Journal of Hazardous Materials 160 (2008) 324–329

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Total concentrations and speciation of heavy metals in municipal sludge from Changsha, Zhuzhou and Xiangtan in middle-south region of China

Ming Chen, Xiao-ming Li*, Qi Yang, Guang-ming Zeng, Ying Zhang,
De-xiang Liao, Jing-jin Liu, Jing-mei Hu, Liang Guo

*College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China*

ARTICLE INFO

*Article history:*
Received 24 May 2007
Received in revised form 16 November 2007
Accepted 3 March 2008
Available online 20 March 2008

*Keywords:*
Municipal sludge
Heavy metal
Sequential extraction
Metal speciation
Bioavailability

ABSTRACT

The presence of heavy metals in municipal sludge restricts its use for agricultural purposes. In this paper, the bioavailability and eco-toxicity of heavy metals in municipal sludge was evaluated, taking into consideration both the speciation of metals and the local environmental characteristics. The dewatered municipal sludge samples were collected from five sewage plants in Changsha, Zhuzhou and Xiangtan respectively, which are representative cities with characteristics of the middle-south region of China. Some agricultural significant parameters and total metal concentrations in the sludge were determined and the metal speciation was studied by using BCR sequential extraction procedure. It was found experimentally that in general the municipal sludge collected from the five sewage plants was rich in organics, N and P. Except that the sludge from Xia Wan Sewage Treatment Plant showed higher concentrations of heavy metals, the sludge from other plants all showed a low total content of heavy metals with only Cd slightly exceeding the permitted values of the national application standard of acid soil in China (GB18918-2002). The sequential extraction results showed that Cu and Zn were principally distributed in the oxidizable fraction, which meant a high potential toxicity, but the bioavailability of Zn might be overestimated to the soil of Hunan. Pb was mainly in the residual fraction. The distribution of Cd showed no obvious characteristics.

## 1. Introduction

In China, the production of municipal sludge has increased sharply due to the demand for better quality water and the imposition of more strict environmental laws. Municipal sludge contains heavy metals, organic compounds, macronutrients, micronutrients and non-essential trace elements, organic micropollutants, microorganisms and eggs of parasitic organisms [1]. So the accumulation of sludge poses a growing environment problem, and the disposal of these bio-wastes may result in secondary environmental pollution if treated improperly [2].

The application of municipal sludge in agriculture is an attractive option of disposal, due to the possibility of recycling valuable components: organic matter, N, P and other plant nutrients [3–5]. However, it has been suggested that applying sewage sludge to soil might provide metals in potentially toxic, labile forms [6]. Thus the heavy metal pollution has become the main obstacle for agricultural use of municipal sludge [7].

Although total metal concentrations may indicate the overall level of metals in sludge, the mobility of heavy metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding [8]. The evaluation of sewage sludge toxicity by chemical speciation and biological testing is therefore very important when deciding on the suitability of sludge for agricultural application.

The speciation of heavy metals can be determined with the selective sequential extraction analysis, which consists of several extraction steps based on the use of different chemical reagents and conditions [9]. During recent decades a great variety of extraction schemes have been developed and some of them have been widely used in the determination of the speciation of metals in soil and sludge [10–12]. However, these methods lack a standard analysis procedure and have a poor comparability, strongly influenced by the choice of extractants [13]. In order to harmonise and validate different fractionation schemes the Community Bureau of Reference (now Measuring and Testing Programme) proposed the BCR three-step procedure [14–16] in 1992, which reaches a compromise between analysis time and the amount of information obtained. In recent years, this method has been used extensively for the study of the heavy metal in the soil and sludge [17–20].

Besides the speciation of metals, the local environmental characteristics also influenced a lot to the estimation of metal mobility,

---

* Corresponding author. Tel.: +86 731 8823967; fax: +86 731 8822829.
*E-mail addresses:* xmli@hun.cn, dahu2000@163.com (X.-m. Li).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.03.036

**Table 1**
Municipal sludge collected from municipal wastewater treatment plant

| Name and address of sewage plant | Population equivalents (PE) | Primary clarifier | Origin of the wastewater | Sample number |
|---|---|---|---|---|
| Jinxia sewage plant, Changsha | 90,000 | Yes | Sewage | S1 |
| Xianghu sewage plant, Changsha | 70,000 | Yes | Sewage | S2 |
| Hexi sewage plant, Xiangtan | 80,000 | Yes | Sewage | S3 |
| Longquan sewage plant, Zhuzhou | 1,00,000 | Yes | Sewage | S4 |
| Xiawan sewage plant, Zhuzhou | 65,000 | Yes | Sewage and a part of industry wastewater | S5 |

bioavailability and related eco-toxicity. Changsha, Zhuzhou and Xiangtan are three adjacent representative cities in Hunan province (the middle-south of China). The main soil type in the area is red soil according to Chinese soil classification [21] and Hunan Province, including Changsha and many other cities, is under the severe $H_2SO_4$-type acid rain pollution, which results from industrial activities in the cities [22]. The evaluation of the biotoxicity of metals to environment is essential to predict changes in metal behavior in response to these environmental conditions. So the feasibility of the agricultural utilization of the municipal sludge in this region mainly depends on two factors: the speciation of heavy metals in the sludge and the environmental characteristics in middle-south of China.

