DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN BIVALVES FROM THE HOMA LAGOON

by
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İZMİR

ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN BIVALVES FROM THE HOMA LAGOON

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Mustafa BİLGİN

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M.Sc THESIS EXAMINATION RESULT FORM

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ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN BIVALVES FROM THE HOMA LAGOON

ABSTRACT

In this study, the concentrations of heavy metals (mercury, cadmium, lead, chromium, copper, manganese, zinc, nickel, iron, cobalt and, aluminium) were determined in two bivalve species from the Homa Lagoon. The selected study area, the Homa Lagoon is the only active lagoon in İzmir and has a wide biodiversity. It is affected by Gediz River and is located in north side of İzmir Bay.

Mytilus galloprovincialis and *Tapes decussatus* samples were collected seasonally in May 2013 to February 2014. Hepatopancreas were dissected from soft tissues of samples. The heavy metal analyses were performed by AAS and ICP-MS. The statistical analyses (ANOVA and correlation tests) were applied to describe significant differences. The results showed that the maximum concentrations of metals in *M. galloprovincialis and T. decussatus* were generally found in February and July, respectively. For tissues, the heavy metal levels in hepatopancreas were generally higher than soft tissues.

The results were compared with the Provisional Tolerable Weekly Intake (PTWI) limits of international regulations. Only the levels of aluminium in *Tapes decussatus* from the Homa Lagoon exceeded the permissible limits.

Keywords: *Mytilus galloprovincialis*, *Tapes decussatus*, heavy metal, aquatic pollution, Homa Lagoon

HOMA DALYANI BİVALVELERİNDEKİ AĞIR METAL KONSANTRASYONLARININ DEĞERLENDİRİLMESİ

ÖZ

Bu çalışmada, Homa Dalyan'ında bulunan iki bivalve türünde ağır metallerin (civa, kadmiyum, kurşun, krom, bakır, mangan, çinko, nikel, demir, kobalt ve alüminyum) konsantrasyonları incelenmiştir. Çalışma alanı olarak seçilen Homa Dalyanı İzmir'de yer alan tek aktif dalyandır ve geniş bir biyoçeşitlilk içerir. Dalyan, Gediz Nehri'nin etkisindedir ve İzmir Körfezi'nin kuzeyinde yer alır.

Mytilus galloprovincialis and *Tapes decussatus* örnekleri Mayıs 2013-Şubat 2014 arasında mevsimsel olarak toplanmıştır. Örneklerin hepatopankreas dokusu, yumuşak dokularından ayrılmıştır. Ağır metal analizleri AAS ve ICP-MS cihazları ile gerçekleştirilmiştir. Anlamlı farklılıkların belirlenmesi için istatistiksel analizler yapılmıştır (ANOVA ve korelasyon testi). Sonuçlara göre *Mytilus galloprovincialis*'te saptanan en yüksek metal konsantrasyonları genelde Şubat ayında ve *Tapes decussatus'ta* ise Temmuz'da bulunmuştur. Doku bakımından, hepatopankreas dokusunun, yumuşak dokuya göre daha yüksek seviyede metal biriktirdiği saptanmıştır.

Elde edilen sonuçlar uluslararası otoritelerin haftalık kabul edilebilen tüketim miktarı (PTWI) değerleri ile karşılaştırılmıştır. Sadece *Tapes decussatus* türündeki alüminyum seviyeleri, izin verilen değerlerin üzerindedir.

Anahtar kelimeler: *Mytilus galloprovincialis, Tapes decussatus*, ağır metal, sucul kirlilik, Homa Dalyanı

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

After increasing of industrial development and unplanned urbanization, our earth faces the main problem; environmental pollution. Discharge and disposal of anthropogenic waste loads effect the environment and especially water sources. Heavy metals are one of the most common pollutants of water pollution. They dissolve in the water naturally and distribute by adsorbing water molecules (Selvin, Priya, Kiran & Bhosle, 2008). There are colloidal, dissolved and particulate forms of heavy metals in aquatic ecosystems. This contamination affects both the ecosystem and the human health that on top of the food chain negatively (Tao, Yuan, Xiaona & Wei, 2012). Dissolved heavy metal compounds can be accumulated rapidly in the environment by marine organisms. Aquatic species can tolerate these metals until they reach to the harmful levels. These levels are individual for all species; it means that different levels of intakes cannot express the same impact (Blanco, Cattoni, Carriquiriborde, Grigera & Chara, 2013).

Because of filter feeding dietary type and inadequate movement capability, bivalves tend to face the aquatic pollution. If tolerance limits exceed, abnormalities can be observed. Therefore, this situation makes them the bioindicator species. They concentrate the metals into their soft tissues, detoxicate and then discharge them partially by urinary system or reproduction way (Zuykov, Pelletier & Harper, 2013).

Lagoon systems are very special geographical structures that are particularly complex environments in which the transition between marine and continental waters is gradual, due to the continuity of the aquatic habitat (Frontalini, Chatelet, Debenay, Coccioni & Bancala, 2011). Lagoons are shallow water bodies that are effected by various physical parameters (e.g salinity and temperature) ranges separated with barriers on the coastline and involve channels to interact between seawater and freshwater (Food and Agricultural Organization [FAO], 1998). Nowadays, due to the water pollution, lagoons are also threatened by anthropogenic activities. Homa Lagoon which is selected for this study is one of the important lagoons of Turkey.

It’s the only active lagoon of five in İzmir Bay. It is affected by Gediz River and is located on Aegean Sea coast, in north side of İzmir Bay (Yürür, 2008). Homa Lagoon has a wide biodiversity that lots of marine plants, vertebrates and invertebrates species inhabit.

In this study, bivalves that one of the bioindicator and commercial species were used for assessment of heavy metal pollution of Homa Lagoon. Mussel (*Mytilus galloprovicialis* Lamarck, 1819) and clam (*Tapes decussatus* Linne, 1787) of bivalves were collected seasonally for analysis.

Aims of these studies are:

- to determine heavy metal concentrations on selected bivalves from study area
- to set a seasonal database of heavy metal levels
- to observe of variances among bivalve species, tissue types and seasonal changes.
- to compare with standard limits for health risk assessment

CHAPTER TWO
HEAVY METALS

2.1 Heavy Metals

Heavy metals are group of elements that includes transition metals, lanthanides, actinides, and the metalloids, also their atomic density greater than 4g/cm^3 or 5 times or greater than water. Their atomic number starts with 21 of Scandium (Hogan, 2011). Heavy metals are physically shiny, good electrical and thermal conductors, have generally higher melting and boiling point than nonmetals, ductile, and malleable materials (Atkins & Jones, 2010). Heavy metals are shown in the periodic table on Figure 2.1.

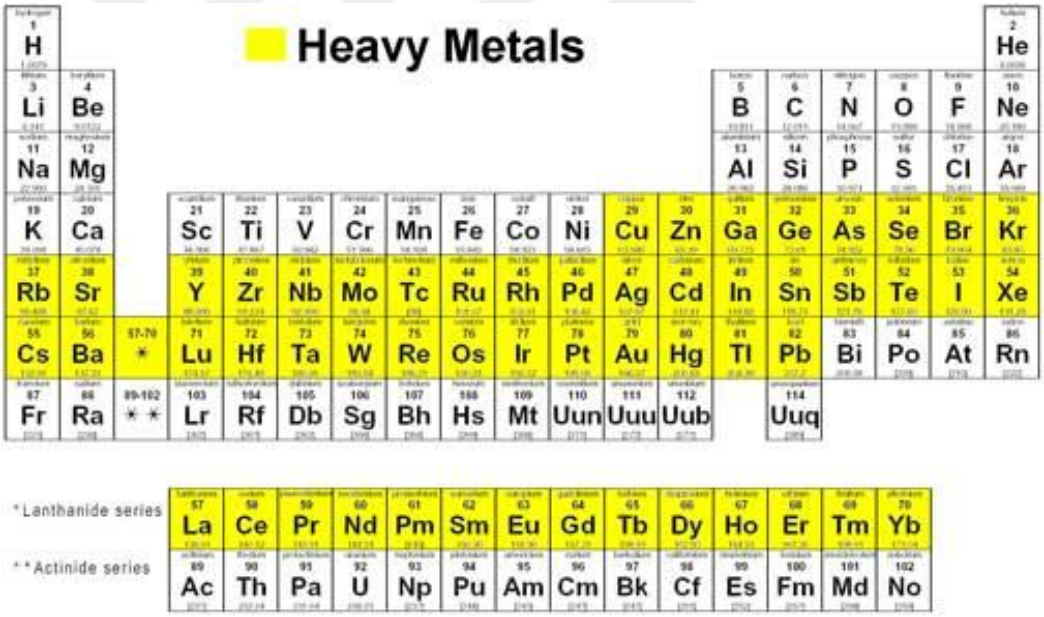


Figure 2.1 Periodic table of elements with heavy metals highlighted.

In general, the organic derivatives of the metals are more toxic than the parent inorganic metal, with the alkyl derivatives bearing greater, such as organotins. They are organometallic compounds that involve tin – carbon bonding. Especially tributyltin (TBT) compounds used as biocide and antifungal material on the industry.

TBT accumulates rapidly on the food chain. Areas that TBT distributed widely, the health deficiencies are recorded on marine biota (de Mora, Fowler, Cassi & Tolosa, 2003). Also methyl mercury and tetraethyl lead, some of the metallo-organic compounds are the toxic compounds. The danger of discharging some of the metals into the environment in inorganic form lies in their conversion into the highly poisonous metallo-organic compounds through biological action, as was discovered not too long ago with mercury (Waldichuk, 1974). On the contrary that other organometallic compound can be less hazardous then their inorganic derivatives such as organoarsenic compounds. For example arsenobetaine is a non-toxic substances in marine environment, but another organoarsenic compounds like dimethylarsine and trimetylarsine are toxic (Pellerito et. al, 2002).

When an organism bioaccumulated the heavy metal compounds, impacts could be quite different on its body. Some metals are essential for life (e.g. iron, manganese, zinc, and copper) but, excessive limits of them might be toxic. By the way, they also called “toxic metals”. Heavy metals like mercury, arsenic and lead can be harmful for living organisms when their bioconcentration increase (Mason, 2013). Metal toxicity is strongly correlated with its ionic form (oxidized or reduced state), complexation condition such as chelationg agent, and adsorbed on an inorganic or organic particulate material (Waldichuk, 1974).

2.2 Heavy Metals and Aquatic Systems

Heavy metals have a global cycle in our world. It begins from their ores and transports to the atmosphere or surface waters. At first, volcanic activities and oceanic hydrothermal inputs are the main sources for metal extraction, but nowadays anthropogenic facilities are the basic explanation for the metal cycle. Also, erosion activities and depositions are firmly effective of transportation heavy metals to the seawater. Detailed progression chains are given on Figure 2.2.

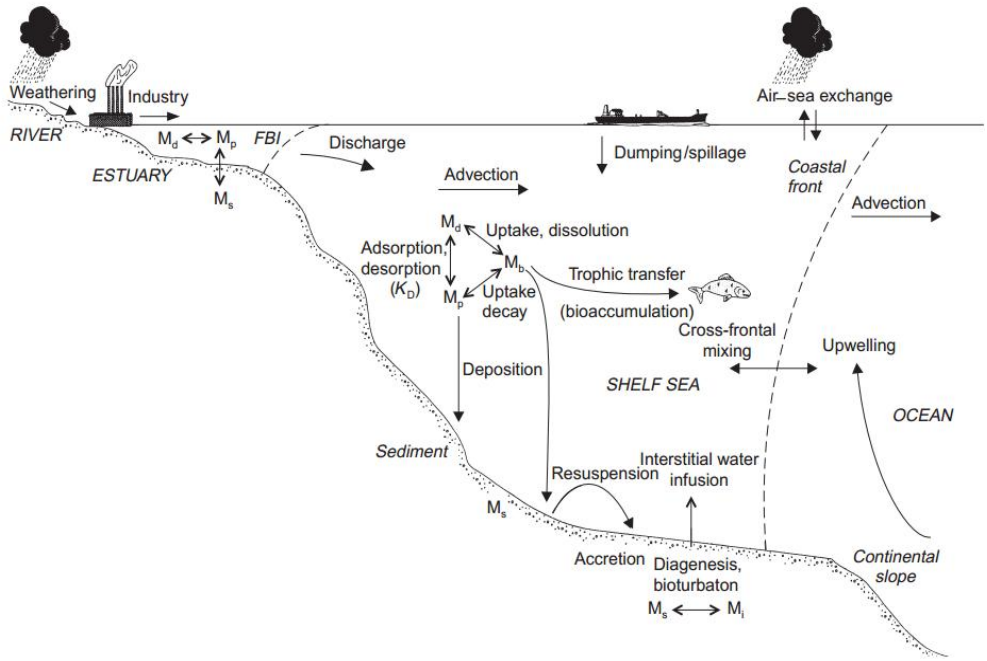


Figure 2.2 Processes affecting the transport and biogeochemistry of metal pollutants in estuaries and shelf seas. (Millward & Turner, 2001).

Metals often move on earth crust as a particulate shape. These are suspended solids in water bodies and aerosols in the atmosphere. Also some metals like selenium, mercury or arsenic are volatile enough, so they transport easily as gas phase (Mason, 2013).

In aquatic systems, amount of metal concentrations depend on their complexation affinity, solubility, tend to organic and inorganic compounds, and form complexes with dissolved natural organic matter (Mason, 2013). Due to their major role in the transport and storage of contaminants, sediment quality is vital for aquatic environment. Today, sediments are used for identify the toxicants, dispersion patterns and locate contaminant discharges in aquatic systems. Also, sediments are widely used for a monitoring tool to detect environmentally changes (Birch, Taylor & Matthai, 2001).

Human activities are the main cause of metal pollution. According to USEPA's results, 19 percent of metals come directly to the water treatment plants from the household products. Also current water treatment plants have an ability to remove 70 to 90 percent of metals mostly from the water bodies (Garbarino et al, 1995).

As a result, using of metal and metalloid compounds in industrial, agricultural or anthropogenic ways has taken part in global contamination of watersheds. On the predictable near future, this continuum won't be able to decrease swiftly. For this reason, metal inputs on water sources and their bioaccumulation by marine organisms have to be observed continuously to see the impact on environment. (Mason, 2013).

