

**DOKUZ EYLÜL UNIVERSITY**  
**GRADUATE SCHOOL OF NATURAL AND APPLIED**  
**SCIENCES**

**HEAVY METALS BIOLEACHING IN THE**  
**SEDIMENTS OF İZMİR INNER BAY**

by  
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**November, 2008**

**İZMİR**

# **HEAVY METALS BIOLEACHING IN THE SEDIMENTS OF IZMIR INNER BAY**

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Graduate School of Natural and Applied Sciences of Dokuz Eylül University  
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**by  
Elif Duyuşen GÜVEN**

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**İZMİR**

## Ph.D. THESIS EXAMINATION RESULT FORM

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# HEAVY METALS BIOLEACHING IN THE SEDIMENTS OF İZMİR INNER BAY

## ABSTRACT

In recent years, heavy metals pollution in aquatic sediments has gained great importance through environmental concerns. The presented study aims to point out the current heavy metal pollution in the sediments of Izmir inner Bay followed by the remediation of metal contaminated sediments with bioleaching method.

In the first part of the study, sediment samples collected from 7 points in Izmir inner Bay are characterized by means of their bulk selected metals (Cr, Cu, Pb, Zn) concentrations and the chemical distributions of these metals according to their binding fractions are determined. Total metal contents of the sediments are determined by using microwave acid digestion and the BCR Sequential Extraction Scheme is used for determination of metals bound as four fractions: exchangeable, reducible, oxidizable, and residual.

In the second part, bioleaching experiments are conducted in flasks by using the sediment samples. Two major Thiobacilli strains (*Thiobacillus ferrooxidans* & *Thiobacillus thiooxidans*) are used for bioleaching and the effects of bacteria type, solid/liquid ratio, sulfur addition rate (as substrate), and the sediment particle size are studied. The changes in chemical distribution of the heavy metals after bioleaching are also observed and reported.

The characterization studies point out that there is high pollution of heavy metals in the sediments of Izmir inner Bay and the binding forms of metals are different from each other. Bioleaching experiments lasted for 48 days under 30°C. The use of *T. thiooxidans*, optimum solid content, optimum sulfur addition, and fine particles perform satisfactory results for heavy metals removal.

**Keywords:** sediment, İzmir Bay, heavy metals, chemical distribution, remediation, bioleaching, Thiobacilli

# İZMİR İÇ KÖRFEZ SEDİMENTİNDE BULUNAN AĞIR METALLERİN BİYOLOJİK SIZDIRMA YÖNTEMİYLE GİDERİLMESİ

## ÖZ

Sucul sedimentlerdeki ağır metal kirliliği, son yıllarda önem kazanan araştırma konularından biri haline gelmiştir. Sunulan çalışmanın amacı İzmir iç Körfez sedimentlerindeki mevcut ağır metal kirliliğini belirlemek ve bu metallerin kirli sedimentlerden biyolojik sızma yöntemiyle arıtılmasını sağlamaktır.

Çalışmanın ilk kısmında, İzmir iç Körfezi'ndeki 7 farklı istasyondan alınan sediment örneklerinde toplam Cr, Cu, Pb, ve Zn konsantrasyonları tespit edilmiş, daha sonra da bu örneklerin kimyasal bağlanma formları incelenmiştir. Toplam metal konsantrasyonlarını belirlemek için mikrodalga parçalama yöntemi, bağlanma formları için ise BCR Ardışık Ekstraksiyon yöntemi kullanılmış ve değişken, indirgenebilir, organiklere bağlı (oksitlenebilir) ve kalıntı formlara bağlı metallerin konsantrasyonları ve oranları belirlenmiştir.

İkinci bölümde, sediment örnekleri kullanılarak biyolojik sızma deneyleri erlenler içinde gerçekleştirilmiş olup deneylerde *Thiobacillus ferrooxidans* ve *Thiobacillus thiooxidans* türü bakteriler kullanılmıştır. Biyolojik sızma deneylerinde test edilen parametreler bakteri türü, katı/sıvı oranı, sülfür (substrat) miktarı ve partikül boyutudur. Deneylerin sonunda metallerin kimyasal bağlanma formlarındaki değişimler de incelenmiştir.

Karakterizasyon çalışması, İzmir iç Körfez sedimentlerinde yoğun bir metal kirliliği olduğunu ortaya koymaktadır. Metallerin bağlanma formları birbirilerine göre farklılık gösterdiği tespit edilmiştir. Biyolojik sızma deneyler 30 °C'de 48 gün boyunca sürdürülmüştür. Kullanılan bakterilerden *T. thiooxidans*, optimum katı/sıvı oranı ve sülfür ilavesi, ve ince partikül boyutundaki sediment örnekleri ile başarılı sonuçlar elde edilmiştir.

**Keywords:** sediment, İzmir Körfezi, ağır metaller, kimyasal bağlanma formları, giderim, biyolojik sızma, Thiobacilli

# CONTENTS

	Page
THESIS EXAMINATION RESULT FORM .....	ii
ACKNOWLEDGEMENTS .....	iii
ABSTRACT .....	iv
ÖZ .....	v
<b>CHAPTER ONE - INTRODUCTION .....</b>	<b>1</b>
1.1 Problem Statement .....	1
1.2 Purpose of the Presented Study .....	2
<b>CHAPTER TWO – BACKGROUND INFO &amp; LITERATURE REVIEW .....</b>	<b>4</b>
2.1 Heavy Metals in Sediments.....	4
2.2 Heavy Metals Transport in Aquatic Environments.....	4
2.3 Heavy Metals in Natural Levels.....	5
2.4 Most Common Heavy Metals in Aquatic Sediments.....	7
2.4.1 Lead (Pb).....	7
2.4.2 Chromium (Cr).....	8
2.4.3 Zinc (Zn) .....	9
2.4.4 Cadmium (Cd).....	9
2.4.5 Copper (Cu).....	9
2.4.6 Mercury (Hg) .....	10
2.5 Chemical Distribution (Speciation) of Metals in Sediments.....	10
2.6 Heavy Metal Pollution in Izmir Bay .....	12
2.7 Remediation Technologies for Metal Contaminated Soils and Sediments.....	15
2.7.1 Isolation Technologies .....	15

2.7.2 Immobilization Technologies .....	15
2.7.2.1 Solidification/Stabilization (S/S) .....	15
2.7.2.2 Vitrification .....	16
2.7.3 Physical Separation .....	16
2.7.4 Extraction .....	17
2.7.4.1 Soil Washing .....	17
2.7.4.2 Pyrometallurgical Extraction .....	18
2.7.4.3 In-Situ Soil Flushing .....	18
2.7.4.4 Electrokinetic Treatment .....	18
2.7.5 Toxicity and/ or Mobility Reduction .....	19
2.7.5.1 Chemical Treatment .....	19
2.7.5.2 Biological Treatment .....	20
2.8 Bioleaching of Metals from Sediments .....	21
2.8.1 Bioleaching Mechanism and Heavy Metals Removal .....	21
2.8.2 Microorganisms Used in Bioleaching Processes .....	23
2.8.2.1 Thiobacillus thiooxidans .....	23
2.8.2.2 Thiobacillus ferrooxidans .....	24
2.8.3 Factors Effecting Bioleaching Process .....	24
2.8.3.1 pH .....	25
2.8.3.2 Oxidation Reduction Potential (ORP) .....	25
2.8.3.3 Nutrients .....	25
2.8.3.4 Substrate .....	26
2.8.3.5 O <sub>2</sub> and CO <sub>2</sub> .....	26
2.8.4 Bioleaching Techniques .....	26
2.8.5 Bioleaching Studies from the Literature .....	27

**CHAPTER THREE – CHARACTERIZATION STUDIES FOR SEDIMENT  
SAMPLES .....** **34**

3.1 Sample Collection and Processing .....	34
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3.2 General Characterization Studies .....	37
3.2.1 pH.....	37
3.2.2 Water Content .....	37
3.2.3 Organic Matter Content .....	37
3.2.4 Grain Size Distribution .....	37
3.3 Heavy Metal Content .....	38
3.3.1 Microwave Acid Digestion .....	39
3.3.1.1 Microwave Digestion System .....	39
3.3.1.2 Digestion Procedures .....	39
3.3.2 The BCR Sequential Extraction Procedure.....	41
3.3.3 Instrumental Analysis .....	41
<b>CHAPTER FOUR – BIOLEACHING EXPERIMENTS .....</b>	<b>44</b>
4.1 Microorganisms.....	44
4.1.1 Growth of the Bacteria.....	44
4.1.2 Enumeration of the Bacteria .....	45
4.1.3 Acclimation of the Bacteria .....	46
4.1.4 Bioleaching Tests.....	46
4.1.4.1 Sediment Sample.....	46
4.1.4.2 Flask Experiments.....	47
4.1.4.3 Initial pH .....	47
4.1.4.4 Bioleaching period .....	48
4.1.5 Parameters that Effect Bioleaching.....	49
4.1.5.1 Bioleaching Experiments with Different Bacteria Type.....	49
4.1.5.2 Bioleaching Experiments with Different Solid/Liquid Ratio .....	50
4.1.5.3 Bioleaching Experiments with Different Sulfur Concentration.....	50
4.1.5.4 Bioleaching Experiments Acc. to the Grain Size of the Sediment ....	51

<b>CHACHAPTER FIVE - HEA VY METALS IN THE SEDIMENTS OF IZMIR INNER BAY .....</b>	<b>53</b>
5.1 General Characteristics .....	53
5.2 Total Metal Concentrations.....	54
5.2.1 Accuracy of the Method.....	55
5.3 Distribution of Metals According to Binding Forms .....	56
5.4 Discussion .....	58
<b>CHAPTER SIX - RESULTS OF BIOLEACHING EXPERIMENTS AND DISCUSSION .....</b>	<b>61</b>
6.1 General .....	61
6.2 Sediment Sample.....	61
6.3 Initial pH and Bioleaching Period.....	63
6.4 Bioleaching Tests.....	64
6.4.1 Bioleaching Test 1- The Effect of the Bacterial Strain.....	64
6.4.1.1 Cell Concentrations of the Bacteria .....	64
6.4.1.2 Acclimation of the Bacteria .....	64
6.4.1.3 pH and ORP Changes.....	65
6.4.1.4 Sulfate Production.....	67
6.4.1.5 Metal Solubilization and Removal Efficiency in Residual Sediment	68
6.4.1.6 Changes in Bounding Fractions .....	71
6.4.1.7 Discussion .....	73
6.4.2 Bioleaching Test 2 -The Effect of the Solid/Liquid Ratio.....	74
6.4.2.1 Cell Concentrations of the Bacteria .....	74
6.4.2.2 Acclimation of the bacteria .....	74
6.4.2.3 pH and ORP Changes.....	75
6.4.2.4 Sulfate Production.....	76

6.4.2.5 Metal Solubilization and Removal Efficiency in Residual Sediment	76
6.4.2.6 Changes in Bounding Fractions .....	78
6.4.2.7 Discussion .....	80
6.4.3 Bioleaching Test 3- Effect of Sulfur Concentration .....	82
6.4.3.1 Cell Concentrations of the Bacteria .....	82
6.4.3.2 Acclimation of the Bacteria .....	82
6.4.3.3 pH and ORP Change .....	82
6.4.3.4 Sulfate Production.....	84
6.4.3.5 Metal Solubilization and Removal Efficiency in Residual Sediment	84
6.4.3.6 Changes in Bounding Fractions .....	87
6.4.3.7 Discussion .....	88
6.4.4 Bioleaching Test 4- Effect of Grain Size .....	90
6.4.4.1 Sediment Samples .....	90
6.4.4.2 Cell Concentrations.....	90
6.4.4.3 Acclimation of the Bacteria .....	92
6.4.4.4 pH and ORP Changes.....	92
6.4.4.5 Sulfate Production.....	93
6.4.4.6 Metal Solubilization and Removal Efficiency in Residual Sediment	94
6.4.4.7 Changes in Bounding Fractions .....	96
6.4.4.8 Discussion .....	99
6.5 Leaching with H <sub>2</sub> SO <sub>4</sub> .....	101
6.6 Recommended Future Work .....	103
<b>CHAPTER SEVEN - CONCLUSIONS .....</b>	<b>105</b>
<b>REFERENCES.....</b>	<b>109</b>

# CHAPTER ONE

## INTRODUCTION

### 1.1 Problem Statement

Metal contaminated sediments are considered to be one of the main sources of pollution in the aquatic environments. Under certain conditions, metals in sediments can be released to overlying waters and taken up by the organisms. They become a number of reactions in the system including sorption and precipitation and they are greatly influenced by redox conditions in the sediments (Allen, 1995). That's why the aquatic sediments deserve special consideration through the environmental studies.

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. In Canada, Council of Resource and Environmental Ministers, legislated the Canadian Water Quality Guidelines in 1987 which points the Interim Marine Sediment Quality Levels (ISQGs) and Probable Effect Levels (PELs; dry weight) (CCRM, 1999). Furthermore, EPA's Office of Solid Waste and Emergency Response (OSWER) published Ecotox Thresholds to give limit values for the contaminants to determine the sediment quality in waters (United States Environmental Protection Agency [USEPA], 1996). (Table 1.1)

Table 1.1 Sediment Quality Criteria for Canada and USEPA

Metals (mg kg <sup>-1</sup> )	ISQG(s)	PELs	Ecotox Thresholds
As	7.24	41.6	8.2
Cd	0.7	4.2	1.2
Cr	52.3	160	81
Cu	18.7	108	34
Pb	30.2	112	47
Hg	0.13	0.70	0.15
Zn	124	271	150

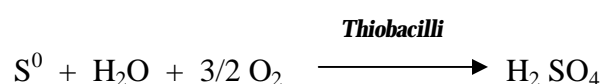
Izmir Bay has been polluted by urban and industrial wastewater discharges for several years. Continued discharges have caused a serious pollution of organics and heavy metals in the sediments in this area. In past, partial dredgings of the sediments were done and the dredged material was dumped in a natural ditch in the Outer Bay. In 2001, the Big Channel Project by Izmir Metropolitan Municipality was completed, and a sewage network was connected to a major collector followed by an urban wastewater treatment plant for the city. But the sediment layer at the bottom of the inner Bay still has organic and inorganic contaminants and creates potential hazard. There are various studies in the literature about the heavy metal pollution in the sediments of Izmir Bay (Aksu *et al.*, 1998; Atgün *et al.*, 2000; Cihangir & Küçüksezgin, 2003).

## 1.2 Purpose of the Presented Study

The treatment of metal contaminated sediments can be achieved by physical or chemical methods. These techniques show limitations such as low efficiency or high cost. Therefore, the bioremediation of heavy metals from contaminated soils and sediments has received a great interest, recently.

Bioleaching process, which causes acidification and the solubilization of metals based on the activity of the chemolithoautotrophic bacteria (mainly *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*) is one of the promising methods for removing heavy metals from contaminated soils and sediments. Under aerobic conditions, the bacterial activity of the *Thiobacillus* species leads to the production of sulfuric acid, extracting metals from the sediment, or to the direct solubilization of metal sulfides by enzymatic oxidation stages (Seidel *et al.*, 1995).

Sulfur oxidation by Thiobacilli follows the general equation:



The bioleaching process is adapted from the mining industry and used in various metal removal studies for soils, sediments, and sludges.

The presented study targets to achieve two goals:

- To investigate the current heavy metal pollution in the sediments of Izmir inner Bay
- To offer an effective treatment method for the removal of metals from the sediments taken from the inner Bay

In the first part of the study, samples taken from different points in Izmir inner Bay were characterized for their total and chemically distributed metal contents. The results present the current heavy metal pollution in the sediments of Izmir inner Bay. In the second part, the performance of bioleaching process depending on various parameters was investigated by using the samples taken from the Bay. The parameters investigated in the scope of this study are;

- The bacteria type (trials with *T. thiooxidans* , *T. ferrooxidans* , and the mixed culture of them)
- Quantity of the substrate (the use of elemental sulfur in different concentrations),
- Solid content in suspension (trials with various solid/liquid ratios),
- Grain size of the sediments (bioleaching trials with fine, medium and coarse samples).

Depending on their environmental impacts and high toxicities, the metals studied in this thesis are chosen as chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn).

## **CHAPTER TWO**

### **BACKGROUND INFORMATION AND LITERATURE REVIEW**

#### **2.1 Heavy Metals in Sediments**

Major indicators of pollution in aquatic environments are contaminated sediments that can be defined as soils, sand, organic matter, or minerals accumulated at the bottom of a water body (United States Environmental Protection Agency [USEPA], 1998). Under certain conditions, contaminants in sediments can be released to overlying waters, that's why the sediments can be important sources of contaminants in aquatic environments.

Sediments of rivers, lakes, and estuaries in a 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. They become part of a number of reactions in the system including sorption and precipitation, and they are greatly influenced by redox conditions in the sediments (Allen, 1995). 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 (Garbarino *et al.*, 1995). They can remain in solution or in suspension and precipitate on the bottom or can be taken up by organisms (Topçuoğlu *et al.*, 2002).

#### **2.2 Heavy Metals Transport in Aquatic Environments**

Association of inorganic contaminants with solids in soils or sediments is typically dominated by adsorption process. Precipitation may play a large role in governing aqueous metal concentrations where high concentrations of sulfide can result in the precipitation of metal sulfides. On the other hand, contaminants are released to the water body through physical/chemical processes and biologically mediated release processes. Physical/chemical releases occur due to the changes in; water saturation soil or sediment, water and gas chemistry, and soil or sediment surface properties. Biologically mediated release processes depend on; microbial

surfactants which are produced by the microorganisms and have a potential to separate the hydrophobic organic contaminants from solids, the microbial effects which cause the reduction of some chemicals and release of inorganic contaminants, and the bioturbation and resuspension processes which lead the biota to move sediments from one location to another (Dean & Dalrymple, 2001). The transport of the sediment-borne particles in a water body is given with Figure 2.1.

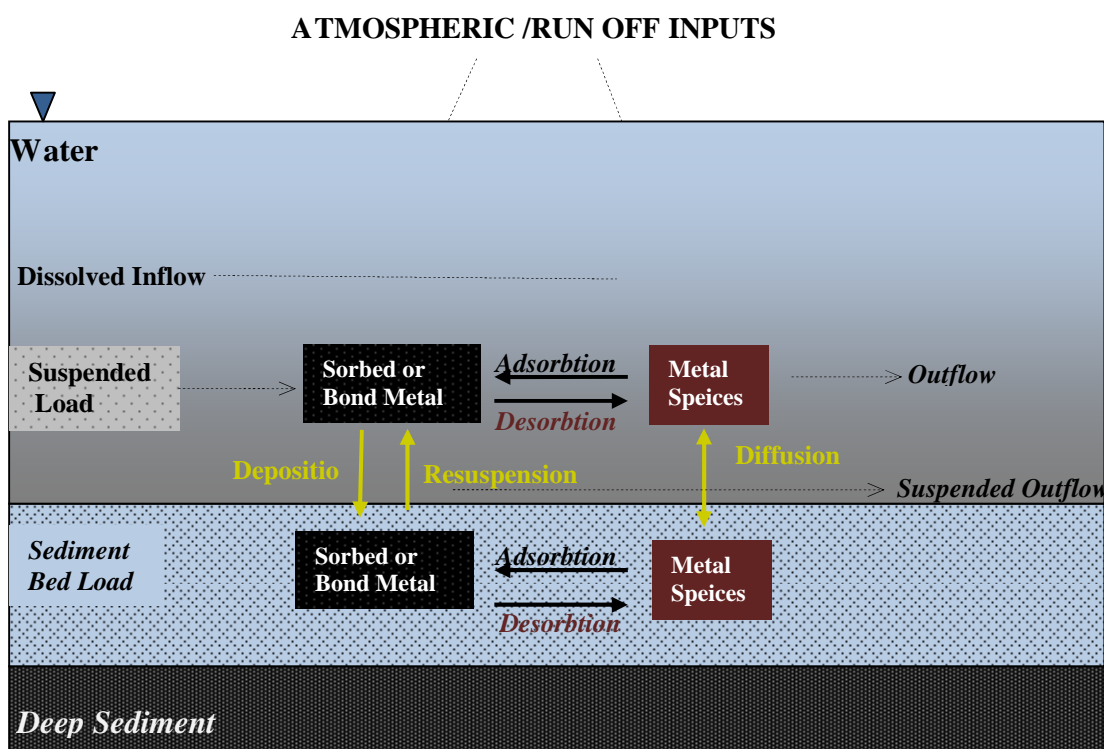


Figure 2.1 Contaminant transport in a water body

### 2.3 Heavy Metals in Natural Levels

The natural background levels of the metals should be known to evaluate the level of pollution in the sediments. Natural background concentrations of heavy metals in sediments are determined by means of different approaches in the literature. The determination of the metal concentrations from an unpolluted area is one of the approaches. Also, metal concentrations in subsurface sediments (sampling 25 cm below the surface) may give the background levels of heavy metals. Natural background levels of heavy metal concentrations in different water bodies are given with Table 2.1.



Table 2.1 Natural background concentrations of heavy metals in sediments from various sites (mg kg<sup>-1</sup>)

Sampling sites	Metals								
	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Reference
Natural decomposition of sea water	0.0026	0.0001	0.0002	0.0009	0.0002	0.0066	0.00003	0.005	Turekian, 2003
Pre-Industrial sediments from Norwegian fjords and coastal waters, Norway	<20	<0,25	<70	<35	<0,15	<30	<30	<150	Sivertsen, 2000
Pre-Industrial sediments from Puddefjorden Solheimsviken, Germany	<5	<1,5	<28	<5	<0,01	<9	<15	<34	Sivertsen, 2000
Pre-Industrial sediments from Vagen, Germany	<8	<10	<32	<9	<0,05	<17	<12	<33	Sivertsen, 2000
Pre-Industrial sediments from Swedish pelagic areas, Sweden	<10	<20	<40	<15	<0,04	<30	<25	<85	Sivertsen, 2000
Bottom Sediments of Volga Delta, Russia	nd	nd	96	50	nd	<36	24	23	Lychagin <i>et al.</i> , 1995
Reference point in Marmara Sea, Turkey	53,5	<5	nd	6,5	0,3	nd	25	nd	Tolun <i>et al.</i> , 2001
Subsamples from Izmir Bay, Turkey	10	0,03	175	17	0,05	nd	8,5	65	Aksu <i>et al.</i> , 1998

(nd: not determined)

## 2.4 Most Common Heavy Metals in Aquatic Sediments

The heavy metal content of sediments comes from natural sources (rock weathering, soil erosion, dissolution of water-soluble salts) as well as anthropogenic sources such as municipal wastewater-treatment plants, agricultural activities and manufacturing industries including mining activities, plating industries, leather tanning plants, battery recycling plants etc. Typical pollutants generated from these activities are lead (Pb), zinc (Zn), chromium (Cr), copper (Cu), cadmium (Cd), mercury (Hg), aluminum (Al), iron (Fe), manganese (Mn), and nickel (Ni) which are considered as the most frequently found metals in sediments. Heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), and zinc (Zn) are regarded as serious pollutants of aquatic ecosystems because of their environmental persistence, toxicity, and ability to be incorporated into food chains (Förtsner & Wittman, 1983). Among them; cadmium, lead and mercury are highly toxic at relatively low concentrations because they can accumulate in body tissues over long periods of time (Garbarino *et al.*, 1995). The fate and transport of a metal in soil or aquatic environment depends significantly on the chemical form and speciation of the metal (Allen & Torres, 1991). The chemical form and speciation of some of the more important metals found at contaminated sediments are discussed below.

### 2.4.1 Lead (Pb)

The primary industrial sources of lead (Pb) contamination include metal smelting and processing, secondary metals production, lead battery manufacturing, pigment and chemical manufacturing, and lead-contaminated wastes. Widespread contamination due to the former use of lead in gasoline is also of concern. Lead released to groundwater, surface water and land is usually in the form of elemental lead, lead oxides and hydroxides, and lead metal oxyanion complexes (Smith *et al.*, 1995).

Lead occurs most commonly with an oxidation state of 0 or +II. Pb(II) is the more common and reactive form of lead and forms mononuclear and polynuclear oxides and hydroxides. Under most conditions  $Pb^{2+}$  and lead-hydroxy complexes are

the most stable forms of lead. In water bodies, a significant fraction of lead is undissolved and occurs as precipitates ( $\text{PbCO}_3$ ,  $\text{Pb}_2\text{O}$ ,  $\text{Pb}(\text{OH})_2$ ,  $\text{PbSO}_4$ ), sorbed ions or surface coatings on minerals, or as suspended organic matter. Lead carbonate solids form above pH 6 and  $\text{PbS}$  is the most stable solid when high sulfide concentrations are present under reducing conditions. The primary processes influencing the fate of lead in soil include adsorption, ion exchange, precipitation, and complexation with sorbed organic matter. These processes limit the amount of lead that can be transported into the surface water or groundwater (Evanko & Dzombak, 1997).

#### **2.4.2 Chromium (Cr)**

Chromium (Cr) is one of the less common elements and does not occur naturally in elemental form, but only in compounds. Chromium is mined as a primary ore product in the form of the mineral chromite,  $\text{FeCr}_2\text{O}_4$ . Major sources of Cr contamination include releases from electroplating processes and the disposal of chromium containing wastes (Evanko & Dzombak, 1997).

Cr (VI) is the dominant form of chromium in water bodies where aerobic conditions exist. Major Cr(VI) species include chromate ( $\text{CrO}_4^{2-}$ ) and dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) which precipitate readily in the presence of metal cations (especially  $\text{Ba}^+$ ,  $\text{Pb}^{2+}$ , and  $\text{Ag}^+$ ). Cr(III) is the dominant form of chromium at low pH (<4).  $\text{Cr}^{3+}$  forms solution complexes with  $\text{NH}_3$ ,  $\text{OH}^-$ ,  $\text{Cl}^-$ ,  $\text{F}^-$ ,  $\text{CN}^-$ ,  $\text{SO}_4^{2-}$ , and soluble organic ligands. Cr(VI) is the more toxic form of chromium and is also more mobile (Chrotowski *et al.*, 1991). Chromium mobility depends on sorption characteristics of the soil, including clay content, iron oxide content and the amount of organic matter present. Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form. Most of chromium released into natural waters is particle associated, however, and is ultimately deposited into the sediment (Smith *et al.*, 1995).

### **2.4.3 Zinc (Zn)**

Zinc (Zn) does not occur naturally in elemental form. It is usually extracted from mineral ores to form zinc oxide (ZnO). The primary industrial use for Zinc is as a corrosion-resistant coating for iron or steel (Smith *et al.*, 1995).

Zinc is one of the most mobile heavy metals in surface waters and groundwater because it is present as soluble compounds at neutral and acidic pH values. Zinc usually occurs in the +II oxidation state and forms complexes with a number of anions, amino acids and organic acids. Zn may precipitate as Zn(OH)<sub>2</sub>(s), ZnCO<sub>3</sub>(s), ZnS(s), or Zn(CN)<sub>2</sub>(s). Sorption to sediments or suspended solids, including hydrous iron and manganese oxides, clay minerals, and organic matter, is the primary fate of zinc in aquatic environments (Evanko & Dzombak, 1997).

### **2.4.4 Cadmium (Cd)**

Cadmium (Cd) occurs naturally in the form of CdS or CdCO<sub>3</sub>. Cadmium is recovered as a by-product from the mining of sulfide ores of lead, zinc and copper. Sources of cadmium contamination include plating operations and the disposal of cadmium-containing wastes (Smith *et al.*, 1995).

The most common forms of cadmium include Cd<sup>2+</sup>, cadmium-cyanide complexes, or Cd(OH)<sub>2</sub> solid sludge (Smith *et al.*, 1995). Hydroxide (Cd(OH)<sub>2</sub>) and carbonate (CdCO<sub>3</sub>) solids dominate at high pH. Under reducing conditions when sulfur is present, the stable solid CdS(s) is formed. Cadmium will also precipitate in the presence of phosphate, arsenate, chromate and other anions. Under acidic conditions, Cd may form complexes with chloride and sulfate (Evanko & Dzombak, 1997).

### **2.4.5 Copper (Cu)**

Copper is mined as primary ore product from copper sulfide and oxide ores. Mining activities are the major sources of copper contamination in ground water and surface waters (Evanko & Dzombak, 1997). Copper is also widely used in metal and automotive industries, therefore wastewater discharges coming from the industrial

zones including these activities may contribute to copper pollution in aquatic environments.

In aerobic, sufficiently alkaline systems,  $\text{CuCO}_3$  is the dominant soluble copper species. The cupric ion,  $\text{Cu}^{2+}$ , and hydroxide complexes,  $\text{CuOH}^+$  and  $\text{Cu}(\text{OH})_2$  are also commonly present. Copper forms strong solution complexes with humic acids. Copper mobility is decreased by sorption to mineral surfaces.  $\text{Cu}^{2+}$  sorbs strongly to mineral surfaces over a wide range of pH values (Dzombak & Morel, 1990). The cupric ion ( $\text{Cu}^{2+}$ ) is the most toxic species of copper. Copper toxicity has also been demonstrated for  $\text{CuOH}^+$  and  $\text{Cu}_2(\text{OH})_2^{2+}$ . (Evanko & Dzombak, 1997).

#### **2.4.6 Mercury (Hg)**

Mercury (Hg) is usually recovered as a by-product of ore processing (Smith *et al.*, 1995). At the present time, the most significant anthropogenic activities giving rise to Hg pollution in land, water and air are; mining and smelting of ores (Cu and Zn), burning of fossil fuels (mainly coal), and industrial production processes (chloralkali industry, batteries, paint industries) (Alloway, 1995).

After release to the environment, mercury usually exists in mercuric ( $\text{Hg}^{2+}$ ), mercurous ( $\text{Hg}_2^{2+}$ ), elemental ( $\text{Hg}^0$ ), or alkylated form (methyl/ethyl mercury). Mercury is most toxic in its alkylated forms which are soluble in water and volatile in air (Smith *et al.*, 1995). Under acidic conditions,  $\text{Hg}^{2+}$  is stable at a redox potential above 0.4 V, and normally occurs as the  $\text{HgCl}_2^0$  complex. Above pH 7, the complex  $\text{Hg}(\text{OH})_2^0$  is the corresponding stable form. Another important property of Hg is the ability to bind strongly to the sulfide ion. Under strongly reducing conditions,  $\text{Hg}^0$  is stable in the presence of  $\text{H}_2\text{S}$  or  $\text{HS}^-$ , but at increasing redox potential,  $\text{HgS}$  will precipitate (Alloway, 1995).

### **2.5 Chemical Distribution (Speciation) of Metals in Sediments**

Chemical speciation can be defined as the identification and quantification of different species, forms or phases present in a material, or the description of these (Fytianos & Laurantou, 2004). Chemical distribution of the metals gives a better

indication of the environmental impact of them since each form has separate binding properties. In order to evaluate the possible toxicity or risk of environmental pollution of heavy metals present in the sediments, the types of association between metals and the sediment must be assessed (Gümğüm & Öztürk, 2001).

In the past, a major method improved by Tessier and his colleagues was used to determine different forms of metals in sediments. The most widely used methods at present are based on sequential extraction procedures whereby several reagents are used consecutively to extract operationally defined phases from the sediments in a certain order (Morillo *et al.*, 2004). Recently, a scheme developed by the European Commission for Standards, Measurement and Testing (ECTS&T, previously BCR) has been started to be widely used which divides metals into four bounding fractions;

- § *Exchangeable and acid soluble fraction*: This phase presents the weakly absorbed and retained metals on the sediment surfaces. These metals can be released easily by ion exchange processes and affected by pH changes. These are loosely bound and labile materials which are most available for plant uptake (Dean, 2003).
- § *Reducible fraction*: These are the metals bound to iron and manganese oxides which may be released if the sediment changes from oxic to anoxic state. This may occur, for example as a result of the activity of microorganisms present in the sediments (Morillo *et al.*, 2004).
- § *Oxidizable fraction (bound to organics)*: The degradation of organic matter under oxidizing conditions can lead to a release of soluble trace metals bound to this component. Trace metals bound to sulfides might be extracted during this step (Dean, 2003).
- § *Residual fraction*: These are the metals bound within the crystal matrix, and they are not expected to be released under normal conditions in nature.

The exchangeable and acid soluble fraction is considered to be the most soluble/bioavailable and the last fraction is the least bioavailable or non-

anthropogenic. Since all forms of a given metal don't have an equal impact on the environment, the determination of potentially-available metals by sequential chemical extraction offers a more realistic estimate of the actual environmental impact. That's why sequential extraction procedures are of great concern in determination of heavy metal pollution in the aquatic sediments.

## 2.6 Heavy Metal Pollution in Izmir Bay

Izmir is one of the largest, most industrial, and also one of the most polluted cities in the eastern Mediterranean (Aksu *et al.*, 1998). A ship port with a heavy commercial and touristic traffic is located in Izmir Bay. From the topographic and hydrographic points of view, the Bay is divided into inner, middle, and outer regions (Figure 2.2).

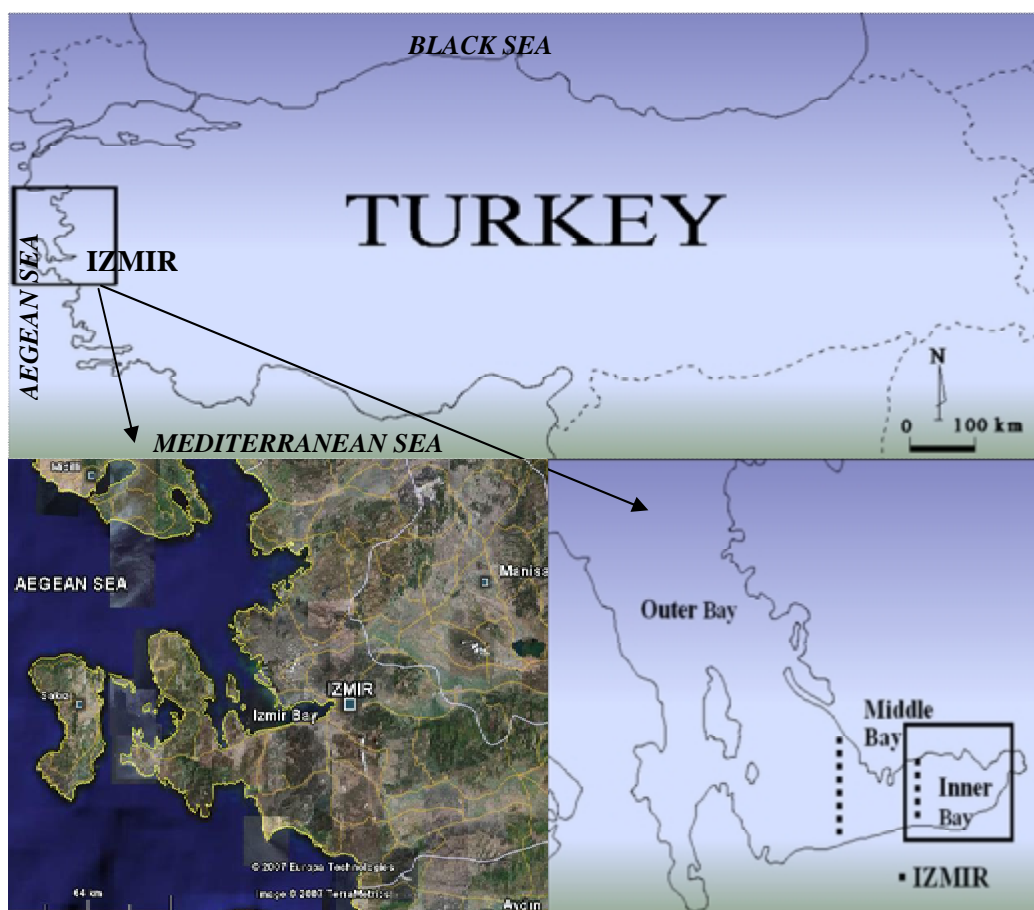


Figure 2.2 Location of Izmir Bay in Turkey

Its average depth is about 20-25 m. The inner Bay, which is shallower, reaches a maximum depth of 20 m, and exhibits a limited water exchange with the outer Bay (Balci & Türkoğlu, 1993). Many industries are located on the edge and the catchment basins of the creeks which flow into the inner Bay. Until 1985, these creeks were highly contaminated by the illegal discharges of the wastewaters coming from industries without any pre-treatment facilities. In Izmir, prevalent industries with heavy metal content in their wastewaters are: textile (on Manda and Sepetci Creeks), chemicals (on Melez and Sepetci Creeks), metal (on Manda, Melez, Ilica, and Bostanli Creeks), automotive (on Manda Creek) industries, the tanneries (on Manda and Melez Creeks), and the industrial zones (on Melez and Old Gediz 1 Creeks) (Izmir Chamber of Commerce [IZTO], 1995). In addition, abandoned landfill areas receiving industrial, medical and domestic solid wastes of Izmir have been operated by dumping and spreading the waste through the sea bank. The operation of these sites which were close to the Bornova and Bostanli Creeks was abandoned in 1991 and a landfill site on the catchment basin of Harmandali Creek with relatively low permeability was taken into operation.

