BIOAVAILABILITY AND BIOACCUMULATION OF HEAVY METALS FROM CONTAMINATED COASTAL SEDIMENTS

(沿岸域底質に残留する重金属の生物利用能と生物蓄積に関する研究**)**

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Declaration by Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
BMF	Biomagnification Factors
BSAFs	Biota Sediment Accumulation Factors
DW	Dry Weight
ERM	Effect Range median
ERL	Effect Range Low
HNO_3	Nitric Acid
HCl	Hydrochloric Acid
PLI	Pollution Load Index
RAC	Risk Assessment Code
TOC	Total Organic Carbon
ND	Not Detected

ABSTRACT

This study consists of three major objectives; at first, the distribution and geochemical speciation of metals in the contaminated coastal sediments in Osaka Bay, Japan were investigated as a case study; secondly, bioavailability of metals in sediments were predicted from the above chemical data; and at last, the agreement between predicted bioavailability and actual bioavailability through the accumulation tests with contaminated coastal sediments by the polychaete (*Perinereis nuntia)* was examined.

In the first objective, the distributions and geochemical speciation of heavy metals such as cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in sediments were investigated at 25 sites in Osaka Bay, Japan. High concentrations of Zn (76-967 mg/kg dry weight (DW)) were observed followed by Cu (not detected (ND)-399 mg/kg DW), Pb (ND-73 mg/kg DW) and Cd (0.2-2.9 mg/kg DW). A considerable fraction of Cd was exchangeable and carbonate-bound. Cu occurred predominantly in the organic material-bound fraction. Both Pb and Zn occurred predominantly in the Fe-Mn oxide fraction. From those results, especially high bioavailability of Cd and Zn was predicted. Then, polychaete, *P. nuntia,* was exposed to sediments collected from two sites of Kagoshima Bay, Japan, for 28 days. The polychaete adsorbed metals such as copper (Cu) and zinc (Zn) from sediments. The prediction of bioavailability of metals through chemical analysis could roughly reflect the actual absorbance of metals in the polychaete. Therefore, the prediction of metal bioavailability through chemical analysis examining depending on geochemical speciation was concluded as useful to reliably estimate actual bioaccumulation in the benthic organism.

ABSTRACT (JAPANESE)

これまで、海底質の重金属モニタリング調査は数多くなされてきた。しかし、底質中の金属の存在形態は様々であり、その全てが生物に取り込まれるわけではない。重金属の生物利用能については良く分かっていないことが多く、様々な評価方法があるが、実際の生物を用いた検証はあまりなされていない。本研究ではまず、日本でも有数の都市部に接する大阪湾をモデル地域の 1 つとして選び、その底質について、重金属の分布とその存在形態を化学分析により調査することを第 1 の目的とした。次に、これらの大阪湾底質の分析データから生物利用能を予測し、その正当性を過去のデータと比較して検証することを第 2 の目的とした。最後に、ゴカイを鹿児島湾で採取した底質に暴露し、化学分析により得られた底質中重金属データから予測した生物利用能と、ゴカイ中に実際に蓄積された重金属のデータを比較して、その相同性を検証した。

大阪湾底質は海岸線から 25 ヶ所で採取し、重金属類は主にカドミウム（Cd）、銅（Cu）、鉛（Pb）、亜鉛（Zn）を対象としてその濃度測定を行った。その結果、最も高濃度で検出されたのは Zn であり、その濃度は 76-967 mg/kg dry weight（DW）であった。以下、Cu：検出限界以下（ND）-399 mg/kg DW、Pb：ND-73 mg/kg DW 、Cd：0.2-2.9 mg/kg DW の範囲で検出された。さらにこの 25 ヶ所の底質について、底質中の存在形態に着目し、土壌中の存在形態についてその比率を算出した。大阪湾底質では、カドミウムはイオン交換態と炭酸塩態のものが多く、銅は有機物態、鉛と亜鉛は酸化物態のものが多い結果となった。これらの存在形態比率から生物利用能を予測し、過去のデータと確認した結果、この予測データはある程度生物利用能を表現できているのではないかと考えられた。さらに鹿児島湾から比較的重金属濃度が高い底質を採取し、この中でゴカイを 28 日間飼育して重金属を蓄積させた。このときの金属の存在形態を化学分析から明らかにし、さらに、実際のゴカイへの蓄積データを比較したところ、大概だがそれぞれが一致することを見出した。これらの結果から、底質中の重金属の存在形態に基づいた化学分析データは、実際の生物利用能を反映することが明らかにでき、さらにはその地点の底質の金属がどの程度、底生生物の蓄積されるかを予測するのに有効な方法であることを示した。

CHAPTER 1

GENERAL INTRODUCTION

1 .1 Background

1.1.1 Heavy Metals in Coastal Sediments

Coastal and estuarine ecosystems are thought to be one of the most important ecosystems on the earth economically and ecologically, as these ecosystems provide breeding, feeding and sheltering ground for commercially important fish and shellfish species (Little 2000). The areas close to coastal areas are important place of human settlement and, therefore, coastal sediments receive the organic and inorganic pollutants from a variety of anthropogenic sources. Metals are some of the major common pollutants in the coast and estuaries throughout the world (NSW EPA 2000).

Heavy metals are commonly defined as metals having densities at least higher than that of water. Density of heavy metals ranged from 3.5 to 7.0 g/cm^3 (Hawkes 1997). Heavy metals are common in the earth's crust and produced with the natural process such as erosion of rocks and eruption from volcanic activities. These are persistent and non biodegradable, and are common in our surrounding environment (Claisse and Alzieu 1993; Larison et al. 2000; Nusetti et al. 2001; Juhasz et al. 2002). Heavy metals affect the organisms certainly. Consequently, they pose potential threat to aquatic biota because they have potentials to be shifted to higher trophic level through the food web (Hsu et al. 2006).

Sediments serve as the largest pool of metals in aquatic environments, and more than 90% of the metal contaminations has been related to the suspended material or sediment (Islam et al. 2018). Therefore, their concentrations in sediments are often 10–100 times higher than those in the surrounding water (Temara et al. 1998). In general, metals that are common in the contaminated areas include lead (Pb), chromium (Cr), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), arsenic (As) and nickel (Ni). Safe limits for the metals in sediment proposed by the United States Environmental Protection Agency (US EPA) are summarized in the Table 1.1

Table 1.1 Pollution Criterions of Heavy Metals in Marine Sediments (Modified from, Pazi 2011).

Metal	Average conc. on earth crust	Non-polluted	Moderately Polluted	Heavily polluted
As	1.8 mg/kg	<3 mg/kg	3-8 mg/kg	>8 mg/kg
Cu	55 mg/kg	<25 mg/kg	25-50 mg/kg	>50 mg/kg
Cr	100 mg/kg	<25 mg/kg	25-75 mg/kg	>75 mg/kg
Ni	75 mg/kg	<20 mg/kg	20-50 mg/kg	>50 mg/kg
Pb	12.5 mg/kg	<40 mg/kg	40-60 mg/kg	>60 mg/kg
Zn	70 mg/kg	<90 mg/kg	90-200 mg/kg	>200 mg/kg

Due to several physical and chemical and biological processes metals in the coastal sediment may release in to the water column and the pore water from sediments (Hamzeh et al. 2014). Therefore, there is a need to assess the risks induced by metals in sediment. So far there has been several indices to estimate the risks of metals in sediments such as enrichment factor (EF), geo accumulation index, contamination factors (CF), pollution load index (PLI), and evaluation of biological risk through effect range low (ERL)/ effect range median (ERM) (Mohiuddin et al. 2011; Essien et al. 2009).

1.1.2 Defining Bioavailability

Bioavailability of metal refers to fractions of the total metal that can be released from sediments to the pore water and thus can reflect bioaccumulation. The international standard of organization (ISO) proposes the definition of the bioavailability is as follows;

"Bioavailability is the degree to which chemicals present in the soil may be absorbed or metabolized by human or ecological receptors or are available for interaction with biological systems" (ISO 11074: 2005).

Juhasz et al. (2002) has been proposed three important criterions to be considered as bioavailable for a contaminant; a) a target organism is exposed to sediments contaminants; b) a proportion of contaminants is available for uptake; and c) an organism is able to accumulate those contaminants.

The wide ranges of physical, chemical and biological factors are responsible to influence bioavailability of contaminants including metals in sediments. In the aquatic ecosystems, physical factors such as the rate of sedimentation, re-suspension and diffusion process are responsible to influence bioavailability of heavy metals. Similarly, a range of chemical factors such as redox condition, pH, organic carbon content in sediment and organic matter characteristics are the major chemical characteristics that are responsible for the bioavailability. Biological characteristics of organisms such as behavior and feeding mechanisms are responsible for controlling bioavailability.

1.1.3 Bioaccumulation of Heavy Metals in Coastal Organisms

According to USEPA and USACE (1998), bioaccumulation is defined as, accumulation of contaminants in the tissue of organisms through any route such as respiration, ingestion, or direct contact with contaminated water, sediment, pore water, and dredged material. Accumulation of metals in the aquatic organisms is not only a threat to them, but sometimes may impact the human as well as proven by the incidents caused in Minamata Bay, Japan during 1960s. In Minamata disease sediments in the bay were contaminated by Hg, discharged from the industries nearby to Minamata city. As a result of the consumption of fish and shellfish much accumulated Hg collected from the Minamata Bay, humans were influenced seriously by the toxicities of Hg .

Bioaccumulation of contaminants is related to a function on bioavailability of contaminants in accordance with species-specific uptake and elimination process. The knowledge of bioaccumulation cannot be obtained only by chemical concentrations determined in the biota or sediments, since the different physical-properties and sediment characteristics will affect bioavailability. It has been reported that the surrounding conditions such as temperature and dissolved oxygen (DO) can influence the uptake rate of contaminants into the organism (Ankley et al. 1992).

The ideal species evaluated bioaccumulation of contaminants contaminated in marine sediments should be based on the following characteristics such as feeding types, adaptability to salinity, pollution tolerance and evidences of being used in many previous investigations related to bioaccumulation (Lee 1992).

1.2 Problem Statement and Objectives

There has been a large number of literatures describing the risk assessments with the data of total contents of metals, though total concentrations of metal in sediments do not corresponds to the bioavailable ones, because their formulations as geochemical forms of metals play a vital rule on bioavailability (Olajire et al. 2003). As a result, only the total concentration of heavy metal could not accurately reflect its absorbance and effects in the organisms. We are required to estimate the accurate bioavailability for metals with the adequate methods (Noah and Oomori 2001; Chaharlang et al. 2016; Yang et al. 2017). However, there are scarcities of information related to bioavailability of metals in sediments.

In aquatic organism, bioaccumulation is the process of absorbing contaminants. Only determination of contaminants in the sediments does not offer us any idea regarding potential absorbance of contaminants in to the aquatic organisms. It is therefore, important to know bioavailability of target contaminants through actual exposure test to estimate bioaccumulation in the benthic organisms, for example using the field sediment. However, there are only limited literatures available regarding their estimations of bioavailability for metals through actual data and the experiments for bioaccumulated processes (Remaili et al. 2016; Väänänen et al. 2019). Besides, there are scarcities of information related to the relationship between predicted bioavailability of metals in sediments through chemical analysis and actual bioaccumulation in the benthic organisms. Therefore, the objectives of the study were as follows;

I. To investigate the distribution and formulation such as geochemical speciation of metals in coastal sediments.

II. To predict bioavailability of metals considering the formulation of metals such as geochemical speciation.

III. Relating metal bioaccumulation from contaminated coastal sediment to polychaete, *Perineris nuntia* with their bioavailability.

CHAPTER 2

INTRODUCTION TO GEOCHEMICAL SPECIATION OF METALS

2.1 Geochemical Speciation of Metals

Total contents of metals are the amount of metals extracted from sediments using strong acid mixtures such as aqua regia (a 3:1 mixtures of HNO_3 (nitric acid) and HCl (hydrochloric acid)) or using mixtures of hydrofluoric acid (HF) and perchloroic acid ($HClO_4$) (Rao et al. 2008).

In sediments, the total contents of metals are partitioned into different formulations such as geochemical fractions (Tessier et al. 1979; Li et al. 2001; Olajire et al. 2003) which are as follows;

(a) Exchangeable Fractions: Exchangeable metals are found in this fraction. A metal bound in this fraction is loosely adsorbed into sediment, and therefore, can easily bioaccumulate in to the aquatic organisms.

(b) Carbonate Bound Fractions: Here is metal bounded to carbonates. The metals in these fractions are associated with sediment carbonate and slight pH change in sediments can release metals bounded in this fractions.

(c) Fe-Mn Oxides Fraction: Here is metal bounded to Fe-Mn oxides. These oxides are acting as cement between sediment particle and scavenger of heavy metals.

(d) Organic Material Bound Fraction: Here is metal bounded to organic matter. The metals found in the plants and/or animal detritus. Under oxidizing conditions these metals can be released into the environment

(e) Residual Fractions: these metals are mainly geological/geographic origin and contain mainly primary or secondary minerals

Bioavailability of metals is strongly associated with these geo chemical fractions of the total content of metals (Figure 2.1).

Exchangeable fraction of the metal can easily be absorbed into the biota, however, metal bound in the carbonate fractions, Fe-Mn oxide fractions and organic material fractions can be available when the sediment characteristics such as pH, salinity, reduction and oxidation changes (Table 2.1).

