DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

MONITORING OF HEAVY METAL LEVELS IN MACROALGAE FROM THE TURKISH COAST OF THE AEGEAN SEA

by

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September, 2009
İZMİR

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **“MONITORING OF HEAVY METAL LEVELS IN MACROALGAE FROM THE TURKISH COAST OF THE AEGEAN SEA”** completed by **İDİL AKÇALI** under supervision of **PROF. DR. FİLİZ KÜÇÜKSEZGİN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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ABSTRACT

Marine organisms were evaluated as possible biomonitors of heavy metal contamination in marine coastal areas. The concentrations of different metals (Hg, Cd, Pb, Cr, Cu, Zn and Fe) were measured in red, brown and green macroalgae species seasonally at eight coastal stations along the Turkish coat of the Aegean Sea. Sediment and seawater samples were also collected from the sampling stations to detect their metal contents in order to gain more information on the environmental conditions of the area and possible bioaccumulation patterns. .The aim of this study is, to gather more information on the use of selected species as cosmopolitan biomonitors for the eastern Aegean; to provide information on the marine environmental quality throughout the use of macroalgae and to determine which algae species are suitable as biomonitoring species for the study area.. Main oceanographic parameters were measured at the sampling stations to evaluate the results more sensitively. The relative abundance of metals in macroalgae decrease in the order: Fe-Zn-Cu-Cr-Cd-Hg-Pb and in seawater: Fe-Zn-Pb-Cu-Cr-Cd-Hg. The distribution order of metals in macroalgae and seawater had the same trend except Pb. In sediment the distribution order from higher to lower was Fe-Cr-Zn-Pb-Cu-Hg-Cd. The highest metal concentrations in seawater were measured from the inner part of the İzmir Bay. The concentrations of heavy metals in sediment samples were lower in the sampling regions than the polluted areas of the Mediterranean Sea. Metal concentrations in macroalgae showed significant correlations with the corresponding ones in seawater and sediment. In all of the macroalgae species Zn and Fe were significantly correlated with each other except for *Codium fragile*. Regarding net accumulation, *Cystoseira* and *Entromorpha* were the strongest accumulators of Cd, Cr, Fe and Hg, Pb, Zn, respectively. *Ulva* turned out to be the highest Cu accumulator. The brown algae *Cystoseira* sp., the green algae *Ulva* sp. and *Entromorpha* sp. possess high potential as cosmopolitan biomonitors for trace metals in Aegean Sea.

Keywords: Biomonitoring, heavy metals, macroalgae, accumulation, Aegean Sea

EGE DENİZİ'NİN TÜRKİYE KIYILARINDA BULUNAN MAKROALGLERDEKİ AĞIR METAL SEVİYELERİNİN İZLENMESİ

ÖZ

Denizel organizmalar, kıyısal alanlarda ağır metal kirliliğinin izlenmesi amacıyla biyoizleyici olarak kullanılmaktadır. Bu çalışmada, Türkiye'nin Ege Denizi kıyısı boyunca sekiz istasyonda bulunan kırmızı, kahverengi ve yeşil makroalg türlerinde mevsimsel olarak bazı ağır metallerin (Hg, Cd, Pb, Cr, Cu, Zn, Fe) birikim düzeyleri saptanmış ve bu makroalg türlerinin biyoizleyici olarak kullanılma potansiyelleri araştırılmıştır. Çevre kalitesi ve biyolojik biriktirme açısından daha fazla bilgi edinmek amacıyla bu istasyonlardan eş zamanlı olarak sediment ve su örnekleri alınmış ve bu örneklerdeki ağır metal miktarlarına bakılmıştır. Örnekleme bölgelerinde sonuçları daha hassas değerlendirmek amacıyla temel oşinografik parametreler ölçülmüştür. Yapılan ölçümler sonucunda, ağır metal konsantrasyonlarının büyükten küçüğe doğru sıralaması makroalgde Fe-Zn-Cu-Cr-Cd-Hg-Pb, su örneklerinde ise Fe-Zn-Pb-Cu-Cr-Cd-Hg olarak belirlenmiştir. Alg ve su örneklerindeki ağır metal konsantrasyonlarının sıralaması Pb hariç aynı düzendedir. Sedimentte ise büyükten küçüğe doğru sıralama Fe-Cr-Zn-Pb-Cu-Hg-Cd şeklinde bulunmuştur. Deniz suyundaki en yüksek metal konsantrasyonları, İzmir Körfezi'nin iç kısımlarında tespit edilmiştir. Çalışma sırasında alınan sediment örneklerindeki ağır metal konsantrasyonlarının Akdeniz'in kirli bölgelerinde ölçülen değerlerden daha düşük olduğu görülmüştür. Makroalg türlerinin ağır metal içerikleri ile deniz suyu ve sedimentteki aynı metal miktarları arasındaki ilişki anlamlı bulunmuştur. *Codium fragile* hariç tüm alg türlerinde Zn ve Fe arasında anlamlı bir ilişki olduğu belirlenmiştir. Net birikim olarak bakıldığında *Cystoseira* ve *Entromorpha* türlerinin sırasıyla Cd, Cr, Fe ve Hg, Pb, Zn açısından güçlü biriktiriciler olduğu tespit edilmiştir. *Ulva* türlerinin yüksek konsantrasyonlarda Cu biriktirdiği görülmüştür. Çalışma sonucunda, Ege Denizi kıyıları boyunca ağır metal kirliliğini izlemede kahverengi alg *Cystoseira* sp., yeşil alg *Ulva* ve *Entromorpha* türlerinin kozmopolit biyoizleyici olarak kullanım potansiyelleri yüksek türler olduğu saptanmıştır.

Anahtar Kelimeler: Biyolojik izleme, ağır metaller, makroalg, akümülasyon, Ege Denizi

CONTENTS

CHAPTER ONE
INTRODUCTION

Urban and industrial activities introduce large amounts of pollutants into the marine environment, causing significant and permanent disturbances in marine systems and, consequently, environmental and ecological degradation. This phenomenon is especially significant in the coastal zones that are the main sinks of almost all anthropogenic discharges of pollutants. It has long been recognised that metals in the marine environment have a particular significant in the ecotoxicology, since they are highly persistent and can be toxic in traces (Claisse and Alzieu, 1993; Langston, 1990;). Certain kinds of contaminants, such as heavy metals, occur naturally in the environment and it is important to be able to distinguish between anthropogenic contamination and background or natural levels to enable accurate evaluation of the degree of contamination in an area.

The use of marine organisms as bioindicators for trace metal pollution is very common these days. Algae and molluscs are among the organisms most used for this purpose (Rainbow, 1995). Macroalgae are able to accumulate trace metals, reaching concentration values that are thousands of times higher than the corresponding concentrations in sea water (Bryan and Langston, 1992; Föster, 1976; Rai *et al.,* 1981). Algae bind only free metal ions, the concentrations of which depend on the nature of suspended particulate matter (Luoma, 1983; Seeliger and Edwards, 1977; Volterra and Conti, 2000) which, in turn, is formed by both organic and inorganic complexes.

The use of biological species in the monitoring of marine environment quality allows to evaluate the biologically available levels of contaminants in the ecosystem or the effects of contaminants on living organisms. The analysis of environmental matrices such as water or sediments provides a picture of the total contaminant load rather than of that fraction of direct ecotoxicological relevance. Thus, the use of biomonitors eliminates the need for complex studies on the chemical speciation (and hence presumptive bioavailability) of aquatic contaminants (Phillips and Segar, 1986).

Despite recognition of the fact that various intrinsic and extrinsic factors can influence metal uptake, determination of the metal concentrations in seaweed is still considered to provide useful information about the levels of metal contamination and environmental quality of an area, albeit of a qualitative nature (Lobban and Harrison, 1997). Moreover, many macroalgae have a relatively long life span and therefore integrate short-term temporal fluctuations in environmental concentrations (Phillips, 1994).

Within the Mediterranean Sea, there have been several studies using seaweed to assess the degree of metal pollution in different regions, e.g., the northern Adriatic Sea (e.g., Munda and Hudnik, 1991) and Lebanon (e.g., Shiber, 1980). However, the coastlines of Greece have been only sporadically investigated and these studies are usually limited to just a few sites, e.g., the Gulf of Thermaikos (Fytianos *et al.*, 1997; Haritonidis and Malea, 1999), Pylos, Ionian Sea (Haritonidis and Nikolaidis, 1990), and the Gulf of Antikyra (Malea *et al.*, 1995). No data are available in macroalgea in the eastern Aegean Sea.

The objective of this work is to gather more information on the use of selected species as cosmopolitan biomonitors for the eastern Aegean; to provide information on the marine environmental quality throughout the use of macroalgea thought bioindicators of the pollution degree; to establish relationship between metal concentrations in macroalgae and sediment, water; to investigate the seasonal changes in metal concentrations in macroalgea and to determine which algae species are suitable as biomonitoring species for the study area.

CHAPTER TWO
HEAVY METALS

2.1 Definition and Properties of Heavy Metals

Heavy metals are elements having atomic weights between 63.546 and 200.590, and a specific gravity greater than 4.0. The term **heavy metal** refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb).

The position of these elements in the periodic table and the different oxidation states of metals determine their toxicity to aquatic organisms. So-called electro negativity no doubt has some bearing on its ecological effects with respect to toxicity to aquatic organisms. The electronic orbital being filled in the atom of the elements is a factor, which may determine its toxicity. Whether it is in ionic form, in an oxidized or reduced state, complexation by organic substance such as chelating agents in added or natural form, adsorbed on inorganic or organic particulate material, or whether it is acting singly or in combination with other cations are the factors determining its uptake by aquatic organisms and its toxicity to them (Waldichuk, 1974).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Trace metals are not usually eliminated from the aquatic ecosystems by natural processes; in contrast to most organic pollutants, most metal pollutants are enriched in mineral and organic compounds. Toxic metals such as Hg, As, Cu, and many other species tend to accumulate in bottom sediments from which they may be released by various process of remobilization, and – in changing form- can move up the biologic chain, thereby reaching human being where they produce chronic and acute ailments (Förstner & Wittmann, 1983). Nonetheless, there is no doubt that all metals are potentially hazardous to living organisms, and not necessarily at large exposure levels.

All heavy metals exist in surface waters in colloidal, particulate, and dissolved phases, although dissolved concentrations are generally low (Kennish, 1992). The colloidal and particulate metal may be found in 1) hydroxides, oxides, silicates, or sulfides; 2) adsorbed to clay, silica, or organic matter. The soluble forms are generally ions or unionized organometallic chelates or complexes. The solubility of trace metals in surface waters is predominately controlled by the water pH, the type and concentration of ligands on which the metal could adsorb, and the oxidation state of the mineral components and the redox environment of the system (Förstner, 1989; Zoumis, Schimidt, Grigorova & Calvano, 2001; Jain, 2004).

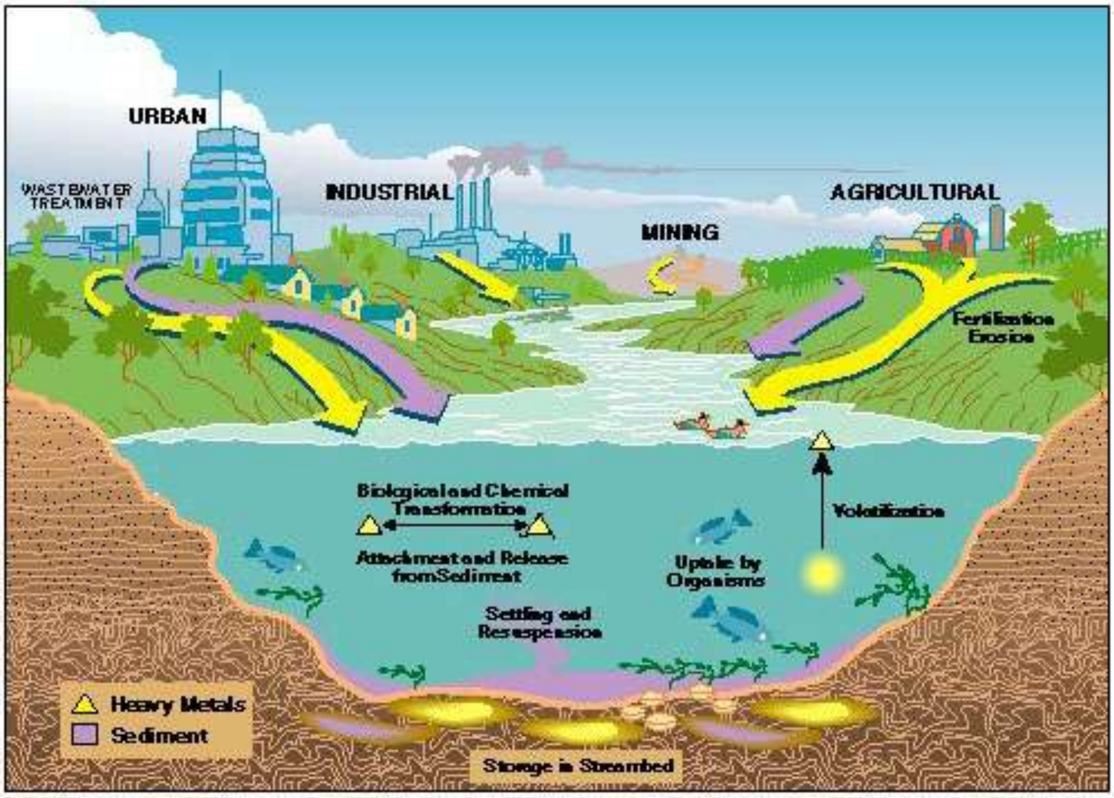

Figure 2.1 The sources of heavy metals (Garbarino, e*t al.*,1995)

2.2 Sources and Inputs of Heavy Metals in the Aquatic Environment

In general, it is possible to distinguish between five different sources from which metal pollution of the environment originates: (1) geologic weathering, (2) industrial processing ores and metals, (3) the use of metals and metal components, (4) leaching of metals from garbage and solid waste dumps, and (5) animal and human excretions (Figure 2.1) (Förstner & Wittmann, 1983).

2.2.1 Geologic Weathering

This is the source of baseline or background levels. It is to be expected that in areas characterized by metal-bearing formations, these metals will also occur at elevated levels in the water of the particular area. The general problem arises of how to distinguish between natural weathering and metal enrichment attributable to human activities (Förstner & Wittmann, 1983).

2.2.2 Mining Effluents

The origin of the high heavy metal values in waters and sediments has been attributed to four supply sources. These are: (1) natural geologic weathering of mineralized zones; (2) erosion and dissolution of mine spoil heaps; (3) surface runoff from soils; (4) dispersion of heavy metals from smelters. Mine drainage does not occur only mine itself but also from waste rock dumps and tailing areas. The latter two sources often contain a high concentration of sulfides and/ or sulfo salts which are associated with most ore and coal bodies. The most commonly occurring sulfides are those of iron, namely pyrite, pyrigotite, and marcasite (Förstner & Wittmann, 1983).

2.2.3 Domestic Effluents and Urban Stormwater Runoff

Metal enrichment which results from residential areas is treated in accordance with its source of origin. Thus, on the one hand there are domestic effluents which are usually discharged from a relatively well-defined point source. On the other hand, urban stromwater runoff is characterized by a diffuse drainage pattern –only partially contributory towards the metal content of domestic effluents– and together with rural areas belongs to the most important nonpoint sources of metal loads in inland waters (Förstner & Wittmann, 1983).

2.2.4 Industrial Effluents

The major industrial effluents of various economically important heavy metals have been compiled Table 2.1. An inspection of this table reveals that most heavy metals under consideration are employed in widely diversified fields such as petroleum refining, steel and fertilizer production, etc. On the other hand, several industries function on a basis where only one specific heavy metal is involved, for example, the use of chromium in the tanning industry. However, in general, the multipurpose usage of numerous heavy metals may lead to difficulties in tracing the source of origin of water pollution conclusively (Förstner & Wittmann, 1983).

Table 2.1 Heavy metals employed in major industries (after Dean *et al.*, 1972)

	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Ni	Sn	Zn
Pulp, papermills, paperboard, building paper, board mills		X	X		X		X	X		X
Organic chemicals, petrochemicals	X	X		X	X		X		X	X
Alkalis, chlorine, inorganic chemicals	X	X		X	X		X		X	X
Fertilizers	X	X	X	X	X	X	X	X		X
Petroleum refining	X	X	X	X			X	X		X
Basic steel works foundries	X	X	X	X	X		X	X	X	X
Motor vehicles, aircraft-plating, finishing	X	X	X		X			X		
Basic nonferrous metal works, foundries	X	X	X		X		X			X
Flat glass, cement, asbestos products, etc.		X								
Textile mill products		X								
Leather tanning, finishing		X								

Chemical and electrochemical methods are employed in the metal finishing and allied industries for the purpose of production and/or the decoration of variety of the metal surfaces. Most processes are allowed by rinsing operations to remove the excess chemicals and other waste material from the treated surfaces, thus giving rise to effluents. Notably, pickling and electroplating give rise to high waste metal concentrations.

It is often overlooked that heavy metal pollution results from the industrial usage of organic compounds containing metal additives. Apart from the well-known case of gasoline (containing tetraethyl lead as additive), there are numerous other examples to support this contention. Thus, oil often contains lead as an additive, whereas lubricating oil is usually supplemented by molybdenum sulfide. Heavy metals are also added to various stearates: For examples, Zn, Sn, Pb and Cd are employed as stabilizers an additives in the manufacture of synthetic rubber and PVC; lead stearate as softener in the manufacture of nitrocellulose; copper stearate for mineral flotation; chrome streate as anti-corrosion agent, etc. (Förstner & Wittmann, 1983).

2.3 Heavy Metals and Organic Life

Heavy metals are often referred as trace metals and the term trace metal might imply the presence of an essential requirement by organisms. Of these, the major metals sodium, potassium, calcium, and magnesium are generally not considered to be heavy metals by any definition. Other metals described as essential to at least some organisms, usually in trace amounts, include aluminium, arsenic, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc. Aluminium falls outside most definitions of heavy metals; arsenic and selenium have variable designations, but the remaining essential trace metals are normally listed among heavy metals. List of nonessential heavy metals usually include cadmium, gold, lead, mercury, and silver, as well as rare, more obscure, metals (including radionuclides) of higher atomic weight (Clark, 1997).

Many metals are essential for living organisms. For example,

- The respiratory pigment hemoglobin, found in vertebrates and many invertebrates, contains iron,
- The respiratory pigment of many molluscs and higher crustaceans, haemocyanin, contains copper,
- Many enzymes contain zinc,
- Vitamins B_{12} contains cobalt,

Metals of biological concern may be divided into three groups:

- Light metals (such as sodium, potassium, calcium), which are normally transported as mobile cations in aqueous solutions,
- Transitional metals (such as iron, copper, cobalt, manganese) which are essential at low concentrations but may be toxic at high concentrations,
- Metalloids (such as mercury, lead, tin, arsenic), which are generally not required for metabolic activity and are toxic to cell at quite low concentrations (Clark, 1997).

Adsorption of heavy metals from solutions is depending on active transport systems in some microorganisms and in sea urchin larvae. Generally, it is by passive diffusion across gradients created by adsorption at the surface and binding by constituents of the cells surfaces and body fluids in plants and animals. An alternative and important pathway for animals is collection of particulate or colloidal material by a food collecting mechanism such as the bivalve gill. There is considerable variation in the extent to which plants and animals can regulate the concentration of metals in the body. Plants and bivalve molluscs are poor regulators of heavy metals, decapods crustaceans and fish are generally able to regulate essential metals such as zinc and copper, but non-essential metals such as mercury and cadmium are less well regulated (Clark, 1997).

Marine organisms tend to accumulate heavy metals from the environment. The accumulation of metals in biota occurs via several pathways, including the ingestion of food and suspended particulate material containing sorbed metals, the uptake of metals either directly from sediments or interstitial waters and the removal of metals from solution. The major routes of metal uptake by invertebrates are solution and food. The drinking of water and consumption of food are primary routes of metal uptake by fish. The gills play a significant role in the entry of dissolved metals. Many factors influence the uptake of trace metals by organisms, most notably physico-chemical factors controlling the metal bioavailability (dissolved metal concentration, temperature, salinity, presence or absence of chelating agents, presence or absence of other metals) and intra-interspecifically variable factors such as surface impermeability, nutritional state and osmotic flux, many of which are in turn affected by other physicochemical factors (Kennish, 1997).

2.4 Heavy Metal Toxicity

Metals in the environment arise from natural sources or directly or indirectly from human activities are potential hazards to aquatic, animal, and human life because of their toxicity and bioaccumulative and nonbiodegradable nature. Acute metal poisoning in humans causes severe dysfunction in the renal, reproductive, and nervous systems, and chronic exposures even at low concentrations in the environment can prove to be harmful to human health. In addition, heavy metals that are discharged from a wide variety of industries such as electroplating, metal finishing, leather tanning, chrome preparation, production of batteries, phosphate fertilizers, pigments, stabilizers, and alloys to the aquatic environment have adverse impacts on aquatic species because they are conserved pollutants that are not subject to bacterial attack or other breakdown and remain as permanent additions to the marine environment. They are dangerous to aquatic animals because they tend to bioaccumulate and cause physiological defects and histopathological manifestations in tissues, resulting in reduced reproduction (Table 2.2). Once mobile in the environment in ionic form, they find their way into the human body through drinking water, food, and air (Krishnani & Ayyappan, 2006; Wyatt et al. 1998; Zuane, 1990).

Metals in their pure state present little hazard, except those having a high vapour pressure, for example, mercury. Nonessential metals such as Hg, Cd, Cr, Pb, As and Sb are toxic in their chemically combined forms as well as the elemental form. It is the water soluble compounds of the metals that create the problems in the aquatic environments. Methyl mercury and tetraethyl lead, some of the metallo-organic compounds are the toxic compounds. The danger of discharging some of the metals into the environment in inorganic form lies in their conversion into the highly poisonous metallo-organic compounds through biological action, as was discovered not too long ago with mercury (Krishnani & Ayyappan, 2006; Waldichuk, 1974).