Izmir Bay has been polluted by urban and industrial wastewater discharges for several years. Continued discharges have caused a serious pollution of organics and heavy metals in the sediments in this area. In order to remove highly contaminated sediments and to ease docking of the ships entering the Bay, partial dredgings of the sediments were done between the years of 1976 and 1990. The dredgings were done mainly along the centre line (from east to west) of the inner Bay. The dredged material was dumped in a natural ditch in the outer Bay. In 2001, as a part of a project of Izmir Metropolitan Municipality, the creeks entering the Bay were taken under control and the sewage network was connected to a major collector followed by urban wastewater treatment plant for the city. But still, there are some direct or indirect illegal discharges into the Bay which cannot be prevented. Previous studies mention about high concentrations of heavy metals and organic pollutants in the sediments of Izmir inner Bay (Aksu *et al.*, 1998; Atgin *et al.*, 2000; Cihangir & Küçüksezgin, 2003). Table 2.2 gives brief information about the increasing concentrations of heavy metals in sediments of Izmir Bay and its two big tributaries; Büyük Menderes and Gediz Rivers.



Table 2.2 Heavy metal concentrations in sediments of Büyük Menderes River, Gediz River and Izmir Bay (mg kg<sup>-1</sup>)

	<b>Büyük Menderes River, 2003</b>	<b>Gediz River, 2003</b>	<b>Izmir Outer Bay, 97 (min-max)</b>	<b>Izmir Inner Bay, 97 (min-max)</b>	<b>Izmir Inner Bay, 99 (mean ± sd)</b>	<b>Izmir Outer Bay, 99 (mean ± sd)</b>	<b>Izmir Inner and Middle Bay, 2001 (min-max)</b>	<b>Izmir Outer Bay, 2001 (min-max)</b>
<b>Cr</b>	165±7	200±6	150-300	250-600	300±100	210±64	171-295	38-199
<b>Cu</b>	137±5	140±3	15-40	20-80	70±38	34±24	32-121	4,1-31
<b>Fe</b>	18500±1000	25500±1000	nd	nd	46000±5300	44400±13000	nd	nd
<b>Mn</b>	388,5±15	510±25	nd	nd	454±80	479±137	nd	nd
<b>Ni</b>	315±15	106±10	nd	nd	125±32	148±39	nd	nd
<b>Pb</b>	54±8	128±15	15-30	20-60	62±29	41±14	61-110	25-73
<b>Zn</b>	120±10	160±10	50-150	50-350	260±100	99±37	nd	nd
<b>Ag</b>	nd	nd	0,2-0,5	0,2-1	nd	nd	nd	nd
<b>Cd</b>	nd	nd	0,1-0,6	0,2-0,8	0,42±0,22	0,26±0,16	0,051-0,545	0,027-0,054
<b>As</b>	nd	nd	20-50	30-60	nd	nd	nd	nd
<b>Hg</b>	nd	nd	0,2-0,6	0,2-1,5	nd	nd	0,38-0,82	0,41-0,62
<b>Zn</b>	nd	nd	50-150	50-350	nd	nd	86-286	20-94
<b>Reference</b>	Akçay et al., 2003		Aksu et al., 1998		Atgın et al., 2000		Cihangir & Küçüksezgin, 2003	

## **2.7 Remediation Technologies for Metal Contaminated Soils and Sediments**

Several technologies exist for the remediation of metal contaminated soil and sediments. These technologies are classified as follows: **isolation, immobilization, toxicity reduction, physical separation and extraction** (Evanko & Dzombak, 1997). These remediation methods of metals from soils and sediments may also be divided as in-situ and ex-situ technologies with different advantage and disadvantages. In situ treatment allows soil to be treated without being excavated and transported where ex-situ treatment methods provides more certainty about the uniformity of treatment because of the ability to homogenize, screen, and continuously mix the soil.

### ***2.7.1 Isolation Technologies***

Isolation technologies attempt to prevent the transport of contaminants by containing them within a designated area (Evanko & Dzombak, 1997). Capping systems and subsurface barriers are used to prevent further contamination of groundwater when other treatment options are not physically or economically feasible for a site.

### ***2.7.2 Immobilization Technologies***

Immobilization technologies are designed to reduce the mobility of contaminants by changing the physical or leaching characteristics of the contaminated matrix (Evanko & Dzombak, 1997). Immobilization methods can be categorized as solidification/stabilization and vitrification processes.

#### ***2.7.2.1 Solidification/Stabilization (S/S)***

Solidification involves the formation of a solidified matrix that physically binds the contaminated material. Stabilization, also referred to as fixation, usually utilizes a chemical reaction to convert the waste to a less mobile form. The general approach for solidification/stabilization treatment processes involves mixing or injecting treatment agents to the contaminated soils. Inorganic binders, such as cement, fly

ash, or blast furnace slag, and organic binders such as bitumen are used to form a crystalline, glassy or polymeric framework around the waste (Evanko & Dzombak, 1997). The dominant mechanism by which metals are immobilized is precipitation of hydroxides within the solid matrix (Bishop *et al.*, 1982). S/S technologies are not useful for some forms of metal contamination, such as species that exist as anions (e.g., Cr(VI), arsenic) or metals that don't have low-solubility hydroxides (e.g., mercury) (Evanko & Dzombak, 1997). These technologies are used for a wide variety of metals, including chromium, lead, arsenic, and cadmium. S/S technologies may be both in situ or ex-situ.

#### *2.7.2.2 Vitrification*

In the vitrification process, the mobility of metal contaminants can be decreased by high-temperature treatment of the contaminated area that results in the formation of vitreous material, usually an oxide solid (Evanko & Dzombak, 1997). Depending on the thermal energy, vitrification technologies may be both in situ and ex-situ.

Typical stages in ex situ vitrification processes may include excavation, pretreatment, mixing, feeding, melting and vitrification, off-gas collection and treatment, and forming or casting of the melted product. In situ vitrification (ISV) involves passing electric current through the soil using an array of electrodes inserted vertically into the contaminated region. Each setting of four electrodes is referred to as a melt (Evanko & Dzombak, 1997).

#### *2.7.3 Physical Separation*

Physical separation is an ex situ process that attempts to separate the contaminated material from the rest of the soil matrix by exploiting certain characteristics of the metal and soil. Physical separation techniques are based on particle size, particle density, surface and magnetic properties of the contaminated soil. These techniques are most effective when the metal is either in the form of discrete particles in the soil or if the metal is sorbed to soil particles that occur in a particular size fraction of the soil (Evanko & Dzombak, 1997). Separation is performed through hydro cyclones

with centrifuginal forces, fluidized bed separators, floatation separators and magnetic separators.

#### **2.7.4 Extraction**

Metals-contaminated sites can be remediated using techniques designed to extract the contaminated fraction from the rest of the soil, either in situ or ex situ. Metal extraction can be achieved by contacting the contaminated soil with a solution containing extracting agents (soil washing and in situ soil flushing) or by electrokinetic processes. The contaminated fraction of soil and/or process water is separated from the remaining soil and disposed or treated (Evanko & Dzombak, 1997).

##### **2.7.4.1 Soil Washing**

Soil washing is a process in which contaminants sorbed onto fine soil particles are separated from bulk soil in an aqueous-based system on the basis of particle size because fine particles in the soil matrix contain majority of the contaminated material. The wash water may be augmented with a basic leaching agent, surfactant, pH adjustment, or chelating agent to help remove organics and heavy metals. Soil washing is an ex situ process that requires soil excavation prior to treatment. In soil washing processes; **Preliminary Screening** (to separate large rocks and debris from the contaminated matrix); **Secondary Screening** (to segregate the particles into different size fractions, usually between 5 mm and 60 mm); **Chemical Treatment** (to solubilize the contaminants from the most contaminated fraction of the soil); **Physical Treatment** (to separate the contaminated fraction, usually the fine materials, from the rest of the soil matrix); **Dewatering** (to separate the contaminated liquid phase from soil matrix) and **Water Treatment** (to remove the contaminants from the extractant water) are the basic process steps (Evanko & Dzombak, 1997).

In a case study, a process named ACT\*DE\*CON was developed for the removal of radioactive and heavy metals from soils and surfaces. This process was based on the use of carbonate solution containing an oxidant and chelant. The contaminated

dissolution liquor was treated for recovery and reuse by the MAG\*SEP process, depending on the concentration and nature of the contaminants (Bradbury & Scrivens, 1995).

#### *2.7.4.2 Pyrometallurgical Extraction*

Pyrometallurgical technologies use elevated temperature extraction and processing for removal of metals from contaminated soils. Soils are treated in a high-temperature furnace to remove volatile metals from the solid phase. Subsequent treatment steps may include metal recovery or immobilization (Evanko & Dzombak, 1997). This technology is most applicable to large volumes of highly-contaminated soils (metal concentrations >5%-20%, especially when metal recovery is expected). Low metal concentrations can be processed, especially for mercury since it is easy to volatilize and recover (Smith *et al.*, 1995).

#### *2.7.4.3 In-Situ Soil Flushing*

Soil flushing is a technology used for extracting contaminants from the soils and sediments. It works by applying water to the soil. The water has an additive that enhances contaminant solubility. Contaminants that are dissolved in the flushing solution are leached into the groundwater which is then extracted and treated.. In many instances; surfactants (i.e., detergent-like substances) or solvents are used as the additive. The effectiveness of this process is dependent on hydro geological variables (e.g., type of soil, soil moisture) and type of contaminant. Low permeability soils, such as clays, are difficult to treat with this method (CPEO, 2002).

#### *2.7.4.4 Electrokinetic Treatment*

Electrokinetic remediation is a process in which a low-voltage direct-current electric field is applied across a section of contaminated soil to move the contaminants. The principle of electrokinetics remediation is similar to a battery. After electrodes (a cathode and anode) are introduced and charged, particles (e.g., ions) are mobilized by the electric current. Ions and water move toward the electrodes (CPEO, 2002). Positively charged metal ions migrate to the negatively

charged electrode, while metal anions migrate to the positively charged electrode. Electrokinetic treatment concentrates contaminants in the solution around the electrodes. The contaminants are removed from this solution by a variety of processes, including electroplating at the electrodes, precipitation/co precipitation at the electrodes, complexation with ion exchange resins, or by pumping the water from the subsurface and treating it to recover the extracted metals (Smith *et al*, 1995). This method is used both in-situ and ex-situ.

### ***2.7.5 Toxicity and/ or Mobility Reduction***

Chemical and/or biological processes can be used to alter the form of metal contaminants in order to decrease their toxicity and/or mobility.

#### *2.7.5.1 Chemical Treatment*

Chemical reactions can be initiated that are designed to decrease the toxicity or mobility of metal contaminants. The three types of reactions that can be used for this purpose are oxidation, reduction, and neutralization reactions. Chemical oxidation changes the oxidation state of the metal atom through the loss of electrons. Commercial oxidizing agents are available for chemical treatment, including potassium permanganate, hydrogen peroxide, hypochlorite and chlorine gas. Reduction reactions change the oxidation state of metals by adding electrons. Commercially available reduction reagents include alkali metals (Na, K), sulfur dioxide, sulfite salts, and ferrous sulfate. Changing the oxidation state of metals by oxidation or reduction can detoxify, precipitate, or solubilize the metals (NRC, 1994). Chemical neutralization is used to adjust the pH balance of extremely acidic or basic soils and/or groundwater. This procedure can be used to precipitate insoluble metal salts from contaminated water, or in preparation for chemical oxidation or reduction (Evanko & Dzombak, 1997). Chemical treatment can be performed ex situ or in situ.

### 2.7.5.2 Biological Treatment

Biological treatment technologies are available for remediation of metals-contaminated sites. These technologies are commonly used for the remediation of organic contaminants and are beginning to be applied for metal remediation, although most applications to date have been at the bench and pilot scale. Biological treatment exploits natural biological processes that allow certain plants and microorganisms to aid in the remediation of metals (Evanko & Dzombak, 1997).

*Bioaccumulation;* Bioaccumulation involves the uptake of metals from contaminated media by living organisms or dead, inactive biomass. Active plants and microorganisms accumulate metals as the result of normal metabolic processes via ion exchange at the cell walls, complexation reactions at the cell walls, or intra- and extra cellular precipitation and complexation reactions (Evanko & Dzombak, 1997).

*Phytoremediation;* Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in the soil (CPEO, 2002). These plants include the species of *Thlaspi*, *Urtica*, *Chenopodium*, *Polygonum*, *Sachalase* and *Allyssim* with the ability of accumulating cadmium, copper, lead, nickel and zinc on the leaves or the roots. After the phytoremediation applications, the plants accumulated by high concentrations of metals are disposed with the methods like drying, gasification, pyrolysis, acid extraction and anaerobic digestion (Evanko & Dzombak, 1997).

*Bioleaching;* This process is being adapted from the mining industry for use in metals remediation. Bioleaching uses microorganisms to solubilize metal contaminants either by direct action of the bacteria, as a result of interactions with metabolic products, or both. Bioleaching can simply be described as the solubilization of metals that is based on the activity of the chemolithotrophic bacteria mainly *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Under aerobic conditions, the bacterial activity of *Thiobacillus* spp lead to the production of sulfuric acid, extracting metals from the sediment, or to the direct

solubilization of metal sulfides by enzymatic oxidation stages (Seidel *et al.*, 1995).

## **2.8 Bioleaching of Metals from Sediments**

The treatment of metal contaminated sediments can be achieved by either physical or chemical methods. Although these techniques have been extensively applied in practice, they show some limitations such as low efficiency and high cost. The bioremediation of heavy metals has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry (Stoll & Duncan, 1996). For example, a variety of bioleaching processes have been successfully applied to remove heavy metals from ores, industrial wastes and sewage sludges (Chen & Lin, 2001). Bioleaching process, which causes acidification and solubilization of heavy metals, is one of the promising methods for removing heavy metals from contaminated soils and sediments (Chen & Lin, 2001). It is an innovative, environmental friendly, simple, economical and effective method, which has gained interest over the past decade.

### ***2.8.1 Bioleaching Mechanism and Heavy Metals Removal***

Bioleaching can simply be defined as the solubilization of metals based on the bacterial activity. In aquatic sediments, under unoxic conditions, metals are immobilized as sulfides (MeS). After erosion and oxidation of the material, the metals are transformed into unstable forms (Allen, 1995). At the present time, bioleaching processes are based more or less exclusively on the activity of Thiobacilli strains which convert heavily soluble metal sulfides into soluble metal sulfates (Bosecker, 1997). The main mechanisms involved in bioleaching of heavy metals by *Thiobacillus* species can be explained by the following equations (Chen & Lin, 2001):

#### ***(1) Direct Mechanism***

Under acidic conditions, bioleaching is achieved by the conversion of insoluble metal sulfides into soluble metal sulfates. There is a physical contact between the



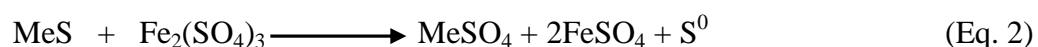
bacteria cell and mineral sulfide surface where MeS is the insoluble metal sulfide and the MeSO<sub>4</sub> is the soluble metal sulfate. The oxidation to sulfate takes place via several enzymatically catalyzed steps (Bosecker, 1997).



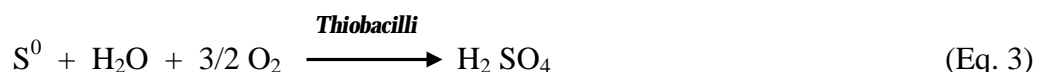
There is some evidence that the bacteria have to be in intimate contact with the mineral surface. Obviously the bacteria do not attach the whole mineral surface but prefer specific sites of crystal imperfection and metal solubilization due to electrochemical interactions (Bosecker, 1997).

## (2) *Indirect Mechanism*

In indirect bioleaching the bacteria may generate a lixiviant which chemically oxidizes the sulfide mineral. In acid solution, this lixiviant is ferric iron, and metal solubilization can be described according to the following reaction (Bosecker, 1997):



The sulfur arising simultaneously may be oxidized to sulfuric acid by the bacteria and the following reaction occurs:



During the indirect mechanism, elemental or reduced sulfur compounds are oxidized to sulfuric acid by the leaching bacteria, resulting in the acidification of the sediments (Eq.3). Subsequently, protons released into the liquid phase can replace heavy metals adsorbed on the sediment particles (Eq. 4) During this stage, thiobacilli oxidize metal sulfides to sulfate and the metals are solubilized (Chen & Lin, 2001).

### 2.8.2 Microorganisms Used in Bioleaching Processes

The bacteria most active in bioleaching belong to the genus *Thiobacillus*. These are gram-negative, non-spore forming rods, which grow under aerobic conditions. Most thiobacilli use the carbon dioxide from the atmosphere as their carbon source for the synthesis of new cell material. The energy derives from the oxidation of reduced or partially reduced sulfur compounds, including sulfides, elemental sulfur and thiosulfate, and the final oxidation product is sulfate (Vichniac & Santer, 1957). The thiobacilli are the mesophilic bacteria, which grow best at temperatures of 25-35 °C.

The three important environmental conditions for the active growth of thiobacilli are the acid pH values (to support acidification), high redox potential (maintained by aeration), and the availability of substrate (sulfur) (Seidel *et al.*, 1995).

Some species of thiobacilli can be counted as *Thiobacillus ferrooxidans*, *T.Thiooxidans*, *Thiobacillus thioparus*, *Thiobacillus denitrificans*, *Thiobacillus thiocyanoxidans*, and *Thiobacillus novellus* (Vichniac & Santer, 1957). Among the bioleaching microorganisms, *Leptospirillum ferrooxidans* and *thermophilic bacteria* can be counted in addition but they have different limitations compared with the thiobacillus species (Bosecker, 1997).

Bacterial leaching is carried out in an acid environment (pH values maintained between 1.5-3) at which most ions remain in solution. Therefore, the acidophilic species *Thiobacillus ferrooxidans* and *T.Thiooxidans* are of particular importance. Other thiobacilli are also able to oxidize sulfur and sulfide but they grow only at higher pH values at which metal ions do not maintain in solution (Bosecker, 1997).

#### 2.8.2.1 *Thiobacillus thiooxidans*

This is a species which is distinguished by its ability to oxidize elemental sulfur at a rate comparable to its oxidation of thiosulfate, in contrast to *T. thioparus* and *T. denitrificans* which oxidize elemental sulfur more slowly (Vishniac & Santer, 1957).

The cells in these species are rod-shaped, appearing singly or in pairs; 0.5x1.0 to 2.0  $\mu\text{m}$ ; gram negative, motile, monopolar flagellated, aerobic, nonspore-forming, mesophilic (10 to 37 $^{\circ}\text{C}$ , optimum 28 to 30  $^{\circ}\text{C}$ ), acidophilic (pH 0.5 to 5.5, optimum pH 2 to 3.5), and chemolithoautotrophic using reduced forms of inorganic sulfur but not ferrous iron energy sources, and carbon dioxide as carbon source (Franson *et al*, 1992). They may decrease the pH in the medium to 1.5 to 1 and even lower.

#### 2.8.2.2 *Thiobacillus ferrooxidans*

*T. ferrooxidans* differs from all other thiobacilli by the fact that besides deriving energy from the oxidation of reduced sulfur compounds, ferrous iron is used as the electron donor. In the absence of oxygen, *T. ferrooxidans* is still able to grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor (Bosecker, 1997).

The cells in these species are rod-shaped, appearing singly or in pairs, 0.5 to 0.5 x1.0 to 1.7  $\mu\text{m}$ , gram negative, motile, monopolar flagellated, aerobic, nonspore-forming, mesophilic (10 to 37 $^{\circ}\text{C}$ , optimum 30 to 35  $^{\circ}\text{C}$ ), acidophilic (pH 2.3 to 4.5, optimum pH 2.5 to 2.8), and chemolithoautotrophic species using reduced forms of inorganic sulfur (elemental sulfur, thiosulfate, tetrathionate), ferrous iron and sulfidic minerals as energy sources, and carbon dioxide as carbon source (Franson *et al*, 1992).

#### 2.8.3 *Factors Effecting Bioleaching Process*

The bioleaching of heavy metals from contaminated sediments is a complex process. The leaching effectiveness 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. Various physicochemical and biological parameters affecting the bioleaching process are discussed below:

### 2.8.3.1 pH

The metal solubilization in the bioleaching process is highly dependent on **pH**. The adjustment of correct pH is necessary to provide the optimum conditions for the growth of the bacteria. Also metal solubilization depends on the low pH values which means the acidification of the medium.

It is found that sludge **pH** is the single most important parameter that influences metal solubilization during the metal bioleaching of sewage sludge. The pH in bioleaching process of contaminated sediment depends on the buffering capacity of the sediment, which is effected by the solid contents of the sediment. High solid content means high buffering capacity, so, solid content does not directly influence the process of metal solubilization but increasing solid contents increase the time to reach the final solubilization rate (Chen & Lin, 2001).

### 2.8.3.2 Oxidation Reduction Potential (ORP)

Concepts of oxidation and reduction are based upon the idea of atomic structure and electron transfer. An atom, molecule, or ion is said to undergo oxidation when it loses an electron, and to undergo reduction when it gains an electron (Sawyer *et al*, 2003)

Solubilization of heavy metals requires an optimum adjustment of pH and Oxidation-Reduction Potential (**ORP**) of the sediment so that the chemical equilibrium will be shifted in favor of soluble metallic ion formation.

### 2.8.3.3 Nutrients

Microorganisms used for metal extraction are the chemolithoautotrophic bacteria and therefore only inorganic compounds are required for growth. Mineral nutrients are necessary for the growth of bacteria to maintain metal extraction from sulfide minerals. For optimum growth, iron and sulfur compounds may be supplemented together with ammonium, phosphate and magnesium salts (Bosecker, 1997).

#### 2.8.3.4 Substrate

Elemental sulfur is an essential **substrate** for the growth of *Thiobacillus* species and bacterially catalyzed metal solubilization in the bioleaching process. Chen & Lin (2001) determined the optimal sulfur concentrations to achieve the maximum solubilization rates. So, the substrate concentration can be counted as one of the major factors affecting the bioleaching process.

#### 2.8.3.5 O<sub>2</sub> and CO<sub>2</sub>

An adequate supply of oxygen is a prerequisite for good growth and high activity of the leaching bacteria. In the laboratory this can be achieved by aeration, stirring, or shaking. Carbon dioxide is the only carbon source required, but there is no need for addition of CO<sub>2</sub> (Bosecker, 1997).

Nature of contaminated particles, temperature and the composition of the medium are the other factors influencing the bioleaching process.

### 2.8.4 Bioleaching Techniques

The industrial leaching processes include;

- *Dump leaching*: This process is used for mining activities. The top of the dump is sprinkled continuously or flooded temporarily with acid. Dump leaching is the oldest process (Bosecker, 1997).
- *Heap leaching*: This procedure is similar to that of dump leaching and mainly used for fine-grained ores that cannot be concentrated by floatation.
- *Underground leaching*: This is usually done in abandoned mines. Galleries are flooded or unmined ore mine waste in side tunnels are sprinkled or washed under pressure (Bosecker, 1997).

The laboratory investigations can be counted as;

- *Percolator leaching*: The experiments are carried out in air lift percolators. The percolator consists of a glass tube provided in its bottom part with a sieve plate. The ore packing is irrigated or flooded with a nutrient inoculated with bacteria. The leach liquor trickling through the column is pumped up (Bosecker, 1997).
- *Column leaching*: It operates on the principle of the percolator leaching and is used as a model for heap and dump leaching processes. Depending on their size, the columns may be made of glass, plastic, lined concrete, or steel (Bosecker, 1997).
- *Submerged leaching*: This technique requires the use of fine grained material which is suspended in the leaching liquid and kept in motion by shaking or stirring providing adequate oxygen for the bacteria. The reaction time is shorter than the percolator leaching due to the growth of bacteria. Bioleaching experiments may be performed in Erlenmeyer flasks (Tsai *et al.*, 2003; Gourdon & Funtowicz, 1995) or they can be carried out in completely mixed bioreactors (CMB), (Chen & Lin, 2001). This is the submerged leaching that requires fine grained material (<100 $\mu$ m) and the sediment is suspended in leaching liquid and it is kept in motion by shaking or stirring. Higher rates of aeration and a more accurate monitoring and control of the various parameters favor the growth and the activity of bacteria so that the reaction times are considerably shortened and the metal extraction increases (Bosecker, 1997).

### ***2.8.5 Bioleaching Studies from the Literature***

Various studies have been carried on by scientists searching for the effects of different parameters on bioleaching with different techniques. The studies are usually laboratory investigations and the solids used for the experiments are the contaminated sediments, soils, and sewage sludge polluted by high concentrations of heavy metals.

In a study conducted by Chen & Lin (2000), the relation between the solid content and the solubilization of metals was investigated. Samples taken from a highly polluted river (Ell Ren River) in Taiwan were placed in a completely mixed batch reactor (CMB) containing selective amounts of dry sediments (10, 20, 40, 70, and 100 g/l) with tydallized elemental sulfur. *Thiobacillus thiooxidans* and *Thiobacillus thioparus* were inoculated and transferred to the bioleaching media. The sets were stopped when the pH of the system was about 2.4. It was seen that it took 3, 6, 14, 20, and 30 days to reach pH 2.4 with increasing solid content. These results indicate the higher sediment content, the longer experimental procedure to reach the required pH value. TCu, Zn, and Mn solubilizations were not influenced by the solid contents and they were detected as 82-95%, 58-70% and 55-73%, respectively. The efficiencies of solubilization of Pb (33-72%), Ni (35-65%) and Cr (9-20%) were affected by the solid contents of the sediments (Chen & Lin, 2000).

Another study of Chen & Lin (2001) was about the effect of substrate concentration on bioleaching efficiency. A 3 liter CMB with an air diffuser and mixer was used by adding the acclimated *Thiobacillus thiooxidans* and *Thiobacillus thioparus* to suspended sediments (Figure 2.3). Various contents (0.1, 0.25, 0.375, 0.5, 0.75 and 1% (w/v)) of tyndallized elemental sulfur were fed in to the reactor. Final pH of the system was 2.5. Most of the metals in the contaminated sediment were cleaned satisfactorily. The efficiency of metal solubilization from the sediment was in decreasing order: Cu>Zn>Mn>Pb>Ni>Cr. Sulfur concentration greater than 0.5% was found to be inhibitory to bacterial activity and metal solubilization of the sediment (Chen & Lin, 2001).

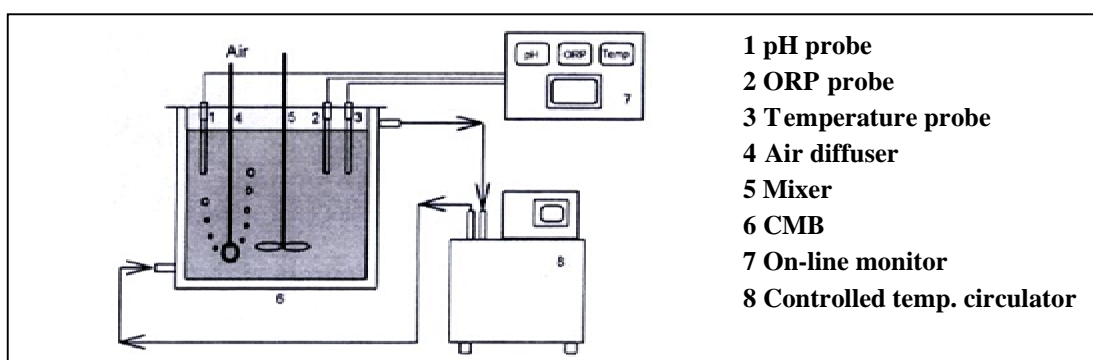


Figure 2.3 Schematic diagram of the CMB bioreactor (Chen & Lin, 2001)

Tsai *et al.* (2003) carried on flask experiments to determine the effect of the ratio of sulfur added to total sediment solids (SA/TS) on the remobilization of heavy metals from contaminated sediment. They also studied the partitioning of the metals in binding fractions before and after bioleaching (Tessier *et al.*, 1979; Belzile *et al.*, 1989). The indigenous sulfur-oxidizing bacteria were enriched by adding bacteria-containing sediment from the Ell Ren River to a culture medium. It was seen in this study that the SA/TS ratio significantly affected the rate of decrease in sediment pH. At the end of 33 days, remobilization of total extractable Zn, Cu, and Ni was significantly higher than Cr and Pb at the same SA/TS ratio. No obvious difference in remobilization of total extractable Zn, Ni, or Cu was found at different SA/TS. But, higher SA/TS ratios were correlated with higher remobilization of Cr and Pb. Binding behaviours of each heavy metal in the sediment were complicated during the bioleaching process. Different metals showed different binding behaviors at various SA/TS

Another bioleaching study was implemented by Lombardi & Garcia (2002). They used municipal sewage sludge from a wastewater treatment plant in Brazil to see the bioleaching effect on partitioning of metals. *Thiobacillus ferrooxidans* were used as leaching microorganism and the experiments were conducted in Erlenmeyer flasks in a rotary shaker. The solubilization efficiency approached 80% for Mn and Zn, 24% for Cu, 10% for Al, 0.2% for Ti, and 77% for Zn. After the bioleaching process, the partitioning of Mn and Zn has changed from organically bound fraction to the exchangeable fraction. During bioleaching, there was no significant change in partitioning of Al and Cu. The study concluded that those metals which had higher solubilization efficiency were those which had their chemical fractionation mostly effected.

The application of two different types of elemental sulfur—commercial sulfur powder (technical) and microbially produced sulfur (biological sulfur)— were studied to evaluate the efficiency on bioleaching of metals from contaminated sediments (Seidel *et al.*, 2005). The biological sulfur was a waste product taken from a gas purification paper mill. The highly polluted sediments taken from Weisse Elster River in Germany were used for bioleaching studies both in suspended flask



experiments and a solid bed reactor in a percolator system. For suspended leaching, biological  $S^0$  did improve the rate and extent of metal solubilization. Adding 2% biological  $S^0$  resulted in a final metal solubilization of 68% where technical  $S^0$  could solubilize 62%. For solid bed leaching biological sulfur had no uniform favorable effect on the bioleaching extent of individual metals. Zn, Cd, Ni, Mn, and Co were solubilized to a large extent where Cu was solubilized to a ratio of only 11-25%. It was concluded that, the use of biological sulfur yielded considerably better results than technical sulfur powder. The equilibrium in acidification, sulfur oxidation, and metal solubilization was reached after 10-14 days of leaching. Generally, solid bed leaching required more time.

Gomez & Bosecker (1999) studied with 4 different environmental samples; the river sediment, sludge from a rainwater collecting basin, soil and rubble, and soil from a tannery site. The bioleaching tests were carried out in flasks with the addition of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* isolated from the fresh soil sample. The pulp density in the flasks varied between 5% and 20% (w/v). Leaching with *Thiobacillus ferrooxidans* resulted in total extraction of Cd, Co, Cu and Ni in soil samples. With the use of *Thiobacillus thiooxidans*, more than 80% of Co, Cu, Zn and Cd were dissolved in the bioleaching experiment. Dissolution of Zn was the best in the bacterial leaching experiment, which had a leaching efficiency about twice that of abiotic leaching test.

Seidel *et al* (2006) studied the effects of oxygen limitation on solid bed leaching of heavy metals in a laboratory percolator system using contaminated sediment supplemented with 2% elemental sulfur. The oxygen supply varied between 150 and 0.5 mol<sub>O<sub>2</sub></sub> mol<sub>S</sub><sup>-1</sup> over 28 days of leaching. Moderate oxygen limitation led to temporarily suspension of acidification, rate of sulfate generation, and metal solubilization. Lowering the oxygen supply to 0.5 mol<sub>O<sub>2</sub></sub> mol<sub>S<sub>0</sub></sub><sup>-1</sup> resulted in retarding acidification over a period of 3 weeks and in poor metal solubilization. The maximum metal solubilization was reached at an oxygen supply of 7.5 mol<sub>O<sub>2</sub></sub> mol<sub>S<sub>0</sub></sub><sup>-1</sup>.

The use of recoverable sulfur particles in bioleaching was studied by Chen *et al* (2003). Three different forms of sulfur particles; powder, pastilles, and pellets were

used to observe the utilization and recovery of sulfur utilized as energy source for bacteria. *Thiobacillus thiooxidans* and *Thiobacillus thioparus* were inoculated and added to the completely mixed batch reactor containing 2% solid content. The specific surface area of sulfur particles was found to be the determining factor in the bioleaching process. The rates of pH reduction, sulfate production, and metal solubilization increased with increasing specific surface area of the particles. The efficiency of metal solubilization with recovered sulfur particles was comparable to sulfur powder. The solubilization efficiencies of heavy metals were in the range 95-96% for Cu, 72-81% for Zn, 16-60% for Pb, and 10-47% for Ni.

Löser *et al.* (2006) studied the abiotic and microbial solid bed leaching comparatively in a percolator system using ripened permeable heavy metal polluted river sediment (Figure 2.4). Influence of process parameters were pH value, percolation flow rate, and solid bed height. For abiotic leaching, 2.5 M sulfuric acid was used and for bioleaching elemental sulfur was added to the system. The pH was set to value of 3.5 or 2.8. In case of abiotic leaching; the rate of metal solubilization was proportional to the percolation rate and inversely proportional to the solid-bed height. Within the 42 days, equilibrium was almost completely achieved in two of the experiments, both carried out with a low bed and the largest percolation rate possible. In case of bioleaching; metal solubilization is mainly determined by  $S^0$  oxidation. The microbial sulfur oxidation is relatively slow and requires some weeks, but proceeds independently of the bed geometry and is not influenced by the percolation flow. Only 21 days were required for solubilization of 60% of the toxic metals by bioleaching in a solid bed with 1.3 m height.

