DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOACCUMULATION OF HEAVY METALS IN RAZOR SHELL (*Solen marginatus*) FROM THE HOMA LAGOON

by
Selin SEVGİ

September, 2019
İZMİR

BIOACCUMULATION OF HEAVY METALS IN RAZOR SHELL (*Solen marginatus*) FROM THE HOMA LAGOON

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ABSTRACT

In this thesis, the concentrations of heavy metals (aluminum, cadmium, chromium, copper, lead, iron, manganese, mercury and zinc) were investigated in razor shell from the Homa Lagoon. The study area, the Homa Lagoon is an active lagoon where is located in north of İzmir Bay. The Homa Lagoon is an important coastal wetland ecosystem and in terms of pollution, it is under the influence of Gediz River and Izmir Bay.

Solen marginatus samples were collected seasonally in May 2015 to November 2017 from the Homa Lagoon. During 2015-2016 sampling period, whole tissues were separated from shells of *S.marginatus*. In 2017 sampling period, hepatopancreas and soft tissues were dissected. For calculation of bioaccumulation factor (BAF), sediment samples were collected at sampling location from lagoon in May 2017. The heavy metal analyses were performed by using atomic absorption spectrophotometer (AAS). The statistical analyses (ANOVA, Kruskal-Wallis and correlation tests) were applied to assess significant differences of metal concentrations between seasons and tissues. In this study, the maximum levels of metal were generally observed in spring and summer periods. Hepatopancreas generally more accumulate metals than soft tissues.

According to BAF ratio, significant values were found for mercury, cadmium and zinc in both tissues. In point of human health risk assessment, metal results were compared with PTWI (Provisional Tolerable Weekly Intake) values of international regulations. The concentrations of chromium, copper, iron and aluminum in *Solen marginatus* were found above permissible values.

Keywords: *Solen marginatus,* Homa Lagoon, metal pollution, bioindicator, aquatic pollution

HOMA DALYANI'NDAKİ SÜLÜNESLERDE (*Solen marginatus*) AĞIR METALLERİN BİRİKİMİ

ÖZ

Bu tezde, Homa Dalyan'ındaki sülünesde ağır metal konsantrasyonları (alüminyum, kadmiyum, krom, bakır, kurşun, demir, mangan, cıva ve çinko) araştırılmıştır. Çalışma alanı olan Homa Lagünü, aktif bir lagün olup İzmir Körfezi'nin kuzeyinde yer almaktadır. Bu lagün önemli bir kıyısal sulak alandır ve kirlilik açısından Gediz Nehri ve İzmir Körfezi'nin etkisi altındadır.

Solen marginatus örnekleri Mayıs 2015- Kasım 2017 arasında mevsimsel olarak toplanmıştır. 2015-2016 örnekleme periyodu boyunca, sülüneslerin tüm dokuları kabuktan ayrılmıştır. 2017 örnekleme periyodunda, hepatopankreas ve yumuşak dokular dissekte edilmiştir. Biyoakümülasyon faktörü (BAF) hesaplanması için sedimentler, lagündeki örnekleme lokasyonlarından Mayıs 2017'de toplanmıştır. Ağır metal analizleri atomik absorbsiyon spektrofotometresi kullanılarak yapılmıştır. İstatiksel analizler (ANOVA, Kruskal-Wallis ve korelasyon testleri), mevsim ve dokular arasındaki metal konsantrasyonlarının anlamlı farklılıklarını değerlendirmek için kullanılmıştır. Bu çalışmada, maksimum metal seviyeleri genellikle bahar ve yaz döneminde gözlemlenmiştir. Genel olarak hepatopankreas, yumuşak dokudan metalleri daha fazla biriktirmiştir.

BAF oranlarına göre, anlamlı değerler her iki dokuda da cıva, kadmiyum ve çinko için bulunmuştur. İnsan sağlığı risk değerlendirmesi bakımından, metal sonuçları uluslararası yönetmeliklerin PTWI değerleri (haftalık kabul edilebilen tüketim miktarı) ile karşılaştırılmıştır. Sülünesdeki krom, bakır, demir ve alüminyum konsantrasyonları izin verilen değerlerin üzerinde bulunmuştur.

Keywords: *Solen marginatus*, Homa Dalyanı, metal kirliliği, biyoindikatör, sucul kirlilik

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

In recent years, organic and inorganic compounds disperse environment which cause serious environmental problems due to increasing industrialization, unplanned coastal urbanization and acceleration of agricultural activities. Besides, humans are also affected given that being at top of food web. Increasing environment contamination leads to irreversible changes in ecosystem and particularly aquatic environments. One of the aquatic environment pollutants is metal which has been extracted from earth crust and has been used for industrial factories for thousands of years (Hu, 2000). Unfortunately, discharging of metals shows a linear trend with growing population which is used in mining sites, metallurgical facilities, pulp and paper mills, chemical plant, plating.

In the contrast to other various marine pollutant groups, metals are natural components at the same time they can be introduced in aquatic ecosystems by anthropogenic sources. Rocks, volcanic activities and forest fires are natural origin of metal pollution. Anthropogenic sources are industrial wastes, solid wastes of waste-water treatment systems, mining sites and combustion residues. The transport and deposition of metals from land to seawater may occur through atmospheric phenomena such as acid rains, wind of dusts, river discharge and hydrothermal sources in marine zones (Mason, 2013). Metals can be presented as ionic, complexed, colloidal or particulate forms in aquatic ecosystems (Florence & Batley, 1977). Furthermore, through aquatic environment, these conservative elements can be transported different forms, such as dissolved in water column or deposited in the sediments (Duruibe, Ogwuegbu & Egwurugwu, 2007).

Metals are natural ingredient of aquatic environments; also, these non-degradable elements can accumulate on marine trophic chain in a long term. Some metals are essential for marine organisms such as iron, copper, zinc, cobalt, which have been required for biochemical process; especially enzyme and protein structures. On the other hand, another part of metals is hazardous. Even their low exposure levels negatively affected on organism health (e.g., mercury, cadmium, lead and arsenic) (Viarengo, 1985).

Determining of metals in water column and sediment can be utilized which has been shown to pollution situation in aquatic environment. However, this situation is not strong enough to proof for pollution effects on organisms (Zhou, Zhang, Fu, Shi & Jiang, 2008). One approach to assessing the quality of the marine ecosystem health is biomonitoring. This tool includes of using various marine biota; such as planktonic or benthic communities of invertebrates, plants and fish species (Cerveny et al., 2016). Marine organisms may indicate the level of chemical pollution of marine environment. Bivalves are widely accepted bioindicators to estimate the level of contamination in aquatic systems (Páez-Osuna, Osuna-López, Izaguirre-Fierro & Zazueta Padilla, 1993; Boening, 1999). In generally, these organisms are sedentary and filter feeder which bivalves take dissolved pollutants from the water column. Therefore, they tend to readily accumulate metals in their habitat.

Lagoons are usually located along the coastal zone, separated from the sea by a line of rock or sand barriers which carried by rivers, waves and tides (Brito, Newton, Tett & Fernandes, 2012). Geomorphological structure of lagoons, transportation of nutrients and high rate of primary productivity provide essential habitat for aquatic species in these areas. Presence of wide diversity of floral and faunal communities makes lagoons more ecologically and commercially valuable areas (Brito et al., 2012). Urban and industrial progress nearby lagoons increases dramatically releasing of pollutant through these areas.

The study area, Homa Lagoon is located between Gediz Delta and Çamaltı Saltpan in İzmir Bay (Atılgan & Egemen, 2001). Homa Lagoon is the third largest lagoon and one of the ten most productive lagoons in the Aegean Sea (İlkyaz, Fırat, Saka & Kınacıgil, 2006). The main source of pollution in Homa Lagoon is transportation of industrial factories, agricultural activities and domestic wastes from Gediz Delta and İzmir Bay.

In this study, razor shell (*Solen marginatus*, Pulteney, 1799) was utilized as new bioindicator species for assessment of heavy metal pollution in the Homa Lagoon. The studies about bioaccumulation of metals on razor shells are a few. Also, there is limited reports about razor shells as bioindicator species in Turkey.

Aims of this thesis are;

- To determine heavy metal levels on razor shell from Homa Lagoon
- To research seasonal variation of heavy metal levels
- To assess metal pollution for potential health risks that can be reached to the humans.

CHAPTER TWO
HEAVY METALS

2.1 Heavy Metals

Heavy metals are group of metals and metalloids with atomic density greater than 4 g/cm^3, 5 times or more, greater than water (Hawkes, 1997; Duruibe et al., 2007). Heavy metals include elements with an atomic weight greater than 40, thus atomic number starts with scandium (Rand, 1995; Duffus, 2002). Physical properties of metals are being malleable and ductile, characteristically have a metallic luster, which ability to conduct electricity and heat (Housecroft & Sharpe, 2008; Appenroth, 2010).

Metal-carbon bonds are formed by result of reaction between organic molecules and inorganic metal species which have important industrial and environmental uses as a biocide, gasoline additives or polymer stabilizers. Generally, organometallic compounds can be found in terrestrial or water zones, also these compounds are more toxic than inorganic metal compounds, except arsenic (Cima, Craig & Harrington, 2003).

Especially in aquatic systems, organometallic compounds are known to be harmful (Abel, 2002). Among organometallic compounds, tributyltin (TBT) is commonly used for various industrial purposes as heat stabilizers in plastics, pesticides, antifungal agents and marine antifouling paints. Having high gravity and low solubility, TBT readily precipitate from water column to sediment and this situation negatively affects marine benthic fauna (Landmeyer, Tanner & Watt, 2004). Also, because of marine organisms' dietary and respiration type, TBT can easily accumulate in the marine trophic chain. Particularly, dissolved TBT may lead growth and reproduction problems in bivalve species (Gibbs & Bryan, 1986; Landmeyer, Tanner & Watt, 2004). Similarly, owing to methylmercury's lipophilic character and tetraalklylead's high vapor pressure make these organometallic compounds toxic to aquatic organisms.

In several environmental areas, metals can be found limited concentrations, therefore metals are known as trace elements (Tchounwou, Yedjou, Patlolla & Sutton, 2012). As regards to biological requirement of organisms, these elements can be classified in two groups as essential and non-essential. Non-essential elements tend to involve in the metabolic reactions and this situation has a negative influence on molecular level at living organisms (Chiarelli & Roccheri, 2014). Toxicity of trace elements is closely related to chemical form of metals, states of metal ions, type and abundance of ligands, and biological properties of organisms (Kaiser 1980; Duffus 2002; Tchounwou et al., 2012).

2.2 Heavy Metals in Aquatic Systems

Metals, metalloids or metallic compounds are largely dispersed in the global environment which composed of atmosphere, lithosphere and hydrosphere. Emission of heavy metals in marine environment can occur by natural sources and anthropogenic affects. Metal rich ores, volcanic activities, forest fires, weathering of rocks may constitute natural sources of heavy metals. In recent years, with industrial development, metal concentrations are exceeded natural background levels. Also, both natural and anthropogenic contamination of metals can transfer in ecosystem by surface waters, winds and rainfalls (Agarwal, 2009).

Industrial effluents and drainage waters from treatment facilities are major sources of metal pollution in aquatic systems (Agarwal, 2009). Generally, fate of trace elements in aquatic environment depend on vertical alterations in turbidity, occurrence of ligands, conditions of redox, mixing intensity, densities of aquatic organisms. Furthermore, sediment and water column have influence on distribution of metals in marine environment (Moore & Ramamoorthy, 2012).

Metal concentrations in aquatic systems show great variations depend on some physical-chemical factors. Distribution of metal compounds is highly variable in different zones; sediment may adsorb high level of metals, also water column can include small amounts of dissolved free metal ions. Therefore, marine organisms can significantly accumulate heavy metals (Tomlinson, Wilson, Harris & Jeffrey, 1980).

Sediment is important part of the aquatic environment, which is used to determine metal pollution (Yu, Tsai, Chen & Ho, 2001). In coastal zones, concentrations of trace elements in the sediment are 3 or 5 times more than in water column (Bryan & Langston, 1992; Foster & Charlesworth, 1996). Metal concentrations in sediment are affected by variable factors, such as amount, size and surface of particulate and organic matter content (Tomlinson, Wilson, Harris & Jeffrey, 1980; Foster & Charlesworth, 1996). Participation of heavy metals in sediment is closely related to fine-grained and oxidized particles (Luoma & Davis, 1983; Bryan & Langston, 1992).

2.3 Heavy Metals and Marine Organisms

In marine environment, presence of organic and inorganic xenobiotics creates risks for marine organisms and ecosystem's health. Marine organisms may easily uptake pollutants through distributed pollutant in water column and sediment. Also, they may accumulate contaminants in their tissues and organs. Metabolism functions and life activities of marine organisms can be affected by many xenobiotics. This situation has a negative influence on different levels of marine ecosystems, such as community, population etc.

Organisms may uptake metals with respiration, feeding and adsorption from seawater and sediments. Marine organisms are surrounded by seawater that have permeable body surfaces; therefore, these organisms easily absorb trace elements from the seawater. Also, some molluscs and gastropods may uptake the dissolved metals from seawater via their gills, pedal sinuses and pharynxes (Kalk, 1963; Hobden, 1967; Depledge & Phillips, 1986; Depledge & Rainbow, 1990). However, metal bioaccumulation can affect by many factors such as different species, pollutant concentrations and physico-chemical parameters of environment (Dallinger & Rainbow, 1993).

Metal penetration in membranes of the cells occurs by different pathways. Taking metals are involved in an interaction with cellular and molecular targets, consequently they are irrecoverably accumulated in the cells (Chiarelli & Roccheri, 2014). The uptake of metals across membrane of the cells rarely needs active transport mechanisms. On the other hands, protein binding of metal ions is transported without consume energy by facilitated diffusion (Dallinger & Rainbow; 1993). Transport of metal ions may require energy-dependent, this process occurs via membrane proteins, intrinsic proteins of membrane or endocytosis (Simkiss & Taylor, 1989; Viarengo, 1989; Depledge & Rainbow, 1990; Marigómez, Soto, Cajaraville, Angulo & Giamberini, 2002).

2.4 Bioaccumulation of Heavy Metals in Marine Organisms

Marine organisms may reflect the change in chemical components of seawater, due to interactive relation each other. To provide organisms' nutritional, physical or chemical requirements; many chemicals are taken up and retention by marine organisms. Therefore, bioaccumulation of essential elements is a necessary for organisms (Chapman, Allen, Godtfredsen & Z'Graggen, 1996).

Among other pollutants, especially amounts of metal compounds tend to show great variation in bioavailability for marine organisms (Neff, 2002). The chemical availability of metals has an influence on metal bioaccumulation and toxicity to organisms and metal transportation on trophic chain (Siebielec, Stuczyński & Korzeniowska-Pucułek, 2006). Ionic forms of metals can be taken by organisms in aquatic environment; however, pure metal, heavy mineral, or precipitates form of metals are not bioavailable to aquatic organisms (Neff, 2002).

Bioavailability and concentrations of metal have an important role on the accumulation by marine organisms. Besides, this accumulation process depends on various environmental and biological factors, such as temperature, pH, salinity, presence of organometallic components and dissolved oxygen content in water column (Phillips, 1976; Förstner & Wittman, 1983). Also, variation of metal bioaccumulation in marine organism depends on age, size, sex, reproduction period, and feeding type. (Dallinger, 1994; Boening, 1999). The bioaccumulation of trace metals by marine invertebrates occur three stages. These stages are metal uptake by organisms via several routes; transportations and distribution of metal in biological systems and last stage is excretion of metals from organisms. Last stage sometimes may not occur (Dallinger & Rainbow, 1993).

The seawater, sediment and nutrients constitute potentially metal uptake sources for marine organisms. When organism uptake and accumulate only from water, and other uptake sources are not considered, this process defined as bioconcentration (Neff, 2002). In marine systems, high-level consumer organisms are able to accumulate metals from nutritional sources rather than from water column (Neff, 2002; Mason, 2013). Firstly, the accumulation of metals on the marine trophic chain starts with phytoplankton, microorganisms and benthic algae that uptake bioavailable metals from their environment. Primary and higher trophic level consumers may accumulate metals via food sources that lead increased bioconcentrations through trophic chain (Mason, 2013). This process is called biomagnification. Generally, biomagnification is resulted with reach higher concentration of chemical in organisms than their food sources (Gray, 2002; Neff, 2002; Arnot & Gobas, 2006).

Marine organisms are able to regulate metal levels in their body to attempt to protect themselves from metal stress which cause of serious damage in their tissues. The cellular organelles of organisms, such as mitochondria, endoplasmic reticulum, lysosome, are negative effected by metals. Toxic metals (e.g. arsenic, chromium, lead and mercury) are provide the production of reactive oxygen (Tchounwou et al., 2012). When heavy metal concentrations exceeded threshold level, excretion or isolation from the organism may increase, or metals bind to specific complexes (metallothioneins) to detoxify from organism (Naimo, 1995; Chiarelli & Roccheri, 2014). The degrees of effective detoxification depend on organism species and their metabolic process (Viarengo & Nott, 1993; Naimo, 1995).

2.5 Marine Organisms as Bioindicators

Organisms may easily respond to biotic and abiotic changeable factors in their environment.