Various agencies have recommended safe levels for heavy metals for the protection of drinking water, fish and other aquatic life. Several countries bordering the Mediterranean have laws that set a limit for the Hg-Total concentration in seafood because mercury is of special importance for the Mediterranean and many fish and shellfish caught exceeds this limit (UNEP, 1990). The mercury concentration in various compartments of the Mediterranean are derived both from natural and anthropogenic sources. The major natural sources of the atmospheric mercury are land and ocean degassing. The following global values have been suggested by Matheson (1979): land degassing 17800 t/year, open ocean degassing 7600 t/year, coastal water degassing 1400 t/year, and volcanic activity 20 t/year. In framework of MEDPOL, estimated on inputs of mercury in the Mediterranean is domestic 0.75 t/year, industrial 6.92 t/year, rivers 122.3 t/year (UNEP, 1984). No systematic survey of cadmium sources has been carried out in the Mediterranean. General data can not be divided into natural and anthropogenic sources.

Table 2.2 Applications, sources of contamination and potential health effects of heavy metals (Krishnani, Ayyappan, 2006).

Metal	Applications	Sources of contaminant in drinking water	Potential health effects
As	Pesticides, wood preservatives	Erosion of natural deposits, runoff from glass and electronics production wastes	Nausea, vomiting, damage to skin and blood vessels, circulatory problems, cancer
Hg	Batteries, lamps, thermometers, as amalgam in dentistry, pharmaceutical	Erosion of natural deposits, discharge from refineries and factories, runoff from landfills and croplands	Abdominal pain, headache, diarrhea, hemolysis, chest pain, kidney damage, neurotoxicological disorders
Pb	Batteries, petrol additives, alloys, pigments	Corrosion of household plumbing systems; erosion of natural deposits	Anemia, vomiting, loss of appetite, convulsions, damage of brain, liver and kidney, high blood pressure, delays in physical or mental development in children
Cd	Nickel cadmium battery, pigments anticorrosive agent, stabilizers for PVC	Corrosion of galvanized pipes; erosion of natural deposits; discharge from metal refineries; runoff from waste batteries and paints	Diarrhea, growth retardation, bone deformation, kidney and lung damage, testicular atrophy, anemia, injury of central nervous system and liver, hypertension, cancer
Cr	Metal alloys, paints, cement, paper, rubber	Discharge from steel and pulp mills; erosion of natural deposits	Nephritis, gastrointestinal ulceration, diseases in central nervous system, cancer, allergic dermatitis
Cu	Additives to control fungal growth, electrical pipes	Corrosion of household plumbing systems, erosion of natural deposits	Hypertension, uremia, anemia, coma, sporadic fever, gastrointestinal distress, liver or kidney damage
Sb	Flame retardant, battery, pigments ceramics, glass	Discharge from petroleum refineries, fire retardants, ceramic, electronics; solder	Nausea, vomiting, diarrhea; increase in blood cholesterol, decrease in blood sugar, suspected human carcinogens
Se	Photoelectric cells, TV cameras, glass industry	Discharge from petroleum refineries: erosion of natural deposits, discharge from mines	Hair or fingernail loss, numbness in fingers or toes, damage to kidney, nervous system and circulatory tissues, irritability

Arnold et al, (1983) estimate the atmospheric fallout of cadmium about 140 t/year. This value refers both to natural and anthropogenic cadmium. The main anthropogenic sources relate to ore mines, metallurgical industries and to the disposal of sewage sludge. Cadmium is also found in sewage (domestic and mixed) in high proportions relative to other trace metals but the reason for this irregularity is not clear (Huttson, 1982).

There is an increasing concern about metal pollution in the aquatic environment according to Förstner and Whittmann (1983) because first of all they are not usually eliminated from the aquatic systems by natural processes, in contrast to most organic pollutants secondly, most metal pollutants are enriched in mineral and organic substances and they may be released by various processes of remobilization, in changing form, can move up to food chain where they can reach human beings. This development gives rise to greater concern, especially at a time when serious consideration is being given to the exploitation of the oceans as future sources of protein for the growing world population.

2.5 Heavy Metals in the Macroalgea

Seaweeds require inorganic carbon, water, light and various ions for photosynthesis and growth. Their nutrient requirements include the essential elements for growth, completing their vegetative or reproductive cycles (Table 2.3).

Seaweeds require some heavy metals as essential elements for normal growth, as can be seen from the table below. The principal roles of Cu, Zn and Ni are as enzyme cofactors. Manganese plays a vital role in the oxygen evolving system of photosynthesis and is a cofactor in several Krebs-cycle enzymes. Copper is present in plastocyanin, one of the photosynthetic electron transfer molecules and is a cofactor in some enzyme reactions (Bidwell, 1979). Zinc is an activator of several important dehydrogenases and is involved in protein-synthesis enzymes in higher plants. It is essential in algae because it probably plays similar roles (O’Kelley, 1974).

Fe has a low solubility in seawater. Organisms do not respond to the total iron concentration, but rather to the biologically available iron which the ratio is greatly affected by the level of chelators present. Iron has several specific roles in cell metabolism in addition to its role in cell growth. It is at the center of the cytochromes and ferredoxin, which transfer electrons in the respiratory chain and in photosynthesis. The importance of iron in electron transport lies in its ability to change valence between Fe^{2+} and Fe^{3+}, but it is also present in a number of oxidizing enzymes (such as catalase) in which it does not change valance (Lobban& Harrison, 1997).

Table 2.3. Functions and compounds of the essential elements in seaweeds (Lobban & Harrison, 1997).

Element	Probable functions	Examples of compounds
Nitrogen	Major metabolic importance in compounds	Amino acids, purines pyrimidines, amino sugars, amines
Phosphorus	Structural, energy transfer	ATP,GTP, etc., nucleic acids phospholipid, coenzymes(including coenzyme A), phosphoenolpyruvate
Potassium	Osmotic regulation, pH control, protein conformation and stability	Probably occurs predominantly in the ionic form
Calcium	Structural, enzyme activation, cofactor in ion transport	Calcium alginate, calcium carbonate
Magnesium	Photosynthetic pigments, enzyme activation, cofactor in ion transport, ribosome stability	Chlorophyll
Sulfur	Active groups in enzymes and coenzymes, structural	Methionine, cystine, glutathione, agar, carrageenan, sulfolipids, coenzyme A
Iron	Active groups in porphyrin molecules and enzymes	Ferredoxin, cytochromes, nitrate reductase, nitrite reductase, catalase
Manganese	Electron transport in photosystem II, maintenance of chloroplast membrane structure	
Copper	Electron transport in photosynthesis, enzymes	Plastocyanin, amine oxidase
Zinc	Enzymes, ribosome structure	Carbonic anhydrase
Molybdenum	Nitrate reduction, ion absorption	Nitrate reductase
Sodium	Enzyme activation, water balance	Nitrate reductase
Chlorine	Photosystem II, secondary metabolites	Violacene
Boron	Regulation of carbon utilization, ribosome structure	
Cobalt	Component of vitamin B_{12}	B_{12}
Bromine[a] Iodine[a]	Toxicity of antibiotic compounds	Wide range of halogenated compounds, especially in Rhodophyceae

[a]Possibly an essential element in some seaweeds

Some heavy metals such as manganese, iron, copper and zinc are essential micronutrients and frequently are referred to as trace metals. They may limit algal growth if their concentrations are too low, but they can be toxic at higher concentrations; frequently the optimum concentrations range for growth is narrow. Other heavy metals, such as mercury and lead, are not required for growth, and they can become toxic to algae at very low concentrations (e.g., 10-50 μgL^{-1}). Hg, Pb, Cd and Cr are nonessential metals mostly introduced to aquatic environment as a result of human activities. From the standpoint of environmental pollution, metals may be classified into the following groups: 1-noncritical, 2-toxic, but very insoluble or very rare, 3-very toxic and relatively accessible (Table 2.4) (Lobban & Harrison, 1997; Wood, 1974).

Table 2.4 . Classification of elements according to their toxicity and availability (Wood, 1974)

No critical	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

Metals in minerals and rocks are generally harmless, becoming potentially toxic only when they dissolve in water. They can enter the environment through natural weathering of rocks, leaching of soils and vegetation and volcanic activity. Some of the highest mercury levels are found not in coastal waters but in the deep sea, near the mid-ocean ridges, deposited there by submarine volcanic activity. Therefore, in assessing marine pollution, a distinction must be made between natural sources and those due to human activities. Humans contribute metals to the environment during a variety of pursuits: mining and smelting ores, burning fossil fuels, disposing of industrial waste and processing raw materials for manufacturing. Most of the metal load is transported by water in a dissolved or particulate state and most of it reaches the oceans via rivers or land runoff. Also, rainwater carries significant amounts of cadmium, copper, zinc and especially lead from the atmosphere to the oceans. These metals in the atmosphere come from the burning of fossil fuels. Metals in sediments may be reduced or oxidized, primarily by bacteria and release into the overlying water (Lobban & Harrison, 1997).

Metals in a aquatic environment may exist in dissolved or particulate forms. They may be dissolved as free hydrated ions or as complex ions (chelated with inorganic ligands such as OH^-, Cl^- or CO_3^{2-} or they may be complexed with organic ligands such as amines, humic and fluvic acids and proteins. Particulate forms may be found in a variety of situations as colloids or aggregates (e.g., hydrated oxides); adsorbed onto particles; precipitated as metal coatings onto particles incorporated into organic particles such as algae held in the structural lattice in crystalline detrital particles (Beijer & Jerenlöv, 1979). The physical and chemical forms of metal in seawater are controlled by environmental variables such as pH, redox potential ionic strength, salinity, alkalinity, the presence of organic and particulate matter and biological activity as well as by the intrinsic properties of the metal. Changes in these variables can result in transformation of the metals chemical forms and can contribute to the availability accumulation and toxicity of these elements to aquatic organisms (Stokes, 1983; Mance, 1987).

In coastal waters, the concentrations of heavy metals decrease with distance from river mouths. This is the result not only dilution but also of the salting-out process of high molecular weight fractions and flocculation of organic matter as salinity increases; metals may absorb to these newly formed particles and sink to the sediments. On the other hand, some metals previously attached to particles in the river water may be displaced by chloride ions and become available for uptake by algae (Lobban & Harrison, 1997).

Metals are taken up both passively and actively by algae. Some, such as Pb and Sr may be passively adsorbed by charged polysaccharides in the cell wall and intercellular matrix (Morris & Bale, 1975; Eide *et al.,* 1980). Other metals such as Zn, Cd are taken up actively against large intercellular concentration gradients (Eide *et al.,* 1980).

Macrophytes concentrate metal ions from seawater and the variations in the concentrations of metal in the thallus often are taken to reflect the metal concentrations in the surrounding seawater. On that basis macroalgae (especially Phaeophyceae) have frequently been used as indicators of trace metal pollution (Morris & Bale, 1975; Philips, 1977, 1991). The rationale for using seaweeds as indicators of metal contamination has three main bases (Lobban & Harrison, 1997). The first is that metal concentrations in solution often are near the limits of analytical detection and may be variable with time. Seaweeds concentrate metals from solution and integrate short term temporal fluctuations in concentrations. Second, empirical methods for distinguishing the biologically available fraction of the total concentration of a dissolved metal have not been developed for natural systems. By definition, seaweeds will accumulate only those metals that are biologically available. Finally, because plants do not ingest particulate bound metals, plants will accumulate metals only from solution (Lobban & Harrison, 1997).

The accumulated heavy metals may have toxic effects on algal metabolism (Figure 2.2). The order of metal toxicity to algae varies with the algal species and the experimental conditions but generally the order is Hg > Cu > Cd > Ag > Pb > Zn (Rice *et al.,* 1973; Rai *et al.,* 1981).

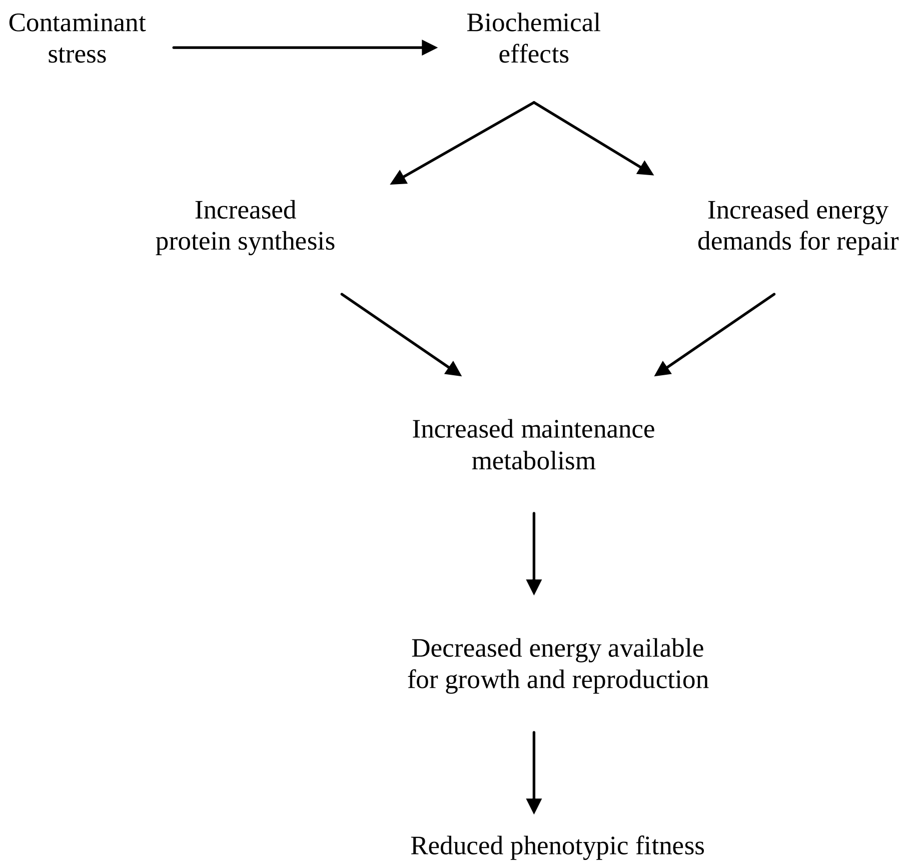

Figure 2.2 Reductions in physical fitness due to the biochemical and physiological effects of a contaminant (Lobban & Harrison, 1997).

Mercury, the most toxic metal interacts with enzyme systems and inhibits their functions especially enzymes with reactive sulfhydryl (-SH) groups (Van Assche & Clijsters, 1990). The toxic effects of mercury on algae generally include 1- cessation of growth in extreme cases 2 –inhibition of photosynthesis 3 –reduction in chlorophyll content and 4 –increased cell permeability and loss of potassium ions from the cell (Rai *et al.,* 1981). There have been studies of the physiological effects of mercury on marine macroalgae. Hopkin & Kain (1978) studied on *Laminaria hyperborea* and found that the growth of gametophytes were affected most by mercury. Respiration rates for sporophytes increased only at the highest concentrations of mercury. A study of the effects of Hg on increases in length for five intertidal Fucales showed that exposure to an average Hg concentration of 100-200 μgL^{-1} for 10 days gave a %50 reduction in growth rate (Strömgren, 1980b). Even at 5-9 μgL^{-1} a reduction in growth was seen in adults of *Fucus spiralis* (Lobban & Harrison, 1997).

Copper, even though it is an essential micronutrient is the second most toxic metal and copper sulfate has been used to control nuisance algae in fresh waters. Copper toxicity is dependent on the ionic activity (concentration of free Cu^{2+}) and not the total copper concentrations (Sunda &Guillard 1976). However some organic copper complexes (specially the lipid soluble ones) are much more toxic than ionic Cu (Stauber & Florence, 1987), because these lipid soluble complexes can diffuse directly through the membrane into the cell. The effects of Cu on macrophytes are important because it is used in antifouling paints and the effects of Cu follow a pattern similar to mercury, have negative impact on reproduction and growth. Cd at sublethal concentrations lead to sharp reductions in photosynthesis and growth rates. Pb and Zn are less toxic to algae, may reduce growth at very high concentrations at very very high concentrations (Lobban & Harrison, 1997).

CHAPTER THREE
MATERIAL AND METHODS

3.1 Study Area

The Aegean Sea is one of the eastern Mediterranean sub basins located between the Greek and Turkish coast and the island of Crete and Rhodes. It is an elongated basin and in the northeast it is connected to the Sea of Marmara through the Strait of Çanakkale and Black Sea through the Strait of Istanbul (Figure 3.1).

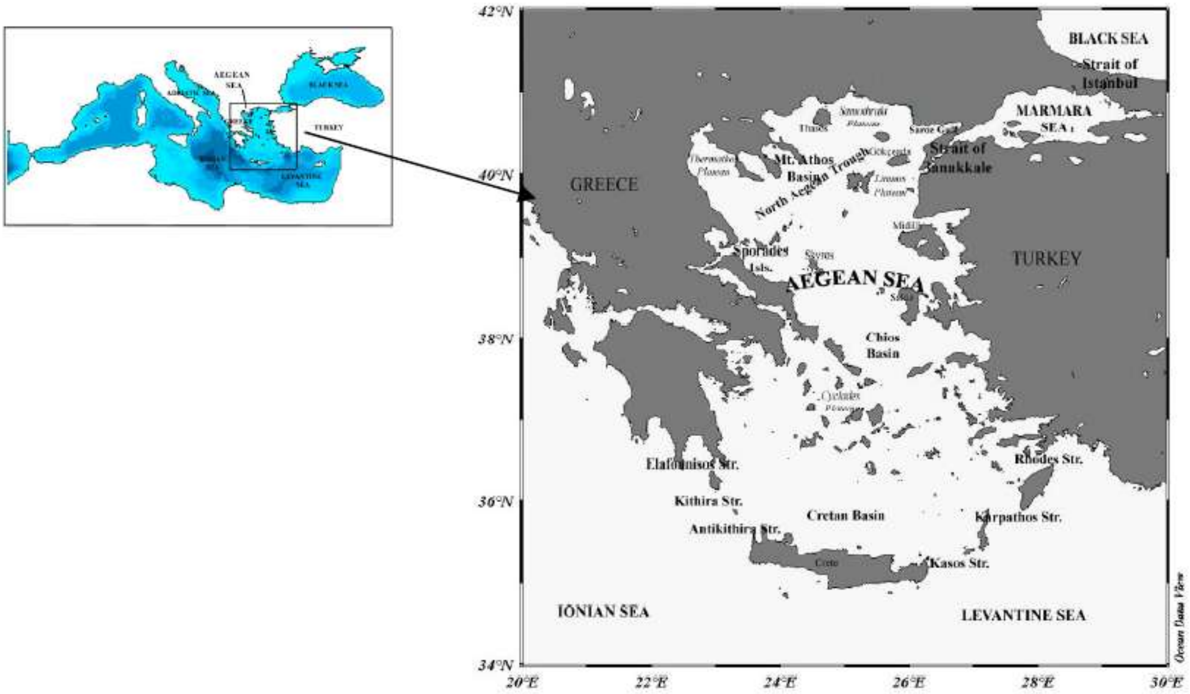

Figure 3.1 The location of the study area, major basins and islands

In the south it is bounded with the Cretan Island and several passages. It is connected to the Levantine Sea to the southeast via the Kassos Strait (sill depth: 1000 m, width 67 km), the Karpathos Strait (sill depth: 850 m, width: 43 km), and the Rhodes Strait (sill depth: 350 m, width 17 km). It joins to the Ionian Sea through three wide passages including the Antikithira Strait (sill depth: 700 m, width: 32km), the Kithira Strait (sill depth: 160 m, width: 33 km) and the Elafonissos Strait (sill depth: 180 m, width: 11 km) (Balopoulos *et al*,. 1999).

It contains more than 200 islands forming small basins and narrow passages with very irregular coastline and topography. It covers an area of 2x105 km^2 and has a volume of 74.000 km^3 and a maximum depth of 2500 m. Bottom topography of the Aegean Sea is very complicated because of the fault block that occurred in the beginning of the Kuvaterner period. The North Aegean Trough is the deepest region, existing in the northern Aegean. It. begins from Saroz Gulf, continues to the northeast-southwest direction including three depressions Samothraki Plateau, Mount Athos basin, and Sporades basin. The sea further extends through the northwest-southeast direction and then it is curled to the northern part of the Create Island. Thus it is ‘S’ shaped and depths reach more than 1000 meters. The Cretan Sea is the deepest basin in the south Aegean reaching a depth of 2500 m. The Cretan Sea is bounded by the Cyclades Plateau with a 100-400 m in depth.

Several major rivers discharge into the Aegean Sea, such as Meric (Maritza River), Nestos, Strimon, Axios and Pinios discharge in the north and Bakırcay, Gediz, Buyuk and Kucuk Menderes in the east. These rivers drain southeastern Europe and western Turkey with a combined annual water discharge ranging between 400 and 2400 m^3 /s, or ~33 km^3 /yr through the Dardanelles. Most of this outflow occurs during the summer (peak in August), closely correlating with the maximum discharge of large rivers draining into the Black Sea, such as Dnieper, Dniester, Don, Danube and Bug.

The Aegean Sea is characterized by a typical Mediterranean type of climate. It is cool and rainy from November to March, hot and dry from May to September. April and October can be characterized as transient months between winter and summer (Poulos, Drakopoulos and Collins, 1997). In the last decade, climatic and oceanographic studies have shown that there have been significant changes in the Aegean Sea. These changes lead to the variations in physical properties not only in the Aegean Sea also in the Eastern Mediterranean.

The grain size distribution of sampling stations were classified according to MED-POL (2006). Marmaris is covered by silty sand; Izmir Bay outer part sandy silt, middle part and inner part are floored clayey silt while, Çanakkale Strait is covered by sand in the Aegean Sea.

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. 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.

3.2 Sampling

The macroalgae, water and sediment samples were collected in eight stations in February, April, July and October 2006 along the Aegean coast. Sampling stations were chosen according to their position along the Aegean coastline. Çanakkale represents the Northern part, İzmir represents the Middle part and Marmaris (Muğla) represents the Southern part of the Aegean coast. The location of sampling points and coordinates are given in Figure 3.2, and Table 3.1, respectively.