2.3 Heavy Metals and Marine Organisms

Many chemical components are available with low amounts in natural waters, marine sediments and aquatic organisms. Nevertheless, discharge of anthropogenic based polluted water increase the chemical concentrations. Chemicals that distributed the seawater may be consumed by marine organisms and accumulated by their tissues and organs. Interactions between tissues and chemical can harm the organism, also it can reach to human with biomagnification at food chain (Neff, 2002).

Aquatic organism contact chemicals in feeding, absorption or adsorption way firstly. When the organism exposes the chemicals, bioavailability starts for it. Bioavailability can be described as portion of chemicals that transport to organisms' circulatory system (Semple et. al, 2004). Pollutant molecules can be found as a part of solution component or amount of contaminant in organisms. Therefore, we can define them as bioavailable. Metal molecules in bodies can react variously according to their amounts. They can be a part of enzyme substance as a cofactor or the can harm cells and tissues mortally (Roosa et. al, 2014).

There are some differences about absorbing metal for organisms. For example, advanced terrestrial animals uptake metals on their food and transport them to their digestive system, and invertebrates usually absorb metals over the body surface. But, aquatic organism gain toxic metals from water and transport them gills and entire bodies. Distribution of metals in bodies depends on metals' species and their amounts usually stem from changes in the physicochemical characteristics of water.

Some metal molecules (e.g. cadmium and copper) can be absorbed as hydrated ions, but their bioavailability can occur toxicity (Bjerregaard & Andersen, 2007). Biological membranes can be diffused lipophilic metal components or organometals

easily, but metal complexes with free hydrated ions cannot pass membranes of cells. They need the other diffusion systems such as endocytosis, facilitated diffusion and ATP-dependent uptake as shown on Figure 2.3.

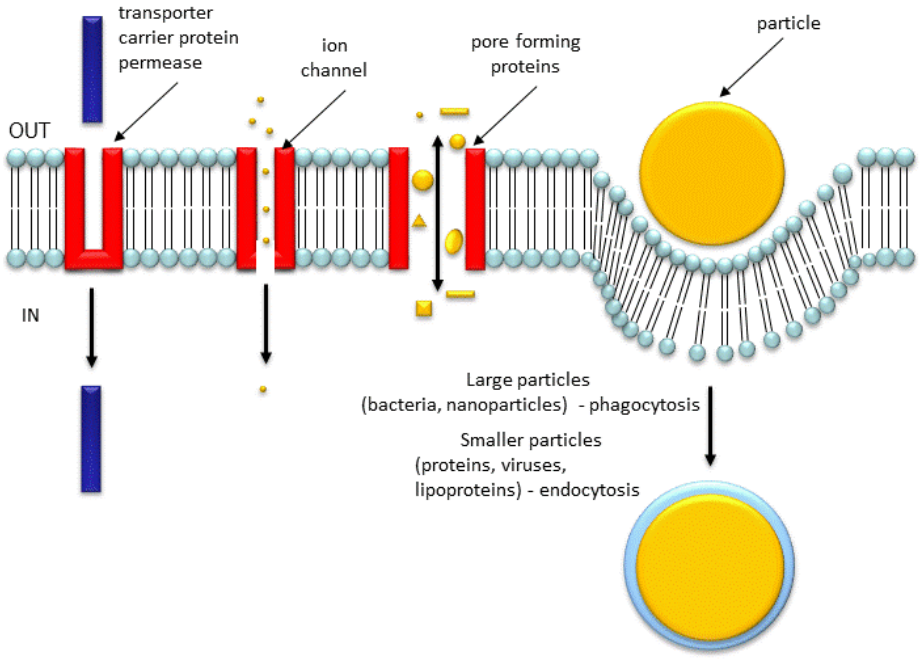


Figure 2.3 Movement of molecules and particles through Membranes (Jakubowski, 2014).

2.3.1 Metal Exposure and Bioaccumulations at Marine Organisms

Chemical molecules and marine organisms are always having an interaction with each other in seawater. When an organism takes into metal ions by feeding or by adsorption, bioaccumulation process has started for it. Bioaccumulation can be defined as uptake and retention of chemical components into organism from all available sources (Neff, 2002). Bioaccumulation quantities depend on metals' concentration and speciation in the environment. Their amounts on water bodies are strongly related waste water loads caused by urbanization and environmental factors (Hernández-Almaraz et al., 2014). Bioaccumulation is caused by some biotic factors such as mass, dietary type, metabolism, sex and, age. Also, it has some physical factors like positions in the trophic web or temperature, pH and salinity of water (Jakimska, Konieczka, Skóra & Namieśnik, 2011).

Whenever metal molecules are bioavailable in water, they can be bioaccumulated. It happens through the food chain. Food chain defined as "a series of organisms interrelated in their feeding habits, the smallest being fed upon by a larger one, which in turn feeds a still larger one, etc." (Collins English Dictionary, 2014).

Microorganisms are unable to decrease the metal molecules directly. On the contrary that, they can accumulate on the food chain with increasing concentrations as described Figure 2.4. It is called "biomagnification". Therefore, increasing amount of metals can be hazardous for aquatic organisms and humans because of their persistent structure (Zhang et al., 2013).

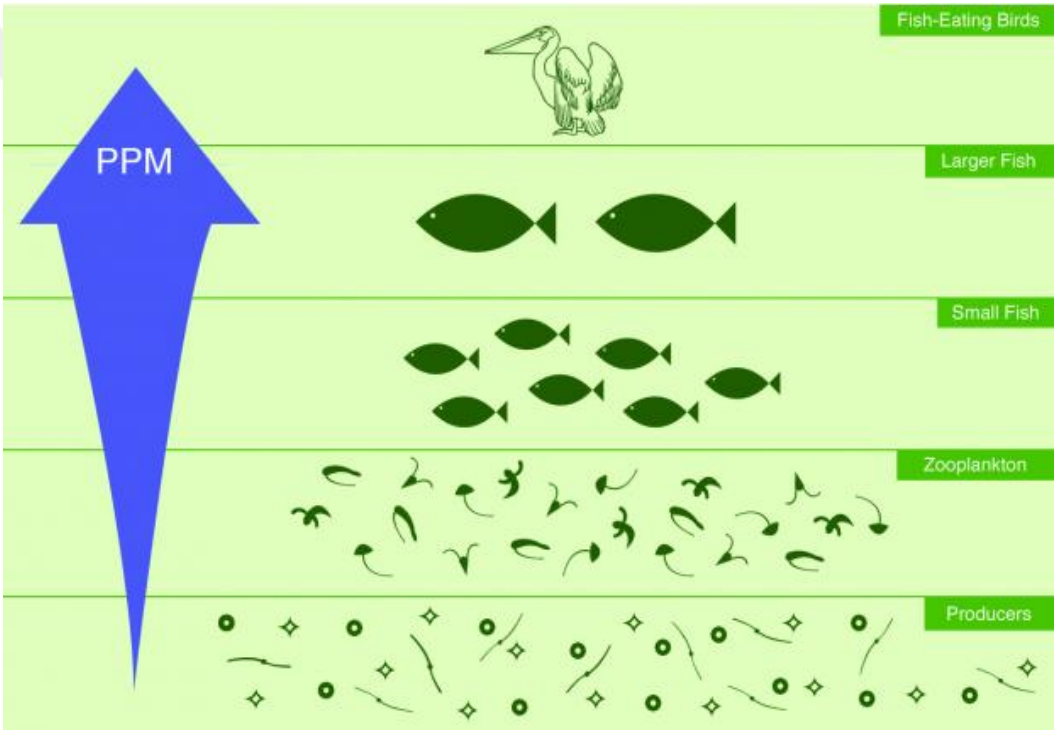


Figure 2.4 Bioaccumulation and biomagnification through the food chain (Gitalis, 2012).

Bioaccumulation initiates from all environmental sources such as rocks, air sediments and pass through the food chain. But, bioconcentration is specific part of bioaccumulation that involves uptakes and retentions only on water (Neff,2002).

In the progress of metal bioaccumulation, organisms tend to evacuate the components, but they cannot manage it fully. Organisms keep some metal particles in their bodies to cumulate or using for biological reactions. If the bioaccumulation is intense and continuous, organisms may have a harmful exposure. Bioavailability of

metal components shows various effects in aquatic organisms. For example, lead involves in lipid substances on the cellular membranes and it causes serious problems on membrane integrity, dysfunctional behaviors and increasing susceptibility on the membranes. Excessive consumption of lead also induces reactive oxygen species production which is a vital part of antioxidant mechanism on the body. Because damaged antioxidant system is unable to repair DNA-damage. If lead accumulation spreads the mitochondria, cells will eventually lose their energetic balance and cell deaths will happen (Taylor & Maher, 2012).

2.3.2 Using Marine Organisms as a Bioindicator

Over the years, researchers tried to figure out a way that observing the changes of environment continuously. Therefore, they realize that organisms which live in their habitat can describe something about condition of the area and the called them "bioindicators" (Lazorchak et. al, 2003).

Bioindicator species is taken a part of observing environment, quality of the environment and how it changes within time. Varieties can be related about anthropogenic sources or natural stressors. After development of the researches, bioindicators assist widely to determine environmental factors (Holt & Miller, 2010).

Biomagnifications in marine biota effect directly human health through the food chain. Therefore, observing exposure process in aquatic organisms were getting crucial. Past studies showed that mussel/bivalve species are known as the most common bioindicators. They accumulate the pollutants into their bodies naturally because of their filter feeding dietary type. Their bioaccumulation amounts are strongly related to metal concentrations of environmental conditions they live (Catsiki & Florou, 2005).

The condition of aquatic environment can be monitored using by various methods depend on analyzing water, sediment or biota. These methods are chemical-based. These chemical processes are both expensive and time consuming and specialized research centers. Also, they required frequent sampling to detect any unpredicted evolution quickly. For these reasons, the use of bioindicators to determine the pollutant level in the habitat is gained importance. Therefore, bioindicator species

state both condition of environment and their harmful effects of pollutants on ecosystem (Selvin, et al., 2008).

Pollutants on estuaries and seawater are accumulated by most of aquatic organisms, especially invertebrates. Bivalve species such as mussels, clams and oysters are the one of the first choice on selecting bioindicators. Their filter feeding dietary type and inadequate motion capability can reflect availabilities of pollutants on their habitat. Also, because of their superior adaptation ability they hardly ever effect on pollutants' lethal effect. Their accumulation is usually caused by from three sources which are water, food and particulate matters. Selected organism should express the same correlation between the bioaccumulated pollutants in their body and amounts of their surrounding environment for using them as a bioindicator. (Yusof, Yanta & Wood, 2003).

2.4 Toxicity of Heavy Metals

Industrial using and discharging metals bring along some serious problems, especially heavy metal toxicity. All organisms can coincide metal toxicity, when its consumption reaches the specific excessive limit for any time. First recognition of metal toxicity was found on the terrestrial animals, surprisingly. Aim of the first studies was about determining the mercury contamination caused by farming facilities. But, understanding of metal toxicity was developed after researches on aquatic organisms. They detected acute metal exposure at first from fish gills. After that, selective analytical methods were developed to quantify bioavailable amounts of total pollutants. (Bjerregaard & Andersen, 2007).

Metals can respond as both ions and compounds. Metal toxicity generally stems from metals' oxidation state and solubility. Metal compounds can interact many types of metabolic structures such as cell membranes, enzyme substances and specific cell components. Also, metals tend to bound on blood cells. This tendency causes the metal transport to target organs and eventually organ failure. For example, lead can deposit easily on skeletal system and transport to central nervous system. Cadmium can accumulate on kidneys and liver. Generally metals can ingest with food, but

rarely organisms can take metal components by inhalation. Besides these, some metals are essential for being healthy (e.g., copper, chromium and selenium). Also, metal toxicity affects harder on younger and older population members (Gad, 2014a).

Metallic compounds can respond number of options that tendency of effects. Feeding, constitutional and physiological factors can change the susceptibility. Metal interaction into cells explains some answers as metabolic changes. They can cause some interfering environmental factors in dose-response relationship (Nordberg, Gerhardsson, Broberg, Mumtaz, & Ruiz, 2007).

Aluminium is the third most abundant element on Earth's crust which includes 8% of the proportion. Aluminium cycle contains geochemical formations to marine environments, adsorption on sediment particulates, and depositions from air to soil or water. Aluminium has +3 oxidation state and it doesn't exist in elemental state. Most common compound is aluminium oxide. Also it can exist various ligand exchange reactions. It naturally occurs in water bodies, air and soils. Aluminium concentrations are usually stabile but ore facilities, productions and processing can reflect higher levels (Gad, 2014b). Humans can accumulate aluminum from dialysis treatment, aluminium-contained drugs or feeding that usually originated from canned products. Also aluminum is a neurotoxicant material. Almost half of aluminium store in skeletal system in the body. Lung contains 25% of them and the rest of aluminium distributes the body burden (Sjögren, Iregren, Elinder & Yokel, 2007).

Cadmium exists as cadmium oxide and generally originated form zinc-ore precessing. Cadmium ions and compounds settle easily into sediment and increase their amounts continuously. Therefore, bioaccumulation and biomagnification are progressing through the food chain (Gad, 2014c). Free cadmium ions are more potential threat of lethal effects. Resistance to cadmium is lower in freshwater bodies then the marine ecosystems. It is well known that marine invertebrates are deeply resistant (Eisler, 2000). Cadmium ions obstruct plasma membrane calcium channels and Ca-ATPase compounds. It also prevents DNA to repair itself. Metallothienin forms of cadmium are very harmful on kidneys (described as nephrotoxic). In ecosysytems, cadmium is generally transported by rivers. Besides these, humans can face cadmium toxicity from spring water because of using galvanized zinc and Cd-

containing pipes to reveal water from the source (G.F. Nordberg, Nogawa, M. Nordberg & Friberg, 2007).

