

*Full Length Research Paper*

# Study of heavy metal in sewage sludge and in Chinese cabbage grown in soil amended with sewage sludge

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**The study was performed to investigate the heavy metal content and availability for crops in sewage sludge and its accumulation in Chinese cabbage grown in sewage sludge amended soil. We determined the total and chemical fraction of As, Cr, Cd, Pb, Ni, Cu, Zn, Fe, Mg and Mn in sewage sludge and the total content of these metals in Chinese cabbage grown in soil amended with sewage sludge. Total content of all metals (except for As) was below the top limits for land application of sewage sludge in China. The majority of As, Cd, Pb, Zn and Fe was present in the residual fraction (70 – 95%) of the total concentration, and 21% of Cd, 59% of Cr and 56% of Cu were present in oxidizable fraction in sewage sludge. Most of the content of metals increased in Chinese cabbage with the increase in sludge amendments ratio, and the content of heavy metal As, Cd, Cr and Zn exceeded the top limits of metals content in China. Our results suggested that application of sewage sludge could enhance the output of vegetable while the risk of heavy metal should be of concern.**

**Key words:** Sewage sludge, BCR procedure, heavy metals, Chinese cabbage, vegetables.

## INTRODUCTION

Anthropogenic activities have altered the environment significantly throughout the world including mining, industry and agriculture as well as increase of the urbanization level. For example, on one hand many chemical fertilizers from various ore that cannot be regenerated are decreasing gradually. On the other hand, however the discharge of wastewater containing abundant nutrition have been considered as the important causes contributing to the eutrophication of water (Wang et al., 2008). In order to reduce the wastewater discharging, abounded wastewater treatment plants have been constructed in recent several decades. In 1980s, only about 20 small-scale treatment plants that provided low levels of treatment were constructed and by 2000, there were about 400 wastewater treatment plants in China. The rapid increase in the number of new wastewater treatment

plants is expected to yield more than four million tonne of municipal dry sewage sludge in coming years (Lee et al., 2002; Dai et al., 2007). Consequently the accumulation of sewage sludge from wastewater treatment is a serious environmental problem in China (Solís et al., 2002; Wang et al., 2006b).

Disposal of sludge through incineration and landfill has been performed in many countries for many years. However, incineration of sludge always needs supplementary fuel, which makes this method less economical (Dolgen et al., 2006), and landfill of sludge is an inexpensive method. In addition, landfill is a kind of waste because there are lots of organic materials and plant nutrition including N, P and other essential microelements in sewage sludge (Wei and Liu, 2005). For these reasons, recycling of sludge for agricultural purposes seems to be an appealing solution that enables valuable components to be recycled (Dolgen et al., 2006). Thus, sewage sludge has been used as an amendment to agricultural soils (Kidd et al., 2007) and the application of sludge also increase soil organic matter content that contributing to

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the structural stability of the soil and to its resistance to erosion (Hernández, et al., 1991; Ortiz and Alcañiz, 2006).

Sewage sludge potentially carries pollutants, since many wastewater treatment plants receive discharges not only from residential area but also from industry (Dai et al., 2007; Bright and Healey, 2003; Berti and Jacobs, 1998). The pollutants such as heavy metals are transferable and are not biodegradable, and at some levels, they become toxic and tend to accumulate along the food chain, where man is the last link (Dudka and Miller, 1999; Amir et al., 2005). In order to minimize the prospective health risks of sludge during land application, many studies have been performed using various methods to study the chemical fraction and remediation of heavy metal in sewage. Wang et al (2006b) have investigated the distributions of total and chemical fractions of eight heavy metal elements in sludge at different process stage. Many reports (Hamon et al., 1995; Otte et al., 1995; Su et al., 2004) have shown that solubility of heavy metals in sewage sludge amended soil is associated with the soil pH and the availability and distribution of heavy metals in the vicinity of roots in soils amended with sludge. The chemical fractionation and heavy metal accumulation were also investigated in the plant grown on soil amended with tannery sludge (Gupta and Sinha, 2006; McBride et al., 2004). However, extensive study should be performed because the species and fraction of heavy metals is different in different sewage sludge source and there is a complex mechanism among sludge, amended soil and plants.