> Therefore, environmental changes are monitored via bioindicator organisms which are defined as a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community, or ecosystem, or is indicative of the diversity of a subset of taxa, or of the wholesale diversity, within an area (Hodkinson & Jackson, 2005, p.559).

Generally, bioindicators are used as assessing the environmental conditions or identify the reason of environmental changes (Dale & Beyeler, 2001). Bioindicator selection should optimized to management purposes and its feasibility. Also, the criteria of bioindicator selection may take a shape according to natural abundance of species, sensitivity and response time to stressor, baseline biological data of species. (McGeoch, 1998; Niemi & McDonald, 2004).

In marine environment, levels of xenobiotics may change mg/L between µg/L, in some cases pollutant may be undetectable concentrations, in this situation marine organisms may be used as a bioindicator (Zuykov, Pelletier & Harper, 2013; Mezzelani et al., 2016). Benthic organisms are the most used groups for biomonitoring of marine environment pollution. Macro benthic organisms are sessile or slow-moving organisms which complete their life cycle on or inside the sediment. Also, these organisms' life span ranging from weeks to months. Because of above these reasons, benthic organisms continuously exposure to marine pollutants.

Among benthic organisms, bivalve species have been widely used to assess the level of pollutants in the marine environment (Phillips, 1977; Páez-Osuna et al., 1993). Bivalve species are sessile filter-feeders; therefore, they can be useful tool for control to both sediment and water column quality (Costa, Carreira, Costa & Caeiro, 2013). These organisms rapidly accumulate pollutants in their tissues through filtering the water. Also, they are cosmopolitan species in marine environment, hereby their collecting and availability is become easy (Farrington, Goldberg, Risebrough, Martin & Bowen, 1983; Costa et al., 2013). Generally, these organisms' populations have stable location, thus sampling of bivalves is repeatable in long-periods that provides collecting data of seasonally changes in bioaccumulation concentrations (Farrington et al.,1983). Mussel, clam, oyster and cockle are the most used bivalves as indicators for biomonitoring of heavy metal pollution in marine environment (Hossen, Hamdan & Rahman, 2015).

2.6 Metal Toxicity

Metals are natural components of marine systems, but, in some cases metal concentrations exceed their background levels in marine environment due to anthropogenic inputs. In recent years, anthropogenic inputs have widespread increased through urbanization and industrialization. Some heavy metals may interact with organic molecules, and organometallic compounds are formed. Heavy metals and these organometallic compounds have high toxicity that are easily accumulated by organisms and biomagnified in trophic chain (Bian, Zhou & Fang, 2016). In the last years, many researches have been focused on metal pollution in marine systems and its impact on ecosystem health.

Even low levels, some heavy metals, such as cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), mercury (Hg), are well known to cause cellular and DNA damages, inhibition of enzymatic activities, inflammations, tissues, organs and their functions harms that generally resulted with carcinogenesis or mutagenesis (Raikwar, Kumar, Singh M. & Singh A., 2008; Lee, Son, Pratheeshkumar & Shi, 2012). According to Bryan (1971), aquatic organisms' morphologies and behaviors may change when they are exposed to metals. On the other hand, some metals/metalloids (e.g. Fe, Zn, Cu and Se) are vital to living organisms. Essential metals have two major roles on physiological process; participating redox reaction and being a part of enzymes (Nordberg, Sandstrom, Becking & Goyer, 2002). Abundance or absence of essential metals may occur detrimental effects to organisms (Inoue, 2013). The metal toxicity depends on dose, exposure, chemical form of metals and environmental factors (pH, salinity, temperature etc.) (Wright & Welbourn, 2002; Akkajit, Fajriati & Assawadithalerd, 2018).

Aluminium is the most abundant element with 8% proportion on the earth crust and it is found naturally in the water, soil and air. However, aluminium concentrations are very changeable because of anthropogenic sources, such as processing and mining of aluminium. Al ions generally occurs as Al^{3+} in natural waters that is precipitate and accumulate in sediment as hydroxide forms (Savory, Martin, Ghribi & Herman, 2002). Aluminium (Al) is non-essential element for biological process. Also, dissolved Al ion (Al^{3+}) is more toxic to organisms than insoluble Al due to its bioavailability (Driscoll & Schecher, 1989; Quiroz-Va´zquez, Sigee & White, 2010). Especially among other aquatic organisms, fishes are most affected organisms because of gill breathing. Gills are target organ where gas and ion exchange occur. Enzyme activity is greatly inhibited by aluminium and it cause osmoregulation problems (Rosseland, Edhuset & Staurnes, 1990). Aluminium toxicity is greatly affected by pH of water and organic matter. When pH decreased, aluminium toxicity increases (Jaishankar, Tseten, Anbalagan, Mathew & Beeregowda, 2014).

Cadmium (Cd) is not found alone on the earth crust's, this metal associated with zinc, copper and lead ores (Bryan & Hummerstone 1973; ATSDR, 2012). Cadmium has only one oxidation state (+2) and it is generally found as a mineral that bonds with oxygen, sulphur and chlorine (Raikwar et al., 2008). Soluble cadmium salts, such as cadmium chloride or cadmium sulphate, are in water column. On the other hand, the insoluble forms precipitate and accumulate in sediment (ATSDR, 2012). Cd is one of the most toxic heavy metals that may accumulate in many organs such as kidney, lungs, liver and skin (Lee et al., 2012). In short-term exposure, liver is the target organ of Cd bioaccumulation, and when its exposure is got chronic, Cd is moved to kidney (Jakimska, Konieczka, Skóra & Namieśnik, 2011). Cd and Zn have similar chemical properties (e.g. the same oxidation state); hence Cd may replace Zn where present in metallothionein which include the cysteine-rich proteins (Wang & Rainbow, 2005). Since Cd bind with metallothioneins, concentrations of Cd increase in the organism

(Jaishakar et al., 2014). Marine organisms, especially bivalves have capability actively accumulate Cd that cannot be regulated (Wang & Rainbow, 2005). The Cd-metallothionein is known as hepatotoxic and nephrotoxic for organisms. Besides, cadmium has an effect on skeletal system and iron deficiency (Raikwar et al., 2008; Jasihakar et al., 2014). Cd toxicity is affected by many parameters of marine environment. Cd's toxicity may be inversely related with salinity (Engel & Fowler, 1979).

Chromium (Cr) naturally occurs on the earth and its compounds are widely used in many industrial factories, such as, wood preservation, metallurgy, paper, pigment and paint production. Cr has several oxidation states that ranging between Cr^{2-} and Cr^{6+}. In natural waters, trivalent (+3) and hexavalent (+6) are the most abundant and important oxidation states of Cr (Moore & Ramamoorthy, 2012; Monalisa & Kumar, 2013). However, Cr (VI) is greatly used in industries. The lower levels of Cr (III) compounds are known as an essential element for organisms. Due to its higher membrane permeability and strong oxidizing capacity, Cr (VI) compounds are more toxic and readily enter the cell (Katz & Salem, 1993). Besides, Cr (VI) compounds play key a role on production of ROS (reactive oxygen species) which causes genetic effects such as DNA damage and chromosomal problems (Casadevall & Kortenkamp, 2002).

Copper (Cu) is naturally found in ecosphere and an essential trace element that is widely required for sustain of biological functions. Cu^{2+} is generally found as a free copper ion in the seawater, also other present copper species are $Cu(OH)_2$, $CuCO_3$, Cu(OH)Cl, $CuHCO_3^-$ and $Cu(OH)^+$ (Eisler 1998; Neff 2002). Moreover, Cu tend to complexation with organic ligands (dissolved, colloidal and particulate) in seawater and sediments (Wright & Welbourn, 2002). In the aquatic systems, Cu complexes have been used as algaecide, fungicide long years, and also anti-parasitic treatment in the gills of fishes. It exists three oxidation states, however Cu (I) and Cu (II) have ability electron donor or receptor between each other. Thus, copper become important part of several metabolic processes (Harvey & McArdle, 2008; Stern, 2010). Cu uptake greatly depends on salinity and temperature of water. Also, copper toxicity is affected by changes in pH, suspended solids, dissolved organic carbon, and hardness of water (Neff, 2002). Copper participates as cofactor and component of metalloenzymes, there is known more than thirty enzymes which include Cu. Also, Cu is an inseparable part of hemocyanin which is assigned as dioxygen carriers in most of mollusks and crustaceans. Although Cu is biological importance, it is potentially toxic for aquatic organisms, in exceeded levels. Many studies have been reported that high level of Cu may lead to gill damage, osmoregulation problems, disruption of energy metabolism, inhibition of mucous productivity, reduction of spawning and growth (Calabrese, MacInnes, Nelson, Greig & Yevich, 1984; Viarengo et al., 1981, 1990; Strømgren & Nielsen, 1991; Davies, 1992).

Even though iron (Fe) is one of the most abundant elements on the Earth, it is very low concentrations in the seawater (Liu & Millero, 2002). Thus, in several marine region, Fe is one of the minor elements which has limited effect on primary productivity. In seawater, iron exists as free ions, ferrous (Fe^{2+}), ferric (Fe^{3+}) or complexed with other ligands. As with Cu, Fe ions have ability to change their valence. Hence, Fe becomes one of the important essential trace elements which has crucial effects on enzymes, redox processes and growth of organisms. Particularly, Fe participate in many biological components as transferrin (protein that transport Al^{3+}, Fe^{3+}, and Mn^{2+}), ferritin (storage protein of iron) and, hemoglobin and myoglobin (metalloprotein that oxygen transport) (Goyer & Clarkson, 1996). Vuori (1995) has been reported that Fe pollution has negative effects on abundance of aquatic organisms such as benthic invertebrates, fish species, periphyton. Precipitated Fe forms, such as iron-hydroxide and iron-humus, may lead to damage on fish breathing, egg development by clogging surfaces or pores. On the other side, when Fe cannot to bind to proteins, free radicals are occurred which cause DNA, cellular and membrane damage (Vuori, 1995; Jaishankar et al., 2014). Furthermore, bivalve species may regulate and accumulate Fe. Fe bioaccumulation in bivalves is inversely related with salinity, in some cases (Phillips, 1978).

Lead (Pb) has been significantly used in many industrial areas due to its stability and resistance of corrosion. So many years, several organolead compounds (called as tetraalklylead-TAL) have been used in gasoline as an additive that is major sources of anthropogenic of lead pollution (Mason, 2013). Therefore, leaded fuel is banned owing to control of Pb pollution in many countries. It naturally occurs in the environment and Pb has mainly two oxidation states that are +2 and +4 (Moore & Ramamoorthy, 2012; Chiarelli & Rochheri, 2014) Besides, Pb is one of the toxic metals that has acute and chronic effects for all organisms. Generally, organometallic compounds of Pb are more toxic than elemental forms. The ions of Pb have an ability to replace with bivalent (e.g. Ca^{2+}, Fe^{2+} , Mg^{2+}) and monovalent cations (such as Na^{+}) which occur toxic effects on cellular level (Flora, Gupta & Tiwari, 2012). Also, Pb is highly toxic for central nervous system, and it has effect on skeletal, hematopoietic and renal system. In marine organisms, high accumulation of Pb was found in gills, kidney and digestive gland (Schulz-Blades, 1974; Alexander & Young, 1976; Moore & Ramamoorthy, 2012).

Manganese (Mn) is an essential trace element which exists in various oxidation states that from -3 to +7. In natural waters, Mn is mostly found as soluble Mn (II) ion, and insoluble Mn (III) and Mn (IV) oxides. Also, Mn forms compounds with sulphur, chlorine and oxygen (Pinsino, Matranga, Trinchella & Roccheri, 2010). The soluble of Mn may be affected by changes in organic matter, pH, Eh (redox potential), and dissolved oxygen (Sanders, Du Preez & Van Vuren, 1998). In the seawater, Mn mainly exists as Mn^{2+} or $[MnCl]^{+}$. Besides, marine organisms may uptake and accumulate Mn^{2+} ions from Mn-rich sediments (Baden & Neil, 2003). Mn is required for sustain many biological processes such as reproduction, formation of bone marrow, metabolism of lipid and carbohydrate (Lima et al., 2008). On the other hand, Mn is especially important for enzymes, it can part of various enzymes as co-factor. Among chemical forms of manganese, Mn-chloride is the most toxic form for organisms due to its high solubility. When Mn concentration exceeds the acceptable levels, the central nervous system and lungs are target organs for Mn-toxicity (Lima et al., 2008).

Mercury (Hg) is widespread pollutant which is considered as the most toxic heavy metal and it easily biomagnifies in the trophic chain. Differently from other heavy metals, mercury has a high vapor pressure and ability of methylation, and it can exist as organic, inorganic and elemental forms. This metal exists three oxidation states; Hg^0 (elemental or vapor mercury), Hg^{+1} (mercurous) and Hg^{+2} (mercuric), however Hg (II) is predominantly most found mercury forms in the environment. Hg (II) highly tends to form hydroxo and chloro complexes. While $HgCl_2$ and $Hg(OH)_2$ are most abundant complexes in freshwater, HgCl is the predominantly found in seawater. Under anaerobic conditions, HgS occurs and precipitates in the sediment (Stein, Cohen & Winer, 1996). In marine environment, inorganic mercury forms are transformed to methylmercury by microorganisms that present in sediment and subsequently methylmercury undergoes biomagnification in food web. For the biomethylation of inorganic mercury, low pH and high level of Hg in sediment are generally optimum conditions. All of Hg forms are bioaccumulated by organisms. However, methylmercury is highly bioavailable and is bioaccumulated more rapidly than inorganic mercury compounds. Both organic and inorganic Hg compounds are toxic since they lead to irreversible damages in organisms. $HgCl_2$ is known as cytotoxic and it may cause DNA single strand breaks (Bhan & Sarkar, 2005). Also, methylmercury has neurotoxic effects which leads lipid peroxidation (MDA) and mitochondrial damages (Patric, 2002; Jaishankar et al., 2014). The toxicity of Hg is affected by many parameters in seawater, such as temperature, dissolved oxygen, water hardness, and salinity (Boening, 2000).

Zinc (Zn) is one of the most found metals in the earth crust and it is widely used in industry. This metal mainly exists as zinc oxide (ZnO) or sphalerite (ZnS) in nature (ATSDR, 2005). Generally, Zn exists two oxidation states Zn (0) and Zn (II) in the environment. However, Zn^{2+} is the most abundant form that is highly bioavailable in the aquatic environment (Bryan & Langston, 1992). Zn may form complexes with inorganic and organic ligands. The solubility of Zn complexes is affected by pH of water. Thus, solubility of Zn increases with decreasing pH. Furthermore, Zn has not an important redox reaction in seawaters (Mason, 2013). Zn is an essential metal that is required for several biological processes. The synthesis of more than 300 metalloenzymes (such as alkaline phosphatase, carbonic anhydrase, and alcohol dehydrogenase) are induced by Zn that is component of many proteins which include biosynthesis of genetic materials (ATSDR, 2005; Moore & Ramamoorthy, 2012). In marine system, among marine organisms, filter-feeding bivalve species may accumulate high concentrations of Zn (Eisler, 2000). Most of organisms have an ability to regulate Zn at considerable degree in their tissue (Bryan & Langston, 1992). Digestive gland, stomach and kidney are target organs for Zn accumulation in marine organisms (Eisler, 1981).

CHAPTER THREE
STUDY AREA

3.1 Description of Study Area

Lagoons are along to coastal line, connected to the sea by limited inlets and its deep not exceed a few meters due to restricted water changes (Kjerfve, 1994). There are approximately 72 lagoons along the coast of Turkey and 40% of these lagoons are located by the Aegean Sea (Elbek, Emiroğlu & Saygı, 2003).

The study area, Homa Lagoon is located between 38° 31’ 10” N and 26°49’50” E where is the northwest of the Izmir Bay and within the Gediz Delta. The satellite image of Homa Lagoon was given on Figure 3.1. It consists two parts; main (Homa) lagoon and small (Kırdeniz) lagoon. However, Homa and Kırdeniz Lagoons became shallow due to alluviums carried by Gediz River. Kırdeniz had lost feature to be a lagoon. Total surface area of lagoon is 1800 hectares. The Homa Lagoon has 3 km width and 7 km length. Mean depth varies between 0.5–1 meter and maximum depth is 1.5 meters.

Figure 3.1 Satellite image of Homa Lagoon (Google Maps)

Izmir Metropolitan Municipality had carried out deepening and water circulation improvement works in the lagoon at 2014 (Uluturhan et al., 2019). After that, Homa Lagoon linkage with sea is provided by two channels (Uçmaklıoğlu, 2016).

The Homa lagoon is very close the Bird Paradise in the Gediz Delta where is declared as protected wetlands since 1980, according to Bern and RAMSAR Conventions (Ermert, 2003; Parlak, Çakır, Boyacıoğlu & Arslan, 2006). This delta is an important coastal wetland ecosystem due to hosting wide biodiversity of fauna and flora (Somay & Filiz, 2003). The Homa Lagoon has been allocated to Ege University-Faculty of Fisheries (Tosunoğlu, Kaykaç & Ünal, 2017).