Figure 3.2 The location of sampling points in the Aegean Sea

Table 3.1 Species, sampling sites and the sampling periods of macroalgae

Sampling Sites	**Station**	**Species**	**Winter**	**Sampling Spring**	**Period Summer**	**Fall**
Canakkale City	1a	*Cystoseira* sp	+	+	-	+
	1a	*Ulva* sp.	+	+	+	+
	1a	*Enteromorpha* sp.	-	-	-	-
	1a	*Padina pavonica*	-	-	-	-
	1a	*Gracilaria gracilis*	-	-	-	-
	1a	*Codium fragile*	-	-	-	-
Canakkale Dardanos	1b	*Cystoseira* sp.	+	+	+	+
	1b	*Ulva* sp.	+	+	+	+
	1b	*Enteromorpha* sp.	-	+	+	-
	1b	*Padina pavonica*	-	-	-	-
	1b	*Gracilaria gracilis*	-	-	-	-
	1b	*Codium fragile*	-	-	-	-
Izmir Bay Foca	2a	*Cystoseira* sp.	-	+	+	-
	2a	*Ulva* sp.	+	+	+	-
	2a	*Enteromorpha* sp.	+	+	+	-
	2a	*Padina pavonica*	+	+	+	+
	2a	*Caulerpa racemosa*	+	+	+	+
	2a	*Gracilaria gracilis*	-	-	-	-
	2a	*Codium fragile*	-	-	-	+
Izmir Bay Bostanlı	2b	*Cystoseira* sp	-	-	-	-
	2b	*Ulva* sp.	-	+	+	+
	2b	*Enteromorpha* sp.	+	+	-	-
	2b	*Padina pavonica*	-	-	-	-
	2b	*Gracilaria gracilis*	+	+	+	+
	2b	*Codium fragile*	-	-	-	-
Izmir Bay Narlidere	2c	*Cystoseira* sp	-	-	-	-
	2c	*Ulva* sp.	+	+	-	-
	2c	*Enteromorpha* sp.	+	+	-	-
	2c	*Padina pavonica*	-	-	-	-
	2c	*Gracilaria gracilis*	-	+	-	-
	2c	*Codium fragile*	+	+	+	+
Izmir Bay Urla	2d	*Cystoseira* sp.	+	+	+	+
	2d	*Ulva* sp.	+	+	+	-
	2d	*Enteromorpha* sp.	+	-	-	-
	2d	*Padina pavonica*	+	+	+	+
	2d	*Gracilaria gracilis*	-	-	-	-
	2d	*Codium fragile*	-	-	-	-
Marmaris Turunç	3a	*Cystoseira* sp.	+	+	+	+
	3a	*Padina pavonica*	-	+	+	+
Marmaris İçmeler	3b	*Cystoseira* sp	+	+	+	+
	3b	*Padina pavonica*	-	-	-	-

The macroalgae samples were handpicked in the sublittoral zone (0.5-3 m). At each station, different species of algae present during the season (some of the species were not found in all seasons) were sampled and epiphyta covered individuals were rejected. The samples were washed at the sampling site in sea water, transferred to the laboratory in polyethylene bags containing seawater. In the laboratory, the algae samples were first washed with tap water and then with distilled water. Then the samples were dried to a constant weight at 45^{o}C, pulverized and stored in airtight bags at room temperature.

Water quality parameters such as pH, dissolved oxygen (DO), salinity and temperature were measured *in situ* with WTW pH/Cond 304i/Set and Winkler method (Strickland and Parsons, 1972).

3.3 Biology of Macroalgae Species

Algae, with 200,000 species, belonging to the Class Thallaopyta of the plant kingdom, are a group of unicellular, multicellular and macrophytic (seaweeds) organisms, which occur in aquatic ecosystems all over the world, including the Arctic zone. Although these grow in a wide range of habitats, the greatest diversity is seen on rocky seashores and coral reefs. The amount of sun’s energy trapped by algae is believed to be 10 times the amount trapped by all terrestrial plants. They are the major primary producers of organic compounds and play a key role in food chains (Kaur and Bhatnagar, 2002).

Algae can be aquatic or subaerial, when they are exposed to the atmosphere rather than being submerged in water. Aquatic algae are found almost anywhere from freshwater spring to salt lakes, with tolerance for a broad range of pH, temperature, turbidity, and O_2 and CO_2 concentration. They can be planktonic, like most unicellular species, living suspended throughout the lighted regions of all water bodies including under ice in polar areas. They can be also benthic, attached to the bottom or living within sediments, limited to shallow areas because of the rapid attenuation of light with depth. Benthic algae can grow attached on stones (epilithic), on mud or sand (epipelic), on other algae or plants (epiphytic), or on animals (epizoic). In the case of marine algae, various terms can be used to describe their growth habits, such as supralittoral, when they grow above the high-tide level, within the reach of waves and spray; intertidal, when they grow on shores exposed to tidal cycles: or sublittoral, when they grow in the benthic environment from the extreme low-water level to around 200 m deep, in the case of very clear water (Barsanti and Gualtieri, 2006).

The term seaweeds traditionally include only macroscopic, multicellular marine red, green and brown algae. However, each of these groups has microscopic, if not unicellular, representatives. All seaweeds at some stage in their life cycles are unicellular, as spores or zygotes, and may be temporarily planktonic. The seaweeds have a valuable ecological role and economic potential. The marine plants, like their terrestrial counterparts, are benign and need to be investigated for their economic value and conserved for posterity. (Kaur and Bhatnagar, 2002; Lobban and Harrison, 2000).

The seaweeds are primarily attached plants, fixed to the substrate by some kind of holdfast, an organ of primary structural importance. The holdfast may sometimes resemble root of higher plants, but both its structure and its functions are distinctly different. Above the holdfast, the thallus (the plant body that is not differentiated into stems and leaves) may consist of a simple filament, a branched filament, a hollo tube or bladder, a bushy tuft of cylindrical or flattened branches, or of a simple or compound blade, sometimes called a lamina (Dawson, 1996; Debelius and Baensch, 1997).

Marine algae are evolutionarily quite diverse. The four traditional divisions (or phyla, phylum) – often contain a reference to the color of organisms included in them: Cyanophyta, blue-green algae; Rhodophyta, red algae; Phaeophyta (Heterokontophyta), brown algae; Chlorophyta, green algae – are assigned to two or more kingdoms depending on the systematist. Cyanophyta are clearly placed in the Kingdom Eubacteria, but the others are either in Plantae (because they are basically multicellular) or in Protista (because they are closely related to unicellular algae). A new kingdom, Chromista, golden algae, has recently been proposed to encompass the “brown-algal line,” namely,Phaeophyta, Chrysophyta and Pyrrhophyta. Some authors would recognize this group at the level of a division ,Chromophyta (Lobban and Harrison, 1997; Van den Hoek, Mann and Jahns, 1995).

Several characteristics are used to classify algae, including the nature of the chlorophyll(s), the cell wall chemistry and flagellation. One common characteristic is that all types of algae contain chlorophyll *a*. However, the presence of phytopigments other than chlorophyll *a* is characteristic of a particular algal division (phylum). The nature of the reserve polymer synthesized as a result of photosynthesis is also a key variable used in algal classification. It is important to point out, however, that there have been many classification schemes employed to date. Table 3.2 is a summary of algal divisions, restricted to those which possess a cell wall and their most significant characteristics. There are important differences in between common algal divisions in the storage products they utilize as well as in their cell wall chemistry. The presence and chemistry of the cell wall is very important for the biosorption mechanism(s). Biosorption in algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation can play a role. Typical algal cell walls of Phaeophyta (Heterokontophyta), Rhodophyta, and many Chlorophyta are comprised of a fibrillar skeleton and an amorphous embedding matrix. The most common fibrillar skeleton material is cellulose (Fig. 3.3). It can be replaced by xylan in the Chlorophyta and Rhodophyta in addition to mannan in the Chlorophyta. The Phaeophyta algal embedding matrix is predominately alginic acid or alginate (the salt of alginic acid) with a smaller amount of sulfated polysaccharide (fucoidan) whereas the Rhodophyta contains a number of sulfated galactans (e.g. agar, carregeenan, porphyran, etc.). Both the Phaeophyta and Rhodophyta divisions contain the largest amount of amorphous embedding matrix polysaccharides. This characteristic, combined with their well known ability to bind metals, makes them potentially excellent heavy metal biosorbents (Davis, Volesky and Mucci, 2003).

Table 3.2 Three algal divisions and their characteristics (Davis *et al.*,2003)

Division (Phylum)	Common name	Pigments	Storage product	Cell wall	Flagella
Chlorophyta	Green algae	Chlorophyll a,b; α-,β- and γ-carotenes and several xanthophylls	Starch (amylose and amylopectin) (oil in some)	Cellulose in many (β-1,4-glucopyroside), hydroxyproline glucosides; xylans and mannans; or wall absent; calcified in some	Present
Phaeophyta (Heterokontophyta)	Brown algae	Chlorophyll a,c;β-carotene andfucoxanthin and several other xanthophylls	Laminaran (β-1,3-glucopyranoside, predominantly); mannitol	Cellulose, alginic acid, and sulphated muco-polysaccharides (fucoidan)	Present
Rhodophyta	Red algae	Chlorophyll a (in some Florideophyceae); R- and C-phycocyanin, allophycocyanin; R- and B-phycoerythrin. α- and β-carotene and several xanthophylls	Floridean starch (amylopectin-like)	Cellulose, xylans, several sulphated polysaccharides (galactans) calcification in some; alginate in corallinaceae	Absent

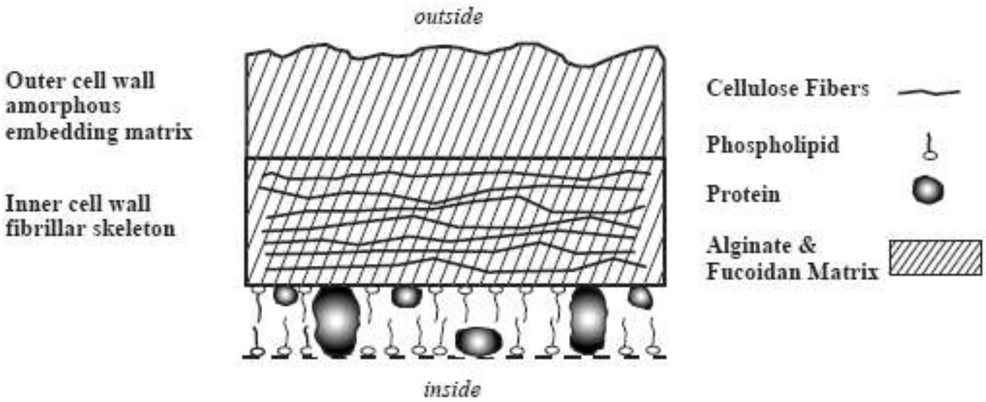

Figure 3.3 Cell wall structure in brown algae (Schiewer and Volesky, 2000)

The biological characteristics of the marine algae species that are sampled are given below (www.algaebase.com):

Phylum: **Rhodophyta**
  Class: **Florideophyceae**
    Order: **Gracilariales**
      Family: Gracilariaceae
        - *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine&W.F. Farnham

Phylum: **Heterokontophyta** (**Phaeophyta)**
  Class: **Phaeophyceae**
    Order: **Dictyotales**
      Family: Dictyotaceae
        - *Padina pavonica* (Linnaeus) Thivy

    Order: **Fucales**
      Family: Sargassaceae
        - *Cystoseira* sp.

Phylum: **Chlorophyta**
  Class: **Bryosidophyceae**
    Order: **Bryopsidales**
      Family: Caulerpaceae
        - *Caulerpa racemosa var. cylindracea* (Sonder) Verlaque, Huisman & Boudouresque

      Family: Codiaceae
        - *Codium fragile* (Suringar) Hariot

  Class: **Ulvophyceae**
    Order: **Ulvales**
      Family: Ulvaceae
        - *Enteromorpha sp.*
        - *Ulva sp.*

3.3.1 Rhodophyta

The red algae are mostly macroscopic seaweeds which share the rocky, coastal waters with green and brown algae. They are usually smaller and more delicate than the brown algae. Red algae tend to be more abundant in subtropical and tropical waters than in temperature and polar seas and they are often more abundant in deeper waters than the green and brown algae which generally prefer colder water and the intertidal zone. There are 5000-5600 species of red algae, which are distributed among 500-600 genera. The average size of the plants differs according to geographic regions. The larger species of fleshy red algae occur in cool-temperate areas, whereas in trophical seas, the Rhodophyta (except for massive calcareous forms) are mostly small, filamentous plants. In addition to the more conspicuous fleshy or membranous forms, red algae also occur as unicells, small- branched filaments, crustose forms and as small parasites on other red algae. The cell walls of a number of red algae are heavily impregnated with $CaCO_3$. Rhodophyta mostly inhabit in marine ecosystems but they are also fairly common in fresh water and present in terrestrial environment. They have a number of characteristics, in spite of their size and extremely complex life cycles, that may be considered primitive including the lack of flagellated cells, simple chloroplast structure and the presence of phycobilin pigments (Darley, 1982; Dawson, 1966; van den Hoek *et al.*, 1995).

There is only chlorophyll a present in Rhodopyta, chlorophyll b and c are absent, and the green of the chlorophyll is masked by the red accessory pigment phycoerythrin. The blue pigment phycocyanin also occurs in the chloroplasts of Rhodopyhta. The more definitive characteristics of the red algae are seen in their manner of sexual reproduction. Unlike brown and green algae, reproductive cells have no flagella, the nonflagellated male gametes reach a fixed female reproductive cell by passive movement in the water medium. The marine Rhodophyta are all multicellular plants with only two exceptions which are *Porphyridium* and *Rhodosorus*. The nuclei are usually small and inconspicuous. In the Florideophyceae, the relatively large protoplasmic strands between adjoining cells (pit connectios) is common place. The most important storage product is a polysaccharide, floridean starchand and the grains of this material are formed in the cytoplasm (Dawson, 1966; van den Hoek *et al.*, 1995).

Some red algae have considerable economic importance. A number of species are utilized for food in the Far East and other countries. The membranous *Porphyra* in particular is intensively cultivated in Japan and eaten as ‘nori’. Agar the familiar gelling agent for culture media, is a complex polysaccharide found in cell walls containing galactose and galactose derivatives. Carrageenan is a sulphated galactan which is utilized as a stabilizer and emulsifier in dairy products, toothpastes, cosmetics and a number of other products (Darley, 1982).

3.3.1.1 Gracilaria gracilis

The Gracilariaceace family have six genera of which *Gracilaria*, with over 100 species, has the largest number. The species of *Gracilaria* widely distributed throughout temperate and tropical waters of the world. The Gracilariaceace which are characterised by multiaxial construction with the medullary cells being parenchymatous and there are no filamentous cells in the mature vegetative thallus (Dawes, 1998; Dawson, 1966).

Gracilaria gracilis has a bushy, reddish brown to purple color thalli with cylindric branches (0.5-3 mm diameter). They are usually seen in sheltered sites where substrata are soft and unstable, comprising sand-silt mixtures (Figure 3.4) (Fischer, Schneider, Bauchot 1987; Critchley, 1993).

Gracilaria is a major agarophyte, currently providing greater than half of the world’s supply of agar. *Gracilaria* has been harvested from naturally occurring stocks in a number of countries in the developing world, but due to over-harvesting and declining populations, cultivation is an increasingly important source of raw material. The cultivation of *Gracilaria,* both in the sea and in tanks, has been a principal factor in making this genus a source of agar-containing seaweeds. In Taiwan, *Gracilaria* is farmed in brackish-water ponds as a main food source for the cultivation of the shellfish abalone Haliotis. Human consumption of species of the red alga *Gracilaria* has been linked to “ogonori” poisoning. The symptoms are hypotension (abnormally low blood pressure), vomiting, nausea, and death resulting from hypotensive shock. Ogonori poisoning is caused by prostaglandin E2 (Fig. 4.43). Soaking Gracilaria in freshwater results in the production of prostaglandin E2. This is usually compounded by eating seafood which is rich in prostaglandin E2. *Gracilaria gracilis* is also used as fertilizer and for preparation of medicine because of its antimicrobial property. (Critchley, 1993; Fischer *et.al.*, 1987 ; Lee, 2008).

Figure 3.4. *Gracilaria gracilis* (www.algaebase.com)

3.3.2 Heterokontophyta (Phaeophyta)

The brown algae, except for a few genera, are exclusively marine, usually dominating the rocky, intertidal vegetation in temperate to subpolar seas and being much less conspicuous in tropical regions. They are an important assemblage of plants that are classified in about 265 genera with more than 1500 species. They derive their characteristic colour from the large amounts of the carotenoid fucoxanthin (which yields a brown colour) contained in their chloroplasts and the presence of various phaeophycean tannins (Figure 3.5) (Darley, 1982; Davis *et al.*, 2003).

The chloroplasts also have chlorophylls a, c_1, and c_2. There are two membranes of chloroplast E.R., which are usually continuous with the outer membrane of the nuclear envelope. The storage product is laminarin. There are no unicellular or colonial organisms in the order, and the algae are basically filamentous, pseudoparenchymatous, or parenchymatous. They are found almost exclusively in the marine habitat, there being only four genera containing freshwater species. Phaeophycean cell walls are generally composed of at least two layers, with cellulose making up the main structural skeleton. The amorphous component of the cell wall is made up of alginic acid and fucoidin, whereas the mucilage and cuticle are composed primarily of alginic acid. Calcification of the wall occurs only in some species of Padina where calcium carbonate is deposited as needle-shaped crystals of aragonite in concentric bands on the surface of the fan-like thallus (Lee, 2008).

Brown algae are commercially important in a number of ways. In the past they have been used as a soil conditioner and fertilizer, providing trace elements, potassium, nitrogen and phosphorus. They are also used as fodder and as supplement in human diet in some countries although they are not used as extensively as the red algae. The most important commercial use of brown algae today is a source of alginic acid, an uronic acid gel which is used as a filler or stabilizer in a number of products (Darley, 1982).

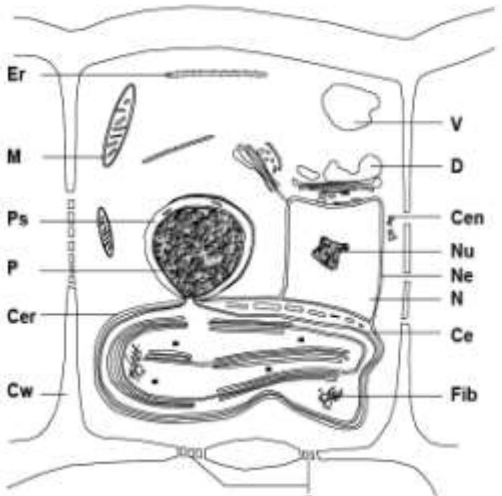

Figure 3.5 Schematic diagram of a brown algal cell. (Ce) chloroplast envelope; (Cer) chloroplast endoplasmic reticulum; (Er) endoplasmic reticulum; (Ne) nuclear envelope; (Fib) DNA fibrils; (Nu) nucleolus; (N) nucleus; (P) prenoid; (Ps) prenoid sac; (D) dictyosome (also known as golgi apparatus or golgi dictyosome); (M) mitochondrian; (V) vacuole; (F) plasmodesma pit field; (Cw) cell wall; (Cen) centrioles (Davis, Volesky, Mucci,2003)

3.3.2.1 Padina pavonica

Dictyotales is a distinctive order with a widespread occurrence in tropical and subtrophical regions. This order has organisms that grow by means of an apical cell or by a marginal row of apical cells. There is an isomorphic alternation of erect, flattened, parenchymatous thalli. A distinctive character of this order is the modification of the unilocular sporangia to produce four to eight large aplanospores. The Dictyotales are common in warmer waters throughout the world (Dawson, 1966; Lee, 2008).

The only calcified genus in the Phaeophyceae, *Padina*, is in the Dictyotales. *Padina pavonica* grows abundantly in the Mediteranean. This alga is abundant from June to September, on the coast from the surface down to 60 m. below. *Padina pavonica* has a fan shaped thalli. The color of thalli changes from yellowish brown to light brown or slightly whitish (Figure 3.6). The reason of the whiteness is the deposition of calcium carbonate.

Calcium carbonate is deposited on the outside of the plant in the form of the aragonite crystals. They attach to solid substrates or they are epiphytic on large macrobenthic algae and seagrass (Fischer *et.al.*, 1987; Lee, 2008; www.icpconcepts.com). *Padina pavonica* is used as a source of algin (Fischer *et. al.*, 1987).

Figure 3.6. *Padina pavonica*

3.3.2.2 Cystoseira sp.

The algae belonging the order Fucales produce only one type of thallus during the life cycle: macrothalli. These grow to moderate sizes. The shape of the thallus varies greatly from genus to genus and even from species to species (van den Hoek *et. al.*, 1995).

The genus *Cystoseira* has a worldwide distribution, 80% of the species occur along the Mediterranean and the adjoining Atlantic coasts, especially in the upper infralittoral zone (0-1 m depth) of the Mediterranean coasts. The *Cystoseira* sp. are usually the dominant element of the benthic vegetation on unpolluted hard substratum except *Cystoseira barbata* because this species can adapt the changes in salinity, temperature and addition of organic pollutants (Fischer *et. al.*, 1987; Montesanto & Panayotidis, 2000).

The alga has cylindrical fronds. The stem is thick and long and furnished with elliptical knobs each producing a brach, many times dischotoma-pinnate and filiform (Figure 3.7). They have air vessels to support the upright position (Greville, 1839). *Cystoseira* sp. are rich in alginates and sterols (Fischer *et. al.*, 1987).

Figure 3.7. *Cystoseira* sp.

3.3.3 Chlorophyta

The green algae are one of the larger groups of algae in terms of numbers of species and are almost as widely distributed and well adapted to extreme habitats. The green algae have also evolved the greatest diversity of body form found among the algae. A great range of somatic differentiation occurs within the Chlorophyta, ranging from flagellates to complex multicellular thalli differentiated into macroscopic organs. The different level of thallus organization (unicellular, colonial, filamentous, siphonous and parenchymatous) have traditionally served as the basis of classification of this division.

The division Chlorophyta contains around 500 genera and approximately 8000 species. A lot of these live in freshwater but there are also many marine and terrestrial species. Some orders are predominantly marine (Caulerpales, Dasycladales, Siphonocladales), whereas others are predominantly freshwater (Ulotrichales, Coleochaetales) or exclusively freshwater (Oedogoniales, Zygnematales). The class Ulvophyceae is almost entirely restricted to marine habitats. On rocky sea coasts green algae are particularly abundant and dominantin the upper part of the intertidal zone. Rocks are often completely covered with green algae, including species of the genera *Ulva*, *Enteromorpha* and *Ulothrix* (only in spring). Species of *Ulva* and *Enteromorpha* can also form dense growths on sandy shores and mudflats in protected, still environments. The *Caulerpa* species can grow on sandy or muddy bottoms, they are anchored in the sediment by creeping stolons (Lee, 2008; van den Hoek *et al.*, 1995).

