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Chemical Speciation and Phytotoxicity of Heavy Metals in Sewage Sludge for the Germination of Chinese Cabbage Seeds

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ABSTRACT

Single extractions with distilled water and diethylene-triamine-pentaacetic acid (DTPA), and a sequential extraction procedure were conducted in this study to determine the chemical speciation of Cd, Cr, Cu, Ni, Pb and Zn in four municipal sewage sludges and subsequently evaluate the phytotoxicity of the metals by a seed germination test of Chinese cabbage (*Brassica chinensis* L.). Analytical results indicated that a high percentage of organic carbon was found in all the sludges, and thus beneficial to application on agricultural land. Total concentrations of the heavy metals were much lower than the pollutant concentration limits for land application of sewage sludge in the USA and Europe. However, Cu and Zn were the most abundant elements in the sewage sludges in this study. In most cases, the water extractable contents of the heavy metals in the sludges were undetectable. However, the heavy metal contents with DTPA extraction were much higher than those with water extraction, respectively. Additionally, the Fe-Mn oxide fraction of Zn was the dominant solid phase, but Zn was the most mobile metal in all the sludges. Lead and Cr were concentrated in residual fractions, while Cu showed the organic fraction as being dominant. The seed germination test of Chinese cabbage showed no significant ($p<0.05$) inhibition by the sludge extracts with different dilution ratios, but the original sludge extracts inhibited the root growth of Chinese cabbage. Copper, Ni and Zn might cause toxicity to plant growth, as demonstrated in this study.

Keywords: bioavailability; inorganic pollutant; land application; sequential extraction; waste disposal

Abbreviations: Carb, Carbonate fraction; **DTPA**, diethylene-triamine-pentaacetic acid; **Exch**, Exchangeable fraction; **FAAS**, flame atomic absorption spectrometer; **Fe-MnOX**, Fe-Mn oxide fraction; **OM**, Organic fraction; **Resi**, Residual fraction; **TEA**, triethanolamine; **WTP**, wastewater treatment processes

INTRODUCTION

The accumulation of sewage sludges from WTP which incorporate a secondary treatment step is a growing environmental problem. The production of sewage sludge in the EU reached around 8 million tons of dry waste matter between 1992 and 2000, while a similar amount was produced in the USA in 2000 (Fuentes *et al*. 2004). In Taiwan, the annual production of sewage sludge from municipal wastewater plants is estimated to be 6.6×10^7 m^3 (Chiou *et al*. 2006). The treatment methods of sewage sludge such as incineration, landfilling or ocean and river disposal are common worldwide.

Sewage sludge can be beneficially recycled as a source of nutrients (particularly N and P) for plant growth and as a soil conditioner to improve physical and microbiological properties. However, land application of sewage sludge has been limited by its enriched pathogens and heavy metal content. Heavy metals are the primary concern in sewage sludge use to land and may be toxic to humans or animals that through soil transfer to plants by different pathways (McBride 1995; Karvelas *et al*. 2003). In sewage sludge, the potentially available and reactive phases containing heavy metals constitute only a few percent of the total content of heavy metals such as Cd, Cr, Ni and Pb, while the percentage for Cu and Zn are expected to be higher (Flyhammar 1997; Álvarez *et al*. 2002). Excessive application of sewage sludge has led to the accumulation of heavy metals in the soil surface and can result in phytotoxicity increased through the food chain to harm human beings (McBride 1995; Karvelas *et al*. 2003).

Risk assessments and regulations for the application of sewage sludge in many countries are based on the total concentrations of heavy metal in the soils. However, measurements of total concentrations of heavy metal can not provide a precise prediction of the bioavailability and/or toxicity of a heavy metal. Both bioavailability and toxicity of the soil or sewage sludge are critically dependent on the chemical speciation of the heavy metal (Fernández *et al*. 2000; McBride 2003). Generally, plant uptake of heavy metals is correlated to extractable forms of the metals rather than to the total metal contents in the soils. However, it is necessary to know the heavy metal distribution among the different solid phases of sewage sludge in which the heavy metal is bound that determines its behavior and its mobilization capacity (López-Sánchez *et al*. 1996).

During recent decades various extraction schemes have been developed involving both single and sequential extraction, although some methods have been widely used (Tessier *et al*. 1979; Förstner and Salomons 1980). The application of sequential extraction proposed by Tessier *et al*. (1979) provides the relevant information about the possible mobility and bioavailability of heavy metals in the environment. Such procedures provide information about the speciation of heavy metals and can yield detailed information about the origin, mode of occurrence, bioavailability, mobilization and transport of heavy metals. Several sequential extraction methods have been used to partition metals into fractions defined as soluble, exchangeable, organically bound, precipitated, oxide bound and residual and to correlate metals in these fractions with plant concentrations or uptake (Shuman 1985; Sims 1986). A positive significant correlation between the metal amounts extracted with DTPA and the sum of exchangeable, carbonate and organic matter fractions in the sequential extraction have been found in sewage sludges (Obrador *et al*. 1997; Fuentes *et al*. 2006).