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) belonging to Cruciferae is one of the main vegetables in China (Zhang et al., 2007). Chinese cabbage has been cultured in soil amended with sewage sludge (Wei and Liu, 2005). The aim of this study is to investigate the species and fraction of ten metals in sewage sludge and the uptake of heavy metal in Chinese cabbage grown in sewage sludge amended soil. The results showed that content of As exceed the limits for land application of sewage sludge and content of As, Cd, Cr and Zn in edible parts of Chinese cabbage was above the top limits in China.

## MATERIALS AND METHODS

### Nutrition characterization of sewage sludge and soil

Samples of the sewage sludge and surface soil (2-15 cm) were respectively collected from Loujiang wastewater treatment plant (Suzhou, China) and from garden of Hohai University (Nanjing, China). All the samples were air-dried at room temperature, finely powdered and sieved to 2 mm mesh size in sequence. Powder samples were used to make the various amendments for experimental studies or to determine the metal content.

pH of sludge and soil was measured by detecting the homogenate of samples and ddH<sub>2</sub>O at the ratio of 1:10 (w/v) using a digital pH meter. Organic matter of the sewage sludge was determined according to the procedure of loss on ignition (LOI) at 450°C for 3 h in muffle. Kjeldahl nitrogen (TN, total nitrogen) in the samples of sludge and soil was determined using the Kjeldahl method.

### Extraction of the metals in soil and sludge sample

To determine the metal contents in the sludge, 0.2 g of powdered sample was digested with 8 ml HNO<sub>3</sub> and 2 ml HClO<sub>4</sub> in a 25 ml Teflon PFA (Perfluoroalkoxy) vial for 6 h at room temperature. 1 ml of HF acid was added, mixed and heated until near dryness. The cooled residue was dissolved in 5 ml 5% HNO<sub>3</sub>, and suitable ddH<sub>2</sub>O was added up to 25 ml in total.

### Chemical fraction of metals in sludge using BCR program

The sequential extraction analysis of metals in sludge was performed according to three-step sequential extraction procedure (Ure et al., 1993; Rauret et al., 1999). The extraction procedure is as follows.

For exchangeable/acid soluble fraction (Step 1), 0.5 g of powdered sludge sample was well homogenized with 20 ml acetic acid (0.11 mol l<sup>-1</sup>) in a 50 ml polypropylene centrifuge. The tubes were shaken at 200 rpm for 16 h at room temperature. The homogenate was centrifuged at 4000 rpm for 25 min, and the extract was transferred into a polyethylene tube for keeping at -4°C. The residue was rinsed with 16 ml of ddH<sub>2</sub>O by shaking for 15 min at 200 rpm and the liquid supernatant was removed after centrifuging.

For reducible fraction (step 2), the residue from the step 1 was extracted with 20 ml 0.1 M hydroxylamine hydrochloride (pH 2) for 16 h in a centrifuge tube, which was shaken at 200 rpm for 16 h at room temperature. The extract was transferred into a polyethylene tube for keeping at -4°C after centrifuging at 4000 rpm for 25 min. The residue was rinsed with 16 ml of ddH<sub>2</sub>O by shaking for 15 min at 200 rpm and the liquid supernatant was removed after centrifuging.

For oxidizable fraction (step 3), residue from step 2 was oxidized with 8 ml hydrogen peroxide (30%) for 4 h. Another 8 ml of hydrogen peroxide was added and digestion was allowed to proceed to near dryness for at 85°C by heating the uncovered tube in a water bath. 40 ml 0.1 M ammonium acetate (adjusted to pH 2 with nitric acid) was added to the cooled residue, which was then shaken for 16 h at room temperature. The extract was transferred into a polyethylene tube for keeping at -4°C after centrifuging. The residue was rinsed with 16 ml of ddH<sub>2</sub>O by shaking for 15 min at 200 rpm and the liquid supernatant was removed after centrifuging.

For residual fraction (Step 4), the metal content in residual fraction was extracted according to the procedure described in 2.3.

### Green house pot experiment

The garden soil mixed with 0 (control), 5, 10, 15, 20 and 25% sewage sludge. The amended soils were allowed to equilibrate for one month. Seeds of Chinese cabbage were sterilized with 5% NaClO for 10 min, rinsed several times with distilled water. The soaked seeds were evenly sown in pots (12 inch diameter) that filled with different amendments of sewage sludge along with one set of control, each in three replicates. Eight germinated seeds were sown in each pot to a depth of 0.5 cm. Plants were watered as needed and plastic saucers were placed below each pot to prevent leaching from the pots. The experiments were conducted in a green house under condition of natural light. The temperature of the air ranged from 18 to 30°C. Plants were harvested on the 15th day after transplanted. Leaves and roots were separated and washed with ddH<sub>2</sub>O. Samples were dried and weighted for the determination of heavy metals.