Chromium may be found at 9 oxidation states that are from -2 to +6 in nature. But, in seawater, chromium ions often occur +3 and +6 states. Cr(VI) is more soluble than the Cr(III). Especially metallurgical facilities in industry are the main reason of hexavalent chromium production on water sources. This production can cause hazardous impacts on marine life. Chromium is able to attach sediment structure strongly. For this reason, Cr-contaminated aquatic foods generate high risk for human health. Past studies show that chromium deposits firstly in liver, kidneys, heart, and lungs. Cr can be found both newborns and adults (Gad, 2014d). When marine organisms bioaccumulate the hexavalent chromium, they readily reduce the ions to trivalent chromium (Neff, 2002). The uptake of chromium through the air is quicker than the digestive way. Air-based Cr is primarily +6 states (Langard & Costa, 2007). Newborns can make a contact to chromium through the breast milk. Besides these, Cr(III) is an essential ion that provides the body use carbohydrates, protein, and fat compounds (Gad, 2014d).

Cobalt isn't one of the most abundant elements on Earth's crust, but it's an essential for being a part of vitamin B_{12} (cobalamin). Cobalt exists particulate form in air. In seawater Co(II) can be occurred chloro, sulfato or carbonato complexes.In shallow waters, Co can be found usually both on particulate matter and the sediment (Nolan, Fowler & Teysie, 1992). When Co molecules reach to surface water, they directly move into sediment. Also rivers transport the 75% of total Co inputs on seawater. Co can occurs +1 to +5 ion forms, but Co(II) and Co(III) are the most stable ones (Gad, 2014). Co intake usually comes from solubility. Dietary accumulation is the rare way. Workers in alloy production and ore facilities generally encounter Co-based diseases (Lison, 2007). Cobalt sources are both anthropogenic and the natural. Appearing of Co on marine food chain is very limited. Co molecules hardly ever occurs biomagnification process. If Co compounds accumulate by an aquatic organism, they often store the non-edible parts of the body (Gad, 2014e).

Copper is an essential nutrition for aquatic life. Therefore, it's named as "trace minerals". Cu ions take part of numerous enzyme structures which are Cu-dependent.

They generally exist in organometallic form in the body (Lin, Shih, Megan & Shiau, 2010). Free ionic Cu^{2+} exists about 5% of total Copper level. Most abundant compound is $CuCO_3$. Copper concentrations are inversely correlated with the salinity level in seawater. Dissolved Cu concentrations tend to be higher in estuaries and coastal waters. Also, high level Cu concentrations can be observed on polluted zones. Copper has a strong affinity for complexation, such as humic substances and dissolved organic matter. Most of aquatic organisms have evolved their metabolic process to regulate the Cu concentrations inside their body so far, because copper is essential for organisms. It is well known that bivalves can accumulate the maximum available concentration without any hazardous threat (Neff, 2002). Although it is named as essential, Cu can be taken part of potential toxicant on elevated levels in seawater. Some research showed that Cu can be existed as ionoregulatory toxicant that reducing sodium ions and causing metabolic inhibitions (Giacomin, Gillis, Bianchini & Wood, 2013).

Iron is the most abundant trace element on Earth. Fe ions tend to interact oxygen molecules and change their valence to form a complex. This complexation happens nearly all aerobic organisms. Iron ions occur almost never by itself in nature. They generally form compounds. Most of iron compounds are water soluble. They provide relative higher concentrations on seawater (Gad, 2014f). On the other hand, analyzing iron forms in marine ecosystem is very hard. They can be found as coating on other minerals. Even less well known marine microorganisms have the generation of iron phases. Bacterial cells can include different types of iron forms that almost same as abiotic precipitation from inorganic solutions (Gillan & De Ridder, 2001). Iron is also an essential mineral. Its deficiency can cause some serious health problems. Fe complexes generally exists ferritin (storage protein), transferrin (Fe^{3+} carrier protein) and hemoglobin (oxygen transport metalloprotein) in the body (Ponka, Tenenbein & Eaton, 2007).

Lead is one most hazardous metal in the Earth. Lead poisoning has been known for centuries. It can cause both acute and long term adverse health effects. Environmental effects from lead are the one of main problem because lead concentrations are still toxic enough for human health. Also, lead can cause

mutagenic and teratogenic impacts when it is taken at excessive amounts (Eisler, 2000). Decades ago main source of lead pollution was leaded fuel. Leaded fuel contained huge amount of tetraethyl and tetramethyl lead and these organolead components could be taken rapidly by humans. Therefore, worldwide lead pollution has increased dramatically after Pb-contained petrol banned on many countries (Skerfving & Bergdahl, 2007). Lead concentrations are lower on open seas then coastal water bodies. Industrialization is the main input source of lead. In seawater, Pb generally tends to move sediments. By the way, many high level measurements were found on past studies. Invertebrates, especially benthic organisms can face lead bioaccumulation more often. Most bioaccumulated lead form is $PbCO_3$. Marine organisms store lead components in liver, hepatopancreas or kidney mostly. Toxic levels on lead can cause numerous lethal and sub-lethal effects, especially abnormal development on larvae of marine organisms, according to past studies (Neff, 2002). Lead particles can also bring about some problems to all ecosystem. Water fowls may become poisoned from lead consumption. Poison can be lead to coma and death (Gad & Pham, 2014).

In natural waters Mn is found as Mn(II), Mn(III) and Mn(IV) ions. In the highly oxidizing, Mn can be found particulate form of MnO_2. Also, most abundant water soluble Mn oxidation state is Mn^{2+} (Von Langen, Johnson, Coale & Elrod, 1997). Manganese inputs on water are usually related to industrial facilities. Some manganese compounds are readily soluble that they can contaminate the drinking water sources. Also, manganese ions can absorb to sediment particles or make an oxidation then settle to the bottom on the sea. Manganese can be bioconcentrated significantly at lower levels. Manganese is of the essential element for humans, but long term exposure on higher concentrations can cause some neurological effects and lungs are the second target organs on manganese exposure. Therefore, some reports presents that lower organisms such as algae have larger bioconcentration factors (BCF) than the higher organisms such as vertebrates. Mn in water can be effected oxidation at high pH level; it can cause microbial activity (Saric & Lucchini, 2007).

Mercury and its compounds haven't known any metabolic reactions. Their existance in the organisms only derived from anthropogenic sources. All types of

mercury contaminations must be probably toxic (Eisler, 2000). Mercury occur three oxidation states which are zero (elemental mercury), +1 (mercurous compounds) and +2 (mercuric compounds). Hg(II) is most abundant form of mercury in marine ecosystems. Generally Hg(II) can be occurred as $HgCl_2$ and some relative chloride compounds. According to some researches, $Hg(OH)_2$ can become the dominant species if mercury ions exist in low salinity estuarine waters. Mercury can be found both inorganic and the organic form. Inorganic Hg is more abundant than organic mercury in seawater and sediments; organic mercury usually is more abundant than inorganic mercury in air and tissues of marine organisms (Neff, 2002). Mercury partioning is show in detail on Figure 2.6.

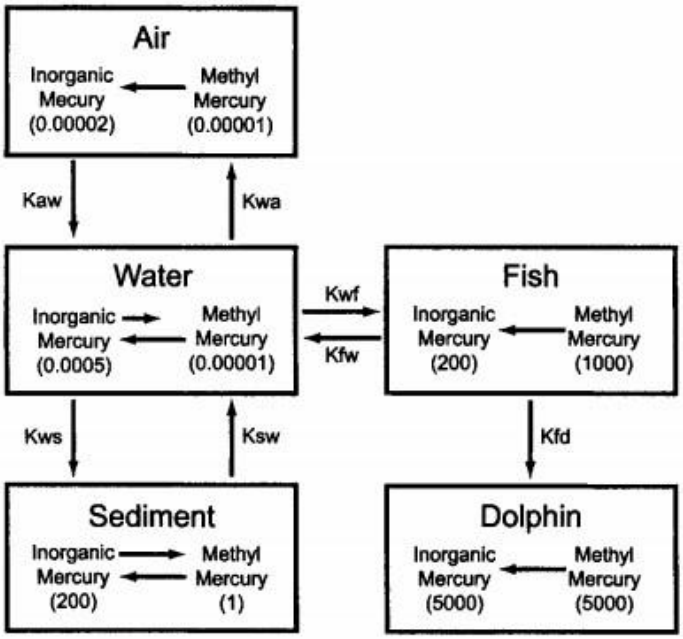


Figure 2.5 Compartmental model of partitioning of inorganic mercury and methyl mercury among air, water, sediments, and marine animals of the Mediterranean Sea (Neff, 2002).

Hg forms have strong affinity to lipids; they can generate complexation with lipids and make a movement together across the cell membranes which can interfere with cell metabolism. Mercury compounds may be bioaccumulated lots of marine organisms and transferred with biomagnification along the food chain. This process is one of the main threats for human health (Coelho et. al, 2010).

Nickel is one of the heavy metals that listed on European Commission as dangerous substances, because of its toxicity. Nickel can be reached to environment natural or human-based activities. Anthropogenic Ni entrances are mainly coastal mining, sewage disposal and atmospheric deposition. It's well known that Ni concentrations are tend to reduce on the offshore (Leonard et. al, 2011). Chemical processes in industrial areas provide them to transport biological mechanisms of living organisms. +2 oxidation state is most stable Ni ions and nickel can be generally found its salts. Presence of nickel salts can make an impact on bioavailability and bioaccumulation (Eisler, 2000). Ni compounds have used globally for decades, but Ni toxicity has started decreasing recently. Nickel can affect sodium balance and lipid metabolism on body. It can prevent metallothionein synthesis mechanism. T-cell system and suppresses the activity of natural killer cells. Therefore, immune system may be harmed significantly (Gad, 2014g).

Zinc is environmentally ubiquitous and essential for life. Common oxidation state of Zn is +2. It has a tendency to react non-metals such as oxygen and sulfur. At mid-range pH, Zn occurs hydroxides which are less soluble in water. At the extreme of pH, its solubility is increased. In water Zn tend to form inorganic (e.g. $Zn(H_2O).5Cl^+$), or organic (e.g. Zn-humate) complexes (Sandstead & Au, 2007). Zn occurs in marine sediments widely. Because of being essential element, marine organisms accumulate zinc components from the seawater. Although zinc is essential, excessive levels on zinc can interfere copper and iron mechanism on tissues; the latter leads to anemia. According to reports zinc concentration's trend tends to decrease over the years (Gad, 2014). They use zinc as a cofactor nearly 300 different enzyme reactions. They can regulate the level of zinc concentrations in their body naturally. Complexed organic ligands are less available to benthic organisms. Aquatic organisms can bioaccumulate zinc compounds from food, water and sediments. Some species such as *Neanthes arenaceodentata* can accumulate free ionic zinc but not organozinc complexes. In mollusks, zinc is found on their digestive gland primarily (Neff, 2002).

CHAPTER THREE
STUDY AREA

3.1 General Description of Study Area

Lagoon is shallow body of water separated from the sea by a barrier which lies parallel to the shoreline. There are more than 76 lagoons in our coasts. One of them is Homa Lagoon which is affected by Gediz River and it is located at the northwest side of İzmir Bay.

It has two divisions called Main Lagoon (Homa Lagoon) and Small Lagoon (Kırdeniz Lagoon). The lagoon has a freshwater cycle between the lagoon and the sea, and the cycle is supported by channels with various widths. Gediz River, one of the most important rivers in Aegean Region, is located in West Anatolia and it sets off one of the biggest basin in our country. Besides, the large agricultural lands in the basin, there are a lot of industrial complexes like metal processing, mining, chemistry, food, paper and, leatherwork. Organic and inorganic pollutants originated from Gediz River are strongly affected Homa Lagoon and Gediz Delta (Uluturhan, Kontaş & Can, 2011). Homa Lagoon is neighbor to İzmir Bird Paradise which is one of the protected areas according to Ramsar Convention. The lagoon has been allocated to Ege University - Fisheries Faculty for teaching and researching facilities since 1986 (Tosunoğlu & Ünal, 2012). Also, Çamaltı Saltpan which is one of the main table salt production areas are located in the south of Homa Lagoon.

Homa Lagoon and Kırdeniz Lagoon are taken up approximately 1800 hectares area combined. Mean depth is 50-150 cm and the maximum depth is about 150 cm. In lagoon, annual fish production mean is about 50 tons, but recent years the production rate has decreased dramatically to 15 tons due to insufficient freshwater input, hypersaline and shoaling factor. Fishing is carried out from June to January. In January, six lagoon straits are opened to provide fish inputs. Then, straits are closed in June and grown fish are carried out fishing in fish traps. Several commercial fish species are recorded for fishing such as grey mullets (*Mugil cephalus, Liza saliens, Liza ramada, Liza aurata, Chelon labrosus*), gilt-head sea bream (*Sparus aurata*), european sea bass (*Dicentrarchus labrax*), common sole (*Solea solea*) and, eel

(*Anguilla anguilla*). Also, caviar production from *Mugil cephalus* has been carried out in the lagoon (Tosunoğlu & Ünal, 2012).

3.2 Previous Studies

Many studies have been performed in the Homa Lagoon. Therefore, the main topic of the studies is about fisheries. Besides these, there are lots of studies such as physics, chemistry, biology, zoology and, marine sciences.

There are 19 Master and PhD thesis titled "Homa Lagoon" in the Council of Higher Education's (YÖK) archive. These are Sıkı (1985), Korkut (1989), Gurbet (1989), Kocabaş (1990), Önen (1990), Sunlu (1994), Tekinay (1995), Tolon (1997), Perçin (1999), Akyol (1999), Cihaner (2001), Önsoy (2002), Yazıcı (2005), Balık (2006), Yürür (2008), Sabancı (2008), Kutlu (2009), Can (2009) and, Sapancı (2013).

Studies about heavy metal analyses can be divided two main groups which are analyses in sediments and organisms from Homa Lagoon. Atılgan and Egemen (2001) were detected the Cu and Zn levels in sediments ranged between 12.8-22.8 mg/kg and 34.2-86.2 mg/kg respectively.

Uluturhan, Kontaş & Can (2011) were investigated some heavy metals in sediments from several stations in this lagoon. They found heavy metals concentrations ranged between 0.22-0.48 mg/kg for Hg, 0.06-.0.19 mg/kg for Cd, 2.43-17.2 mg/kg for Pb, 83.9-129 mg/kg for Cr, 10.3-25.8 mg/kg for Cu, 410-729 mg/kg for Mn, 58.1-108 mg/kg for Ni, 46.2-91.9 mg/kg for Zn, 17054-30234 mg/kg for Fe, 12663-42637 mg/kg for Al and, 11.4-37.5 for Li.

