# Metal Speciation, Mixtures and Environmental Health Impacts

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# METAL SPECIATION, MIXTURES AND ENVIRONMENTAL HEALTH IMPACTS

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**Metal Speciation, Mixtures and Environmental Health Impacts**

**Abstract**

Numerous applications of heavy metal have caused to their wide contamination in the environmental system and raised serious concerns over potential harmful effects on public health and the environment. Water, sediment, and dietary food are the main exposure media of heavy metal pollution and key determinants of adverse human and environmental health effects. Heavy metal(s) toxicity and speciation involve various mechanistic features with specific media and some of them are not clearly investigated. In particular, biological effects such as toxicity are not related to the total concentration of heavy metals in media, and many laboratory and field studies have supported this supposition. Organisms respond to the bioaccessible and bioavailable fraction of metals only, not the total concentration. The bioaccessibility and bioavailability of toxicants are dependent on chemical properties of the contaminant, the many exposure pathways, and temporal variability of these variables with respect to uptake by the target organism. Usually, bioavailable fractions are estimated using chemical or biological approaches. For this study, biological approaches were performed to better ascertain the toxic effects of heavy metals on organisms. A better understanding of bioaccessibility and bioavailability can be a useful tool in exposure and risk assessment. Therefore, this study presents experimental designs focusing on

assessing of the bioaccessibility and bioavailability of metals in aquatic, benthic organisms and dietary food. This study also examines the role of metal mixtures on the adverse effects of metals.

# TABLE OF CONTENTS

## CHAPTER 1: INTRODUCTION

## CHAPTER 2: COMBINED EFFECTS OF PH, DISSOLVED ORGANIC CARBON, AND HARDNESS ON ACUTE METAL TOXICITY TO DAPHNIA MAGNA

## CHAPTER 3:   EFFECT OF MIXTURES ON THE ACUTE TOXICITY OF METALS IN ESTUARINE SEDIMENTS

## CHAPTER 4:  BIOACCESSIBLE ARSENIC IN THE DIET OF BANGLADESH

**CHAPTER 5: CONCLUSIONS**

# LIST OF FIGURES

**CHAPTER 4: BIOACCESSIBLE ARSENIC IN THE DIET OF BANGLADESH**

# LIST OF TABLES

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# CHAPTER 1: INTRODUCTION

**Introduction**


Heavy metals pollution has been an important concern for human health and the environment (Ruhling and Tyler, 1969). Although the harmful health effects of metals have been studied, exposure to toxic metals increases continuously. In August 2015, an old acid mine blew out during cleaning, releasing a tremendous amount of toxic sludge into Colorado's water streams. Increased amounts of heavy metals will subsequently be stirred up with sediments and during the wet season. The United States Environmental Protection Agency (US EPA) announced that such substances contain As and Pb, which initially posed a threat to human health via water and sediment. Additionally, in Colorado, there are approximately 200 abandoned mines, which are leaking heavy metals such as Cd, Pb, Zn and those are significantly contaminating water and sediments.


Arsenic is another historical and major toxicant and it is been an issue for water and soil contaminations, especially in Bangladesh. Moreover, rice plants uptake As from As contaminated soil and may lead potential harmful effects to human. Many studies have been performed to understand how As can be ingested and be toxic in the human body. Public health agencies and researchers have studied exposure to inorganic As via drinking water for a long time, but recently, more studies have focused on inorganic As exposure via food intake (Kile et al. 2007A). Particularly, in 2013, inorganic As in rice and rice products were announced to contain high levels of inorganic As. However, there has been no federal limit for the amount of

As in foods. The amount of As in US public drinking water is limited to 10 parts per billion (ppb) only by the US EPA.

As a result, in 2013, the Food and Drug Administration (FDA) set the limit for the allowable amount of As in juice, but not for rice and rice products and is currently collecting data and analyzing more samples. However, until now, few human data are available and further studies are necessary to understand the pathway of As exposure through food intake.

For this, numerous ecotoxicological studies have demonstrated that exposure to heavy metal is associated with adverse effects to humans and the environment. Ecotoxicological studies have investigated the association between the total concentrations of heavy metal and toxic effects. However, exposure and risk assessments are particularly difficult because there is large variability in the concentrations of heavy metals depending on physicochemical and biological parameters for both humans and the environmental systems.

Thus, studies of bioavailability and bioaccessibility have been applied to research for heavy metal pollution (Reeder and Schoonen. 2006). Based on a deep understanding of environmental toxicology, we can estimate an exact risk of harmful metals. Even though organisms are exposed to heavy metals, it may not necessarily be toxic. For example, bivalve populations from San Francisco Bay had impaired reproduction from toxic metals with an elevation of bioaccumulated metal (Rainbow and Luoma, 2010). Depending on the different species of organisms, different metals, and environments, the relationship between harmful effects and biological impact is quite complicated. But to understand the toxicology, it is helpful more than using the concept of total

metal concentration. Interactions between metals and environmental factors are extremely complicated but important to explain the bioavailability of concerned metals to organisms, since the toxicity of metals by bioavailability can be decreased or increased depending on the environmental factors such as metal uptake specificity, uptake route, and environmental influences (change of organic matters concentration by resuspension).

For several decades, a number of studies conducted in many countries have suggested the relationships between total concentration and bioavailability of heavy metals. These defined relationships enable us to estimate more practical effects of toxic metals with appropriate control of pollutants. This may be useful to avoid overestimate or underestimate of harmful effects from toxicants. Previous studies have mostly described total metal concentrations and single metal toxicity. Several studies investigated bioavailable or bioaccessible concentrations of target metals (He et al. 2012; Laparra et al. 2005). Simpson et al. (2012) investigated the bioavailability of metals in sediment using benthic organisms. For As study, Kile et al. (2007A and 2007B), showed in a comprehensive research paper that various analysis of adverse effects of inorganic As to human by intake of food have been proposed.

Still, there are several limitations of research about bioavailable and bioaccessible metal concentrations. First, all organisms have different sensitivies with different parameters. It is quite difficult to collect enough ecotoxicological data for variable conditions of environment or media. Second, although inorganic As concentration is a better estimator to predict carcinogenic effects, bioaccessibility or bioavailability of As may provide for more information about As

toxicity in human body than that of total inorganic As concentration. These two limitations have heretofore limited the ability of As to be applied for ecotoxicological studies. Therefore, this research is to address the limitations and improve the comprehension of metal toxicity, leading to more accurate exposure assessments. We investigate the adverse effect of target heavy metals on environmental media (fresh water, seawater, and sediment) and human with various changes of exposure conditions.

**Thesis objectives**

The specific aim 1 of this research (Chapter 2) is to predict the change of Cu and Cr toxicity with variable aquatic parameters using freshwater test species. In this study, we hypothesize that there are interactive effects of target metal toxicity on physicochemical properties. Thus, we assume that Cu(II) and Cr(VI) toxicities depending on changes of pH, dissolved organic carbon (DOC) and hardness may be changed. For this, we utilize the five-level central composite design (CCD) to test toxicities more practically using test species. This study is unique in that we can predict the interactions between target metals with various levels of parameters, using a minimum number of toxicity tests. Since a bioassay test is significantly labor intensive and less cost effective to perform as a number of combinations, our statistical study design can be simple, using a minimized number of tests to estimate more variables. Hence, this approach is expected to improve the prediction of metal toxicity with variables. However, seawater has different conditions related with metal activity and this is a limitation for its application using a freshwater study.

5

For an estuarine environmental study, the specific aim 2 is to study heavy metal toxicity in the sediment and seawater. Most contaminants introduced to the ocean and estuarine are adsorbed by and transported with suspended sediments. For this reason, many researchers have started to emphasize sediments. Accumulation of pollutants in important species poses a risk to human consumers of seafood. Direct contact with contaminated sediments may also pose a risk to human health.

In general, the risks of heavy metal contamination to organisms in water are well studied, but potential harmful effects of heavy metals to sediment-dwelling organisms are less studied. In addition, sediment toxicity has focused on the most bioavailable forms of metal as determined by binding phases in the previous studies. Recently, metals in interstitial water are concerned with the most bioavailable form, and solid phase metals are also bioavailable to a certain degree, particularly to sediment-dwelling organisms with multiple exposure pathways. A variety of geochemical factors can affect the species of metals in sediment and therefore affect biological uptake. Therefore, the crucial component of this study is an investigation of the biological influence of metal mixtures in sediment depending on metal species with the concept of simultaneous extractable metals and acid volatile sulfide using marine organisms. This is of paramount importance for assessing metal toxicity in sediment due to an understanding of different environmental systems.

The specific aim 3 (Chapter 4) is to examine the difference between total and bioaccessible As concentrations for human exposure via food intake in Bangladesh. Adverse effects of heavy metal to the human body also work differently depending on individual variability and metal

species. This study investigates not only environmental media like water and sediment, but also humans. To the best of our knowledge, most studies about bioaccessible As focus on crop species. Therefore, this study is expected to enhance our understanding of harmful effects of heavy metal and the effectiveness of associated heavy metal control strategies.

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# CHAPTER 2: COMBINED EFFECTS OF PH, DISSOLVED ORGANIC CARBON AND HARDNESS ON ACUTE METAL TOXICITY TO DAPHNIA MAGNA

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**Abstract**

Effects of pH, dissolved organic carbon (DOC) and hardness on toxicities of copper and chromium to Daphnia magna were investigated using a three-factor and five-level central composite design (CCD). There were no interactive effects on Cu(II) and Cr(VI) toxicities between water quality parameters. For Cu(II) toxicity, both pH and DOC showed significant effects, and DOC was the most important parameter, representing 89.3% of the variation in 48-h $EC_{50}$ values. In contrast with Cu(II) toxicity, hardness was the most significant parameter to Cr(VI) toxicity, explaining 78.8% of the variation in 48-h $EC_{50}$ values. In addition, pH explained 17.3% of the variation in Cr(VI) toxicity. On the basis of toxicity data, empirical models were developed to predict Cu(II) or Cr(VI) toxicity under different physicochemical conditions studied in this work using a backward regression analysis.

# 1. Introduction

Copper and chromium are often found in wastewaters from metal plating industries (Hunsom et al. 2005; Venkateswaran and Palanivelu, 2005; Baral et al. 2006). To account for their risks to humans and the environment, many researchers have evaluated the biological toxicity of copper and chromium in both synthetic and industrial wastewaters (Villaescusa et al. 1997; Dirilgen and Dogan, 2002; Long et al. 2004). Although it is well known that metal toxicity is affected by water quality parameters, such as pH, dissolved organic carbon (DOC) and hardness, studies on their combined effects are limited (De Schamphelaere and Janssen, 2004). In particular, the interactive effects of those parameters on anionic metals such as Cr(VI) have not been reported in the literature.

Therefore, the aims of the present study are: (1) to investigate the extent to which the changes in pH, DOC and hardness affect Cu(II) and Cr(VI) toxicities to Daphnia magna, and (2) to establish a regression model that is able to predict Cu(II) and Cr(VI) toxicities as a function of pH, hardness and DOC concentration. In order to efficiently obtain information on the combined effects of these parameters, a three-factor and five-level central composite design (CCD) was employed in this study. The CCD can provide the optimized experimental designs which can evaluate the direct relationship between variables with small numbers of combinations (Lock and Janssen, 2002; De Schamphelaere and Janssen, 2004).

## 2. Materials and Methods

### 2.1. Preparation of test solutions

Test solutions for acute toxicity on D. magna were prepared with 64.8 mg/L $NaHCO_3$ and 5.75 mg/L KCl, and appropriate amounts of $CaCl_2$ and $MgSO_4$ (Ca:Mg=1:1) to obtain the required hardness (Hunsom et al. 2005). Subsequently, the DOC concentration was adjusted by adding humic acid (Fluka, Germany) stock solution (100 mg/L). 3-N-Morpholino-propanesulfonic acid was used as a pH buffer, and the desired pH was adjusted with 0.1 N HCl or 0.1 N NaOH. Concentrated Cu(II) and Cr(VI) solutions made with $CuCl_2 \cdot 2H_2O$ (J.T. Baker, The Netherlands) and $K_2Cr_2O_7$ (Merck, Germany), respectively, were then added to the test solutions. Before and after each toxicity test, pH was measured using an Orion 720A pH meter (Thermo Electron Inc., USA). In addition, hardness was determined by an EDTA titrimetric method according to the Standard Methods for the Examination of Water and Wastewater (1998), and DOC was analyzed by TOC-5000 analyzer (Shimadzu, Japan).

### 2.2. Experimental design

Individual effects of pH, DOC and hardness on the toxicities of Cu(II) and Cr(VI) to D. magna, respectively, were first evaluated with ranges of water quality parameters as follows (APHA, 1998). The pHs were 6, 7 and 8, DOC concentration were 0, 10, 20, 40 and 80 mg/L, and hardness were 0, 100, 200, 300, 400 and 500 mg/L as $CaCO_3$. A CCD is the most widely used response surface methodology (RSM) and remarkably effective in cutting down the number of

13

mixture combinations for predicting the effects of variables. Using a CCD, combined effects of pH, DOC and hardness were then investigated using a three-factor and five-level CCD as given in Table 2-1. The linear, quadratic and interactive effects of water quality parameters on metal toxicities were analyzed with a Design-Expert 7.0 program (Stat-Ease Inc., USA). To develop a mathematical prediction model, a backward regression analysis ($p < 0.05$) was also applied to the toxicity data of Cu(II) and Cr(VI). Factors explaining less than 5% of 48-$EC_{50}$ variability were excluded from the prediction model, although they showed statistically significant effects.

## 2.3. Toxicity tests

Acute toxicity tests were performed using D. magna according to the Organization for Economic Co-operation and Development (OECD) guideline 202 (OECD, 2004). The test animals were obtained from the Korea Institute of Toxicology (Daejeon, Korea). All experiments were conducted using polyethylene vessels to decrease effects of adsorption of heavy metals. Toxicity tests were performed using five daphnids in each test vessel with 20 mL test solution with four replicates, and conducted at $20 \pm 2$ °C with a 16:8 h light:dark photoperiod for 48 h. Immobilization data were used to calculate the effective concentration ($EC_{50}$) by probit analysis (USEPA, 2002).

## 3. Results and Discussion

## 3.1. Individual effects of pH, DOC and hardness

Effects of pH on Cu(II) and Cr(VI) toxicities are illustrated in Figure 2-1. The toxicity of Cu(II) slightly decreased with an increase of pH. This agrees well with the works of other researchers (Kim et al. 1999; Long et al. 2004; Markich et al. 2005). As pH decreases, the toxicity of Cu(II) increases due to increase of toxic free copper ions ($Cu^{2+}$) (Long et al. 2004). For Cr(VI), toxicity to D. magna was increased at both pH 6 and 8 compared with that of pH 7. It is known that the toxicity and bioavailability of Cr(VI) largely depends on chemical species of Cr(VI) (Villaescusa et al. 1997; Dirilgen and Dogan, 2002). Villaescusa et al. (1997) reported that the toxicity of Cr(VI) to luminescent bacteria decreased with increasing pH from 4.6 to 7.5, and then increased above pH 7.5. The reduction of Cr(VI) toxicity was due to decreasing concentration of major toxic species, $HCrO_4^-$.

Figure 2-2 shows dependence of Cu(II) and Cr(VI) toxicities on DOC concentration. The toxicity of Cu(II) decreased drastically with increasing DOC concentration. This reduction of Cu(II) toxicity is caused by the increase of complex formation between Cu(II) and DOC (Kim et al. 1999). In general, copper associated with organic matter is considered to be non-bioavailable or non-toxic to aquatic organisms (Long et al. 2004). Contrary to Cu(II) toxicity, Cr(VI) toxicity was not influenced by DOC concentration. Kaczynski and Kieber (1994) reported that organic matter complexation with chromium was insignificant, and Kislenko and Oliynyk (2004) also showed that chromium was hardly bound with humic acid at pH values higher than 4.

