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# Environmental investigation of bio-modification of steel slag through microbially induced carbonate precipitation

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## ABSTRACT

Steel slag (SS) is one of byproduct of steel manufacture industry. The environmental concerns of SS may limit their re-use in different applications. The goal of this study was to investigate the leaching behavior of metals from SS before and after treated by microbially induced carbonate precipitation (MICP). Toxicity characteristic leaching procedure, synthetic precipitation leaching procedure and water leaching tests were performed to evaluate the leaching behavior of major elements (Fe, Mg and Ca) and trace elements (Ba, Cu and Mn) in three scenarios. The concentrations of leaching metals increased with the content of SS. After it reached the peak concentration, the leaching concentration decreased with the content of SS. The leachability of all elements concerned in this study was below 0.5%. The carbonate generated from the MICP process contributed to the low leachability of metals. After bio-modified by MICP process, the leaching concentrations of Ba from TCLP, SPLP and WLT tests were below 2.0 mg/L, which was the limit in drinking water regulated by U.S. EPA. The concentrations of Cu leached out from MICP-treated SS-sand samples were below 1.3 mg/L which is the limit regulated by national secondary drinking water. Compared with the regulations of U.S.EPA and Mississippi Department of Environment Quality (MDEQ), MICP-treated samples were classified as non-hazardous materials with respects to the leaching of metals. Meanwhile, maximum contaminant limits regulated by U.S.EPA states that MICP-treated SS are eco-friendly materials that can be reused as construction materials.

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## Introduction

Steel slag (SS) is one of the byproducts of steel industry. Slagging agent is added to trap the impurities from the metal in the steel making process, and then, the slagging agent and

impurities are combined to form SS. According to the report of the U.S. Geological Survey, it is estimated production of SS in the range of 190 million to 290 million tons in 2018. It is estimated that about 7.7 to 8.3 million tons of SS are being beneficially reused each year in the United States. The solution pH of SS is generally in the range of 8 to 10. However, the pH of SS can exceed 11 which can be corrosive to aluminum or galvanized steel pipes in direct contact with slag

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**Table 1 – Chemical components of steel slag and sand (wt.%).**

Components	MgO	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	SO <sub>3</sub>	CaO	TiO <sub>2</sub>	MnO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	NiO
Steel slag	15.92	4.77	23.47	0.97	40.90	0.39	1.64	11.76	0.18
Sand	ND*	<0.1	99.7	ND	ND	ND	ND	<0.1	ND

\* Not detected.

(U.S. Department of Transportation, 2016). The leaching tests also illustrated that SS could potentially release toxic chemical elements (Gomes et al., 2018; FaËllman, 2000). Therefore, the recycling of SS should be in caution, especially for its environmental effects.

Literature study shows the promising future of applying microbiology into the modification of solid waste (Mwandira et al., 2017; Chen and Achal, 2019; Kavamura and Esposito, 2010; Kumari et al., 2014; Jiang et al., 2019). MICP utilizes the bacteria which can enzyme urea to carbonate ions and ammonia, and thus, changing the environmental condition and producing mineral precipitates (Li et al., 2016; Zhao et al., 2014; Wen et al., 2018). Several researchers have successfully applied the urea hydrolyzing bacteria in the solidification and stabilization of metals in solid waste. Mwandira et al. (2017) used MICP technology to bioremediate lead, and their lab-scale experiments have removed 1036 mg/L of Pb<sup>2+</sup> completely. Jiang et al. (2019) illustrated bioimmobilization efficiency within a wide range of Pb concentration. Wang et al. (2018) used MICP technology to immobilize Cd in soil, and their results showed the immobilization efficiency reached 33.14%. Kumari et al. (2014) employed MICP technology to immobilize Cd in soil, and more than 90% of Cd in the tested soil was converted to carbonate-bound fraction from soluble-exchangeable fraction in 14 days. However, there are insufficient literatures refer to the effect of MICP on the modification of SS which holds a complex metal system. Moreover, the leaching behavior of metals in SS is an important factor for the beneficial uses of bio-modified SS.

To investigate the environmental influence of bio-modified SS through MICP, TCLP, SPLP and WLT were conducted. The bio-modification efficiency and the potential environmental effects are evaluated based on the leaching behavior of major elements (Fe, Mg and Ca) and trace elements (Ba, Cu and Mn).

## 1. Materials and methods

### 1.1. Materials

Steel slag used in this study was provided by a local company in Jackson, MS. Particle size distribution of SS has been determined through sieve analysis, and its median sized (D<sub>50</sub>) is about 0.65 mm. The particles which are between 0.425 and 2.0 mm were utilized in this study (Fig. 1a). The chemical components have been detected by Energy Dispersive X-Ray Analysis (EDX). As shown in Table 1, SS consists of 40.90% CaO, indicating its cementation potential. The amounts of SiO<sub>2</sub>, MgO and Fe<sub>2</sub>O<sub>3</sub> in SS are 23.47%, 15.92% and 11.76%, respectively. The pH of SS is 10.12 according to EPA method 9045b-soil and waste pH (U.S. EPA, 1986). Silica sand was used to prepare SS-

sand mixtures. The chemical components in Table 1 indicate that sand consists of 99.7% SiO<sub>2</sub>. SS-sand mixtures were prepared by 0% (sand only), 10%, 20%, 30%, 40%, 50%, 75% and 100% SS on weight basis.

### 1.2. MICP process

*Sporosarcina pasteurii* (ATCC 11,859) was used to perform the MICP process in this study. The nutrition solution for bacteria consists of Tris buffer (pH = 9.0), 10.0 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 20.0 g/L yeast extract. Bacteria liquor was mixed with the autoclaved nutrition solution, and then cultured at 23 ± 2 °C in a shaking bed at 200 rpm. Mature bacteria were harvested after 40 hours' culturing. The optical density at 600 nm (OD<sub>600</sub>) was utilized to illustrate the bacteria concentration as a quick method. The bacteria concentration was adjusted to OD<sub>600</sub> = 0.8 with fresh nutrition solution before MICP process. After agar plate culture, the concentration of bacteria was confirmed to be 7.5 × 10<sup>7</sup> CFU/mL.