Taş, Ergen & Sunlu (2009) were searched some heavy metals in sediments and ragworms (*Hediste divesicolor*) which is one of the indicator species from the Homa Lagoon. They determined the maximum levels in sediments; 1.16 mg/kg for Cd, 90 mg/kg for Cr, 51.30 mg/kg for Cu, 19.10 mg/kg for Pb, 95.50 mg/kg for Zn and, 27800 mg/kg. In ragworms, they found 0.31-0.34 mg/kg for Cd, 51-62 mg/kg for Cr, 27-29 mg/kg for Cu, 11-13 mg/kg for Pb and 57-59 mg/kg for Zn.

Çelik & Oehlenschlager (2005) were determined Zn and Cu bioaccumulations on some commercial fish species in this lagoon. They found the mean Zn levels 5.56±0.02 mg/kg on gilt-head sea bream (*Sparus aurata*), 4.40±0.03 mg/kg on horse mackerel (*Tracharus tracharus*), 4.07±.0.43 mg/kg on derbio (*Trachinotus ovatus*), 3.71±0.10 mg/kg leaping grey mullet (*Lisa saliens*), 4.33±0.18 mg/kg on Thin-lipped grey mullet (*Lisa ramada*) and, 3.12±0.10 mg/kg on red pandora (*Dentex machroptalmus*). For mean Cu levels; 0.30±0.11 mg/kg on gilt-head sea bream (*Sparus aurata*), 1.14±0.03 mg/kg on horse mackerel (*Tracharus tracharus*), 0.27±.0.01 mg/kg on derbio (*Trachinotus ovatus*), 0.48±0.05 mg/kg leaping grey mullet (*Lisa saliens*), 0.41±0.02 mg/kg on Thin-lipped grey mullet (*Lisa ramada*) and, 0.22±0.05 mg/kg on red pandora (*Dentex machroptalmus*).

CHAPTER FOUR
MATERIAL AND METHODS

4.1 Bivalves

4.1.1 Mediterranean Mussel (Mytilus galloprovincialis)

Phyllum : Mollusca

Subpyhllum : Bivalvia

Classis : Pteriomorphia

Ordo : Mytiloida

Familya : Mytilidae

Species : *Mytilus galloprovincialis*, (Lamarck, 1819)

Mediterranean mussel (*Mytilus galloprovincialis*) is one of the common commercial bivalve species (Figure 4.1). As it evident from its name, *M. galloprovincialis* is generally found in Mediterranean Sea and Black Sea. Also, it was recorded in the coast of South Africa, British Isles and Eastern China (Encyclopedia of Life [EOL], 2012).



Figure 4.1 The image of *Mytilus galloprovincialis*

Due their tolerance capability, *M. galloprovincialis* is one of the most invasive species in marine ecosystem. It can be the dominant species in very short time in

certain locations. *M. galloprovincialis* can grow up to 140 mm. Its average size is about 60 mm. In its native range, *M. galloprovincialis* can be found from exposed rocky outer coasts to sandy bottom and it holds the surface by using byssus fibers (Pagad, 2006). Distribution of *M. galloprovincialis* map was given on Figure 4.2.

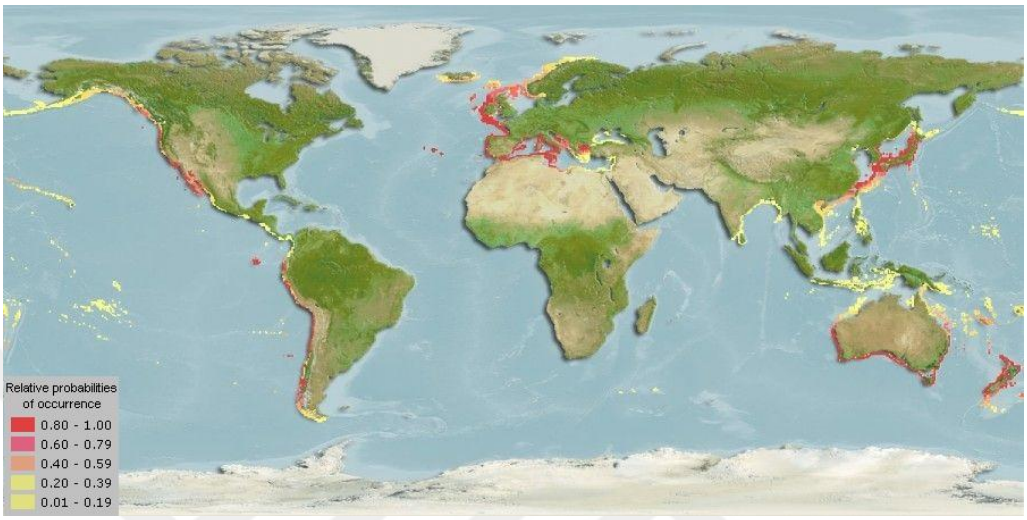


Figure 4.2 Distribution map for *Mytilus galloprovincialis* (EOL, 2012)

It often presumes as *M. edulis*, but *M. galloprovincialis* has blue-violet color which is darker than *M. edulis*. *M. galloprovincialis* has filter feeding dietary type, so it can feed by filtering planktons into its body. The spawning period of *mussels* continues with rising in water temperature between spring to summer (Cáceres-Martínez & Figueras, 1998).

4.1.2 Carpet Shell Clam (Tapes decussatus)

Phyllum : Mollusca

Subpyhllum : Bivalvia

Classis : Heredonta

Ordo : Euheredonta

Familya : Veneroida

Species : *Tapes decussatus*, (Linneaus, 1758)

Carpet Shell Clam (*Tapes decussatus*) is one of the coastal and lagoonal bivalve species that provides quite profitable products (Figure 4.3). *T. decussatus* has broadly oval broaden shape. It distributes widely, especially in the Mediterranean region, it generally shows spread in coasts of Aegean Sea and Iberian Peninsula.



Figure 4.3 The image of *Tapes decussatus*

T. decussatus can grow up to 75 mm. Its average size is about 40 mm. It lives in muddy-sand sediments in the coastal zone. Also, it has feet for moving along the habitat or for digging the surface. It hasn't to live as commune. Distribution map are given on Figure 4.4.

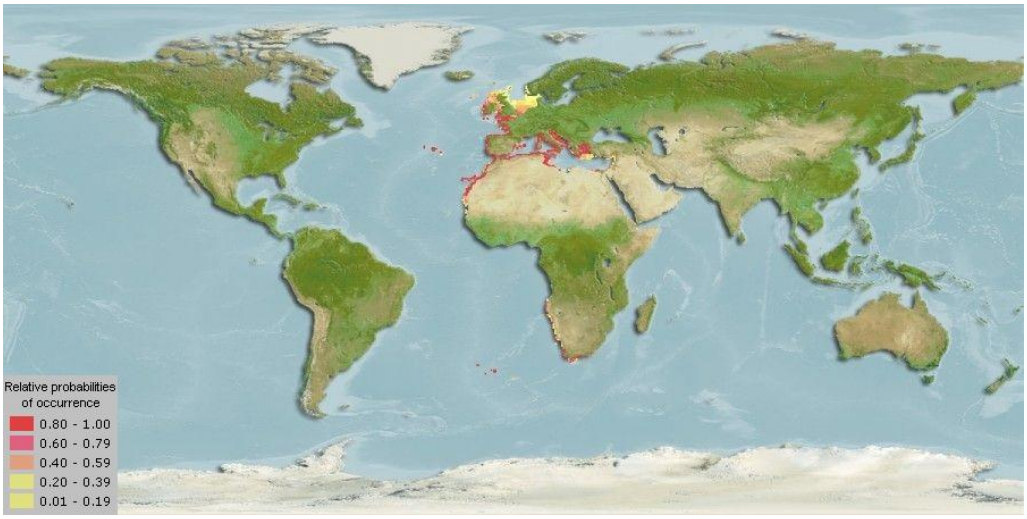


Figure 4.4 Distribution map for *Tapes decussatus* (EOL, 2012)

It often presumes as *T. philippinarum,* but *T. decussatus*' shell has crossed (decussated) pattern which *T. philippinarum* hasn't. Like *M. galloprovincialis, T. decussatus* has filter feeding dietary type, too. The spawning period of clam starts in July and continues into August and September at İzmir Bay (Serdar & Lök, 2009).

4.2 Sampling Designs and Analyses

4.2.1 Sampling and Preliminary Preparations

Bivalve samples were seasonally collected from the Homa Lagoon (May 2013, July 2013, October 2013 and, February 2014). Study area and the sampling points were given in Figure 4.5. Samples were transported to the laboratory by using vessels with fully seawater. Mussels were rinsed with sterile distilled water to remove epiphytes on the shell.



Figure 4.5 Map of the Homa Lagoon and the sampling points

Water quality parameters such as salinity, pH, and temperature and conductivity of seawater were measured with WTW and dissolved oxygen (DO) was measured in situ on sampling points at the Homa Lagoon. Results were shown in Table 4.1.

Table 4.1 Physicochemical parameters of sampling point from the Homa Lagoon

Season	Temp (^{o}C)	Salinity (ppt)	pH	Cond. (mS)	DO(mg/L)
May '13	25.6	38.0	8.42	57.0	6.95
July '13	23.5	39.9	8.37	59.6	8.08
Oct '13	17.0	40.8	8.66	61.5	7.40
Feb '14	13.7	34.9	8.26	53.9	7.83



Figure 4.6 Meristic measurements of bivalves from the Homa Lagoon

Meristic measurement (Table 4.2 and 4.3) were recorded, all samples were divided into the groups (Table 4.4) within their shell size and weight and hepatopancreas were dissected from soft tissues of the samples. Soft tissues contain gonad, gills, heart, mantle, foot and muscles.

Table 4.2 Meristic measurement ranges of *M. galloprovincialis*

Season	Size (mm)	Total Weight (g)	Soft Tissue Weight (g)	Hepatopancreas Weight (g)
May '13	38-58	5.6-15.1	0.5-3.6	0.1-1.0
July '13	44-82	9.3-50.1	1.0-8.2	0.2-1.2
Oct '13	51-70	10.2-35.6	1.2-5.6	0.3-1.3
Feb '14	39-55	5.6-19.5	0.1-2.1	0.1-0.4

Table 4.3 Meristic measurement ranges of *T. decussatus*

Season	Size (mm)	Total Weight (g)	Soft Tissue Weight (g)	Hepatopancreas Weight (g)
May '13	27-50	4.3-31.0	0.8-5.0	0.1-1.0
July '13	36-51	11.7-31.8	1.3-5.2	0.2-1.2
Oct '13	32-49	7.2-31.8	1.2-3.9	0.1-0.8
Feb '14	30-53	6.3-31.1	0.9-5.6	0.1-0.8

Table 4.4 Grouping intervals of bivalves from the Homa Lagoon

Seasons	*Mytilus galloprovincialis*		*Tapes decussatus*	
	n	**size range(mm)**	**n**	**size range(mm)**
May '13	20	51-58	12	44-50
	20	45-53	11	35-48
	20	38-46	13	41-44
			11	27-37
Jul '13	20	61-82	18	44-51
	20	54-62	18	42-46
	20	44-55	18	36-43
Oct '13	30	51-68	15	38-49
	25	62-70	20	32-38
	30	51-57		
Feb '14	13	43-55	22	42-53
	21	39-43	18	30-39

Separated soft tissues and hepatopancreas were weighted and recorded. After that, they were placed in Labconco Freeze Dryer System for at least 24 hours for dehydration. Dried samples were homogenized by using hand blender. Samples were packaged and stored in the refrigerator until the analyses. Samples were weighted approximately 1 gram and were digested with acid mixture solution (5:1 HNO_3:$HClO_4$ acid mixture) on Milestone 1200 Mega Microwave Digestion (Bernhard, 1976; United Nations Standard Programme [UNEP], 1982, 1984, 1985).



Figure 4.7 Freeze-dryer and microwave digestion systems

4.2.2 Heavy Metal Determinations

All metal analyses were performed by Varian Atomic Absorption Spectrometer (AA280FS & AA280Z). Cu, Mn, Zn, Fe and, Al concentrations were determined by flame technique. Cd, Cr and Pb concentrations were determined by graphite furnace technique. Finally, Hg concentrations were determined by cold vapor technique. All methods were exhibited according to UNEP (1985) methods. Ni and Co analyses were performed by ICP-MS. The detection limits for heavy metals are; Hg:0.05 µg/kg, Cd:0.10 µg/kg; Pb:0.10 µg/kg, Cr:0.10 µg/kg, Cu:0.03 mg/kg, Zn:0.01 mg/kg, Mn:0.02 mg/kg, Ni:0.004 mg/kg, Co:0.004 mg/kg. Fe:0.06 mg/kg, Al:0.30 mg kg. Accuracy and validity of the analytical processes tested with a reference material (National Institute of Standards and Technology [NIST] SRM 2976, USA). The reference and obtained values were given on Table 4.5.



Figure 4.8 Atomic Absorption Spectrometer

Table 4.5 Heavy metal levels of reference material NIST SRM 2976 mussel tissue (mean± SD mg/kg dry weight).

Metals	Reference Value	Obtained Value
Hg	0.06±0.004	0.07±0.005
Cd	0.82±0.16	0.76±0.18
Pb	1.19 ± 0.18	1.33±0.09
Cr	0.50 ± 0.16	0.61±0.03
Cu	4.02 ± 0.33	4.15±0.10
Mn	33.0 ± 2.0	35.8±0.67
Ni	0.93±0.12	0.97±0.04
Zn	137 ± 13	146±3
Fe	171 ± 4.9	171 ± 1.7
Co	0.61±0.02	0.54±0.01
Al	134 ± 34	132 ± 1.2

4.2.3 Statistical Analyses

Pearson correlations between size and heavy metal concentrations in tissues of bivalves were calculated (R> |0.50|). Statistical investigations were made for observing differences in heavy metal concentrations. All statistical calculations were made by using Statistica© 8.0 for Windows (StatSoft, 2007). Two-Way ANOVA test was performed for detecting significant differences among seasons, species and tissue types. Also, One-Way ANOVA was performed for detecting statistical differences between seasons and tissue types for each species. In all cases, the level of significant was set at $P< 0.05$.

CHAPTER FIVE
RESULTS

The seasonal minimum and maximum concentrations of heavy metals in bivalves were represented on Table 5.1 and 5.2.

