LEVELS OF HEAVY METALS IN DIFFERENT ORGANS OF *Pagellus erythrinus* (RED PANDORA) WITH ENVIRONMENTAL PARAMETERS IN THE AEGEAN SEA

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İZMİR

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ABSTRACT

In this study, the heavy metal (Hg, Cd, Pb, Cr, Zn, Cu, Fe, Ni and Mn) contents were investigated in different organ tissues of Red Pandora *(Pagellus erythrinus*, Linnaeus, 1758) from the Aegean Sea. Biota samples were collected by trawling in May 1996, September 1997, January and July 1998. In muscle tissues, the concentrations of heavy metals were ranged between varied with Hg: 16-716; Cd: nd-9.6; Pb: nd-1397; Cr: nd-1180; Zn: 1352-6693; Cu: nd-383; Fe: 125-26382; Ni: nd-1575; Mn: 34-969 μg/kg wet weight. The ranges of metal levels were Hg: 125-5451; Cd: 1.4-2245; Pb: nd-8311; Cr: nd-10162; Zn: 2710-78705; Cu: nd-21986; Fe: 11210-553862; Ni: nd-106707; Mn: nd-21224 μg/kg wet weight in liver. Obtained values on metal contents in gonad were Hg: 2.0-1858; Cd: nd-192; Pb: nd-2927; Zn: 7273-205242; Cu: nd-20499; Fe: 321-525762; Ni: nd-41513; Mn: nd-10515 μg/kg wet weight. In stomach, the concentrations of heavy metals found varied with Hg: 2.0-948; Cd: 2.3-1139; Pb: 14-18197; Cr: nd-1006600; Zn: 3206-49803; Cu: nd-22034; Fe: 453-892809; Ni: 526-66039; Mn: 301-94340 μg/kg wet weight. There were no significant correlations between fork lengths and metal concentrations in muscle, liver and stomach tissue. ANOVA were utilized to investigate effect of length, season and station on variations in metal concentrations. The highest metal concentrations (Hg except) were generally found in the Southern Aegean Sea. The highest Hg, Cd, and Cu concentrations were found in liver, while the lowest were detected in muscle tissues. The high concentrations of Pb, Fe, Ni and Mn in stomach were associated with its food and food habits. The metal concentrations were lowest in muscle and obtained levels were below the proposed limit for human consumption. The heavy metals ratios between liver and muscle indicated that liver accumulated higher levels of metals than muscle tissues.

Keywords: Metals, fish, accumulation, organ distribution, *Pagellus erythrinus*, Aegean Sea

ÖZET

Bu çalışmada, Ege Denizi'ndeki Kırma Mercanın (*Pagellus erythrinus*, Linnaeus, 1758) farklı organ dokularındaki ağır metal (Hg, Cd, Pb, Cr, Zn, Cu, Fe, Ni and Mn) içerikleri araştırıldı. Biota örnekleri trolle Mayıs 1996, Eylül 1997, Ocak ve Temmuz 1998'de toplandı. Kas dokusundaki ağır metal konsantrasyonları Hg: 16-716; Cd: nd-9.6; Pb: nd-1397; Cr: nd-1180; Zn: 1352-6693; Cu: nd-383; Fe: 125-26382; Ni: nd-1575; Mn: 34-969 µg/kg yaş ağırlık aralıklarında bulundu. Karaciğerdeki metal seviyelerinin aralığı Hg: 125-5451; Cd: 1.4-2245; Pb: nd-8311; Cr: nd-10162; Zn: 2710-78705; Cu: nd-21986; Fe: 11210-553862; Ni: nd-106707; Mn: nd-21224 µg/kg yaş ağırlıktır. Gonadlarda bulunan metal içeriği değerleri Hg: 2.0-1858; Cd: nd-192; Pb: nd-2927; Zn: 7273-205242; Cu: nd-20499; Fe: 321-525762; Ni: nd-41513; Mn: nd-10515 µg/kg yaş ağırlık aralığındadır. Midedeki ağır metal konsantrasyonları Hg: 2.0-948; Cd: 2.3-1139; Pb: 14-18197; Cr: nd-1006600; Zn: 3206-49803; Cu: nd-22034; Fe: 453-892809; Ni: 526-66039; Mn: 301-94340 µg/kg aralığında değiştiği bulundu. Kas, karaciğer ve mide dokularındaki, metal konsantrasyonları ve çatal boy arasında önemli ilişki yoktur. Metal konsantrasyonlarındaki değişimler üzerinde mevsim, istasyon ve boyun etkilerini araştımak için ANOVA testi kullanıldı. En yüksek metal konsantrasyonları (Hg hariç) genelde Güney Ege'de bulundu. En yüksek Hg, Cd, ve Cu konsantrasyonları karaciğerde saptanırken, en düşük konsantrasyonlar kas dokusunda ölçüldü. Midedeki yüksek Pb, Fe, Ni ve Mn konsantrasyonları balığın beslenme alışkanlığı ile ilişkilidir. En düşük metal konsantrasyonları kas dokusunda olup, bulunan metal seviyeleri insanların tüketmesi için müsade edilen limitlerin altındadır. Karaciğer ve kas arasındaki ağır metal oranları, kas dokusuna oranla karaciğerin daha yüksek seviyede metal akümüle ettiğini gösterdi.

Anahtar Kelimeler: Metaller, balık, akümülasyon, organlardaki dağılım, *Pagellus erythrinus,* Ege Denizi.

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CHAPTER ONE
INTRODUCTION

1.1. Introduction

Among the more dangerous pollutants are the so called heavy metals (mercury, cadmium, lead, silver, nickel) introduced into marine environment by waste and sewage. The contamination by most of heavy metals in coastal environments reflects impacts from point or multipoint discharges from municipal and industrial sources. Because of their permanent in the environment, their toxicity at high concentrations, and their tendency to accumulate in the tissues of biota, heavy metals pose potentially hazardous conditions for man. Hence, they have been the subjects of ever expanding research activities to control their concentrations in marine habitats (Kennish, 1992). The trace metals concentrations in marine environment are controlled by many advective transports, mixing and differential settling of sediment-deposited metals give rise to substantial variations in trace metals composition. Many metal complex with organic compounds influence to their chemical speciation.

Some heavy metals are present in extremely small concentration in marine environment. Marine plants and animals accumulate these metals without harm to themselves. The variability in metal concentrations of marine organisms depends on many factors, either environmental, or purely biological (Phillips, 1995). Fish are widely used as sentinels of contamination in aquatic environment. The levels of metals accumulated in some marine organisms may be many orders of magnitude above background of certain species as bioindicators of heavy metal pollution (Pastor et al., 1994, 1996). Fish muscle provides low metal content because of its low metabolic activity. However it is very important to study the metal levels in this tissue in order to estimate metal quantities that enter to human by the consumption of fish.

Red pandora (*Pagellus erythrinus* Linnaeus, 1758) is very important commercial species and one of the most consumed fish, so it is selected in this study. Fish tissues (muscle, liver, stomach, etc.) are commonly used as indicators of degree of contamination of marine environment with trace metals. Liver analysis is used to determine recent metal accumulations, while gonad analysis is assessing possible transfer and/or influence of metals through the reproductive processes and also human consumption. Due to consumption of food are primary routes of metal uptake by fish, metal analysis is carried out in stomach.

Main purpose of this study is;

- To find metal concentrations in different organs of Red Pandora in the Aegean Sea,
- To find which organ contains maximum heavy metal concentration,
- To estimate which region contains maximum heavy metal concentration,
- To compare with metal concentration of fish in Mediterranean Sea.

The Mediterranean Sea is semi-enclosed sea which contamination from both anthropogenic and natural sources could generate pollution problems affecting the whole Mediterranean basin. The Aegean Sea is part of the Eastern Mediterranean. The present environmental problems are due to unmanaged shipping activity, river run-off (Gediz is the biggest river along the eastern Aegean), and untreated sewage discharge by coastal settlements, dumping of toxic and industrial wastes from the western part of Turkey.

CHAPTER TWO

HEAVY METALS

2.1. Toxic Metals

It is difficult enough to define a metal, even more so to define heavy metals. The heavy metals have atomic weights ranging from 63.546 to 200.590 gram and characterized by similar electronic distribution in the external shell (e.g. copper, zinc, cadmium). A metal generally is recognized as a substance having attributed to free electrons, high electrical and thermal conductivity, brilliance, great opacity and high reflectivity for light, a combination of toughness and malleability which permits a metal to be drawn into wire. Metals in their normal, pure state are crystalline (Waldichuk, 1974). Heavy metals are often referred to as trace metals, occurring as they do in low concentrations in organisms, although the term trace metal might imply the presence of an essential requirement by organisms for that metal.

Metals in their pure state present little hazard, except those having a high vapour pressure, such as mercury. It is the water soluble compounds of the metals that create the problems in the aquatic environments. Such as methyl mercury and tetraethyl lead, some of the metallo-organic compounds are the toxic compounds. The danger of discharging some of the metals into the environment in inorganic form lies in their conversion into the highly poisonous metallo-organic compounds through biological action, as was discovered not too long ago with mercury (Waldichuk, 1974).

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).

Viewed from the standpoint of environmental pollution, metals may be classified according to three criteria: 1. noncritical, 2. toxic but very insoluble or very rare, 3. very toxic and relatively accessible. Such as classification has been made by Wood (1974) and is listed in Table 2.1.

Table 2.1. Classification of elements according to toxicity and availability

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

2.2. Heavy Metals and Aquatic Systems

Heavy metals occur in the following chemical forms during transport into the sea:

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

Although heavy metals exist in dissolved, colloidal and particulate phases in seawater, the concentration of dissolved forms is low. The heavy metals rapidly sorb onto suspended particulate matter as they enter waters. Interaction with suspended particulates in estuaries is pronounced in the reactive turbidity maximum and finally, most of the metals are removed to bottom sediments in estuaries (Kennish, 1997).

The heavy metals removal during estuarine mixing are accelerated through precipitation or interactions with particle surfaces or flocculating colloids, co-precipitation with organic, iron and manganese hydrous oxides, increased affinity of the metals for anions in sea water, and uptake by organisms. Sediment-adsorbed heavy metals often are suspended from the bottom during storms and other turbulent periods (Kennish, 1997). Because of their large burden of trace metals, sediment exerts strong control on the biogeochemical cycling of some heavy metals. Sediments contain from three to five times the concentration of metals in waters; thus the bioavailability of even a small fraction of the total sediment burden assumes considerable importance. The bioavailability and concentrations of metals in estuarine sediments depend on many different processes, such as:

- Mobilization of metals to interstitial waters and their chemical speciation.
- Transformation of metals
- Control exert by major sediment components (e.g., iron oxides and organics) to which metals are preferentially bound
- Competition between sediment metals for uptake sites in organisms
- Influences of bioturbation, salinity, redox or pH on these processes (Kennish, 1997).

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).

Because certain metals are required in life process, most organisms have a capability of concentrating them. This capability is enhanced by certain feeding and metabolic processes, which can lead to enormously high concentration factors. Because of the ability of many metals to form complexes with organic substances, there is a tendency for them to be fixed in the tissue and not to be excreted. In other words, they have a large biological half-life. This is perhaps one of the major problems that metals pose with respect to their effects on aquatic organisms.

Several trace metals are chemically highly reactive hence their utilization as catalysts) and while they are essential and show a "beneficial" dose response curve at low concentrations, with increasing concentration, they may become inhibitory and ultimately toxic. There are several reasons for this effect; the catalytic activities of metallo-enzymes are future of their specific three-dimensional configuration. If a different metal is bound, the geometry of the active site and hence enzyme activity will be altered metal ions bind to organic ligands with different affinities, following the Irving-Williams series. The relative stability constants for cysteine-SH co-ordination are $Cu^{++}>Ni^{++}=Zn^{++}>Co^{++}>Fe^{++}>Mn^{++}$; thus Cu will displace Zn, Fe etc. When free metal ion concentrations rise, these metal substitution reactions will become significant and then inhibition of enzyme activity or destabilization of structural components of cellular molecules may occur. Toxicity may also be indirect, the displaced metal itself exerting toxic effects by binding to other cellular components. These substitution and displacement reactions are particularly important for metal ions, which show closely related chemical behaviour, such as Ag^{+}, Cu^{+}, Hg^{+}, Cd^{++} and Zn^{++}. Thus the toxicity of nonessential metals is due to Cu/Zn competition as well as to spill over onto other binding ligands. For other metals, toxic effects are quite specific. For example, Cr^{+3} appears to function only in the glucose tolerance factor; toxicity is due to covalent binding to proteins and nucleic acids. On the other hand lead binds to nucleic acids and is substituted for Ca in bone and the toxic effects of organometallic compounds, such as methyl Hg and tributyl tin are similarly specific (George, 1990).

Metallothioneins are low- molecular weight proteins, rich in cysteine with an ability to bind group IB and IIB metals (Cu, Zn, Cd, Hg). This property serves a protective function during exposure to heavy metals by sequestering most of the free metal ions within the cell, thereby preventing them from binding to the sulfhydryl groups of other functionally important proteins. They are thought to function primarily as regulators of the intercellular metabolism of Cu and Zn and have role in heavy metal detoxification (Pickering & Potteinger, 1995). Despite their potential for regulating metals concentration in organisms, metallothioneins and other metal-binding proteins have a finite metal-binding capacity. When this capacity is exceeded, the toxic effects of the contaminants may be manifested. Metallothioneins are most abundant in liver, kidney, gills and intestine of fish species but their value varies with species, reproductive condition age and diet. The liver tissue is highly active in uptake and storage of heavy metals. Fish responds to heavy metals exposure by producing metallothionein, particularly in liver.

The mercury species is the most toxic inorganic chemical form, but all three forms of inorganic mercury may have common molecular mechanisms of damage in which Hg^{2+} is the toxic species. Depending on pH, alkalinity, redox and other variables, a wide variety of chemical species can be formed, each having different electrical charges and solubility. Methylmercury is the most hazards mercury species due to its stability, it is lipid soluble and its possession of ionic properties that lead to an ability to penetrate membranes in living organisms. Methylmercury is produced by methylation of inorganic mercury present in sediments by bacteria and it accumulates in aquatic food chains in which top-level predators usually contain the highest concentrations. Organomercury compounds other than methylmercury decompose rapidly in environmental and behave much like inorganic mercury compounds. Such as carnivorous fishes, in organisms near the top of food chain almost all mercury accumulated is in the methylated form, primarily as a result of the consumption of prey containing methylmercury; methylation also occurs at the organisms level by way of mucus, intestinal bacteria and enzymatic processes but these pathways are not important as diet. However, methylmercury has low affinity for metlallothioneins and is strongly lipophilic, inorganic mercury compounds are strongly bound to metlallothioneins and high-molecular-weight ligands in tissues (Eisler, 2000).