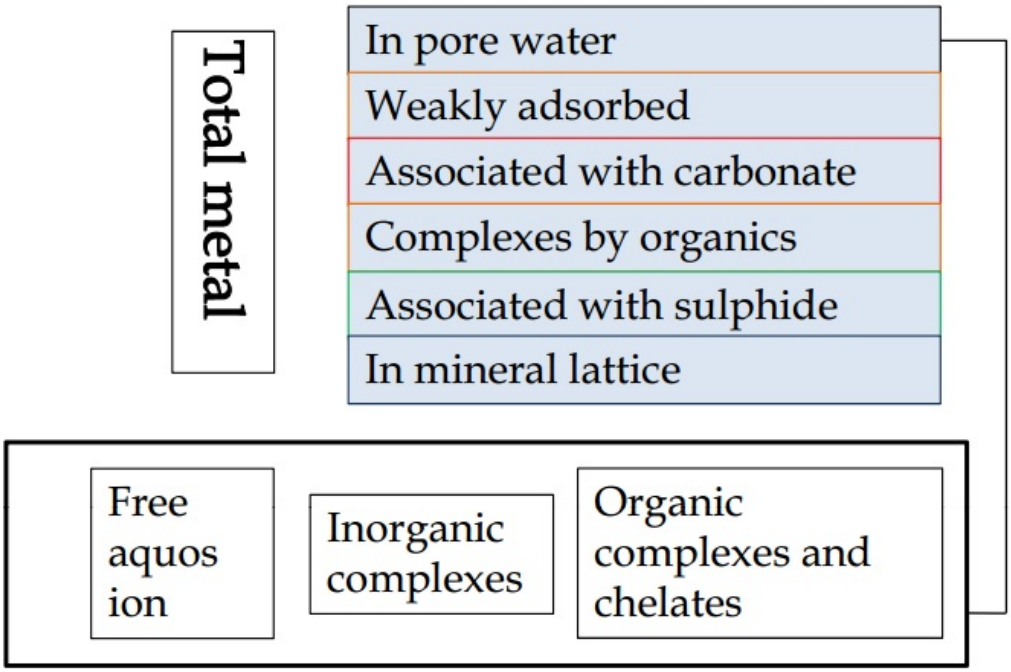

Figure 2.1 Chemical Forms of Metals in Sediment Fractions (Modified from John and Leventhal 1995).

Table 2.1 Relative Mobility and Availability of Heavy Metals in Different Geochemical Fractions (Modified From Salomons 1995)

Metal Species and Association	Mobility/Bioavailability
Exchangeable	High. Changes in major cationic composition (estuarine environment) may cause to release these metals from sediment
Carbonate bound	Medium. Changes in slight pH condition may release these metals from sediments
Metals bound in the Fe-Mn oxides	Medium. Changes in reduction and oxidation conditions may cause a release
Metals associated with organic matter	Medium/High. With time, decomposition/oxidation of organic matter occurs
Residual metal	Low. Only available after weathering or decomposition

Residual condition of metal remains inactive because metal bound in this phase are bound to the crystalline lattices of sediment particles (Tessier et al. 1979). In the field of environmental chemistry, the release of metals from sediment is important for establishing the bioavailable fraction. The exchangeable fraction and carbonate bound fraction, is believed to the most bioavailable fraction because metals bound in these fraction can easily be liberate or re-adsorbed from sediment. Generally, the order of bioavailability in different geo-chemical fractions is as follows: exchangable > carbonate bound > Fe-Mn oxides > organic material bound > residual (Liu et al. 2016; Jafarabadi et al. 2018).

CHAPTER 3

GENERAL METHODOLOGY

3.1 List of Chemical Reagents

During the research project following chemical standards and reagents were used. The manufactures are cited next to each reagent.

a) Zinc (Zn) Standard Solution, Wako Pure Chemicals Industries, LTD
b) Copper (Cu) Standard Solution, Wako Pure Chemicals Industries, LTD
c) Lead (Pb) Standard Solution, Wako Pure Chemicals Industries, LTD
d) Cadmium (Cd) Standard Solution, Wako Pure Chemicals Industries, LTD
e) Hydroxilamine hydrochloride (NH_2OH-HCl, >97.0%), Tokyo Chemical Co, LTD
f) Hydrogen Peroxide (H_2O_2, 30%), Fujifilm Wako Pure Chemical Corporation
g) Acetic Acid (CH_3-COOH, 99.7%), Wako Pure Chemicals Industries, LTD
h) Magnesium Chloride Hexahydrate ($MgCl_2$. $6H_2O$), Wako Pure Chemicals Industries, LTD
i) Sodium Acetate Trihydrate (CH_3COONa. $3H_2O$), Wako Pure Chemicals Industries, LTD
j) Nitric Acid (HNO_3, 60%), Fujifilm Wako Pure Chemical Corporation
k) Hydrochloride Acid (HCl, 37%), Nacalai Tesque, INC.

3.2 Analysis of Total Metals

Total contents of metals in sediments were measured following Chen and Ma (2001). Twelve milliliters of aqua regia, mixed a solution of nitric acid (HNO_3) and hydrochloric acid (HCl) in a 3:1 (v/v) mixture, was added to 0.5 g of dried sediment.

The sample was then digested on a hot-plate kept at 110°C for 3 h (Fig 3.1). After nearly evaporating all of solvent, 20 mL of 2% HNO_3 was added to the residue, filtered through a glass filter (pore size, 1.2 µm), and diluted to 45 mL with Milli-Q water.

Figure 3.1 Digestion of Sediment Digestion with Aqua Regia on Hot Plate

The contents of Cd, Cu, Pb, and Zn in this solution were then measured by an atomic absorption spectrophotometer (Hitachi Z-2300, Hitachi; Figure 3.2) and expressed as mg/kg DW. The absorption wavelengths were 324.8, 228.8, 283.3, and 213.9 nm for Cu, Cd, Pb, and Zn, respectively. The detection limits were as follows: 0.02 mg/kg for Cd, 0.006 mg/kg for Cu, 0.03 mg/kg for Pb, and 0.02 mg/kg for Zn. An air-acetylene type of flame was used for the determination. The analysis of target metals, which was carried out in twice (n=2) for each site, and the results are presented as mean concentrations. The reliability of the results was frequently checked using blanks and

standard solutions of each respective metal. In this study, the metal recoveries from the analytical procedures were between 75% and 105%.

Figure 3.2 Atomic Absorption Spectrophotometer (AAS, Hitachi Z-2300, Hitachi) Used in Present Study

3.3 Analysis of Metals in Individual Fractions

The 5 formation of metals in each geochemical fraction were individually obtained following the method by Tessier et al. (1979), which is illustrated through a flow

diagram in Figure 3.3. As shown in the Figure 3.3, 1 g of dried sediment was added to 8 mL of 1M magnesium chloride ($MgCl_2$) solution (pH 7) and left at 25°C for 1 h. The solution layer, comprising the fraction of exchangeable metals, F1, was then collected.

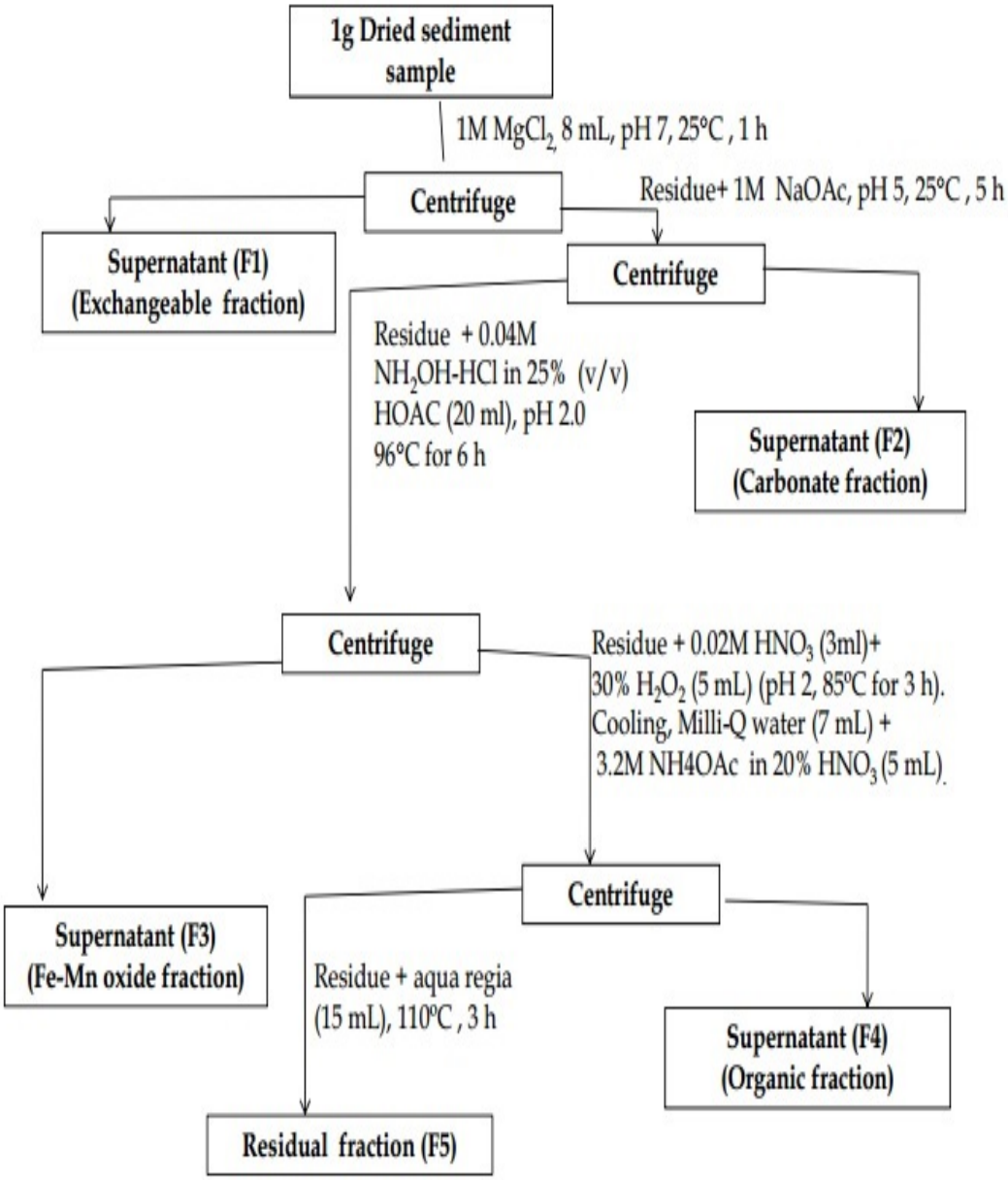

Figure 3.3 Flow Chart for the Analysis of the Heavy Metal in the Different Geochemical Fraction (F1-F5) Following Tessier et al. (1979).

The residues was treated by 1M sodium acetate solution (pH 5) at 25°C for 5 h, and then the solution layer, comprising the fraction for metals bound to carbonate, F2, was collected. After collecting the F2 solutions, the residue was added to 20 mL of 0.04 M ammonium hydroxide hydrochloride (NH_2OH-HCl) in 25% acetic acid, and then heated at 96°C for 6 h. The upper layer solution, comprising the fraction of metals bound to Fe-Mn oxide, F3, was collected. After collecting the F3 solutions, the residue metals were extracted by 5 mL of 30% hydrogen peroxide (adjusted to pH 2 by 0.02 M HNO_3) and heated to 85°C for 3 h. After cooling the mixture to 25°C, 7 mL of Milli-Q water was added, followed by 5 mL of 3.2M ammonium acetate in 20% HNO_3. The mixture was left for 30 min, and after collecting the upper layer comprising the fraction of metals bound to organic matter, F4, the residual metals were extracted by 15 mL of aqua regia at 110°C for 3 h to extract the residual metals, F5 (Figure 3.3).

Fractions F1 to F4 were centrifuged at 1000 rpm for 30 min, followed by filtration of the supernatants through a glass filter (pore size 1.2 μm). The filtered supernatants were acidified to pH 2 by adding a few drops of HNO_3 to avoid precipitating the metal in the solutions. Each metal was measured by an AAS using conditions similar to those described in Section 3.2.

3.4 Validation and Accuracy of Fractionation Method

Validation of the extraction efficiencies for each fraction was accomplished by comparing a cumulative yield of the individual metal obtained from the individual fraction (Σ (F1+F2+F3+F4+F5)) and a whole metal concentration obtained from the extraction by aqua regia as follows (Bastami et al. 2014);

$$\text{Recovery (\%)} = 100 \times (\text{F1} + \text{F2} + \text{F3} + \text{F4} + \text{F5})/(\text{whole metal concentration})$$

In the most of case in the present study, the cumulative concentrations of each metal obtained from the five fractions were similar to their corresponding total concentration. Hence, methods of fraction used in the present study can be regarded as reliable.

CHAPTER 4

Distribution, Geochemical Speciation and Bioavailable Potencies of Heavy Metals in Sediments from Urban Coastal Environment in Osaka Bay, Japan

4.1 Introduction

Rapid industrial and urban development has increasingly exposed coastal environments to several pollutants, including heavy metals (Du Laing et al. 2009; Bhuiyan et al. 2018) which are among the more serious pollutants of the aquatic ecosystems (Pini et al. 2015; Sarmiento et al. 2016; Jafarabadi et al. 2018; Sharifinia et al. 2018). Heavy metals are introduced directly by the direct effluents, for example, from petrochemical, ship building, and electrochemical industries; and indirectly, such as from river input and atmospheric deposition (Bastami et al. 2014; Chaharlang et al. 2016; Jaysankar et al. 2009).

Coastal sediments, which provide the habitat and food for a number of benthic organisms, have also become a large repository of contaminants that serve as toxicants to marine biota (Hamzeh et al. 2014; Bonnail et al. 2016). However, the total contents of metal in sediment provide little information regarding their bioavailability, mobility, toxicity, and potential reactivity (Yang et al. 2015; Chaharlang et al. 2016). The bioavailability of metals in sediments is controlled by the several physical potencies and chemical characteristics (Yang et al. 2017). Based on previous studies, metals distributed in sediments are partitioned into five geochemical fractions: exchangeable, carbonate-bound, Fe-Mn oxides-bound, organic material-bound and residual fractions (Tessier et al. 1979; Olajire et al. 2003). The bioavailability of metals depends on their association

with these geochemical fractions, which controls their absorption from sediments into organisms (Jafarabadi et al. 2018).