A volume of 40 mL bacteria solution was mixed with 150 g SS-sand mixtures which was hold by full contact flexible geotextile mold. The full contact flexible geotextile mold was developed by Zhao et al. (2014) to prepare the MICP-treated soil samples. Then, the samples were placed on the shelf carefully, and immersed into the batch reactor. The cementation media was prepared by 0.5 mol/L CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.5 mol/L Urea, 2.12 g/L NaHCO<sub>3</sub>, 3.0 g/L nutrition broth, and 10.0 g/L NH<sub>4</sub>Cl. The system was aerated with aeration stone. After 7-day reaction, the loosely banded SS and sand particles were cemented together as shown in Fig. 1b. The harvested MICP-treated SS-sand specimens were washed by deionized water (DI) water to remove the surplus cementation media until the electrical conductivity of leachate below 1000 µs. Then, MICP-treated SS-sand specimens were used to perform unconfined compressive stress test. The broken samples were grinded until particle size below 2 mm to investigate their environmental effects.

### 1.3. Environmental effects

#### 1.3.1. Acid digestion test

U.S. EPA method 3050B was used to conduct acid digestion test (ADT) for the determination of metal contents in SS (U.S.EPA, 1996). The strong acid digestion process of this test could make almost all elements environmentally available. In this test, concentrated trace metal grade nitric acid (HNO<sub>3</sub>) and 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were repeatedly added to an aliquot of 1.0 g sample until the general appearance is unchanged. A volume of 10 mL concentrated hydrochloric acid (HCl) was also added. The mixture was covered with a watch

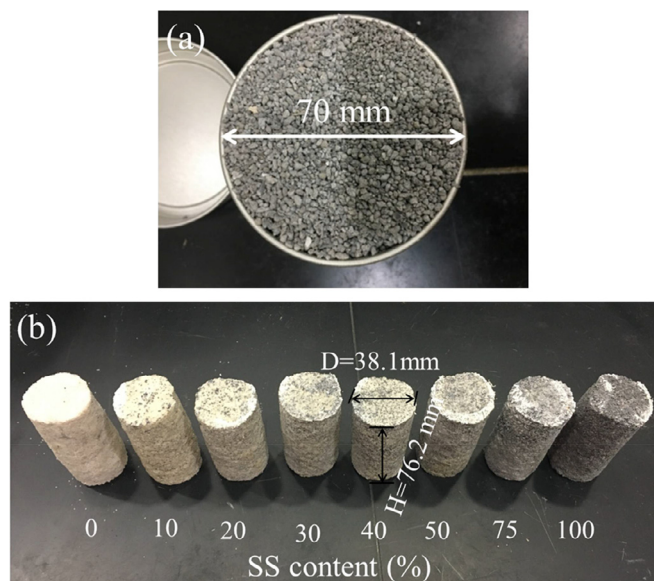


Fig. 1 – Materials used in this study: (a) raw SS, (b) MICP-treated SS-sand samples.

glass, placed on a hot plate and refluxed at  $95 \pm 5^\circ\text{C}$ . The digest was filtered and diluted with DI water to 100 mL for metal analysis.

### 1.3.2. Toxicity characteristic leaching procedure

U.S. EPA method 1311 - Toxicity Characteristic Leaching Procedure (TCLP) (U.S. EPA, 1992) is mainly used to determine whether or not a waste material is hazardous according to its leaching toxicity. It is also used to examine the effectiveness of stabilization of hazardous waste (Li et al., 2009). Some states including Mississippi use the TCLP test results to determine whether an industrial solid waste can be beneficially used (MDEQ, 2005).

TCLP test specifies two kinds of extraction fluids. Extraction fluid #1 is prepared by 5.7 mL glacial  $\text{CH}_3\text{CH}_2\text{OOH}$  and 64.3 mL of 1 mol/L NaOH with DI water to 1 L volume. The pH of this fluid is around  $4.93 \pm 0.05$ . Extraction fluid #2 contains 5.7 mL glacial  $\text{CH}_3\text{CH}_2\text{OOH}$  with DI water to a volume of 1 L with a final pH of  $2.88 \pm 0.05$ .

To determine the appropriate extraction fluid for TCLP, an aliquot of 5.0 g solid waste is weighted to mix with 96.5 mL DI water in 500 mL baker. Then, the mixture is stirred vigorously for 5 min. The pH of slurry is measured and recorded. If the pH is  $< 5.0$ , extraction fluid #1 is used. If the pH is  $> 5.0$ , 3.5 mL 1 mol/L HCl is added to the slurry, heated to  $50^\circ\text{C}$  and hold at  $50^\circ\text{C}$  for 10 min. When the solution cools to  $23 \pm 2^\circ\text{C}$ , the pH is measured and recorded. If pH is less than 5.0, extraction fluid #1 is used. Otherwise, extraction fluid #2 is used. Through the pretest for extraction fluid of TCLP, extraction fluid #2 was used in this study.

An aliquot of 75 g solid samples were submerged in the extraction fluid with liquid-to-solid ratio (L:S) of 20:1 (L/Kg) in a 2 L HDPE bottle. The bottle was then agitated in a rotary extractor at 30 rpm for a period of  $18 \pm 2$  hr. The leachate was filtered through  $0.75 \mu\text{m}$  glass-fiber filter, and then, the pH and ORP were measured and recorded, immediately. Then, the pH

was adjusted to less than 2 for preservation and analysis of concentrations of the concerned constituents.

### 1.3.3. Synthetic precipitation leaching procedure

U.S. EPA Standard Method 1312 was introduced to conduct synthetic precipitation leaching procedure (SPLP) (U.S. EPA, 1994). It was designated to determine the leachability of contaminants in wastes and soils. The extraction fluid consists of 60/40 wt percent mixture of sulfuric and nitric acid to DI water until pH reaches to  $4.20 \pm 0.05$ . This extraction fluid is designated for wastes and soils from a site located east of the Mississippi River. An aliquot of 10 g solid samples were mixed with 200 mL extraction fluid, and then rotated at  $30 \pm 2$  r/min for  $18 \pm 2$  hr. After agitation, the sample was filtered through  $0.6\text{--}0.8 \mu\text{m}$  glass-fiber filter. The pH and ORP of leachates were measured immediately. After that, the pH was adjusted to below 2.0 for metals determination.

### 1.3.4. Batch water leaching test

Water leaching tests (WLT) were performed according to ASTM D 3987 – Standard Test Method for Shake Extraction of Solid Waste with Water (ASTM, 2006). An aliquot of 10 g samples was mixed with DI water at L/S ratio of 10:1. After  $18 \pm 0.25$  h agitation period, the leachate was filtered. pH and ORP of leachate were measured. Then the pH was adjusted with 1 N HCl solution to pH below 2.0 for preservation and determination of metals. The comparisons of TCLP, SPLP and WLT about extraction fluid, solid-to-liquid ratio and extraction time are listed in Table S1.