All Ulvophyceae known to date are sessile organisms having walled vegetative cells, except for a small group of species, the thalli are usually multicellular or coenocytic during at least some part of the life history. Many species have microscopic, filamentous thalli, but most are macroscopic seaweeds, capable of considerable morphological differentiation. Unbranched and branched filamentous forms are common among the green algae. Some species have a well developed or even dominant, basal, prostrate, pseudoparenchymatous system of filaments from which the erect filaments develop (Darley, 1982; Barsanti and Gualtieri, 2006).

Cell walls usually have cellulose as the main structural polysaccharide, although xylans or mannans often replace cellulose in the Caulerpales. The cell wall composition thought to be dynamic, changing according to the age of the plant and at different stages in the life cycles of species. Chloroplast pigments are similar to those of higher plants; chlorophyll a and b are present. The main carotenoid is lutein. The siphonaceous genera, as well as the unicells *Tetraselmis* and *Mesostigma,* are the only green algae to have siphonoxanthin and its ester siphonein. Starch is formed within the chloroplast, in association with a pyrenoid, if one is present. The starch is similar to that of higher plants and is composed of amylose and amylopectin. The photosynthetic pathways are similar to those of higher plants (Lee, 2008; van den Hoek *et al.,* 1995).

In Japan *Ulva* and *Enteromorpha* species are grown for food. They are culvitated in nutrient-rich bays or estuaries. Japanese eat *aonori*, which is a mixture of sea lettuce (*Ulva*), green laver (*Enteromorpha*) and *hitoegusa* (*Monostroma latissimum*) (Lobban& Harrison, 1997; van den Hoek *et al.*, 1995).

3.3.3.1 Ulva sp.

The genus *Ulva* is very common in the world’s oceans. In Europe there about ten species of *Ulva,* of which *U. lactuca* is the best known.. The leaf-like thalli of sea lettuce can vary in length from several centimetres to more than a meter. The large surface of thallus to volume ratio allows it to have a high nutrient uptake. The plants grow attached to solid substrata, anchored by an attachment disc. Many species can continue to grow even when detached. The thallus is two cells thick (distromatic), with each cell having a large cup-shaped chloroplast toward the exterior of the cell (Fig. 3.8). The holdfast is formed by the cells of the thallus.

Ulva species are normally marine species although it can be found in brackish waters, particularly in estuaries. It normally grows on rocks in the middle to low intertidal zone, although the fronds are not situated at the same level throughout the year. During the colder months the plants grow mainly in the middle intertidal zone, covering wide vertical areas. In the warmer months the *Ulva* is lower in the intertidal zone and in a narrower band. *Ulva* contain high pigment concentration, so they can absorb all wavelengths of light and do better in shallow waters. These species also prefer areas where waterwave shearing forces are low and herbivory is reduced. (Lee, 2008; van den Hoek *et al.*, 1995; www. mbari.org).

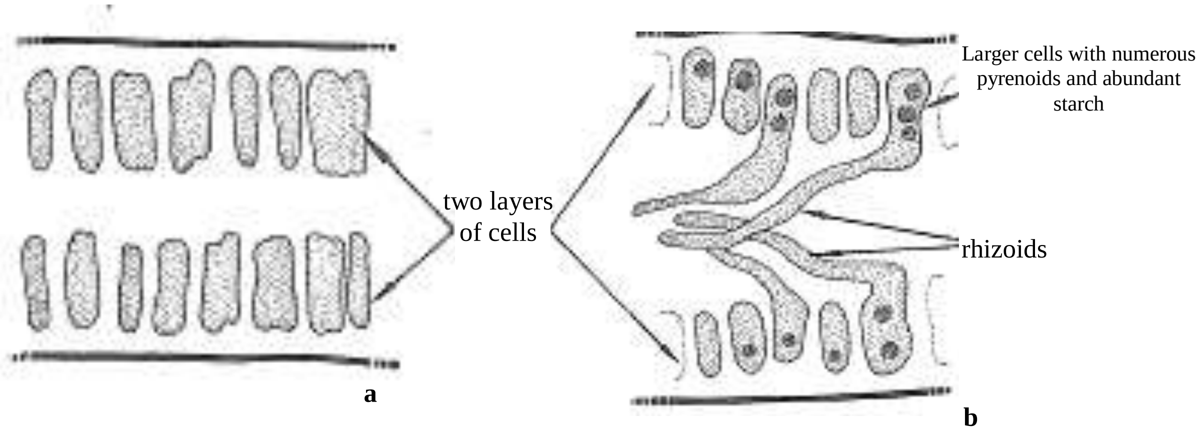

Figure 3.8 Cross-section of thallus a) upper part of frond, b) basal part of frond (Fischer, Hureau, 1985)

Ulva is an opportunistic alga, capable of rapid colonization and growth when conditions are favorable. This occurs primarily because of a rapid growth rate and the ability to take up and store nutrients available in pulsed supply. Because of its ability to quickly respond to enhanced nutrient supply, *Ulva* has proliferated in many areas that have received anthropogenic (related to man) nutrient enrichment. The presence of *Ulva* species often indicates freshwater input or pollution. The cosmopolitan distribution, simple morphology and ease of growth assessment, along with a graded tolerance and response to stress induced by pollutants make *Ulva* species good bioindicators. A feature of nuisance growths of *Ulva* in enclosed and semienclosed waters is that *Ulva* comprises a large proportion of drift plants, which may smother other benthic communities or be cast ashore where they decompose, causing considerable aesthetic nuisance (Lee, 2008).

Ulva is commonly known as the sea lettuce and has been eaten as a salad, used in soups, added to *Enteromorpha* and *Monostroma* as a part of aonori and can be used as sea vegetables for human consumption, mixed with other vegetables (Figure 3.9). The chemical composition of dried *U. latuca* is 15% protein, 50% sugar and starch, less than 1% fat, and 11% water, making it usable as roughage in the human digestive system. They also can be used as medicine and as a source of vitamin E, A, B1 (Barsanti and Gualtieri, 2006; Carpenter, Niem 1998; Lee, 2008).

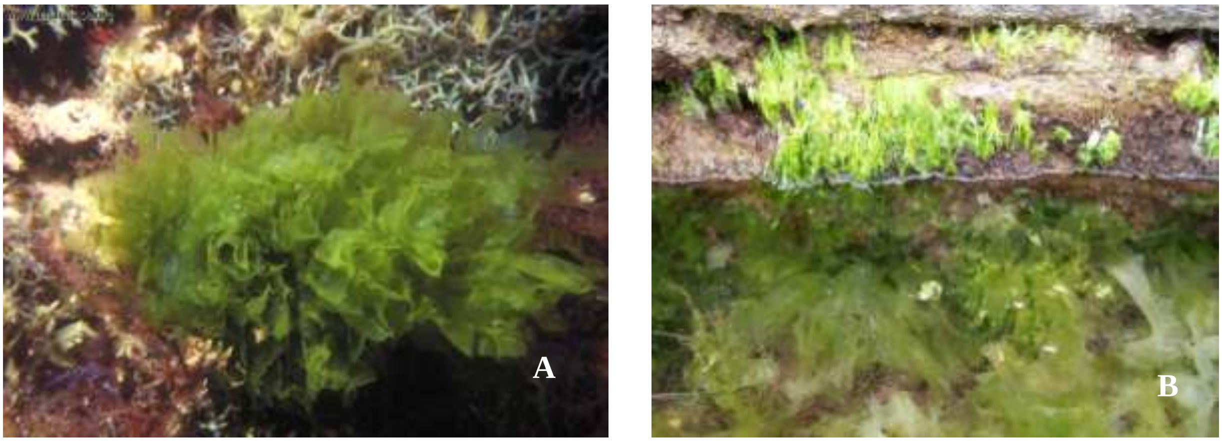

Figure 3.9 **A**. Detail of *Ulva* sp. (www.algaebase.org), **B**. Photograph of *Ulva* sp. from the Foça station

3.3.3.2 Enteromorpha sp.

The species of *Enteromorpha* with *Ulva* sp. are the most conspicuous of the green algae in the oceans. They are usually bright green and often so large and abundant as to form a prominent component of most marine floras. They occur in all seas and generally grow at brackish or polluted areas or in salt marshes. *Enteromorpha* occupies the same kinds of habitats as *Ulva* and they sometimes grow together in great abundance. About 25 species of *Entromorpha* have been found in Europe, in brackish and marine waters. A few species penetrate into fresh water (Dawson, 1996; van den Hoek *et al.*, 1995).

It can be difficult to identify the species of the genus *Enteromorpha* in the field and they have similarities to *Ulva*, so it is sometimes difficult to differentiate between the two different genuses. *Enteromorpha* can be recognized by their hollow, membranous construction. The thalli of *Enteromorpha* are tubular (although the tubes are often partially compressed) and may be branched or unbranched. The walls of the tube are only one cell layer thick (monostromatic) (Fig. 3.10), while *Ulva* has flat blades that are two cell layers thick. *Enteromorpha* is attached to the substrate by a disc-like holdfast. These species can grow on a wide variety of substrates: sand, mud, rock, wood, concrete or metal. They can also grow without any substrate, the detached *Enteromorpha* from the substrate rise to the surface and continue to grow, covering the surface of the water (Dawson, 1996; www.mbari.org; van den Hoek *et al.,* 1995).

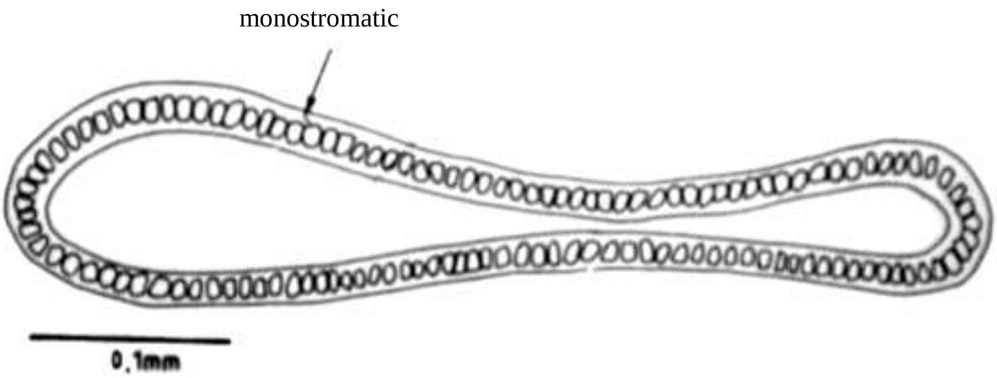

Figure 3.10. Cross-section of *Enteremorpha* sp. thallus (FAO, 1987)

Enteromorpha is one of the green macroalgae genera cultivated in Japan and known as green laver (Figure 3.11). It contains about 20% protein, little fat, low sodium, and high iron and calcium. Its vitamin B-group content is generally higher than most vegetables, and while its vitamin A is high, it is only half of that found in spinach. They can be used for human consumption (contains vitamin A, B1, B2), animal feed, fertilizer and as medicine because they have an antibacterial property (Barsanti and Gualtieri, 2006; Carpenter, Niem, 1998).

Figure 3.11.*Enteromorpha* sp. (www.algaebase.org).

3.3.3.3 Codium fragile

The genus *Codium* including more than 80 species is distributed from tropical to temperate areas. Most *Codium* species have narrow geographic ranges, while *Codium fragile* (Suringar)

Hariot originally described from Japan is widely distributed in temperate areas throughout the world. Moreover, one subspecies, *C. fragile* subsp. *tomentosoides* (van Goor) Silva, thought to be native to Japan, rapidly spread in temperate areas throughout the world during the 20th century (Nanba, Kado, Ogawa, Nakagawa&Sugiura, 2005).

Codium species are siphonous green algae that form large multinucleate thalli which are at least technically single cells interweaving of numerous narrow siphons. The thallus of *Codium* has cylindrical branches. *Codium* species have two morphologically different thalli which are called spongy and filamentous (Figure 3.12). The spongy thalli composed of pigmented utricles and intertwined colorless medullary filaments are amorphously creeping, spherical or branched erect. On the other hand, the filamentous thalli are composed of fine branched filaments. The filamentous thalli had been considered as early developmental stage of *Codium* spongy thalli. The *Codium* species trap gas among the filaments to support and keep their selves upright. This speciality of the *C. fragile* subsp. t*omentosoides* has become a nuisance in some countries because it becomes buoyant enough to carry the cultivated shellfish to which they have attached (Lobban& Harrison, 1997; Nanba *et al.,* 2005).

Whole plants of *C. fragile* are able to fix nitrogen, owing to an association between the alga and a nitrogen-fixing bacterium (*Azotobacter*) on the surface of the alga. The alga secretes 0.7 to 1.3 mg glucose per gram of dry weight of the alga per hour, or 16% to 31% of the carbon assimilated to the outside of the thallus. The bacterium uses the secreted glucose and in turn fixes the nitrogen. The nitrogen fixation occurs only under conditions of nitrogen deficiency and is probably an important factor in the growth of *Codium* in shallow bays under oligotrophic conditions. *Codium fragile* shows a number of adaptations to its habitat. During winter months when the availability of dissolved inorganic nitrogen is at its highest in the water, *C. fragile* accumulates reserves of nitrogen which are utilized in times of relative nitrogen deficiency. The period of maximum carbon fixation, pigment content, and chloroplast size occurs during the early winter when competition from other algae is minimal and variation in tidal amplitude is decreased. In the summer, the physical environment is more extreme, owing to increased drying in the intertidal zone and increased competition from other algae. *Codium fragile* effectively retires from much of this competition by undergoing reproduction in the summer. This is accompanied by the development of frond hairs which may increase nutrient uptake. Symbiotic associations between a number of molluscs and flatworms with chloroplasts of the Codiales are fairly common. Some molluscs normally feed on siphonaceous Chlorophyceae such as *Codium* and *Caulerpa* by puncturing the cells and sucking out the contents. The chloroplasts are not always digested, and many chloroplasts lodge in the body of the animal and actively photosynthesize (Lee, 2008).

Codium fragile has economic value: it is cultivated for human consumption in Asia, used as invertebrate food by the mariculture industry, is a pest for natural and cultivated shellfish beds, is a source of bioactive compounds(antibacterial, antibiotic, anticarcinogenic, immuno-suppressive, anti-insect and antihelminthic activity) and accumulates heavy metals, thus providing a model indicator of pollution (Trowbridge, 1998; Fischer *et. al.*, 1987).

Figure 3.12. Detail of *Codium fragile* (www.algaebase.org).

3.3.3.4 Caulerpa racemosa

Caulerpa is the only genus in the Caulerpaceae family and is a common inhabitant of intertidal and infratidal tropical and semitropical marine waters. The plants have a creeping green rhizome (stolon), that do not have internal divisions segregating the algae into individual cells, with root-like colorless rhizoids and frond-like erect shoots. The erect shoots exhibit a considerable variation in morphology after which some of the species are named. The thallus looks like a higher plant that has roots, stems and leaves, but it is not anatomically equal. The tube- shaped thallus consists of a single immense cell, which is multinucleate and contains one to several vacuoles and a thin layer of protoplasm. The thallus derives support from turgor pressure and from wall ingrowths, the trabeculae. The walls have a β-1, 3 linked xylan as the main structural component (Debelius and Baensch, 1997; Lee, 2008; van den Hoek *et al.*, 1995).

The development of aquaculture, aquariums and international shipping has led to worldwide exposure of marine environments to non-indigenous species of algae. More than 60 macroalgal species have been introduced into the Mediterranean Sea. Species of *Caulerpa* are the more aggressive of these introduced algae, quickly overgrowing native algae by the rapid elongation of its stolons (2 cm d^{-1}). The spread of *Caulerpa* is also due to a unique method of asexual reproduction. No zoospores are formed; instead breakage of the thallus results in new viable plants. The *Caulerpa* spp. in the Mediterranean Sea may be Red Sea migrants through the Suez Canal (Lessepsian species) (Lee, 2008).

Caulerpa racemosa was first collected in Tunisia at the Mediterranean Sea in 1926. Later its presence was reported throughout the eastern basin of the Mediterranean Sea, but without any comment about a potential invasive tendency. Until 1990, these populations remained stationary. At the beginning of the 1990s, however, the situation abruptly changed and *Caulerpa racemosa* continuously spread throughout most of the Mediterranean Sea, generally resulting in the development of extensive populations. The reason for this extensive distribution is thought to be the response to global sea water warming (Verlaque, Boudouresque, Meinesz &Gravez, 2000).

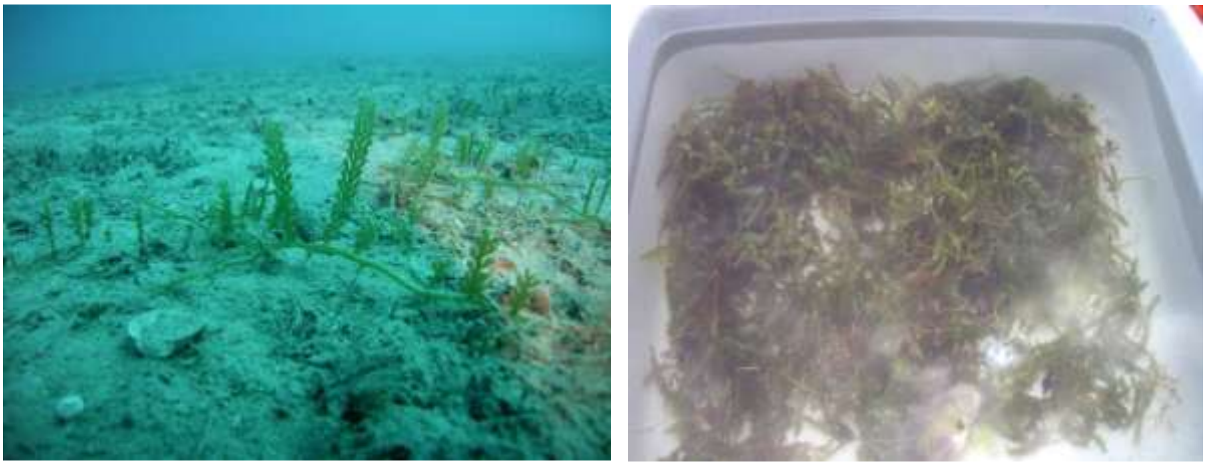

Figure 3.13. Detail of *Caulerpa racemosa*.

The typical *racemosa* variety characterised by the possession of short erect branches bearing crowded ramuli with short stalks and oval or spherical tips (Figure 3.13). The arrangement and shape of the ramuli differ among the numerous varieties. The ramuli can be sparse or dense, arranged radially, alternately, pinnately or irregularly on the erect branch. Plants growing on sandy substrate in calm, turbid water tend to have long erect branches while those growing on rocky-wave exposed portions possess strong short erect branches (Carpenter, Niem, 1998).

These species are being used for human consumption, as a source of caulerpin which is a substance with anaesthetic effect, as a source of caulerpicin which has an toxic effect and as medicine (antifungal, lowers blood pressure) (Carpenter, Niem, 1998).

3.4 Analytical Procedures

The water samples were collected in (about 10 l) preconditioned polyethylene bottles. They were acidified by adding 1 N HNO_3. Ion exchange technique, using Chelex-100 resin was applied for heavy metals in water samples (Dorfner, 1972; Khym, 1974). A few drops of HNO_3 were added as preservative to another unfiltered samples (500 ml) for determining total mercury.The samples (500 ml) for determining total mercury were measured by BrCl oxidation (Fitzgerald, Clarkson, 1991), followed by $SnCl_2$ reduction. Total Hg analyses in water samples were carried out using a mercury concentration accessory. Mercury metal readily forms an amalgam with metals and the trapped mercury is subsequently released by electro thermally heating the mercury amalgamation trap to generate a concentrated mercury vapour, which is delivered to the atomic absorption spectrometer (AAS). The detection limit for total Hg using amalgamation technique in water samples is 10 ngl^{-1}.

Sediment samples were collected using with a small stainless steel Van-Veen grab sampler operated by handling from the middle of the grab to a depth of 1 cm, wrapped in polyethylene bag and stored at -20°C for subsequent analysis in order to avoid degradation. The sediment samples were freeze-dried and homogenized and reduced to a fine powder. In the laboratory, three subsamples of each sample (approximately 1 g dry weight, DW) were digested in microwave digestion system (Milestone 1200) with HNO_3-HF-$HClO_4$-HCl acid mixture solution and diluted to the desired volume with double distilled water.

The macroalgae samples were freeze-dried and homogenized and reduced to a fine powder. In the laboratory, three subsamples of each macroalgae sample (approximately 1 g dry weight) were digested in microwave digestion system (Milestone 1200) with HNO_3-HF-$HClO_4$-HCl acid mixture solution and diluted to the desired volume with double distilled water.

All the analyses were performed by Varian (Spectra AA-300 Plus) atomic absorption spectrophotometer. Mercury concentration was measured by cold vapour technique; other heavy metals were measured by flame and graphite furnace using the manufacturer's conditions and with background correction (UNEP, 1982; 1985). The detection limits for heavy metals are Hg:0.05 μgl^{-1}, Cd:0.10 μgl^{-1}, Pb:0.10 μgl^{-1}, Cu:0.03 mgl^{-1}, Zn:0.01 mgl^{-1} and Fe:0.06 mgl^{-1}.

3.5 Quality Assurance

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 mg kg^{-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), 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), 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.

Accuracy of atomic absorption spectrophotometer and validity of the processes tested with a reference material (sea plant homogenate, IAEA-140/TM, from the International Laboratory of Marine Radioactivity, IAEA, Monaco). The values obtained for the analysis of three replicates of this sample (certified: observed values in mg/kg dry weight ± standard deviation) were as follows: Hg, 0.038 ± 0.006 : 0.037 ± 0.002; Cd, 0.54 ± 0.037 : 0.057 ± 0.049; Pb, 2.19 ± 0.28 : 2.27 ± 0.39; Cu, 5.05 ± 0.28 : 5.73 ± 0.25; Zn, 47.3 ± 2.0 : 47.4 ± 0.40 and Fe, 1256 ± 35 : 1233 ± 31.

