

DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES

TEMPORAL AND SPATIAL BIOMONITORING
OF HEAVY METALS IN EASTERN AEGEAN
COASTAL WATERS USING
Amphibalanus amphitrite

by
Sinem ÖNEN

August, 2011
İZMİR

**TEMPORAL AND SPATIAL BIOMONITORING
OF HEAVY METALS IN EASTERN AEGEAN
COASTAL WATERS USING
*Amphibalanus amphitrite***

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
In Partial Fulfillment of the Requirements for the Degree of Doctor of
Philosophy in Coastal Engineering, Marine Sciences and Technology**

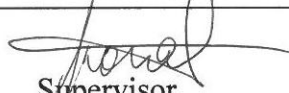
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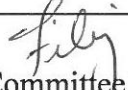
Ph.D. THESIS EXAMINATION RESULT FORM

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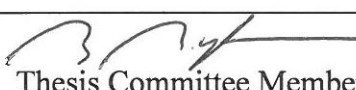
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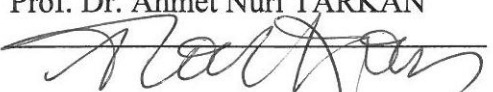
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Sinem ÖNEN

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ABSTRACT

This biomonitoring study presents the spatial and temporal variations of heavy metals (Hg, Cd, Pb, Cr, Cu, Zn, Mn and Fe) in the soft tissues of *Amphibalanus amphitrite* collected from different sites along the eastern Aegean coast. *A. amphitrite* has been chosen as a strong candidate for monitoring heavy metals. Sediment and seawater samples were collected to detect their metal contents to gain more information on the environmental conditions and possible bioaccumulation patterns. ANCOVA was used to show possible temporal and spatial differences in the bioavailabilities of heavy metals to barnacles. And the relationships of metal concentrations in the soft tissues of barnacles were also compared as a function of barnacle size. The physico-chemical characteristics have been measured in order to characterize the sampling area.

The accumulation order of mean metal concentrations in barnacles showed that barnacles accumulate Cu in a higher degree than both sediment and seawater. The highest mean values of Cu, Cr, Fe, Mn, Pb and Zn were obtained from Kusadasi-Setur Marina and Izmir-Pasaport where recreational boats are densely located and the use of metal-based antifouling paints are high. There was a significant negative relationship between concentrations of Hg, Cd, Cu, Fe, Mn and Zn and soft tissue dry weight of barnacles. According to concentration factor, *A. amphitrite* has ability to accumulate metals several times more than seawater and sediments. It is concluded that barnacles proved to be a good choice to be used as a bioindicator because of their strong accumulation capacity for many trace metals.

Keywords: Biomonitoring; antifouling; metal contamination; *Amphibalanus amphitrite*; barnacle; concentration factor; Eastern Aegean coast.

DOĞU EGE KIYI SULARINDA *Amphibalanus amphitrite* KULLANILARAK AĞIR METALLERİN MEVSİMSSEL VE BÖLGESEL BİYÖİZLENMESİ

ÖZ

Bu izleme çalışması, Doğu Ege kıyıları boyunca dört farklı bölgede sert substrat üzerinde yaşayan başlıca fouling türü *Amphibalanus amphitrite*' in yumuşak dokularındaki ağır metallerin (Hg, Cd, Pb, Cr, Cu, Zn, Mn, ve Fe) bölgesel ve mevsimsel değişimini sunar. *Amphibalanus amphitrite*, ağır metallerin izlenmesi için güçlü bir aday olarak seçilmiştir. Su ve sediment örnekleri, metal içeriklerini saptamak, çevresel durum ve olası biyoakümülyasyon yolları hakkında daha fazla bilgi edinmek için toplanmıştır. Balanuslar tarafından biriktirilen ağır metallerin, biyolojik bulunurluluklarındaki mevsimsel ve bölgesel farklılıkları göstermek için kovaryans analizi kullanılmıştır. Ayrıca balanusların yumuşak dokularındaki metal konsantrasyonlarını biriktirmesi ile vücut büyüklüğü arasındaki ilişki karşılaştırılmıştır. Örnekleme alanını karakterize edebilmek için her örnekleme mevsiminde dört örnekleme istasyonunun fiziko-kimyasal parametreleri ölçülmüştür.

Balanuslardaki ortalama metal konsantrasyonlarının akümülyasyon sırasına göre, bu organizmaların bakırı (Cu), su ve sedimente göre daha yüksek konsantrasyonda biriktirdiğini göstermiştir. Cu, Cr, Fe, Mn, Pb ve Zn deki en yüksek ortalama metal değerleri, dinlenme amaçlı kullanılan yat ve teknelerin yoğun bir şekilde bulunduğu ve metal içerikli antifouling boyaların kullanımının yüksek olduğu Kuşadası-Setur Marina ve İzmir-Pasaport'tan elde edilmiştir. Hg, Cd, Cu, Fe, Mn ve Zn konsantrasyonları ve balanusların yumuşak dokularının kuru ağırlığı arasında negatif yönde bir ilişki bulunmuştur. Konsantrasyon faktörüne göre *Amphibalanus amphitrite*, metalleri su ve sedimentten birkaç kat daha fazla biriktirme yeteneğine sahiptir. Birçok iz elementi biriktirmedeki güçlü biriktirme kapasitesinden dolayı balanusların biyoindikatör olarak kullanmak için iyi bir seçim oldukları sonuca varılmıştır.

Anahtar Kelimeler: Biyolojik izleme; antifouling; metal kirlenmesi; *Amphibalanus amphitrite*, balanus, konsantrasyon faktörü, Doğu Ege kıyıları.

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Chemical contamination of marine environment is a world wide problem, but it is particularly serious along the coasts of industrialized countries, where wastes derived from a number of human activities reach the sea. Some of these wastes can represent a threat to marine life and possibly to man as a consumer of seafood.

In contrast to conditions in the open ocean, shallow estuarine and coastal marine waters continue to be extensively degraded by point and nonpoint sources of pollution. Systems characterized by a slow rate of exchange relative to their volume (e.g. semi enclosed estuaries, embayments, marinas) are most susceptible to contaminant inputs. These systems typically have a very limited assimilative capacity for pollutants, consequently certain unassimilated materials, such as syntethic toxic organic compound can accumulate and persist for a long periods of time posing a potential long term danger to marine food webs. The most common anthropogenic wastes found in estuarine and coastal marine environments are dredged spoils, sewages, and industrial and municipal discharges, a terrestrial source from mining, intensive aquaculture, antifouling paints from ships, untreated effluents, harbour activities, urban and agricultural runoff along major rivers and estuaries and bays.

These wastes generally contain a wide range of pollutants notably heavy metals, petroleum hydrocarbons, chlorated hydrocarbons, toxic organic compounds and other substances. The continued discharged of industrial wastes including PCBs, heavy metals and other toxics, and the indirect release of nitrates, phosphates and pesticide products often result in toxic accumulations in the marine food chain.

Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern.

This is due to their persistence as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants and also their toxicity and their ability to accumulate in the biota.

Metals coming from corrosion and certain additives such as the products antifouling (biocides and their halogenous organic compounds of degradation), antiscaling (carbonic acids, polyphosphates), antifoaming (detergents), anticorrosive agents and others (sodium sulphite and sulphuric acid). The potential impact on the biotic communities of the estuaries appears by death, at various levels, of invertebrates and fish.

The aim of this study is;

- to evaluate the concentration of Hg, Cd, Pb, Cr, Cu, Zn, Mn and Fe in selected stations and marinas which have got large amounts of contaminated wastes derived from different heavy metal sources especially antifouling paints,
- to assess the relationships between metal concentrations in the soft tissue of *A. amphitrite* which were collected from hard substrate such as piers, docks and ports and particularly the water column, the surficial sediments and physicochemical parameters in the surrounding environment,
- to determine trends of spatial and temporal variations of heavy metal concentrations in water column, sediment and *A. amphitrite*,
- to obtain a database now being compiled on barnacle species for using future ecotoxicological fieldwork,
- to get information about environmental parameters such as dissolved oxygen, salinity, conductivity, pH and temperature,
- to clarify if there is any correlation between the metal concentration, and environmental parameters and the correlation between sediment samples with grain size and total organic carbon (TOC).

There are not any further studies that could be carried out on the same or similar topics along the eastern Aegean coast.

CHAPTER TWO

HEAVY METALS

2.1 Description of Heavy metals

Heavy metals are one of the most serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems (Tam & Wong, 2000). The term heavy metal has general or more specific meanings. According to one definition, the heavy metals are a group of elements between copper and lead on the periodic table of the elements; having atomic weights between 63.546 and 200.590 gr and specific gravities greater than 4.0 gr/cm^3 .

Heavy metals are metallic chemical elements that have a relatively high density and they are highly toxic or poisonous at low concentrations. These anthropogically derived inputs can accumulate in sediments particularly in coastal areas, in invertebrates and in food webs. There is a less knowledge about the uptake of heavy metals by ingestion with food or by close contact with contaminated sediments (Harris & Santos, 2000).

Table 2.1 Classification of elements according to toxicity and availability

Noncritical	Toxic but very insoluble or very rare	Very toxic and relatively accessible
Na, C, F, K, P, Li, Mg, Fe, Rb, Ca, S, Sr, H, Cl, Al, O, Br, Si, N	Ti, Ga, Hf, La, Zr, Os, W, Rh, Nb, Ir, Ta, Ru, Re, Ba	Be, As, Au, Co, Se, Hg, Ni, Te, Tl, Cu, Pb, Pd, Zn, Ag, Sb, Sn, Cd, Bi, Pt

The position of heavy metals in the periodic table and their different oxidation stages and electronic orbital being filled in the atom of the elements determine the toxicity level for aquatic organisms.

The classification has been made according to their toxicity by Wood (1974) and metals can be classified as (Table 2.1):

- 1- Noncritical
- 2- Toxic but very insoluble or very rare
- 3- Very toxic and relatively accessible.

Heavy metals are often referred to as trace metals when occur in low concentrations in organisms. Although the trace metal term may imply the presence of an essential requirement by organisms (Waldichuk, 1974), some definitions including “heavy” explain accumulation comes from industrial and mining activities into coastal waters and estuaries at many sites (Harris & Santos, 2000). Nevertheless, the term of heavy metal is used synonymously with trace metal and includes both essential and non-essential metals for organisms.

2.2 Heavy Metals in Aquatic Ecosystem

Heavy metals can be found in the following forms in the aquatic system:

- In solution as inorganic ion and both inorganic and organic complexes,
- adsorbed onto surface,
- in solid organic particles,
- in coatings on detrital particles after coprecipitation with and sorption onto mainly iron and manganese oxides,
- in lattice positions of detrital crystalline material,
- precipitated as pure phases, possibly on detrital particles (Kennish, 1997).

Although heavy metals exist in dissolved, colloidal and particulate phases in seawater, the concentration of dissolved forms is low. The heavy metals rapidly sorb onto suspended particulate matter as they enter waters (Kennish, 1997).

The heavy metals removal during estuarine mixing are accelerated through precipitation or interactions with particle surfaces or flocculating colloids,

coprecipitation with organic, iron and manganese hydrous oxides, increased affinity of the metals for anions in sea water and uptake by organisms. Sediment which adsorbed heavy metals often is suspended from the bottom during storms and other turbulent periods (Kennish, 1997). Because of their large load of trace metals, sediments play an important role to control the biogeochemical cycling of some heavy metals. Thus, the bioavailability of even a small fraction of the total sediment load assumes considerable importance.

According to Kennish (1997) the bioavailability and concentrations of metals in sediments depend on many different processes, such as:

- Mobilization of heavy metals to interstitial waters and their chemical speciations,
- transformation of metals,
- control exert by major sediment components (e.g., iron oxides and organics) to which metals are preferentially bound,
- competition between sediment metals for uptake sites in organisms,
- influences of bioturbation, salinity, redox potential or pH on these processes.

2.3 Heavy Metal Inputs

Heavy metals derived from natural inputs and anthropogenic emissions are ubiquitous in the global environment (Nriagu, 1989, 1990; Blackmore, Morton & Huang, 1998; De Wolf, Ulomi, Backeljau, Pratap & Blust, 2001). Heavy metals represent a common type of chemical pollution in aquatic environment. They can be found naturally in bedrock and sediment or they could enter the aquatic environment from both natural and anthropogenic sources.

Natural sources include weathering of minerals and soils (Merian, 1991) and also rock erosion, and volcanic activity. Heavy metals enter the aquatic environment naturally through weathering of the earth crust. In addition to geological weathering, human activities have also introduced large quantities of metals to localized area of

the sea, in some cases upsetting the natural steady state balance (Forstner & Wittmann, 1983). Anthropogenic inputs are mainly from industrial effluents, domestic effluents, rural and urban storm water runoff and spoil heaps (Agbozu & Ekweozor, 2001) and sludge from treatment plants, consumer waste, or even from acidic rain.

Metals are introduced into the marine environment through river runoff, atmospheric deposition, hydrothermal venting, diagenetic remobilization and anthropogenic activities (Libes, 1992). Industrial and agricultural activities, as well as urban effluents, are the major anthropogenic sources, which supply important loads of toxic metals to the sea. The major biogeochemical processes, which regulate the distribution and behavior of trace metals in seawater, are mixing of water bodies, particle-water interaction (through biological uptake and adsorption-desorption) and diagenetic processes in sediments (Bruland, 1983; Chester, 1990a). In coastal waters, trace metal concentrations do not tend to correlate well with nutrients, since external inputs (terrestrial, anthropogenic, sedimentary) and local hydrography appears to play a more important role than biogenic processes. Due to several biogeochemical and sedimentological processes that occur there, the coastal zone may act as a source or sink of trace metals (Martin, Elbaz-Poulichet, Guieu, Loye-Pilot & Han, 1989).

Metal mobility will depend on a variety of processes including chemical (dissolution, desorption, complexation, precipitation and adsorption), biological (degradation, transformation, accumulation, faeces production and filtration) and physical ones (diffusion, phytolysis, aggregation and burying) (Luoma, Cain, Ho & Hutchinson, 1983; Forstner, 1986).

Heavy metals occur under different forms in sea water and only a fraction of their total concentrations is readily available to organisms (Rainbow, 1985). Therefore, measuring the levels of heavy metals in water and sediments may not reflect the actual toxicity of a given element. The total metal concentration in sea water, its bioavailability and therefore its concentration in marine organisms will be a function of the element involved (e.g. essential vs non-essential metal), type of metal source (e.g. urban vs industrial), total metal load and the organism studied. Moreover, the

conjunction of these factors will cause completely different metal distribution patterns among different areas. The study of metal concentrations in organisms themselves can give more reliable informations on the bioavailability and consequently of the potential damage to the organism's metabolism and to the environment in general (Bryan, Langston & Hummerstone, 1980).

2.3.1 Heavy Metals in Water Column

Heavy metals can be transported between sediment and water column. The behavior of heavy metals in the aquatic environment is strongly influenced by adsorption to organic and inorganic particles. The dissolved fraction of the heavy metals may be transported through the water column via the processes of advection and dispersion, while the particulate fraction may be transported with the sediments, which are governed by sediment dynamics (Bourg, 1987; Turner & Millward, 1994; Turner, Millward & Le Roux, 2001; Turner & Millward, 2002; Wu, Falconer & Lin, 2005).

Heavy metals are not fixed permanently in the sediment. In fact, the variation of the physico-chemical characteristics of the water column (pH, salinity, temperature, redox potential and the concentration of different organic ligands) can release part of the metal content trapped into the sediment to the water column (Van Ryssen, Leermakers & Baeyens, 1999; Wright & Mason, 1999) and become available to living organisms.

Fine sediments, acting as a source (or sink) for the organic chemical and heavy metals entering (or leaving) the water column with sediments contaminated by the heavy metals, pose a potential threat to the aquatic environment. Resuspension of contaminated bed sediments caused by strong tidal currents may release a significant amount of heavy metals into the water column, and this desorption of contaminants from their particulate phase can have a pronounced impact on the aquatic environment and ecosystem (Chen, Leva & Olivieri, 1996; Lung & Light, 1996; Ng, Turner, Tyler, Falconer & Millward, 1996; Mwanuzi & Smedt, 1999; Wu, Falconer

& Lin, 2005; Hartnett, Lin, Jones & Berry, 2006; Zagar, Knap, Warwick, Rajar, Hovat & Cetina, 2006).

Coastal sediments and estuarine have been widely used to evaluate water quality because of a higher stability and lower variability of the sediments compared to the water column. Furthermore, the sediments integrate the concentration of pollutants throughout time and therefore, this can be useful to study the historic evolution of contamination and to predict its future effects (Calmano, Ahlf & Forstner, 1996; Soares, Boaventura & Machado, 1999; Ruiz-Fernández, Hillaire-Marcel, Páez-Osuna, Ghaleb & Soto-Jiménez, 2003).

2.3.2 Heavy Metals in Sediment

The study of the distribution of metals in sediments is very important from the point of view of environmental pollution because sediment concentrates metals from aquatic systems, and represents an appropriate medium to monitor contamination (Moore & Ramamoorthy, 1993; Foster & Charlesworth, 1996). Due to human activities sediments are often polluted from industrial effluents, domestic effluents, atmospheric deposition and antifouling paints from ships (Ottosen & Villumsen, 2006) and reported that the enrichment of trace elements in marine sediments may, in general, originate from the following sources super and subjacent sediments, through diagenesis; suboxic shelf and slope sediments, hydrothermal input, aeolian input, fluvial runoff, seawater (Nijenhuis, Bosch, Sinninghe Damste, Burmsack & De Lange, 1999).

Industrial and urban activities contribute to the introduction of significant amounts of pollutants (among them trace metals) into the marine environment and affect directly the coastal systems where they are quite often deposited (GESAMP/UNESCO, 1987, 1994; Salamons & Forstner, 1984).

Heavy metals, pesticides and other toxic substances can be absorbed from the water column onto surfaces of fine particles and they move with the sediments. They

participate in various biogeochemical mechanisms and can affect the ecosystems through bioaccumulation and biomagnification processes (GESAMP/UNESCO, 1987, 1994; Salomons & Forstner, 1984).

Particularly heavy metals accumulate in organically rich sediments. Therefore, metal concentrations in sediment can be measured easily and are much less susceptible to accidental contamination. In addition, sediments offer a degree of time integration, overcoming effects of temporal changes of heavy metal availability (Luoma, 1990). During transport and/or deposition, metals are subject to a variety of processes associated with floods, tides and wave action; they can be adsorbed by clays and can form organic complexes or co-precipitate as inorganic mineral phases (Thornton, 1983).

Marine sediments can be a sensitive indicator for both spatial and temporal monitoring of contaminants in the marine environment (Ergin, Saydam, Basturk, Erdem & Yoruk, 1991; Rowlett & Lovell, 1994; Balls, Hull, Miller, Pirie & Proctor, 1997). Heavy metals tend to be trapped in the aquatic environment and accumulate in the sediments and may be directly available to benthic fauna or released to the water column through sediment resuspension, adsorption-desorption reactions, reduction-oxidation reactions and degrading organisms. Such processes enhance the dissolved concentration of trace metals in the environment and threaten the ecosystem (Rivail Da Silva, Lamotte, Donard, Soriano-Sierra & Robert, 1996; Jones & Turki, 1997; Fang & Hong, 1999; Wright & Mason, 1999).

Organic matter and fine grained particles are known to adsorb heavy metals. For instance, metal ions adsorbed on small grain size particulate matter are often considered to be “bioavailable”, whereas metals complexed with organic matter or included in amorphous metal oxides through precipitation or coprecipitation are likely to be less bioavailable; metals present in crystalline structures are generally unavailable for uptake (Dicks & Allen, 1983). Transport and sedimentation of suspended materials is thus determining where the pollution is mainly found (Ottosen & Villumsen, 2006). Its role is very important in marine biogeochemical cycles and

can influence the partitioning of heavy metals and their potential bioavailability by forming either soluble complexes or insoluble flocks (Forstner & Wittmann, 1983; Chester, 1990a).

2.3.2 Heavy Metals in Aquatic Organisms

Heavy metals are accumulated by many marine organisms in their body tissues. These accumulated concentrations are easily measured and provide a time-integrated measure of metal supply over weeks, months or even years, according to the species analysed.

Such organisms are biomonitors and are now used widely to establish geographical and/or temporal variations in the bioavailable concentrations of heavy metals in coastal and estuarine waters (Bryan, Langston & Hummerstone, 1980; Bryan, Langston, Hummerstone & Burt, 1985; Phillips, 1980; Phillips & Rainbow, 1993). The term 'metal biomonitor' preferred to alternatives such as 'bioindicators', 'sentinel organisms' and 'biological monitors'. It is used to describe a species which accumulates heavy metals in its tissues, and analysed as a measure of the bioavailability of the metals in the ambient habitat (Goldberg, Bowen, Farrington, Harvey, Martin, Parker, Risebrough, Robertson, Schneider & Gamble, 1978; Martin & Coughtrey, 1982; Hellawell, 1986; Phillips & Rainbow, 1993). The term 'bioindicator' is defined a species that denotes an ecological effect by its mere presence or absence, and 'biological monitors' denote degrees of ecological change by behavioural, physiological or biochemical responses such as changes in scope for growth, respiration rate or degree of lysosome latency (Phillips & Rainbow, 1993).

Biomonitors should fulfil several criteria (Butler, Andren, Bonde, Jernelov & Reisch, 1971; Bryan, Langston & Hummerstone, 1980; Phillips, 1980; Phillips & Rainbow, 1993) such as they should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, large enough to provide sufficient tissue for (individual) analysis, resistant to handling stress caused by laboratory studies or field transplantations, tolerant of exposure to environmental variations in

physico-chemical parameters such as salinity and they should be a net accumulator of the relevant metal. Barnacles have been shown to fulfil many of these criteria and used to assess the bioavailability of metals in the coastal waters of many parts of the world (Walker, Rainbow, Foster & Crisp, 1975; White & Walker, 1981; Anil & Wagh, 1988; Powell & White, 1990).

For choosing suite biomonitors, the knowledge of their biology is must be identified. For example their methods of feeding, extent of production of respiratory or irrigatory currents, life history and breeding season, length of life, age structure of population, etc. can be investigated. This knowledge can not be sufficient to understand the kinetics of metal accumulation. So additional questions can be answered for example, does metal accumulation continue throughout life sequentially adding a new metal to an existing body load (Rainbow, 1987; Rainbow, 1990) or does the body metal content equilibrate to new higher values with increasing metal bioavailability, as apparently in the amphipod crustacean *Gammarus pulex* (Shutes, Ellis, Revitt & Bascombe, 1993).

Studies conducted during the last 10 years to develop biomarkers of pollutant exposure in the aquatic environment have indicated the need to integrate interactions of abiotic factors (temperature, salinity, turbidity, diet, etc.) and biotic factors (reproduction cycle, growth, age, sex, etc.) (Norton, Cormier, Smith & Jones, 2000).

Heavy metals are used widely in industry and can enter the environment via low-dose continual influx. Over time this can lead to significant 'enrichment' of ecosystems via bioaccumulation in plankton and filter feeders and biomagnification through the food chain. Heavy metals may damage biological systems by replacing essential metals as cofactors, inhibiting enzymes, altering membrane integrity and causing physiological damage. Many organisms have developed mechanisms to deal with toxic metal loads, but these biological systems can become stressed and overloaded leading to cellular damage, usually via oxidative processes (Phillips & Rainbow, 1993). Heavy metal pollution in aquatic environment and subsequent uptake in the food chain by aquatic organisms and humans put public health at risk,

because it can result in morphological abnormalities, neurophysiological disturbances, genetic alteration of cells (mutation), teratogenesis and carcinogenesis. In addition, heavy metals can affect enzymatic and hormonal activities, as well as growth rate and increase mortality (Bubb & Lester, 1991).

Subsequently, diagenesis, physical disturbance or change in the physico-chemical conditions (redox, temperature, low oxygen, high sulphur, ammonia concentrations and salinity, etc.) may release them to become available to living organisms, either through ingestion or by absorption across integuments or respiratory surfaces. Metals may also adsorb on to the surfaces of plants and animals. Furthermore, many estuarine invertebrates process sediments as a food source. While all metals are naturally present in the aquatic environment (Rainbow, 1990), it is their presence at elevated concentrations which presents a potential threat to aquatic life (Turner, 1990). Toxic metals in invertebrates is of concern because they are the food of many fish species consumed by man, especially for birds, many of which feed on invertebrates.

Bioconcentration of metals from water and biomagnification of metal concentrations through food chains is a factor of exposure time, exposure concentration, and size of the organism, water hardness, acclimation, feeding level, and trophic level (Brooks & Mahnken, 2003).

The levels of heavy metals accumulated by marine organisms are function not only of water quality, but also of seasonal factors, temperature, salinity, diet, spawning and individual variation, among other factors. Moreover, the levels of metals accumulated in some marine organisms may be many orders of magnitude above background concentrations, thus demonstrating the potential of certain species as bio-indicators of heavy metal pollution (Chan, 1989).

A trace metal has the potential to bind to any molecule with an affinity for that metal. Since trace metals typically have an affinity for sulphur and nitrogen (Nieboer & Richardson, 1980), and proteins are made up of amino acids, many of which

contain sulphur and/or nitrogen, there is no shortage of potential binding sites for trace metals within cells (Rainbow, 1997a). Such affinities make all trace metals potentially toxic, binding to proteins or other molecules and preventing them from functioning in their normal metabolic role.

The trace metal has been detoxified (Mason & Jenkins, 1995), detoxification often involving binding to proteins such as metallothioneins or to insoluble metaliferous granules (Viarengo, 1989; Mason & Jenkins, 1995; Langston, Bebianno & Burt, 1998). Many trace metals cannot be immediately excreted or detoxified, for they are required to play essential roles in metabolism. Zinc for example is a key component of many enzymes including carbonic anhydrase, and copper is a functional part of the respiratory protein haemocyanin, found in certain molluscs and arthropods, particularly malacostracan crustaceans. Thus a certain quantity of each essential metal is required in the body (in metabolically available form) to meet essential metabolic needs and it is possible to make theoretical estimates of these quantities (White & Rainbow, 1985, 1987; Rainbow, 1993). Any further accumulation of these essential trace metals in metabolically available form, however, has the potential to be toxic, with the subsequent need for excretion and/or detoxification.

Non-essential metals, like cadmium, lead or mercury, would have no required minimum concentration and need to be detoxified or excreted forthwith. Cadmium has in fact been shown to play a metabolic role in carbonic anhydrase in certain oceanic diatoms (Cullen, Lane, Morel & Sherrell, 1999), but is still considered non-essential for other organisms including aquatic invertebrates.

Trace metals are also accumulated from other sources including food; in this case the absorption of bioavailable forms the metal after digestion in the alimentary tract. A variable proportion of any trace metal taken up by a marine invertebrate will remain passively adsorbed onto the outside of the animal and not be available to metabolic processes.

Biomonitoring programmes should preferably analyse site differences using data for individual species. Nevertheless, it is still possible, and may be necessary, to make interspecific comparisons. These should, however, involve comparisons of rank orders of sites, and each rank being derived from data for a single biomonitoring species (Phillips & Rainbow, 1993).

Several biomonitoring programmes (such as US Mussel Watch, French RNO and RINBIO) are based on a quantitative bio-indicator concept, using the ability of marine bivalves (usually mussels) to concentrate and, under certain conditions, accumulate contaminants in their tissues with respect to the ambient level.

Mussels genera used as biomonitors in the development of biomonitoring programmes for heavy metals, especially species of *Mytilus* as in the US Mussel Watch Program (Goldberg *et al.*, 1978; Goldberg, Koide, Holdge, Flegal & Martin, 1983; Lauenstein, Robertson & O'Connor, 1990) and in NW Europe, for example Scandinavia (Phillips, 1977, 1978). There are several biomonitoring species used for biomonitoring, include the mussels of the genera *Mytilus* (e.g. *M. edulis* and *M. galloprovincialis*, *M. trossulus*) and *Perna* (e.g. *P. viridis* and *P. canaliculus*), oysters of the genera *Ostrea* (e.g. *O. edulis*) and *Crassostrea* (e.g. *C. gigas* and *C. virginica*, *C. margaritacea*, *C. brasiliensis*, *C. angulata*), Barnacles (e.g. *Balanus amphitrite*, *B. uliginosus*, *T. squamosa*) and *C. mitella* (Phillips & Rainbow, 1988; Chan, Rainbow & Phillips, 1990; Rainbow & Smith, 1992; Rainbow, 1993), the upper shore barnacle *Chthamalus stellatus* (Weeks, Rainbow & Depledge, 1995) and the talitrid amphipod *Platorchestia platensis* (Rainbow & Phillips, 1993), the tellinids, *Scrobicularia plana* and *Macoma balthica* (Bryan, Langston & Hummerstone, 1980; Bryan *et al.*, 1985), the terebellid *Lanice conchilega*, the ragworm *Nereis (Hediste) diversicolor* (Bryan & Hummerstone, 1973), the talitrid amphipods *Orchestia gammarellus* (Rainbow, Moore & Watson, 1989b; Moore, Rainbow & Hayes, 1991), *Talorchestia quoyana* and *Orchestia tennisi* (Rainbow, Emson, Smith, Moore & Mladenov, 1993a).

Bivalve molluscs are the organisms most often used for biomonitoring metal contamination (Fialkowsky & Newman, 1998). Filter-feeding macrobenthic invertebrates can be conveniently used to monitor the paths and fates of pollutants entering various bodies of water. The most widely employed are bivalve molluscs, upon which the Mussel Watch Program has been based (Goldberg *et al.*, 1983; Goldberg *et al.*, 1978). However, in recent years there has been growing interest in finding other organisms that can be used as biomonitors, since it is not always possible to find bivalve molluscs in all polluted areas (Paulson, Sharack & Zdanowicz, 2003; Kang, Choi, Oh, Wirght & Koh, 1999). Barnacles are smaller and more difficult to dissect than bivalves. The species occurs gregariously on almost all hard substrata throughout the sea, including the shells of their dead, and specimens are easy to collect. Nonetheless, they have been employed in several monitoring programmes (Rainbow, 1995).