### 1.3.5. Metal analysis

The metal concentrations in leachate were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (ICPE-9000, Shimadzu, Japan). Commercial produced multi-element standard solutions were used to provide calibration curves. The minimum detection limits ( $\mu\text{g/L}$ ) for metals are: Al (2.5), As (50), B (10), Ca (0.5), Cd (5), Cr (5), Cu (1.5),

Fe ( ), Mg (0.5), Mn (1), Ni (5), V (5), Pb (50), Sn (50) and Zn (1), respectively.

### 1.3.6. Leachability factor

As only certain part of total constituent of metals which is leachable and available for organism that have a toxic or deleterious effect, the leachability factor is employed to illustrate the mobility and availability of metals before and after MICP process. The leachability factor is calculated by the total constituent concentration divided by the leachable constituent concentration, as shown in Eq. (1) (Marshack, 1989):

$$\text{Leachability factor} = \frac{\text{Total constituent concentration}}{\text{Leachable constituent concentration}} \quad (1)$$

### 1.4. Pearson correlation coefficient

The Pearson correlation coefficient can show the strength and direction of the association between two variables. Leaching concentrations from TCLP, SPLP and WLT for the concentration of Ba, Cu, Fe, Mg and Mn, SS content, and pH of effluent were set as the variables. Pearson correlation coefficients were calculated with SPSS 9.0 software. Two-sided P value was used to test the hypothesis. If P value is less than 0.05 and more than 0.01, the correlation between two variables is statistically significant. If P value is less than 0.01, the correlation between two variables is statistically highly significant.

### 1.5. X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was employed to determine the mineral phase of SS. X-ray diffractometer (Regiku Miniflex 600) (UTAR two, PerkinElmer) was used with Cu-K $\alpha$  radiation. The 2 $\theta$  ranged from 10° to 80° with a step width of 1°.

## 2. Results and discussion

### 2.1. Properties of raw steel slag

#### 2.1.1. XRD analysis

XRD results and minerals information of raw SS are shown in Fig. 2. According to XRD analysis, it can be seen that SS was enriched in metals and the main crystalline compounds were silica oxide (SiO<sub>2</sub>), magnesium calcite-synthetic (Mg<sub>0.1</sub>Ca<sub>0.9</sub>CO<sub>3</sub>), copper selenate (CuSeO<sub>4</sub>), magnesioferrite aluminian ((MgAl<sub>0.74</sub>Fe<sub>1.26</sub>)O<sub>4</sub>), calcium gold germanium (CaAu<sub>1.24</sub>Ge<sub>0.76</sub>) and iron silicon oxide (Fe<sub>5.36</sub>Si<sub>0.64</sub>O<sub>8</sub>). These results agreed with the EDX results (in Table 1.) which showed SS consisted of 23.47% SiO<sub>2</sub>, 40.90% of CaO, 15.90% of MgO and 11.76% of Fe<sub>2</sub>O<sub>3</sub>.

#### 2.1.2. Contents and leaching characteristic of metals

Leaching results of raw SS from ADT, TCLP, SPLP and WLT tests are shown in Table 2. The related limits for metals from Mississippi Department of Environmental Quality (MDEQ) and U.S. EPA are also listed in Table 3. The metal contents (mg/kg) of raw SS followed the order of Fe (146,750) > Mg (50,125) > Mn (21,275) > Ca (18,933.3) > Al (9772.5) > Sn (1907.5) > Cr (1797.5) > Zn (408) > Ba (323.5) > V (282.75) > Ni (91.5) > Cd (0.48).

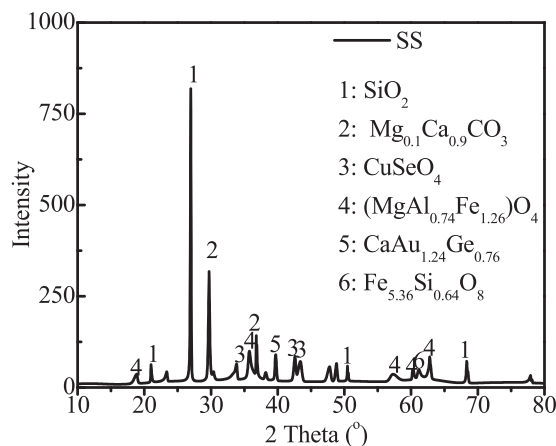


Fig. 2 – XRD patterns of raw SS.

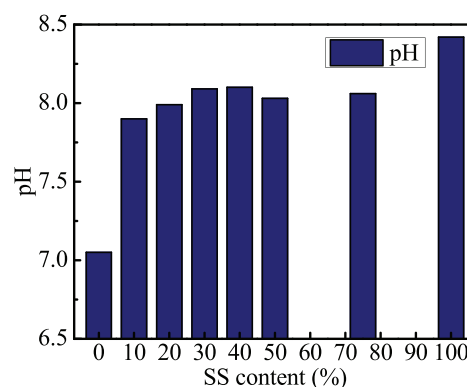


Fig. 3 – pH value of MICP-treated SS-sand samples.

The leaching results from TCLP, SPLP and WLT revealed that As, Be, Cd, Cr, Ni, Pb, Se and Zn were not detectable. The leaching concentration of Ba, Cu and Fe met the regulation thresholds. Al, Ca, Fe, Mg, Mn and Sn are not concerned by any regulations. Therefore, SS can be an eco-friendly material to be recycled.

