

Assessment of Trace and Heavy Metal Distribution by Four Sequential Extraction Procedures in a Contaminated Soil

QINGSONG HE¹, YUE REN², IBRAHIM MOHAMED^{3,4}, MAHA ALI³, WASEEM HASSAN⁴
and FANGUI ZENG⁵

¹College of Mining Technology, Taiyuan University of Technology, Taiyuan, Shanxi, China; ²Hubei Geological Research Laboratory, Wuhan, China; ³Soil Science Department, Faculty of Agriculture, Benha University, Benha, Egypt; ⁴College of Resources and Environment, Huazhong Agricultural University, Wuhan, China; ⁵Key Laboratory of Coal Science and Technology of Ministry of Education and Shanxi Province, Department of Earth Science and Engineering, Taiyuan University of Technology, Taiyuan, Shanxi, China

Abstract

HE Q., REN Y., MOHAMED I., ALI M., HASSAN W., ZENG F. (2013): **Assessment of trace and heavy metal distribution by four sequential extraction procedures in a contaminated soil.** Soil & Water Res., 8: 71–76.

Four sequential extraction procedures (Sposito, Tessier, Silveira and Bureau Communautaire de Reference (BCR)) were used to evaluate the distribution of some metals (Fe, Cu, Cd and Zn) in a contaminated soil around a mining area. The results showed that Fe and Zn were mainly recovered in the recalcitrant soil fractions, while Cd was primarily localized in the exchangeable fraction. Soil Cu was highly associated with organic matter fraction. The amorphous Fe fraction in Silveira could be recognized as part of the Fe-Mn oxide fraction in Tessier and BCR procedures, while the crystalline Fe oxide fraction was classified into the residual fraction in Sposito, BCR and Tessier schemes. Although the same reagent was used to extract target fraction, less carbonate-bound Cu, Cu and Zn were extracted in Tessier procedure as compared to Silveira method, while Tessier scheme yielded a higher proportion of Fe, Cu and Zn in the Fe-Mn oxide fraction than BCR method. Due to the lack of uniformity of experimental conditions and the differences in extraction reagents, the extraction efficiency of metal species varied with the sequential extraction schemes. Therefore, care should be taken when comparing the results obtained by different sequential extraction procedures.

Keywords: fractionation; metals; mining activities; pollution

The metals in soils can be found in soil solution, on exchange sites, occluded into soil oxide material, incorporated into organic plant litter or in the lattice structure of primary and secondary soil minerals (COTTENIE & VERLOO 1984; RAO *et al.* 2007). The speciation of heavy metals exerts

strong influences on the mobility, bioavailability and toxicity of heavy metals in contaminated soils (URE & DAVIDSON 2002; HASS & FINE 2010). Among other techniques available to estimate the geochemical partitioning of heavy metals in soils, sequential extraction procedure has been widely

accepted (BACON & DAVIDSON 2008). Sequential extraction methods are based on the rational use of a series of more or less selective reagents chosen to solubilise successively the different mineralogical fractions thought to be responsible for retaining the larger part of metals (GLEYZES *et al.* 2002).

Several sequential extraction procedures have been proposed for determining the speciation of particulate heavy metals. For example, TESSIER *et al.* (1979) developed a five-stage extraction to evaluate the fractions of Cd, Cu, Fe, Pb, Mg, Ni and Zn in river sediments. SPOSITO *et al.* (1982) used a four-stage sequential extraction scheme to evaluate the fractionation of Ni, Cu, Zn, Cd, and Pb in arid-zone field soils amended with sewage sludge. In an attempt to provide an internationally accepted sequential extraction protocol, a modified BCR (Community Bureau of Reference or now the Standards, Measurements and Testing Program of the European Commission) sequential extraction procedure was developed (RAURET *et al.* 1999). SILVEIRA *et al.* (2006) proposed a sequential fractionation procedure for Zn and Cu suitable for tropical soils, which distinguished metals associated with amorphous or poorly crystalline oxides from crystalline forms.

