Gazi University Journal of Science
GU J Sci
25(2): 363-370 (2012)

ORIGINAL ARTICLE

Assessment of Metal Pollution in Contaminated Sediment after Bioleaching with Different Solid Contents

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Received: 18.07.2011 Revised: 07.12.2011 Accepted: 14.12.2011

ABSTRACT

The effect of solid concentration on bioleaching of metals from sediment was tested in this study in order to assess the Cr, Cu, Pb, and Zn pollution levels after the process. The solid contents of 10, 5, and 2.5% (w/v) were used in the bioleaching tests. After 48 days of flask experiments, Zn, Cr, and Cu, solubilizations were over 90% with 2.5% solid content. The metal pollution remained in the sediment after bioleaching is assessed by using the Pollution Index (PI) due to the values of Ecotox Thresholds of EPA. Bioleaching with 5% and 2.5% solid contents provided low PI values as 0.88 and 0.5 at the end of the experimental period.

Key Words: *sediment, heavy metals, bioleaching, Acidithiobacillus thiooxidans, pollution index.*

1. INTRODUCTION

Sediments of rivers, lakes and estuaries in large number of locations have been contaminated by inorganic and organic materials. Among the inorganic materials, metals are frequent and important contaminants in aquatic sediments [1]. Heavy metals are transported as either dissolved species in water or as an integral part of suspended solids. They may be volatilized to the atmosphere or stored in riverbed sediments [2]. They can remain in solution or in suspension and precipitate on the bottom or be taken up by organisms [3]. That is why; metal contaminated sediments deserve special consideration among the environmental issues.

Bioleaching process, which is adapted from the mining industry for use in metals remediation, can simply be described as the solubilization of metals that is based on

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[♠] Corresponding author, e-mail: duyusen.kokulu@deu.edu.tr the activity of the chemolithotrophic bacteria mainly *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Under aerobic conditions, the bacterial activity of Thiobacilli leads to the production of sulfuric acid, extracting metals from the sediment, or to the direct solubilization of metal sulfides by enzymatic oxidation stages [4]. A variety of bioleaching processes have been successfully applied to remove heavy metals from sewage sludges, contaminated soils and sediments [5,6,7,8,9].

The bioleaching of heavy metals from contaminated soils, sediments, and sludge is a complex process. It depends largely on the efficiency of microorganisms and maximum extraction of the metals can be achieved when the leaching conditions correspond to the optimum growth of the bacteria. Besides, various physicochemical and biological parameters have effects on the bioleaching process. Among them, solid content, the initial pH, oxidation-reduction potential (ORP), nutrients, substrate concentration are of particular importance and their influences on bioleaching efficiency have been studied in the literature [10, 11, 4, 6, 12].

Solid content is an important factor influencing heavy metals bioleaching efficiency because of the abrasion of solid particles on the cell membrane. In addition, solid content affects the buffering capacity which causes the pH variations in the system. The current study aims to assess the Cr, Cu, Pb and Zn concentrations remained in the sediment after bioleaching with different solid concentrations. Single culture of *Acidithiobacillus thiooxidans* were used in the experiments depending on its high ability of oxidizing sulfur to sulfuric acid (Akinci and Guven, 2011) [13]. The changes in the chemical distribution of the metals remained in the sediments were also measured in order to make a more realistic estimate of the bioleaching efficiencies depending on the solid/liquid ratio. In Turkey, there are no legal obligations related to the sediment quality criteria presenting the limit levels of organic and inorganic contaminants in aquatic sediments. Therefore, the sediment quality values of Ecotox Threshold published by EPAǿs Office of Solid Waste and Emergency Response (OSWER) are used to assess the pollution level after bioleaching.

2. EXPERIMENTAL

2.1. Sediment

The sediment sample was taken from a stationary point in the middle of Izmir inner Bay located on the eastern zone in the Aegean Sea and known to be polluted by high concentrations of heavy metals [14]. A Van Veen Grab sampler was used for sediment sampling. A ten centimeter thick top layer of the sediment sample was taken by the spatula in order to determine the recent pollution level. The sample was then wet sieved through 2 mm, dried, homogenized, and deposited in a plastic bag to be used in bioleaching experiments.