3.6 Statistical Analyses

Data will be processed using the STATISTICA 5.1 (Stat.Soft. Inc.1998, Tulsa) in order to obtain mean values, standard errors and confidence levels. One-way and two-way ANOVA are going to be used to compare the heavy metal concentrations among seasons and sampling stations. Spearman Rank Order Correlation test will be used to check for significant relationships heavy metals in algae, sediment and seawater. The different statistical methods will be performed with a 95% confidence interval (significance, $p<0.05$). Statistical analysis were performed considering all samples and elements. These analysis addressed whether the studied heavy metals differentiate in seaweeds according to their type and/or location and which seaweed is more appropriate to use in monitoring studies.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Physico-Chemical Properties

The physico-chemical parameters have been analyzed in order to characterize the Aegean coastal waters and to find possible correlations between metal concentrations and some of these parameters. Table 4.1 illustrates the basic physico-chemical parameters of the sampling points during different periods. These parameters included pH, salinity, temperature and dissolved oxygen (DO). The pH of the aquatic system is an important indicator of the water quality and the extent of the pollution in the watershed areas. A pH range of 6.5-8.5 is normally acceptable as per guideline suggested by WHO (1993). The pH values obtained for the Aegean coastal waters were 8.10-8.85 indicates the moderately alkaline nature of the seawater. Low temperature values were measured as 7.4-14.0 °C at Çanakkale/City and Dardanos during winter and spring periods. The highest temperature values (24.7-26.5°C) were found at Marmaris/İçmeler and Turunç stations during summer and fall periods.

Conductivity reflects the status of major ions inorganic pollution and is a measure of total dissolved solids and ionized species in the water. Conductivity levels ranged between 35.5-47.7 mSi/cm in Çanakkale/City and Çanakkale/Dardanos stations due to Black Seawaters. There were no big differences in conductivity values in other sampling stations. It ranged between 52.8-59.5 mSi/cm during sampling periods. The salinity observed with values between 22.3-30.5 psu in Çanakkale/City and Dardanos same as conductivity. Seasonal salinity variations are very low in the other sampling stations.

During the cold period DO showed higher values than the other periods in the Çanakkale/City and Dardanos. The significant decrease in DO during spring, summer and fall periods were observed at Çanakkale/City, İzmir/Narlıdere and Marmaris/İçmeler sampling stations. During the warm periods, high DO concentrations were observed in İzmir/Narlıdere and İzmir/Urla stations. DO concentrations varied from 7.93 to 9.04 mgl^{-1} İzmir/Foça and Marmaris/Turunç stations.

Table 4.1 Physico-chemical parameters of the sampling sites

Region/Site	**Season**	**DO (mg/l)**	**Salinity (psu)**	**pH**	**Temperature (°C)**	**Conductivity mSi/cm**
Çanakkale/City	Winter	10.25	26.4	8.25	7.4	42.4
	Spring	9.07	25.2	8.49	12.8	40.2
	Summer	6.95	22.3	8.50	23.3	35.5
	Autumn	6.57	23.0	8.46	20.4	36.6
Çanakkale/Dardanos	Winter	10.95	28.9	8.66	11.3	45.9
	Spring	7.86	30.5	8.71	14.0	47.7
	Summer	8.08	29.8	8.85	22.3	46.1
	Autumn	8.44	28.5	8.48	21.6	44.4
İzmir Bay/Foça	Winter	7.93	38.6	8.27	14.7	58.8
	Spring	8.55	38.7	8.32	17.4	58.6
	Summer	9.04	39.4	8.49	24.0	58.9
	Fall	8.12	38.9	8.41	18.3	58.8
İzmir Bay/Bostanlı	Winter	8.33	37.4	8.40	16.2	57.3
	Spring	13.09	38.6	8.85	22.8	58.0
	Summer	12.39	39.5	8.49	28.4	58.8
	Fall	10.34	39.3	8.47	20.5	59.2
İzmir Bay/Narlıdere	Winter	8.12	39.1	8.62	15.4	59.5
	Spring	8.54	38.4	8.17	18.6	58.1
	Summer	6.55	39.5	8.49	24.8	59.2
	Fall	6.93	38.8	8.19	20.4	58.6
İzmir Bay/Urla	Winter	7.95	38.2	8.80	15.1	58.4
	Spring	7.96	38.7	8.10	17.3	58.7
	Summer	10.23	40.1	8.53	23.2	59.1
	Fall	9.94	38.9	8.36	19.1	58.7
Marmaris/Turunç	Winter	8.32	37.1	8.45	16.2	53.5
	Spring	7.72	39.2	8.78	18.1	59.2
	Summer	7.50	39.5	8.45	24.7	59.5
	Fall	7.23	39.9	8.36	26.5	59.5
Marmaris/İçmeler	Winter	8.30	34.3	8.19	15.8	52.8
	Spring	8.16	39.0	8.61	18.6	58.9
	Summer	7.10	39.3	8.24	25.0	59.1
	Fall	6.30	39.9	8.27	25.7	59.6

4.2 Metal Content in Seawater

Table 4.2 and 4.3 show the metal concentrations of water samples taken from the stations during 2006. The maximum Hg concentration (0.49 μgl^{-1}) was found in Bostanlı for all sampling periods. The minimum concentration of Hg was obtained in Foça, İzmir Bay, which was 0.08 μgl^{-1}. The lowest concentration of Cd was measured as 0.09 μgl^{-1} in Marmaris/Turunç, while the highest Cd (0.49 μgl^{-1}) was observed in Bostanlı/İzmir. The maximum levels of Pb (3.20 μgl^{-1}), Cr (1.53 μgl^{-1}), Cu (5.57 μgl^{-1}) and Zn (10.36 μgl^{-1}) were measured in Bostanlı. The maximum heavy metal levels were found in Bostanlı.

The minimum concentrations of Pb, Cr, Cu and Zn were observed at station Dardanos (Çanakkale). The lowest concentration of Fe was observed in Turunç, while the highest value was found at Bostanlı station. The highest Fe levels were also measured in İzmir region. Generally, the minimum metal concentrations were measured in Çanakkale/Dardanos, since there is no industrial or domestic pollution at this station. The highest metal concentrations were found in İzmir Bay due to the industrial activities from the inner part of the İzmir Bay.

The mean metal concentrations in seawater decreased in the following order: Fe>Zn>Pb>Cu>Cr>Cd>Hg in the Aegean coast (Table 4.4). The orders were different in Bostanlı (Fe>Zn>Cu>Pb>Cr>Cd>Hg) and Marmaris/İçmeler (Fe>Cu>Zn>Pb>Cr>Cd>Hg) from the other sampling stations. Regional order differed only in Marmaris (Fe>Cu>Zn>Pb>Cr>Cd>Hg). The concentrations of all metals in seawater were lower than the plant and sediment.

The Spearman correlation coefficient of metal concentrations in seawater demonstrated a positive correlation ($p<0.05$) of Hg with Fe ($R=0.505$) and Cu with Zn ($R=0.401$). Metal concentrations in macroalgae showed significant correlations with the corresponding ones in seawater (Table 4.16). The one-way ANOVA revealed that the temporal variations of metal concentrations in seawater were significant for Hg ($F=4.721$, $p=0.009$), Pb ($F=9.146$, $p=0.0002$) and Cr ($F=21.109$, $p=0.00$) while no significant variations were found for all metals in sampling stations.

Table 4.2 Heavy metal levels in seawater samples from the Aegean coast in February and April 2006 (μgl^{-1})

Region/Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
February 2006							
Çanakkale/City	0.16	0.25	0.94	0.75	0.50	1.44	6.89
Çanakkale/Dardanos	0.30	0.31	0.87	1.12	0.98	1.26	6.18
İzmir Bay/Foça	0.28	0.24	0.68	0.71	0.45	0.92	2.45
İzmir Bay/Bostanlı	0.49	0.38	3.20	1.53	5.57	10.36	103.60
İzmir Bay/Narlıdere	0.30	0.44	0.94	1.31	0.78	2.06	11.13
İzmir Bay/Urla	0.17	0.38	1.00	1.29	0.79	1.68	2.76
Marmaris/Turunç	0.12	0.34	0.94	1.00	1.03	3.44	6.34
Marmaris/Içmeler	0.14	0.28	0.88	0.81	2.88	1.88	5.97
April 2006							
Çanakkale/City	0.12	0.33	0.19	0.41	0.76	3.27	7.15
Çanakkale/Dardanos	0.21	0.35	0.14	0.58	0.66	0.92	42.00
İzmir Bay/Foça	0.08	0.38	0.95	1.11	0.90	1.60	5.76
İzmir Bay/Bostanlı	0.26	0.28	0.92	0.72	0.58	1.33	12.58
İzmir Bay/Narlıdere	0.16	0.41	0.24	0.47	0.74	1.53	48.18
İzmir Bay/Urla	0.14	0.36	0.29	0.39	0.70	1.67	9.51
Marmaris/Turunç	0.12	0.42	0.19	0.44	1.00	0.83	2.17
Marmaris/Içmeler	0.09	0.45	0.16	0.42	1.08	0.68	2.76

Table 4.3 Heavy metal levels in seawater samples from the Aegean coast in July and October 2006 (µgl^{-1})

Region/Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
			July 2006				
Çanakkale/City	0.14	0.39	1.30	0.21	1.20	2.42	6.41
Çanakkale/Dardanos	0.17	0.18	0.78	0.20	0.40	0.40	24.88
İzmir Bay/Foça	0.24	0.40	1.21	0.26	1.42	3.06	17.65
İzmir Bay/Bostanlı	0.31	0.35	1.30	0.24	0.84	1.33	61.69
İzmir Bay/Narlıdere	0.29	0.43	1.46	0.31	1.14	0.91	39.75
İzmir Bay/Urla	0.31	0.36	1.55	0.33	1.91	1.41	16.41
Marmaris/Turunç	0.15	0.09	1.70	0.28	1.16	1.22	3.86
Marmaris/Içmeler	0.23	0.41	1.27	0.28	0.75	1.00	4.89
			October 2006				
Çanakkale/City	0.12	0.33	2.58	0.60	0.62	1.16	57.10
Çanakkale/Dardanos	0.15	0.38	1.20	0.79	0.85	1.02	26.20
İzmir Bay/Foça	0.11	0.39	1.42	0.66	0.66	1.13	4.16
İzmir Bay/Bostanlı	0.11	0.49	1.72	0.83	0.80	1.52	2.75
İzmir Bay/Narlıdere	0.09	0.40	1.73	1.09	0.69	1.68	2.38
İzmir Bay/Urla	0.16	0.39	1.56	0.70	0.57	0.96	2.85
Marmaris/Turunç	0.15	0.43	1.59	0.78	0.61	0.98	4,95
Marmaris/Içmeler	0.13	0.42	1.55	0.76	0.70	0.90	5.94

Table 4.4 The mean±*SE* and minimum-maximum concentrations of the heavy metals in seawater during sampling periods (μgl^{-1})

Station	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale City	0.13±0.01	0.33±0.03	1.25±0.50	0.49±0.12	0.77±0.15	2.07±0.48	19.4±12.6
	0.12-0.16	0.25-0.39	0.19-2.58	0.21-0.75	0.50-1.20	1.16-3.27	6.41-57.1
Çanakkale Dardanos	0.21±0.03	0.31±0.04	0.75±0.22	0.67±0.19	0.72±0.13	0.90±0.18	24.8±7.33
	0.15-0.30	0.18-0.38	0.14-1.20	0.20-1.12	0.40-0.98	0.40-1.26	6.18-42.0
İzmir/ Foça	0.18±0.05	0.35±0.04	1.07±0.16	0.69±0.17	0.86±0.21	1.68±0.48	7.51±3.45
	0.08-0.28	0.24-0.40	0.68-1.42	0.26-1.11	0.45-1.42	0.92-3.06	2.45-17.7
İzmir/Bostanlı	0.29±0.08	0.38±0.04	1.79±0.50	0.83±0.27	1.95±1.21	3.64±2.24	45.2±23.4
	0.11-0.49	0.28-0.49	0.92-3.20	0.24-1.53	0.58-5.57	1.33-10.4	2.75-104
İzmir/Narlıdere	0.21±0.05	0.42±0.01	1.09±0.33	0.80±0.24	0.84±0.10	1.55±0.24	25.4±11.0
	0.09-0.30	0.40-0.44	0.24-1.73	0.31-1.31	0.69-1.14	0.91-2.06	2.38-48.2
İzmir/Urla	0.20±0.04	0.37±0.01	1.10±0.30	0.68±0.22	0.99±0.31	1.43±0.17	7.88±3.25
	0.14-0.31	0.36-0.39	0.29-1.56	0.33-1.29	0.57-1.91	0.96-1.68	2.76-16.4
Marmaris/Turunç	0.13±0.01	0.32±0.08	1.11±0.35	0.63±0.16	0.95±0.12	1.62±0.61	4.33±0.88
	0.12-0.15	0.09-0.43	0.19-1.70	0.28-1.00	0.61-1.16	0.83-3.44	2.17-6.34
Marmaris/İçmeler	0.15±0.03	0.39±0.04	0.97±0.30	0.57±0.13	1.35±0.52	1.12±0.26	4.89±0.75
	0.09-0.23	0.28-0.45	0.16-1.55	0.28-0.81	0.70-2.88	0.68-1.88	2.76-5.97

4.3 Metal Content in Sediment

The metal concentrations of sediment samples were given in Table 4.5 and 4.6. The highest Hg value (0.08 μgg^{-1}) was found in Bostanlı and the lowest value (0.02 μgg^{-1}) was found in İçmeler, Marmaris. The maximum concentrations of Cd (0.047 μgg^{-1}) and Cu (32.4 μgg^{-1}) were obtained at Turunç, Marmaris station and the minimum Cd concentration (0.004 μgg^{-1}) was obtained at station Dardanos, Çanakkale. The highest concentration of Pb was measured as 51.10 μgg^{-1} in Foça and the lowest measured concentration was 1.75 μgg^{-1} (Dardanos). The maximum Zn (83.6 μgg^{-1}) and Fe (36545 μgg^{-1}) values were observed in Narlıdere and the minimum concentrations of Zn (8.90 μgg^{-1}) and Fe (4524 μgg^{-1}) were found in Dardanos, Çanakkale.

The mean metal concentrations in sediment decreased in the following order: Fe>Cr>Zn>Pb>Cu>Hg>Cd in the Aegean coast (Table 4.7). Hg, Cd and Zn levels in algae were higher than the sediment. Mercury, cadmium and zinc levels in macroalgae were 2.3, 5.8 and 1.6 times higher than the sediment, respectively. The order in Marmaris region was different from the general abundance of metals in sediment (Fe>Cr>Zn>Cu>Pb>Hg>Cd). The highest levels of Hg, Pb, Zn and Fe were measured in İzmir Bay, while Cd, Cr and Cu were found in Marmaris due to the geological characteristics.

Metal concentrations in sediment showed significant positive correlations (Spearman rank-order correlation coefficient, $p<0.05$) between Hg-Zn (R=0.351), Cd-Cu (R=0.464), Cr-Cu (R=0.571), Cr-Fe (R=0.414), Cu-Zn (R=0.619), Cu-Fe (R=0.642) and Zn-Fe (R=0.873). Negative correlations were found between Hg-Cr (R=-0.408) and Pb-Cr (R=0.413) in sediment.

The one-way ANOVA demonstrated that no metal in the sediment showed significant temporal variation. The analysis of variance revealed that all metals (except lead); Hg (F=3.271, p=0.014), Cd (F=2.723, p=0.031), Cr(F=24.493, p=0.00), Cu (F=7.18, p=0.0001), Zn (F=3.972, p=0.005) and Fe (F=15.031, p=0.00) showed significant local distribution

Table 4.5 Heavy metal levels in sediment samples from the Aegean coast in February and April 2006 (µgg^{-1} dry weight)

Region/Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
			February 2006				
Çanakkale/City	0.046	0.012	36.1	27.7	15.1	46.2	16353
Çanakkale/Dardanos	0.028	0.004	15.5	38.3	4.10	10.4	4524
İzmir Bay/Foça	0.046	0.015	36.5	21.9	7.30	26.4	8251
İzmir Bay/Bostanlı	0.074	0.034	8.70	40.8	10.7	34.9	22525
İzmir Bay/Narlıdere	0.059	0.007	19.8	163.1	18.0	73.0	36545
İzmir Bay/Urla	0.065	0.005	29.1	31.6	7.70	30.8	11987
Marmaris/Turunç	0.030	0.035	11.1	91.1	32.4	29.3	15662
Marmaris/Içmeler	0.026	0.018	13.8	145.8	24.0	27.7	16033
			April 2006				
Çanakkale/City	0.036	0.007	10.4	53.9	5.20	13.0	5785
Çanakkale/Dardanos	0.048	0.003	32.1	47.1	18.0	69.2	18524
İzmir Bay/Foça	0.052	0.008	51.1	29.3	8.70	31.5	8420
İzmir Bay/Bostanlı	0.080	0.030	11.6	63.9	18.6	56.9	22781
İzmir Bay/Narlıdere	0.045	0.006	12.7	117.5	16.1	69.1	32946
İzmir Bay/Urla	0.022	0.007	24.3	37.0	3.90	21.4	8940
Marmaris/Turunç	0.027	0.047	6.60	63.7	29.7	32.9	14290
Marmaris/Içmeler	0.020	0.018	6.20	211.1	27.9	63.1	13152

Table 4.6 Heavy metal levels in sediment samples from the Aegean coast in July and October 2006 (μgg^{-1} dry weight)

Region/Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
			July 2006				
Çanakkale/City	0.020	0.034	18.70	77.2	25.2	58.9	21260
Çanakkale/Dardanos	0.018	0.005	1.75	69.1	6.68	8.90	4813
İzmir Bay/Foça	0.050	0.021	19.37	41.3	7.60	23.9	7447
İzmir Bay/Bostanlı	0.041	0.006	13.22	64.2	8.02	21.1	12630
İzmir Bay/Narlıdere	0.033	0.014	11.57	141.0	18.7	68.6	32058
İzmir Bay/Urla	0.055	0.005	8.10	37.0	3.70	17.6	8025
Marmaris/Turunç	0.027	0.004	9.78	83.3	29.5	23.4	12287
Marmaris/Içmeler	0.019	0.028	11.47	231.0	19.9	27.4	10995
			October 2006				
Çanakkale/City	0.026	0.015	10.90	41.9	6.40	14.6	5722
Çanakkale/Dardanos	0.026	0.018	18.00	40.0	12.0	15.0	6357
İzmir Bay/Foça	0.035	0.018	13.70	32.6	6.90	30.4	7021
İzmir Bay/Bostanlı	0.043	0.042	14.00	18.6	13.1	46.6	22139
İzmir Bay/Narlıdere	0.027	0.011	9.50	140.0	17.1	83.6	30437
İzmir Bay/Urla	0.021	0.006	11.80	34.2	3.20	20.5	6870
Marmaris/Turunç	0.027	0.023	18.30	64.5	23.4	30.6	12087
Marmaris/Içmeler	0.013	0.016	24.10	136.0	5.80	18.3	12163

Table 4.7 The mean±*SE* and minimum-maximum concentrations of the heavy metals in sediment during sampling periods (µgg^{-1} dry weight)

Station	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale City	0.032±0.01 0.02-0.046	0.017±0.01 0.007-0.034	19.0±6.00 10.4-36.1	50.2±10.5 27.7-77.2	13.0±4.64 5.2-25.2	33.2±11.5 13.0-58.9	12280±3899 5722-21260
Çanakkale Dardanos	0.030±0.01 0.018-0.048	0.007±0.004 0.003-0.018	16.8±6.22 1.75-32.1	48.6±7.09 38.3-69.1	10.2±3.08 4.10-18.00	25.9±14.5 8.90-69.2	8555±3347 4524-18524
İzmir/ Foça	0.046±0.004 0.035-0.052	0.015±0.003 0.008-0.021	30.2±8.50 13.7-51.1	31.3±4.02 21.9-41.3	7.63±0.39 6.90-8.70	28.1±1.76 23.9-31.5	7785±331 7021-8420
İzmir/Bostanlı	0.060±0.010 0.041-0.080	0.028±0.01 0.006-0.042	11.9±1.17 8.70-14.0	46.9±10.9 18.6-64.2	12.6±2.25 8.00-18.6	39.9±7.70 21.1-56.9	20019±2466 12630-22781
İzmir/Narlıdere	0.041±0.007 0.027-0.059	0.01±0.002 0.006-0.014	13.4±2.24 9.5-19.8	140±9.31 118-163	17.5±0.56 16.1-18.7	73.6±3.48 68.6-83.6	32996.5±1292 30437-36545
İzmir/Urla	0.041±0.011 0.021-0.065	0.006±0.0004 0.005-0.007	18.3±4.99 8.1-29.1	34.9±1.3 31.6-37.0	4.63±1.04 3.2-7.7	22.6±2.86 17.6-30.8	8956±1096 6870-11987
Marmaris/Turunç	0.0278±0.001 0.027-0.030	0.0272±0.009 0.004-0.047	11.4±2.47 6.6-18.3	75.7±6.86 63.7-91.1	28.8±1.89 23.4-32.4	29.1±2.03 23.4-32.9	13582±853 12087-15662
Marmaris/İçmeler	0.0195±0.003 0.013-0.026	0.0199±0.003 0.016-0.028	13.9±3.76 6.2-24.1	181.0±23.6 136-231	19.4±4.82 5.8-27.9	34.1±9.9 18.3-63.1	13086±1077 10995-16033

4.4 Metal Content in Macroalgaea

Metal concentrations determined in different species of macroalgae from all sampling sites are given in Table 4.9-4.13. The concentrations of metals are expressed as the minimum/maximum and mean value ± *SD* obtained from the specimens collected from different regions. As regards macroalgae, among essential elements, metal concentrations were in the order Fe>Zn>Cu. The mean concentrations of Fe, Zn and Cu varied from 13.7 to 625.8, 9.8 to 160.5 and 1.2 to 35.6 μgg^{-1} dry wt., respectively. Significant seasonal differences of concentration were detected in the macroalgae species except Cr (ANOVA Test, Table 4.8). There were significant differences between all sampling stations.

Table 4.8 Values of one-way analysis of variance in all macroalgae species for all sampling periods

		Season			***Station***	
	df	***F***	***P level***	***df***	***F***	***P level***
Macroalgae						
Hg	3	5.0242	**	7	5.2383	***
Cd	3	4.7993	**	7	18.3011	***
Pb	3	5.0003	**	7	8.2423	***
Cr	3	0.5417	Ns	7	71.9403	***
Cu	3	3.1347	*	7	19.7923	***
Zn	3	30.5660	***	7	2.9917	**
Fe	3	22.1271	***	7	6.4527	***

n.s. not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

As for the elements which are generally not essential for metabolic activities, the concentrations were in the order Cr>Cd>Hg>Pb. The mean levels of Cd, Hg and Pb varied from 4.8 to 571.8, 9.6 to 202 and 3.5 to 571.8 μgkg^{-1} dry wt., respectively (Table 4.13).

The Zn concentration in *Entromorpha* (63.9-73.8) collected from Çanakkale/Dardanos is significantly more than the Zn concentration in *Ulva* (28.7-36.6) collected at the same periods. A similar trend is found for Fe in between these two seaweed species for spring and summer periods.