On the other hand, the municipal sludge which is the main residue and carrier of pollutant during sewage treatment procedure also indicates the pollution degree of inlet wastewater of treatment plants. So, the overall investigation of sewage sludge from the three cities would be beneficial for monitoring sewage permitted discharge in this region.

In this paper, the agricultural significant parameters and total concentrations of the heavy metals (Cu, Zn, Pb and Cd) of the sludge from sewage treatment plants at Changsha, Zhuzhou and Xiangtan were determined, and the speciation of each heavy metal was studied by BCR sequential extraction procedure. The bioavailability and eco-toxicity of heavy metals (Cu, Zn, Pb and Cd) were evaluated, which took into consideration both their speciation and local environmental characteristics, and the industrial layout was discussed preliminarily, based on the indication of the municipal sludge to the environment.

## 2. Experimental

### 2.1. Sample collection and pre-treatment

Sludge samples obtained from five sewage plants at Changsha, Zhuzhou and Xiangtan in Hunan Province are listed in Table 1 and the location of the three cities in China is shown in Fig. 1. The samples were dried at 55 °C, ground and homogenized in an agate mortar, sieved through a siever (mesh size 100), stored in jars at room temperature.

The agricultural significant parameters of sludge from sewage plants such as pH values, contents of organic carbon, total N, total K, total P, and cation exchange capacity (CEC) values were determined following the standard analytical methods [23].

### 2.2. The sequential extraction

Sequential extraction was performed using the BCR three-step procedure recommended by Community Bureau of Reference, and the analysis of the residual fractions was supplemented as step four.

*Step one*: *acid soluble/exchangeable fraction* (*F1*). Sludge samples (0.5 g) were introduced in a 50-mL polypropylene centrifuge tubes containing 20 mL of acetic acid (0.1 mol $L^{-1}$) and then shaken for 16 h at room temperature. The solution and solid phases were separated by centrifugation at 4000 rpm for 20 min. Subsequently, the suspension was filtered through a 0.45-μm membrane filter and the solid residues were preserved for the subsequent extractions.

*Step two*: *reducible fraction* (*F2*). The residues from step one were slurried with a portion of a 20-mL volume of 0.1 mol $L^{-1}$ hydrox-