Barnacles are considered excellent metal biomonitors (Ruelas-Inzunza & Páez-Osuna, 2000). Laboratory research has shown that barnacles are net accumulators of metals (Rainbow & White, 1989). Barnacles are present in different types of locations, with different degrees of pollution, so this group of organisms may be considered ideal for biomonitoring programmes (Ruelas-Inzunza & Páez-Osuna, 1998). It is possible to assume that higher metal bioavailability in seawater leads to greater accumulation of metals in barnacles. Therefore, in the locations where barnacles show high metal levels, the waters should contain relatively elevated bioavailability (Páez-Osuna, Bójorquez-Leyva & Ruelas-Inzunza, 1999). *Amphibalanus amphitrite* has two important properties to be a suitable biomonitor of metal contamination: (1) it has a strong predisposition to uptake and retain metals and (2) accumulates metals above environmental levels (Barbaro, Francescon, Polo & Bilio, 1978).

2.4 Using of Antifouling in Marine Ecosystem

Structures such as ships and marine platforms, as well as offshore rigs and jetties, are under constant attack from the marine environment. These structures need to be

protected from the influences of the key elements of the marine environment such as saltwater, biological attack and temperature fluctuations. Besides injectable biocides in closed systems, methods of protecting marine structures must be capable of expanding and contracting with the underlying surface, resist the ingress of water and control the diffusion of ions. Protective organic coatings can offer these functions (Munger, 1984) and consequently are largely used in the shipping industry to increase the working life of systems and improve its reliability.

The use of antifouling coatings for protection from the marine environment has a long history. The antifouling paints are commonly used to prevent the undesired fouling. Especially for ships and boats biofouling is a big problem. Because biofouling increased drag which affects fuel consumption dramatically, increases carbon dioxide pollution and other air pollutants, and increases the workload on the machinery for maintaining speed; increased dry-dock cleaning and maintenance costs when the ship is immobile; loss of manoeuvrability of the ship; and increased risk of ecologic contamination by alien species (Pınar, 1978; Rouhi, 1998; Callow & Callow, 2002; Yebra, Kiil & Dam-Johansen, 2004).

It is assumed that vessel bottoms may gather up to 150 kg of fouling per m² in six months, increasing the fuel consumption of up to 50% when no antifouling paint is applied (Haak, 1996; Reincke, Krinitz & Stachel, 1999; Nehring, 2000).

Biofouling can also lead to biocorrosion, reducing the lifetime of technical structures in the marine environment. Paint coatings on ships are used for a wide range of functions such as corrosion resistance, ease of maintenance, appearance, non-slip surfaces on decking as well as the prevention of fouling on the hull by unwanted marine organisms.

2.4.1 Description of Fouling and Colonization Process

Fouling is the unwanted growth of biological material, e.g., barnacles, algae or molluscs, on the water-immersed surface of a vessel. When vessel hulls are clean and

smooth, they travel faster through water and consume less fuel. Fouling can be removed when a vessel is dry-docked, which occurs every two to five years.

The settlement and accumulation of marine organisms on an inanimate substrate can cause large penalties to engineered structures. The biological fouling has a process. Growth stages which include an initial accumulation of adsorbed organics, the settlement and growth of pioneering bacteria creating a biofilm matrix and the subsequent succession of micro and macrofoulers (Figure 2.1).

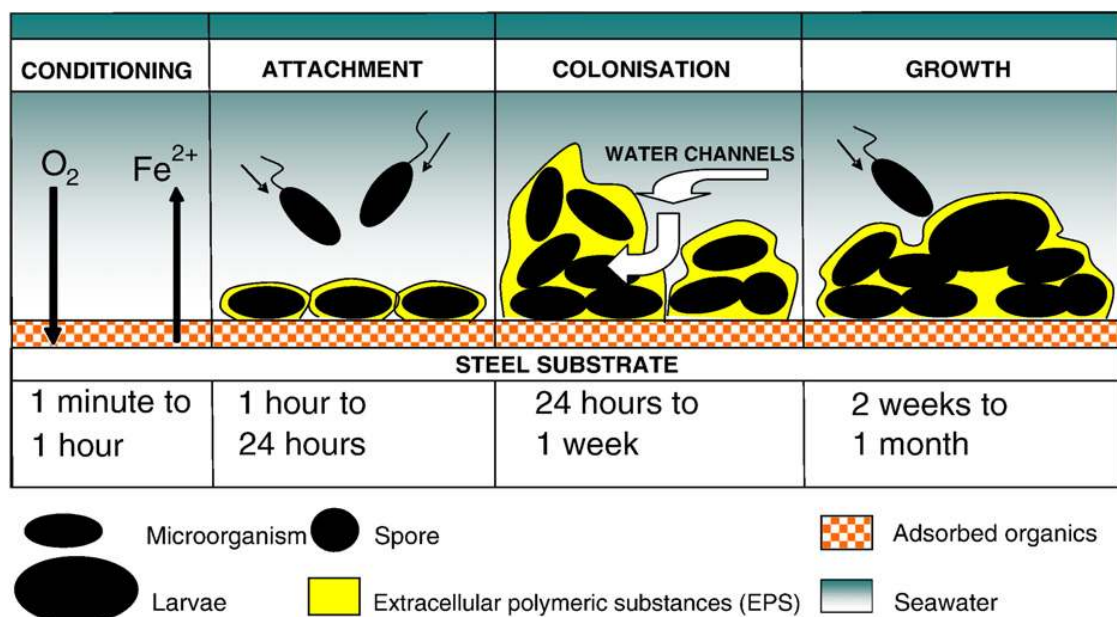


Figure 2.1 Schematic of critical biofouling stages (Chambers, Walsh, Wood & Stokes, 2006).

The sequence of biofouling is not predictable due to the exploitation of substrate niches by higher fouling organisms. Biofilm formation is often a precursor to subsequent fouling by macrofoulers. The succession of biofouling has been experimentally tested by removing initial algal layers resulting in limited further fouling. The presence of a biofilm has been recorded to have a positive influence on the settlement of some algal zoospores (Patel, Callow, Joint & Callow, 2003; Faimali, Garaventa, Terlizzi, Chiantore & Cattaneo-Vietti, 2004) recorded that an aging biofilm inhibited the settlement of barnacles. In general it is agreed that there is a sequence of events to biofouling and the first stage is usually taken to be the formation of a biofilm (Costerton, 1999).

Table 2.2 Stages of attachment of marine organisms on surfaces immersed in sea water

Processes involved	Attached organisms	Nature of film formed	Approximate initiation time
Stage 1			
Essentially physical forces, such as electrostatic interactions, Van der Waals forces and Brownian movement.	“Adhesion” of organic molecules, such as proteins, polysaccharides, proteoglycans and possibly, some inorganic molecules.	Conditioner.	1 min
Stage 2			
Reversible “adsorption” of mentioned species, especially by physical forces, and subsequent adhesion their interacting together with rotifers.	Bacteria, such as <i>Pseudomonas putrefaciens</i> and <i>Vibrio alginolyticus</i> and diatoms (single-cell algae) such as <i>Achnanthes brevipes</i> , <i>Amphora coffeaeformis</i> , <i>Amphiprora paludosa</i> , <i>Licmophora abbreviata</i> and <i>Nitzschia pusilla</i> .	Microbial biofilm.	1–24 h
Stage 3			
Arrangement microorganisms with greater protection from predators, toxicants and environmental alterations, making it easier to obtain the nutrients necessary for the attachment of other microorganisms.	Spores of <i>Ulothrix zonata</i> and <i>Enteromorpha intestinalis</i> and protozoans, including <i>Vaginicola</i> sp., <i>Zoothamnium</i> sp. and <i>Vorticella</i> sp.	Biofilm.	1 week
Stage 4			
Increase in the capture of more particles and organisms, such as the larvae of marine macroorganisms, as a consequence of the pre-existence of the biofilm and the roughness created by the irregular microbial colonies that comprise it.	Larvae of macroorganisms, such as <i>Amphibalanus amphitrite</i> (Crustacea), <i>Laomedea flexuosa</i> (Coelenterata), <i>Electra crustulenta</i> (Bryozoa), <i>Spirobrachia borealis</i> (Polychaeta), <i>Mytilus edulis</i> (Mollusca) and <i>Styela coriacea</i> (Tunicata).	Film consisting of attachment, development of marine invertebrates growth of Macroalgae seaweed.	2-3 weeks

When a chemically inert substrate is immersed in seawater an almost immediate accumulation of organic carbon residues adsorb onto the wetted surface and ions, glycoproteins, humic and fulvic acids available in the liquid phase (Table 2.2). The forces that promote the adsorption and conditioning of the surface include electrostatic interactions and Van der Waals forces. Pioneering microorganisms can now attach to the surface forming a biofilm. Contact and colonisation between the microorganism and the surface is promoted by the movement of water through

Brownian motion, sedimentation and convective transport, although organisms can also actively seek out substrates due to propulsion using flagella. Bacteria and other colonising microorganisms secrete extracellular polymeric substances (EPS) to attach them strongly to the substrate thereby altering the local surface chemistry which can stimulate further growth such as the recruitment and settlement of macroorganisms.

The biofilm generated is a mass of microorganisms and their EPS which creates a gel matrix providing enzymatic interaction, exchange of nutrients, protection against environmental stress (Videla, 1996) and an increased resistance to biocides (Morton, Greenway, Gaylarde & Surman, 1998). Biofilms also interrupt the flow of ions from water and from the substrate surface by acting as a diffusion barrier.

The adhesion of species to a substrate is an important aspect of biofouling for if this process could be prevented, fouling could be controlled. Adhesion and settlement is also often a key stage in the life cycle of marine organisms, so the evolutionary pressure to colonise a surface is great. The driving force of adhesion can be considered as being made up of contributions from the interfacial tension between the organism and the substratum, organism and the liquid and between the substratum and the liquid.

Biochemical conditioning describes the adsorption of dissolved chemical compounds (mostly macromolecules) to any surface in the first moments after contact with natural seawater. This instant (called 'immersion') may be the extrusion of a growing seagrass blade from its sheath, the appearance of a new crustacean carapace after moulting, the emergence of a fresh rock surface after breakage or the experimental immersion of a glass slide, etc. The concentration process of organic molecules at interfaces (solid/liquid, liquid/gas) is purely physical and 'spontaneous' (Baier, 1984). As this event reduces the randomness of molecular distribution, the accompanying loss of entropy must be compensated by a notable diminution of the total free energy of the system. This latter phenomenon is apparently due to the replacement of a high energy solid/liquid interface by a lower energy organic layer

(Dexter 1976, 1978; Dexter & Lucas, 1985). The adsorption of macromolecules begins within seconds after immersion and a dynamic equilibrium is reached within a few hours.

Substrata originally exhibiting a wide range of surface free energy values seem to adsorb the same kind of macromolecules (mostly glycoproteins, proteoglycans and polysaccharides) from seawater. By this process their physical and chemical surface properties converge so that low-energy (hydrophobic) surfaces experience an increase and high-energy (hydrophilic) surfaces a decrease of their gamma-values (Baier, 1981).

2.4.2 Behaviour of Antifouling Paints in Aquatic System

Little attention has been paid to the influence of the different sea water parameters on the performance of chemically active antifouling paints. It has recently been shown that chemical reactions and diffusion are key mechanisms in the performance of biocide-based antifouling paints, and that these can be markedly affected by sea water conditions (Kiil, Weinell, Pedersen, Dam-Johansen, Arias & Codolar, 2002).

The above-mentioned paints are based on the release of several biocides, which are linked or, more often, embedded in a film-forming organic matrix (Figure 2.3). Sea water has to penetrate into the paint, dissolve such biocides and diffuse out into the bulk phase again. To avoid the build-up of long diffusion paths and consequently decreasing release rates, the organic matrix is designed for slow reaction with sea water (and sea water ions) within the paint pores. Once this reaction has reached a certain conversion at the sea water-paint interface, the binder phase is released, thus controlling the thickness of the biocide-depleted layer (leached layer).

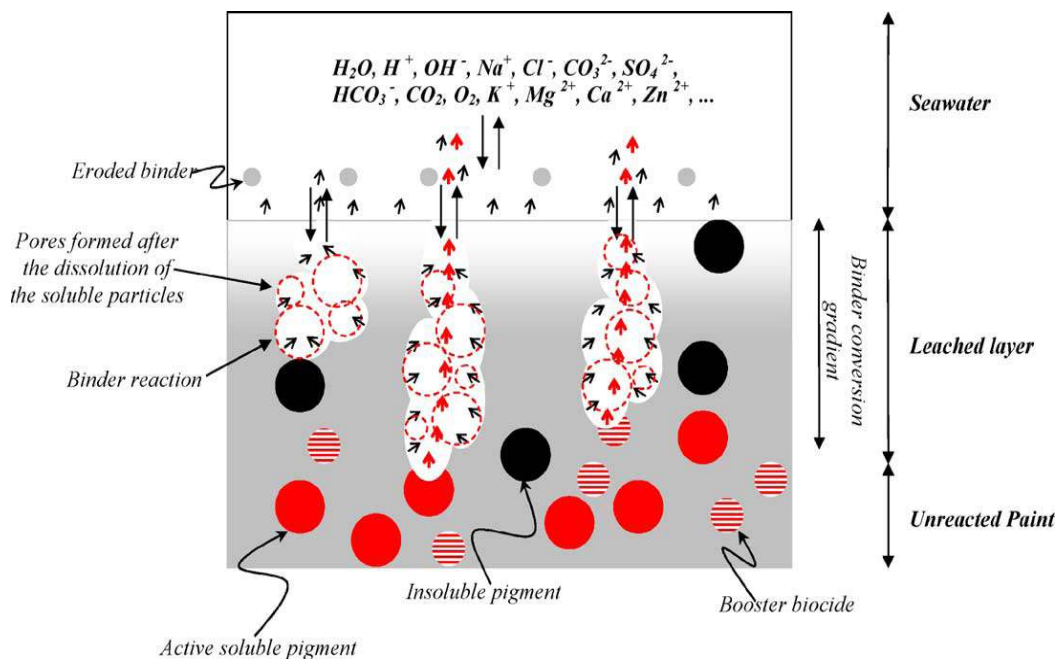


Figure 2.3 Schematic illustration of the behaviour of a biocide-based antifouling system exposed to sea water.

Many references to the influence of sea water parameters on the performance of A/F paints can be found in the open literature. For example, the salinity value influences the dissolution of the most typical biocidal pigment (Cu_2O) the dissolution of the most typical biocidal pigment (Cu_2O) particles the reaction of important binder components such as rosin (Rascio, Giúdice & B. del Amo, 1990) and the cleavage of the TBT groups in TBT-SPC paints (Kiil *et al.*, 2002; Kiil, Weinell, Pedersen & Dam-Johansen, 2001, 2002).

The influence of temperature is also significant as it affects the rate of all chemical reactions, dissolution rates and transport processes associated with the activity of chemically active A/F paints. The effect of sea water pH on the release rate of TBT groups from TBT-SPC paints was measured by Hong-Xi, Mei-ying, Huai-min & Jing-hao, (1988) and subsequently used by Kiil *et al.*, (2001); (2002) in the modelling and analysis of such paints. In addition, the severity of the biofouling and, consequently, the antifouling requirements, and the environmental fate of the released toxicants are affected by most of these parameters. Despite these facts, most studies dealing with the development of new chemically active antifouling binders or coatings lack studies on the behaviour of such systems in waters under conditions

different from the “standard” or “average” ones. This could eventually lead to biocide-based paints performing excellently under certain conditions but failing in waters with different characteristics. Consequently, it is useful to characterise the environment faced by antifouling coatings by determining the range of values of the most significant sea water variables.

2.4.3. Prevention of Fouling - Antifouling Paints

The hulls of ships and other artificial structures have to be protected against marine biofouling, i.e. the settlement and growth of animals and plants on submerged surfaces (Evans & Hoagland, 1986; Kiil, *et al.*, 2001). Some of the most important problems related to biofouling on ships and boats are: (i) increased drag which affects fuel consumption dramatically, increases carbon dioxide pollution and other air pollutants, and increases the workload on the machinery for maintaining speed; (ii) increased dry-dock cleaning and maintenance costs when the ship is immobile; (iii) loss of manoeuvrability of the ship; and increased risk of ecologic contamination by alien species (Rouhi, 1998; Callow & Callow, 2002; Yebra, Kiil & Dam-Johansen, 2004).

In recent decades and nowadays there are several types antifouling systems used to protect ship hulls from marine fouling. First technology used prior to mid 19th century such as wax, tar and asphalt (Callow, 1990) was used as antifouling products. In 700 B.C. Phoenicians and Carthagians used Cu and Greek and Romans are also used Cu and they investigated the use of lead sheathing. In the 18th century wooden sheathing covered with mixture of tar, fat and pitch and studded with metal nails, whose heads, closely in contact with each other seem to have formed a sort of second metallic sheath. Several countries use copper sheathing with copper and zinc nails, sheathing of zinc, lead, nickel, galvanized steel and copper - coated wood sheathing. Non-metallic sheathing were also preferred such as rubber, vulcanite, cork and others. In the mid 19th century, containing copper, arsenic or mercury oxide as toxicants dispersed linseed oil, shellac or rosin (Lunn, 1974).

First antifouling paints used on steel hulls prior to 1960. The first type of antifouling paints was based on the idea of dispersing a powerful toxicant in a polymeric binder. The others based on different bituminous products and natural resins. Meanwhile new products were emerging, including “hot plastic paints” with natural binders and copper or other toxicants, “rust preventine compounds” which were shellac-based products containing toxicants and with the development of polymer chemistry “cold plastic paints” which used different synthetic resins or natural products alone or in mixtures (Ekama, Londen & Wolf, 1962). The latter which was easier to apply by means of “airless” spraying, which was also developed around that time, allowed dry docking intervals of up to 18 months. Main types of products used on steel hulls in the second half of the 20th century. These paints are based on dispersion of toxicants in different types of polymeric binders.

2.4.3.1 Toxic Pigments

All traditional antifouling paint types use copper complexes as their pigments. However copper ions are not equally efficient against all types of fouling organisms, and the latter’s sensitivity to copper ions decreases in the following order: microorganisms, invertebrates, algae and macrophytes (Voulvoulis, Scrimshaw & Lester, 1999). Apart from copper oxides, use is at times made of zinc (II), iron (III) and titanium (IV) oxides, and in colour paints, copper thiocyanate, in view of their good solubility characteristics (Vetere, Pérez, Romagnoli, Stupak & del Amo, 1997). Among the latter, attention is drawn to tributyltin (TBT) and its derivatives, which are highly toxic to oysters, molluscs and crustaceans (Kroustein, 1973; Chromy & Uhacz, 1978).

In marine areas throughout the world where recreational boats are densely located, concentrations of copper in the water are being found to be in excess of government standards, due to the hull coatings used on these boats. Copper-based hull coatings are intended to be antifouling in that they retard the growth of algae, barnacles and tubeworms; but alternatives exist that can eliminate the harm that copper contamination does to marine organisms. A variety of policy options are available to

mandate or provide economic incentives to switch to these less harmful alternatives.

Toxic hull paints are used worldwide to control the growth of organisms such as algae and barnacles on boats. This growth, known as fouling, creates friction that can decrease a boat's speed, maneuverability, and fuel efficiency. To prevent these adverse effects of fouling, most bottom paints contain a copper biocide. Copper-based antifouling paints are designed to leach copper slowly into the water immediately surrounding a boat's hull. Copper is also released into the water when boat hulls are cleaned. Unfortunately, the copper is toxic not only to the potentially fouling organism but also to other organisms in the marine environment. This is particularly true when copper is present in high concentrations and there is growing concern that copper pollution poses a major threat to the marine environment. The problem is largely centered on major harbors where large numbers of recreational boats are densely located.

During the past two decades, organotin compounds have been the focus of much research and concern, however, as late as 1983 the opinion that they were "unlikely to create a serious long-term pollution hazard" was still being expressed (Bennett, 1983). Wide distribution, high hydrophobicity, and persistence of organotin compounds have raised concern about their bioaccumulation, their potential biomagnification in the food webs, and their adverse effects to the human health and environment (Galloway, 2006; Nakanishi, 2007; Takahashi, Mukai, Tanabe, Sakayama, Miyazaki & Masuno, 1999; Veltman, Huijbregts, van den Heuvel-Greve, Vethaak & Hendriks, 2006).

CHAPTER THREE

STUDY AREA

3.1. Location of Study Area

The study area includes four different sites; Candarli (C), Izmir-Pasaport (P), Izmir-Levent Marina (L) and Kusadasi-Setur Marina (K) (Figure 3.1). The study area extends from Candarli in the north to Kusadasi in the southeastern Aegean.

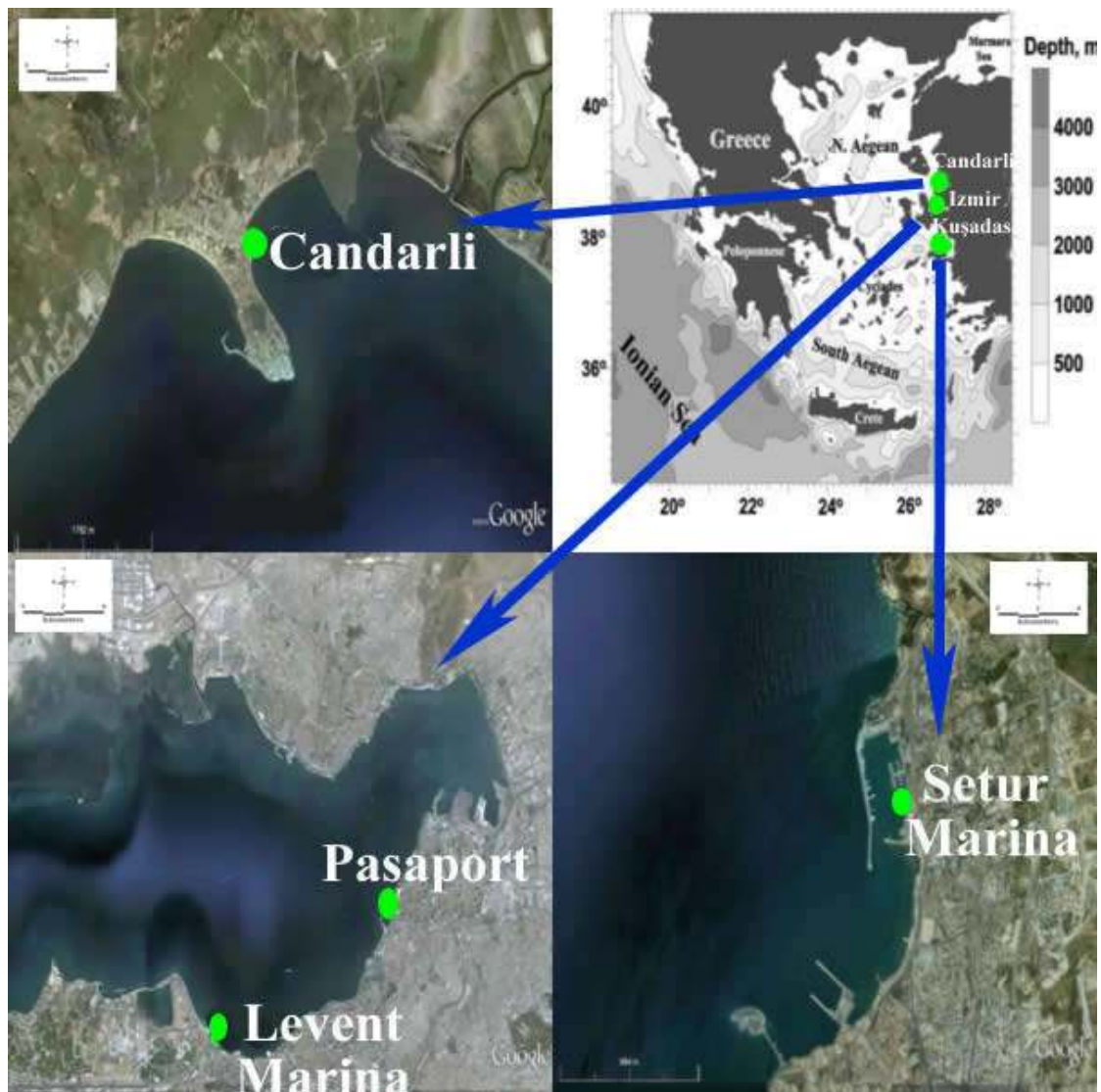


Figure 3.1 The location of sampling points in the Aegean Sea.

The Aegean Sea situated between Turkey and Greece and constitutes one of the main parts of the Eastern Mediterranean. It comprises both the territorial waters of these two countries and also international waters. It can be described as a confluence zone, where colder Black Sea waters coming from the Dardanelles Strait (Canakkale) meet the warmer waters of Eastern Mediterranean origin. These enter the basin through the southern straits. The Aegean Sea is connected with the Sea of Marmara through the Dardanelles Strait, with the Ionian Sea through the Kithira, Antikithira and Elafonisos Straits and with the Levantine Sea through the Rhodes, Kassos and Karpathos Straits.

Gulf of Izmir is situated on the eastern coast of the Aegean Sea. The gulf is roughly ‘L’ shaped. It has been divided into three areas according to their physical characteristics; Outer Section, Middle Section and Inner Section. The Outer Section is further divided into three sub-regions: Outer Section (A), Outer Section (B) and Outer Section (C). There are a series of islands parallel to the west coast of the gulf. The narrow Mordogan Strait, which is situated between Uzunada Island and the west coast of the Bay, has a sill depth of 14m. Surface water of the Aegean Sea can flow in the surface layer through the narrow Mordogan Strait into the small Gulbahce Bay, which is situated at the southwest end of the Gulf of Izmir. Another very important feature is the narrow Yenikale strait between the Inner Bay and the Middle Bay. The physical and chemical characteristics of water change drastically both sides of the Yenikale sill. The depth in the Outer Bay is about 70 m. It decreases significantly towards the Inner Bay to about 10 m depth Gediz Delta and saltpan area are in the centre of the gulf.

The bottom sediments of the eastern Aegean Sea are composed of material covering a wide range of grain size from silty clay to sandy gravel. Muds, are called mixture of silt and clay, occur mainly off river mouths. The coarse-grained sediments are mainly represented by abundant sand and gravel size fractions, which are composed of biogenic and terrigenous components. Those dominated by biogenic components are largely derived from the calcareous remains of species of pelecypods, gastropods, foraminifers, ostracods, bryozoans, algae, echinoids and

pteropods. The terrigenous components are generally consistent with geological source on land and coast (Ergin, Bodur, Ediger, Ediger & Yilmaz, 1993).

Candarli Bay is located between $38^{\circ} 55' 55''$ N latitude and $26^{\circ} 56' 55''$ E longitude in the eastern Aegean coast. The Gulf, in fact, has been strongly affected by growing population and industrialization. Great industry settlements, located in the coastal area of Candarli, have been discharging their solid and liquid wastes into Bakircay or Candarli Gulf after limited treatment. The intensive maritime traffic and untreated domestic discharges from 200,000 inhabitants around the bay area are the other factors that influence sea contamination. In the present study, Candarli Station is far away from industrial and the majority of urban sewage, receiving only local wastes. It serves mainly the fisheries, containing a number and variety of boats. Pasaport ($38^{\circ} 55' 55''$ N, $26^{\circ} 56' 55''$ E) is located near the harbour area and influenced by maritime traffic and untreated domestic discharges.

Levent Marina and Kusadasi Setur Marina is located between ($38^{\circ} 24' 24''$ N and $37^{\circ} 52' 00''$ N) latitudes and ($27^{\circ} 04' 09''$ E and $27^{\circ} 15' 30''$ E) longitudes, respectively. Kusadasi Setur Marina, one of the most important marinas of Turkey, which provides various services such as boat docking, boat repair and all kinds of painting and maintenance, varnishing, epoxy-polyester works, boat covering, sprayhood manufacture and repair, all kinds of main machinery, generator and outboard repair, plane, stainless metal and galvanizing works, electric and electronic equipment repair, bilge control, mounting and maintenance are available.

3.2. Hydrochemical Characteristics

The Aegean Sea is one of the most oligotrophic parts of the Mediterranean Sea. Although nitrogen and phosphorus levels are low in general, concentrations of nutrients are higher than the Mediterranean Sea in some regions.

Very few published data are available on nutrient concentrations in the Aegean Sea. Distribution of nutrients was investigated by Friligos (1986); Kucuksezgin, Altay & Kontas (1995) in the Aegean Sea. Nutrient levels are generally higher in the

northern Aegean than in the southern part. This situation may result from water originating from the Marmara and the Black Sea. Nutrient values increase with increasing depth. There are many rivers, which transport nitrogen and phosphorus into the northern Aegean. The order of magnitude of fresh water inputs is $1000 \text{ m}^3/\text{s}$ in total along the Aegean coastline and this value is higher than in other Mediterranean regions (IMST, 1997).

3.3. Hydrophysical Characteristics

Not only hydrochemical but also hydrophysical characteristics are different in the southern and northern parts of the Aegean. Lower salinity and temperatures were observed in the Northern Aegean due to the influence of black Sea. Salty and warm waters of the eastern Mediterranean Sea affect Southern Aegean waters. In the beginning of the summer, Black Sea originated water masses move towards the Edremit Bay ((IMST, 1997).

Surface water mass in the Aegean Sea forms a counter-clockwise gyre. At present, warm ($16\text{-}25^\circ\text{C}$) and high salinity ($39.2\text{-}39.5 \text{ ppt}$) Mediterranean water moves northward along the west coast of Turkey. This water mass is placed westward south the Strait of Dardanelle by the cooler and low salinity water mass initially moves west-northwest along the Northern Aegean Sea, then flows southwards along the east coast of Greece (Yasar, 1994). Water masses:

1- Aegean Sea surface water forms a 40-50 m thick veneer, with summer temperatures ranging between $21\text{-}26^\circ\text{C}$ and winter temperatures change between $10\text{-}16^\circ\text{C}$ in the Aegean Sea. Similar North-South gradient is also represented with summer salinity values ranging between 30-39.5 psu and winter salinities changed between 36.1-39.2 psu.

2- Aegean Sea intermediate water mass (40-50 m to 200-300 m) has a smaller north-south temperature gradient ranging between $15\text{-}18^\circ\text{C}$ and $11\text{-}16^\circ\text{C}$ from the

northern to southern Aegean Sea, respectively. Seasonal salinity variations are very low and the salinity changes between 39.0-39.2 psu.

3- Aegean Sea Bottom Water (below 200-300 m) is very uniform in temperature (13-14 °C) and salinity (39.1-39.2 psu) with little variations between in summer and winter (Yasar, 1994).