In another study of Löser *et al.* (2007), four various leaching procedures were compared with each other; abiotic suspended leaching, microbial suspended leaching, abiotic solid bed leaching, and microbial solid bed leaching. Abiotic suspended leaching was fastest, but suspending the solids exhibits some disadvantages. Abiotic solid bed leaching implies the supply of acid by percolating water which proceeds slowly due to a limited bed permeability. In microbial solid bed leaching, the removal of 98% of the mobile metals lasted 57-61 days.

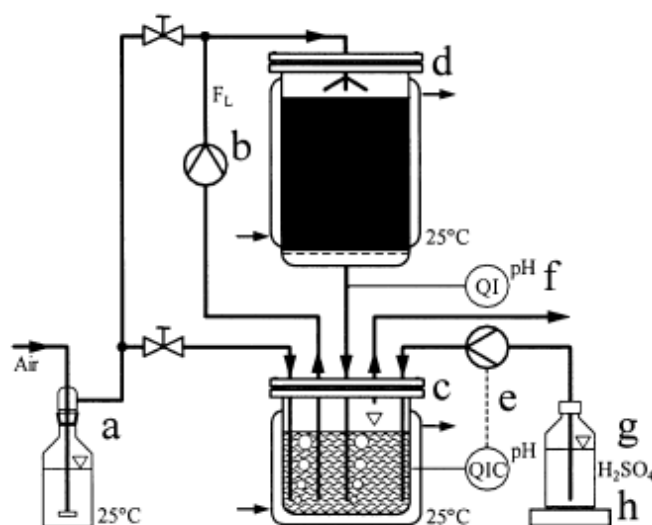


Figure 2.4 Percolator system for solid-bed leaching of sediment (Löser *et al.*, 2006)

Tsai *et al.* (2003) studied the effect of temperature on bioleaching of heavy metals from contaminated sediments. Indigenous sulfur oxidizing bacteria was enriched by adding bacteria-containing sediment from the Ell Ren River (in Taiwan) to a culture medium. Bioleaching experiments were performed in 500 ml flasks at 3 controlled temperatures; 25, 37, and 55 °C. Except for Pb, the solubilization of the metals was highest at 37°C. Solubilization efficiencies of Ni, Zn and Cu at 25°C were similar to those at 37°C. The order of average solubilization efficiencies of total extractable heavy metals is; Ni, Zn, Cu > Cr > Co, Pb. Even though less heavy metal was released at 55 °C, transfer of heavy metals between binding fractions was most apparent at this temperature during bioleaching.

Chen & Lin (2004) investigated the optimum sulfur concentration in an air-lift bioreactor (Figure 2.5). The indigenous sulfur-oxidizing bacteria were activated by using sediment slurry and tyndallized elemental sulfur. Increasing the sulfur concentration from 0.5 to 5 g/l enhanced the rates of pH reduction, sulfate production, and metal solubilization. After 8 days of bioleaching, 97-99% of Cu, 96-98% of Zn, 62-68% of Ni, and 31-50% of Pb were solubilized from the sediment. From economical consideration, the recommended sulfur dosage for the bioleaching of metals from the sediment is 3g/l.

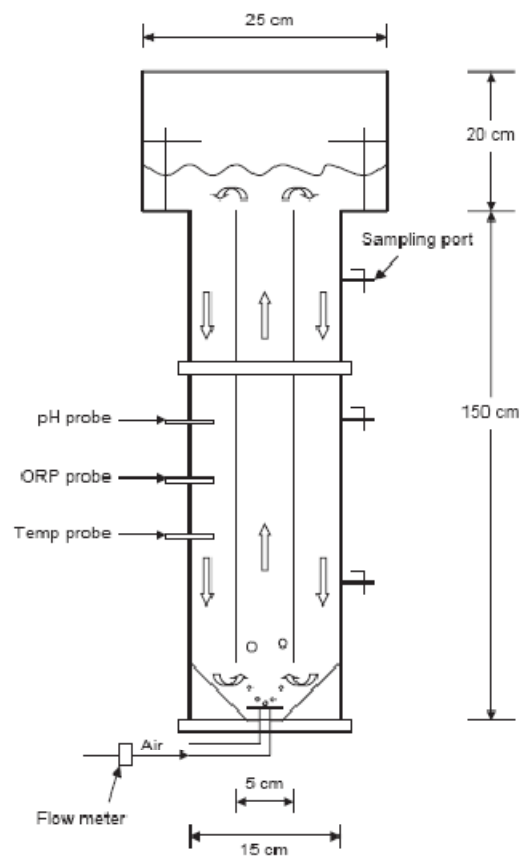


Figure 2.5 Schematic diagrams for air-lift reactor of bioleaching experiment (Chen & Lin, 2004)

## **CHAPTER THREE**

### **CHARACTERIZATION STUDIES FOR SEDIMENT SAMPLES**

Sediment samples taken from 7 different stations in Izmir inner Bay were analyzed for their pH, water content, grain size distribution, total organic matter content and, the total Cu, Cr, Pb and Zn concentrations. Total heavy metal contents of the sediment samples were determined by using microwave acid digestion which is a common method with a closed vessel extraction system. The accuracy of the digestion method was tested by using a standard reference material. The chemical distribution of the metals were determined by means of the BCR (Community Bureau of Reference) Extraction Procedure (developed by European Commission for Standards, measurement and Testing) which allows the determination of metals bound as four fractions; exchangeable and acid soluble, reducible (bound to iron-manganese oxides), oxidizable (bound to organics) and residual.

#### **3.1 Sample Collection and Processing**

The sediment samples were collected from 7 stations located in the inner Bay of Izmir using RV Koca Piri Reis, a research boat which belongs to Dokuz Eylul University, Institute of Marine Sciences and Technology. The sampling points are named as Harbor, 23, 24, 44, 45, 46, and 47. The sampling points, Harbor, 23, 24, 44 and 47 are on the center line of the bay, and 45 and 46 are located at southern and northern parts of the bay, respectively. Table 3.1 presents the water depths and the coordinates of the sampling sites. As can be seen from the table, sampling point Harbor is the shallowest point in the inner Bay. The deepest point is the sampling point 44 with the 17 m water depth and it is on the center line of the inner Bay. Sampling point 23 is the furthest point from the Izmir Port and it is the nearest point to the middle Bay with 10 m water depth. The increasing depth through the center line suddenly decreases at this point depending on a natural wetland near this area.

Table 3.1 Water depths and coordinates of the sampling stations

Sampling site	Depth (m)	Coordinates
Harbor	8	38 <sup>0</sup> 27' 20'' N, 27 <sup>0</sup> 08' 51'' E
47	11	38 <sup>0</sup> 26' 56'' N, 27 <sup>0</sup> 07' 80'' E
24	15	38 <sup>0</sup> 25' 88'' N, 27 <sup>0</sup> 06' 95'' E
44	17	38 <sup>0</sup> 26' 01'' N, 27 <sup>0</sup> 06' 11'' E
45	15	38 <sup>0</sup> 24' 67'' N, 27 <sup>0</sup> 06' 03'' E
46	12	38 <sup>0</sup> 26' 71'' N, 27 <sup>0</sup> 06' 11'' E
23	10	38 <sup>0</sup> 25' 38'' N, 27 <sup>0</sup> 02' 80'' E

The sampling points were selected considering the heavy metal concentrations in sediments from different water depths of the bay. The locations of the sampling stations are given in Figure 3.1.

A Van Veen Grab sampler was used for sediment sampling. In order to determine the recent pollution on the sediment surface, ten-centimeter thick top layer of the sediment samples was collected with spatulas and deposited into plastic bags. Samples were stored at 4<sup>0</sup>C until processing and analysis.

The collected samples were separated into two identical sub-samples for implementing different studies. Wet sediment samples were used for the determination of water content and grain size distribution. On the other hand, half of the wet sediment samples were dried overnight at 65<sup>0</sup>C in an oven to obtain dry samples necessary for chemical analysis. To provide the homogenization for the further operations, the samples were granulated and grinded to the <60 μm. All bulk metal analysis were done with dry and <60 μm grain size sediment samples. Granulated samples were kept in plastic bags for the chemical analysis (Figure 3.2).

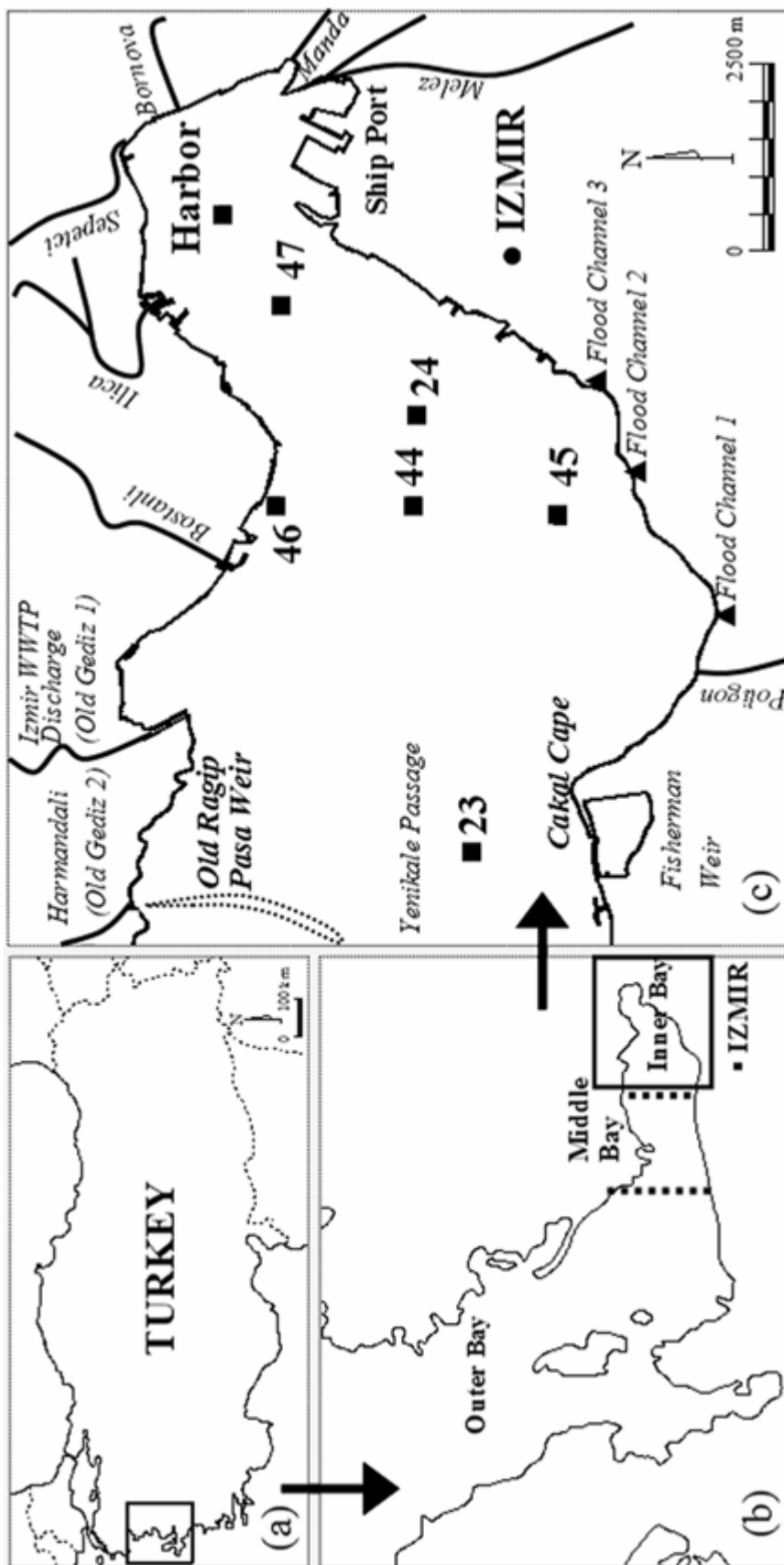


Figure 3.1 (a) Location of Izmir Bay in Turkey (b) Divisions of the Bay (c) Creeks and coastal structures around the inner Bay and the locations of the sampling points

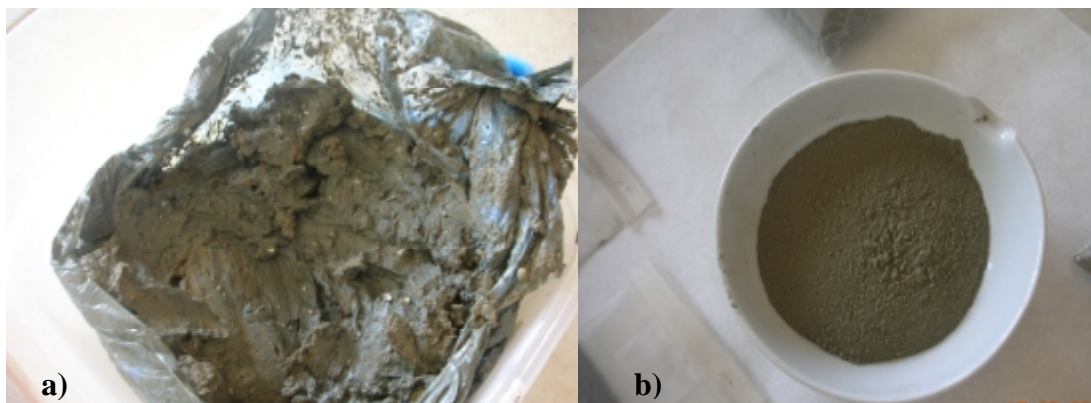


Figure 3.2 General appearance of sediment (a) wet sample ( b) dry and granulated sample

## 3.2 General Characterization Studies

### 3.2.1 pH

The pH values of the sediment samples were determined by using wet samples according to the EPA Method 9045 C (USEPA, 1995). The values were monitored by using a WTW pH 720 pH meter.

### 3.2.2 Water Content

Water content was determined via gravimetric analysis by drying the wet sediment samples overnight at 105<sup>0</sup>C. The moisture content of the sample is determined by using the difference between the weight of wet and dry samples sediments.

### 3.2.3 Organic Matter Content

The determination of organic matter content of the sediment samples were conducted according to the Standard Methods (Franson *et al.*, 1992). This method depends on the ignition of dry sediment samples in an oven at 500±50<sup>0</sup>C.

### 3.2.4 Grain Size Distribution

The grain size distributions of the sediment samples were determined by wet sieving using 4 sieves with different hole sizes. Particles larger than 2000µm were



eliminated since they are counted as rock and gravels. Slurry sediment samples were poured on the top of the sieve set and the sieves were rocked to collect the fractions under the tap water (pH 7.8-8.2). Particles accumulated on the sieves were dried in the oven at 65<sup>0</sup>C and dry sediment samples with different size fractions were obtained on the sieves. Dry sediment samples on the sieves were then swept and weighed. The detected size fractions are given with Table 3.2.

Table 3.2 Fractions used to determine grain size distribution

<b>Fraction</b>	<b>Soil Type</b>
2000 $\mu\text{m}$ > F <sub>A</sub> > 300 $\mu\text{m}$	sand
300 $\mu\text{m}$ > F <sub>B</sub> > 90 $\mu\text{m}$	sand
90 $\mu\text{m}$ > F <sub>C</sub> > 45 $\mu\text{m}$	sand+silt
45 $\mu\text{m}$ > F <sub>D</sub>	Silt+clay

### 3.3 Heavy Metal Content

Examining the heavy metal contents of the sediments in the inner Bay, the bulk concentrations of metals and the chemical distribution (partitioning) of these metals are of great importance. Conventional (open) and microwave (closed) digestion techniques are used to determine; the total metal content in the samples. In open digestion, temperature is limited by the reactive's boiling point and depending on low temperature, the reaction time increases. Microwave digestion provides high temperature and short digestion time (Berghoff, 2004). That's why; the microwaves are preferred to conventional methods in many of the heavy metal monitoring studies of solids. (Cook *et al.*,1997; Sandroni *et al.*, 2003; Sastre *et al.*, 2002).

Total heavy metal contents of the sediment samples were determined by using microwave acid digestion and the partitioning of the metals in the sediment samples were conducted by using sequential extraction procedure.

### 3.3.1 Microwave Acid Digestion

The total Cr, Cu, Pb and Zn concentrations of the sediment samples were determined by using microwave acid digestion method with a closed extraction system. The accuracy of the method was determined by testing it on the standard reference material.

#### 3.3.1.1 Microwave Digestion System

The Questron MicroPrep Q20 Microwave Digestion System with four digestion vessels was used for the extraction (Figure 3.3). The system includes a microwave oven, four high pressure digestion vessels and a vessel module. The maximum microwave power the system can reach is 1000 watts. Samples were placed in liners (TFM Teflon, softening point 250<sup>0</sup>C) which are mounted in ultem caps. The vessels support an operating pressure of 350 psi and a maximum temperature of 260<sup>0</sup>C and they are resistant to HF (Questron Technologies, 2005). Rupture disks were placed in the Over Pressure Valve Stems in the vessels to become a pressure control device in the digester. The system allows the digestion of four samples at the same time and different programmes can be operated by using the control panel.



Figure 3.3 The Questron microwave digestion system (a) microwave oven (b) digestion vessels

#### 3.3.1.2 Digestion Procedures

A heating procedure was improved and tested for accuracy by using the digester. Three different digestion programmes labeled as P1, P2 and P3 were tested with the

standard reference material (SRM). A 0.1 gram of sample was used for each digestion. Combinations of nitric acid (%65 HNO<sub>3</sub>- used for easily oxidizable material) and hydrofluoric acid (%40 HF- used for extraction of inorganic matrixes) and hydrochloric acid (37% HCl) were added to each of the digestion vessels. For microwave assisted digestion procedures, the total extraction time was set as 26 minutes. The highest power applied for all procedures was 600 watts, held for 1 minute in P1 and P2, and 2 minutes for P3. 0.8 ml of HCl was added to the acid mixture for P2 and P3. The details of the heating programs used are given in Table 3.3. In order to determine the accuracy of the extraction procedure, a standard reference material (SRM-1646-A Estuarine sediment obtained from National Institute of Standards and Technology) (NIST, 2004) was digested using the same protocol. For precision, all extractions with SRM and the samples were done in triplicate and the mean values were reported. The digestate obtained were then filtered through 0.45 µm membrane paper, diluted to 20 ml for storage and further analyses. For accurate results, all the extraction procedures were applied in triplicate to the samples.

Table 3.3 Microwave heating procedures used for the digestion of the samples

Program	Sample Weight (g)	Reactives and Consumptions (ml)	Prog. Step	Power (W)	Time (min)
P1 (26 min.)	0.1	65% HNO <sub>3</sub> (3 ml) 40% HF (1 ml) (Total volume :4ml)	1	200	8
			2	400	7
			3	600	1
			4	0	10
P2 (26 min.)	0,1	65% HNO <sub>3</sub> (3 ml) 40% HF (1 ml) 37% HCl (0.8 ml) (Total volume :4.8 ml)	1	200	8
			2	400	7
			3	600	1
			4	0	10
P3 (26 min.)	0,1	65% HNO <sub>3</sub> (3 ml) 40% HF (1 ml) 37% HCl (0.8 ml) (Total volume :4.8 ml)	1	200	8
			2	400	6
			3	600	2
			4	0	10

### 3.3.2 The BCR Sequential Extraction Procedure

The scheme developed by the European Commission for Standards, Measurement and Testing (ECTS&T) that offers a practical procedure using 3 main stages was used to determine the partitioning of metals (Dean, 2003; Rauret *et al.*, 1999). In this technique, metals are divided into 4 fractions such as; exchangeable and acid soluble, reducible, oxidizable, and residual forms. The exchangeable and acid soluble metals are extracted from the sediment mass by the application of  $\text{CH}_3\text{COOH}$  (0.11 mol/L acetic acid). 1 gram of sediment sample was extracted with 40 ml of the reagent in shaking incubator at 80 rpm for 16 hours. The sample was then centrifuged at 2500 rpm for 30 minutes; the supernatant was carefully removed to a test tube to analyze the exchangeable and acid soluble fraction of the metal content. The reducible fraction of the metals was determined by using  $\text{NH}_2\text{OH}\cdot\text{HCl}$  (0.5 mol/L hydroxylamine hydrochloride) as extraction chemical. The process of shaking, centrifugation and washing was repeated as in the first step. The third stage consists of the application of  $\text{H}_2\text{O}_2$  (30% hydrogen peroxide) solution and  $\text{CH}_3\text{COONH}_4$  (1 mol/L ammonium acetate solution). This step allows the extraction of oxidizable fraction (metals bound to organic matter). The difference between the total metal concentration and the sum of the three fractions above gives the metals in the residual fraction.

The extractions were conducted in 50 ml polypropylene centrifuge tubes to prevent or minimize losses of solid materials. The sequential extraction procedure was applied twice to the samples. The summary of sequential extraction scheme is summarized with Figure 3.4.

### 3.3.3 Instrumental Analysis

The analysis Cr, Cu, Pb and Zn in the solutions obtained from both microwave digestion and sequential extraction procedures were done using an inductively coupled plasma-optical emission spectrometer (ICP OES) (Perkin Elmer Inc. Optima 4300 DV, USA). A summary of the experimental procedure for the characterization of the sediment samples is given with Figure 3.5.



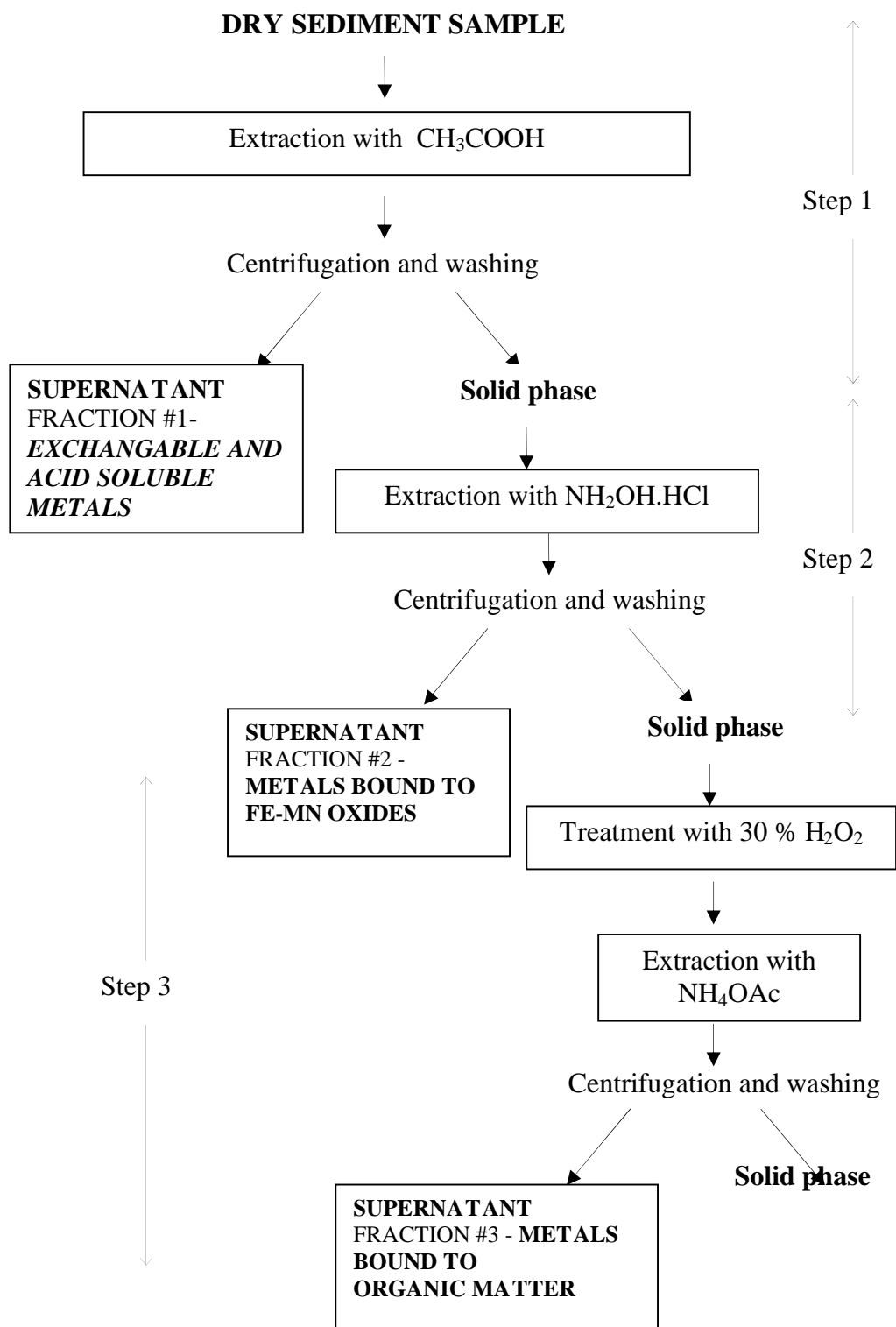


Figure 3.4 The BCR Sequential Extraction Scheme

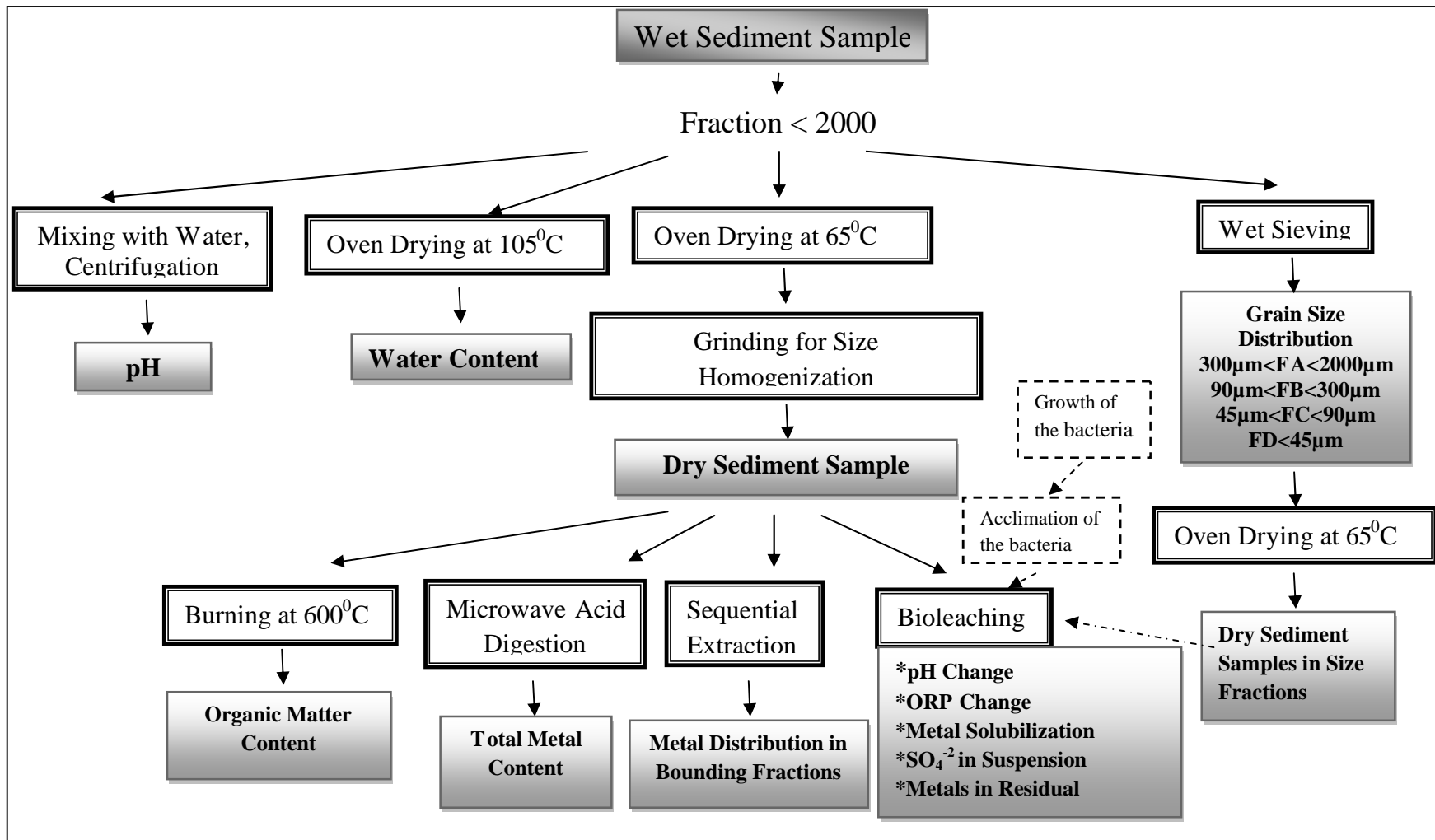


Figure 3.5 Experimental procedure for sediment characterization followed by bioleaching

## CHAPTER FOUR

### BIOLEACHING EXPERIMENTS

The content of this chapter involves the materials and methods used to implement the bioleaching experiments. Two subtopics are handled in this part; the growth and use of the microorganisms and the bioleaching tests conducted in flasks.

#### 4.1 Microorganisms

The microbial cultures of *Thiobacillus ferrooxidans* (11477) and *Thiobacillus thiooxidans* (11478) were supplied from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and stored in cool dark place as emphasized in the instructions. The two of the bacterial strains were studied, either separately or mixed.

##### 4.1.1 Growth of the Bacteria

The liquid media-Medium 271 (DSMZ, 2004) was used to cultivate both species. For *Thiobacillus ferrooxidans*, 1 l of basal medium contains 2 g of  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 g of  $\text{K}_2\text{HPO}_4$ , 0.5 g of  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ , 0.1 g of KCl, 0.01 g of  $\text{Ca}(\text{NO}_3)_2$ , 8 g of  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  and 1000 ml of distilled water. The pH was adjusted to 2.0 with dilute  $\text{H}_2\text{SO}_4$  (1 mol/l). The basal medium and the ferrous sulfate (8.0 g  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  in 50 ml distilled water) were autoclaved separately and mixed together after being cooled.

For *Thiobacillus thiooxidans*, 1 l of basal medium consists of 2 g of  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 g of  $\text{K}_2\text{HPO}_4$ , 0.5 g of  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ , 0.1 g of KCl, and 0.01 g of  $\text{Ca}(\text{NO}_3)_2$ . The pH was adjusted to 2.0 with dilute  $\text{H}_2\text{SO}_4$  (1 mol/l). This time, elemental sulfur was used as the substrate. For 1 l of media, 10 g of sulfur was sterilized in screw-capped tubes in a water bath on 3 successive days for 3 h at 90-100 °C. Before use, the sterilized sulfur was layered onto the surface of autoclaved liquid basal medium.

The sterilization was operated under 121 °C for 15 minutes by using Alp CL- 40L Autoclave. The cultures were inoculated for maintaining subcultures in 500 ml

flasks. The flasks were being incubated in shaking incubator under 30°C with 170 rpm speed for 15 days. The growing cultures were inoculated periodically to regenerate the subcultures. The growing cultures in the flasks can be seen in Figure 4.1.



Figure 4.1 Growing cultures of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* in the flasks

#### **4.1.2 Enumeration of the Bacteria**

Plate count method was used for the enumeration of the bacteria. TSM 1 Medium for enumeration of *Thiobacillus ferrooxidans* on plates containing 3 g of  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 g of  $\text{K}_2\text{HPO}_4$ , 0.5 g of  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ , 0.1 g of KCl, 0.01 g of  $\text{Ca}(\text{NO}_3)_2$ , 22 g of  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ , 5 g of agar and 1000 ml of distilled water. The ferrous sulfate, basal medium and agar in 250 ml water were autoclaved separately and mixed at 60°C after sterilization (Franson *et al*, 1992).

To determine the cell concentrations of *Thiobacillus thiooxidans*, Medium of Starkey was used. This media contains 0.2 g of  $(\text{NH}_4)_2\text{SO}_4$ , 0.25 g of  $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ , 3 g of  $\text{K}_2\text{HPO}_4$ , 0.5 g of  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ , 0.01 g of  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$ , 5 g of agar and 1000 ml of distilled water. 10 g of elemental sulfur was sterilized in a water bath As



mentioned above, the basal medium and agar was autoclaved separately and mixed by adding the sterilized elemental sulfur (Franson *et al*, 1992).

For both of the cultures, the media was then poured in to sterile petri plates on diluted cultures. The cultures were then incubated at 30°C for 14 days in Memmert INB 500 Incubator and the cell concentrations were counted.

#### ***4.1.3 Acclimation of the Bacteria***

Before the bioleaching experiments, the subculture of the microorganisms must be acclimated to contaminated sediment sample. The growth of bacteria in sediment slurry is monitored by measuring the decrease in pH with sulfur oxidation. For the acclimation process, the inoculum of 1%(v/v) of bacteria (*Thiobacillus ferrooxidans* or *Thiobacillus thiooxidans* or mixed culture) was transferred to 150 ml of water containing 2% (w/v) total solids of autoclaved sediment. To be used as substrate, 0,5% (w/v) elemental sulfur was added to the suspension and the cultures were incubated in 500 ml flasks in the shaking incubator at 30 °C and 170 rpm. It was planned to end the acclimation process until pH dropped to 2 (Chen & Lin, 2001). The pH values were determined during the acclimation process by taking samples from the flasks at regular intervals. The bacteria obtained from acclimation process were used for the bioleaching experiments

#### ***4.1.4 Bioleaching Tests***

##### ***4.1.4.1 Sediment Sample***

Large amount of sediment sample taken from a point near Station 23 was used for all the bioleaching sets. The sample was characterized as mentioned in the previous chapter and the dry sample was kept in plastic bags in a cool dark place prior to the use.

#### 4.1.4.2 *Flask Experiments*

The bioleaching experiments were carried out in 1000 ml flasks with 250 ml reaction volume containing the dilution medium necessary for the bacteria growth. A 5% (v/v) growing culture inoculums of the bacteria obtained from the acclimation process was added to the each main volume consisting of contaminated sediment. The bioleaching experiments were conducted in shaking incubator (Thermo Electron 420) at 170 rpm. Temperature was maintained at 30°C which is the optimum growing temperature of the bacteria. Aeration of the flasks was maintained by orbital shaking of the incubator (Figure 4.2).



Figure 4.2 Bioleaching flasks in the shaking incubator

#### 4.1.4.3 *Initial pH*

The initial pH in the flasks was determined by pre-bioleaching experiments by testing pH:7, pH:5.5, and pH:4 as initial values. The pH adjustments were maintained by using dilute H<sub>2</sub>SO<sub>4</sub> (1 mol/l). Due to the pH decrease depending on the

bacterial growth, the initial pH value was chosen and used in the bioleaching experiments while the rest of them were ignored.

#### *4.1.4.4 Bioleaching period*

The bioleaching period was determined by pre-bioleaching experiments. Depending on the cease of the pH decreases, the bioleaching duration was fixed and used in the experiments.

- § The pH in the bioleaching flasks were monitored twice a week.
- § The ORP in the bioleaching flasks were monitored twice a week.
- § The Cr, Cu, Pb and Zn concentrations in solution were monitored by sampling once a week.
- § The sulfate ( $\text{SO}_4^{-2}$ ) concentration in solution was monitored by sampling once a week.
- § The Cr, Cu, Pb, and Zn remained in the residual sediment were determined by sampling on the 18<sup>th</sup>, 32<sup>nd</sup>, and 48<sup>th</sup> days of the experiments. The solid samples taken from the bioleaching flasks were dried and digested by using the microwave digestion procedure explained in 3.3.1.2.