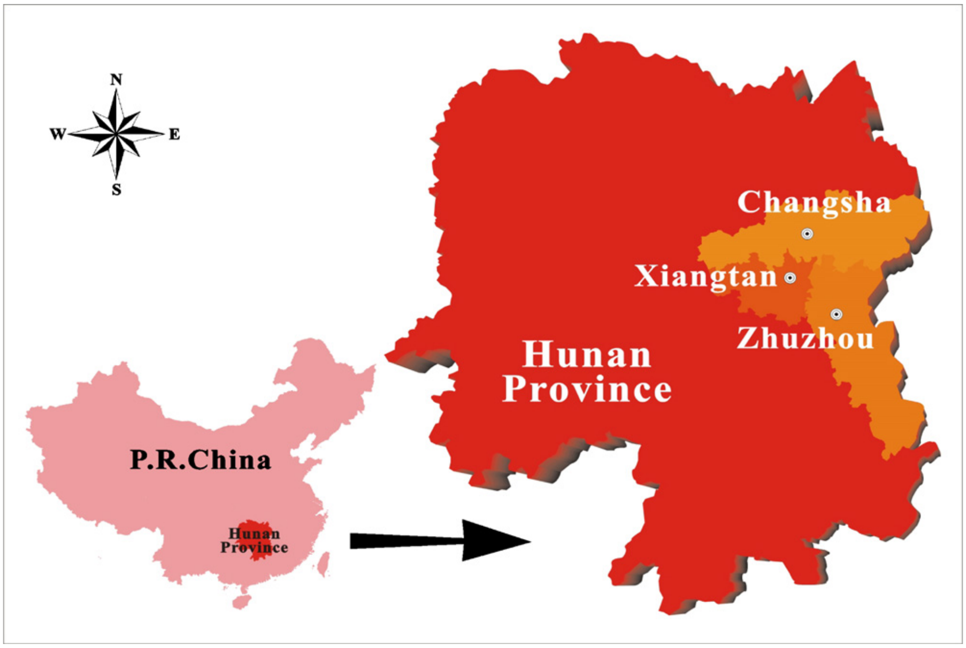


**Fig. 1.** The location of Changsha, Zhuzhou and Xiangtan in China.

**Table 2**
Agronomic parameters of municipal sludge

| Sample | pH | Total N (%) | Nitrate N (mg kg$^{-1}$) | Total P (g kg$^{-1}$) | Total K (g kg$^{-1}$) | CEC (cmol kg$^{-1}$) | Organic carbon (%) |
|---|---|---|---|---|---|---|---|
| S1 | 6.9 | 1.94 | 2.53 | 13.05 | 4.42 | 95.7 | 37.8 |
| S2 | 6.5 | 1.73 | 2.74 | 15.82 | 5.68 | 88.4 | 40.5 |
| S3 | 6.2 | 2.55 | 2.15 | 9.56 | 6.29 | 74.5 | 35.2 |
| S4 | 6.6 | 2.12 | 1.96 | 5.68 | 5.42 | 76.8 | 27.1 |
| S5 | 6.7 | 2.03 | 3.27 | 11.55 | 10.55 | 68.7 | 31.5 |

ylammonium chloride (adjusted to pH 2 with nitric acid) for 16 h. The extraction procedure described above was followed.

*Step three*: *oxidizable fraction* (*F*3). The residues from step two were dispersed in 5 mL volume of hydrogen peroxide (30%) and digested at room temperature for 1 h with occasional shaking. A second 5-mL aliquot of hydrogen peroxide was introduced and digested at 85 °C (water bath) for 1 h. The contents were evaporated to a small volume (1–2 mL). Twenty-five milliliters of ammonium acetate (1.0 mol L$^{-1}$, adjusted to pH 2 with nitric acid) was added to the cool and moist residue, shaken, centrifuged and the extract separated described in step one.

*Step four*: *residual fraction* (*F*4). Five milliliters of $HNO_3$ were added to the residues from step three. The contents were heated on a hot plate and evaporated to near dryness. After cooling, the residues were dissolved in 5% $HNO_3$. The resultant solutions were subsequently used to determine the heavy metals.

The concentrations of Zn, Cu, Pb and Cd in different fractions and the resultant solutions of step four were determined by atomic absorption spectrophotometry analysis (AAnalyst-700, PerkinElmer). Each experiment was conducted in triplicate and the results reported were the average values.

## 3. Result and discussion

### 3.1. Physicochemical analysis

Some agricultural significant parameters of the sludge samples are listed in Table 2 and a part of physicochemical properties of the northeast black soil that is the most fertile land in China are as follows: organic carbon 4–6%, total N 1.99 g kg$^{-1}$, total P 0.74 g kg$^{-1}$ and total K 16.73 g kg$^{-1}$ [24]. Though the nutrients of the each sludge sample varied from the other, compared with the black soil in northeast China, the municipal sludge generally contained high organic contents and was rich in N and P, which posed a good prospect for agricultural application

**Table 3**
Total concentrations of Cu, Zn, Pb and Cd in sludge and permitted values in discharge standards

| Sample | Heavy metal (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | Cu | Zn | Pb | Cd |
| S1 | 111.0 | 424.8 | 152.0 | 7.2 |
| S2 | 130.4 | 450.9 | 53.6 | 10.7 |
| S3 | 159.6 | 444.6 | 71.8 | 15.7 |
| S4 | 67.0 | 361.0 | 98.4 | 7.9 |
| S5 | 659.0 | 1105.9 | 1270.2 | 903.8 |
| Domžale, Slovenia | 227 | 2180 | 105 | 4.2 |
| Murcia, Spain | 204 | 487 | 58 | 1.1 |
| Threshold values | | | | |
| Germany | 1000 | 3000 | 900 | 10 |
| France | 800 | 2500 | 900 | 10 |
| EU | 1750 | 4000 | 1200 | 40 |
| China pH < 6.5 | 800 | 2000 | 300 | 5 |
| China pH ≧ 6.5 | 1500 | 3000 | 1000 | 20 |