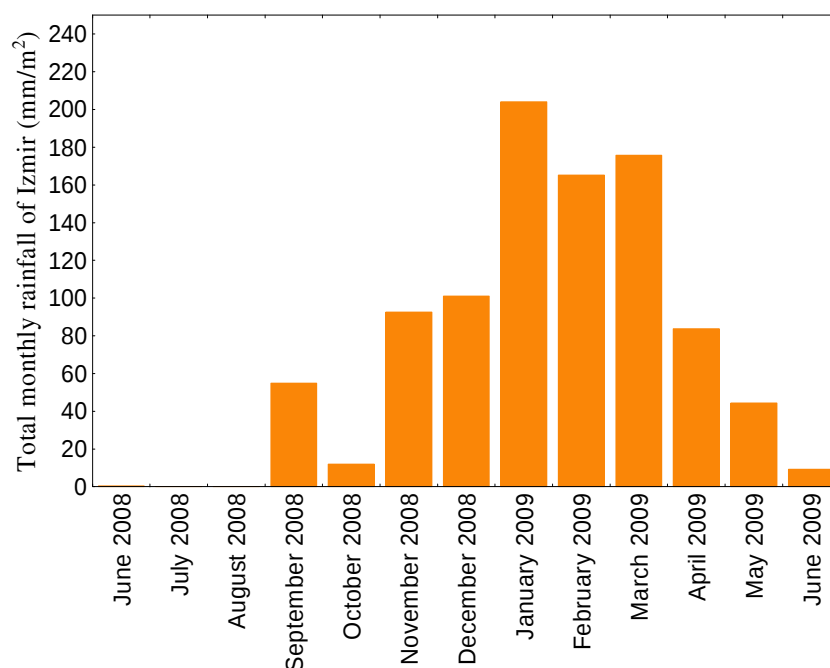


Figure 3.2 Total monthly rainfall of Izmir (June 2008-June 2009) (TurkStat, Turkey's Statistical Yearbook, 2008; 2009).

The annual rainfall distributions of Izmir from July 2008 to June 2009 were given in Figure 3.2. As for the seasonality of rainfall of Izmir, July-December 2008 was represented dry to moderately rainy season and January-June 2009 was showed heavily rainy to moderately rainy season (TurkStat, Turkey's Statistical Yearbook, 2008; 2009).

3.4. Geological Characteristics of Sampling Sites

Grain size analyses were performed using standard sieving and settling procedures (Hakanson & Jansson, 1983) in the eastern Aegean Sea coast. Hydrometer method;

based on records of the variation in density of settling suspensions using a hydrometer.

Table 3.1 Grain size distribution and organic matter content of the sampling stations of surficial sediments

Station	Gravel	Sand	Silt	Clay	Organic matter (%)
<i>Summer 2008</i>					
Candarli	6.24	57.94	23.76	12.06	40.18
Izmir-Pasaport	0.40	11.52	54.94	33.14	37.65
Izmir-Levent Marina	1.12	27.59	62.82	8.47	27.97
Kusadasi-Setur Marina	0.23	12.17	58.11	29.49	17.60
<i>Autumn 2008</i>					
Candarli	3.67	64.16	21.33	10.83	35.74
Izmir-Pasaport	0.76	13.93	53.22	32.09	41.46
Izmir-Levent Marina	1.13	23.67	67.76	7.44	12.22
Kusadasi-Setur Marina	0.26	11.38	58.62	29.74	5.41
<i>Winter 2009</i>					
Candarli	9.19	64.45	17.40	8.96	30.47
Izmir-Pasaport	0.90	12.09	57.71	29.29	36.66
Izmir-Levent Marina	0.97	24.74	66.93	7.35	31.97
Kusadasi-Setur Marina	0.51	12.52	57.70	29.27	2.62
<i>Spring 2009</i>					
Candarli	6.09	59.66	24.76	9.50	34.58
Izmir-Pasaport	0.54	11.79	63.37	24.30	39.50
Izmir-Levent Marina	1.23	22.86	57.87	18.03	38.03
Kusadasi-Setur Marina	0.25	13.57	55.16	31.02	3.66

These sedimentation methods require inexpensive apparatus and cover a wide range of grain sizes. The hydrometer method is not applicable if less than 10% of the sample passes the 63 μm mesh. Textural classification of the sediment samples was based on the relative percentages of clay (<0.002 mm), silt (0.002-0.063 mm), sand (0.063-2 mm) and gravel (>2 mm). The amount of organic carbon and organic matter are determined by spectrophotometrically in dried sediment samples following the sulfochromic oxidation method. The accuracy of this method is ± 0.017 % organic matter (HACH Publication 3061, 1988).

The grain size composition of the Eastern Aegean coast surficial sediments was given in Table 3.1. The classification was performed by grain size distribution.

According to this classification Candarli was covered by sand while Izmir-Pasaport, Izmir-Levent Marina and Kusadasi-Setur Marina were covered by silt during all sampling periods. With respect to organic matter (%) (OM), the highest and the lowest (%) OM contents varied from 41.46 % in Izmir-Pasaport in autumn to 2.62% in Kusadasi-Setur Marina in winter and represented with the highest percentage (average 38.82%) in Izmir-Pasaport and the lowest percentage (average 7.32%) in Kusadasi-Setur Marina.

3.6. Previous studies

A lot of studies have been carried out on the concentrations and distribution of heavy metals accumulation of seawater, sediments and marine biota in the world and in the Aegean Sea.

Dassenakis, Andrianos, Depiazi, Konstantas, Karabela, Sakellari & Scoullou, (2003) investigated the heavy metal (Cu, Fe, Mn, Zn) levels of sediments taken from Euvoikos Gulf, Greece by using a sequential extraction schema and two single-step methods for the determination of trace metals. Bertolotto, Tortarolo, Frignani, Bellucci, Albanese, Cuneo, Alvarado-Aguilar, Picca & Gollo, (2005) analysed As, Cd, Cr, Cu, Hg, Pb and Zn in sediment samples collected from Ligurian Sea, Italy. García-Cobelo & Prego, (2004) were measured heavy metals (Cu, Co, Cr, Fe, Ni, Pb and Zn) taken from 35 surface sediment samples in the Ferrol Ria (Galicia, NW Spain). Morillo, Usero & Gracia, (2004) investigated sediment samples taken from the southwest coast of Spain. Their study provides valuable information on the potential mobility of heavy metals in marine sediments. Adamo, Arienzo, Imperato, Naimo, Nardi & Stanzione, (2005) studied the heavy metal pollution in the marine sediments within the port of Naples, Italy. They analysed As, Cd, Co, Cr, Cu, Mn, Mo, Pb, Ti, V and Zn in the sediment samples. Ferraro, Sprovieri, Alberico, Lirer, Prevedello & Marsella, (2006) analysed 90 surficial sediments taken from three docks of the Naples Harbour (Levante, Granili, and Diaz) which permits to compare the distribution modes of heavy metals with grain sizes, total organic carbon content (TOC) and distribution patterns of benthic foraminifera. Mendiguchía, Moreno,

Mánuel-Vez & García-Vargas, (2005) studied the metals (Zn, Cu, Pb) and organic matter concentrations in an arm-of-the-sea of the Bay of Cádiz (SW Spain) where several intensive marine aquaculture facilities are located and they conclude that marine aquaculture can increase the levels of organic matter and heavy metals in the sediments.

Several studies have been carried out in the Aegean Sea, Aksu, Yasar & Uslu, (1998) analysed the concentrations of 42 elements in 84 samples established that surface sediments in Inner Izmir Bay display significant enrichments in Ag, As, Cd, Cr, Cu, Hg, Mo, P, Pb, Sb, Sn, V, and Zn, associated with notably high concentrations of total organic carbon and sulphur. Combined inorganic and organic geochemical data indicated that Inner Izmir Bay surface sediments are extremely polluted and probably pose high risk to the resident marine biological community. Except for a few small regions, the Outer and Central Izmir Bays show low levels of heavy metal enrichments. Kucuksezgin, Kontas, Altay, Uluturhan & Darılmaz, (2006) investigated Hg, Cd, Cr and Pb and concentrations in the sediment of Izmir Bay.

Heavy metal concentrations in marine organisms were investigated different parts of the Aegean Sea, Campanella, Conti, Cubadda & Sucapane, (2001) measured Cd, Cr, Cu, Pb and Zn levels in the seagrass *Posidonia oceanica* (L.), *Padina pavonica*, two gastropod molluscs *Monodonta turbinata* Born and *Patella caerulea* L. Lionetto, Caricato, Giordano & Schettino, (2004) studied two fish species, such as *Mullus barbatus* and *Trachurus mediterraneus* and in mussels (*Mytilus galloprovincialis*). Usero, Morillo & Gracia, 2005 determined trace metals (As, Cr, Cu, Hg, Pb and Zn) in the two most abundant species of bivalve molluscs along the Atlantic coast of southern Spain (*Donax trunculus* and *Chamelea gallina*) and in the sediments where they live. Hamed & Emara, (2006) studied the levels of the heavy metals; copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), iron (Fe) and manganese (Mn) were determined in coastal water, sediments and soft tissues of the gastropod limpet, *Patella caerulea*, the bivalve and *Mullus barbatus* from seven different stations in the western coast of the Gulf of Suez.

The heavy metal concentrations in several fish species from Black Sea and Aegean Sea were determined. Guner, Dincer, Alemdag, Colak & Tufekci, (1998); Kucuksezgin, Uluturhan, Kontas & Altay, (2002); Topcuoglu, Kirbasoglu & Gungor, (2002); Dalman, Demirak & Balci, (2006) investigated the heavy metals (Pb, Cd) and trace elements (Cu, Zn) in fish and sediments in the Bay of Gulluk in Southeastern Aegean Sea (Turkey). Kucuksezgin *et al.*, (2006) studied the nutrient concentrations of the water and heavy metal levels of sediment and fish samples (*Mullus barbatus*) taken from Izmir Bay. The concentrations of heavy metals found in fish varied for Hg: 4.5-520, Cd: 0.10-10 and Pb: 0.10-491 $\mu\text{g kg}^{-1}$ in Izmir Bay. Heavy metal levels were lower than the results in fish tissues reported from polluted areas of the Mediterranean Sea.

Much attention has been paid to the use of different kinds of marine organisms like barnacles as bioindicators for trace metal pollution in marine waters. The researchers have been done several studies on the accumulation of heavy metal in barnacle species in different parts of the the world. Philips & Rainbow, (1988) collected *Perna viridis*, *Capitulum mitella* (Linnaeus), *Tetraclita squamosa* Bruguiere (not distinguished into subspecies *T. squamosa squamosa* Bruguiere and *T. squamosa japonica* Pilsbry), and *Balanus amphitrite amphitrite* Darwin for determining cadmium, chromium, copper, lead, zinc for up to 18 sites in Hong Kong coastal waters. Blackmore, Morton & Huang, (1998) were found 0.87-5.50 Cd, 28.97-2205 Cu, 38.83-277 Mn, 1521-10000 Zn $\mu\text{g kg}^{-1}$, dry mass in soft tissues of *Amphibalanus amphitrite* taken from coastal waters of Xiamen, China. Fialkowski & Newman, (1998) observed accumulations of Cd, Cu, Fe, Hg, Pb, Sn and Zn in body tissues and egg masses of *Amphibalanus amphitrite*. Rainbow, Amiard-Triquet, Amiard, Smith & Langston, (2000) measured accumulated trace metal concentrations (Cu, Zn, Fe, Cd, Pb, Mn, Ni) in the mussel *Mytilus trossulus* and the barnacle *Balanus improvisus* collected in the Gulf of Gdansk, Poland. Blackmore & Rainbow, (2001) investigated in 19 species of intertidal invertebrates collected relatively unpolluted area in Hong Kong for cadmium, copper and zinc concentrations. Rainbow & Blackmore, (2001) found 9.82-457 As, 0.69-9.45 Cd, 52.4-1810 Cu,

14.5-95.4 Mn, 1.25-98.9 Ni, 2860-23300 Zn mg kg⁻¹, dry mass in *Amphibalanus amphitrite* from Curimataú estuary, Natal, Brasil. Silva, Smith & Rainbow, (2006) reported 5.13 mg kg⁻¹Cd, 23.6 mg kg⁻¹Cu, 9.6 mg kg⁻¹ Mn, 9.1 mg kg⁻¹Ni, 1185 Zn mg kg⁻¹ dry wt in *Amphibalanus amphitrite*. Morillo & Usero (2008) used barnacle *Amphibalanus amphitrite* for biomonitoring of metals (As, Cd, Cu, Hg, Mn, Ni, and Zn) in two different littoral habitats in southern Spain, Huelva estuary and Algeciras Bay. Water samples were collected from the same sites as the barnacles to assess metal concentrations and to gain additional information on the environmental conditions. However, no detailed information was available for barnacle species taken from Aegean margin and along the Turkish coasts. Turkmen, M., Turkmen, A., Akyurt & Tepe, (2005) showed the levels of cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, and zinc in soft tissues of the *Patella caerulea* and *Balanus sp.* collected from the Iskenderun Bay.

CHAPTER FOUR

MATERIAL AND METHODS

4.1 Biology and Distribution of *Amphibalanusa amphitrite* (Darwin, 1854)



Figure 4.1 *Amphibalanus* (= *Balanus*) *amphitrite*, (Darwin, 1854).

Kingdom : Animalia

Phylum : Arthropoda

Subphylum : Crustacea

Class : Maxillopoda

Subclass : Thecostraca

Order : Sesilia

Suborder : Balanomorpha

Superfamily : Balanoidea

Family : Balanidae

Subfamily : Balaninae

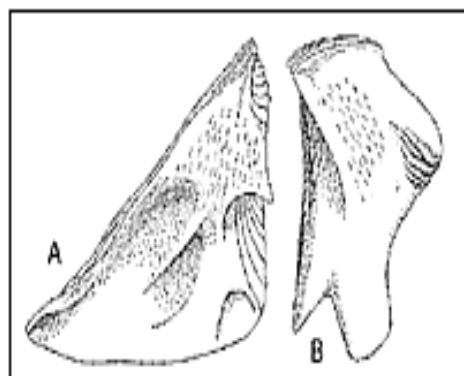
Species : *Balanus amphitrite*

(= *Amphibalanus amphitrite*). (Darwin, 1854).

Amphibalanus amphitrite is a small, conical, sessile barnacle (to about 1.5 cm diameter) (Figure 4.2a). Color is white or very light pink, each plate carrying one or a group of several vertical purple or dark pink stripes that are broader at the basis. Surface of test plates are longitudinally ribbed. The interlocking tergum and scutum, the paired structures which cover the animal inside are as pictured below (Figure 4.2b). A similar species, *Balanus reticulatus* Utinomi, is also an introduced species and commonly occurs with *B. amphitrite*. It also has longitudinal purple or brown stripes, but these stripes are intersected by horizontal grooves, giving the surface of the test plates a rough reticulated striation, unlike *B. amphitrite*. It can also be distinguished by examination of the tergum and scutum pictured below. Note the more sharply pointed apex of the tergum and the elongated and narrower tergum spur of *B. reticulatus*.



a



Balanus amphitrite. (A) Scutum. (B) Tergum.

b

Figure 4.2a *Amphibalanus amphitrite* (= *Balanus amphitrite*) (Darwin, 1854). (b) Scutum and tergum.

In a barnacle, the body consists of the thorax with six pairs of thoracic limbs (cirri), a tiny abdomen and part of the head (the oral cone with mouthparts). It sits the mantle cavity and is attached to the remainder of the head that forms the mantle cavity, the tissue lining the shell plates (including the opercular plates) and any egg masses lying in the mantle cavity.

The opercular opening is diamond shape, but with rounded corners. The scuta carry a single purple or pink stripe, narrowing towards the terga. The tergoscute flaps are distinctive. They are held flat, and the ground colour is mostly white but tinged with faint pink or light yellow in old specimens. The flaps are crossed by four strongly marked transverse bands of black or purple, the centre band at the micropyle corresponds to the coloured dots in this location in other barnacle species (Southward, 2008).

Barnacles use the three posterior thoracic limbs in captorial feeding of larger particles and microfiltration is performed using setae on the smaller anterior cirri (Anderson, 1980; Hunt & Alexander, 1991). Barnacles have specialized paired appendages, called cirri that they use as a scoop net, reaching out into the water and extracting food particles. When the cirri are drawn back, food is scraped off into the mouth.

Barnacles are microphagous feeders, may ingest many potentially metal-rich food sources such as plankton and detritus, (Rainbow, Phillips & Depledge, 1990) they also pass large volumes of water across the permeable surfaces by means of their cirri and this process further facilitates metal uptake. Aquatic invertebrates will take up trace metals into the body either from solution through permeable body surfaces or from the gut. Recently it has become increasingly appreciated that uptake of trace metals from the diet may be the major source of metals for many aquatic invertebrates (Wang, 2002), including barnacles (Wang, Qiu & Qian, 1999a, 1999b; Rainbow & Wang, 2001, 2005).

Barnacles are the most sedentary crustaceans and in temperate regions barnacles are relatively easy to age for they typically release larvae and thereafter recruit to the parent population over a restricted period once a year. Moreover, barnacles are strong net accumulators of trace metals adding metals newly absorbed from water or suspended particle sources to existing permanent body stores of detoxified metal (Rainbow, 1987; Rainbow & White, 1989). Accumulated body metal contents are

therefore on integrated measure of the bioavailable supply of the metal over a known preceding period, in this case the lifetime of the barnacle.

It is well known that one of the most important factors that play a significant role in heavy metal accumulation in marine animals is the metabolic activity (Langston, 1990; Roesijadi & Robinson, 1994). It is also known that the metabolic activity of a young individual is normally higher than that of an older individual. Thus, metal accumulation was shown to be higher in younger individuals than the older ones (Rainbow, Huang, Songkai & Smith, 1993).

These barnacles are hermaphrodites, but cross-fertilization occurs in dense populations. In such cases, males deposit sperm directly into the mantle cavity of adjacent functional females via a long tube. Fertilized eggs are brooded in the mantle cavity, and it may be several months before the free-swimming planktonic larvae are released (Edmonson, 1933). Fertilization occurred from March to September and settlement to May to August. It is a very common fouling species, now widely distributed intertidally and sublittorally in sheltered bays, estuaries and ports in all tropical seas (Southward, 2008). Barnacles are sessile and representatives occur in most salinities, most shore types and at varying degrees of exposure to wave action (Wu, 1975).

The larval development of these organisms includes six naupliar instars and a non feeding presettling cyprid instar. The anatomy of the cyprid is different from the preceding naupliar instars. The cypris larvae explore the substratum hunting a place for attachment.

The attaching antennular segment consists of large, thin, circular sucking disc from the edge of which cement is secreted, and the antennular disc becomes attached to the substratum. Two types of barnacle adhesion to a substratum have been observed, namely temporary and permanent adhesion. The cyprid employs the antennular disc, an adhesive organ, for temporary attachment to the substratum. Barnacle cement is used for permanent settlement and is an underwater adhesive of

insoluble protein complex. The cyprid after settling on a surface molts its carapace and the body exoskeleton except for the embedded parts of the antennule and metamorphoses into an adult.

Barnacles lack a hepatopancreas and they accumulate metals into granular deposition in the body cells of the stratum perintestinale (midgut epithelium). These deposits are amorphous with spherical shape and diameter ranging between 1-10 micrometer (Masala, McInnes & O' Brien, 2002). Feeding behavior of barnacles may be determined by various factors such as water flow direction, flow velocity, type and location of substratum, predators, systematics, and more. The granules contain pyrophosphate as the main anion and calcium as the major cation. In addition, a variety of other metals, such as Al, Fe, Mn and Zn accumulate in these materials. $\text{Ca}_2\text{P}_2\text{O}_7$ (calcium pyrophosphate) acts as traps for foreign metal ions that could be exchanged with calcium and then incorporated inside the granules.

This ion exchange appears to be facilitated by the amorphous structure of the deposits since the random network structure enables the movement of ions from the granule into the solution and vice versa. It has been also suggested that the formation of these granules in intracellular membrane-bound compartments is responsible for their specific composition, morphology and amorphous nature.

4.2 Water Collection and Analysis

Water samples were taken four times in June 2008- June 2009 from four sampling sites, using 1 litre acid-leached polyethylene bottles. For water samples, 1 N HNO_3 was used to acidify the ion exchange technique; using Chelex-100 resin was applied for determining heavy metals in water samples. Mercury concentration was measured by anodic stripping voltammeter (ASV) at the rotating gold electrode in seawater. With a deposition time of 90 s the calibration curve is linear from 0.4 to 15 $\mu\text{g l}^{-1}$; the limit of quantification is 0.4 $\mu\text{g l}^{-1}$. The method has primarily been drawn up for investigating water samples. After appropriate digestion the determination of mercury is possible even in samples with a high load of organic substances. The

concentration in the sample is determined by standard addition (METROHM Application Bulletin 96/5 e, Determination of mercury at the rotating gold electrode by anodic stripping voltammetry).

4.3 Sediment Collection and Analysis

Sediment samples were taken by using Van-Veen Grab from surface sediments. After collection, the sediments were placed in polyethylene bags and frozen (-20 °C). Thereafter, samples were dried in oven at 40 °C, homogenized and reduced to a fine powder by using a sieve (63 µm). Samples were digested in microwave digestion system with a HNO₃-HF-HClO₄-HCl acid mixture solutions (UNEP 1985b, c, d, e) and they were analysed by flame and graphite furnace AAS (Varian Spectraa-300 plus), using manufacturer's conditions and with background correction.

4.4 Barnacle Collection and Analysis

Amphibalanus amphitrite were sampled seasonally during June 2008 to June 2009 from four different locations. Sixty organisms of the different size (rostrum-carinal axis) were selected and collected from the intertidal zone at each station; they were removed from the substratum with a stainless steel scraper and placed in individual clean polythene bags then transported in closed, refrigerated containers and frozen at -20 °C. In the laboratory, identification of individuals was done by direct examination of morphological characteristics considering Southward (2008). Their rostrum-carinal axis were measured and made different size groups. At a later date, barnacles were defrosted, their bodies dissected out with new stainless steel instruments and the soft parts (the body) were separated. Each replicate sample was weighed and then dried to constant weight at 60 °C and weighed again (Ruelas-Inzunza & Páez-Osuna, 2000; Rainbow, Wolowicz, Fialkowski, Smith & Sokolowski, 2000; Rainbow & Blackmore, 2001).

The samples were digested in an automatic microwave digestion system (Milestone 1200) because of the advantages of this technique, which include speed of

digestion and less possibility of contamination during the process. A portion of the dry, finely powdered solid tissues (0.5-1 g) were accurately weighed in a dry, pre-cleaned Teflon digestion vessel. Then samples digested with 5:1 HNO₃/HClO₄ in a microwave oven (UNEP, 1982, 1984, 1985a). All analyses were performed by flame (Pb, Cr, Cu, Mn, Zn and Fe), graphite furnace (Cd) and cold vapour technique (Hg) in AAS (Varian Spectraa-300 plus) for sediment samples. (Cd, Pb, Cr, Cu, Mn, Zn and Fe) by flame AAS (Varian Spectraa-300 plus) for water samples and analyses were performed by flame (Cu, Fe, Mn and Zn), cold vapour (Hg) and graphite furnace technique (Cd, Cr and Pb) in AAS (Varian Spectraa-300 plus) for barnacle species by using the manufacturer's conditions and with background correction. The detection limits for trace metals were: Hg: 0.05 µg kg⁻¹, Cd: 0.10 µg kg⁻¹, Pb: 0.10 µg kg⁻¹, Cr: 0.06 µg g⁻¹, Cu: 0.03 µg g⁻¹, Zn: 0.01 µg g⁻¹.

4.5 Quality Assurance

Accuracy and validity of the analytical processes were tested with a reference material (homogenate muscle sample, IAEA-407, from the International Laboratory of Marine Radioactivity, IAEA, Monaco). The values obtained from the analysis of three replicates of this sample (certified: observed values in µg g⁻¹ dry wt + standard deviation) were as follows: Hg, 0.22±0.024: 0.21±0.0015; Cd, 0.19±0.019: 0.18±0.004; Pb, 0.12±0.06: 0.10±0.012; Cr, 0.73±0.22: 0.74±0.062; Cu, 3.28±0.40: 3.39±0.36; Zn, 67.1±3.8: 69.4±1.3.

Intercalibration sediment (IAEA-433) sample (from the International Laboratory of Marine Radioactivity, IAEA) were used as a control for the analytical methods. The values obtained (in µg g⁻¹ dry wt.) for the analysis of six replicates of this sample were as follows: Hg (certified 0.168, s.d: 0.017; found 0.167, s.d: 0.012), Cd (certified 0.153, s.d: 0.033; found 0.140, s.d: 0.025), Cr (certified 136, s.d: 10; found 136.7, s.d: 2.0), Cu (certified 30.8, s.d: 2.6; found 30.8, s.d: 2.3), Pb (certified 26.0, s.d: 2.7; found 27.0, s.d: 3.1), Zn (certified 101, s.d: 8.0; found 103, s.d: 1.9), Mn (certified 316, s.d: 16; found 317, s.d: 1.7), Fe (certified 40800, s.d: 1900; found

40412, s.d: 293). Total Hg and Cd analyses were performed by cold vapor and graphite furnace AAS, respectively in sediment samples.

4.6. Statistical Analyses

Metal concentrations in the bodies of marine organisms are dependent on the rates of accumulation, excretion and diluting body growth. There is, therefore, the potential for an effect of size on the body metal concentration that has to be allowed for in meaningful analyses of data. *A. amphitrite* has been shown to demonstrate significant effects of size on body metal concentrations (Phillips & Rainbow, 1988; Chan, Rainbow & Phillips, 1990; Rainbow & Smith, 1992; Rainbow, Huang, Songkai & Smith, 1993). Accumulated concentrations are modelled by the power function:

$$y = ax^b$$

where y is the metal concentration ($\mu\text{g g}^{-1}$), x is the mean body dry weight (g) and a and b are constants. The data are, therefore, by definition, multiplicative. All data were transformed logarithmically (to the base 10) to reduce deviations of the data sets from normal distributions, creating an additive data set with skews removed. Log-transformed regression relationships between body burden and size were used to compare the species. Data for Hg, Cd, Pb, Cr, Cu, Zn, Mn and Fe at each site will be checked for significant regressions between metal concentration (y) in $\mu\text{g g}^{-1}$ and body dry weight (x) in g.

Statistical analysis was performed using STATISTICA for Windows, Release 5.0, Copyright StatSoft, Inc. 1995. ANCOVA compares metal concentrations of bodies of a barnacle species from several sites at standardized body dry weights. If there was a significant effect of size on accumulated metal concentration for any data set for any one site or for the whole data set of a particular metal, then analysis of covariance (ANCOVA) was used to compare concentrations of that metal in that organism between sites and sampling seasons, having checked first that there was no significant difference between regression coefficients of the data sets being compared.

Homogeneity of variances was tested with the Cochran test of heavy metal contents in tissues.

Spearman Rank Order Correlation test will be used to check for significant relationships heavy metals in barnacle, sediment and seawater. Pearson product moment correlation test was applied to find the relationship between physicochemical parameters and metals in seawater and barnacle.

Multi Dimensional Scaling (non metric MDS) was used to compare the similarity between sample weights and heavy metal profiles. A two dimensional plot was obtained which satisfies all the conditions imposed by rank, i.e. if sample 1 had a higher similarity to sample 2 than sample 3, it is placed closer to the former on the plot than the later. PRIMER v3.1b was used for ordination. Hierarchical cluster analysis (HCA), which identifies homogenous groups of samples, was engaged, according to the group average, between groups of metals in samples. Prior to analysis the raw data was transformed using the $\log(x+1)$ transformation.

CHAPTER FIVE

RESULTS

5.1 Physico-Chemical Properties

The physico-chemical characteristics of four sampling stations for each season have been measured in order to characterize the water quality parameters and to find possible correlations between metal concentrations and some of these parameters. These parameters included pH, salinity, conductivity, temperature and dissolved oxygen (DO) and the results were given in Table 5.1. Pearson correlation coefficients between physicochemical parameters and heavy metals in seawater, sediment and *A. amphitrite* were presented in Table 5.2, 5.3 and 5.4, respectively.

Table 5.1 Physicochemical parameters of the Eastern Aegean coast

Site	Period	DO (mg/l)	Salinity (psu)	Conductivity (mSi/cm)	pH	Temperature (°C)
Candarli	Summer	6.5	39.8	59.00	8.30	25.1
	Autumn	6.9	39.7	59.60	8.38	20.2
	Winter	7.9	38.7	58.60	8.47	16.7
	Spring	7.1	39.5	59.40	8.19	22.7
Izmir-Pasaport	Summer	3.2	40.8	60.70	7.98	26.0
	Autumn	5.6	39.5	59.90	8.18	16.6
	Winter	9.4	38.2	57.20	8.52	13.6
	Spring	6.9	38.1	57.10	8.47	25.1
Izmir-Levent Marina	Summer	4.7	39.2	58.70	8.24	24.0
	Autumn	7.3	39.9	60.00	8.32	19.8
	Winter	8.1	38.8	59.30	8.38	11.9
	Spring	6.8	37.3	58.10	8.37	24.6
Kusadasi-Setur Marina	Summer	6.7	39.6	59.10	8.27	25.2
	Autumn	7.0	38.2	57.70	8.19	20.7
	Winter	8.6	38.1	57.90	8.39	16.8
	Spring	6.8	38.2	57.80	8.09	22.8

The pH which acceptable range normally changed between 6.5-8.5 according to guideline suggested by WHO (1993) is an important indicator of the water quality and the pollution in the aquatic system. The pH values obtained from the stations ranged between 7.98-8.52 indicate the moderately alkaline nature of the seawater. Low temperature value was measured as 11.9 °C at Izmir-Levent Marina during

winter period while the highest value (26 °C) was found at the Izmir-Pasaport in the summer period.