Sequential extractions schemes have not been standardized and the results of different procedures are not always comparable due to the lack of uniformity in experimental conditions and the differences in extraction reagents (FILGUEIRAS *et al.* 2002). Previous works mainly focused on the development of new extraction schemes and application of sequential extraction for characterization of metal mobility for different variety of environmental samples. However, there is less information available about the comparison of different sequential extractions schemes for metal fractions in the soil. The objective of this study was to compare four sequential extraction procedures (Sposito, BCR, Tessier and Silveira scheme) to determine the fractionation of trace metals (Fe, Cu, Cd and Zn) in a mining-contaminated soil.

MATERIAL AND METHODS

Soil sampling and analysis. The soil sample was collected from the surface layer (0–15 cm) at the city of Huangshi (30°16'N and 114°93'E), Hubei province, China. The soil was contaminated with heavy metals due to smelting and mining activities. The soil was a loamy soil with 38.7% sand, 39.2% silt and 22.1% clay. The soil was air-dried and ground to pass through a 2 mm sieve for subsequent analysis. The pH of soil was measured using a ratio of 1:2.5 (w/v) soil/water. Soil organic carbon was analysed by potassium dichromate oxidation and titrated with ferrous ammonium sulphate (HESSE 1972). After sulphuric acid-perchloric acid digestion (JACKSON 1973), total N and P concentrations in the digested solution were determined by FIA-star 5000 analyser (FOSS Tecator, Höganäs, Sweden), and the concentrations of total K, Fe, Cu, Cd and Zn were analysed by atomic adsorption spectrophotometer (Varian AA240FS, Varian, Mulgrave, Australia). Some chemical characteristics of soil are listed in Table 1.

Four sequential extraction procedures (Sposito, BCR, Tessier and Silveira scheme) were used to evaluate the fractionation of soil metals. Details of each procedure are presented in Table 2. Sequential extractions were carried out in triplicate, using 1 g air-dried soil. Soil samples were placed in 50 ml polycarbonate centrifuge tubes, mixed in a stepwise fashion with various reagents, and the suspensions were equilibrated as described in Table 2. Following equilibration, the solution and solid phases were separated by centrifugation at 1225 g for 10 min. Between all successive extractions, the solid residues were suspended in 5 ml of 0.1M NaCl, shaken by hand, and centrifuged to displace the extraction solution remaining from the previous step. The supernatant was added to the former extractant. This step was intended to reduce sample dispersion and to minimize the re-adsorption of the metal. The supernatants were filtered through a 0.45 µm membrane, and the solid residues were preserved for the subsequent extractions. The concentrations of Fe, Cu, Cd and Zn

Table 1. Chemical characteristics of soil

Properties	pH	OM	Total N	Total P	Total K	Total Fe	Total Cu	Total Cd	Total Zn
		g/kg				mg/kg			
Value	6.56	33.67	1.50	1.26	19.56	31.77	440.43	5.01	146.94

OM – Organic matter

in the various extracts were determined by atomic adsorption spectroscopy (Varian AA240FS).

RESULTS AND DISCUSSIONS

Iron sequential fractionation

The distribution of Fe among various soil fractions is presented in Figure 1a. In general, the exchangeable and organically bound Fe fractions in Sposito, BCR, Tessier and Silveira procedure

were relatively low (average < 1%). In Silveira procedure, the Fe associated with poor crystalline Fe fraction, crystalline Fe fraction and residual fraction accounted for 18.4, 58.7 and 22.7% of total Fe in soil, respectively. The Sposito, BCR and Tessier procedure yielded similar results with the highest proportion of Fe in the residual fraction. The comparison of BCR method with Silveira procedure indicated that the second step of BCR dissolved most of the poorly crystalline Fe fraction, while it had a small effect on the crystalline Fe. Meanwhile, the crystalline Fe fraction in Silveira

