行政院國家科學委員會補助專題研究計畫成果報告

以硫氧化菌淋溶二仁溪底泥重金屬之研究(III):總固體物之影響
Bioleaching of heavy metals from contaminated sediments of the Ell-Ren river using sulfur-oxidizing bacteria: effect of total solids content

計畫類別：■個別型計畫　　□整合型計畫
計畫編號：**NSC- 90-2313-B-041-004**
執行期間：90 年 08 月 01 日至 91 年 07 月 31 日

主持人：余光昌　教授

執行單位：嘉南藥理科技大學環境工程衛生系

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1、摘要

本計畫之目的乃欲瞭解硫之添加量與底泥總固體物含量之比值(SA/TS)不同對重金屬以硫氧化菌自底泥中再釋出之影響以及此生物淋溶程序前後，此比值之不同對重金屬在底泥中鍵結型態之變化之影響。

結果顯示，在愈高之 SA/TS 比值下，底泥 pH 之下降率及硫氧化物之增加率愈大。在愈高之 SA/TS 比值下，總可萃取之鉛及鉻之釋出效率明顯較大。生物淋溶之後，錳氧化物成為一個比較強之可鍵結位置。此外，鉛及鋅與錳氧化物之鍵結百分比以及鉻及銅與有機物之鍵結百分比隨著 SA/TS 比值之降低而增加。不同重金屬在不同 SA/TS 比值下展現不同鍵結行為。

關鍵詞：生物淋溶；重金屬；底泥；逐步萃取程序；硫氧化菌；總固體物

Abstract

The aim of this study was to determine the effect of the ratio of sulfur added to total sediment solids (SA/TS) on the remobilization of heavy metals from contaminated river sediment by sulfur-oxidizing bacteria (SOB). Also, the difference in metal binding fractions before and after bioleaching was explored.

It was found that sediment pH decreased at a significantly faster rate at higher SA/TS ratios (0.413 and 0.199) than at lower ones. Sulfate concentrations increased at a faster rate at these higher SA/TS ratios. At the lower SA/TS ratios, more acid must be produced and therefore it took more time for SOB to lower the sediment pH. Remobilization efficiency of total extractable Pb and Cr was significantly higher at higher SA/TS ratios. After bioleaching, Mn-oxides became a stronger binding group, and the percentage of Pb and Zn bound to Mn-oxides and Cr and Cu bound to organic matter increased with the decrease of SA/TS. Different heavy metals showed different binding behavior at the various SA/TS ratios.

Keywords: Bioleaching; Heavy metal; Sediment; Sequential extraction procedure; Sulfur-oxidizing bacteria; Total solids

2、緣由與目的 (Introduction)

Because riverbed sediments act as both carriers and potential sources of contaminants in an aquatic environment, they not only play an important role in influencing river water pollution, but can also provide a record of the river’s pollution history. Heavy metals contained in human, industrial and urban waste discharged into river systems present a problem that is of major concern worldwide. Several chemical and microbial methods for their removal from sewage sludge and from sediments have been reported (Seidel et al., 1998; Ito et al., 2000; Tyagi et al., 1990, 1993, 1994, 1997; Blais et al. 1992a, b and c; Couillard and Chartier, 1991; Kitada et al., 2000). Although mineral acids and chelating agents have commonly been used to mobilize heavy metals, such chemical methods are more costly than microbial ones. Tyagi et al. (1993) indicated a microbial method of metal leaching to be 80% cheaper than an acid-requiring chemical one due to a lesser requirement for acid and lime. Operational difficulties in using acids, including the need for acid-resistant apparatus and safe storage and transportation facilities for the acid, also make such chemical methods unattractive. In the past decade, the ability of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* to remove trace metals from sewage sludge and from sediments has been studied with regard to sediment characteristics, initial pH of sediment, percentage of inoculum, retention time, total solid content, percentage of substrate added, temperature, and nutrition.

Sreekrishnan et al. (1993) linked sulfate concentration and solids concentration in sewage sludge with decrease in pH, which in turn induced metal bioleaching. They found that the buffering capacity index (BCI), defined as the slope of pH versus the sulfate concentration curve at pH 4.0, was positively correlated with sulfate concentration and sludge solids. Chen and Lin (2000, 2001) found that the rate of sediment pH reduction decreased with increasing solid content due to higher buffering capacity, such that it took more time for sediments with higher solids contents to attain the pH values necessary for metal solubilization.