Marine biota generally contained significantly higher cadmium residues than their fresh water because total cadmium levels are higher in seawater. Cadmium tends to concentrate in viscera of vertebrates, especially the liver and kidneys and biomagnifies in food chains. Free cadmium ions may inactivate various metal dependent enzymes; however, cadmium not bound to metallothionein may have the capacity to directly damage renal tubular membranes during uptake (Eisler, 2000).

Soluble waterborne lead is toxic to aquatic biota and that free cationic forms are more toxic than complexed forms. Organolead compounds are more toxic than inorganic lead compounds. Ethyl derivatives are more toxic than methyl derivatives and toxicity increased with increasing degree of alkylation, tetraalkylead being the most toxic. Among aquatic organisms, lead concentrations were usually highest in algae and benthic organisms and lowest in upper trophic level predators. In generally, lead does not biomagnify in food chains (Eisler, 2000).

Chromium in biological materials in usually in the +3 form and is the form that functions as an essential element in mammals. In generally the toxicity of trivalent chromium is low because its membrane permeability is poor and there is little tendency for Cr^{+3} to biomagnify in food chains in the inorganic form. Hexavalent chromium is more toxic than the +3 form because its oxidizing potential is high and it easily penetrates biological membranes. Cr^{+6} is usually the major species in seawater and thus mobile the aquatic environment. Trivalent chromium tends to form stable complexes with negatively charged inorganic or organic compounds and if anionic or particulate compounds, thus is unlikely to be found uncomplexed in aqueous solution (Eisler, 2000).

Copper is essential for the successful growth and development of many species of aquatic organisms but its extent of accumulation and retention are modified by numerous biological and abiotic variables. Copper is part of several essential enzymes including tyrosinase (melanin production), dopamine beta-hydroxylase (catecholamine production), copper-zinc superoxide dismutase (free radical detoxification) and cytochrome oxidase and copper protein complexes. Excess copper causes a variety of toxic effects, including altered permeability of cellular membranes. Bioavaibility and toxicity of copper to aquatic organisms depends on the total concentration of copper and its speciation. Copper toxicity is related primarily to the dissolved cupric ion (Cu^{+2}) and possibly hydroxyl complexes. In fishes the gill surface's low affinity for metal allows greater entry of the metal to the intracellular compartment. Once there, more complex binding sites are present. Binding to these ligands causes one or more the following toxic mechanisms: 1. blocking of the essential biological functional groups of biomolecules, 2. displacing the essential metal ion in molecules, 3. modifying the active conformation of biomolecules. These mechanisms may account for the specific inhibition of ion transport from ionic (Cu^{+2}) copper exposure. Copper detoxifying mechanisms in fishes include the induction of metallothioneins. Hepatic metallothionein contents of individual fishes usually reflect the accumulation of copper in that organ. This strongly supports the use of metallothionein as an indicator of copper stress (Eisler, 2000).

Dissolved zinc usually consists of the toxic aquo ion ($Zn(H_2O)_6^{+2}$) and various organic and inorganic complexes. Zinc is ubiquitous in tissue of organisms and essential for normal growth, reproduction and wound healing. More than 200 different enzymes require zinc for maximum catalytic activity, including carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, acid phosphatese, lactic dehydrogenase, and carboxypeptidase and superoxide dismutase. Zinc has its major metabolic effect on zinc-depent enzymes that regulate RNA and DNA. Metallothioneins play important role in zinc homeostasis and in protection against zinc poisoning in animals. In fish, gill epithelium is the primary target site. Biological effects of excess zinc are modified by numerous variables, especially by interactions with other chemicals. Concentrations of zinc in tissues of aquatic organisms are usually far in excess of that required for normal metabolism. Much of the excess zinc is bound to macromolecules or present as insoluble metal inclusions in tissue (Eisler, 2000).

2.4. Toxic Effects of Metals

The exposure of organisms to toxic levels of metal contaminants elicits a range of pathological responses, such as tissue inflammation and degeneration, lack of repair and regeneration of damaged tissue, neoplasm formation and genetic derangement. Growth inhibition may arise, as well as changes in physiology, reproduction and development. Feeding behaviour, respiratory metabolism, and digestive efficiency also can be adversely affected (Kennish, 1997). Signs of acute mercury poisoning in fish included flaring of gill covers, increased frequency of respiration, loss equilibrium, excessive mucus secretion and darkening coloration. Signs of chronic mercury poisoning included emaciation, brain lesions, diminished response to change in light intensity, inability to capture food, abnormal motor coordination. Minimata was regognized in 1953 in Japan. The patients who had consumed fish and shellfish from Minimata Bay that region suffered from a weakening of muscles, loss of vision, impairment of cerebral functions and eventual paralysis that in numerous cases resulted in coma and death. This desease became known as the "Minimata" that the deaths were caused by the consumption of fish and other foodstuffs contaminated with methylmercury (Wittmann, 1983).

Lead poisoning included spinal curvature, anaemia, darkening of the dorsal tail region, degeneration of the caudal fin, destruction of spinal neurons, reduced ability to swim against a current, destruction of the respiratory epithelium, elevated lead concentrations in blood, bone, gill, liver and kidney, muscular atrophy, renal pathology, growth inhibition, retardation of sexual maturity, altered blood and lipid chemistry, testicular and ovarian histopathology (Eisler, 2000). During 1947 an unusual and painful disease of a "rheumatic nature" was recorded in Japan and it became known as the "itai-itai" disease in accordance with patients' shrieks resulting from painful skeletal deformities. The itai-itai disease is caused by chronic cadmium poisoning, on condition of the existence of such inducing factors as pregnancy, lactation, imbalance internal secretion, aging, defiency of calcium, etc. (Witmann, 1983). For marine organisms, high cadmium concentrations resulted in decreases in growth, respiratory disruption, molt inhibition, altered enzyme levels and abnormal muscular contractions (Eisler, 2000).

In aquatic vertebrates, copper causes gill damage at high concentrations and in fishes it interferes with osmoregulation. Elevated concentration of copper interferes with oxygen transport and energy metabolism; tissue hypoxia is the cause of death and is associated with reductions in the activities of regulatory enzymes of ATP-synthesizing pathways. Nickel toxicity reduces photosynthesis, growth and nitrogenase activity of algae; growth rate of marine bacteria. Signs of nickel poisoning in fishes include surfacing, rapid mouth and opercular movements and prior to death, convulsions and loss equlibrium. Destruction of the gill lamellae by ionic nickel decreases the ventilation rate and may cause blood hypoxia and dead. Signs of nickel poisoning include decreased concentrations of glycogen in muscle and liver with increases in levels of lactic acid and glucose in blood. Zinc deficiency has been reported in humans and a wide variety of plants and animals with severe effects on all stages of reproduction, growth and tissues proliferation in the young. Significánt adverse effects of zinc on growth, survival and reproduction occur in representative species of aquatic plants, protozoans, sponges, molluscs, crustaceans, echinoderms, fishes at nominal water concentrations (Zn 10-25 μg/l). In general, zinc was more toxic to embryos and juveniles than to adults or to starved animals at elevated temperatures, at reduced salinities, under conditions of marked oscillations in ambient zinc concentrations, at decreased water hardness and alkalinity and low dissolved oxygen concentrations. At high environmental levels of Cr^{+6}, enzyme activities were altered in a wide variety of organs and tissues in fish species and gill histopotology was documented in catfish. Growth rate of larvae was reduced. The high concentrations chromium showed depletion of glycogen and glucose reserve of liver and kidney and decreased activity of respiratory enzymes. Chromium is causally associated with mutations and malignancy in mammals. In generally, nearly all Cr^{+6} compounds are potent mammalian mutagens and are respiratory carcinogens in humans. However, Cr^{+3} (water-solubilized) has caused cancer and dermatitis (Eisler, 2000).

2.5. Sources and Inputs of Heavy Metals in Mediterranean

A large of heavy metals potentially harmful to the marine environment and through seafood to human was studied in the Mediterranean. Mercury is of special importance for the Mediterranean because several countries bordering the Mediterranean have laws that set a limit for the Hg-Total concentration in seafood, and as will be shown below, many fish and shellfish species 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. Cadmium inputs (metric t/year) to the environment of the European Economic Community from various activities (Huttson, 1982)

	Air	Land	Water
Volcanic action	20	ND	ND
Non-ferrous metal production	23	255	70
Production of cadmium	3	90	108
Iron and steel production	34	349	ND
Waste disposal	31	1434	ND
Sewage sludge disposal	2	130	33
Phosphate fertilizers	-	346	62

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. Table 2.2. shows the concentration of cadmium in sewage from some Mediterranean cities (Huttson, 1982).

Table 2.3. Zinc and copper loads in the Mediterranean Sea (tonnesx10^3 per year) (UNEP, 1993)

Inputs	Zinc	Copper
Inputs through straits of Gibraltar	28.5	9.2
Inputs through straits of Bosphorus	0.08-1.3	0.5-11
Domestic load	1.9	2.3
Industrial load	5	6
Loads carried by rivers	0.6-1.6	0.2-0.6
Atmospheric inputs	42.5	5.3

Zinc and copper inputs into the marine environment include surface runoff, river outflow, domestic and industrial effluents discharged directly to the sea through outfalls, dumping of sewage and industrial sludges and discharge via the atmosphere. Zinc and copper inputs are shown at Table 2.3. (UNEP, 1993).

CHAPTER THREE
STUDY AREA

3.1. Description of the 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's maximum depth 2500 m north of Crete. In the southeast, the Aegean Sea joins the Levantine Sea through three passages between the island of Crete and Karpathos (Cassos strait), the island of Karpathos and Rhodes (Karpathos strait) and Rhodes and Turkey. In the southwest, it joins the Ionian Cretan Seas through three wide passages between the island of Crete and Peloponnese (Laskaratos, 1983). There is considerable exchange of waters through each of these straits, thus linking strongly the water circulation in the Aegean Sea to the hydrodynamics of the eastern Mediterranean (Unluata, 1986).

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 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.

3.2. Geological Characteristic

The bottom sediments of the eastern Aegean Sea are composed of material covering a wide range of grain size: from silty clay to sandy gravel. Muds, are called mixture of silt and clay, occur mainly off river mouths. The coarse-grained sediments are mainly represented by abundant sand and gravel size fractions, which are composed of biogenic and terrigenic components. Those dominated by biogenic components are largely derived from the calcareous remains of species of pelecypods, gastropods, foraminifers, ostracods, bryzoans, algae, echinoids and pterepods. The terrigenous components are generally consistent with geological source on land and coast (Ergin et al., 1993).

3.3. Hydrochemical Characteristic

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

Very few published data are available on nutrient concentrations in the Aegean. Distribution of nutrients was investigated by Friligos (1986), Kucuksezgin et al. (1995), UNEP, (1996) in the Aegean Sea. Nutrient levels are generally higher in the northern Aegean than in the southern part. This situation may result from water originating from the Marmara and the Black Sea. Nutrient values increase with increasing depth. Seasonal distributions of nutrient in Aegean Sea are shown at Table 3.1. There are many rivers, which transport nitrogen and phosphorus into the northern Aegean. The order of magnitude of the fresh water inputs is $1000\,m^3/s$ in total along the Aegean coastline and this value is higher than in other Mediterranean regions (MEDPOL, 1997).

Table 3.1. Seasonal distribution of nutrients (µM) in Aegean Sea

Period	(NO_3+NO_2)-N	o-PO_4–P	NH_4-N	Si
		Northern Aegean		
Spring				
Range	0.16-0.89	0.01-0.05	0.10-0.94	0.30-1.9
Mean±SE	0.48±0.013	0.03±0.001	0.31±0.014	0.94±0.048
Summer				
Range	0.14-0.95	0.01-0.08	0.10-0.61	0.43-2.9
Mean±SE	0.45±0.03	0.03±0.002	0.19±0.014	1.2±0.071
Autumn				
Range	0.14-1.8	0.01-0.09	0.10-0.69	0.33-3.3
Mean±SE	0.60±0.037	0.04±0.002	0.20±0.014	1.1±0.048
Winter				
Range	0.17-2.7	0.01-0.14	0.10-0.95	0.30-2.9
Mean±SE	0.82±0.072	0.04±0.002	0.39±0.021	1.02±0.037
		Southern Aegean		
Spring				
Range	0.12-0.72	0.01-0.06	0.10-0.63	0.30-1.7
Mean±SE	0.38±0.017	0.03±0.002	0.33±0.015	0.93±0.052
Summer				
Range	0.12-1.7	0.01-0.09	0.10-0.57	0.43-2.9
Mean±SE	0.59±0.050	0.04±0.003	0.16±0.010	1.3±0.080
Autumn				
Range	0.21-1.2	0.01-0.06	0.10-0.69	0.30-1.8
Mean±SE	0.48±0.046	0.03±0.003	0.25±0.022	0.83±0.086
Winter				
Range	0.15-2.0	0.01-0.13	0.10-0.89	0.35-4.8
Mean±SE	0.74±0.038	0.05±0.003	0.31±0.016	1.5±0.78

3.4. Hydrophysical Characteristic

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

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

1. Aegean Sea Surface Water forms a 40-50 m thick veneer, with summer temperatures ranging between 21-26 °C and winter temperatures range between 10-16 °C in the Aegean Sea. Similar north-south gradient is also observed in salinities with summer salinity values ranging between 30-39.5 psu and winter salinities ranging between 36.1-39.2 psu.
2. Aegean Sea Intermediate Water mass (40-50 m to 200-300 m) has a smaller north-south temperature gradient ranging between 15-18 °C and 11-16 °C from the northern to southern Aegean Sea, respectively. The salinity observed with values between 39.0-39.1 psu Seasonal salinity variations is very low.
3. Aegean Sea Bottom Water (below 200-300 m) is very uniform in temperature (13-14 °C) and salinity (39.1-39.2 psu) with little variations between in summer and winter (Yaşar, 1994).