### 3.2. Total concentration of Cu, Zn, Pb and Cd in sewage sludge

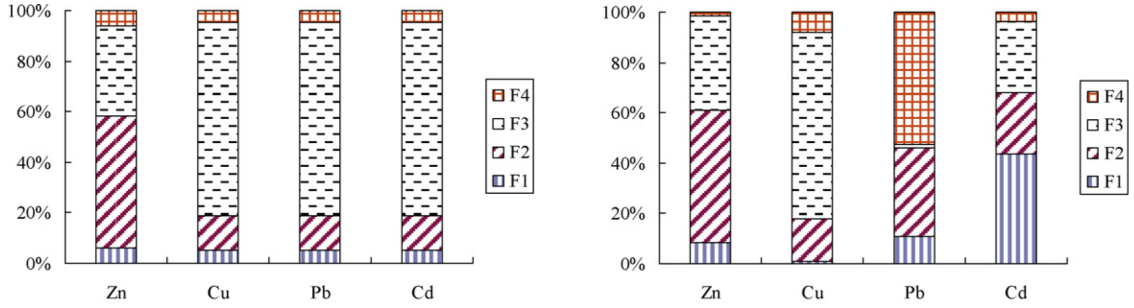
The total content of the heavy metals Cu, Zn, Pb and Cd and discharge standards of pollutants for municipal wastewater treatment plant [25–27] are listed in Table 3. Compared with the sludge from other cities (Domžale and Murcia), in samples S1, S2, S3 and S4, the concentrations of Cu, Zn and Pb were relatively low. The concentration of Cd was higher and slightly exceeded the acid soil standard, but still in the basic soil control range. In general, the total content of heavy metal contained in the samples S1, S2, S3 and S4 was low and had a bright foreground in agricultural application.

In sludge sample S5, the total concentrations of Cu, Zn, Pb and Cd were all far beyond the discharge standards of pollutants for municipal wastewater treatment plant (GB18918-2002). Compared with the sludge from Domžale (Slovenia), the content of Cu, Pb and Cd in S5 was about 3, 12 and 215 times of that in sludge from Domžale, respectively. This was because that the sample S5 was collected from Qingshuitang area in Zhuzhou, where many chemical plants were centralized. The effluent from some factories did not meet the standard and the pollutant settled and accumulated in the sludge, which caused a high heavy metal concentration in sewage sludge. The sludge samples S1, S2, S3 and S4 could be used as good organic fertilizers while the S5 should not be used directly unless bioremediation [28] and chemical remediation [29] were performed. Some recent studies also describe thermal and chemical methods for reducing the heavy metal content of sewage sludge. For example, Dewil et al. [30] enhanced the use of waste-activated sludge as biofuel through selectively reducing its heavy metal content. Dominica et al. [31] removed heavy metals from contaminated sewage sludge using *Aspergillus niger* fermented raw liquid from pineapple wastes. Marinos and Demetra [32] removed heavy metals from sewage sludge by acid treatment. The sludge from different areas may have different characteristics such as the content of heavy metal and organics, and we should dispose the sludge with proper method based on these characteristics. So, the overall analysis and investigation to the municipal sludge is the important basis for the rational and integrated utilization of the sludge.

As the basic filtrate and carrier during the municipal sewage treatment process, the sludge is a good indicator of the sewage permitted discharge. That only Cd in S1, S2, S3 and S4 slightly exceeded the permitted values of the national application standard of acid soil (GB18918-2002) indicates that with the more and more strict regulation to the sewage discharge and the development of modern intensive industry, the degree of sewage permitted discharge is satisfying in this region. However, the higher concentrations of heavy metals in S5 reveal that the effluent from some factories did not meet the standard in Qingshuitang area in Zhuzhou, where

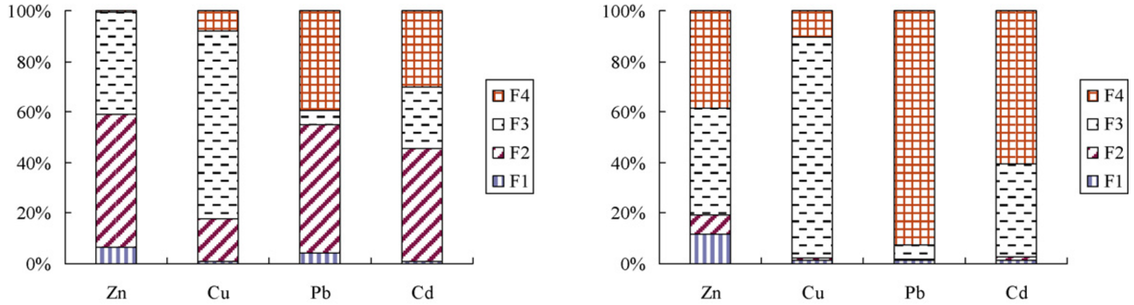
**Table 4**
The relation among fraction of heavy metals, eco-toxicity and bioavailability

| Fraction of heavy metals | Eco-toxicity | Bioavailability |
|---|---|---|
| Acid soluble/exchangeable fraction (*F*1), reducible fraction (*F*2) | Direct toxicity | Direct effect faction |
| Oxidizable fraction (*F*3) | Potential toxicity | Potential effect fraction |
| Residual fraction (*F*4) | No toxicity | Stable fraction |