Table 5.2 Pearson correlation coefficients between physicochemical parameters and heavy metals in seawater (*Marked correlations are significant at $p < 0.05$*)

	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe
DO	-0.67	0.11	-0.34	-0.46	0.04	0.31	0.21	0.12
Salinity	0.33	0.36	0.48	0.53	0.25	0.02	-0.03	0.12
Conductivity	0.19	0.10	0.20	0.24	0.14	0.10	-0.01	0.12
pH	-0.38	-0.04	-0.25	-0.26	-0.39	-0.29	0.34	-0.09
OM	0.03	0.29	0.05	0.17	-0.43	-0.49	0.31	-0.29
Temperature	0.26	-0.17	0.44	0.51	0.28	-0.51	-0.17	-0.23

Conductivity reflects the status of major ions derived from inorganic pollution and is a measure of total dissolved solids and ionized species in the water column. Conductivity levels ranged between 57.10-60.70 mSi/cm at Izmir-Pasaport station. There were not found big differences at other sampling stations.

Table 5.3 Pearson correlation coefficients between physicochemical parameters and heavy metals in sediment samples (*Marked correlations are significant at $p < 0.05$*)

	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe
DO	-0.15	0.07	-0.08	-0.23	-0.14	-0.19	-0.05	0.12
Salinity	0.54	-0.11	-0.11	0.11	0.23	-0.01	0.01	0.12
Conductivity	0.43	-0.30	-0.25	0.10	0.30	0.02	-0.08	0.12
pH	0.07	0.29	0.29	0.23	0.33	0.21	0.04	-0.09
Temperature	0.01	-0.17	0.04	0.02	0.05	-0.11	0.09	0.23

The lowest salinity value was measured 37.3 psu in Izmir-Levent Marina in spring period while the highest value was recorded in Izmir-Pasaport (40.8 psu) in summer period. Seasonal salinity variations were very low at the other sampling stations. Salinity and conductivity values showed either increasing or decreasing trend with each other due to high correlation ($r=0.87$, $p<0.001$) between them.

DO concentration values at the stations varied from 3.20 to 9.40 mg l⁻¹. During the cold period dissolved oxygen (DO) concentrations were higher than the values measured at the other periods. In this period the highest DO level (9.4 mg l⁻¹) was observed at the Izmir-Pasaport where the significant decrease was determined for the

summer period. Moreover, temperature and DO concentrations showed a negative correlation ($r=-0.65$, $p<0.01$) with each other.

Table 5.4 Pearson correlation coefficients between physicochemical parameters and heavy metals in *A. amphitrite*. (Marked correlations are significant at $p<0.05$)

	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe
DO	-0.78	-0.09	-0.19	-0.25	-0.24	-0.30	-0.26	-0.27
Salinity	0.36	0.38	0.23	-0.43	0.30	0.23	0.10	0.01
Conductivity	0.22	0.37	-0.00	-0.33	0.31	0.35	0.02	-0.03
pH	-0.14	-0.23	-0.10	-0.37	-0.33	-0.21	0.10	-0.32
OM	0.00	0.19	-0.11	-0.51	-0.13	0.24	0.27	-0.33
Temperature	0.17	-0.01	0.18	0.31	0.07	-0.20	0.28	0.07

5.2 Heavy Metals in Sea Water

The results for the metal concentrations of water samples taken from the stations during the sampling periods were summarised in Table 5.5. Mercury concentrations presented with the highest values in the summer period and the maximum values in seawater samples were recorded in Izmir-Pasaport ($0.033 \mu\text{g l}^{-1}$) and Levent Marina ($0.032 \mu\text{g l}^{-1}$), while the lowest one ($0.0103 \mu\text{g l}^{-1}$) was obtained from Izmir-Levent Marina in autumn period. Mercury levels measured in all seasons have a similar distribution trend except summer data which caused statistically significant seasonal differences (Figure 5.1a).

The mean cadmium concentration in water showed slight fluctuation from a minimum mean value of $0.17 \mu\text{g l}^{-1}$ at Izmir-Levent Marina and maximum mean one $0.24 \mu\text{g l}^{-1}$ at Izmir-Pasaport station. The highest value was measured in Izmir-Pasaport ($0.30 \mu\text{g l}^{-1}$) in winter and the lowest value was determined in Kusadasi-Setur Marina ($0.083 \mu\text{g l}^{-1}$) in the same period where seasonal and spatial patterns were not evidenced clearly (Figure 5.1b).

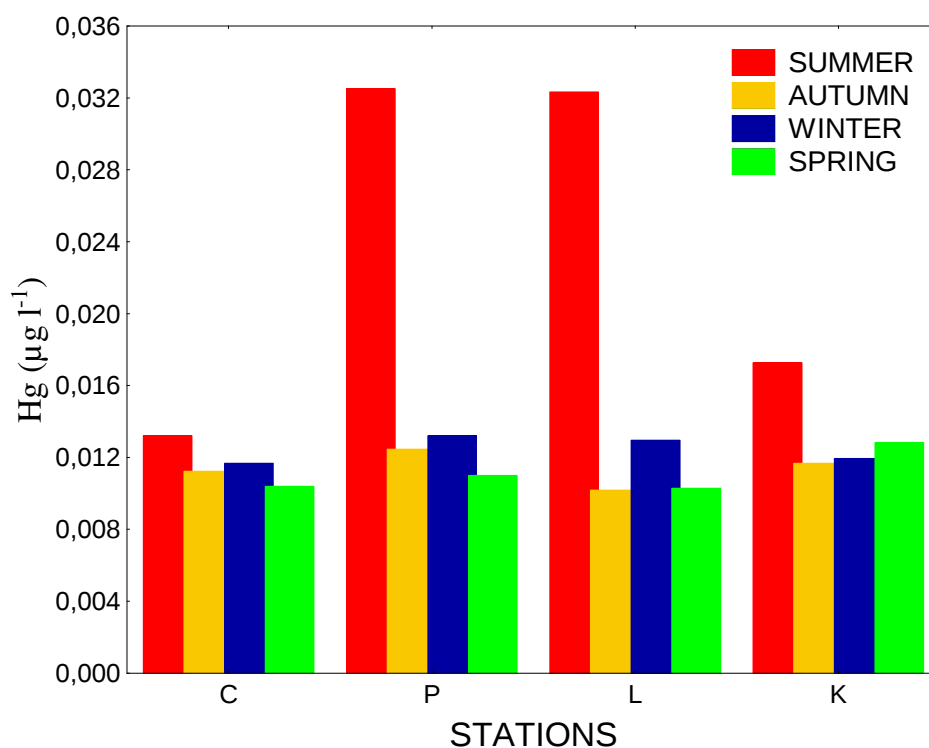


Figure 5.1a Mercury levels in seawater during the different sampling periods ($\mu g l^{-1}$).

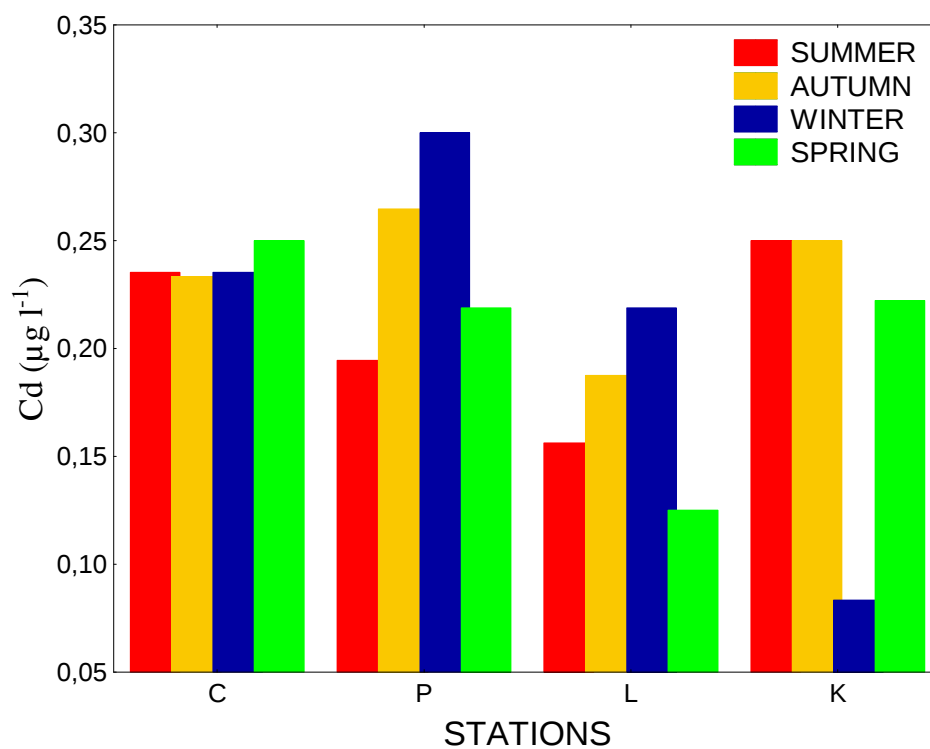


Figure 5.1b Cadmium levels in seawater during different sampling periods ($\mu g l^{-1}$).
(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Table 5.5 The mean $\pm$ SE and minimum-maximum concentrations of the heavy metals in seawater during sampling periods ($\mu\text{g l}^{-1}$)

	Candarli	Izmir-Pasaport	Izmir-Levent M.	Kusadasi-Setur M.
Hg	0.012 $\pm$ 0.001 0.010-0.013	0.017 $\pm$ 0.005 0.011-0.033	0.016 $\pm$ 0.005 0.010-0.032	0.013 $\pm$ 0.002 0.012-0.017
Cd	0.24 $\pm$ 0.004 0.23-0.25	0.24 $\pm$ 0.020 0.19-0.30	0.17 $\pm$ 0.040 0.13-0.22	0.20 $\pm$ 0.040 0.083-0.25
Pb	1.5 $\pm$ 0.36 1.0-2.5	1.4 $\pm$ 0.29 0.91-2.2	1.1 $\pm$ 0.29 0.66-1.9	1.8 $\pm$ 0.85 0.53-4.3
Cr	1.2 $\pm$ 0.71 0.37-3.3	0.98 $\pm$ 0.43 0.44-2.4	0.77 $\pm$ 0.48 0.25-2.1	1.0 $\pm$ 0.70 0.19-3.1
Cu	3.0 $\pm$ 0.85 1.0-4.9	0.83 $\pm$ 0.36 0.50-1.2	1.4 $\pm$ 0.15 0.84-2.4	4.9 $\pm$ 1.45 2.2-8.1
Zn	3.7 $\pm$ 0.88 2.2-6.0	4.8 $\pm$ 2.24 1.6-8.2	5.4 $\pm$ 1.61 1.8-11	8.5 $\pm$ 1.25 5.9-12
Mn	4.5 $\pm$ 1.80 2.0-9.7	2.4 $\pm$ 0.29 1.4-3.7	2.2 $\pm$ 0.47 1.8-3.0	1.4 $\pm$ 0.38 0.56-2.2
Fe	6.9 $\pm$ 1.61 3.3-9.8	8.9 $\pm$ 2.60 3.9-16	17.3 $\pm$ 6.31 4.6-35	21.5 $\pm$ 8.90 7.1-45

The highest value of lead ($4.3 \mu\text{g l}^{-1}$) and chromium ($3.3 \mu\text{g l}^{-1}$) were measured in Kusadasi-Setur Marina and Candarli, respectively and both of them recorded in the summer period whereas the lowest value of Pb ($0.53 \mu\text{g l}^{-1}$) and Cr ($0.19 \mu\text{g l}^{-1}$) were determined in Kusadasi-Setur Marina in the winter period (Figure 5.1c-d). Concerning Pb, the highest mean level was found at Kusadasi-Setur Marina station ($1.8 \mu\text{g l}^{-1}$) and the lowest mean was measured ($1.1 \mu\text{g l}^{-1}$) in Izmir-Levent Marina. Moreover, the highest mean level for chromium was obtained from Candarli station ($1.2 \mu\text{g l}^{-1}$) and lowest mean concentration was measured at Izmir-Levent Marina ($0.77 \mu\text{g l}^{-1}$). Seasonal differences in the concentrations of two metals were significant (Table 5.7).

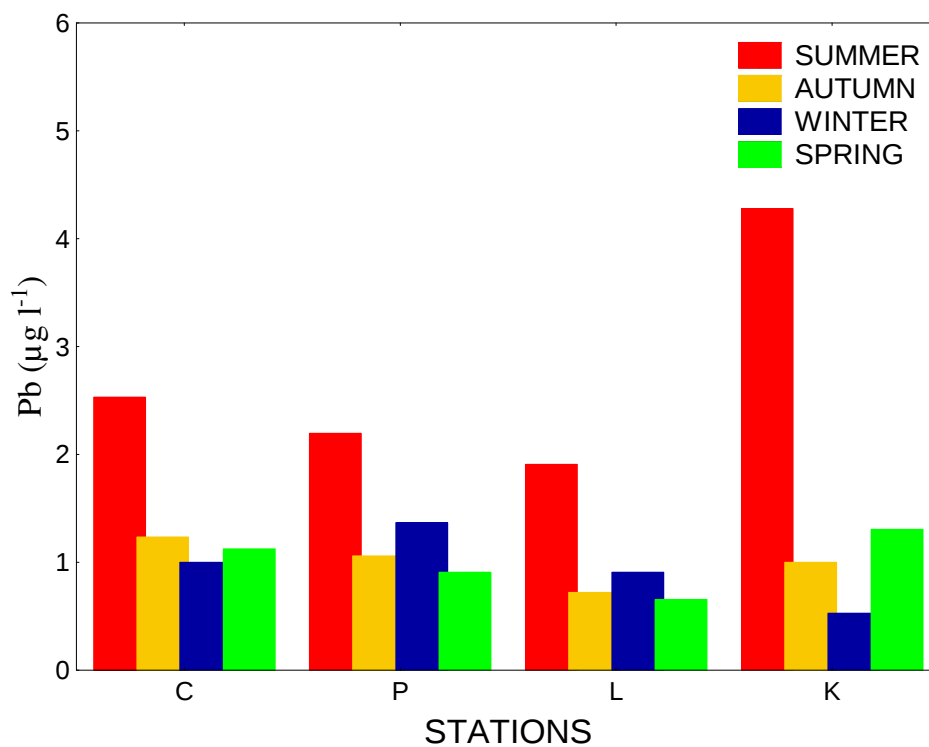


Figure 5.1c Lead levels in seawater during different sampling periods (µg l⁻¹).

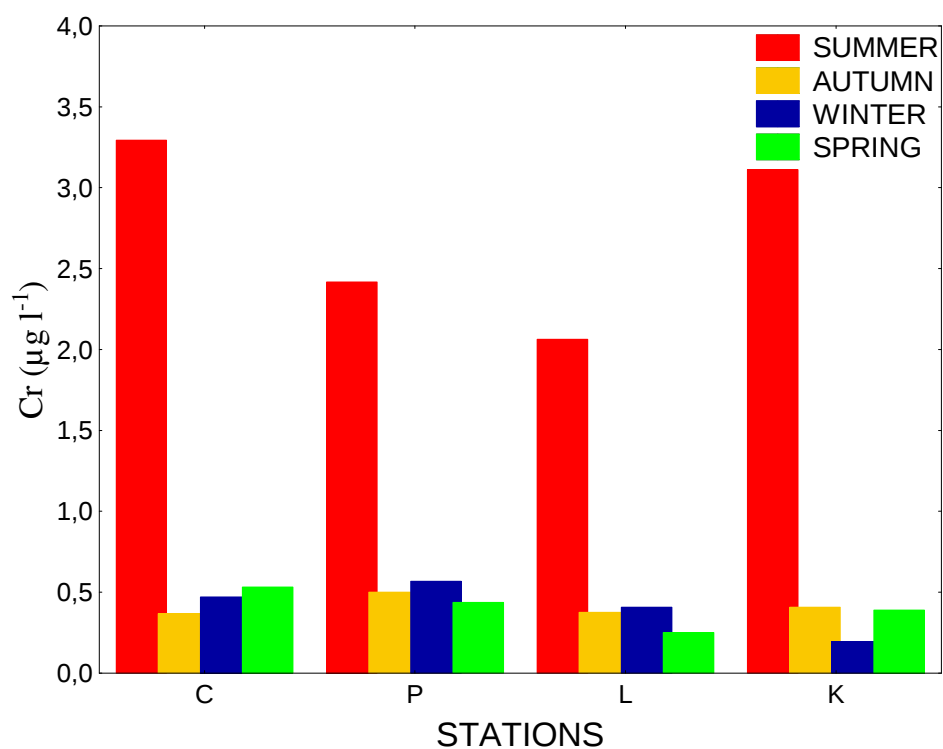


Figure 5.1d Chromium levels in seawater during different sampling periods (µg l⁻¹).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

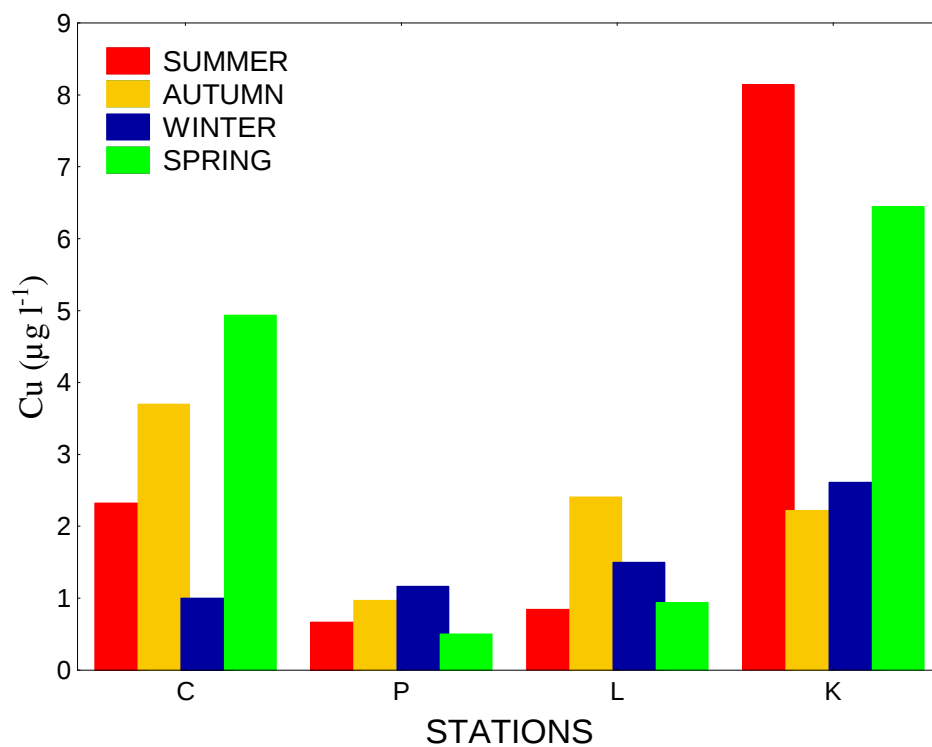


Figure 5.1e Copper levels in seawater during different sampling periods ($\mu\text{g l}^{-1}$).

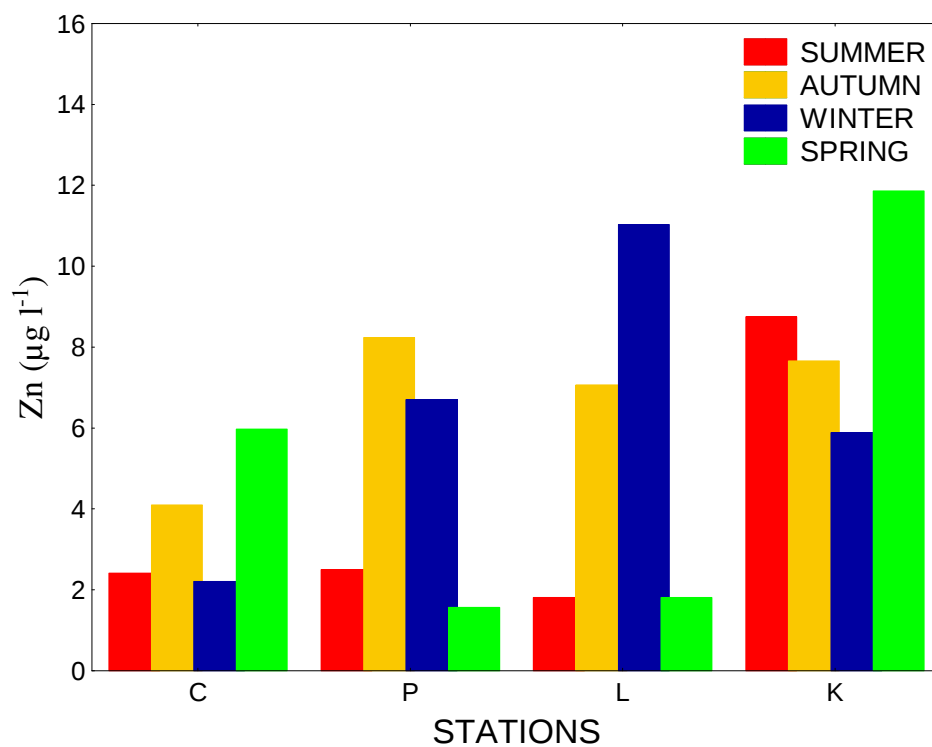


Figure 5.1f Zinc levels in seawater during different sampling periods ($\mu\text{g l}^{-1}$).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

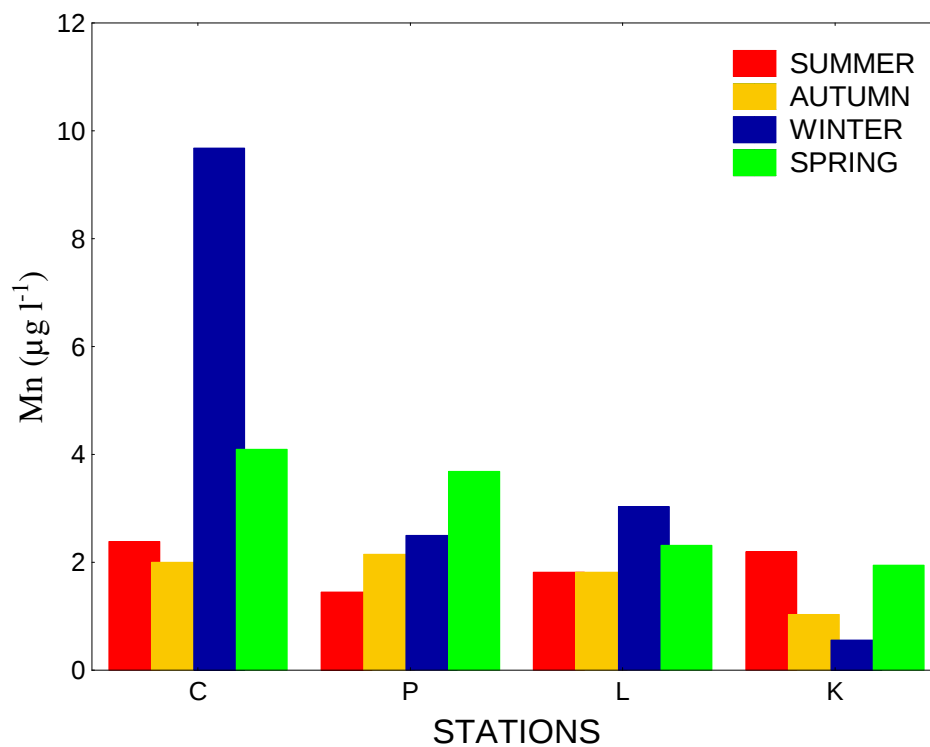


Figure 5.1g Manganese levels in seawater during different sampling periods ($\mu\text{g l}^{-1}$).

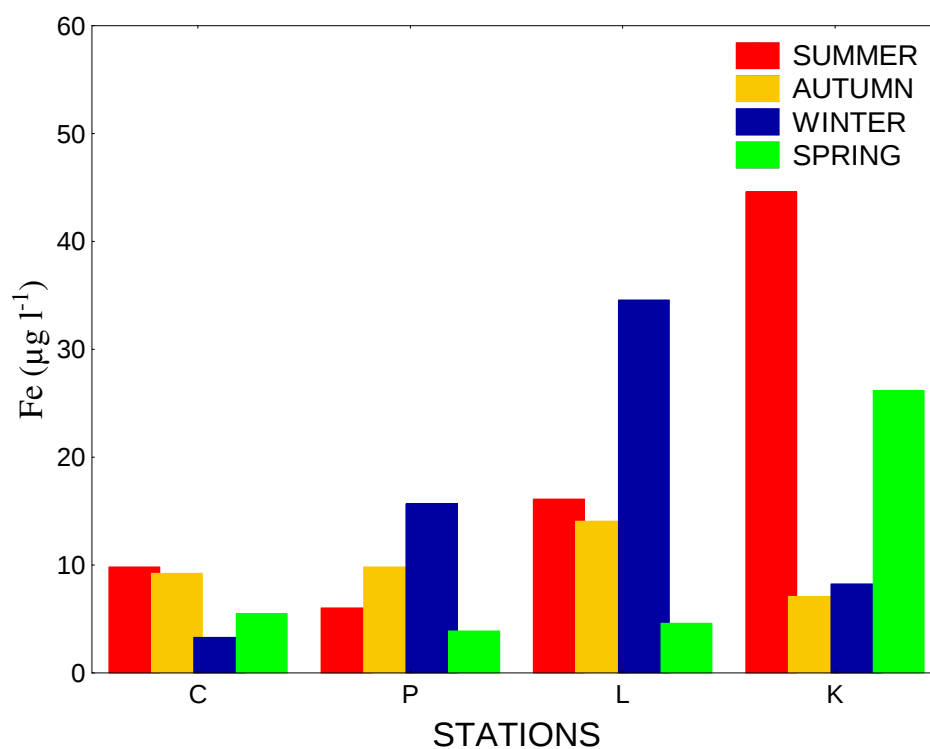


Figure 5.1h Iron levels in seawater during different sampling periods ($\mu\text{g l}^{-1}$).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

The copper concentrations in water indicated important variations among stations and the highest Cu value was determined in Kusadasi-Setur Marina where the maximum concentration ($8.14 \mu\text{g l}^{-1}$) was found in the summer period (Figure 5.1e). The lowest mean concentration of copper was recorded at Izmir-Pasaport station ($0.83 \mu\text{g l}^{-1}$) while the highest mean was found in Kusadasi-Setur Marina ($4.85 \mu\text{g l}^{-1}$). In addition to this, statistically significant temporal pattern was not observed.

The highest ($8.5 \mu\text{g l}^{-1}$) and the lowest ($3.7 \mu\text{g l}^{-1}$) mean value for zinc was determined at Kusadasi-Setur Marina and Candarli, respectively. The minimum Zn value was detected in Izmir-Pasaport ($1.6 \mu\text{g l}^{-1}$) and maximum level ($12 \mu\text{g l}^{-1}$) was measured in Kusadasi-Setur Marina, both of them were recorded in spring period (Figure 5.1f). During sampling periods, manganese concentration peaked only one time in winter at Candarli ($9.7 \mu\text{g l}^{-1}$) station and minimum level was found in Kusadasi-Setur Marina ($0.56 \mu\text{g l}^{-1}$). Concentrations of Mn reached the highest mean level in Candarli ($4.5 \mu\text{g l}^{-1}$) and the lowest mean ($1.4 \mu\text{g l}^{-1}$) in Kusadasi-Setur Marina. On the other hand, no significant temporal and spatial variations were found along the eastern Aegean coast.

Fe concentrations showed the highest mean value in Kusadasi-Setur Marina ($21.5 \mu\text{g l}^{-1}$) and the lowest in Candarli ($6.9 \mu\text{g l}^{-1}$). Furthermore, the presence of this metal in seawater was recorded minimum in Candarli ($3.3 \mu\text{g l}^{-1}$) in winter and maximum ($45 \mu\text{g l}^{-1}$) in summer where no significant difference was found. Concerning zinc (Figure 5.1f) and iron (Figure 5.1h), their concentrations showed an obvious increase in the selected stations from northern to southern part of the Aegean Sea.

Generally, the highest metal concentrations in different seasons were determined in Kusadasi-Setur Marina, Candarli and Izmir-Pasaport where the source of metals could be derived from not only marinas but also industrial or domestic pollution.

The mean metal concentrations in seawater generally decreased in the following order: Fe>Zn>Mn>Cu>Pb>Cr>Cd>Hg in the Aegean coast (Table 5.5). The orders were different for Candarli (Fe>Mn>Zn>Cu>Cr>Pb>Cd>Hg), Izmir-Pasaport (Fe>Zn>Mn>Cr>Pb>Cu>Cd>Hg), Izmir-Levent Marina (Fe>Zn>Mn>Cu>Cr>Pb>Cd>Hg) and Kusadasi-Setur Marina (Fe>Zn>Cu>Pb>Cr>Mn>Cd>Hg).

Table 5.6 Spearman rank-order correlation coefficients for relationship between heavy metals in seawaters (*Marked correlations are significant at $p<0.05$*)

Variable	Hg							
Hg	1.000	Cd						
Cd	0.199	1.000	Pb					
Pb	0.694	0.507	1.000	Cr				
Cr	0.647	0.569	0.790	1.000	Cu			
Cu	-0.041	0.260	0.274	-0.048	1.000	Mn		
Mn	0.225	0.363	0.098	-0.019	0.565	1.000	Zn	
Zn	-0.154	0.371	0.020	0.325	-0.121	-0.166	1.000	Fe
Fe	0.582	0.099	0.395	0.179	0.355	0.693	-0.287	1.000

Spearman's rank correlation analysis was performed to evaluate the relationship between metal concentrations in seawater (Table 5.6). This analysis demonstrated a positive correlation between some metal pairs: Pb with Hg, Cd, Cr and Cr with Hg, Cd and Mn with Cu, Fe and Hg with Fe.

Table 5.7 Values of main effects ANOVA in water column

Water Column	Season			Station		
	<i>df</i>	<i>F</i>	<i>p level</i>	<i>df</i>	<i>F</i>	<i>p level</i>
Hg	3	6.920	*	3	0.911	ns
Cd	3	0.238	ns	3	1.019	ns
Pb	3	9.845	**	3	1.090	ns
Cr	3	40.920	***	3	1.457	ns
Cu	3	0.248	ns	3	5.505	*
Zn	3	1.046	ns	3	1.473	ns
Mn	3	0.829	ns	3	2.774	ns
Fe	3	0.518	ns	3	1.449	ns

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

The main effects ANOVA results revealed that the temporal variations of metal concentrations in seawater were significant for Hg, Pb and Cr concentrations, while no significant spatial variations were observed for all studied metals except Cu (Table 5.7).