The effect of hardness on Cu(II) and Cr(VI) toxicity is shown in Figure 2-3. $EC_{50}$ value of Cu(II) was slightly increased (from 0.006 to 0.028 mg/L) with increasing hardness. This is in agreement with the work of Long et al. (2004), which showed that low hardness affected Cu(II) toxicity to

15

D. magna. Similarly, Erickson et al. (1996) reported that increase in hardness significantly reduced Cu(II) toxicity to Pimephales promelas. Generally, hardness cations, such as $Ca^{2+}$ and $Mg^{2+}$, decrease Cu(II) toxicity through competition (Welsh et al. 1993). Decrease in Cr(VI) toxicity upon increase in hardness was more significant than that for Cu(II) toxicity. In the report published by USEPA (1985), it is demonstrated that an increase in water hardness decreases the toxicity of Cr(VI) to D. magna. Persoone et al. (1989) also reported that 24-h $EC_{50}$ of potassium dichromate to D. magna increased from 0.08 to 2.17 mg/L with an increase of hardness from 80 to 560 mg/L as $CaCO_3$. These results are consistent with our findings in this work.

### 3.2. Combined effects of pH, DOC and hardness

Copper and chromium $EC_{50}$ values for different combinations of pH, DOC and hardness (Table 2-1) were statistically analyzed to calculate percentage of experimental variability explained by each water quality parameter (Table 2-2). The linear effect of DOC is the most significant to Cu(II) toxicity, explaining 76.3% of the observed variability of 48-h $EC_{50}$ values. This result is in agreement with the work of De Schamphelaere and Janssen (2004), showing that DOC is the most important parameter in determining chronic Cu(II) toxicity to D. magna. However, there were neither linear nor quadratic effects of hardness, which may seem to contradict the result of individual effects of hardness (Figure 2-3(a)). De Schamphelaere and Janssen (2004) also concluded that hardness was not a significant factor in the combined effects of pH, DOC and hardness on chronic Cu(II) toxicity to D. magna. Linear effect of pH was insignificant ($p > 0.05$) and quadratic effect explained only 2.2% of 48-h $EC_{50}$ variation. These results were a little different from the work of De Schamphelaere and Janssen (2004), showing that the effect of pH

provided an explanation for about 14% of the variation in 21-d NOEC and 21-d EC$_{50}$. This discrepancy might be caused by differences in DOC concentrations used in the experiments. It is thought that a wide range of DOC concentrations (0 - 80 mg/L) used in this work lessens the effect of pH on Cu(II) toxicity. On the basis of above results, only DOC concentration was included as a factor in regression analysis of Cu(II) toxicity data, and the following equation was obtained:

Equation (1):

Cu 48-h EC$_{50}$ = 0.0286 + 0.00835*(DOC) – 0.0000608*(DOC$^2$)

$$(r^2 = 0.904, p < 0.0001)$$

with EC$_{50}$ in copper at mg/L and DOC in mg/L. The graphical representation of Equation 1 is given in Figure 2-4.

Similar to the result of Cu(II) toxicity, there were no interactive effects of pH, DOC and hardness on Cr(VI) toxicity (Table 2-2). However, Cr(VI) toxicity was analyzed to be significantly affected by linear and quadratic effects of all water quality parameters studied. Unlike the Cu(II) toxicity results, hardness was the most important parameter in determining acute Cr(VI) toxicity to D. magna. Thus, the linear and quadratic effects of hardness explained 64.1% and 14.7% of the variability in 48-h EC$_{50}$ values, respectively. In addition, pH had a significant effect on Cr(VI) toxicity, explaining 17.3% of the variability in 48-h EC$_{50}$ values. Though DOC concentration showed a statistically significant effect, it only explained less than 5% of EC$_{50}$ variability. These results are well consistent with that for individual effects of pH, DOC and

17

hardness on Cr(VI) toxicity. Therefore, DOC concentration was not included as a factor in regression analysis of Cr(VI) toxicity data, and the following equation was obtained:

Equation (2):

Cr 48-h $EC_{50}$

$$= -9.61 + 2.65*(pH) + 0.00299*(Hardness) - 0.182*(pH^2) - 0.00000360*(Hardness^2)$$

$$(r^2 = 0.924, \ p < 0.0001)$$

with $EC_{50}$ in chromium at mg/L and hardness in mg/L as $CaCO_3$. The surface response plot of the effect of pH and hardness on Cr(VI) toxicity is given in Figure 2-4(b).

## 4. Conclusions

In this study, effects of water quality parameters, such as pH, DOC and hardness, on Cu(II) and Cr(VI) acute toxicity to D. magna were investigated. DOC and hardness were the most important parameters for Cu(II) and Cr(VI) toxicity, respectively, and interactive effects between pH, DOC and hardness were not shown. The empirical models developed in this study are able to predict D. magna acute toxicity of natural waters and wastewaters containing Cu(II) or Cr(VI) as toxicants. However, a model should be developed to predict mixture toxicity under different physicochemical conditions because toxicants in the aquatic environment are encountered as mixtures.

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**Figure 2-1.** Effects of pH on (a) Cu(II) and (b) Cr(VI) toxicity to D. magna. with 95% confidence interval.

(a)
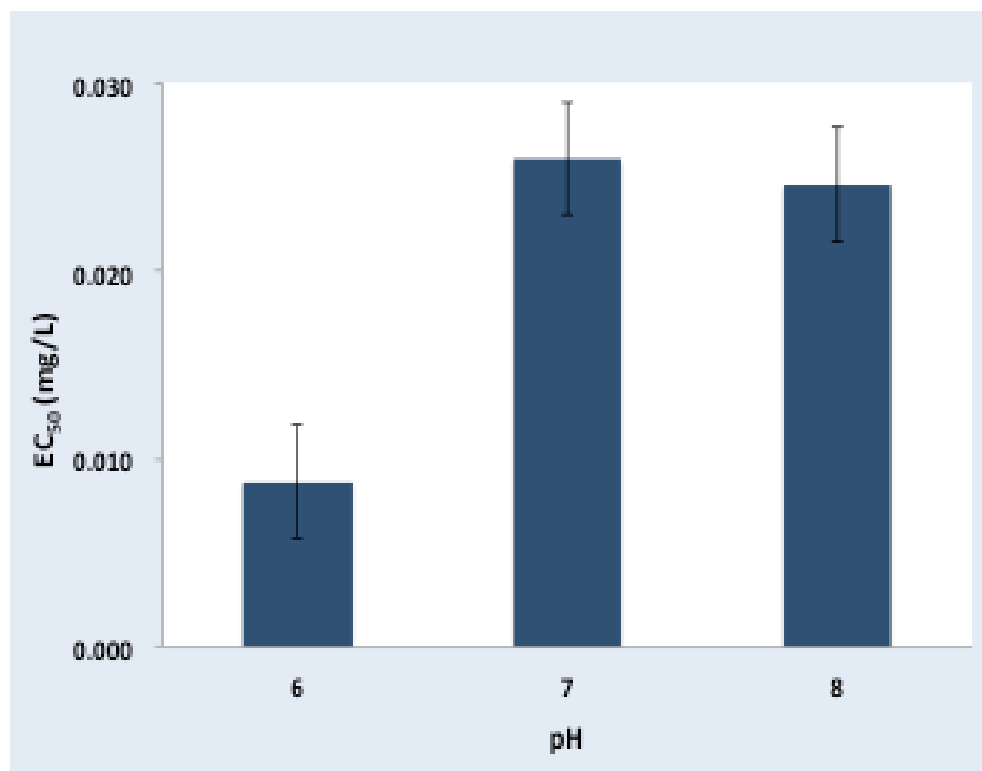


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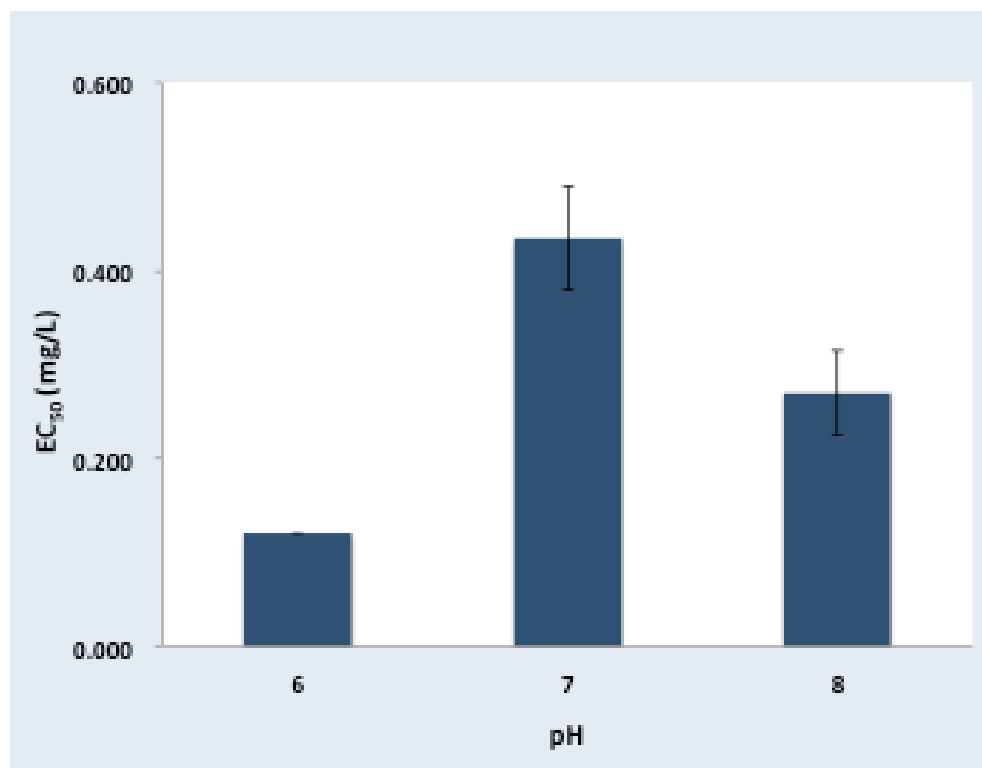
**Figure 2-2.** Effects of DOC on (a) Cu(II) and (b) Cr(VI) toxicity to D. magna. with 95% confidence interval.

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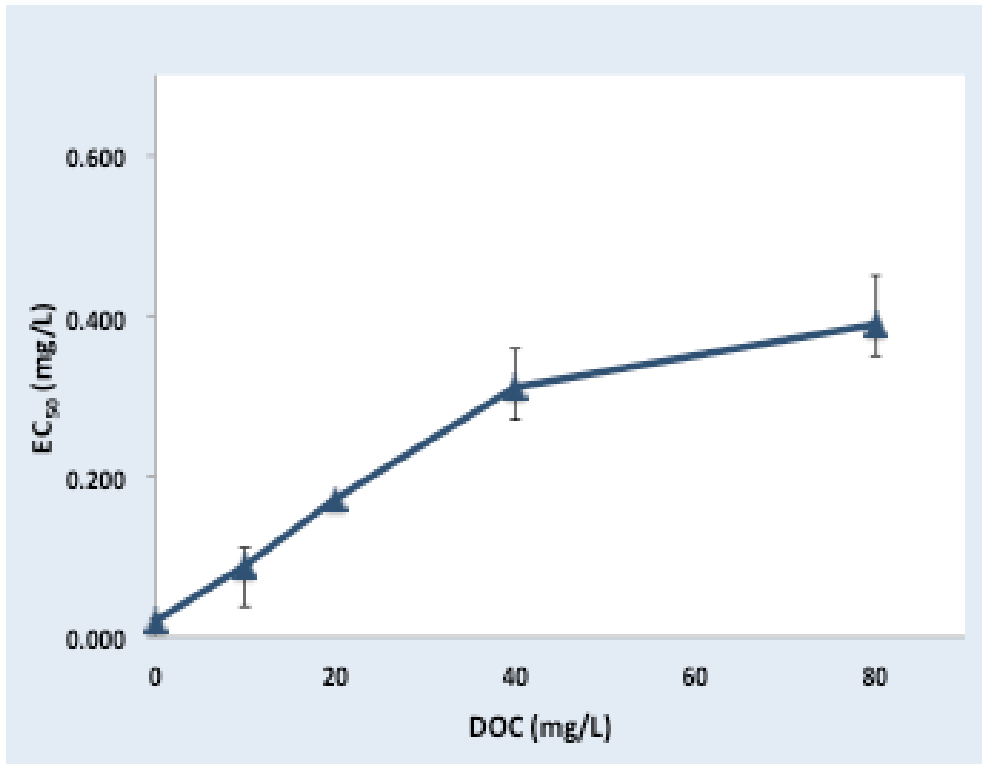


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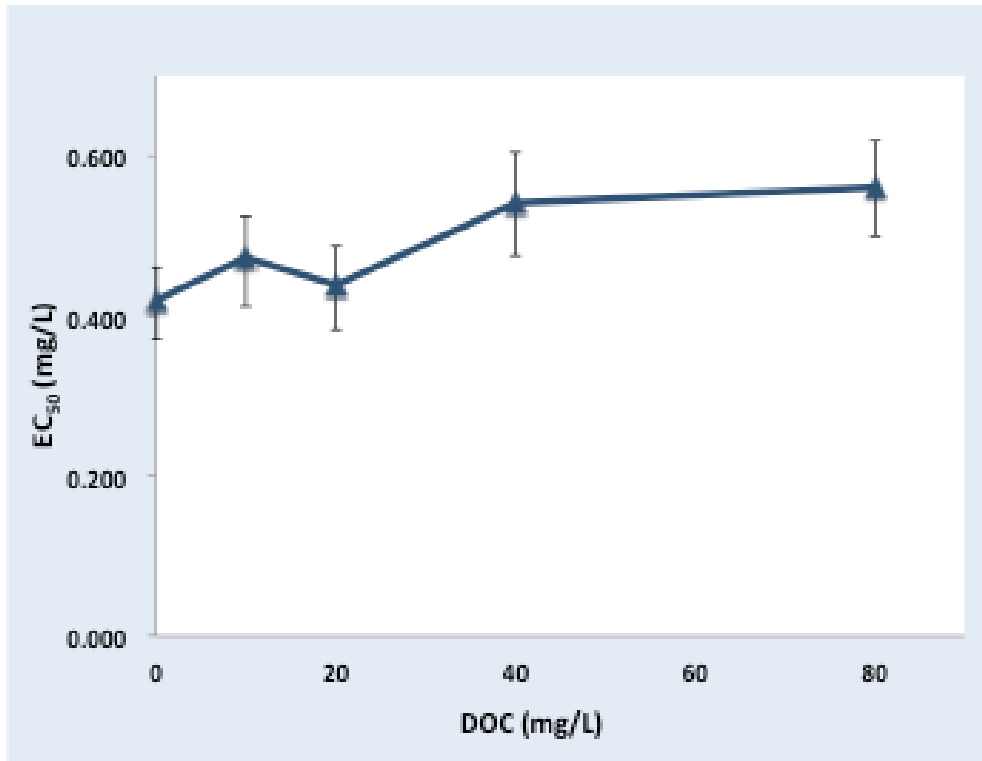
**Figure 2-3.** Effects of hardness on (a) Cu(II) and (b) Cr(VI) toxicity to D. magna. with 95% confidence interval.