Table 4.9 Concentrations (Hg. Cd and Pb µg kg^{-1} ; Cr, Cu, Zn and Feµg g^{-1} dry weight) of metals in the samples of most common seaweed species collected in winter from the sampling sites (mean±*SD*. n=3)

Region/Site	**Species**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	*Cystoseira* sp.	179.3±7.29	92.5±1.36	3.26±0.17	nd	2.84±0.28	46.1±0.56	239.9±5.06
	Ulva sp.	44.3±2.77	123.7±6.12	9.45±0.56	1.60±0.43	8.82±0.17	78.4±5.89	304.5±5.94
Çanakkale/Dardanos	*Cystoseira* sp.	50.5±2.40	416.5±4.56	1.84±0.18	2.55±0.21	2.53±0.28	38.0±0.78	139.5±4.53
	Ulva sp.	200.0±5.31	145.8±1.02	1.95±0.26	4.02±0.098	7.37±0.22	43.5±0.41	103.4±2.09
İzmir Bay/Foça	*Ulva* sp.	44.6±1.58	20.8±1.14	nd	nd	6.84±0.16	125.1±2.02	236.8±5.53
	Enterophmorpha sp.	117.9±3.32	26.2±2.25	3.06±0.29	nd	12.94±0.22	132.9±3.18	571.0±2.19
	Padina pavonica	75.6±1.09	123.7±2.06	3.69±0.25	nd	4.96±0.22	116.4±1.83	323.5±6.73
	Caulerpa racemosa	84.0±4.54	37.3±1.53	10.96±1.70	5.16±0.32	8.54±0.89	81.4±3.23	586.6±4.85
İzmir Bay/Bostanlı	*Enterophmorpha sp.*	82.0±1.72	33.1±0.71	1.90±0.07	5.93±0.21	13.4±0.29	141.8±1.78	602.5±3.83
	Gracilaria gracilis	166.9±2.89	21.4±0.66	5.18±0.23	1.86±0.07	8.01±0.14	74.5±0.57	561.8±5.81
İzmir Bay/Narlıdere	*Ulva* sp.	60.3±1.19	35.9±1.28	nd	6.72±0.57	5.52±0.06	75.9±1.12	471.9±6.38
	Enterophmorpha sp.	43.7±3.60	37.2±1.84	3.30±0.12	5.79±0.19	4.46±0.08	96.5±2.84	491.6±5.00
	Codium fragile	38.6±2.08	33.9±1.15	2.73±0.08	9.75±0.07	2.76±0.10	18.5±0.06	467.2±5.64
İzmir Bay/Urla	*Cystoseira sp.*	60.4±2.25	236.5±2.58	nd	2.54±0.37	1.21±0.06	59.6±2.86	275.0±8.10
	Ulva sp.	41.3±2.68	56.3±1.83	nd	0.97±0.02	6.87±0.14	115.0±2.94	268.9±3.29
	Enterophmorpha sp.	67.9±3.34	42.7±1.17	nd	3.15±0.03	4.43±0.10	97.5±1.41	205.1±5.47
	Padina pavonica	40.4±2.38	159.9±4.30	0.66±0.07	3.23±0.38	2.49±0.29	66.4±3.84	318.7±8.71
Marmaris/Turunç	*Cystoseira sp.*	161.2±1.17	156.3±2.48	nd	nd	8.95±0.70	70.3±1.58	576.2±4.07
Marmaris/Içmeler	*Cystoseira sp.*	31.2±1.87	224.6±1.65	6.22±0.30	83.81±1.90	16.7±0.94	94.6±1.8	625.8±3.36

Table 4.10 Concentrations (Hg. Cd and Pb µg kg^{-1} ; Cr, Cu, Zn and Fe µg g^{-1} dry weight) of metals in the samples of most common seaweed species collected in spring from the sampling sites (mean±*SD*. n=3)

Region/Site	**Species**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	*Cystoseira sp.*	122.77±2.21	56.98±1.23	5.20±0.36	1.50±0.10	3.78±0.26	56.19±1.46	314.01±2.97
	Ulva sp.	64.60±2.39	62.95±1.58	4.48±0.36	nd	8.72±0.80	55.17±4.19	173.50±2.93
Çanakkale/Dardanos	*Cystoseira sp.*	49.54±2.66	36.52±1.13	4.53±0.36	8.75±1.34	2.67±0.14	25.84±0.11	164.35±2.17
	Ulva sp.	79.83±1.62	32.98±3.19	7.21±0.58	8.06±0.20	6.97±0.31	28.67±1.34	154.17±1.58
	Enterophmorpha sp.	85.21±2.47	62.00±1.11	3.24±0.94	13.71±1.42	8.84±0.06	63.93±0.87	534.37±3.71
İzmir Bay/Foça	*Cystoseira sp.*	33.96±2.07	5.17±0.22	3.24±0.22	6.15±0.12	4.00±0.07	10.81±0.29	97.62±3.01
	Ulva sp.	9.62±1.27	7.96±0.10	2.48±0.26	4.20±0.14	8.57±0.12	16.82±0.24	84.69±1.99
	Enterophmorpha sp.	30.99±1.74	7.81±0.43	5.32±0.42	2.05±0.15	6.95±0.21	29.20±0.20	299.64±4.34
	Padina pavonica	23.75±0.83	126.76±2.77	4.59±0.24	6.37±0.19	5.05±0.057	43.02±0.74	412.52±1.86
	Caulerpa racemosa	39.2±1.58	6.87±0.64	5.66±0.49	2.95±0.47	6.24±0.23	13.05±0.37	53.84±1.73
İzmir Bay/Bostanlı	*Ulva* sp.	35.54±2.08	27.54±0.30	3.90±0.34	3.33±0.18	8.87±0.14	22.89±0.88	125.66±2.76
	Enterophmorpha sp.	80.27±1.23	25.93±0.75	7.50±0.48	7.97±0.37	13.08±0.38	73.35±0.54	202.88±1.52
	Gracilaria gracilis	39.67±0.64	6.27±0.37	4.68±0.27	2.11±0.16	5.65±0.25	29.15±0.65	155.97±1.60
İzmir Bay/Narlıdere	*Ulva* sp.	337.51±0.57	8.06±0.22	7.63±0.38	2.85±0.38	10.90±0.41	46.78±0.46	143.0±1.35
	Enterophmorpha sp.	628.99±1.98	4.83±0.33	6.23±0.35	4.18±0.34	8.82±0.21	76.04±0.38	216.42±1.56
	Codium fragile	53.51±0.88	28.35±1.11	13.94±0.53	4.25±0.31	3.86±0.16	9.81±0.48	65.06±1.59
	Gracilaria gracilis	223.36±2.82	69.34±0.90	7.73±0.27	3.53±0.32	3.93±0.28	57.38±1.38	158.68±2.00
İzmir Bay/Urla	*Cystoseira sp.*	100.23±0.75	188.78±1.87	8.28±0.24	4.35±0.47	2.25±0.17	27.17±0.83	212.14±1.85
	Ulva sp.	124.02±1.33	7.54±0.56	1.12±0.14	0.71±0.025	7.37±0.17	49.64±1.07	32.80±0.62
	Padina pavonica	56.63±1.16	88.47±0.83	6.21±0.26	5.32±0.19	4.84±0.05	37.37±0.88	107.85±0.96
Marmaris/Turunç	*Cystoseira sp.*	97.34±0.87	100.63±1.09	2.78±0.10	1.80±0.13	4.95±0.28	35.64±0.58	398.16±1.41
	Padina pavonica	48.28±1.90	134.86±0.92	1.69±0.058	0.96±0.036	3.85±0.11	22.80±1.03	63.60±1.51
Marmaris/Içmeler	*Cystoseira sp.*	104.03±1.52	173.33±1.80	2.22±0.26	25.58±1.37	11.28±0.49	62.48±1.03	526.38±2.15

Table 4.11 Concentrations (Hg. Cd and Pb µg kg^{-1}; Cr, Cu, Zn and Fe µg g^{-1} dry weight) of metals in the samples of most common seaweed species collected in summer from the sampling sites (mean±*SD*. n=3)

Region/Site	**Species**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	*Ulva* sp.	62.47±0.99	3.54±0.07	1.69±0.03	1.21±0.025	4.27±0.06	24.33±0.44	132.56±3.47
Çanakkale/Dardanos	*Cystoseira sp.*	62.00±1.46	438.57±1.40	1.44±0.01	2.40±0.021	2.16±0.02	39.73±0.31	145.88±2.16
	Ulva sp.	111.58±1.40	37.35±0.85	0.97±0.02	2.19±0.033	5.60±0.09	36.63±0.44	159.42±1.13
	Enterophmorpha sp.	93.56±1.54	26.30±0.34	2.65±0.013	5.55±0.028	8.93±0.045	73.76±1.77	344.59±1.77
İzmir Bay/Foça	*Cystoseira sp.*	79.74±1.65	30.47±1.23	2.67±0.028	0.43±0.045	6.81±0.071	36.71±0.50	96.03±1.55
	Ulva sp.	90.90±1.68	15.95±0.58	2.67±0.027	0.52±0.051	26.31±0.13	59.82±0.65	67.18±1.84
	Enterophmorpha sp.	120.29±0.94	3.85±0.039	21.57±1.21	11.24±0.21	12.37±0.68	62.23±1.33	434.74±1.51
	Padina pavonica	40.06±1.68	56.82±1.55	5.34±0.027	0.26±0.033	12.38±0.30	59.82±1.82	160.42±1.17
	Caulerpa racemosa	202.22±1.88	20.85±0.96	5.60±0.039	2.19±0.015	17.93±0.27	47.29±0.50	334.11±1.19
İzmir Bay/Bostanlı	*Ulva* sp.	116.91±1.67	16.20±0.71	1.47±0.013	3.18±0.029	8.47±0.35	34.52±0.091	321.81±2.52
	Gracilaria gracilis	99.34±1.06	11.67±0.61	0.73±0.0053	1.14±0.14	3.59±0.17	28.09±0.79	257.77±1.93
İzmir Bay/Narlıdere	*Codium fragile*	97.81±1.39	39.72±1.35	8.29±0.058	21.44±0.15	10.48±0.25	74.11±0.87	400.14±1.16
İzmir Bay/Urla	*Cystoseira sp.*	91.52±0.90	451.73±1.32	1.68±0.021	2.24±0.12	2.40±0.030	40.45±0.97	115.60±1.26
	Ulva sp.	53.43±0.76	10.73±0.58	1.21±0.0086	1.21±0.0046	9.11±0.34	78.30±1.27	136.61±2.16
	Padina pavonica	45.04±0.74	246.42±1.17	1.45±0.0056	1.05±0.14	3.07±0.13	60.99±1.07	99.56±1.32
Marmaris/Turunç	*Cystoseira sp.*	60.59±0.78	62.66±0.99	1.91±0.033	2.24±0.12	4.22±0.21	54.39±1.14	94.02±1.05
	Padina pavonica	58.72±0.92	113.56±1.23	1.48±0.052	0.99±0.035	4.43±0.13	25.64±0.099	13.73±0.040
Marmaris/Içmeler	*Cystoseira sp.*	129.49±0.89	122.56±1.23	4.07±0.090	25.66±0.23	14.50±0.24	59.22±1.12	387.76±2.09

Table 4.12 Concentrations (Hg. Cd and Pb µg kg^{-1} ; Cr, Cu, Zn and Fe µg g^{-1} dry weight) of metals in the samples of most common seaweed species collected in fall from the sampling sites (mean±*SD*. n=3)

Region/Site	**Species**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	*Cystoseira* sp.	71.20±1.81	120.38±1.91	10.25±0.19	4.84±0.05	13.56±0.39	124.17±1.58	352.96±2.35
	Ulva sp.	41.48±1.12	26.53±2.40	6.99±0.08	0.27±0.04	12.22±0.02	42.44±0.56	179.81±1.91
Çanakkale/Dardanos	*Cystoseira* sp.	53.18±0.57	221.75±2.23	1.45±0.01	5.32±0.03	5.16±0.13	44.00±0.95	296.67±1.53
	Ulva sp.	39.06±1.16	11.50±0.60	0.24±0.00	0.68±0.07	5.99±0.06	58.31±1.00	94.80±0.84
İzmir Bay/Foça	*Padina pavonica*	46.99±0.93	107.45±2.11	5.32±0.04	2.66±0.02	10.48±0.21	81.16±1.35	301.27±2.63
	Codium fragile	26.06±0.40	14.92±1.13	2.02±0.14	1.73±0.06	6.05±0.05	18.80±0.09	187.21±1.90
	Caulerpa racemosa	91.08±1.93	23.86±1.45	6.69±0.08	1.70±0.02	15.47±0.06	59.15±0.88	328.51±2.98
İzmir Bay/Bostanlı	*Ulva* sp.	26.00±0.81	12.52±0.46	0.48±0.01	1.95±0.05	7.54±0.30	63.80±0.85	247.49±1.59
	Gracilaria gracilis	69.87±0.90	5.32±0.05	1.93±0.02	0.97±0.01	7.57±0.10	126.00±1.63	359.23±0.65
İzmir Bay/Narlıdere	*Codium fragile*	29.35±1.44	29.11±0.48	2.89±0.00	6.82±0.13	4.09±0.01	16.04±0.85	380.05±1.47
İzmir Bay/Urla	*Cystoseira* sp.	52.16±1.67	368.76±1.35	1.93±0.02	1.45±0.01	3.62±0.03	34.89±0.85	317.58±1.61
	Padina pavonica	34.02±1.24	196.69±0.81	4.59±0.05	3.14±0.03	4.59±0.05	48.76±0.38	421.53±1.57
Marmaris/Turunç	*Cystoseira* sp.	33.04±1.57	109.53±1.25	0.96±0.01	1.68±0.02	4.64±0.11	45.76±0.51	246.26±1.22
	Padina pavonica	23.13±0.62	194.24±2.10	0.48±0.00	0.24±0.00	2.90±0.02	30.46±0.79	39.90±0.97
Marmaris/Içmeler	*Cystoseira* sp.	77.39±0.85	571.84±2.56	2.92±0.02	44.04±1.83	21.74±0.82	101.01±1.61	396.61±0.95

Table 4.13 The mean±*SE* and minimum-maximum concentrations of the heavy metals in macroalgae species during sampling periods ((Hg, Cd and Pb µg kg^{-1} ; Cr, Cu, Zn and Fe µg g $^{-1}$ dry weight)

Species	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
***Cystoseira* sp.**	81.0±5.06 31.2-179	199±19.9 5.17-572	3.28±0.31 nd-10.25	10.8±2.49 nd-83.8	6.66±0.70 1.21-21.7	52.7±3.33 10.8-124	287±19.6 94.0-626
Ulva sp.	83.3±9.87 9.62-338	35.0±5.07 3.54-146	2.91±0.38 nd-9.45	2.30±0.29 nd-8.06	8.75±0.61 4.27-26.3	55.7±3.84 16.8-125	181±13.8 32.8-472
Enterophmorpha sp	135±31.0 31.0-629	27.0±3.23 3.85-62.0	5.58±1.05 nd-21.6	5.96±0.61 nd-13.7	9.42±0.61 4.43-13.4	84.7±5.95 29.2-142	390±27.6 203-603
Padina pavonica	44.8±2.60 23.1-75.6	141±9.19 56.8-246	3.23±0.36 0.48-6.21	2.21±0.36 nd-6.37	5.37±0.53 2.49-12.4	53.9±4.66 22.8-116	206±25.7 13.7-422
Gracilaria gracilis	120±17.8 39.7-223	22.8±6.40 5.32-69.3	4.05±0.66 0.73-7.73	1.92±0.25 0.97-3.53	5.75±0.49 3.59-8.01	63.0±9.64 28.1-126	299±40.5 156-562
Codium fragile	49.1±7.00 26.1-97.8	29.2±2.21 14.9-39.7	5.97±1.22 2.02-13.9	8.80±1.83 1.73-21.4	5.45±0.73 2.76-10.5	27.4±6.30 9.81-74.1	300±40.1 65.1-467
Caulerpa racemosa	104.1±18.1 39.2-202	22.2±3.27 6.87-37.3	7.23±0.70 5.60-11.0	3.00±0.40 1.70-5.16	12.1±1.45 6.24-17.9	50.2±7.46 13.1-81.4	326±56.8 53.8-587

Heavy metal concentrations recorded in this study are in the same order of magnitude of those reported for relatively contaminated areas in green algae species. The order was given as: Fe>Zn>Cu>Cr>Hg>Cd>Pb for *Ulva, Gracilaria, Entromorpha* and *Caulerpa* species. In *Cystoseira*, *Padina* and *Codium* the distribution order of metals were Fe>Zn>Cr>Cu>Cd>Hg>Pb, Fe>Zn>Cu>Cr>Cd>Hg>Pb and Fe>Zn>Cr>Cu>Hg>Cd>Pb, respectively.

Maximum mercury level was observed in *Entromorpha* (629 µgkg^{-1}) from İzmir Bay/Narlıdere during spring. Extremely high Cd levels reaching up to 571.8 µgkg^{-1} in *Cystoseira* was found from Marmaris/İçmeler during fall. Maximum lead level was reported for *Entromorpha* (21.57µgkg^{-1}) during summer in İzmir Bay/Foça.

According to mean metal concentrations determined among the sampling stations indicates that minimum Pb, Fe were measured in *Ulva*; Hg, Cr, Cu were found in *Padina;* Cd, Zn in *Caulerpa* while maximum levels of Hg Zn and Fe were found in *Entromorpha*; Cd, Cr in *Cystoseira*; Pb and Cu in *Caulerpa* (Table 4.13).

Concentrations in *Codium* and *Caulerpa* differed significantly among seasons for all metals ($p<0.05$) (Table 4.14). Hg, Pb, Cu, Zn, Fe were shown with a Post-Hoc Tukey HSD analysis that levels in spring for *Codium* and for *Caulerpa* all metals except Hg differed significantly from other sampling periods. The significant differences between sampling sites were found for cadmium and chromium in *Codium*.

The ANOVA test showed the significant seasonal differences for chromium (except *Cystoseira*), Zn, Fe ($p<0.05$) for all macroalgae. The significant differences among stations were found for all metals in *Ulva* and *Cystoseira*. Results obtained in winter were differed from other seasons for all metals except Cr and Cu in *Ulva* (Post- Hoc Tukey HSD test). Post-Hoc HSD analysis showed that Hg, Zn and Fe levels were differed in winter significantly from other seasons in *Cystoseira*. Zn, Cr, Pb concentrations for *Enteromorpha*, Hg, Zn, Fe for *Padina* and Pb, Cu, Zn, Fe for *Gracilaria* were different in winter from summer due to post-hoc test.

Table 4.14 Values of one-way analysis of variance for all macroalgae species

		Season			***Station***	
	df	***F***	***P level***	***df***	***F***	***P level***
Ulva	*(n=57)*					
Hg	3	2.071	ns	5	6.002	***
Cd	3	13.678	***	5	2.895	*
Pb	3	3.380	*	5	5.458	***
Cr	3	3.007	*	5	7.399	***
Cu	3	1.700	ns	5	3.737	**
Zn	3	17.276	***	5	3.515	**
Fe	3	9.500	***	5	4.665	**
Enteromorpha	*(n=30)*					
Hg	3	1.9029	ns	4	3.591	*
Cd	3	2.947	ns	4	6.519	**
Pb	3	8.895	**	4	2.645	ns
Cr	3	4.065	*	4	2.603	ns
Cu	3	0.593	ns	4	14.701	***
Zn	3	30.372	***	4	1.528	ns
Fe	3	3.709	*	4	1.741	ns
Cystoseira	*(n=63)*					
Hg	3	2.782	*	5	4.899	***
Cd	3	5.002	**	5	9.436	***
Pb	3	2.454	ns	5	6.634	***
Cr	3	0.988	ns	5	28.026	***
Cu	3	2.437	ns	5	34.997	***
Zn	3	6.829	***	5	12.942	***
Fe	3	5.693	**	5	15.446	***
Padina	*(n=33)*					
Hg	3	3.970	*	2	0.136	ns
Cd	3	1.367	ns	2	7.344	**
Pb	3	1.414	ns	2	13.655	***
Cr	3	6.860	**	2	4.422	*
Cu	3	1.476	ns	2	15.787	***
Zn	3	10.799	***	2	17.215	***
Fe	3	4.453	*	2	16.630	***
Gracilaria	(n=15)					
Hg	3	1.17	ns	1	19.5991	***
Cd	3	1.62	ns	1	213.38	***
Pb	3	19.90	***	1	15.8433	***
Cr	3	10.40	***	1	41.3662	***
Cu	3	34.18	***	1	4.3538	ns
Zn	3	55.57	***	1	0.0802	ns
Fe	3	12619.78	***	1	3.5370	ns
Codium	(n=15)					
Hg	3	1083.093	***	1	3.104	ns
Cd	3	8.295	**	1	38.329	***
Pb	3	656.715	***	1	2.986	ns
Cr	3	62.683	***	1	4.686	*
Cu	3	65.055	***	1	0.160	ns
Zn	3	2047.555	***	1	0.452	ns
Fe	3	18.451	***	1	2.141	ns
Caulerpa	(n=12)					
Hg	3	1893.65	***			
Cd	3	323.33	***			
Pb	3	24.55	***			
Cr	3	85.90	***			
Cu	3	402.76	***			
Zn	3	842.25	***			
Fe	3	15444.56	***			

Hg (except *Padina*) and Cd differed significantly for *Enteromorpha*, *Gracilaria* and *Padina* at all sampling sites. Hg, Cr and Fe levels for *Ulva* at İzmir/Narlıdere were different from other stations, while Pb, Cu and Zn concentrations significantly differed at Çanakkale site from İzmir Bay. Post-Hoc test for *Cystoseira* indicated that the differences in Marmaris/İçmeler were significant for Cr, Cu, Zn and Fe. In the case of Hg, Cd and Pb, Çanakkale/City significantly differed from Çanakkale/Dardanos and İzmir/Urla.

The mean concentrations of Hg, Cd, Pb, Cr, Cu, Zn and Fe were shown in Figure 4.1-4.7. The highest mean mercury concentration was measured in *Enteromorpha* sp., while minimum levels were found in *Padina pavonica* and *Codium fragile*.