At the end of bioleaching period, the chemical distributions of Cr, Cu, Pb and Zn in the remaining sediments were monitored to see the change in bounding fractions. To achieve this, solid samples were dried and extracted by applying the sequential extraction procedure.

For precision, the tests were duplicated and the mean values of the detected parameters are reported. The metal analyses of the bioleaching tests and sediment extracts were conducted by Perkin Elmer Optima 4300 DV ICP-OES.  $\text{SO}_4^{-2}$  analyses were conducted by Dionex IC-3000 Ion Chromatography System.

#### 4.1.5 Parameters that Effect Bioleaching

Various physicochemical and biological parameters affect the process of bioleaching. Among these parameters; the solid/liquid ratio (sediment concentration) in the solution, bacteria type used in the process, sulfur concentration in suspension, and the grain size of the sediment samples were investigated in this study to determine the optimum values for the highest bioleaching efficiency. All of the bioleaching flasks were autoclaved for sterilization before the bacteria was transferred to the contaminated sediment. The initial pH values in the flasks were arranged as 4.

##### 4.1.5.1 Bioleaching Experiments with Different Bacteria Type

As mentioned before, two of the bacterial cultures (*Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*) were used, both separately and together to determine the effect of microbial activity and obtain the optimum bioleaching efficiencies (Table 4.1). Mixed cultures of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* (1:1 (v/v)) were grown together in the dilution medium. The solid liquid ratio in the flasks was kept constant in all the flasks with 5%(w/v) and the sulfur concentration was 0.5%(w/v). The cultures were acclimated separately to be used in the bioleaching tests.

Table 4.1 Composition of the bioleaching tests for different bacteria type

Flasks	Bacteria type	Solid/Liquid (w/v)	Sulfur Concentration	Reaction Volume	Grain Size
F1, F2	<i>Thiobacillus ferrooxidans</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Bulk
F3, F4	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Bulk
F5, F6	<i>Mixed culture</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Bulk
F7 – Blank	-	5 % (50g/l)	0.5% ( 5g/l)	125 ml with methanol	Bulk

#### 4.1.5.2 Bioleaching Experiments with Different Solid/Liquid Ratio

The sediment concentration in water (solid liquid ratio) is of great importance in bioleaching studies. In this set, the sulfur concentration added to suspension and the bacteria type inoculated in the flasks were kept constant where three different sediment concentrations were tested to observe their effect on bioleaching of metals (Table 4.2). *Thiobacillus thiooxidans* were and used in this set of experiments.

Table 4.2 Composition of the bioleaching tests for different solid/liquid ratio

Flasks	Bacteria type	Solid/Liquid (w/v)	Sulfur Concentration	Reaction Volume	Grain Size
F1, F2	<i>Thiobacillus thiooxidans</i>	10 % (100g/l)	0.5% ( 5g/l)	250 ml	Bulk
F3, F4	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Bulk
F5, F6	<i>Thiobacillus thiooxidans</i>	2.5 % (25g/l)	0.5% ( 5g/l)	250 ml	Bulk
F7 – Blank	-	5 % (50g/l)	0.5% ( 5g/l)	125 ml with methanol	Bulk

#### 4.1.5.3 Bioleaching Experiments with Different Sulfur Concentration

Elemental sulfur is an essential **substrate** for the growth of Thiobacilli and bacterially catalyzed metal solubilization in the bioleaching process. So, the substrate concentration added to the reaction volume can be counted as one of the major factors affecting the bioleaching process. In our study, elemental sulfur is used with the concentrations of 0.25%, 0.5%, 1%, and 1.5% (w/v). The sediment concentration in all the bioleaching flasks was 5% (w/v). Since sulfur is necessary only for the growth of *Thiobacillus thiooxidans*, this strain was used as the inoculums in this set of experiments (Table 4.3).

Table 4.3 Composition of the bioleaching tests for different sulfur concentrations

Flasks	Bacteria type	Solid/Liquid (w/v)	Sulfur Concentration	Reaction Volume	Grain Size
F1, F2	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	0.25% ( 2.5g/l)	250 ml	Bulk
F3, F4	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Bulk
F5, F6	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	1% ( 10g/l)	250 ml	Bulk
F7, F8	<i>Thiobacillus thiooxidans</i>	5 % (50g/l)	1.5% (15g/l)	250 ml	Bulk
F9 – Blank	-	5 % (50g/l)	-	125 ml with methanol	Bulk

#### 4.1.5.4 Bioleaching Experiments According to the Grain Size of the Sediment

Particle size distribution of the sediment sample was determined by wet sieving and the following size fractions were obtained prior to the use of bioleaching experiments;

2 000  $\mu\text{m}$  > **Coarse** > 300  $\mu\text{m}$       sand  
 300  $\mu\text{m}$  > **Medium** > 45  $\mu\text{m}$       Sand + silt  
 45  $\mu\text{m}$  > **Fine**      silt+clay

In order to determine the particle size effect on bioleaching process, 3 subsamples (fine, medium, and coarse particles) were used in the flasks. Since the previous sets of experiments allowed to collect enough data in order to compare the results with literature studies, the last set of experiments were conducted with mixed culture to enrich the content of the study.

The subsamples were extracted by using the microwave and BCR Extraction procedures explained in Chapter 3. Mixed culture of the bacteria was inoculated to the reaction volume. The solid liquid ratio was constant in the flasks with 5% and the sulfur concentration was fixed to 0.5% (Table 4.4).

Table 4.4 Composition of the bioleaching tests for different grain size distributions of the sediment

<b>Flasks</b>	<b>Bacteria type</b>	<b>Solid/Liquid (w/v)</b>	<b>Sulfur Concentration</b>	<b>Reaction Volume</b>	<b>Grain Size</b>
F1, F2	<i>Mixed culture</i>	5% (50g/l)	0.5% ( 5g/l)	250 ml	Fine
F3, F4	<i>Mixed culture</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Medium
F5, F6	<i>Mixed culture</i>	5 % (50g/l)	0.5% ( 5g/l)	250 ml	Coarse
F7 – Blank	-	5 % (50g/l)	0.5% ( 5g/l)	125 ml with methanol	Medium

**CHAPTER FIVE**  
**HEAVY METALS IN THE SEDIMENTS OF IZMIR INNER BAY**

**5.1 General Characteristics**

The sediment samples collected from seven points in Izmir inner Bay were usually dark grey in color with no smell. The amount of the particles larger than 2000 $\mu\text{m}$  was small, except sample 44 which contains considerable amount of seashells. General sediment features in Izmir inner Bay is given with Table 5.1.

Table 5.1 General features of sediment samples in Izmir inner Bay

General features		Sampling Sites						
		Harbor	23	24	44	45	46	47
pH		8.15	8.20	8.14	7.70	8.30	8.20	7.90
Moisture (%)		60.5	50.2	43.3	57.7	65.3	61.7	47.4
Total organic matter (%)		9.4	7.3	12.3	9	7.7	9.1	9.9
Grain Size Distribution	>2000 $\mu\text{m}$ (%)	8.7	2.7	5.8	31.6	1.3	4	3.2
	2000-300 $\mu\text{m}$ (%)	27.2	22.8	35	10.9	31.9	32	24.3
	300-90 $\mu\text{m}$ (%)	29.2	11.1	30	7.3	31.2	19.4	15.2
	90-45 $\mu\text{m}$ (%)	8.6	12.3	7.3	4.8	9.2	7.7	4.1
	<45 $\mu\text{m}$ (%)	26.3	51.1	21.9	45.4	26.4	36.9	53.2

As can be seen in Table 5.1, the pH values of the sediment samples change between 7.7 and 8.30 which present alkali conditions. The sediment samples were usually solid since the moisture content varied between 47.4% and 65.3%.

High organic content in the samples indicates the organic pollution in the sediments of Izmir inner Bay. The highest organic matter content was observed at sampling point Harbor (23.8, dry mass) where it is lowest at point 23 (14.7, dry mass). These values are in agreement with the literature (Kontas, 2006). Organic



content in the samples tend to decrease from sampling point Harbor to point 23, this indicates that organic pollution decreases from inner to outer regions of the Bay.

## 5.2 Total Metal Concentrations

The total concentrations of Cr, Cu, Pb and Zn in the inner bay sediments show fluctuations at different sampling points and the maximum concentrations of metals are found at sampling point Harbor. Cr concentrations change between 404 and 1563 mg kg<sup>-1</sup>. The concentrations of Cu vary between 66 and 150 mg kg<sup>-1</sup>, only the sample from point Harbor has 993 mg kg Cu. The Pb concentrations in the sediment samples were close to each other and vary from 82 to 203 mg Pb/kg dry mass. Only sample 23 contains 18 mg kg<sup>-1</sup> of Pb. The same condition is observed for Zn with the highest and lowest values at samples Harbor and 23, 1031 and 213 mg Zn/kg dry mass respectively (Figure 5.1).

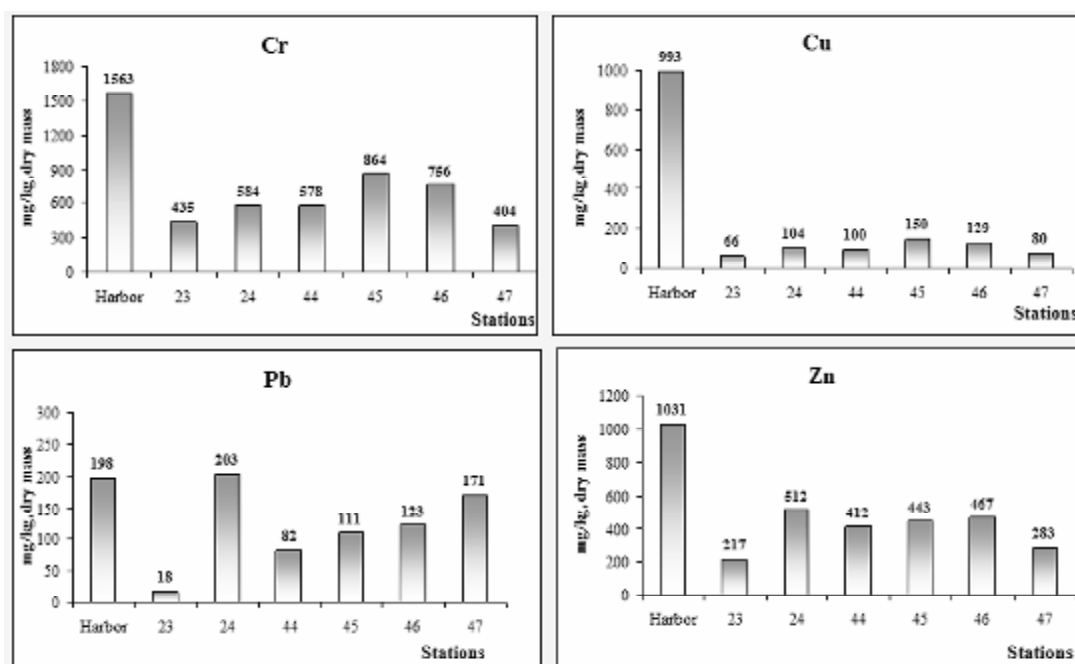


Figure 5.1 Concentrations of Cr, Cu, Pb and Zn in the sediment samples (mg kg<sup>-1</sup>, dry mass)

### 5.2.1 Accuracy of the Method

The standard reference material, Estuarine Sediment -1646 A (SRM) obtained from NIST was used to determine the accuracy of the selected microwave extraction procedure and the recoveries are given with Table 5.2.

Table 5.2 Recoveries of metals obtained from Estuarine Sediment 1646A

Metals	Certified Value (mg kg <sup>-1</sup> ±sd)	P1 mean (min-max), mg kg <sup>-1</sup>	P2 mean (min-max), mg kg <sup>-1</sup>	P3 Mean (min-max), mg kg <sup>-1</sup>
<b>Cr</b>	<b>40.9±1.9</b>	35.9 (33.6-38.6)	37.1 (33.0-39.8)	38.9 (36.6-43.2)
<b>Cu</b>	<b>10.0±0.3</b>	5.1 (4.6-5.8)	11.3 (10.0-12.8)	11.1 (10.4-12.2)
<b>Pb</b>	<b>11.7±1.2</b>	7.2 (6.8-7.8)	13.1 (8.2-16.4)	11.4 (10.9-11.7)
<b>Zn</b>	<b>48.9±1.6</b>	41.7 (35.2-51.6)	34.3 (28.4-37.8)	47.1 (45.1-48.2)

By using P1, poor recovery rates were obtained for Cu (50.6%) and Pb (61.5%) where Zn was recovered satisfactorily with 85.3% as well as Cr (recovery rate 87.9%). The difference of P2 from P1 is the HCl addition into the HNO<sub>3</sub>-HF mixture. Recovery values for the Estuarine Sediment showed differences according to the metals. High recovery rates were obtained for Cu and Pb (113.7% and 111.7%, respectively) where Zn was poorly recovered with 70.1%. Cr was recovered satisfactorily with 90.6% in the sediment sample. In P3, 600 watts power was applied to the system for 2 minutes. Different than P2, this procedure showed consistent results for Estuarine Sediment. For all the metals studied, accurate recovery values were obtained (Cr; 99.8%, Cu; 103.9%, Pb; 97.4 %, Zn; 96.3 %). This may be explained by the longer application of 600 watts power for the complex matrix of the sediment.

Comparison of recovery rates of Estuarine Sediment – 1646A for the digestion procedures P1, P2, and P3 are shown in Figure 5.2.

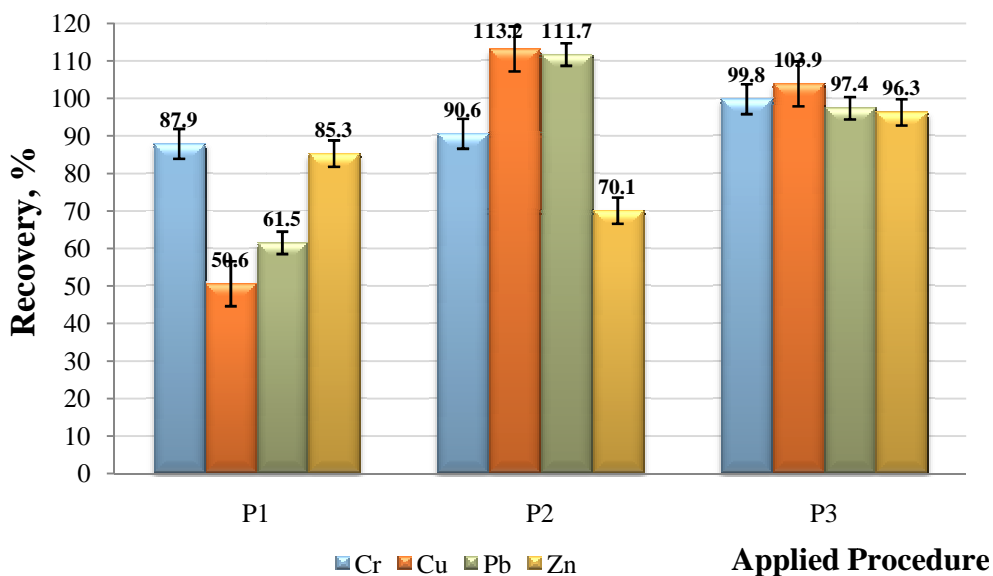


Figure 5.2. Comparison of recovery rates of the digestion procedures for Estuarine Sediment-1646A

As can be seen from Figure 5.2, the Estuarine Sediment gave the most adequate results with P3. The recovery values for all the metals were over 95%. This shows that the extraction procedure P3 is acceptable to determine the total heavy metals in the sediment samples. This procedure was decided to be used for the determination of Cr, Cu, Pb and Zn in the solid sediment samples during all the study.

### 5.3 Distribution of Metals According to Binding Forms

Differences are observed in the chemical partitioning of heavy metals with respect to the location of the sampling points (Figure 5.3). Cr exists highly as the oxidizable form (71% to 92%) except for sample 23, where it is found in 68% as the residual form. The highest value for the sum of acid soluble and reducible forms of Cr in the samples is below 7% of total concentrations.

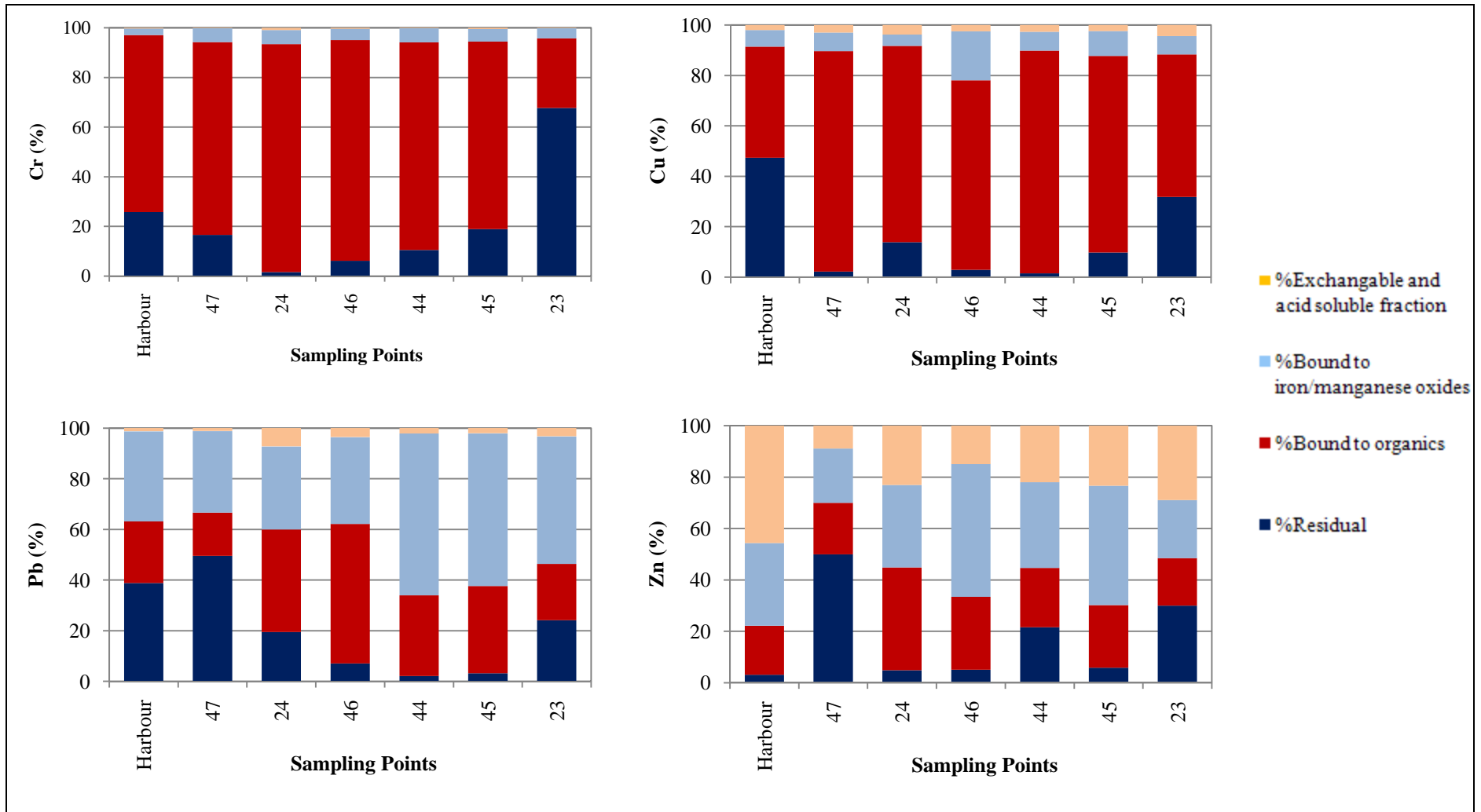


Figure 5.3 Distributions of Cr, Cu, Pb and Zn according to the binding forms in the sampling points of Izmir inner Bay

The chemical distribution of Cu in the samples is similar to Cr, and Cu tends to accumulate mostly in the organic fraction of the sediment samples (in 44% - 88%), this means that Cu and Cr can be released under oxidizable conditions. Cu is found in the residual fraction in samples from Harbor and 23; 47% and 32%, respectively. In the sample from point 46, Cu in the reducible fraction is 20%, this may also be considered significant. The sum of acid soluble and reducible fractions of Cu in the samples does not exceed 13% except for sample from point 46.

Contrary to the case of Cr and Cu, the concentration of Pb in the reducible fraction is high (mean value is 44%) in the sediment samples. For samples from points 44, 45, and 23; Pb concentrations in the reducible fraction are 64%, 60%, and 50%, respectively. In samples 24 and 46, Pb is mainly observed in the oxidizable fraction, with concentrations of 41% and 55%, respectively. But in samples Harbor and 47, the residual fraction contains higher Pb values (39% -50%) than the other samples.

Zn is mostly found in the acid soluble and reducible fractions (mean value for the sum of these fractions is 63%) except for sampling point 47. In sample 47, Zn is bound mostly to the residual fraction (50%).

#### **5.4 Discussion**

The total concentrations of Cr, Cu, Pb and Zn and their chemical distributions in the sediments of Izmir inner Bay were determined in this part. Due to the findings, the highest values of the metals were found in sampling point Harbor which is the shallowest sampling point. The water depth at this point is 8 m and it is located just across the port in Izmir Harbor. As can be seen in Figure 3.1-c, sampling point Harbor is under the effect of Ilica, Sepetci, Bornova, Manda, and Melez Creeks. The total catchment area of these Creeks is 362 km<sup>2</sup>, with the major components of Melez (173 km<sup>2</sup>) and Manda (108 km<sup>2</sup>) Creeks (IZTO, 1995). The amount and the content of the materials carried by the creeks from this large catchment area is the major reason of the shallow depth and the high concentrations of heavy metals in the sediments from sampling point Harbor.

The farthest sampling point from the port; point 23, which is located at the western part of the Bay, contains the lowest metal concentrations. This point is far from the creek discharges that may affect it, so the lowest concentrations of metals were found at this point. The water is shallow at this point because a natural wetland is located near this area.

Sampling points 24, 44, 45, 46, and 47, which are located in the middle parts of the inner Bay, do not seem to be influenced by the effect of surrounding creeks and flood channels. The distribution of the pollutants in this area was effected by the water circulation in the Bay and sedimentation has occurred according to the mass of the particles and the depth of the water.

With respect to their total bulk concentrations, the metals can be ranked in decreasing order as follows: Cr > Zn > Cu > Pb.

Cr is found in the highest concentration in the sediments of the sampling area. This may be the result of past illegal discharges from the tanneries which are on the catchment basins of Manda and Melez Creeks. Zn and Cu are widely used in metal and automotive industries. The organized industrial zones which are located on the catchment basins of Manda and Old Gediz 1 Creeks contain a number of metal industries, and automotive parts manufacturing plants, so, Cu and Zn may reach the inner bay by means of these creeks. Pb enters the Bay from the automotive, chemical (especially dye production), and metal industries and from the organized industrial zones located on the basins of Sepetci, Manda, and Melez Creeks.

In the past, various studies were conducted to determine the heavy metals pollution in the sediments of Izmir Bay. As can be seen in Table 3, the highest Cr concentration in the inner Bay was measured in 1997 as  $600 \text{ mg kg}^{-1}$  (Aksu *et al.*, 1998). In the same study, the highest Zn concentration found in the sediments of inner Bay was  $350 \text{ mg kg}^{-1}$ . The highest Cu and Pb values were determined as  $121 \text{ mg kg}^{-1}$  and  $110 \text{ mg kg}^{-1}$ , respectively by Cihangir and Küçüksezgin, 2003.

Compared with the literature values reported for the heavy metal content of the sediments in Izmir Bay, the values determined in this study are higher. This may not

be solely due to an increase of the heavy metal concentrations in time but also to the different sampling points chosen. Also, different extraction procedures were used in the previous studies and this may have contributed to the differences

Table 5.3 Heavy metal concentrations in the sediments of Izmir Bay; a comparison of published results and this study ( $\text{mg kg}^{-1}$ , dry mass)

Metals	Izmir Inner Bay, 1997 (min-max)	Izmir Inner Bay, 1999 (mean $\pm$ sd)	Izmir Inner and Middle Bay, 2001 (min-max)	This work Izmir Inner Bay (min-max)
<b>Cr</b>	250-600	300 $\pm$ 100	171-295	404 - 1563
<b>Cu</b>	20-80	70 $\pm$ 38	32-121	66 - 993
<b>Pb</b>	20-60	62 $\pm$ 29	61-110	18 - 203
<b>Zn</b>	50-350	nd	86-286	217 - 1031
<b>Ref.</b>	Aksu <i>et al.</i> , 1998	Atgin <i>et al.</i> , 2000	Cihangir and Küçüksezgin 2003	2006

The present study also reveals the chemical distribution of the metals. According to our results, Cr is mostly bound to the oxidizable fraction, only in sample 23, Cr exists in the residual fraction (68%) indicating low Cr mobility in this area.

Cu can easily form complexes with organic matter due to the high stability constant of organic Cu compounds (Morillu *et al.*, 2004). Similar to Cr, Cu is mainly found in oxidizable form (44% - 88%) in the sediments so it can easily be released upon degradation of organic matter.

Pb is mostly concentrated in the reducible fraction. This fraction is unstable and the metals can be transferred to available forms due to the changes in redox potential.

In contrast to the other metals, Zn is distributed almost evenly on each fraction. The amount of Zn in the exchangeable and acid soluble fraction is the highest and therefore, Zn will probably have the highest mobility in the sediments.

**CHAPTER SIX**  
**RESULTS OF BIOLEACHING EXPERIMENTS AND DISCUSSION**

**6.1 General**

The bioleaching studies were conducted as four sets to observe the effects of main parameters on metal solubilization. These parameters are the applied bacteria type, solid/liquid ratio, sulfur concentration, and the grain size of sediments. The four sets of bioleaching experiments are presented with Table 6.1.

Table 6.1 A summary of the bioleaching tests

<b>Tested Parameter</b>	<b>Bacteria type</b>	<b>Solid/Liquid Ratio (w/v)</b>	<b>Sulfur Conc. (w/v)</b>	<b>Grain Size</b>
<b>Bacterial Strain</b>	<i>T. ferrooxidans</i> <i>T. thiooxidans</i> <i>Mixed culture</i>	5% (50 g/l)	0.5 % (w/V)	Bulk
<b>Solid/Liquid Ratio</b>	<i>T. thiooxidans</i>	<b>10%(100g/l)</b> <b>5% (50g/l)</b> <b>2.5%(25 g/l)</b>	0.5 % (w/V)	Bulk
<b>Sulfur Concentration</b>	<i>T. thiooxidans</i>	5% (50g/l)	<b>0,25 %</b> <b>0,5%</b> <b>1%</b> <b>1.5%</b>	Bulk
<b>Grain Size of the Sediments</b>	<i>Mixed culture</i>	5% (50 g/l)	0.5%	<b>Fine</b> <b>Medium</b> <b>Coarse</b>

**6.2 Sediment Sample**

The bulk sediment sample used in the bioleaching experiments was characterized for its general features and metal content (Table 6.2). The subsamples of different grain sizes (fine, medium and coarse) were characterized separately for their total and chemically distributed metal concentrations and the results are presented. The metals in binding fractions of the bulk sediment sample are presented with Figure 6.1.



Table 6.2 Characteristics of the bulk sediment sample used in bioleaching experiments

Characteristics		Bulk Sediment Sample
pH		7.81
Moisture,(%)		46.4
Total organic matter, (%)		8.8
Grain Size Distribution	Coarse 2000-300 $\mu$ m (%)	10
	Medium 300-45 $\mu$ m (%)	39
	Fine < 45 $\mu$ m (%)	51
Total Cr Concentration, (mg kg <sup>-1</sup> $\pm$ sd)		527 $\pm$ 45.6
Total Cu Concentration, (mg kg <sup>-1</sup> $\pm$ sd)		115.4 $\pm$ 13.3
Total Pb Concentration, (mg kg <sup>-1</sup> $\pm$ sd)		141.2 $\pm$ 13.8
Total Zn Concentration, (mg kg <sup>-1</sup> $\pm$ sd)		565 $\pm$ 19.8

(sd: standard deviation)

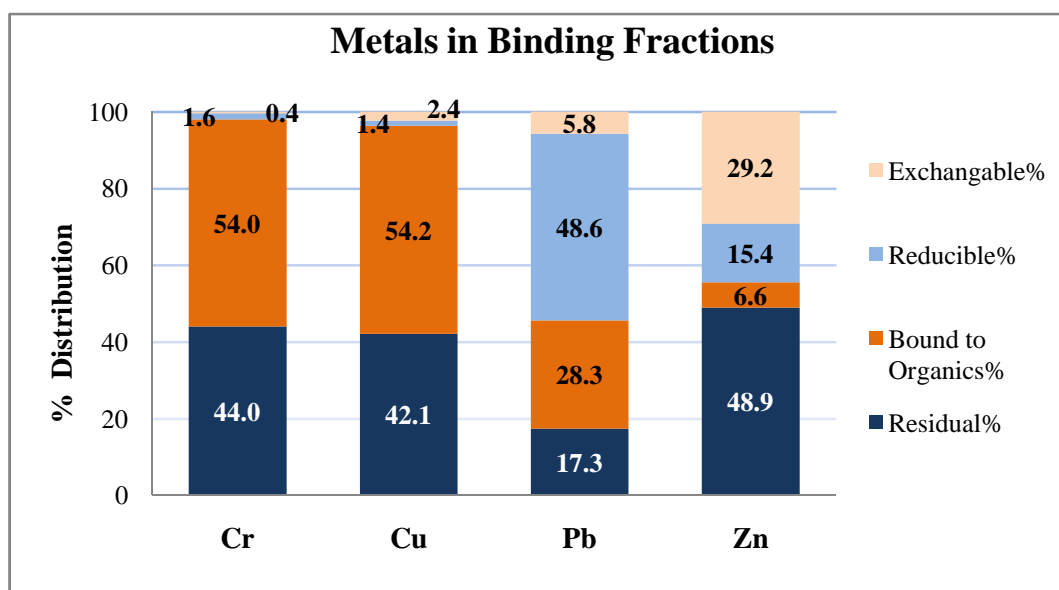


Figure 6.1 Distributions of Cr, Cu, Pb and Zn according to the binding fractions

As can be seen in Figure 6.1, binding fractions of metals in the bulk sediment sample are different from each other. Cr and Cu tend to accumulate mostly on the organic fraction where Pb can be found in the reducible fraction with the highest

proportion. Most of the Zn is found in the residual fraction but the amount of Zn in the exchangeable fraction is the highest of all metals studied. The distributions of the binding fractions of the bulk sediment sample are in agreement with the results given in Chapter 5.

The total metal concentrations in the sediment sample are much higher than the limits indicated in EPA-OSWER Ecotox Thresholds, and CCREM's Probable Effect Levels (Table 1.1). This situation shows necessity of a treatment method to be applied to the sediments in Izmir inner Bay.

### 6.3 Initial pH and bioleaching period

As mentioned in Chapter 4, the initial pH in the flasks was determined by pre-bioleaching experiments by setting the initial pH in the flasks with the values of pH:7, pH:5.5, and pH:4. The pH change during 50 days were examined and shown in Figure 6.2.

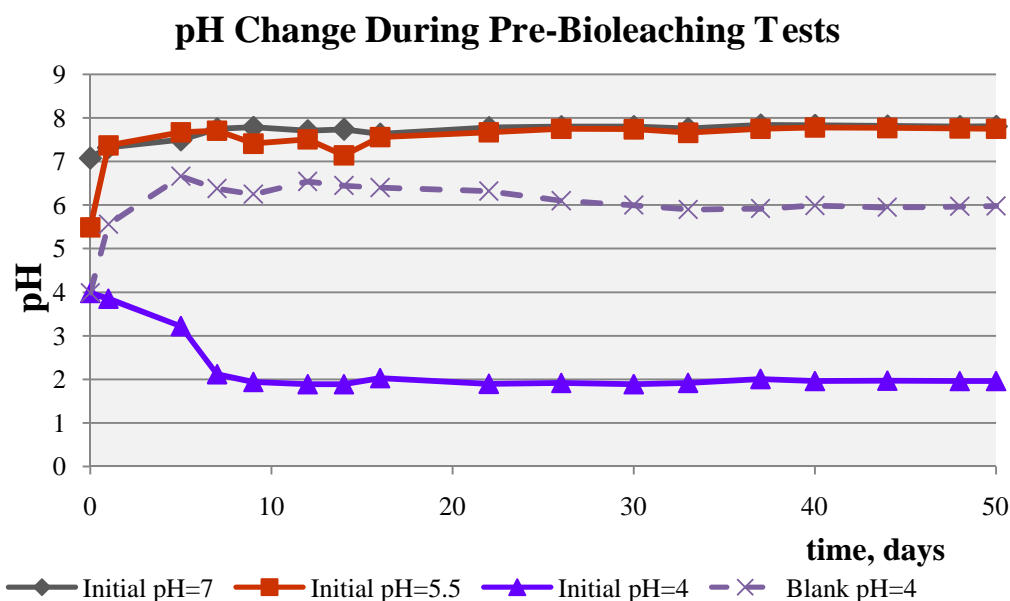


Figure 6.2. pH change depending on the initial pH values

As seen in Figure 6.2, in the flasks where the initial pH value was 7, this value changed between 7.5 and 8 during the 50 days. Because the thiobacilli can only grow

under acidic conditions, it's concluded that no growth of bacteria was obtained in those flasks. Depending on the buffering capacity of the sediments in the flasks with initial pH 5.5, the pH increased rapidly at the end of the first day, and did not decrease in time. It's clear that the bacteria cannot grow under high pH values, so this trial was eliminated, too. For the samples with initial pH=4, the pH increased in the first day then it started to decrease rapidly. At the end of 10 days, the pH values were under 2, so in these flasks it's considered that the microbial activity was carried out. Finally, it's been decided to start the experiments with pH 4 in the bioleaching experiments.

On the other hand, at the end of 48 days, no pH mobility was observed in any of the flasks, so 48 days was chosen as the bioleaching period.

## **6.4 Bioleaching Tests**

### ***6.4.1 Bioleaching Test 1- The Effect of the Bacterial Strain***

The bacterial strains of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, and a mixed culture of them were used to determine their effect on metal solubilization. The solid/liquid ratio was kept constant with 5% (w/v) and the sulfur concentration added to the flasks was 0.5% (w/v) of the dilution medium. Initial pH in the flasks was adjusted to  $4\pm 0.2$ . Bulk sediment samples were used for the experiments.

#### *6.4.1.1 Cell Concentrations of the Bacteria*

The cell concentrations of *T. ferrooxidans* and *T. thiooxidans* in the inoculums were determined as  $3 \times 10^4$  and  $7 \times 10^6$  cfu/ml, respectively. Mixed culture consisted of  $4 \times 10^4$  cfu/ml of *T. ferrooxidans* and  $6 \times 10^5$  cfu/ml of *T. thiooxidans*.

#### *6.4.1.2 Acclimation of the bacteria*

For the acclimation process, 5 days old culture of the bacteria (1% (v/v)) was used in the acclimation flasks containing elemental sulfur as the substrate and autoclaved sediment with 2% (w/v) solid content. The acclimation process was carried out in the

same way for all the bioleaching tests. The pH in the acclimation flasks were arranged to the value of  $4 \pm 0.2$ .

The acclimation processes had lasted for 13 days for all the three trials with the bacteria and at the end of acclimation interval, pH dropped to 1.48, 0.85, and 1.57 with *T. ferrooxidans*, *T. thiooxidans*, and the mixed culture (Figure 6.3).