(a)
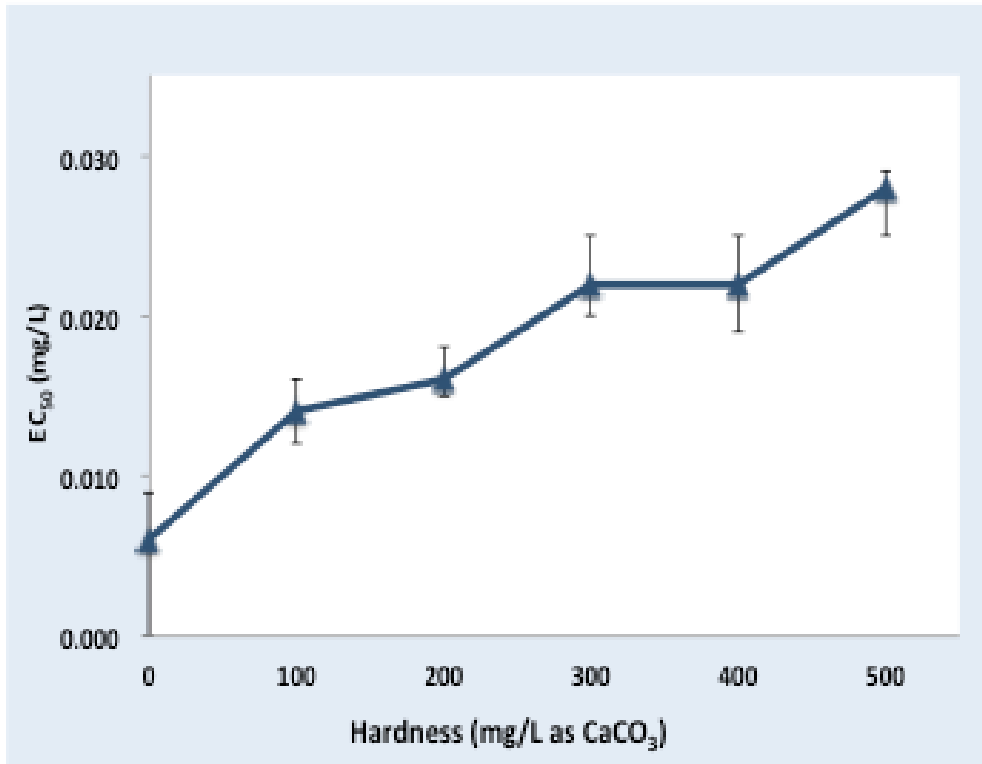


(b)
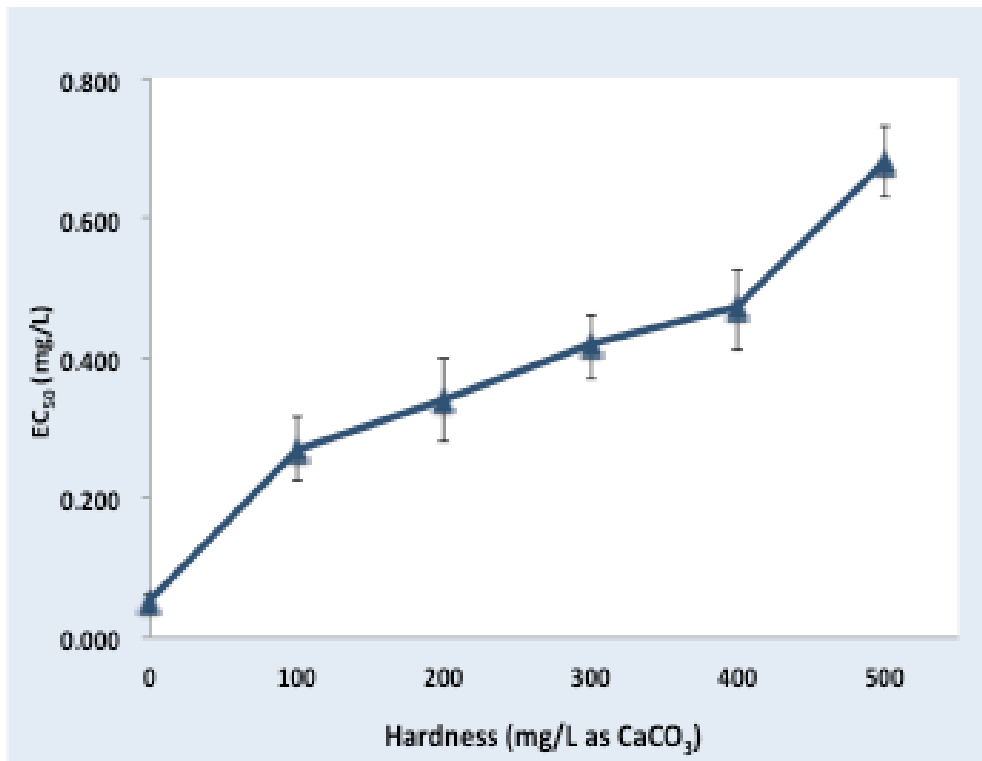
**Figure 2-4.** Graphical representation of the effect of water quality parameters on (a) Cu(II) and (b) Cr(VI) toxicity to D. magna. The plot is generated according to Equation 1 and 2 (see text).

(a)
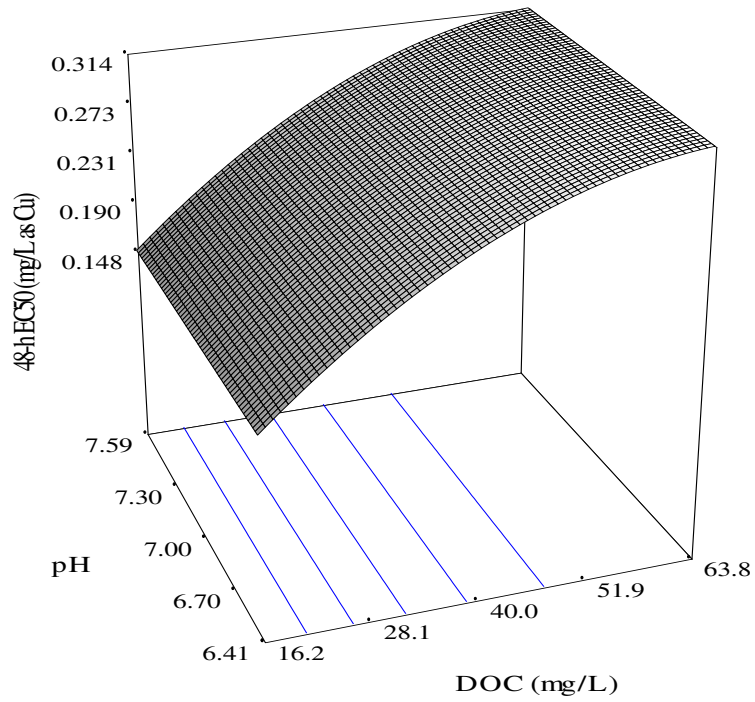


(b)
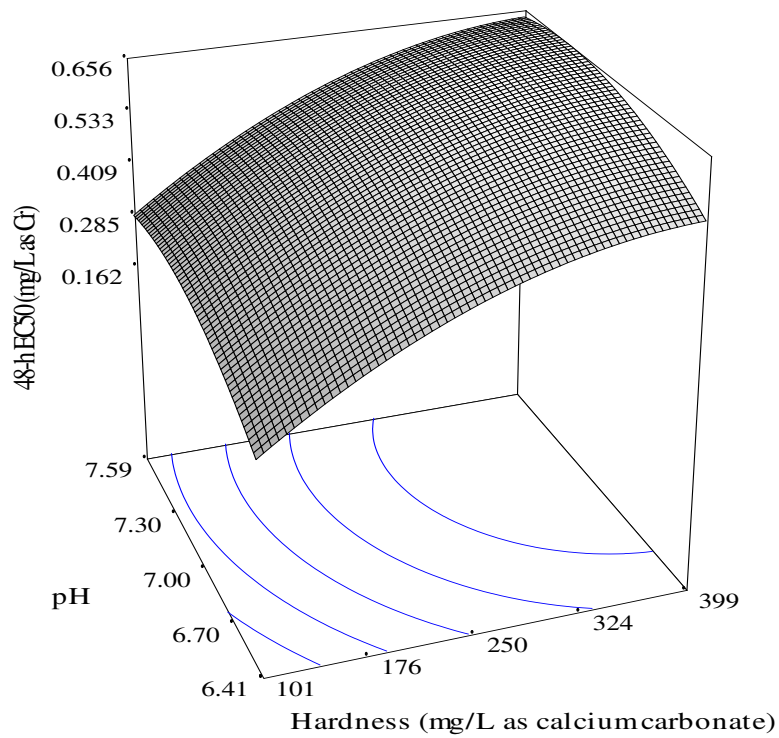
**Table 2-1.** Physicochemical property of D. magna test solutions and corresponding $EC_{50}$ values

| Test solution | pH | DOC (mg/L) | Hardness (mg/L as $CaCO_3$) | Cu(II) 48-h $EC_{50}$ (mg/L) | Cr(VI) 48-h $EC_{50}$ (mg/L) |
|---|---|---|---|---|---|
| 1 | 6.41 | 16.2 | 101 | 0.130 | 0.160 |
| 2 | 7.60 | 16.2 | 101 | 0.150 | 0.250 |
| 3 | 6.41 | 63.8 | 101 | 0.280 | 0.160 |
| 4 | 7.60 | 63.8 | 101 | 0.280 | 0.250 |
| 5 | 6.41 | 16.2 | 399 | 0.160 | 0.410 |
| 6 | 7.60 | 16.2 | 399 | 0.160 | 0.630 |
| 7 | 6.41 | 63.8 | 399 | 0.310 | 0.490 |
| 8 | 7.60 | 63.8 | 399 | 0.320 | 0.650 |
| 9 | 6.00 | 40.0 | 250 | 0.290 | 0.316 |
| 10 | 8.00 | 40.0 | 250 | 0.332 | 0.480 |
| 11 | 7.00 | 0.00 | 250 | 0.017 | 0.400 |
| 12 | 7.00 | 80.0 | 250 | 0.340 | 0.590 |
| 13 | 7.00 | 40.0 | 0.00 | 0.270 | 0.040 |
| 14 | 7.00 | 40.0 | 500 | 0.300 | 0.670 |
| 15 | 7.00 | 40.0 | 250 | 0.250 | 0.590 |
| 16 | 7.00 | 40.0 | 250 | 0.250 | 0.550 |
| 17 | 7.00 | 40.0 | 250 | 0.250 | 0.590 |
| 18 | 7.00 | 40.0 | 250 | 0.250 | 0.550 |
| 19 | 7.00 | 40.0 | 250 | 0.250 | 0.550 |
| 20 | 7.00 | 40.0 | 250 | 0.250 | 0.590 |

**Table 2-2.** Percent of experimental variance on 48-h $EC_{50}$ values explained by linear and quadratic effects of pH, DOC and hardness

|     | pH    | DOC  | Hardness | $pH^2$ | $DOC^2$ | $Hardness^2$ | Total |
|-----|-------|------|----------|--------|---------|--------------|-------|
| Cu  | -[a]  | 76.3 | -        | 2.2    | 13.0    | -            | 91.5  |
| Cr  | 7.5   | 1.9  | 64.1     | 9.8    | 2.4     | 14.7         | 96.7  |

a. Not significant ($p > 0.05$)

# CHAPTER 3:  EFFECT OF MIXTURES ON THE ACUTE TOXICITY OF METALS IN ESTUARINE SEDIMENTS

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**Abstract**

Many studies using sediment from freshwater and seawater have shown that sediment characteristics such as the presence of sulfides, organic carbon, and/or solid particles can modify the bioavailability and thus the toxicity of metals in sediments. The acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) approach for predicting the toxicity of metal mixtures in sediments arises from this concept. Metals in sediment do often occur as mixtures, and each metal will have a different thermodynamic affinity for those different binding phases. The exact nature of the nominal 'excess SEM' in sediments is therefore a function of the specific mixture of metals and sorbing phases that are present. To test the impact of metal mixtures in sedimenton toxicity, bioavailability and acute toxicity were done on 10-day tests using the estuarine amphipod Leptocheirus plumulosus. Single metal toxicity tests were conducted with the metals Cd, Cu, and Zn. Cd, Cu, and Zn and single toxicities to L. plumulosus were consistent with previous studies. However, with certain metal mixtures, metal bioavailability and toxicity differed from what would be expected from the summed toxicity of the single metals. The results suggest that competition amongst spiked metals for binding to sediment phases can protect or enhance toxicity to benthic organisms. This demonstrates that an understanding of the fundamental chemical reactions that govern the speciation, bioavailability and subsequent toxicity in sediments may require a refined geochemical understanding of the underlying speciation.

## 1. Introduction

In aquatic environments, toxic heavy metals discharged from industrial or sewage effluents may be rapidly removed from the water column and transported to bottom sediment. After complicated cycles of deposition, resuspension, and biological and chemical interactions, pollutants on particles may be eventually buried in bottom sediments, which become the ultimate contaminant sink. Many metals are preferentially sorbed to particles due to their high particle reactivity and hydrophobicity. Metals accumulate in the sediment reservoir as a result of physical interactions with the solid phase, such as the interaction of metals with mineral oxides, sulfides, carbonates, and clays, and adsorption of hydrophobic non-ionic organic compounds to organic matter (Shea, 1988). The complexation of contaminants with particle surfaces or organic matrices influences their bioavailability, and thus their potential toxicity. As a result, metal concentrations in sediment are often higher by magnitude orders than those in ambient water. There has been increasing awareness that sediment may play an important role as the source of metal bioaccumulation in aquatic animals. Benthic organisms that live in contact with sediment are at risk of acute or chronic toxicity due to sediment contaminants. In addition, benthic organisms bioaccumulate the metals, which may magnify along the food web from benthic dwellers to consumer species, such as fish and crustaceans. Additionally, because of widespread metals, human beings are exposed to the risk of ingestion of them via food and water through diverse pathways. This contamination can also increase bioaccumulation in organisms which reside the contaminated areas via the food web (Casado-Martinez et al. 2010). For this reason, it is essential to measure the level of heavy metals in media and figure out the transformation of those metals between media.

It has been generally been understood that quantification of metal bioavailability from contaminated sediment is challenging due to the complex metal geochemistry in sediment. One important biological parameter quantifying metal bioavailability from the environment is the sediment toxicity test. The sediment toxicity test is an indicator of the amount of metal from the aquatic environment (Figure 3-1). But it remains unclarified between media and for most benthic organisms.

Accordingly, many studies have been performed regarding heavy metal contamination for water and sediment. In particular, currently, to assess direct effects of metal mixtures to people, who may be exposed to toxicants, bioavailability and bioaccessibility are studied actively. Since the bioavailability and bioaccessibility of metals are affected by physicochemical and biological characteristics of the given conditions, total metal concentration is regarded as an incomplete indicator for potential toxicity caused by metal (Janssen et al. 2003). For this, the metal mixture toxicity test has been throroughly investigated using aquatic organisms. L. plumulosus acquires sediment as a food source and is a good subject for biomonitoring the bioavailability of metals from the sediment (Lee et al. 2006).

Owing to this, a study on bioavailability and bioaccessibility of heavy metals was conducted for the exact prediction of potential heavy metal effects to people. Bioavailability and bioaccessibility of metals have been researched associated with the environmental quality criteria (EQC) since the 1980s. In general, most procedures for water quality standards and methods of risk assessment were based on the total or dissolved metal concentrations. However, currently, many studies report that aquatic toxicity occurring by metals were not accounted for by total or

dissolved metal (Campbell et al. 1995; Woodward et al. 1995; Janssen et al. 2000). Various model have been developed to evaluate the effects of mixtures on benthic organisms. Braek et al. (1980) showed that mixtures of Cd and Zn acted either synergistically or antagonistically depending on the algal species. Many studies have examined single metal toxicity, but few studies were performed for the effects of metal mixtures on benthic organisms despite the fact that these elements are generally present together in benthic environments. Nevertheless, no studies have evaluated the combined effects of copper, cadmium, and zinc on the acute toxicity test in L. plumulosus.

Therefore, the main goal is to assess the contribution of heavy metal and its impact in aquatic ecosystems and to predict more reliable influences using observed values from interstitial waters (IW), sediments, and aquatic organisms via exposure tests. For this, we investigated different heavy metals and metal mixtures have differing bioavailability and toxicity based on competition for uptake at the chemical binding site in sediment. Then, we measured the acute toxicity of metal mixtures in water-only tests and how changes in toxicity occurred by metal mixtures in sediments.

## 2. Materials and methods

### 2.1. Sediment sampling and storage

Control sediments were used 1) to provide a material to dilute contaminated sediments, 2) to provide a controlled environment to evaluate toxicity mechanisms, and 3) to compare exposure

tests to control. Control sediments were collected from the Fox Creek (42°676473, -70°785567) near Ipswich Bay in the Massachusetts sampling site. This sampling site was analyzed and qualified by Vinturella (2004) (Table 3-1). Although reference sediments have been used in toxicity testing, comparison of the sensitivity of marine amphipod species to toxicants in reference sediment has not been done. For a validation of reference toxicity test using L. plumulosus, we compared the 50% lethal concentration ($LC_{50}$) from Aquatic Research Organisms (ARO, Hampton, NH) and the was quality assured.