5.1 Heavy Metal Concentrations in *Mytilus galloprovincialis*

5.1.1 Soft Tissue

In soft tissues; the lowest concentrations of Hg (0.095 mg/kg), Cd (0.221 mg/kg), Zn (79.75 mg/kg), and Co (0.298 mg/kg) were detected in May 2013. Concentrations of Pb (0.808 mg/kg), Cu (2.087 mg/kg), Mn (4.098 mg/kg), Ni (0,918 mg/kg), Fe (81.91 mg/kg), and Al (16.77 mg/kg) were found lowest in July 2013. The minimum level of Cr was obtained as 0.102 mg/kg in October 2013.

Highest Cr levels were determined in May 2013 as 6.206 mg/kg. The maximum concentrations of Cd (0.507 mg/kg) and Pb (2.473 mg/kg) were found in October 2013. Besides these, most of heavy metals reached their highest levels in February 2014. The maximum concentrations of Hg (0.154 mg/kg), Cu (5.627 mg/kg), Mn (13.57 mg/kg), Ni (2.441 mg/kg), Zn (231.5 mg/kg), Fe (476.5 mg/kg), Co (1.079 mg/kg), and Al (415.6 mg/kg) were detected at this month.

5.1.2 Hepatopancreas Tissue

For hepatopancreas tissues; Cd (0.287 mg/kg) and Zn (60.99 mg/kg) were measured as lowest concentrations in May 2013. Apart from these, the minimum concentrations of Hg (0.139 mg/kg), Pb (0.988 mg/kg), Cr (2.443 mg/kg), Cu (4.206 mg/kg), Mn (11.53 mg/kg), Ni (5.556 mg/kg), Fe (298.3 mg/kg), Co (1.365 mg/kg), and Al (344.7 mg/kg) were determined in July 2013.

Cr concentrations were reached the highest values in May 2013 with 12.89 mg/kg. The highest levels of Cd (0.482 mg/kg), Mn (35.39 mg/kg), Zn (146.9 mg/kg), and Al (1527 mg/kg) were obtained in October 2013. Besides, maximum concentrations of Hg (0.251 mg/kg), Pb (2.765 mg/kg), Cu (11.92 mg/kg), Ni (18.20 mg/kg), Fe (1647 mg/kg), and Co (3.660 mg/kg) were detected in February 2014.

5.2 Heavy Metal Concentrations in *Tapes decussatus*

5.2.1 Soft Tissue

The lowest concentrations of Pb (0.803 mg/kg), Cu (4.796 mg/kg), Mn (7.600 mg/kg), Ni (2.585 mg/kg), Fe (267.9 mg/kg), Co (0.504 mg/kg), and Al (259.7 mg/kg) was detected in May 2013. Zn and Cr concentrations were obtained 52.93 and 0.499 mg/kg respectively as minimum level in October 2013. Concentrations of Hg (0.073 mg/kg) and Cd (0.098 mg/kg) were found lowest in February 2014.

For soft tissue samples of *Tapes decussatus*, all highest concentrations of analyzed heavy metals were reached in the same period. Hg (0.132 mg/kg), Cd (0.329 mg/kg), Pb (1.354 mg/kg), Cr (13.37 mg/kg), Cu (9.566 mg/kg), Mn (18.22 mg/kg), Ni (6.276 mg/kg), Zn (82.21 mg/kg), Fe (805.6 mg/kg), Co (1.387 mg/kg), and Al (1009 mg/kg) were determined in July 2013.

5.2.2 Hepatopancreas Tissue

The results showed that; the minimum concentration of Pb (0.879 mg/kg), Cr (2.352 mg/kg), Fe (503.8 mg/kg), and Al (278.6 mg/kg) were detected in May 2013. Concentrations of Cd (0.124 mg/kg), Cu (13.72 mg/kg), Zn (80.41 mg/kg), and Co (1.135 mg/kg) were obtained as lowest levels of heavy metals in July 2013. Besides, the minimum values of Hg (0.146 mg/kg) and Ni (6.235 mg/kg) were determined in October 2013. Finally, the lowest Mn levels were measured 22.29 mg/kg in February 2014.

According to data, the maximum concentrations of Hg (0.281 mg/kg) and Zn (96.09 mg/kg) were detected in May 2013. Cd (0.527 mg/kg), Mn (31.23 mg/kg), Fe (2331 mg/kg), Co (1.942 mg/kg), and Al (2170 mg/kg) concentrations were reached the highest point in July 2013. The maximum Cr concentration was found 13.28 mg/kg at February. The highest concentrations of Pb (3.947 mg/kg), Cu (24.39 mg/kg) and Ni (10.31 mg/kg) were determined in February 2014.

Table 5.1 The minimum and maximum concentrations of heavy metals in *Mytilus galloprovincialis* (mg/kg dry weight)

	May 2013		**July 2013**	
	Soft Tissue	**Hepatopancreas**	**Soft Tissue**	**Hepatopancreas**
Hg	0.095-0.104	0.162-0.177	0.099-0.146	0.139-0.171
Cd	0.22-0.29	0.29-0.32	0.29-0.48	0.34-0.45
Pb	0.83-1.00	1.35-1.39	0.81-0.97	0.99-1.16
Cr	4.25-6.21	6.51-12.9	1.39-2.62	2.44-3.61
Cu	4.24-4.48	6.83-7.77	2.09-3.06	4.21-4.85
Mn	7.02-7.48	14.9-17.2	4.10-6.48	11.5-16.2
Ni	1.14-1.48	7.00-9.17	0.92-1.39	5.56-7.10
Zn	79.8-102	61.0-72.8	111-146	97.4-134
Fe	126-145	555-675	81.9-120	298-444
Co	0.30-0.38	1.46-1.67	0.48-0.74	1.37-1.98
Al	94.7-131	610-773	16.8-67.0	345-517
	October 2013		**February 2014**	
	Soft Tissue	**Hepatopancreas**	**Soft Tissue**	**Hepatopancreas**
Hg	0.128-0.139	0.190-0.217	0.127-0.154	0.241-0.251
Cd	0.44-0.51	0.39-0.48	0.34-0.47	0.38-0.42
Pb	1.00-2.47	1.40-2.04	1.09-1.53	2.45-2.77
Cr	0.10-0.32	6.86-12.4	1.78-3.32	6.77-9.40
Cu	3.18-3.42	6.78-8.11	5.28-5.63	11.0-11.9
Mn	6.90-8.80	20.8-35.4	10.6-13.6	23.9-32.1
Ni	1.20-2.11	9.31-13.5	1.88-2.44	15.2-18.2
Zn	118-189	93.5-147	126-232	88.4-98.6
Fe	194-286	648-1449	261-477	972-1647
Co	0.56-0.89	1.93-2.65	0.65-1.08	3.34-3.66
Al	117.3-235.3	730-1527	218-416	937-1520

Table 5.2 The minimum and maximum concentrations of heavy metals in *Tapes decussatus* (mg/kg dry weight)

	May 2013		**July 2013**	
	Soft Tissue	**Hepatopancreas**	**Soft Tissue**	**Hepatopancreas**
Hg	0.085-0.129	0.183-0.281	0.127-0.132	0.17-0.21
Cd	0.12 - 0.26	0.13-0.35	0.22-0.33	0.12-0.53
Pb	0.80-1.32	0.88-2.43	1.07-1.35	1.28-2.08
Cr	3.33-5.75	2.35-8.91	6.81-13.4	5.23-9.05
Cu	4.80-5.95	14.7-18.9	8.86-9.57	13.7-15.9
Mn	7.60-11.0	13.5-25.8	14.2-18.2	27.8-31.2
Ni	2.59-4.13	6.39-10.3	5.49-6.28	6.28-9.03
Zn	54.7-66.6	85.2-96.1	63.0-82.2	80.4-89.0
Fe	268-510	504-1580	633-806	1090-2331
Co	0.50-1.10	1.32-1.68	1.12-1.39	1.13-1.94
Al	260-438	279-1293	790-1009	1081-2170
	October 2013		**February 2014**	
	Soft Tissue	**Hepatopancreas**	**Soft Tissue**	**Hepatopancreas**
Hg	0.089-0.100	0.146-0.162	0.073-0.092	0.169-0.172
Cd	0.23-0.28	0.16-0.37	0.10-0.20	0.13-0.18
Pb	0.96-1.05	0.89-0.91	0.80-0.91	1.54-3.95
Cr	0.50-0.65	5.81-13.3	2.91-3.27	6.22-6.82
Cu	5.00-5.44	16.4-21.4	4.82-4.83	23.5-24.4
Mn	11.9-18.0	23.0-23.5	11.9-14.0	22.3-24.6
Ni	4.18-4.53	6.24-6.36	4.25-4.66	6.95-10.3
Zn	52.9-54.8	85.7-89.6	55.6-57.1	87.6-90.5
Fe	494-769	963-999	500-614	982-1133
Co	0.81-1.13	1.19-1.68	0.70-1.22	1.29-1.42
Al	473-786	816-880	421-619	862-1040

5.3 The Comparison of Metal Bioaccumulation between Bivalve Species

The mean concentrations of heavy metals with standard deviations for each species were showed in from Figure 5.1 to 5.6.

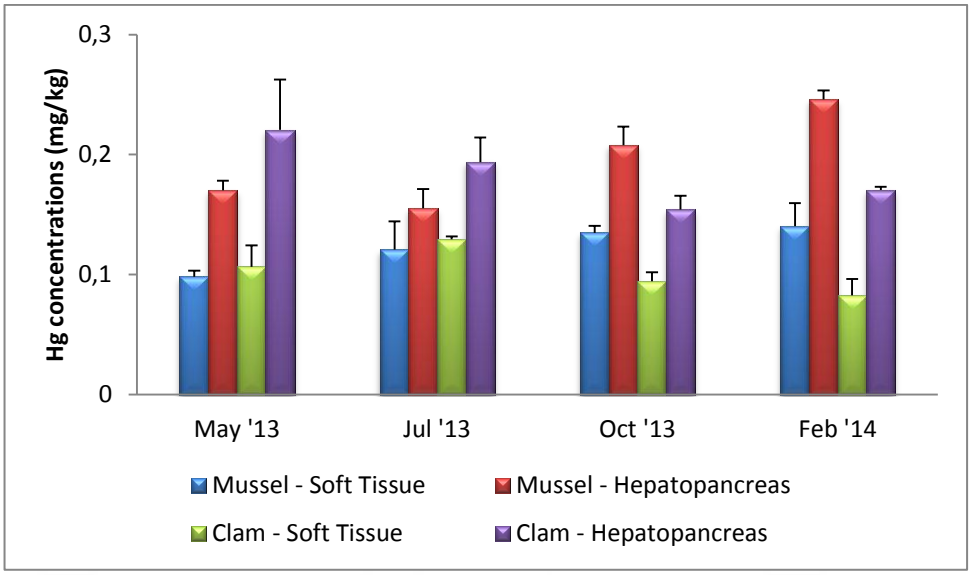
The results showed that; the mean concentrations of Hg, Cd and Zn in *M. galloprovincialis* were detected higher than *T. decussatus* in both tissues. On the other hand; the mean values of Cu, Mn, Co, Fe and, Al in *T. decussatus* were higher than *M. galloprovincialis* for both tissues.

For soft tissues, Ni and Cr mean concentrations in *T. decussatus* were higher than *M. galloprovincialis*. On contrary that, in hepatopancreas tissues, these mean metal values in *M. galloprovincialis* were detected higher than *T. decussatus*.

The mean concentrations of Pb were found higher in *M. galloprovincialis* for soft tissue. For hepatopancreas tissues, Pb mean values were determined higher in *T. decussatus*.

For *M. galloprovincialis*, the bioaccumulation pattern in soft tissues was Fe>Al>Zn>Mn>Cu>Cr>Ni>Pb>Co>Cd>Hg, meanwhile in hepatopancreas tissues was Al>Fe>Zn>Mn>Ni>Cu>Cr>Co>Pb>Cd>Hg.

For *T. decusssatus*, the bioaccumulation ordering in soft tissues as was Al>Fe>Zn>Mn>Cu>Cr>Ni>Pb>Co>Cd>Hg and in hepatopancreas tissues, the bioaccumulation pattern was found Fe>Al>Zn>Mn>Cu>Ni>Cr> Pb>Co>Cd>Hg.



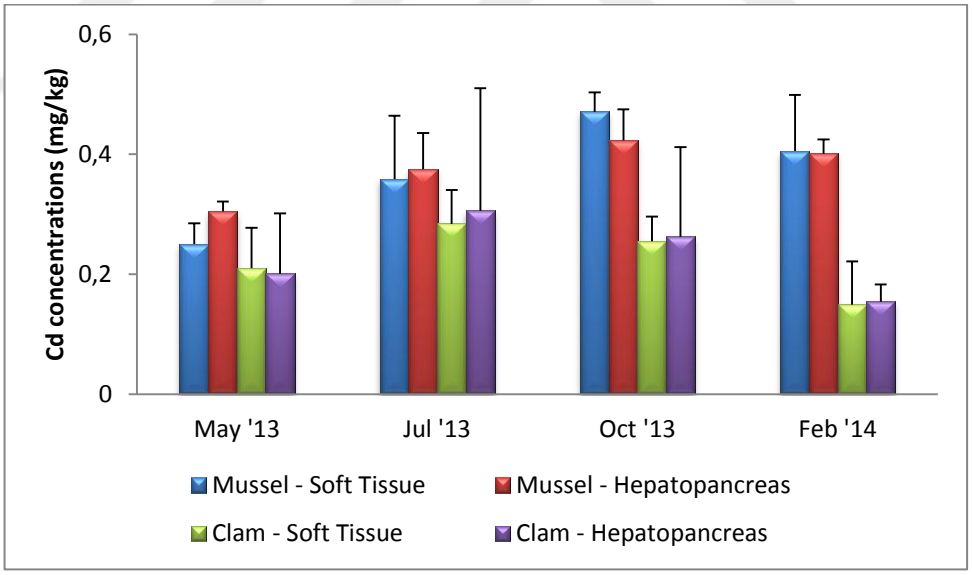
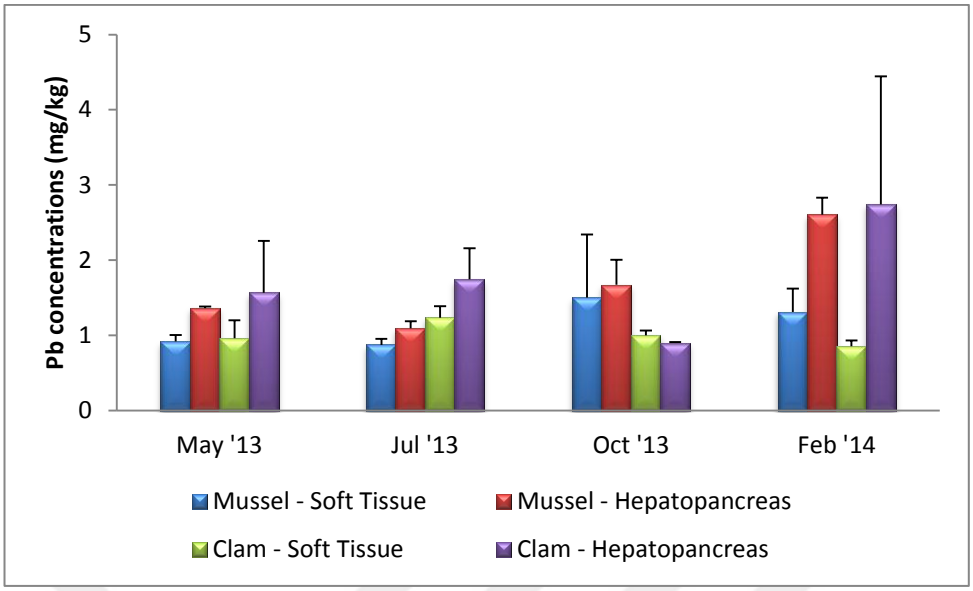


Figure 5.1 Seasonal variations of Hg and Cd values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.