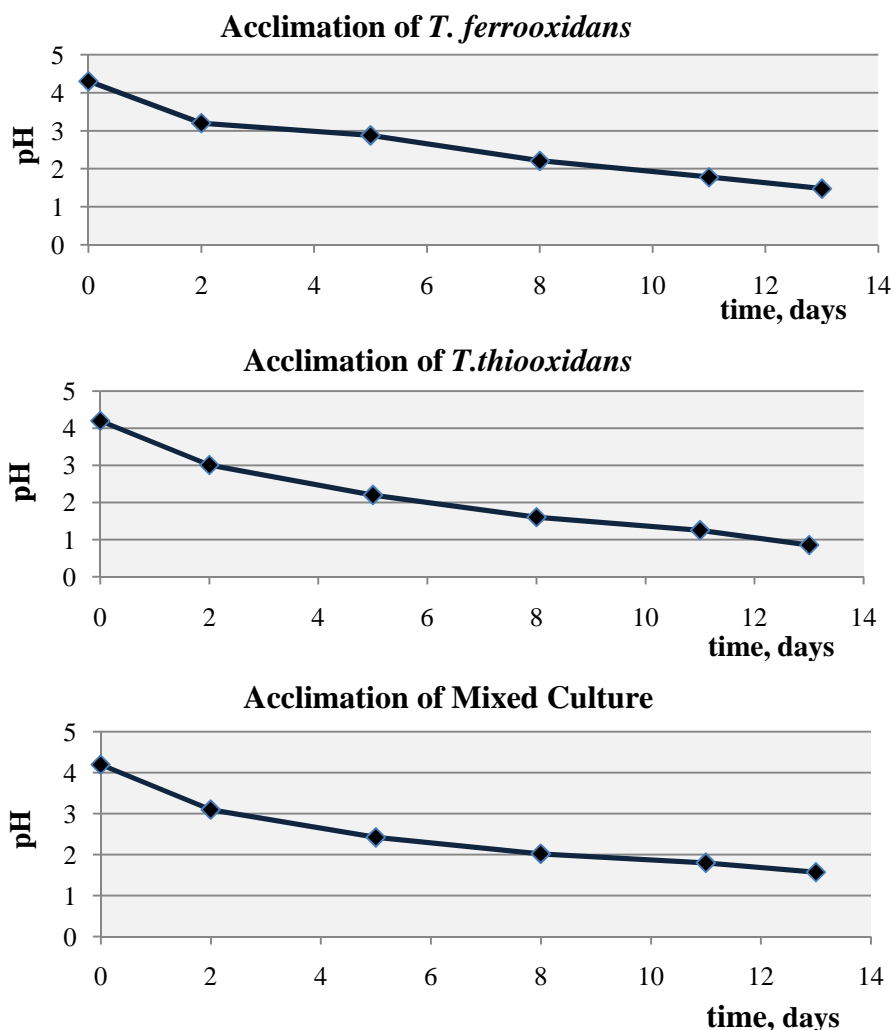


Figure 6.3 The pH changes during the acclimation of the bacteria

#### 6.4.1.3 pH and ORP Changes

Following the acclimation process, the cultures were transferred to the bioleaching flasks containing autoclaved sediment and dilution medium. The initial pH values showed deviations on a small scale depending on the sterilization in the

autoclave (pH  $4 \pm 0.4$ ). The initial ORP values in the flasks changed between 123 and 206. The pH and ORP changes during the bioleaching experiments are presented with Figure 6.4 and 6.5.

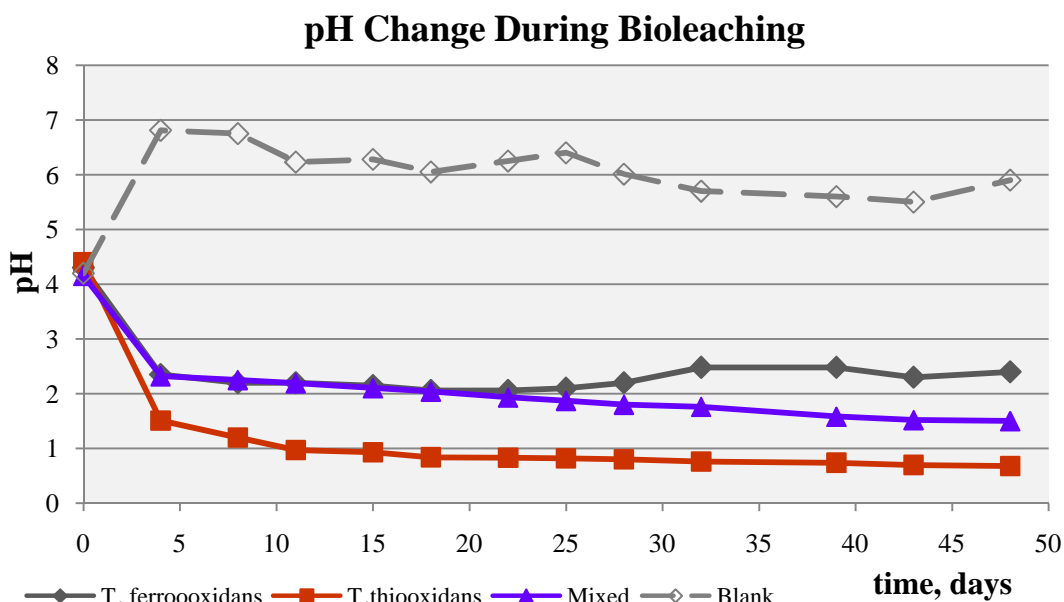


Figure 6.4 Variations of pH in the bioleaching tests depending on bacteria type

There was a sudden decrease in pH in the flasks with *T. thiooxidans* in the first ten days of the experiments and final value reached to pH 0.7 at the end of 48 days. On the other hand, the tests with *T. ferrooxidans* could reach pH 2.4 and pH in the mixed culture dropped to 1.5. The control test without bacteria could not reach the acidic environment (pH was around 6).

During the bioleaching processes conducted with different bacteria strains, decrease of the pH caused an increase in the oxidation-reduction potential (ORP) in sediment. The ORP values were higher with *Thiobacillus ferrooxidans* compared to *Thiobacillus thiooxidans* and the mixed culture. The maximum ORP value reached to 565 mV with *T. ferrooxidans* on the 15<sup>th</sup> day and then decreased slowly. The oxidation reduction potential in the control tests varied between 123 and 190 mV.

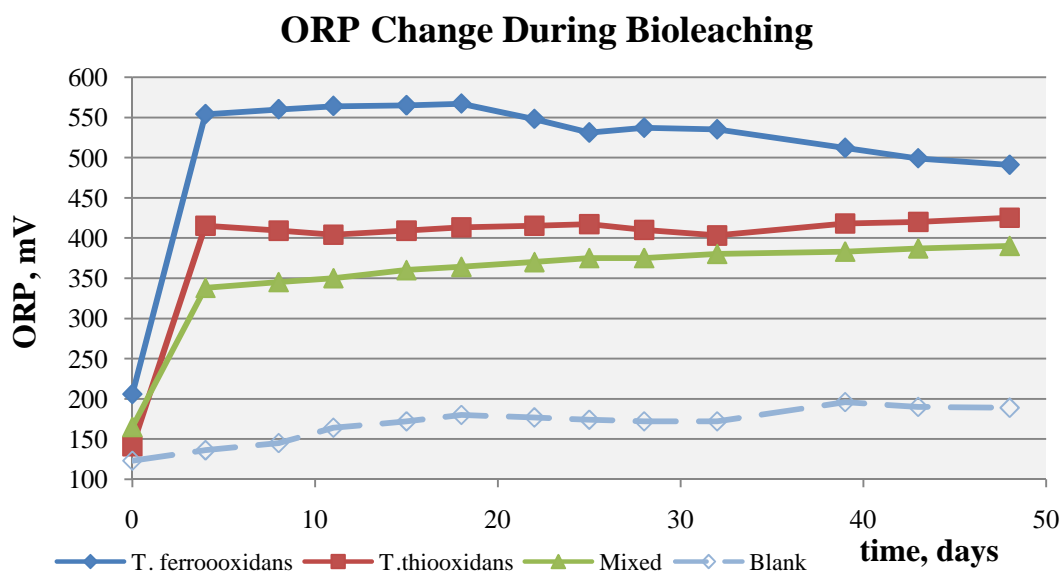


Figure 6.5 Variations of ORP in the bioleaching tests depending on bacteria type

#### 6.4.1.4 Sulfate Production

Sulfate production depending on the bacteria type is given with Figure 6.6. Maximum sulfate production was observed with *T.thiooxidans* at the end of 48 days (21650 mg/l).

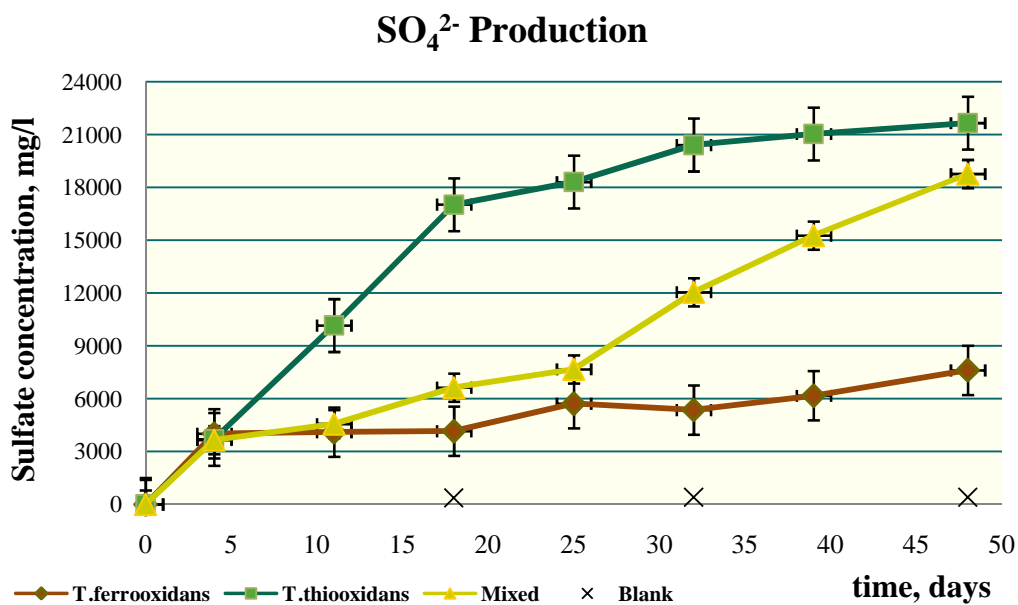


Figure 6.6 Sulfate production during bioleaching according to the bacteria type

#### 6.4.1.5 Metal Solubilization and Removal Efficiency in Residual Sediment

Periodic sampling and analysis of the sediment suspension for soluble heavy metals (Cr, Cu, Pb, Zn) were conducted once a week till the end of 48<sup>th</sup> day. The solubilization (removal from the sediment) ratios of the metals were calculated by Equation 5.

$$\text{Metal Solubilization, \%} = \frac{\text{Load in water (mg)}(L_w)}{\text{Load in sediment (mg)}(L_s)} \times 100 \quad (\text{Eq. 5})$$

According to Equation 5, load in water denotes the mass of soluble metals in the aqueous phase ( $L_w$ ) and the load in sediment ( $L_s$ ) presents the mass of metals in the sediment at the beginning of the bioleaching (Equation 7 and Equation 8).

$$L_w \text{ (mg)} = \text{Metal concentration in water (mg/l)} \times \text{Total volume (l)} \quad (\text{Eq. 6})$$

$$L_s \text{ (mg)} = \text{Metal conc. in sediment (mg/kg)} \times \text{sediment mass (kg)} \quad (\text{Eq. 7})$$

The sediment samples taken on the 18<sup>th</sup>, 32<sup>nd</sup> and 48<sup>th</sup> days of the bioleaching tests were analyzed for their metal content and the removal efficiencies were determined to be compared with the solubilization ratios of the metals. The removal efficiencies were calculated depending on the metal concentrations before starting the experiments and the concentrations detected in the residual sediment samples (Equation 8).

$$\text{Removal Eff., \%} = \frac{\text{Metal in bulk sed. (mg/kg)} - \text{Metal in res.sed. (mg/kg)}}{\text{Metal conc.in bulk sediment (mg/kg)}(t=0)} \times 100 \quad (\text{Eq. 8})$$

Solubilizations and removal efficiencies of Cr, Cu, Pb, and Zn are given with Figures 6.7 and 6.8.

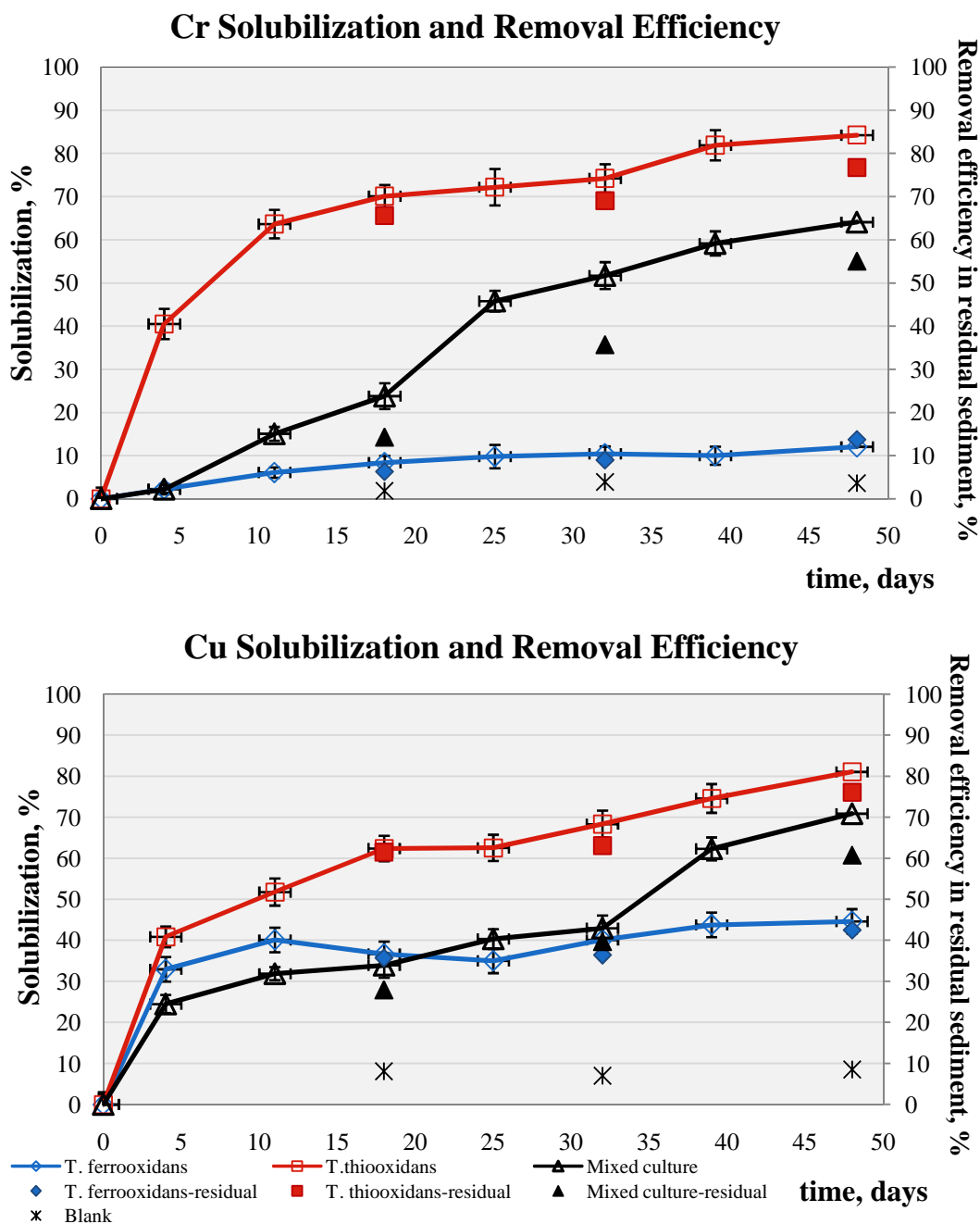


Figure 6.7 Cr and Cu solubilizations and removal efficiencies depending on the bacteria type

As a result, Cr solubilization with *T. ferrooxidans* was not sufficient with 12%. 84% of Cr was solubilized with *T. thiooxidans* and 64% of solubilization was achieved with the mixed culture. Cu was solubilized to a ratio of 44% with *T. ferrooxidans*. With the use of *T. thiooxidans*, Cu solubilization reached to 81%, and mixed culture provided 70% of Cu solubilization. The solubilization efficiencies of



Cr and Cu are in agreement with the removal efficiencies in the residual sediment (Figure 6.7).

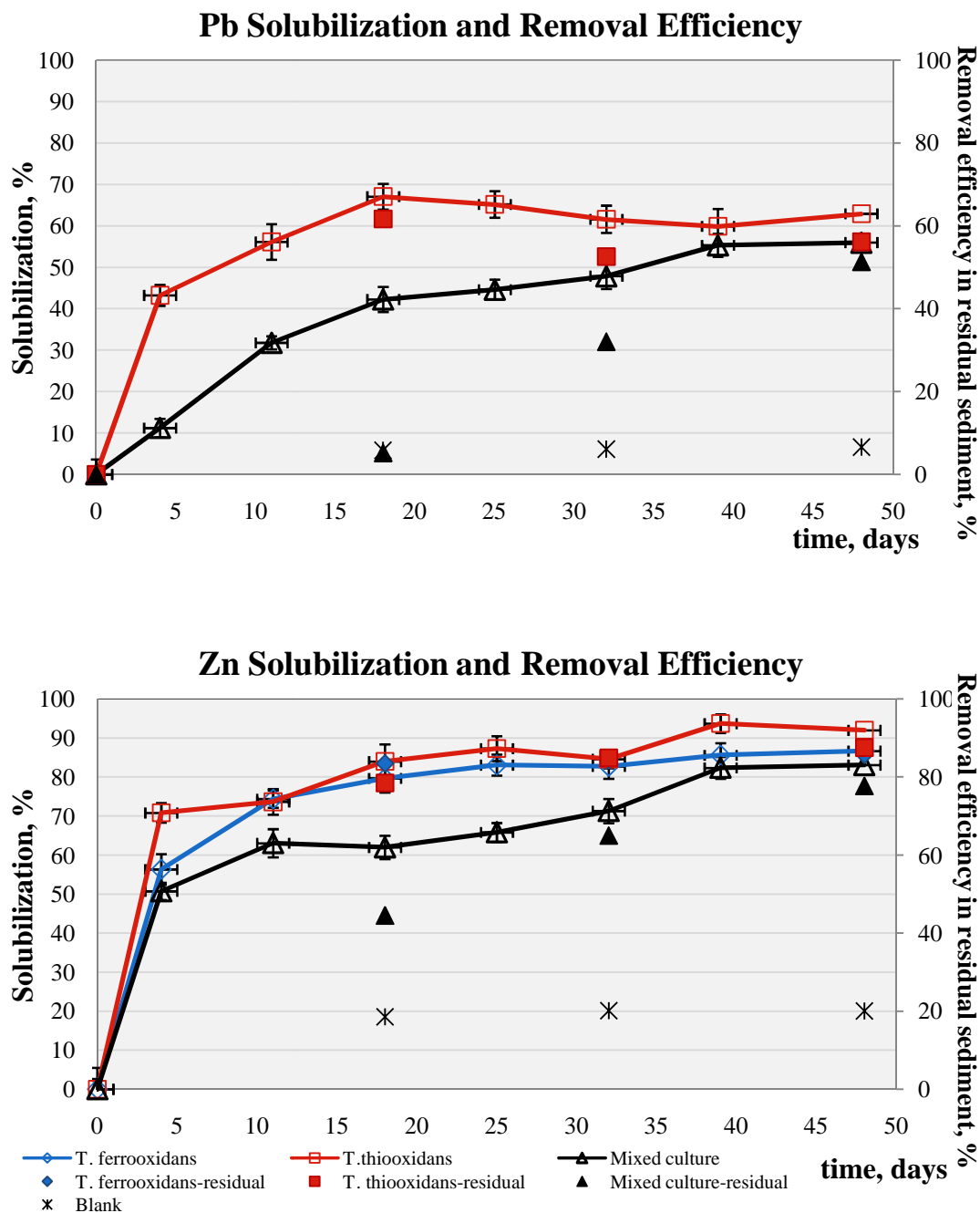


Figure 6.8 Pb and Zn solubilizations and removal efficiencies depending on the bacteria type

Pb solubilization could not be achieved with the use of *T. ferrooxidans*, but removal with *T. thiooxidans* was 63% and the mixed culture provided 56% of Pb

solubilization. Zn was solubilized satisfactorily in all two trials with *T. ferrooxidans*, *T. thiooxidans*, and in the mixed culture, where the solubilization rates are 87%, 92%, and 83%, respectively. The results in residual sediment are in agreement with the results in water (Figure 6.8).

#### 6.4.1.6 Changes in Bounding Fractions

Cr, Cu, Pb, and Zn concentrations in the binding fractions changed after bioleaching. Figure 6.9 and 6.10 illustrate the metal concentrations in the chemical binding fractions of sediments before and after bioleaching.

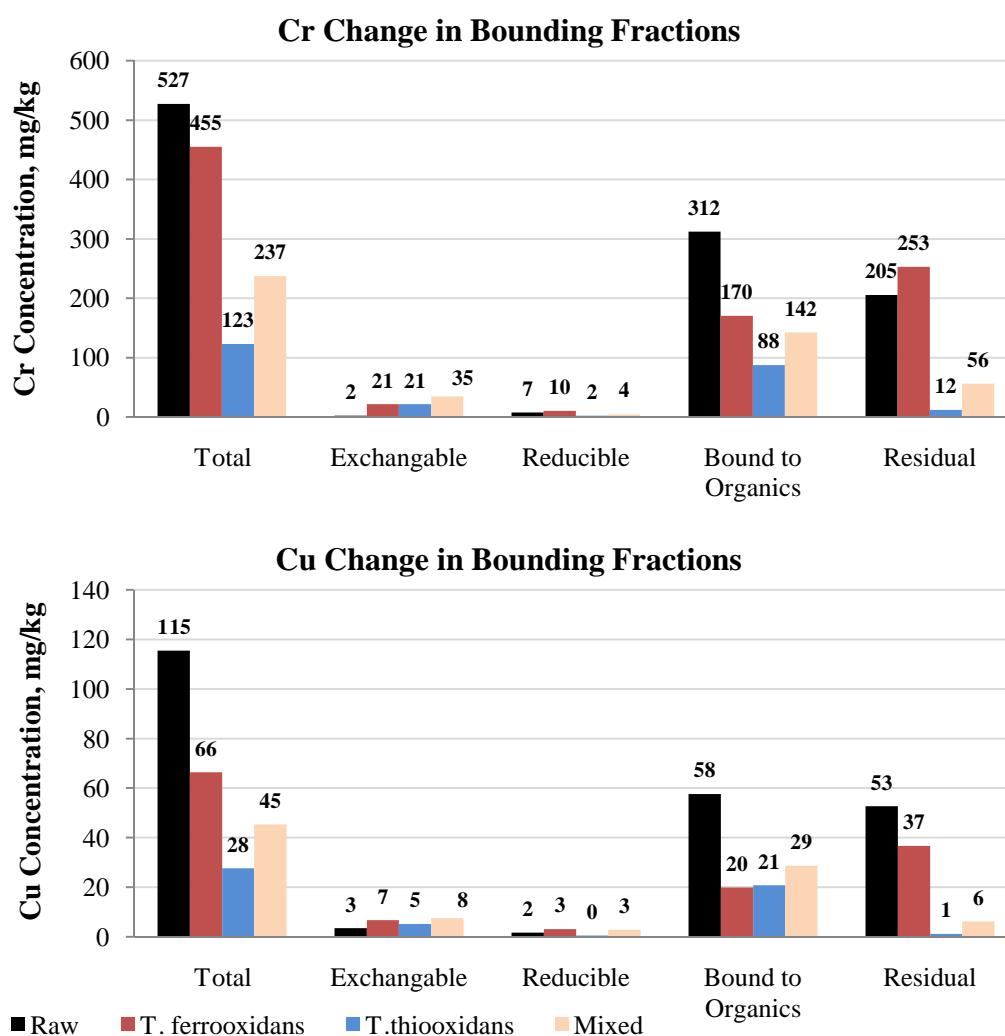


Figure 6.9 Cr and Cu in the binding fractions before and after bioleaching with different bacteria type

After bioleaching, Cr and Cu concentrations increased in the exchangeable fraction for all bacteria types. Reducible fraction was remained almost stable, where the concentrations in the organic fraction decreased significantly. Releases from the residual fraction were also observed for Cr and Cu, only in the application of *T. ferrooxidans* Cr concentration in the residual fraction increased.

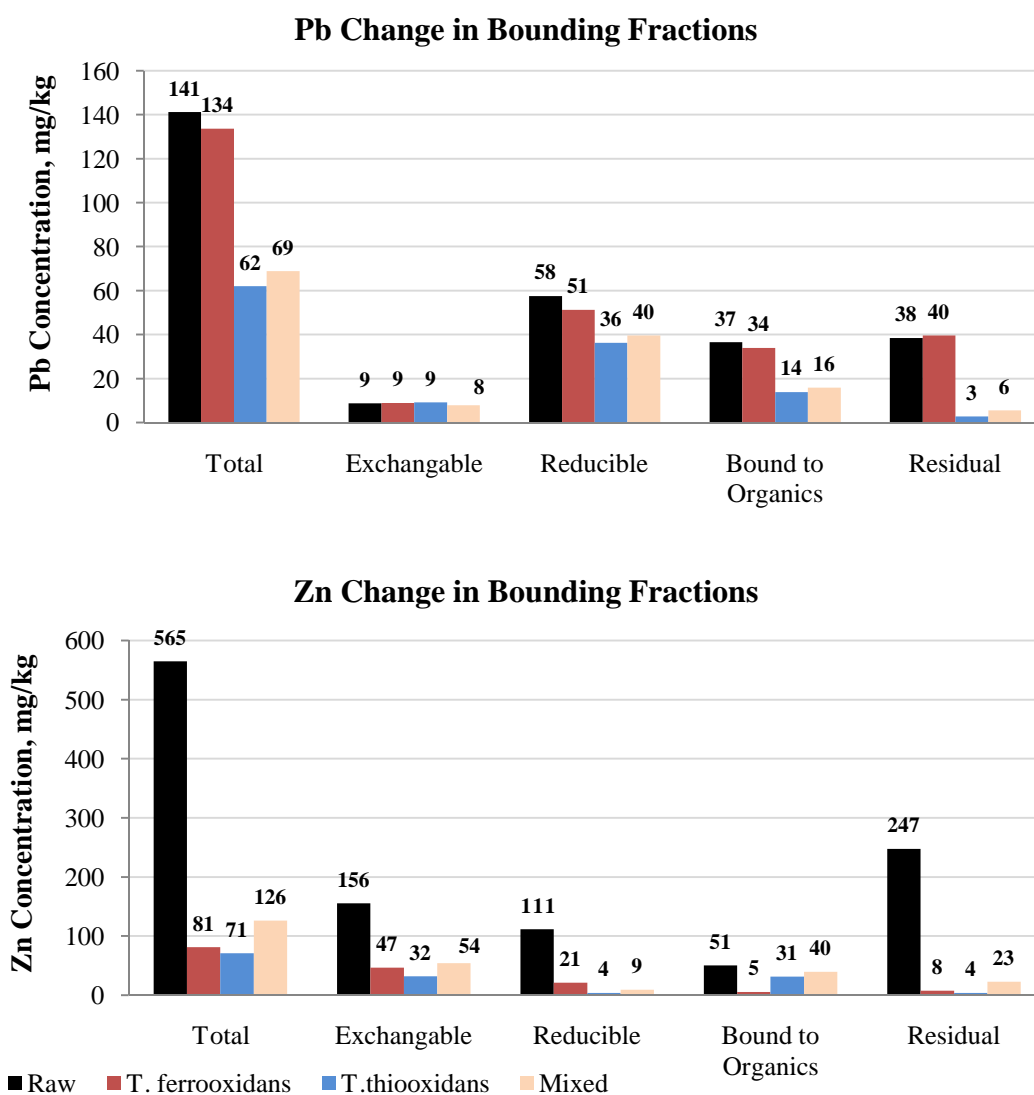


Figure 6.10 Pb and Zn in the binding fractions before and after bioleaching with different bacteria type

Pb release was mostly observed in the residual fraction except bioleaching with *T. ferrooxidans*. Exchangeable fraction of Pb was remained constant for all trials. Significant Zn releases were observed in all of the fractions, since there was a high solubilization of Zn in the bioleaching experiments (Figure 6.10).

#### 6.4.1.7 Discussion

The effect of bacterial strains on bioleaching of metals was studied in this first set of the experiments. Significant pH decrease was provided by *Thiobacillus thiooxidans*. Depending on the rapid sulfur oxidation and bacterial production of acid, within the 11 days of the experiments, pH in the leaching suspension dropped from 4.4 to 0.97. The pH decreases with *T. ferrooxidans* and the mixed culture were slower. Sulfate production in the flasks was in correlation with the pH decreases. This may depend on the cell concentrations of the bacteria in the bioleaching flasks. Highest sulfate production was observed in bioleaching with *Thiobacillus thiooxidans*. The decrease in pH coincided with an increase in metal solubilization. Satisfactory solubilization efficiencies were provided for Cr, Cu, Pb, and Zn with *Thiobacillus thiooxidans*.

Cr solubilization efficiency was 84% with *Thiobacillus thiooxidans* where the mixed culture could solubilize Cr to a ratio of 64%, and *Thiobacillus ferrooxidans* to a ratio of only 12%. This shows Cr requires extremely acidic conditions to be solubilized from the sediments. Since Cr is mostly found in the organic and residual fractions before bioleaching, Cr release was observed in these forms with *Thiobacillus thiooxidans* and the mixed culture. Another point is the increase in the exchangeable fraction after bioleaching with all bacteria type. Releases from the residual fraction may result as the accumulation of them in the exchangeable form which is the most mobile fraction.

Cu was solubilized mostly with *Thiobacillus thiooxidans* (81%). The solubilization efficiency with the mixed culture was 70% and *T. ferrooxidans* could release 44% of Cu. The most considerable changes in bounding fractions were determined in the organic and residual fractions for all the strains studied.

Pb solubilization could not be determined with *Thiobacillus ferrooxidans*. Since Pb is a hardly soluble metal, the efficiencies with *Thiobacillus thiooxidans* and the mixed culture were lower compared with other metals 63% and 56%, respectively).

As mentioned in Chapter 2, Zn is present as the soluble compounds in nature. High solubilization efficiencies of Zn were observed in the bioleaching flasks. *T. thiooxidans* achieved 92% of Zn solubilization where *T. ferrooxidans* solubilized 87% and mixed culture could remove 83% of the zinc. Furthermore, Zn was released from every binding fraction, mostly from the residual form.

For all the metals studied, removal efficiencies in the residual sediment were in agreement with the solubilization ratios in water. Deviations to a ratio of 10% depend on the heterogeneity of the sediments.

#### 6.4.2 Bioleaching Test 2-The Effect of the Solid/Liquid Ratio

In this set, the sulfur concentration was kept constant (0.5%(w/v)) and the inoculums of *T. thiooxidans* were used in the bioleaching experiments. The solid content in the flasks were arranged as 10%, 5%, and 2.5% (w/v).

##### 6.4.2.1 Cell Concentrations of the Bacteria

The cell concentrations of *Thiobacillus thiooxidans* in the inoculums were determined as  $5 \times 10^6$  cfu/ml.

##### 6.4.2.2 Acclimation of the bacteria

The acclimation of the bacteria lasted for 13 days reaching the pH of 0.9 (Figure 6.11).

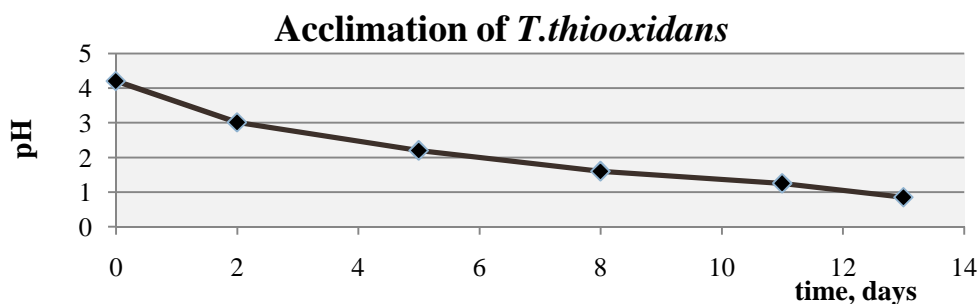


Figure 6.11 The pH change during the acclimation of *T. thiooxidans*

### 6.4.2.3 pH and ORP Changes

The pH value in suspension dropped from 4 to 0.8 and 0.7 with solid concentrations of 5% and 2.5% at the end of 48 days. This final value was pH 1.45 for higher solid content (10%) (Figure 6.12).

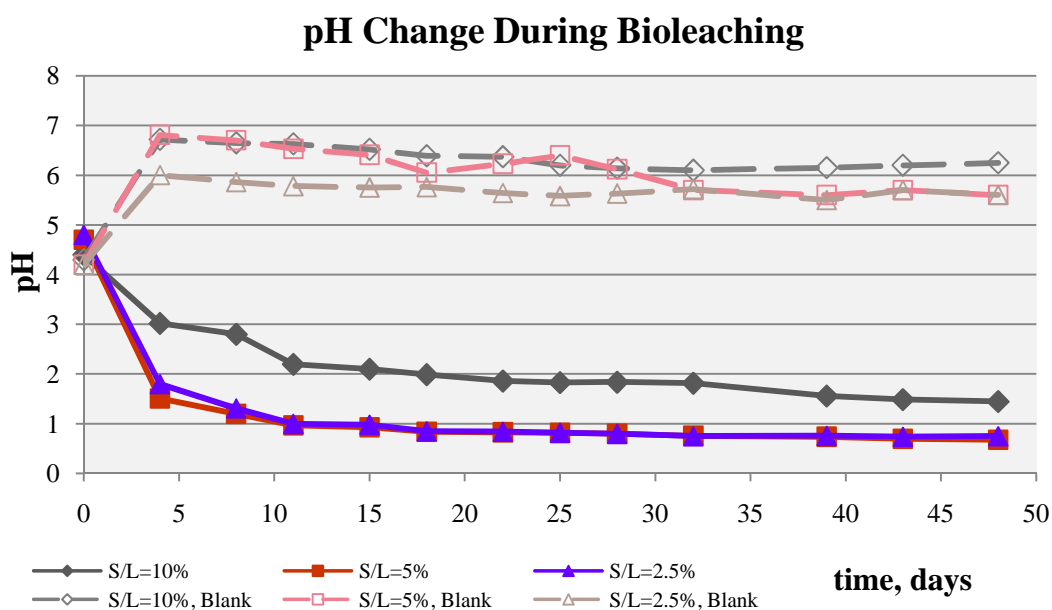


Figure 6.12 Variations of pH in the bioleaching tests depending on solid content

Figure 6.13 shows the changes in ORP during bioleaching with solid contents.