### 2.2. pH of MICP-treated steel slag-sand mixtures

Effluent pH of solid waste as an important safety criterion has been highlighted in many regulations. If the pH of a waste either higher than 12.5 or lower than 2.0 would be classified as hazardous waste due to the apparent corrosive characteristic based on the results of EPA 9045D (U.S. EPA, 1986). From Fig. 3, the pH value of raw steel slag was 10.12 which meant there is no concern about corrosive. With the treatment of MICP process, the pH value of MICP-treated SS-sand samples were in the range of 6.5–8.5 which met the regulation of U.S.EPA National Secondary Drinking Water. As shown in Fig. 3, the pH value of MICP-treated SS-sand samples increased with the increase of SS content, except samples that containing 50% and 75% SS. As stated by Mahedi et al. (2019), pH of material highly depends on the ratio of CaO and MgO contents and their dissolution rates in the aqueous solution. For the MICP-treated SS-sand samples, SS is the unique origin of CaO and MgO. There-

**Table 2 – Contents and leaching concentrations of raw SS.**

Elements	ADT (mg/kg)	TCLP (mg/L)	SPLP (mg/L)	WLT (mg/L)	EPA <sup>1</sup> (mg/L)	MDEQ <sup>2</sup> (mg/L)	MCL <sup>3</sup> (mg/L)
Al	9772.5	ND <sup>4</sup>	0.289	0.269	NA <sup>5</sup>	NA	NA
As	ND	ND	ND	ND	5.0	0.5	0.01
Ba	323.5	1.41	0.543	0.553	100	10	2
Be	ND	ND	ND	ND	NA	NA	0.004
Ca	18,933.3	81.7	73.9	40	NA	NA	NA
Cd	0.48	ND	ND	ND	1.0	0.1	0.005
Cr	1797.5	ND	ND	ND	5.0	0.5	0.1
Cu	201	0.112	0.832	0.124	NA	NA	1.3
Fe	146,750	0.0142	0.0306	0.125	NA	NA	NA
Mg	50,125	88.2	0.304	0.943	NA	NA	NA
Mn	21,275	0.761	ND	0.0345	NA	NA	NA
Ni	91.5	ND	ND	ND	NA	NA	NA
Pb	ND	ND	ND	ND	5.0	0.5	0
S	5927.5	23.1	IA <sup>6</sup>	48.3	NA	NA	NA
Se	ND	ND	ND	ND	1.0	NA	0.05
Sn	1907.5	4.76	ND	ND	NA	NA	NA
V	282.75	ND	ND	0.0582	NA	NA	NA
Zn	408	ND	ND	ND	NA	NA	NA

<sup>1</sup> EPA: U.S. EPA Regulatory levels for classifying a waste as hazardous based on TCLP results.

<sup>2</sup> MDEQ: Mississippi Department of Environmental Quality (MDEQ) regulatory levels for beneficial use of solid waste.

<sup>3</sup> MCL: Maximum Contaminant Level (MCL) - the highest level of a contaminant that is allowed in drinking water according to National Primary Drinking Water Regulation.

<sup>4</sup> ND: Not detected.

<sup>5</sup> NA: Not available.

<sup>6</sup> IA: Inappropriate.

**Table 3 – Leaching concentrations of MICP-treated SS-sand samples in TCLP leachates.**

Metal	SS content (%)								EPA <sup>1</sup>	MDEQ <sup>2</sup>
	0	10	20	30	40	50	75	100		
Ag	ND <sup>3</sup>	ND	ND	ND	ND	ND	ND	ND	5.0	0.5
Al	ND	0.384	ND	ND	ND	ND	ND	ND	NA <sup>4</sup>	NA
As	ND	ND	ND	ND	ND	ND	ND	ND	5.0	0.5
Ba	0.452	0.697	0.796	0.480	0.440	0.023	0.039	0.048	100	10
Ca	55.2	66.8	75.9	57.5	50.5	3.8	6.48	6.23	NA	NA
Cd	ND	ND	0.003	0.009	0.001	ND	ND	ND	1.0	0.1
Cr	ND	0.020	0.002	0.001	0	ND	ND	ND	5.0	0.5
Cu	0.120	0.214	0.127	0.188	0.116	0.173	0.057	0.030	NA	NA
Fe	1.05	7.35	2.32	0.182	0.689	0.0045	0.0011	0.0006	NA	NA
Mg	4.25	36.3	45.6	66.9	55.1	4.41	12.0	15.8	NA	NA
Mn	2.05	8.06	7.83	6.00	6.83	0.314	0.676	0.680	NA	NA

<sup>1</sup> EPA: U.S. EPA Regulatory levels for classifying a waste as hazardous based on TCLP results.

<sup>2</sup> MDEQ: Mississippi Department of Environmental Quality (MDEQ) regulatory levels for beneficial use of solid waste.

<sup>3</sup> ND: Not detected.

<sup>4</sup> NA: Not available.

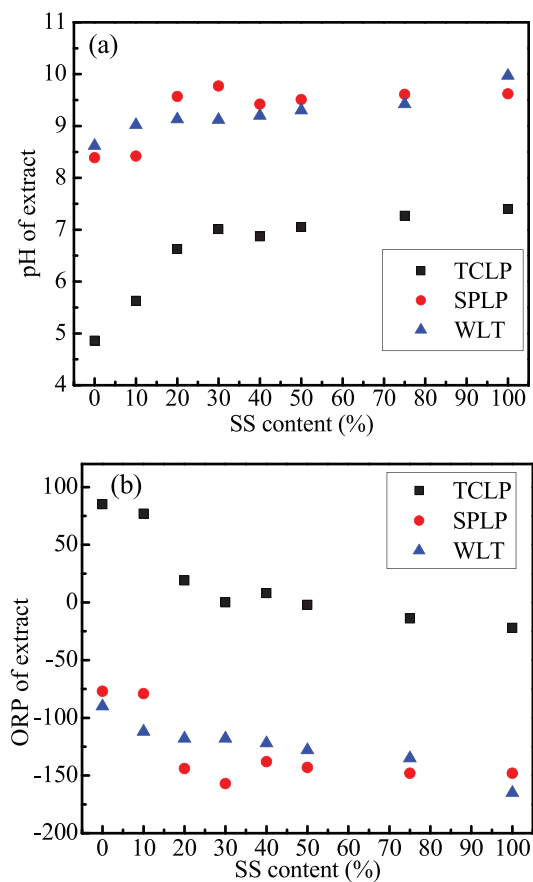
fore, it is reasonable that the pH of MICP-treated SS-sand samples were highly associated with the content of SS.

### 2.3. pH and redox potential (Eh) of extract of TCLP, SPLP and WLT tests

The effluent pH could greatly influence the leaching behavior of metals, and thus, pH is an important factor to analyze the leaching trend of metals. Fig. 4a indicates that the effluent pH of MICP-treated SS-sand samples increased significantly with the increase of SS content. When the SS content

was higher than 30%, the effluent pH of MICP-treated SS-sand sample kept steady which means there was a dynamic equilibrium between H<sup>+</sup> provided by extraction fluid and alkalinity of MICP-treated SS-sand samples. The final pH was near 7.0, for TCLP effluent, 9.6 for SPLP effluent and 9.7 for WLT effluent. Compared with the original pH of extraction fluid, the huge increase of pH illustrates the strong acid neutralization characteristic and ability to resist acid corrosion for MICP-treated SS-sand samples.