The pH of the sediment sample was determined according to the EPA Method 9045 C [15]. Sediment water content was determined by drying the wet samples overnight at 105 ^{0}C. The dried samples were combusted at 550 ^{0}C for two hours in order to detect the organic matter content [16].

Total heavy metals in the sediment sample were extracted with Questron MicroPrep Q20 Microwave Digestion System by using the acid mixture of concentrated HNO_3, HF, and HCl. A simple program was used in order to digest the solid samples and the program was tested with Estuarine Sediment-1646-A as the standard reference material [17]. The chemical partitioning of Cr, Cu, Pb and Zn were determined by using the BCR Extraction Procedure which is developed by European Commission for Standards, Measurement and Testing [18]. The technique includes the applications of acetic acid (CH_3COOH), hydroxylamine hydrochloride ($NH_2OH.HCl$), hydrogen peroxide (H_2O_2), and ammonium acetate (CH_3COONH_4) solutions as the extracting agents and the processes of shaking, centrifugation and washing were applied to the sample in each step of the extraction procedure [19]. This method allows the determination of metals bound as four fractions which are exchangeable and acid soluble, reducible (bound to iron-manganese oxides), organic (oxidizable) and residual forms.

2.2. Microorganisms

The pure culture of *Acidithiobacillus thiooxidans* (11478), supplied from DSMZ (Deutche Sammlung von Mikroorganismen und Zellkulturen GmbH), was used in this study. The liquid media-medium 271 was used to cultivate the bacteria [20]. The indigenous *Acidithiobacillus thiooxidans* were inoculated in shaking incubator at 170 rpm under 30^{0}C for maintaining subcultures.

2.3. Bioleaching Tests

Laboratory-scale bioleaching experiments were carried out in 1000 ml Erlenmeyer flasks prepared with a working volume of 250 ml consisting 5% (v/v) growing culture inoculums of the bacteria obtained from the acclimation process. The solid content in the flasks were arranged as 10%, 5%, and 2.5% (w/v). The sulfur concentration added to the suspension was kept constant as 0.5 % (w/v). The initial pH in the reaction mixtures were adjusted to 4±0.4 by using dilute H_2SO_4. The bioleaching experiments were conducted in a shaking incubator at 170 rpm, and the temperature was maintained at 30^{0}C. The process was monitored by periodic sampling and analysis of the mixture for pH, ORP, sulfate and soluble Cr, Cu, Pb, and Zn. Sulfate and soluble metals were measured once a week where pH and ORP values were monitored every other day.

The concentrations of metals remained in the treated sediment were determined by taking solid samples from the flasks on the 18th, 32nd, and 48th days of the bioleaching tests, in order to control the mass balance in the system and determine the pollution levels remained in the sediment. Besides, the chemical speciations of Cr, Cu, Pb and Zn in the treated sediments were monitored at the end of 48 days to see the changes in binding fractions. The control set without any bacteria addition was conducted parallel with the experiments with 5% solid content. For precision, all analyses were duplicated in the two parallel bioleaching experiments and the mean values of the parameters were reported.

The analyses of heavy metals in solution and in the sediment extracts were conducted by Perkin Elmer Optima 4300 DV ICP-OES. Sulfate ions in the suspensions were analyzed by using Dionex IC-3000 Ion Chromatography System.

3. RESULTS AND DISCUSSION

3.1. Sediment Features

The general characteristics of the sediment sample including the proportions of the chemical distributions of the metals are given in Table 1. The sediment pH was measured as 7.81 and the water content was found as 46.4%. High organic content of the sample indicated (8.8%) the organic pollution of the station where the sediment was taken from. High concentrations of Cr and Zn in the sediment sample depend on the past illegal discharges into the inner Bay from the metal and manufacturing industries and the tanneries. Cu and Pb are widely used in metal and automotive industries and the concentrations of these two metals in the sediments may have been originated from the industrial zones located on the basins of creeks entering the Bay [14].

Table 1. Characteristics of the sediment sample used in bioleaching experiments.