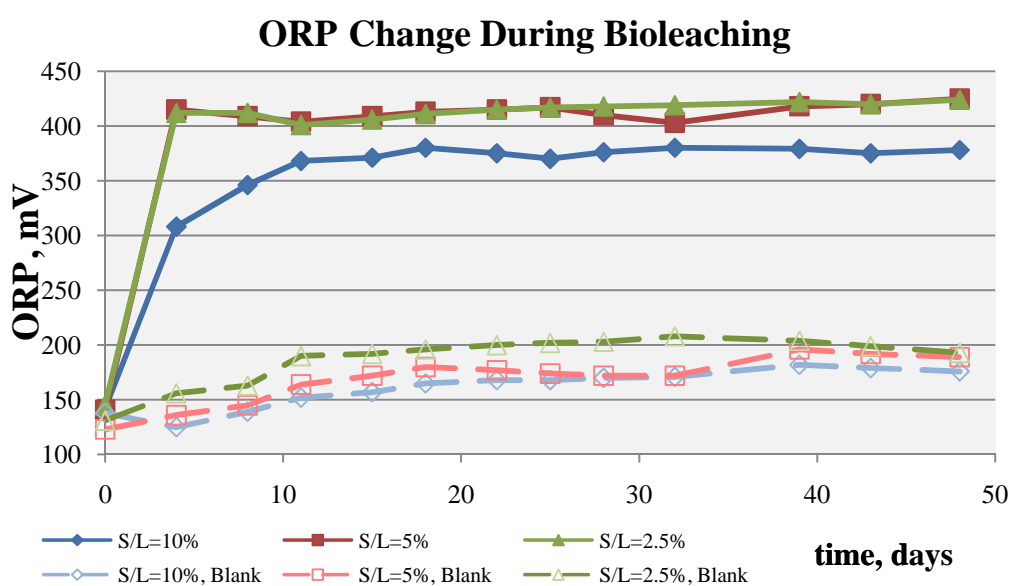


Figure 6.13 Variations of ORP in the bioleaching tests depending on solid content

The ORP values with lower solid contents were nearly the same and the reaching 420-425 mV finally. High solid content provided 378 mV ORP in the bioleaching flasks.

#### 6.4.2.4 Sulfate Production

The sulfate production in time is illustrated with Figure 6.13. Lowest production was observed with 10% solid content (10090 mg/l) and the highest production was achieved with 5% sediment concentration (21659 mg/l sulfate production) ( Figure 6.14).

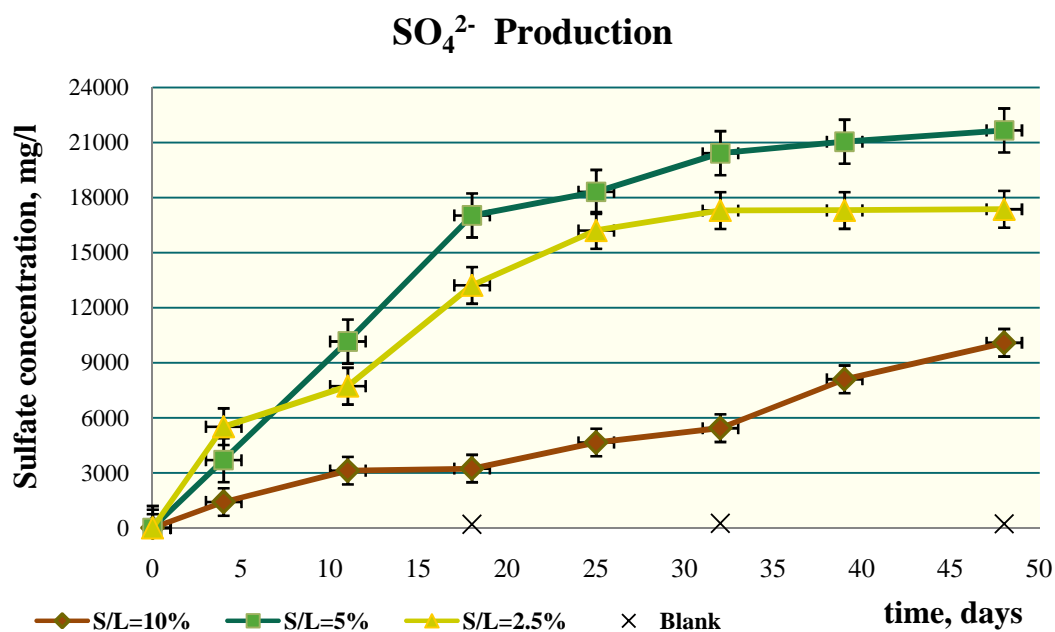


Figure 6.14 Sulfate production during bioleaching according to the solid/liquid ratio

#### 6.4.2.5 Metal Solubilization and Removal Efficiency in Residual Sediment

Cr and Cu concentrations in suspension during bioleaching are shown in Figure 6.15. The solubilization efficiencies of Cr for different solid contents are 65%, 84%, and 92%. The results of Cu are nearly the same with Cr with 65%, 81%, and 95% of solubilizations according to the decreasing solid contents.

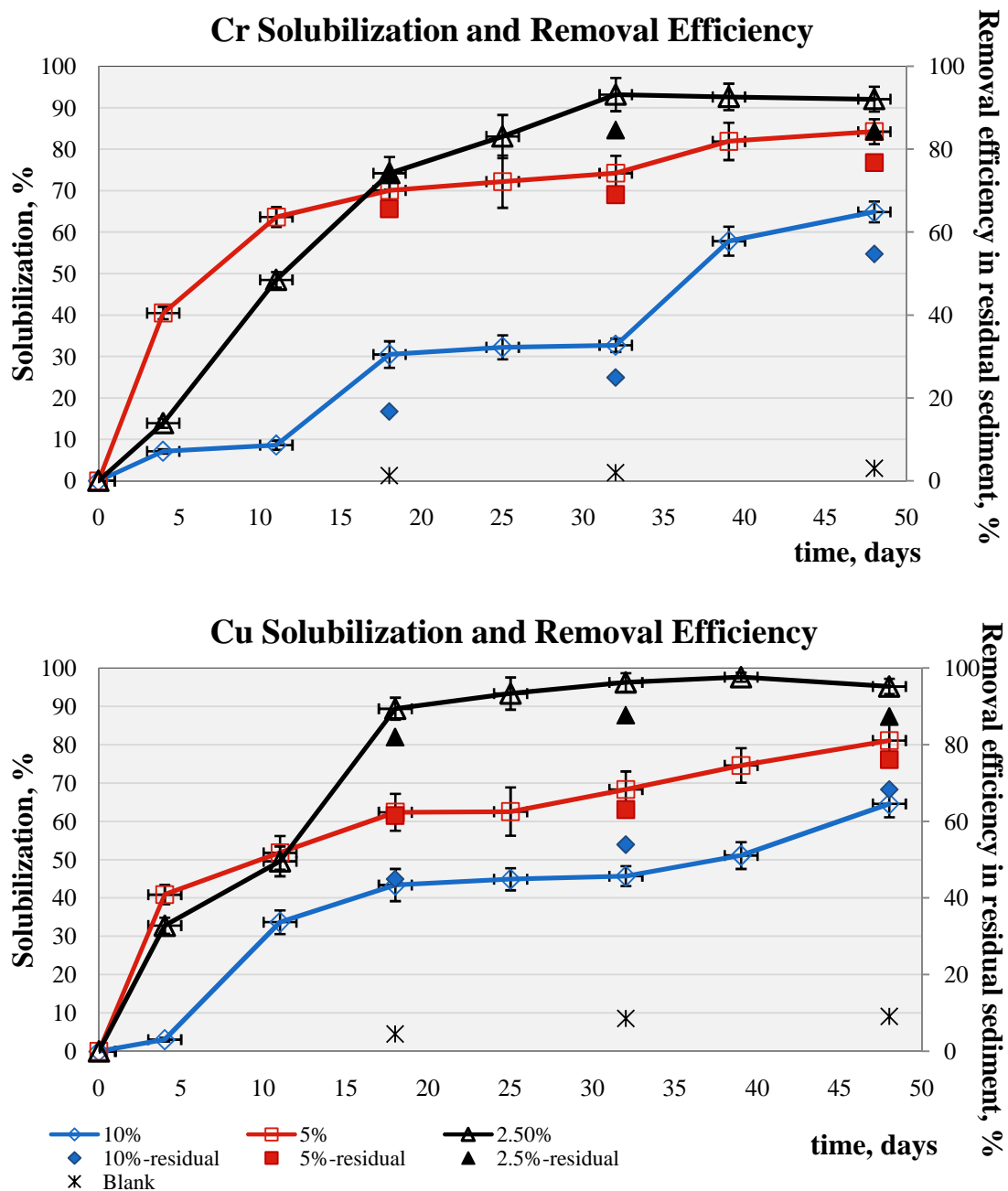


Figure 6.15 Cr and Cu solubilizations and removal efficiencies depending on the solid/liquid ratio

The results of Pb and Zn are given with Figure 6.16. Pb was solubilized to the ratios of 54%, 63%, and 77% according to the decreasing solid content. Zn solubilization efficiencies were determined as 77%, 92%, and 97% according to 10%, 5%, and 2.5 % (w/v) of solid contents.



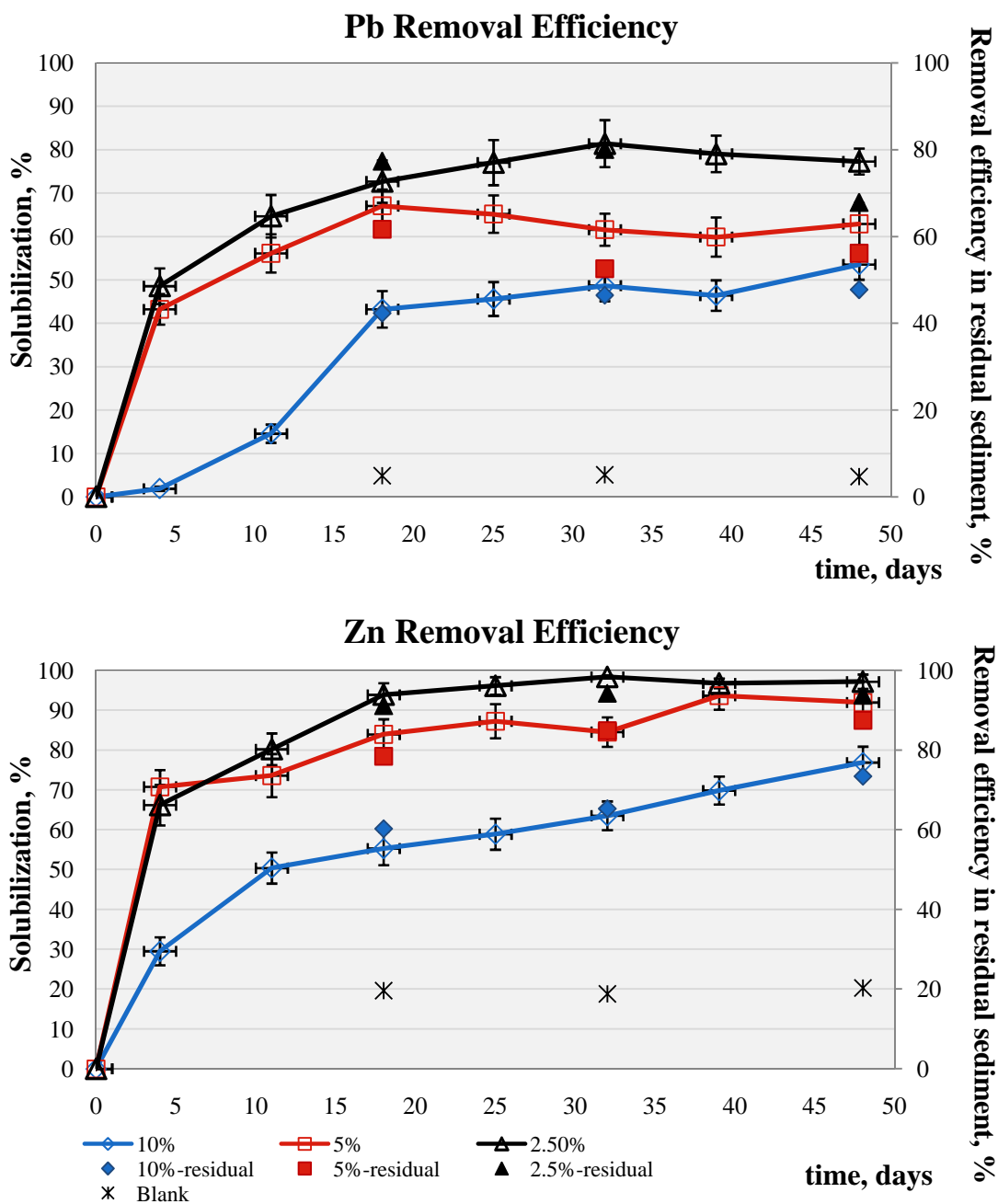


Figure 6.16 Pb and Zn solubilizations and removal efficiencies depending on the solid/liquid ratio

6.4.2.6 Changes in Bounding Fractions

Figure 6.17 and 6.18 show the changes of metal concentrations in the binding fractions of sediments with different solid contents..

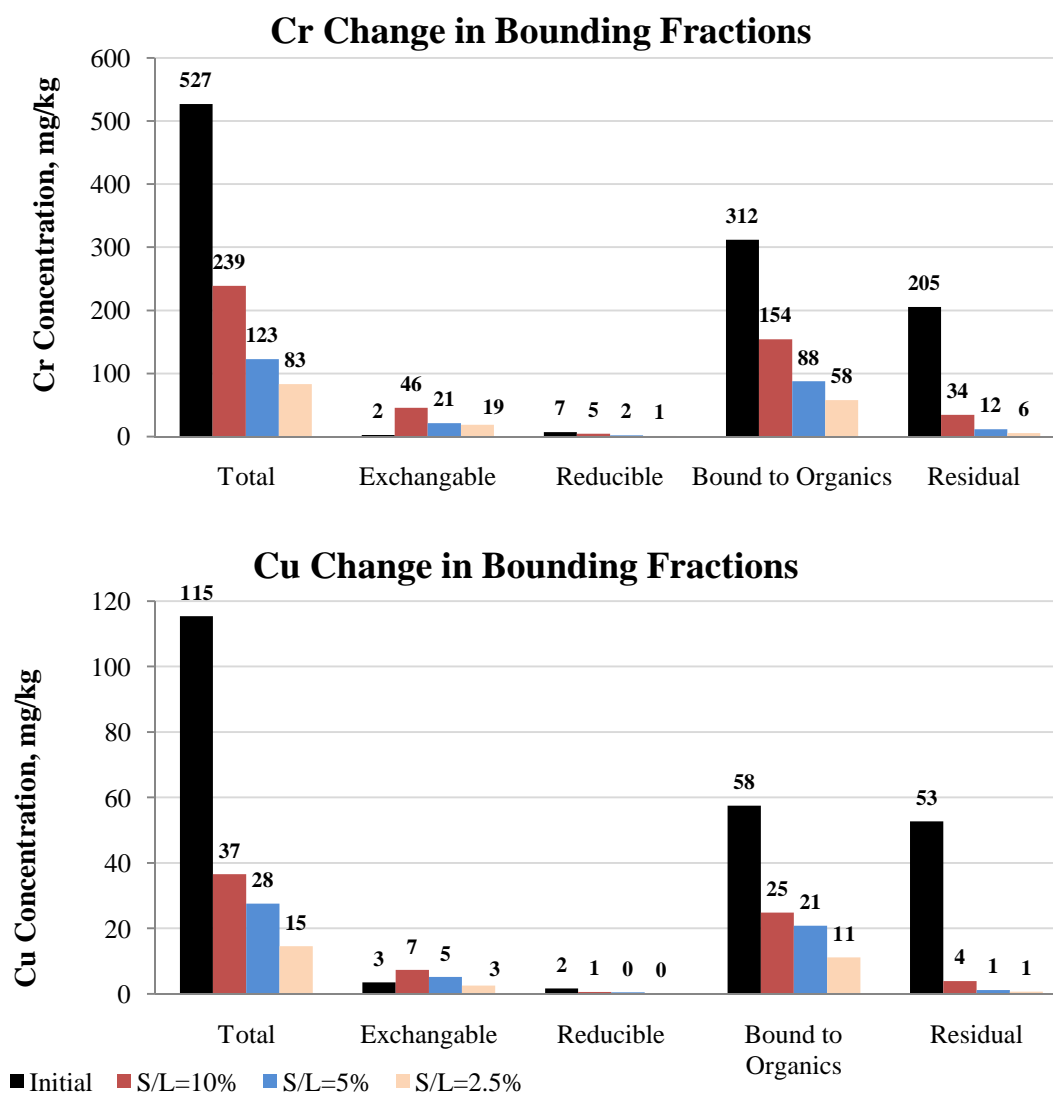


Figure 6.17 Cr and Cu in the binding fractions before and after bioleaching with different solid content

For all the solid concentrations, Cr and Cu concentrations in residual fraction decreased in the highest ratios (Figure 6.17) where concentrations in the exchangeable fraction increased for both Cr and Cu. Another considerable decrease in concentrations of Cr and Cu was observed in the organic fraction. Pb concentrations in reducible and organic fractions decreased significantly after bioleaching. In addition to this, Pb in residual fraction were almost solubilized (Figure 6.18). Zn concentrations in every binding form decreased after bioleaching.

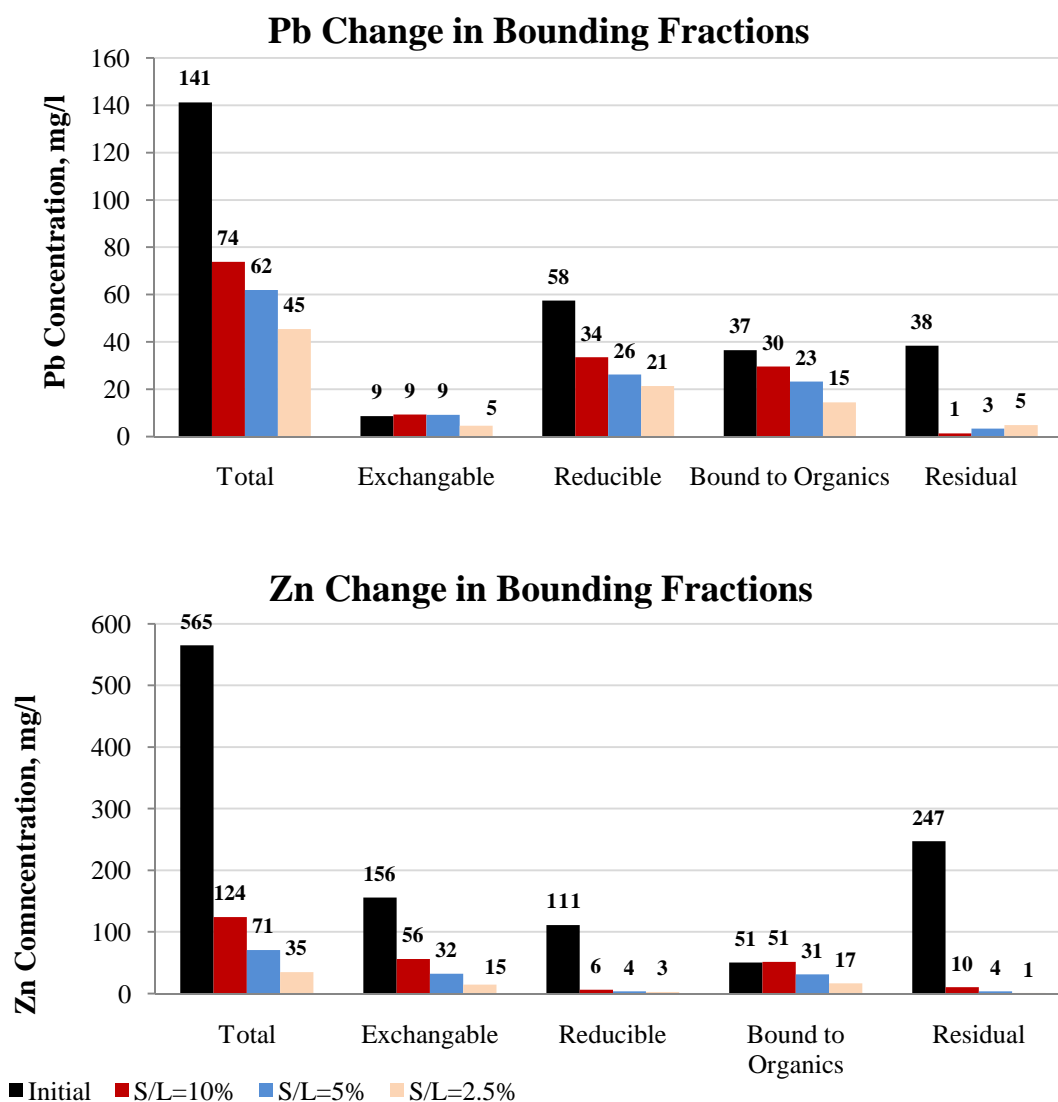


Figure 6.18 Pb and Zn in the binding fractions before and after bioleaching with different solid content

#### 6.4.2.7 Discussion

The effect of solid concentration on bioleaching of metals from sediments was studied in this part by using 10, 5, and 2.5% (w/v) of solid concentrations. The sediment pH dropped to lower values with low solid contents. This is attributed to the higher sediment contents with higher buffering capacity. Sediment solid content plays an important role in the variation of pH during the microbial leaching process. The ORP values in the bioleaching flasks decrease with the increases in the sediment solid content.

Sulfate production depending on solid content reached to a maximum value (21650 mg/l) with 5% solid content. This situation may be explained depending on the cell concentrations of the bacteria per solid sediment amount in suspension. In 10% solid content, bacteria in the medium was not sufficient to solubilize metals and produce sulfate where smaller amount of solid mass could not contain enough metal load to produce  $\text{SO}_4^{2-}$ .

The efficiencies of solubilization of different metals were compared according to the solid/liquid ratio. Satisfactory efficiencies were achieved with *Thiobacillus thiooxidans*. For Zn, Cr, and Cu, solubilization efficiencies 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. Previous studies report that; the mobilization of the metals increase with the decreasing solid/liquid ratio (Bordas & Bourg, 2001). This depends on the increasing solid-liquid interaction area depending on the decreasing solid mass and increasing  $\text{H}^+$  ions in suspension.

The solubilization efficiencies of metals in the decreasing order are:  $\text{Zn} > \text{Cu} > \text{Cr} > \text{Pb}$ . The solubilization ratios in water are compatible with the removal efficiencies determined from the residual sediments.

Metal concentrations in bounding fractions changed 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. Reducible fraction seemed not to be changed considerably for Cr and Cu. Pb showed a different behavior and it was released from reducible and organic fractions depending on solid content. Furthermore, nearly all Pb from residual fraction was removed and there was no significant change in the exchangeable fraction of lead after bioleaching.

Zn is the metal with the highest solubility and it was released from all fractions significantly, except the organic form.

### 6.4.3 Bioleaching Test 3- Effect of Sulfur Concentration

Various contents of elemental sulfur (0.25, 0.5, 1, and 1.5% (w/v)) were added to the bioleaching flasks. The sulfur used in the experiments was the rhombic elemental sulfur; a yellow, crystalline solid with a density of 2.06. The solid content was kept constant with 5% (w/v) and the inoculums of *T. thiooxidans* were used to provide the sulfur oxidation during the experiments.

#### 6.4.3.1 Cell Concentrations of the Bacteria

The cell concentrations of *T. thiooxidans* in the inoculums were determined as  $9 \times 10^5$  cfu/ml.

#### 6.4.3.2 Acclimation of the Bacteria

*T. thiooxidans* were acclimated to the sediment, the pH of the acclimated bacteria dropped from 4.1 to 1.0 in 13 days (Figure 6.19).

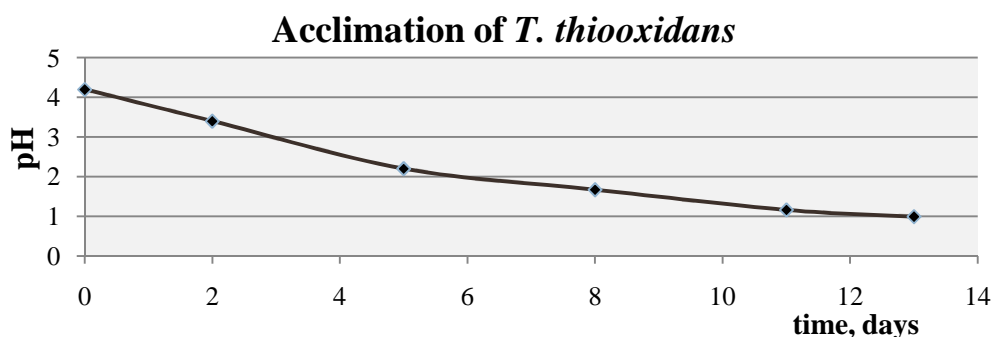


Figure 6.19 The pH change during the acclimation of *T. thiooxidans*

#### 6.4.3.3 pH and ORP Change

Rapid increase in pH was observed in all the flasks within the 15 days. The lowest final pH was determined with 5% (w/v) sulfur addition (pH dropped from 4 to 0.86). No acidification was observed in the control test without sulfur addition. (Figure 6.20).

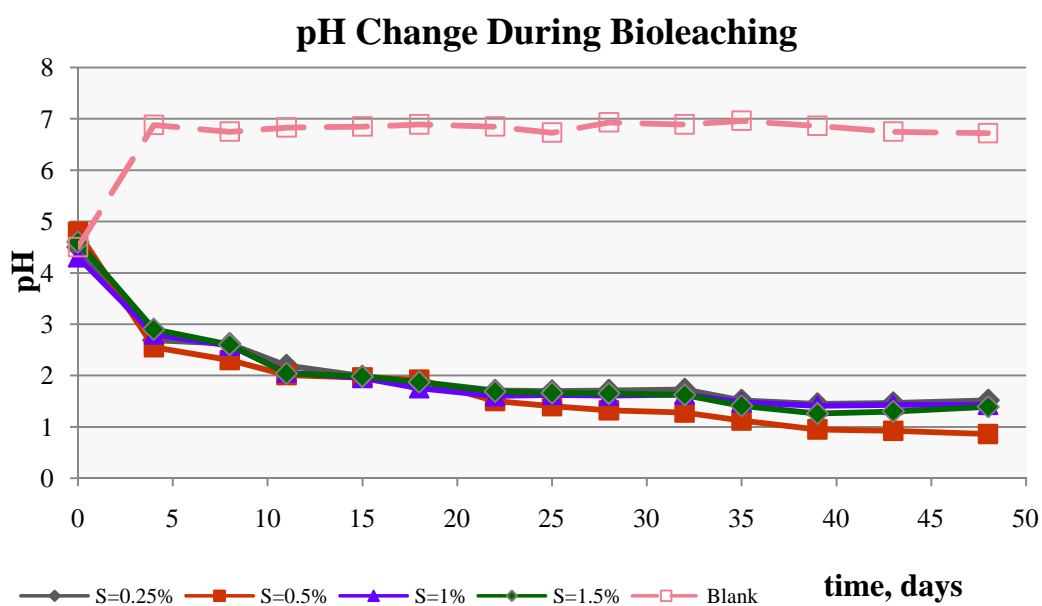


Figure 6.20 Variations of pH in the bioleaching tests depending on sulfur concentrations

During the tests, acidification and aeration increased the ORP significantly. The ORP values in the flasks reached to 400-425 mV. Highest values were determined with 0.5% (w/v) sulfur content (Figure 6.21).

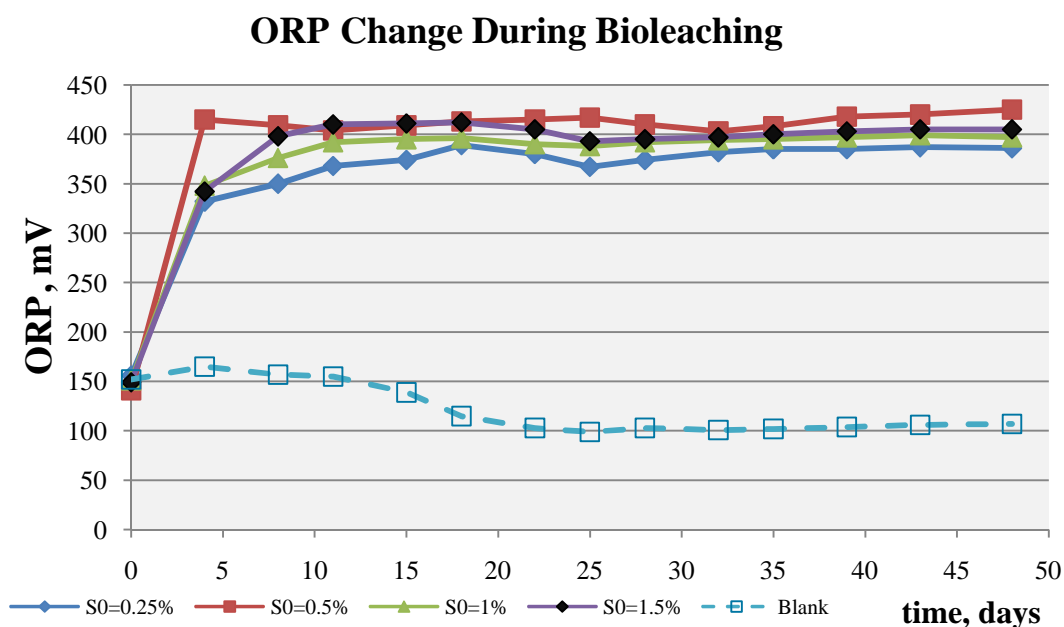


Figure 6.21 Variations of ORP in the bioleaching tests depending on sulfur concentrations

#### 6.4.3.4 Sulfate Production

Sulfate production was determined by taking the difference between the sulfate concentration in suspension and the sulfate content in the initial dilution medium (Figure 6.22).

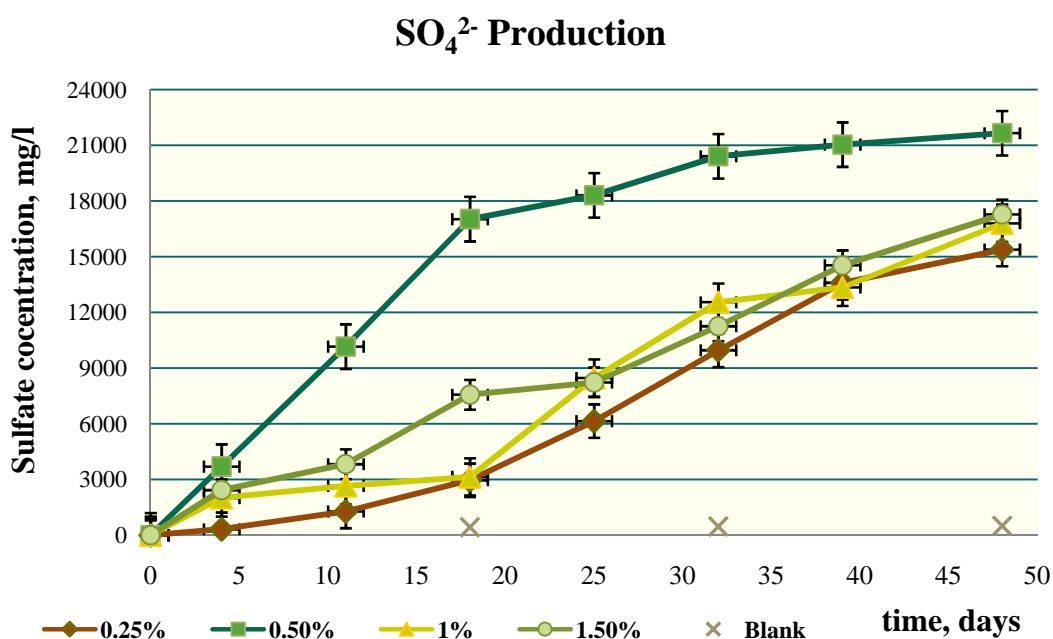


Figure 6.22 Sulfate productions during bioleaching according to the sulfur concentration

There was a slight increase in sulfate production as the sulfur concentration increased from 0 to 1.5% (w/v) sulfur content. But the observations for 0.5% (w/v) sulfur addition exhibits significant differences. As can be seen from Figure 6.21, both SO<sub>4</sub><sup>2-</sup> production rate and final concentration with 0.5% sulfur addition show the highest values.

#### 6.4.3.5 Metal Solubilization and Removal Efficiency in Residual Sediment

Solubilization and removal efficiencies of Cr, Cu, Pb, and Zn are illustrated with Figures 6.23 and 6.24.

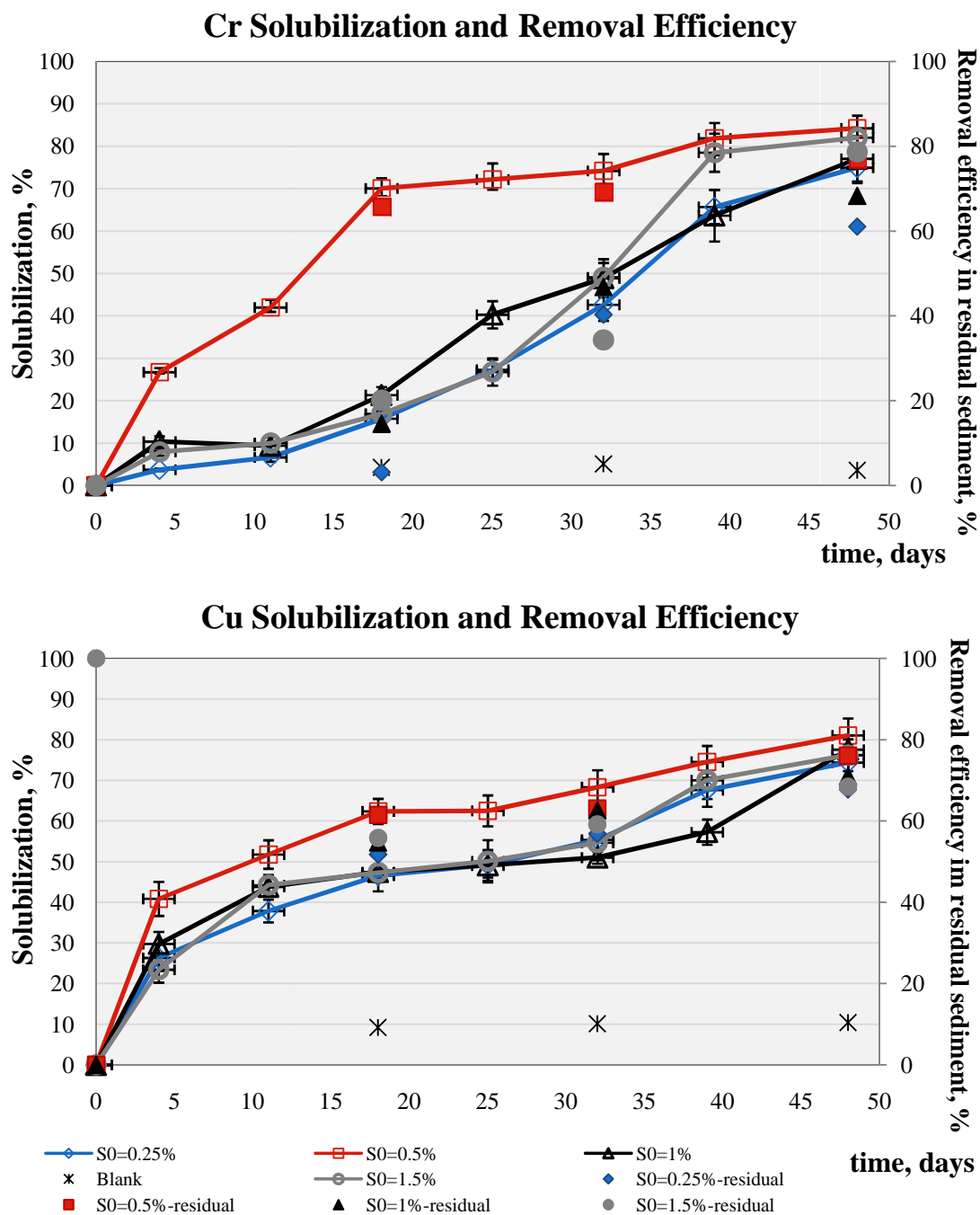


Figure 6.23 Cr and Cu solubilizations and removal efficiencies depending on sulfur concentrations

The determined Cr and Cu solubilizations changed between 75-84% and 74-81%, respectively. The highest efficiencies were detected with 0.5% sulfur content and the lowest with 0.25% sulfur content. Removal efficiencies with 1-1.5% sulfur content were 77- 80% for Cr, and 78-76% for Cu.