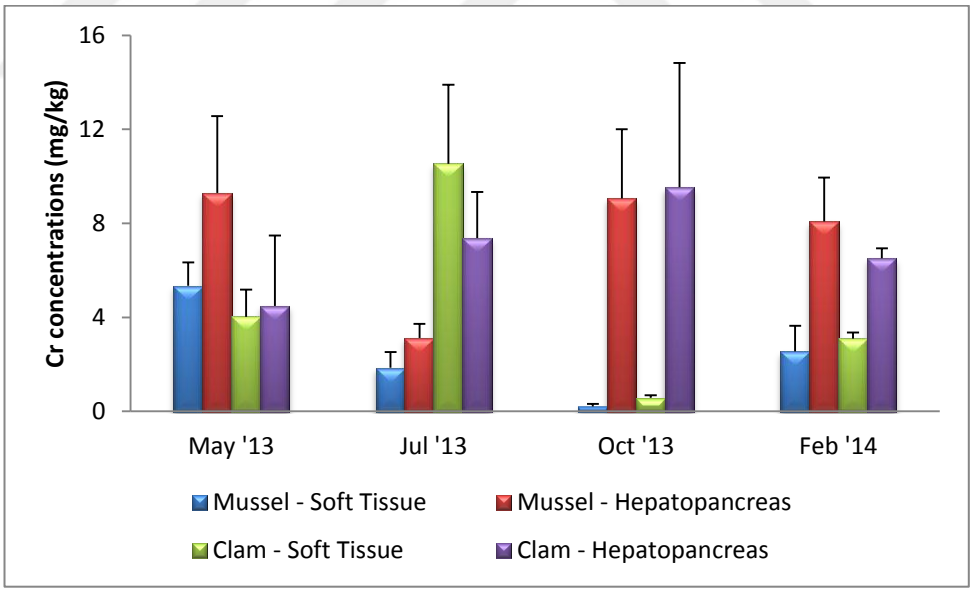
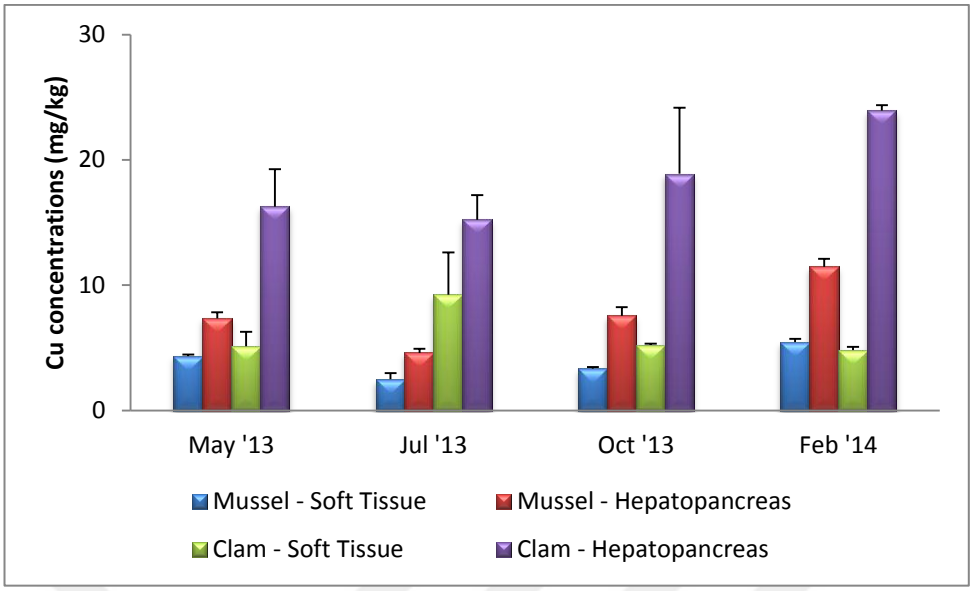


Figure 5.2 Seasonal variations of Pb and Cr values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.



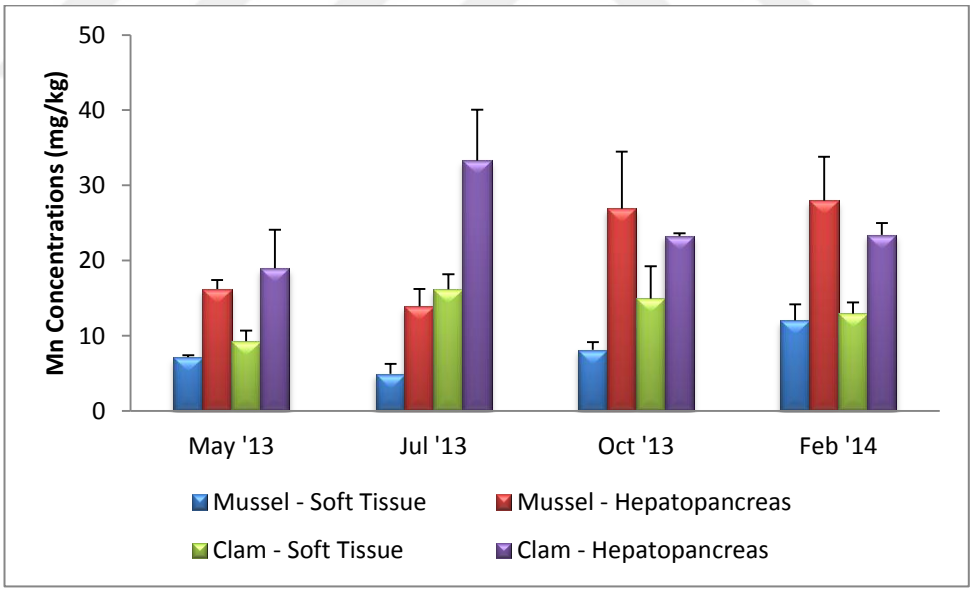
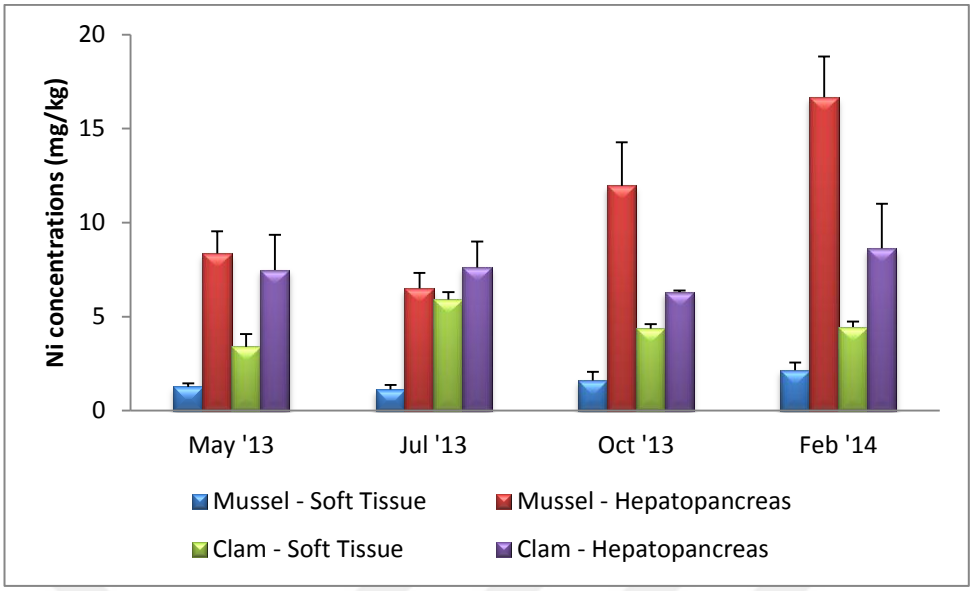


Figure 5.3 Seasonal variations of Cu and Mn values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.



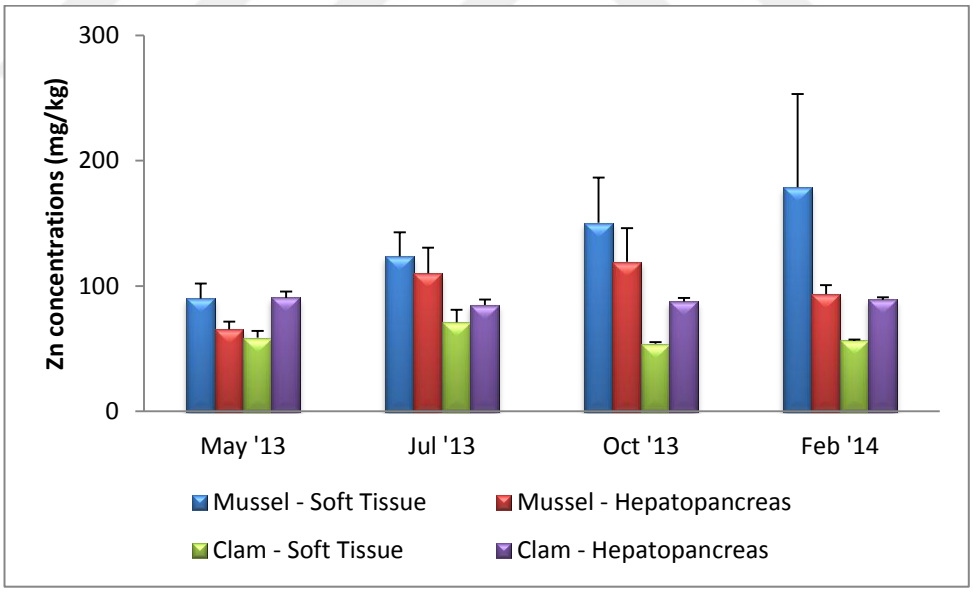
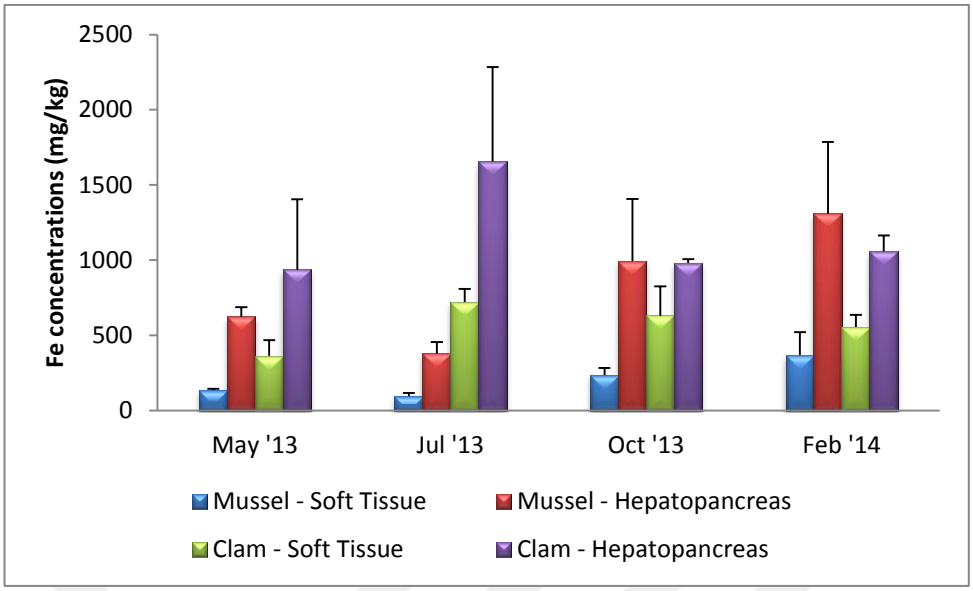


Figure 5.4 Seasonal variations of Ni and Zn values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.