Osaka Bay is located in the eastern part of the Seto Inland Sea, Japan. The bay is oval-shaped, with an area of about 1450 km^2, bounded on the west by Awaji Island and with two narrow openings comprising the Kitan and Akashi channels. The Bay is surrounded by big cities such as Osaka and Kobe. Recent studies carried out in the coastal environments of Osaka Bay describe the concentrations of several contaminants such as poly aromatic hydrocarbons (PAHs) and alkylated PAHs (Miki et al. 2014), perfluoroalkyl acids (Beškoski et al. 2017), and endocrine disrupting chemicals (Koyama et al. 2013; Nurulnadia et al. 2014). There are only few investigations in the distribution of heavy metals in the coastal environment of Osaka Bay (Kitano and Fujiyoshi 1980). Hosono et al. (2010) reported that the concentrations of Cu, Cd, and Pb in sediments at Osaka Bay peaked in the 1970s, followed by a sharp decline due to the pollution controls imposed on Japanese industries starting from the 1970s. Nagaoka et al. (2004) reported that the distributions of total metal Zn, Pb, Cu, and Cd in the sediments of Osaka Bay. However, there is no recent data on the geochemical speciation characteristics of metals in Osaka Bay.

The overall objective of the present study was to improve our understanding of the distribution, geochemical speciation, and bioavailability pattern of heavy metals (Cd, Cu, Pb, and Zn) in sediments collected from 25 sites along the shoreline of Osaka Bay. Specific objectives of this study included I) investigations of the distribution and formulations such as geochemical speciations of metals in sediments collected from study areas, and II) prediction for bioavailability of metals in sediments collected from sampling sites with considering the geochemical speciation.

4.2 Materials and Methods

4.2.1 Sediment Samples

Sediments were collected using an Ekman–Birge grab sampler from 25 sampling sites along the shore of Osaka Bay between 2014 and 2016 (Figure 4.1). The samples were brought to the laboratory, sifted through a1 mm mesh sieve, and then dried at 25 ^{o}C.

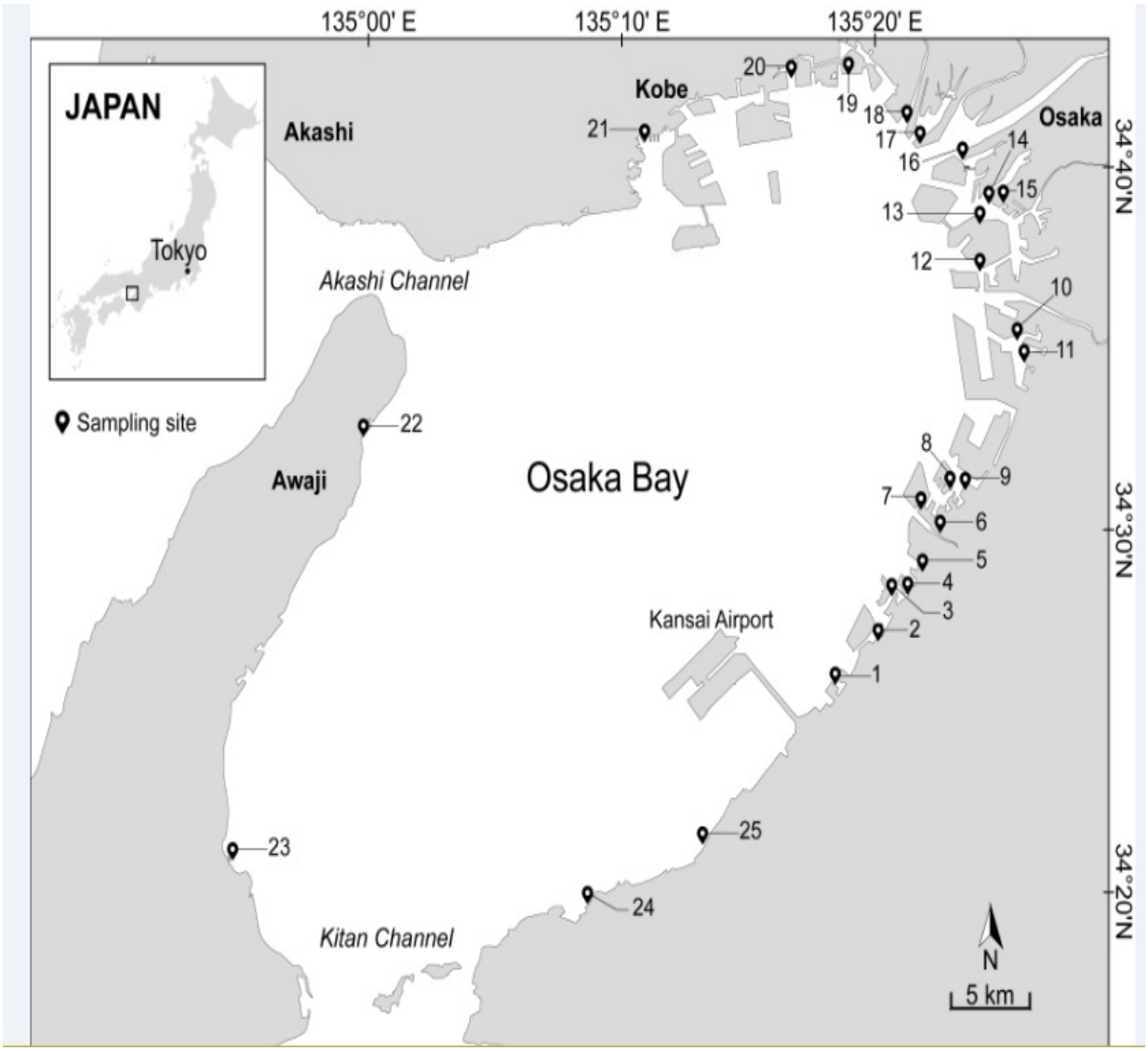

Figure 4.1 Sampling Sites in Osaka Bay, Japan

4.2.2 Analysis of Total Metals

Analysis of total metals were carried out following the methods described in the section 3.2

4.2.3 Analysis of Metals in Individual Fractions

Analysis of metals in individual geochemical fraction is determined following the methods described in the section 3.3

4.2.4 Validation and Accuracy of Fractionation Method

Validation and accuracy of the fraction methods were confirmed following the methods described in the section 3.4

4.2.5 Risk Assessment Code (RAC)

Metals in sediment are partitioned in a variety of fractions with various adsorptive capacities. Therefore, the adsorption characteristics of metals within each geochemical fraction possibly reflect risks associated with these metals. The criteria determining the adsorptive characteristics are known as the Risk Assessment Code (RAC; Perin et al. 1985), which is calculated by the following formula:

$$RAC=100 \times (F1 + F2)/\text{total concentrations}$$

Where F1 and F2 are concentrations of metals found in the exchangeable and carbonate-bound fraction, respectively (Perin et al. 1985; Bastami et al. 2014). The risks

associated with RAC values are divided into the following 5 categories: 'no risk' with RAC of < 1%; 'low risk' with RAC from 1% to 10%; 'medium risk' with RAC from 10% to 30%;'high risk' with RAC from 30% to 50% and 'very high risk' with RAC > 50%.

4.2.6 Spatial Distributions

Arc GIS Arc MAP version 10.5 and Adobe illustrator CC was used for mapping the spatial distribution of metals in the 25 sampling sites on the Osaka Bay.

4.2.7 Pollution Load Index (PLI)

In order to understand the sediment contamination status, Tomllinson et al. (1980) proposed the pollution load index (PLI), which reflects the pollution condition in sediments due to metals and is calculated using the following formula:

$$\mathrm{PLI}=(\mathrm{CF1} \times \mathrm{CF2} \times \mathrm{CF3} \times \mathrm{CF4})^{1/n} \qquad (1)$$

Where *n* is the number of kinds of the analytical target metals. In the present study, we treated four metals, and therefore *n* was set as four. *CF* is calculated from the ratio of the measured concentration (C_{mc}) of a metal being investigated and the concentration at a reference site (background, C_{bg}) as expressed in the formula below:

$$\mathrm{CF}= C_{mc}/C_{bg} \qquad (2)$$

Where C_{bg} is the natural abundance of metals obtained from an area that has not been polluted by human activities and have been generally used by previous research. In the present study, C_{bg} of Cd, Cu, Pb, and Zn are the following: 0.3, 45, 20, and 95

mg/kg, respectively. These values were obtained from levels found in deep-sea sediments (Turekian and Wedepohl 1961).

PLI values thus propose 4 classes: Class 0 (unpolluted), 0 < PLI < 1; Class 1 (moderately polluted), 1 < PLI < 2; Class 2 (heavy polluted), 2 < PLI < 3; and Class 3 (extremely heavy polluted), 3 < PLI (Tomllinson et al. 1980).

4.3 Results and Discussion

4.3.1 Distributions of Total Concentration of Metals in Sediments

The distributions of Cd, Cu, Pb, and Zn in the individual sites are shown in the Figs. 4.2-a, 4.2-b, 4.2-c, and 4.2-d, respectively. All metals had relatively low concentrations in sites St. 22 and 23 on Awaji Island, and St. 24 and 25 at southeastern parts of Osaka Bay. However, in other sites, the metals tended to reach relatively high concentrations.

The concentrations of Cd ranged from 0.2 to 2.9 mg/kg DW (Fig. 4.2-a). Especially high concentrations were detected at St. 1 (2.7 mg/kg DW), St. 3 (2.7 mg/kg DW), and St. 5 (2.9 mg/kg DW). The Cd concentrations in sediments of Osaka Bay were remarkably higher than those in Tokyo Bay, Japan (not-detected (ND) to 0.5 mg/kg) (Uno et al. 2017), in Yangtze estuary, China (0.19 mg/kg) (Wang et al. 2015), and the Spanish coast (0.22 mg/kg) (Bhuiyan et al. 2018). On the other hand, Cd concentration measured in the coast of Aliaga, Turkey, were similar to that in Osaka Bay (1.70 mg/kg) (Neser et al. 2012) (Table 4.1).

The concentrations of Cu ranged from ND to 399 mg/kg DW (Fig. 4.2-b). High concentrations were detected at St. 4 (398 mg/kg DW) and St. 3 (200 mg/kg DW). The Cu concentrations are roughly similar to those found in Tokyo Bay (33 to 106 mg/kg)

(Uno et al. 2017), but higher than those measured in the Luanhe estuary, China (10-36 mg/kg) (Liu et al. 2016)) and Miri estuary, Malaysia (4-35 mg/kg) (Billah et al. 2017) (Table 4.1).

The Pb concentrations ranged from ND to 73 mg/kg DW (Figure 4.2-c). High concentrations of Pb were detected at St. 1 (64 mg/kg DW), St.16 (73 mg/kg DW), and St. 21 (50 mg/kg DW). The levels of Pb found in this study were similar to those measured at the coastal areas of the Persian Gulf, Iran (19 to 45 mg/kg) (Sharifinia et al. 2018), although they were higher than those found in the tidal flats of Yamaguchi prefecture, Japan (27 to 29 mg/kg) (Rahman and Ishiga 2012) (Table 4.1).

Table 4.1 Comparison of Metal Concentrations (mg/kg) Detected in Sediment Collected from the Osaka Bay with Those Obtained in Coastal Areas in the World

Study site	Cd	Cu	Pb	Zn	Reference
Osaka Bay, Japan	0.2-2.9	ND-399	ND-73	82-967	Present study
Luanhe River Estuary, Bohai Sea	0.02-0.2	9.6-36	22-44	13-95	Liu et al.(2016)
Tokyo Bay, Japan	ND-0.5	17-106	14-60	113-410	Uno et al. (2017)
Miri estuary, Malaysia		4 to35		9 to 45	Billah et al. (2017)
Caspian Sea	NA	4 to 15	4 to 16	16-36	Bastami et al. (2014)
Sancti Petri channel	0.08-0.4	13-91	10 to 71	32-162	Bhuiyan et al. (2018)
Osaka Bay, Japan	1.23	45	58	242	Nagaoka et al. (2004)
Coastal sediment, Yamaguchi Pref., Japan	na	16-34	27-29	80-151	Rahman et al. (2012)
Seine River Estuary, France	6.16	133	138	448	Hamzeh et al. (2014)
Aliağa Bay (Turkey)	1.47	321	284	86.4	Nes¸er et al. (2012)
Shantou Bay	0.67	49	52	153	Qiao et al. (2013)