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Such extraction provides more information about metal availability and tends to be correlated with metal uptake by plants. However, it has been observed that DTPA-extractable metals from soil decrease with time due to the decrease in soil pH induced by mineralization of substrates in sewage sludge, especially after sewage sludge applications have ceased (Su *et al*. 2004). Toxicity evaluation of heavy metals in sewage sludge by chemical characterization and biological tests is very important in screening the suitability of sewage sludge for land application (Wong *et al*. 2001). Therefore, the objectives of this study were to (1) determine the chemical speciation of Cd, Cr, Cu, Ni, Pb and Zn in four different sewage sludges, and (2) evaluate the mobility and bioavailability of the heavy metals in the sewage sludge for plant growth by crop seed germination test.

MATERIALS AND METHODS

Collection of sewage sludge

Four common sewage sludges were sampled from the WTP through Taiwan in this study. They include two aerobic sewage sludges both from the Ba-Li (BL) WTP of Taipei County and the Ming-Sen (MS) WTP of Taipei City, anaerobic nightsoil sludge (DS) from the slop WTP of Kaohsiung City and anaerobic sludge (NPUST) from the WTP of National Pingtung University of Science and Technology. Activated sludge process is currently used to treat sewage for all these WTP where the sewage was produced by the municipal population of approximate 5 million in Taiwan. Dewatered digested sludges were air-dried, then ground to pass through a 2-mm sieve for chemical analysis. The pH was measured using a mixture of sludge and deionized water (1:1, w/v) with a glass electrode (McLean 1982). Electrical conductivity (EC) was measured on the extract at a sludge/distilled water ratio of 1:5 (w/v) using a conductivity meter, respectively. Total organic carbon content was determined via the Walkley-Black wet oxidation method (Nelson and Sommers 1982). Moreover, total N was measured using the Kjeldhal method (Bremner and Keeney 1966). Total P was determined by the sodium carbonate fusion method (Kuo 1996).

Aqua regia, water and DTPA extractions

Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the sewage sludges were determined as total content and single extractions (water and DTPA). Ščančar *et al*. (2000) indicated that *aqua regia* could be efficiently used for the determination of total metal concentrations in sewage sludge. Therefore, *aqua regia* was used in the present study for the pseudo-total concentrations of the heavy metals. The *aqua regia* procedure was conducted by weighing 3 g (dry wt.) sample into a 500 ml round-bottomed flask and 22.5 ml conc. HCl and 7.5 ml conc. HNO_3 were added. The attack was allowed to proceed for 16 h at room temperature and then for 2 h in an open reflux condition at 180°C. After digestion, the solution was filtered with a Whatman No. 42 filter paper and <0.45 μm Millipore filter paper and further transferred quantitatively to a 50-ml volumetric flask by adding distilled water (ISO 1995). Soluble heavy metals in the sewage sludges were extracted by shaking 5 g (dry wt.) sample with 50 ml distilled water for 4 h, and then the extracts were filtered with a Whatman No. 42 filter paper and <0.45 μm Millipore filter paper. DTPA-extractable heavy metals were obtained by shaking of 5 g (dry wt.) sample with 50 ml of 0.05 M DTPA, 0.01 M $CaCl_2$ and 0.1 M TEA buffered at pH 5.3 (Lindsay and Norvell 1978). The extracts were filtered with a Whatman No. 42 filter paper and <0.45 μm Millipore filter paper. Three replicated samples were measured in all cases. Metal concentrations in all the solutions were determined by FAAS (Hitachi Z-8100, Japan).

Sequential extraction

The method of sequential extraction used in this study was developed by Tessier *et al*. (1979), but the extractant for residual fraction was replaced by *aqua regia*. Each of the chemical fractions of Cd, Cr, Cu, Ni, Pb and Zn in the sewage sludges is operationally defined as follows:

(1) Exch: 1 g soil (dry wt.) extracted with 8 ml of pH 7, unbuffered, 1.0 M $MgCl_2$ in Teflon centrifuge tubes for 1 h at 25°C with continuous agitation.

(2) Carb: Residue from exchangeable fraction extracted with 8 ml of pH 5, 1.0 M NaOAc for 6 h at 25°C with continuous agitation. The carbonate fraction indicates the acid soluble forms.

(3) Fe-MnOX: Residue from carbonate fraction extracted with 8 ml of 0.04 M $NH_2OH{\cdot}HCl$ in 25% acetic acid (v/v) for 6 h at 96°C with occasional agitation.

(4) OM: Residue from Fe-Mn oxide fraction extracted with 2 ml of 0.02 M HNO_3 and 3 ml of pH 2, 30% H_2O_2 for 2 h at 85°C with occasional agitation, an additional 3 ml of pH 2, 30% H_2O_2 for 3 h at 85°C with occasional agitation, and then 3 ml of 3.2 M NH_4OAC in 20% HNO_3 (v/v) was added and agitated continuously for 0.5 h at 25°C. The organic fraction indicates the oxidized forms.

(5) Resi: residue from organic fraction was removed into beakers, digested with *aqua regia*. Three replicated samples were measured in all cases.

Metal concentrations in all the solutions were determined by FAAS.

