity; Heavy metals (Cu, Zn, Pb, Cd): acid dissolution (HF-HNO_3-$HC1O_4$) for total contents; DTPA-$CaC1_2$-TEA as a leaching system for available contents. The leaching system was adjusted by 6 $mmol \cdot L^{-1}$ HCl to pH = 7.3 and to a soil:liquid ratio = 1:2, then shaken at 180±20 rpm and 25ºC for a leaching time of 2 h, and after filtration the supernatant was collected. Total amounts and available contents were determined by atomic absorption (or graphite furnace) spectrophotometry. China's national standard samples were used to ensure the high quality of measurements.

All reagents used in this study were of superior purity, and deionized redistilled water was added to a constant volume. All vessels were soaked in 2% detergents overnight, and after washing with tap water were soaked in 15% (V/V) HNO_3 over 24 h, and then washed by deionized water several times, followed by drying at constant temperature and storage in preservation bags until used.

Data Processing and Analysis

Single-factor analysis of variance (AVONA) was performed on SPSS 17.0 and least significant difference (LSD) was used for comparison of different treatments and for statistical analysis ($p<0.05$). Excel 2003 was used for graph processing.

Results and Discussion

Long-Term Effects of Fertilization on Basic Soil Physicochemical Properties

Long-term effects of fertilization on basic soil physicochemical properties are listed in Table 1. Apparently, pH decreased to different degrees from the initial values, where pH was reduced by 1.36 after NP treatment. Organic carbon contents after treatments ranked in the order of M>HMNP>MNP>NP>CK, and increased by 13.9%, 75.0%, 33.3%, 52.0%, and 2.3%, respectively. Total nitrogen after treatments ranked in the order of M >HMNP>MNP>NP>CK, and increased by 21.8%, 78.2%, 38.5%, 69.2%, and -14.1%, respectively. Alkali-hydrolyzable nitrogen after treatments ranked in the order of HMNP>M>MNP>NP>CK, and increased by 61.5%, 110.3%, 81.9%, 122.6%, and -39.8%, respectively. Reportedly, long-term application of organic and inorganic fertilizers could significantly increase organic carbon and total nitrogen contents in soil, as well as contents of active organic matters, such as soil microbial biomass and soluble organic matter [15]. Dong [16] studied the long-term effects of fertilization on the physicochemical properties of red soil, and in comparison with the control group, organic matter and total nitrogen contents significantly increased, especially after treatment of organic manure; these results are consistent with our results.

After HMNP and M treatments, total P was 2 times higher than that with no fertilization. Total K content was

Table 2. Effects of different fertilizer treatments on total heavy metal contents of Shajiang Black Soil.

Treatments	Cu (mg·kg^{-1})	Zn (mg·kg^{-1})	Pb (mg·kg^{-1})	Cd (mg·kg^{-1})
NP	17.81±2.80c	90.87±4.94c	37.57±5.38a	0.335±0.058a
M	27.88±4.60a	115.89±10.41a	23.85±4.26c	0.194±0.049c
MNP	24.39±3.73b	107.79±10.84ab	32.43±6.14b	0.258±0.047b
HMNP	22.83±2.84b	102.42±12.06b	31.15±3.73b	0.230±0.021bc
CK	15.17±1.14c	82.47±4.75c	21.49±1.81c	0.131±0.011d
soil background content in Anhui	20.20	56.30	26.00	0.083
soil background content in China	22.6±11.4	74.2±32.8	26.0±12.4	0.097±0.0612
soil environmental standard in China	35/100 (second level)	100/250	35/300	0.2/0.3

The different small letters in the same column indicate that different fertilizer treatments were significantly different ($p<0.05$)

reduced only after NP treatment, but increased to different degrees in other treatments. Available K content changed in the same way as total K. In general, soil fertility and crop quality were improved by fertilization. Reportedly, wheat production after 22 years of fertilization was increased by 280.7%, 280.1%, 242.8%, and 84.8% after treatments of HMNP, M, MNP, and NP, respectively [13]. Organic C and total N contents were highly correlated (correlation coefficient r = 0.89), and the use of organic manure was beneficial for conservation of soil N, which are consistent with another study [17].