Table 2. Sequential extraction procedures

Fraction	Solution	Equilibrium conditions
Procedure 1 (TESSIER 1979)		
Exchangeable	8 ml 1M MgCl ₂ (pH 7)	1 h, room temperature
Carbonates	8 ml 1M NaOAc (pH 5)	5 h, room temperature
Fe and Mn oxides	20 ml 0.04M NH ₂ OH/HCl in 25% (v/v) HOAc	6 h, 96°C
Organic matter	3 ml 0.02M HNO ₃ + 5 ml 30% H ₂ O ₂ (pH 2)	3 h, 85°C
	5 ml 3.2M NH ₄ OAc in 20% (v/v HNO ₃ + 7 ml water)	20 min, room temperature
Residual	HNO ₃ -HCl digestion	
Procedure 2 (SPOSITO 1982)		
Exchangeable	10 ml 0.5M KNO ₃	16 h, 25°C
Organic matter	10 ml 0.5M NaOH	16 h, 25°C
Fe and Mn oxides	10 ml 0.05M Na ₂ EDTA	6 h, 25°C
Residual	10 ml 4M HNO ₃	16 h, 25°C
Procedure 3 (BCR 1999)		
Exchangeable, water and acid-soluble	40 ml 0.11M CH ₃ COOH	16 h, room temperature
Reducible (Fe and Mn oxides)	40 ml 0.5M NH ₂ OH-HCl (pH 1.5)	16 h, room temperature
Oxidisable (Organic matter)	10 ml 8.8M H ₂ O ₂	1 h, room temperature
	10 ml 8.8M H ₂ O ₂	1 h, 85°C
	50 ml 1M NH ₄ OAc (pH 2)	16 h, room temperature
Residual	HNO ₃ -HCl digestion	
Procedure 4 (SILVEIRA 2006)		
Soluble-exchangeable	15 ml 0.1M CaCl ₂	2 h, room temperature
Surface adsorbed	30 ml 1M NaOAc (pH 5)	5 h, room temperature
Organic matter	5 ml NaOCl (pH 8.5)	30 min, 90–95°C
Mn oxides	30 ml 0.05M NH ₂ OH-HCl (pH 2)	30 min, room temperature
Poor crystalline Fe oxides	30 ml 0.2M oxalic acid + 0.2M NH ₄ oxalate (pH 3)	2 h, dark
Crystalline Fe oxides	40 ml 6M HCl	24 h, room temperature
Residual	HNO ₃ -HCl digestion	