Seedling test of Chinese cabbage

Germination is a complicated physiological process of plant growth. The main environmental conditions that affect it are: water, temperature, oxygen, radiation, carbon dioxide and nutrient status. The seedling test is generally used for evaluating the phytotoxicity of soil or solid waste by extracted solution, and its result mainly reflects the chemical characteristics of the tested materials (Zucconi *et al*. 1981). Therefore, sludge extracts of this study were prepared by shaking 2 g (dry wt.) sewage sludge with 20 ml of distilled water, and the suspensions were filtered with a Whatman No. 42 filter paper and <0.45 μm Millipore filter paper. The extract was diluted to 50% (× 2), 20% (× 5), 10% (× 10) and 5% (× 20) of the original extract concentration. Twenty-five seeds of Chinese cabbage (*Brassica chinensis* L.) were placed in each Petri dish containing 10 ml of the extract, which were then placed in a dark incubator at 28°C for 5 days. Then, seed germination and root length of seeds in each plate were measured (Zucconi *et al*. 1981). The variance and significant differences of seed germination test in different treatments were analyzed by ANOVA (SAS Institute 1982). The statistical significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

The traditional treatment of sewage sludge such as incineration and landfill is the mainstream measure of wastes disposal in Taiwan. The risks of air and groundwater pollution, however, are the major side effects following these methods applied. Sewage sludges enriched abundant nutrients are beneficial to agricultural soils. Even though, land application of sewage sludge to cultivated soils has stay in research stage and not approved legislatively in Taiwan because of uncertainly risks derived from persistent pollutants in sewage sludges to the soil ecosystem.

Selected properties of sewage sludge

Some important agronomic characteristics of the sewage sludges are shown in **Table 1**. The sludge pH values varied from 5.22 to 6.36. A high percentage of organic carbon was found in the sludges, especially in the NPUST sludge in which the level was high, i.e. 496 g kg^{-1}. Generally, estimates of organic carbon (OC) pools in Taiwan's culti-

Table 1 Characteristics of the four sewage sludges.

Parameters	BL	MS	DS	NPUST
pH (1:1)	5.22	5.28	6.36	5.80
Organic C (g kg^{-1})	202	254	262	496
EC (dS m^{-1})	0.51	0.22	0.24	0.34
Total N (g kg^{-1})	16.0	54.0	39.2	68.0
C/N	5.68	4.70	6.68	7.29
Total P (mg kg^{-1})	38.4	58.5	64.1	70.8

Table 2 Heavy metal contents (mg kg^{-1}) of the four sewage sludges.

Element	BL	MS	DS	NPUST	Ceiling limits in US[a]	Threshold values[b] pH < 7	Threshold values[b] pH > 7
Cd	2.96	3.16	5.56	0.67	85	20	40
Cr	393	47.4	40.8	102	3000	1000	1500
Cu	909	235	547	551	4300	1000	1750
Ni	407	53.3	45.7	47.7	420	300	400
Pb	103	81.5	59.2	63.5	840	750	1200
Zn	941	1424	2030	620	7500	2500	4000

[a] USEPA (2000)
[b] Council of the European Communities (1986)

Table 3 Water and DTPA extractable heavy metal contents (mg kg^{-1}) in the four sewage sludges.

Heavy metal	BL	MS	DS	NPUST
Water extraction				
Cd	0.27 ± 0.02 (9.12 %)[a]	< 0.03	< 0.03	< 0.03
Cu	5.06 ± 0.13 (0.56%)	1.42 ± 0.30 (0.60%)	1.07 ± 0.09 (0.20%)	0.85 ± 0.01 (0.15%)
Pb	< 0.19	0.81 ± 0.13 (0.99%)	0.45 ± 0.01 (0.76%)	< 0.19
Zn	129 ± 1.10 (13.7%)	7.06 ± 0.75 (0.50%)	3.06 ± 0.58 (0.15%)	3.13 ± 0.59 (0.50%)
Ni	97.3 ± 0.81 (23.9%)	1.08 ± 0.23 (2.02%)	1.16 ± 0.04 (2.54%)	3.19 ± 0.16 (6.68%)
Cr	< 0.19	< 0.19	< 0.19	< 0.19
DTPA extraction				
Cd	1.45 ± 0.04 (49.0%)	1.12 ± 0.01 (35.4%)	3.11 ± 0.12 (55.9%)	0.23 ± 0.01 (34.2%)
Cu	186 ± 0.48 (20.5%)	88.8 ± 0.55 (37.8%)	173 ± 3.85 (31.6%)	58.3 ± 1.05 (10.6%)
Pb	15.4 ± 0.11 (15.0%)	20.8 ± 0.45 (25.5%)	20.0 ± 0.29 (33.8%)	10.8 ± 0.00 (17.0%)
Zn	454 ± 2.80 (48.2%)	578 ± 7.36 (40.6%)	1081 ± 13.8 (53.3%)	199 ± 6.82 (32.1%)
Ni	201 ± 1.62 (49.4%)	10.9 ± 0.32 (20.5%)	13.2 ± 0.27 (28.9%)	8.76 ± 0.19 (18.2%)
Cr	< 0.41	< 0.41	< 0.41	< 0.41

[a] values in parentheses are proportions of extractable to total content of heavy metal.

Table 4 Chemical fractions of heavy metal contents (mg kg^{-1}) of the four sewage sludges.