### Extraction of heavy metals in plant

All the harvested plant materials were oven dried at 105°C and then

**Table 1.** Select physicochemical properties of garden soil and sewage sludge (TOC, total organic C).

Parameter	Sewage sludge	limits of sewage sludge usage <sup>a</sup>	Soil	SEPA limits for soils <sup>b</sup>
pH <sup>c</sup>	7.45±0.12	-	7.72±0.09	-
TOC(%)	42.55±2.16	-	2.79±0.47	-
TN (mg/kg)	6.48±0.73	-	4.29±0.34	-
TP (g/kg)	16.53±0.7875	-	7.29±1.08	-
K (g/kg)	4.9±0.94	-	19.5±1.77	-
As (mg/kg)	322.76±31.77	75	30.12±2.33	30
Cd (mg/kg)	5.06±0.65	20	0.57±0.22	0.6
Cr (mg/kg)	48.85±5.22	1200	29.07±2.23	250
Pb (mg/kg)	41.19±4.78	1000	12.85±1.11	350
Ni (mg/kg)	25.32±1.28	200	21.88±1.72	60
Cu (mg/kg)	105.08±4.57	1500	18.96±1.22	100
Zn (mg/kg)	1872.23±22.71	3000	113.44±5.43	300
Fe (g/kg)	24.99±3.05	-	34.19±2.62	-
Mg (g/kg)	2.11±0.74	-	2.24±0.79	-
Mn (mg/kg)	629.46±33.52	-	265.10±23.11	-

Data are the mean values and standard errors of triplicate experiments. <sup>a</sup>Permissible limits of sewage sludge usage in agriculture in China. <sup>b</sup>State Environmental Protection Administration (SEPA) in China. <sup>c</sup>soil:water = 1:10.

kept at 80°C for 72 h. Dried materials were weighed, and then were ground for the analysis of the heavy metals content. Briefly, 0.2 g of ground powder plant samples were digested with 10 ml HNO<sub>3</sub>-HClO<sub>4</sub> solution (8:2, by volume) and heated at 100-200°C until near dryness. The cooled residue was dissolved in 5 ml 5% HNO<sub>3</sub>, and suitable ddH<sub>2</sub>O was added up to 25 ml in total.

#### Metal determination and statistical analyses

Total phosphorus (TP) and potassium (K) were determined by the inductively-coupled plasma atomic emission spectrometry (ICP-AES) in garden soil and sewage sludge. The contents of As, Cd, Cr, Pb, Cu, Ni, Zn, Fe, Mg and Mn in extracts from sludge or plant were determined by using ICP-AES. All reagents used were of analytical grade. The transport factor (expressed as %) was calculated as the ratio of the metal content in plant leaves to roots. Data are the mean values and standard errors of triplicate experiments -

## RESULTS

### Characteristics of soil and sewage sludge

Select physicochemical properties of heavy metal concentrations within the sampled sewage sludge and garden soil are presented in Table 1. The pH of the sewage sludge and garden soil were both over 7.4. The content of TOC, TN and TP was higher in sewage sludge than that in garden soil significantly. The contents of heavy metal elements in sewage sludge such as Cd, Cr, Pb, Ni, Cu and Zn were lower than the permissible limits of sewage sludge usage in agriculture in China except for As, which was three times higher than the limits content. Like in sewage sludge, only content of As was higher

slightly than the limits regulations of State Environmental Protection Administration (SEPA) for soil in China. Among the metal elements, only K, Fe and Mg concentrations were higher in garden soil than those in sewage sludge.

### Chemical fraction of metal elements in sewage sludge

The fraction of heavy metals in sewage sludge should be further analyzed, since total content of most heavy metal (As, Cd, Cr, Pb, Ni, Cu and Zn) was higher significantly than that in garden soil. Figure 1 shows the distribution percentages of various fractions of heavy metals or plant necessary nutrition in sewage sludge samples. Exchangeable fraction (water and acid-soluble) of Cd, Cr, Pb, Cu and Fe was lower than 5% and that of As, Ni and Zn ranged from 11% to 17%. Reducible fraction of metals ranged 0.1 to 10% except for Mg and Mn. 21% of Cd, 59% of Cr and 56% of Cu were present in oxidizable fraction while the majority of As, Cd, Pb, Zn and Fe was present in the residual fraction (70 – 95%) of the total concentration.