3.5. Previous Studies

Very little is known about the levels and distribution of trace elements in the Aegean Sea. Zeri et al. (2000) and Voutsinou et al. (1997) and Voutsinou & Zeri (2001) reported on dissolved trace elements in seawater. The concentrations in the northern Aegean Sea range between Cd: 0.02-0.42 nM, Cu: 0.74-5.93 nM, Ni: 2.22-8.46 nM, Mn: 0.07-44.98 nM (Zeri et al., 2000). Voutsinou et al. (1997) reported from 0.3 to 5.0 µg/l Cu, from 1.3 to 1.5 µg/l Mn, around 0.008 µg/l Cd, from 0.7 to 1.0 µg/l Ni, from 0.6 to 2.0 µg/l Pb, about 5.2 µg/l Cr in the Aegean Sea. In north and south dissolved trace elements were compared and Cd concentration was found 0.047-0.128 nM and 0.071-0.103 nM in north and south, respectively. Copper showed similar concentration levels in both areas with values fluctuating around 2.0 nM Ni concentrations were slightly higher at northern (5.25-6.94 nM) than southern (3.58-5.25 nM). Lead showed similar concentration values in both areas 0.2 nM

(Voutsinou & Zeri, 2001). Brondi et al. (1985) and Hamilton (1990) measured value of 6.5 ng/ l for dissolved forms of mercury in the coastal waters of the Ionion Sea.

Voutsinou (1982); Ergin et al. (1993); Angelidis & Aloupi (1998) and Batki et al. (1999) investigated heavy metals concentration in the Aegean sediments. Batki et al. (1999) reported the distribution Cd concentrations change 0.20-0.42 ppm in marine sediments along the eastern Aegean Sea shelf. The distribution of Zn and Cu concentration range between 27-106 and 14-40 ppm, respectively. The Pb concentrations were observed between 30-50 ppm. The concentrations of Cr range between 38-97 ppm in this region. Voutsinou et al. (1999) measured contents ranged between Cr:121-305 ppm, Ni:60-120 ppm, Mn:460-1360 ppm, Zn:73-220 ppm, Co:14-20 ppm, Cu:17-60 ppm and Pb:20-180 ppm in sediment of Thermaikos Bay in northern Aegean Sea. Surface sediments from South Aegean Sea were researched (Voutsinou et al., 2001) and were reported the distribution Pb concentrations change 20-43, Cu concentration 11-43, Zn concentration 50-81, Cr concentration 68-78, Mn concentration 442-1652, Ni concentration 53-106.

The heavy metal concentrations are investigated many organisms in the Aegean Sea. Mediterranean mussels (*Mytillus galloprovincialis* Lamark, 1819) and the striped mullet (*Mullus barbatus* Linnaeus, 1758) are currently favoured organisms for monitoring coastal and off-shore metal levels. Tsoukali-Papadopoulou et al. (1989); Demirkurt et al. (1990); Parlak & Demirkurt (1990); Bei et al. (1992); Uysal (1992); Strogyloudi et al. (1998); Kucuksezgin (1999), Kucuksezgin et al. (2001) and Kucuksezgin et al. (2002) studied metal concentrations in selected marine fishes in this region. In other marine species, heavy metal concentrations were observed by Bei et al. (1990 and 1998); Catsiki et al. (1998 and 2001); Cotou et al. (1998) and Malea (1998). Kucuksezgin (1999) determined the concentration of trace metals in red mullet from Eastern Aegean Sea. The concentration of trace metals found with Hg ranging from 17-116 μg/kg, Cd ranging from 2-12 μg/kg, Pb is ranging from 85-158 μg/kg (wet weight). The concentrations of trace metals varied with Hg: 16-200 μg/kg, Cd: 0.57-4.5 μg/kg and Pb:40-207 μg/kg wet weight in *Mullus barbatus* (Kucuksezgin et al., 2001). In some edible fishes (red mullet, red Pandora, hake, annular sea bream and common sole), the range of trace metal concentrations (µg/kg wet weight) in Izmir Bay were Hg: 5-829, Cd: 0.10-10, Pb: 7.1-918 (Kucuksezgin et al., 2002). Strogyloudi et al. (1998) studied metal concentration in flesh and gills of striped mullet in different areas of Aegean Sea (Greece). They found that gills have higher levels than flesh. Cu ranged between 0.28-20.56 ppm and Ni values varied from 0.08 to 52.68 ppm in tissue. Bei et al. (1992), found mean values of Cu:2.5-3.37 ppm for demersal fishes 2.74-5.94 ppm for pelagic fishes Mean Cd concentrations ranged from 0.64-0.77 ppm for demersal and 0.65-0.78 ppm for pelagic fishes. Of all the research on the levels of trace metals in Red Pandora has not been carried out.

CHAPTER FOUR

MATERIAL AND METHODS

4.1. Biology and Distributions of Red Pandora

Phyllum : Vertebrata

Subpyhllum : Pisces

Classis : Osteichthyes

Ordo : Perciformes

Familya : Sparidae

Species : *Pagellus erythrinus*, (Linnaeus, 1758)

Red Pandora body is ellipse and oblong. Upper profile of head is straight and rather strong. Its snout is conical and eye diameter is shorter than snout and occipital scales extend for-wards to the anterior edge of eye. Molar teeth are in 2-3 (4) upper, 2 (3) lower series and gillrakers are 8-10 lower and 5-6 upper. Its formulas of dorsal fin are D XII + 10-11 and formulas of anole fine is A III + 8-9. Lateral line scales are 55-65 to caudal base. Bright pink with small blue spots scatter on upper part of sides and upper edge of opercula is carmine and a reddish spot is on pectoral base. Sometimes Bases of last dorsal rays have dark red marks and the transverse red bands are mentioned in the literature are a fight coloration. Maximum size reaches to 60cm (SL) and usually 20-25 cm (UNESCO, 1986). Its geographical distribution extends from rare in Black Sea, throughout Mediterranean Sea, along the west coast of Europe and Africa from Angola to Norway although it is notably rarer in the northern part of its range. Its bathymetric range extends from the shallow sublittoral down to about 100 m (200 m at Mediterranean, 300 m at Atlantic), but it is most common at depths of about 60 m and inhabits deeper water in winter; young extends near the coast. Red Pandora is demersal species. It dwells on rocks, gravels, sand and mud bottoms (UNESCO, 1986).

It is omnivorous, mainly carnivorous and predominantly a diurnal feeder. It feeds benthic invertebrates and small fish. The food habits and stomach contents of red Pandora are investigated in various regions of the Mediterranean Sea (Ardizzone & Messina, 1983; Andaloro & Giarritta; 1985; Caragitsou & Papaconstantinou, 1988; Özaydın, 1997; Benli et al. 2001). The diet was found to consist primarily of polychates, especially glyceridae, and crustacea, mainly natantia, occasionally fishes and cephalopods. Isopods, cumacea, mysids, and anisopods represented negligible sources of food.

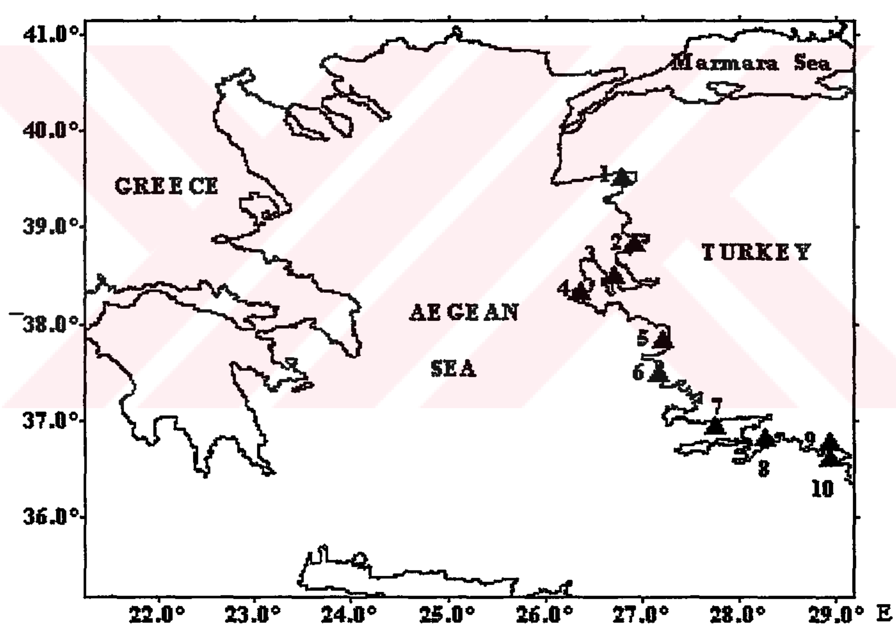

Figure 4.1. Location of sampling areas in the Aegean Sea

This fish is hermaphroditic protogynous and reaches maturity at 1-2 years. According to hydrological conditions, it spawns from spring to autumn and has possibly two spawning periods in the southern Mediterranean. It's not spawning in Black Sea and in the waters of northern Europe (UNESCO, 1986; Papaconstantınou et al. 1988; Uluturhan, 1996; Özaydın, 1997).

4.2. Sampling Design and Analyses

Table 4.1. The coordinates and sediment types of sampling areas during sampling periods

Sampling Station	Area	Longitude E (°)	Latitude N (°)	Sediment Type
1	Edremit	26.8617	39.5358	Sandy silt
2	Aliağa	26.925	38.8483	Sand-silt-clay
3	İzmir Bay	26.7	38.52	Clayey silt
4	Çeşme	26.3575	38.3533	Clayey silt
5	Kuşadası	27.2023	37.8365	Clayey silt
6	Didim	27.16	37.49	Silt
7	Gökova	27.7393	36.956	Gravely mud
8	Marmaris	28.2673	36.8195	Silt
9	Göcek	28.9253	36.7853	-
10	Fethiye	28.9262	36.6243	Sand-silt-clay

Sampling area and station are given in Figure 4.1. Location of sample stations and sediment types stations are presented in Table 4.1. Biota samples were collected by trawling from sampling stations in May 1996, September 1997, January and July 1998 during cruises of R/V *K. Piri Reis* (Institute of Marine Sciences and Technology). The samples are weighed, measured and the sex is determined. The numbers of sampled and main biometric parameters are represented in Table 4.2.

Table 4.2. Main biometric parameters of Red Pandora during sampling periods

Sampling Periods	*Sampling Number*	*Fork Length (mm)* Mean±S.D.	*Fork Length (mm)* Range	*Body Weight (g)* Mean±S.D.	*Body Weight (g)* Range
May 1996	27	143.8±19.16	119-172	55.3±18.7	31-81
September 1997	20	151.8±33.8	95-228	74.4±52.6	21-221
January 1998	113	152.9±24.6	105-210	68.3±35.1	19-162
July 1998	96	148.4±23.5	114-220	73.9±40.2	35-222

In this study, the length frequency composition of Red Pandora was based on observed length from 95 and 228 mm. Biota samples showed age groups from 0 to VII according to average body lengths of various age groups in the Agean Sea. The mean size of first maturity was at 155 mm (Uluturhan, 1996) and first maturity was at 1-2 years old (Zupanovic & Rijavec, 1980). The number of female, male and sex ratios are presented in Table.4.3. The sex ratios of females to males showed a female predominance.

Table 4.3. Sex ratios of Red Pandora during sampling periods

Sampling Periods	*Unknown*	*Female*	*Male*	*Sex Ratios*
May 1996	-	27	0	-
September 1997	13	6	1	1:0.17
January 1998	6	94	13	1:0.14
July 1998	-	86	10	1:0.12

After dissection, gonadal (Wg) and liver weights (Wh) were measured and after removal of the organs from abdominal cavity, "gutted weight" (Wa) was measured. Gonadal weight and "gutted weight" were used to calculate the gonadosomatic index (GSI):(Wg/Wa)x100 (De Vlaming et al. 1982). Liver weight and "gutted weight" were used to determine the hepatosomatic index (HSI):(Wh/Wa)x100 (Craik, 1978). GSI and HSI mean values were used as criteria to establish the seasonal changes in liver and gonads. The GSI maxima of female were found in July and the maximum HSI were obtained in January in this study.

Table 4.4. Gonadosomatic index of Red Pandora during sampling periods

Sampling	*Female (GSI)*		*Male (GSI)*	
Periods	*Mean±S.D.*	*Range*	*Mean±S.D.*	*Range*
September 1997	1.799±0.264	1.286-2.461	-	-
January 1998	0.8044±0.524	0.066-2.411	0.5847±0.201	0.3199-0.834
July 1998	2.102±1.304	0.119-4.212	0.562±0.277	0.162-0.760

Table.4.5. Hepadosomatic index of Red Pandora during sampling periods

Sampling	*HSI*	
Periods	*Mean±S.D.*	*Range*
January 1998	1.184±0.481	0.664-3.265
July 1998	0.977±0.224	0.5019-1.311

The dorsal muscle tissue, liver, stomach, gonad are separated and then placed one by one in plastic bags and stored at below -20°C until analysis. In the laboratory, approximately 5-7 g of biota muscle is digested with 5:1 HNO_3:$HClO_4$ in microwave digestion system and diluted to the desired volume with double distilled water (Bernhard, 1976). All the analyses are performed by Varian (Spectra AA-300 Plus) atomic absorption spectrophotometer. Mercury concentration is measured by cold vapour technique, Cd, Pb are determined by graphite furnace, Cu, Cr, Zn, Mn, Fe and Ni are measured by flame and background corrections are used as required. The detection limits for trace metals were; Hg:0.05 μg/l, Cd: 0.10 μg/l; Pb: 0.10 μg/l, Cr: 0.06 mg/kg, Cu: 0.03 mg/kg, Zn: 0.01 mg/kg, Mn: 0.02 mg/kg, Ni: 0.10 mg/kg. Accuracy of atomic absorption spectrophotometer and validity of the processes tested with a reference material (homogenate muscle sample, IAEA-407, from the International Laboratory of Marine Radioactivity, IAEA, Monaco). The values obtained for the analysis of three replicates of this sample were given at Table 4.6. The bioaccumulation factors (BAFs) were calculated according to following formula (Barron, 1995); BAF= (metal concentration in whole organisms/metal concentration in sediment)x100%.