Redox potential (Eh), also known as oxidation–reduction potential, is defined as the potential of a solution or envi-



**Fig. 4 – pH and Eh of TCLP, SPLP and WLT effluents for MICP-treated samples: (a) pH, (b) Eh.**

ronment to engage in either oxidation or reduction reactions (Zhang et al., 2016). The Eh of MICP-treated SS-sand mixtures are illustrated in Fig. 4b. With the treatment of MICP process, the conditions of SPLP and WLT extract solutions were reductive with the Eh value varying from  $-160$  to  $-60$  mV, while TCLP extract solution was near neutral with the Eh value in the range of  $-20$  to  $100$  mV.

Metals leaching behavior as a function of the pH of TCLP, SPLP and WLT effluents are illustrated in Fig. S1a, S1b, S1c. To clarify the effects of the pH of effluent on metal leaching behavior, correlations of leachate pH and metal concentrations in TCLP, SPLP and WLT tests were calculated based on the Pearson correlation tests as shown in Table S2. Komonweeraket et al. (2015) found that the dissolution of CaO and MgO minerals from fly ashes is considered to contribute to the increase in pH solution while the dissolution of  $B_2O_3$ ,  $Fe_2(SO_4)_3$  and  $Al_2O_3$  constituents in fly ashes decrease the alkalinity of the aqueous solutions. In this study, the coefficients of leaching concentrations of Ba and Mg with the pH of TCLP and SPLP leachate were 0.804 and 0.893, respectively, with a high significance as shown in Table S2, which means the leaching of Ba and Mg from MICP-treated SS-sand samples is highly correlated with TCLP and SPLP leachate pH. The leaching concentration of Cu from MICP-treated SS-sand mixture in TCLP, SPLP and WLT effluents was negatively related to the pH of leachate solution. The leaching concentrations of Fe

and Mn did not show any noticeable correlation to other factors. As Ca presented in this study was not merely from SS, it cannot be treated as the pH-controlling element.

#### 2.4. Metal leaching behavior evaluation

According to U.S. National Primary Drinking Water Regulation, Ba and Cu that may cause concerns to the drinking water system, and thus, both were measured in this study. There are also environmental concerns and the human health hazards associated with the leaching of Fe and Mn. For instance, excess amount of Fe may cause tissue damage (Mahedi et al., 2019). The leaching concentrations of Mg and Ca were also measured for their abundant amounts in raw steel slag, and both may influence the precipitation of other metals.

The leaching concentration of Ag, Al, As, Ba, Ca, Cd, Cr, Cu, Fe, Mg and Mn in TCLP, SPLP and WLT leachate are listed in Tables 3, 4, 5. The selective regulations for TCLP and SPLP leachate are U.S. EPA regulation for classifying a waste as hazardous based on TCLP results and MDEQ regulation for beneficial use of solid wastes based on TCLP results. The regulation compared with WLT results is U.S. EPA regulation for the highest level of a contaminant which is allowed in drinking water. As shown in Tables 3, 4, 5, the leaching concentrations of metals met the regulation limits, which means MICP-treated SS-sand samples are environmentally friendly materials and can be beneficially used. The components detected in WLT effluents were below the regulation of MCL. Although there is no government to determine the solid waste toxic or not based on WLT results, the positive results in this study by comparing the WLT results to MCL limit still could illustrate that MICP-treated samples are environmentally friendly samples.

##### 2.4.1. Major elements

The concentrations of Fe leached from MICP-treated SS-sand samples are shown in Fig. 5a. Komonweeraket et al. (2015) revealed that Fe behaves as amphoteric metals that it can readily dissolve in leachate under both acidic and alkaline conditions. As shown in Fig. 5a, the highest leaching concentration of Fe was 7.35 mg/L obtained by TCLP effluent of MICP-treated sample containing 10% SS. As the content of SS increased, the leaching concentration of Fe decreased. Moreover, the leaching concentrations of Fe in TCLP, SPLP and WLT effluents decreased to near zero, when the contents of SS in MICP-treated samples were over 50%. The increased Fe content in samples and the decreased leaching concentration of Fe in effluents resulted in the low leachability of Fe. From Fig. 5b, it is apparent that the highest leachability of Fe was 0.01% achieved by biomodified sample containing 10% SS. The leachability for the rest of samples was below 0.003%. This phenomenon illustrates negligible proportions of Fe were leachable. Fe in SS might present as amorphous oxyhydroxide, carbonate salt, magnetite, hematite, and glassy matrix. The spinel structures are highly stable and resistant to acid weathering, and therefore, they are not easily released to the environment (Moreno et al., 2005). Moreover, leaching of Fe is highly dependent on the pH variation of extraction fluid. Kim et al. (2003) stated that less than 10 mg/kg of Fe are leachable in near neutral and alkaline solutions. As shown in Fig. 3, the pH of samples modified by MICP process are higher than

**Table 4 – Leaching concentrations of MICP-treated SS-sand samples in SPLP leachates.**

Metal	SS content (%)								EPA <sup>1</sup>	MDEQ <sup>2</sup>
	0	10	20	30	40	50	75	100		
Ag	ND <sup>3</sup>	ND	ND	ND	ND	ND	ND	ND	5.0	0.5
Al	ND	ND	ND	ND	ND	ND	ND	ND	NA <sup>4</sup>	NA
As	ND	ND	ND	ND	ND	ND	ND	ND	5.0	0.5
Ba	0.088	0.119	0.140	0.165	0.164	0.149	0.169	0.175	100	10
Ca	2.76	4.27	4.48	4.67	5.57	4.71	5.31	4.50	NA	NA
Cd	ND	0.032	0.044	0.062	0.068	0.069	0.080	ND	1.0	0.1
Cr	ND	ND	ND	ND	ND	ND	ND	ND	5.0	0.5
Cu	0.029	0.110	0.279	0.293	0.203	0.193	0.212	0.181	NA	NA
Fe	0.028	0.032	0.028	0.190	0.061	0.013	0.032	0.026	NA	NA
Mg	4.25	36.3	45.6	66.9	55.1	4.41	12.0	15.8	NA	NA
Mn	2.05	8.06	7.83	6.00	6.83	0.314	0.676	0.680	NA	NA

<sup>1</sup> EPA: U.S. EPA Regulatory levels for classifying a waste as hazardous based on TCLP results.