Element	Fractions Exch	Carb	Fe-MnOX	OM	Resi	∑[a]	Total[b]	Recovery
BL								
Cd	1.65	0.35	1.30	0.24	< 0.35	3.54	2.96	120
Cu	40.8	55.2	15.2	750	52.9	914	909	101
Pb	1.51	3.18	40.0	12.1	35.9	92.7	103	90.0
Zn	413	139	501	47.9	41.1	1143	941	121
Ni	176	46.9	153	55.7	26.6	459	407	113
Cr	< 0.59	1.37	272	75.1	44.7	393	393	100
MS								
Cd	1.18	0.23	1.19	0.31	< 0.35	2.91	3.16	92.1
Cu	3.27	4.95	4.97	199	13.7	226	235	96.1
Pb	< 0.58	< 0.58	6.36	6.39	58.9	71.7	81.5	88.0
Zn	189	116	878	190	64.1	1437	1424	101
Ni	1.49	1.78	14.6	6.45	14.1	38.4	53.3	72.0
Cr	< 0.59	< 0.59	1.66	13.3	21.7	36.6	47.4	77.2
DS								
Cd	1.66	0.32	1.79	0.61	0.56	4.94	5.56	88.9
Cu	4.86	3.71	1.37	435	45.3	490	547	89.5
Pb	< 0.58	< 0.58	2.82	3.72	45.8	52.4	59.2	88.4
Zn	81.0	115	1105	516	203	2020	2030	99.5
Ni	< 0.83	2.03	13.6	16.1	11.6	43.4	45.7	94.9
Cr	< 0.59	< 0.59	1.29	13.0	20.5	34.7	40.8	85.1
NPUST								
Cd	0.20	< 0.11	0.28	0.22	< 0.35	0.70	0.67	104
Cu	2.93	2.66	4.52	495	11.5	517	551	93.8
Pb	< 0.58	< 0.58	2.90	42.3	17.1	62.3	63.5	98.1
Zn	25.4	41.8	419	118	8.51	612	620	98.8
Ni	4.44	2.49	13.0	11.2	25.6	56.7	47.7	119
Cr	< 0.59	< 0.59	5.22	30.8	42.4	78.4	102	76.8

[a] Sum of heavy metals content in each fraction.
[b] Pseudo-total content of heavy metals.

vated soils are low, ranging from 3.50 to 3.68 kg m^{-2} (Chen and Hseu 1997). The sewage sludges were enriched in organic carbon (> 200 g kg^{-1}); therefore, the land application of sewage sludges should increase the OC content of Taiwan's soils. The C/N ratios ranged from 4.70 to 7.29, suitable in compost maturity (Zucconi *et al*. 1981).

Laws in various industrialized countries include a limit on heavy metal pollution of soils through sewage sludge application (Düring and Gäth 2002). However, no regulations exist in Taiwan that address the application of sewage sludge on land. Copper and Zn were the predominant elements in the sludges in this study, however, the total concentrations of heavy metal in all the sludges were much lower than the pollutant concentration limits for land application of sewage sludge in the USA (USEPA 2000) and Europe (Council of the European communities 1986) (**Table 2**). Therefore, the application of sewage sludge with relatively low heavy metal content and abundant organic carbon is

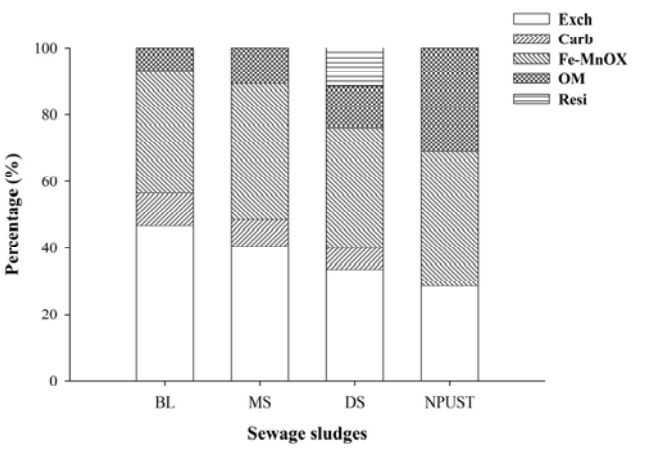

Fig. 1 Percentages of chemical fractions of cadmium in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

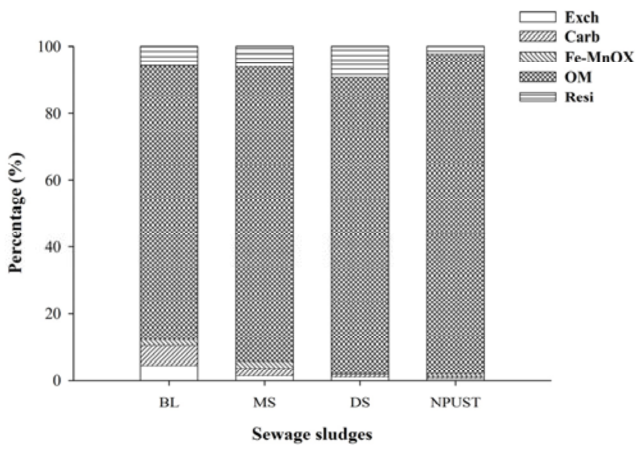

Fig. 2 Percentages of chemical fractions of copper in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

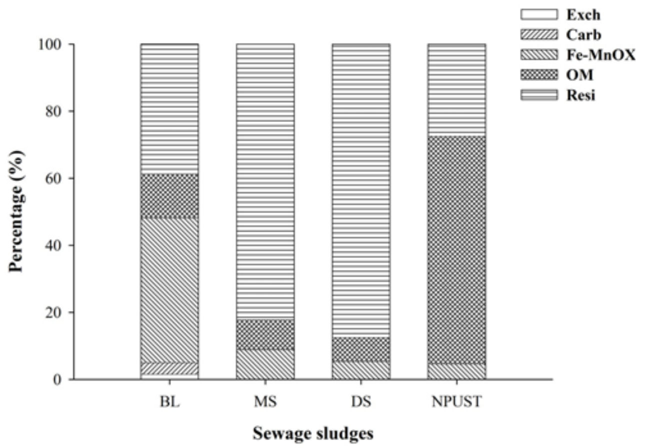

Fig. 3 Percentages of chemical fractions of lead in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