It is reported that many commercial species was caught in the Homa Lagoon such as species of Mugilidae family (*Mugil cephalus, Chelon labrosus, Liza saliens, Liza aurata, Liza ramada*), species of Sparidae family (*Sparus aurata, Diplodus sargus, Lithognathus mormyrus, Diplodus annularis, Diplodus vulgaris*), cuttlefish (*Sepia officinalis*), sea bass (*Dicentrarchus labrax*), anchovy (*Engraulis encrasicholus*), sole (*Solea solea*), eel (*Anguilla anguilla*), flounder (*Platichthys flesus*), red mullet (*Mullus barbatus*), European pilchard (*Sardina pilchardus*) and shrimp (*Penaeus kerathurus*) (Özden, Saka, Fırat & Suzer, 2015).

Gediz River is the one of the biggest rivers (secondly) in the Aegean Region. There are large agricultural areas and many industrial factories such as metal, mining, chemistry, soil products, food, paper industry and leather processing facilities in the Gediz Delta. The Homa Lagoon and Gediz Delta are negatively influenced by organic and inorganic pollutants originated from domestic, agricultural and industrial wastewaters from Gediz River and Izmir Bay (Uluturhan, Kontaş & Can, 2011).

3.2 Previous Studies

Several researches have been carried out in the Homa Lagoon. Many of them are about fisheries studies. In addition to these, studies about physics, chemistry and biology of the Homa Lagoon have carried out.

According to Council of Higher Education's (YÖK) archive, there are 23 Master and PhD thesis titled "Homa Lagoon". 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), Sapancı (2013), Bilgin (2015), Uçmaklıoğlu (2016), Kaya (2017) and Başdemir (2017).

Uluturhan et al (2019) were investigated biomarker in tissues of bivalves response to metal and pesticide pollution in the Homa Lagoon. In this study, heavy metal concentrations found as Hg: 0.07-0.16 mg/kg, Cd: 0.07-0.32 mg/kg, Pb: 0.27-1.57 mg/kg, Cr: 2.90-17.0 mg/kg, Cu: 4.06-7.09 mg/kg, Mn: 8.70-21.6 mg/kg, Zn:43.6-51.3 mg/kg and Fe: 257-1083 mg/kg.

The study about seasonal variations of heavy metal levels were investigated in hepatopancreas and soft tissues of *T. deccussatus* and *M. galloprovincialis* from this lagoon by Bilgin & Uluturhan-Suzer (2017). The heavy metal levels in whole tissues of *M.galloprovincialis* were determined as Hg: 0.11-0.15 mg/kg, Cd: 0.24-0.49 mg/kg, Pb: 0.84-2.41 mg/kg, Cr: 0.32-7.27 mg/kg, Cu: 2.44-5.49 mg/kg and Zn: 75.9-201 mg/kg. On the other hand, metal levels of *T. deccusatus* were found as Hg: 0.07-0.14 mg/kg, Cd: 0.12-0.34 mg/kg, Pb: 0.85-1.49 mg/kg, Cr: 0.77-12.0 mg/kg, Cu: 4.73-10.8 mg/kg and Zn: 58.7-83.5 mg/kg.

The concentrations of heavy metals in sediment from different stations in Homa Lagoon were investigated by Uluturhan, Kontaş & Can (2011). The metal concentrations were determined Hg: 0.22-0.48 mg/kg, Cd: 0.06-.0.19 mg/kg, Pb: 2.43-17.2 mg/kg, Cr:83.9-129 mg/kg, Cu: 10.3-25.8 mg/kg, Mn: 410-729 mg/kg, Ni: 58.1-108 mg/kg, Zn: 46.2-91.9 mg/kg, Fe: 17054-30234 mg/kg, Al: 12663-42637 mg/kg and Li: 11.4-37.5 mg/kg, respectively.

Taş, Ergen & Sunlu (2009) researched concentrations of metal in *Hediste diversicolor* and sediments from two different stations the Homa Lagoon. The study results for *H. diversicolor* were found Cd: 0.31-0.34 mg/kg, Cr: 51-62 mg/kg, Cu: 27-

29 mg/kg, Pb: 11-13 mg/kg, Zn: 57-59 mg/kg and Fe: 21220-18150. The heavy metal concentrations of sediment were determined as Cd: 0.064-0.066 mg/kg, Cr: 1.396-0.890 mg/kg, Cu: 3.535- 4.042 mg/kg, Pb: 9.948-10.408 mg/kg, Zn: 14.580-15.182 mg/kg and Fe: 221.367-253.195 mg/kg.

Zn and Cu accumulation on the several commercial fish species were investigated by Çelik & Oehlenschläger (2005). The Zn concentrations were found for *Sparus aurata* 5.56±0.02 mg/kg, *Tracharus tracharus* 4.40±0.03 mg/kg, *Trachinotus ovatus* 4.07±.0.43 mg/kg, *Lisa saliens* 3.71±0.10 mg/kg, *Lisa ramada* 4.33±0.18 mg/kg and, *Dentex machroptalmus* 3.12±0.10 mg/kg. On the other hand, Cu concentrations were determined as 0.30±0.11 mg/kg on *Sparus aurata*, 1.14±0.03 mg/kg on *Tracharus tracharus*, 0.27±.0.01 mg/kg on *Trachinotus ovatus*, 0.48±0.05 mg/kg *Lisa saliens*, 0.41±0.02 mg/kg on *Lisa ramada* and, 0.22±0.05 mg/kg on *Dentex machroptalmus*.

CHAPTER FOUR
MATERIAL AND METHODS

4.1 Razor Shell (*Solen marginatus*)

Phyllum: Mollusca

Class: Bivalvia

Subclass: Heterodonta

Ordo: Adapendonta

Superfamilya: Solenoidea

Familya: Solenidae

Species: *Solen marginatus* (Pulteney, 1799)

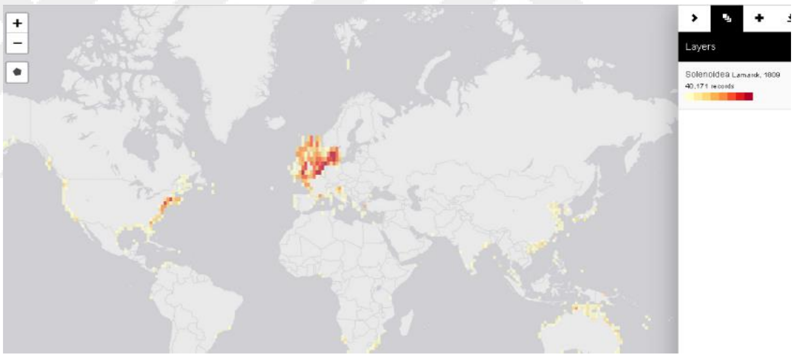

Figure 4.1 General distribution of Solenidae familia (Ocean Biogeographic Information System)

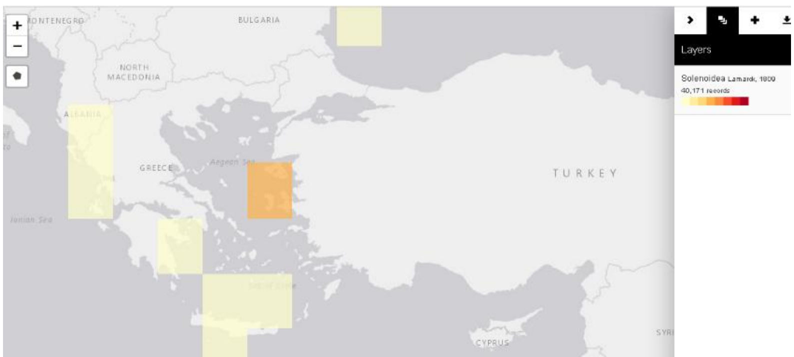

Figure 4.2 Distribution of Solenidae familia in Mediterrenean Sea (Ocean Biogeographic Information System)

The razor shell (*Solen marginatus*) is one of the commercial bivalve species worldwide. Razor shell is geographically distributed from Mediterranean Sea to coasts of the southern of Norway and England. Distribution of Solenidae familia was given on Figure 4.1 and 4.2. *S. marginatus* has characteristic shape that narrow and long shells with gap at both semicylindrical ends and it has one strong foot (Figure 4.3) (Da Costa, Nóvoa, Ojea & Martínez-Patiño, 2011; Sfriso et al., 2018).

Figure 4.3 The image of *Solen marginatus* (Personal archive, 2017)

Razor shells have been well adapted to inhabit soft seabed and tidal (intertidal-subtidal) areas. Due to its strong foot and short siphon, *S. marginatus* generally burrows into (up to 30 cm deep) sandy-muddy sediments. As a filter-feeder, razor shells feed by mainly planktons. The reproductive cycle of *Solen marginatus* base on two different strategies; razor shell adopts restricted reproductivity in the cold season, and an opportunistic reproductive strategy in summer and autumn (Ayache et al., 2016). *S. marginatus* is edible bivalve that has high commercial value at international markets in European countries (Spain, Italy, Portugal and Ireland) (Da Costa et al., 2009; Souissi et al., 2019). Also, it is used as bait by fishers, hence there is the high demand for razor shells. Since the stock of *Solen marginatus* is threatened due to fishing pressure. Ministry of Agriculture and Forestry released regulations about protecting population of *S. marginatus* in the Turkey coasts.

4.2 Sampling Designs and Analyses

4.2.1 Sampling and Preliminary Preparations

Razor shell samples were seasonally collected from May 2015 to November 2017 at the Homa Lagoon (Figure 4.4). Samples were carried to DEU-IMST marine chemistry laboratory in seawater with vessels. Also, sediment samples were collected at biota sampling location in only May 2017 from lagoon, they were transported with plastic bags. During sampling, the physicochemical parameters (pH, salinity, temperature) of seawater were measured by multiparameter meter (WTW) and dissolved oxygen was determined by Winkler titration method. The physicochemical parameters were given at Table 4.1.

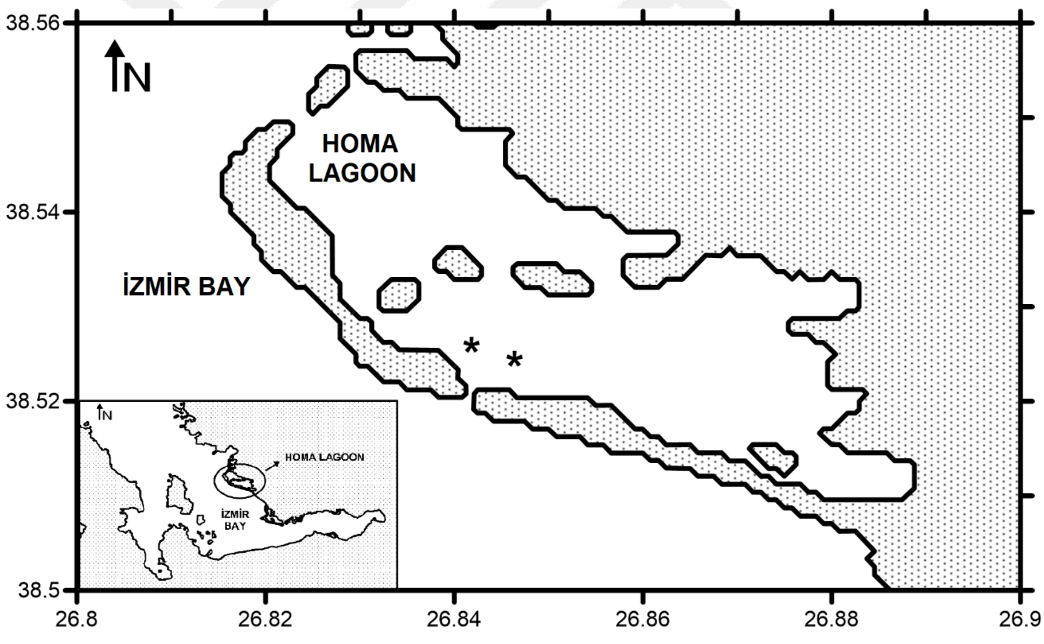

Figure 4.4 Homa Lagoon, *:sampling area

Table 4.1 The physicochemical parameters in Homa Lagoon

Season	Temp (ºC)	Salinity(ppt)	pH	DO (mg/L)
May ‘15	20.0	38.6	8.35	7.52
Aug ‘15	25.3	39.1	8.20	6.28
Nov ‘15	16.1	38.7	8.26	7.73
Feb ‘16	11.4	37.0	8.21	6.93
Feb ‘17	11	36.0	8.20	6.81
May ‘17	20	38.5	8.39	7.41
Aug ‘17	22	39.8	8.25	5.74
Nov ‘17	16	38.7	8.24	7.97

Razor shells were washed with seawater to remove particles or sediment. Biomeristic measurements of *S. marginatus* were made and recorded (Figure 4.5).

Figure 4.5 Biomeristic measurements of *Solen marginatus* (Personal archive, 2017)

Collected samples in May’15 – Feb’16 were got into one group, and then whole tissues dissected from shells, weight of razor shell samples could not measured only in Feb‘16 (Table 4.2). On the other hand, collected samples in Jan’17 – Nov’17 were divided into two groups according to their shell lengths (Table 4.3) and hepatopancreas were dissected from soft tissues which include gills, gonad, heart, foot, muscles, mantle and siphons (Figure 4.6).

Figure 4.6 Dissection of *Solen marginatus* (Personal archive, 2017)

Table 4.2 Biomeristic measurements of *S.marginatus* in May'15-Feb'16

Season	n	Size (mm)	Total weight (g)
May '15	31	77.9-96.1	7.40-16.80
Aug '15	35	80.8-111.6	8.7-23.9
Nov '15	27	84.5-107.9	11.2-25.1
Feb '16	46	71.1-100.2	-

After dissection process, tissues samples were weighted and recorded. Tissues specimen were dried by Labconco Freeze-Dryer and then dried tissues specimens were homogenized by using hand blender. Samples (1 gram) were digested with 5:1 HNO_3:$HClO_4$ by microwave system (Milestone 1200). After digestion of samples, they were diluted to final volume with ultra-pure water (Bernhard, 1976; United Nations Standard Programme [UNEP], 1982, 1984, 1985).

Table 4.3 Biomeristic measurements of *S.marginatus* in Feb '17- Nov'17 within groups

Season	n	Size (mm)	Total weight (g)	Soft tissue weight (g)	Hepatopancreas weight(g)
Feb '17	12	83.13-93.86	11.3-20	4.1-6.6	0.2-0.8
	18	62.07-81.65	5.3-11.7	2-4.8	0.1-0.3
May '17	16	90.35-104.8	10.7-18.3	2.9-7.1	0.3-1.5
	22	74.67-89.6	6.5-15.5	1.3-4.2	0.1-1.1
Aug '17	14	91.05-104.3	12.4-24.9	5.3-10	0.3-0.6
	20	74.21-84.82	7.4-13.5	2.6-6.2	0.1-0.7
Nov '17	17	90.24-103.1	8.5-14.9	4.1-7.1	0.3-0.8
	20	78.14-89.7	5.6-10.7	2.3-5	0.1-0.4

Sediment samples were dried by Labconco Freeze-Dryer System. Then, sediment samples were sieved to pass 63 µm and after that samples were homogenized. Sediment samples were weighted approximately 0.4 gram and digested with HNO_3, $HClO_4$, HCl and HF (3:0.7:0.8:1) acid mixture by microwave digestion system. After digestion of samples, they were diluted to final volume with ultra-pure water (UNEP, 1982, 1985).

4.2.2 Heavy Metal Analyzes

Heavy metal levels were analyzed by using Varian Atomic Absorption Spectrometer (AA280FS & AA280Z). Hg concentrations were detected by cold vapor technique. Cd, Cr and Pb concentrations were detected by graphite furnace technique. Al, Cu, Fe, Mn and Zn levels were detected by flame technique. The detection limits for 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, Fe:0.06 mg/kg, Al:0.30 mg/kg. Reference material (Trace elements and methylmercury in homogenate mussel tissue, National Institute of Standards and Technology [NIST] SRM 2976, USA and Sediment

International Laboratory of Marine Radioactivity, IAEA-158, Monaco) were used to test accuracy of Varian-AAS and validity of the processes.

The result of obtained reference material and certificated values were given in Table 4.4 and Table 4.5.

Table 4.4 Heavy metal levels of reference material NIST SRM 2976 (mean± SD mg/kg dry weight)

Metals	Metals Reference Value	Obtained Value
Hg	0.06±0.04	0.06±0.01
Cd	0.82±0.16	0.79±0.07
Pb	1.19 ± 0.18	1.08±0.16
Cr	0.50 ± 0.16	0.80±0.19
Cu	4.02 ± 0.33	4.02±0.31
Mn	33.0 ± 2.0	36.34±1.99
Zn	137 ± 13	129.72±14.02
Fe	171 ± 4.9	170.1 ± 11.98
Al	134 ± 34	185.65

Table 4.5. Heavy metal levels of reference material IAEA-158 (mean± SD mg/kg dry weight)

Metals	Metals Reference Value	Obtained Value
Hg	0.132±0.014	-
Cd	0.372±0.039	0.3725±0.02
Pb	39.6±4.7	-
Cr	74.4±5.8	87.9± 8.86
Cu	48.3 ± 4.2	48.9
Mn	356±24	330.22±1.60
Zn	140.6 ± 9.5	137.356

4.2.3 The Bioaccumulation Factor

In the aquatic environment, one of the approaches for the assessing ecological risk of pollutants is to determine the biota-sediment accumulation factor (BSAFs) (Burkhard, Cook & Lukasewycz, 2004). The bioaccumulation factor is calculated by following formula according to Barron & Woodburn, 1995 (Eq. 4.1).

$$BAF = \frac{Metal\ concentration\ in\ whole\ organism}{Metal\ concentration\ in\ sediment} \times 100\% \tag{4.1}$$

4.2.4 The Human Health Risk Assessment

The assessment of risk of metals to human health was performed by utilizing provisional tolerable weekly intake (PTWI). PTWI standards (Agency for Toxic Substances and Disease Registry (ATSDR), Food and Agriculture Organizationof the United States and the World Health Organization (FAO/WHO) and Joint FAO/WHO Expert Committee on Food Additives (JECFA)) were compared with metal levels in total tissues of razor shell at both sampling periods from Homa Lagoon. Also metal concentrations in *Solen marginatus* were transformed to wet weight and conversion ratio is determined 0.230. PTWI values were calculated to refer 70 kg adult individual (Velez, Freitas, Soares & Figueira, 2016; Chiesa et al., 2018).