### Contents of metal elements of Chinese cabbage grown in soil amended with sewage sludge

In order to investigate the effects of sewage sludge application, Chinese cabbage was planted in soil amended with different content of sewage sludge. The plant was washed with ddH<sub>2</sub>O post harvest, and the weight of

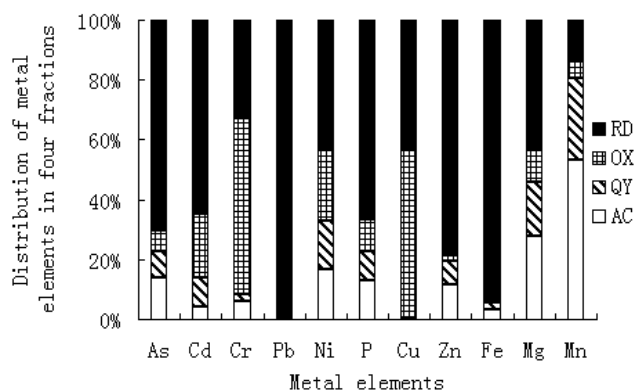
**Table 2.** Concentration of partial metal elements (mg/Kg) in leaves of Chinese cabbage grown in soil amended with various content of sewage sludge.

Metal	Ck	5% <sup>a</sup>	10% <sup>a</sup>	15% <sup>a</sup>	20% <sup>a</sup>	25% <sup>a</sup>	Lims <sup>b</sup>
As	2.1±0.21	5.8±0.88	5.9±0.97	7.4±1.08	10±0.59	7.9±0.97	0.05
Cd	0.14±0.07	0.15±0.07	0.25±0.02	0.25±0.07	0.41±0.01	0.24±0.09	0.2
Cr	0.7±0.15	2.4±0.47	3.1±0.25	3.2±0.34	5.5±0.53	5.8±0.79	0.5
Pb	0.08±0.01	0.17±0.4	0.24±0.6	0.27±0.2	0.19±0.02	0.22±0.5	0.3
Ni	1.2±0.2	0.6±0.2	1.6±0.4	1.6±0.6	2.1±0.3	3.1±0.1	-
Cu	2.6±0.5	4.7±0.7	5.6±1.1	4.2±0.8	3.6±0.9	4.2±1.1	10
Zn	43.4±5.8	63.3±9.3	65.9±6.6	78.9±11.6	72.5±11.1	69.5±10.7	20
Fe	185.2±29.7	551.9±84.5	781.7±90.1	991.8±119.3	510.1±121.7	673.1±147.4	-
Mg	1450.1±141	1550.4±152	1460.5±111.8	1477.8±192.6	1650.3±117	1664.1±141	-
Mn	17.2±4.2	26.7±4.9	34.1±3.1	28.7±1.2	28.1±2.8	24.1±3.5	-

Data are the mean values and standard errors of triplicate experiments.

<sup>a</sup>Percentages of sewage sludge in soil.

<sup>b</sup>Tolerance limit levels of metal contaminants in foods (Pb, Cd, As, Cr-GB 2762-2005; Cu-GB 15199-94; Zn-GB13106-91) as stipulated in the regulations.

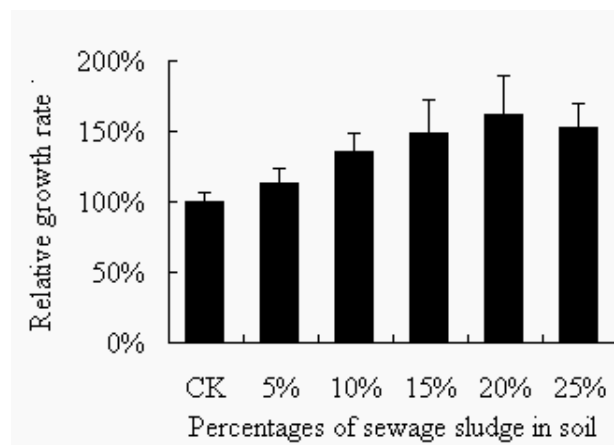


**Figure 1.** The distribution percentages of various fractions of heavy metals or plant necessary nutrition in sludge samples. AC = exchangeable fraction; QY= reducible fraction; OX = oxidizable fraction; RD = residual fraction

plants in every pot was weighed after dried in oven at 80°C. As showed in Figure 2 application of sewage sludge to garden soil enhances the relative growth rate of Chinese cabbage significantly compared with CK.