Table 4.6. Heavy metal levels of reference material fish homogenate (mg/kg dry weight ± standard deviation).

Metals	**Certified values**	**Observed values**
Hg	0.222 ± 0.024	0.214 ± 0.0015
Cd	0.189 ± 0.019	0.179 ± 0.004
Pb	0.12 ± 0.06	0.10 ± 0.012
Cr	0.73 ± 0.22	0.74 ± 0.062
Cu	3.28 ± 0.40	3.39 ± 0.36
Zn	67.1 ± 3.8	69.4 ± 1.3
Mn	3.52 ± 0.32	3.40 ± 0.16
Fe	146 ± 14	144 ± 12

4.3. Statistical Data Analyses

The graphs presenting the whole set of data obtained for ten stations show the average concentrations calculated for each organ. Statistical analysis was performed using STATISTICA for Windows, Release 5.0, Copyright StatSoft, Inc.1995. Pearson's Product-Moment Correlation test used to check for significant relationships nine heavy metals in tissues of muscle, liver, stomach and gonads. In all case, the level of significant was set at $P< 0.05$. One- and two-way analysis of variance (ANOVA) were utilised to investigate effect of length, season and station on variations in metal concentrations in tissues. Post hoc Tukey HSD test were applied to determine statistically significant differences ($P< 0.05$) following ANOVA. Levene Test was used to examine the homogeneity of variances on trace metal content in tissues. Data used were plotted on graphs to see their distributions. Data showed mostly non normal distribution or close to normal distribution and these data were logarithmically transformed in order to reach normal distribution but non normal distribution again was found at the end of statistical analysis.

CHAPTER FIVE
RESULTS

5.1. Tissues concentrations of Heavy Metals

The mean concentrations and associated standards errors of heavy metals for each organ are summarized in Table 5.1-5.5. The observed minimum and maximum metal levels of different organs and stations were represented in Table 5.6-5.7.

Muscle

Obtained values on Hg, Cd and Fe contents in muscle tissue are high at station 3 (İzmir Bay) while lowest concentrations of Hg, Cd and Cu were always found at station 4 (Çeşme) for May 1996. Marmaris shows maximum concentrations of Pb, Zn and Cu (152, 5436 and 372 µg/kg) in this term. The Cr and Mn levels reached high values as 257 and 513 µg/kg at station 7 (Gökova). The muscle contains minimum levels of Pb and Ni at stations 8 (Marmaris). The lowest Cr and Zn were encountered in İzmir Bay while Fe and Mn levels were detected in Göcek and Edremit, respectively.

The results show that mercury and chromium seem to be more concentrated (616 and 1180 µg/kg) at station 3 (İzmir Bay) in September 1997. The maximum concentration of Pb (1397 µg/kg) was found at Marmaris while the highest Cd, Zn, Cu, Fe, Ni and Mn were encountered at station 7 (Gökova). The minimum levels of Hg and Cd were detected at Marmaris. Gökova shows lowest Pb, Zn, Cu and Fe values. Ni content was measured below detection limit at station 1, 2, 7 and 8 (Edremit, Aliağa, Gökova and Marmaris).

For January 1998, the concentrations of Hg, Zn and Mn were reached maximum levels (680, 3656 and 969 µg/kg) at Kuşadası while maximum Cd, Cr, Fe and Ni levels were detected at Didim. The muscle contains high levels of Pb and Cu (702 and 301 µg/kg) at Aliağa and Edremit, respectively. The minimum Pb value was found below detection limit at Çeşme and Didim. The lowest Hg and Cr were encountered in at Edremit while the minimum levels of Cd and Zn were found at Aliağa in this term. The lowest Fe and Mn were obtained at station 8 and 3 while Ni and Cu content were measured below detection limit at station 1, 2 and 4.

İzmir Bay shows maximum concentrations of Hg, Zn, Cu, Fe, Ni and Mn in July 1998. The higher Cd and Pb (8.8 and 444 µg/kg) were found at Kuşadası and Çeşme while maximum values of Cr (116 µg/kg) was measured at Gökova. In muscle tissues, the Cd and Pb values decreased below detection limits at Gökova, Marmaris and Didim. The lowest quantities of Hg, Cr, Zn, Cu, Fe and Mn were encountered at Fethiye, Didim, Çeşme, İzmir Bay, Marmaris and Gökova, respectively. Ni content was measured below detection limit at Çeşme and Didim in this term.

Liver

The Hg concentration is high at Kuşadası while Çeşme shows maximum values of Cd, Zn and Mn. The Pb and Cu levels were reached high values at Gökova in January 1998. On the other hands Edremit demonstrates maximum concentrations of Cr, Fe and Ni. The minimum values of Hg and Ni were found at Marmaris while Cd and Pb were measured at Çeşme. The lowest Zn and Fe concentrations were encountered at Aliağa and Didim. Generally Cr, Cu and Mn contents were measured below detection limit at Göcek, Gökova Çeşme and Edremit. The results show that maximum concentration of Hg (2057 µg/kg wet weight) was found at Kusadasi Bay same as January while lowest mercury concentration (172 µg/kg) was detected at Gökova in July 1998. The concentrations of Cd and Cu (743 and 21986 µg/kg) are high at Marmaris. Göcek shows maximum values of Pb and Ni. The Zn and Mn levels were reached high concentrations at Gökova while the maximum value of Cr was found at Fethiye in this term. Generally Gökova show the lowest quantities of Cd, Pb, Cu and Fe. The minimum Cr, Zn and Mn levels were found at İzmir Bay, Kuşadası and Didim, respectively. Ni content was measured below detection limit at Kuşadası, Göcek and Fethiye in July 1998.

Gonads

For September 1997, the highest Hg and Cd in ovaries were encountered at Kuşadası while the maximum levels of Pb, Zn, Cu, Fe were measured in Gökova. High concentrations of Ni and Mn were found at Gökova and İzmir Bay, respectively. The concentrations of Hg and Mn are low at Gökova while the minimum value of Zn is found at Kuşadası. İzmir Bay shows lowest quantities of Cu and Fe. The Cd, Pb and Ni levels decreased below detection limits at İzmir Bay and Gökova in this term.

Obtained values on Hg and Pb contents (1858 and 2927 µg/kg) in ovaries are high at station 2 (Aliağa) while the maximum concentration of Cd (180 µg/kg) was found at Edremit for January 1998. The levels of Cu, Ni and Mn reached maximum values at Çeşme. The highest concentrations of Zn and Fe were obtained at Gökova and Göcek. The minimum Hg and Fe values were found at Çeşme while the lowest of Zn level was measured at Aliağa in this term. The Cd and Ni levels decreased below detection limits at Çeşme and Edremit, respectively. The concentration of Pb was measured detection limit at Çeşme, Marmaris and Göcek while Edremit, Aliağa, Gökova, Marmaris and Göcek showed lowest level of Cu. The Mn values decreased below detection limit at Çeşme, Kuşadası, Gökova, Marmaris and Göcek in this organ tissue.

Ovaries present that maximum concentration of Hg (563 µg/kg) was at İzmir Bay while lowest mercury concentration (20 µg/kg) was at Gökova July 1998. The concentrations of Cd, Zn, Cu, Fe, Ni and Mn were reached maximum levels at Gökova. The maximum Pb level was detected at Marmaris in this term. The concentration of Cd and Pb at İzmir Bay, Didim and Gökova and Ni contents decreased in the ovaries below detection limits at Çeşme. İzmir Bay demonstrated minimum concentrations of Cu, Fe and Mn. The lowest of Zn level was obtained at Didim.

For January 1998, Çeşme presented high concentrations of Hg, Cd (1564 and 192 µg/kg), while the maximum values of Pb and Ni (172 and 7186 µg/kg) were measured at Marmaris in the testis tissues. The concentrations of Zn, Cu, Fe and Mn reached maximum levels at Didim. The minimum values of Hg, Zn and Fe were found at Marmaris. Didim showed lowest concentration of Cd while Pb and Cu, Mn, Ni contents decreased below detection limits at Marmaris and Çeşme in this term. Mercury, cadmium, lead and manganese seem to be more concentrated (379, 34, 227 and 2354 µg/kg) at Göcek in July 1998. The highest Zn, Cu and Fe were encountered at Didim while the maximum level of Ni detected at İzmir Bay. The lowest concentrations of Hg and Zn were measured at Marmaris. The minimum level of Cu, Fe and Mn were found at İzmir Bay. Cd content decreased below detection limit at İzmir Bay while the minimum concentration of Pb measured at İzmir Bay, Didim and Marmaris. The level of Ni decreased below detection limit at Marmaris and Göcek in this organ tissue.

Stomach

The results show that, mercury, chromium and copper seem to be more concentrated (541, 14690 and 6145 µg/kg) at Gökova in September 1997. The maximum concentrations of Cd, Pb and Fe were obtained in stomach, which were 428, 2417, and 405805 µg/kg at İzmir Bay. The levels of Ni and Mn were reached maximum values at Marmaris while lowest Cd, Pb, Cu and Mn values were found at the same station. The minimum levels of Hg, Cr and Ni were detected at İzmir Bay. The lowest Zn and Fe concentrations (49803 and 405805 µg/kg) were encountered at Kuşadası and Gökova.

For January 1998, the highest Hg (948 µg/kg) and Zn (44978 µg/kg) levels were observed at Kuşadası and Çeşme while Göcek showed maximum concentrations of Cd, Pb, Cr, Cu, Fe, Ni and Mn. The lowest quantities of Cd, Pb, Ni and Mn were obtained at Edremit. The minimum Hg concentration (5.0 µg/kg) was measured at Marmaris. The Zn and Fe values decreased minimum levels at Kuşadası. Cr and Cu contents were measured below detection limit at Aliağa and Çeşme.

İzmir Bay presented high concentrations for Hg, Cd, Pb Cr, Zn, and Fe in July 1998. The maximum values of Cu, Ni and Mn were found at Marmaris, Fethiye and Çeşme, respectively. The lowest Hg, Cd, Cr and Ni (35, 14 and 231 µg/kg) were encountered at Gökova while minimum concentrations of Zn and Mn were found at İzmir Bay. The lowest quantities of Pb, Cu and Fe were detected at Marmaris, Çeşme and Didim, respectively.

A general examination of the statistical summary (Figure 5.1-5.3) shows that processes of uptake and distribution of metals within different organs. The summary statistics of metal contents in organs indicates that dots represent mean values (as µg/kg wet weight); lower and upper box edges represent mean ± SE; outlying bars are mean ± 1.96 SE. According to results liver accumulated higher levels of Hg, Cd and Cu than other organs.

Table 5.1. Minimum, maximum and mean±standard error values of the heavy metal concentrations in muscle of Red Pandora (µg/kg wet weight)

	May-1996		September 1997	
	Mean	*Min-Max*	*Mean*	*Min-Max*
Hg	302±67.95	148-716	201±37.76	39-616
Cd	1.7±0.297	0.61-3.2	2.63±0.67	*nd*-9.6
Pb	71±17.79	27-152	278±90.22	15-1397
Cr	118±24.96	48-257	237±70.71	38-1180
Zn	3384±347	2558-5436	300±286.7	1352-6693
Cu	152±41.98	33-372	236±13.39	157-383
Fe	5137±602	3829-8119	5643±734.4	125-11708
Ni	256±66.4	76-660	444±135	*nd*-1575
Mn	257±55.4	156-513	229±37.24	85-610
	January-1998		**July-1998**	
	Mean	*Min-Max*	*Mean*	*Min-Max*
Hg	259±21.7	52-680	154±21.9	16-403
Cd	1.1±0.14	0.2-5.9	1.6±0.41	*nd*-8.8
Pb	136±25.8	*nd*-702	110±22.8	*nd*-444
Cr	103±8.90	*nd*-321	67±5.15	18-116
Zn	2946±54.3	2032-3656	2623±83.8	2072-4151
Cu	91.5±12.4	*nd*-301	194±8.30	137-307
Fe	5271±725.8	2418-26382	3050±272.6	1672-6958
Ni	480±45.9	*nd*-1033	226±27.5	*nd*-521
Mn	328±30.4	34-969	211±12.4	69-429

Table 5.2. Minimum, maximum and mean±standard error values of the heavy metal concentrations in liver of Red Pandora (µg/kg wet wt)

	January-1998		July-1998	
	Mean	*Min-Max*	*Mean*	*Min-Max*
Hg	1064±161.9	125-5451	643±94.7	172-2057
Cd	796±85.3	45-2245	204±27.7	1.4-743
Pb	2657±280.8	*nd*-8311	2007±408.3	402-7964
Cr	661±243.3	*nd*-10162	602±109.1	74-2424
Zn	32440±1729.0	2710-78705	32636±1768	18450-48599
Cu	4081±440.5	*nd*-11634	7754±1001	1807-21986
Fe	211781±14557	23850-553862	172427±18025	11210-371210
Ni	6516±2378.6	561-106707	1187±286.3	*nd*-6489
Mn	2979±517.8	*nd*-21224	3458±916.4	177-21203

Table 5.3. Minimum, maximum and mean±standard error values of the heavy metal concentrations in ovaries of Red Pandora (µg/kg wet weight)

	September-1997		January-1998		July-1998	
	Mean	*Min-Max*	*Mean*	*Min-Max*	*Mean*	*Min-Max*
Hg	370±70.8	186-584	324±61.9	2.0-1858	141±25.8	20-563
Cd	4.79±1.94	*nd* -9.8	24±6.92	*nd* -180	15.9±5.48	*nd* -121
Pb	179±75.6	*nd* -393	377±107.3	*nd*-2927	127±41.1	*nd* -800
Zn	36103±7674.4	18438-61688	81756±7090.3	13275-205242	44088±9198	9681-168655
Cu	1145±227.2	414-1948	998±363.5	*nd* -13838	3496±1174	213-20499
Fe	31316±14442	4974-87270	37484±7777	2912-196117	71709±29897	321-525762
Ni	4001±1907.3	*nd* -10065	5041±1339.6	*nd* -41513	1295±334.3	*nd* -5591
Mn	1149±410.8	303-3068	593±241.9	*nd* -9225	1785±548.9	170-10515

Table 5.4. The minimum and maximum values of the heavy metal concentrations in testis of Red Pandora (µg/kg wet weight)