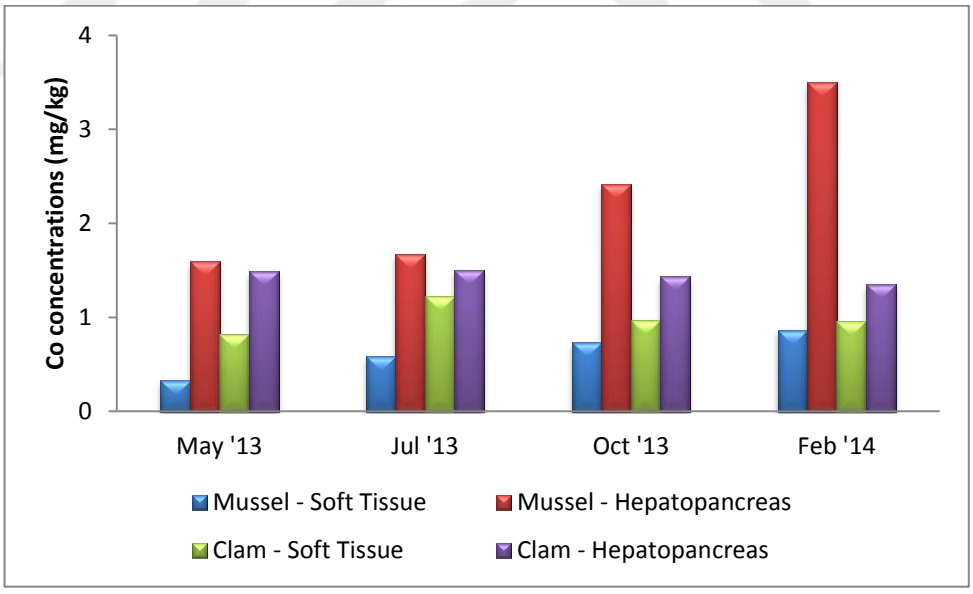


Figure 5.5 Seasonal variations of Fe and Co values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.

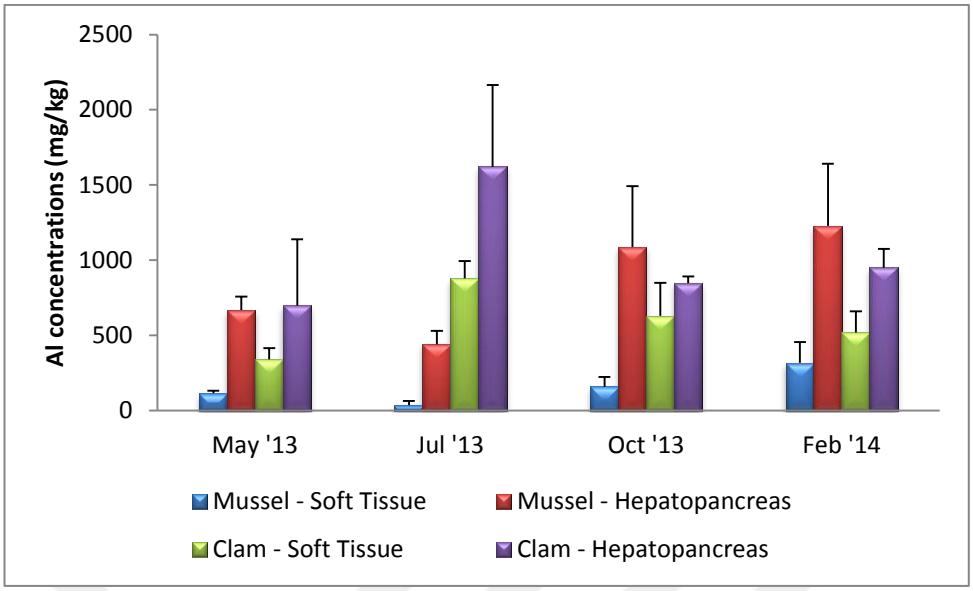


Figure 5.6 Seasonal variations of Al values (means+SD mg/kg dw) in mussel (*Mytilus galloprovincialis*) and clam (*Tapes decussatus*) from the Homa Lagoon.

5.4 Statistical Analyses

5.4.1 Correlations between Size and Heavy Metal Concentrations in Bivalves

In *M. galloprovincialis*, the significant correlations between size and metal concentrations were detected for Cr (R=-0.56), Cu (R=-0.80) and Mn (R=-0.57) in soft tissues; Cu (R=-0.55) and Zn (R=0.70) in hepatopancreas.

In *T. decussatus*, the significant correlations between size and metal concentrations were found for Hg (R=0.60), Cd (R=0.74) and Co (R=0.84) in soft tissues; Cd (R=0.54) and Zn (R=-0.64) in hepatopancreas (Table 5.3).

Table 5.3 Correlations between size and heavy metal concentrations in bivalves from the Homa Lagoon (R> |0.50| values are bolded)

	Mytilus galloprovincialis		***Tapes decussatus***	
	Soft Tissue	**Hepatopancreas**	**Soft Tissue**	**Hepatopancreas**
Hg	-0.017	-0.357	**0.604**	-0.268
Cd	0.144	0.416	**0.736**	**0.541**
Pb	0.295	-0.371	0.207	0.299
Cr	**-0.556**	-0.394	0.321	-0.286
Cu	**-0.802**	**-0.552**	0.297	-0.378
Mn	**-0.573**	0.009	-0.201	0.162
Ni	-0.459	-0.306	0.398	0.004
Zn	-0.049	**0.695**	0.146	**-0.642**
Fe	-0.312	-0.118	-0.033	0.302
Co	-0.041	-0.258	**0.836**	0.319
Al	-0.446	-0.023	-0.100	0.331

5.4.2 Variations of Heavy Metal Concentrations among Seasons, Species and Tissues on Bivalves

Factorial ANOVA tests were performed on each metal for detecting significant differences among species, seasons and tissues. Results were given on Table 5.4.

According to *p-values*; Cu, Mn, Co, and Al concentrations exhibited significantly differences for each independent variables which are seasons, species and tissues. For Hg and Fe, significant differences were determined in species and tissues; but significant differences were not detected between seasons. For concentrations of Cd and Zn significant differences were detected in seasons and species. But, there weren't any statistical differences between tissues. Ni and Pb values showed significant differences at seasons and tissues, but no significant differences were found between species. Finally, Cr concentrations exhibited only significant differences between tissues. No effects were observed for seasons and species.

Table 5.4 Variations of heavy metal concentrations among seasons, species and tissues on bivalves from the Homa Lagoon ($p > 0.05$). (Obtained significant differences were shown as bolded.)

Metals	**Seasons**			**Species**			**Tissues**		
	df	**F**	**p**	***df***	**F**	**p**	***df***	**F**	**p**
Hg	3	0.742	0.5362	1	6.566	**0.0161**	1	166.2	**0.0000**
Cd	3	3.909	**0.0189**	1	28.97	**0.0000**	1	0.040	0.8427
Pb	3	3.831	**0.0204**	1	0.075	0.7861	1	17.07	**0.0003**
Cr	3	0.486	0.6948	1	1.465	0.2363	1	28.61	**0.0000**
Cu	3	20.96	**0.0000**	1	404.8	**0.0000**	1	633.1	**0.0000**
Mn	3	6.534	**0.0017**	1	14.67	**0,0007**	1	116.0	**0.0000**
Ni	3	10.54	**0.0001**	1	0.246	0.6241	1	262.1	**0.0000**
Zn	3	4.742	**0.0085**	1	43.82	**0.0000**	1	0.695	0.4117
Fe	3	2.392	0.0897	1	15.97	**0.0004**	1	48.96	**0.0000**
Co	3	10.58	**0.0001**	1	9.671	**0.0043**	1	184.5	**0.0000**
Al	3	3.589	**0.0259**	1	14.39	**0.0007**	1	50.29	**0.0000**

5.4.3 Variations of Heavy Metal Concentrations between Seasons and Tissues on Mytilus galloprovincialis

Two-Way ANOVA tests were performed for each metal detecting significant difference among seasons and tissues. Results were given on Table 5.5.

According to data, significant differences for seasons were observed for all metals. Also, statistical differences were detected for all tissues except Cd. It's the only element that no significant effects on tissues.

Table 5.5 Variations of heavy metal concentrations between seasons and tissues on *Mytilus galloprovincialis* from Homa Lagoon ($p > 0.05$). (Obtained significant differences were shown as bolded.)

Metals	**Seasons**			**Tissues**		
	df	**F**	**p**	***df***	**F**	**p**
Hg	3	19.95	**0.0000**	1	138.7	**0.0000**
Cd	3	8.661	**0.0017**	1	0.032	0.8611
Pb	3	7.336	**0.0034**	1	11.55	**0.0043**
Cr	3	7.086	**0.0039**	1	38.28	**0.0000**
Cu	3	100.6	**0.0000**	1	403.7	**0.0000**
Mn	3	10.17	**0.0008**	1	75.32	**0.0000**
Ni	3	20.09	**0.0000**	1	320.7	**0.0000**
Zn	3	5.057	**0.0140**	1	9.697	**0.0076**
Fe	3	7.852	**0.0026**	1	46.44	**0.0000**
Co	3	24.50	**0.0000**	1	258.3	**0.0000**
Al	3	7.146	**0.0038**	1	65.24	**0.0000**

5.4.4 Variations of Heavy Metal Concentrations between Seasons and Tissues on Tapes decussatus

Two-Way ANOVA tests were performed for each metal detecting significant difference among seasons and tissues. Results were presented on Table 5.6.

For concentrations of Hg, Cr, Cu, Mn, and Al, significant differences were detected both seasons and tissues. Pb, Ni, Zn, Fe, and Co concentrations showed significant differences only between tissues, but there were not any significant differences between seasons. Cadmium exhibited no statistical differences both seasons and tissues.

Table 5.6 Variations of heavy metal concentrations between seasons and tissues on *Tapes decussatus* from the Homa Lagoon. ($p > 0.05$) (Obtained statistical differences were shown as bolded.)

Metals	**Seasons**			**Tissues**		
	df	**F**	**p**	***df***	**F**	**p**
Hg	3	4.406	**0.0222**	1	62.86	**0.0000**
Cd	3	1.650	0.2230	1	0.017	0.8982
Pb	3	1.549	0.2460	1	7.590	**0.0155**
Cr	3	4.435	**0.0217**	1	4.648	**0.0490**
Cu	3	6.118	**0.0070**	1	395.8	**0.0000**
Mn	3	8.732	**0.0016**	1	44.58	**0.0000**
Ni	3	1.938	0.1698	1	28.87	**0.0001**
Zn	3	1.580	0.2387	1	132.3	**0.0000**
Fe	3	3.090	0.0616	1	15.87	**0.0014**
Co	3	0.875	0.4777	1	14.86	**0.0017**
Al	3	6.694	**0.0050**	1	10.35	**0.0062**

CHAPTER SIX
DISCUSSION

6.1 Distribution of Heavy Metals on Bivalves

As we know, lagoons are ecologically and economically vital ecosystems which are involved in fisheries and productivity. The Homa Lagoon, the study field, is affected by agricultural activities and pollution transported by the Gediz River. Therefore, heavy metal biomonitoring researches are getting vital on our earth. Bivalves which are part of benthic organisms have been preferred to observe pollutants because of their filter feeding dietary type, long-term expose ability and inadequate movement capability. These abilities make bivalves one of the bioindicator species. In this thesis, heavy metal assessment had been carried out to determine heavy metal pollution on selected bivalve species from Homa Lagoon.

Seasonal metal bioaccumulation depends on many factors such as environmental, spatial, temporal, and physicochemical variations. Also, biologically available metal levels on the habitat and local characteristics of ecosystem are strongly related. Previous researches showed that growth, gonadal improvement, age and sex are commonly emphasized. Therefore; location, season and species based biomonitoring studies are needed to observe metal bioaccumulation (Sokolowski, Bawazir & Wolowicz, 2004).

Mercury is one of the highly toxic contaminants that can be found naturally or anthropogenic way on water bodies. Obtained Hg concentrations showed that, each measurement on hepatopancreas values were found higher than soft tissues. It can be inferred that highly toxicant metals such as Hg, accumulates on target organ such as hepatopancreas and liver in marine organisms. For both tissues, Hg concentrations on *M. galloprovincialis* are generally higher than *T. decussatus*. Many researchers suggest that each bivalve has its own capacity to bioaccumulate metals (Usero, Gonzalez-Regalado & Gracia, 1997). The maximum concentrations for *M. galloprovincialis* and *T. decussatus* were found February and May respectively. It's quite possible that bioaccumulation of Hg reaches the peak point before spawning period of each bivalve. Najdek & Sapunar (1987) explained that pre-spawning period

occurs greater accumulation of Hg and also other biochemicals to set a better condition. Previous studies suggest that main reasons of hazardous Hg levels in aquatic ecosystems are anthropogenic activities such as dumping and mining facilities (Baseda, Andrade, Schultze & Gonzalez, 2005). On lagoonal environment, the level of Hg contaminants also reported that due to density of domestic discharges in lagoons (Joiris, Azokwu, Otchere & Ali, 1998).

Cadmium is a non-essential element and also shows toxic reactions in small amounts. Excessive Cd concentrations are originated generally human activities such as industrial and agricultural facilities. These Cd sources may lead to contamination and it causes increased uptake on animals and plants which are most of them are related to human consumption (Jovic, Onjia & Stankovic, 2012). According to Cd levels, bioaccumulations were measured higher in *M. galloprovincialis* at this study. On their Cd exposure study, Bebianno & Serafim (1998) also suggest that *M. galloprovincialis* has tend to accumulate Cd more, to compare with *T. decussatus*. The maximum concentrations were detected in October for *M. galloprovincialis* and in July for *T. decussatus*. These results are coherent with previous studies involved in seasonal determination of these bivalves (Fattorini et al., 2008; Cravo et al., 2012).

Lead is a toxic heavy metal with unknown metabolic function. Many previous studies explained that Pb contamination in aquatic ecosystem is strongly related to anthropogenic activities. It is known that Pb pollution in environment has been dramatically decreased, after many countries abandoned leaded fuel. Pb concentrations were recorded the highest level in autumn and winter season. Giarratano & Amin (2010) were found similar seasonal results in their work. Pb levels on *M. galloprovincialis* were determined higher than levels on *T. decussatus*. Beiras & Albentosa (2003) also detected the same results on their studies about early stages of these bivalves. Results showed that Pb concentrations on hepatopancreas tissue found higher than soft tissues'. Scuderio, Creti, Trinchella & Esposito (2014) were found similar results on their research.

Chromium is considered that one of the hazardous heavy metal, although it's an essential and part of glucose metabolism (Joksimovic, Tomic, A. Stankovic, Jovic & S. Stankovic, 2011). Obtained values showed that Cr concentration in *T. decussatus* is higher than in *M. galloprovincialis*. Ther maximum levels of *M. galloprovincialis* and *T. decussatus* on hepatopancreas were found May and October respectively. These months are post-spawning period for these species. Richir & Gobert (2014) were found similar pattern of Cd bioaccumulation on their study. They explained this situation as Cr molecules are one of the less abundant materials during the spawning period. According to tissue concentrations *M. galloprovincialis* are tend to accumulate Cr molecules into their digestive gland. For *T. decussatus* samples, Cr levels in hepatopancreas slightly higher than in the soft levels. Researches explained this as metabolism differences of species (Irato, Santovito, Cassini, Piccinni & Albergoni, 2003).