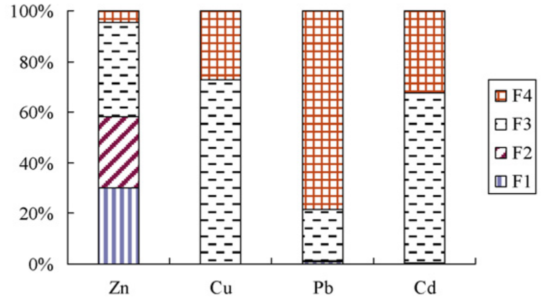
(a) Metal speciation in sample 1

(b) Metal speciation in sample 2



(c) Metal speciation in sample 3

(d ) Metal speciation in sample 4



(e) Metal speciation in sample 5

**Fig. 2.** Speciation of heavy metals in S1 (a), S2 (b), S3 (c), S4 (d) and S5 (e).

many chemical plants are centralized. From the point view of environment protection and present environmental characteristics in Zhuzhou region, taking strict management and implementing clean production is necessary to improve the environment in this area.

### 3.3. *The speciation of heavy metals in the municipal sludge*

The bioavailability and eco-toxicity of metals mainly depends on their speciation in sludge (Table 4). Heavy metals that are distributed in acid soluble/exchangeable fraction ($F1$) and reducible fraction ($F2$) are readily to be absorbed in plants or in water system causing pollution. So, these fractions should be identified as direct effect fraction. Because the red soil dominated in Hunan Province is acidic [21] and this region is located in acid rain area [22], the soil has highly oxidizing property. The oxidizable fraction ($F3$) in this oxidizing condition is easily mobilized and transformed into acid soluble/exchangeable fraction ($F1$) or reducible fraction ($F2$), potential of that eco-toxicity should not be ignored. So, the oxidizable fraction can be identified as potential effect fraction. The heavy metal bound to residual fraction is often considered “unreactive”, and not affected by environment changes, is identified as stable fraction.

The speciation of Cu, Zn, Pb and Cd estimated by BCR procedure in municipal sludge represented as percent of total concentrations in sludge is shown in Fig. 2, and the statistical results of each fraction of heavy metals in samples are listed in Table 5.

It is noted that the content of $F1$ and $F2$ fraction of copper was low in all sludges, especially acid soluble/exchangeable fraction ($F1$) was low to less than 5%, showing less direct toxicity to environment. 67.7–91.5% of total Cu present in oxidizable fraction ($F3$) in all sludges, and this result was in agreement with Bibak on Cobalt’s study [33]. Cu–organic matter complexes were generally considered relatively stable [34,35]. However, the percentage of Cu associated with organic matter fraction is primarily depending on the quantity of Fe oxides in soil [36] and the evaluation of the bioavailability of metals to environment is essential to predict changes in metal behavior in response to environment conditions. Red soil dominated in Hunan Province is rich in Fe oxide and Cu in oxidizable fraction ($F3$) can be mobilized and available in this oxidizing conditions. So, the Cu in all sludge samples had a high potential eco-toxicity and bioavailability to the soil of Hunan.

Zn had higher concentration in reducible (28.0–317.2 mg $\text{kg}^{-1}$) and oxidizable (151.9–419.8 mg $\text{kg}^{-1}$) fractions, seemed to have high bioavailability and potential eco-toxicity. However, Zn appeared to be preferentially bound to Fe oxides in soil [36] and