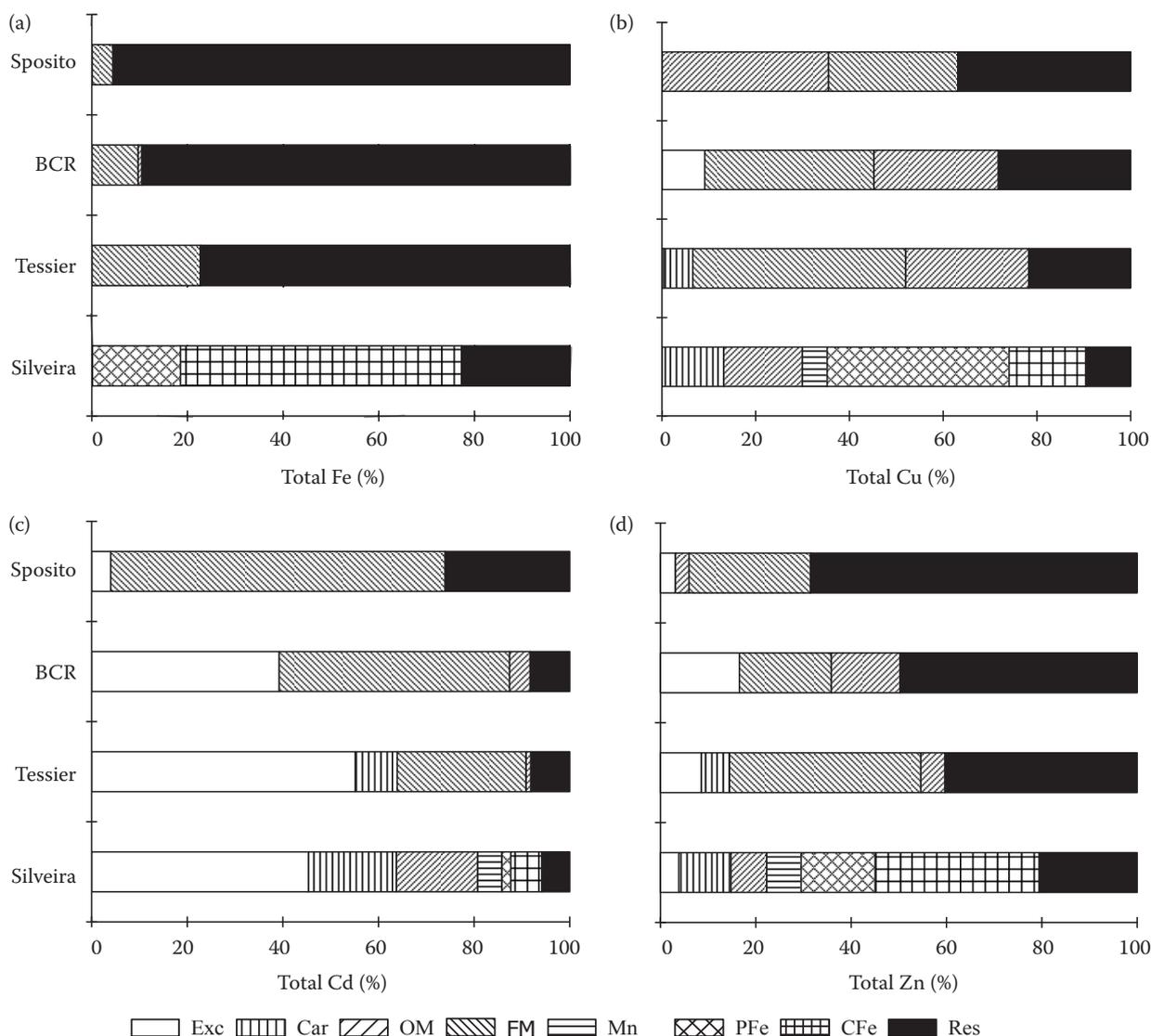


Figure 1. Relative distributions of Fe (a), Cu (b), Cd (c), and Zn (d) in soil according to four sequential extraction procedures; Exc – exchangeable; Car – carbonates; OM – organic matter; FM – Fe and Mn oxides; Mn – Mn oxides; PFe – poor crystalline Fe oxides; CFe – crystalline Fe oxides; Res – residual

procedure could be classified into the residual fraction in BCR and Tessier schemes. The poorly crystalline Fe fractions are a more active component in the geochemical cycling of trace metals than the crystalline Fe fractions (CHAO & ZHOU 1983). The Silveira procedure could distinguish metals associated with amorphous or poorly crystalline oxides from crystalline forms and provided more detailed information on the distribution of Fe and related metals in soils (SILVEIRA *et al.* 2006). Different sequential extraction procedures partitioned trace metals into various fractions. Therefore, the selection of extraction scheme should be specifically optimized based on the characteristics of the target sample and the purpose of work.

Copper sequential fractionation

The total concentration of total Cu in the soil is 440.43 mg/kg, which exceeds the Cu limits for agricultural lands in China according to the soil environmental quality standard (< 50 mg/kg) (SEPAC 1995). As shown in Figure 1b, approximately 17~36% of total Cu was bound by organic matter in Sposito, BCR, Tessier and Silveira procedure. In Silveira procedure, the sum of Cu associated with crystalline Fe fraction and residual fraction was higher than 26% of the total Cu. Meanwhile, the percentages of residual Cu assessed by BCR and Tessier were 28.2 and 21.7%, respectively, which indicated that the Cu associated with crystalline

Fe fraction can be considered as a part of the residual fraction in BCR and Tessier schemes. In BCR procedure, exchangeable Cu was 9.1% of total Cu in soil, which was more than the exchangeable Cu assessed by Sposito, Tessier and Silveira procedure (< 0.5%). This might be attributed to the reagent (0.11 mol/l CH₃COOH) used in the first step of BCR scheme. MILLER *et al.* (1986) and BERTI and JACOBS (1996) reported the lack of selectivity of CH₃COOH because of its ability to dissolve a wide range of soil minerals.