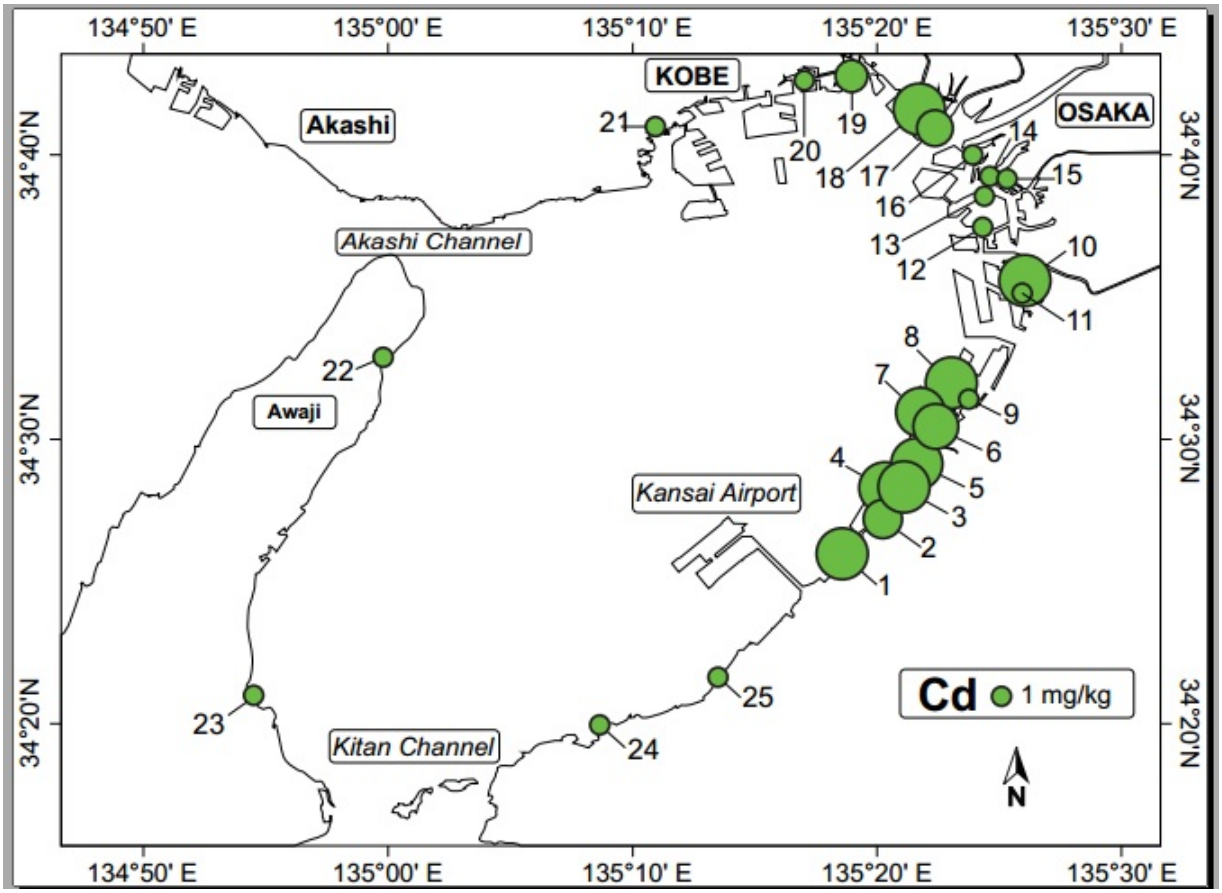

Figure 4.2-a Distribution of Cd in Sediments at Each Site of Osaka Bay

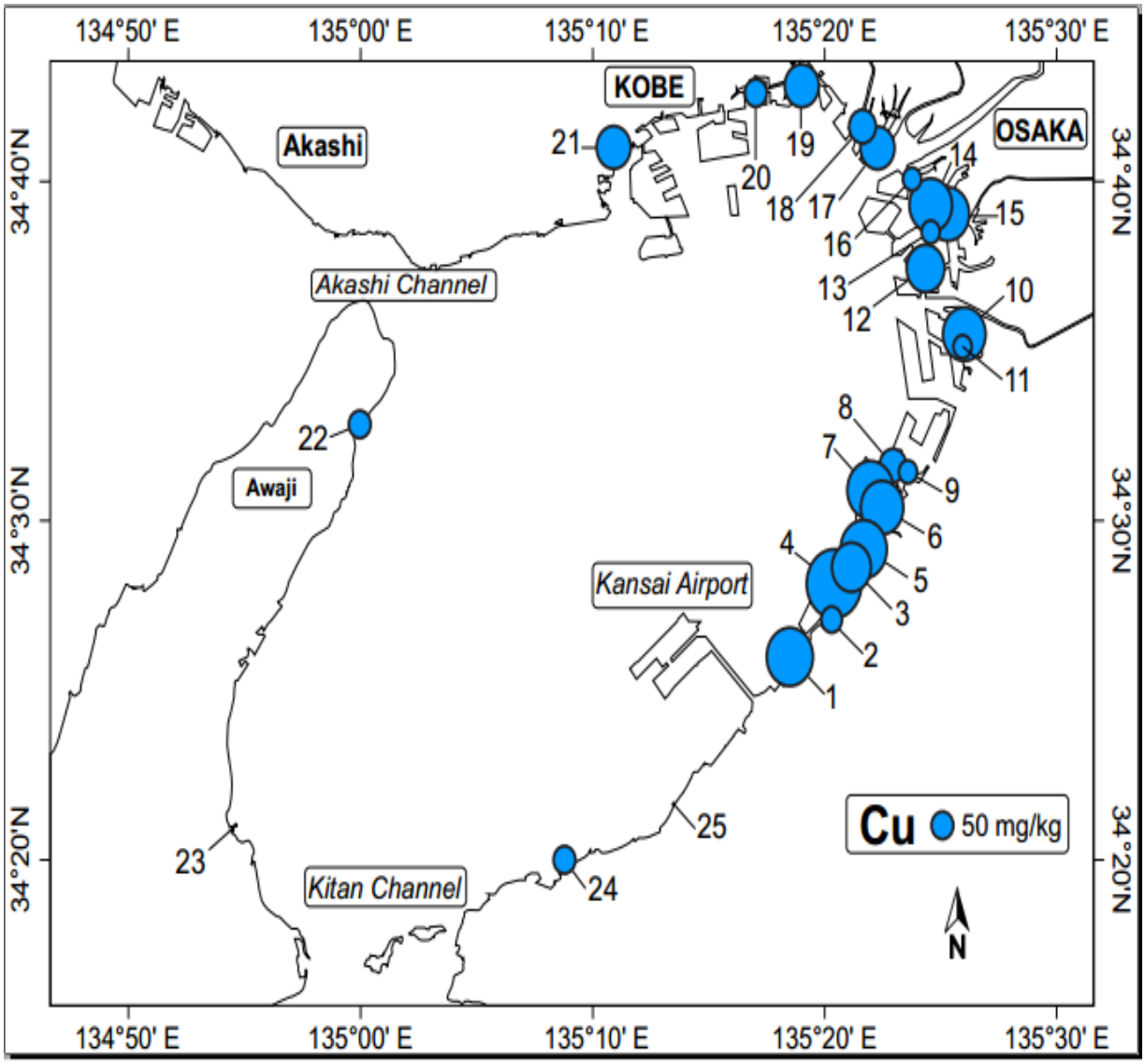

Figure 4.2-b Distribution of Cu in Sediments at Each Site of Osaka Bay

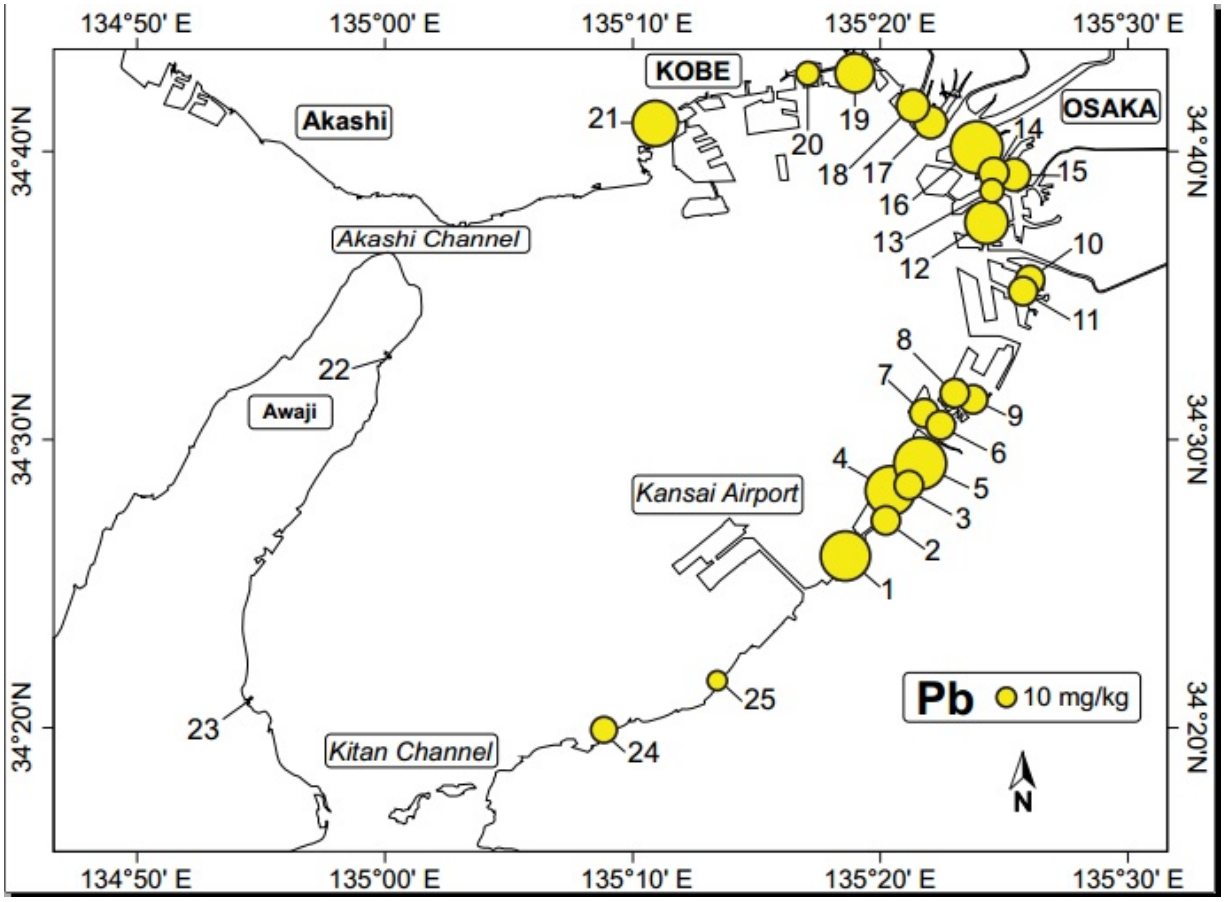

Figure 4.2-c Distribution of Pb in Sediments at Each Site of Osaka Bay

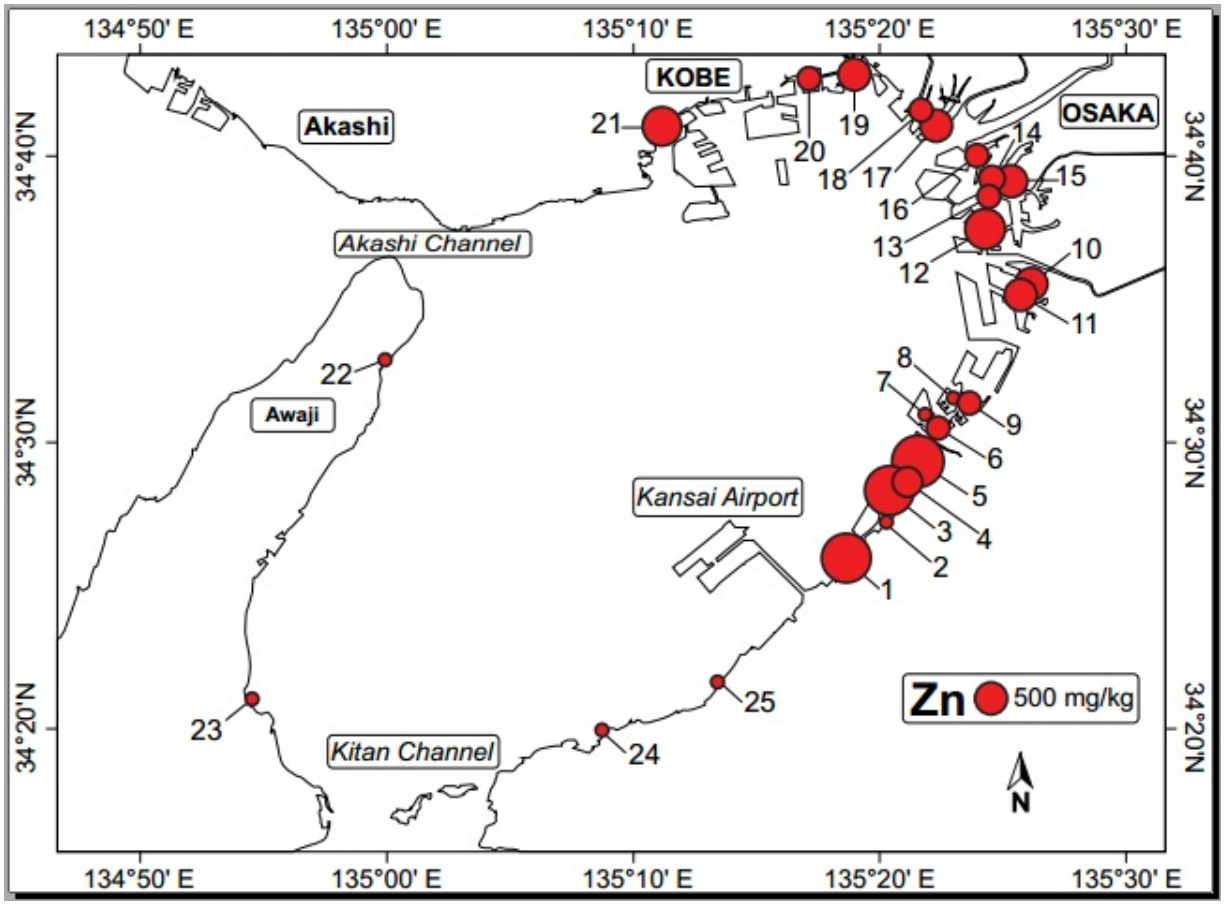

Figure 4.2-d Distribution of Zn in Sediments at Each Site of Osaka Bay

The range of the concentrations of Zn was from 76 to 967 mg/kg DW (Figure 4.2-d). High concentrations of Zn were found at St. 1 (670 mg/kg DW), St. 3 (686 mg/kg DW), St. 5 (967 mg/kg DW), St. 12 (510 mg/kg DW), and St. 21 (521 mg/kg DW). However, low concentrations were also detected at the St. 23 (88 mg/kg DW), St. 24 (90 mg/kg DW), and St. 25 (76 mg/kg DW). The concentrations of Zn found in the present study are remarkably higher than those detected in Tokyo Bay (113 to 372 mg/kg) (Uno et al. 2017) and the Yangatze estuary (83 mg/kg) (Wang et al. 2015). On the other hand, a similar concentration was detected in the Seine estuary, France (448 mg/kg) (Hamzeh et al. 2014) (Table 4.1).

The differences of the metal concentrations profile around the sampling sites can be explained considering the sources of pollution, sediment textures and organic carbon contents. The above results show that four metals were present in relatively high levels in the sediment of Osaka Bay. These levels may be the result high levels of metals discharged from various kinds of human activities.

4.3.2 Geochemical Speciation of Metals and Their Distributions

The partitioning of metals in sediments into geochemical fractions, i.e., exchangeable (F1), carbonate-bound (F2), Fe-Mn oxide-bound (F3), organic matter-bound (F4), and residual fractions (F5), is deeply related to their bioavailability and toxicity to aquatic organisms. Therefore, information on metal concentrations in the individual fractions is necessary to understand their bioavailability and potential toxicities (Lian et al. 1999; Naji et al. 2010; Guo et al. 2015). Generally, metal bioavailabilities in different fractions increase in the following order: exchangeable > carbonate bound > Fe-Mn oxides > organic material bound > residual (Jafarabadi et al. 2018). The contents of metals found in the different fractions are shown in Figure 4.3.