4.2.5 Statistical Analyses

Pearson correlations between size, physicochemical parameters and heavy metal concentrations in tissues of *S. marginatus* were calculated ($R > |0.50|$) to investigate inter-relations of metal levels and environmental indices. Statistical analyses were performed for obtaining differences in heavy metal concentrations. At first, Shapiro-Wilk test were applied to dataset for normality. Kruskal-Wallis test was applied in 2015-2016 and Two-Way ANOVA test was exhibited in 2017 sampling period for detecting significant differences among seasons and tissue types. ANOVA tests were adjusted by using Statistica© 12.0 for Windows (StatSoft, 2014). Non-parametric Kruskal-Wallis test was performed by R statistical software (v. 3.5.1). The significant level was set at $p < 0.05$.

CHAPTER FIVE
RESULTS

5.1 Heavy Metal Concentrations in *Solen marginatus*

The seasonal minimum and maximum levels of heavy metals in *Solen marginatus* were represented at Table 5.1.

5.1.1 Total Tissue (sampling in May'15-Feb'16)

In whole tissues of *Solen marginatus*; concentrations of Hg, Cd, Cr, Cu, Fe and Al were determined that decreasing in the same month. The lowest concentrations of Hg (0.035 mg/kg), Cd (0.323 mg/kg), Cr (12.30 mg/kg), Cu (5.728 mg/kg), Fe (949 mg/kg) and Al (611.6 mg/kg) were found in November 2015. The minimum level of Pb (1.421 mg/kg) and Mn (21.61 mg/kg) were determined in February 2016. Only lowest concentration of Zn (58.57 mg/kg) was obtained in August 2015.

The maximum concentrations of Hg (0.129 mg/kg), Cd (0.925 mg/kg) and Cu (10.66 mg/kg) were detected in May 2015. Highest levels of Pb (3.094 mg/kg), Cr (32.36 mg/kg), Mn (67.38 mg/kg) and Fe (1858 mg/kg) were determined in August 2015. Besides, Zn (71.85 mg/kg) and Al (1308 mg/kg) were detected as highest concentrations in February 2016.

5.1.2 Soft and Hepatopancreas Tissue (sampling in Feb'17-Nov'17)

The seasonal minimum and maximum levels of heavy metals in *Solen marginatus* were represented at Table 5.2.

5.1.2.1 Soft Tissue

In soft tissues, minimum level of Cd (0.054 mg/kg), Cu (5.149 mg/kg), Mn (7.244 mg/kg) were determined in February 2017. The lowest concentrations of Pb (0.792

mg/kg), Cr (2.485 mg/kg) and Al (315.1 mg/kg) were found in May 2017. The minimum concentration of Hg, Zn and Fe were detected respectively as 0.057 mg/kg, 55.834 mg/kg and 510.5 mg/kg in November 2017.

According to analyses; Pb (6.5 mg/kg), Cr (6.033 mg/kg), Zn (94.90 mg/kg), Fe (913.7 mg/kg) and Al (718.3 mg/kg) were determined that reached the highest level in soft tissue in February 2017. While the highest concentration of Hg (0.247 mg/kg), Cd (0.182 mg/kg) and Cu (20.19 mg/kg) were determined in May, the highest level of Mn was detected as 30.49 mg/kg in November 2017.

5.1.2.2 Hepatopancreas Tissue

In hepatopancreas tissue of *Solen marginatus*, minimum concentrations of Hg (0.09 mg/kg), Cd (0.521mg/kg), Pb (4.324 mg/kg), Cu (11.20 mg/kg) and Fe (6039 mg/kg) were found in May 2017. Besides, Cr (9.314 mg/kg), Mn (8.797 mg/kg) and Al (1333 mg/kg) were detected lowest in February 2017. The only Zn (69.22 mg/kg) concentration were obtained as minimum concentration in August 2017.

In February 2017, Hg (0.509 mg/kg), Pb (15.20 mg/kg), Cu (18.57 mg/kg), Zn (111.2 mg/kg) and Fe (9108 mg/kg) were obtained that reached maximum concentration in hepatopancreas tissue. Also, Cd (1.563 mg/kg), Cr (30.05 mg/kg), Mn (77.75 mg/kg) and Al (3667 mg/kg) were detected as maximum concentrations in November 2017.

5.2 The Comparison of Metal Bioaccumulation

The heavy metal mean concentrations with standard deviations were showed in from Figure 5.1 to 5.23. In 2015-2016, the bioaccumulation pattern in whole tissues of *Solen marginatus* was found Fe>Al>Zn>Mn>Cr>Cu>Pb>Cd>Hg.

In 2017, the bioaccumulation pattern in soft tissues was determined Fe>Al>Zn>Mn>Cu>Cr>Pb>Cd>Hg and in hepatopancreas tissues, the bioaccumulation ordering was detected Fe>Al>Zn>Mn>Cr>Cu> Pb>Cd>Hg. Generally, nearly all metal accumulation was found in hepatopancreas.

Table 5.1 The minimum and maximum concentrations of heavy metals in whole tissues of *Solen marginatus* at 2015-2016 (mg/kg dry weight)

Season	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe	Al
May ’15	0.120-0.129	0.861-0.925	1.92-2.38	16.0-25.3	9.3-10.7	63.1-65.5	43.9-53.3	1409-1664	995-1128
Aug ‘15	0.066-0.069	0.809-0.846	2.18-3.09	30.2-32.4	7.7-9.2	58.6-63.1	46.4-67.4	1273-1858	1236-1244
Nov ‘15	0.035-0.052	0.323-0.351	1.47-2.04	12.3-16.1	5.7-6.5	59.3-60.8	33.4-27.7	948-1266	611-805
Feb ‘16	0.077-0.089	0.521-0.562	1.42-2.52	23.6-30.9	9.9-10.7	69.6-71.9	21.6-28.4	995-1523	1306-1308

Table 5.2 The minimum and maximum concentrations of heavy metals in soft and hepatopancreas tissues of *Solen marginatus* at 2017 (mg/kg dry weight)

Season	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe	Al
Soft Tissue									
Feb ‘17	0.100-0.150	0.054-0.086	5.3-6.5	2.8-6.0	5.2-8.5	66.6-94.9	7.2-10.3	549-913	397-718
May ‘17	0.131-0.247	0.136-0.182	0.79-1.2	2.5-4.7	16.9-20.2	65.5-68.0	14.5-17.7	585-846	315-544
Aug ‘17	0.067-0.085	0.113-0.162	0.89-1.5	3.4-4.7	13.5-15.5	56.8-57.7	25.8-27.9	706-762	439-570
Nov ‘17	0.057-0.066	0.106-0.129	1.3-2.05	2.6-5.5	6.9-10.4	55.8-58.5	17.9-30.5	510-872	388-656
Hepatopancreas									
Feb ‘17	0.106-0.509	1.4-1.5	6.6-15.2	9.3-19.6	12.6-18.6	88.7-111	8.8-40.3	6323-9108	1333-1748
May ‘17	0.090-0.203	0.52-1.4	4.3-5.5	16.5-26.1	11.2-12.6	76.6-86.0	40.5-54.2	6039-8768	1654-3147
Aug ‘17	0.361-0.431	0.99-1.2	4.4-8.6	13.7-23.4	12.8-14.1	69.2-70.0	44.9-70.5	6571-7096	1575-3163
Nov ‘17	0.218-0.281	1.3-1.6	5.8-9.3	17.4-30.1	12.9-15.4	73.4-76.5	35.3-77.8	6477-7313	2153-3667

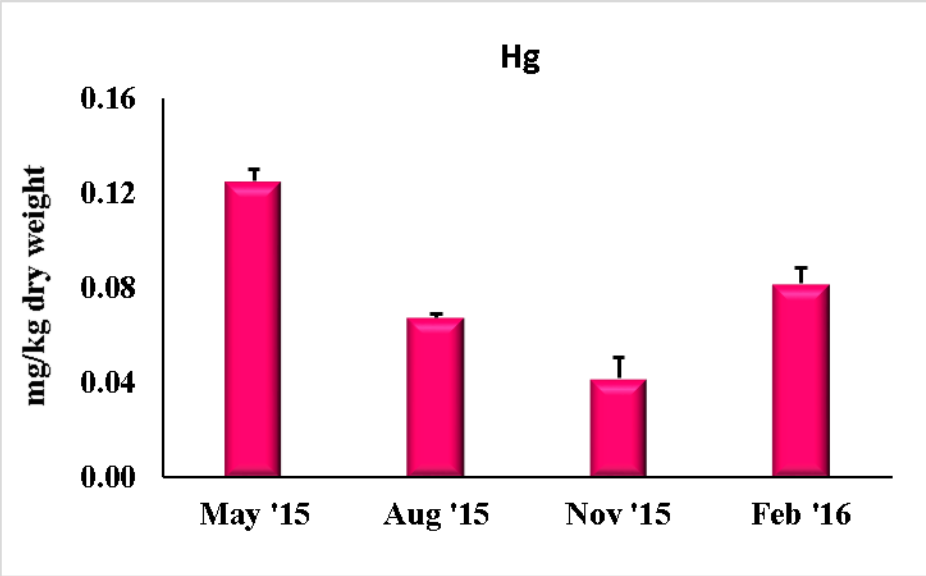

Figure 5.1 Seasonal variations of Hg concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

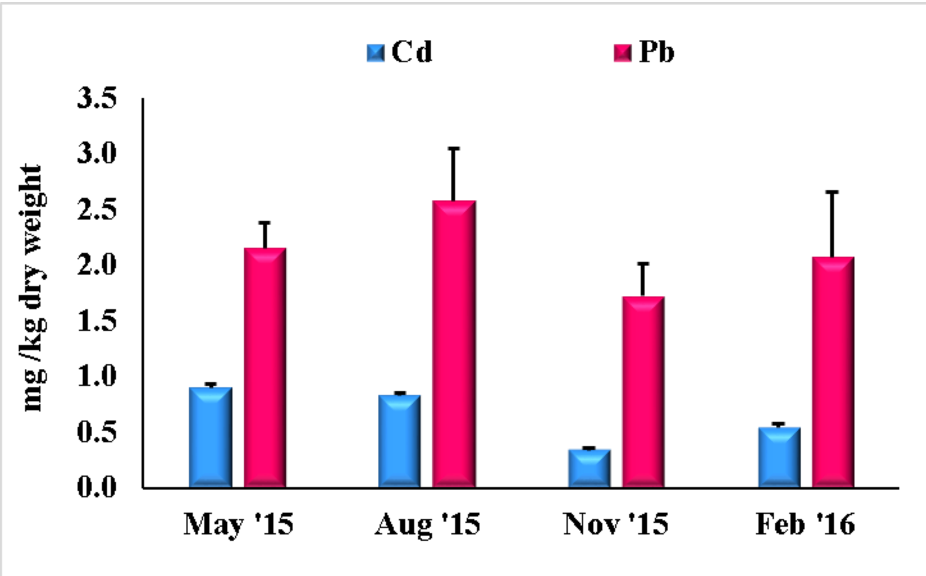

Figure 5.2 Seasonal variations of Cd and Pb concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

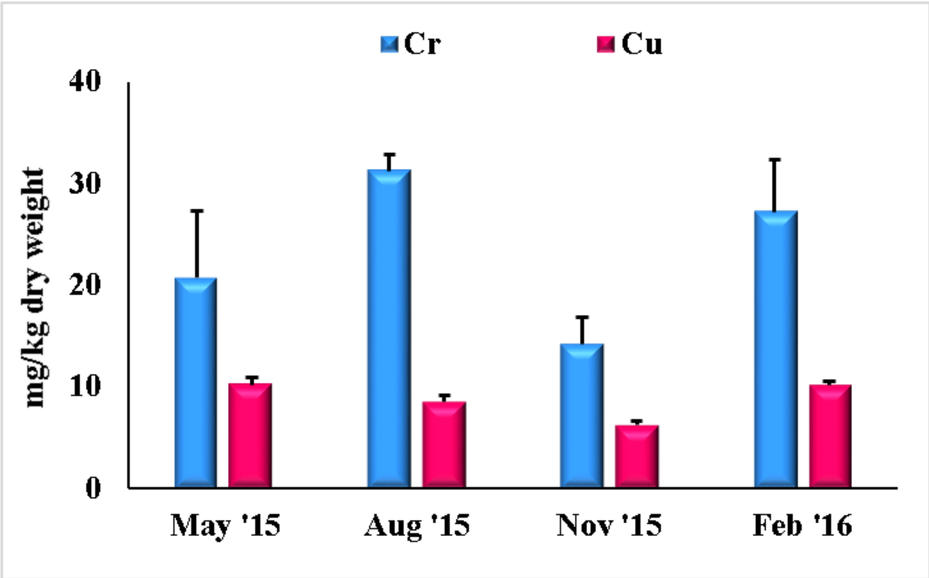

Figure 5.3 Seasonal variations of Cr and Cu concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

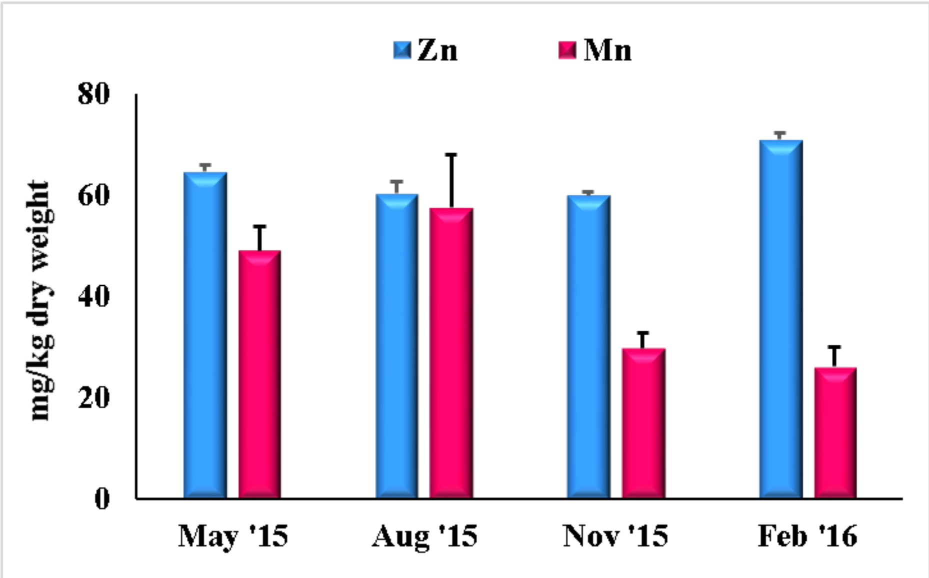

Figure 5.4 Seasonal variations of Zn and Mn concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

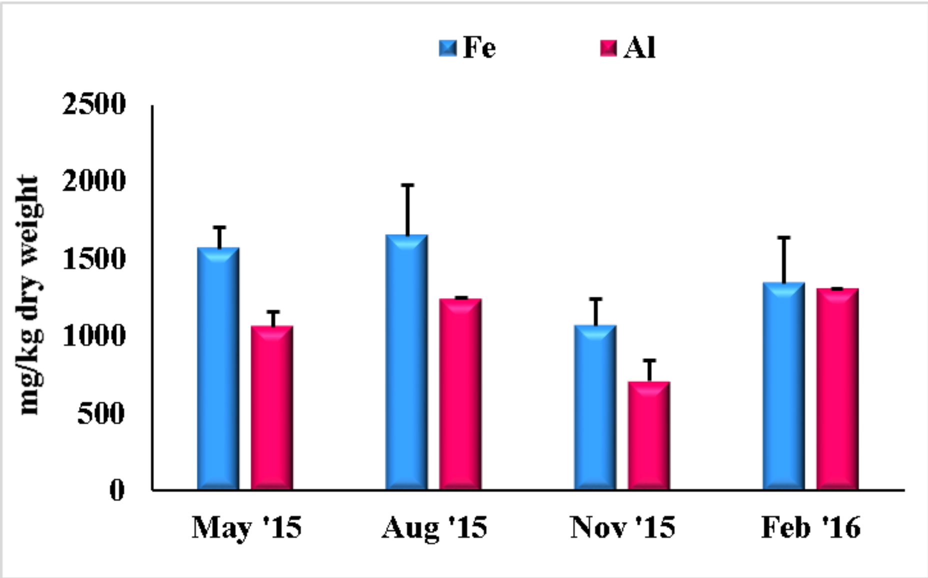

Figure 5.5 Seasonal variations of Fe and Al concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