Surficial sediment was collected from the sampling site. Sediments were collected at approximately 0-5, 5-10, and 10-15 cm depths using a Ponar grab sampler and were sealed in polyethylene bags using plastic spatulas. Sampling methods were performed in accordance with US EPA technical guidance (US EPA, 2005a). After being collected from the field, the sediment was sieved by (500 um), washed with dechlorinated tap water, and stored at 4°C before testing. Samples were taken to determine AVS content and sediment density. The organic matter content of the sediment was measured by a CHN element analyzer (Perkin-Elmer 2400 Series II CHNS/O).

## 2.2. Spiked sediment preparation

The sediment suspension technique (Cairns et al. 1984; Schuytema et al. 1984; Stemmer et al. 1990) was used to spike sediment with Cd, Cu and Zn. All manipulations to sediments were performed in a $N_2$ gas-filled glove box and the sediment containers were purged with nitrogen before closing.

The sediment was mixed with an equivalent volume of deoxygenated saline water, and then oxidized by aeration continuously with air for two weeks. The AVS concentration after two week of aeration decreased to approximately <1 umol/g. Estuarine sediments were spiked with an appropriate volume of Cd, Cu, and Zn stock solutions to prepare for concentrations of 1) 6-levels of single toxicity tests and 2) 16 combinations of metals mixture (Cd-Cu, Cd-Zn, and Cu-Zn) of acute toxicity test (Figure 3-1). The stock solutions of three metals were prepared by dissolving $CdCl_2$, $CuCl_2$, and $ZnCl_2$ in deionized water. Manipulated sediments, homogenized well, were placed in 1-L glass beaker with the volume of 175 ml with 800 ml of overylying water prior to 24 hrs. Then, 10-day toxicity test were conducted utilizing L. plumulosus.

**2.3. 10-day toxicity test**

The 10-day acute mortality test was conducted following the guidelines described in the American Society for Testing and Materials standard methods documents (ASTM) (1993) and the US Environmental Protection Agency (US EPA) guideline (1994). Experimental marine organisms, L. plumulosus, were collected from the Chesapeake Bay and acclimated to test temperature before testing. Overlying water (OW) was prepared using Instant Ocean® (Aquarium Systems, Mentor, OH, USA) with deionized water. Figure 3-2 shows an illustration of the bioassay.

As a reference test, acute 96 hrs $LC_{50}$ values from aqueous Cd were determined for L. plumulosus using water only. Mean 96 hrs L. plumulosus was detected as 0.96 ug/L and was

corresponded with the number of ARO's dataset. This indicated similar sensitivity of test species to metals for bioassay.

A seven-day period prior to adding test organisms allowed excess Cd, Cu and Zn to partition into and exit the flowing, OW. Sediments were added to exposure chambers and the seawater system started 24 hrs prior to the random addition of 20 amphipods with four replicates. The overall size range of experimental organisms was 2 to 4 mm (juvenile) and were maintained in a salinity of 20 psu with continuous aeration system. Exposures were kept at 25°C with continuous aeration under 24 hrs light cycle (no dark) with 500-1000 lux. Animals were not fed during the test. Ancillary OW quality (salinity, pH, dissolved oxygen, and temperature) was monitored daily. On day 10, the sediment in each beaker was sieved (0.25 mm) and the surviving amphipods were recovered. Reconstituted seawater was maintained with conductivity ($\leq$1 uS/cm) and TOC ($\leq$2 mg/L). Total ammonia concentration in the OW collected above the sediments was also below the toxic level to test species. After 10 days bioassay, amphipods were sieved and counted for mortality (%) and $LC_{50}$ value calculation. The remained beaker was kept for chemical analysis.

Chemical analysis for heavy metals were carried out in the <250 mesh fraction of sediments. All glassware, container, and supplier used in this study were acid-washed in 10% $HNO_3$ for 24 hrs and then cleaned with deionized water to avoid carry-over contamination.

Sediment concentrations were determined based on the calculated SEM:AVS ratios. Samples for AVS, SEM, and IW were taken from the test chamber at each day 0 and day 10. Sediment samples analyzed for all metals were digested in strong acid according to the procedures

described in Simpson (2002) and US EPA (1994 and 1996). The analysis of AVS and SEM was conducted by an $N_2$ gas purging and trapping method using an ion-specific sulfide electrode within 7 days of sample collection (Allen et al. 1993; Cline, 1969; and Simpson, 2002). Sediment IW was extracted suing the procedure described by Simpson et al. (2002). In this procedure, sediment samples were centrifuged (5 min, 3000 rpm) under a $N_2$ atmosphere followed by membrane filtration (0.45 um) of the displaced IW. The filtered liquid was acidified with Ultrex® (Baker, phillipsberg, NJ, USA) HCl to pH 1-2 and stored for PW analysis. Metal samples were analyzed utilizing blank and duplicate samples along with matrix spikes in order to assess the precision and accuracy of the analytical work. Total metal concentration from sediment, metal concentration of SEM, and IW samples analysis were completed by inductive coupled plasma mass spectrometer, Elan 6100 (ICP-MS, Perkin-Elmer, USA). Quality assurance (QA) samples were included in each sample batch and included method blanks, duplicates, matrix spikes, and a certified reference material. It was calculated $\pm 5\%$, $\pm 5\%$, $\pm 12\%$, and $\pm 8\%$, respectively.

**2.4. Data analysis**

For the toxicity test, two-tailed Student's t-tests were performed to assess a significant reduction in the amphipods' mortality in test sediments compared to the control, between day 0 and 10 using STATA, version 13. Trimmed Spearman-Karber method was used for 10-day $LC_{50}$ values. Significance in all statistical tests was set at the $p <0.05$ level.

## 3. Results and discussions

### 3.1. Physicochemical parameter of sediment and artificial OW

For this study, field sediment samples of Fox Creek, Ipswich Bay were collected. We analyzed ammonia concentrations of sediments after sampling, then the average of $NH_3$ concentration (mean = 1 mg/L) was compared with previous values as shown in Table 3-1. For single metal toxicity (water only), artificial OW was prepared and physicochemical parameters of seawater were tested for confirmation of no-effect to test organisms (Table 3-2). pH, temperature, conductivity, salinity, and dissolved oxygen (DO) met the appropriate conditions for L. plumulosus and were kept during bioassay.

### 3.2. Water only test of target metals

The L. plumulosus values from the water-only tests are shown in Table 3-3. The 10-day water-only tests for Cd, Cu and Zn were conducted individually using L. plumulosus. The $LC_{50}$s of 10-day for each metal were detected as 0.42 uM (Cd), 0.34 uM (Cu), and 22.0 uM (Zn).

### 3.3. Single metal toxicity test in spiked sediment

Significant reductions of metal toxicity were observed for all three 10-d exposure sediment tests. The data were considered acceptable for the current comparative toxicity study (Lee et al, 2004). The overall mean measured concentrations of SEM/AVS in metal spiked sediment for acute mortality bioassays were in general lower than the nominal concentrations.

Acid volatile sulfide, SEM and dry weight metals sediment chemistry measurements are shown Table 3-4. The IW values were consistent as the SEM and AVS values and demonstrated a high degree of variability within treatments. Some of the IW metal concentrations were analyzed below detection limits with below a 1 value of SEM/AVS ratio. In all experiments, a half of the SEM/AVS ratio of sediments was over 1.0 in Cd, Cu, and Zn. Higher (>1.0) SEM/AVS ratios of separately metal-spiked sediment were observed with explicit increments of IW concentrations up to Cd as 158153.56 ug/L, Cu as 7134.60 ug/L, and Zn as 124027.50 ug/L. For the lower SEM/AVS ratios of sediment, relatively low IW metals concentrations in sediments may be explained by analytical variability. Also, other binding phases in sediments may be considered.

Comparing IW metal concentration of sediment to average SEM concentration, in all ranges, metal concentrations appeared higher in IW than SEM. This indicates that significant amounts of metals were dissolved in sediments then binding to sulfides which could cause toxicity to test organisms. Most of sediments showed decreases in AVS concentrations. This could be related to formation of metal sulfides in the sediment. Berry et al. (1996) mentioned that a short time of AVS extraction could lead incomplete dissolution of sulfides.

## 3.4. Metal mixture bioassay

The mortality of L. plumulosus as a function of total metal concentrations followed a similar pattern in each of the three single metal and the mixed metals toxicity tests. Figure 3-3, 3-4, and 3-5 show 10-day mortality for amphipods exposed to metal mixture-spiked sediments. Mortality was increased with increasing metal concentration (umol/g dry weight) for each sediment.

Greenstein et al. (2008) assessed the correlation between toxicity and sediment parameters including metals using 8 representative test species of benthic environments. All of the significant correlations were negative, illustratring that as the concentration increased the toxicity increased. Among 8 organisms, L. plumulosus reacted relatively sensitively, especially to Cd, Cu and Zn with -0.401, -0.375, and -0.434, respectively.

Although in this study we assessed one sediment sample, the metal concentration-response curves were not overlapped in any combination for all three metal mixtures. Generally, different sediment and compositions have various physicochemical characteristics including AVS concentration. Concentration of SEM, which causes a toxic effect on organisms, is changed depending on sediment. Therefore, it is hard to predict mortality of amphipods in similar or different sediments using total metal concentrations only.

The 10-d metal mixture acute mortality results are presented with measured SEM Cd/AVS, SEM Cu/AVS, and SEM Zn/AVS in Figure 3-3, 3-4, and 3-5. Mortality did not appear to be metal mixture specific, with similar trend curves. In Figure 3-3, when metal mixture concentration was 0.49 uM of SEM-Cu, the mortality of amphipods was significantly increased from lower SEM-Cu concentrations, not depending on the concentration of SEM-Cd. The L. plumulosus value for Cu is 0.34 uM, so the toxicity of Cu affected mortality more highly than that of Cd. This suggests that copper is more toxic to amphipods. It is consistent with the value for *L. plumulosus* for Cd and Cu in Table 3-3. Considering the affinity of each metal to sulfide, Cu could affect the mortality more significantly than Cd and Zn.

Comparing to metal mixture sediments of SEM-Cd and SEM-Cu, mortality of Cd and Zn spiked sediment was increased continuously. Between Cd and Zn, mortality of amphipods was more affected by increases of SEM-Cd than SEM-Zn. Otherwise, in mortality of Cu and Zn spiked sediments, it was similar with the mortality curve of Cd and Cu mixtures. When the concentration of SEM-Zn was low, it was appeared more significantly. The concentration of SEM-Cu was slightly over the value for L. plumulosus of Cu (0.32 uM), and the mortality of amphipods was steeply incremented. However, in all experiments, 100% mortality of L. plumulosus was observed only with the highest concentration of both metals, though the SEM concentration of each metal exceeded both L. plumulosus values. This suggests that some binding factors affect the bioavailability of metals, and that combined toxicity was decreased lower then 100% mortality.

According to Di Toro et al. (1992) and Spehar and Fiandt (1986), mortality in the individual and metal mixtures was sediment independent, changing with SEM/AVS ratio. In this study, when the results of all of the toxicity tests were drawn together the mortality of L. plumulosus as a indicator of SEM/AVS ratio appears to be sediment and metal independent for each of the three individual metals and metal mixtures. Figure 3-6 shows the association between SEM/AVS ratio and mortality of amphipods. When the ratio was over 1, the mortality of amphipods was not 100% for all three metals until a certain ratio. For each metal, the amphipods all died when the SEM/AVS ratio was 12.25 for Cd, 23.71 for Cu, and 9.98 for Zn (Table 3-4). Although SEM concentration was over the capacity of sulfide to bind SEM, some amount of metal became more bioavailable to organisms, and it was lower than $LC_{50}$ values of each metal.

Mortality was also not sediment- or metal specific when we observed the relationship between mortality and interstitial water toxic unit (IWTU) values. Low mortality was shown in sediments with IWTUs of 10 (Figure 3-6). Amphipod mortality was significantly higher with IWTU of 10 and increased exponentially for three metals. As shown in Figure 3-7, when we observed the relationship between mortality and SEM/AVS and ratios were over 1.0, there were several sediments with the IWTU values over 10 (not toxic effects). This was especially true in the range of IWTU of over 0.5 but lower than 10.0. This may be a result of water-only toxicity tests. Additionally, it is supposed that all IW metal concentrations are not bioavailable to organisms. The results of metal mixture experiments showed that the sum of the IWTUs can be used to make predictions about amphipod mortality for any combination of metals tested in these experiments. Therefore, IWTU and SEM/AVS ratio can be utilized to predict which sediments are not toxic to test species more exactly.

A more complete understanding of the relationship of SEM/AVS ratio and IW metal concentration is shown Figure 3-8. It showed that the IW metal concentrations of sediments were lower when the SEM/AVS ratio was low. When the SEM/AVS ratio approached 1, the IW metal concentration was much increased for all three metals. Especially, the IW concentration of Cu was always the lowest and Cd and Zn were easier to detect by analysis, not depending on SEM/AVS ratio. Considering the higher solubility of Cu, the result was considered plausible.

**4. Conclusions**

These laboratory studies and analyses highlight the value of an integrated approach to develop hypotheses that can be tested using experimental models. The results suggest that competition between spiked metals for binding to sediment phases can reduce or increase the toxicity to benthic organisms and the environment. It also demonstrates that it is not possible to predict the toxicity of sediments spiked with metals using the total metal concentration. The association between 1) mortality of L. plumulosus and SEM/AVS ration and 2) the mortality of L. plumulosus and IWTUs were described not depending on specificity of sediment and metal. This result corresponds with previous studies using different test organisms and different test sites. Therefore, it can support the use of SEM/AVS ratios, IWTU, and bioavailability to predict the toxicity of sediment to microorganisms.

Currently, SEM, AVS, and IWTU are utilized to predict and estimate metal toxicity in sediment. However, SEM/AVS ratios have been used in sediment assessments to rule out metals as probable toxicants. Also, the SEM/AVS approach to predicting the bioavilability and toxicity of Cd, Cu, and Zn is applicable only to anaerobic sediments with AVS, because in aerobic sediments, other binding factors control bioavailability. Measurement of IW metal may be useful for evaluations of these heavy metals in aerobic sediments widely. Although many studies have been performed to predict more exact impacts of toxicity in benthic and aquatic environments, it is quite complicated with the specificity of various organisms and different conditions. However, the approach using SEM, AVS, and IWTU is more applicable than total metal concentrations.