Characteristics	Sediment Sample	Exchangeable Form (%)	Reducible Form (%)	Oxidizable Form (%)	Residual Form (%)
pH	7.81				
Moisture,(%)	46.4				
Total organic matter, (%)	8.8				
Total Cr Concentration, (mg.kg^{-1}±sd)	527±45.6	0.4	1.6	54.0	44.0
Total Cu Concentration, (mg kg^{-1}±sd)	115.4±13.3	2.1	1.6	54.2	42.1
Total Pb Concentration, (mg kg^{-1}±sd)	141.2±13.8	5.8	48.6	28.3	17.3
Total Zn Concentration, (mg kg^{-1}±sd)	565±19.8	27.5	9.7	9.0	43.8

3.2. pH, ORP, and Sulfate Changes in the Sediment Solution

Figure 1 presents the pH, ORP, and sulfate change in the bioleaching flasks. The pH values in suspension dropped from 4 to 0.8 and 0.7 with the solid concentrations of 5% and 2.5% at the end of 48 days. On the other hand, the suspension with 10% solid content reached a higher final pH value as 1.45. The sediment pH dropped to lower values with low solid contents. This is attributed to the higher sediment contents with higher buffering capacity. The ORP values with 5% and 2.5% (w/v) were nearly the same at the end of the bioleaching period, reaching 420-425 mV, finally. On the other hand, the ORP in 10% solid content flask could reach to 378 mV. The ORP values in the bioleaching flasks decreased with the increasing sediment solid content (Figure 1). The lowest sulfate was produced with 10% solid content as 10090 mg.L^{-1} and the highest sulfate production (21659 mg.L $^{-1}$) in the bioleaching flasks was observed with 5% solid content. This situation may be explained with the cell concentrations of the bacteria per solid sediment amount in suspension. In 10% solid content, high pulp density may cause the death of the bacteria and prevent the sufficient solubilization of the metal load and production of higher sulfate. On the other hand, 2.5% sediment solid mass did not contain enough metal load to produce the highest sulfate concentration.

3.3. Metals Solubilized in Suspension

Cr, Cu, Pb and Zn concentrations in suspension are shown in Figure 2. The final solubilization efficiencies of Cr were 65%, 84%, and 92% for the bioleaching flasks with 10%, 5%, and 2.5% sediment solid contents, respectively. The solubilizations of Cu were similar to Cr with 65%, 81%, and 95% according to the decreasing solid content in the flasks. On the other hand, Pb was solubilized to the ratios of 54%, 63%, and 77% where these results for Zn were determined as 77%, 92%, 97% for 10%, 5%, and 2.5% (w/v) sediment solid contents in suspension. Relatively low solubilization ratios were observed for Pb because of its low soluble compound, $PbSO_4$. In contrast to Pb, Zn salts and compounds are soluble in water and high removal efficiencies were observed in the bioleaching media.

For all the metals studied, solubilization efficiencies increased with the decreasing solid content. This finding is compatible with the literature [21, 10]. Previous studies also report that, the mobilization of metals increase with the decreasing solid/liquid ratio. This depends on the increasing solid-liquid interaction area depending on the decreasing solid mass and increasing H^+ ions in suspension.