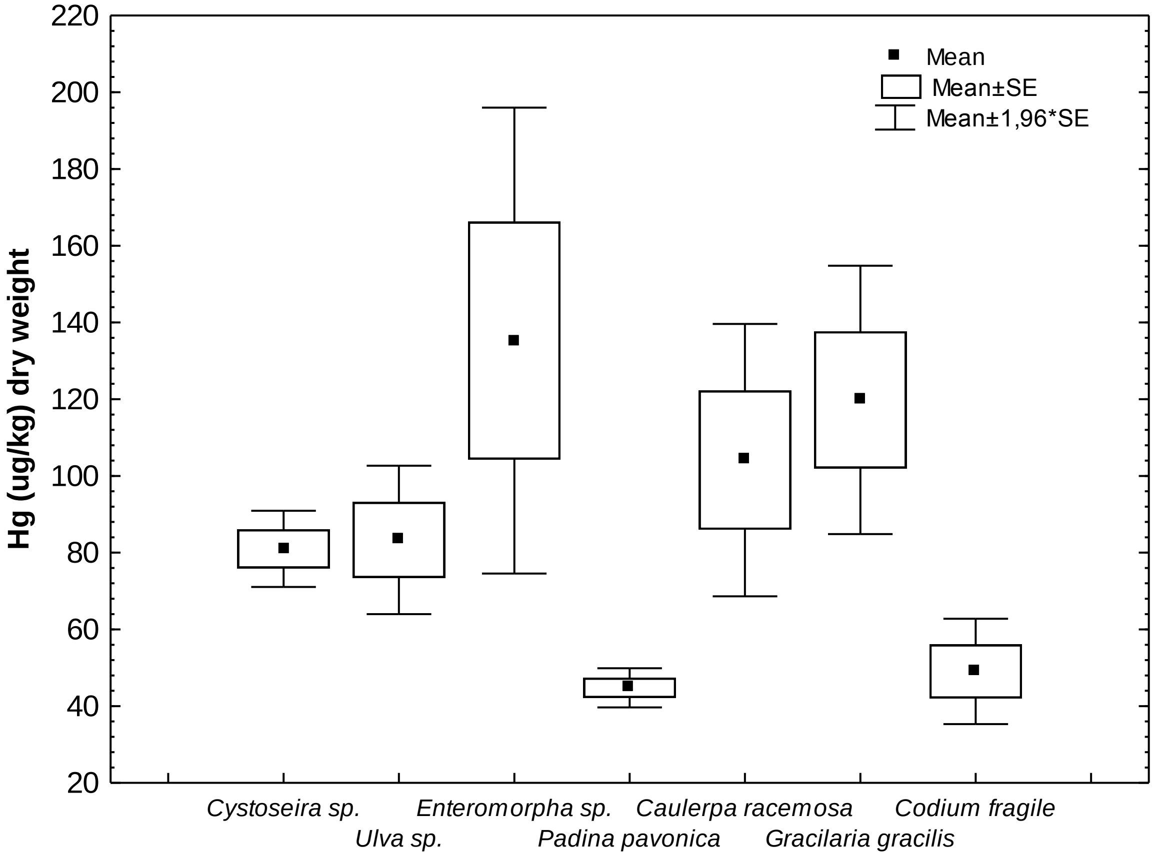

Figure 4.1 Means, mean±standard error and confidence intervals (1.96σ) of Hg concentrations in macroalgae species along the Aegean coast

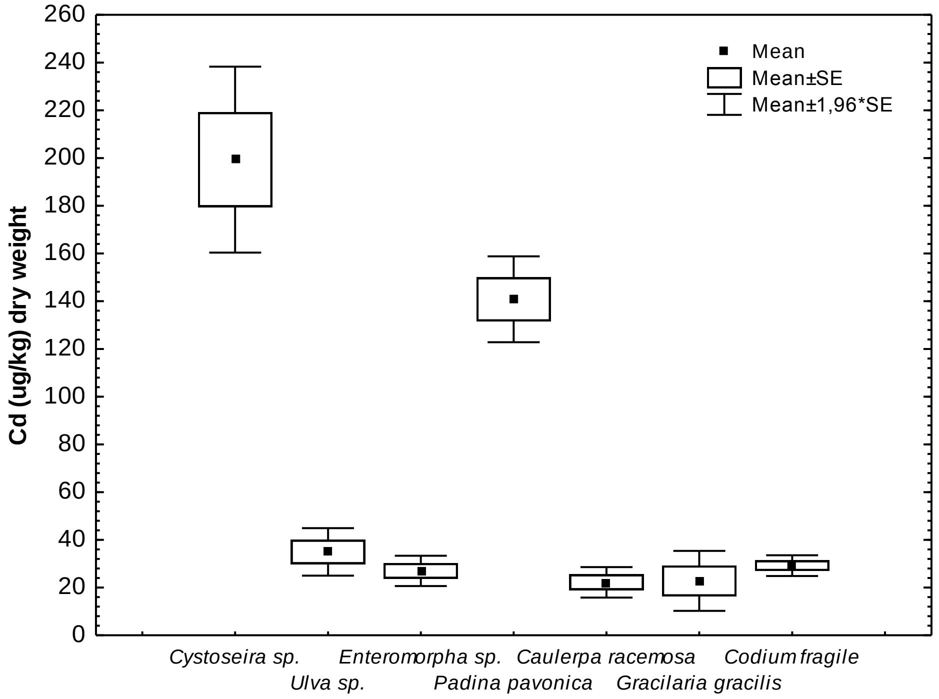

Figure 4.2 Means, mean±standard error and confidence intervals (1.96σ) of Cd concentrations in macroalgae species along the Aegean coast

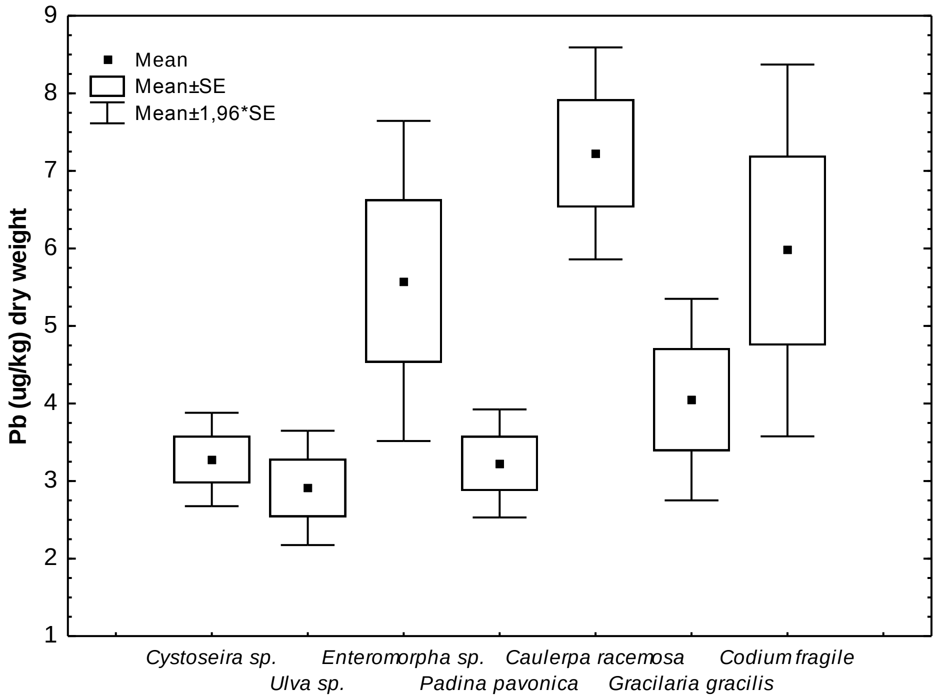

Figure 4.3 Means, mean±standard error and confidence intervals (1.96σ) of Pb concentrations in macroalgae species along the Aegean coast

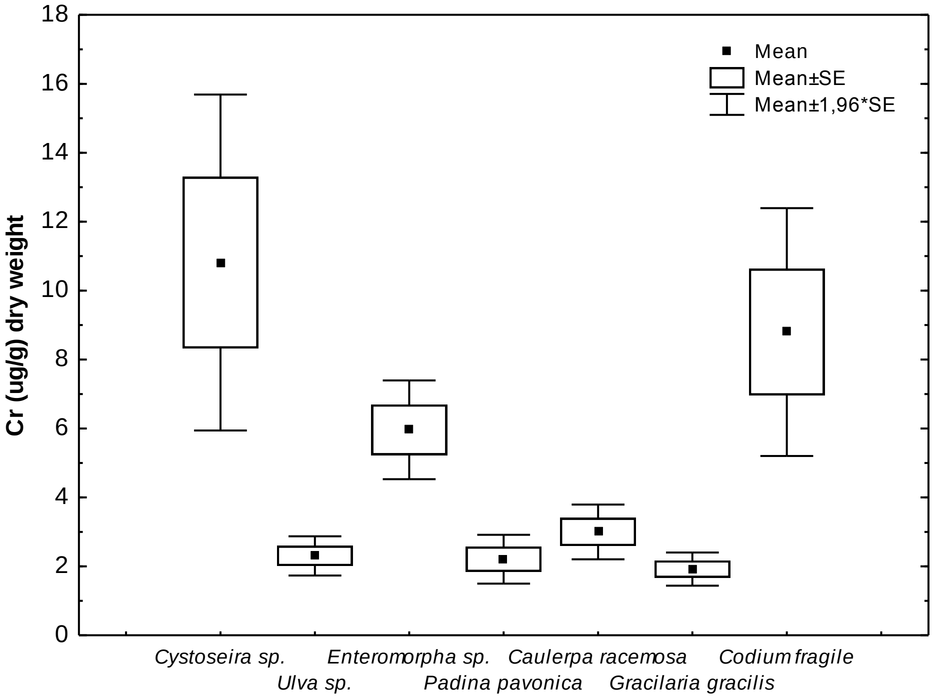

Figure 4.4 Means, mean±standard error and confidence intervals (1.96σ) of Cr concentrations in macroalgae species along the Aegean coast

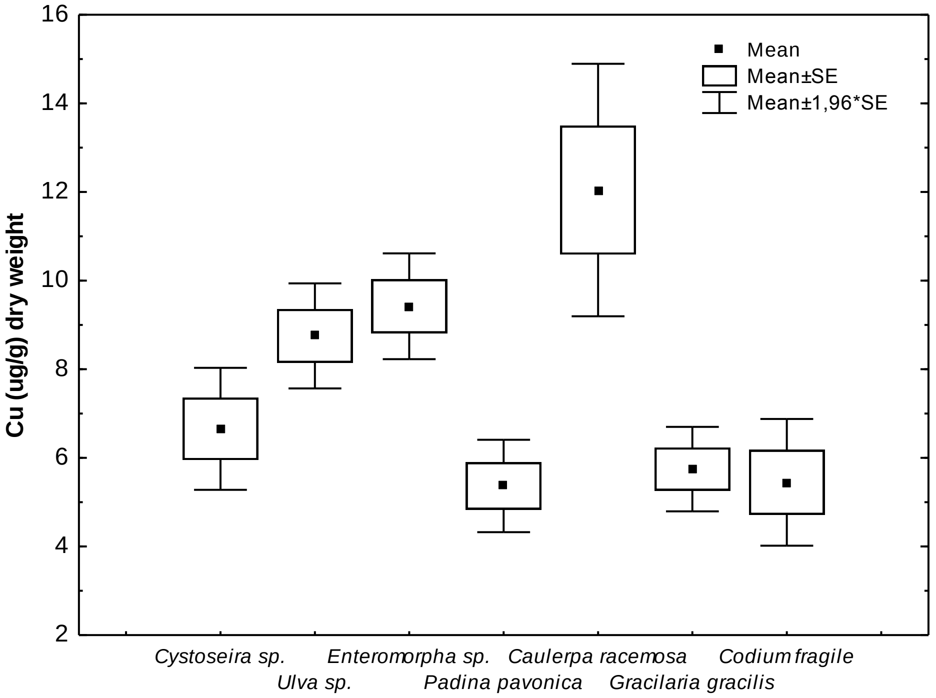

Figure 4.5 Means, mean±standard error and confidence intervals (1.96σ) of Cu concentrations in macroalgae species along the Aegean coast

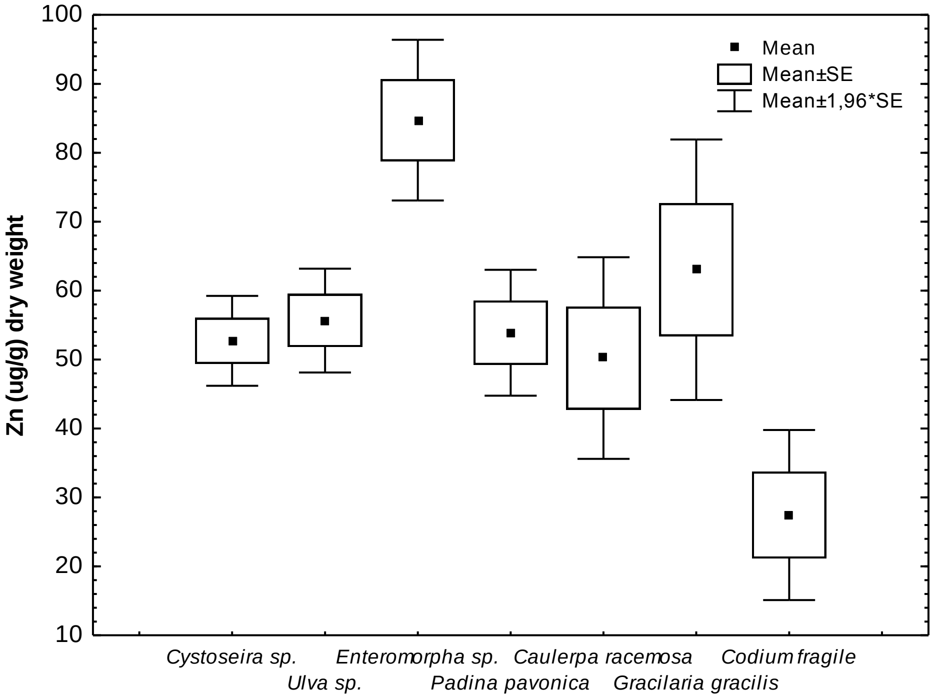

Figure 4.6 Means, mean±standard error and confidence intervals (1.96σ) of Zn concentrations in macroalgae species along the Aegean coast

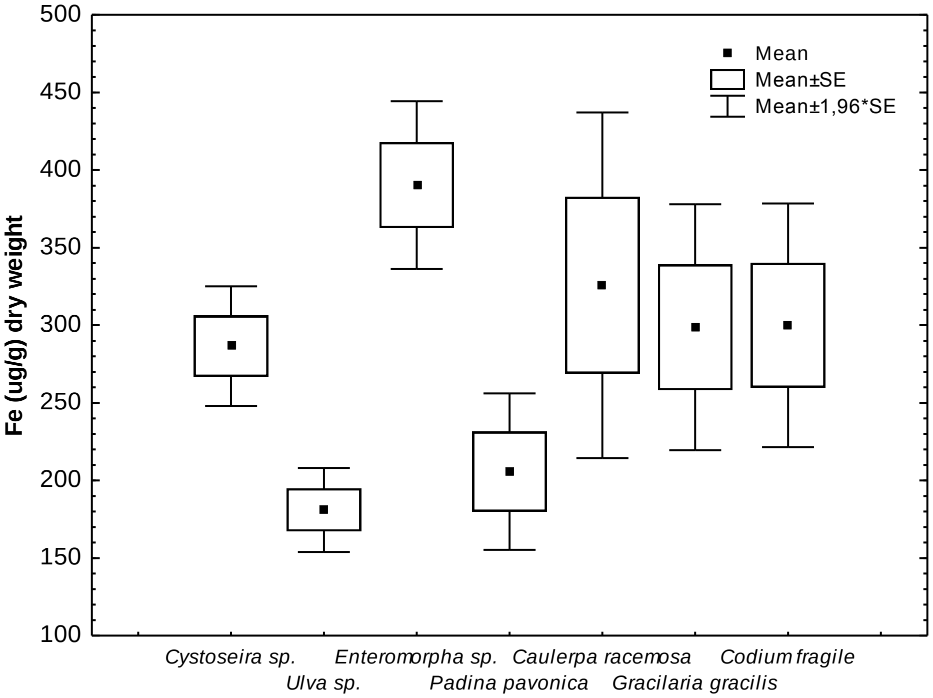

Figure 4.7 Means, mean±standard error and confidence intervals (1.96σ) of Zn concentrations in macroalgae species along the Aegean coast

The mean Cd, Cr; Pb, Cu and Zn, Fe levels were higher in *Cystoseira* sp., *Caulerpa racemosa* and in *Enteromorpha* sp. than other species, respectively. The lower Pb, Fe; Cu, Zn and Cd concentrations were found in *Ulva* sp., *Codium fragile* and *Caulerpa racemosa,* respectively.

The data of macroalgae metal concentrations showed that some relations may exist between metals and species, so data were tested for Spearman correlation (Table 4.15a and b). In all species Zn and Fe were significantly correlated with each other except in *Codium fragile*. The correlation between Fe and Zn may also be explained by the effect of environmental nutrients on the accumulation of metals in algae.

Significant positive correlations ($p<0.05$) were found between Pb-Cr, Cu-Fe in *Cystoseira* sp., *P. pavonica* and *G. gracilis*. There were positive correlations between Cd-Cr, Cu-Zn in *Cystoseira* sp., *G. gracilis* and *Codium fragile*, which may be due to a common origin for these elements: urban effluents are usually rich in nutrients and metals. Cadmium and zinc were positively correlated in *Cystoseira* sp., *Enteromorpha* sp. and *C. racemosa*. The level of lead in *Ulva* sp. and *Enteromorpha* sp. was negatively correlated with zinc. Mercury and cadmium were positively correlated in *Enteromorpha* sp., *G. gracilis* and *C. fragile* while these metals were negatively correlated in *P. pavonica*. Moreover, significant positive correlations ($p<0.05$) were observed between Cd-Pb in *G. gracilis* and *C. racemosa*, as this metal pair was negatively correlated in *Cystoseira* sp., *Enteromorpha* sp. and *P. pavonica*. The analysis for correlation of element pairs did not reveal any definite pattern among taxonomic groups. There were pairs of metals that were significantly correlated only in a single species.

These positive correlations may be explained by a common origin for the elements or by synergic interaction among them (Ho, 1990; Haritonidis and Malea, 1999). The negative correlations between pairs of metals in some species may be due to different origins, or environmental behavior, or to competition for uptake sites (Villares *et al.,* 2005).

Metal concentrations in macroalgae showed significant correlations with the corresponding ones in seawater and sediment (Table 4.16). Although a root system is absent in macroalgae, significant correlations were found between metal concentrations in algae and sediment.

Table 4.15a Spearman rank-order correlation coefficients for relationship between heavy metals in macroalgae species (Marked correlations are significant at $p<0.05$)

***Cystoseira* sp.**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Hg	1.000						
Cd	-0.086	1.000					
Pb	0.122	**-0.337**	1.000				
Cr	**-0.326**	**0.259**	**0.384**	1.000			
Cu	0.100	-0.103	0.198	**0.328**	1.000		
Zn	**0.263**	**0.256**	0.047	0.207	**0.594**	1.000	
Fe	**0.253**	0.245	0.158	0.245	**0.621**	**0.610**	1.000
***Ulva* sp.**							
Hg	1.000						
Cd	0.053	1.000					
Pb	0.215	0.157	1.000				
Cr	0.098	0.072	0.199	1.000			
Cu	0.019	-0.050	**0.614**	-0.179	1.000		
Zn	-0.112	0.251	**-0.427**	**-0.469**	-0.022	1.000	
Fe	-0.129	**0.474**	-0.103	0.062	-0.148	**0.398**	1.000
***Enteromorpha* sp.**							
Hg	1.000						
Cd	**-0.425**	1.000					
Pb	0.269	**-0.716**	1.000				
Cr	0.098	0.155	0.312	1.000			
Cu	**0.488**	-0.283	0.218	0.253	1.000		
Zn	0.081	**0.397**	**-0.608**	-0.294	0.174	1.000	
Fe	0.179	0.256	-0.277	0.155	0.353	**0.366**	1.000
Padina pavonica							
Hg	1.000						
Cd	**-0.424**	1.000					
Pb	0.189	**-0.658**	1.000				
Cr	-0.243	0.018	**0.345**	1.000			
Cu	0.179	**-0.737**	**0.840**	0.044	1.000		
Zn	0.111	-0.042	0.182	-0.080	0.283	1.000	
Fe	-0.264	0.064	**0.391**	**0.438**	**0.349**	**0.594**	1.000

Table 4.15b Spearman rank-order correlation coefficients for relationship between heavy metals in macroalgae species (Marked correlations are significant at p<0.05)

Gracilaria gracilis	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Hg	1.000						
Cd	**0.886**	1.000					
Pb	**0.572**	**0.672**	1.000				
Cr	0.393	**0.689**	**0.772**	1.000			
Cu	-0.139	-0.243	0.200	-0.271	1.000		
Zn	0.114	-0.182	0.273	-0.339	**0.711**	1.000	
Fe	0.254	-0.039	-0.132	**-0.593**	**0.632**	**0.586**	1.000
Codium fragile							
Hg	1.000						
Cd	**0.707**	1.000					
Pb	**0.768**	0.332	1.000				
Cr	**0.679**	**0.950**	0.293	1.000			
Cu	0.100	0.054	0.004	0.082	1.000		
Zn	0.114	0.343	-0.368	0.379	**0.693**	1.000	
Fe	0.182	**0.714**	-0.264	**0.779**	-0.089	0.486	1.000
Caulerpa racemosa							
Hg	1.000						
Cd	0.210	1.000					
Pb	-0.168	**0.825**	1.000				
Cr	-0.573	0.168	0.315	1.000			
Cu	**0.923**	0.147	-0.189	-0.545	1.000		
Zn	0.196	**0.916**	**0.867**	0.175	0.217	1.000	
Fe	0.392	**0.762**	0.497	0.391	0.385	**0.755**	1.000

Similar results have been reported in the past (e.g. Boyden, 1975; Luoma *et al.*, 1982; Malea *et al.*, 1994). The significant correlation between metal levels of macroalgae and sediment may appear unusual as the uptake of metals by seaweeds is mainly of metals in solution if there is not any particulate contamination. However, it should be considered that, to a certain extent, the concentrations of metals in sediments reflect the degree of contamination in the water column therefore, the significant correlations between certain metal concentrations in sediment and in algae is not surprising. Furthermore, it may be that part of the metal was scavenged from the sediment (Luoma *et al.*, 1982).