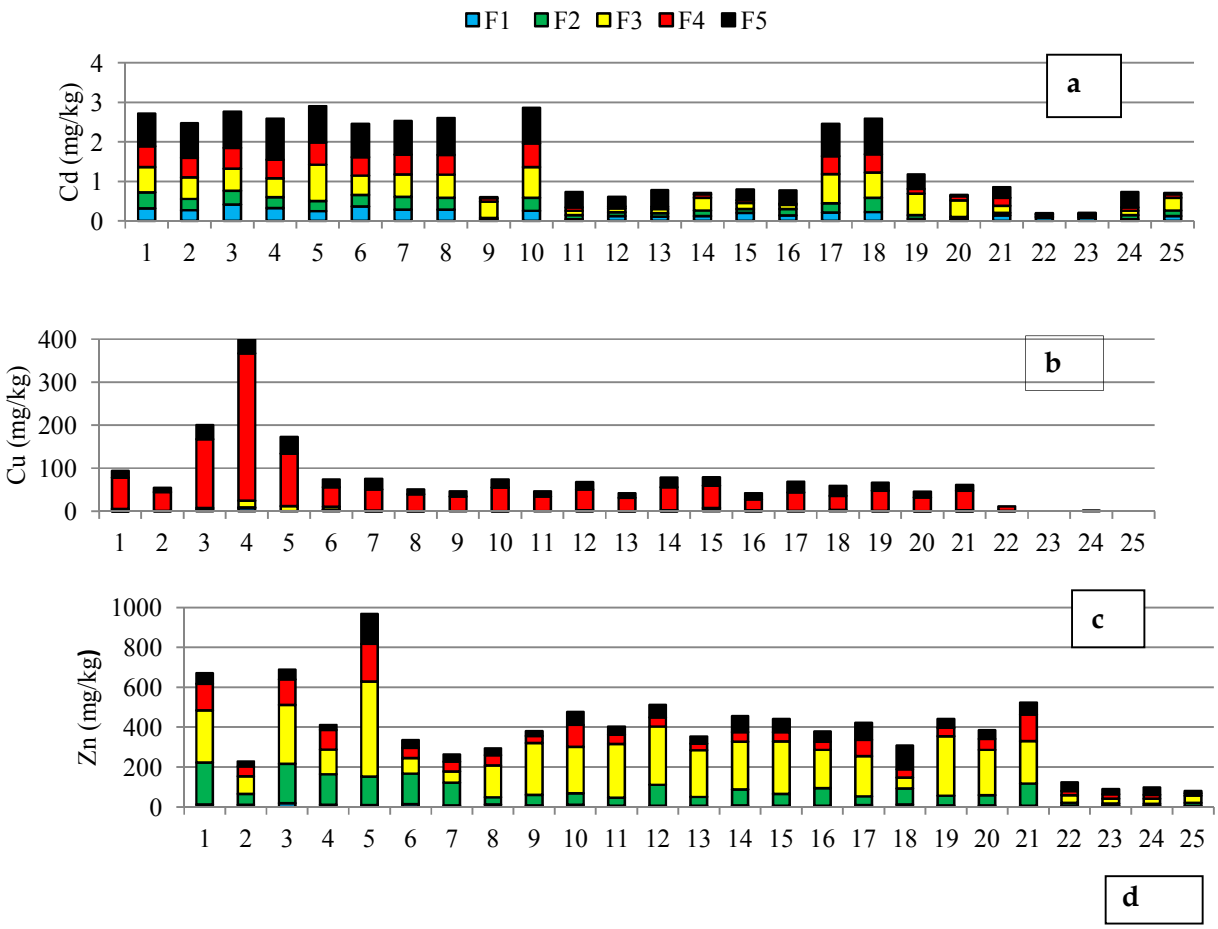

Figure 4.3 Profiles of Geochemical Fractions for Cd (a), Cu (b), Pb (c) and Zn (d) in Sediments of Osaka Bay (F1: Exchangeable, F2: Carbonate-Bound, F3: Fe-Mn Oxide-Bound, F4: Organic-Bound, and F5: Residual).

4.3.2.1 Cadmium

Considerable amounts of Cd were found in both the F1 (ND-0.4 mg/kg DW) and F2 (0.03-0.4 mg/kg DW) fractions (Fig. 4.3-a). The ranges of the percentage of total Cd found in the F1 and F2 fractions were 4-40% and 6-22%, respectively. These results are similar to those from research carried out on sediments in other coastal areas (Lopez-Sachez 1996; Sundaray et al. 2011). In the bay, similar amounts (around 0.2 mg/kg DW) of Cd were distributed throughout the F1 fractions from most sites. The abundance of the Cd in the carbonate fractions in the present study can be explained by the alkaline nature (pH range 7.1-8.2) of Osaka Bay sediments (Tokunaga et al. 1995). At St. 9, 20, and 25, there were relatively low concentrations of Cd in the F5 fraction.

4.3.2.2 Copper

Cu was dominant mainly in the F4 fraction (ND-348 mg/kg DW) (Fig. 4.3-b). The range of percentage values of total Cu found in F4 was 55-100%. The high abundance of Cu in the organic matter fraction has also been reported by other research carried out in marine sediments (Vicente-Martorell et al. 2009). One reason for this association is the ability of Cu to easily form complexes with available organic compounds in the coastal environments (Jaysankar et al. 2009). Reduced oxygen levels lead to the release of Cu bound to the organic compounds into the aquatic ecosystems. In all sites, similar amounts of Cu were found in the F5 fraction (except at St. 22 and 24), while Cu concentrations in the F1, F2 and F3 fractions were very low. These results suggest that most of Cu in the sediments of Osaka Bay is not absorbed into aquatic organisms.

4.3.2.3 Lead

Pb dominated mainly in fraction F3 (3-41 mg/kg DW) (Fig. 4.3-c). The range of percentages of total Pb in F3 was 28-93%. Pb can form stable complexes with the Fe-Mn oxide fraction, resulting in the high abundance of Pb in F3 (Li et al. 2001). The higher ratio of Pb in the Fe-Mn oxide fraction was consistent with previous studies carried out in the coastal areas of Okinawa Islands, Japan (Noah and Oomori 2001), the Barcelona Coast, Spain (Lopez-Sanchez et al. 1996), and Tees Estuary, England (Jones and Turki 1997). Importantly, Pb was not found in the F1 fraction (except St. 16). In addition, very low levels of Pb were associated with the F2 fraction in the sediments of Osaka Bay.

4.3.2.4 Zinc

Zn was common in all sampling sites and in all of the fractions from F1 to F5. High abundance levels of Zn were found in the F3 fractions (24-477 mg/kg DW) (Fig. 4.3-d). The range of the percentages of total Zn found in the F3 fraction was 18-68%. The high abundance of Zn in the F3 fraction agrees with many previous studies carried out in marine sediments (Noah and Oomori 2001; Sutherland et al. 2007; Masoud et al. 2010). Fe-Mn oxide is an important component in many minerals and acts as a cementing and coating agent between particles and zinc. As a result, Fe-Mn oxide acts as a scavenger, especially for Zn. Since Zn was dominant in the F2 fraction at St. 4, 6, and 7, it was probable that this was due to the higher accumulation of Zn in the aquatic organisms inhabiting these sites.

4.3.3 Bioavailability of Metals in the Sediment of Osaka Bay

The potential bioavailability levels of metals depend on the percentage of the F1 (exchangeable) fraction and the F2 (carbonate-bound) fraction relative to the total content. Table 4.2 expresses the potential bioavailabilities of each metal in the sediments of Osaka Bay. Metals existing in the bioavailable fractions are considered to be weakly bonded, and thus are easily dissolved in the aqueous phase. In sediments, a slight pH change can potentially release the metals from these fractions to the aquatic environment (Pardo et al. 1990; Zhang et al. 2014; Chaharlang et al. 2016; Ke et al. 2017). In the present study, bioavailable Cd ranged from 13-59% of the total Cd in sediments of Osaka Bay. Similarly, the ranges of bioavailability for Zn, Cu, and Pb were 12-50%, ND-5%, and ND-16%, respectively. Low levels of bioavailable Cu and Pb reflect similar levels reported by previous investigations on Cu (0-7%) (Lestari et al. 2018) and Pb (1-5%) (Bastami et al. 2014) carried out in marine sediments. In this study, the bioavailable Cd ranged from 13% to 50%. The lowest concentration was observed in St. 9 and the highest in St. 23. The high bioavailability of Cd in marine sediment has also been reported several times by previous investigations carried out in Spain (Morillo et al. 2002, 2004; Vicente-Martorell et al. 2009), and China (Qiao et al. 2013). The range of bioavailability for Zn found in sediments of Osaka Bay was remarkably high compared to that recorded in the Caspian Sea (3-6%) (Bastami et al. 2017).

Table 4.2 Ratios of Fractions of Bioavailable (Bio) and Non-Bioavailable (NB) Metals in the Sediments at Osaka Bay (%) (ND; Not Detected).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Cd	Bio	26.5	22.5	27.4	23.3	17.2	26.7	24.2	22.5	12.9	20.5	20.0	35.5	25.0	37.1	38.3	39.5	18.2	22.7	12.5	15.0	23.9	59.1	59.1	20.0	37.0
	NB	73.5	77.5	72.6	76.7	82.8	73.3	75.8	77.5	87.1	79.5	80.0	64.5	75.0	62.9	61.7	60.5	81.8	77.3	87.5	85.0	76.1	40.9	40.9	80.0	63.0
Cu	Bio	0.21	1.60	0.81	2.21	ND	5.00	0.11	0.21	ND	ND	ND	1.11	0.21	0.31	1.50	1.30	ND	ND	ND	ND	0.40	ND	ND	ND	ND
	NB	99.8	98.4	99.2	97.8	100	95.0	99.9	99.8	100	100	100	98.9	99.8	99.7	98.5	98.7	100	100	100	100	99.6	100	ND	100	ND
Pb	Bio	ND	ND	7.71	ND	0.50	4.50	4.70	ND	ND	ND	ND	ND	5.00	ND	ND	16.4	3.40	ND	1.20	10.0	2.41	ND	ND	ND	ND
	NB	100	100	92.3	100	99.5	95.5	95.3	100	100	100	100	100	95.0	100	100	83.6	96.6	100	98.8	90.0	97.6	ND	ND	100	100
Zn	Bio	33.1	29.0	31.4	40.0	15.7	49.9	46.5	16.3	16.0	14.5	11.5	21.7	14.2	19.4	15.0	25.0	12.7	29.8	12.8	15.3	22.3	16.6	19.2	16.7	26.5
	NB	66.9	71.0	68.6	60.0	84.3	50.1	53.5	83.7	84.0	85.5	88.5	78.3	85.8	80.6	85.0	75.0	87.3	70.2	87.2	84.7	77.7	83.4	80.8	83.2	73.4

4.3.4 Pollution Load Index (PLI)

The PLI values are listed in Figure 4.4, showing values ranging from 0 to 5.1. St. 1, 3, 4, and 5 has values belonging to the "extremely heavy pollution" category. These sites are mainly located around the cities of Izumisano and Sakai, which host several fishing ports, a harbor, shipping industries, and a heavy industrial zone. In particular, St. 1, 4, and 5 could be impacted by the steel industry, reflected by their high PLI values above 3. Notably, St. 3 is close to St. 4, and therefore possibly shares a common source of discharge water. In addition, 44% of study sites (St. 6, 7, 10, 12, 14, 15, 16, 17, 18, 19, and 21) were categorized as "heavily polluted" (2 < PLI < 3). Most of these sites are close to the big ports, shipping industries and the estuarine areas of the Yodo, Yamato, and the Muko rivers. Large amounts of waste water and sewage reach Osaka Bay through these rivers (Hosono et al. 2010), which possibly contributes to the high PLI values. St. 2, 8, 9, 11, 13, and 20 belonged to the "moderately polluted" category (2 > PLI > 1).

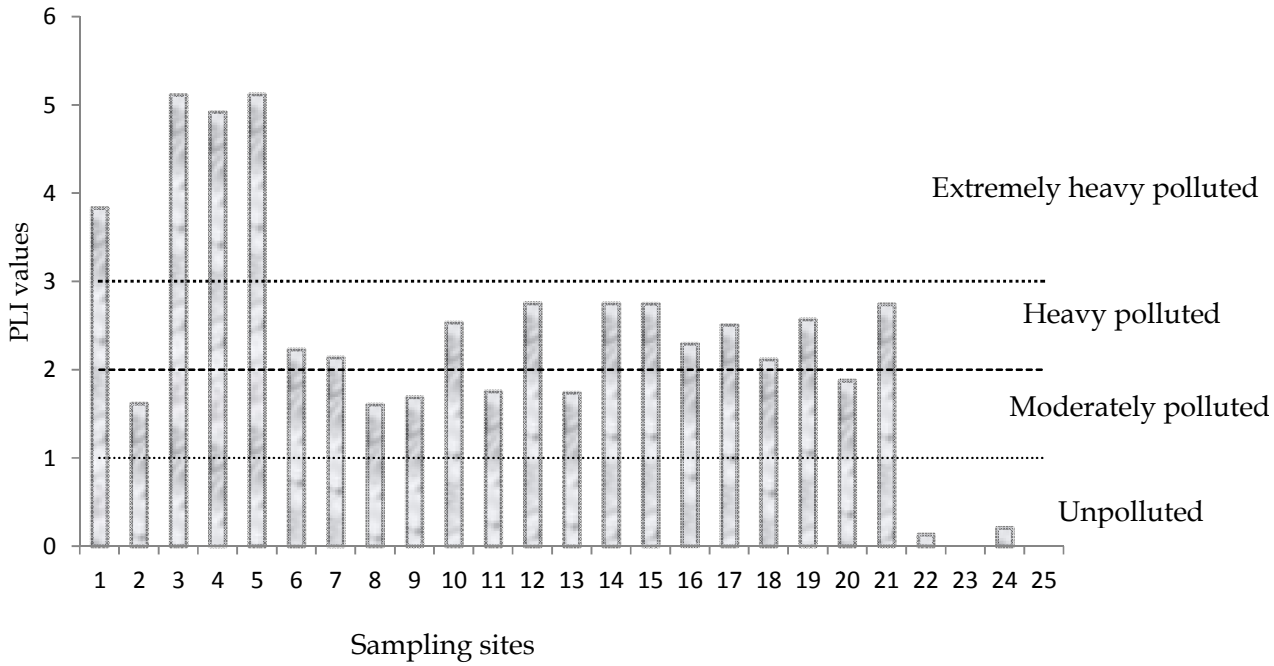

Figure 4.4 Pollution Load Index for Cd, Cu, Pb, and Zn in Sediments of Osaka Bay

4.3.5 Evaluations of Biological Risks

To predict the biological effects of the studied metals adsorbed to sediments of Osaka Bay, their concentrations in all sites were compared to the ERL (10th percentile) and ERM (50th percentile) values described by Long and MacDonald (1998). Generally, adverse biological effects are expected to occur rarely below the ERL, while 10-50% of organisms are possibly affected when the metal concentrations are between the range of ERL to ERM; and most organisms are affected when metal concentrations are above the ERM (Long et al. 1995; Long and MacDonald 1998). The concentrations of Cu were between this metal's ERL and ERM in 80% of the study sites (20 sites; Table 4.3). Pb concentrations were between this metal's ERL and ERM at St. 1, 16, and 21. Remarkably, the levels of Zn at 10 sites (St. 1, 3, 4, 5, 10, 12, 15, 17, 19, and 21) were over the ERM, and thus may have already reached the level of toxicity to aquatic organisms around these sites. Lastly, 44% of the study sites had Cd concentrations between this metal's ERL and ERM.