	January-1998		July-1998	
	Mean	*Min-Max*	*Mean*	*Min-Max*
Hg	566±209.3	132-1564	263±38.5	146-379
Cd	36±26.0	3.2-192	17.1±6.51	*nd* -34
Pb	50±25.1	*nd* -172	63±44.38	*nd* -227
Zn	27252±11318	7273-72589	16642±1807	12382-21038
Cu	253±116.7	*nd* -850	1184±259	614-2111
Fe	29115±13571	10643-109479	12122±4018	479-23219
Ni	2858±919.1	*nd* -7186	1857±1083	*nd* -5523
Mn	281±113.3	*nd* -680	1286±355.2	319-2354

Table 5.5. Minimum, maximum and mean±standard error values of the heavy metal concentrations in stomach of Red Pandora (μg/kg wet weight)

	September-1997		January-1998		July-1998	
	Mean	*Min-Max*	*Mean*	*Min-Max*	*Mean*	*Min-Max*
Hg	263±65.23	93-541	274±29.6	5.0-948	162±27.5	35-563
Cd	167±56.04	2.3-428	382±43.9	19-1139	123±18.7	14-419
Pb	827±329.5	14-2417	3049±313.4	102-8887	4286±721.5	497-18197
Cr	4457±1933	255-14690	129996±41031	*nd*-1006600	926±146	231-4419
Zn	21230±4524	11217-49803	16377±849.7	4763-44978	15675±990.4	3206-31883
Cu	3042±568.6	1806-6145	3626±437.8	*nd*-19141	5611±857.3	726-22034
Fe	224495±53099	453-405805	593417±94327	35431-2471528	376841±46401	11711-892809
Ni	11689±4409	1285-37919	10983±2204	526-66039	8854±1714	809-31252
Mn	9267±1649	5677-17903	18292±2808.3	301-94340	13976±1712	2029-45552

Table 5.6. Observed of the minimum levels of the heavy metal concentrations in different organs of Red Pandora (µg/kg wet weight)

Metals	Station No	Organs	Sex*	Conc.
09/1997				
Hg	8	Muscle	2	39
Cd	8	Muscle	2	*nd*
Pb	7	Gonads	2	*nd*
Cr	3	Muscle	3	48
Zn	7	Muscle	2	1352
Cu	7	Muscle	1	157
Fe	7	Muscle	3	125
Ni	1,2,7,8	Muscle, ovaries	1,2,3	*nd*
Mn	8	Muscle	1	85
01/1998				
Hg	4	Ovaries	2	2.0
Cd	4	Ovaries	2	*nd*
Pb	4,6,8,9	Muscle, liver, gonads,	1,2,3	*nd*
Cr	1,4,5,7,8,9	Muscle, stomach, liver	1,2	*nd*
Zn	2	Liver	2	2710
Cu	1,2,4,6,7,8,9	All organs	1,2,3	*nd*
Fe	8	Muscle	2	2418
Ni	1,2,4,8	Gonads, muscle	1,2,3	*nd*
Mn	4,5,7,8,9	Gonads	1,2	*nd*
07/1998				
Hg	10	Muscle	2	16
Cd	3,7,8	Muscle, gonads	1,2,3	*nd*
Pb	6,7,8	Muscle, gonads	1,1,2	*nd*
Cr	6	Muscle	2	18
Zn	4	Muscle	2	2072
Cu	1	Muscle	2	137
Fe	1	Ovaries	2	321
Ni	4,5,8,9,10	Muscle, liver, gonads	2,2,1,2	*nd*
Mn	7	Muscle	2	69

Sex*: 1:male, 2: female, 3: uncertain

Table 5.7. Observed of the maximum levels of the heavy metal concentrations in different organs of Red Pandora (μg/kg wet weight)

Metals	Ist. No	Organs	Sex*	Conc.
09/1997				
Hg	3	Muscle	2	616
Cd	3	Stomach	2	428
Pb	3	Stomach	2	2417
Cr	7	Stomach	3	14690
Zn	7	Gonads	2	61688
Cu	7	Stomach	2	6145
Fe	3	Stomach	2	405805
Ni	8	Stomach	3	37919
Mn	8	Stomach	3	17903
01/1998				
Hg	5	Liver	2	5451
Cd	4	Liver	2	2245
Pb	9	Stomach	3	8887
Cr	9	Stomach	2	1006600
Zn	8	Ovaries	2	205242
Cu	9	Stomach	3	19141
Fe	9	Stomach	2	2471528
Ni	1	Liver	3	106707
Mn	9	Stomach	3	94340
07/1998				
Hg	5	Liver	2	2057
Cd	8	Liver	2	743
Pb	3	Stomach	2	18197
Cr	3	Stomach	2	4419
Zn	7	Ovaries	2	168655
Cu	8	Stomach	2	22034
Fe	3	Stomach	2	892809
Ni	10	Stomach	2	31252
Mn	4	Stomach	2	45552

Sex*: 1:male, 2: female, 3: uncertain.

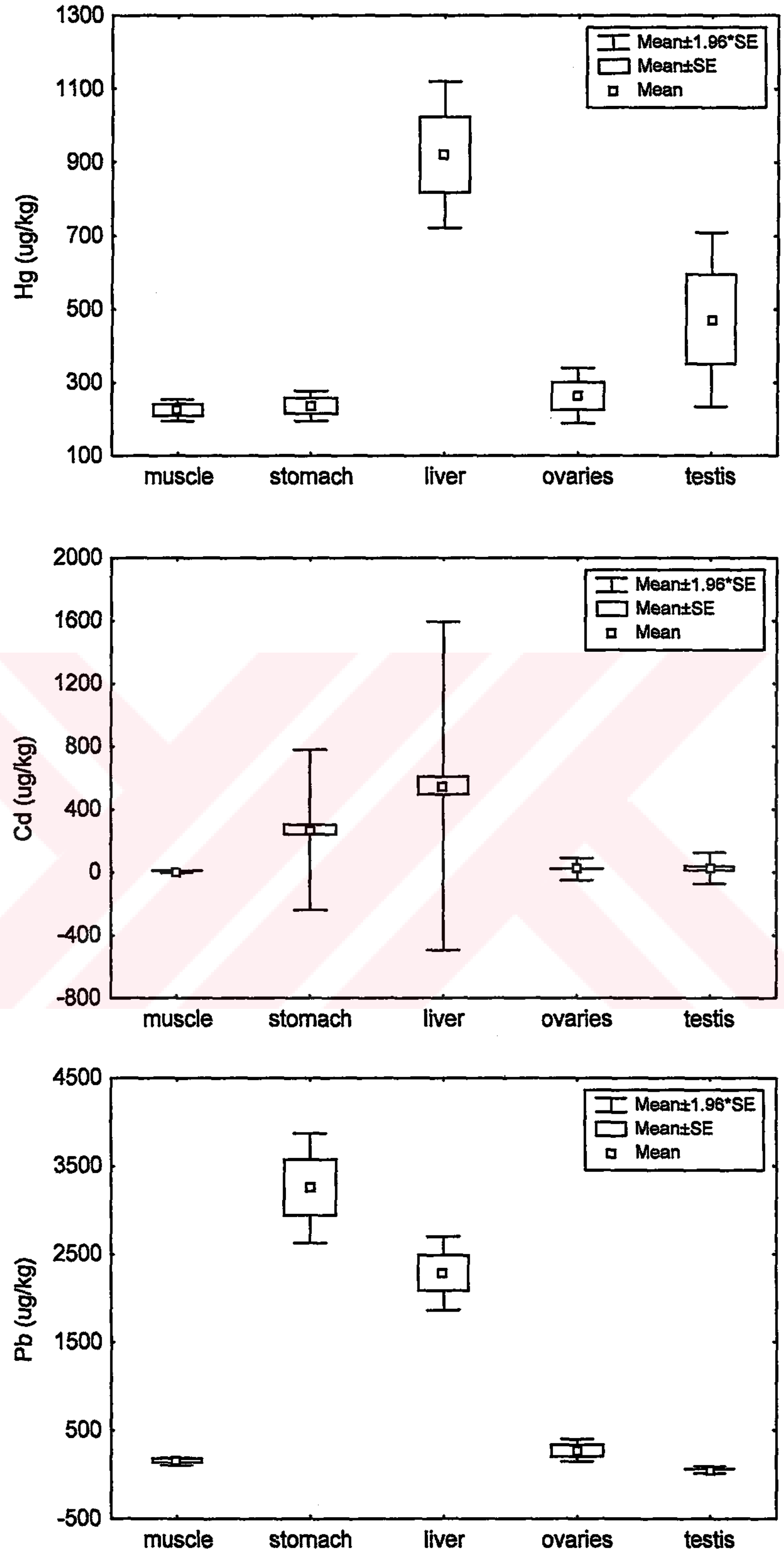

Figure 5.1. Means, mean ± standard error and confidence intervals (1.96σ) of trace metals in Red Pandora.

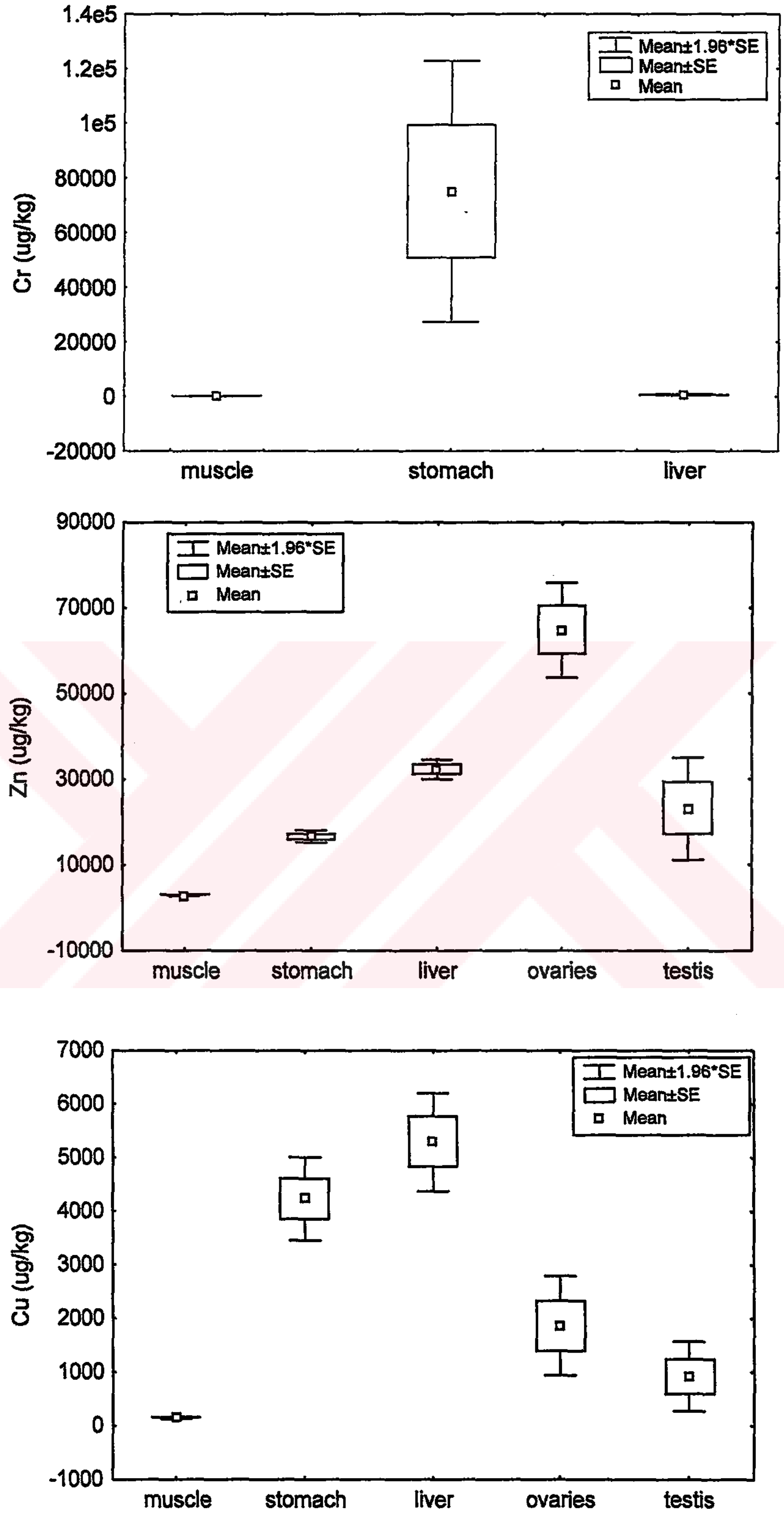

Figure 5.2. Means, mean ± standard error and confidence intervals (1.96σ) of trace metals in Red Pandora.