<sup>2</sup> MDEQ: Mississippi Department of Environmental Quality (MDEQ) regulatory levels for beneficial use of solid waste.

<sup>3</sup> ND: Not detected.

<sup>4</sup> NA: Not available.

**Table 5 – Leaching concentrations of MICP-treated SS-sand samples in WLT leachates.**

Metal	SS content (%)								MCL <sup>1</sup>	
	0	10	20	30	40	50	75	100		
Ag	ND <sup>2</sup>	ND	ND	ND	ND	ND	ND	ND	ND	NA <sup>3</sup>
Al	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA
As	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01
Ba	0.019	0.036	0.180	0.085	0.081	0.104	0.147	0.034	0.034	2.0
Ca	2.42	1.93	1.72	2.26	1.96	1.78	2.19	0.961	0.961	NA
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.005
Cr	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1
Cu	0.095	0.144	0.186	0.209	0.218	0.187	0.190	0.068	0.068	1.3
Fe	0.207	0.021	0.015	0.029	0.087	0.034	0.025	0.025	0.025	NA
Mg	0.519	12.8	14.9	27.1	23.5	18.7	27.9	15.1	15.1	NA
Mn	0.031	0.013	0.013	0.016	0.020	0.012	0.015	0.044	0.044	NA

<sup>1</sup> MCL: Maximum Contaminant Level (MCL) - the highest level of a contaminant that is allowed in drinking water according to National Primary Drinking Water Regulation.

<sup>2</sup> ND: Not detected.

<sup>3</sup> NA: Not available.

7.8. After reaction with extraction fluid, the pH of effluent for TCLP, SPLP and WLT were in near neutral or slight alkalinity condition. These characteristics also promoted the stabilization of Fe in MICP-treated SS-sand samples.

As shown in Fig. 5c, the leaching concentration of Mg is highly related to the extraction fluid. When the content of SS in MICP-treated samples was less than 50%, the concentration of Mg in TCLP effluent were higher than that in SPLP and WLT effluents. However, as the SS content increased continuously, the trend reversed. The leaching concentrations of Mg in TCLP effluents were even lower than that in SPLP and WLT effluents. The highest leaching concentration of Mg was 66.9 mg/L in TCLP effluent obtained by MICP-treated sample containing 30% SS. The concentration of Mg in SPLP effluents presented a highly relation with the SS content in MICP-treated samples, except the sample containing 100% SS. However, the content of releasable Mg in WLT effluent presented a similar trend with that in TCLP effluents. The Mg concentration in WLT effluent increased first, and then decreased with the increase

of SS content. Jones (1995) studied the leaching behavior of Mg from ash, and the results illustrated the release of Mg is pH-dependent. Mg appears to be slightly soluble at acidic pH and insoluble in water and alkalinity reagents. As shown in Fig. 3, the pH of MICP-treated samples which contains more than 30% SS are around 8.0 which present weak alkalinity characteristic. Meanwhile, the effluent of TCLP, SPLP and WLT reached to neutral or alkalinity conditions. These conditions all contributed to the low leaching concentrations of Mg from samples containing more than 30% SS.

Dudas (1981) investigated the leaching of Mg in a column leaching test, and the results revealed that the leaching of Mg was enhanced as Ca was progressively washed out. Querol et al. (2001) also observed the leaching of Mg over time linked to the leaching of Ca. Compared with the leaching behavior of Ca in this study as shown in Fig. 5e, the leaching of Mg over SS contents presented a lag trend to that of Ca leaching. Leaching concentration of Ca increased first, reached the maximum value at samples containing 20% SS, and then de-

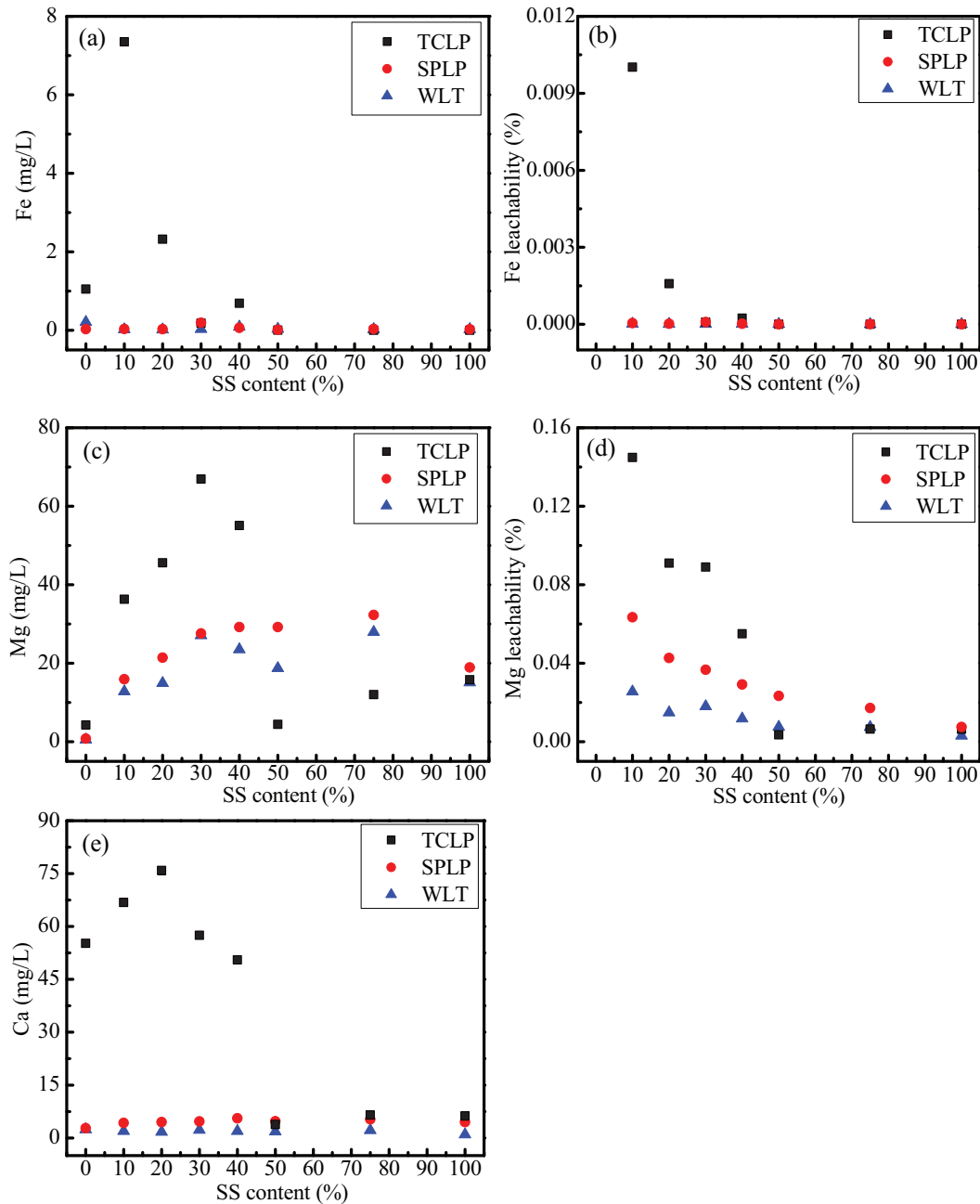


Fig. 5 – Leaching behavior of Fe, Mg and Ca for MICP-treated SS-sand samples.