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**Figure 3-1.** A scheme of metals mixture toxicity test with sediment.
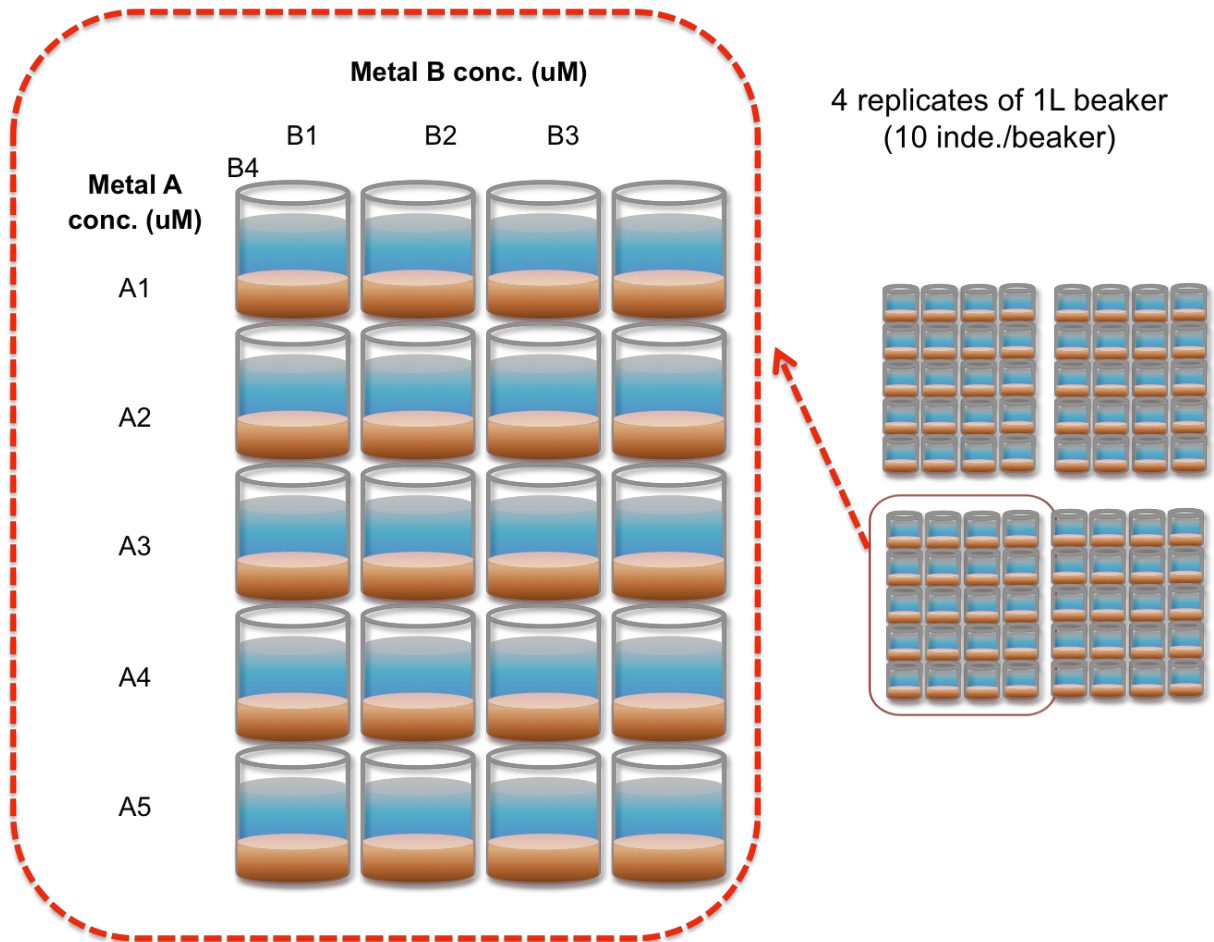
**Figure 3-2.** A concept of bioaccumulation and bioavailability.
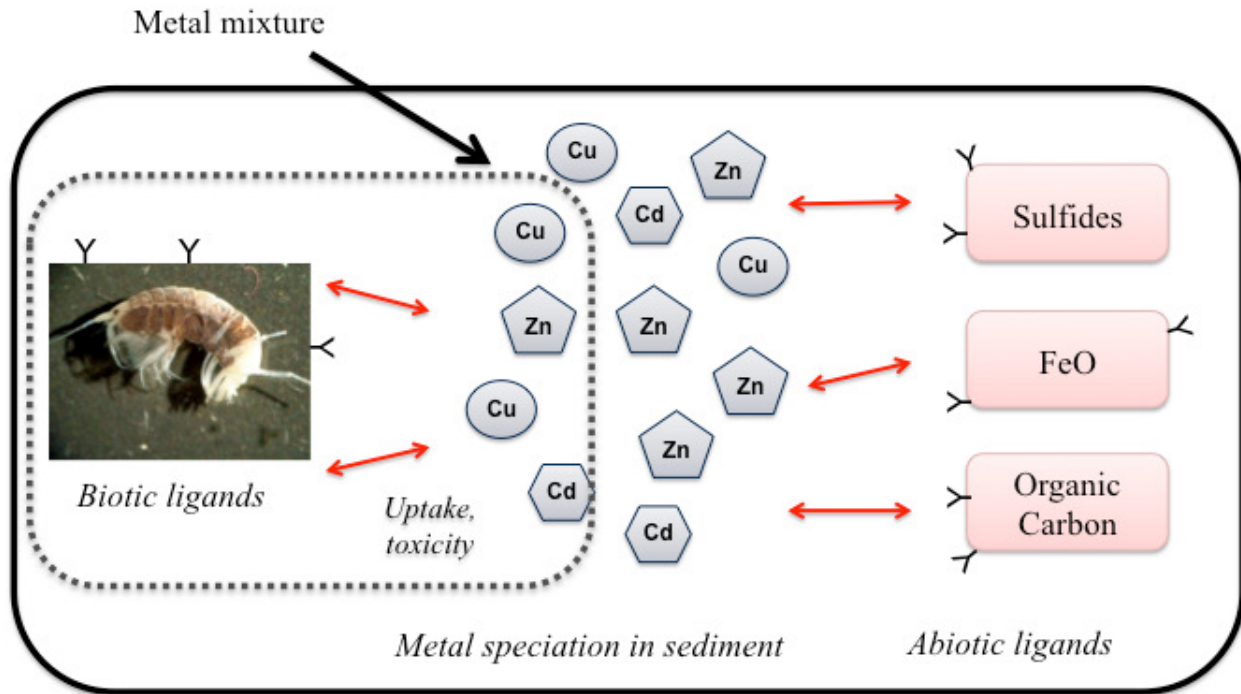
**Figure 3-3.** 10-d mortality for L. plumulosus in exposures to Cd-Cu spiked sediments.
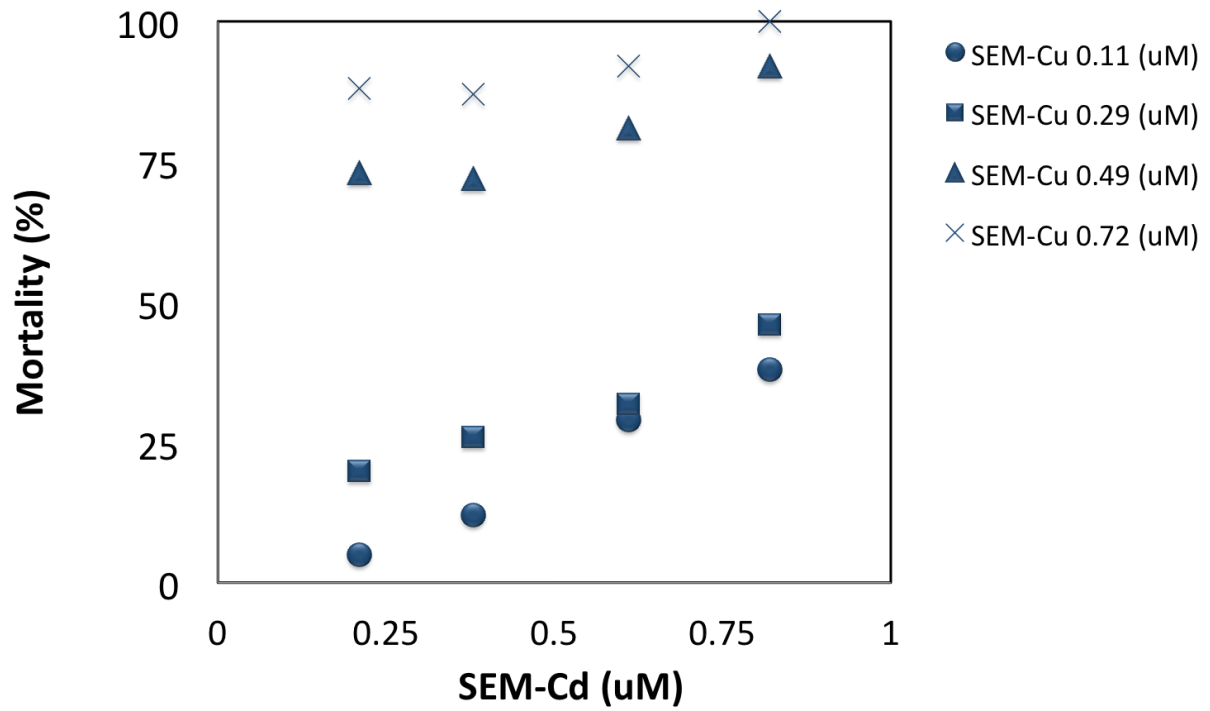
**Figure 3-4.** 10-d mortality for L. plumulosus in exposures to Cd-Zn spiked sediments.
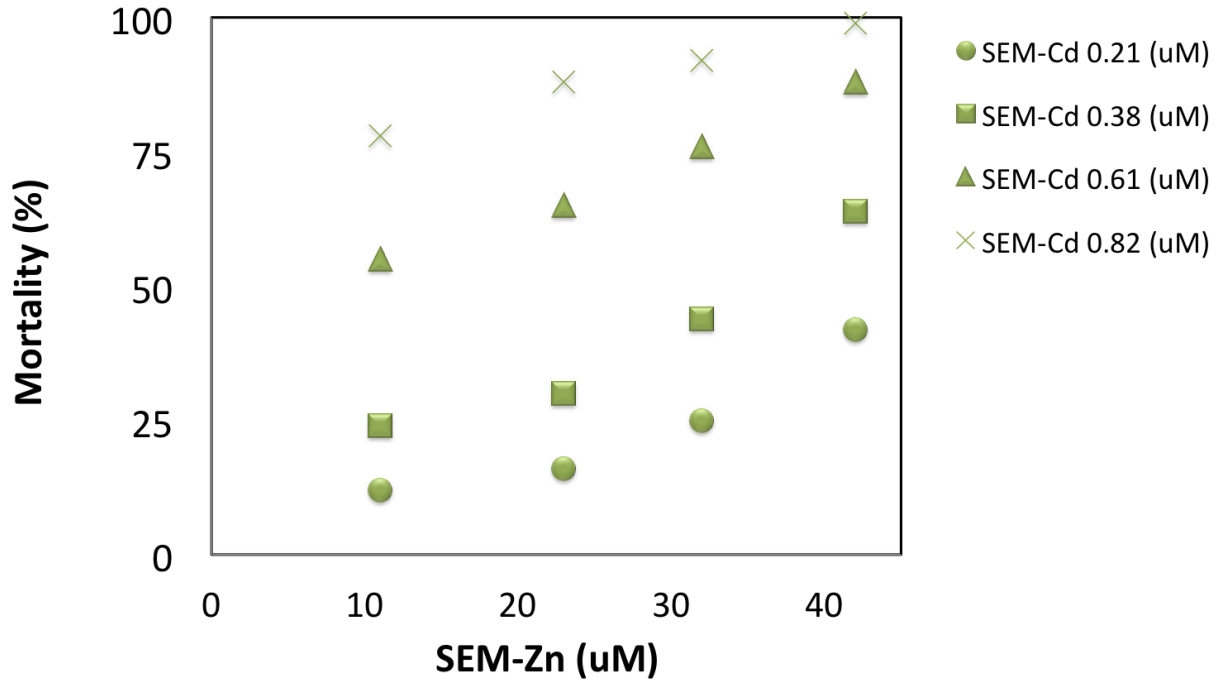
**Figure 3-5.** 10-d mortality *for L. plumulosus* in exposures to Cu-Zn spiked sediments.
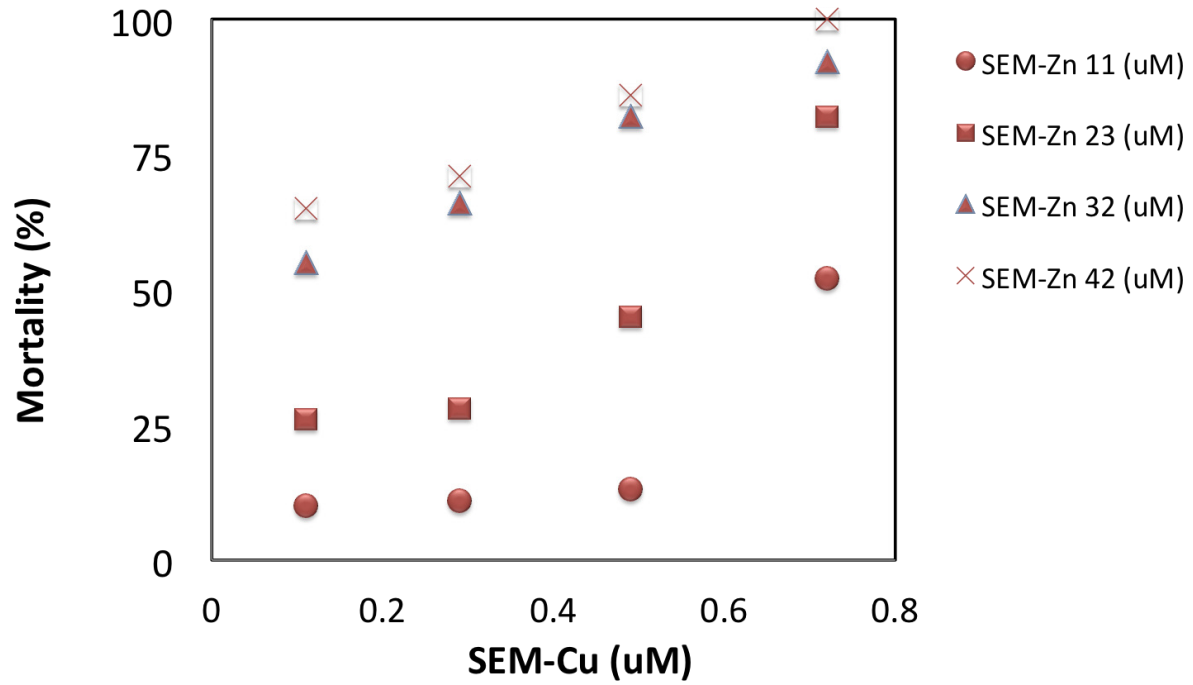
**Figure 3-6.** The relationship between mortality (%) of the amphipod and averaged SEM/AVS ratio.
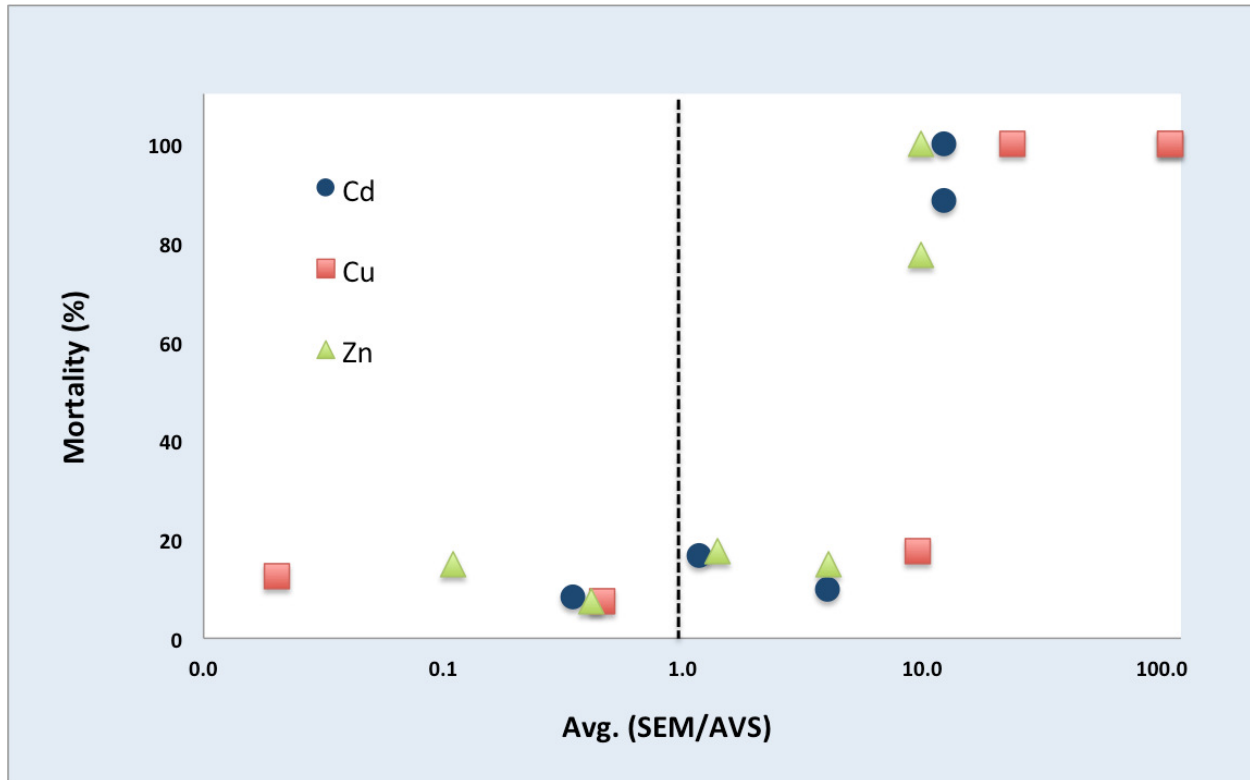
**Figure 3-7.** The relationship between mortality (%) of the amphipod and average of IWTU.
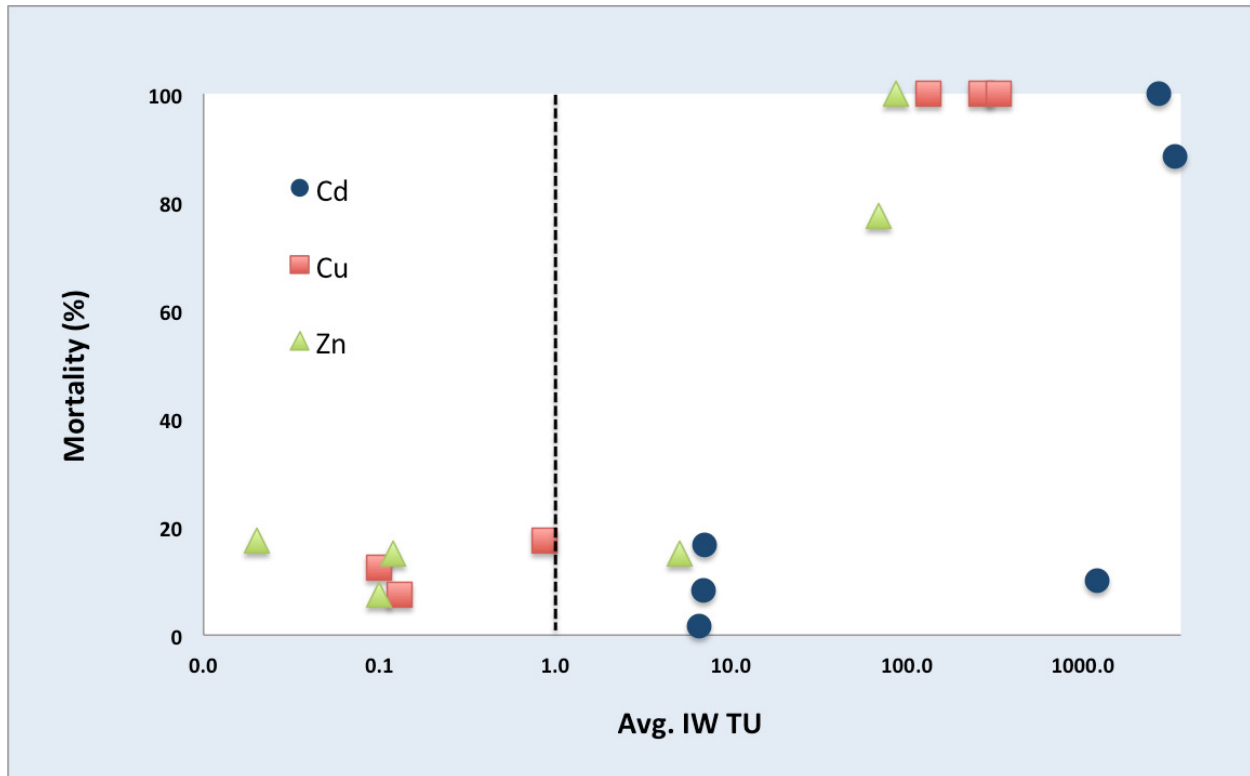
**Figure 3-8.** The relationship between averaged SEM/AVS ratio and averaged IWTU.
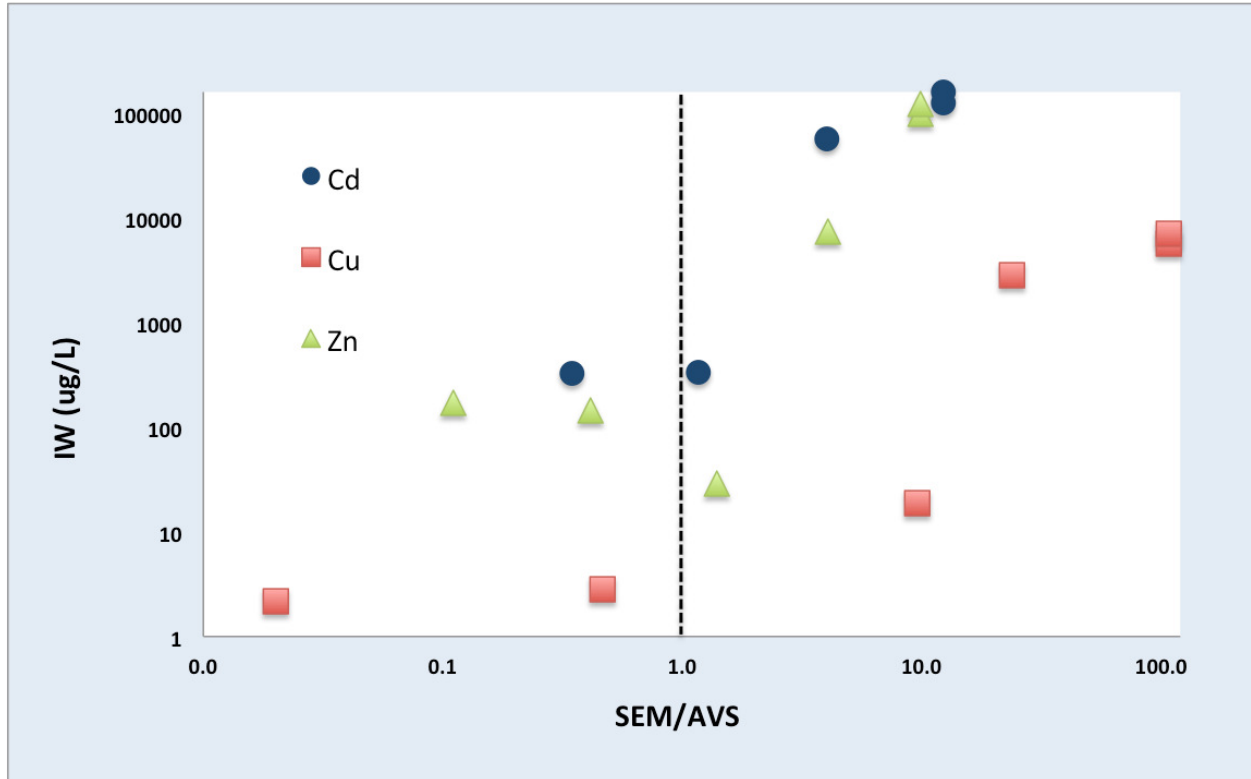
**Table 3-1.** Chemical parameters of sediment used as control in the toxicity test[a]