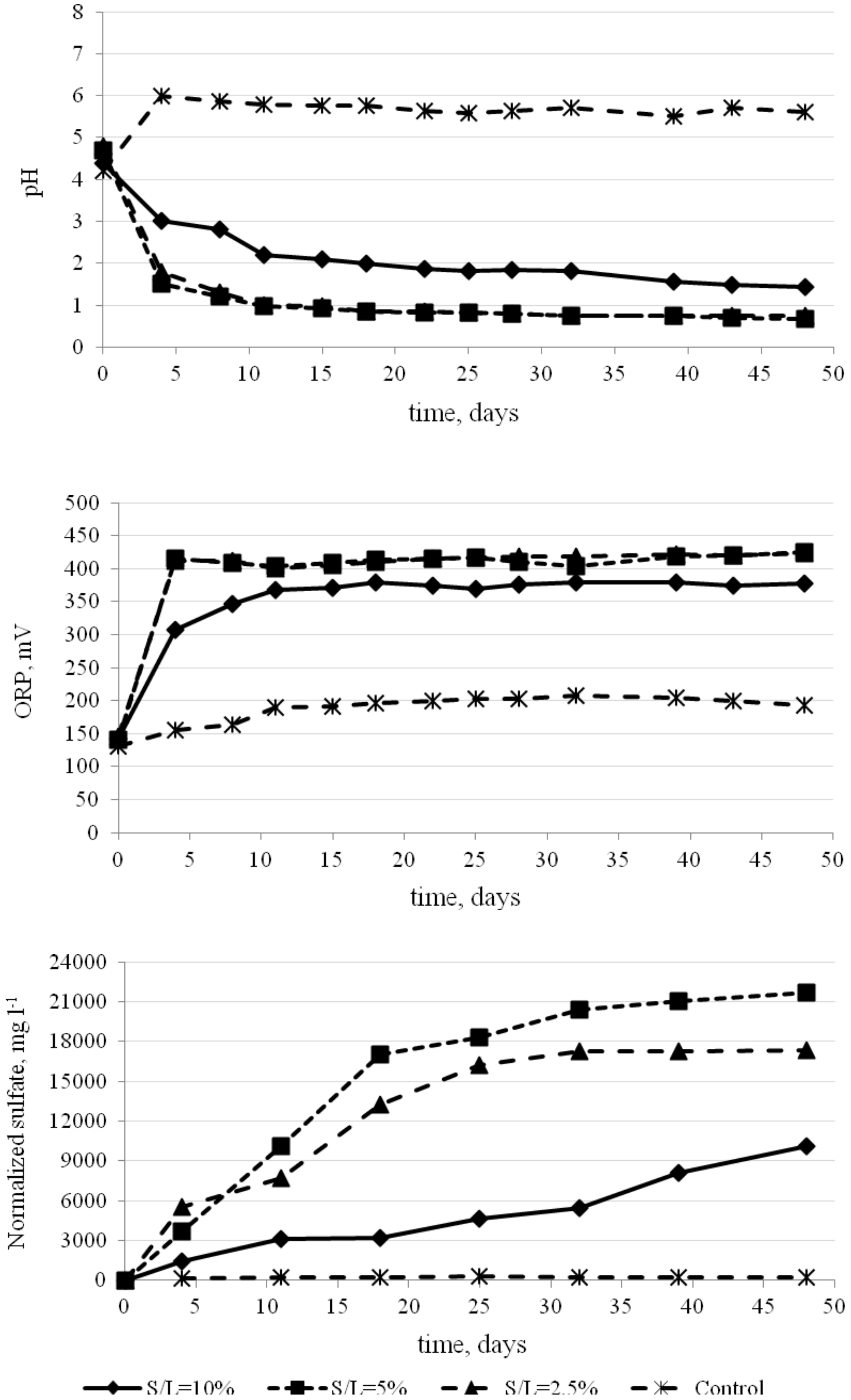

Figure 1. pH and ORP and sulfate change during bioleaching according to the solid content.