### Heavy metal content in leaves of Chinese cabbage and transport factors

Table 2 shows the accumulation of metals in leaves of Chinese cabbage. Most of the content of metals increased in Chinese cabbage with the increase in sludge amendments ratio, and the content of heavy metal As, Cd, Cr and Zn exceeded the limits of metals in the regulation. Leaf:root metal concentration ratio was calculated according to the metal concentrations in leaves and roots



**Figure 2.** Relative growth rate of Chinese cabbage in soil amended with various content of sewage sludge. Data are the mean values and standard errors of triplicate experiments.

of Chinese cabbage, and the range and mean of transport factor in Chinese cabbage was shown in Table 3. Among the metal elements, the transport factor of Mg was 97.64% in Chinese cabbage, while those of other metals detected were below 35% (Table 3).

### DISCUSSION

The usage of sewage sludge has been encouraged in amendment of agricultural soils for many years, and this is a more economically attractive way than landfill and incineration because that these practices enable the waste materials to be recycled and at the same time, the natural resources and energy are saved effectively. In

this study, high level of TOC, TN and TP (Table 1) was detected in sewage sludge. These support the viewpoint that sewage sludge has the high potential agricultural benefits for land application (Fuentes et al., 2004).

Total concentration of heavy metal is one of the important factors on the risk assessments for land application of sewage sludge in many countries (McBride, 1995). Whereas bioavailability of heavy metals in sewage sludge should be also an important factor to assess the risk of heavy metals and determines the ability for plant uptake and potential for contamination of groundwater (Iwegbue et al., 2007). Sequential extraction to fractionate metals in solid and sludge into several groups of different leach ability is widely used to determine the distribution of metals in different phases (Shiowatana et al., 2001; Zhang et al., 2007). In this study, most of metals were not present in the exchangeable and reducible fraction but in oxidizable and residual fraction (Figure 1), suggesting that the majority of metals (Cd, Cr, Pb and Cu) might be linked to organic substances or be co-precipitated with oxides, carbonates and phosphates in sewage sludge (Zhang et al., 2007). Wang et al. (2006) reported that the residual fraction was the predominant fraction for Ni and Cr while the oxidizable fraction was the primary fraction for Cu and Pb in sewage sludge. These differences may be caused by the different physico-chemical properties of sewage sludge (Wang et al., 2006). However, the high content and the high potential mobility and bioavailability of As, Ni and Zn in Table 1 and Figure 1 may be a limitation in agricultural application.

Growth rate of Chinese cabbage was enhanced by application of sewage sludge (Figure 2). Chinese cabbage takes up metals (As, Cd, Cr, Pb, Ni, Cu, and Zn et al.) and accumulates them in their edible and inedible parts with various concentrations (Tables 2 and 3) and most of the metal concentrations increased in plants grown in soils amended with sewage sludge compared with those in control plants. These accorded with the earlier research results from other researcher (Wei and Liu, 2005) and suggested that abundant nutrition in sewage sludge can increase the output of vegetables, but the bio-available concentration of metals was also increased in soils amended with sewage sludge. Kazi et al. (2005) reported that metal mobility and bioavailability increase with decreasing pH below pH 6.5 while decrease with increasing pH above pH 6.5. The pH was not affected significant (pH7.61- pH7.72) in soil amended with sewage sludge (data not shown). Wei and liu (2005) found that land application of sewage sludge main increased the concentrations of bio-available metals (Cu and Zn) in soil. Similar results also found in this study.

For example, concentrations of Pb increased significantly in Chinese cabbage when amended with sewage sludge, while the total content of Pb was lower in soil and the majority of Pb was present in residual status in sewage sludge. Recent report (Lin et al., 2004) showed that bio-available concentration of metals might be asso-