**Table 5**
Statistical results[a] of each fraction of heavy metals in samples (mg kg$^{-1}$)

| Element | Speciation | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|
| Cu | *F*1 | 5.7 ± 0.8 | 1.5 ± 0.3 | 2.3 ± 0.2 | 0.8 ± 0.01 | 0.4 ± 0.02 |
| | *F*2 | 15.4 ± 1.5 | 23.7 ± 1.8 | 26.0 ± 3.8 | 0.7 ± 0.01 | 0.6 ± 0.03 |
| | *F*3 | 86.6 ± 5.5 | 104.6 ± 11.2 | 124.7 ± 13.5 | 61.3 ± 4.2 | 446.1 ± 25.7 |
| | *F*4 | 5.4 ± 1.3 | 11.5 ± 2.1 | 20.3 ± 3.3 | 7.2 ± 0.5 | 167.2 ± 14.5 |
| Zn | *F*1 | 30.6 ± 2.6 | 39.7 ± 3.4 | 33.1 ± 2.5 | 41.6 ± 4.3 | 336.0 ± 10.2 |
| | *F*2 | 257.3 ± 24.5 | 248.1 ± 15.2 | 255.9 ± 11.8 | 28.0 ± 2.5 | 317.2 ± 32.2 |
| | *F*3 | 176.4 ± 13.1 | 177.4 ± 17.5 | 194.9 ± 25.3 | 151.9 ± 13.2 | 419.8 ± 13.7 |
| | *F*4 | 30.2 ± 2.2 | 5.6 ± 1.2 | 3.4 ± 0.3 | 139.2 ± 14.0 | 53.1 ± 5.1 |
| Pb | *F*1 | 29.1 ± 2.8 | 5.4 ± 1.3 | 2.8 ± 0.2 | 1.5 ± 0.2 | 10.2 ± 0.7 |
| | *F*2 | 26.5 ± 3.7 | 17.8 ± 2.2 | 34.4 ± 2.8 | 0.3 ± 0.12 | 4.6 ± 0.5 |
| | *F*3 | 9.8 ± 1.9 | 0.8 ± 0.1 | 3.8 ± 0.4 | 5.8 ± 0.4 | 245.7 ± 30.2 |
| | *F*4 | 88.7 ± 3.5 | 26.5 | 26.6 ± 2.7 | 93.2 ± 7.5 | 947.9 ± 73.9 |
| Cd | *F*1 | 0.3 ± 0.05 | 5.1 | 0.1 ± 0.02 | 0.1 ± 0.03 | 2.0 ± 0.3 |
| | *F*2 | 4.7 ± 0.2 | 2.8 | 7.7 ± 0.6 | 0.1 ± 0.02 | 2.5 ± 0.6 |
| | *F*3 | 2.0 ± 0.3 | 3.3 | 4.2 ± 0.3 | 2.8 ± 0.7 | 612.1 ± 64.7 |
| | *F*4 | 0.1 ± 0.04 | 0.4 | 5.2 ± 0.6 | 4.7 ± 0.2 | 293.0 ± 25.5 |

[a] Results are expressed as the mean ± standard deviations.

Hseu reported that Zn associated with chemical reactive fractions (exchangeable and carbonate fractions) in native tropical soils varied from approximately 2% to 6% of total Zn concentrations [37]. A part of Zn can be occluded inside of crystalline structures and not readily available for plants absorption. Therefore, for the soil in Hunan, which is rich in Fe oxides, the eco-toxicity of Zn in municipal sludge might be overestimated.

Pb was primarily present in the *F*4 fraction (37.0–94.7%). The results were in good agreement with the reports by other studies [5]. It was reported that Pb can be immobilized by insoluble salts, such as phosphates [38] and the importance of soil organic matter in limiting Pb bioavailability has also been demonstrated [39]. These can explain the high Pb concentration of residual fraction in municipal sludge and predict the low direct and potential bioavailability to environment, if the sludge is used for organic amendment in soil.

Cd distribution into various fractions showed different patterns for each sewage sludge sample. The variable total Cd concentrations were found in sludge (7.2–903.8 mg kg$^{-1}$), but acid soluble/exchangeable fraction (*F*1) was relatively low in S1, S3, S4 and S5. Approximately 47.6% of total Cd in S2 was extracted in the first step, showing high bioavailability to environment. In S5, despite the relatively small proportion of Cd in acid soluble/exchangeable fraction (*F*1), the high total Cd concentration of S5 (903.8 mg kg$^{-1}$) meant that much Cd is expected to be bioavailable.

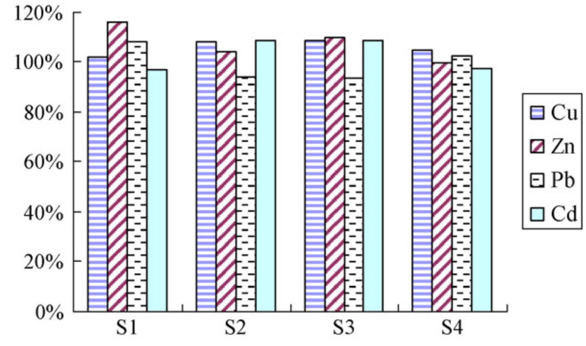


**Fig. 3.** The sum of the percentage of the each fraction.

A check on the results of BCR sequential extraction procedure was performed by comparing the sum of the four fractions (*F*1, *F*2, *F*3 and *F*4) with the total concentrations of heavy metal from $HNO_3$ digestion procedure. The detailed calculations were expressed as follows:

$$\text{Recovery}\,(\%) = \frac{F1 + F2 + F3 + F4}{\text{Total concentration}} \times 100 \qquad (1)$$

The results were statistic and shown in Fig. 3. It can be seen clearly that the sum of the four steps was in good agreement with the total heavy metal concentration with satisfactory recoveries (93.5–116.3%). It indicated that this modified BCR sequential extraction method used in detecting the speciation of Cu, Zn, Pb and Cd in municipal sludge was exact and reliable.