Changes in sulfur concentration would be expected to affect acid production and therefore pH in the process of bioleaching using sulfur-oxidizing bacteria (SOB). However, Sreekrishnan et al. (1996) reported an initial rise in pH to around 8.0 at various sulfur concentrations, although the subsequent fall in the sludge pH was faster and sharper with higher sulfur concentrations. Seidel et al. (1998) found that metal solubilization increased with increasing sulfur in total solids. Acid production and buffering capacity of the sludge or sediment affected the pH, i.e. the extent of pH drop in the bioleaching process was dependant on the ratio of sulfur content to total solids concentration.

In the above studies, heavy metal extracted via bioleaching was termed ‘total metal content’. In addition to the total metal content, the distribution of metals among various fractions in the sediment is a useful measure as the fraction in which it exists determines the behavior of the metal in the environment. Chartier et al. (2001) described the partitioning of trace metals into different fractions before and after their biological removal from sediments. Analysis of partitioning allows an investigation of which metals, bound to which fractions, are solubilized during bioleaching.

The Ell-Ren River, located in southern Taiwan, is heavily polluted with heavy metals. Its sediment has also been contaminated with heavy metals. Over the past few years, our group (Tsai et al., 1998; Yu et al., 2001a, b and c) has explored the binding behavior of heavy metals in the sediments of this river.

This study investigates the effect of the ratio of sulfur added to total sediment solids [SA/TS] on remobilization of heavy metals from contaminated river sediment by sulfur-oxidizing bacteria (SOB). It also explores the changes in metal binding fractions that occurred during the bioleaching process.

3、材料與方法 (Materials and Methods)

3.1 Study area and sampling

The Ell-Ren River, one of the main rivers in southern Taiwan, originates at Sun-Zu-Hu, flows through Kaoshiung County, Tainan County and Tainan City, and eventually into the Taiwan Strait. It drains an area of about 350 km^2 and has a total length of about 65 km. Pollutants entering this river fall into three main categories: domestic waste (about 8%), poultry waste (about 64%), and industrial waste (about 28%). Samples were taken at the confluence of the Ell-Ren and San-Yeh-Kong River (the most polluted tributary of the Ell-Ren River).

3.2 Chemical analysis

Air-dried sediment samples were analyzed for carbonates, Fe-oxides, Mn-oxides, and organic matter (OM). The approximate gravimetric method (Raad, 1978) was used to identify carbonates, the Walkley-Black wet combustion method (Nelson and Sommers, 1982) for OM, and the procedure initiated by Tessier et al. (1979) was used to measure Fe- and Mn-oxide content. Analyses of acid-volatile sulfide (AVS) and its concentration in sediments were performed following the method of Allen et al. (1993), which was recommended by USEPA. Heavy metals (Cr, Cu, Zn, Ni, and Pb) were analyzed using the modified sequential extraction procedure (SEP) developed by Tessier et al. (1979) and Nelson and Sommers (1982), to determine in which of five fractions (exchangeable, bound to carbonates, bound to Mn-oxides, bound to Fe-oxides, and bound to OM) they were held, and in what amounts. After the first extraction (extraction of exchangeable metal) the sediment was washed, by shaking it with deionized water for 1 minute, and centrifuged prior to each subsequent extraction.

The amount of each of the five metals in each fraction was determined using an atomic absorption spectrophotometer (GBC AA960, Australia). The total extractable amount of any specific heavy metal (TEHM) is defined as the sum of the amounts of that metal found in the five fractions.

3.3 Bioleaching process

Experiments were performed in 500mL Erlenmeyer flasks at six controlled SA/TS ratios (0.413, 0.199, 0.100, 0.081, and 0.067), at 120rpm. Sediment (5.2, 10.8, 21.6, 26.5, or 32.3 g/L [wet-weight]) was added to the Erlenmeyer flasks, which contained 250mL of a culture medium consisting of 2.15g/L sulfur (prepared from $Na_2S_2O_3 \cdot 5H_2O$), 1g/L NH_4Cl, 1g/L KH_2PO_4, and 0.5g/L $MgCl_2 \cdot H_2O$. This medium provided nutrition, sulfur substrate and buffer capacity for the growth of sulfur-oxidizing bacteria in the sediments. The indigenous sulfur-oxidizing bacteria were acclimated and then sub-cultured several times until the pH of the sediment solution had steadied. Experimental conditions during acclimation and bioleaching are shown in Table 1.