5.3 Heavy Metals in Sediment

The mean concentrations and maximum-minimum values (as $\mu\text{g g}^{-1}$ dry weight) of heavy metals in sediment samples collected from the stations during four seasonal periods are given in Table 5.8.

The highest Hg level was found in sediment samples collected from Izmir-Levent Marina ($4.3 \mu\text{g g}^{-1}$) in autumn period; whereas the lowest concentration was measured at Kusadasi-Setur Marina ($0.22 \mu\text{g g}^{-1}$) in spring (Figure 5.2a). On a seasonal scale, mean values of Hg did not show significant variation (Table 5.10). However, spatial variation was important and the highest mercury level which was observed at Levent Marina was approximately 20 times higher than the lowest value recorded at Kusadasi-Setur Marina. According to mean Hg levels in sediment samples, higher mean values were obtained from Izmir-Pasaport ($2.4 \mu\text{g g}^{-1}$) and Izmir-Levent Marina ($2.0 \mu\text{g g}^{-1}$) and the lowest mean concentration was recorded in Kusadasi-Setur Marina ($0.37 \mu\text{g g}^{-1}$).

The obtained data from the Izmir-Pasaport where the highest mean value of Cd of ($1.9 \mu\text{g g}^{-1}$) was recorded represented with maximum values in all seasons within stations whereas the lowest Cd concentrations over the four sites was found ($0.11 \mu\text{g g}^{-1}$) at Kusadasi-Setur Marina station (Figure 5.2b). Nonetheless, maximum Cd level was detected in Izmir-Pasaport ($2.3 \mu\text{g g}^{-1}$) in winter period while minimum level recorded in Kusadasi-Setur Marina ($0.084 \mu\text{g g}^{-1}$) in spring. Apart from Izmir-Pasaport, all stations showed a similar distribution trend with low concentration of Cd. Concentrations of cadmium showed marked variations among stations.

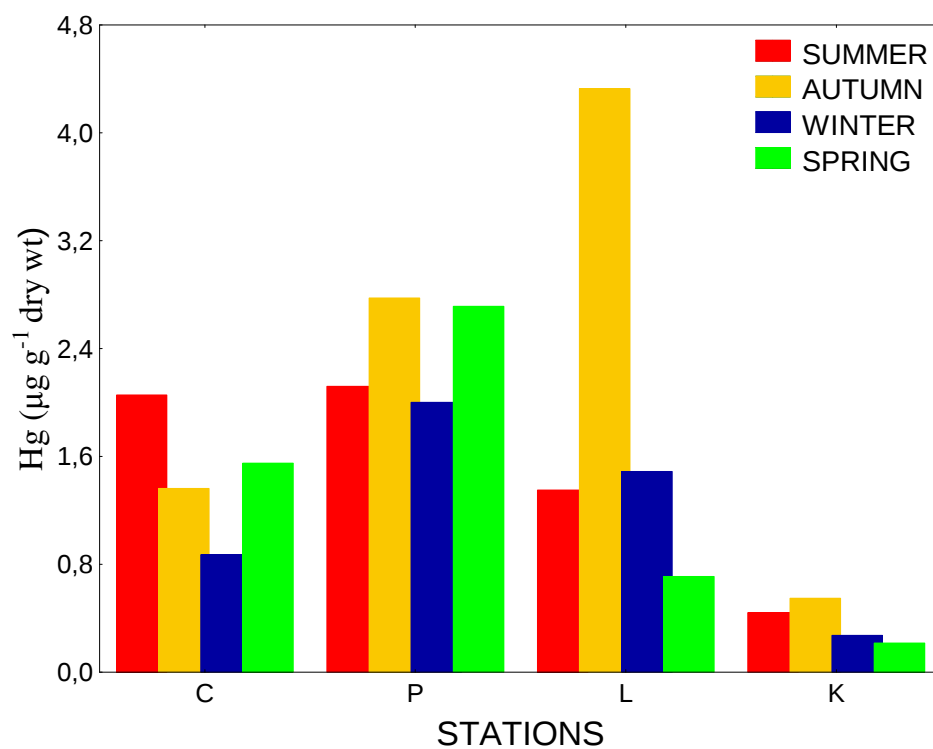


Figure 5.2a Mercury levels in sediment during sampling periods (µg g⁻¹).

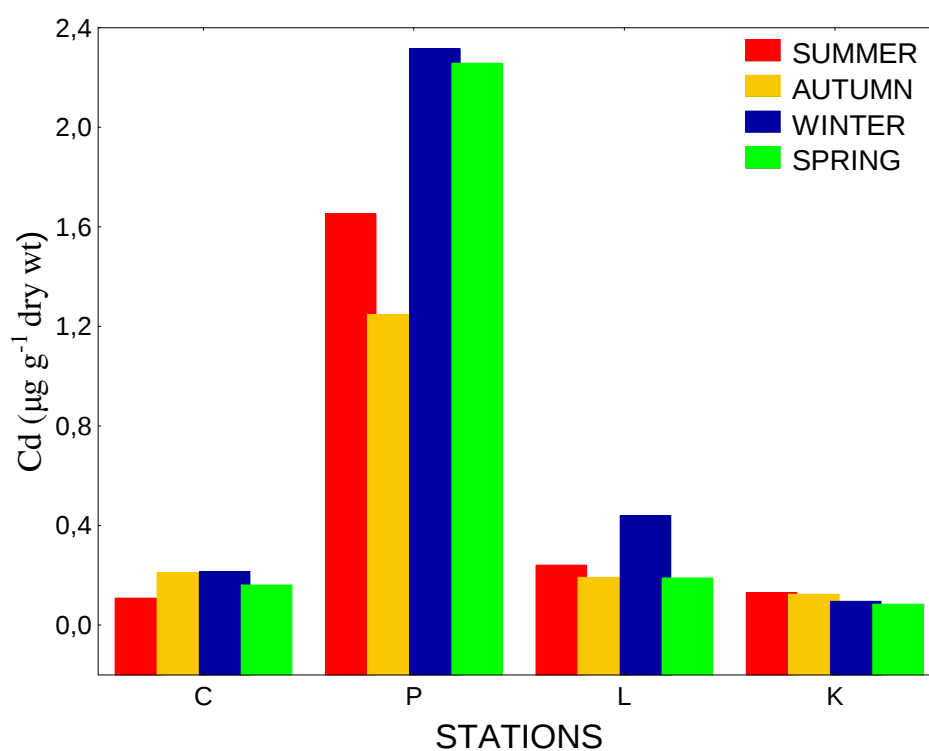


Figure 5.2b Cadmium levels in sediment during sampling periods (µg g⁻¹).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Table 5.8 The mean $\pm$ SE and minimum-maximum concentrations of the heavy metals in sediments during sampling periods ($\mu\text{g g}^{-1}$ dry weight)

	Candarli	Izmir-Pasaport	Izmir-Levent M.	Kusadasi-Setur M.
Hg	1.5 $\pm$ 0.25 0.87-2.1	2.4 $\pm$ 0.20 2.0-2.8	2.0 $\pm$ 0.80 0.71-4.3	0.37 $\pm$ 0.08 0.22-0.55
Cd	0.17 $\pm$ 0.025 0.11-0.22	1.9 $\pm$ 0.26 1.2-2.3	0.27 $\pm$ 0.059 0.19-0.44	0.11 $\pm$ 0.010 0.084-0.13
Pb	85 $\pm$ 14 44-104	335 $\pm$ 78 252-570	89 $\pm$ 7.0 68-100	29 $\pm$ 1.4 26-32
Cr	75 $\pm$ 3.3 69-84	164 $\pm$ 9.2 146-190	116 $\pm$ 1.6 114-120	38 $\pm$ 5.8 23-52
Cu	256 $\pm$ 25 189-308	279 $\pm$ 22 239-343	272 $\pm$ 20 219-316	59 $\pm$ 11.6 28-85
Zn	199 $\pm$ 8.8 181-215	620 $\pm$ 28 557-681	336 $\pm$ 27 289-387	84 $\pm$ 19 48-117
Mn	284 $\pm$ 18 256-335	392 $\pm$ 6.2 374-400	362 $\pm$ 4.7 352-375	354 $\pm$ 21 295-390
Fe	26149 $\pm$ 2859 18074-31253	27875 $\pm$ 1635 25038-31861	31246 $\pm$ 631 29645-32550	9953 $\pm$ 1461 6537-12864

The lead concentration of sediment samples collected from Izmir-Pasaport represented with higher values in all seasons with regarding to other stations. In that case, Izmir-Pasaport shows apparent spatial patterns for the sediment samples. The mean lead value was extremely higher at Izmir-Pasaport station ($335 \mu\text{g g}^{-1}$) where the value approximately 10 times greater than the lowest mean value found at Kusadasi-Setur Marina ($29 \mu\text{g g}^{-1}$) (Figure 5.2c). The maximum lead value was measured in spring period in Izmir-Pasaport as $570 \mu\text{g g}^{-1}$ while the minimum lead level was found in Kusadasi-Setur Marina as $26 \mu\text{g g}^{-1}$ in winter.

Chromium concentrations reached the highest concentration in Izmir-Pasaport ($190 \mu\text{g g}^{-1}$) in spring while the lowest level of Pb found in Kusadasi-Setur Marina ($23 \mu\text{g g}^{-1}$) in the same period (Figure 5.2.d). Furthermore, chromium levels showed significant spatial variations. The higher mean values were measured at two stations from Izmir-Pasaport ($164 \mu\text{g g}^{-1}$) and Izmir-Levent Marina ($116 \mu\text{g g}^{-1}$) located in Izmir Inner Bay, while the lowest mean chromium value was measured at Kusadasi-Setur Marina ($38 \mu\text{g g}^{-1}$).

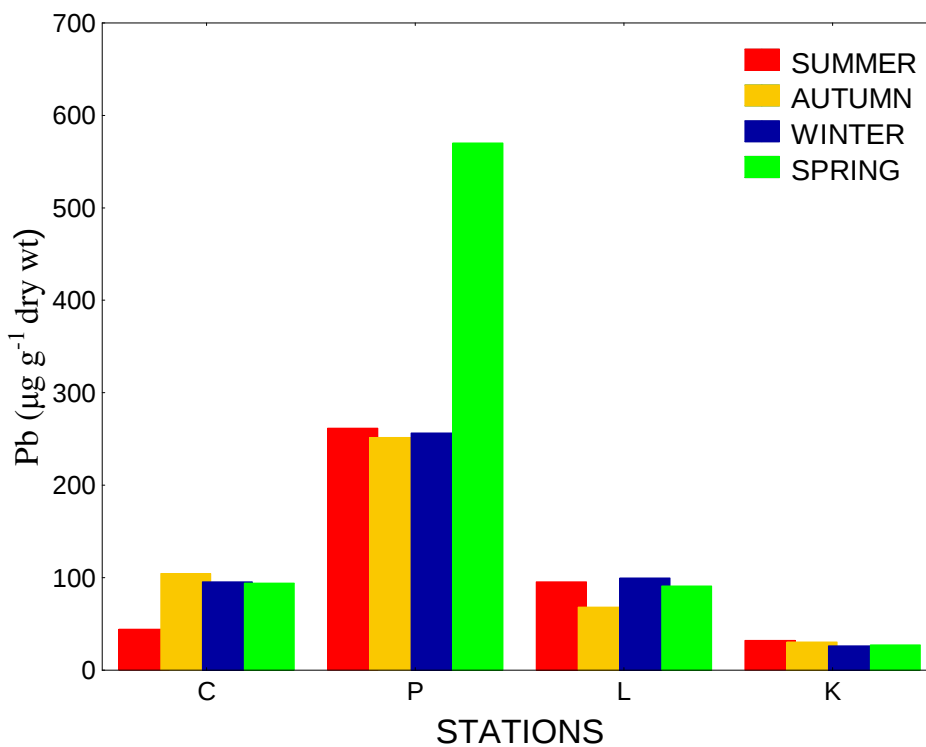


Figure 5.2c Lead levels in sediment during sampling periods ($\mu\text{g g}^{-1}$).

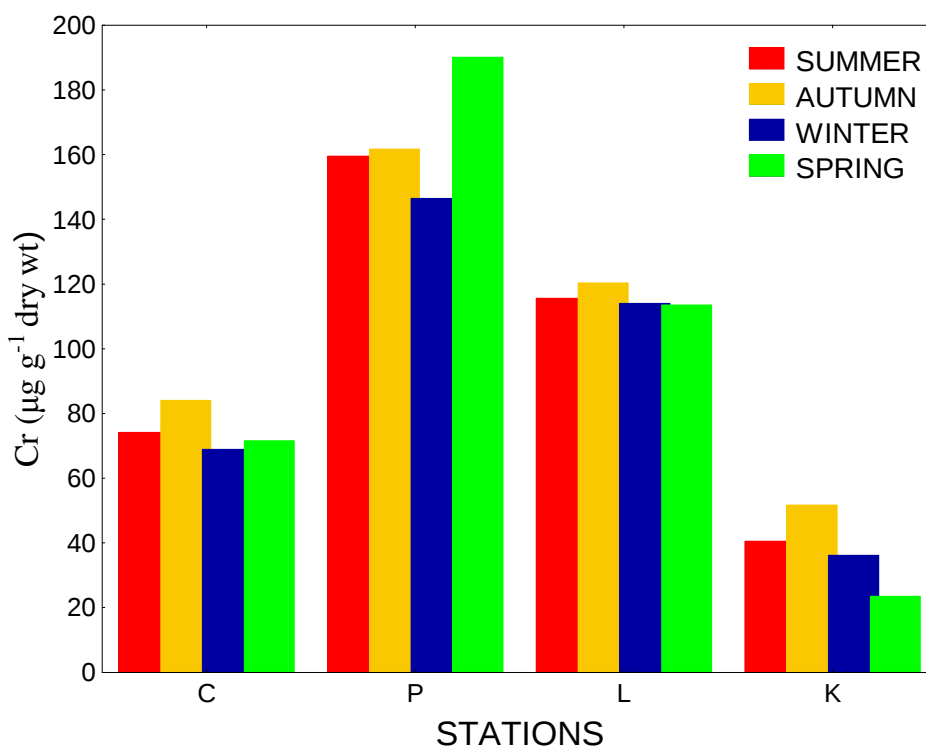


Figure 5.2d Chromium levels in sediment during sampling periods ($\mu\text{g g}^{-1}$).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Mean levels of copper in sediment samples were nearly similar at all stations except Kusadasi-Setur Marina where maxima and minima concentrations were recorded in autumn ($85 \mu\text{g g}^{-1}$) and in winter ($28.27 \mu\text{g g}^{-1}$), respectively (Figure 5.2e). There were no clear temporal patterns for Cu concentration in sediment samples, furthermore, a significant spatial difference was observed. Moreover, higher average values were obtained from Izmir-Pasaort ($279 \mu\text{g g}^{-1}$), Izmir-Levent Marina ($272 \mu\text{g g}^{-1}$) and Candarli ($256 \mu\text{g g}^{-1}$), whereas the lowest level was measured in Kusadasi-Setur Marina ($59 \mu\text{g g}^{-1}$).

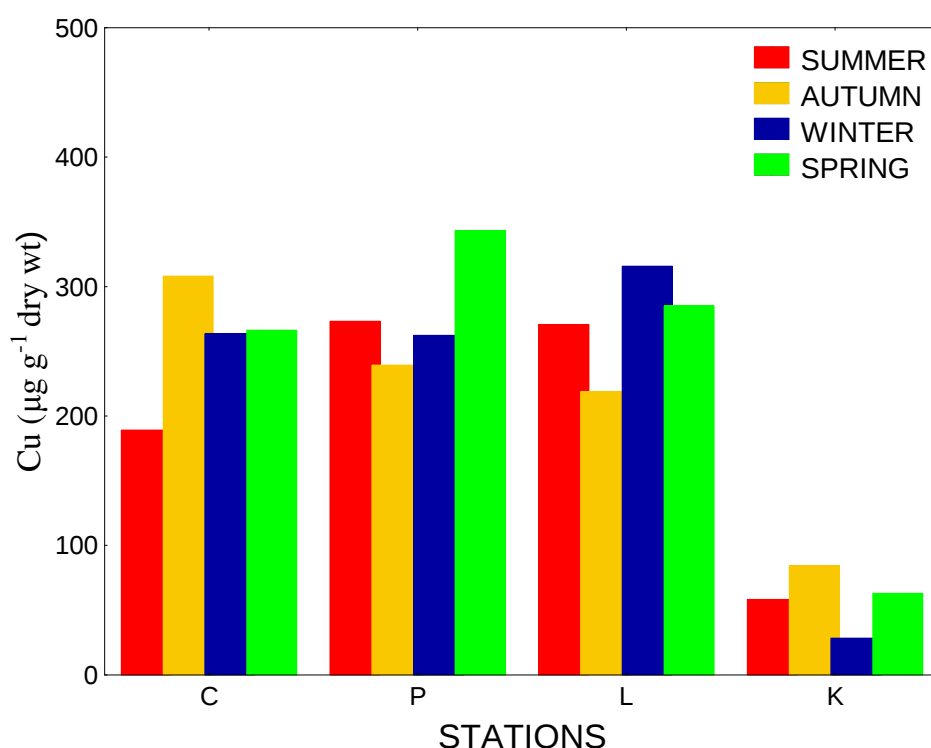


Figure 5.2e Copper levels in sediment during sampling periods ($\mu\text{g g}^{-1}$).

(C: Candarli, P: Izmir-Pasaort, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

The Zn values ($681 \mu\text{g g}^{-1}$) in summer were found higher at Izmir-Pasaort corresponding to the areas closest to the main urban area (Figure 5.2f). The concentration of zinc in the sediments collected from the Kusadasi-Setur Marina was particularly low and the lowest value ($48.20 \mu\text{g g}^{-1}$) was determined in spring period compared with other sampling stations (Figure 5.2f).

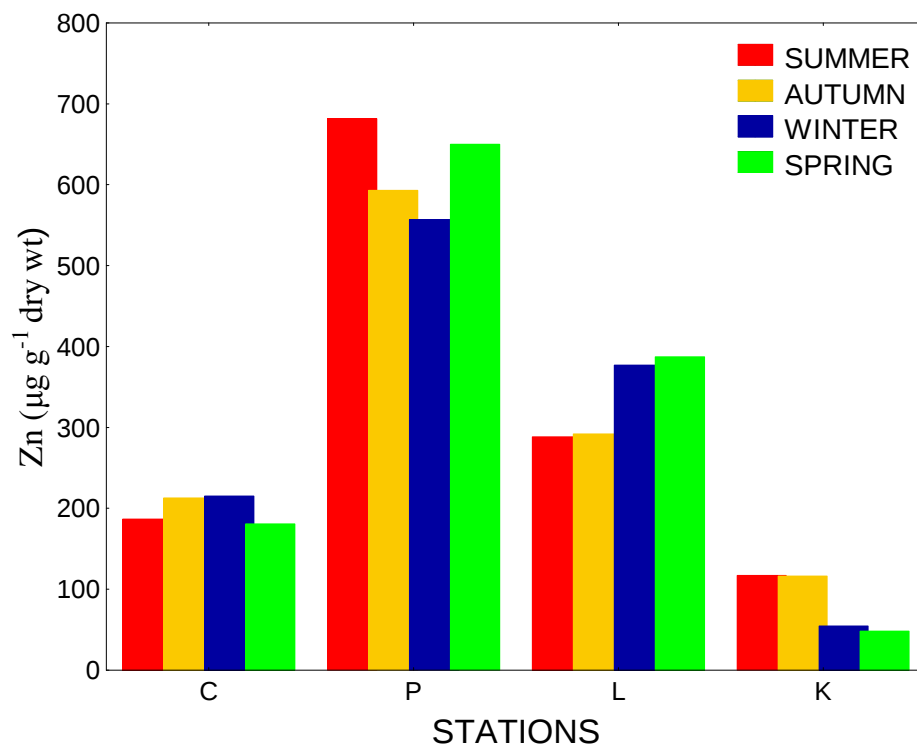


Figure 5.2f Zinc levels in sediment during sampling periods (µg g⁻¹).

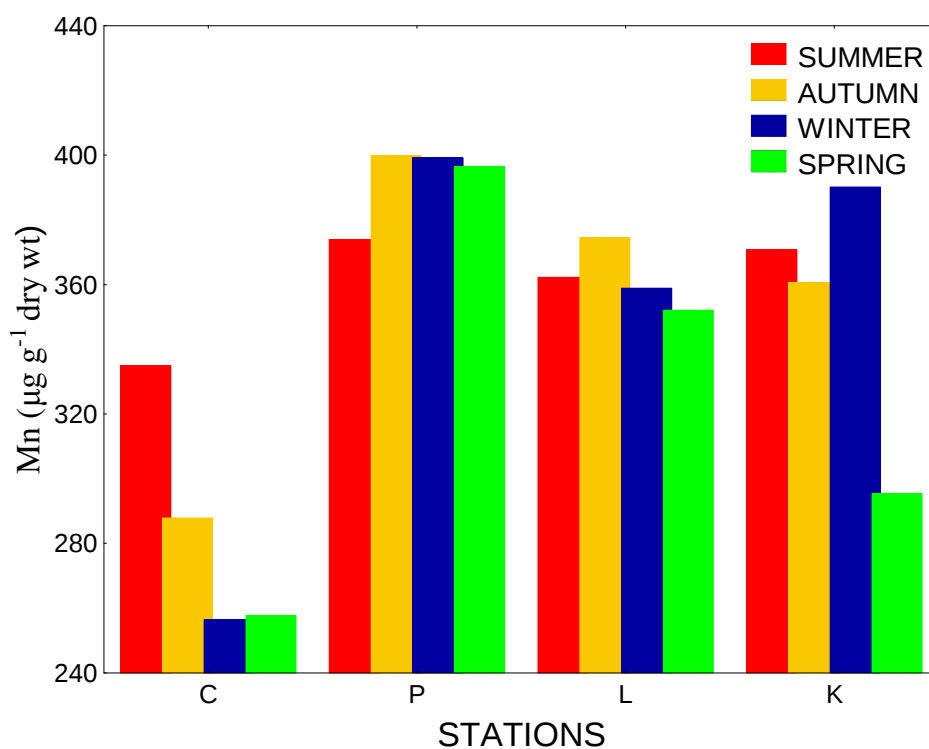


Figure 5.2g Manganese levels in sediment during sampling periods (µg g⁻¹).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

As a result, the highest mean value was obtained from Izmir-Pasaport ($620 \mu\text{g g}^{-1}$) and the lowest mean value was measured at Kusadasi station ($84 \mu\text{g g}^{-1}$). The presence of Zn showed significant variations among stations and the main source of the variation derived from the values measured in Izmir-Pasaport.

Mn concentrations ranged from $256 \mu\text{g g}^{-1}$ at Candarli in winter to $400 \mu\text{g g}^{-1}$ at Izmir-Pasaport station in autumn. The highest mean value was determined at Izmir-Pasaport ($392 \mu\text{g g}^{-1}$) and the lowest in Candarli $284 \mu\text{g g}^{-1}$ (Figure 5.2g). However, the values measured in Candarli were relatively lower than the other stations. There was a significant spatial difference among stations; moreover, no significant temporal difference was determined among sampling periods.

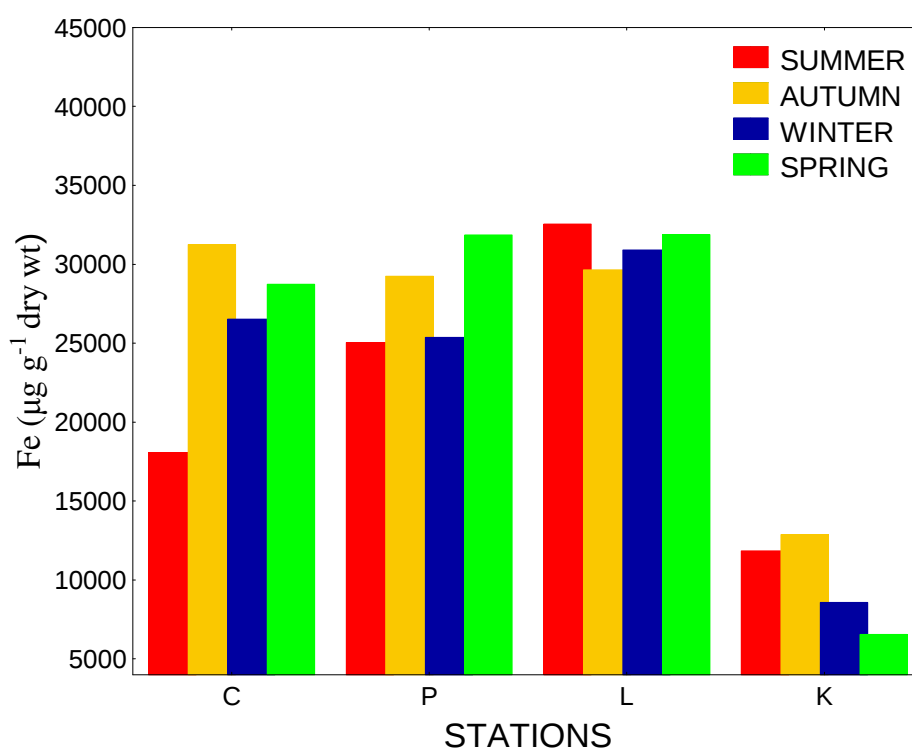


Figure 5.2h Iron levels in sediment during sampling periods ($\mu\text{g g}^{-1}$).

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Concentrations of Fe in sediment samples showed marked changes at all stations. In contrast to this result, iron levels did not show temporal differences. The maximum and minimum iron concentrations were obtained in summer ($32550 \mu\text{g g}^{-1}$) and spring ($6537 \mu\text{g g}^{-1}$) periods at Izmir-Levent Marina and

Kusadasi-Setur Marina, respectively (Figure 5.2h). In addition to this, the highest mean value was obtained from Izmir-Levent Marina ($31246 \mu\text{g g}^{-1}$) while the lowest mean was measured in Kusadasi-Setur Marina ($15703 \mu\text{g g}^{-1}$).

The order of mean metal concentrations in sediments recorded in this study decreased in the following order $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cr} > \text{Hg} > \text{Cd}$ for Aegean Sea. The orders were different in Candarli ($\text{Fe} > \text{Mn} > \text{Cu} > \text{Zn} > \text{Pb} > \text{Cr} > \text{Hg} > \text{Cd}$) while Izmir-Pasaport the sequence is $\text{Fe} > \text{Zn} > \text{Mn} > \text{Pb} > \text{Cu} > \text{Cr} > \text{Hg} > \text{Cd}$. The following sequences were observed $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Pb} > \text{Hg} > \text{Cd}$ and $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Pb} > \text{Hg} > \text{Cd}$ for Izmir-Levent Marina and Kusadasi-Setur Marina, respectively. In generally the order of metals in sediment samples were not similar to the sequences obtained from sea water with the exception of Fe, Pb, Cu and Cr.

Spearman rank-order correlation analysis ($p < 0.05$) demonstrated that positive correlation existed between some metal concentrations in sediment. According to results, Hg with Cd, Pb, Cr, Zn; Cd with Pb, Cr, Cu, Zn, Fe; Pb with Cr, Cu, Zn, Fe; Cr with Cu, Mn, Zn, Fe; Cu with Zn, Fe and Zn with Fe showed positive correlations in the sediment (Table 5.9). The most strong correlation was determined between lead and cadmium ($r = 0.947$). Zinc showed significant correlations with chromium ($r = 0.944$), cadmium ($r = 0.900$) and lead ($r = 0.874$). Chromium also showed marked correlations with cadmium ($r = 0.877$), mercury ($r = 0.853$) and lead ($r = 0.847$). In the sediment samples the relationship between iron and copper was found considerably high ($r = 0.821$) in the study area. Significant correlations were observed between organic matter and heavy metals except Mn, Fe (Table 5.9).

Table 5.9 Spearman rank-order correlation coefficients for relationship between heavy metals in sediment samples (*Marked correlations are significant at $p < 0.05$*)

Variable	Hg	Cd	Pb	Cr	Cu	Mn	Zn	Fe	OM	Gravel	Sand	Silt	Clay
Hg	1.00												
Cd	0.64	1.00											
Pb	0.67	0.95	1.00										
Cr	0.85	0.88	0.85	1.00									
Cu	0.42	0.69	0.78	0.64	1.00								
Mn	0.41	0.45	0.28	0.57	-0.12	1.00							
Zn	0.74	0.90	0.87	0.94	0.69	0.48	1.00						
Fe	0.44	0.57	0.57	0.64	0.82	0.01	0.61	1.00					
OM	0.66	0.56	0.70	0.69	0.55	0.16	0.72	0.45	1.00				
Gravel	-0.32	0.10	0.20	0.13	0.39	-0.51	0.14	0.51	0.37	1.00			
Sand	0.04	-0.12	-0.02	-0.15	0.23	-0.67	-0.16	0.37	0.09	0.85	1.00		
Silt	0.08	0.16	-0.06	0.26	0.09	0.48	0.18	0.27	-0.28	-0.40	-0.44	1.00	
Clay	-0.14	-0.05	-0.02	-0.04	-0.38	0.39	0.01	-0.57	0.11	-0.71	-0.73	-0.21	1.00

Table 5.10 Values of main effects of ANOVA in sediment

Sediment	Season			Station		
	df	F	p level	df	F	p level
Hg	3	2.26	ns	3	16.45	***
Cd	3	1.06	ns	3	65.01	***
Pb	3	0.81	ns	3	40.56	***
Cr	3	1.11	ns	3	53.47	***
Cu	3	0.60	ns	3	29.71	***
Zn	3	0.40	ns	3	38.77	***
Mn	3	1.36	ns	3	10.64	**
Fe	3	0.57	ns	3	24.67	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns: non significant

Main effect ANOVA was applied on log transformed metal data (Table 5.10). According to main effect ANOVA results the metals studied in the sediment did not show any significant temporal changes. However, all metal levels at the sampling sites showed marked variations.

5.4 Heavy Metals in Barnacles

Mean and minimum-maximum concentrations (as $\mu\text{g g}^{-1}$ dry weight, dry wt) for eight metals in soft tissue of *A. amphitrite* were given in Table 5.11. Hg showed slight fluctuations from a minimum value of $0.02 \mu\text{g g}^{-1}$ in Izmir-Levent Marina and maximum one $0.76 \mu\text{g g}^{-1}$ in Candarli in autumn where represented with higher mean mercury level with regard to other sampling stations. In this station seasonal differences can be seen between autumn and winter periods (Figure 5.3a). Hg showed both seasonal and spatial variations during sampling periods. (Table 5.12).