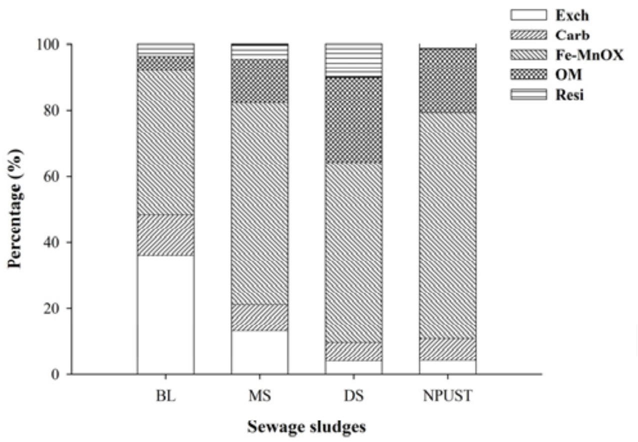

Fig. 4 Percentages of chemical fractions of zinc in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

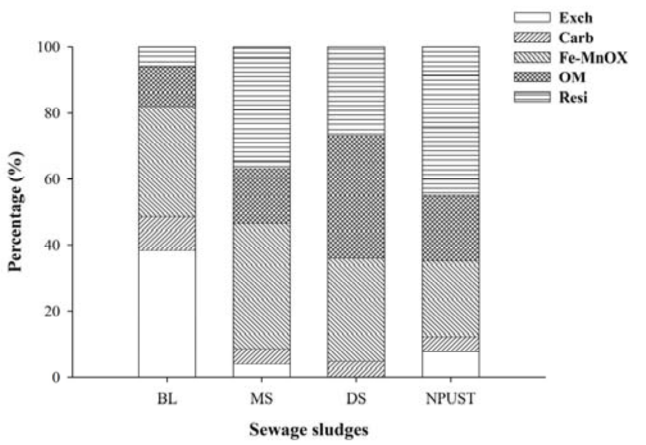

Fig. 5 Percentages of chemical fractions of nickel in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

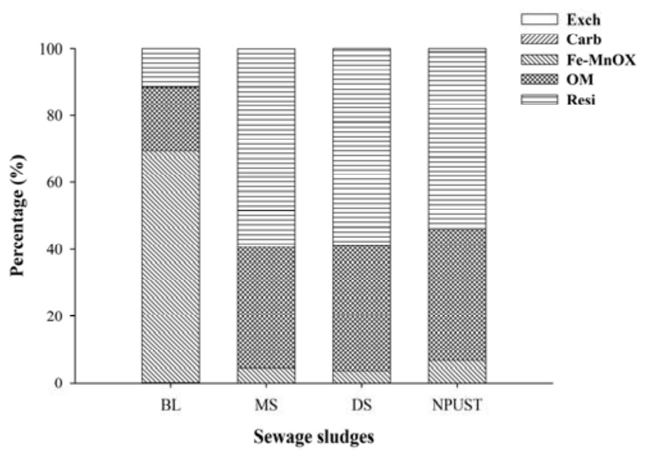

Fig. 6 Percentages of chemical fractions of chromium in the four sewage sludges. Exch: exchangeable; Carb: carbonate; Fe-MnOX: Fe-Mn oxide; OM: organic matter; Resi: residual.

potentially valuable to agricultural soils in Taiwan. The BL sludge always contained the highest heavy metal contents except for Cd and Zn.

Water and DTPA extractable heavy metals

The water extractable contents of Cd, Cr and Pb in the sludges were less than the detection limit, excluding Cd of the BL sludge and Pb of the MS and DS sludges (**Table 3**). Only <1% of total Cu was extracted from the sludges by distilled water. Despite the abundance of Cu in this study (**Table 2**), all sludges are sufficiently rich in organic matter and heterogeneous chelating agents to serve as the metal traps (McBride 1995), thus limiting water extractability. DTPA extraction provides a chemical evaluation of the amounts of metals that are available for plant uptake (Tandy *et al*. 2004; Meers *et al*. 2005). Organic acids in sludge can attract heavy metals and bind them in stable organo-metallic complexes to be more available to plant because they are kept in a soluble, chelated form (Brady and Weil 2002).

Therefore, the DTPA extraction results showed much higher Cu contents ranging from 10.6% to 37.8% of total Cu than those of water extraction results.

The DTPA extractable Pb, ranging from 15.0% to 33.8% of total Pb, was much higher than water extractable Pb in all the sludges. Regarding total content of all the heavy metals in the sludges, the highest extractability of Zn were obtained from water and DTPA treatment, for example, DTPA extracted more than 1,000 mg kg^{-1} of Zn (53.3% of total Zn) in the DS sludge, while 48.2%, 40.6% and 32.1% of Zn can be characterized from the BL, MS and NPUST sludges by DTPA. Therefore, Zn presents a great bioavailability level for the sludges. The concentrations of total Ni in all the sludges ranged from 45.7 mg kg^{-1} to 407 mg kg^{-1}. However, the total Ni level in the BL sludge was approximate 8 times higher than that in the other sludges, and thus water and DTPA extractable Ni contents of the BL sludge are much higher than those of the other sludges.