| Parameter | Control sediment | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Total PAHs (ug/g OC) | 43 | 40 | 39 | 42 | 38 |
| Total organic carbon (wt%) | 1.61 (0.06) | 1.22 (0.06) | 1.09 (0.05) | 1.52 (0.06) | 1.47 (0.04) |
| $NH_3$ (mg/L) | <1 | 2.10 | <1 | 3.10 | 1.80 |

a. Vinturella (2004)

**Table 3-2.** Physicochemical parameters of test water in the toxicity test

| Metal | pH | Temp. (C) | Con. (mS) | Salinity (ppt) | DO (mg/L) |
|---|---|---|---|---|---|
| Cd | 8.26 | 23.82 | 31.86 | 19.86 | 5.83 |
| Cu | 8.11 | 22.80 | 30.02 | 19.56 | 5.85 |
| Zn | 8.04 | 23.62 | 29.00 | 17.85 | 5.82 |

**Table 3-3.** 10-d single metal control tests

| Metal | Water only LC$_{50}$ (uM)[a] | Toxic Unit (TU)[b] |
|---|---|---|
| Cd | 0.42 (0.38-0.44) | 2.38 |
| Cu | 0.34 (0.31-0.37) | 2.94 |
| Zn | 22.0 (18.2-25.4) | 0.05 |

a. LC$_{50}$ values with the lower and upper 95% confidence limits
b. Toxic Unit = $1/LC_{50}$ (uM)

**Table 3-4.** Measured concentrations of SEM, AVS, and IW determined in a 10-d acute toxicity test with sediments spiked with Cd, Cu and Zn

| Nominal conc.[a] ratio | | sum SEM | AVS | SEM/ AVS ratio | IW (ug/L) | IW Toxic Unit[b] | Mortality (%) |
|---|---|---|---|---|---|---|---|
| | | (umol/g/dry sediment) | | | | | |
| Cd | control | 0.00 | 14.90 | 0.00 | 310.47 | 6.58 | 1.65 |
| | 0.50 | 4.82 | 13.90 | 0.35 | 330.20 | 6.99 | 7.35 |
| | 1.00 | 16.70 | 14.30 | 1.17 | 335.00 | 7.10 | 16.70 |
| | 1.50 | 25.85 | 6.45 | 4.01 | 56888.05 | 1205.00 | 10.00 |
| | 2.00 | 35.00 | 2.86 | 12.24 | 126522.80 | 2680.00 | 100.00 |
| | 2.50 | 43.75 | 3.58 | 12.24 | 158153.50 | 3350.00 | 87.40 |
| Cu | control | 0.25 | 12.40 | 0.02 | 2.16 | 0.10 | 12.50 |
| | 0.50 | 1.59 | 3.44 | 0.46 | 2.81 | 0.13 | 8.50 |
| | 1.00 | 11.60 | 1.21 | 9.59 | 18.81 | 0.87 | 18.50 |
| | 1.50 | 23.00 | 0.97 | 23.71 | 2853.84 | 132.00 | 100.00 |
| | 2.00 | 36.00 | 0.33 | 107.78 | 5707.68 | 264.00 | 100.00 |
| | 2.50 | 45.00 | 0.42 | 107.78 | 7134.60 | 330.00 | 100.00 |
| Zn | control | 1.20 | 10.90 | 0.11 | 172.56 | 0.12 | 14.00 |
| | 0.50 | 5.50 | 13.30 | 0.41 | 143.80 | 0.10 | 6.50 |
| | 1.00 | 21.10 | 15.10 | 1.40 | 28.76 | 0.02 | 17.50 |
| | 1.50 | 36.65 | 9.05 | 4.05 | 7333.80 | 5.10 | 16.00 |
| | 2.00 | 31.60 | 3.20 | 9.88 | 99222.00 | 69.00 | 78.50 |
| | 2.50 | 39.50 | 4.00 | 9.88 | 124027.50 | 86.25 | 100.00 |

a. the molar ratio of Cd, Cu, and Zn to AVS

b. interstitial water toxic units, which is the measured Cd water concentration divided by Cd water-only $LC_{50}$ (47.21 ug/L), by Cu water-only $LC_{50}$ (21.62 ug/L), and by Zn water-only $LC_{50}$ (1438 ug/L) (Table 3-3)

# CHAPTER 4: BIOACCESSIBLE ARSENIC IN THE DIET OF BANGLADESH

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**Abstract**

Many studies have investigated the total As concentration in soil and food crops in several countries including Bangladesh. However, few researchers have researched either bioaccessible As concentrations or dietary food samples. Thus, in this study, three consecutive day food samples of 47 female participants in Pabna, Bangladesh were used to investigate the association of total arsenic (As) and bioaccessible As concentration. Bioaccessibility of As was assessed using a simplified bioaccessibility extraction test (SBET) to estimate more precise toxicity of As from food intake and to understand potential for uptake in the human body. Significant changes of total As concentration of food sample were not observed.

The results indicate that total As can predict 78% of bioaccessibility of As in food sample. No seasonal changes of total and bioaccessible As intake were shown. The data also demonstrate that bioaccessible As intake is significantly lower than total As intake via food. In comparison with total As intake, fewer participants (3 in winter, 2 in summer) exceeded the World Health Organiziation's provisional tolerable daily intake (PTDI) of 2.1 ug As/kg-day, when we applied bioaccessible As intake. A bioaccessibility of As was lower than 18% for total As. This represents the importance of estimating the real risk from ingestible As sources like food and drinking water sources.

# 1. Introduction

Grains, fruits and vegetables grown in arsenic contaminated environments show high concentrations of As. Especially, rice takes up As from soil and water much more readily than other grains. Organic As is less toxic but is still in a concern and inorganic As is a carcinogen, with long term health effects. The Food and Drug Administration (FDA) reports that long-term exposure to high levels of arsenic is associated with higher rates of skin lesion, bladder and lung cancer, as well as heart disease. High levels of As exposure during childhood are associated with neurobehavioral effects as well as cancer and lung disease later in life.

Therefore, many studies have been performed regarding As contamination in soil and water in Bangladesh. In particular, to assess direct effects of As to residents in contaminated regions, Kile et al. (2007A) studied the relative contributions of drinking water and dietary intake to exposure in Pabna, Bangladesh using total As concentrations. They reported that an As concentration in the diet plays an important role for As ingestion of residents, especially intake of drinking water with lower As concentration. Juhasz et al. (2006) assessed As bioavailatility in rice using an in vivo swine model for As uptake rate for rice varieties.

However, since the bioaccessibility of metals are affected by physico-chemical characteristics of the given conditions, total As concentration is regarded as an incomplete indicator for potential toxicity caused by As (Janssen et al. 2000). For this reason, a study on bioaccessibility of As was conducted for exact prediction of potential As toxicity to people.

Bioaccessibility and bioavailability of metals have been questioned associated with the environmental quality criteria (EQC) since 1980s. In general, most procedures for water quality standards and methods of risk assessment were based on the total of dissolved metal concentrations. However, currently, many studies report that aquatic toxicity from metals were not accounted for by total or dissolved metal (Paquin et al., 2002; Janssen et al. 2003; Allen & Janssen 2006). For example, Janssen et al. (2003) demonstrated that inorganic arsenic species were dominant in the contaminated water samples and assessed the bioaccessibility and bioavailability of arsenic species using physiologically based extraction tests (PBET) to prove it.

Thus, in this study, bioaccessible As fraction of food samples were analyzed by simplified bioaccessibility extraction test (SBET) and compared with total As concentration of the samples to predict As toxicity to participants.

## 2. Material and Methods

### 2.1. Participant selection and food samples from Bangladesh

All food samples used in this study were collected by Kile. Forty seven women, who prepared family's primary food, in Pabna district, located north of Dhaka in central Bangladesh participated in this study. Duplicate dietary samples were gathered for 3 consecutive days during winter and summer periods, respectively January-March and June-August in 2004. After collecting duplicate portions from the participant's meals, food samples (n = 282) were

homogenized using a blender and kept frozen at -4 °C before analysis. For bioaccessible As analysis, the number of food sample was 249 (missing samples = 19; no remaining samples = 14).

## 2.2 Treatment of food samples for As analysis

For total As, samples were closed-vessel oven-bomb digested with concentrated nitric acid (reagent grade, Merck, Germany) using the microwave digestion method and analyzed for total As using dynamic reaction cell Inductive Coupled Plasma Mass Spectrometry (ICP-DRC-MS) with oxygen as the cell gas (Model 6100 DRC; PerkinElmer, Norwalk, CT, USA).

The SBET method was used to determine As bioaccessibility in contaminated diet by simulation of stomach condition (a fasting human). Contaminated diet (0.25 g, wet weight) and gastric solution (0.4M glycine adjusted to pH 1.5 with concentrated HCl) (Kelly et al. 2002) (25 mL) were added to 50 mL polyethylene test tubes. The pH was noted, then test tubes were placed into an incubator adjusted to 37 °C, while rotating at 40 rev per min on a shaker. Before placing, oxygen was removed and the samples were saturated with argon gas. Samples were extracted by rotating the samples end-over-end for 1 hour. Then, the pH was determined and supernant fluids of duplicate gastric phase samples (15 mL) were collected, filtered through 0.22 μm filters and analyzed using the same method by ICP-DRC-MS.

## 2.3. Analysis of total and bioaccessible As

This analytical method detects As oxide species ($^{75}As^{16}O^+$) at mass 91 and avoids argon chloride interference (Bollinger and Schleisman, 1999). Indium was added as an internal standard upstream of the nebulizer. Every sample was analyzed five times and the average limit of detection (LOD) was 0.012 ug As/g wet weight. The reported As value was corrected for any As detected in the method blank. For total and bioaccessible As samples (n= 9 and n = 7, respectively) which had lower concentration below the mean method LOD of 0.004 μg As/g wet weight, half of LOD value was applied.