4. 結果與討論 (Results and Discussion)

4.1 Effect of the SA/TS ratio on pH, ORP, and sulfates in the sediment solution

The amount of sulfur added (SA) relates directly to acid production by SOB, while total solids in the sediment (TS) equates to the buffer capacity of the sediment solution. The SA/TS ratio therefore represents a comparison of acid produced against buffer capacity. Consequently, a higher SA/TS ratio leads to a greater likelihood of overcoming the sediment buffer, and hence a lower sediment pH. In bioleaching, solubilization of heavy metals from sediments occurs via bio-oxidation and acidification, such that changes in sediment pH over time can be used to represent the degree of bioleaching. Results herein show that the SA/TS ratio significantly affected the rate of decrease in sediment pH (Fig. 1). Using the indicator $-d(pH)/dt$, it was noted that sediment pH decreased faster at the higher SA/TS ratios (0.413 and 0.199) than at the lower ones. This indicates that the activity of sulfur-oxidizing bacteria increased with increased SA/TS ratio. Moreover, we found that it took about 15 days for sediment pH to drop below 2.0 at higher SA/TS ratios, whereas it took about 30 days at lower SA/TS ratios, confirming that the ratio of SA to TS affects the rate of decrease of sediment pH. It was noted that more acid must be produced for buffering and more time is needed for SOB to lower the sediment pH to the same level at lower SA/TS ratios. After 33 days, the sediment pH was lowered to between 1.23 and 1.74. Figures for each SA/TS ratio were as follows: 1.23 (SA/TS 0.413); 1.24 (SA/TS 0.199); 1.44 (SA/TS 0.100); 1.46 (SA/TS 0.081); 1.74 (SA/TS 0.067).

Changes in both oxidation-reduction potential (ORP) and sulfate concentration can also be used as indicators of bioleaching efficiency. It was found that increases in ORP were similar for all SA/TS ratios (Fig. 2). This implies that ORP is not significantly correlated with the SA/TS ratio, which indicates that the ratio of SA to TS does not obviously affect the input and uptake of oxygen. Sulfate concentration increased, as did the rate of decrease in sediment pH, with the increase in SA/TS (Fig. 3).

The noticeable initial rise in sediment pH at the lower SA/TS ratios (Figs. 4 and 5), which corresponded with a retardation of sulfate increase, did not occur at the higher SA/TS ratios of 0.413 and 0.199 (Figs. 6 and 7). This phenomena may be due to the absence of bio-activity of sulfur oxidizing bacteria and the release of basic (or buffering) components as a result of aerobic reactions (Sreekrishnan et al., 1996). At lower SA/TS ratios (i.e., higher sediment solids content), the buffering basic components released from sediment solids were high, which might have resulted in the inhibition of the SOB.

4.2 Effect of the SA/TS ratio on TEHM after bioleaching

Results (shown in Table 2) indicate that the remobilization of total extractable Zn, Cu, and Ni was significantly higher than that of Cr and Pb at the same SA/TS ratio. After bioleaching, no obvious difference in remobilization efficiency of total extractable Zn, Cu, or Ni was found at different SA/TS ratios. Remobilization efficiency of total extractable Cr, and Pb, however, was significantly different at different SA/TS ratios. Higher SA/TS ratios correlated with higher remobilization of Cr and Pb. The difference in percentage remobilization of Cr at SA/TS 0.413 and SA/TS 0.067 was about 40%, in spite of a final sediment pH difference of only 0.51. Similarly, the difference in percentage remobilization of Pb at SA/TS 0.413 and SA/TS 0.067 was about 42%.