The concentrations of cadmium in *A. amphitrite* varied among the sampling sites ($p < 0.001$) with the lowest to the highest measured values being $5.0\text{-}20.0 \mu\text{g g}^{-1}$ in Izmir-Pasaport to Candarli in winter and spring periods, respectively (Figures 5.3b).

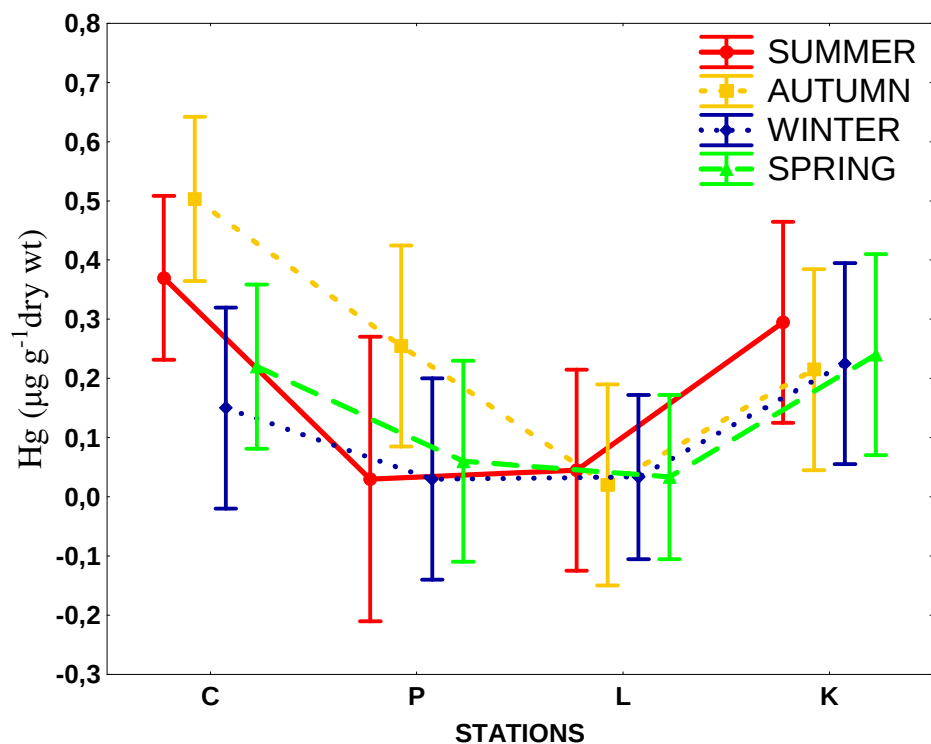


Figure 5.3a Mercury concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrite*.

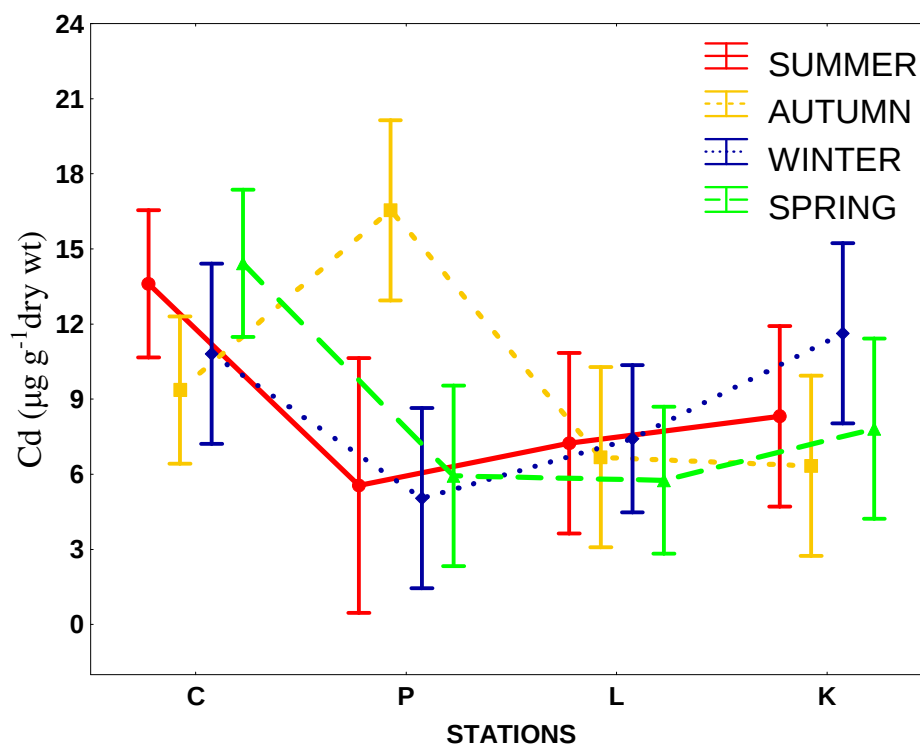


Figure 5.3b Cadmium concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrite*.

(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

The highest mean concentration of Cd in *A. amphitrite* was mainly recorded in Candarli (12.5 $\mu\text{g g}^{-1}$) from the Northern Aegean Sea during the sampling period; significantly the lowest mean value was obtained from Levent Marina (6.7 $\mu\text{g g}^{-1}$) in polluted Izmir Bay. Seasonal variation was only important in Izmir-Pasaport where the highest mean value was recorded in autumn period (16.54 $\mu\text{g g}^{-1}$) (Figure 5.3b).

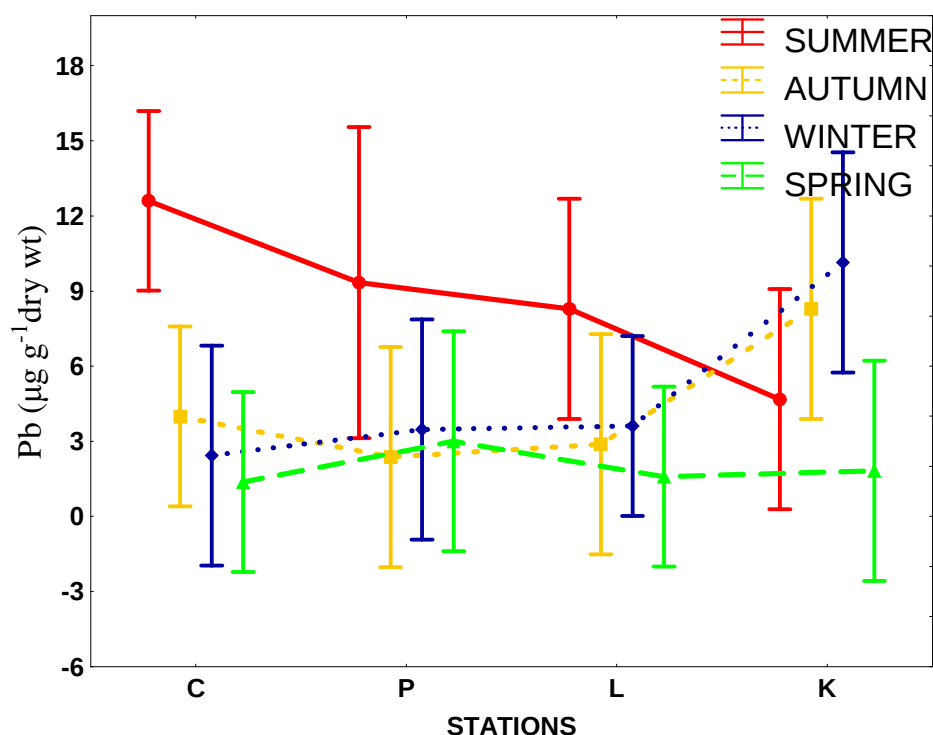


Figure 5.3c Lead concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrite*. (C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

A. amphitrite showed a wide range of Pb concentrations with significant differences between seasons ($p < 0.001$). Lead levels in organisms ranged between 19.6 $\mu\text{g g}^{-1}$ at Candarli in summer and 0.97 $\mu\text{g g}^{-1}$ at Kusadasi-Setur Marina in spring. At all stations, lead concentration was recorded higher in summer period except Kusadasi-Setur Marina where mean Pb concentration reached its highest value in winter (Figures 5.3c). Furthermore, the highest mean value was found as 6.3 $\mu\text{g g}^{-1}$ in Kusadasi-Setur Marina and the lowest mean was detected as 3.67 $\mu\text{g g}^{-1}$ in Izmir-Pasaport and 3.7 $\mu\text{g g}^{-1}$ in Izmir-Levent Marina.

Table 5.11 The mean $\pm$ SE and minimum-maximum concentrations of the heavy metals in *Amphibalanus amphitrite* during sampling periods ($\mu\text{g g}^{-1}$ dry weight)

Region/Site	Length (mm)	Hg	Cd	Pb	Cr	Cu	Zn	Mn	Fe
Candarli	4-6	0.20 $\pm$ 0.05	10.5 $\pm$ 0.97	4.3 $\pm$ 1.67	2.9 $\pm$ 0.56	496 $\pm$ 47.1	203 $\pm$ 8.46	19.0 $\pm$ 3.74	407 $\pm$ 114
		0.12-0.35	8.9-13.3	1.3-7.4	2.0-4.2	429-633	179-217	10.0-25.9	179-717
	6-8	0.36 $\pm$ 0.14	11.2 $\pm$ 2.02	4.5 $\pm$ 2.19	2.7 $\pm$ 0.51	1069 $\pm$ 43.5	286 $\pm$ 80.7	34.9 $\pm$ 12.6	586 $\pm$ 177
8-10		0.18-0.76	7.3-15.9	1.3-10.9	1.7-4.1	549-2371	200-528	20.8-72.6	241-1064
		0.45 $\pm$ 0.04	15.8 $\pm$ 0.92	8.0 $\pm$ 2.15	3.6 $\pm$ 0.45	1060 $\pm$ 89.7	282 $\pm$ 13.4	27.8 $\pm$ 0.67	322 $\pm$ 23.0
	8-10	0.27-0.63	11.4-20.0	1.3-19.6	2.1-6.0	752-1541	235-354	25.3-31.3	216-428
Izmir-Pasaport	4-6	0.09 $\pm$ 0.02	8.6 $\pm$ 1.22	4.7 $\pm$ 0.85	4.1 $\pm$ 0.65	599 $\pm$ 110	353 $\pm$ 53.7	20.5 $\pm$ 3.46	533 $\pm$ 58.1
		0.03-0.20	5.0-15.2	2.4-9.3	2.7-6.9	27.6-1033	197-644	9.9-39.2	224-733
	6-8	0.13 $\pm$ 0.03	9.8 $\pm$ 1.49	3.3 $\pm$ 0.23	5.5 $\pm$ 1.35	612 $\pm$ 185	432 $\pm$ 76.5	24.5 $\pm$ 4.56	528 $\pm$ 63.2
8-10		0.03-0.31	5.1-17.8	2.3-4.5	1.7-12.9	98.1-1615	198-846	9.9-49.1	233-821
		0.06 $\pm$ 0.0	5.4 $\pm$ 0.0	3.0 $\pm$ 0.0	6.2 $\pm$ 0.0	109 $\pm$ 0.0	221 $\pm$ 0.0	48.6 $\pm$ 0.0	471 $\pm$ 0.0
	8-10	0.06	5.4	3.0	6.2	109	221	48.6	471
Izmir-Levent Marina	4-6	0.05 $\pm$ 0.01	6.5 $\pm$ 0.14	3.3 $\pm$ 1.80	7.2 $\pm$ 4.09	345 $\pm$ 44.3	273 $\pm$ 64.6	23.1 $\pm$ 0.58	476 $\pm$ 20.1
		0.04-0.05	5.6-7.4	1.5-5.08	3.1-11.2	301-389	209-337	22.5-23.6	456-496
	6-8	0.03 $\pm$ 0.00	6.6 $\pm$ 0.41	5.1 $\pm$ 2.49	7.0 $\pm$ 2.11	353 $\pm$ 56.4	217 $\pm$ 11.4	23.5 $\pm$ 2.35	481 $\pm$ 131
8-10		0.02-0.04	5.8-7.7	1.4-12.4	2.3-10.6	196-444	197-249	19.9-30.3	314-871
		0.03 $\pm$ 0.01	7.0 $\pm$ 0.60	2.7 $\pm$ 0.49	5.4 $\pm$ 2.02	306 $\pm$ 86.8	228 $\pm$ 23.0	20.3 $\pm$ 2.13	320 $\pm$ 30.1
	8-10	0.02-0.05	5.9-8.7	1.9-4.14	1.7-10.9	125-513	198-297	16.3-26.3	251-397
Kusadasi-Setur Marina	4-6	0.21 $\pm$ 0.03	8.2 $\pm$ 0.98	7.0 $\pm$ 1.49	9.5 $\pm$ 0.82	807 $\pm$ 42.8	222 $\pm$ 1.95	16.6 $\pm$ 1.14	701 $\pm$ 43.0
		0.15-0.28	5.5-10.1	2.7-9.5	7.1-10.8	701-910	219-227	14.7-19.5	584-777
	6-8	0.28 $\pm$ 0.01	8.9 $\pm$ 1.43	5.5 $\pm$ 2.80	11.8 $\pm$ 1.17	1062 $\pm$ 65.8	252 $\pm$ 14.1	18.0 $\pm$ 1.98	695 $\pm$ 85.0
8-10		0.26-0.31	7.2-13.2	0.97-12.7	8.6-14.1	905-1194	227-279	14.0-21.0	501-865
	8-10								

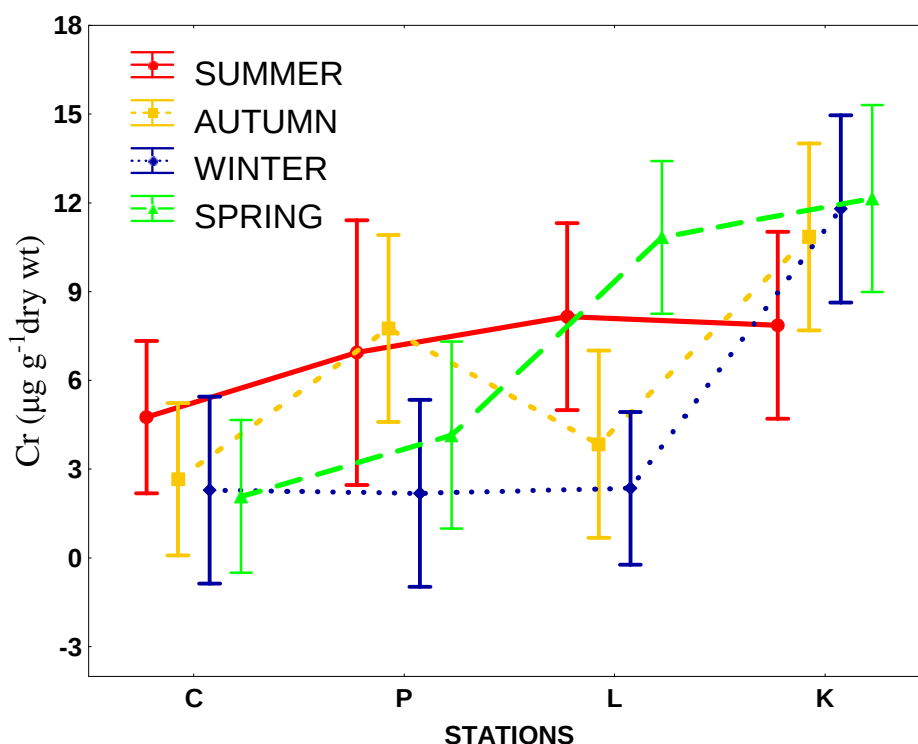


Figure 5.3d Chromium concentrations (mean±0.95 confidence intervals) in *A. amphitrite*. (C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Chromium concentrations in barnacle soft tissues showed higher values in all seasons at Kusadasi-Setur Marina with the maximum value in spring ($14.1 \mu\text{g g}^{-1}$). In addition, the lowest Cr concentration was observed at Candarli ($1.66 \mu\text{g g}^{-1}$) in spring period where a seasonal decreasing trend was detected (Figure 5.3d). The highest mean concentration of Cr was recorded in Kusadasi ($10.65 \mu\text{g g}^{-1}$) while the lowest mean value was obtained from Candarli ($3.06 \mu\text{g g}^{-1}$). There was a significant differences among stations in which Candarli represented with the lowest mean concentration ($3.06 \mu\text{g g}^{-1}$) in the study area.

The barnacle showed a wide range of accumulated body Cu concentrations in soft tissues. The maximum Cu level was recorded at Candarli ($2372 \mu\text{g g}^{-1}$) in autumn while minimum concentration was recorded at Izmir-Pasaport ($27.6 \mu\text{g g}^{-1}$) in winter. According to ANCOVA results, there were apparent consistent spatial ($p < 0.001$) and temporal ($p < 0.01$) patterns (Table 5.9). Higher mean values in autumn period were determined both Izmir-Pasaport ($1324.28 \mu\text{g g}^{-1}$) and Candarli ($1450.97 \mu\text{g g}^{-1}$) (Figure 5.3e).

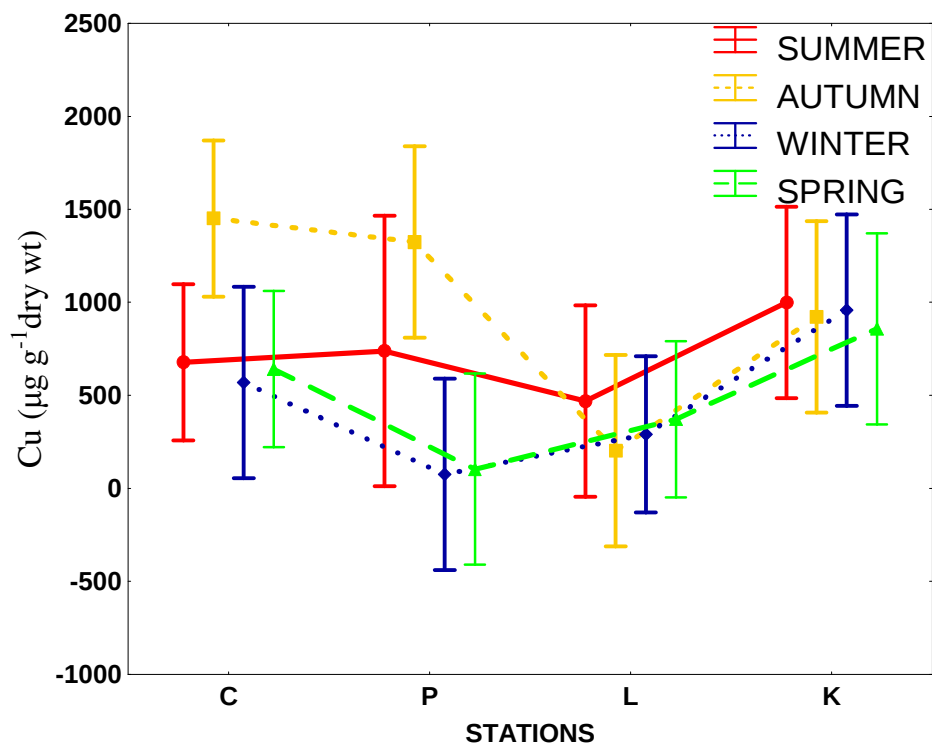


Figure 5.3e Copper concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrute*.

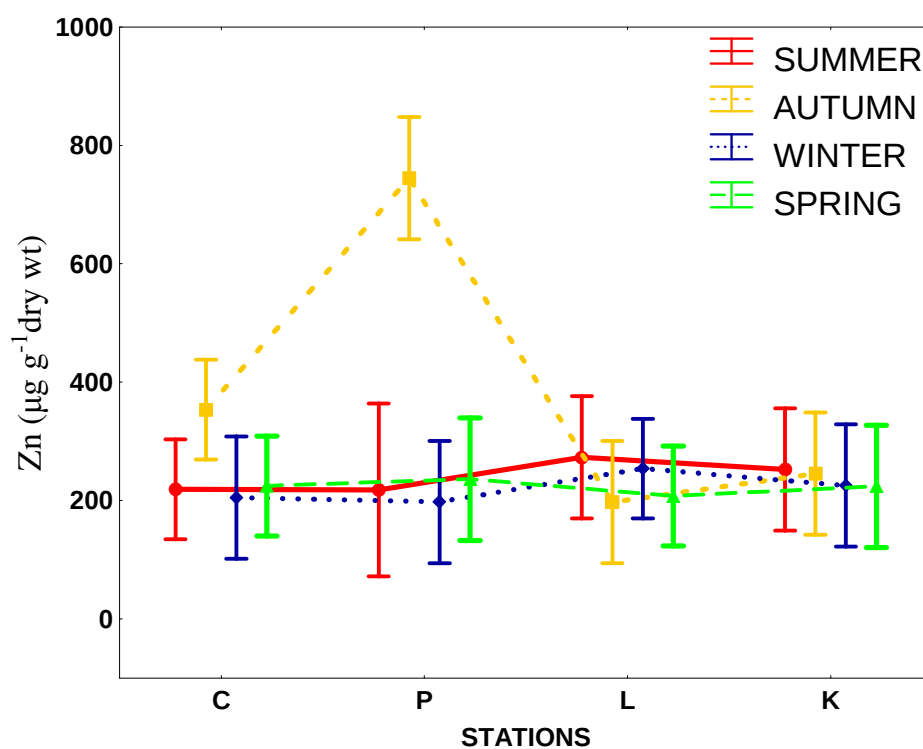


Figure 5.3f Zinc concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrute*.
(C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

The maximum Zn concentration was found at Izmir-Pasaport station ($846 \mu\text{g g}^{-1}$) in autumn period and such body concentration was four times greater than those of measured as minimum value at Candarli station ($179 \mu\text{g g}^{-1}$) during the same period (Figure 5.3f). According to ANCOVA there were not significant differences among stations for the Zn which was showed similar trends within stations. Zn represented with the highest average concentration at Izmir-Pasaport ($335 \mu\text{g g}^{-1}$) and the lowest mean was measured in the body content of barnacles collected from Kusadasi station ($237 \mu\text{g g}^{-1}$) and Levent Marina ($239.3 \mu\text{g g}^{-1}$).

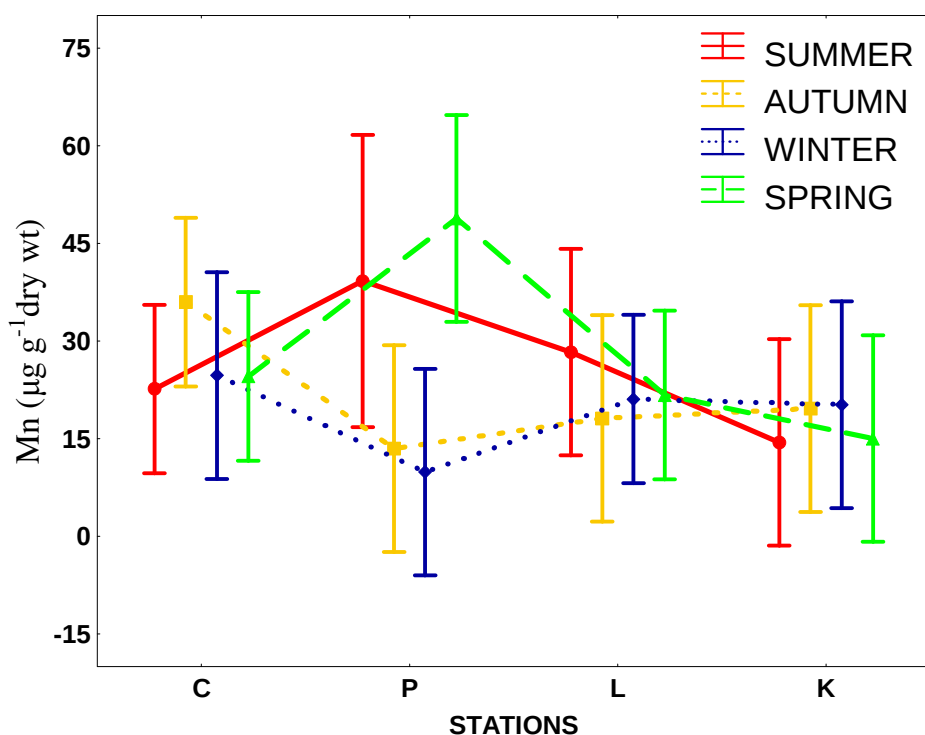


Figure 5.3g Manganese concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrite*. (C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

Minimum ($9.9 \mu\text{g g}^{-1}$) and maximum ($72.6 \mu\text{g g}^{-1}$) concentrations of Mn were found in the data collected from Izmir-Pasaport in winter and Candarli in autumn, respectively. The manganese concentrations did not significantly differ among seasons and stations (Table 5.12). However, inter-seasonal variation can be only observed at Izmir-Pasaport because of the highest mean value in spring period

(Figure 5.3g). The highest mean concentration among stations were found in the body content of barnacles collected from Pasaport with the values $31.2 \mu\text{g g}^{-1}$ and the lowest mean $17.3 \mu\text{g g}^{-1}$ from Kusadasi-Setur Marina.

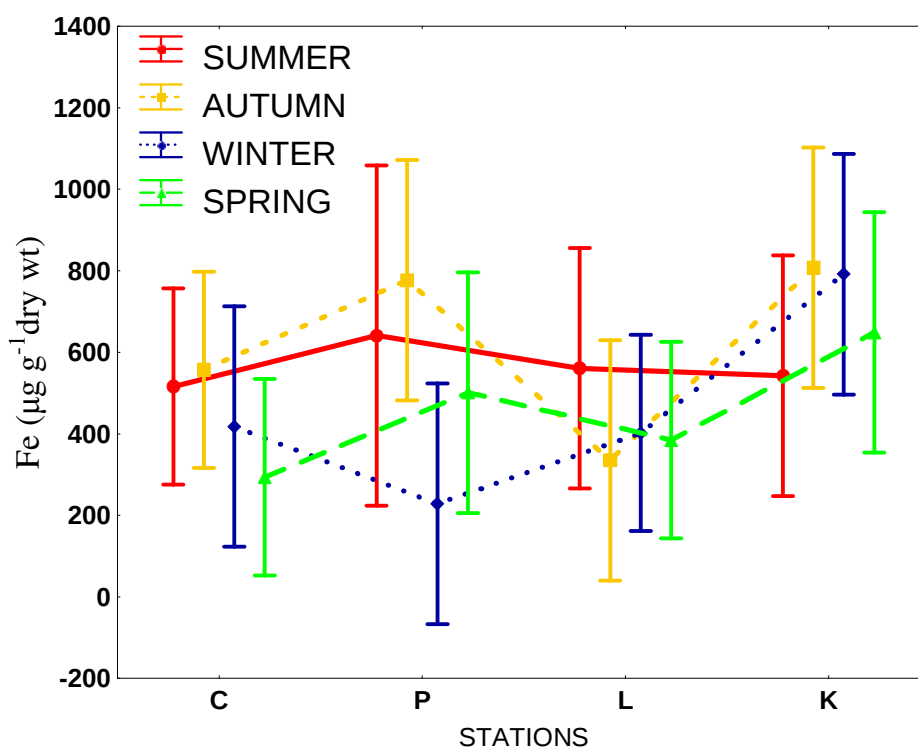


Figure 5.3h Iron concentrations (mean $\pm$ 0.95 confidence intervals) in *A. amphitrite*. (C: Candarli, P: Izmir-Pasaport, L: Izmir-Levent Marina, K: Kusadasi-Setur Marina).

The minimum and maximum concentration of Fe ranged between $179\text{--}1064 \mu\text{g g}^{-1}$ in samples collected from Candarli in autumn (Figure 5.3h). The mean concentrations of Fe in *A. amphitrite* recorded at all sites ranged from $438 \mu\text{g g}^{-1}$ in Candarli to $698 \mu\text{g g}^{-1}$ in Kusadasi-Setur Marina during the study periods. Slightly higher mean levels of Fe in *A. amphitrite* were observed at Izmir-Pasaport and Kusadasi-Setur Marina in autumn. Iron values did not show significant temporal and spatial variations at all stations (Table 5.12).

Analysis of covariance (ANCOVA) was used to detect differences between sites for each of the sampling seasons and seasonal differences at each of the four sites in terms of accumulated body metal burdens. In the data analysis, metal concentrations and season were considered as the dependent and independent variable, respectively,

whereas mean weight was selected as the covariate. The data set was normalized using double log transformation [log body weight (x) against log metal concentration (y)] before ANCOVA test (Table 5.12).

Table 5.12 Effect of inter-season and inter-station variations covariate size on the variability of soft tissues in *Amphibalanus amphitrite*

Variable	df	Season		df	Station	
		F	p level		F	p level
Hg	3	3.367	*	3	59.617	***
Cd	3	1.008	ns	3	14.680	***
Pb	3	9.515	***	3	0.377	ns
Cr	3	2.938	ns	3	19.090	***
Cu	3	7.126	**	3	16.317	***
Zn	3	7.184	**	3	1.914	ns
Mn	3	1.354	ns	3	1.055	ns
Fe	3	1.004	ns	3	2.282	ns

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant

In consequence, the comparison of soft tissue concentrations of metals demonstrates that, there were significant ($p < 0.05$) seasonal variations for Hg, Pb, Cu and Zn. Significant inter-site differences were observed for Hg, Cd, Cr and Cu concentrations in the soft tissues of *A. amphitrite*. The test did not show statistically significant spatial differences for Pb, Mn, Zn and Fe (Table 5.12).

There is a significant correlation ($p < 0.05$) for concentrations of Cd, Cu, Fe, Hg and Zn in barnacles (Table 5.13). This analysis demonstrated a significant positive correlation ($p < 0.001$) of Hg with Cd ($R = 0.638$), Cu ($R = 0.804$) and Zn ($R = 0.561$). The correlation coefficient obtained for Hg and Zn were positive but it had a low confidence level. Fe with Cu ($R = 0.548$) and Zn ($R = 0.572$); Cu with Zn ($R = 0.620$) and Cd ($R = 0.552$). The concentrations of all metals in seawater were lower than in barnacle samples. Comparison of rank orders of sites from seawater and barnacle metal data using Spearman's rank correlation tests revealed that there was no

significant correlation ($p < 0.05$) except Cu/W-Hg/B ($R = 0.518$), Fe/W-Mn/B ($R = -0.506$), Zn/W-Mn/B ($R = -0.610$) and Mn/W-Cr/B ($R = -0.643$).