## 4. Conclusion

Municipal sludge collected from five sewage treatment plants at Changsha, Zhuzhou and Xiangtan had high organic carbon, and was rich in nutrient like N and P, so they could be used as good organic amendment. The analysis results of the total content of heavy metals in the sludge showed that the total concentrations of heavy metals in samples S1, S2, S3 and S4 were below the discharge standard with only Cd slightly exceeding the permitted values of the national application standard of acid soil in China (GB18918-2002), which was very promising in agricultural application. However, the total content of heavy metals in S5 was high, and the treatment of remediation was necessary before its application in agriculture.

Evaluating the bioavailability and eco-toxicity of heavy metals in sludge is essential to take into consideration both the speciation of metals and the soil characteristics. In Hunan Province, Red soil which is rich in Fe oxides and acid rain are influential factors. Under this soil conditions, Cu and Zn were mainly distributed in oxidizable fraction, posing a high potential eco-toxicity, but the bioavailability of Zn might be overestimated for the soil of Hunan. Pb was mainly in the residual fraction, showing low direct and potential eco-toxicity to environment. Cd distributed into various fractions and showed different patterns for each municipal sludge sample, of which the eco-toxicity in S2 and S5 was worth paying attention.

According to the indication of municipal sludge to the sewage permitted discharge and from the point view of environment protection, taking strict management and implementing clean production is necessary to improve the environment in Qingshuitang area.

## Acknowledgements

This research was supported by the National Natural Science Foundation of China (no. 50478054) and Program for New Century Excellent Talents in University (NCET-04-0770).

## References

[1] B.J. Alloway, A.P. Jackson, The behaviour of heavy metals in sewage sludge-amended soil, Sci. Total Environ. 100 (1991) 151–176.

[2] M.J. Wang, Land application of sewage sludge in China, Sci. Total Environ. 197 (1997) 149–160.

[3] T. Hernández, J.L. Moreno, F. Costa, Influence of sewage sludge application on crop yields and heavy metal availability, Soil Sci. Nutr. 37 (1991) 201–210.

[4] S.R. Smith, Agricultural Recycling of Sewage Sludge and the Environment, CAB International, UK, 1996.

[5] J.C. Wong, K. Li, M. Fang, D.C. Su, Toxicity evaluation of sewage sludges in Hong Kong, Environ. Int. 27 (2001) 373–380.

[6] M.B. McBride, Toxic metal accumulation from agricultural use of sludge: are USEPA regulations protective? J. Environ. Qual. 24 (1995) 5–18.

[7] C. Mo, Q. Wu, Q.Y. Cai, et al., The agricultural application of sewage sludge and sustainable development, Chin. J. Appl. Ecol. 11 (2000) 157–160.

[8] C.W. Jin, S.J. Zheng, Y.F. He, G.D. Zhou, Z.X. Zhou, Lead contamination in tea garden soils and factors affecting its bioavailability, Chemosphere 59 (2005) 1151–1159.

[9] H. Domingues, J.V. Silva, Copper and zinc in sandy, granitic and schist soils, Environ. Technol. 11 (1990) 463–468.

[10] A. Tessier, P.C. Campbell, M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, Anal. Chem. 51 (1979) 844–851.

[11] L.N. Benitez, J.P. Dubois, Evaluation of the selectivity of sequential extraction procedures applied to the speciation of cadmium in soils, Int. J. Environ. Anal. Chem. 74 (1999) 289–303.

[12] Y. Lu, Z.T. Gong, G.L. Zhang, The chemical speciation of heavy metals of urban soil in Nanjing, Environ. Chem. 22 (2003) 131–136.

[13] C.M. Davidson, P.S. Ferreire, A.M. Ure, Some sources of variability in application of the three-stage sequential extraction procedure recommended by BCR to industrially-contaminated soil, Fresen. J. Anal. Chem. 363 (1999) 446–451.

[14] A.M. Ure, P. Quevauviller, H. Muntau, et al., Speciation of heavy metals in soils and sediments: an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European communities, Int. J. Environ. Anal. Chem. 51 (1993) 135–151.

[15] P. Quevauviller, G. Rauret, H. Muntau, et al., Evaluation of a sequential extraction procedure for the determination of extractable trace metal contents in sediments, Fresen. J. Anal. Chem. 349 (1994) 808–814.