**Table 3.** Leaf:root metal concentration ratio in Chinese cabbage grown in soil amended sewage sludge (%).

Metal	Range of shoot/ root	Mean (n=6)
As	11.67%-33.05%	21.77±3.69%
Cd	18.75%-48.00%	34.85±5.72%
Cr	14.63%-36.36%	27.94±3.48%
Pb	8.22%-53.33%	29.82±8.39%
Ni	6.52%-33.87%	26.64±5.24%
Cu	9.44%-20.90%	15.86±3.39%
Zn	20.81%-34.26%	29.00±2.55%
Fe	4.11%-20.71%	16.49±3.09%
Mg	88.52%-105.03%	97.64±7.21%
Mn	12.14%-28.93%	25.59±3.17%

ciated with the physical and chemical characteristics of the rhizosphere, which may in turn change the characteristics of trace metals present. However, the interaction mechanism related to the release of bio-available metals should be further studied in plant grown in soil amended with sewage sludge. Cu and Zn are essential nutrients for plants. Like heavy metal Cd, Cr, Pb et al., however, high level of Cu and Zn is toxic to plants under certain conditions (Jefferies and Freestone, 1984). The concentration of these metals normally increased in plants grown in soil amended with sewage sludge and content of As, Cd, Cr and Zn was higher in leaves (Table 2) than the limits of metals in the regulations. Thus, their potential accumulation in human tissues and bio-magnification through the food-chain cause both human health and environmental concerns (Zheng et al., 2007). On the other hand, however, the potential ability of the crucifer plants Chinese cabbage in taking up heavy metals should be of concern although the ability of transport metal elements from roots to shoots is relative lower (the average shoot: root ratio of most metals ranged from ~15 to ~35%) in this study (Table 3). Several reports (Wei and Liu, 2005; Zhao et al., 2006; Huang et al., 1998; Zheng et al., 2007; Grëman et al., 2003; Diego et al., 2005) had shown that Chinese cabbage can uptake the As, Zn, U, Hg, Pb, Cd Cr and Cu and accumulate them in a higher level. Thus, further study should be performed to investigate the potential ability of Chinese cabbage in the remediation of heavy metals contaminated soils.

In conclusion, the heavy metal is one of the limits for land application of sewage sludge, although there is abundant nutrition for plants. High level of heavy metals was detected in Chinese cabbage grown in soil amended with sewage sludge. Therefore, stringent guidelines should be set for the sewage sludge application for edible crop production. One the other hand, Chinese cabbage may be a candidate plants to remediation the soil contaminated with heavy metal.