The bioavailability of metals in sediments depends on geochemical speciation (Morillo et al. 2002; Saeedi et al. 2013); each metal's adsorption characteristics are different among each geochemical fraction. For example, metals contained in the exchangeable and carbonate-bound fractions are weakly adsorbed to sediments, so the metals adsorbed in those fractions are easily released, possibly affecting the aquatic organisms. Therefore, the adsorption characteristics of metals within each geochemical fraction possibly reflect risks associated with these metals. The criteria determining the adsorptive characteristics are known as the Risk Assessment Code (RAC).

Table 4.3

Comparison of Metal Concentrations (mg/kg DW) Measured in this Study with ERL and ERM Guideline Values (ND; not detected).

Metals	ERL[a]	ERM[a]	Metal conc.		Sampling sites	
			Min	Max	Between ERL and ERM	Over ERM
Cd	1.2	9.6	0.2	2.9	From 1 to 8, 10, 17, and 18	--
Cu	34	270	ND	399	1, 2, 3, and from 5 to 21	4
Pb	47	218	ND	73	1,16, and 21	--
Zn	150	410	76	967	2, 6, 7, 8, 9, 11, 13, 16, 18 and 20	1, 3, 4, 5, 10, 12, 15, 17, 19, and 21

The risks associated with RAC values are divided into the following 5 categories: 'no risk' with RAC of < 1%; 'low risk' with RAC from 1% to 10%; 'medium risk' with RAC from 10% to 30%;'high risk' with RAC from 30% to 50% and 'very high risk' with RAC > 50%. The results of the risk assessment at each site are shown in Fig 4.5.

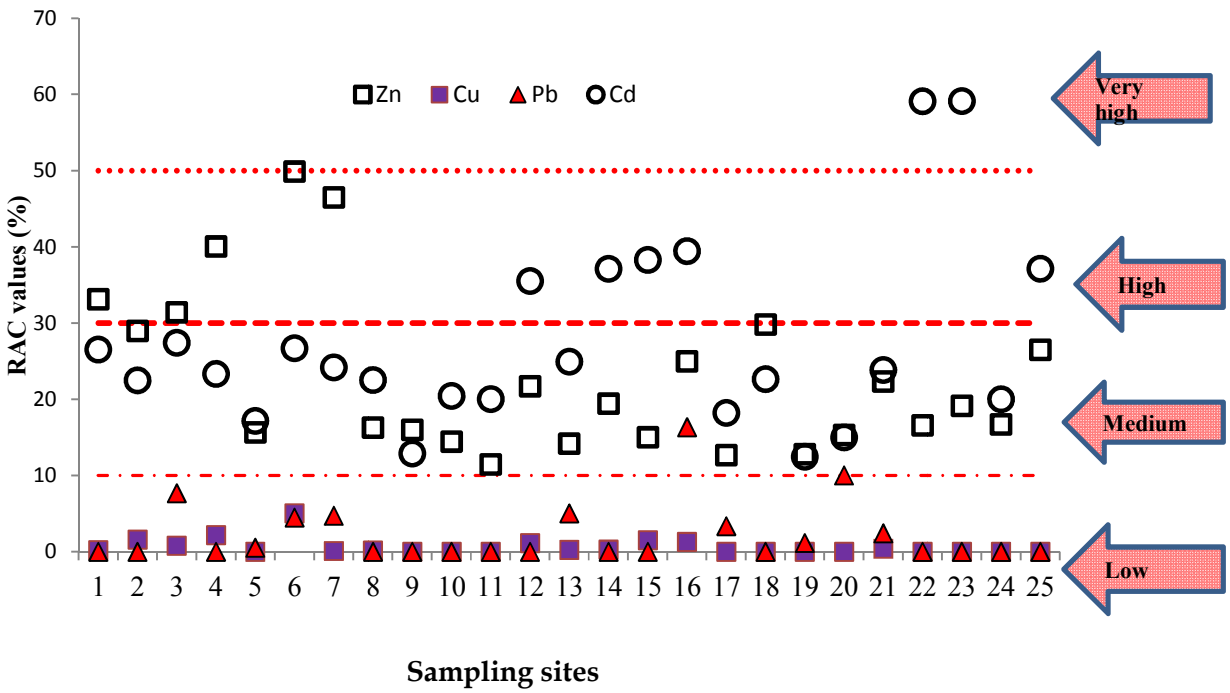

Figure 4.5 Risk assessment codes of Cd, Cu, Pb, and Zn in sediments Collected from Osaka Bay

Cd is associated with medium risk in most of the sampling sites, but is at high risk levels at St. 12, 14, 15, and 16, and very high risk levels at St. 22 and 23. In fact, Cd in sediments, especially those collected from St. 1 to 8, 10, 17, and 18, tended to be slightly high, and may pose some risk to aquatic organisms, as shown in Table 4. 3. On the other hand, St. 12, 14, 15, and 16 are close to densely industrial areas, producing discharges that may affect aquatic organisms. However, St. 22 and 23 on Awaji Island is local site, and the risk assessments based on RAC values should be treated carefully (Figure 4.5). Zn represents a medium risk in all of the sites except for St. 1, 3, 4, 6, and 7, where it is categorized as high risk. Notably, Zn in the F2 fraction was detected at a remarkable high ratio at St. 1, 3, 4, 6, and 7 (Figure 4.3-d), which are sites close to Kishiwada City.

These areas are near to concentrated industries that manufacture and process many kinds of metal products. The high ratio of Zn in the F2 fraction relative to its total concentration is a possible reflection of the impact of these industries. Although these values are not different from the evaluations using ERL and ERM in Table 4. 3, the risks potencies estimated from RAC provide more detail. It should be noted that no or low risks were associated with Cu and Pb using RAC calculations. However, these metals pose certain levels of risk based on ERL and ERM, as shown in Table 2. The factors that caused the inconsistencies between these risk evaluations these should be further explored by conducting actual toxicity tests with aquatic organisms.

4.4 Conclusions

Chapter 4, addresses the first two objectives (see section 1.2) of the thesis. Therefore, chapter 4 is basically highlighting the distribution and geochemical speciation of total metals in the coastal sediment and based on the geochemical speciation data prediction of the bioavailable potentials of metals in sediment are provided.

In spite of the strict environmental rules on the discharge of urban and industrial effluents, relatively high concentrations of Cd, Cu, and Zn were found in most of the sampling sites of Osaka Bay. Based on the pollution load index (PLI), 44% of the sampling sites were categorized as "heavily polluted" with the studied metals. Moreover, the concentrations of Cd, Zn, and Cu exceeded the ERL values considerably in all the sampling sites, except for St. 22, 23, 24 and 25. These results suggest that aquatic organisms have potentially been affected by the metals adsorbed to the sediments.

Geochemical speciation obtained from chemical analysis suggests that Cu and Pb are mainly in the organic-bound (F4) and Fe-Mn oxide-bound (F3) fractions, respectively. On the other hand, Zn and Cd are considerably abundant in the exchangeable (F1), and carbonate-bound (F2) fractions. F1 and F2 are considered the bioavailable fractions, and these contained high levels of Cd (13%-59%) and Zn (12%-50%). As a result, risk assessment based on RAC values indicate that Cd and Zn are at "high risk" levels at several sites. High bioavailabilities of Zn and Cd may have ecotoxicological significance, because bioavailable metals may potentially accumulate from the sediment into aquatic organisms. The approach used in the present study, has been very useful in understanding the patterns and potencies of metal bioavailability in the sediments of any coastal area around the world.

CHAPTER 5

Metal Bioavailability and Bioaccumulation in the Polychaete *Perinereis nuntia*: Relationship between Predictions Using Chemical Analysis and Accumulation through Actual Exposures

5.1 Introduction

Heavy metals are among the most common pollutants in coastal areas. They can enter the environment through a variety of natural processes, such as weathering of rocks and volcanic activities. Additionally, a certain amount of metals is also discharged into the field from human activities, including industrial discharge (e.g., discharge from the steel, chemical, and food industries) (Barjhoux et al. 2012; Kalman et al. 2015; Liu et al. 2019). In aquatic environments, the metals settle on the sediments with time, and persist for long periods. Therefore, sediments are the ultimate sink for metals. In facts, metals can be often detected with remarkably high concentrations (Kalman et al. 2012).

Whole metals in sediments cannot be completely absorbed by organisms, and their accumulations are dependent on their bioavailability. As a result, the total concentration does not provide accurate information on bioaccumulation and toxicities (Delshab et al. 2017; Väänänen et al. 2019). Each metal in sediment is mainly partitioned into the following geochemical fractions: exchangeable fraction (F1), carbonate-bound fraction (F2), Fe-Mn oxide-bound fraction (F3), organic material-bound fraction (F4), and residue fraction (F5) (Tessier et al. 1979). Although, metals in the exchangeable fraction are the most bioavailable, those in residual fractions are the least bioavailable. Therefore, metals in the exchangeable fraction may affect their uptake and assimilation by benthic organisms (Fan et al. 2014). As discussed in the previous chapter (Chapter 4), metals were analyzed in each fraction of sediments collected from the coastal areas in Osaka Bay, Japan, and showed the bioavaialability and distribution patterns of cadmium (Cd),

cupper (Cu), lead (Pb) and zinc (Zn). However, our approach yielded limited information owing to a lack of the description of the validation of the correspondences between chemical estimation and contact assays using real field sediments (Gaw 2009).

Polychaetes are benthic organisms and are known to be among the most common inhabitants in sediments of intertidal coastal areas. They have been used in the monitoring of pollutants such as metals and persistent organic compounds in coastal sediments (Martı´n-Dı´az et al. 2007). In the present study, *Perinereis nuntia,* a common polychaete in Japanese coastal areas was placed in the field sediments collected from the coastal areas, and was exposed to metals contained in the sediments. We aimed to evaluate the correspondences and differences between actual and estimated bioavailability for metals.

5.2 Materials and Methods

5.2.1 Study Area and Sediment Sampling

Kagoshima Bay is a typical semi-enclosed bay in the southern Japan. Elevated Hg concentrations have been reported in the northern part of Kagoshima Bay due to volcanic activities (Sakamoto 1985; Haraguchi et al. 2000). Surface sediment used for this experiment was collected from the two sites (St. 1 and St. 2) on Kagoshima Bay (Fig 5.1). St. 1 is near to the fish market and is characterized with anchoring place for numerous fishing vessels and boats and St. 2 is very close to the Sakurajima ferry terminal and anchoring place of numerous fishing boats. The sediment was collected by Ekman-Berge bottom sampler during September 2018. The sediment was then passed through a sieve to eliminate larger debris and shell. The sediment was then kept in the 4°C in the dark until further treatment.

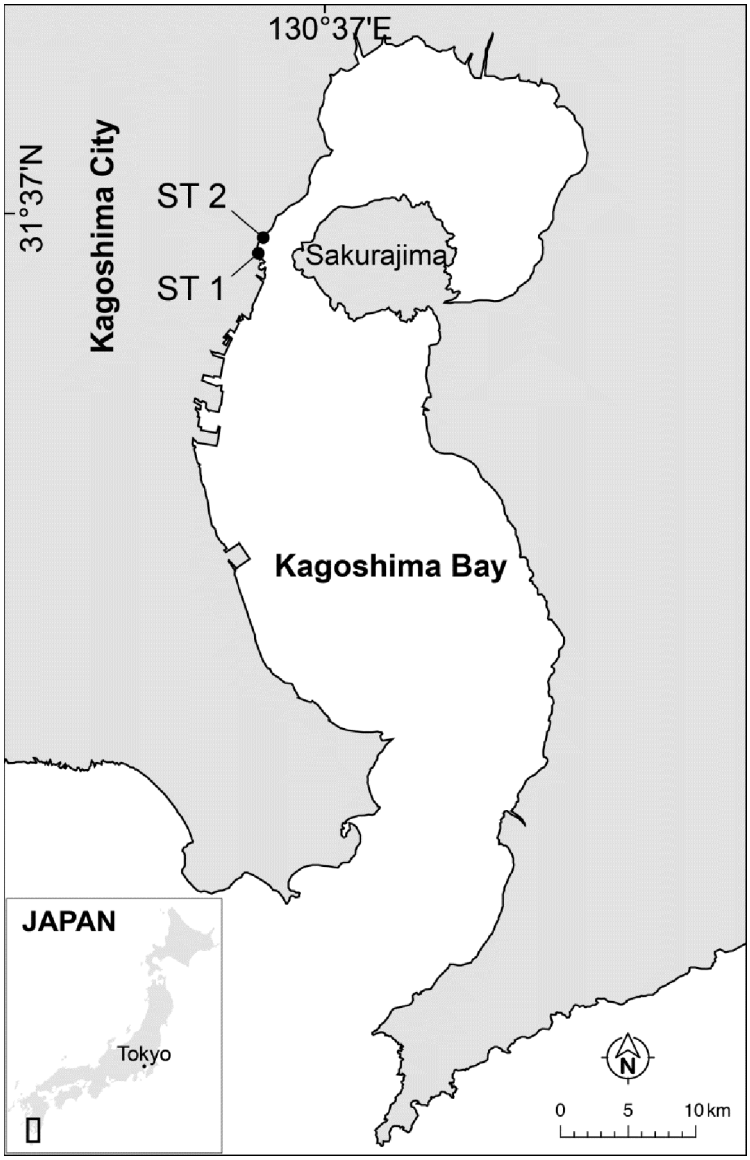

Figure 5.1 Map of the Study Area, Showing the Sampling Sites (ST 1 and ST 2) in Kagoshima Bay, Japan

5.2.2 Test Organisms and Acclimatization

In the present investigation, polychaete worms were collected from the commercial aquaculture facilities of Kochi prefecture, Japan (Figure 5.2). The animals were then acclimatized for 5 weeks in the laboratory conditions until use in the exposure experiment. Throughout the acclimatization and experiment sea water was sourced from the Kagoshima Bay, through natural sand filtration systems.