4.3 Effect of the SA/TS ratio on change in heavy-metal partitioning after bioleaching

As shown in Fig. 8, excluding Ni, the difference in heavy-metal partitioning before and after bioleaching was significant. Sediment pH may have been an important factor in this, as the percentage of bound metal in raw sediment solution at weakly acidic pH (around 6.0) differed significantly from that at strongly acidic pH (1.23-1.74). Mn-oxides, weak competitors in raw sediment, became a stronger binding group after bioleaching. Lead, mainly bound to carbonates, Fe-oxides and OM in raw sediment, was mainly bound to Mn-oxides and carbonates after bioleaching. The percentage of Pb bound to carbonates decreased as the SA/TS ratio decreased (i.e., as the final sediment pH increased), whereas the percentage of Pb bound to Mn-oxides increased. Put another way, Pb gradually converted from associating with Mn-oxides to carbonates as the SA/TS ratio increased from 0.067 to 0.413 (i.e., as the final sediment pH after bioleaching dropped from 1.74 to 1.23). There was no significant difference in percentage of Pb bound to carbonates before and after bioleaching, although the percentage of Pb bound to Mn-oxides did increase significantly after bioleaching.

Zinc, mainly bound to Fe-oxides and OM in raw sediment, was mainly bound to Fe-oxides and Mn-oxides after bioleaching. The percentage of Zn bound to the exchangeable, carbonates, and Mn-oxides fractions increased noticeably after bioleaching, whilst that bound to Fe-oxides and OM decreased. The percentage of Zn bound to Fe-oxides decreased with the decrease of SA/TS, whereas that of Zn bound to Mn-oxides increased with the decrease of SA/TS. That is, the main binding percentage of Zn gradually converted from one bound to Mn-oxides to one bound to Fe-oxides as the SA/TS increased from 0.067 to 0.413. The percentages of both Pb and Zn bound to Mn-oxides reduced as sediment pH fell from 1.74 to 1.23.

Copper, initially mainly bound to OM, was mainly bound to OM and Mn-oxides after bioleaching. The percentage of Cu bound to OM increased with the decrease of SA/TS, whereas the percentage bound to Mn-oxides was almost unchanged at the various SA/TS ratios. Chromium, initially mainly bound to Fe-oxides, was mainly bound to Fe-oxides and OM after bioleaching at low SA/TS ratios, and to Fe-oxides, Mn-oxides, and exchangeable at high SA/TS ratios. The percentage of Cr bound to OM increased with the decrease of SA/TS, as had that of Cu, implying that the percentage of these metals bound to OM reduced when the sediment pH fell from 1.74 to 1.23. There was no obvious difference in the percentage of Ni bound to the various fractions before and after bioleaching, except for exchangeable Ni, which decreased with the decrease of SA/TS. Metals are in fact released from the exchangeable fraction relatively easily. This outcome conforms to the described remobilization efficiency of Ni and reveals that the Ni remaining in the sediment after bioleaching was still more easily remobilized at higher SA/TS.

These results show how the individual heavy metals had their own individual binding behaviors at different SA/TS ratios both before and after bioleaching.

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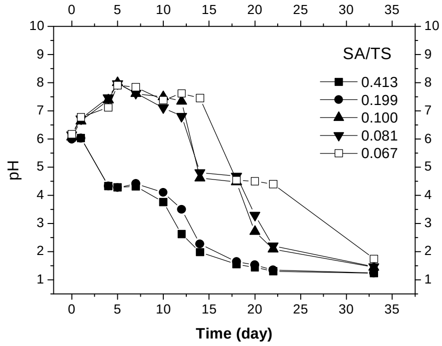

Fig. 1. Change in sediment pH with time at various SA/TS.

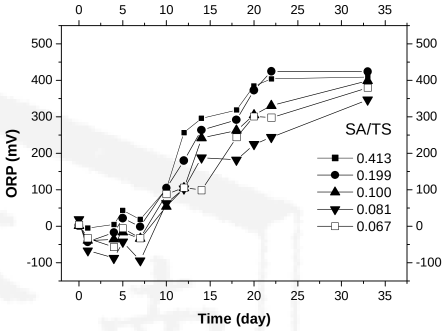

Fig. 2. Change in sediment ORP with time at various SA/TS.