Chemical fractions of heavy metals by sequential extraction

Harrison *et al*. (1981) suggested that the mobility and bioavailability of the metals decreased approximately in the order of sequential extraction. The operationally defined extraction sequence follows the order of decreasing solubility of the geochemical forms of the metals; hence, the exchangeable fraction may indicate which metals are most bioavailable. In this study, the results obtained after application of the sequential extraction scheme to all the sludges and the recovery of each metal are shown in **Table 4**. Fractional totals of heavy metals by sequential extraction ranged from 72 to 120% of total heavy metals for each sample, showing that recovery of heavy metal during sequential extraction was not problematic.

If the results of metal concentrations obtained by sequential extraction are compared with those obtained with DTPA extraction, it could be pointed out that the bioavailable level of the metals is similar. For instance, Cd was principally distributed in the Fe-Mn oxide fraction, which ranged from 0.28 to 1.79 mg kg^{-1} (**Table 4**). The exchangeable fractions of Cd were very low (<2.00 mg kg^{-1}), despite a great percentage of this fraction (**Fig. 1**). As a result, the sums of exchangeable and carbonate fractions resembling that of the DTPA extraction may indicate low bioavailability of Cd in all the sludges.

The sums of exchangeable, carbonate and Fe-Mn oxide fractions of Cu in all the sludges were much lower than those extracted by DTPA. It is estimated that high affinity of organic matter to Cu results in the formation of stable complexes (Norvell 1991), so that over 90% of the Cu was enriched in the organic fraction (**Fig. 2**). The organic fractions of Cu extracted after oxidizing with hydrogen peroxide in the sequential extraction procedure may be associated with a high degree of soil organic matter. However, the organic fractions of Cu were much higher than the Cu concentrations extracted by DTPA. The extractability difference between hydrogen peroxide in sequential extraction and DTPA reagent for Cu in the sewage sludges of this study is higher than the differences reported by Wong *et al*. (2001) and Fuentes *et al*. (2004). Possibly part of Cu strongly associated with the different behavior of organic matter in the sewage sludges between H_2O_2 and DTPA.

The relatively high amount of Pb was in the Fe-Mn oxide, organic and residual fractions, followed by carbonate fraction, while the exchangeable fraction was the lowest (**Fig. 3**). The speciation results of the exchangeable and carbonate fractions indicated the low bioavailability of Pb in the sludges, which further supported the finding of low water and DTPA extractable Pb of <20.8 mg kg^{-1} (**Table 3**). The proportions of Pb present in the residual fractions, ranging from 30% to 85% of this study, were similar to those reported in previous studies (Ščančar *et al*. 2000; Wong *et al*. 2001; Álvarez *et al*. 2002; Fuentes *et al*. 2004). Therefore, Pb was associated with the primary mineral in sewage sludge produced in Taiwan and it explained the very low percentages (<1%) of water extractable contents of total Pb.

The predominant species of Zn in all the sludges was the Fe-Mn oxide fraction which ranged from 419 mg kg^{-1} to 1105 mg kg^{-1} (**Table 4**). This is estimated that Zn is easily formed complex with Fe and Mn oxides (Dixon and White 2002), and thus the proportions of Fe-Mn oxide fraction of Zn were more than 50% of total Zn (**Fig. 4**). Meanwhile, the results from previous studies reported that Zn in sludge was dominated by the Fe-Mn oxide fraction (Wong *et al*. 2001) or in the organic fraction (Fuentes *et al*. 2004). Compared with other metals, the exchangeable and carbonate fractions of Zn were relatively high in all the sludges, which coincided well with the high water and DTPA extractable Zn contents. The sums of exchangeable and carbonate fractions of Zn were lower than DTPA extractable Zn contents except for BL sludges. However, the extractability of $NH_2OH{\cdot}HCl$ in dissolving Fe-Mn oxides used in the third step of sequential extraction was stronger than DTPA extractability in Zn. It is concluded that possibly part of Zn bound with Fe-Mn oxides can not only be extracted by $NH_2OH{\cdot}HCl$, but also by DTPA. Therefore, the chemical characterization of Zn confirmed that Zn was the most mobile metal among the heavy metals in all the sludges, which was in agreement with the report by Planquart *et al*. (1999).

No definite patterns of the chemical fraction were found for Ni in all the sludges (**Fig. 5**). The dominant frac-tion of Ni was the exchangeable form in the BL sludge, it was residual in the NPUST sludge, and it was Fe-Mn oxide bounded both in the MS and DS sludges. Regarding Cr, the poor levels of bioavailability were identified by the low amounts of exchangeable and carbonate fractions (**Table 4**), while resembled the results with the single extractions both by water and DTPA (**Table 3**). Therefore, most of the Cr existed in high unavailable species in all the sludges such as organic and residual phases (**Fig. 6**).