Samples were digested in 15 batches, with each batch containing a method blank, certified rice flour [standard reference material (SRM) 1568A; National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA], certified dogfish liver sample (DOLT-2; National Research Council, Ottawa, Ontario, Canada) and certified lobster hepatopancreas (TORT-1; National Research Council, Canada) for quality control. The average percent recovery for 1568A, DOLT-2, and DOLT-3 were 100.2% ± 6.5%, 98.0% ± 4.2%, and 99.7% ± 8.0, respectively. Furthermore, for replicate analysis, one sample was chosen randomly for every ten samples. The average percent differences in total and bioaccessible As concentrations detected in replicate samples were 4.0% and 5.1%. Both filtered and unfiltered method blanks were compared using average bioaccessible As concentration and there was no difference. The average percent difference in bioaccessible As concentration in replicate samples was 0.54%. For validation of instrument performance, standard reference material (SRM) 1643e (Trace Elements in Water;

NIST) was used and the percent recovery was 93 ± 5%. All quality assurance met the laboratory's acceptance criteria. All glassware and supplier used in this study were acid-washed in 10% $HNO_3$ for 24 hr and then cleaned with deionized water to avoid carry-over contamination. All statistic analysis was conducted for significant difference by two-tailed Student's t-test using Stata/SE for Mac, version 13 (StataCorp, College Station, TX).

## 3. Results and Discussion

### 3.1. Validation test for the comparison

Prior to the comparison between total and bioaccessible As concentrations, a validation test was conducted to verify the correspondence of the comparison between previous total As concentration [conducted by Kile et al. (2007A)] and total As concentration for this study. We calculated statistics of both total As concentrations analyzed in 2004 and 2010 (Table 4-1). Also, both data sets were subjected to a paired t-test. There was no significant difference between the two data sets, P-value (> 0.05).

As shown in Figure 4-1, the degree of agreement between both total As concentrations was high with $R^2$ of 0.9627. Even though there was a time gap between As anlysis, total As concentration of the food sample was considered stable. The comparison between datasets for total As was feasibly considered.

66

## 3.2. Participant and food sample

Physical characteristics of participants were shown in Table 4-2 (Kile et al. 2007A). The average age of participant was 36.6 years old and average body mass index. Body weight ranged from 35 kg to 70 kg with average of 51.4 kg. Weight information is important for comparison to the provisional tolerance daily intake (PTDI) (2.1 ug As/kg-day) of the World Health Organization (WHO) (WHO, 1985). Each food type collected for this study is shown in Table 4-3 (Kile et al. 2007A). Rice and vegetables were the most commonly ingested food sources with 35.5% and 34.3%. All participants bought their food from local markets or domestic products. Based on the observation of collected sample and reports by Kile et al. (2007A), rice was the main component of every meal at 91%. For this study, the average ingested food weight was 1,636 g (wet weight) (Kile et al. 2007B). By season, participants ingested food 1,700 ± 338 g (wet weight) and 1,571 ± 324 g (wet weight) in winter and summer, respectively. The weight of ingested food in winter was significantly higher than that in summer, but there was no difference in food types seasonally.

## 3.3. Comparison of total and bioaccessible As

Using paired data of total As and bioaccessible As concentrations of collected food samples, we performed linear regression. As shown in Figure 4-2, bioaccessible As concentration was correlated with the total As concentration ($R^2$=0.6926). When total As concentration was

increased, the correspondence of bioaccessible As with total As was increased. It is supposed to be related with water contents of food and water sources utilized for food cooking. According to Kile et al. (2007A)'s results, median daily total As intake from food and water were 48 ug As/day and 4 ug As/day, respectively. However, for the higher daily total As exposure, participant's intake of consumption of contaminated drinking water significantly affected total As intake from food. More information of drinking water sources, consumption rate, and bioaccessible As analysis of water would be helpful to predict bioaccessible As using total As.

Fractions of bioaccessibility by winter and summer were compared (Figure 4-3 and 4-4). Mean of bioaccessibility of As in winter and summer were $31.57 \pm 20.57$ (%) and $33.19 \pm 22.10$ (%), respectively. As described in Table 4-3, since the major portion of participants' meal was rice and vegetables and there was no significant difference by season, the rate of bioaccessibility of As was observed similarly. Considering the trend of each participants, most female participants were assumed to have the same dietary characteristics in winter and summer. It means total As intake can be affected more sensitively by drinking water than As intake from food. Though Table 4-3 showed the different amount of fruit consumption with 0.1% in winter and 6.9% in summer, it did not affect the results seriously (only 3.6% of total food meal).

**3.4. Comparison to WHO PTDI standard**

Arsenic has long been recognized as a toxic element with much research on environemtnal behavior and bioavailability reported (Azcue et al., 1994; Naidu et al. 2009). Especially, inorganic As is a main toxin which cause skin cancer (Seow et al 2012). Also, the toxicity and bioavailability of As strongly depends on speciation and environmental conditions with other variables. This is the reason we assessed total As and bioaccessible As for this study.

However, unfortunately, there is no established guideline to assess the health risk due to dietary intake of inorganic As. The WHO's PTDI is the most common standards for total As standard. Thus, to estimate a real risk of As intake to participants and to compare the difference of total and bioaccessible As intakes, As concentrations per wet weight of food to the daily total As intake from food was converted. Then, WHO's PTDI recommendations (WHO, 1985) was compared to this value.

The daily total As intake was the sum of daily total As intake from food and drinking water (Kile et al. 2007B). However, in this study, the association between total As concentration and bioaccessible As concentration was focused and As concentration from drinking water was not considered. First, the average food wet weight of participants for winter and summer were applied, separately. Participants ingested food 1,700 ± 338 g (wet weight) and 1,571 ± 324 g (wet weight) in winter and summer, respectively. We calculated daily As intake from food (ug As/kg-day) for each participant using the averaged wet weight of food intake each day multiplied by concentration of intake As from food measured in the corresponding 24-hr composite samples (ug As/g wet weight), then divided by the average body weight (51.4 kg, shown in Table 4-1).

The equestion is decribed below:

$$Daily\ As\ intake\ from\ food\ (\frac{ug\ As}{kg-day})$$

$$= \frac{The\ average\ food\ intake\ (g)}{The\ average\ body\ weight\ (kg)} * conc.of\ intake\ As\ from\ food\ \left(\frac{ug\ As}{g-day}\right) \qquad \text{-------------- (1)}$$

We then compared these values with WHO's PTDI, 2.1 ug As/kg-day. The result is shown in Figure 4-5 and 4-6. As shown in Figure 4-5, for total As concentration analyzed in 2004 and 2010, 11 and 10 participants exceeded 2.1 ug As/kg-day of PTDI for winter food consumption. On the other hand, bioaccessible As concentration, only 3 participants were observed near standard line. The highest concentration of daily intake of total As from food was observed as 7.9 and 5.95 ug As/kg-day, but it was shown as 2.25 ug As/kg-day for bioaccessible As concentration, which is considerabley lower than the result of total As. In Figure 4-6, for total As concentrations analyzed in 2004 and 2010, 8 and 5 participants exceeded 2.1 ug As/kg-day of PTDI for summer food consumption. The highest concentration of daily intake of total As from food was 4.34 and 3.63 ug As/kg-day, but it was shown as 2.2- ug As/kg-day for bioaccessible As concentration. This points out total As concentration could overestimate the risk of As exposure. However, considering the range of body weight (35 to 70 kg), the scale of change in daily intake of As from food can be increased or decreased. Intake of contaminated drinking water and ingestion rate of individual can also affect the result. Kile et al. (2007A) note one participant was diagonosed with As caused skin cancer. This woman consumed contaminated tube well water source with As concentration of 360 ug/L for 12 years and was recorded with the highest daily intake total As (25.7 ug As/kg-day), unlike another participant who used more

70

contaminated water source with As and showed no symptoms. This means individual differences in intestinal conditions, cooking style and other factors can affect the estimation of exposure. Depending on season, the results of winter was higher than summer. It can be explained by difference of the average food consumption weight between winter and summer as mentioned previously.

## 4. Conclusions

Arsenic is usually recalcitrant and non-degradable in the environment. Thus, when it is exposed to organisms, biological organism would be threatened by high levels of metals (Tong and Lam, 2000). Increase of exposure and risk probability to carcinogens like As is considered more seriously. Thus, this study estimated the bioaccessible As fraction of total As concentration from food intake using SBET. As a result, no significant difference of total As concentration from Pabna food samples was observed between the datasets of 2004 and 2010. Total As concentration by food intake predicted 78% of bioaccessible As concentration. Drinking water source was considered to be a high potential risk and indicated that the carcinogenic risk of As cannot be tolerable (Seow et al. 2012). Therefore, more information such as total and bioaccessible As concentration of drinking water source for each participant and gastrointestinal rate of individual can contribute the bioaccessibility fraction. It is also necessary to estimate more precise As exposure and risk assessment.

No seasonal changes were detected for fraction of bioaccessible As concentration by food in corresponding to the food types. Dietary pattern of participant was considered constant. Contrarily, body weight and drinking water source could affect bioaccessible As fraction more significantly considering the result of daily intake of As from food.  Halder (2013) described the total As concentration from drinking water is equivalent to inorganic As intake from rice consumption, thus bioaccessible inorganic As concentration should be studied more to estimate the effect of As toxicity. Continuous studies about bioaccessibility can provide more applicable As standards and guidelines for exposure assessment and more feasible countermeasurement againt carcinogens.

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**Figure 4-1.** A validation test for the comparison of total As concentration (ug As/g wet weight) analyzed.
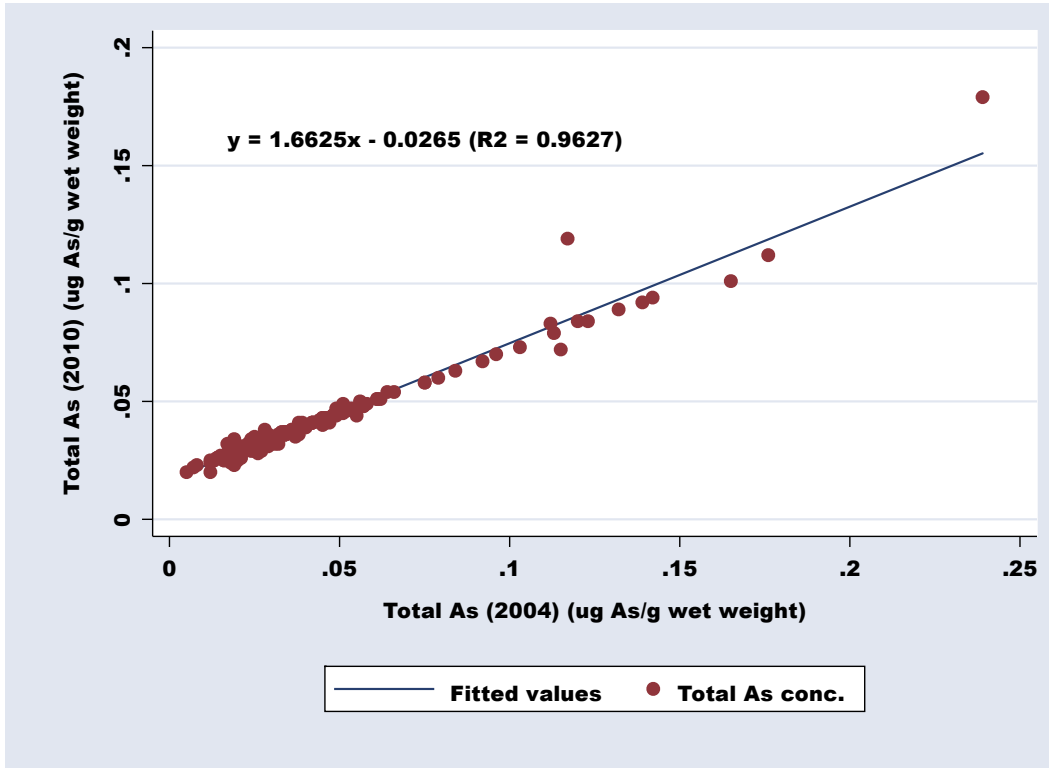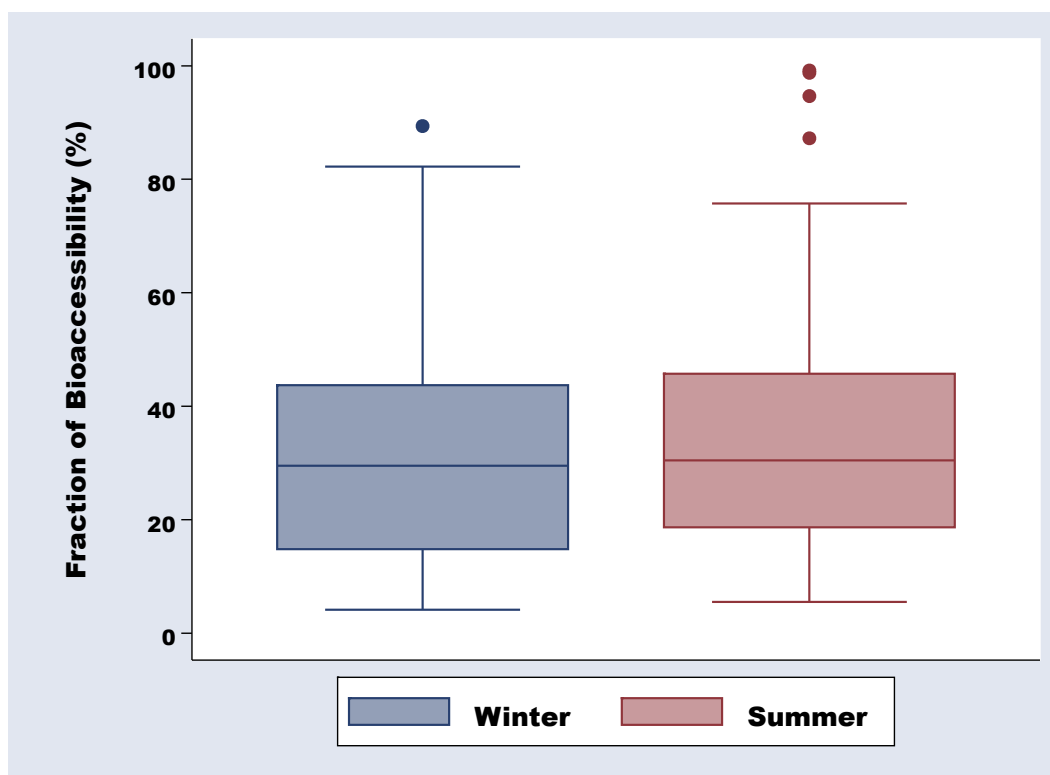
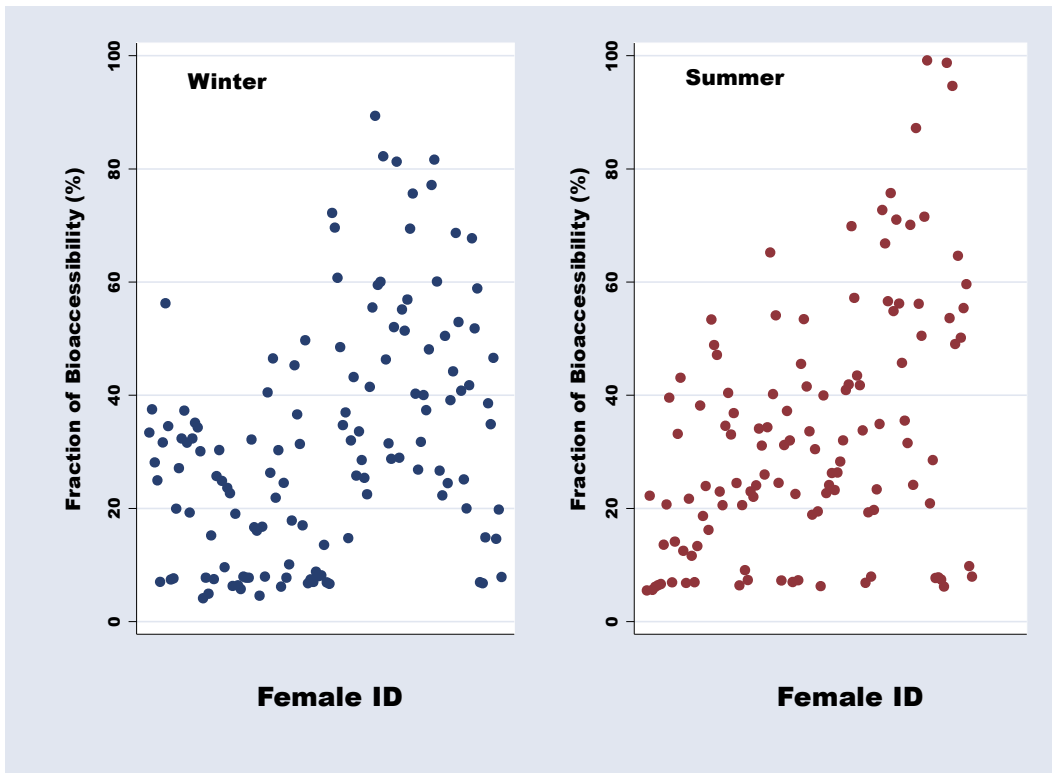**Figure 4-2.** A linear regression of total As concentration vs. bioaccessible As concentration.
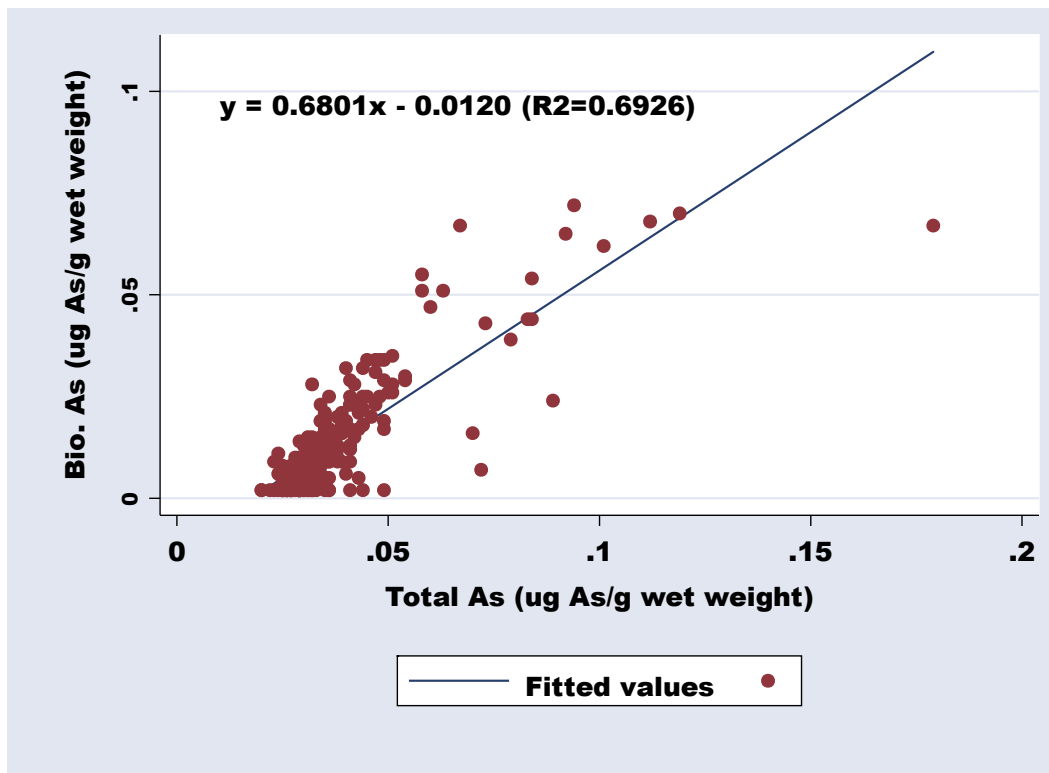
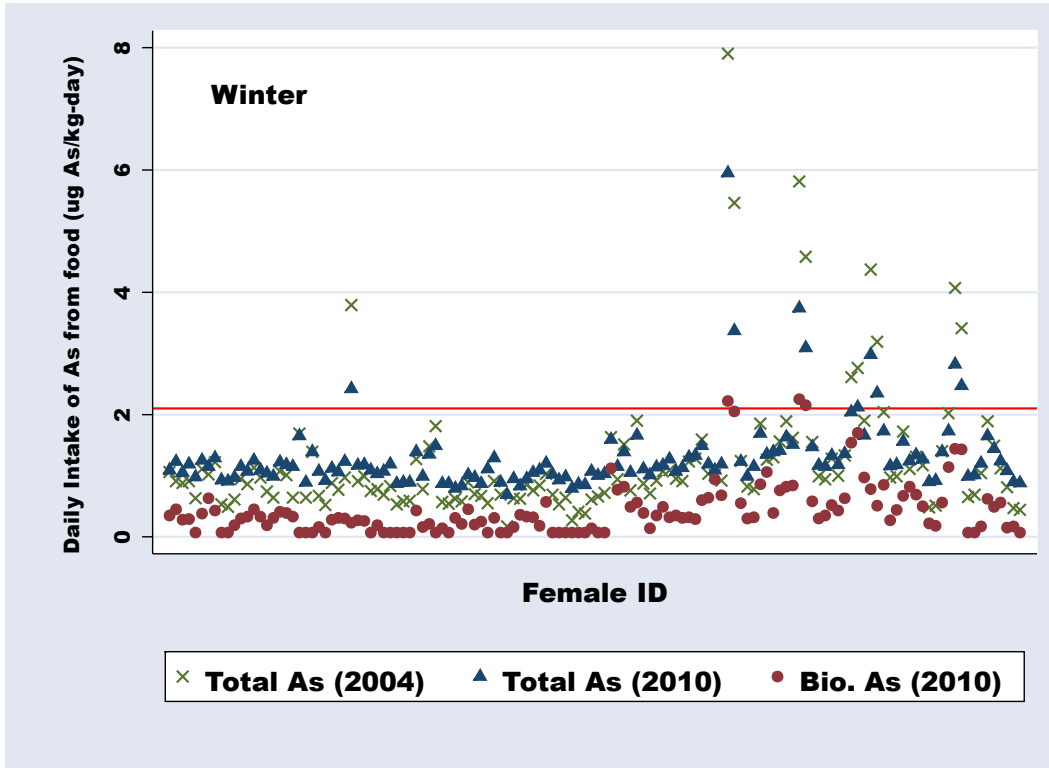**Figure 4-3.** Fraction of bioaccessible As (%) depending on sampling time (winter and summer).