Effects of Different Treatments on Total Contents of Cu, Zn, Pb, and Cd in Shajiang Black Soil

Total contents of Cu, Zn, Pb, and Cd in Shajiang black soil after different treatments are listed in Table 2. Single-factor AVONA showed that total contents of Cu, Zn, Pb, and Cd (total Cu: n=40, F=19.95, $p<0.05$; total Zn: n=40, F=16.95, $p<0.05$; total Pb: n=40, F=16.90, $p<0.01$; total Cd: n=4 0, F=26.64, $p<0.05$) all changed significantly after different treatments. Then LSD was used to compare the total contents of each heavy metal after different treatments. Because the amounts of N or P were equal in all treatments, and if atmospheric precipitation and irrigation were ignored, the major influence factors on total contents of Cu, Zn, Pb, and Cd were the treatments and fertilizer sources. Total Cu contents were significantly different between any of the four treatments and the control (CK) (n=40, $p<0.05$), and were 17.4%, 83.8%, 60.8%, and 50.5% higher than CK, respectively (Table 2). Total Cu content after M treatment was 56.5% higher than after NP treatment, because the high content of Cu in the organic manure exceeded the soil background levels in Anhui and in China (not exceeding SEQS Level I). Total Cu contents were not significantly different after MNP and HMNP treatments, both with organic manure + chemical fertilizers (n=40, $p>0.05$).

Total Zn contents after NP treatment and CK were not significantly different (Table 2), indicating that NP did not bring much Zn into soil. Total Zn contents after M, MNP, and HMNP treatments were all significantly different from CK, indicating the high positive effect of organic manure on total Zn content. Total Zn contents after all treatments exceeded the soil background levels in Anhui and SEQS Level I, indicating that the content of aqua regia-extractable Zn (457 mg·kg^{-1}) exceeded the recommended SEQS level (GB15618-1995) [18].

After different treatments, total Pb and total Cd contents both changed in the order as NP>MNP>HMNP>M>CK (Table 2). Total Pb contents after each treatment and CK were significantly different, which increased by 74.8%, 11.0%, 50.9%, and 45.0%, respectively. Total Pb content was the highest after NP treatment, and was significantly different from other treatments. Total Pb contents after MNP and HMNP were not significantly different. Total Pb contents after NP, MNP, and HMNP treatments exceeded the soil background level in Anhui, but did not exceed SEQS Level I. Total Cd content after NP treatment was the highest and was significantly different from other treatments, indicating the high effect of fertilizers (calcium superphosphate) on total Cd in Shajiang black soil. The raw material for phosphate fertilizers is phosphate rocks. In China, phosphate rocks contain 0.7-4.0 mg/kg total Cd on average, of which 70-80% will finally be transmitted to phosphate fertilizers. Some imported phosphate fertilizers contain 32.2 mg/kg Cd [19]. After application of phosphate fertilizers, total Cd and available Cd in soil would increase [20], and thus the absorption of Cd by plants also increases [21]. Therefore, attention should be given to the potential pollution caused by Cd-containing fertilizers. Exotic Cd after entering soil can easily be absorbed by soil. The environmental capacity of Cd is very low compared with Pb, Cu, and Zn; a slight increase of total Cd in soil would improve the total Cd content in crops and thus potentially harm human health. The UN Environmental Programme identified 12 key harmful substances, and ranked Cd with significant biotoxicity first. In the present study total Cd contents

Table 3. Effect of different fertilizer treatments on available heavy metal contents of Shajiang Black Soil.

Treatments	Available Cu mg·kg^{-1}	Available Zn mg·kg^{-1}	Available Pb mg·kg^{-1}	Available Cd μg·kg^{-1}
NP	2.13±0.08a	1.53±0.24a	4.66±0.16a	120.42±16.38a
M	1.63±0.10b	1.40±0.08ab	3.61±0.16c	103.25±11.33b
MNP	1.52±0.22b	1.27±0.17b	4.16±0.76b	99.95±13.76b
HMNP	2.19±0.26a	1.25±0.11bc	4.05±0.12b	102.97±8.11b
CK	1.13±0.18c	1.10±0.04c	3.52±0.25c	70.63±12.69c

The different small letters in the same column indicate that different fertilizer treatments were significantly different ($p<0.05$)

exceeded the soil background level in Anhui after the five treatments, and exceeded SEQS Level I after NP, MNP, and HMNP.

Cu and Zn are trace elements needed by plants for maintenance of normal living activities, and are the components or catalysts of various enzymes. Cu and Zn are widely involved in various living activities. But Cu and Zn over a certain level will cause negative effects on plants, such as disorders of metabolic processes, blocking of growth and development, and even death. Total Cu and Zn contents after M treatment were both the highest, and were significantly different from other treatments. Reportedly, after only application of organic manure in rice, total Cu and Zn contents were significantly higher than the control group [22], which is consistent with our results.