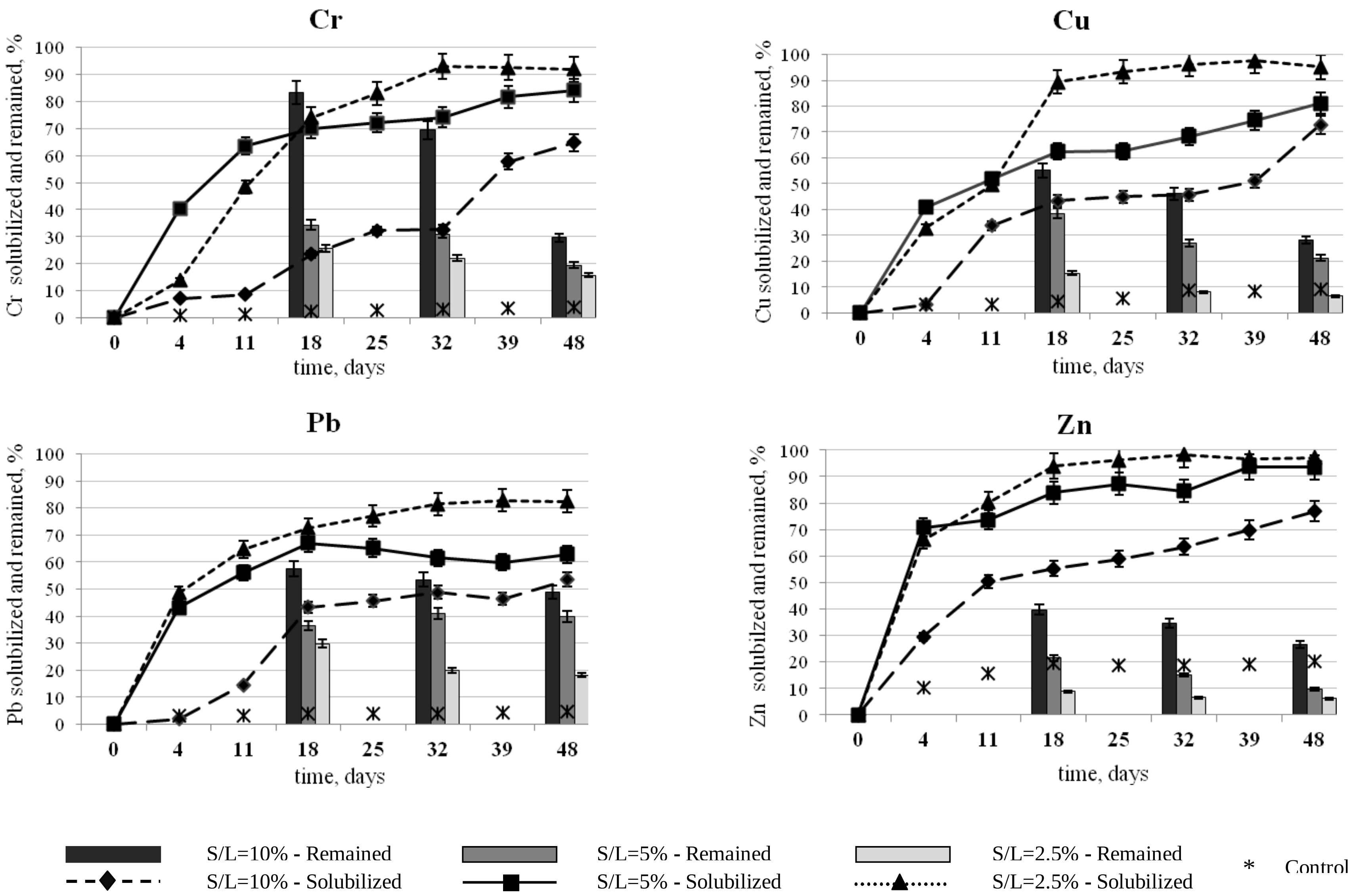

Figure 2. Metals solubilized and remained in the sediment according to solid content.

3.4. Metals Remained in the Sediment and Pollution Index (PI)

One of the factors for pollution assessment in soils and sediments is the pollution index value (PI). PI values have been used by many researchers to estimate the degree of multiple element pollutions [22,23,24]. In this study, the pollution index was calculated by taking the average of the ratios for each metal concentration (mg.kg^{-1}) before and after bioleaching to the limit value given in Ecotox Thresholds (ET) published by EPAôs Office of Solid Waste and Emergency Response. The PI values for 4 elements were calculated by the following equation:

Pollution Index (PI) = [(Cr/81) + (Cu/34) + (Pb/47) + (Zn/150)] / 4

PI values above 1.0 indicate that the sediment or soil samples might be assumed as polluted by anthropogenic effects [24]. In the current study, the metals remained in the solid sediment sample were determined by drawing solid sediment masses from the suspension on the 18th, 32nd, and the 48th days. The metals remained in the sediment were compatible with the metals solubilized in bioleaching suspensions (Figure 2). Table 2 presents the heavy metal concentrations remained in the sediment after bioleaching with different solid contents and the limit values given in ET. It is seen that bioleaching with 10% solid content could not decrease Cr and Pb concentration below ET values where 2.5% of solid content in bioleaching flasks could achieve sufficient removal for all the metals studied. The pollution index values were calculated by considering all the metal concentrations remained in the sediment sample (Figure 3). At the beginning of the experiments, the PI was found to be 4.17 which indicated extreme metal pollution of the sediment sample taken from the stationary point. On the 18th day, bioleaching with 2.5% solid content provided sufficient solubilization when compared to the higher solid contents and the PI was found as 0.85. On the 32nd day of the experiments, there were slight decreases in the PI values for all the bioleaching tests. At the end of 48 days of bioleaching period, the PI values 10%, 5%, and 2.5% solid content were calculated as 1.46, 0.88, and 0.51, respectively.