Figure 5.2 Polychaete *P. nuntia* Used in the Present Accumulation Tests

During the acclimatization, glass balls (1.5 cm diameter) were taken in the polyporpilene vessels as the substrate (~6 cm height) for the polychaete. Two such vessels were then put and submerged in a large vessel (74 × 60 × 30 cm). The water quality parameters (DO; temperature and pH) were checked regularly. Water (about 30%) was changed twice in a day. Any dead individuals were removed immediately after they were found. Organisms were fed twice a day with commercial fish food (Tetra Min, Tetra) at approximately 2% of their body weight.

5.2.3 Exposure Experiment

The exposure experiment set up mainly consisted with of three large plastic containers, of which one was relatively large (Figure 5.3a) having an area of 74 × 60 × 30 cm and the remaining two boxes were of same size (Figure 5.3b) with an area of 60 × 35 × 30 cm. The large plastic box was considered as the main tank to keep sea water, from where the water was pumped to a water cooler. The cooled water was then finally showered on two plastic boxes; where multiple columnar plastic vessels (Figure 5.3c) were submerged in sea water around 2 cm over these vessels. The volume of each columnar vessel was 400 mL (**7** cm diameter and 10 cm height). Each columnar vessel was loaded with sediments of around 5 cm depth in a vessel (180 mL, about 400 g of wet sediments). Besides, the overlying waters were sufficiently aerated to maintain a high DO level (> 6.5 mg/L) throughout the experimental period.

The overlaying water was maintained at 18 ± 2°C under 14h light and 10h dark conditions. On the seventh day of setting the experimental systems, 20 individual polychaetes (average wet weight 13 gm) were loaded to each plastic box. This was conducted after confirming the achievements of very low concentrations of metals in test water. Polychaetes were observed daily to check their mortality, and the dead individuals on the sediment surface were removed from the vessel. Rearing water (approximately 30% of the total amount each time) was changed twice in a day. During the exposure period, DO, pH, and temperature were 6.8 ± 0.5 mg/L, 8.2 ± 1.0 and 18 ± 2°C, respectively. The polychaetes and sediments in the respective plastic vessel for both sites (St 1 and St 2) were sampled at days 3, 7, 14, 21, and 28.

After sampling, polychaetes were rinsed gently with seawater to remove the particles on their body and then continuously rinsed with the Milli-Q water.

The polychaetes were then anesthetized by keeping them at 1 to 4°C for about 10 min and were then dissected to remove the gut (Casado-Martinez et al. 2013). The dissected worms were then freeze-dried (72 h). Sediments were dried in the laboratory at around 25°C.

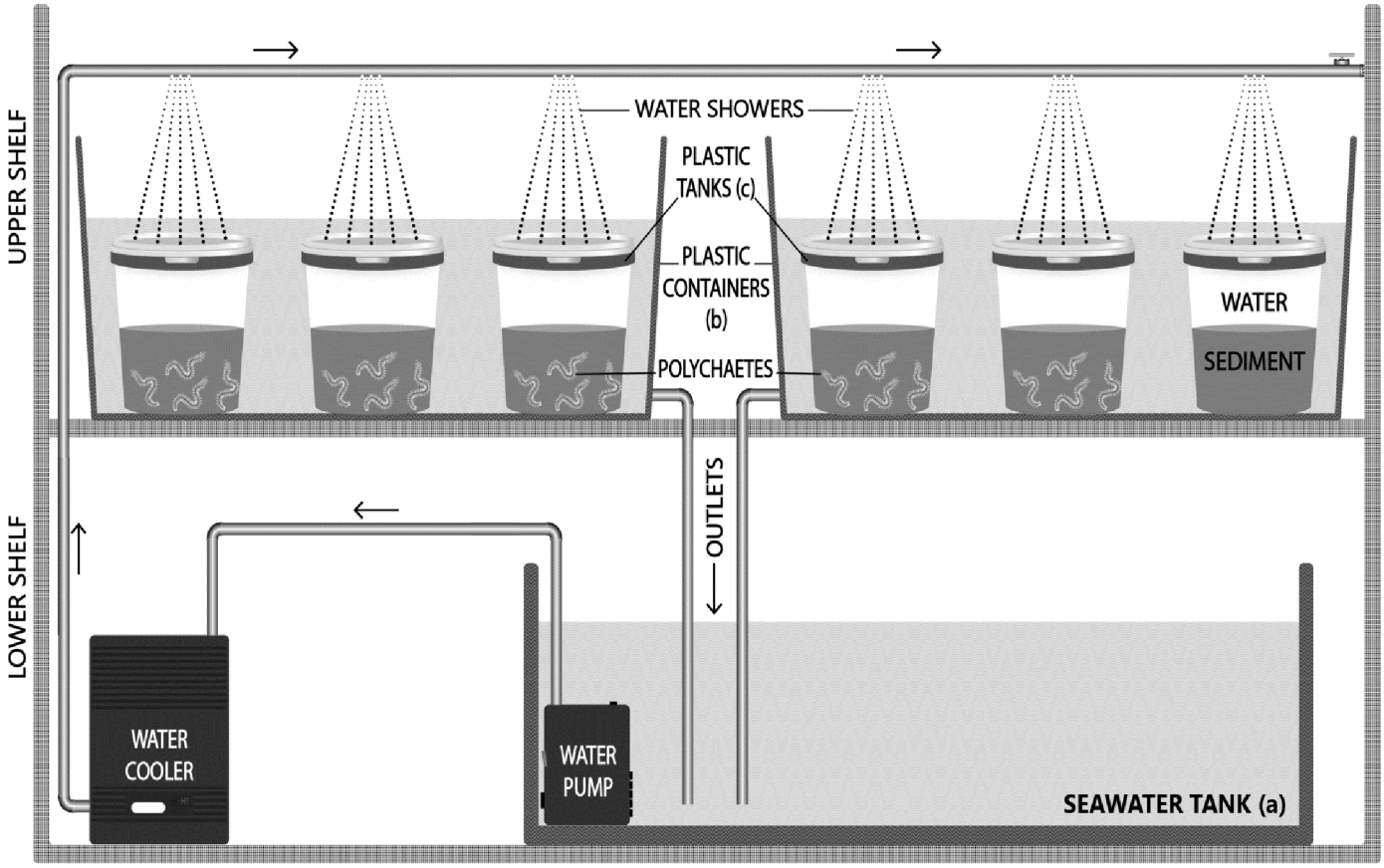

Figure 5.3 Illustration of Exposure Systems for Polychaete Kept in Real Sediments. Different Alphabets (a, b and c) Indicate Different Plastic Boxes.

5.2.4 Analysis of Metals in Polychaete

The freeze-dried polychaete tissues with 0.5–0.8 g dry weight (8–10 individuals of polychaete) were added to 10 mL of concentrated nitric acid. The samples were digested in a hot plate at 100°C for 3 h (Casado-Martinez et al. 2013). After evaporation to near dryness, 10 mL of 2% nitric acid was added to the samples. The digest was then transferred to a polypropylene tube and was adjusted to 25 mL with Milli-Q water. The concentrations of metals such as Cd, Cu, Pb, and Zn were then determined in the solution and were expressed in mg/kg wet weight (mg/kg WW).

Measurements of metals were carried out using an atomic absorption spectrophotometer (AAS; Hitachi Z-2300, Hitachi). The absorption wavelengths were 228.8 nm for Cd, 324.8 nm for Cu, 283.3 nm for Pb, and 213.9 nm for Zn, and the detection limits were as follows: 0.02 mg/kg for Cd; 0.007 mg/kg for Cu; 0.05 mg/kg for Pb; and 0.03 mg/kg for Zn. Blank and standard solutions of the respective metals were frequently used to check the reliability of the results. In this study, the metal recoveries ranged between 75% and 105%.

5.2.5 Analysis of the Total Content of Metal in the Sediment and Metals in Pore Water

Analyses of total concentration of metals are shown in section 3.2. In order to analsyse the metal content in the pore water, the sieved sediment (see section 5.2.1) was centrifuged for 10 min (90 *g*, 4 °C) in order to collect the pore water. 20-mL pore water samples were then acidified by adding few drops of nitric acid. The concentrations of Cd, Cu, Pb, and Zn in the solution were determined using AAS with instrumental conditions similar to those described in the Section 5.2.4.

5.2.6 Analysis of Metals in Five Geochemical Fractions

Analyses of metals in the five geochemical fractions were showed in 3.3.

5.2.7. Analysis of total organic carbon (TOC)

The analysis of total organic carbon (TOC) in the sediment was measured with CHN analyser (JM10, J-Science Lab Co. Ltd.). The observed concentrations (n=3) of TOC are shown by the percentage in dry samples.

5.2.8 Statistical Analysis

In the present investigations, bio magnification factors (BMFs) were calculated based on the following formula:

BMF= (maximum metal concentration found during the exposure period) / (concentration of metal in sediment).

Biota sediment accumulation factor (BSAFs) is a parameter describing bioaccumulation of metals associated with sediments into tissues of organisms based on the following formula (Zhao et al. 2012; Sarmiento et al. 2016):

$$BSAFs = (C_m - C_i)/C_s.$$

Where C_m is the maximum concentration during the exposure period, C_i, the initial concentration in polychaete tissues, and C_s is concentration of metal in sediment.

5.3 RESULTS AND DISCUSSION

5.3.1 Sediment Characteristics

TOC content is the most important factor determining the binding of metals in sediments (Pini et al. 2015). TOC was found to be similar at St 1 (1.5 ± 0.7% (mean ± SD)) and St 2 (1 ± 0.8%). These values are slightly lower than that determined in sediments from other coastal areas (>4%, Essien et al. 2009; Uno et al. 2017). Low levels of TOC in the sites would possibly indicate the limited residues of organic materials both from anthropogenic and/or natural sources (Cho et al. 1999). It can be also being presumed that the sources of metals may not be mostly from anthropogenic sources, but may be related to the natural/geological origin.

5.3.2 Metals in Test Sediments

Total Cu in sediments collected at St. 2 (2400 mg/kg DW) was 16-fold higher than that at St. 1 (150 mg/kg DW) (Table 5.1). The concentration of Cu in the present study ranged between 150 mg/kg DW at St. 1 and 2400 mg/kg DW at St. 2. It is noteworthy to mention that, the study sites are affected by volcanic ashes deposition from the active volcano, Sakurajima. Therefore, high Cu concentrations might be related to these volcanic ash depositions.

Compared to St. 1, markedly higher Cu concentration was found in the sediments of St. 2. This is because, St. 2 is located nearer to Sakurajima ferry port, where a number of ferries operate between Sakurajima and Kagoshima ports throughout the day thereby possibly experiencing high amount of ferry discharges as was pointed out by Noah and Oomori (2001) who found high Cu concentration in sediment (950 mg/kg) around Tomari ferry port, Okinawa, Japan. Furthermore, St. 2 has lower water exchange than St. 1 because St. 2 is surrounded by many coastal dikes, thus limiting the water exchange.

The higher concentration of Cu found in the sediments of St. 2 could therefore be related to the ferry discharges and limited water exchange. However, we could not specifically point out the driving factor behind the extremely high Cu concentration in St. 2, thereby this interesting phenomenon deserves more attention in the future studies. Additionally, concentrations of Zn (300–350 mg/kg DW) and Pb (30–40 mg/kg DW) were similar in the sites, though Cd was not detected in any.

Metals bound in F1 and F2 were loosely adsorbed to the surface of particles in sediments and, therefore, highly available for absorbing by the organisms. From the chemical analysis, the bioavailability for Zn was expected to be higher (16–21% of the total content) than that of Cu (2–4% of the total content) (Table 5.1). It was expected that Zn should be much more accumulated in the polychaetes than Cu. Concentration of Pb, however, was found to be below the detection limit in the exchangeable and carbonate bound fractions (Table 5.1). Thus, it can be predicted that Pb was not available to the polychaetes.

Concentrations of metals in the pore water are included in the risk evaluations of metals (Pini et al. 2015). Cu and Zn content in the pore water were 5 ± 0.04 mg/L and 2 ± 0.02 mg/L, respectively, at St 2, while not detected (ND) and 0.31 ± 0 mg/L, respectively, at St. 1. Pb and Cd were not detected in both the sites. Concentrations of Cu and Zn were roughly similar with those found in the UK estuaries (0.7–2 mg/L for Cu and 0.5–3 mg/L for Zn) (Pini et al. 2015).

Table 5.1 Metals (Cu, Pb and Zn) in Different Geochemical Fractions (F1 to F5) and Total Concentrations Detected in Sediments from St. 1 and St. 2 on Kagoshima Bay (Values Are mean ± SD; *n*=2; and ND= Not Detected).