Fertilizers are used to improve soil quality and thus crop yield and quality. Poor management or overuse of fertilizers will cause pollution to soil, so the toxic heavy metals will enter the human body through food chains, which is undoubtedly harmful to human health. Heavy metals cannot be fully removed from soil, so it is an effective way to prevent heavy metal pollution from the source by controlling the total amount of heavy metals entering soil.

Effects of Different Treatments on Available Contents of Cu, Zn, Pb, and Cd

The most commonly used soil extracting agents include diethylene triamine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), NH_4NO_3, and $CaCl_2$, and the continuous extraction methods include European Communities Bureau of References (BCR) method and Tessier method [23, 24]. In this paper, single extraction was used. DTPA-$CaC1_2$-TEA with a medium extraction rate that can express the available contents of heavy metals was used as an extracting agent. In evaluation of heavy metal pollution, the soluble or available contents are more effective on plants than total contents [25]. The available heavy metals are very active in soil, so studies on available heavy metals will help to investigate the sources and risks of soil heavy metal pollution.

The results of heavy metal contents were processed by single-factor AVONA at 95% confidence level (CI), which showed significant differences among all treatments. Then the available contents were compared using LSD. The results are listed in Table 3. Available Cu contents were significantly different between CK and other treatments, and increased by 88.5%, 44.3%, 34.5%, and 93.8%, respectively, after NP, M, MNP, and HMNP. Available Cu content was the highest after HMNP treatment, but were not significantly different after M and MNP treatments.

There are two views regarding the effects of M treatment on available Cu. First, M treatment could reduce the available Cu content in soil, because soil organic matter has high ability in fixing Cu [26]. Second, M treatment can slightly increase available Cu content, and pig manure with rich Cu might increase available Cu content [27]. In this study, M treatment increased available Cu content in Shajiang black soil; MNP and HMNP both increased available soil Cu contents. These results indicated that the effects of different treatments on available Cu in Shajiang black soil were complex, as the available Cu content depended on the composition and dosage of fertilizers and on land use. Generally, the soil-available Cu contents are classified into very low, low, medium, high, and very high, corresponding to the levels of: <0.1 mg/kg, 0.1-0.2 mg/kg, 0.3-1 mg/kg, 1.1-1.8 mg/kg, and >1.8 mg/kg, but content higher than 1.8 mg/kg may reduce crop production. In this study, the content was 1.72 mg/kg, which was at medium level according to the classification of available soil Cu contents.

LSD results (Table 3) show that in comparison with CK, available Zn content increased by 39.1%, 27.3%, 15.5%, and 13.6% after NP, M, MNP, and HMNP, respectively. The available Zn contents after M, MNP, and HMNP treatments were all significantly different with CK. However, M vs. NP, M vs. MNP vs. HMNP, and HMNP vs. CK did not significantly affect the available Zn contents. Available Zn contents were improved after M, MNP, and HMNP treatments containing organic manure, because the organic manure was rich in Zn and most of Zn was organic complexation, which can be decomposed by microorganisms to supply Zn to soil. Available Zn content was the highest after NP treatment, which was related to the reduction of pH in soil. Research showed that pH reduction in soil could apparently improve soluble Zn content, but did not affect soluble Pb [28]. However, after long-term application of phosphate fertilizers, P would easily react with Zn to form insoluble $Zn_3(PO_4)_2$, which could reduce available Zn content in soil. Therefore, further studies are needed to reveal the effects of different treatments on soil availability. Available Zn at the content of 1.1-2.0 mg/kg in soil is suitable for crop growth, and the level below 0.5 mg/kg is insufficient. Generally, available Zn content in Shajiang black soil was relatively low (only 1.31 mg/kg), mainly because after entering soil the exotic Zn would be fixed by the absorption of soil. Shajiang black soil contains abundant montmorillonite and calcium carbonate, and thus Zn is absorbed by carbonates, or forms and precipitates as $[2ZnCO_3{\cdot}3Zn(OH)_2]$, which is composed of insoluble Zn creosote and Zn hydroxide, or forms insoluble calcium zincate. Montmorillonite is an important clay mineral with a small grain size (about 0.2-1 μm) and can irreversibly fix Zn in soil.