Table 2. The heavy metal concentrations remained in the sediment sample according to the solid content in bioleaching flasks (mg.kg^{-1}).

	Cr			**Cu**			**Pb**			**Zn**		
	10%	**5%**	**2.5%**	**10%**	**5%**	**2.5%**	**10%**	**5%**	**2.5%**	**10%**	**5%**	**2.5%**
t= 18 days	439	181	135	64	44	18	81	64	42	185	122	50
t=32 days	366	163	116	53	43	9	76	59	28	161	85	36
t=32 days	209	102	81	33	24	8	69	56	26	124	55	35
Ecotox Thresholds	81			34			47			150		

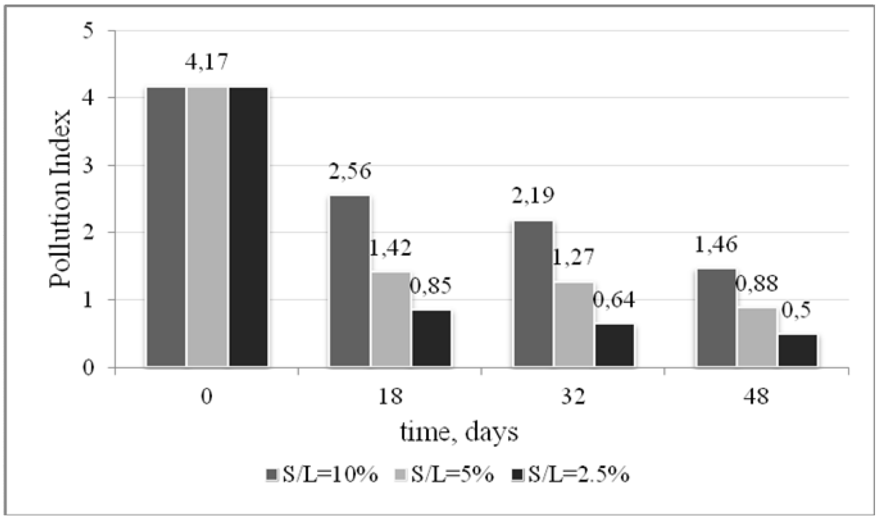

Figure 3. The pollution index values due to the metals remained in the sediment sample.

3.5. Changes in Chemical Distributions after Bioleaching

Figure 4 shows the changes of metal concentrations in the binding fractions of sediments with different solid contents. In this study, no significant changes depending on solid content were observed in chemical distribution behavior of the metals. For all the solid concentrations in suspensions, Cr and Cu concentrations in residual fraction decreased in the highest ratios (average 91% and 96%) where concentrations in the exchangeable fraction increased for both Cr and Cu (Figure 4). Another considerable decrease in concentrations of Cr and Cu was observed in the organic fraction. Pb concentrations in reducible fraction decreased from 58 mg.kg^{-1} to 34, 26, and 21 mg.kg^{-1} with 10%, 5%, and 2.5% solid/liquid ratio where a similar situation was observed for the organic fraction (Figure 4). Pb in residual fraction was almost solubilized. Zn is the metal with the highest solubilization ratios and removal efficiencies and Zn concentrations in every chemical binding form showed considerable decreases after bioleaching, only except for the organic fraction. Relatively low decreases (from 52 mg.kg^{-1} to 51, 31, and 17 mg.kg^{-1} due to the decreasing solid content) in Zn concentration were observed in organic form.

4. CONCLUSION

The effect of solid concentration on bioleaching of metals from sediments was tested in this study in order to assess the Cr, Cu, Pb, and Zn pollution remained in the sediment. The solid contents of 10, 5, and 2.5% (w/v) were used in the bioleaching tests. Satisfactory efficiencies were achieved with *Acidithiobacillus thiooxidans.* For Zn, Cr, and Cu, solubilization ratios were over 90% with 2.5% solid content. For all the metals studied, solubilization efficiencies increase with the decreasing solid content. This finding is compatible with the literature.