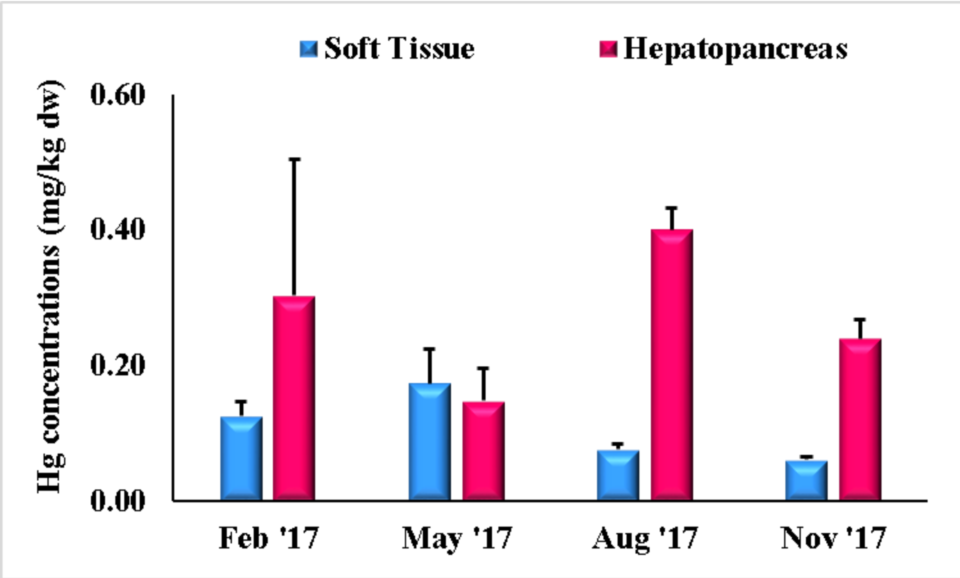

Figure 5.6 Seasonal variations of Hg concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

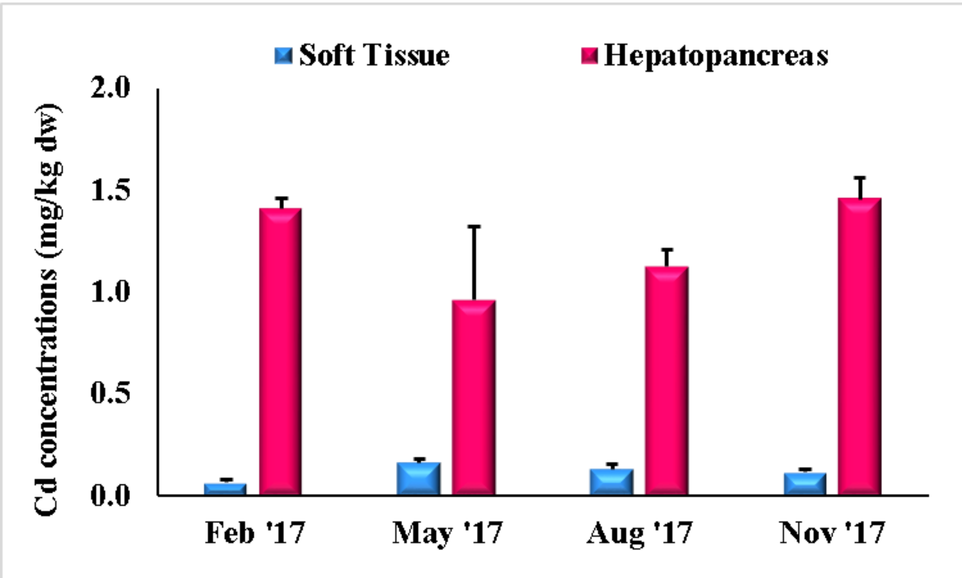

Figure 5.7 Seasonal variations of Cd concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

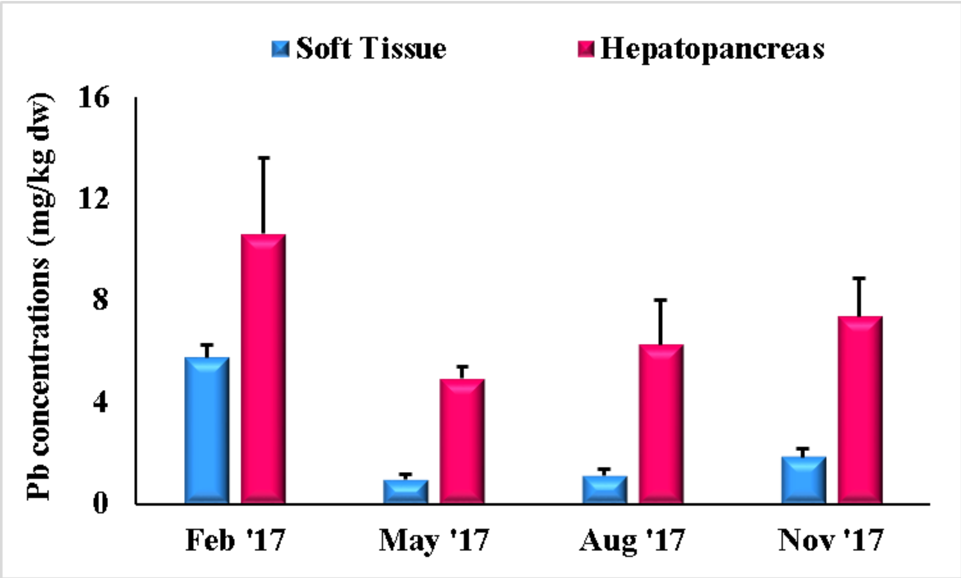

Figure 5.8 Seasonal variations of Pb concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

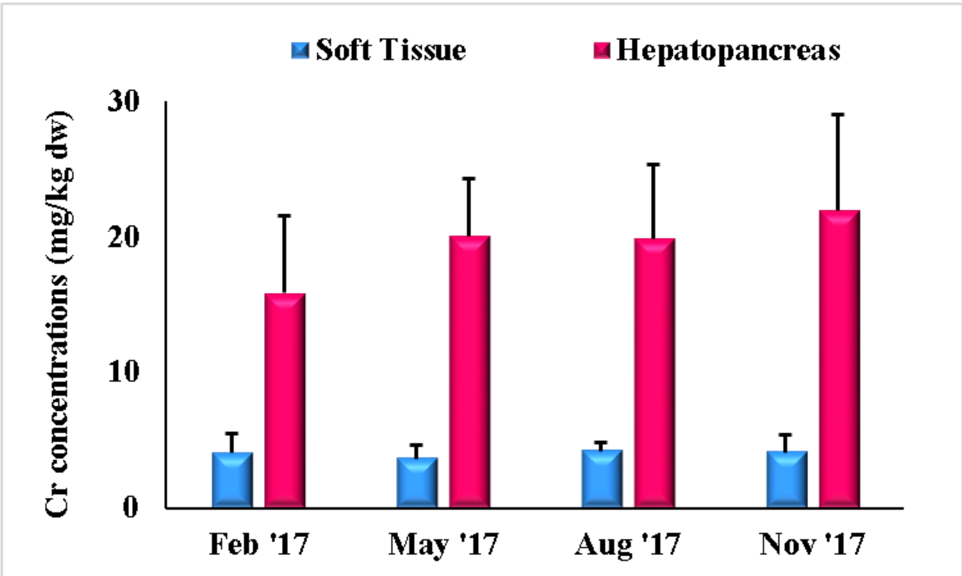

Figure 5.9 Seasonal variations of Cr concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

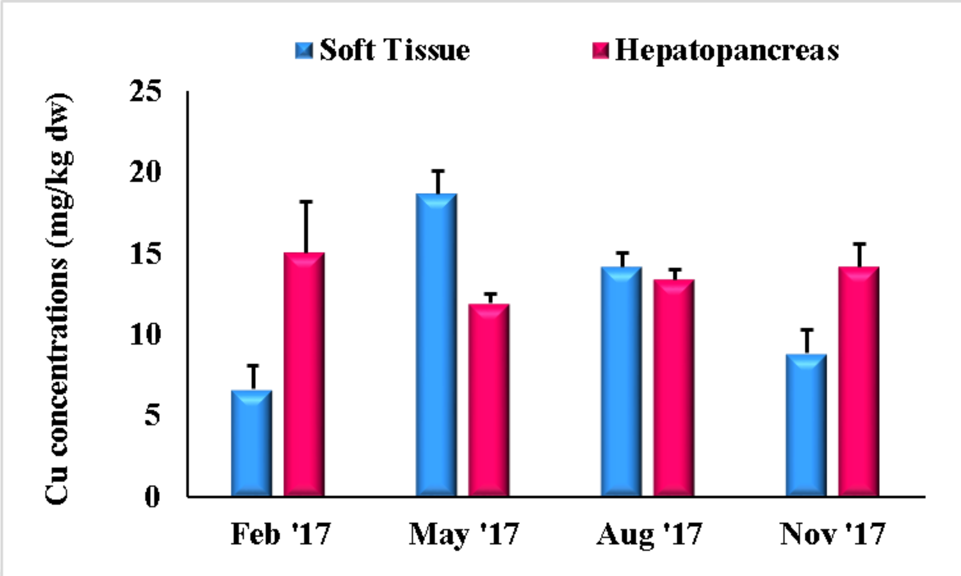

Figure 5.10 Seasonal variations of Cu concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

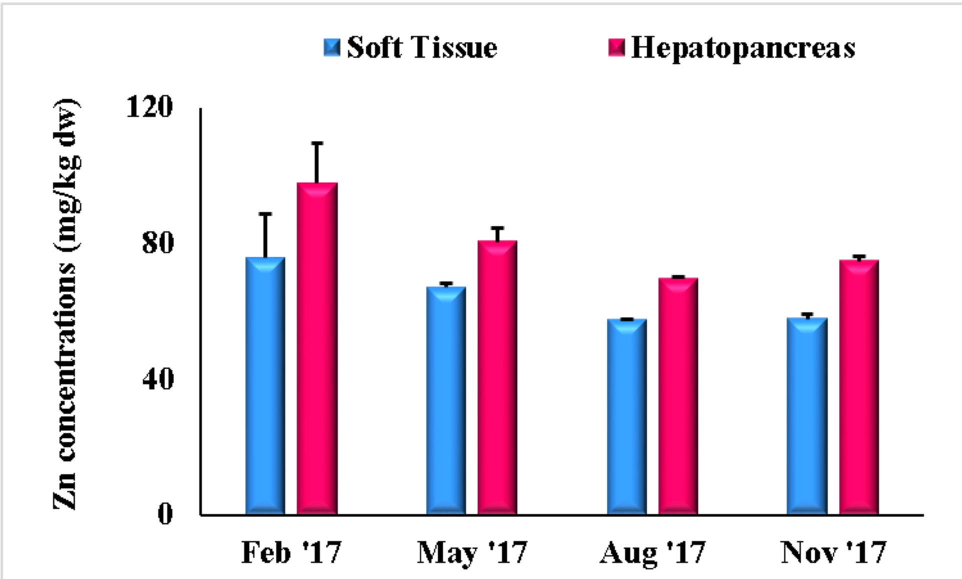

Figure 5.11 Seasonal variations of Zn concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

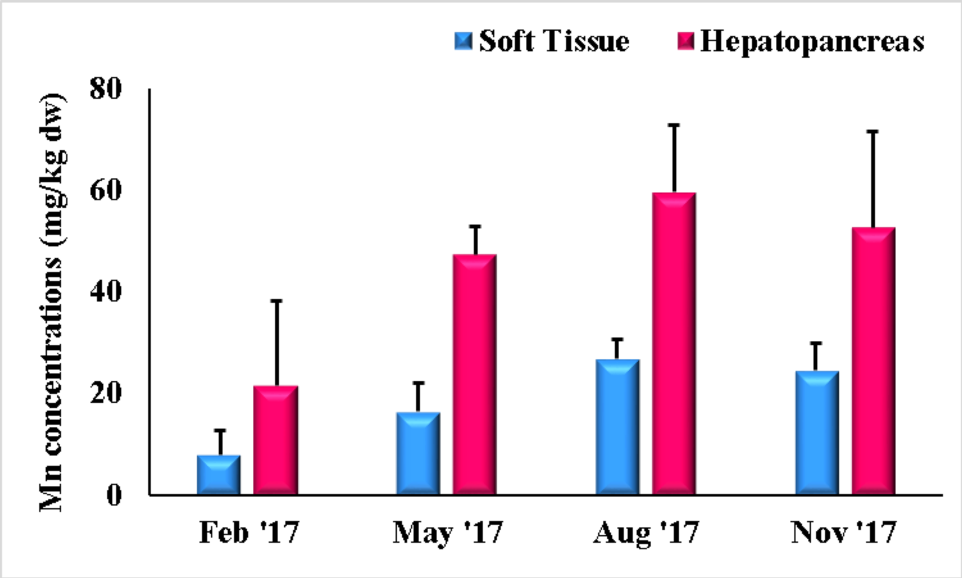

Figure 5.12 Seasonal variations of Mn concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

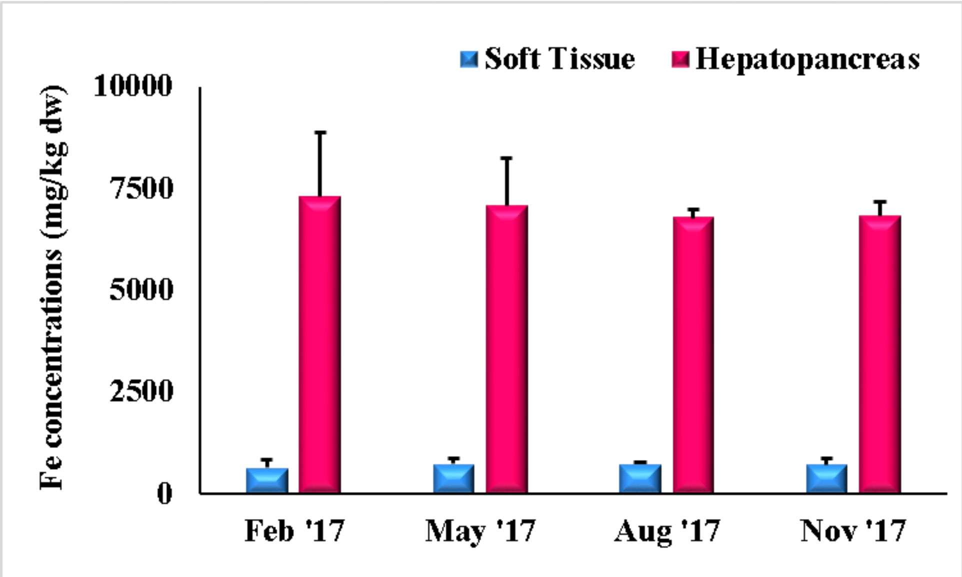

Figure 5.13 Seasonal variations of Fe concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

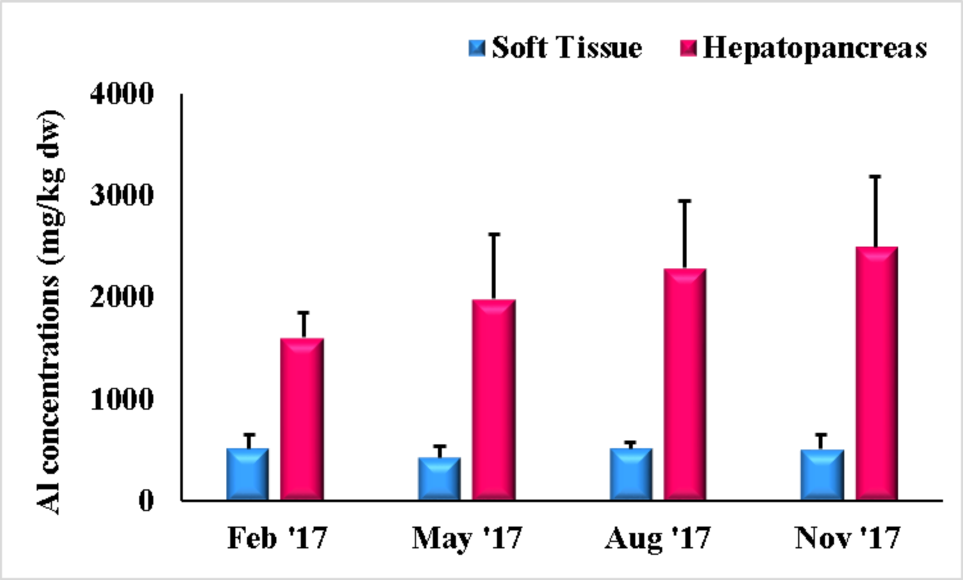

Figure 5.14 Seasonal variations of Al concentrations (means+SD) in soft tissue and hepatopancreas of razor shell (*Solen marginatus*) from the Homa Lagoon