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## REFERENCES

- Amir S, Hafidi M, Merlina G, Revel JC (2005). Sequential extraction of heavy metals during composting of sewage sludge. *Chemosphere*. 59: 801-810.
- Berti WR, Jacobs LW (1998). Distribution of trace elements in soil from repeated sewage sludge application. *J. Environ. Qual.* 2: 1280-1286.
- Bright DA, Healey N (2003). Contaminant risk from biosolids land application: contemporary organic contaminant levels in digested sewage sludge from five treatment plants in greater Vancouver. British Columbia. *Environ. Pollut.* 126: 39-49.
- Dai JY, Xu MQ, Chen JP, Yang XP, Ke ZS (2007). PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China. *Chemosphere*. 66: 353-361.
- Dolgen D, Alpaslana MN, Delen N (2006). Agricultural recycling of treatment-plant sludge: A case study for a vegetable-processing factory. *J. Environ Manage.* doi:10.1016/j.jenvman.2006.06.013.
- Dudka S, Miller WP (1999). Accumulation of potentially toxic elements in plants and their transfer to human food chain. *J. Environ. Sci. Health B*. 34: 681-708.
- Fuentes A, Llorens M, Sáez J, Soler A, Aguilar MI, Ortuño JF, Meseguer VF (2004). Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere*. 54: 1039-1047.
- Gréman H, Vodnik D, Velikonja-Bolta S, Leštan D (2003). Ethylenediaminedisuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. *J. Environ. Qual.* 32: 500-506.
- Gupta AK, Sinha S (2006). Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: Selection of single extractants. *Chemosphere*. 64: 161-173.
- Hamon RE, Lorenz SE, Holm PE, Christensen TH, McGrath SP (1995). Changes in trace metal species and other components of the rhizosphere during growth of radish. *Plant Cell Environ.* 18: 749-756.
- Hernández T, Moreno JL, Costa F (1991). Influence of sewage sludge application on crop yields and heavy metal availability. *Soil Sci. Nutr.* 37: 201-210.
- Huang JW, Blaylock MJ, Kapulnik Y, Ensley BD (1998). Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environ. Sci. Technol.* 32: 2004-2008.
- Iwegbue CMA, Emuh FN, Isirimah NO, Egun AC (2007). Fractionation, characterization and speciation of heavy metals in composts and compost-amended soils. *Afr. J. Biotechnol.* 6(2): 067-078.
- Jefferies DJ, Freestone P (1984). Chemical analysis of some coarse fish from a Suffolk River carried out as part of the preparation for the first release of captive-bred otters. *J. Otter Trust.* 1: 17-22.
- Kazi TG, Jamali MK, Kazi GH, Arain MB, Afridi HI, Siddiqui A (2005). Evaluating the mobility of toxic metals in untreated industrial wastewater sludge using a BCR sequential extraction procedure and a leaching test. *Anal. Bioanal. Chem.* 383: 297-304.
- Kidd PS, Domínguez-Rodríguez MJ, Díez J, Monterroso C (2007). Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere*. 66: 1458-1467.
- Lee DJ, Spinosa L, Liu JC (2002). Towards sustainable sludge management. *Water*. 21: 22-23.
- Lin Q, Chen YX, He YF, Tian GM (2004). Root-induced changes of lead availability in the rhizosphere of *Oryza sativa* L. *Agric. Ecosys. Environ.* 104: 605-613.
- McBride MB (1995). Toxic metal accumulation from agricultural use of sludge: are USEPA Regulations protective? *J. Environ. Qual.* 24: 5-18.
- McBride MB, Richards BK, Steenhuis T (2004). Bioavailability and crop uptake of trace elements in soil columns amended with sewage sludge products. *Plant Soil*, 262: 71-84.
- Ortiz O, Alcañiz JM (2006). Bioaccumulation of heavy metals in *Dactylis glomerata* L. growing in a calcareous soil amended with sewage sludge. *Bioresour. Technol.* 97: 545-552.
- Otte ML, Kearns CC, Doyle OM (1995). Accumulation of arsenic and zinc in the rhizosphere of wetland plants. *Bull. Environ. Contam. Toxicol.* 55: 154-161.
- Rauret G, López-Sánchez JF, Sahuquillo A, Rubio R, Davidson C, Ure A, Quevauviller PH (1999). Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* 1:57-61.
- Shiowatana J, Tantidanai N, Nookabkaew S, Nacapricha D (2001). A Novel Continuous-Flow Sequential Extraction Procedure for Metal Speciation in Solids. *J. Environ. Qual.* 30: 1195-1205.
- Solís GJ, Alosa E, Riesco P (2002). Distribution of metal extractable fractions during anaerobic sludge treatment in southern Spain WWTPs. *Water, Air, Soil Pollut.* 140: 139-156.
- Su DC, Wong JWC, Jagadeesan H (2004). Implications of rhizospheric heavy metals and nutrients for the growth of alfalfa in sludge amended soil. *Chemosphere*. 56: 957-965.
- Ure AM, Quevauviller PH, Muntau H, Griepink B (1993). 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 European Communities. *Int. J. Environ. Anal. Chem.* 5: 135-151.
- Wang C, Li XC, Ma HT, Qian J, Zhai JB (2006a). Distribution of extractable fractions of heavy metals in sludge during the wastewater treatment process. *J. Hazard Mater.* A137: 1277-1283.
- Wang C, Li XC, Wang PF, Zou LM, Ma HT (2006b). Total contents and extractable fraction of metals in sludges from urban wastewater treatment plants of China. *Pedosphere*. 16(6): 756-761.
- Wang C, Zhang SH, Wang PF, Hou J, Li W, Zhang WJ (2008). Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte *Vallisneria spiralis* (Lour.) Hara. *Aquat. Toxicol.* doi:10.1016/j.aquatox.2008.01.009.
- Wei Y, Liu Y (2005). Effects of sewage sludge compost application on crops and cropland in a 3-year field study. *Chemosphere*. 59: 1257-1265.
- Zhang JL, Liu JG, Li C, Nie YF, Jin YY (2007). Comparison of the fixation of heavy metals in raw material, clinker and mortar using a BCR sequential extraction procedure and NEN7341 test. *Cement and Concrete Research.* doi:10.1016/j.cemconres.2007.09.004.
- Zhao R, Zhao M, Wang H, Taneike Y, Zhang X (2006). Arsenic speciation in moso bamboo shoot – a terrestrial plant that contains organoarsenic species. *Sci. Total Environ.* 371: 293-303.
- Zheng N, Wang QC, Zheng DM (2007). Health risk of Hg, Pb, Cd, Zn, and Cu to the inhabitants around Huludao Zinc Plant in China via consumption of vegetables. *Sci. Total Environ.* 383: 81-89.