**Figure 4-4.** Fraction of bioaccessible As (%) depending on participant by winter and summer.

**Figure 4-5.** Comparison between intake total As concentration (ug As/kg-day) and PTDI (2.1 ug/kg-day) for winter.

**Figure 4-6.** Comparison between intake total As concentration (ug As/kg-day) and PTDI (2.1 ug/kg-day) for summer.
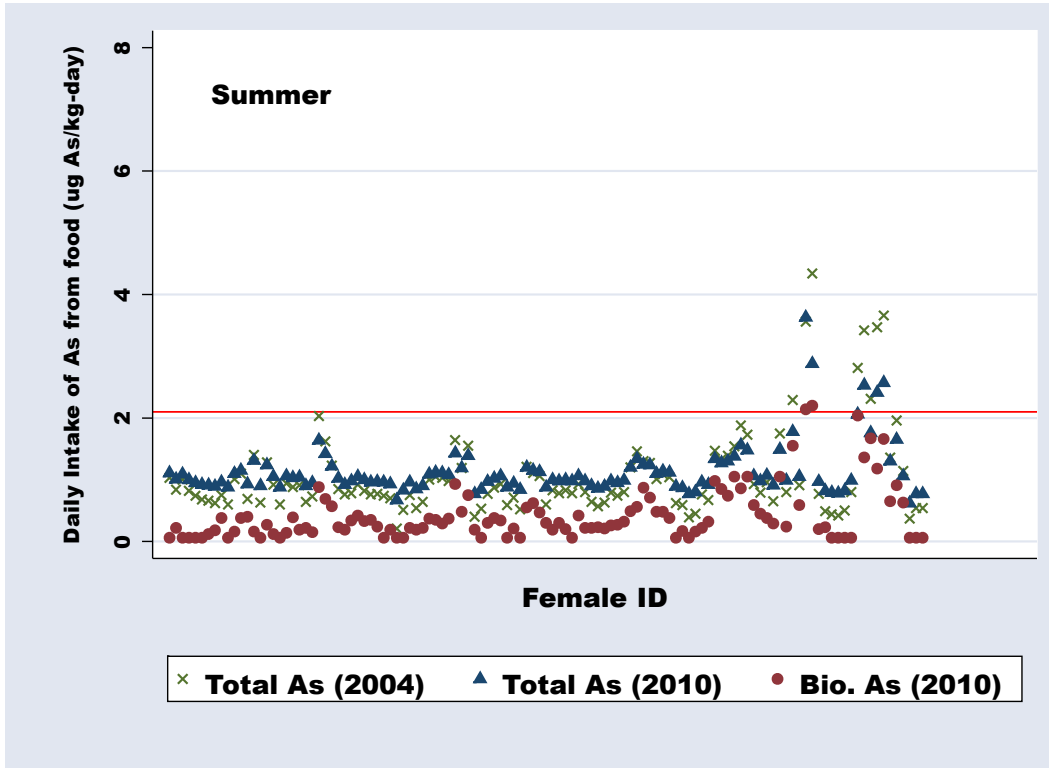
**Table 4-1.** Summary statistics of total As concentration detected in the participant's diet samples

| Total As conc. (ug As/g wet weight) | N | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| Data (2004)[a] | 249 | 0.037 | 0.030 | 0.01 | 0.24 |
| Data (2010) | 249 | 0.038 | 0.020 | 0.02 | 0.18 |

[a](Kile et al. 2007A)

**Table 4-2.** Physical characteristics of the 47 female participants

| Characteristics | Mean ± SD | Range |
|---|---|---|
| Age (years) | 36.6 ± 8.6 | 20-65 |
| Body mass index | 22.5 ± 3.5 | 15.1-30.3 |
| Weight (kg) | 51.4 (8.9) | 35-70 |

**Table 4-3.** Frequency of food types collected in the duplicate diet study[a]

|  | Winter (%) | Summer (%) | Total (%) |
|---|---|---|---|
| Grains |  |  |  |
|   Rice | 393 (35.0) | 390 (33.7) | 783 (34.3) |
|   Bread | 32 (2.9) | 43 (3.7) | 75 (3.3) |
| Proteins |  |  |  |
|   Fish (all freshwater) | 139 (12.4) | 95 (8.2) | 234 (10.3) |
|   Meat (poultry, beef, goat) | 27 (2.4) | 21 (1.8) | 48 (2.1) |
|   Egg | 21 (1.9) | 22 (1.9) | 43 (1.9) |
| Fruits and vegetables |  |  |  |
|   Vegetables | 436 (38.8) | 374 (32.4) | 810 (35.5) |
|   Fruit | 1 (0.1) | 80 (6.9) | 81 (3.6) |
|   Pulses/legumes | 65 (5.8) | 94 (8.1) | 159 (7.0) |
| Others |  |  |  |
|   Condiments (sugar, salt) | 3 (0.3) | 11 (1.0) | 14 (0.6) |
|   Fried snacks | 2 (0.2) | 4 (0.4) | 6 (0.3) |
|   Butter | 0 (0) | 1 (0.1) | 1 (0.0) |
|   Dessert (sweet noodles) | 2 (0.2) | 7 (0.6) | 9 (0.4) |
| Dairy |  |  |  |
|   Milk[b] | 3 (0.3) | 14 (1.2) | 17 (0.8) |

A total of 423 meals were collected for each season, with 846 meals collected in total from 47 participants.

a. (Kile et al. 2007A)

b. was not included in the 24-hr composite and analyzed for As.

**CHAPTER 5: CONCLUSIONS**

**Conclusions**

This research gave ways to better estimate heavy metal toxicity in complicated environment. It also presented how bioavailable or bioaccessible metal concentrations differ from total metal concentrations when it affects adverse health effect to humans and the environment. Although current water and sediment contamination management based on total concentration have provided important information for exposure assessment and risk assessment for several decades, they may not accurately reflect the variability of media and variability of metal species responding to parameters.

In Chapter 2, in freshwater, effects of water quality parameters (pH, dissolved organic carbon (DOC), and hardness) on Cu and Cr mixture showed different patterns of acute toxicity. For Cu toxicity, DOC had the highest effects on the change of toxicity. Otherwise, for Cr toxicity, hardness was the most important factor (Villaescusa et al. 1997). The approach used here is fundamental to understand how metal toxicity to organisms is changed in different conditions . In contrast, previous approaches were more focused on total concentrations of pollutants only. By considering different trends of changed metal toxicity, the importance of metal bioavailability related with harmful effect to humans and the ecosystem was accounted for in the prediction models. This suggests that it may not be accurate to predict the toxicity with total metal concentrations, not considering real bioavailability. Because our final goal is to manage our environment contamination more effectively and to assess more accurate exposure assessment with risk assessment, it was unnecessary to overestimate or underestimate the harmful effects from metals. In addition, there is large variability depending on the metal species, test species,

and aquatic parameters such as pH, DOC, and hardness. Although we assessed Cu and Cr mixture toxicity using Daphnia magna, this cannot be applied for other metals, other test species, and seawater. This is the reason why more researchers have applied a biotic ligand model (BLM) (Drilgin et al. 2002) to predict toxicity of metals.

In Chapter 3, to better understand toxicity of heavy metal in estuarine systems, we investigated heavy metal toxicity in sediment with seawater. Through this investigation, geochemical processes which affect the bioaccumulation of target metal can be explained. Based on single metal toxicity, metal mixture toxicity depending on different ratios of simultaneously extracted metals (SEM)/acid volatile sulfide (AVS) was tested using Leptocheirus plumulosus (marine amphipod). Although single metal toxicity, not with sediment, was 0.42 uM, 0.32 uM and 22 uM for Cd, Cu, and Zn, respectively, metal toxicity in sediment was significantly decreased. Even with the ratio of SEM/AVS over 10, mortality of amphipods was not 100%. This indicates that metal toxicity patterns varied with different environments, different target metals, and different species (Lock et al. 2002). Even with the combination of metal mixture, the differences in various conditions are not enough to predict the bioavailability of heavy metals in sediment. Though the proposed regression models allow assessment by estuarine sediment samples, it is definitely possible that the models may not be applicable to some special sediments, whereas various conditions may become more decisive for certain sediments. Concentrations of metals are extremely sensitive to small changes in media characteristics. However, this study is essential to understand the interactions between metals and variable in the sediments for the next step.

In Chapter 4, because As is a major toxicant among heavy metals, the difference of bioaccessible As concentration and total As concentration were examined by using simplied physiolocial-based extraction technique (SBET). Three consecutive day food samples of 47 female participants in Pabna, Bangladesh were used to estimate the relationship between bioaccessible As and total As via food intake. It showed that only 78% of total As from food produced a harmful effect to humans. Additionally, bioaccessibility of As was approximately 20% lower than total As. This indicates that accurate exposure assessment for metal is difficult between total and bioaccessible metal concentrations, especially for humans. Because it is hard to investigate confounding factors, unlike water or sediment test experiments. which can affect metal species, we should attempt to get more information about different ingestion rates, cooking styles, nationality, body weight, and dietary habits of participants. If this is not adequately figured out, it may lead to less reliable exposure estimates or more exposure errors, Therefore, this research suggests that the SBET technique can improve the assessment of inorganic As via food intake, which may lead to the development of more exact As contamination control in the environmental policymaking process.

Conclusively, this study can bridge the current gap between geochemical and toxicological studies on heavy metals. In addition to the current risk assessment approaches that operationally give the answer of how much the real risk is, our work provides insights on why there is a risk with the combination of chemical analysis. Our research serves to dictate the necessity of additional data collection of site-specific parameters, which might capture the distribution and variation of the bioavailability of metals better than the fixed mean values. This research is

aimed at describing the ongoing kinetics of metal speciation in sediments and will give a try to

establish an uptake rate model for future risk characterization.

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