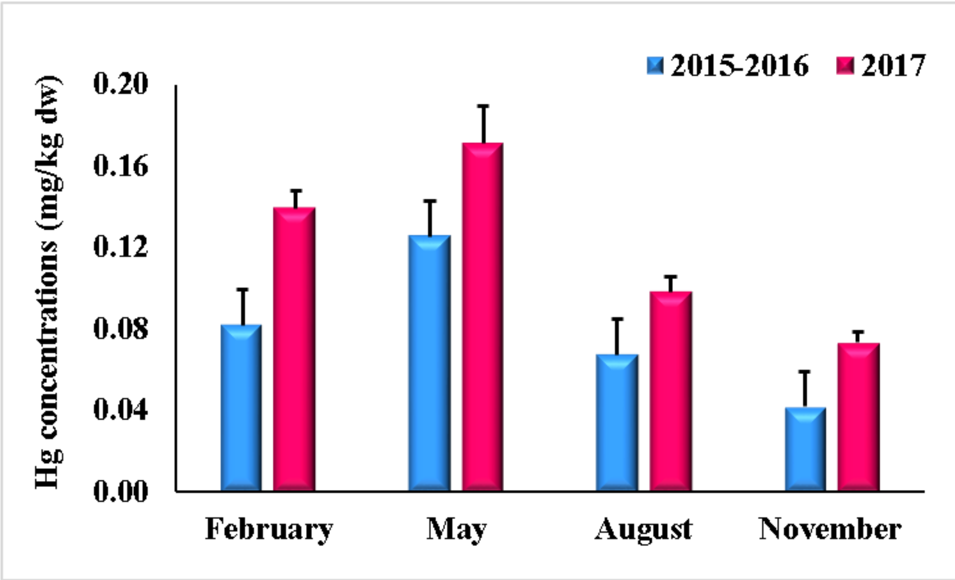

Figure 5.15 Temporal variations of Hg concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

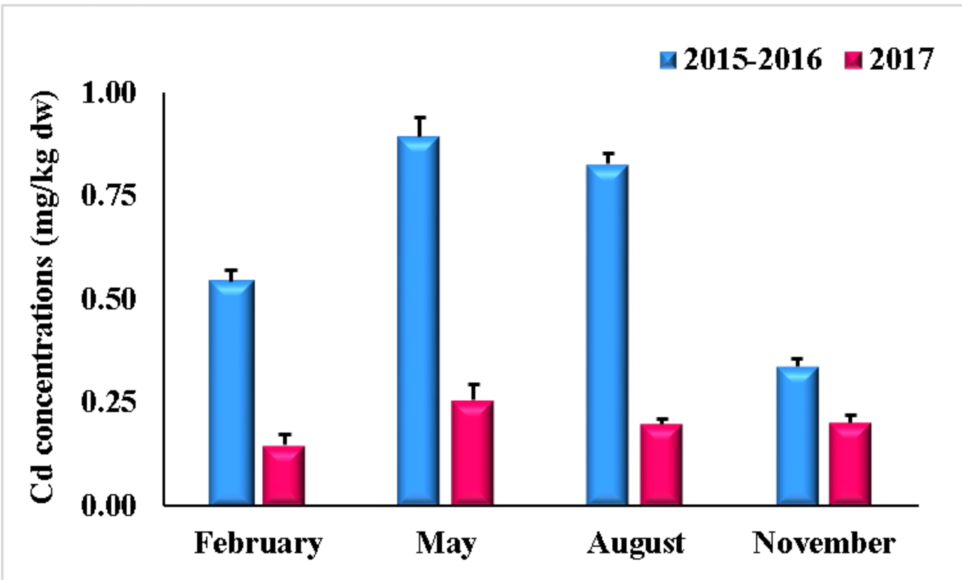

Figure 5.16 Temporal variations of Cd concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

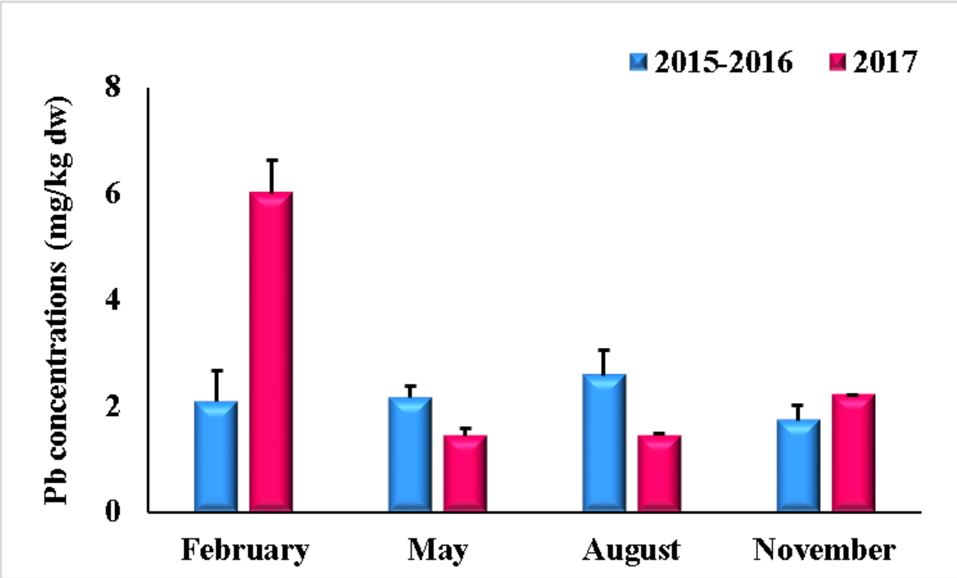

Figure 5.17 Temporal variations of Pb concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

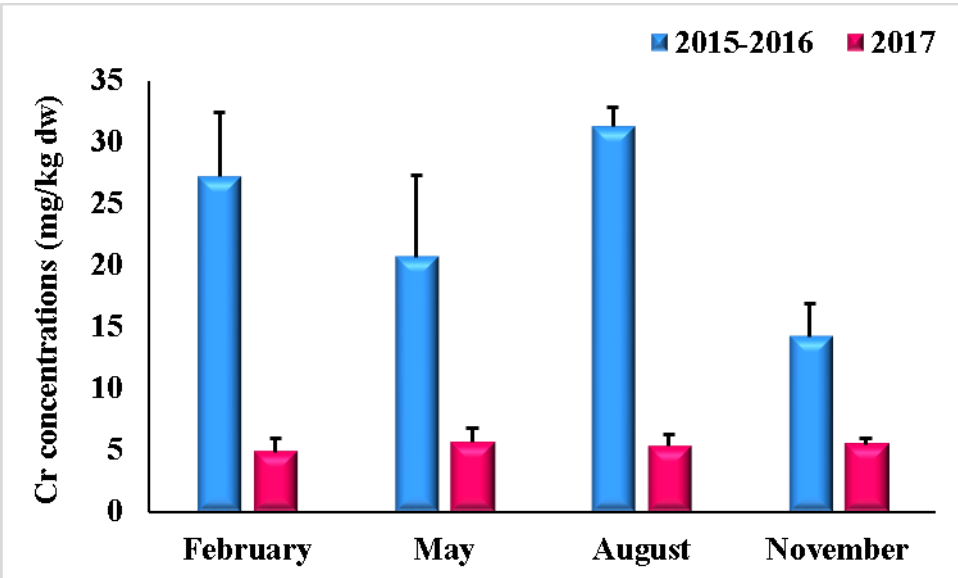

Figure 5.18 Temporal variations of Cr concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

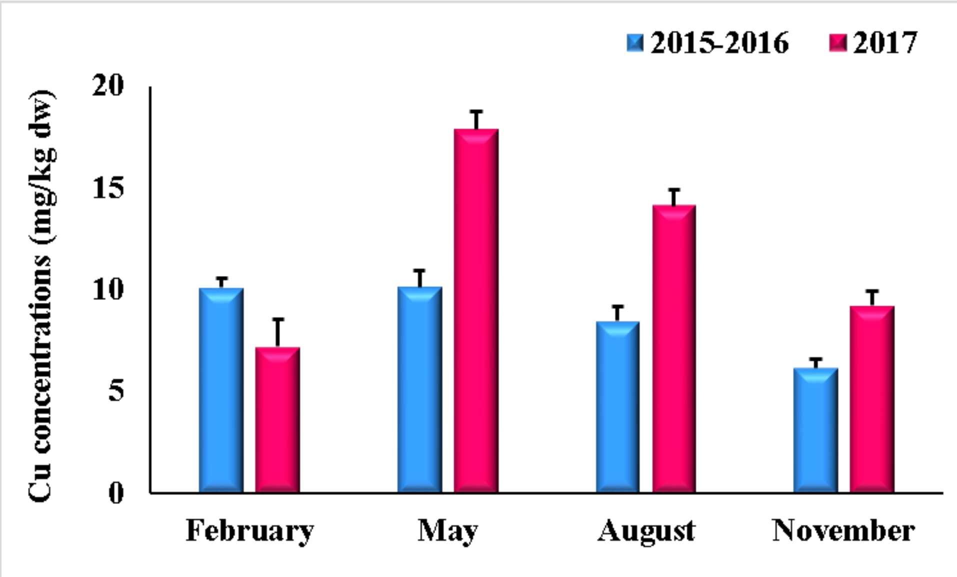

Figure 5.19 Temporal variations of Cu concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) the from Homa Lagoon

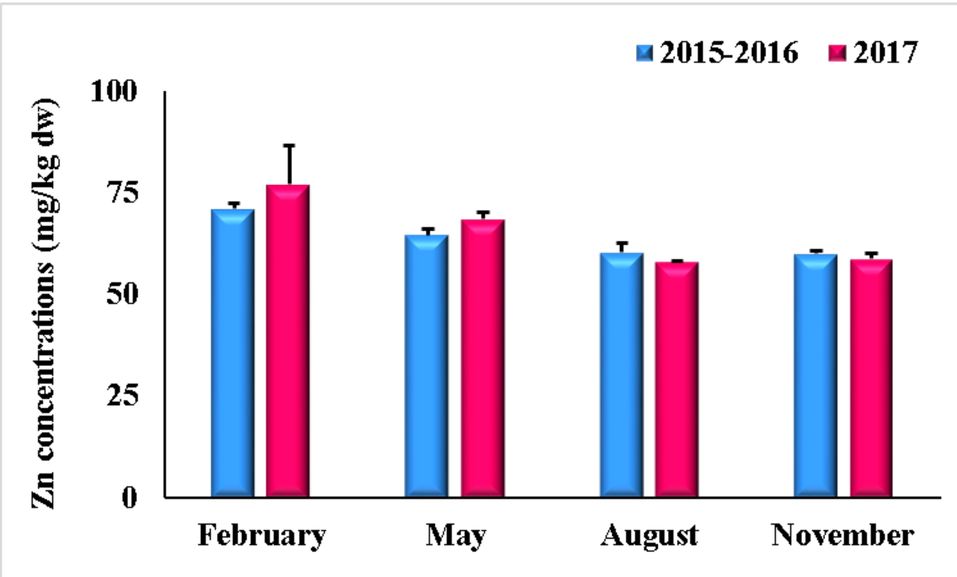

Figure 5.20 Temporal variations of Zn concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

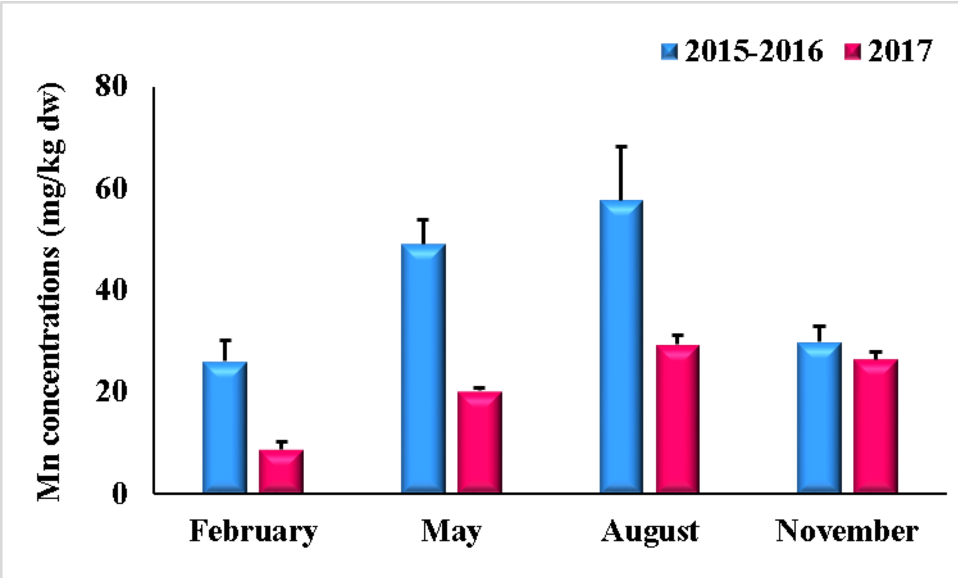

Figure 5.21 Temporal variations of Mn concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

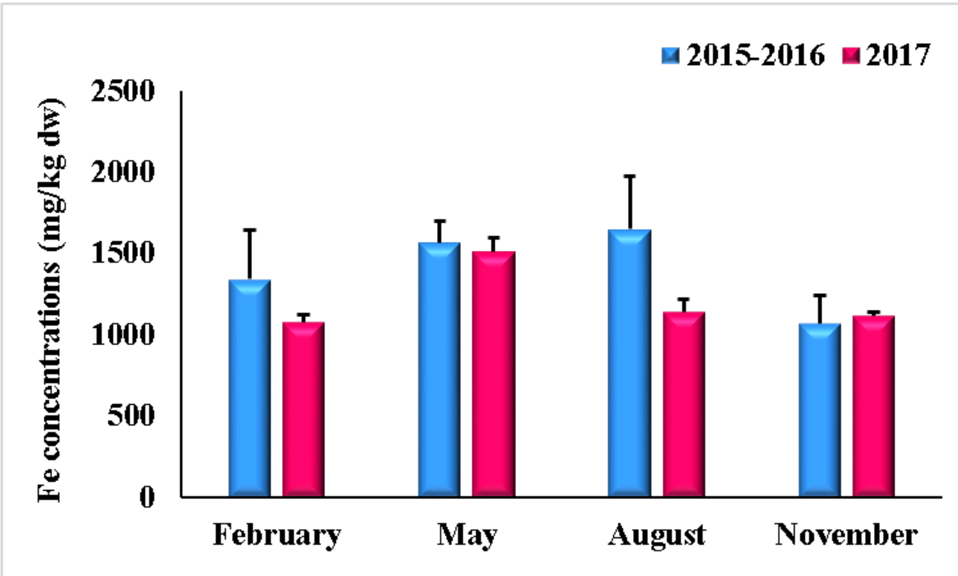

Figure 5.22 Temporal variations of Fe concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*) from the Homa Lagoon

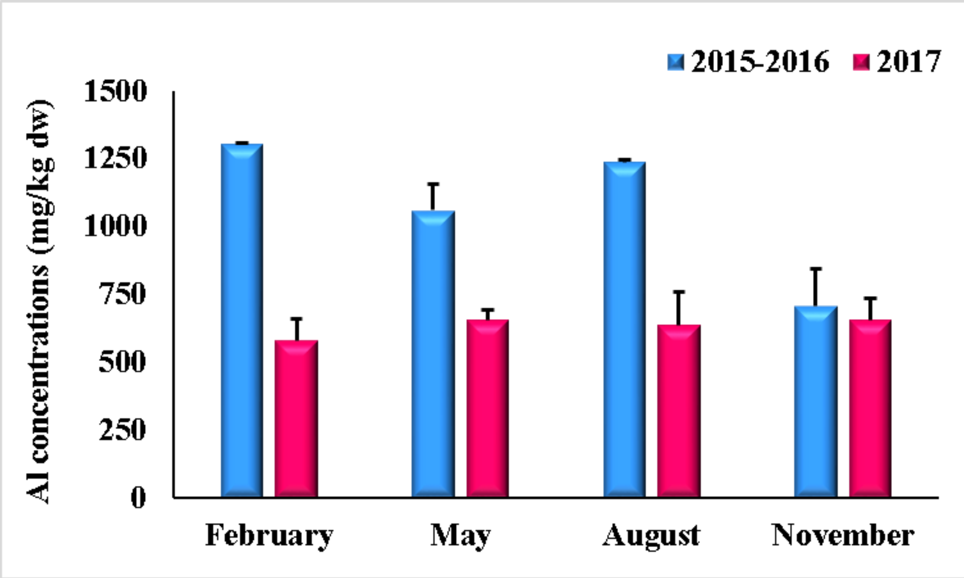

Figure 5.23 Temporal variations of Al concentrations (means+SD) in whole tissue of razor shell (*Solen marginatus*)from the Homa Lagoon

5.3 The Bioaccumulation Factor

The BAF have been calculated for each heavy metal and different tissues in *Solen marginatus* in the Homa Lagoon. BAF results were given in Table 5.3.