Sulfate production depending on solid content reached to a maximum value (21650 mg.L^{-1}) with 5% solid content. This situation may be explained with the cell concentrations of the bacteria per solid sediment amount in suspension.

The Cr, Cu, Pb, and Zn concentrations remained in the sediment after bioleaching were assessed by using the Pollution Index and the limit values of Ecotox Thresholds of EPA were used for the calculations. Depending on the extreme metal pollution of the raw sediment, 10% solid content could not maintain sufficient removal to decrease the pollution index below 1. Bioleaching with 5% and 2.5% solid contents provided low PI values as 0.88 and 0.5. Especially, the pollution index was below 1 for 2.5% solid content starting from the 18th day which means this ratio was adequate for Cr, Cu, Pb, and Zn removal from polluted sediment.

Metal concentrations in bounding fractions showed changes after bioleaching. For all the experiments, Cr and Cu were mostly released from the organic and residual fraction with the same ratio and accumulated on the exchangeable fraction after bioleaching. Pb was released from reducible and organic fractions depending on solid content. In addition, nearly all Pb from residual fraction was removed. Zn is the metal with the highest solubility and it was released from all fractions significantly, except the organic form.

ACKNOWLEDGEMENTS

This work was supported by the Research Fund of Dokuz Eylul University (No.03.KB. FEN.018) and by the Turkish State Planning Organization (No. DPT.2003 K120360).

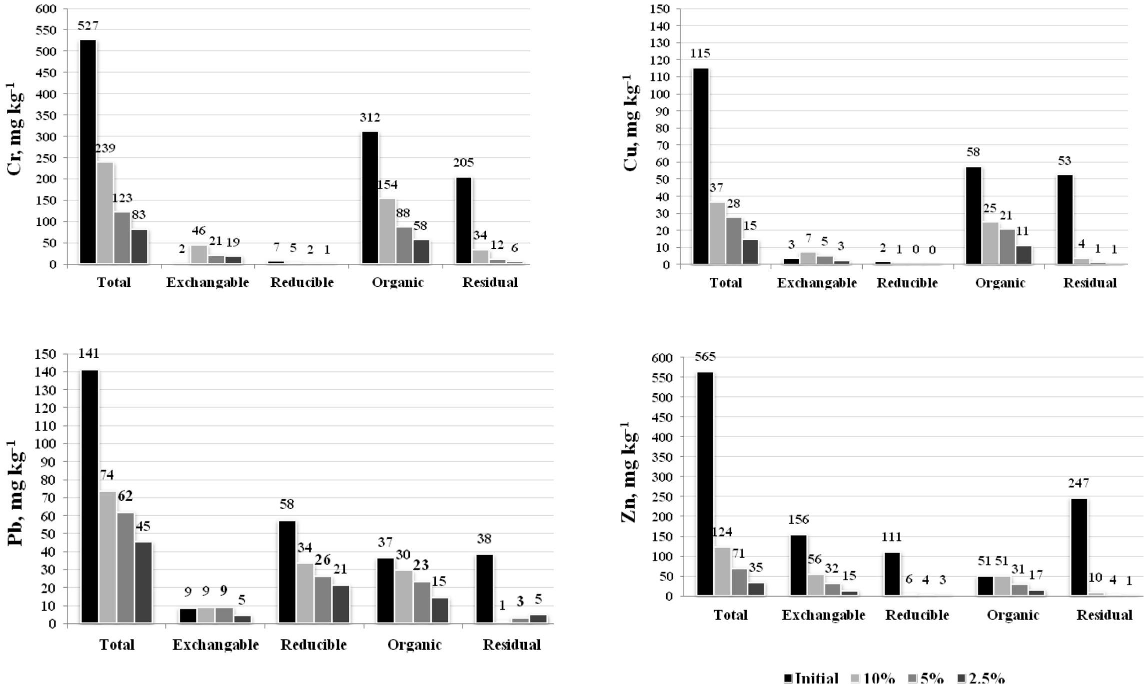

Figure 4. Metals in the chemical binding fractions before and after bioleaching according to the solid content (mgkg^{-1})

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