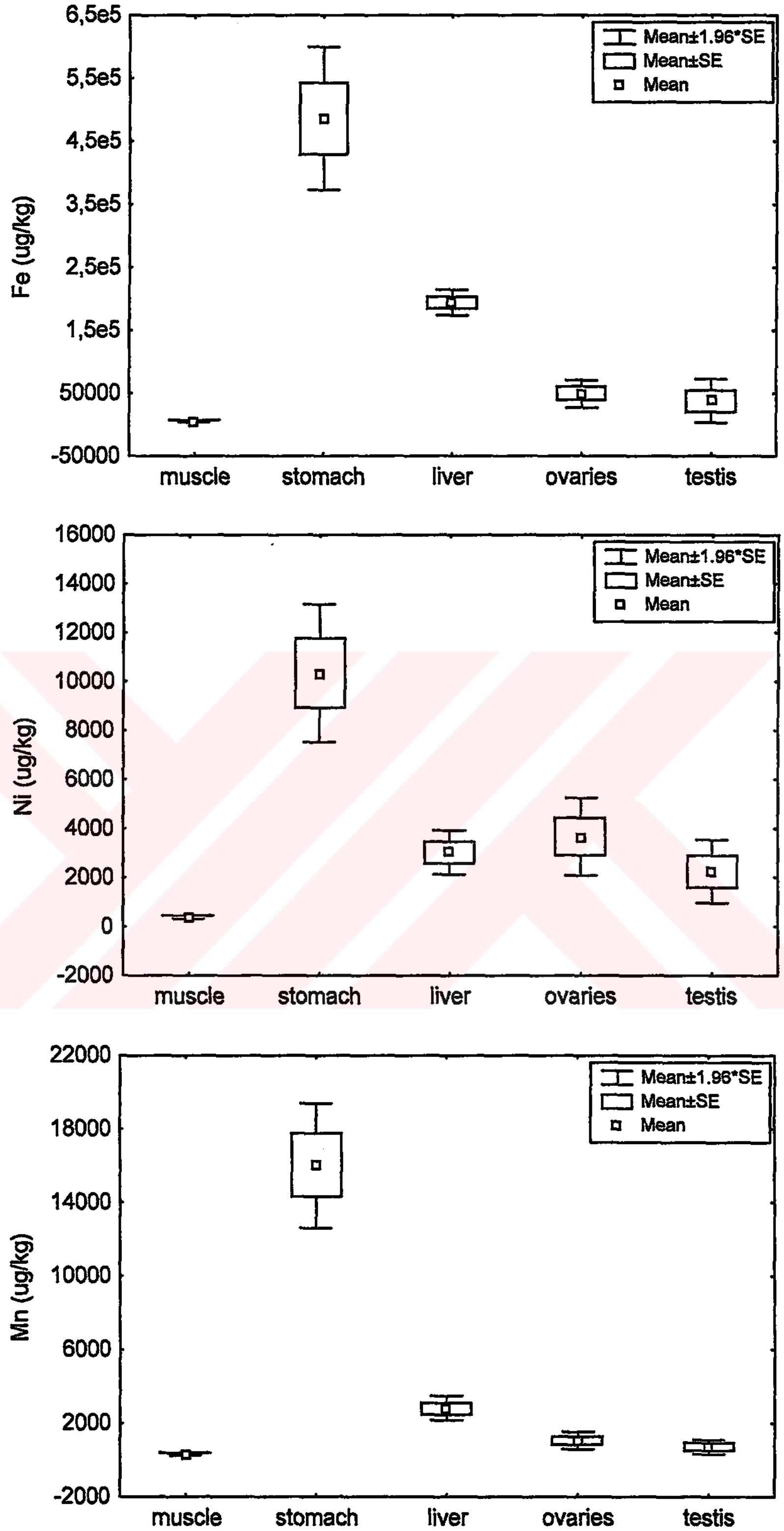

Figure 5.3. Means, mean ± standard error and confidence intervals (1.96σ) of trace metals in Red Pandora.

5.2. Distribution of Heavy Metals

The graphs presenting the whole set of data obtained for ten stations show the mean concentrations calculated for each organ during sampling periods (Figure 5.4.-5.12). In generally the concentration of Hg increased in fall and summer in gonads tissue and the Hg content of gonads were higher than in muscle. In summer, Hg concentrations decreased in muscle, liver and stomach during spawning periods. Highest values were found in liver during all sampling periods.

However the muscle always contains lowest concentrations of Cd than other tissues, liver concentrations of Cd exhibit high level. For liver, this metal concentration reached maximum values in winter. The levels of Cd in stomach are smaller than in liver same as Hg levels and maximum values of Cd is encountered in winter. Cd levels are higher in gonads than muscle at all term and this metal tends to increase in winter in gonads. In generally Pb levels in stomach are higher than other tissues while the gonads contain higher concentration of Pb than in muscle at all term. In liver this metal reached high levels in winter same as Cd and Hg. The values of Cr in stomach are higher than other tissues same as Pb while the muscle Cr is always lower than liver.

The levels of Cu in muscle are lower than other tissues and gonads tend to content less concentrations of Cu than liver and stomach. The values of Cu in liver reached maximum level in summer, during spawning periods. Compared to results for all organs Fe concentrations appeared to be low in muscle. Stomach shows elevated concentrations of this metal and the values of Fe in liver are lower than stomach contents. In generally muscle levels of Zn are lower than stomach and other organs. In winter the gonads contain maximum concentration of Zn than in liver this metal tends to increase in gonads but concentrations of Zn in liver appeared to be high than gonads. For all organs, the lowest concentrations of Ni were found in muscle while the maximum values detected in stomach. In gonads and liver, high quantities of Ni are reached in winter. According to results, the Mn levels in muscle are lower than other organs and in stomach this metal appeared to be high values same as Ni.

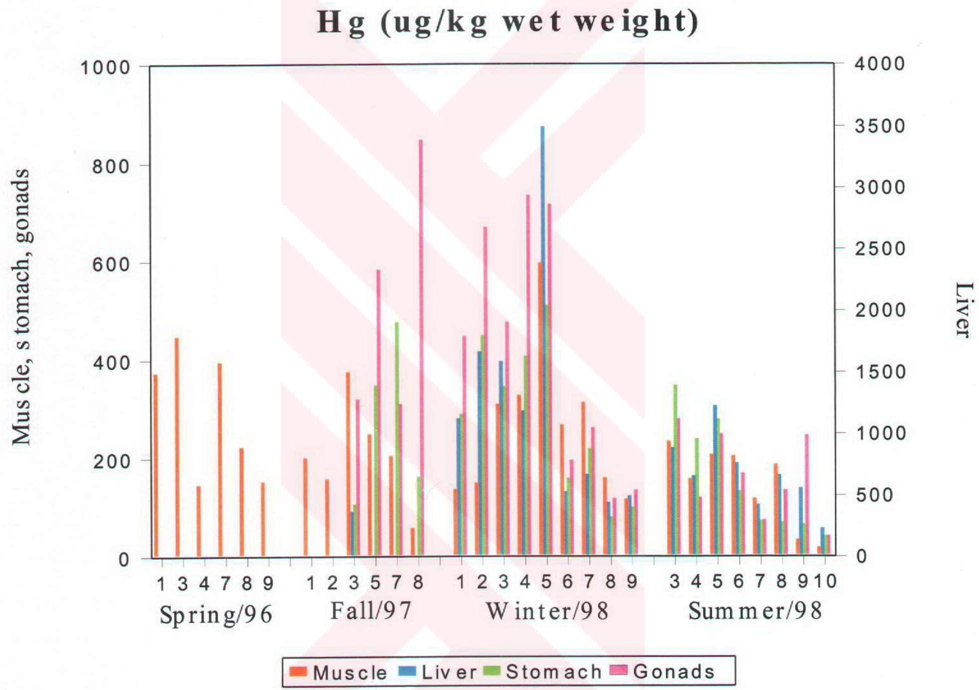

Figure 5.4. Mean Hg concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

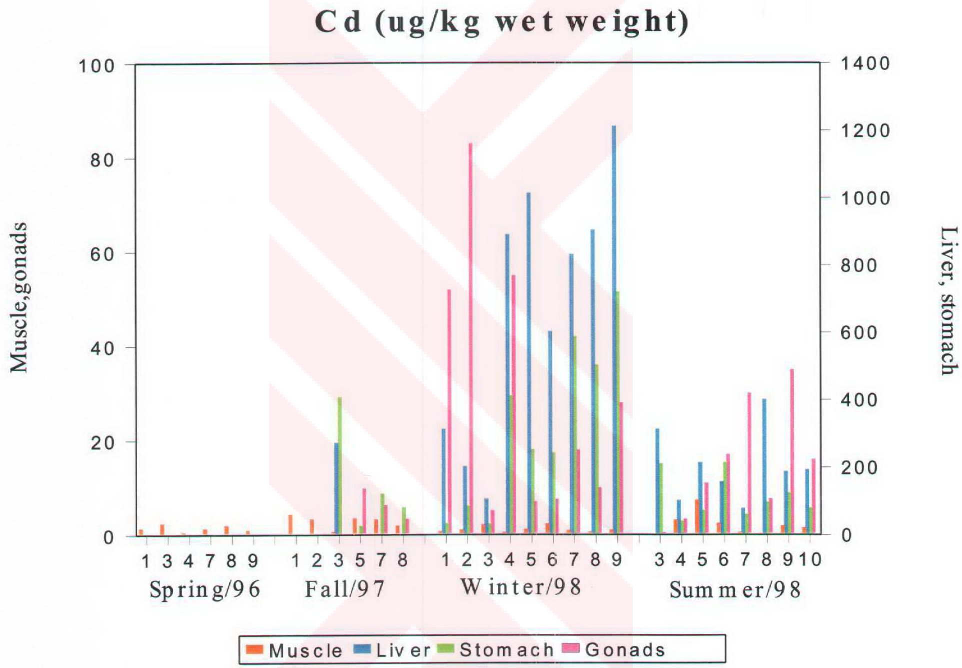

Figure 5.5. Mean Cd concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

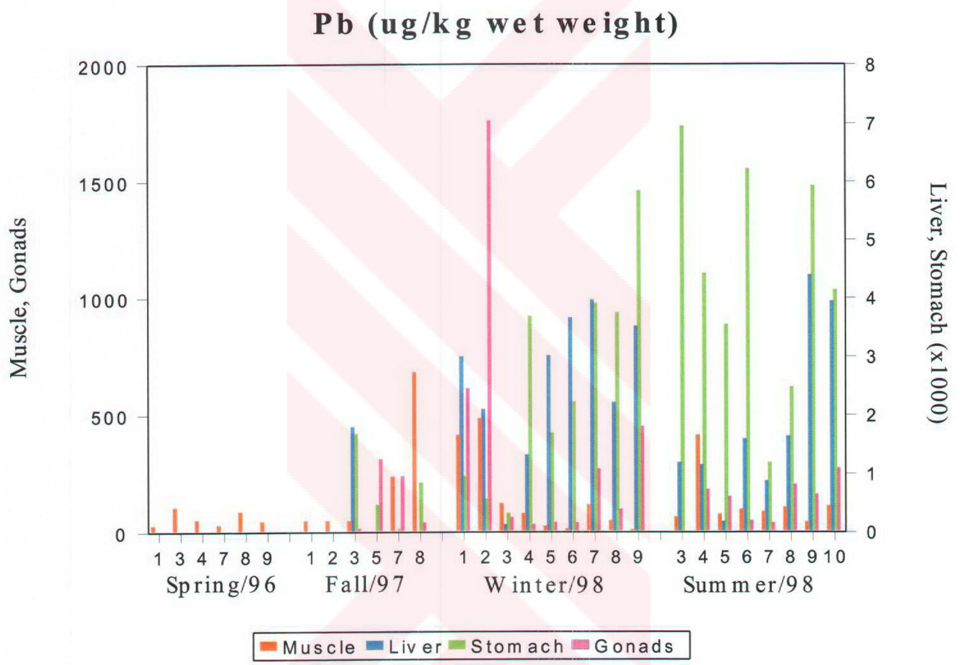

Figure 5.6. Mean Pb concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

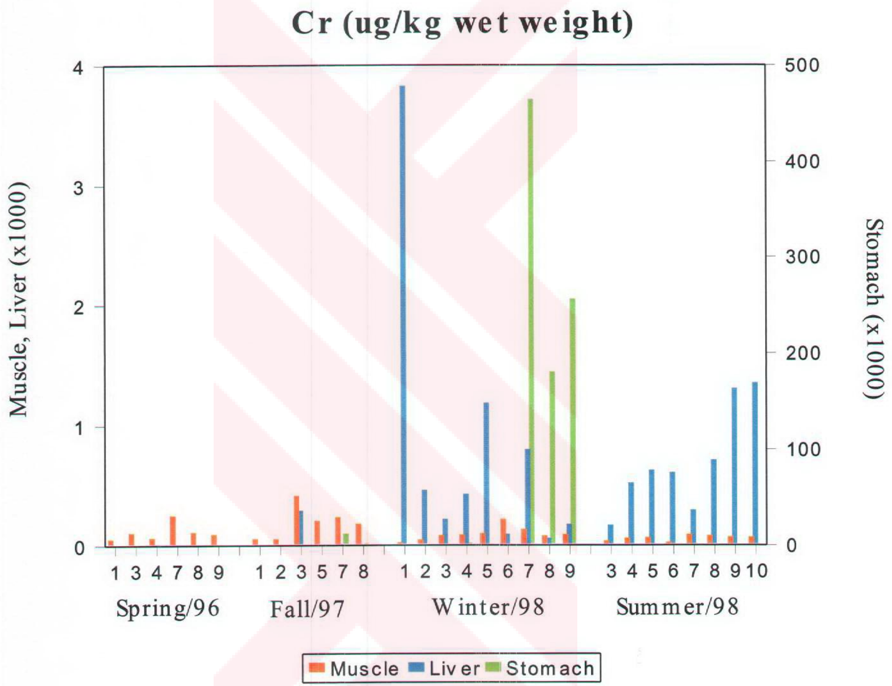

Figure 5.7. Mean Cr concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

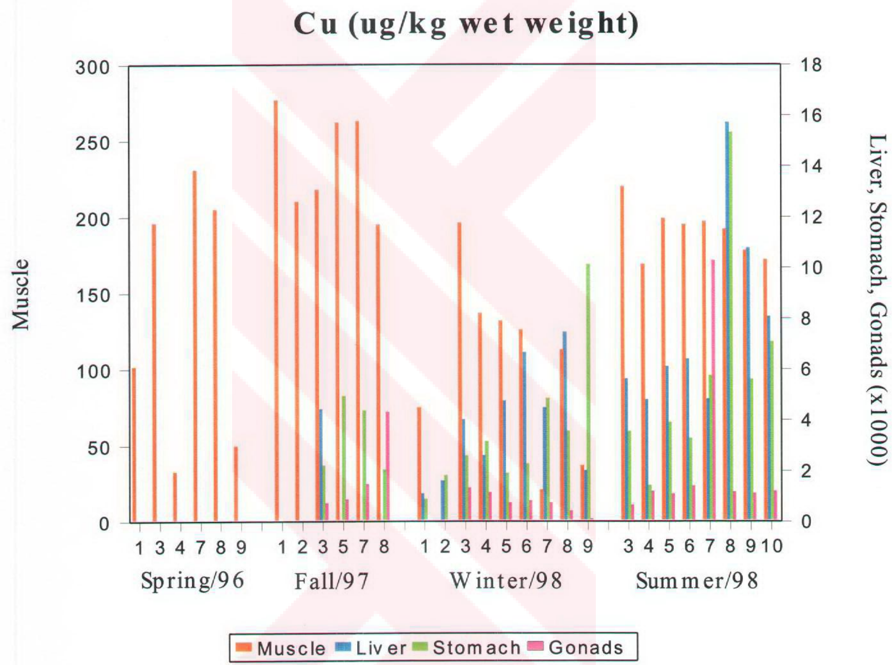

Figure 5.8. Mean Cu concentrations (µg/kg wet weight) in different tissues of Red Pandora from ten stations