According to annually mean BAF results, the high ratio values observed for Hg (BAF=1.580±0.70), Cd (BAF=1.581±0.531) and Zn (BAF=1.255±0.176) in soft tissue and Hg (BAF=4.131±1.95), Cd (BAF=16.460±3.47) and Zn (BAF=1.587±0.272) hepatopancreas. The minimum BAF levels were found Pb (BAF=0.077±0.067), Cr (BAF=0.024±0.006), Mn (BAF=0.047±0.020), Fe (BAF=0.016±0.002) and Al (BAF=0.034±0.007) in soft tissue and Pb (BAF=0.226±0.084), Cr (BAF=0.123±0.029), Mn (BAF=0.112±0.047), Fe (BAF=0.156±0.021) and Al (BAF=0.150±0.041) hepatopancreas, respectively.

Table 5.3 The Bioaccumulation factor ratios of heavy metal in different tissues of *Solen marginatus* and heavy metal concentrations in sediment from the Homa Lagoon (mg/kg dry weight)

Metals (mg/kg)	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Mn**	**Fe**	**Al**
Homa Lagoon sediment	0.069	0.075	31.16	165.8	14.11	51.31	406.4	45440	14708
Soft Tissue (BAF %)									
Feb'17	1.81±0.32	0.87±0.21	0.18±0.01	0.02±0.01	0.47±0.11	1.47±0.21	0.02±0.003	0.01±0.003	0.04±0.004
May '17	2.51±0.31	2.17±0.07	0.03±0.01	0.02±0.01	1.32±0.09	1.31±0.02	0.04±0.003	0.02±0.003	0.03±0.01
Aug'17	1.11±0.13	1.77±0.33	0.04±0.01	0.03±0.004	1.00±0.06	1.12±0.01	0.07±0.002	0.02±0.0003	0.04±0.005
Nov '17	0.89±0.01	1.52±0.15	0.06±0.01	0.02±0.01	0.63±0.07	1.13±0.02	0.06±0.001	0.02±0.003	0.03±0.01
Annually mean BAF	1.580±0.70	1.581±0.531	0.077±0.067	0.024±0.006	0.857±0.361	1.255±0.176	0.047±0.020	0.016±0.002	0.034±0.007
Hepatopancreas (BAF %)									
Feb'17	5.12±3.14	18.62±0.66	0.31±0.14	0.10±0.02	1.13±0.27	1.97±0.28	0.05±0.01	0.17±0.04	0.10±0.02
May '17	2.14±0.04	12.82±4.81	0.16±0.003	0.12±0.02	0.85±0.03	1.56±0.09	0.12±0.0005	0.16±0.02	0.15±0.03
Aug'17	5.79±0.50	14.98±0.78	0.20±0.06	0.13±0.02	0.95±0.01	1.36±0.01	0.15±0.028	0.15±0.002	0.16±0.04
Nov'17	3.47±0.38	19.43±1.48	0.24±0.05	0.14±0.05	1.01±0.12	1.46±0.02	0.13±0.05	0.15±0.008	0.18±0.05
Annually Mean BAF	4.131±1.95	16.460±3.47	0.226±0.084	0.123±0.029	0.983±0.155	1.587±0.272	0.112±0.047	0.156±0.021	0.150±0.041

5.4 Statistical analyses

5.4.1 Statistical Calculations for 2015-2016 Sampling Period

5.4.1.1 The Correlations of Metals among Size, Physicochemical Parameters in Whole Tissue of S. marginatus

The correlation results of metal concentrations among size, physicochemical parameters were given at Table 5.4. During sampling period in 2015-2016, the significant correlations between metal concentrations and size were detected for Hg (R= -0.518), Cu (R= -0.798), Zn (R= -0.996) and Al (R= -0.609).

The significant correlations between metal concentrations and temperature were found for Cd (R= 0.677), Pb (R= 0.719), Zn (R= -0.708), Mn (R= 0.961) and Fe (R= 0.690).

The salinity values were significantly correlated with Cu (R= -0.500), Zn (R= -0.946) and Mn (R= 0.730). On the other hand, Hg (R= 0.656) and Cr (R= -0.589) were statistically correlated with pH.

Also, DO levels were significantly inversely correlation with Pb (R= -0.880), Cr (R= -0.958), Fe (R= -0.657) and Al (R= -0.786).

Table 5.4 Correlations of metals among size, physicochemical parameters in whole tissue of *Solen marginatus* at 2015-2016 sampling period (R> |0.50| values are bolded)

	Size	**Temperature**	**Salinity**	**pH**	**DO**
Hg	**-0.518**	0.098	-0.143	**0.656**	0.073
Cd	-0.066	**0.677**	0.322	0.313	-0.440
Pb	0.047	**0.719**	0.283	-0.303	**-0.880**
Cr	-0.284	0.335	-0.151	**-0.589**	**-0.958**
Cu	**-0.798**	-0.100	**-0.500**	0.191	-0.284
Zn	**-0.996**	**-0.708**	**-0.946**	-0.073	-0.045
Mn	0.444	**0.961**	**0.730**	0.150	-0.498
Fe	-0.076	**0.690**	0.268	0.057	**-0.657**
Al	**-0.609**	0.072	-0.431	-0.411	**-0.786**

5.4.1.2 Variations of Heavy Metal Concentrations with Seasons

For all whole tissue of razor shell, since there were not normal distributions, non-parametric Kruskal-Wallis test was performed on each metal. Results were given on Table 5.5. According to p-values; Hg, Cu, Zn and Mn concentrations showed significant differences between seasons.

Table 5.5 Variations of metal concentrations between seasons on *Solen marginatus* from Homa Lagoon at 2015-2016 sampling period ($p > 0.05$). (Significant differences were bolded.)

Metal	D*f*	F	P
Hg	3	10.3846	**0.015564**
Cd	3	6.666667	0.083316
Pb	3	4.64103	0.200051
Cr	3	4.833333	0.184416
Cu	3	9.358974	**0.024879**
Zn	3	8.692308	**0.033674**
Mn	3	8.948718	**0.029981**
Fe	3	6.179487	0.103197
Al	3	6.666667	0.083316

5.4.2 Statistical Calculations for 2017 Sampling Period

5.4.2.1 The Correlations of Metals among Size, Physicochemical Parameters in Soft and Hepatopancrease Tissue of S. marginatus

The metal correlations among size and physicochemical parameters for both tissues were given at Table 5.6-5.7.

In soft tissues of *S. marginatus* in Feb’17 – Nov’17, there were significant correlations between size and metal concentration in Cd (R=0.860), Pb (R=-0.974), Cu (R= 0.628), Zn (R= -0.874), Mn (R= 0.866) and Fe (R= 0.958).

Temperature values were significantly related with Cd (R=0.881), Pb (R=-0.923), Cu (R=0.838), Zn (R=-0.695), Mn (R=0.741) and Fe (R=0.949).

There were significantly correlation in salinity values with Cd (R= 0.773), Pb (R= -0.935), Zn (R= -0.920), Mn (R= 0.942) and Fe (R=0.943). All metal levels (except Zn and Mn) were significantly related with pH values.

Table 5.6 Correlations of metal concentrations among size, physicochemical parameters in soft tissue of *Solen marginatus* at 2017 sampling period (R> |0.50| values are bolded)

	Size	**Temperature**	**Salinity**	**pH**	**DO**
Hg	-0.252	-0.027	-0.378	**0.687**	0.140
Cd	**0.860**	**0.881**	**0.773**	**0.869**	0.021
Pb	**-0.974**	**-0.923**	**-0.935**	**-0.649**	0.042
Cr	-0.110	-0.122	0.136	**-0.872**	-0.449
Cu	**0.628**	**0.838**	0.601	**0.912**	-0.174
Zn	**-0.874**	**-0.695**	**-0.920**	-0.059	0.111
Mn	**0.866**	**0.741**	**0.942**	0.068	-0.199
Fe	**0.958**	**0.949**	**0.943**	**0.646**	-0.113
Al	-0.336	-0.367	-0.118	**-0.968**	-0.349

In hepatopancreas concentrations at 2017, the significant correlations between size and metal concentrations (except Hg and Cd) were observed.

The temperature values were significantly correlated with metals, except Hg. The significant correlations between salinity levels and metal concentrations were found for Pb (R= -0.825), Cr (R= 0.811), Zn (R= -0.990), Mn (R= 0.994), Fe (R= -0.923) and Al (R= 0.803).

The pH values were significantly correlated with Hg (R=-0.706), Cd (R= -0.843), Pb (R= -0.823) and Cu (R=-0.957). On the other hand, there were significant correlations between DO values and metal levels in solely Hg (R= -0.815).

Table 5.7 Correlations of metal concentrations among size, physicochemical parameters in hepatopancreas tissues of *Solen marginatus* at 2017 sampling period (R> |0.50| values are bolded)

	Size	**Temperature**	**Salinity**	**pH**	**DO**
Hg	-0.190	0.075	0.163	**-0.706**	**-0.815**
Cd	-0.432	**-0.769**	-0.468	**-0.843**	0.346
Pb	**-0.890**	**-0.912**	**-0.825**	**-0.823**	0.026
Cr	**0.949**	**0.615**	**0.811**	0.377	0.329
Cu	**-0.668**	**-0.795**	-0.582	**-0.957**	0.016
Zn	**-0.936**	**-0.857**	**-0.990**	-0.275	0.195
Mn	**0.952**	**0.881**	**0.994**	0.339	-0.180
Fe	**-0.852**	**-0.705**	**-0.923**	-0.029	0.174
Al	**0.931**	**0.582**	**0.803**	0.305	0.322

5.4.2.2 Variations of Metal Concentrations among Seasons and Tissues on S.marginatus

Two-way ANOVA tests were performed on each metal for detecting significant differences among seasons and tissues. Results were given on Table 5.8. Hg, Cd, Pb, Cu, Zn and Mn concentrations showed significant differences both seasons and tissues. Significant differences were found for Cr, Fe and Al concentrations between tissues; however, no significant differences were obtained between seasons.

Table 5.8 Variations of metal concentrations between seasons and tissues on *Solen marginatus* from Homa Lagoon at 2017 sampling period ($p > 0.05$). (Significant differences were bolded.)

Metal	**Seasons**			**Tissues**		
	D*f*	**F**	**P**	**D*f***	**F**	**P**
Hg	3	3.1599	**0.043941**	1	46.2059	**0.000001**
Cd	3	3.6990	**0.027011**	1	442.5765	**0.000000**
Pb	3	16.0373	**0.000008**	1	76.0263	**0.000000**
Cr	3	0.8175	0.498655	1	122.8573	**0.000000**
Cu	3	15.376	**0.000010**	1	8.876	**0.006709**
Zn	3	20.683	**0.000001**	1	53.370	**0.000000**
Mn	3	10.9758	**0.000131**	1	52.5138	**0.000000**
Fe	3	0.195	0.898843	1	703.780	**0.000000**
Al	3	1.9560	0.148792	1	124.6824	**0.000000**

CHAPTER SIX
DISCUSSION

6.1 Bioaccumulation of Heavy Metals on *Solen marginatus*

Lagoons are special habitats and highly productive coastal areas that have controlling of climate changes and provide specific habitats for many species. Also, these areas have socio-economic values because they mediate several natural services such as fisheries productivity (Gönenç & Wolflin, 2005). The study area is last active lagoon that is protected by RAMSAR convention in the Aegean Sea. However, the lagoon is greatly subjected to contaminants which are welded from Gediz River and Izmir Bay. In the marine environment, which is under threat of pollution, should be periodically monitored with benthic species. Among them, bivalves are used to monitor pollutants in the marine environment. Bivalves are one of the most ideal bioindicator organisms, since they are filter-feeder and sedentary species, have an ability to accumulate pollutants such as metals in their bodies in long terms. In this thesis, the assessment of metal concentrations had been carried out in *Solen marginatus* from the Homa Lagoon.

The many previous researches have shown that accumulated metal concentrations in bivalves depend on many variables. The bioavailability of metals, seasonally physicochemical parameters and hydrological conditions in aquatic environment influence on metal bioaccumulation. As well as, organisms' biologic state such as age, size, sex, growth and reproductive cycle are associated with metal up taking and accumulation. Due to the above mentioned reasons, seasonal changes and different bioindicator species are considered for metal pollution monitoring (Szefer, Kim.B, Kim.C, Kim.E & Lee, 2004; Maanan, 2007; Tarique, Burger & Reinfelder, 2019).

Al concentrations in the Mediterranean Sea is relatively high (>100 nM) due to atmospheric inputs (Mason, 2013). It exists several different chemical forms, and its toxicity and bioavailability greatly depend conditions of aquatic environment such as organic matter and pH (Rosseland, Edhuset & Staurnes, 1990; Quiroz-Vázquez, Sigee

& White, 2010). In this thesis, Al concentrations were determined as second highest metal, after Fe. The highest Al concentrations were found in hepatopancreas. Similar result for Al levels in bivalves were determined previous study in Homa Lagoon (Bilgin & Uluturhan-Suzer, 2015). Kádár et al., (2001) have also claimed that hepatopancreas could provide accumulation of metals due to the fact that it plays key role as a storage and regulates metabolic and homeostatic activities.

The Cd levels may exceed background levels in aquatic ecosystem due to its greatly used in many facilities. Cd has no biological function in organisms and also this metal may be greatly toxic both acute and chronic exposure. Temperature, salinity and presence of calcium have an effect on Cd toxicity in the marine environment (Chiarelli & Roccheri, 2014). According to Pearson correlation results in this study, significant correlations between temperature and Cd concentrations were determined. This situation may possibly that metabolic activity's related with temperature that effects on Cd bioaccumulation rate as well. Heugens et al., (2003) have reported that Cd uptake and bioaccumulation level increase with higher temperatures in marine organisms. According to results, the highest Cd concentrations in whole tissues were determined in May for both sampling periods. Also, Kamaruzzaman et al (2010), Kanakaraju, Ibrahim & Berseli (2008) had found similar Cd levels in soft tissues of razor clams. In early summer is pre-spawning period for razor clams (Ayache et al., 2016). While feeding rate increases for reproduction, metal concentrations shows increasing trend in bivalves in this period (Regoli & Orlando, 1994; Szefer et al., 2004). It’s explained that hence bivalve species cannot regulate Cd, this metal could be readily accumulated by these organisms (Li, Yu, Song & Mu, 2006; Kanakaraju, Ibrahim & Berseli, 2008; Kamaruzzaman et al., 2010). When accumulation of Cd was considered, hepatopancreas tends to more accumulate Cd than soft tissue. Due to involving metallothionein and detoxification mechanism, bivalves could more accumulate metals in their hepatopancreas with low harmful effects (Scudiero, Cretì, Trinchella & Esposito, 2014; Uluturhan et al., 2019).

In this study high Cr concentrations in 2015-2016 sampling period are same as levels in razor shells from Malaysia (Hassan & Kanakaraju, 2013; Kanarakaju, Ibrahim & Berseli, 2008). Cr levels in hepatopancreas was higher than in soft tissues. Due to the fact that, it metabolizes and detoxicates pollutants, hepatopancreas is a target organ for metal pollution (Ahearn, Mandal & Mandal, 2004; Bilgin & Uluturhan-Suzer, 2017). During 2017 sampling period, Cr concentrations are similar with bivalves from Homa lagoon in previous study (Bilgin, 2015).

Due to being inseparable part of haemocyanin, Cu is vital importance for some marine organism groups, such as molluscs and crustaceans. It’s reported that organisms' homeostatic mechanisms have a capability regulate essential metals, such as Cu, Zn (Catsiki, Bel & Nicolaidou, 1994). During sampling periods, the highest Cu concentrations were obtained in May when is before spawning period. Organisms meet the necessary energy with nutritional uptake before spawning. Metal uptake and bioaccumulation also occur in this period. Therefore, Cu accumulation may possibly reach the maximum levels before spawning period.

Fe is inseparable part of many metalloenzymes and metalloproteins (such as hemoglobin and myoglobin). In this thesis, Fe values were determined as the highest concentration than other metals. Our results are parallel with Fe concentrations in *T. decussatus* and *M. galloprovincialis* sampled in Homa Lagoon (Bilgin, 2015). In this study, hepatopancreas much more tends to accumulate Fe than soft tissue. Digestive gland acts as major storage for Fe, like other metals. Fe concentrations were decreased in November ‘15 and February ‘17 due to post spawning period. It’s pointed that after spawning period, metal levels show a decrease approximately 30-60% (Cheggour, Chafik, Fisher & Benbrahim, 2005).

Pb is a non-essential metal and has not any known metabolic function, it mostly presents in marine environment cause of anthropogenic sources (Cabrini et al., 2017). In 2017 sampling period, the highest Pb concentration in *S. marginatus* were found in February. These results were found as similar with *T. decussatus* and *M. galloprovincialis* sampled in Homa Lagoon (Bilgin & Uluturhan-Suzer, 2017) and *M. galloprovincialis* from Beagle Channel (Giarratano & Amin, 2010). According to Pb results, hepatopancreas tissues tend to accumulate much more than soft tissue. And also, these findings were determined as similar with other bioaccumulation researches (Scuderio et al., 2014; Bilgin & Uluturhan-Suzer, 2017).

Because its using in several industrial facilities, Mn contamination have attracted attention as a new pollution factor in the aquatic environment (Satyanarayana & Saraf, 2007; Pinsino et al., 2010). The maximum Mn concentrations for both sampling periods were obtained in August. During the summer, if temperature and food uptake conditions are optimum levels, reproduction activities may be continued in razor shells (Ayache et al., 2016). The minimum Mn values were detected in February, the post-spawning period. Generally, metal concentrations tend to decrease in marine organisms after spawning (Cheggour et al., 2005). Like other metals in this study, Mn accumulation have tendency to store in hepatopancreas.