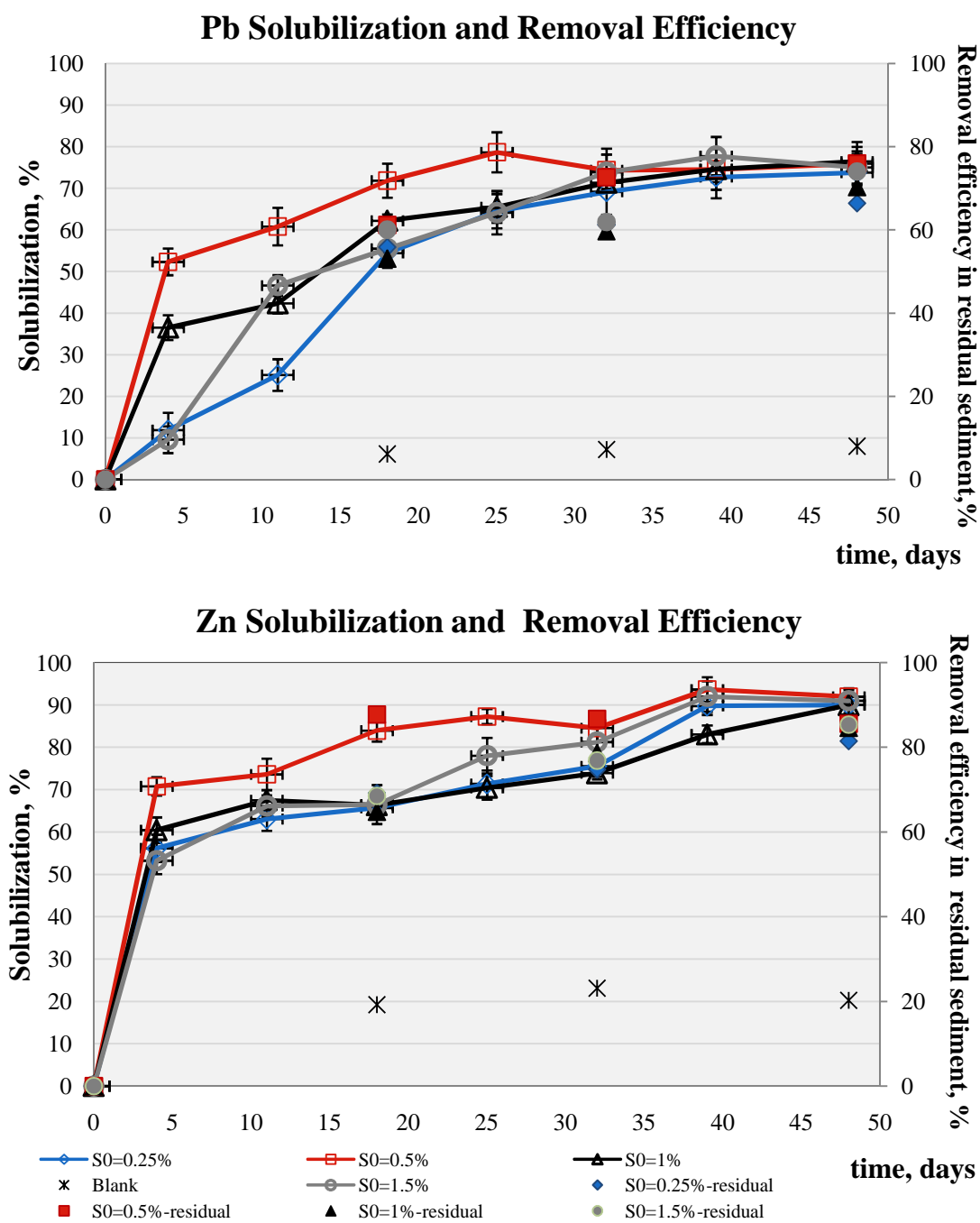


Figure 6.24 Pb and Zn solubilizations and removal efficiencies depending on sulfur concentrations

The efficiencies of solubilization of Pb and Zn depending on sulfur concentration showed slight changes (74-76% and 90-92%, respectively). For both metals solubilization ratios with 0.5% sulfur were the highest.

#### 6.4.3.6 Changes in Bounding Fractions

Metal concentrations in chemical binding forms before and after bioleaching with different sulfur concentrations are given with Figures 6.25 and 6.26.

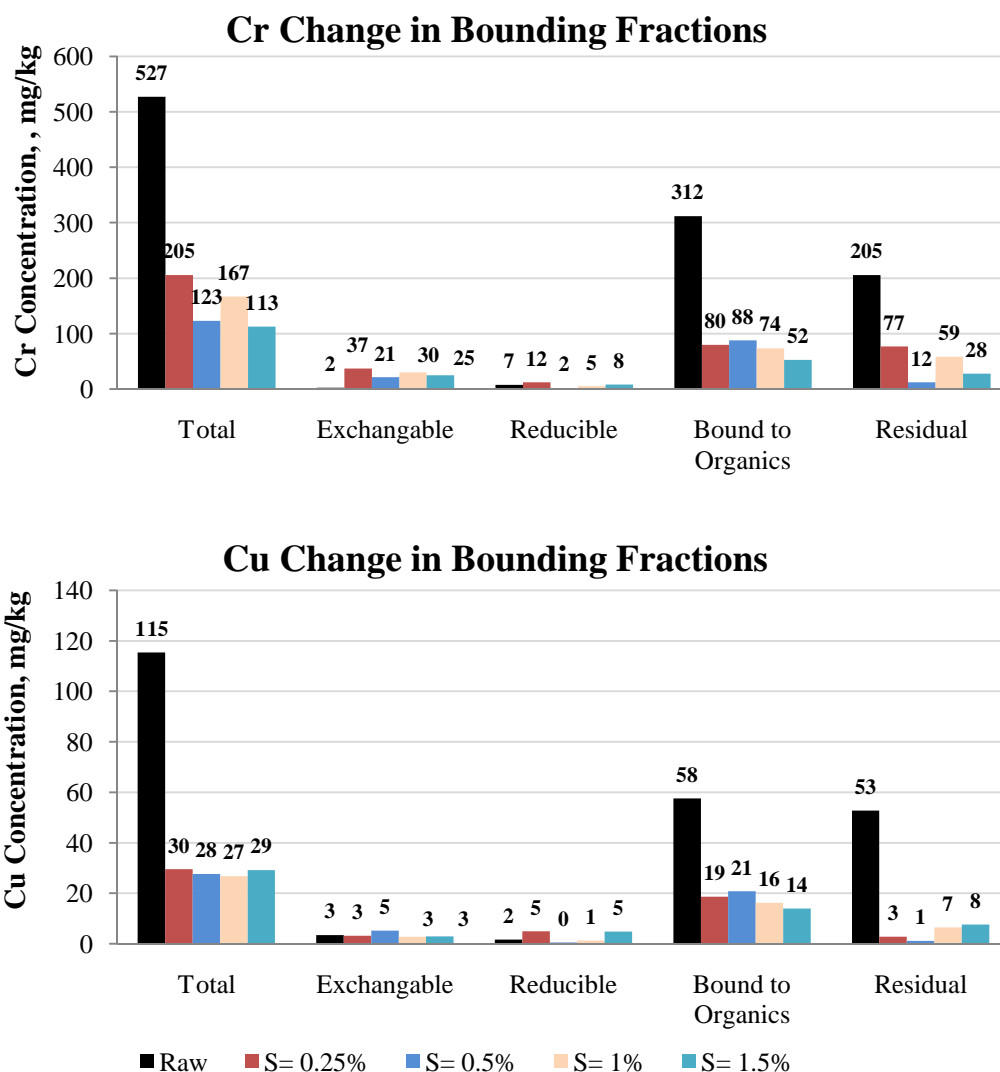


Figure 6.25 Cr and Cu in the binding fractions before and after bioleaching with different sulfur concentrations

Considerable decreases were determined in the organic and residual fractions of sediments for Cr and Cu. The highest removal ratio in residual fraction was detected with 0.5% sulfur content. Cr concentrations increased in the exchangeable fraction after bioleaching and no significant changes were detected for Cr and Cu in the residual fraction.

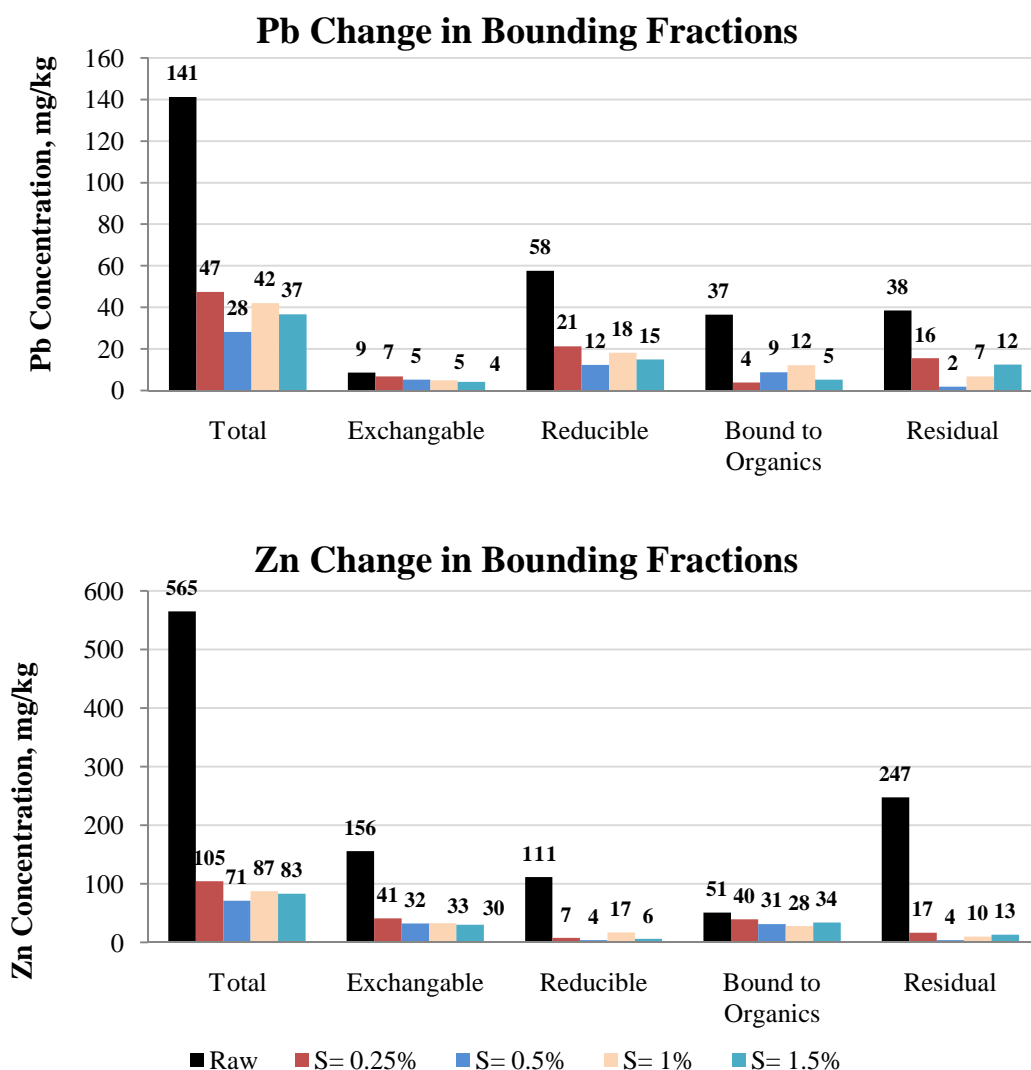


Figure 6.26 Pb and Zn in the binding fractions before and after bioleaching with different sulfur concentrations

Significant decreases were determined for Pb concentrations in reducible, organic, and residual fractions. Same as Cr and Cu, the highest removal ratio of Pb in residual fraction was detected with 0.5% sulfur content. Decreases in exchangeable fraction were low in proportion. Zn concentrations in every binding form decreased after bioleaching, only the accumulations in the organic fraction decreased in small ratios.

#### 6.4.3.7 Discussion

The effects of sulfur concentration on the performance of a bioleaching process by using *T. thiooxidans* were evaluated in this study. Depending on the sulfur oxidation,

rapid pH decreases were observed in the bioleaching flasks. Thus, the oxidation reduction potential increased within the 10 days of the tests. Maximum decrease in pH and increase in ORP were observed with 0.5% (w/v) sulfur content in the flasks. This may be explained by the inhibiting effect of excess substrate on the oxidation of elemental sulfur (Chen & Lin, 2001).

The changes in  $\text{SO}_4^{2-}$  production rates exhibit an exception for the sulfur addition of 0.5% (w/v). The highest sulfate production rate and the maximum  $\text{SO}_4^{2-}$  concentration is found in the flask with 0.5% sulfur addition. It is observed that the higher sulfur addition ratios (1% and 1.5% (w/v)) during sulfate production caused an inhibitory effect on the microbial activity. Besides, 0.25% (w/v) sulfur addition was not sufficient to support the microbial activity which gave the results of lower production rates and concentrations of sulfate.

Satisfactory results were achieved with the metals solubilization in this part of the study. Cr was solubilized to the highest ratio of 84% with the addition of 0.5% sulfur. During bioleaching process, Cr was mostly released from residual fraction. Considerable losses of Cr were also determined from the organic form of the sediments. On the other hand, the concentrations of Cr increased in the exchangeable fraction after bioleaching.

Cu solubilizations depending on sulfur concentrations were on the same level with Cr (74-81%). Most Cu was solubilized from the organic and residual form. With 0.5% (w/v) sulfur addition, almost all Cu was removed from the crystal matrix where the concentrations in exchangeable and reducible fractions were not affected significantly.

Depending on low pH provided by *T. thiooxidans*, Pb solubilization efficiencies were satisfactory (74-76%) when compared to the other sets. Pb was released mostly from the residual and organic fractions.

Zn solubilization efficiencies varied between 90-92% and no significant effect of sulfur concentration on Zn solubility was observed here. Similar like the previous

sets, Zn was released almost from every binding fraction, only low concentration decreases were determined in the organic form.

#### 6.4.4 Bioleaching Test 4- Effect of Grain Size

##### 6.4.4.1 Sediment Samples

Fine, medium size, and coarse sediment particles obtained as subsamples from the wet sediment were used in this set of experiments. Total metal distributions in these particles are given with Table 6.3.

Table 6.3 Cr, Cu, Pb and Zn Concentrations in sediments with different grain sizes

Sediment sample	Distribution %	Cr (mg kg <sup>-1</sup> )	Cu(mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
<b>Fine</b>	51	504	45	118	335
<b>Medium</b>	39	612	113	130	739
<b>Coarse</b>	10	569	326	128	729
<b>Cumulative</b>	<b>100</b>	<b>553</b>	<b>100</b>	<b>124</b>	<b>532</b>
<b>Bulk</b>		<b><u>527</u></b>	<b><u>115</u></b>	<b><u>141</u></b>	<b><u>565</u></b>

The chemical distribution of the metals in the fine, medium, and coarse particles are illustrated with Figure 6.27. For the samples in different grain sizes, Cr was found mostly in the residual fraction with a portion over 50%. Cu was mostly accumulated in the organic form, except the fine particles were detected in the residual fraction over 44%. Pb accumulation showed distinctions for different particle sizes of the sediments, and the accumulating ratio on reducible fraction was the highest of all the metals studied. Zn was found in all the fractions almost evenly. Different than other metals, Zn in all grain sizes of the sediments was found mostly in the exchangeable form.

##### 6.4.4.2 Cell Concentrations

The cell concentrations of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* in the mixed culture were determined as  $7 \times 10^4$  and  $3 \times 10^5$  cfu/ml, respectively.

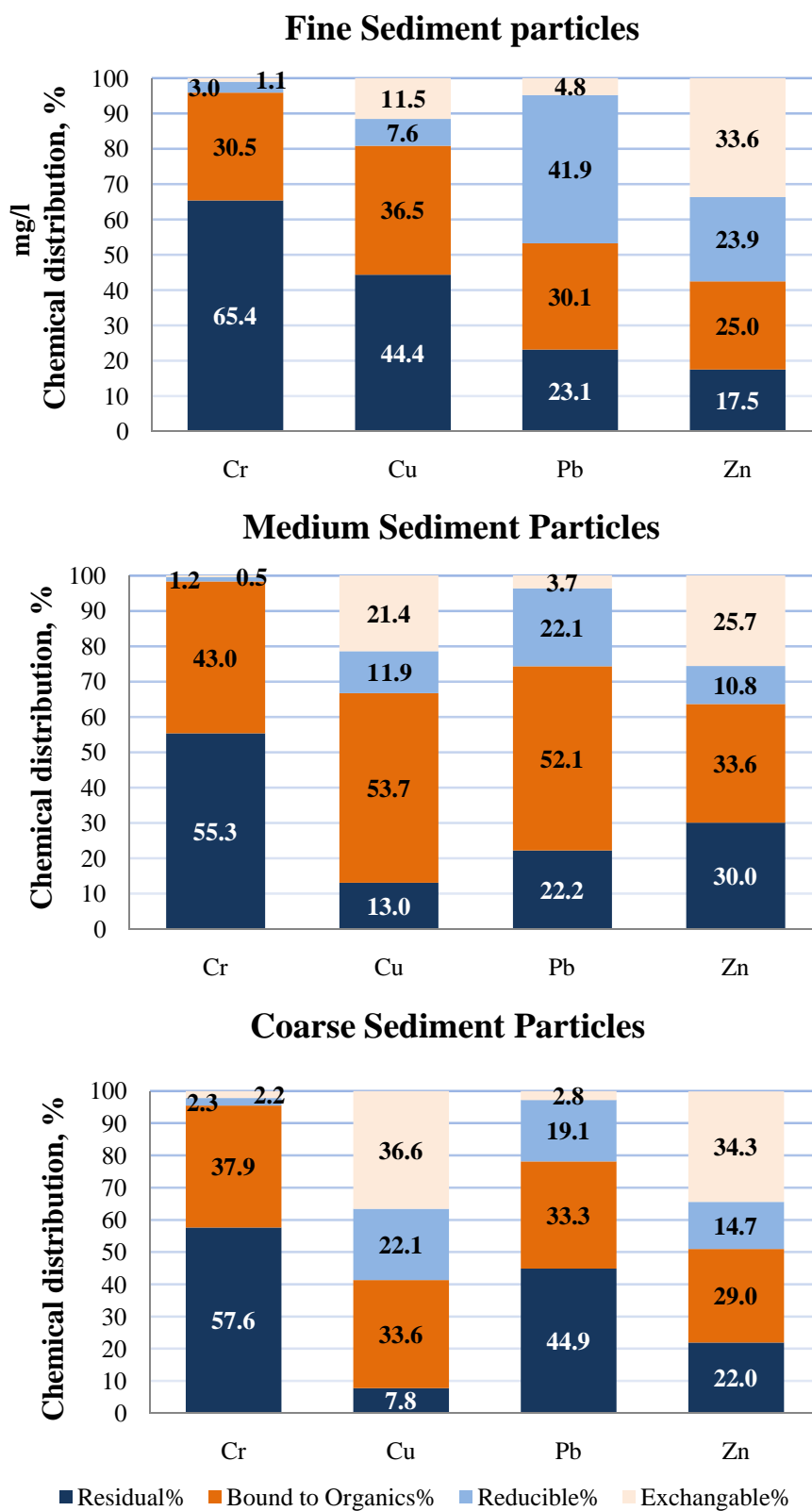


Figure 6.27 Chemical distributions of Cr, Cu, Pb and Zn in fine, medium and coarse particles

#### 6.4.4.3 Acclimation of the Bacteria

The acclimation of the mixed culture was carried on using medium size particles and pH in suspension dropped from 4.3 to 1.47 in 13 days (Figure 6.28).

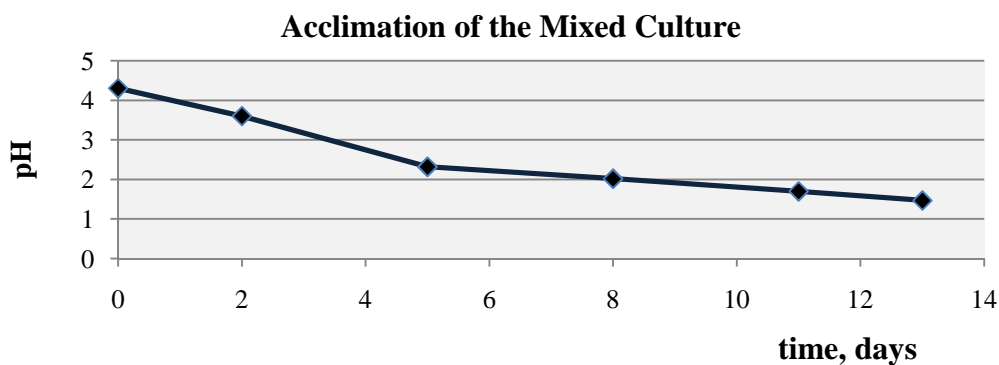


Figure 6.28 The pH change during the acclimation of mixed culture

#### 6.4.4.4 pH and ORP Changes

Depending on the activity of the mixed culture of *T. thiooxidans* and *T. ferrooxidans*, pH decreased rapidly within 10 days. At the end of 48 days, pH values in the flasks were close to each other (pH 1-6-1.7) (Figure 6.29).

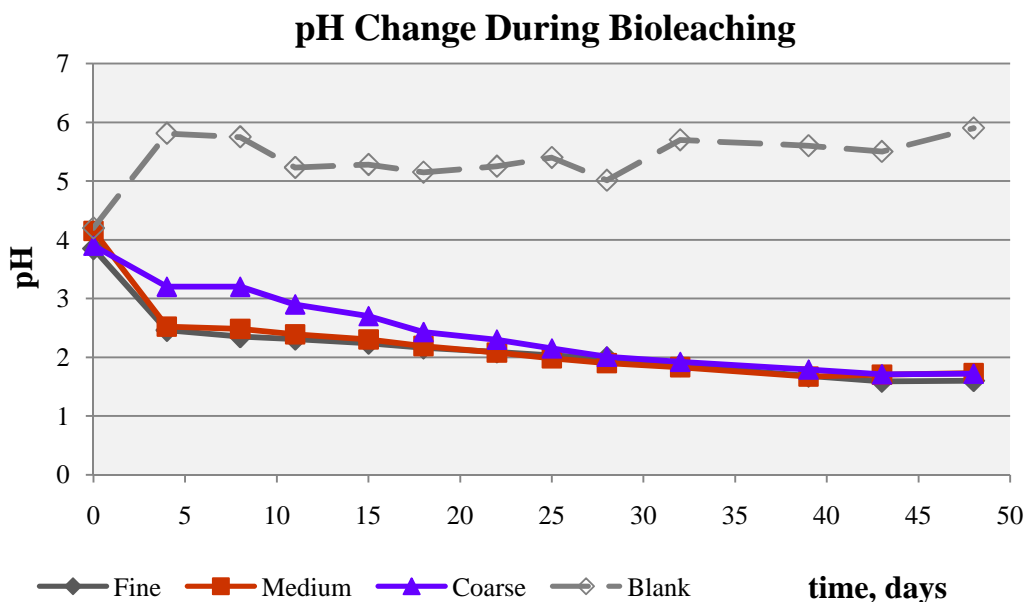


Figure 6.29 Variations of pH in the bioleaching tests depending on grain size of the sediments

The ORP values in the flasks increased with the decreasing pH (Final values reached 396-405 mV). No considerable changes were detected in ORP depending on the grain size of the sediments (Figure 6.30).

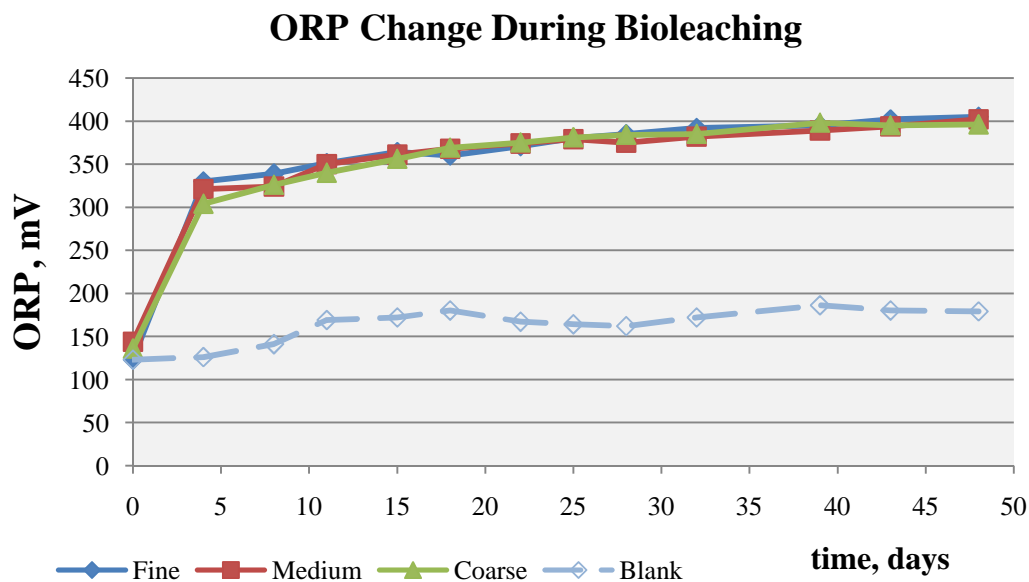


Figure 6.30 Variations of ORP in the bioleaching tests depending on grain size of the sediments

#### 6.4.4.5 Sulfate Production

Figure 6.31 presents the formation of sulfate in time depending on grain sizes.

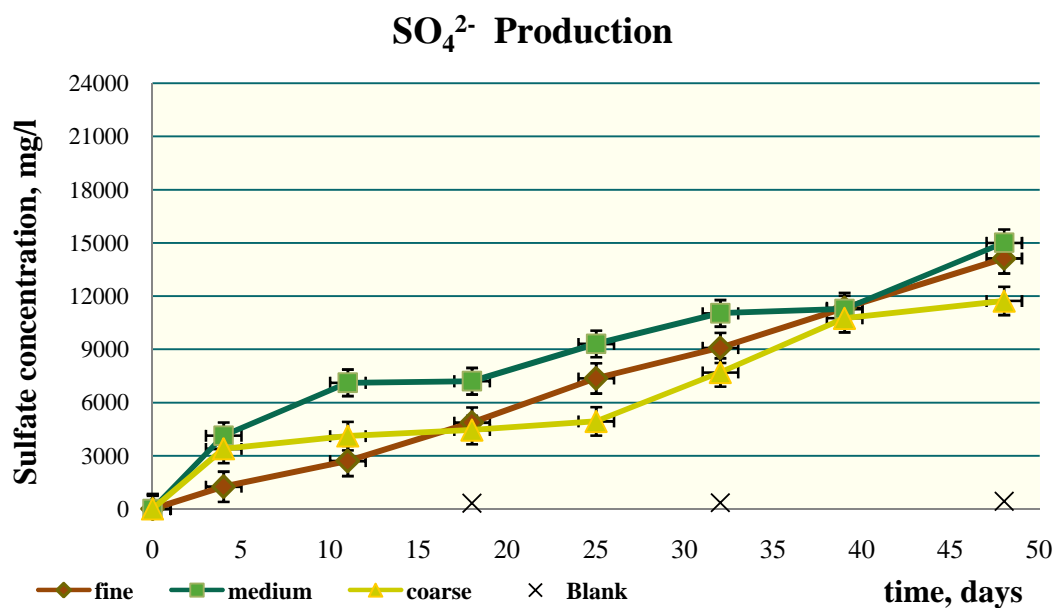


Figure 6.31 Sulfate productions during bioleaching according to grain size of the sediments



Sulfate production with 48 days of bioleaching reached to 14125 mg/l for fine particles, 15000 mg/l for medium particles, and 11750 mg/l for coarse particles.

6.4.4.6 Metal Solubilization and Removal Efficiency in Residual Sediment

Metal solubilization ratios depending on grain size of the sediments are given with Figures 6.32 and 6.33.

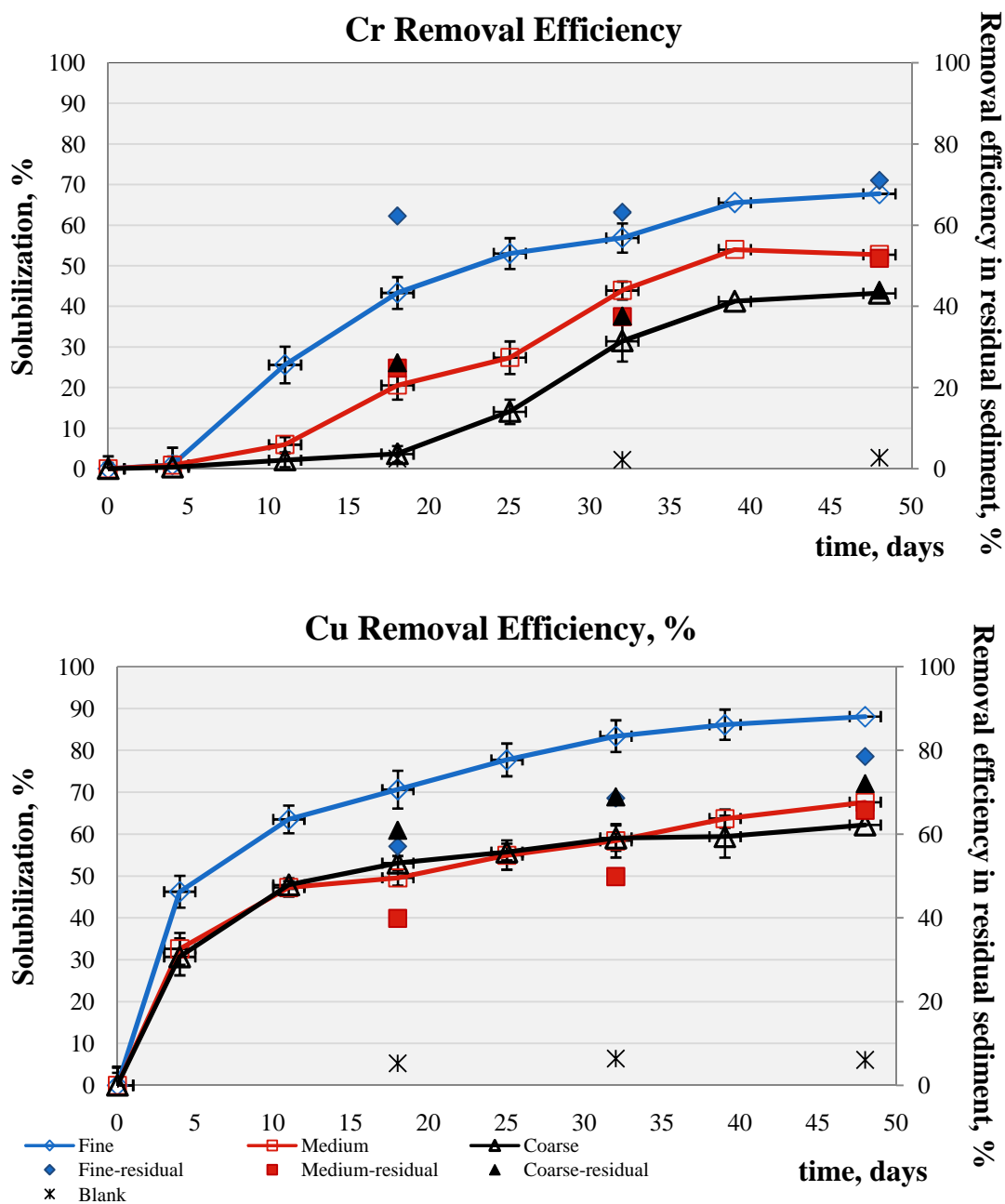


Figure 6.32 Cr and Cu solubilizations and removal efficiencies depending on grain size of the sediments

Cr solubilization efficiencies of bioleaching are 68%, 53%, and 43% for fine, medium and coarse particles. The solubilization ratios of Cu depending on increasing size were determined as 88%, 68%, and 62% (Figure 6.32).