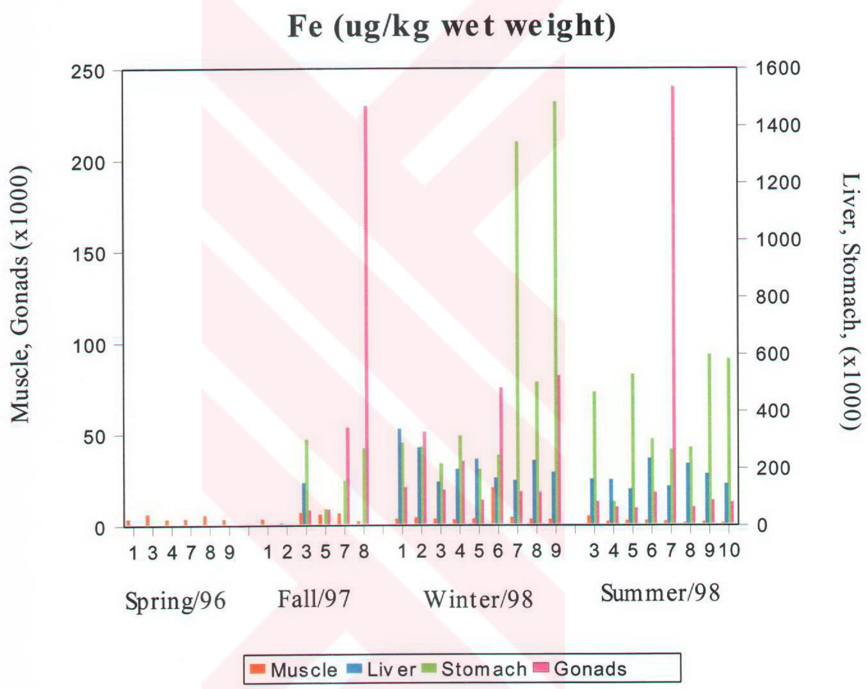

Figure 5.9. Mean Fe concentrations (µg/kg wet weight) in different tissues of Red Pandora from ten stations

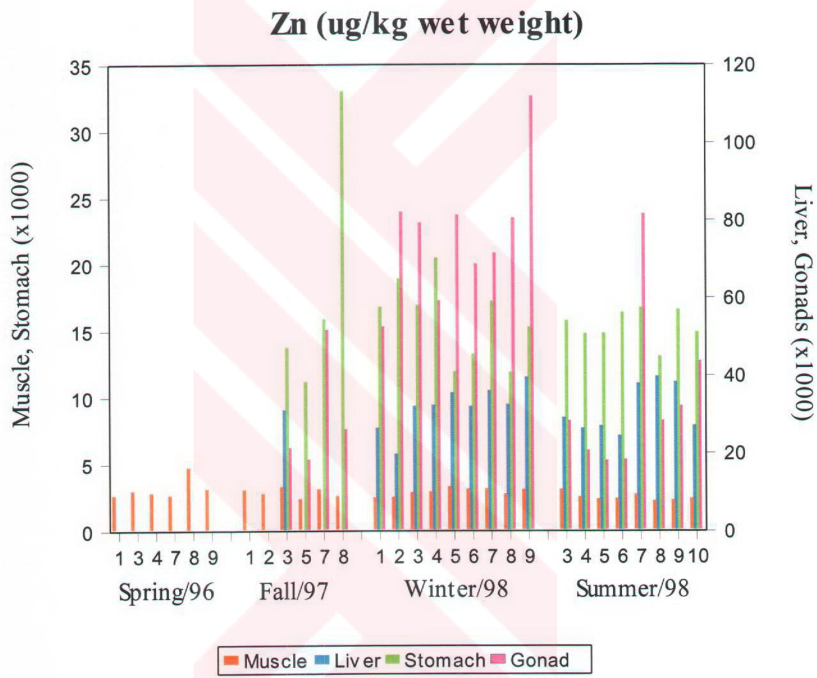

Figure 5.10. Mean Zn concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

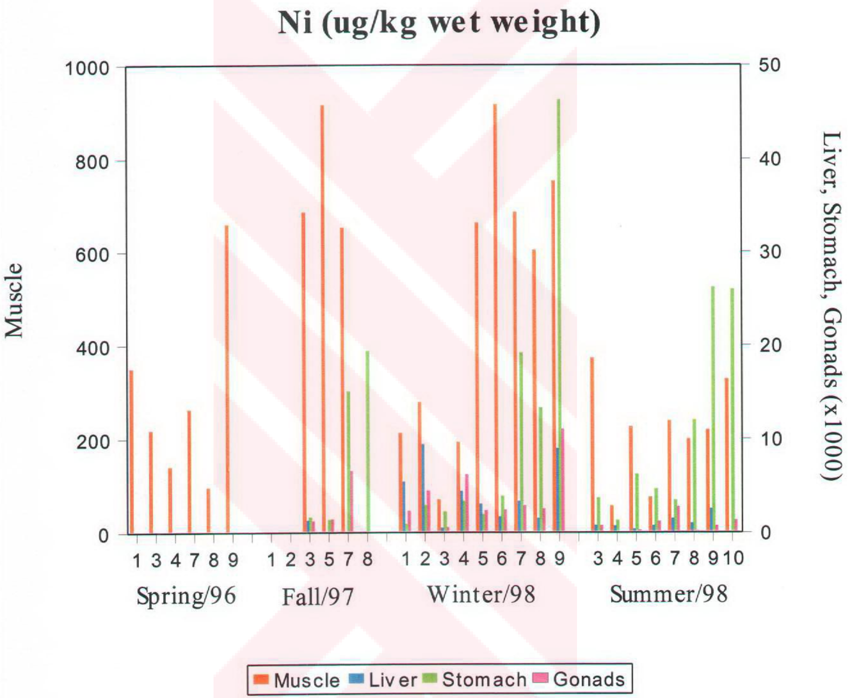

Figure 5.11. Mean Ni concentrations (µg/kg wet weight) in different tissues of Red Pandora from ten stations

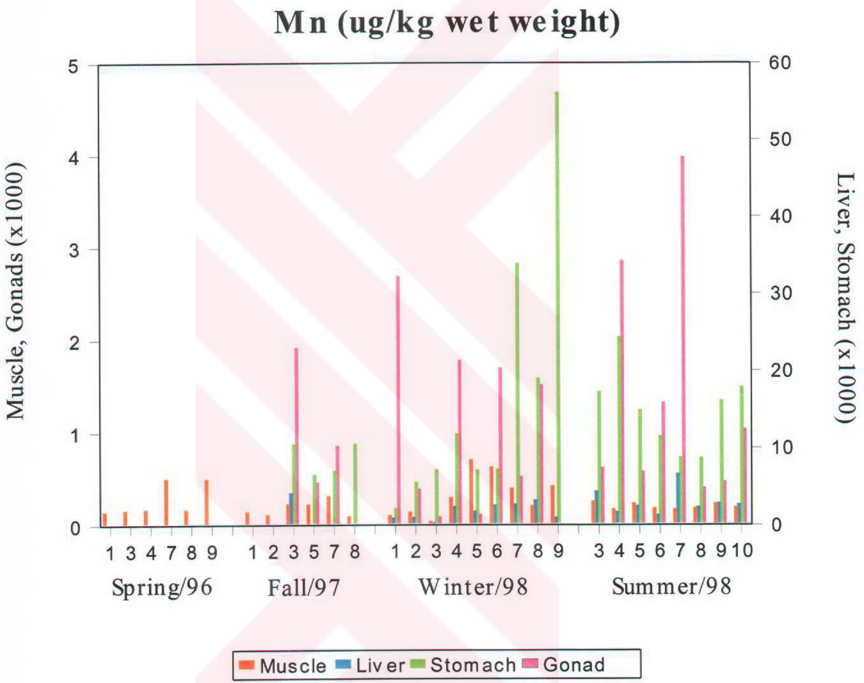

Figure 5.12. Mean Mn concentrations (μg/kg wet weight) in different tissues of Red Pandora from ten stations

5.3. Tissues Ratios of Heavy Metals

Between liver and muscle

The calculated ratios of metals between liver and muscle (L/M) are summarized in Table 5.8.

Table 5.8. Minimum, maximum and mean±standard error values of heavy metals ratios between liver and muscle (L/M)

(L/M)	*Mean*	*Min-Max*
Hg	5.43±0.69	0.37-34.88
Cd	890±157.2	27.1-7100
Pb	857.6±615.4	0.88-3930
Cr	8.50±1.931	0.112-125.46
Zn	11.48±0.49	1.12-29.51
Cu	43.44±4.28	0.78-142.19
Fe	56.48±3.91	1.56-165.62
Ni	8.02±1.3	0.84-60.13
Mn	16.17±4.35	0.83-307.29

Zn/Cu ratios inmuscle and liver

The ratios of zinc and copper (Zn/Cu) in muscle and liver are given in Table 5.9.

Table 5.9. Minimum, maximum and mean±standard error values of zinc and copper ratios in muscle (Zn/Cu)

Period	*Mean*	*Min-Max*
Muscle		
05/1995	16±2.8	11-26
09/1997	14±0.84	9.2-21
01/1998	19±1.2	8.5-27
07/1998	13±0.48	9.4-18
Liver		
01/1998	9.7±0.71	6.1-22
07/1998	5.4±0.53	2.1-11

5.4. Bioaccumulation Factors (BAFs) of Heavy Metals

Metal concentrations (μg/g dry weight) of sediments in this region and calculated BAF in different organs are summarized in Table 5.10.

Table 5. 10. Metal concentrations of sediments and mean BAF±standard error in various fish tissues

Sediment*	Cd	Pb	Cr	Zn	Cu
Edremit	0.21	50	59	78	31
Aliağa	0.31	45	58	88	29
İzmir Bay	0.28	34	59	54	23
Kuşadası	0.28	35	56	56	21
Didim	0.30	37	86	76	36
Gökova	0.25	30	50	55	21
BAF (%)					
Muscle	0.686±0.108	0.406±0.066	0.234±0.041	4.769±0.217	0.667±0.061
Liver	153±24.29	6.587±0.828	1.263±0.426	51.72±3.186	19.189±2.06
Gonads	8.278±2.428	0.742±0.193	-	926±101.9	9.049±2.924
Stomach	83.819±12.84	8.141±1.355	157.12±67.48	25.421±1.293	15.144±1.66

*Sediment concentrations (Batkı et al., 1999)

5.5. Statistical Analysis

5.5.1. Concentrations of heavy metals and body size relationship in different tissues

Matrices of Pearson's correlation coefficients were calculated each metals for tissue of different organs in Red Pandora (Table 5.11-5.12). There are no significant correlations between fork lengths and metal concentrations in muscle, liver and stomach tissue for all stations and seasons. In stomach, less significant correlation are found between Mn and Cd, Cr concentrations. Ni concentrations are less significant correlated with chromium. However numbers of significant positive correlations were detected between iron and chromium, nickel in stomach. Similar relationships were found among manganese nickel and iron.

In gonads, less significant correlations are found between Hg, Cd and fork length. The less significant negative correlation were found between Mn and size while significant positive correlation was encountered between iron and copper in testis tissues. Similar significant relationships were detected among copper and iron, manganese in ovaries.

Muscle

Concentration in muscle tissue differed significantly among season for Hg ($p<0.05$, $df=3$, $F=3.93$), Cr ($p<0.05$, $df=3$, $F=6.33$), Cu ($p<0.05$, $df=3$, $F=20.43$) and Ni ($p<0.05$, $df=3$, $F=3.98$). Hg is showed with a Post hoc Tukey HSD analysis that levels in July differed significantly from January. September is different from January and July for concentrations of Cr in muscle tissue. The copper concentrations are different in January from other months. In same tissue, January is different from July for nickel.

The significant differences between sampling areas for Hg ($p<0.05$, $df=9$, $F=4.59$) Fe ($p<0.05$, $df=9$, $F=5.37$) and Mn ($p<0.05$, $df=9$, $F=4.67$) are found in muscle. Due to Post hoc Tukey HSD analysis, mercury is showed that levels at Göcek is differed significantly from İzmir Körfezi and Kuşadası. Simultaneously Kuşadası is different from Marmaris. The iron concentrations are different in Didim from other stations. Didim were different from the first station (Edremit) in muscle tissue, while the Mn concentration was showing significantly difference in Kuşadası from Edremit, Aliağa and Marmaris.

Liver

The One-way ANOVA test showed the statistically significant seasonal differences for Cd ($p<0.05$, $df=3$, $F=13.31$),Cu ($p<0.05$, $df=3$, $F=6.51$), and Ni ($p<0.05$, $df=3$, $F=6.11$) in liver tissues. For Cd, Cu and Ni levels, January is different from July due to Post hoc Tukey HSD analysis.