Mercury is highly toxic contaminant even at low concentrations. The highest Hg levels were found for both sampling periods in May which is pre-spawning period of *S. marginatus*. On the other hand, during sampling periods, minimum Hg concentrations in whole tissues was obtained in November. It's pointed that bioaccumulated metals could be mobilized through physiological processes such as spawning (Dahlgaard, 1986; Cheoggur et al., 2005). In this study, Hg values were found higher in hepatopancreas. Researchers have reported that, in marine organisms, liver and hepatopancreas are target organs which accumulate high concentrations of metals (Engel, 1983; Mao, Mahaut, Pineau, Barillier & Caplat, 2011; Azad et al., 2019).

Zinc is an essential metal that's known as constituent of many enzymes and protein, therefore Zn concentrations may present high level in molluscs (White & Rainbow, 1985). At the same time, many researches have reported that 100 μg/L and above concentrations of Zn is stated as toxic for marine organisms (Tellis, Lauer, Nadella, Bianchini & Wood, 2014; Le Pabic et al., 2015). However, Zn concentrations may be regulated through metabolic processes or homoeostasis (Jakimska et al., 2011). The peak levels of Zn were found at February in both sampling periods. Similar results were detected in *Mytilus galloprovincialis* from Homa Lagoon (Bilgin &Uluturhan-Suzer, 2017).

According to comparison of sampling periods, Al, Fe, Mn, Cd and Cr were determined as high levels in 2015-2016. Sediment were transferred and mixed up due to deepening and improvement works in Homa Lagoon at 2014. It's known that sediments act as sink for pollutants such as metals. These deepening processes, metal levels could be changed in water column and sediment. Therefore, marine organisms living in sediment could be affected these processes and exposed more to metal pollution (Boening, 1999; Uluturhan et al., 2019). Concordantly, Hg and Cu concentrations were obtained maximum levels in 2017 period. Uluturhan, Kontas & Can (2011) have reported that Homa Lagoon sediments were contaminated metals especially Cr, Pb and Ni due to industrial, agricultural and domestic wastewater through the Gediz River and Izmir Bay.

6.2 The Bioaccumulation Factor (BAF)

The bioaccumulation factor results may help to understand the transfer and accumulation of metals from sediment to organisms (Sfriso et al., 2018).

When the BAF value is found as > 1, metal bioaccumulation in the organism is expected to occur (Cheggour et al., 2001). According to BAF results, the significant values (> 1) were observed for only Hg (BAF=1.58±0.70), Cd (BAF=1.58±0.53) and Zn (BAF=1.26±0.18) in soft tissue of razor shell. The significant BAF values of hepatopancreas were determined as BAF_{Hg} =4.13±1.95, BAF_{Cd} =16.46±3.47 and BAF_{Zn} =1.59±0.27). For *Solen marginatus*, the bioaccumulation factor ordering in soft tissues was as Hg>Cd>Zn>Cu>Pb>Mn>Al>Cr>Fe and in hepatopancreas tissues, the bioaccumulation factor was determined as Cd>Hg>Zn>Cu>Cr>Fe>Al>Pb>Mn. Yusoff & Long (2011) had calculated the BAF in *Solen regularis* from Sarawak-Malaysia and they had found high BAF values for Cd which is parallel to our results.

6.3 The Human Health Risk Assessment

The human health risk assessment was found for each metal in total tissue of *S. marginatus* from the Homa Lagoon at both sampling periods. This assessment can help to understand the potential risks to consumption. The highest metal level in razor shell from the Homa Lagoon and PTWI limits were given at Table 6.1. According to results at 2015-2016 sampling period, Cr and Fe levels exceeded PTWI values. Besides, only Cu concentrations were above PTWI limits in 2017 sampling period. Only Al levels were found as above PTWI values for both sampling periods. Bilgin & Suzer-Uluturhan (2015) detected excessive Al values in *T. decussatus* from Homa Lagoon which showed similar profile to our results. On the other hand, Pb levels in razor shells were detected closely PTWI levels.

Al and Fe are natural components and conservative elements in sediment. It’s pointed that these metals were found as high levels in sediment from Homa Lagoon (Uluturhan, Kontas & Can, 2011). Due to mining and industrial facilities, these metal concentrations increase in aquatic environments especially in lagoons (ATSDR, 2008). Al inhibits enzymes (such as phosphodiesterase, hexokinase, alkalic phosphatase) and also effects phosphorous, calcium, iron and fluorine metabolism in organisms. Furthermore, it’s found that Al is harmful to hemopoietic, osseous and nervous cells. The greatest aluminium complication is neurotoxic effects such as neuronal atrophy and Alzheimer disease (Barabasz, Albinska, Jaskowska & Lipiec, 2002; Jaishankar et al., 2014). Fe free ions could penetrate into brain, liver and heart cells, it causes to lipid peroxidation and damages to microsomes, mitochondria and lysosomes (Albretsen, 2006). Also, it’s pointed that excessive Fe levels lead to DNA damage, malignant transformations and mutations (Grazuleviciene, Nadisauskiene, Buinauskiene & Grazulevicius, 2009).

Table 6.1 Maximum metal concentrations (mg/kg wet weight) in razor shell from the Homa Lagoon and PTWI limits (mg/kg wet weight)

Metals	2015-2016	2017	PTWI*
Hg	0.030	0.042	0.35[a]
Cd	0.213	0.065	0.40[b]
Pb	0.712	1.482	1.75[a]
Cr	**7.44**	1.477	2.45[c]
Cu	2.45	**4.26**	2.45[d]
Zn	16.53	19.27	490[d]
Mn	15.50	7.06	29.4[f]
Fe	**427**	361	392[h]
Al	**301**	**167**	140[e]

*PTWI levels retrieved from a:FAO/WHO (2004), b:FAO/WHO (2010), c:ATSDR (2016), d: FAO/WHO (2007), e: FAO/WHO (2011), f: Who (2008), h: JECFA (1983)

In aquatic ecosystem, Cr is greatly persistent in sediments. Anthropogenic sources of Cr are wood preservatives, leather tanning, cement, rubber, metal alloys, protective metal coatings, paint pigments, paper, and metal plating (Martin & Griswold, 2009). Metal concentrations in razor shells in Homa Lagoon are parallel to metal pollution in surface sediments. It's pointed that high Cr levels related with industrial activities especially textile and leather facilities in Gediz Basin (Uluturhan, Kontas & Can, 2016). The excessive concentrations of Cr may cause erythrocyte glutathione reductase inhibition in organisms and DNA damages such as sister chromatid exchanges, alterations of transcription and replication in DNA (O'Brien, Xu & Patierno, 2001; Jaishankar et al., 2014).

Cu is present in aquatic ecosystems; however anthropogenic activities could lead to increasing Cu levels. The high concentrations of Cu could lead to reduction of reproductivity and growth, productivity of mucous inhibition, gill damages and osmoregulation problems (Calabrese, Maclnnes, Nelson, Greig & Yevich, 1984; Viarengo et al., 1981, 1990; Strømgren & Nielsen, 1991; Davies, 1992).

6.4. The Comparison of Heavy Metal Concentrations

Although, razor shell species are edible and one of the important economic bivalves, there is a few studies have been reported about bioaccumulation of metals on razor shells. Therefore, metal accumulation dataset of whole tissue was compared with *Solen spp* from different areas and *M. galloprovincialis*, *T. decussatus* from Homa Lagoon (Table 6.2).

6.4.1 The Comparison of Metal Concentrations in 2015-2016 period

In the comparison of 2015-16 data set, Hg concentrations of *S. marginatus* were similar with levels in *S. sarawakensis* (K.Selangor-Malaysia) and *S. strictus* (Chiku) (Jeng et al., 2000; Hassan & Kanakaraju, 2013).

The Cd values of *Solen spp* from K.Selangor (*S. sarawakensis*), Sarawak (*S. regularis*), Moyan (*S. regularis*), Serpan (*S. regularis*) and Persian Gulf (*S. dactylus*) were higher than *S. marginatus* in the Homa Lagoon, however Cd levels from Tanjung lumpur (*S. brevis*), Chiku (*S. strictus*) and Persian Gulf (*S. brevis*) were similar with this study (Jeng et al., 2000; Kanakaraju, Ibrahim & Berseli, 2008; Salashur, Bakhtiari & Kochanian, 2012; Kamarruzaman et al., 2010; Yusoff & Long, 2011; Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Hassan & Kanakaraju, 2013).

The Pb concentrations in razor shells from the Homa Lagoon were lower than from Sarawak (*S. regularis*) and Persian Gulf (*S. dactylus* and *S. brevis*) but were similar with *Solen spp.* from Morocco and Tanjung lumpur (Yusoff & Long, 2011;. Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Salashur, Bakhtiari & Kochanian, 2012).

While high Cr values in this study were interestingly found as similar with K.Selangor, Moyan and Serpan, Cr concentrations in razor shells from Sarawak (*S. regularis*) and Persian Gulf (*S. dactylus*) were lower than *S. marginatus* from the Homa Lagoon (Kanakaraju, Ibrahim & Berseli, 2008; Yusoff & Long, 2011; Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Hassan & Kanakaraju, 2013).

Generally, Cu concentrations of razor shells in the Homa Lagoon were determined as similar with in *Solen spp*. from Moyan, Serpan, Tanjung Lumpur, Chiku (Jeng et al., 2000; Kanakaraju, Ibrahim & Berseli, 2008; Kamarruzaman et al., 2010). On the other hand, accumulation of Cu in razor shells from Morocco and Persian Gulf were higher than in this study (Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Mansouri, Fegrouche, Harrak, Allami & Fadli, 2018).

Zn values in this study were similar with in *Solen spp* from Persian Gulf (*S. brevis*) and Chiku (*S. strictus*) (Jeng et al., 2000; Salashur, Bakhtiari & Kochanian, 2012). Also this metal accumulation in razor shells from Tanjung lumpur and Persian Gulf (*S. dactylus*) were higher than in *S. marginatus* from the Homa Lagoon (Kamarruzaman et al., 2010;. Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012) On the contrary, concentrations of Zn in razor shells from Sarawak and Morocco were lower than in this study (Yusoff & Long, 2011; Mansouri et al., 2018).

Mn accumulation in *S. marginatus* from Homa Lagoon were found as lower than in *Solen spp*. K.Selangor, Moyan and Serpan but were determined as higher than Tanjung lumpur and Persian Gulf (Kanakaraju, Ibrahim & Berseli, 2008; Kamarruzaman et al., 2010; Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Hassan & Kanakaraju, 2013). Fe concentrations in razor shell from Homa Lagoon were generally higher than other studies.

According to previous study in Homa Lagoon, Cd, Pb, Cr, Mn, Fe and Al concentrations in *Solen marginatus* were generally higher than in *M. galloprovincialis* and *Tapes decussatus*. Hg accumulation in razor shell were found as similar with in mussel and clam. Cu and Zn levels in razor shell were similar with only *T. decessatus* (Bilgin, 2015).

There was one study about metal accumulation in *Solen marginatus* from Izmir Bay at 2005. Their results showed that metal accumulation of Cd, Pb, Cr, Cu, Zn and Fe found higher in *S. marginatus* from the Homa Lagoon (Sunlu & Taş, 2005).

6.4.2 The Comparison of Metal Concentrations in 2017 period

While Hg concentrations in *S. marginatus* from Homa Lagoon were higher than in *Solen spp* from other areas, Cd and Cr levels were generally found as lower. Also, Pb bioaccumulation in different *Solen spp*. from other study areas were generally similar with *S.marginatus* from Homa Lagoon.

In this study, Zn values in *S. marginatus* were lower than in Tanjung lumpur (*S. brevis*) and Persian Gulf (*S. dactylus*), on the other hand were higher than in Sarawak (*S. regularis*), Chiku(*S. strictus*) Persian Gulf (*S. brevis*) and Morocco (*S. marginatus*) (Jeng et al., 2010; Kamarruzaman et al., 2010; Yusoff & Long, 2011; Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012; Salashur, Bakhtiari & Kochanian, 2012; Mansouri et al., 2018). Cu concentrations in razor shell from Homa Lagoon were generally detected higher than other studies, except Persian Gulf (*S. dactylus*) (Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012).

Mn bioaccumulations in razor shell from the Homa Lagoon were lower than *Solen spp*.from other study areas (except Tanjung lumpur (*S. brevis*) and Persian Gulf (*S. dactylus*)) (Kamarruzaman et al., 2010; Saaedi, Ashja, Hassanzadeh & Zibaseresht, 2012). On the contrary, Fe concentrations in *S. marginatus* from Homa Lagoon were generally found higher than in other studies.

For Homa Lagoon, Hg concentrations in *S. marginatus* were showed similar level with *M. galloprovincialis* and *T. decussatus* from previous study. In razor shell, Cd levels were detected lower than in mussel and clam, but Pb, Cu, Mn and Fe accumulations were higher than in these bivalves. Cr levels in *S. marginatus* were lower than in *T. decussatus* from previous study. Zn concentrations in *S. marginatus* were similar with in *T. decussatus*, on the other hand were found lower than in *M. galloprovincialis*. When Al values in razor shell were higher than in mussel, were showed similar levels with clam (Bilgin, 2015).

The Cd, Pb, Cr, Cu, Zn and Fe accumulation in the *Solen marginatus* from Homa Lagoon were higher than from Izmir Bay (Sunlu & Taş, 2005).

Table 6.2. The heavy metal concentrations in *Solen marginatus* from Homa Lagoon and other Solen species from different areas (mg/kg dry weight)

Species	Location	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe	Al	Reference
S. sarawakensis	K.Selangor	0.05-0.15	21-29	-	24-32	-	-	74-80	82-90	-	Hassan & Kanakaraju (2013)
S. regularis	Moyan	-	0.62-1.1	-	24-34	5.2-8.3	-	57-85	231-296	-	Kanakaraju et al., (2008)
S. regularis	Serpan	-	0.55-1.2	-	25-36	5.9-10.3	-	51-78	566-615	-	Kanakaraju et al., (2008)
S. regularis	Sarawak	-	2.3-2.4	4.4-5.3	6.1-10.1	1.3-3.1	25-29	-	159-196		Yusoff & Long (2011)
S. brevis	Tanjung lumpur	-	0.38-0.96	1.2-2.1	-	6.9-10.4	76-100	17-21	359-472	-	Kamaruzzaman et al., (2010)
S. dactylus	Persian Gulf	-	1-3	4-7	1-6	13-20	60-92	8-56	210-1886	-	Saaedi et al., (2012)
S. strictus	Chiku	0.12	0.54	n.d	-	6	55.5	-	-	-	Jeng et al., (2000)
S. brevis	Persian Gulf	-	0.67	4.38	-	-	63.3	-	-	-	Salashur et al., (2012)
S. marginatus	Morocco	-	-	1.92	-	16.54	26.98	-	18918	-	Mansouri et al, (2018)
S. marginatus	Izmir Bay	-	0.09-0.19	0.01-0.20	0.11-1.4	0.47-4.3	7.3-14	-	39-134	-	Tas & Sunlu (2005)
M.galloprovincialis	Homa Lagoon	0.10-0.15	0.22-0.51	0.81-2.5	0.10-6.2	2.1-5.6	80-232	4.1-1.4	82-477	17-416	Bilgin (2015)
T. decussatus	Homa Lagoon	0.07-0.13	0.10-0.33	0.8-1.4	2.9-13	4.8-9.6	53-82	7.6-18	268-806	260-1009	Bilgin (2015)
S. marginatus 2015-2016	Homa Lagoon	0.04-0.13	0.32-0.93	1.4-3.1	12-32	5.7-10.7	58-71	21-67	949-1858	612-1308	In this study
S. marginatus 2017	Homa Lagoon	0.07-0.18	0.13-0.28	1.3-6.4	4-6	6.3-18	57-83	8-31	1044-1571	527-726	In this study

CHAPTER SEVEN
CONCLUSION

Lagoons are extremely productive coastal areas with highly economic and ecological value. In addition, these special areas are very sensitive to pollution. These situations start from aquatic organisms through the trophic chain, are transported and adversely affect human health. Therefore, bivalves are widely accepted as bioindicators for monitoring of pollutants in aquatic ecological systems and for assessing the quality of the environment.

Seasonal concentrations of metals in *S. marginatus* from the Homa Lagoon were investigated in following two years in this thesis. According to the results, the maximum metal levels in *S. marginatus* were generally obtained in May and August through to the spawning periods. All metal concentrations were determined higher in hepatopancreas of *S. marginatus*.

The metal concentrations in razor shells were compared with authorities' permissible limits. According to results, Cr, Cu, Fe and Al concentrations were found as exceeded PTWI limits. On the other hand, Pb level is closely PTWI value. Therefore, these metals could pose potential risks for human health.

After determination of metal concentrations, bioaccumulation factor calculations and statistical approaches were demonstrated that *S. marginatus* could be selected as a useful new bioindicator species for marine pollution monitoring.

The Homa Lagoon is negatively influenced by organic and inorganic contaminants originated from large agricultural areas and industrial factories in the Gediz Delta. Concordantly, results showed that bioindicator organisms should utilized in keep under control and monitor of metal pollution in ecologically important and productive marine areas such as the Homa Lagoon.

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