Available Zn contents after all treatments ranked NP>MNP>HMNP>M>CK. Available Zn content is highest after NP treatment, about 32.4% higher than after CK treatment. NP could reduce pH in Shajiang black soil and increase available Pb. The available Pb contents after M and CK treatment were not significantly different, indicating that M very slightly affected available Pb in Shajiang black soil. The available Pb content after M treatment was significantly different compared with NP, MNP, and HMNP treatments, but available Pb contents after MNP and HMNP treatments were not significantly different.

Available Cd contents after all treatments ranked as NP>M>HMNP>MNP>CK. In comparison with CK, treatments of NP, M, HMNP, and MNP increased available Cd by 70.5%, 46.2%, 41.5%, and 45.8% respectively, indicating that available Cd contents were significantly improved by different treatments. M, MNP, and HMNP treatments were not significantly different in increasing available Cd contents, indicating that M and HMNP treatments only slightly affected available Cd. Within reasonable years, application of fertilizers in low-Cd soil would significantly affect soil composition and cause soil Cd pollution [29]. Cd with higher toxicity than Zn may replace Zn in several biochemical processes, and thereby destroy the functions of carbonic anhydrases (related to respiration and other physiological processes), various dehydrogenases and phosphatases, proteases involved in protein metabolism, and other enzymes. Cd as the analogues chemical of Zn may replace Zn in the enzyme systems needed in glucose phospho-rylation, and in production and depletion of carbohydrates. Cd can replace Zn inside plants, which will result in lack of Zn and thereby cause slower growth or even death to plants.

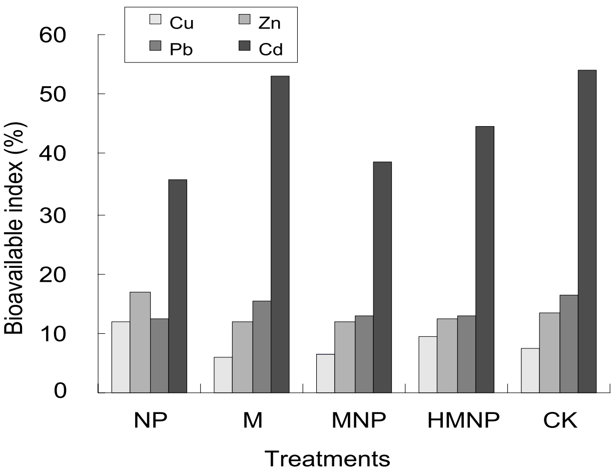

Fig. 2. Bio-available index of the heavy metals in different fertilization experiments. Zn×10

Correlation between Total Contents and Available Contents for Each Heavy Metal After Different Fertilization Treatments

Linear regression analysis showed that the available contents and total contents of Pb after different fertilization treatments were fitted to: $Y=0.0692X+1.9723$ ($R^2=0.9754$), indicating significant linear correlation. The available contents and total contents of Cd after different fertilization treatments were fitted to: $Y=215.33X+50.004$ ($R^2=0.8186$), indicating significant linear correlation. The correlations for Cu and Zn were both insignificant ($R^2=0.0351$ vs. 0.0933). The available heavy metals came from the total contents. The total contents of Pb and Cd significantly affected the available contents. Available heavy metals can be directly absorbed by plants. The available Pb and Cd are highly active, but will easily endanger the plants, and thus can be used as indicators of soil pollution.

Bioavailability Index (BAI) of Heavy Metals

BAI refers to the ratio of available content of a heavy metal to the total content, and the BAIs of the four heavy metals are shown in Fig. 2. Apparently, BAI of Cd is the largest, followed by Pb, Cu, and Zn, which is consistent with other research [30]. This result indicates that in the same soil environment, bioavailabilities of heavy metals were very different. Available Cd and Pb which are highly migratory can be absorbed and enriched by crops, and will threaten human health through food chains. Available Cd and Pb show high risks for ecological pollution and should be paid close attention.

According to the classification by Nieboer E, Cu ions and Pb ions are the most toxic to organisms, followed by the so-called 'borderline' ions Cd and Zn [31]. Soil organic matter can coordinate or chelate with heavy metals to reduce their physiological toxicity and bioavailability, so the stress intensities of heavy metals on organisms could be reduced, and soil quality can be further improved [32]. Fertilization will change the physicochemical soil properties of available heavy metals and further affect the bioavailability of heavy metals. The actual harms of heavy metals contained in fertilizers are related to multiple factors, such as resistance of plants and climate. However, no valid data can prove the maximum contents of trace heavy metals that can be contained in fertilizers to prevent soil from pollution and plants from damage [29].