	Cu		Pb		Zn	
	St. 1	St. 2	St. 1	St. 2	St. 1	St. 2
Exchangable (F1)*	1±0.4**	3±1	ND***	ND	4±3	5±4
Carbonate bound (F2) *	6±4	43±15	ND	ND	45±18	68±20
Fe-Mn oxide fractions (F3)*	8±3	10±1	11±1	16±8	155±6	163±27
Organic bound fraction (F4)*	126±20	2269±427	15±10	6±5	63±38	62±8
Residual fraction (F5)*	12±2	61±6	9±1	9±5	41±31	54±40
Total *	153±23	2387±387	37±13	34±15	307±51	352±56
Bioavailable content (% of total)	4±1	2±2	ND	ND	16±10	21±10

*Concentrations are shown by mg/kg DW;

**Values are mean, where n=2;

***ND: not detected

5.3.3 Bioaccumulation of Metals in Polychaete

Prior to bioaccumulation study (day 0), the concentrations of metals in polychaete were measured, and those of Cu and Zn were 1.6 ± 0.7 and 6.4 ± 0.5 mg kg^{-1} WW, respectively. Cd and Pb were not detected. The concentration of Cu in St. 1 was 3.2 fold (on day 14) of that on day 0, while that in St. 2 (day 7) was 2.4 fold (Figure 5.4). Unfortunately, much mortality of polychaete were observed after day 14, and the unexpected variations of Cu in polychaete might have been caused by the unhealthy conditions.

During the period of exposure in test sediments, the concentrations of Zn in polychaetes at St. 1 and 2 increased until day 14 since the beginning (Figure 5.4). Unhealthy conditions, as described above, might have caused the unexpected variations of Zn in polychaete after day 14. For St. 2, the polychaete were not found to be alive on

day 28. Therefore, as per the present data, both Cu and Zn were reported to be absent on the sampling day (Fig. 5.4). Besides, during the exposures, no Cd and Pb accumulation was detected in the tissues.

In this study, site with low sediment Cu bioavailability (St. 1, 7 mg kg^{-1} DW) showed higher tendency of Cu bioaccumulation (Fig. 5.4), compared with site of high Cu bioavailability (St. 2, 46 mg kg^{-1} DW). Low Cu bioaccumulation tendency in the St. 2 was observed; even though this site had higher bioavailable concentrations of Cu concentration. The reason for these differences are not known to us but may be related to differences in age, size, health and physiological condition of the animals (Bryan and Hummerstone 1971).

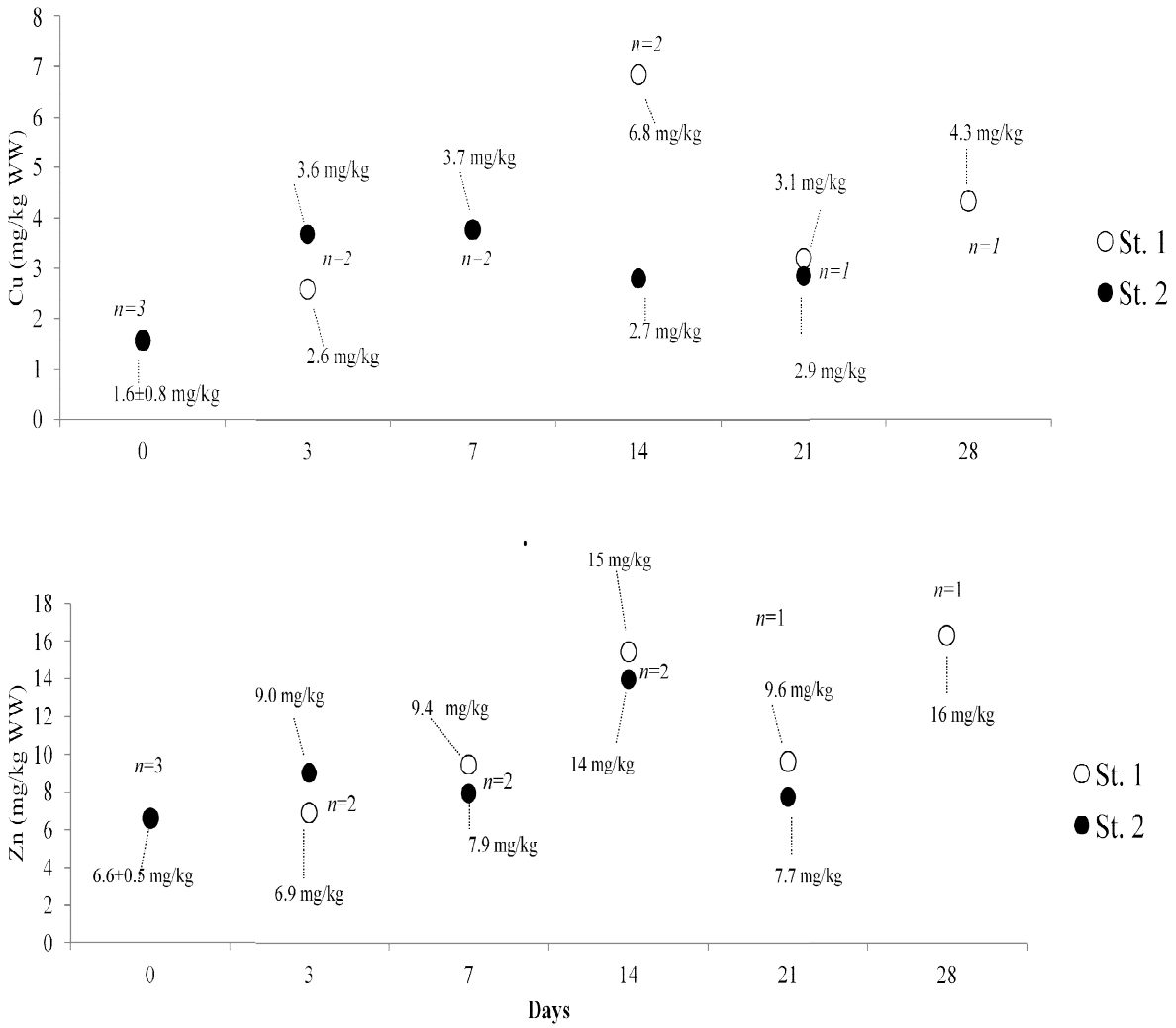

Figure 5.4 Metal Concentrations in *P. nuntia* during Bioassay on Different Sampling Days for Both Stations

5.3.4 BMFs and BSAFs

In this study, BMF values varied between 0.062-0.066 and 0.002-0.052 for Zn and Cu, respectively. Similarly, BSAFs were 0.03-0.04 and 0.002-0.04 for Zn and Cu, respectively (Table 5.2). Biomagnification is defined as the process of the transfer of chemical substances from lower trophic organisms to higher ones. If the ratio of chemical concentration between the prey and the predator is greater than one, the chemical is biomagnified in the predator. In the present investigations, BMF values were lower than 1, suggesting that Cu and Zn were not magnified. However, those metals were transferred from sediments to polychaetes. Low values of the BMF and BSAF, especially for Cu at St 2 is due to relatively high concentrations of Cu in the sediment.

Bioaccumulation efficiency of metals from sediments was evaluated by BSAFs considering the accumulated metal concentrations in the polychaete tissues. The present BSAFs for Cu and Zn were lower than those reported in the field conditions; for example, 0.54-1.29 for Cu and 0.94-2.9 for Zn in the polychaetes collected at the Indian coasts (Alam et al. 2010), and 1.20-2.30 for Cu and 0.75-2.57 for Zn in the polychaetes collected at the estuaries of central Chile (Gaete et al. 2017). In the present study, lower BSAFs are possibly due to high concentrations of Cu and Zn in sediments than those reported in the previous studies. Several researches have confirmed that BSAFs are higher usually at lower concentration of metals in sediments (McGeer et al. 2003; Sun and Zhou 2007).

Table 5.2 The Biomagnification Factors (BMF) and Biota Sediment Accumulation Factors (BSAFs) of Zn and Cu in the Sediment of Two Sites on the Kagoshima Bay.

Metals	Sites	BMF	$BSAF_S$
Zn	St. 1	0.066	0.04
	St. 2	0.062	0.03
Cu	St. 1	0.052	0.04
	St. 2	0.002	0.002

5.3.5 Relationship between Metal Bioavailability and Bioaccumulation in Polychaete

Our major objective was to explore the critical relationship between the bioavailability of metals estimated by chemical analysis and actual accumulation by *P. nuntia*. Determination of metals in different geochemical fractions enable us the prediction of bioavailability, although some limitations have been proposed (Nirel and Morel 1990). The relationship between metal in geochemical speciation and bioaccumulation in the benthic organisms have been explored from the benthic animals, including mussels (Yap et al. 2002), and sipuncula (Tan et al. 2013). Besides, there are investigations showing the relationship between bioavailability and bioaccumulation for several species of polychaetes (Zhou et al. 2003; Baumann and Niohdas 2011; Fan et al. 2014; Pini et al. 2015). However, there are scarcities of information to compare our results regarding the presumed bioavailability from sediment and actual bioaccumulation of Cu and Zn in *P. nuntia*.

It is known to us that metals artificially spiked into sediments do not truly reflect their characteristics during the laboratory accumulation test; their accumulated concentrations are much higher compared to field sediment. Additionally, we already know that the metals artificially spiked into sediments were not similar for their existent forms under the real condition; their accumulated concentrations are much higher compared to field sediment. Therefore, the present study was performed with the field sediments to determine the actual absorption from sediments into the polychaetes.

It is known that metals bound in the exchangeable fractions and carbonate fractions can be loosely adsorbed and thus become readily available in the pore water. In the present chemical analysis, it was estimated that the bioavailability for Cu and Zn was 2–4% and 16–21%, respectively (Table 5.1). Taking into account the low bioavailability for Cu, it was expected that bioaccumulation for Cu in the polychaetes was lower than that for Zn. The accumulation test with the field sediments confirmed this, showing that Cu in polychaetes were relatively in lower concentrations than Zn. These results suggest that bioavailability estimated with chemical analysis can roughly predict actual bioaccumulation of metals in benthic organisms.

Pb in most bioavailable fractions, such as F1 and F2, was not detected, though certain concentration of total Pb was detected to be 32–34 mg/kg DW. Most importantly, Pb was not detected in the polychaete tissues. These results suggest that Pb bioavailability estimated with chemical analysis can roughly predict actual bioaccumulation of metals in *P. nuntia*.

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5.4 CONCLUSION

Heavy metals determined in individual geochemical fractions offer us to know the possibility of bioavailability in the benthic organisms. In the present study, *P. nuntia* was exposed to the contaminated sediment collected from Kagoshima Bay, and Zn and Cu were absorbed by *P. nurita*. Their BMF resulted lower than 1, suggesting that Cu and Zn were not magnified but transferred from sediment into polychaetes. At last, we have compared the results of chemical analysis for the estimation of bioavailability and actual accumulation with the exposure test and found that chemical analysis can predict the actual bioavailability.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

Due to their persistence and ecological risk heavy metals are of environmental concern. Sediments are known to act as important sinks for their contaminants in coastal areas. Geochemical speciation of heavy metals in sediment adjacent to rapid developing areas can be useful to investigate anthropogenic impacts, and may assist in the assessment of risks caused by heavy metals. In comparison with measurements of whole metal concentrations, the speciation of metals in sediments provide more information concerning their bioavailabilities and toxicities, as these characteristics are often dependent on the geo-chemical form. In this regard, in the present study five geochemical fractions (exchangeable (F1), carbonate-bound (F2), Fe-Mn oxide-bound (F3), organic matter-bound (F4), and residual fractions (F5)), were used for speciation of metals in sediments. Usually, among the five geo-chemical fractions, exchangeable and carbonate bound fractions can be easily be absorbed in to the biota.

Relatively high levels of metals (Cd, Cu, Pb and Zn) were found in coastal sediment collected from Osaka Bay, Japan. These high concentrations may be the result of high levels of metals discharged from various kinds of human activities. Geochemical speciation data suggest that, considerable amounts of Cd was detected in both the F1 (ND-0.4 mg/kg DW) and F2 (0.03-0.4 mg/kg DW) fractions. Their rrations of the percentage of whole Cd found in the F1 and F2 fractions were 4-40 and 6-22%, respectively. Cu was dominant mainly in the F4 fraction (ND-348 mg/kg DW). Cu detected in F4 to whole Cu was 55-100%. Pb dominated mainly in the F3 fraction (3-41 mg/kg DW). The range of percentages of total Pb in F3 was 28-93%. Pb can form stable complexes with the Fe-Mn oxide fraction, resulting in the high abundance of Pb in F3.

High concentrations of Zn were found in the F3 fraction (24-477 mg/kg DW), and Zn in the F3 was 18-68% of whole Zn.

In order to establish the relationship between metal bioaccumulation from contaminated coastal sediment with their estimated bioavailability from chemical analysis polychaete, *P. nuntia* was exposed to the sediments from two sampling sites on the Kagoshima Bay for 28 days. In the sediment of the Kagoshima Bay, considerable amount of Zn was found in the most bioavailable fractions ((F1 (4-5 mg/kg DW) and F2 (45-68 mg/kg DW). Similarly, Cu was also found considerable in the F1 fractions (exchangeable fractions, 1-3 mg/kg DW) and F2 fraction (carbonate bound fractions; 6-43 mg/kg DW). The exposure bioassay experiment revealed that, the highest accumulated Zn concentrations varied between 14-16 mg/kg WW and the highest accumulated concentration for Cu varied between 4-7 mg/kg WW. Chemical analysis estimated that, the bioavailability for Cu and Zn was 2–4% and 16–21%, respectively (Table 5.1). Taking into account the low bioavailability for Cu, it was expected that bioaccumulation for Cu in the polychaetes was lower than that for Zn. The accumulation test with the field sediments also confirmed that Cu in polychaetes was relatively in lower concentrations (2-7 mg/kg WW) than Zn (6-17 mg/kg WW) (Figure 5.4). These results suggest that bioavailability estimated with chemical analysis can roughly predict actual bioaccumulation of metals in benthic organisms.

Based on the findings in the present research, future laboratory experiments can be carried out using the different coastal benthic species in order to compare their bioavailability of sedimentary metals in sediments among species. Additionally, future studies can be carried out based on the trophic transfer of metal in the marine food chain using polychaete and polychaete feeding predatory animals.

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