Cadmium sequential fractionation

The total concentration of Cd in soil is 5.00 mg/kg, which also exceeds the Cd limits set down by the soil environmental quality standard in China (< 1.0 mg/kg) (SEPA 1995). The exchangeable Cd assessed by BCR, Tessier and Silveira procedure accounted for 39.2–55.2% of total Cd in soil (Figure 1c). The high percentage of exchangeable Cd in soil indicated that the bioavailability of Cd was high (CAO *et al.* 2000). However, only 3.4 % of total Cd was found in the exchangeable fraction for Sposito procedure. This might be attributed to the low exchange capacity of K⁺ (BOHN *et al.* 2001). Approximately 4.3–18.4% of total Cd was bound by organic matter in BCR and Silveira procedure, while organic matter bound Cd assessed by Sposito was close to zero. This probably resulted from the NaOH solution used in this procedure, which promotes the formation of metal precipitates during extraction (YONG *et al.* 1993). These results indicated that Sposito procedure had low extraction efficiency for exchangeable and organic matter bound Cd, and was not suitable for the fractionation of Cd in this soil.

Zn sequential fractionation

As shown in Figure 1d, Sposito, BCR, Tessier and Silveira procedure yielded similar results with high proportions of Zn in the residual fraction (40.3~68.5%) and Fe-Mn oxide fraction (19.4~40.1%). Approximately 14% of total Zn was bound by organic matter in BCR procedure, while the organic matter bound Zn assessed by Tessier procedure accounted for 5.1% of total Zn in soil. This might be attributed to a large amount of ammonium acetate and longer extrac-

tion time used in BCR procedure (Table 2). LÄ *et al.* (2003) reported that extraction yields were low when the ratio between the soil and extractant solution (w/v) was too low. Although BCR and Tessier both used NH₂OH/HCl to extract Fe-Mn oxide fraction, the percentage of Zn extracted in Tessier procedure (40.1%) was higher than in BCR scheme (19.4%). The same trend was observed in the fractionation of Fe and Cu. This probably resulted from the higher temperature used in Tessier procedure (96°C). However, the results of Cd in the Fe-Mn fraction showed the opposite tendency between the Tessier and BCR schemes. USERO *et al.* (1998) indicated that the levels of metals (Cu, Zn, Mn, Cr, Pb and Fe) bound to iron and manganese oxides (reducible fraction) were influenced by the efficiency and selectivity of the reagents used in the previous stages. Therefore, the results may be too high if the carbonates have not been completely dissolved or too low if part of the iron and manganese oxides has already been extracted. Overall, besides the different reagents used in various extraction procedures, the extraction conditions such as temperature, number of extractions, shaking time and fluid/solid ratio had important influences on the extraction efficiency.

CONCLUSION

The results concluded that soil Fe and Zn were mainly recovered in the recalcitrant soil fractions, while significant proportions of Cu and Cd were associated with organic matter fractions and exchangeable fractions, respectively. The metals bound by amorphous iron in Silveira could be part of the Fe-Mn oxide fraction in Tessier and BCR procedure, and the crystalline oxide fractions in Silveira were classified into the residual fraction in Sposito, BCR and Tessier schemes. Sposito procedure was not suitable for evaluation of Cd species in this soil because of its lower extraction efficiency for exchangeable and organic matter bound Cd. Although the same reagent was used to extract objective fraction, the high fluid/solid ratio led to more carbonate-bound Cu, Cd and Zn extracted in Silveira scheme than in Tessier procedure. Meanwhile, high temperature led to higher association of Fe, Cu and Zn with Fe-Mn oxide fractions in Tessier procedure compared to BCR scheme. Besides the different reagents used in various extraction procedures, the experimental

conditions had an important impact on the efficiency of extraction. Therefore it should be done with caution when comparing the results of different sequential extraction procedures. These findings also reinforce the need for increased adoption of standardized sequential extraction procedures.

Acknowledgements. The authors thank Dr. F. SHAH, College of Recourse and Environment, Huazhong Agricultural University, China for his reading and revision of the manuscript.