Effects of heavy metals on the seedlings of Chinese cabbage

High germination percentages of Chinese cabbage were found in this study, but were not significantly ($p<0.05$) different in most cases of sludge extract with different dilution ratios (**Table 5**). Seed germination of Chinese cabbage has been regarded as a less sensitive method than root elongation when used as a biossay in evaluating phytotoxicity (Wang and Keturi 1990; Wong *et al*. 2001). In this study, only the root growth of Chinese cabbage was significantly inhibited by the original sludge extract (**Table 6**). The reduction in root growth indicates that the factors existent in the sludges imposed adverse effects on root growth. The toxic levels of heavy metals for crops in soil have been recommended as Cd: 3-8 mg kg^{-1}, Cu: 60-125 mg kg^{-1}, Cr: 75-100 mg kg^{-1}, Ni: 100 mg kg^{-1}, Pb: 100-400 mg kg^{-1} and Zn: 70-400 mg kg^{-1} (Kabata-Pendias and Pendias 1992). Therefore, Cu, Ni and Zn in the sludge are evaluated as the potentially crucial elements for plant growth in this study. Planquart *et al*. (1999) found that the sum of exchangeable and carbonate fractions of Zn in the sewage sludge from sequential extraction procedure could show the most labile forms for *Brassica napus* rather than its total Zn concentration. However, the available concentrations of Cu, Ni and Zn evaluated by water, DTPA extractions and the sum of exchangeable and carbonate fraction of sequential extraction in this study were much higher than those of Cd, Cr and Pb. Brady and Weil (2002) indicated that the passive fraction of soil organic matter with C/N ratio ranging between 7 and 10, probably makes a large contribution to an important source of mineralizable plant nutrients. As a result, N and P supply was not deficient for seed germination and growth of Chinese cabbage. It is very likely that the phytotoxicity of Chinese cabbage was due to the high Cu, Ni and Zn contents of all the sludges, particularly the average root length in the 100% BL sludge extract was shorter

Table 5 Seed germination percentages of sludge extract at different dilution ratios.

Dilution ratio	BL	MS	DS	NPUST
Control[a]	97.8 ± 3.8 a[b]	97.8 ± 3.8 a	97.8 ± 3.8 ab	97.8 ± 3.8 ab
× 20	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	97.6 ± 4.1 ab
× 10	100 ± 0.0 a	100 ± 0.0 a	97.8 ± 3.8 ab	95.6 ± 7.7 ab
× 5	97.8 ± 3.8 a	95.6 ± 7.7 a	88.9 ± 10.2 ab	100 ± 0.0 a
× 2	100 ± 0.0 a	93.0 ± 7.1 a	84.1 ± 3.6 b	84.4 ± 3.8 b
Original	95.6 ± 3.8 a	81.0 ± 10.9 b	88.9 ± 10.2 ab	82.1 ± 1.1 b

[a] Seeds germinated in 10 mL of distilled water.
[b] Values followed by different letter within the same column are different significantly at 5% level according to the Duncan's new multiple range test.

Table 6 Average root length of Chinese cabbage in sewage sludge extract at 5 day (cm $seed^{-1}$).

Dilution ratio	BL	MS	DS	NPUST
Control[a]	5.8 ± 1.9 a[b]	5.8 ± 1.9 a	5.8 ± 1.9 a	5.8 ± 1.9 a
× 20	4.7 ± 0.6 a	5.6 ± 0.6 a	6.2 ± 0.4 a	4.6 ± 0.5 ab
× 10	5.0 ± 0.7 a	5.4 ± 0.2 a	6.2 ± 0.6 a	4.4 ± 0.8 ab
× 5	5.1 ± 0.5 a	5.2 ± 0.3 a	5.8 ± 0.5 a	4.6 ± 0.4 ab
× 2	4.4 ± 0.4 ab	4.7 ± 0.6 a	5.5 ± 0.5 a	4.2 ± 0.4 ab
Original	2.8 ± 0.7 b	3.1 ± 0.4 b	3.4 ± 1.0 b	3.8 ± 0.3 b

[a] Seeds germinated in 10 mL of distilled water.
[b] Values followed by different letter within the same column are different significantly at 5% level according to the Duncan's new multiple range test.

than 3.0 cm $seed^{-1}$ with approximate 100 mg kg^{-1} of Ni by water extraction.

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REFERENCES

Álvarez EA, Móchon MC, Sánchez JCJ, Rodríguez MT (2002) Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere* **47**, 765-775

Brady NC, Weil RR (2002) Soil organic matter. In: Brady NC, Weil RR (Eds) *The Nature and Properties of Soils*, Prentice Hall, NJ, USA, pp 498-543

Bremner JM, Keeney DR (1966) Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3 Exchangeable 'ammonium, nitrate, and nitrite' by extraction distillation methods. *Soil Science Society of America Proceedings* **30**, 577-582

Chen ZS, Hseu ZY (1997) Total organic carbon pool in soils of Taiwan. *Proceedings of the National Science Council (Republic of China) Part B, Life Science* **21**, 120-127

Cuiou IJ, Wang KS, Chen CH, Lin YT (2006) Lightweight aggregate made from sewage sludge and incinerated ash. *Waste Management* **26**, 1453-1461

Council of the European communities (1986) Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in Particular of the Soil, When Sewage Sludge is Used in Agriculture. Community Legislation in Force. Document 386L0278

Dixon JB, White GN (2002) Manganese oxides. In: Dixon JB, Schulze DG (Ed) Soil *Mineralogy with Environmental Applications*, Soil Science Society of America, Inc., Madison, WI., USA, pp 367-368

Düring RA, Gäth S (2002) Utilization of municipal organic wastes in agriculture: where do we stand, where will we go? *Journal of Plant Nutrition and Soil Science - Zeitschrift Fur Pflanzenernahrung und Bodenkunde* **165**, 544-556