Table 5.13 Spearman rank-order correlation coefficients for relationship between heavy metals in *Amphibalanus amphitrite* (Marked correlations are significant at $p < 0.05$)

Variable	Hg							
Hg	1.000	Cd						
Cd	0.638	1.000	Pb					
Pb	0.066	0.011	1.000	Cr				
Cr	0.127	-0.102	0.188	1.000	Cu			
Cu	0.804	0.552	0.030	0.310	1.000	Mn		
Mn	0.047	0.011	0.050	-0.164	-0.038	1.000	Zn	
Zn	0.561	0.297	0.049	0.230	0.620	0.267	1.000	Fe
Fe	0.433	0.141	0.242	0.483	0.548	0.065	0.572	1.000

The mean metal concentration in barnacles recorded in this study decreased in the following order; Cu>Fe>Zn>Mn>Cd>Cr>Pb>Hg for the Aegean Sea coast. This order showed changes according to stations. In Izmir-Pasaport and Candarli, mean value of metals sorted in decreasing order (Cu>Fe>Zn>Mn>Cd>Cr>Pb>Hg) and (Cu>Fe>Zn>Mn>Cd>Pb>Cr>Hg), respectively and showed nearly similar tendency with the Aegean Sea. The orders were different in Izmir-Levent Marina (Fe>Cu>Zn>Mn>Cr>Cd>Pb>Hg), while Kusadasi-Setur Marina the sequence is (Cu>Fe>Zn>Mn>Cr>Cd>Pb>Hg). In the southern part of the Aegean Sea the order changed by elevated Cr concentrations due to geological characteristic of this region.

The dendrogram was realized using transformed ($\log x + 1$) metal data obtained from the different sites of the sampling area. Two clusters with sub groups were identified; the first one (G1), Hg constitute a group alone and joined to other group with weak similarity and the second one has two main sub groups (G2 and G3) of metals which constitute subgroups linked to each other with higher similarity values. The group G2 consists of Zn, Cu and Fe which are essential elements in organism life and are closely linked to each other more than %90 similarity (Figure 5.4).

In another group (G3) including Cd, Pb and Cr can be considered as xenobiotic metals, with tendency to bioaccumulation, although distributed in the marine environment at low concentrations. Mn joined to this group with %80 similarity although it comes mainly from soil sources.

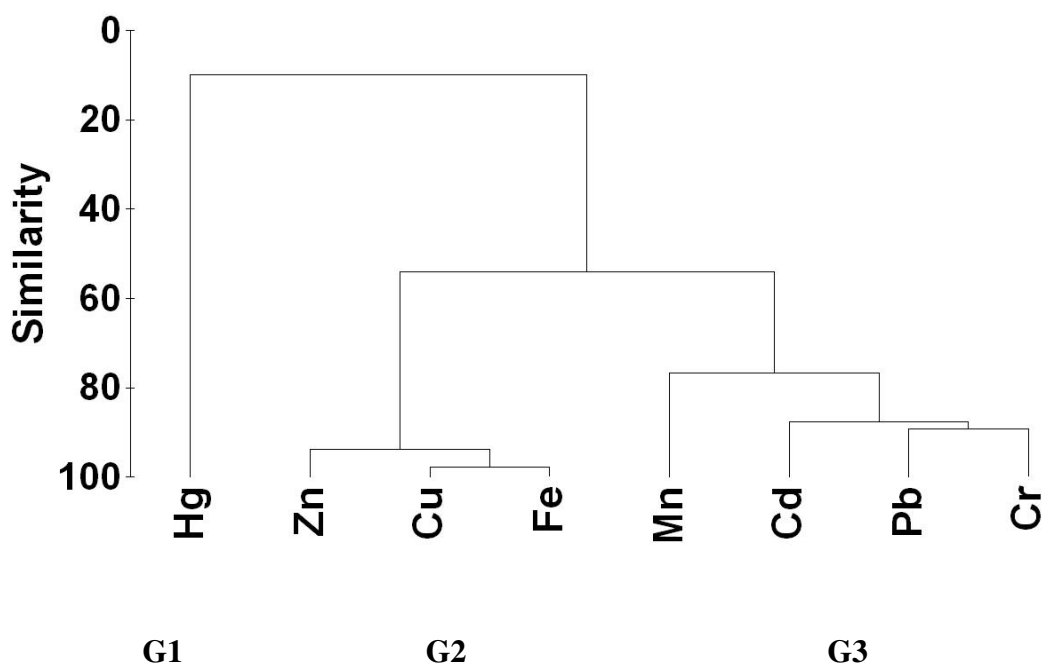


Figure 5.4 Hierarchical dendrogram for heavy metals in sampling sites from the Eastern Aegean coast.

5.5 Discussion

The pollution levels of the aquatic environment by heavy metals can be estimated by analysing water, sediments, marine organisms, physicochemical parameters, OM (%) content and grain size. In the present investigation, higher values of dissolved oxygen were recorded in winter which might be due to the cumulative effect of higher wind velocity coupled with heavy rainfall and the resultant freshwater mixing (Govindasamy, Kannan & Azariah, 2000; Rajasegar, 2003; Saravanakumar, Rajkumar, Serebiah & Thivakaran, 2008). Seasonal variation of dissolved oxygen is mainly affected by freshwater flow, temperature and salinity changes (Saravanakumar *et al.*, 2008).

In this study, the surface water temperature showed an increasing trend from winter (11.9 °C) to summer (26 °C) and was influenced by the intensity of solar

radiation, evaporation, freshwater influx and cooling and mixing water. The highest salinity value was found in summer season (40.8 psu) and the lowest (37.3 psu) in spring period among all stations. The recorded higher values could be attributed to the low amount of rainfall (Turkish State Meteorological Service, 2009), higher rate of evaporation and also due to neritic water dominance (Balasubramanian & Kannan, 2005; Sridhar, Thangaradjou, Senthil Kumar & Kannan, 2006; Asha & Diwakar, 2007). Conductivity levels ranged between 57.10-60.70 mSi/cm from spring to summer in Izmir-Pasaport station, respectively and there were no big differences found at other sampling stations. pH in surface waters remained alkaline throughout the study period and the pH represented with the highest value (8.52) in summer and the lowest in winter (7.98). Generally, its seasonal variation could be due to CO₂ removal by photosynthesis through bicarbonate degradation, dilution of seawater by freshwater influx, low primary productivity, reduction of salinity and temperature, and decomposition of organic matter (Bragadeeswaran, Rajasegar, Srinivasan & Kanagarajan, 2007). The recorded high summer pH value in the sampling stations might be due to the high biological activity (Govindasamy, Kanan & Azariah, 2000) and the occurrence of high photosynthetic activity (Sridhar *et al.*, 2006; Saravanakumar *et al.*, 2008).

Concerning the seasonal variations, higher mean Hg levels in water column were obtained from Pasaport and Levent Marina, located in the inner part of the Izmir Bay in summer period. In our study the distribution of Hg levels in water column (0.010-0.033 µg l⁻¹) were found similar with the data obtained from Kalloni Bay in Eastern Aegean Sea (Gavriil & Angilidis, 2005). However, Hg values (0.08-0.49 µg l⁻¹) in water samples collected inner part of the Izmir Gulf (Akcali & Kucuksezgin, 2011) were higher than our results. Furthermore, sediment samples showed stationational variations and both sediment and water column samples were represented with higher mean values of Hg at the same sampling stations. This means that anthropogenic contribution to the heavy metal concentrations at the inner part of the Izmir Bay was clearly noticed in water and sediment samples. Spatial and temporal variations of Hg in *A. amphitrite* species were much more evident. At Pasaport station the highest mean accumulation of Hg can be recognized in water, and

sediment contrary to soft tissue dry weight (mean level $0.03\text{--}0.45 \mu\text{g g}^{-1}$). Fialkowski & Newman, (1998) obtained considerably higher values ($11\text{--}13 \mu\text{g g}^{-1}$) in Salton Sea, Southern California than those reported in this study. Another study in Huelva Estuary, the Hg values ($0.2\text{--}2.1 \mu\text{g g}^{-1}$) were also found higher in barnacle soft tissues (Morillo & Usero, 2008) (Figure 5.14).

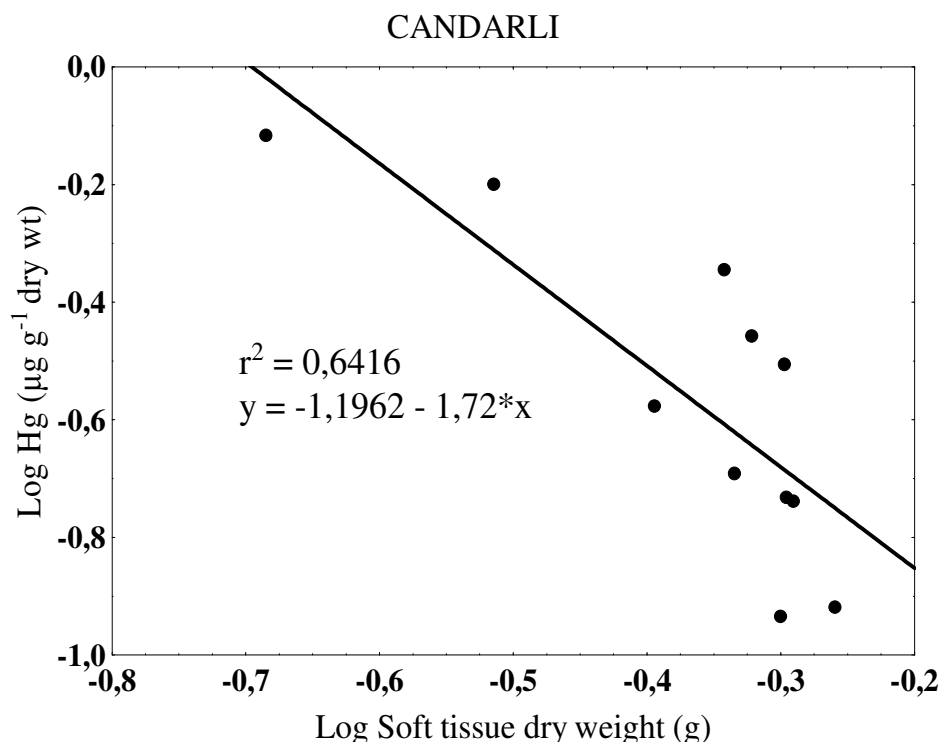


Figure 5.5a Linear regression between Hg ($\mu\text{g g}^{-1}$ dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Candarli.

Many parameters can affect mercury accumulation such as specimen size, sexual maturity, sensitivity to seasonal changes, feeding habits, trophic position, water quality and environmental contamination (Szefer, Kim, B.-S., Kim, C.-K., Kim, E.-H. & Lee, 2004). Metal concentrations in the bodies of marine organisms are dependent on the rates of accumulation, excretion and diluting body growth. Therefore, there is potential for an effect of size on the body metal concentration (Blackmore, Morton & Huang, 1998). In an effort to investigate the relationship between the tissue metal content of *A. amphitrite* and its soft tissue dry weight, linear regression analyses were conducted, taking into account all metal pairs at each site. In the present study, a significant negative correlation was found between Hg concentration in barnacle soft tissue and its weight at all stations except Kusadasi-Setur Marina (Figure 5.5a-c).

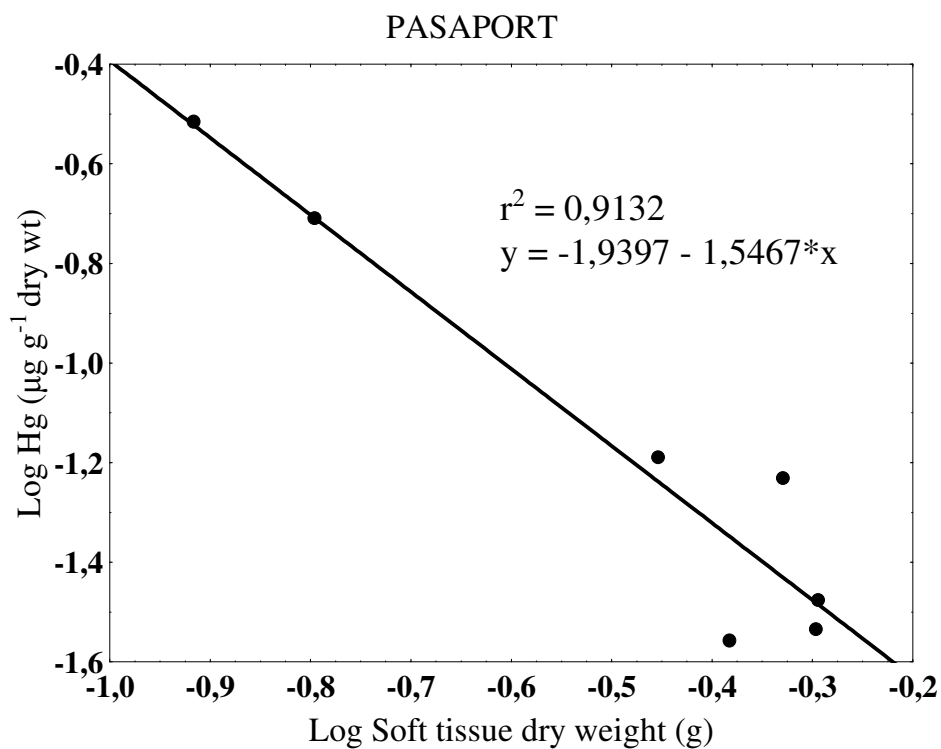


Figure 5.5b Linear regression between Hg ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Pasaport.

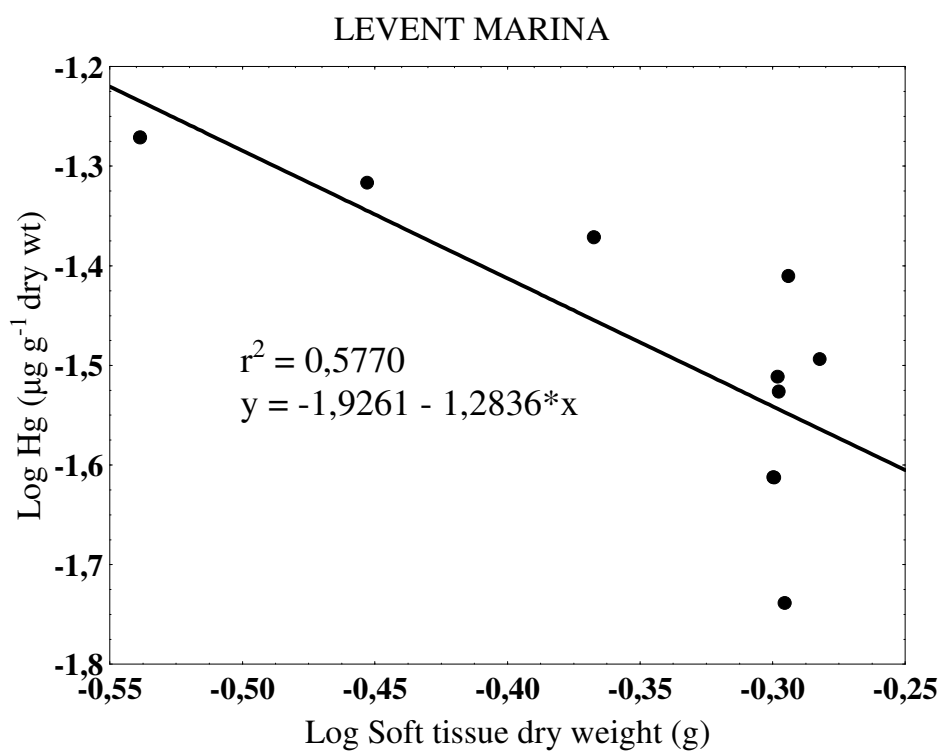


Figure 5.5c Linear regression between Hg ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Levent Marina.

Cd levels in sediment samples collected from all stations showed marked station differences and this is obviously seen that samples taken from Izmir-Pasaport included higher concentrations than other stations for all seasons. Moreover, the higher mean Cd levels in water samples were also found in Izmir-Pasaport and Candarli. In the present study, the detected Cd levels in sediment samples are close to those found previously in sediments of port of Naples-Italy, (Adamo *et al.*, 2005) and Huelva Estuary-Spain (Morillo, Usero & Gracia, 2004). In our study, Cd concentrations in sediment found a bit higher than the values obtained from Ligurian Sea-Italy (Bertolotto *et al.*, 2005) and Izmir Bay (Kucuksezgin *et al.*, 2006). However, this results were lower than those obtained from Mar Piccolo, a semienclosed basin located near the city of Taranto-Italy (Calace *et al.*, 2005) and other previous studies (Ferraro *et al.*, 2006; Vicente-Martorell, Galindo-Riaño, García-Vargas & Granado-Castro, 2009). In the current study, the recorded Cd values in water column were lower than the previous studies carried out in different localities (Morillo, Usero & Gracia, 2005; Vicente-Martorell, *et al.*, 2009; Akcali & Kucuksezgin, 2011). A major source of cadmium in the marine environment is sewage and industrial activities such as manufacture of paints, plastics, ceramics and batteries, alloys, agricultural fertilizers, fossil fuels (Maruham 1994; Waalkes & Misra, 1996). At Izmir-Pasaport station, high values of cadmium concentration should be derived from the polluted inner Izmir Bay, is subjected to urban sewage, industrial activities and port operations. One factor that can affect the bioavailability of many trace metals without a change in total dissolved metal concentration is salinity (Rainbow, 1997; Blackmore, Morton & Huang, 1998). For example, the metals zinc and cadmium are complexed by chloride; thus in higher salinity there is greater chloride complexation and a reduction in the availability of the free metal ion, widely considered to be the metal species most available for uptake from solution (Campbell, 1995). Thus Zn and Cd bioavailabilities may be higher in lower salinities simply as a physico-chemical effect of salinity change (Rainbow, 1997). Variation in salinity did not significantly affect Cd uptake in *A. amphitrite* collected from the low salinity site and both water and body content of *A. amphitrite* showed a weak correlation with salinity ($p < 0.05$) (Table 5.4). Source of Cd in barnacles include dissolved metals in water column and organically metal rich food particles.

Especially *A. amphitrite* takes more of the smaller suspended particles (Anderson, 1994). Therefore, it should be expected that once the main Cd flux into *A. amphitrite* is from food, the biomonitor should reflect the Cd concentration in the dissolved phase and ultimately in the total Cd concentration in the environment. It may be that the smaller particles taken by *A. amphitrite* have more significant geographical differences in metal loadings for uptake and accumulation. In this study, a significant negative correlation was found between Cd concentration in barnacle soft tissue and its weight for only Izmir-Pasaport station (Figure 5.5d).

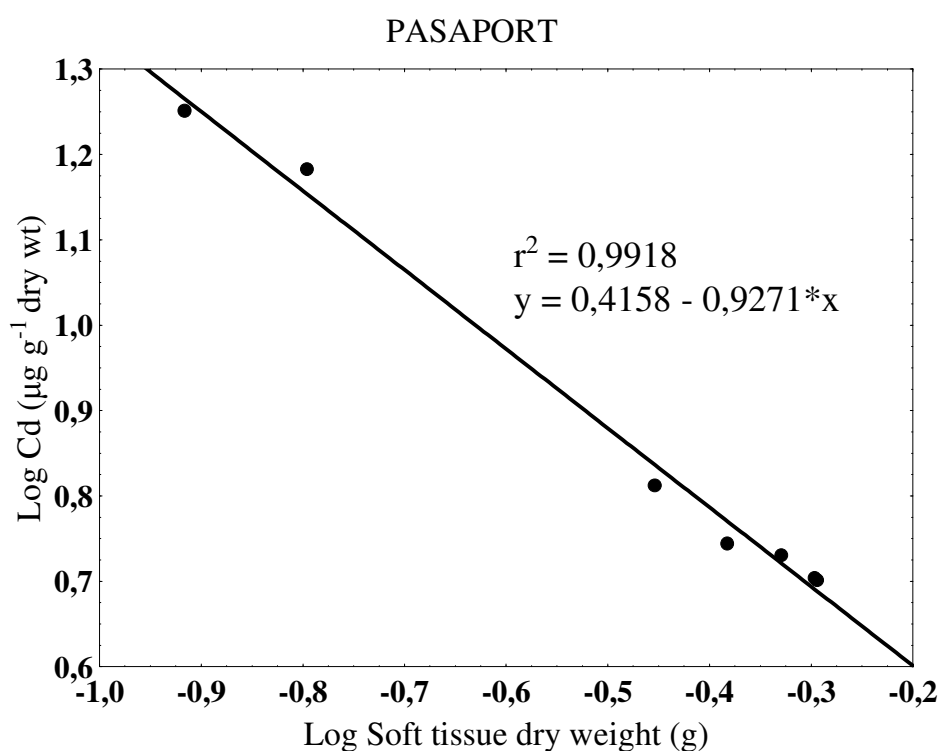


Figure 5.5d Linear regression between Cd ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Pasaport.

Although Cd values in *A. amphitrite* were found slightly higher than those obtained from other studies (Phillips & Rainbow, 1988; Blackmore, Morton & Huang, 1998; Fialkowsky & Newman, 1998; Rainbow & Blackmore, 2001; Blackmore, 2001; Silva, Smith & Rainbow, 2006), Cd values ($5.4\text{--}15.8 \mu\text{g g}^{-1}$) were approximately four times lower than those determined at Mission Bay ($58 \mu\text{g g}^{-1}$) (Fialkowsky & Newman, 1998) and eleven times lower than that found in the Huelva Estuary ($168 \mu\text{g g}^{-1}$) (Morillo & Usero, 2008) (Table 5.14).

Table 5.14 Comparative metal concentrations ($\mu\text{g g}^{-1}$ dry wt) in *A. amphotrite* in different parts of the world

Area	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Zn	References
Hong Kong coast	2.1-10.1		59.3-3472					2726-11990	Phillips&Rainbow, 1988
Coast of Xiamen	0.87-5.50		28.9-2205	817-3126		38.8-277		1521-10000	Blackmore et al, 1998
Southern California	3.71-58		40-3750		11-13			620-37900	Fialkowsky&Newman, 1998
Cape d'Aguilar Hong Kong	3.71		57.8					9.73	Blackmore, 2001
Hong Kong coast	0.69-9.45	0.62-3.32	52.4-1810			14.5-95.4	0.40-9.08	2860-23300	Rainbow&Blackmore, 2001
NE Mediterranean	2.38-7.31	6.19-22.3	7.62-19.9	19.9-72.5		13.9-21.8	23.1-107	46.2-126	Turkmen et al., 2005
South America	1.34-2.36		18.0-39.0	152-519		28.1-60.9		967-2028	Augusto et al., 2006
Hong Kong coast	4.15-11.1		188-6317	1843-5929		17.8-223	19-106	3148-11298	Blackmore, 1996
Curimatau estuary Brasil	5.13		23.6	466		9.6		1185	Silva et al., 2006
Southern Spain	2.1-168		94-9430		0.2-2.10	26-469		852-48500	Morillo&Usero, 2008
Eastern Aegean	5.4-15.8	2.7-11.8	108-1069	320-701	0.03-0.45	6.6-48.6	0.97-19.6	203-432	This study

On a seasonal scale, the high values of Pb were recorded in early summer period for water samples at all stations. This seasonal difference within the water column might be result of the rainy period (Figure 3.2) and this can also be attributed to atmospheric transport of the metal through wet deposition because atmosphere is a very important Pb pathway to the sea and even to enclosed marine systems. It is estimated that the atmospheric fluxes of Pb are two orders of magnitude higher than the fluvial fluxes (Martin *et al.*, 1989). The highest mean lead value in sediment samples was obtained at Izmir-Pasaport station where spatial variation was notable. The appearance of Pb may be the result of local anthropogenic sources, i.e. urban sewage runoff, industrial, the presence of port facilities, the deposition of atmospheric Pb and the roads washing from rainfall during the winter and the spring time of the year, contribute significantly to the Pb loading of marine sediments (Stamatis, Nikolaos & Sylaios, 2006). The atmospheric pathway may represent a major source of Pb to marine sediments. The highest mean Pb levels in *A. amphitrite* and in water column showed similar pattern and maximum concentrations were recorded at Kusadasi-Setur Marina station for both of them. Concerning Pb, accumulation in barnacle ($0.97\text{--}19.6\text{ }\mu\text{g g}^{-1}$) was lower than the findings obtained from studies conducted in contaminated Hong Kong coastal waters and Turkish coasts (Blackmore, 1996; Turkmen, *et al.*, 2005) where they found maximum values as approximately 107 mg kg^{-1} . Pb values in *A. amphitrite* were higher than the results given by Rainbow & Blackmore, 2001 (Table 5.14). In water samples Pb values were lower than $2.6\text{--}17.8\text{ }\mu\text{g l}^{-1}$ reported by Vicente-Martorell *et al.* (2009). In general, we can state that the concentration of Pb in sediment samples were higher than that given for Inner Izmir Bay (Aksu, Yasar & Uslu, 1998; Cihangir & Kucuksezgin, 2003; Kucuksezgin, *et al.*, 2006; Duyusen & Akıncı, 2008). In accordance with our results, Ferraro *et al.*, (2006) found higher Pb values in Naples Harbour's sediments. The results for sediment samples collected from the estuary of Tinto and Odiel rivers in Huelva-Spain, one of the most metallic polluted estuaries in Europe (Vicente-Martorell, *et al.*, 2009) were two times higher than the results given in this study.

Chromium levels in water samples at all stations showed a significant seasonal variation like Pb and it can be noticed that summer values at all stations were obviously presented with high Cr values in sea water. Besides, higher mean values both water and sediment samples were obtained from Izmir-Pasaport and Levent Marina stations where OM (%) contents represented with high levels (Table 3.1) and the lowest mean Cr concentration found at Kusadasi-Setur Marina station. Moreover, Cr levels tended to correlate positively ($p < 0.01$) with OM (%). Our findings for sediment were generally lower than that reported by Aksu, Yasar & Uslu, (1998); Cihangir & Kucuksezgin, (2003); Bertolotto *et al.*, (2005). However, in some previous studies, Cr levels in sediment samples exceeded our measurements (Ferraro *et al.*, 2006; Kucuksezgin *et al.*, 2006; Adamo *et al.*, 2005; Duyusen & Akıncı, 2008). In the current study, barnacles showed significant station differences and the average concentrations of Cr in barnacle soft dry tissue ranged between 3.06-10.65 $\mu\text{g g}^{-1}$. In the previous study, carried out in Iskenderun Bay and Hong Kong coastal waters higher values were found than our results (Turkmen *et al.*, 2005; Rainbow & Blackmore, 2001) (Table 5.14).

Mean Cu levels both water and barnacle samples were found relatively higher in Kusadasi-Setur Marina and Candarli. The lowest mean level was recorded in Izmir-Pasaport for both water column and barnacles. The correlation between Cu and Zn in water and sediment indicates a common contamination source, which is typical for other anthropogenically impacted coastal areas. These can be related to the element pairs may have similar anthropogenic sources and distributions. Natural background concentrations of Cu in seawater are estimated between 0.5 and 3 $\mu\text{g l}^{-1}$, but concentrations up to 21 $\mu\text{g l}^{-1}$ in contaminated areas (Brooks & Waldock, 2009). In our study, the highest Cu levels in seawater (2.2-8.1 $\mu\text{g l}^{-1}$) which exceed the natural background concentrations was measured in Kusadasi-Setur Marina and these obtained levels increased winter through to summer period (Figure 5.1e). This may be due to boat docking, boat repairing activities and corrosion of ships' hulls which generally have the antifouling coating (Dolgen, Alpaslan & Serifoglu, 2003). Kusadasi-Setur Marina used as landing place has a yacht capacity rising from 410 yachts in winter conditions through to 525 yachts in summer conditions.

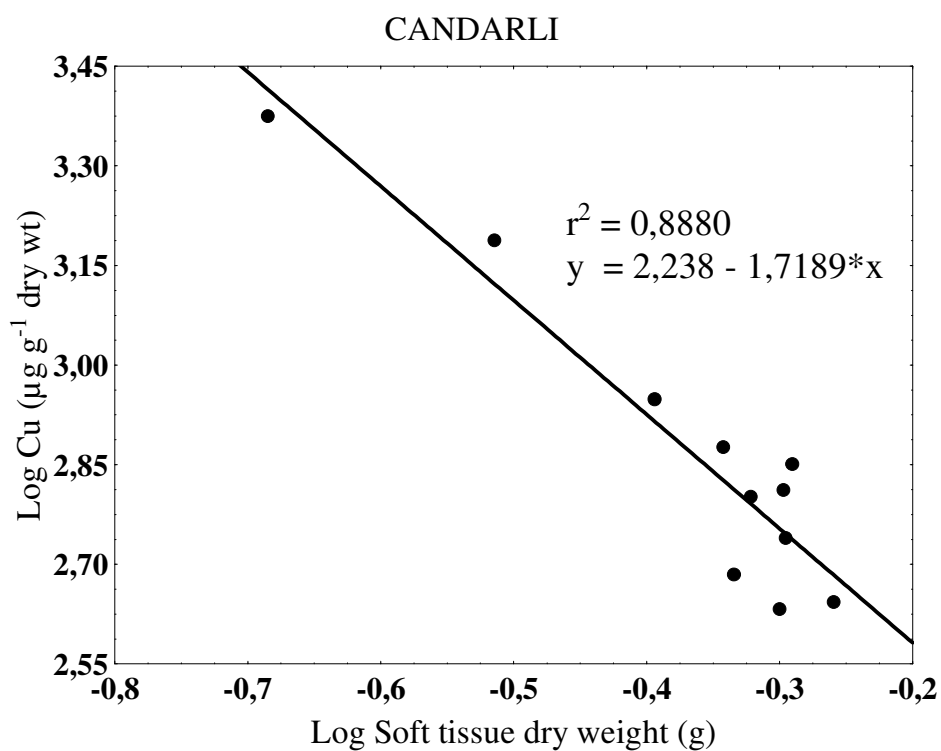


Figure 5.5e Linear regression between Cu ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Candarli.