Conclusions

1. Different fertilizations altered soil physicochemical properties such as pH and organic carbon content, and thereby affect the availability of heavy metals in Shajiang black soil.
2. Fertilization improved total contents of heavy metals in Shajiang black soil to different degrees. After fertilization, contents of Cd, Cu, Pb and Zn were all within SEQS Level I. The effects of different treatments on available Cu in Shajiang black soil were complex. M treatment resulted in the highest available Cu content and reduced available Zn content. Available Zn contents were relatively low after all treatments; available Pb and Cd contents were the highest.
3. Under different fertilizer treatments, BAIs of heavy metals in Shajiang black soil were different, and availability was affected by pH, organic matter, and total content. BAIs were high for non-essential elements such as Cd and Pb, but were low for essential elements such as Cu and Zn. The order was Cd > Pb > Cu > Zn. Available Cd and Pb which are highly migratory can be easily absorbed and enriched by crops, and will finally affect human health through food chains. They show high risks for ecological pollution and should be given great attention.
4. Available contents can better indicate soil pollution and bioavailability than total content, and thus we suggest setting up soil environmental quality standards by using the available contents of heavy metals.

Acknowledgements

This research was financed by the National Natural Science Foundation of China (41371444), the 2013 national Spark Program projects, Anhui Provincial Natural Science Major Project (No. KJ2013ZD07), and Huaibei key research project (No. 20110215).

References

1. CHIVENGE P., VANLAUWE B., SIX J., Does the combined application of organic and mineral nutrient sources influence maize productivity? A meta-analysis. Plant soil, **342**, 1, **2011**.
2. MULVANEY R., KHAN S., ELLSWORTH T., Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. J. Environ. Qual., **38**, 2295, **2009**.
3. JU X.T., XING G.X., CHEN X.P., ZHANG S.L., ZHANG L.J., LIU X.J., CUI Z.L.,YIN B., CHRISTIE P., ZHU Z.L., ZHANG F. S., Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proc. Natl. Acad. Sci. U.S.A., **106**, 3041, **2009**.
4. LOPES C., HERVA M., FRANCO-URÍA A., ROCA E., Inventory of heavy metal content in organic waste applied as fertilizer in agriculture: evaluating the risk of transfer into the food chain. Environ. Sci. Pollut. Res. Int., **18**, 918, **2011**.
5. DONG D.M., ZHAO X.M., HUA X.Y., LIU J.F., GAO M., Investigation of the potential mobility of Pb, Cd and Cr (VI) from moderately contaminated farmland soil to groundwater in Northeast, China. J. Hazard. Mater., **162**, 1261, **2009**.
6. YU W.T., ZHOU H., ZHU X.J., XU Y.G., MA Q., Field balances and recycling rates of micronutrients with various fertilization treatments in Northeast China. Nutr. Cycl. Agroecosys, **90**, 75, **2011**.
7. NIE S.W., GAO W.S., CHEN Y.Q., SUI P., ENEJI A.E., Review of current status and research approaches to nitrogen pollution in farmlands. Agric. Sci. China, **8**, 843, **2009**.
8. AJAYI S., ODESANYA B., AVWIOROKO A., OKAFOR G.A.B., Effects of long term fertilizer use on trace metal levels of soils in a farm settlement. E3 J.Agri. Res. Dev., **2**, 44, **2012**.
9. NIE S.W., GAO W.S., CHEN Y.Q., SUI P., ENEJI A.E., Use of life cycle assessment methodology for determining phytoremediation potentials of maize-based cropping systems in fields with nitrogen fertilizer over-dose. J. Cleaner Prod., **18**, 1530, **2010**.
10. JIAO W., CHEN W., CHANG A.C., PAGE A.L., Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. Environ. Pollut., **168**, 44, **2012**.
11. IMPELLITTERI C.A., SAXE J.K., COCHRAN M., JANSSEN G.M., ALLEN H.E., Predicting the bioavailability of copper and zinc in soils: Modeling the partitioning of potentially bioavailable copper and zinc from soil solid to soil solution. Environ. Toxicol. Chem., **22**, 1380, **2003**.
12. URE A., Single extraction schemes for soil analysis and related applications. Sci. Total Environ., **178**, 3, **1996**.
13. CAO C.F., KONG L.C., ZHANG C.L., ZHAO B., ZHAO Z., ZHANG Y.L., DU S.Z., Effect of fertilization on soil fertility, wheat yield and quality in Shajiang black soil. Chin. J. Eco-agri., **16**, 73, **2008**.
14. LIU E.K., YAN C.R., MEI X., ZHANG Y.Q., FAN T.L., Long-Term Effect of Manure and Fertilizer on Soil Organic Carbon Pools in Dryland Farming in Northwest China. PloS one **8**, e56536, **2013**.
15. LIANG B., YANG X.Y., HE X.H., ZHOU J.B., Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. Biol. Fertil Soils, **47**, 121, **2011**.
16. DONG W.Y., ZHANG X.Y., WANG H.M., DAI X.Q., SUN X.M., X.M.,QIU W.W., YANG F.T., Effect of different fertilizer application on the soil fertility of paddy soils in Red Soil Region of Southern China. PloS one **7**, e44504, **2012**.
17. WANG W., XIE X.L., CHEN A.L., YIN C.M., CHEN W.C., Effects of long-term fertilization on soil carbon, nitrogen, phosphorus and rice yield. J. Plant Nutr., **26**, 551, **2013**.
18. WU L.H., CHENG M.M., LI Z., REN J., SHEN L., WANG S.F., LUO Y.M., CHRISTIE P., Major nutrients, heavy metals and PBDEs in soils after long-term sewage sludge application. J. Soil. Sediment., **12**, 531, **2012**.
19. MODAIHSH A.S., AL-SEWAILEM M.S., MAHJOUB M.O., Heavy metals content of commercial inorganic fertilizers marketed in the Kingdom of Saudi Arabia, Agric. Mar. Sci., **9**, 21, **2004**.
20. HUANG B., KUO S., BEMBENEK R., Cadmium uptake by cucumber from soil amended with phosphorus fertilizers. J. Am. Soc. Hortic. Sci., **128**, 615, **2003**.
21. SELLES F., CLARKE J., ZENTNER R., CAMPBELL C., Effects of source and placement of phosphorus on concentration of cadmium in the grain of two durum wheat cultivars. Canadian J. Plant. Sci., **83**, 475, **2003**.
22. DUAN G.L., ZHANG H.M., LIU Y.X., JIA Y., HU Y., CHENG W.D., Long-term fertilization with pig-biogas