References

- BACON J.R., DAVIDSON C.M. (2008): Is there a future for sequential chemical extraction? *Analyst*, **133**: 25–46.
- BERTI W., JACOBS L. (1996): Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge applications. *Journal of Environmental Quality*, **25**: 1025–1032.
- BOHN H.L., MCNEAL B.L., O'CONNOR G.A. (2001): *Soil Chemistry*. John Wiley & Sons Inc., New York.
- CAO X., WANG X., ZHAO G. (2000): Assessment of the bio-availability of rare earth elements in soils by chemical fractionation and multiple regression analysis. *Chemosphere*, **40**: 23–28.
- CHAO T.T., ZHOU L. (1983): Extraction techniques for selective dissolution of amorphous iron oxides from soils and sediments. *Soil Science Society of America Journal*, **47**: 225–232.
- COTTENIE A., VERLOO M. (1984): Analytical diagnosis of soil pollution with heavy metals. *Fresenius Journal of Analytical Chemistry*, **317**: 389–393.
- FILGUEIRAS A.V., LAVILLA I., BENDICHO C. (2002): Chemical sequential extraction for metal partitioning in environmental solid samples. *Journal of Environment Monitoring*, **4**: 823–857.
- GLEYZES C., TELLIER S., ASTRUC M. (2002): Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. *Trends in Analytical Chemistry*, **21**: 451–467.
- HASS A., FINE P. (2010): Sequential selective extraction procedures for the study of heavy metals in soils, sediments, and waste materials – a critical Review. *Environmental Science and Technology*, **40**: 365–399.
- HESSE P. (1972). *A Textbook of Soil Chemical Analysis*. Chemical Pub Co. Inc., New York.
- JACKSON M.L. (1973): *Soil Chemical Analysis*. Prentice Hall Inc., New Delhi.
- LÃ O.R., BARRA C.M., AMARAL SOBRINHO N.M.B., MAZUR N., VELLOSO A.C.X. (2003): Evaluation of Tessier, Keller and Miller sequential extraction methods in the determination of native iron in three types of soil: histosol, oxisol, and mollisol. *Quimica Nova*, **26**: 323–330.
- MILLER W., MARTENS D., ZELAZNY L., KORNEGAY E. (1986): Forms of solid phase copper in copper-enriched swine manure. *Journal of Environmental Quality*, **15**: 69–72.
- RAO C.R.M., SAHUQUILLO A., LOPEZ SANCHEZ J.F. (2007): A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water Air and Soil Pollution*, **189**: 291–333.
- RAURET G., LOPEZ-SANCHEZ J., SAHUQUILLO A., RUBIO R., DAVIDSON C., URE A. (1999): Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *Journal of Environment Monitoring*, **1**: 57–61.
- SEPAC (1995): *Environmental Quality Standard for Soils (GB15618-1995)*. Standards Press of China, Beijing. (in Chinese)
- SILVEIRA M.L. ALLEONI L.R.F., O'CONNOR G.A., CHANG A.C. (2006): Heavy metal sequential extraction methods – A modification for tropical soils. *Chemosphere*, **64**: 1929–1938.
- SPOSITO G., LUND L., CHANG A. (1982): Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases. *Soil Science Society of America Journal*, **46**: 260–264.
- TESSIER A., CAMPBELL P., BISSON M. (1979): Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, **51**: 844–851.
- URE A.M., DAVIDSON C.M. (2002): *Chemical Speciation in the Environment*. Blackie Academic and Professional, Chapman and Hall, London.
- USERO J., GAMERO M., MORILLO J., GRACIA I. (1998): Comparative study of three sequential extraction procedures for metals in marine sediments. *Environment International*, **24**: 487–496.
- YONG R.N., GALVEZ-CLOUTIER R., PHADUNGCHEWIT Y. (1993): Selective sequential extraction analysis of heavy-metal retention in soil. *Canadian Geotechnical Journal*, **30**: 834–847.

Received for publication March 16, 2012

Accepted after corrections November 23, 2012

Corresponding author:

Dr. FANGUI ZENG, Taiyuan University of Technology, Department of Earth Science and Engineering, Key Laboratory of Coal Science and Technology of Ministry of Education and Shanxi province, Taiyuan, Shanxi, China
e-mail address: turang_hzau@yahoo.com, zengfangui@tyut.edu.cn