Fernández A, Pérez-Cid B, Fernández E, Falqué E (2000) Comparison between sequential extraction procedures and single extractions for metal portioning in sewage sludge samples. *Analyst* **125**, 1353-1357

Flyhammar P (1997) Estimation of heavy metal transformations in municipal solid waste. *Science of the Total Environment* **198**, 123-133

Förstner U, Salomons W (1980) Trace metal analyses on polluted sediments, Part I: Evaluation of environmental impact. *Environmental Technology Letters* **1**, 506-517

Fuentes A, Lloréns 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

Fuentes A, Lloréns M, Sáez J, Aguilar MI, Pérez-Marín AB, Ortuño JF, Meseguer VF (2006) Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilized sewage sludge. *Environmental Pollution* **143**, 355-360

Harrison RM, Laxen DPH, Wilson SJ (1981) Chemical associations of lead, cadmium, copper, and zinc in street dusts and roadside soils. *Environmental Science and Technology* **15**, 1378-1383

ISO 11466 International Standard (1995) Soil quality-extraction of trace elements soluble in *aqua regia*, pp 3-9

Kabata-Pendias A, Pendias H (1992) *Trace Elements in Soils and Plants*, CRC Press, Boca Raton, FL, USA

Karvelas M, Katsoyiannis A, Samara C (2003) Occurrence and fate of heavy metals in the wastewater treatment process. *Chemosphere* **53**, 1201-1210

Kuo S (1996) Phosphorus. In: Sparks DL (Ed) *Methods of Soil Analysis, Part 3: Chemical Methods* (SSSA book series No. 5), ASA and SSSA, Madison, WI, USA, pp 869-919

Lindsay WL, Norvell WA (1978) Development of DTPA soil test for Zn, Fe, Mn, Cu. *Soil Science Society of America Journal* **42**, 421-428

López-Sánchez JF, Rubio R, Samitier C, Rauret G (1996) Trace metal partitioning in marine sediments and sludges deposited off the coast of Barcelona (Spain). *Water Research* **30**, 153-159

McBride MB (1995) Toxic metal accumulation from agricultural use of sludge: Are USEPA Regulations protective? *Journal of Environmental Quality* **24**, 5-18

McBride MB (2003) Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risk? *Advances in Environmental Research* **8**, 5-19

McLean EO (1982) Soil pH and lime requirement. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis, Part 2: Chemical and Microbiological Methods* (2nd Edn), Agron. Monogr. 9, ASA and SSSA, Madison, WI, USA, pp 199-224

Meers E, Ruttens A, Hopgood MJ, Samson D, Tack FMG (2005) Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. *Chemosphere* **58**, 1011-1022

Nelson DW, Sommers LE (1982) Total carbon, OC, and organic matter. In: Page AL, Miller RH, Keeney DR (Eds) *Methods of Soil Analysis, Part 2: Chemical and Microbiological Methods* (2nd Edn), Agron. Monogr. 9, ASA and SSSA, Madison, WI, USA, pp 539-577

Norvell WA (1991) Reactions of metal chelates in soils and nutrient solutions. In: Mortvedt JJ, Cox FR, Shuman LM, Welch RM (Eds) *Micronutrients in Agriculture* (SSSA book series No. 4), ASA and SSSA, Madison, WI, USA, pp 187-227

Obrador A, Rico MI, Mingot JI, Álvarez JM (1997) Metal mobility and potential bioavailability in organic matter-rich soil-sludge mixtures: effect of soil type and contact time. *Science of the Total Environment* **206**, 117-126

Planquart P, Bonin G, Prone A, Massiani C (1999) Distribution, movement and plant availability of trace elements in soils amended with sewage sludge composts: application to low metal loading. *Science of the Total Environment* **241**, 161-179

SAS Institute (1982) *SAS User's Guide*, Statistics. SAS Institute Cary, NC, USA

Ščančar J, Milačič R, Stražar M, Burica O (2000) Total metal concentrations and partitioning of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge. *Science of the Total Environment* **250**, 9-19

Shuman LM (1985) Fractionation method for soil microelements. *Soil Science* **140**, 11-22

Sims JT (1986) Soil pH effects on the distribution and plant availability of manganese, copper and zinc. *Soil Science Society of America Journal* **50**, 367-373

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

Tandy S, Bossart K, Mueller R, Ritschel J, Hauser L, Schulin R, Nowack B (2004) Extraction of heavy metals from soils using biodegradable chelating agents. *Environmental Science and Technology* **38**, 937-944

Tessier A, Campbel PGC, Bisson M (1979) Sequential extraction procedures for the speciation of particulate trace metals. *Analytical Chemistry* **51**, 844-851

USEPA (US Environmental Protection Agency) (2000) *A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule*, EPA 832-B-93-005 (Revision). Office of Wastewater Management, U.S. Environmental Protection Agency, Washington, DC

Wang W, Keturi PH (1990) Comparative seed germination tests using ten plants species for toxicity assessment of a metal engraving effluent sample. *Water, Air and Soil Pollution* **52**, 369-376

Wong JWC, Li K, Fang M, Su DC (2001) Toxicity evaluation of sewage sludges in Hong Kong. *Environment International* **27**, 373-380

Zucconi F, Forte M, Monaco A, de Bertoldi M (1981) Biological evaluation of compost maturity. *Biocycle* **22**, 27-29