residues results in heavy metal accumulation in paddy field and rice grains in Jiaxing of China. Soil Sci. Plant Nutr., **58**, 637, **2012**.

23. FENG M.H., SHAN X.Q., ZHANG S.Z., WEN B., A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$, and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley. Environ. Pollut., **137**, 231, **2005**.
24. PEIJNENBURG W.J., ZABLOTSKAJA M., VIJVER M.G., Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. Ecotoxicol. Environ. Saf., **67**, 163, **2007**.
25. WILLIAMS D.E., VLAMIS J., PUKITE A.H., COREY J.E., Trace element accumulation, movement, and distribution in the soil profile from massive applications of sewage sludge. Soil Sci., **129**, 114, **1980**.
26. LIU X.L., GAO Z., LIU C.S., SI L.Z., Effect of combined application of organic manure--and fertilizers on crop yield and soil fertility in a located experiment. Acta Pedologica Sin., **2**, 138, **1996**.
27. ZHU Y.M., BERRY D.F., MARTENS D., Copper availability in two soils amended with eleven annual applications of coppe-enriched hog manure. Communications in Soil Science & Plant Analysis **22**, 769, **1991**.
28. CUI Y.S., DONG Y.T., LI H.F., WANG Q.R., Effect of elemental sulphur on solubility of soil heavy metals and their uptake by maize. Environ. Int., **30**, 323, **2004**.
29. SENESI N., POLEMIO M.,Trace element addition to soil by application of NPK fertilizers. Fertilizer res., **2**, 289, **1981**.
30. VANĚK A., ETTLER V., GRYGAR T., BORŮVKA L., ŠEBEK O., DRÁBEK O., Combined chemical and mineralogical evidence for heavy metal binding in mining- and smelting-affected alluvial soils. Pedosphere, **18**, 464, **2008**.
31. NIEBOER E., RICHARDSON D.H., The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ. Pollut. Ser. B, Chemical and Physical, **1**, 3, **1980**.
32. MORA A.P.D., ORTEGA-CALVO J.J., CABRERA F., MADEJÓN E. Changes in enzyme activities and microbial biomass after "in situ" remediation of a heavy metal-contaminated soil. Appl. Soil Ecol., **28**, 125, **2005**.