Table 4.16 Correlation (Spearman rank-order correlation coefficient) between metal concentrations in algae, sediment and seawater (*p*<0,05)

	Hg	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Cystoseira sp.-sediment	-0.073	-0.177	**0.254**	**0.360**	**0.429**	-0.109	**0.333**
Cystoseira sp.-seawater	-0.115	0.008	-0.210	0.108	**0.269**	0.105	**-0.294**
Ulva sp.-Sediment	**-0.392**	**-0.383**	-0.113	**0.305**	-0.249	-0.047	**0.306**
Ulva sp.-Seawater	**0.359**	**-0.533**	-0.222	**0.268**	0,071	0.150	-0.043
Enteromorpha sp.-sediment	**-0.638**	**-0.414**	0.030	**0.465**	-0.081	0.097	-0.099
Enteromorpha sp.-seawater	-0.017	-0.157	-0.143	-0.155	-0.060	0.203	0.227
Padina pavonica-sediment	-0.153	-0.211	0.219	-0.328	-0.234	-0.164	**-0.643**
Padina pavonica-seawater	-0.289	-0.062	**-0.390**	0.254	-0,062	**0.398**	-0.133
Gracilaria gracilis-sediment	-0.196	**-0.604**	**-0.584**	**0.687**	0.033	0.164	-0.502
Gracilaria gracilis-seawater	0.196	0.000	-0.311	-0.196	0.338	**0.655**	0.382
Codium fragile-sediment	0.098	-0,142	-0.491	**0.884**	0.098	**-0.589**	0.393
Codium fragile-seawater	**0.589**	**0.807**	-0.196	-0.098	0.000	**-0.589**	-0.196
Caulerpa racemosa-sediment	-0.389	0.194	-0.130	**-0.777**	-0.389	-0.389	-0.194
Caulerpa racemosa-seawater	0.389	-0.389	-0.324	**0.583**	0.389	**-0.777**	-0.389

Enteromorpha is more susceptible to contamination by particulate material (Villares *et al.*, 2001), so as expected higher correlations between metals in sediments and in this seaweed than *Ulva* (Table 4.16). Besides that, the coefficients were mostly not very high confirming that contamination by particulate material was of little importance.

However, stronger correlations were found in *Gracilaria* for Cd and Cr showing that chromium was better correlated with the metal level in sediment. The negative correlation between Cd in sediment and *Gracilaria* was significant. The significant correlations between *Cystoseira* -sediment and *Caulerpa*-seawater were found along the Aegean coast. Positive correlations were observed in *Codium*-seawater for Hg, Cd and *Codium*-sediment for Cr.

The concentrations of heavy metals in seawater are affected by a number of processes, namely biological uptake, scavenging by particulate matter, release from bottom sediments, advection and mixing of water masses and aeolian transport of terrestrial materials (Leal *et* *al.,* 1997). In some areas, the concentrations of heavy metals (Cd and to a lesser extent Cu and Pb) are typically related to the concentrations of nutrients and exhibit marked seasonality.

4.5 Discussion

There are several reasons for such differences in the accumulation of metals by different species. The concentrations of metals in the various seaweeds species may depend on their morphology, with those having a larger surface area having a greater internal content. Growth rates can affect accumulation patterns, with faster growing material appearing to have lower concentrations. The results obtained in this study also indicate that different species of seaweeds have different affinities for different heavy metals. This may reflect competition between metals for binding or uptake sides in the seaweeds (Lobban and Harrison, 1997).

The high Fe concentration encountered in all seaweeds as compared to the other trace metals, e.g., Zn and Cu, is probably due to several factors: the established need of Fe for normal growth of marine plants (Storelli M. M., Storelli, A., Marcotrigiano, 2001), ability of most algal species to biomagnify Fe from the surrounding environment and contamination from industrial activity (Eisler 1981). In general, Zn and Cu are readily accumulated from seawater by algae (Ho, 1988).

In benthic macrophytes, Zn levels not exceeding 100 μgg^{-1} are suggested as background for nonpolluted areas (Moore and Ramamurti, 1987). In the opinion of these authors, higher concentrations are characteristic of the regions subjected to anthropogenic contamination. The Zn concentrations recorded in our study for algae considered to exceed the background level in *Cystoseira* collected from Marmaris İçmeler and *Enteromorpha* collected from İzmir Bay/Bostanlı. In literature, Cu levels of 200-300 μgg^{-1} have been recorded in species from polluted areas (Hawk *et al.,* 1974). The relatively low levels of Cu recorded in the present study. No contamination is found by this metal along the Aegean coast. Burdon-Jones *et al.* (1975) and Agadi *et al.* (1978) accounted that Pb levels greater than 100 μgg^{-1} dry weight are from contaminated waters. The high Hg values of 20 μgg^{-1} and 14 μgg^{-1} dry wt. were reported from several polluted areas (Storelli *et al.,*2001). Hg and Pb concentrations recorded in this study are very much lower than those reported for contaminated areas.

The accumulation ratios of the metals in the macroalgea decreased in the order: Fe>Zn>Cu>Cr>Cd≥Hg>Pb. The metal concentrations of algae in different countries has been summerised by various authors (Hou and Yan, 1998, Villares *et al.*,2005, Haritonidis and Malea, 1999), but it is difficult to compare results of these studies because of differences in sample treatments and analytical procedures.

In this study, Cr, Zn, Fe and Cd, Cu levels increased during winter and fall periods, respectively. Minimum metal levels were generally found in spring period. There may be different reasons for the seasonal differences found, including: environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH etc.; metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors. The algal growth rate effects the seasonal variations in the metal concentrations, for example in macroalgae the metal concentrations decrease during growing periods and increase during the winter period (Phillips, 1994). Haritonidis and Malea (1995; 1999) attributed the seasonal pattern of concentrations of several metals in *Ulva rigida* and *Enteromorpha linza* to the growth effect and other factors such as the age of the tissue examined, and abiotic factors such as salinity and temperature, as well as variation in metal concentrations in the environment.

Hg and Pb levels increased during spring period in this study due to higher rates of photosynthesis and respiration, which would favour the assimilation of metals. The mobilization of metals from the sediment in areas covered by large amounts of macroalgae could also contribute to this accumulation. Catsiki and Papathanassiou (1993) also noted similar pattern and observed greater accumulation of several metals in *Ulva lactuca* in summer.

Comparison of metal concentrations in different macroalgae species from other geographical areas were given in Table 4.17. In other studies, the seasonal variations in metal concentrations in seaweeds have been exclusively attributed to variations in the levels of these elements in water. In a study of five species of macroalgae, Lacerda *et al.*, (1985) found maximum values in the rainy season (summer), which they attributed to potentially higher concentrations of metals in water because of an increase in terrestrial inputs.

Table 4.17 Comparison of bibliographical data on metal concentrations (μg g^{-1} dry wt) of macroalgae species from different geographical areas

Species	**Geographic area**	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**	**Reference**
Cystoseira barbata	Aegean Sea, Greece	-	0.06-2.6	0.02-2.5	-	0.7-8.8	8.8-58.1	-	Sawidis *et al.*, 2001
Cystoseira barbata	Black Sea, Şile/Sinop, Turkey	-	<0.02-0.09	<0.1-3.5	<0.06-1.2	1.7-6	6.5-192	130-593	Topcuoglu *et al.*, 2003
Cystoseira sp.	Aegean Sea, Turkey	0.03-0.18	0.005-0.57	ND-0.01	ND-83.8	1.21-21.7	10.8-124	94-626	Present study
Ulva lactuca	Mediterranean shore of Israil		ND-0.38	ND-8.63	-	6.69-28	19.9-798	182-2572	Hornung *et al.*,1992
Ulva rigida	Thermaikos Gulf, Greece		0.1-2.5	6.3-29.8	-	1.1-4.3	39-82.5	84.7-119	Haritonidis and Malea, 1999
Ulva sp.	Aegean Sea, Turkey	0.01-0.34	0.004-0.15	ND-0.009	ND-8.06	4.27-26.3	16.8-125	32.8-472	Present study
Enteromorpha linza	Thermaikos Gulf, Greece	-	0.1-2.5	7.8-87.4	-	1.9-4.4	49.7-141	53.3-135	Malea and Haritonidis, 1999
Enteromorpha sp.	British North Sea coast	0.02-0.23	0.07-4.8	19-437	17-57	-	-	-	Say *et al.*, 1990
Enteromorpha prolifera	South Adriatic Sea,Italy	ND-0.29	0.30-1.27	ND-1.81	-	6.07-15.1	32.63-94.59	139-1971	Storelli *et al.*, 2001
Enteromorpha sp.	Aegean Sea, Turkey	0.03-0.63	0.004-0.06	ND-0.02	ND-13.7	4.43-13.4	29.2-142	203-603	Present study
Padina pavonica	Favignana Island (Sicily), Italy	-	0.66-2.06	5.2-11.4	2.20-3.55	10.4-13.3	44-84	-	Campanella *et al.*, 2001
Padina pavonica	Gulf of Gaeta, central Italy	-	0.39-0.66	3.04-4.82	2.88-3.96	11.8-13.2	45-56	-	Conti and Cecchetti,2003
Padina pavonica	Aegean Sea, Greece	-	1.2-1.6	0.02-2.1	-	3-3.7	19.3-26.3	-	Sawidis *et al.*, 2001
Padina pavonica	Aegean Sea, Turkey	0.02-0.08	0.06-0.25	0.0005-0.006	ND-6.37	2.49-12.4	22.8-116	13.7-422	Present study
Gracilaria verrucosa	Aegean Sea, Greece		0.06-0.9	0.02-14.7	-	2.1-14.9	38-155	-	Sawidis *et al.*, 2001
Gracilaria gracilis	Venice lagoon, Italy		0.1-0.6	2.8-20.6	0.3-1.7	4-12	36-240	224-1080	Caliceti *et al.*, 2002
Gracilaria verrucosa	İzmir Bay, Turkey	0.035-0.099	0.05-0.076	0.5-4.56	-	2.4-2.55	5.7-5.85	9.9-145.7	Cirik *et al.*, 1989
Gracilaria gracilis	Aegean Sea, Turkey	0.04-0.22	0.005-0.07	0.0007-0.008	0.97-3.53	3.59-8.01	28.1-126	156-562	Present study
Codium vermilara	South Adriatic Sea, Italy	ND-0.26	0.12-0.29	ND-4.12	-	3.49-15.2	24.8-104	114-1403	Storelli *et al.*, 2001
Codium vulgarae	Aegean Sea, Greece	-	0.09	0.02	-	0.7	11.2	-	Sawidis *et al.*, 2001
Codium fragile	Aegean Sea, Turkey	0.03-0.1	0.01-0.04	0.002-0.014	1.73-21.4	2.76-10.5	9.81-74.1	65.1-467	Present study
Caulerpa racemosa	Yucatán, Mexico	-	-	30.7	3.15	5.35	2.96	405	Robledo and Pelegrín, 1997
Caulerpa racemosa	Aegean Sea, Turkey	0.04-0.20	0.007-0.04	0.006-0.011	1.70-5.16	6.24-17.9	13.1-81.4	53.8-587	Present study

The comparison of metal concentrations in macroalgae from other geographical areas revealed that: Cr, Cu, Fe concentrations in *Cystoseira*; Hg, Zn levels in *Enteromorpha*; Cr and Zn in *Padina*; Cr in *Gracilaria*; Cr, Cu, Zn in *Codium* and *Caulaerpa* were higher than those other areas in the world. The metal levels in *Ulva* were lower than the concentrations from other countries.

In order to assess the concentration factors (CFs) for observation of the bioaccumulation ability of every single species, analysis of the waters were performed which were collected at the same sites and collection time. 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 plant (µg g^{-1} dry weight) to the concentration of metal in seawater (mg l^{-1}). Table 4.18-4.24 reports the calculation of CFs for the species examined. These data must be viewed cautiously, as CFs could be influenced by the passage of contaminant through the trophic chain.

Concentration factors for Fe, Cu, Zn and Cr were observed as 10^3, while Hg, Cd, Pb varied from 10^0 to 10^2. The data shown in the tables of CFs confirm the high aptitude of the examined species as bioaccumulators. The macroalgae has the ability to accumulate metals several times more than marine water, so it is important to remark that macroalgae is also a food source for invertebrates and fish (Gerking, 1994). These species are commonly consumed sea food in many countries. The passage of trace metals from the abiotic section to the tissue of these species is highly interesting for food safety.

Table 4.18 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Cystoseira sp.* along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	9.6×10^{2}	2.7×10^{2}	5.0	4.4×10^{3}	8.7×10^{3}	36.5×10^{3}	15.6×10^{3}
Çanakkale/Dardanos	2.6×10^{2}	9.0×10^{2}	3.1	7.1×10^{3}	4.4×10^{3}	41.0×10^{3}	7.5×10^{3}
İzmir/ Foça	3.2×10^{2}	0.5×10^{2}	2.8	4.8×10^{3}	6.2×10^{3}	14.1×10^{3}	12.9×10^{3}
İzmir/Urla	3.8×10^{2}	8.4×10^{2}	3.1	3.9×10^{3}	2.4×10^{3}	28.3×10^{3}	29.2×10^{3}
Marmaris/Turunç	6.8×10^{2}	3.4×10^{2}	1.3	2.2×10^{3}	6.0×10^{3}	31.8×10^{3}	75.9×10^{3}
Marmaris/İçmeler	5.7×10^{2}	7.0×10^{2}	4.0	78.6×10^{3}	11.9×10^{3}	70.8×10^{3}	99.0×10^{3}
Mean Aegean coast	5.3×10^{2}	5.2×10^{2}	3.2	16.8×10^{3}	6.6×10^{3}	37.1×10^{3}	40.0×10^{3}

Table 4.19 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Ulva* sp. along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/City	4.1×10^{2}	1.6×10^{2}	4.5	1.6×10^{3}	11.1×10^{3}	24.2×10^{3}	10.2×10^{3}
Çanakkale/Dardanos	5.1×10^{2}	1.8×10^{2}	3.5	5.6×10^{3}	9.0×10^{3}	46.4×10^{3}	5.2×10^{3}
İzmir/ Foça	2.7×10^{2}	0.4×10^{2}	1.6	2.3×10^{3}	16.2×10^{3}	40.4×10^{3}	17.3×10^{3}
İzmir/Bostanlı	2.1×10^{2}	0.5×10^{2}	1.1	3.4×10^{3}	4.3×10^{3}	11.1×10^{3}	5.1×10^{3}
İzmir/Narlıdere	9.5×10^{2}	0.5×10^{2}	3.9	6.0×10^{3}	9.8×10^{3}	39.6×10^{3}	12.1×10^{3}
İzmir/Urla	3.6×10^{2}	0.7×10^{2}	0.9	1.4×10^{3}	7.9×10^{3}	56.6×10^{3}	18.5×10^{3}
Mean	4.5×10^{2}	0.9×10^{2}	2.6	3.8×10^{3}	9.7×10^{3}	36.4×10^{3}	11.4×10^{3}

Table 4.20 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Enteromorpha* sp. along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
Çanakkale/Dardanos	4.2×10^{2}	1.4×10^{2}	3.9	14.4×10^{3}	12.3×10^{3}	76.5×10^{3}	17.7×10^{3}
İzmir/ Foça	5.0×10^{2}	0.4×10^{2}	9.3	6.4×10^{3}	12.5×10^{3}	44.5×10^{3}	57.9×10^{3}
İzmir/Bostanlı	2.8×10^{2}	0.8×10^{2}	2.6	8.4×10^{3}	6.8×10^{3}	29.6×10^{3}	8.9×10^{3}
İzmir/Narlıdere	16.0×10^{2}	0.5×10^{2}	4.4	6.2×10^{3}	7.9×10^{3}	55.6×10^{3}	14.0×10^{3}
İzmir/Urla	3.4×10^{2}	1.2×10^{2}	0.9	4.6×10^{3}	4.5×10^{3}	68.2×10^{3}	26.0×10^{3}
Mean	6.3×10^{2}	0.9×10^{2}	4.2	8×10^{3}	8.8×10^{3}	54.9×10^{3}	24.9×10^{3}

Table 4.21 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Padina pavonica* along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
İzmir/ Foça	2.6×10^{2}	3.0×10^{2}	4.4	3.4×10^{3}	9.6×10^{3}	44.7×10^{3}	39.9×10^{3}
İzmir/ Urla	2.2×10^{2}	4.7×10^{2}	2.9	4.7×10^{3}	3.8×10^{3}	37.3×10^{3}	30.1×10^{3}
Marmaris/Turunç	3.3×10^{2}	4.6×10^{2}	1.1	1.2×10^{3}	3.9×10^{3}	16.2×10^{3}	9.0×10^{3}
Mean	2.7×10^{2}	4.1×10^{2}	2.8	3.1×10^{3}	5.8×10^{3}	32.7×10^{3}	26.3×10^{3}

Table 4.22 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Gracilaria gracilis* along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
İzmir/ Bostanlı	3.2×10^{2}	0.3×10^{2}	1.7	1.8×10^{3}	3.2×10^{3}	17.7×10^{3}	7.4×10^{3}
İzmir/ Narlıdere	10.6×10^{2}	1.7×10^{2}	7.1	4.4×10^{3}	4.7×10^{3}	37.0×10^{3}	6.2×10^{3}
Mean	6.9×10^{2}	1×10^{2}	4.4	3.1×10^{3}	4.0×10^{3}	27.4×10^{3}	6.8×10^{3}

Table 4.23 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Codium fragile* along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
İzmir/ Foça	1.4×10^{2}	0.4×10^{2}	1.9	2.5×10^{3}	7.0×10^{3}	11.2×10^{3}	24.9×10^{3}
İzmir/ Narlıdere	2.6×10^{2}	0.8×10^{2}	6.4	13.2×10^{3}	6.3×10^{3}	19.1×10^{3}	12.9×10^{3}
Mean	2.0×10^{2}	0.6×10^{2}	4.2	7.9×10^{3}	6.7×10^{3}	15.2×10^{3}	18.9×10^{3}

Table 4.24 Mean concentration factors for Hg, Cd, Pb, Cr, Cu, Zn and Fe in *Caulerpa racemosa* along the Aegean coast

Site	**Hg**	**Cd**	**Pb**	**Cr**	**Cu**	**Zn**	**Fe**
İzmir/ Foça	5.8×10^{2}	0.6×10^{2}	6.8	4.3×10^{3}	14.3×10^{3}	29.9×10^{3}	43.4×10^{3}
Mean	5.8×10^{2}	0.6×10^{2}	6.8	4.3×10^{3}	14.3×10^{3}	29.9×10^{3}	43.4×10^{3}

CHAPTER FIVE
CONCLUSIONS

The use of biomonitors turned out to be very valuable for the study of a coastal area with fairly significant basal contamination levels. The brown algae *Cystoseira* sp., the green algae *Ulva* sp. and *Enteromorpha* sp. possess high potential as cosmopolitan biomonitors for trace metals in Aegean Sea. These species have the necessary prerequisites for use as biomonitors: they are easy to identify and to sample, are available all year round and are present in almost all coastal areas of the Aegean Sea. Samples of the above mentioned species and the others were collected at eight stations along the Aegean coast.

The highest metal concentrations in seawater were measured from the inner part of the İzmir Bay due to the industrial activities, İzmir harbour and inputs of rivers reaching the bay. The minimum concentrations in seawater were generally found in Çanakkale/Dardanos since this station situated away from industry and urbanisation. The maximum levels of Hg, Pb, Zn and Fe in sediment were measured in İzmir Bay, while Cd, Cr and Cu were found in Marmaris due to the geological characteristics of the region. The minimum levels were found at stations out of the urban area (Dardanos, Foça, Urla, İçmeler, Turunç). The concentrations of heavy metals in sediment samples were lower in the sampling regions than the polluted areas of the Mediterranean Sea.

The metal accumulation in sediments is related to different parameters, such as sediment characteristics, particle size and organic carbon content. The determination of metal concentrations in sediment provides information about the total content but not on the bioavailable fraction. In this study, metal levels in the sediment showed no significant temporal variation, while all metals (except lead) showed significant local distribution. Hg, Cd and Zn levels in algae were higher than the sediment.

The relative abundance of metals in macroalgae decrease in the order: Fe>Zn>Cu>Cr>Cd>Hg>Pb and in seawater: Fe>Zn>Pb>Cu>Cr>Cd>Hg. The distribution order of metals in macroalgae and seawater had the same trend except Pb. In sediment the distribution order was Fe>Cr>Zn>Pb>Cu>Hg>Cd. These results showed that algae accumulate Pb in a lower degree than both seawater and sediment. Cu and Cr mean levels were in the same order of magnitude in both macroalgae and seawater showing that they were accumulated from seawater.

According to mean metal concentrations determined among the sampling stations indicates that minimum Pb, Fe were measured in *Ulva*; Hg, Cr, Cu were found in *Padina;* Cd, Zn in *Caulerpa* while maximum levels of Hg Zn and Fe were found in *Entromorpha*; Cd, Cr in *Cystoseira*; Pb and Cu in *Caulerpa* (Table 4.13).

Comparing metal concentrations in algae among the studied sampling stations clearly indicates that the degree of accumulation depends not only to human activities, but also on the geology of the specific area. The examined species showed a great ability to accumulate concentrations of metals several thousand times more than those detected in marine waters except Pb. The concentration factor for Pb was very lower than the other metals. The correlation analysis of pairs of metals did not reveal any definite pattern among taxonomic groups. In all of the species Zn and Fe were significantly correlated with each other except for *Codium fragile*. There were many pairs of metals significantly correlated with each other only in some of the species or in a single species.

The examined species of macroalgae having the ability to accumulate metals several times more than marine water and since macroalgae is located at the base of the food web in the Aegean Sea, macroalgae is probably the main source of metals for many animals like invertebrate and fish feeding on them. These species are commonly consumed sea food in many Aegean countries. Therefore, the investigation of trace metal concentrations in the tissues of algae species may provide useful information on the transfer of potentially toxic elements from abiotic compartments (water, sediments) to higher consumers, including man.

Regarding net accumulation, *Cystoseira* and *Entromorpha* were the strongest accumulators of Cd, Cr, Fe and Hg, Pb, Zn, respectively. *Ulva* turned out to be the highest Cu accumulator where as this species accumulate Pb, Zn, Fe at the minimum aptitude. However, even if the use of the above mentioned species as biomonitors for heavy metals seems advantageous for many reasons, further studies are needed in order to strengthen the routine use in marine biomonitoring, aiming to fully clarify their actual accumulation pattern.

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