[16] C.M. Davidson, A.L. Duncan, D. Littlejohn, et al., A critical evaluation of the three-stage BCR sequential extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially contaminated land, Anal. Chim. Acta 363 (1998) 45–55.

[17] Y.S. Cui, Q.R. Wang, Y.T. Dong, et al., Effect of element sulfur on speciations of Pb and Zn of contaminated oil, Environ. Chem. 23 (2004) 46–50.

[18] I. Ko, Y.Y. Chang, C.H. Lee, K.W. Kimc, Assessment of pilot-scale acid washing of soil contaminated with As, Zn and Ni using the BCR three-step sequential extraction, J. Hazard. Mater. A127 (2005) 1–13.

[19] I. Walter, F. Martinez, V. Cala, Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses, Environ. Pollut. 139 (2006) 507–514.

[20] M. Sulkowski, A.V. Hirner, Element fractionation by sequential extraction in a soil with high carbonate content, Appl. Geochem. 21 (2006) 16–28.

[21] J.M. Zhang, F.Q. Cai, T.K. He, The soil of China, The Commercial Press, Beijing, 1997.

[22] Z.Y. Chen, Z.R. Liu, Acid Rain Research in China, China Environmental Science Press, Beijing, 1997.

[23] G.S. Liu, Soil Physical and Chemical Analysis and Description of Soil Profiles, Standard Press of China, Beijing, 1996.

[24] Q. Ma, W.T. Yu, S.H. Zhao, et al., Comprehensive evaluation of cultivated black soil fertility, Chin. J. Appl. Ecol. 15 (2004) 1916–1920.

[25] Environmental Protection Administration (EPA), PR China, Ministry of Rural and Urban Construction PR China. Discharge standards of pollutants for municipal wastewater treatment plant (GB 18918-2002), 2002.

[26] Š. Janez, M. Radmila, S. Marjeta, et al., Total metal concentrations and partitioning of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge, Sci. Total Environ. 250 (2000) 9–19.

[27] F. Ana, L. Mercedes, S. Josě, Simple and sequential extractions of heavy metals from different sewage sludges, Chemosphere 54 (2004) 1039–1047.

[28] W.C. Wong, L. Xiang, L.C. Chan, Removal of heavy metals from anaerobically digested sewage sludge by isolated indigenous iron oxidizing bacteria, Chemosphere 41 (2000) 283–287.

[29] A.-M. Veeken, H.-M. Hamelers, Removal of heavy metals from sewage sludge by extraction with organic acids, Water Sci. Technol. 40 (1999) 129–136.

[30] R. Dewil, J. Baeyens, L. Appels, Enhancing the use of waste activated sludge as bio-fuel through selectively reducing its heavy metal content, J. Hazard. Mater. 144 (2007) 703–707.

[31] D. Dominica, D. Mundo, B. Sandhya, Removal of heavy metals from contaminated sewage sludge using *Aspergillus niger* fermented raw liquid from pineapple wastes, Bioresour. Technol. (2007), doi:10.1016/j.biortech.2007.04.002.

[32] A.S. Marinos, K. Demetra, H. Katherine-Joanne, et al., Effect of acid treatment on the removal of heavy metals from sewage sludge, Desalination 215 (2007) 73–81.

[33] A. Bibak, Cobalt, copper and manganese adsorption by aluminum and iron oxides and humic acid, Commun. Soil Sci. Plant Anal. 25 (1994) 3229–3239.

[34] I. Walter, G. Cuevas, Chemical fractionation of heavy metals in soil amended with repeated sewage sludge application, Sci. Total Environ. 226 (1999) 113–119.

[35] V. Illera, I. Walter, P. Souza, V. Cala, Short-term effects of biosolid and municipal solid waste applications on heavy metals distribution in a degraded soil under a semi-arid environment, Sci. Total Environ. 255 (2000) 29–44.

[36] M.L. Sliveira, L.F. Alleoni, G.A. O'Connor, et al., Heavy metal sequential extraction methods—a modification for tropical soils, Chemosphere 64 (2006) 1929–1938.

[37] Z.Y. Hseu, Extractability and bioavailability of zinc over time in three tropical soils incubated with biosolids, Chemosphere 63 (2006) 762–771.

[38] D.J. Walker, R. Clemente, A. Roig, M.P. Bernal, The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils, Environ. Pollut. 122 (2003) 303–312.

[39] D.G. Strawn, D.L. Sparks, Effects of soil organic matter on the kinetics and mechanisms of Pb(II) sorption and desorption in soil, Soil Sci. Soc. Am. J. 64 (2000) 144–156.