Although copper is an essential element for organism, its excessive amounts cause a hazardous impact. In aquatic ecosystems, bivalves have the richest Cu sources with crustaceans. Cu plays a role on syntesis of hemoglobin (Sivaperumal, Sankar & Viswanathan Nair, 2007). Some previous studies suggest that main reason of high Cu levels in seawater is anthropogenic activities around the environment (Baseda, et al., 2005; Funes, Alhama, Navas, Lopez-Barea & Peinado, 2006). According to Cu concentrations on bivalves, maximum values were reached in February for these species. Sokolowski et al. (2004) explained the case as Cu increase was probably related with faster metal uptake during intense food consumption in this season. Results show that Cu concentrations in *T. decussatus* samples were generally higher. Also Cu levels in hepatopancreas were made much more differences to soft tissues. Bebianno & Serafim (2003) stated that Cu is an essential element and it takes part of many metalloenzyme reactions. Therefore, digestive glands act as a storage organ for Cu molecules.

Zinc is vital element for organisms, but it may be harmful when it reached the excessive values. Seafood provides humans a major source of Zn (Jovic, et al., 2012). Previous studies claimed that Zn pollution in seawater is originated from anthropogenic activities (Giusti & Zhang, 2002; El Nemr, Khaled, Moneer & El

Sikaily, 2012). Like many other essential elements, the maximum Zn concentrations in *Mytilus galloprovincialis* and *Tapes decussatus* were obtained in February and July respectively. Similar patterns were observed in previous studies. They suggested that metals in tissues showed variations during spawning period (Regoli & Orlando, 1994; Cravo et al., 2012). According to Zn values, *M. galloprovincialis* accumulated Zn more than *T. decussatus*. Irato, et al., (2003) described this situation as interspecies differences. To compare tissue differences, hepatopancreas tissues were higher massively. Digestive gland has ability to storage metal concentration during the annual reproduction cycle of mussels (Ivanković, Pavičić, Erk, Filipović-Marijić, & Raspor, 2005).

Manganese is an essential element for both aquatic organisms and humans. Several metabolic roles have been identified which Mn involved in (Joksimovic et al., 2011). Researchers reported that Mn concentration is strongly related with pollution amounts in the environment (Mubiana, Qadah, Meys & Blust, 2005). The highest concentrations of Mn were found for *M. galloprovincialis* and *T. decussatus* were generally found on February and July respectively, which are pre-spawning seasons. Catsiki & Florou (2006) explained that during the reproductive cycle, metal content increased by bivalves before spawning period. Like Cu, another essential element, values of Mn in hepatopancreas is higher than soft tissue. Mn takes part of many enzyme syntesis as cofactor such as Mn-SOD. For interspecies comparison, Mn levels were found higher in *T. decussatus*. As mentioned on Cr, it's related to biological and metabolically differences of species (Irato et al., 2003).

It's well-known that nickel is an essential element for several organisms, but shows carcinogenic effects in case of excessive consumption (Joksimovic, et al., 2011). Many studies reported that source of high Ni abundance in environment is related to mining and industrial facilities (Usero, et al., 1997; Andral et al., 2004). The minimum concentrations in soft tissues were found for *M. galloprovincialis* and *T. decussatus* in July and May respectively. Fattorini et al. (2008) associate this observing seasonal infiltration of gonadic tissues in mussels. Besides, the highest concentrations were obtained pre-spawning season for each bivalve. Jovic, et al. (2012) explained that Ni takes part of normal growth and reproduction on animals,

plants and humans. Statistical results show that significant differences were observed in *M. galloprovincialis*, but *T. decussatus* weren't. Researchers claimed that this situation comes from bioavailability of metal components and bioaccumulation rate differences of species (Mubiana et al., 2005; Zuykov, et al., 2013). Higher concentrations were found on hepatopancreas tissues in this study. Researchers suggest that bivalves were shown efficiently Ni ingested with their food and retain it efficiently. Therefore, they tend to bioaccumulate in their digestive glands (Hédouin, Pringault, Metian, Bustamante & Warnau, 2007).

Cobalt is an essential element for organisms, and also is a vital part of Vitamin B_{12} which also known as "cobalamin" (Sivaperumal et al., 2007). Also, it is known that Co has been implicated in blood pressure regulation. Researchers claimed that high Co values are generally associated with pollutants discharge in environment (El Nemr, et al., 2012). The highest concentrations of Co were found for *M. galloprovincialis* and *T. decussatus* were generally found on February and July respectively. As mentioned before on other essential elements, Co particles tend to accumulate increasingly before reproduction cycle. This situation is originated with bioaccumulation rate of the seasons. Results presented that Co concentrations in *M. galloprovincialis* is higher than *T. decussatus* in their hepatopancreas. Researchers found similar results on their study and they were associated with physiological, metabolic and biological differences across species (Irato et al., 2003).

Iron is the most abundant element on earth crust. Also Fe compounds were by far the most available metal in sediments (Joksimovic, et al., 2011). In this study, Fe concentrations were obtained the highest levels across analyzed heavy metals. Both species were given highest levels before spawning. The highest Fe values were obtained in February for *M. galloprovincialis*, and in July for *T. decussatus*. Then, dramatically decreases were observed in following months. Similar trends were recorded by several researches. They explained that bivalves bioaccumulate metals according to their seasonal dynamics. Metal uptake was increased during spawning season. Then, they enter a resting period (Cardelicchio, Buccolieri, Di Leo, Giandomenico & Spada, 2008; Vernocchi, Maffei, Lanciotti, Suzzi & Gardini, 2007). Results show that, *T. decussatus* bioaccumulate Fe compounds more. Like

most of case, the major percentage of metal deposition was associated species-specific relationships (Irato et al., 2003). The highest concentrations were found in hepatopancreas for each bivalve. Like other essential elements, Fe ions bioaccumulate into the digestive gland for storage or detoxification. Many researchers were showed similar patterns (Jebali, Chouba, Banni & Boussetta, 2014).

Like iron, Al is also one of the most available metals on earth. Al and Fe considered as major element for earth, they always being the high values or even lower than those found in different regions (Giarratano, Gil & Malanga, 2013). Thus, many studies suggest that Al compounds in environment are generally originated with natural sediment structure and anthropogenic activities, especially harbor and shipyard areas (Liu & Kueh, 2005; Ščančar, Zuliani, Turk, & Milačič, 2007). In this study, Al concentrations were found second highest metal, after Fe. The highest Al values were obtained in February for *M. galloprovincialis*, and in July for *T. decussatus*. In addition, *M. galloprovincialis* samples accumulate more Al in hepatopancreas. Researchers explained these; Al accumulation is parallel with behavioral changes. Reason of higher values in hepatopancreas is digestive gland of marine bivalves in response to xenobiotics and heavy metals for storage and detoxification. It can inferred that bivalves have ability to regulate their uptake mechanisms (Kádár et al., 2001).

6.2 Metal Bioaccumulation in Bivalves

The results demonstrated that two bivalve species studied accumulated metals with some differences in soft tissues and hepatopancreas. *M. galloprovincialis* accumulates Hg, Cd and Zn more, especially in both tissues. Whereas, *T. decussatus* accumulates Cu, Mn, Co, Fe and Al more, in particular in both soft tissues and hepatopancreas from the Homa Lagoon. Many researches demonstrated that different species of bivalve have different capacities for accumulating metals. The differences observed also seemed to be species-specific (Usero et al., 1997; Irato et al., 2003). It is known that *M. galloprovincialis* can be found from exposed rocky outer coasts to sandy bottoms and it attaches firmly to rocks by means of strong byssus. It typically requires rocky coastlines with a high rate of water flow. On contrary that, *T. decussatus* lives in muddy-sand sediments of tidal flats or shallow coastal areas.

In this study, the metal bioaccumulation patterns were generally similar in these species. The trends were closely related to lithogenic sources and anthropogenic inputs of heavy metals into the Homa Lagoon. Therefore, it can be inferred that bivalves accumulate heavy metals with similar patterns depending on their different bioaccumulation rates.

6.3 Comparison of Heavy Metal Levels in Bivalves from the Mediterranean

Some studies were selected to compare the obtained values at this study (Table 6.1). Comparison of data set revealed that Cd, Cu, Mn and, Ni values in the Homa Lagoon were generally lower than other study areas. On the contrary that Cr values were generally higher than other researches. In this study, Hg concentrations were similar with Adriatic Sea and coast of Algeria, but Hg concentrations from Morocco and Balearic Islands were higher than the Homa Lagoon. For Zn concentrations, detected values in bivalve from Homa Lagoon were similar with the results from the Adriatic Sea, Gulf of Thermaikos and Morocco.

6.4 The Assessment of Health Risks

To assess potential health risk from consumption, the maximum concentrations of metal at soft tissues of bivalves were evaluated with international standards of Provisional Tolerable Weekly Intake (PTWI) values. The levels of metal in bivalve from the Homa Lagoon and PTWI limits were given at Table 6.2. According to values, determined metal concentrations in bivalves from the Homa Lagoon were generally below the health risk level (except Al). On the other hand, the levels of Al in *T. decussatus* were found above the international regulation limits at summer and autumn. Also the maximum concentrations of Cr in *T. decussatus* were detected very close to the PTWI limits in summer.

Table 6.1 The heavy metal levels in bivalves from Homa Lagoon and different areas from Mediterranean

Location	Hg	Cd	Pb	Cr	Cu	Mn	Ni	Zn	Fe	Co	Al	Reference
M.galloprovincialis												
Adriatic Sea	0.02-0.19	0.41-1.6	0.29-2.9	0.39-2.3	1.1-8.1	4.4-16	1.8-6.5	23-153	59-469	-	-	Fattorini et al., (2008)
Adriatic Sea	-	-	-	2.1-4.2	4.6-17	7.3-86	3.4-19	132-345	128-604	1.1-9.1	-	Joksimovic et al., (2011)
Venice	-	1.2-6.6	1.1-4.3	0.72-2.8	3.6-11	4.7-12	-	135-400	3.8-308	-	-	Nesto et al., (2007)
Thermaikos	-	-	-	0.20-3.4	1.8-6.4	7.2-25	1.2-7.5	21-115	81-715	-	-	Catsiki & Florou (2006)
Balearic Is.	0.13-2.21	0.25-1.7	3.3-19	0.7-15	5.9-58	-	0.7-35	49-317	-	-	-	Deudero et al., (2007)
Perna perna												
Algeria	0.01-0.18	nd-0.51	nd-1.75	0.7-2.4	5.71-27	5.2-23	3.3-10	127-362	372-938	-	-	Belabed et al., (2013)
T.decussatus												
Egypt	-	0.04-0.11	0.05-0.3	5.2-11	1.5-3.5	-	9.0-10	8.4-14	-	1.1-2.5	24-53	El Nemr et al., (2012)
Morocco	0.08-0.58	1.4-3.7	1.2-6.7	4.3-15.3	6.2-18.3	12-28	15-34	67-157	-	-	-	Maanan (2008)
M.galloprovincialis	0.10-0.15	0.22-0.51	0.81-2.5	0.10-6.2	2.1-5.6	4.1-14	0.9-2.4	80-232	82-477	0.3-1.1	17-416	***This Study***
T.decussatus	0.07-0.13	0.10-0.33	0.80-1.4	2.9-13	4.8-9.6	7.6-18	2.6-6.3	53-82	268-806	0.5-1.4	260-1009	***This Study***

Table 6.2 The maximum heavy metal concentrations in bivalves from the Homa Lagoon and PTWI values. (Obtained excessive values were given as bolded)

Metals	***M.galloprovincialis***	***T.decussatus***	**PTWI***
Hg	0.15	0.13	1.94[a]
Cd	0.51	0.33	2.24[a]
Pb	2.5	1.4	9.72[a]
Cr	6.2	13.4	13.61[b]
Cu	5.6	9.6	13.61[a]
Mn	14	18	163.3[c]
Ni	2.4	6.3	13.61[c]
Zn	232	82	2722[a]
Fe	477	806	2177[a]
Co	1.1	1.4	4.36[c]
Al	416	**1009**	777.8[d]

*PTWI values retrieved from a: Jovic, Onjia & Stankovic, (2012); b: Agency for Toxic Substances and Disease Registry, (2013); c: Jovic & Stankovic, (2014); d: Food and Agricultural Organization / World Health Organization, (2011).

CHAPTER SEVEN
CONCLUSION

In this biomonitoring study, we evaluated seasonally heavy metal concentrations in bivalves from the Homa Lagoon. The results showed that the maximum concentrations of metal were generally found at February and July in *M. galloprovincialis* and *T. decussatus*, respectively. For tissues, heavy metal levels in hepatopancreas were generally higher than soft tissues in both bivalves. Bioindicator organisms generally increase their accumulations of metal with feeding and as the spawning period approaches. Therefore, the maximum concentrations of metal were usually found during pre-spawning season for each bivalve. The results showed that *M. galloprovincialis* and *T. decussatus* are good bioindicator for biomonitoring studies. *M. galloprovincialis* is widely utilized as a bioindicator in research marine environmental metal pollution, also *T. decussatus* is another bioindicator for monitoring in the Homa Lagoon due to it can accumulate metals to a considerable degree. It's concluded that their bioaccumulations depend on their physical, biological and chemical properties.

The levels of metal in bivalve from the Homa Lagoon were compared with the international permissible limits. Only the concentrations of Al in *T. decussatus* were exceeded the acceptable values. When it is considered to our national seafood products consumption, heavy metal poisoning risk is thought to be low but the potential risk is available. Because Cr levels are very close to the international limits, it also takes attention for health risk.

In the Gediz Delta, there are large agricultural lands, a lot of industrial complexes like metal processing, mining, chemistry, food, paper and, leatherwork. Organic and inorganic pollutants originated from Gediz River are strongly affected Homa Lagoon and Gediz Delta. Therefore, biomonitoring researches by using bioindicator organisms should be continuous to keep under control pollution levels in these ecologically protected areas such as the Homa Lagoon.

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