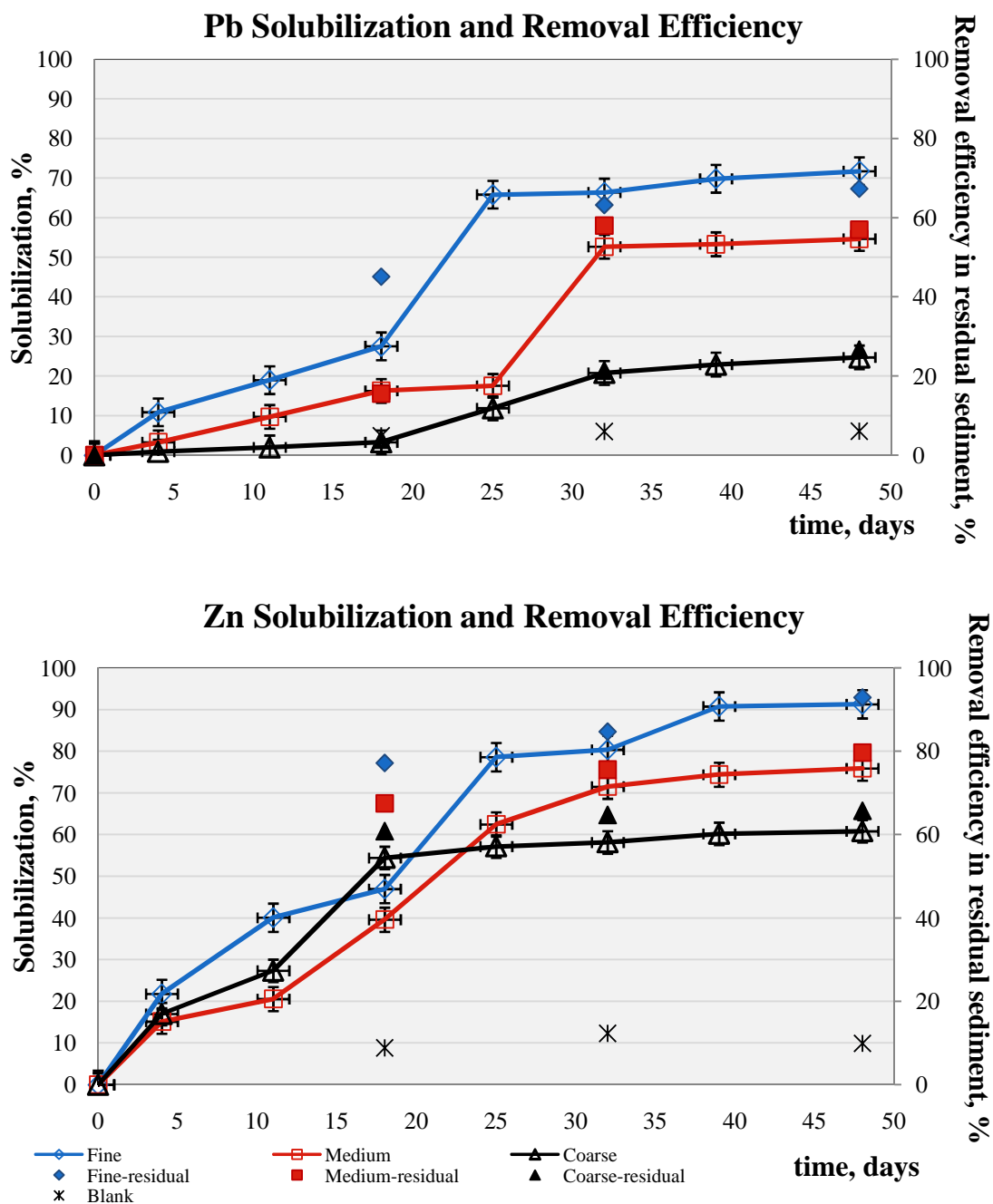


Figure 6.33 Pb and Zn solubilizations and removal efficiencies depending on grain size of the sediments

Pb was solubilized in 72%, 55%, and 25% for fine, medium and coarse particles. The solubilization ratios of Zn depending on the increasing grain size were detected as 91%, 76%, and 61% (Figure 6.33).

#### 6.4.4.7 Changes in Binding Fractions

Cr was solubilized mostly from the reducible and organic fraction for all grain sizes. The increases in exchangeable fraction were observed after bioleaching. However the losses of Cr in residual fraction decreased as the grain size of the particles increased (Figure 6.34).

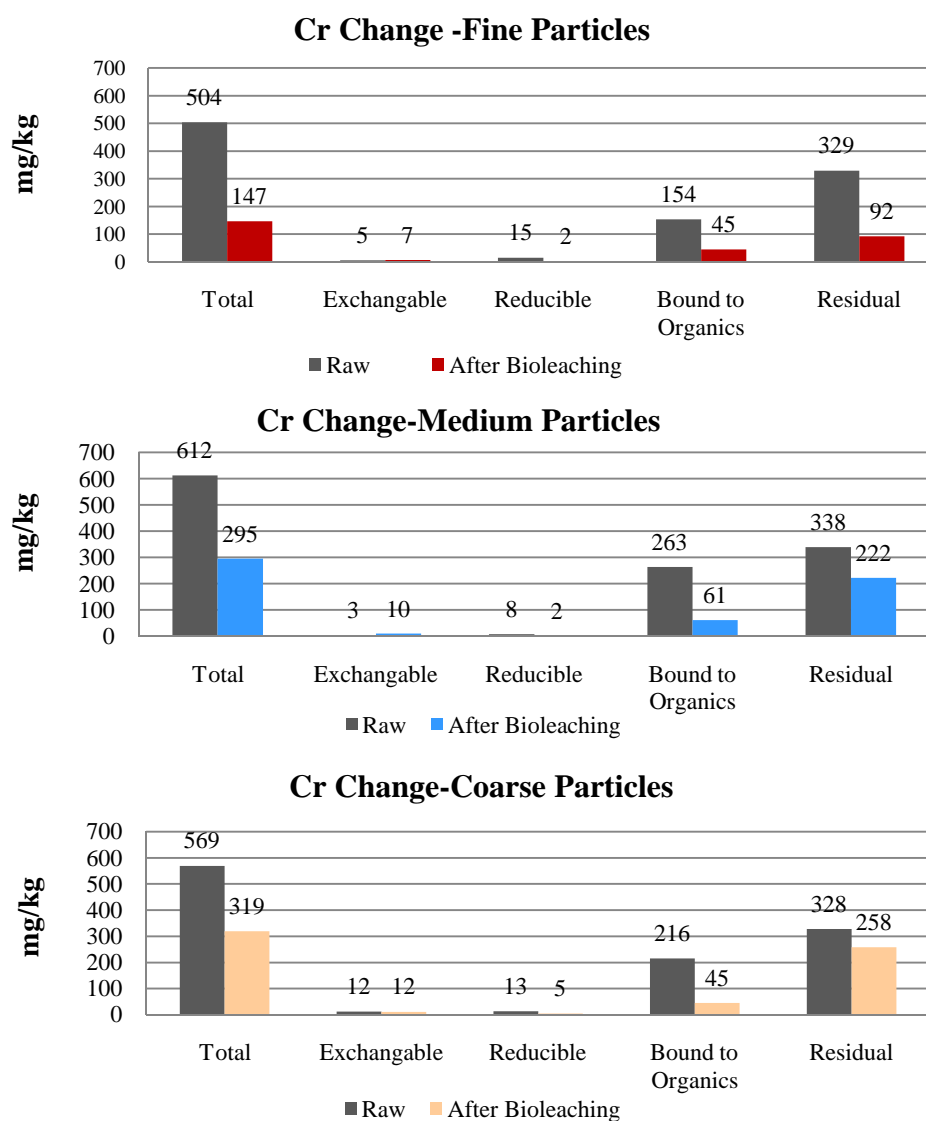


Figure 6.34 Cr in the binding fractions before and after bioleaching depending on grain size

Except the residual fraction, Cu was removed from every fraction considerably. There was nearly no Cu found in the reducible fraction after bioleaching and the organic fraction released most of the Cu load in the sediment. However, the concentrations of Cu in the residual fraction increased for medium and coarse sediments at the end of 48 days. To see the increasing level, the concentrations distributed in the fractions are presented in Figure 6.35.

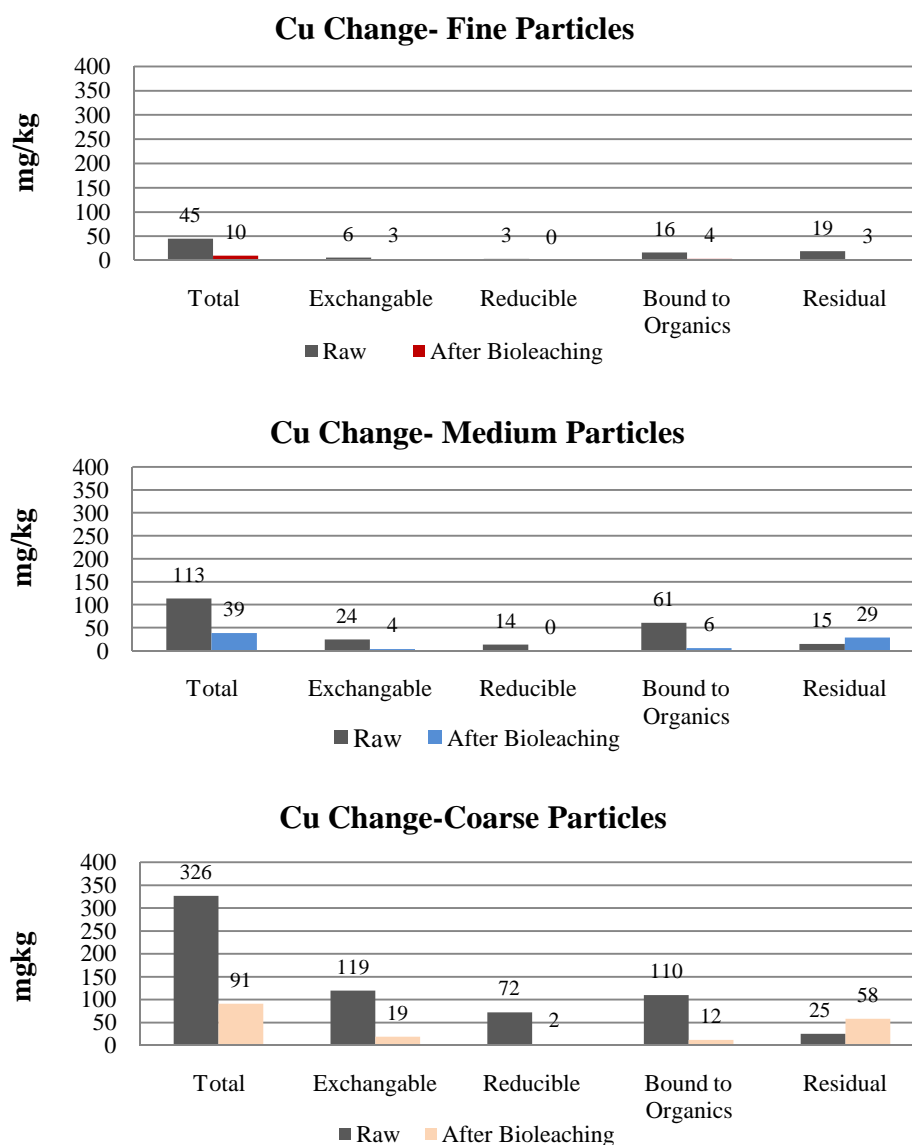


Figure 6.35 Cu in the binding fractions before and after bioleaching depending on grain size

Pb releases in this set were mostly observed in the reducible and organic fractions of the sediments. Slight increases in concentrations were determined in the exchangeable fraction. Similar like Cr and Cu, increases in Pb concentrations were observed for medium and coarse particles. This situation was not observed for fine sediment particles (Figure 6.36).

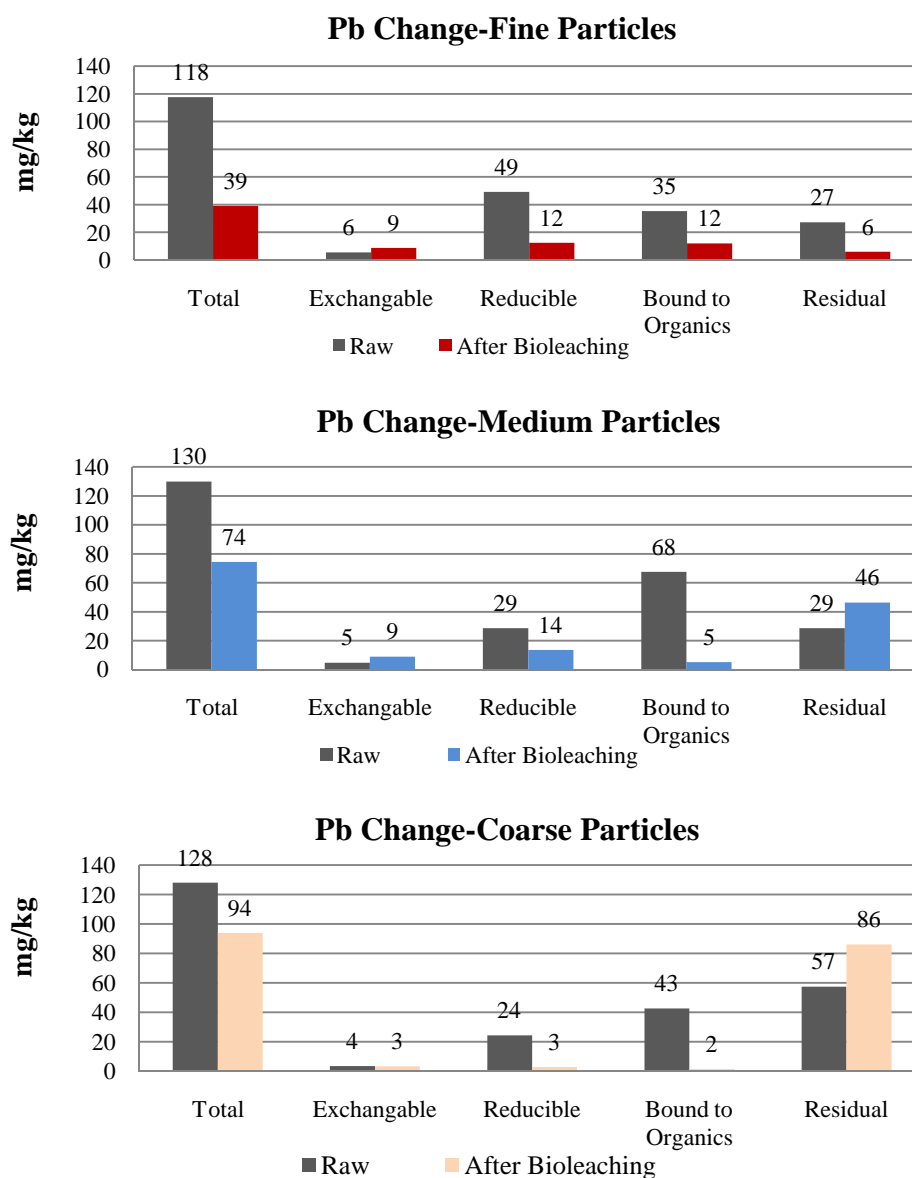


Figure 6.36 Pb in the binding fractions before and after bioleaching depending on grain size

Similar to the other sets, Zn was released from every binding fraction in significant ratios. Only the coarse particles contained increasing amount of Zn after bioleaching (Figure 6.37).

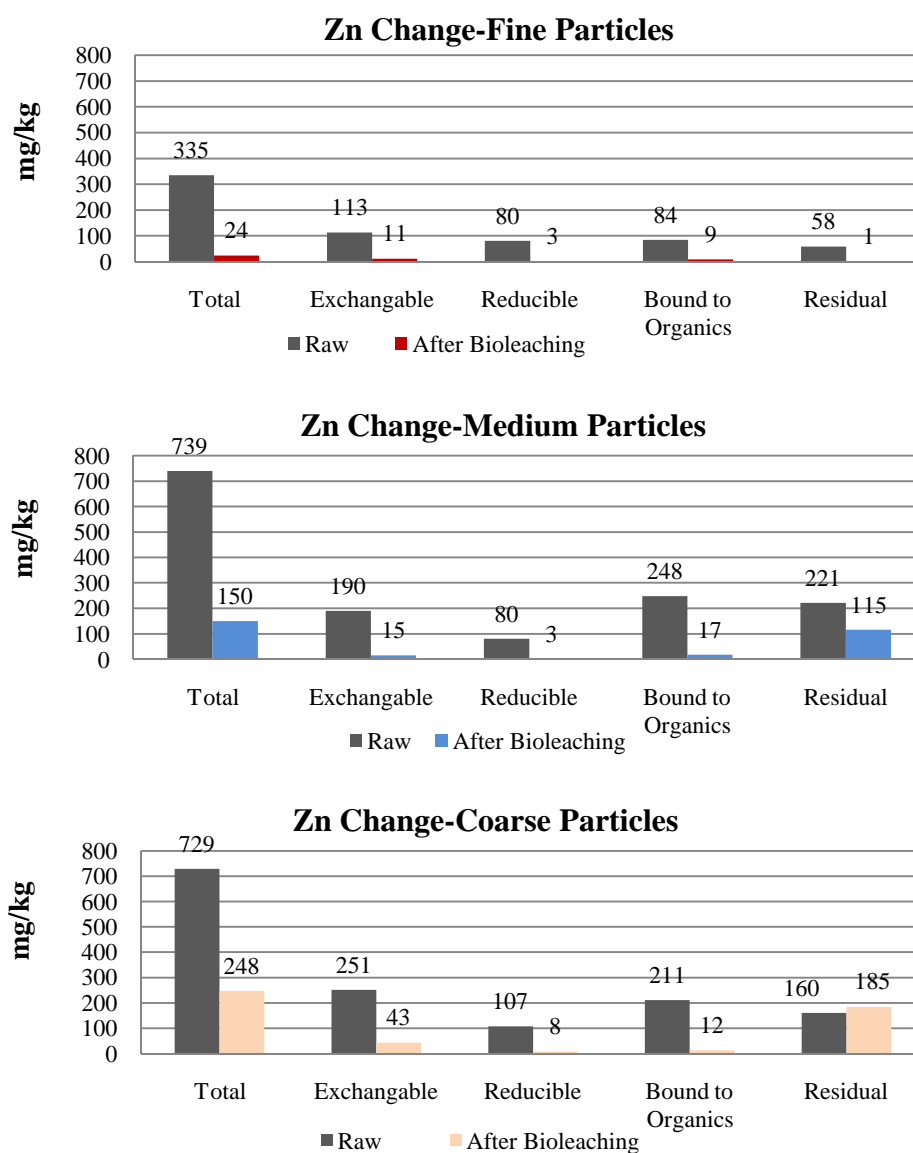


Figure 6.37 Zn in the binding fractions before and after bioleaching depending on grain size

#### 6.4.4.8 Discussion

The effect of grain size of sediments on metal solubilization during bioleaching was tested in this set of experiments. Fine (<45 $\mu\text{m}$ ), medium size (45-300 $\mu\text{m}$ ), and coarse (300-2000 $\mu\text{m}$ ) particles obtained by wet sieving of the raw bulk sample were used in the bioleaching experiments. Mixed culture was used as the inoculums and other parameters were kept constant.

Rapid pH decreases were observed in the flasks within the 10 days of bioleaching and the rate was slower afterwards. The ORP values increased depending on the pH decreases. Since the solid content (5%(w/v)) and sulfur concentration (0.5%, (w/v)) in the flasks were kept constant, no significant differences depending on the buffering capacity or sulfur oxidation were observed in pH or ORP variations.

Sulfate productions with fine and medium size particles were higher than the formation of  $\text{SO}_4^{2-}$  with coarse sediment samples. Since sufficient solid-liquid interaction area is necessary to support the bacterial activity, higher surface area of fine particles could lead the bacteria do more work and produce more sulfate.

As can be seen in Figures 6.32 and 6.33, for all the metals studied, solubilization efficiencies increased with the decreasing grain size of the samples. Metals were solubilized from fine particles sufficiently and the lowest efficiencies were determined with the coarse particles. This depends on the larger surface area provided by the smaller particles. The efficiency of metal solubilization from contaminated sediment particles is in the decreasing order:  $\text{Zn} > \text{Cu} > \text{Cr} > \text{Pb}$ . These results are compatible with the ones obtained from bulk samples. Higher solubilization of Zn depends on its mobility and formation of highly soluble compounds. The efficiency of Pb was not high in the process because of its low solubility compound;  $\text{PbSO}_4$ .

The changes in the chemical forms of metals are presented individually since each metal was found in different concentrations in the fine, medium, and coarse particles (Figures 6.34, 6.35, 6.36, 6.37). For all the metals studied, releases were observed from reducible and organic fraction independent from grain size. However, the accumulation in the residual fraction increased depending on the increasing grain size. This shows that larger particles cannot release metals from the residual fraction. Changes in the exchangeable fraction did not show a regular trend according to the size of the particles.

## 6.5 Leaching with H<sub>2</sub>SO<sub>4</sub>

As an alternative to bioleaching with thiobacilli, leaching heavy metals with 1 molar H<sub>2</sub>SO<sub>4</sub> (sulfuric acid) was tested to determine the acid consumption and metal solubilization ratios. Same as the bioleaching flasks, dry sediment samples (without the dilution medium this time) were added to the 1 l flasks with 250 ml water. Since the acid consumption depends on the amount of solid in suspension (depending on the buffering capacity of the sediment samples), the tests were done with 3 different solid/liquid ratios; 10%, 5%, 2.5%. It was aimed to decrease the pH to 1.1 with repeated additions of 1 molar sulfuric acid because the previous bioleaching tests showed that satisfactory solubilization ratios of metals were detected under this pH value. The acid consumptions for each of the flasks are presented in Figure 6.38.

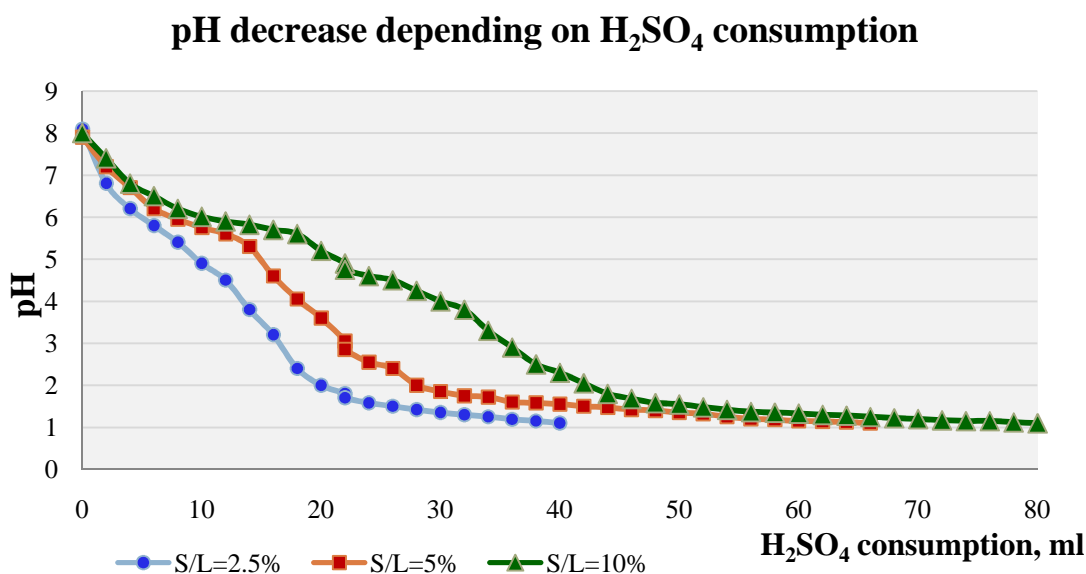


Figure 6.38 Decrease in pH versus sulfuric acid addition depending on solid content

As can be seen in Figure 6.38, acid consumption increases with higher solid concentration. For 5% solid/liquid ratio, approximately 70 ml of acid solution is required for 250 ml sediment/water suspension. For bioleaching, the initial pH was set to  $4 \pm 0.2$  and approximately 18 ml acid solution was used to achieve this. Acid consumption in leaching is four times greater than bioleaching which shows that leaching with sulfuric acid is not economic.



The solubilization of metals with sulfuric acid leaching is shown in Figure 6.39.

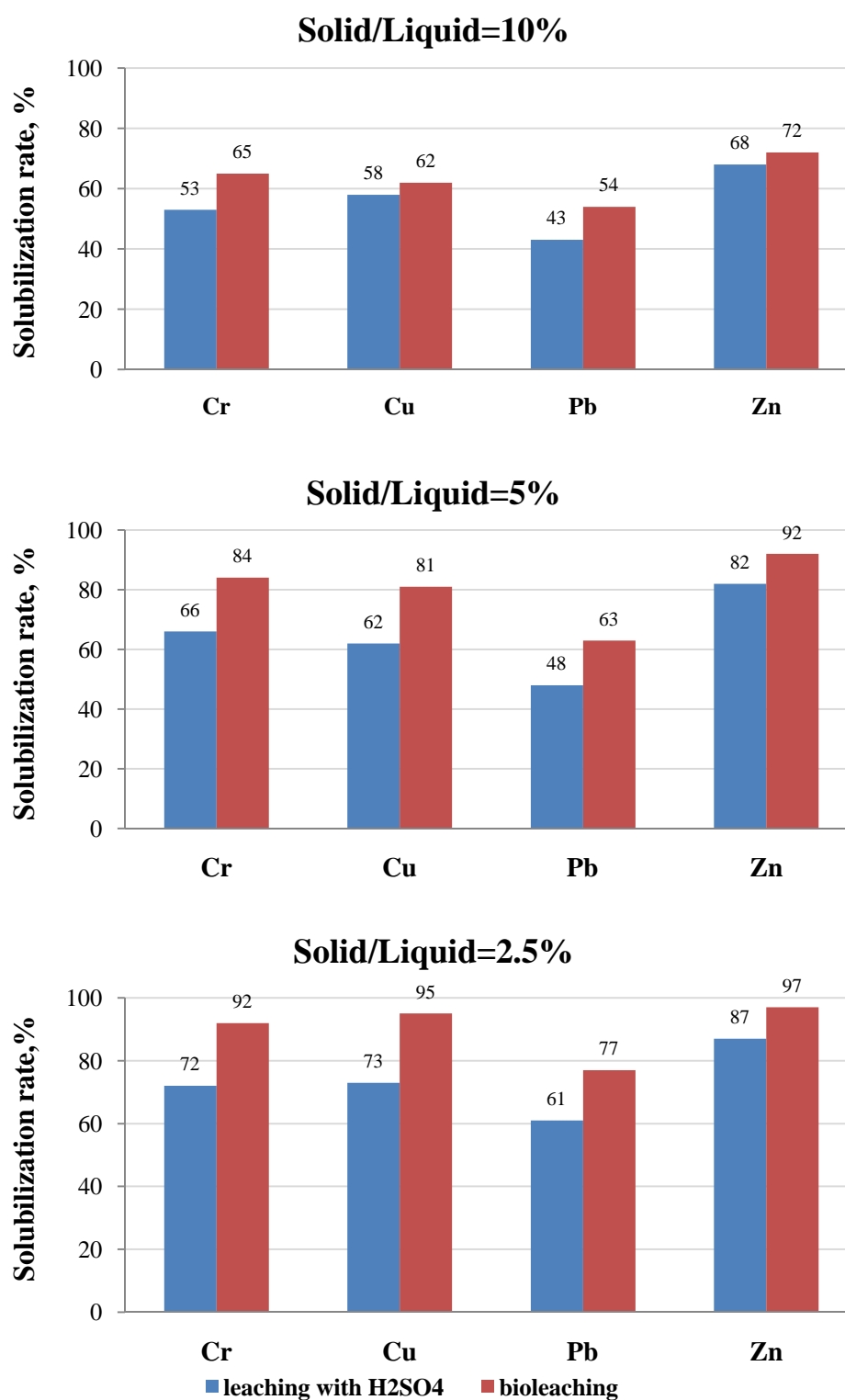


Figure 6.39 Comparison of metal solubilization with sulfuric acid leaching and bioleaching

When the results of leaching and bioleaching are compared with each other, it is seen that bioleaching gives better solubilization of metals in suspension. This may depend on the continuous decrease of pH in time (48 days) with the help of microbial activity. So, the bioleaching efficiency is higher than leaching with sulfuric acid in addition to its economical benefit.

## **6.6 Recommended Future Work**

In the presented study, the bioleaching efficiencies depending on different parameters are expressed in numbers and discussed. The experiments were conducted as suspension leaching in 1 liter flasks with a volume of 250 ml. When sediment remediation in Izmir Bay is concerned, suspension leaching may not be applicable for large amounts of sediment mass. So, a future work has to be recommended on the basis of the laboratory data.

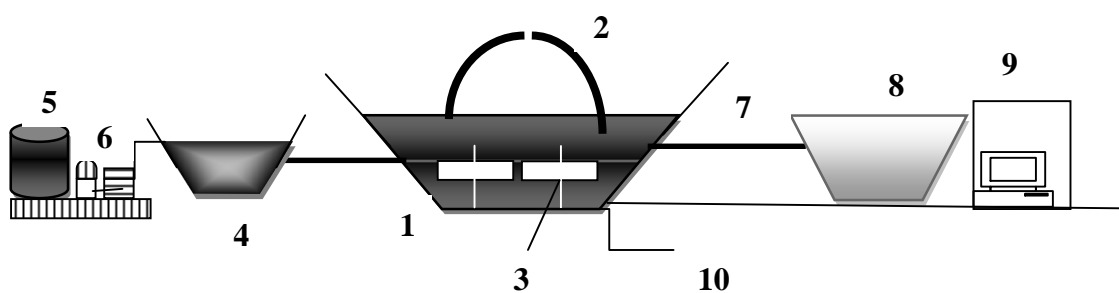
In order to leach the heavy metals from large amounts of sediments, a field scale leaching plant may be erected at a disposal site near the inner Bay to demonstrate scale up of the leaching process and confirm the laboratory data.

The leaching plant includes; a basin with a capacity of 25-30 m<sup>3</sup> equipped with drainage, a tank for leachate, a buffer tank for the additives (sulfuric acid) and a tank for the acclimation process, a tank for leachate, pumps to transport the process water, sprinklers to sprinkle the sediment, and a monitoring system to measure temperature, pH and redox potential.

The basin is filled with dredged sediment from Izmir inner Bay due to the optimum solid/liquid ratios. The mixers placed in the basin provide the mixing and aeration at the same time providing the speed of 120-150 rpm. The acclimation tank is placed near the bioleaching basin to provide the acclimated organisms ready for the bioleaching process. The microorganisms from the acclimation tank are transferred to the bioleaching basin and sulfur is mixed the sediment during the charging. The sediment is sprinkled with process water at intervals. To support acidification, sulfuric acid may be added to the process water. The microbial process leads to the direct solubilization of metal sulfides and production of sulfuric acid, by

extracting heavy metals in the sediment. Continuous monitoring of the pH is carried on. Depending on low pH values, metals solubilized in the leachate are detected, and optimum bioleaching time is fixed. The pilot plant must be tested for at least 4 months to study its technical functions. The remediated sediment is drawn from the bioleaching basin with the collection pipe. The leachate with heavy metals is taken from the bioleaching basin by the leachate pipe and transferred to the leachate tank for further metal removal processes (electrokinetic processes or precipitation with lime can be applied for the metal contaminated leachate).

A simple diagram of the field scale pilot plant is figured with Figure 6.40.



- |                      |                                  |
|----------------------|----------------------------------|
| 1) bioleaching basin | 6) pump                          |
| 2) sprinkler         | 7) leachate pipe                 |
| 3) mixers            | 8) leachate tank                 |
| 4) acclimation tank  | 9) monitoring                    |
| 5) additives tank    | 10) collection pipe for sediment |

Figure 6.40 Diagram of the recommended pilot plant

## CHAPTER SEVEN

### CONCLUSIONS

This thesis study has been conducted by using the sediment samples obtained from different locations of Izmir inner Bay. The study includes the characterization of the samples by means of their bulk selected heavy metals (Cr, Cu, Pb, Zn) concentrations and distributions of these heavy metals according to their binding forms which is followed by the remediation of metals contaminated sediments with bioleaching method. Two major Thiobacilli strains are used for bioleaching tests and the effects of solid/liquid ratio, sulfur addition rate, and the sediment particle size are studied as well as the effect of bacteria type used. The changes in chemical distribution of the heavy metals are also searched in the content of the study.

The major findings and conclusions of the thesis for sediment characterization part and the heavy metals bioleaching section are presented below, respectively.

The findings for the sediment characterization studies:

- Heavy metals content of the seven samples collected from inner Bay are highly above the EPA-OSWER Ecotox Thresholds. High organic matter content (7.7-12.3%) found in the sediment samples is another indication of pollution. With respect to their total bulk concentrations, the metals can be ranked in decreasing order as follows: Cr>Zn>Cu>Pb.
- The higher metal concentrations are found in the sediment samples which are closer to the river discharge locations to the inner Bay.
- Chemical partitioning of the heavy metals in the sediment samples change with the location of the sediment and the type of the heavy metal. It is found that heavy metals load in soluble fractions are much higher than the insoluble forms for the extend of the samples and the heavy metals.
- The depth of the water, the water circulation availabilities in the area, the number and extent of the previous dredgings completed in the area, the

distance from the discharging rivers, the catchment areas of the river basis, the nature of the pollutant received by the media, and the time of pollution are the effects on the chemical distribution of the heavy metals in the sediments. The differences in total metal concentrations in sediment samples and their different chemical distribution are the results of the mentioned effects.

The findings for the bioleaching studies:

- For all the sets studied, the bacteria acclimation can be achieved in 13 days for bioleaching.
- The treatment of heavy metals contaminate sediments can be achieved in 48 days by suspended leaching. The temperature of 30°C is optimum for the bacterial growth.
- The strain of *Thiobacillus thiooxidans* is found to be the most effective bacteria with respect to *Thiobacillus ferrooxidans* and the mixed culture depending on its ability of oxidizing sulfur rapidly.
- There is a great influence of solid/liquid ratio in the treatment media on bioleaching efficiency. Lower content of solid provides higher removal efficiency. Most satisfactory results were obtained with the 2.5% solid content (90% of average metal solubilization was achieved). However, low solid/liquid ratio means less amount of sediment to be treated. So, 5% solid content in suspension seems to be the optimum ratio, since average 80% of the metals were solubilized from the sediments.
- It is found that lower and excess sulfur additions to the medium result with lower bioleaching efficiencies. The ratio of 0.5% elemental sulfur exhibited the highest metal solubilizations and removal efficiencies.
- The size of the sediment samples has an important effect on the solubilization and removal efficiencies. Bioleaching with the smallest

particles of the sediment resulted with the highest metal removal efficiencies. This is related with the larger surface area of unit amount of particles with lower diameters. Depending on the replacement of the metals, larger interaction area of particles, water, and bacteria concentration in suspension resulted better solubilization achieved by the microbial activity. This shows that sediments with silt/clay structures can be leached more satisfactorily with the microbial activity.

- The general particle size characteristics of the sediment samples (Table 5.1) show that small and medium particles contribute to the bulk sediment with an average ratio of 70%. Even if the coarse particles have considerable concentrations of heavy metals, the load of metals they carry is lower than that of medium and small size particles and overall treatment efficiency allows us to remove metals from the sediments to obtain the limits indicated in EPA-OSWER Ecotox Thresholds.
- In the bioleaching studies, both solubilization ratios in water and the removal efficiencies depending on the heavy metal concentrations left in the residual sediment particles are calculated to check the mass balance during microbial leaching. The results are compatible with each other. Deviations to a ratio of 10% can be neglected.
- According to the results of the bioleaching trials, it is determined that Zn is the metal mostly removed from the contaminated sediments. Lowest removal is detected with Pb, because the solubilized Pb can only form into low solubility of  $\text{PbSO}_4$ . The efficiency of metal solubilization from contaminated sediment is in the decreasing order:  $\text{Zn} > \text{Cu} > \text{Cr} > \text{Pb}$ .
- The changes of chemical distributions of heavy metals during bioleaching were very similar to each other for all of the trials. It is observed that, the stable forms of heavy metals are transferred and accumulated in more soluble forms during bioleaching and they directly solubilize in the water

when they are finally transferred to the exchangeable and/or reducible form.

- When the changes in the binding forms are considered according to the grain size of the sediments, it is found that coarse particles cannot release metals from the residual fraction, oppositely higher accumulations in the crystal matrix are observed after bioleaching. This shows that, even the metals cannot be solubilized from the coarse particles sufficiently; they are trapped in the residual fraction from which they don't release under natural conditions.
- The experiments in the study were conducted as suspension leaching in small volumes. When sediment remediation in Izmir Bay is concerned, suspension leaching may not be applicable for large amounts of sediment mass. So, a future work has been recommended on the basis of the laboratory data.
- It can be concluded that bioleaching is an effective and satisfactory method for the removal of heavy metals from contaminated sediments, soils, and sludges. It is also economical when compared to leaching with  $H_2SO_4$ . But it transfers the metals from solid to liquid phase which is the highly metal polluted, acidic wastewater to be treated. The left water can be treated via chemical processes (precipitation with lime) or electrokinetic treatment facilities which provides the metals recovery.

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