creased when SS content increased continuously. While the leaching of Mg reached its peak point at samples containing 30% SS, and then decreased. It is confirmed with the conclusion of Dudas and Querol et al. researches (Dudas, 1981; Querol et al., 2001). The relative leachability of Mg in TCLP, SPLP and WLT effluents for samples containing different SS decreased as the increase of SS. The leachability of Mg is below 0.16%, which means most of Mg was sealed in MICP-treated SS-sand samples.

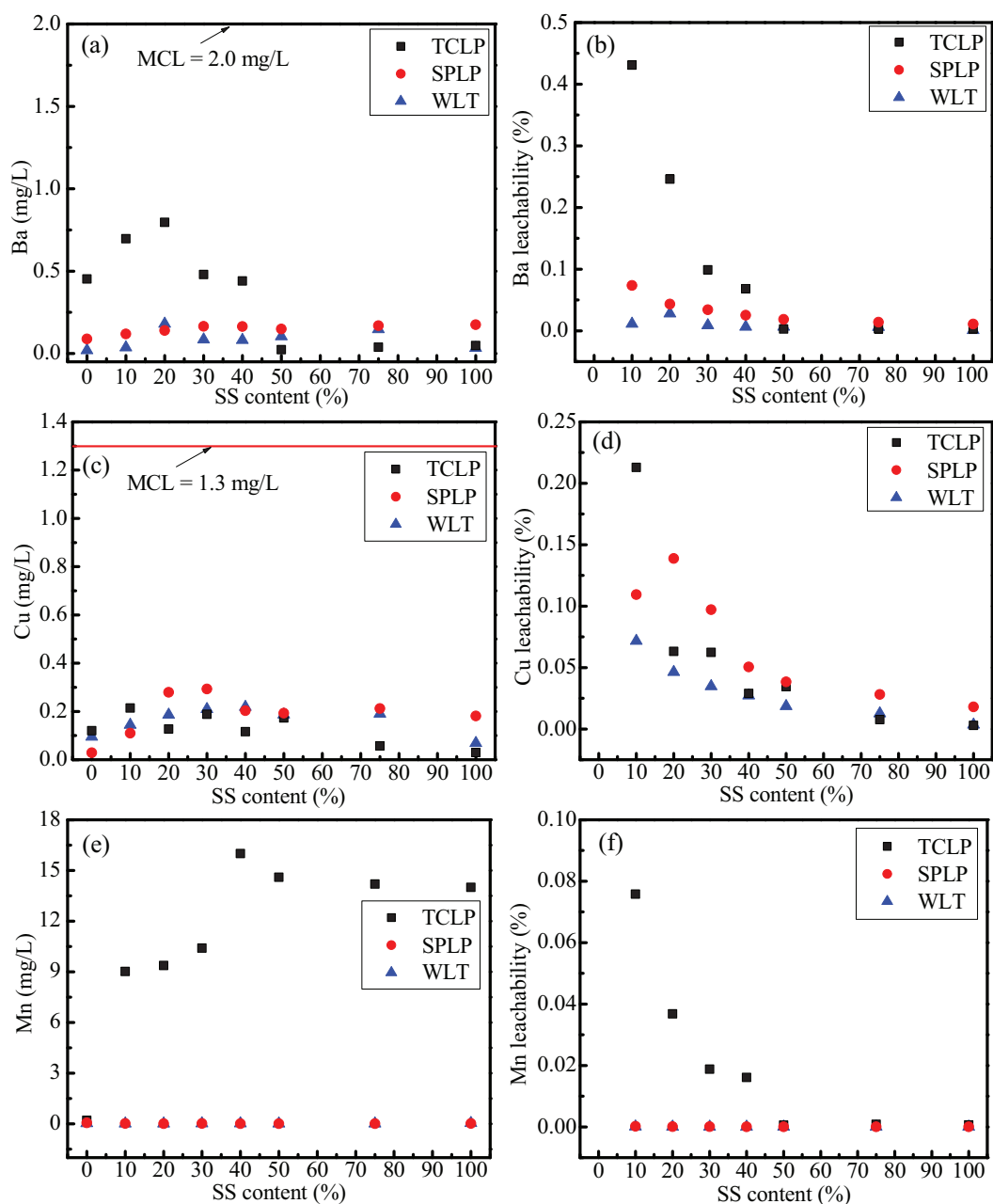
Former researches indicated that Ca in SS might present in multiple mineral phases, primarily lime, anhydrite, calcite and glassy matrix (Kim et al., 2003). Ca is the most readily released cation regardless of the extraction fluid used. As shown in Table 2, the concentration of Ca for raw SS in TCLP, SPLP

and WLT leachates were 81.7, 73.9 and 40.0 mg/L, respectively. Considering the high amount of Ca in raw SS, its leachability in TCLP, SPLP and WLT test were about 8.63%, 7.81% and 2.11%, respectively. Despite of the different extraction fluids were used in TCLP, SPLP and WLT tests, similar trend in leaching behavior of Ca were observed. It sounds meaningless to calculate the Ca leachabilities of MICP-treated SS-sand samples considering the CaCl<sub>2</sub> as one of the reagents in reaction solution.

2.4.2. Trace elements

The leaching behaviors of Ba from MICP-treated SS-sand samples were demonstrated in Fig. 6. For raw SS, the leaching concentrations of Ba in TCLP leachate solution were higher than





**Fig. 6 – Leaching behavior of Ba, Cu and Mn for MICP-treated SS-sand sample.**

that in SPLP and WLT extract. Several researchers revealed that high CaO, MgO and Fe-complexing oxides content could form calcium silicates lattice (Mombelli et al., 2016). And Ba could bind to this compound as  $\beta$ -Ca<sub>2</sub>SiO<sub>4</sub> (Izquierdo et al., 2012; Mombelli et al., 2016). Due to high reactivity of calcium silicate with water, bounded Ba was released, and thus enhanced its leachability.