Table 1 Experimental conditions for bioleaching

Run No.	Inoculum Dosage (%, v/v)	Sulfur Added (g S / L)	Total Solids (g/L)	SA/TS
A	2	2.15	5.2	0.413
B	2	2.15	10.8	0.199
C	2	2.15	21.6	0.100
D	2	2.15	26.5	0.081
E	2	2.15	32.3	0.067

Table 2 Changes in TEHM$_S$ after bioleaching at different SA/TS ratios

		TEHMs				
Status	SA/TS	Ni (mg/kg)	Zn (mg/ kg)	Cu (mg/ kg)	Pb (mg/ kg)	Cr (mg/ kg)
Raw sediments		198.7	1241.5	1087.9	373.6	336.8
Sediment after bio-leaching	0.413	9.2 (95.4%)[a]	14.1 (98.9%)	10.8 (99.0%)	52.0 (86.1%)	20.4 (93.9%)
	0.199	15.8 (92.0%)	12.9 (99.0%)	8.1 (99.3%)	52.1 (86.1%)	12.9 (96.2%)
	0.100	19.3 (90.3%)	46.0 (96.3%)	74.4 (93.2%)	198.1 (49.5%)	63.5 (64.3%)
	0.081	17.7 (91.1%)	45.4 (96.3%)	106.9 (90.2%)	210.3 (43.7%)	63.6 (64.3%)
	0.067	32.9 (83.4%)	70.3 (94.3%)	62.9 (94.2%)	210.0 (43.8%)	151.1 (55.1%)

[a]: solubilization percentage of TEHMs via bioleaching

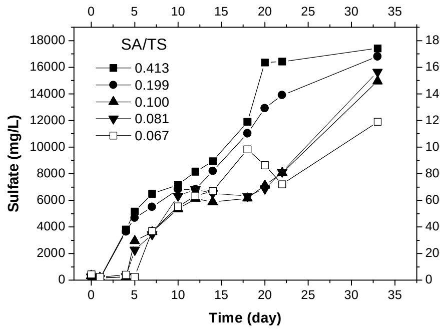

Fig. 3. Change in sulfates content with time at various SA/TS.

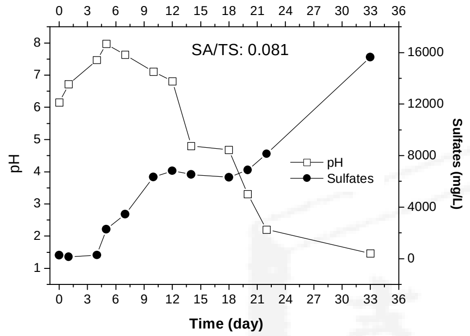

Fig. 4. Changes in sediment pH and sulfates content with time at SA/TS of 0.081.

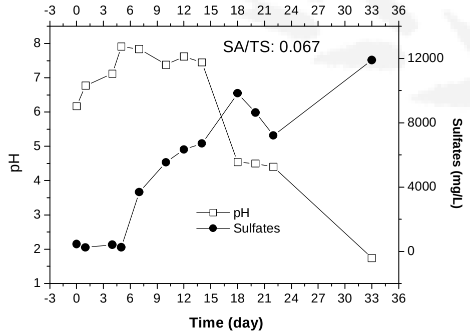

Fig. 5. Changes in sediment pH and sulfates content with time at SA/TS of 0.067.

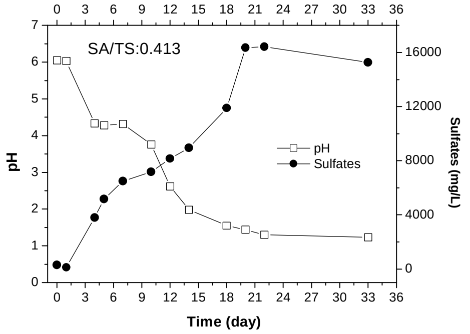

Fig. 6. Changes in sediment pH and sulfates content with time at SA/TS of 0.413.

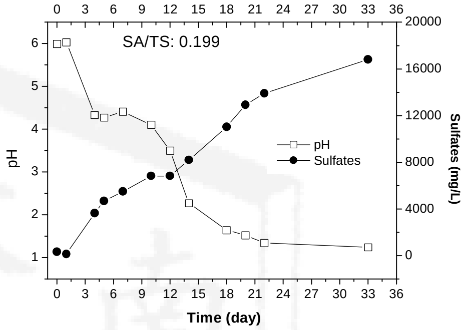

Fig. 7. Changes in sediment pH and sulfates content with time at SA/TS of 0.199.