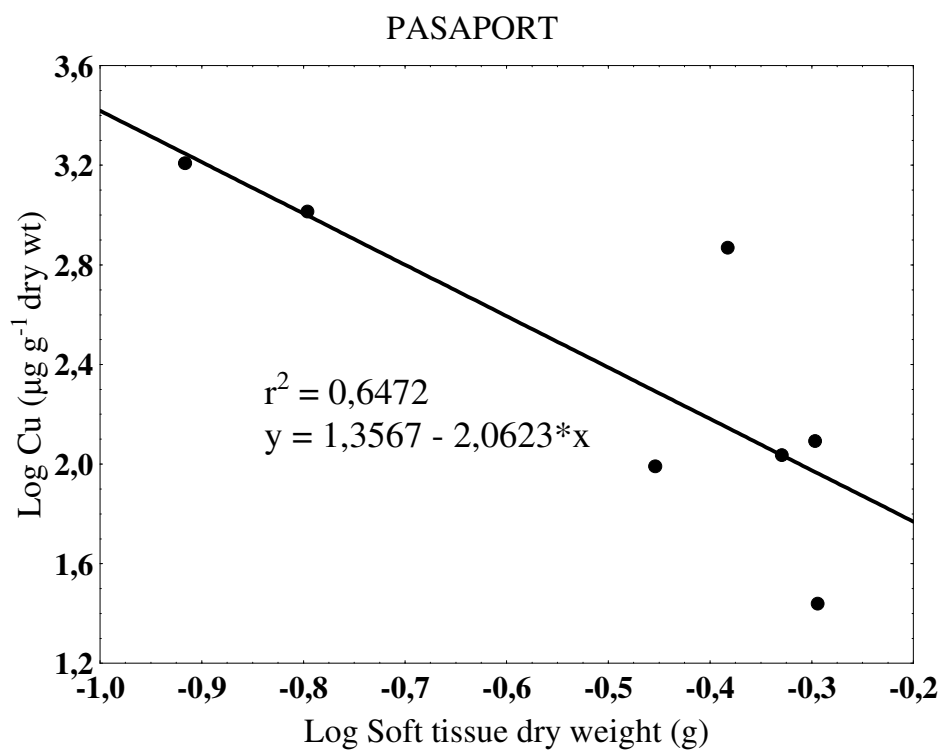


Figure 5.5e Linear regression between Cu ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Pasaport.

The copper distribution in soft dry tissue of *A. amphitrite* presented both spatial and temporal variations. There were similarly size-effects were determined for Cu in Candarli, Izmir-Pasaport (Figure 5.5e and f). Barnacles have a higher potential for metal accumulation than other invertebrates (Rainbow & White, 1989), for instance, copper is accumulated from copper- and sulphur-rich deposits by barnacles (in extreme copper rich environment). End products including copper-containing metallothioneins accumulated as a result of the lysosomal breakdown (Rainbow, 1987, 1998). Concentrations of Cu ($108\text{--}1069\ \mu\text{g g}^{-1}$) levels in present study were generally low relative to previously reported values for barnacles (Phillips & Rainbow, 1988; Blackmore, Morton & Huang, 1998; Blackmore, 1996; Fialkowski & Newman, 1998; Rainbow & Blackmore, 2001; Morillo & Usero, 2008) (Table 5.14). Cu is the main component of antifouling paints (Jones & Bolam, 2007) and is also considered as an important anthropogenic source of Cu to the aquatic environment (Warnken & Dunn, 2004). Other substances leached from the paints that may have potentially adverse effect with one another such as Zn and Pb (Karlsson & Eklund, 2004; Karlsson, Breitholtz & Eklund, 2006; Karlsson, Ytreberg & Eklund, 2010).

Measured copper and zinc levels in sediment samples were nearly similar along the Aegean coast. Compared to other stations this can be attributed to organic matter because the lowest organic matter value was recorded at Kusadasi-Setur Marina. It is worth noting that there were a significant positive relationships between organic matter (%) and Cu ($p < 0.05$), Zn ($p < 0.001$). Decomposition of the organic matter are found to release heavy metals back to sediments and accumulated; and this process might be responsible for the strong association of Zn and Cu with organic carbon (Bardarudeen, Damodaran, Sajan & Padmalal, 1996). Besides, the release of organically bound heavy metals through influx from land runoff might have also contributed elevated level of Zn and Cu, despite they are meager in amount. Zn and Cu are generally good indicators of anthropogenic inputs (Forstner & Wittman, 1979).

In this study, Cu levels for water samples were similar reported by Vicente-Martorell *et al.*, (2009); and Cu results in water close to results obtained from Algericas Bay (Spain) given by Morillo & Usero, (2008). Cu values were lower than the results obtained from Huvelva Estuary (Morillo, Usero & Gracia, 2005; Morillo & Usero, 2008) and from Naples Harbour-Italy (Adamo *et al.*, 2005). Results in this study indicate that Cu levels in the sediment were higher than the previous studies (Aloupi, Angelidis, Gabriel, Karantanelli, Koulousaris, Nikolaou, *et al.*, 2007; Cihangir & Kucuksezgin 2003; Aksu, Yasar & Uslu, 1998; Bertolotto *et al.*, 2005; Dassenakis *et al.*, 2003; Calace *et al.*, 2005). Especially higher Cu levels were found in sediment samples taken from Harbour areas (Ferraro, *et al.*, 2006) and polluted areas (Vicente-Martorell *et al.*, 2009) in the Mediterranean Sea.

In this study, Zn values in sea water which exceeded the clean offshore levels were recorded in marinas. The levels of Zn in seawater can be as high as $450 \mu\text{g l}^{-1}$ especially in polluted harbours. However, in clean offshore areas the values range from 1 to $5 \mu\text{g l}^{-1}$, and can even go down to $0.002 \mu\text{g l}^{-1}$ (UNEP, 1996d). The highest average Zn concentrations in *A. amphitrite* was found in Izmir-Pasaport but lowest value was measured in Kusadasi-Setur Marina. The metal concentration in barnacle showed seasonal changes in flesh weight during the development of gonadic tissues (Joiris, Holsbeek & Otchere, 2000). In addition, barnacles have higher metal levels just before spawning in which fertilization occurred from March to September (Southward, 2008). And increase may be due to life cycle differences that influence uptake, storage and or excretion or body conditions. However, zinc bioavailability is affected by biotic (e.g. organism age and size, prior history of exposure, food availability, metal bioavailability, season of sampling, hydrodynamics of the environment, size, sex, changes in tissue composition seasonal variations in weight and reproductive condition) and abiotic factors (e.g. temperature, salinity, pH, metal concentration water hardness, dissolved organic carbon) (Blackmore, Morton & Huang, 1998; Brock, 1998; Wang, Qiu & Qian, 1999a, 1999b). Barnacles are particularly strong net accumulators of zinc in body concentrations compared to most invertebrates (Rainbow, 1987, 1998).

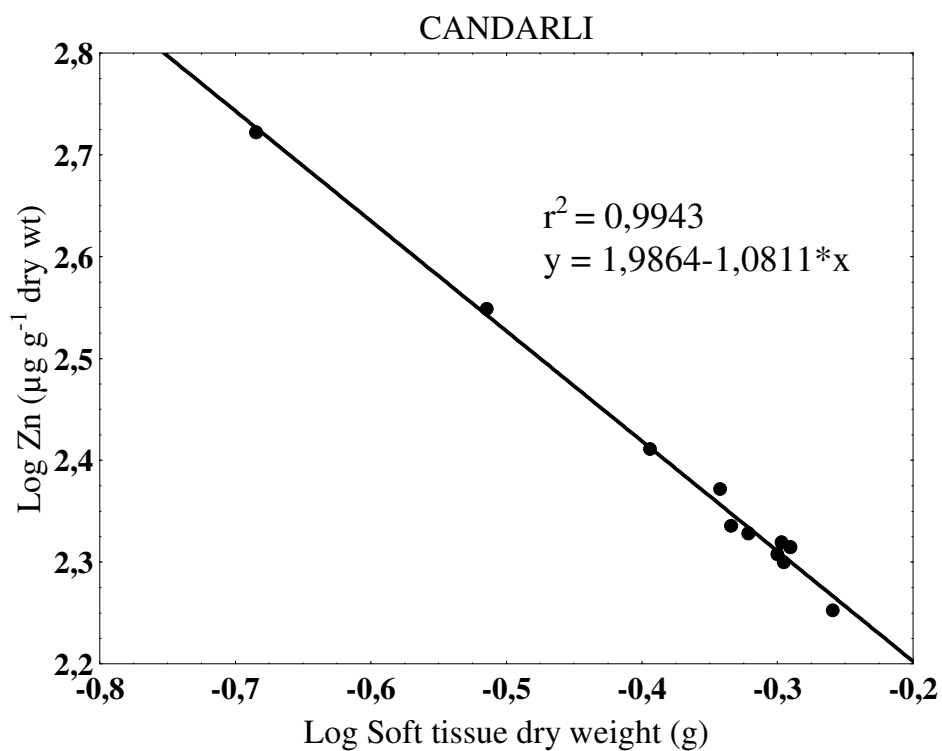


Figure 5.5g Linear regression between Zn ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Candarli.

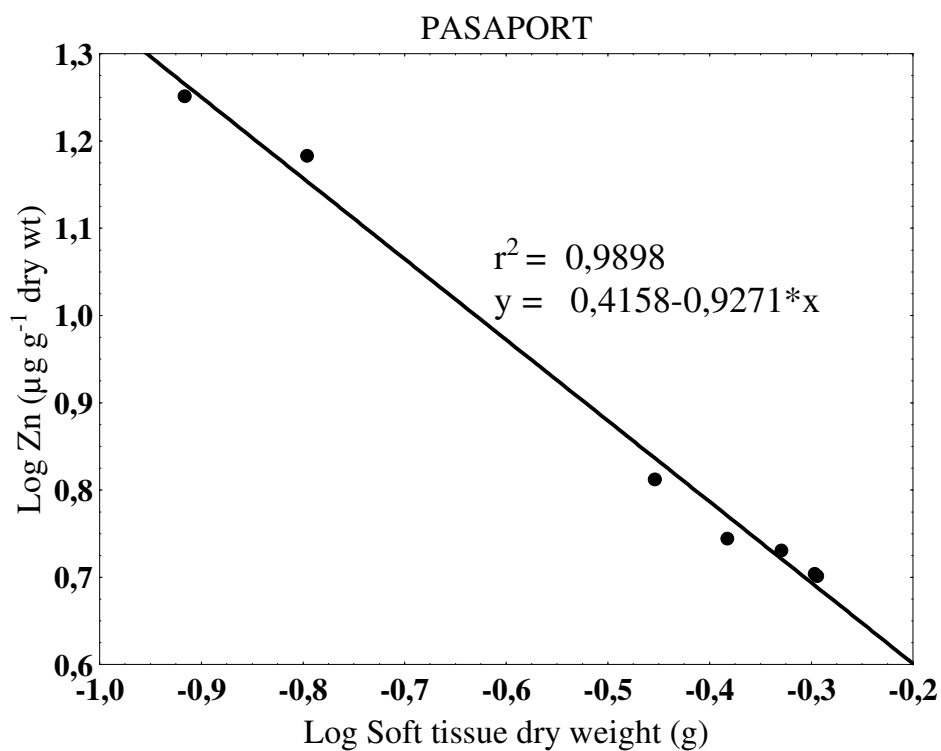


Figure 5.5h Linear regression between Zn ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Pasaport.

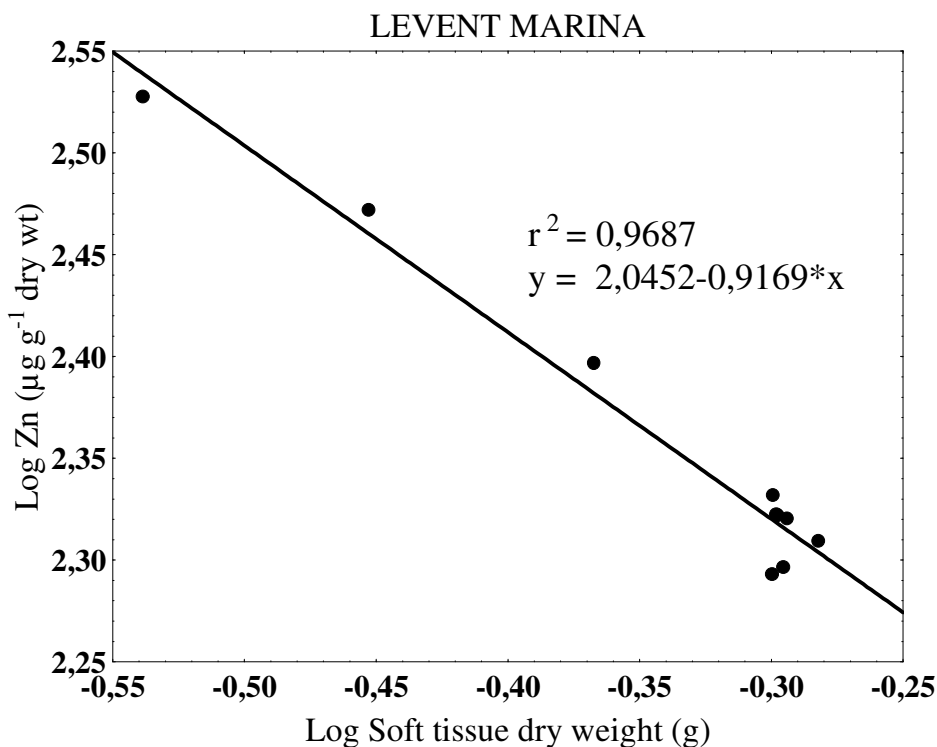


Figure 5.5i Linear regression between Zn ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Levent Marina.

Various metal concentrations in *A. amphitrite* were higher not only in polluted environment such as Kusadasi-Setur Marina, Izmir-Pasaport and Izmir-Levent Marina but also in less urbanised area, Candarli. The soft dry tissues of *A. amphitrite* also showed negative relationships ($p < 0.01$) between weight (g) and Zn ($\mu\text{g g}^{-1}$) at all stations except Kusadasi-Setur Marina (Figure 5.5g-i).

Due to lack of a hepatopaneas in barnacle (Masala, McInnes & O' Brien, 2002) it takes up available zinc from solution (Rainbow & White, 1989) and has an ability to detoxify in form of zinc pyrophosphate granules ($\text{Ca}_2\text{P}_2\text{O}_7$) in the body tissue which are stored in the mid-gut region (Rainbow, 1987, 1998; Pullen & Rainbow, 1991). These granules are not excreted, and this allows barnacles to accumulate extremely high body concentrations of zinc e.g. $100,000 \text{ mg Zn g}^{-1}$ dry weight (Rainbow, 1987, 1998) with apparently no physiological effects. Our Zn values for water were lower than the previous studies given by Morillo, Usero & Gracia, (2005); Morillo & Usero, (2008) and Vicente-Martorell *et al.*, (2009). Zn levels in sediment were similar to values measured in coastal area of Ligurian Sea which is

densely populated and characterised by the presence of a variety of pollution sources which are likely to release heavy metals into the environment (Bertolotto *et al.*, 2005) and sediments taken from southwest coast of Spain (Morillo, Usero & Gracia, 2004) and were higher than Dassenakies *et al.*, (2003); Calace *et al.*, (2005); Cihangir & Kucuksezgin, (2003); Aksu, Yasar & Uslu, (1998); lower than Adamo *et al.*, (2005); Duyusen & Akinci, (2008).

Considering Mn, there was a significant stational variation for sediment samples; moreover, there were no clear spatial and temporal differences in water column. The high dissolved Mn concentrations in the bottom waters must be considered as a result of high availabilities of manganese (perhaps together with iron) which may be associated with elevated suspended load of organically rich sediment particles (Rainbow & Blackmore, 2001). In this study, the Mn concentrations in water column were similar to the concentrations reported for other coastal sites (Vicente *et al.*, 2009) and our results were lower than the results given by Morillo, Usero & Gracia, (2005); Morillo & Usero, (2008). The most abundant metal-sorption phases in sediment oxic horizons are Mn and Fe oxides. Organic carbon has also been shown to sorb metals in both aerobic and anaerobic sediment horizons, whereas in anoxic horizons, acid-volatile sulphides are the most important binding phase for divalent metals (Hagopian-Schlekat, Chandler & Shaw, 2001). Mn accumulation showed negative correlations with gravel and sand and no significant correlations were found for silt and clay (Table 5.9). Total Mn levels showed positive correlation with Cr in sediment ($R=0.565$, $p<0.05$) (Table 5.9), and implies that Mn-oxides in the surface sediments may partly control the Cr level.

In sediment, comparatively, Mn levels found in this study were higher than the levels reported by Adamo *et al.*, (2005); Calace *et al.*, (2005) and our results were similar that found by Dassenakis *et al.*, (2003). There was no clear spatial and temporal differences observed for water and barnacle manganese concentrations. The potential of Mn for size-effects with regard to barnacles collected from Candarli was significant (Figure 5.5g). In this study, the Mn values for *A. amphitrite* (16.6-48.6 $\mu\text{g g}^{-1}$) represented with lower values than those published by other authors (Blackmore,

Morton & Huang, 1998; Blackmore, 1996; Rainbow & Blackmore, 2001; Morillo & Usero, 2008; Augusto, Silva, Smith & Rainbow, 2006) (Table 5.14).

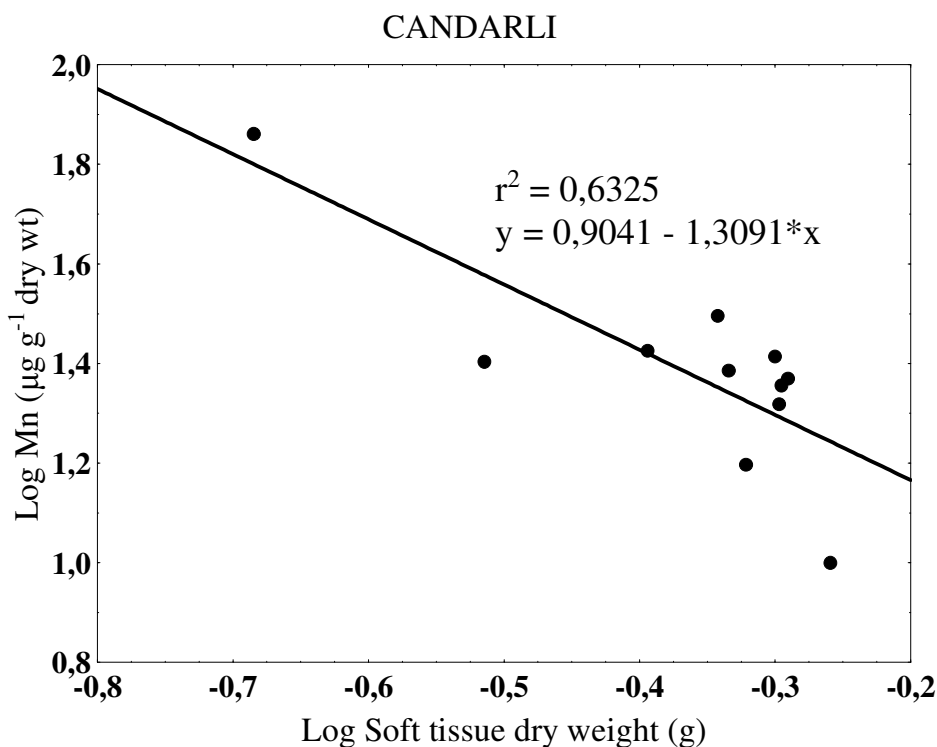


Figure 5.5j Linear regression between Mn ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Candarli.

No significant temporal variations were observed for Fe levels in water, sediment and barnacle while there was a significant spatial variation was observed for only sediment samples (Table 5.10). Measured Fe levels in sediment samples were high except Kusadasi-Setur Marina where OM (%) levels represented at low concentrations compared to other stations. This can be related to the distribution profiles of iron and manganese adsorbed onto the surface of suspended particles (Ali & Fishar, 2005). In this study, lower Fe levels in water samples were found according to the results given by other authors (Morillo, Usero & Gracia, 2005). Comparing our findings, Fe levels measured in sediment samples were lower than the results reported by Bertolotto *et al.*, 2005. Similar values were found with the study performed on Fe analyses of sediment taken from Euvoikos Gulf, Greece, which is a semi-enclosed system receiving pollution loads from several urban and industrial sources and is affected by a strong tidal current (Dassenakis *et al.*, 2003). The reported Fe values in our study were found higher than the levels given by other published studies (Calace *et al.*,

2005; Morilo, Usero & Gracia, 2004; Aloupi *et al.*, 2007). The highest mean level of Fe for water and barnacle was measured at same sampling station (Kusadasi-Setur Marina). On the other hand, the data set for iron was showed significant size-effect for only Izmir-Pasaport (Figure 5.5k). The Fe values in barnacles were lower in comparison with the obtained data taken from Hong Kong and Xiamen coastal waters (Blackmore, 1996; Blackmore, Morton & Huang, 1998) (Table 5.14). The highest Fe values measured in this study exceeded the levels given by other studies carried out in Iskenderun Bay (Turkmen *et al.*, 2005); in Curimataú Estuary-Natal, Brasil (Silva, Smith & Rainbow, 2006) and in South America, N. Brazil (Augusto *et al.*, 2006).

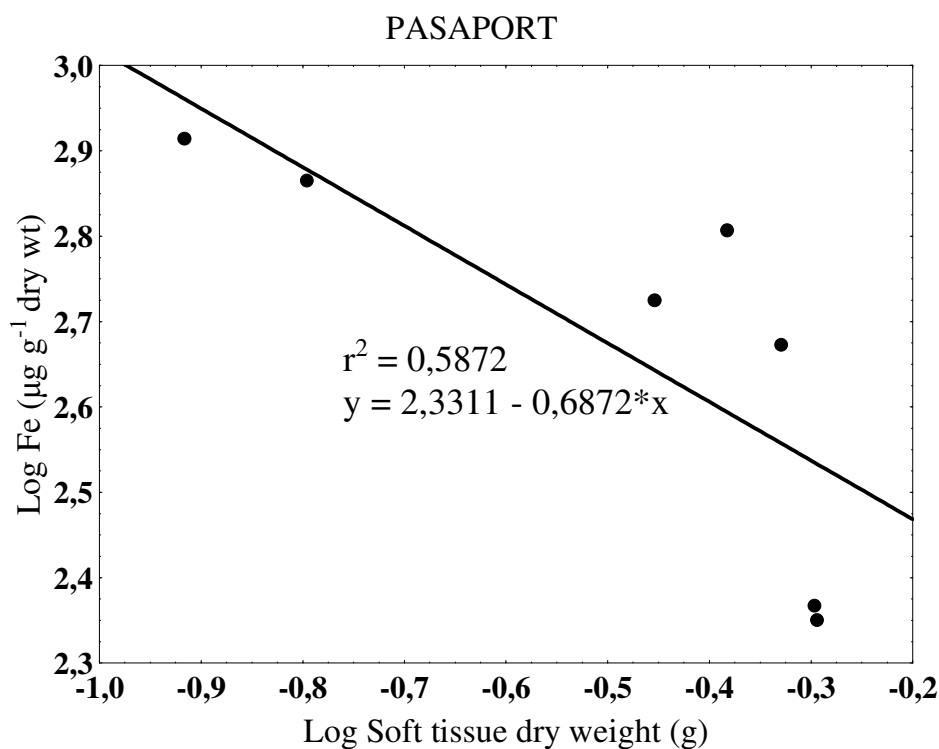


Figure 5.5k Linear regression between Fe ($\mu\text{g g}^{-1}$, dry weight) and soft tissue dry weight (g) of *A. amphitrite* in Izmir-Pasaport.

The negative relationships between metal concentrations and size found in this study may be the difference in metabolic activity between younger and older barnacles. The net accumulation of heavy metals in an organism is a result of the difference between uptake and depuration and this is the most important factor in metal accumulation. Therefore, results of this study may also lead us to say that heavy metal concentrations in the studied stations in such levels that the barnacles

may control their tissue levels with the growth (Canli & Atli, 2003). If we compare the metal levels and weight of the soft tissue, it can be seen that the slope for Hg, Cd, Cu, Zn, Mn and Fe were generally greater in Izmir-Pasaport and Candarli stations, which could be explained either by different bioavailability.

In order to assess the concentration factors (CFs) for *Amphibalanus amphitrite*, analysis of the waters were performed which were collected at the same sites and collection period. The CF may be used to evaluate the state of conservation of an ecosystem or to monitor its state (Conti and Cecchetti, 2001). CF is the ratio of metal concentration in the barnacle ($\mu\text{g g}^{-1}$ dry wt) to the concentration of metal in seawater (mg l^{-1}) over the sampling periods. CFs could be influenced by the passage of a contaminant through the trophic chain.

Table 5.15. Concentration factors for Hg, Cd, Pb, Cr, Cu, Mn, Zn and Fe in *Amphibalanus amphitrite* along the Aegean coast

Site	Hg	Cd	Pb	Cr	Cu	Mn	Zn	Fe
Candarli	2.8×10^4	5.1×10^4	3.6×10^3	2.6×10^3	2.9×10^5	6.0×10^3	6.9×10^4	7.5×10^4
Izmir-Pasaport	5.9×10^3	3.7×10^4	2.8×10^3	5.1×10^3	6.5×10^4	1.1×10^4	7.7×10^4	5.8×10^4
Izmir-Levent M.	2.1×10^3	2.2×10^4	4.8×10^3	8.3×10^3	2.3×10^5	9.9×10^3	4.3×10^4	2.4×10^4
Kusadasi-Setur M.	2.4×10^4	4.2×10^4	3.5×10^3	1.0×10^4	9.5×10^4	1.2×10^4	2.8×10^4	3.2×10^4

Concentration factor (CF) for Cd, Zn and Fe observed as 10^4 while Hg, Cr, Pb and Mn varied from 10^3 to 10^4 (Table 5.15). Concentration factor for Cu changed between 10^4 and 10^5 in *A. amphitrite* has the ability to accumulate metals several times more than sea water because heavy metals, pesticides and other toxic substances can be absorbed from the water column onto surfaces of fine particles (suspended material and benthic fauna) and they move with the sediments. They participate in various biogeochemical mechanisms and can affect the ecosystems through bioaccumulation and biomagnification processes (GESAMP/UNESCO, 1987, 1994; Salomons & Forstner, 1984).

CHAPTER SIX

CONCLUSIONS

The use of biomonitors is well established in determining geographical and/or temporal variations in the available concentrations of trace contaminants. Barnacles are excellent biomonitors for using because they should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, tolerant of exposure to environmental variations in physico-chemical parameters such as salinity and they should be a net accumulator of the relevant metal.

The highest mean metal concentrations of Hg and Cd in seawater were measured in Izmir-Pasaport due to the industrial activities. The maximum levels of Pb, Cu, Zn, Fe and Cr, Mn were measured in Kusadasi-Setur Marina and Candarli, respectively. For sediment the highest mean concentrations of Hg, Cd, Pb, Cr, Cu, Zn, Mn were recorded in Izmir-Pasaport. The highest mean levels of Fe was found at Izmir-Levent Marina. The concentrations of heavy metals in sediment samples were generally lower in the sampling regions than the polluted areas of the Mediterranean Sea except mercury. In this study, metal levels in the sediment showed no significant temporal variation, while all metals showed significant local distributions.

The highest soft tissue mean concentrations of Hg, Pb and Cd were recorded in barnacles collected from Candarli whereas the lowest mean concentrations were measured in Izmir-Levent Marina and Izmir-Pasaport, respectively. The highest mean values of Cr, Fe and Mn, Zn were obtained from Kusadasi-Setur Marina and Izmir-Pasaport, respectively. The maximum mean level of Cu in barnacles was measured in Candarli. While mercury and copper showed significant spatial and temporal differences in barnacle soft tissue, zinc and lead showed only seasonal; cadmium and chromium indicated spatial variations in this study according to ANCOVA results. The manganese and iron values did not show significant differences both the stations and sampling seasons. As barnacles increase in size, metal burdens are diluted, i.e. larger barnacles have lower metal concentrations.

Barnacles collected from Eastern Aegean coast may have been larger and, therefore, may have comparatively lower body metal concentrations.

Whilst microphagus feeders, such as barnacles, may ingest potentially metal-rich particles, they also pass large volumes of water across the permeable surfaces of their cirri and this further facilitates uptake (Rainbow and White, 1989). According to our results, it is possible to assume that higher metal bioavailability in seawater leads to greater accumulation of metals in barnacles.

Since sediments and most of the barnacles show non significant temporal variation in metal concentrations it would seem likely that environmental factors (pH, salinity, suspended matter, temperature etc.) are not having a greater overall influence on seasonality than biological factors (metabolism, reproduction, fluctuations in tissue weight, etc.).

The mean metal concentrations in seawater generally decreased in the following order: Fe>Zn>Mn>Cu>Pb>Cr>Cd>Hg and in sediment: Fe>Mn>Zn>Cu>Pb>Cr>Hg>Cd. The order of mean metal concentrations in barnacles recorded in this study decreased in the following order Cu>Fe>Zn>Mn>Cd>Cr>Pb>Hg for the study area. These results showed that barnacles accumulate Cu in a higher degree than both sediment and seawater.

Generally, in the Mediterranean Sea, organic and trace metal pollution has increased over the last 20 years and this has had a perceptible effect on marine environments. The land based activities and the intensive maritime traffic and untreated domestic discharges, urban activities are the main source of heavy metals in the study area. The study area can be divided into different type of regions, with different degree of environmental pollution. In addition to metal levels investigated in this region, our results indicate that the inner of Izmir Bay especially harbour area is mostly affected by human activities.

In marine areas throughout the world where recreational boats are densely located, concentrations of copper in the water are being found to be in excess of government standards, due to the hull coatings used on these boats. The results suggest that the amounts of Zn and Cu leached from anti-fouling paints may attain toxic concentrations in areas with high boat density. To fully account for potential ecological risk associated with anti-fouling paints. Cu and Zn concentration were high in all the barnacle species from the sampling sites. It is concluded that the barnacle is suitable candidates to be used in biomonitoring surveys of the Eastern Aegean coast. Therefore, for monitoring programs, the interpretation of variations in metal concentration in biomonitors would be improved by looking at the potential sources of metal and dietary routes of contaminant transfer in organisms This would represent an important advance in the understanding of metal concentrations in biomonitor organisms.

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