After bio-modified by MICP process, the leaching concentrations of Ba from TCLP, SPLP and WLT tests were below 2.0 mg/L, which was the limit in drinking water regulated by U.S. EPA. As shown in Fig. 6b, leachability of Ba from MICP-treated SS-sand samples decreased with the increase of SS content. The high carbonate ions produced in MICP process could capture the dissociative Ba ions and formed BaCO<sub>3</sub>.

Leachability of Ba in WLT effluents for MICP-treated sample containing 20% SS increased, but it is acceptable considering the pretty low values.

The limit regulated by national secondary drinking water for Cu is 1.3 mg/L, while concentrations of Cu leached out from MICP-treated SS-sand samples were below this value. With the increase of SS content in MICP-treated samples, the leaching concentrations of Cu increased. The maximum leaching concentration of Cu achieved by MICP-treated sample containing 30% SS. When SS percent increased continually, leaching concentration of Cu slightly decreased and fluctuated in the range of 0.05 to 0.15. The concentrations of leached Cu were not directly related to the ratio of SS. This trend was also observed by Chen et al. (2012) which studied

the leaching behavior of Cu from recycled construction aggregates (RCA) based on the results of pH-dependent tests. Engelsen et al. (2010) found that leaching of Cu was controlled by the dissolution/precipitation of tenorite (CuO) and malachite ( $\text{Cu}_2(\text{CO}_3)(\text{OH})_2$ ) in fly ash. Moreover, Cu has a very high affinity to organic ligands. Former researches showed existing dissolved organic carbon (DOC) could enhance the leachability of Cu under alkaline conditions (Mahedi et al., 2019; Engelsen et al., 2010). During MICP process, organic ligands were produced through bacteria metabolism, and the high affinity between organic ligands and Cu forced the Cu dissociate from the solid phase of MICP-treated samples. Therefore, the leachability of Cu increased. However, as the SS content increased continually, the limited organic ligands could not provide enough attaching points for Cu. The ratio of Cu released through the bioleaching mechanism decreased. Meanwhile, produced  $\text{CO}_3^{2-}$  and high alkalinity of MICP reaction solution promoted the form of  $\text{CuCO}_3$  and  $\text{Cu}(\text{OH})_2$ , and thus the leaching concentrations and leachabilities decreased.

Leaching behavior of Mn in TCLP, SPLP and WLT leachates are shown in Fig. (e, 6f). It is apparent that the concentrations of Mn in SPLP and WLT effluents were very low, while the concentrations in TCLP effluents were high. State-of-the-art illustrated that Mn compounds are insoluble under near neutral to alkaline conditions (Kukier et al., 2003; Querol et al., 2001). The concentrations of Mn in TCLP effluents were not directly related to the content of SS. The highest Mn concentration was achieved by samples containing 40% SS. Costa et al. (2016) studied the leaching behavior of Mn in SS, and found the release of Mn was controlled by Mn carbonates, either in the amorphous or in the crystalline form. During the MICP process, carbonate was produced and it might bond with the dissociated Mn in solution, and thus decreased the leachability of Mn.

### 2.5. Pearson correlation analysis

As shown in Tables S3, S4 and S5, SS content was negatively related to the leaching concentrations of metals in TCLP leachate. Leaching concentration of Ba was positively related to the concentration of Ca and Mn. Meanwhile, the leaching concentration of Ca was highly and positively related to the leaching concentration of Mn. That coincides with the results illustrated in Section 3.4. SS content was positively related to the leaching of Ba in SPLP leachate, which means the released Ba in SPLP leachate increased with the increase of SS content. Considering that Ca was not merely from SS, and reaction solution was also an important source, this correlation was negligible. Meanwhile, the correlations of SS content and the concentrations of Cu, and Fe were negative. And the correlation between SS content and concentration of Mg was positive. But these correlations were not significant. It means the leaching concentrations of Cu, Fe and Mg were not controlled by the total mass in samples. There are more complicated mechanisms behind the leaching process.

Fruchter et al. (1990) reported that Ba formed sparingly soluble compounds with carbonates and sulfates in fly ash. And its leachability was not pH-dependent, but was rather controlled by the ubiquitous Ca due to the competition for sulfates. As shown in Table S4, released Ba in SPLP leachate was

positively related to the dissociated Ca ions in the leachate, even though it was a moderate correlation. Due to the sufficient  $\text{CO}_3^{2-}$  produced in MICP process, the competition pressure for  $\text{SO}_4^{2-}$  between Ba and Ca weaken. Meanwhile, leaching concentrations of Mg were also strongly correlated to Ca concentrations. The correlation of Ca and Cu was negative and significant.

In WLT leachate, the correlation between SS content and metals were different from that in SPLP effluent. There was no metal leaching significantly correlated to SS content. It means SS content has little effect on the leaching of metals under water erosion pressure. The correlation of Ba and Cu and the correlation of Ba and Mg were positive and significant. The Pearson correlation coefficient between Fe and Mn was 0.905, which is statically significant. It indicated that the leaching concentration of Mn might promote the leaching of Fe.

## 3. Conclusions

The aim of this study focused on the environmental effects of MICP-treated SS-sand samples. TCLP, SPLP and WLT tests were performed to simulate three scenarios and to present the leaching behaviors of bio-modified samples. The leaching concentrations of Ba, Ca, Cu, Fe, Mg and Mn under TCLP, SPLP and WLT tests were analyzed, and their leachability were calculated to illustrate the work of MICP process. Meanwhile, the effects of SS content and the potential mutual influence of metals were also discussed based on Pearson correlation coefficient. The following conclusions were obtained in this study:

- 1) After modified by MICP, the pH values of SS-sand samples were in the range of 6.5 to 8.5 which is acceptable according to U.S. EPA Secondary Drinking Water Regulation compared to 10.12 of raw SS.
- 2) Produced carbonate in MICP process provided the possibilities for metals to precipitate. The low solubility product made metals deviated from the solution, especially for Ba, Ca.
- 3) The byproduct produced by bacteria introduced the bioleaching technology. The release of Cu was influenced by the bioleaching.

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## Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.08.023.

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