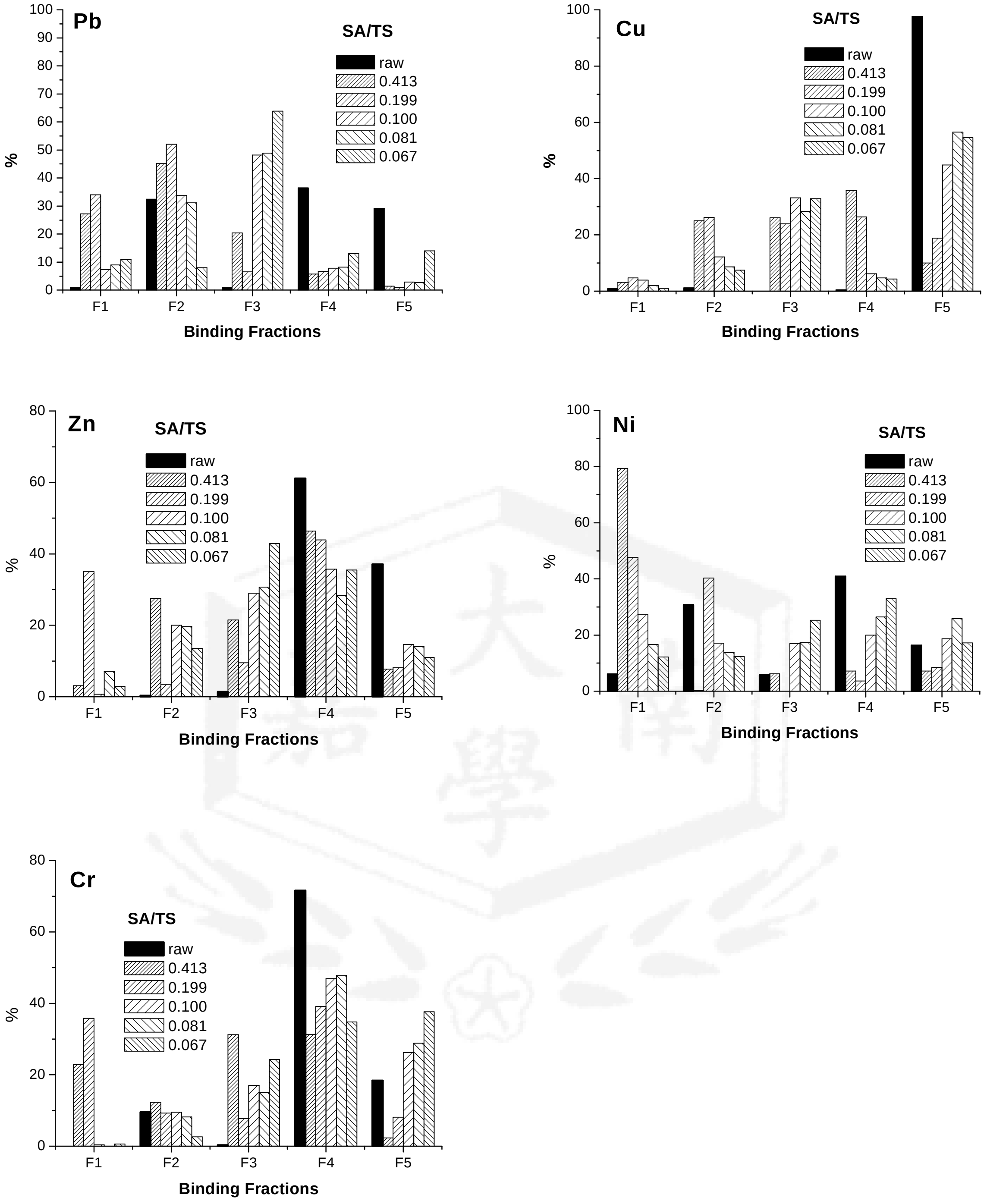

Fig. 8. Effect of SA/TS on the binding distribution of heavy metals. (F1: exchangeable, F2: bound to carbonates, F3: bound to Mn-oxides, F4: bound to Fe-oxides, F5: bound to OM)