Table 5.11. Pearson's correlation coefficients for relationships between nine heavy metals and average length in tissue of muscle, liver and stomach in Red Pandora (Marked correlations are significant at p< 0.05)

Muscle	Lenght	Hg	Cd	Pb	Cr	Zn	Cu	Fe	Ni	Mn
Lenght	1.00									
Hg	**0.26**	1.00								
Cd	-0.09	-0.03	1.00							
Pb	-0.08	**-0.28**	0.03	1.00						
Cr	0.09	0.07	0.08	**0.22**	1.00					
Zn	-0.08	**0.24**	-0.17	-0.04	0.15	1.00				
Cu	-0.04	-0.03	**0.24**	0.06	0.20	0.07	1.00			
Fe	0.10	0.17	0.13	-0.06	**0.32**	**0.27**	0.03	1.00		
Ni	-0.02	0.19	0.13	**-0.33**	**0.32**	-0.08	-0.14	**0.36**	1.00	
Mn	-0.19	**0.36**	0.07	**-0.32**	0.06	**0.26**	-0.17	**0.28**	**0.43**	1.00
Liver	Lenght	Hg	Cd	Pb	Cr	Zn	Cu	Fe	Ni	Mn
Lenght	1.00									
Hg	**0.33**	1.00								
Cd	0.17	**0.33**	1.00							
Pb	-0.15	0.04	**0.34**	1.00						
Cr	-0.20	0.15	0.04	**0.51**	1.00					
Zn	-0.09	-0.02	**0.34**	**0.23**	-0.01	1.00				
Cu	0.18	-0.10	-0.06	-0.03	-0.13	**0.29**	1.00			
Fe	0.22	**0.31**	**0.27**	0.22	**0.41**	0.15	-0.05	1.00		
Ni	**-0.27**	0.07	**0.29**	0.19	0.21	0.12	**-0.38**	**0.24**	1.00	
Mn	-0.03	-0.16	-0.17	-0.17	-0.14	0.20	0.17	-0.03	-0.21	1.00
Stomach	Lenght	Hg	Cd	Pb	Cr	Zn	Cu	Fe	Ni	Mn
Lenght	1.00									
Hg	**0.33**	1.00								
Cd	-0.03	-0.07	1.00							
Pb	0.05	-0.09	**0.40**	1.00						
Cr	**-0.24**	-0.16	**0.37**	0.10	1.00					
Zn	-0.06	0.17	-0.03	-0.10	-0.06	1.00				
Cu	-0.18	**-0.26**	0.19	0.19	0.11	-0.04	1.00			
Fe	**-0.27**	-0.14	**0.45**	**0.27**	**0.81**	0.05	**0.29**	1.00		
Ni	**-0.30**	**-0.30**	**0.40**	**0.27**	**0.53**	0.09	**0.42**	**0.74**	1.00	
Mn	-0.21	-0.12	**0.57**	**0.31**	**0.54**	0.06	**0.39**	**0.79**	**0.74**	1.00

Table 5.12. Pearson's correlation coefficients for relationships between heavy metals and size in gonads in Red Pandora (Marked correlations are significant at p< 0.05)

Ovaries	Lenght	Hg	Cd	Pb	Zn	Cu	Fe	Ni	Mn
Lenght	1.00								
Hg	**0.46**	1.00							
Cd	**-0.24**	0.09	1.00						
Pb	-0.08	0.19	**0.74**	1.00					
Zn	**-0.21**	0.08	**0.26**	0.15	1.00				
Cu	**-0.28**	**-0.25**	0.13	**-0.20**	**0.37**	1.00			
Fe	**-0.33**	-0.03	**0.28**	-0.11	**0.38**	**0.82**	1.00		
Ni	-019	-0.10	0.01	-0.04	**0.51**	**0.33**	**0.29**	1.00	
Mn	**-0.28**	**-0.21**	**0.20**	-0.14	**0.34**	**0.85**	**0.72**	**0.37**	1.00
Testis	Lenght	Hg	Cd	Pb	Zn	Cu	Fe	Ni	Mn
Lenght	1.00								
Hg	**0.51**	1.00							
Cd	**0.43**	**0.33**	1.00						
Pb	-0.2	-0.08	0.02	1.00					
Zn	-0.07	**0.41**	**-0.23**	**-0.24**	1.00				
Cu	-0.10	0.07	**-0.23**	-0.03	0.07	1.00			
Fe	0.01	0.17	-0.13	-0.02	**0.31**	**0.80**	1.00		
Ni	**0.26**	-0.05	-0.02	0.19	0.01	**-0.45**	**-0.25**	1.00	
Mn	**-0.57**	-0.32	-0.17	**0.34**	-0.13	**0.37**	0.03	**-0.46**	1.00

The significant differences among stations are found for the concentrations of Hg ($p<0.05$, df=9, F=3.76), Cr ($p<0.05$, df=9, F=3.26), Cu ($p<0.05$, df=9, F=3.76) and Ni ($p<0.05$, df=9, F=3.49) in liver tissue. Cr is showed with a Post hoc Tukey HSD test that levels at first station differed significantly from other stations. For mercury levels in Kuşadası is different from Didim, Gökova, Marmaris, and Göcek. While Cu level at Marmaris was showing difference from Çeşme and Gökova, Aliağa is different from İzmir Körfezi and Didim for concentrations of Ni in liver tissue.

Stomach

In stomach tissues, statistically significant seasonal differences were detected only for Cd ($p < 0.05$, df=3, F= 11.67) and Pb ($p < 0.05$, df=3, F=5.15) levels. Due to Post hoc Tukey HSD analysis, the concentrations of Pb were calculated at September is different from July. The January is significantly different from July for Cd levels.

The concentrations in stomach tissue differed significantly among stations for Hg ($p < 0.05$, df=9, F=6.13), Ni ($p < 0.05$, df=9, F=11.54) and Mn ($p < 0.05$, df=9, F=3.33). In generally Ni and Mn showed with a Post hoc Tukey HSD analysis that levels at Göcek differed significantly from other stations. The calculated concentrations of Hg at Marmaris and Göcek are different from Aliağa, Çeşme and Kuşadası.

Ovaries

Concentrations in ovaries tissue differed significantly among seasons for only Zn ($p<0.05$, df=3, F=3.98). Zn showed with a Post hoc Tukey HSD analysis that levels in July differed significantly from January.

The significant differences between stations for Cd ($p<0.05$, df=9, F=5.37) and Pb ($p<0.05$, df=9, F=4.67) were found in this tissue. Due to Post hoc Tukey HSD analysis, for cadmium, Aliağa is different from İzmir Körfezi, Çeşme, Kuşadası, Didim and Marmaris. Aliağa was different from all station, except the first station, while Pb concentration was showing significantly difference in Edremit from İzmir Körfezi and Didim.

Testis

The ANOVA test showed the statistically significant seasonal differences for Cu ($p<0.05$, df=3, F=13.31), Fe ($p<0.05$, df=3, F=6.51), and Mn ($p<0.05$, df=3, F=13.31) in testis tissues. For Cu, July was different from September due to Post hoc Tukey HSD analysis, while value January was showing difference from September and July. September is significantly different from January and July for Fe level. Mn level in July differed significantly from January. The significant differences among stations were found for only Ni ($p<0.05$, df=9, F=3.49) in this tissue. Mn showed with a Post hoc Tukey HSD test that levels at İzmir Bay differed significantly from Didim.

5.5.2. Two-Way ANOVA Analysis

Analysis of covariance (ANCOVA) was used to compare the seasonal differences in the heavy metal concentrations of tissues, with metal concentrations as the dependent variable, season as the independent variable and mean length as the covariate. In consequence, the comparison of muscle concentrations of Hg, Cd, Pb, Cr, Zn, Cu, Ni and Mn demonstrate that, there are significant ($p<0.05$) seasonal variations. For liver, there are significant ($p<0.05$) seasonal variations of Cd, Cu and Ni. The significant seasonal variations of Zn and Ni were found in ovaries tissues while seasonal variations of Cu and Fe were detected in testis. Significant season-length interactions were observed for Cd, Pb and Cr concentrations in stomach (Table 5.13).

Data analysed with station as the independent variable and mean length as covariate that regional variations of Hg, Pb, Cu, Fe, Ni and Mn were found in muscle and stomach tissues. The test showed statistically significant regional differences for Hg, Cr, Cu, Fe and Ni concentrations of liver tissues. For ovaries, there are significant regional variations of Cd and Pb (Table 5.14).

Analysis of covariance was performed to compare the regional differences in the metal concentrations of tissues, with station as independent variable and season as the covariate, the covariance analysis show that there are regional differences of Hg, Pb, Cu, Fe, Ni and Mn in muscle tissues. The comparison of liver concentrations of Hg, Cr, Cu, and Ni demonstrate that, there are significant ($p<0.05$) regional variations. The significant regional variations of Cd and Pb were found in ovaries tissues while regional variations were not detected in testis. Significant station-season interactions were observed for Hg, Cu, Fe, Ni, Mn concentrations in stomach.

Table 5.13. Effect of inter-season and inter-station variations covariate size on the variability of organs tissues in Red Pandora

	Season			Station		
	df	*F*	*P level*	*df*	*F*	*P level*
Muscle						
Hg	3	4.03	**0.00963**	9	3.920	**0.000315**
Cd	3	3.07	**0.03205**	9	1.352	0.223496
Pb	3	3.08	**0.03108**	9	2.205	**0.028915**
Cr	3	6.25	**0.000655**	9	1.052	0.406533
Zn	3	3.29	**0.023951**	9	0.578	0.812060
Cu	3	20.16	**0.0000**	9	2.32	**0.021685**
Fe	3	2.33	0.0789	9	5.27	**0.000009**
Ni	3	4.07	**0.009176**	9	1.99	**0.049975**
Mn	3	3.96	**0.010473**	9	4.69	**0.000044**
Liver						
Hg	2	2.44	0.0942	9	4.376	**0.000172**
Cd	2	13.08	**0.000014**	9	1.655	0.118108
Pb	2	1.41	0.2516	9	1.643	0.121408
Cr	2	0.076	0.9264	9	2.831	**0.007234**
Zn	2	0.0263	0.9741	9	1.697	0.107457
Cu	2	7.211	**0.001387**	9	3.639	**0.000976**
Fe	2	1.8195	0.1694	9	2.716	**0.009343**
Ni	2	7.143	**0.001468**	9	3.053	**0.004090**
Mn	2	2.186	0.1197	9	1.016	0.436855
Stomach						
Hg	2	2.948	0.058247	9	5.287	**0.000005**
Cd	2	11.667	**0.00038**	9	1.776	0.088703
Pb	2	5.464	**0.005966**	9	2.036	**0.047047**
Cr	2	4.053	**0.02058**	9	1.220	0.296124
Zn	2	2.634	0.078047	9	0.606	0.788023
Cu	2	2.869	0.062633	9	2.750	**0.007977**
Fe	2	2.733	0.071098	9	2.089	**0.041326**
Ni	2	0.677	0.511261	9	9.904	**0.000000**
Mn	2	1.399	0.252695	9	2.861	**0.006034**

Table 5.14. Effect of inter-season and inter-station variations and covariate size on the variability of gonad tissues in Red Pandora

	Season			*Station*		
	df	*F*	*P level*	*df*	*F*	*P level*
Ovaries						
Hg	2	2.282	0.110569	9	1.353	0.232075
Cd	2	0656	0.522489	9	4.244	**0.000328**
Pb	2	2.062	0.136014	9	12.96	**0.000000**
Zn	2	7.419	**0.001278**	9	0.554	0.828289
Cu	2	2.912	0.061728	9	1.056	0.409039
Fe	2	0.773	0.465920	9	0.796	0.621238
Ni	2	3.353	**0.04137**	9	1.076	0.394979
Mn	2	2.624	0.080446	9	0.556	0.826417
Testis						
Hg	2	0.426	0.665356	5	2.428	0.155211
Cd	2	0.152	0.860790	5	0.845	0.563740
Pb	2	0.041	0.959736	5	3.059	0.102982
Zn	2	0.563	0.588275	5	0.913	0.530033
Cu	2	37.940	**0.000041**	5	0.310	0.890432
Fe	2	25.404	**0.000199**	5	0.158	0.969453
Ni	2	0.503	0.620568	5	0.576	0.720809
Mn	2	2.183	0.168635	5	3.171	0.096242

CHAPTER SIX
DISCUSSION

6.1. Tissue concentrations

Few studies were found on metal levels in Red Pandora. Therefore obtained results were compared with literature data for other fish species. Related publications on heavy metals in different organs of fish species are summarized in Table 6.1 and 6.4.

Muscle

In generally the Hg concentrations in Red Pandora from Eastern Aegean Sea are similar to those reported by previous studies carried out in Mediterranean. Obtained values on Cd and Cu content in the muscle tissues are lower than most values for the Mediterranean. Our results of Pb and Zn are nearly close within same range of reported data. Fe levels are higher than other area (except Italy) (Demirkurt et al., 1990; Hornung & Kress, 1991; Bei et al., 1992; Pastor et al., 1994; Focardi et al., 1998; Kucuksezgin et al., 2001; Gaspic et al., 2001,2002; Yazkan et al., 2002; Topcuoglu et al., 2002; Kucuksezgin et al., 2002, Yılmaz, 2003).

Liver

Gaspic et al. (2001; 2002), Kucuksezgin et al. (2002) and Yazkan et al. (2002) researched heavy metal levels in liver tissue in Mediterranean. When comparing the previous results, the concentrations of Hg, Pb Cd, Zn and Cu are higher in Red Pandora from Aegean Sea than the other areas (Table 6.2). The liver tissue in fish are more offen recommended as an environmental indicator of water pollution than any other fish organs. This is possibly attributed to the tendency of liver to accumulate pollutants of various kinds at higher levels from their environment (Galindo et al., 1986). The specific metabolism process and the enzyme-catalyzed reaction taking place in liver involving Zn, Cu, Mn and Cd may also account for this behavior (Jaffar & Shahid, 1986).

Gonads

Related publications on heavy metals in gonads of some species fish include Vas & Gordon (1993); Chalabi et al. (1992); Das et al. (2000); Mormede et al. (2001a) and Yılmaz (2003) (Table 3). The concentrations of Hg, Cd, Ni, Zn and Mn found during this study are higher than other results from Mediterranean and Atlantic (Table 6.3). Compared to the results obtained in this study, Cu level are similar to those found in the previous study although Pb levels are lower than the other sites.

Stomach

Generally, few studies were found on metal levels in stomach from marine environment. When comparing the other results, the heavy metal levels (except Zn) in this study tended to be higher than these reported in the current literature (Table 6.4).

Even trough the heavy metal concentrations in the fish muscular are usually low, the amounts found in the viscera and waste products of fish often exceed those permitted for human consumption. This is of relevance when one consider that both viscera and waste products play an important role in food industry (e.g., cod liver oil, animal feed) and still present a danger to man.

Dietary habits have an impact on metal concentrations in marine organisms and heavy metals biomagnifies in variety aquatic food webs. Red Pandora is omnivorous, mainly carnivorous. It feeds benthic invertebrates and small fish. The diet was found to consist primarily of polychates, especially glyceridae, and crustacea, mainly natantia, occasionally fishes and cephalopods. Isopods, cumacea, mysids, and anisopods represented negligible sources of food (Ardizzone & Messina, 1983; Andaloro & Giarritta, 1985; Caragitsou & Papaconstantinou, 1988; Özaydın, 1997; Benli et al., 2001). Carnivorous fishes feed particularly on mollusk and crustaceans that are known to accumulate high levels of heavy metals in their body (GESAMP, 1985; Heat, 1987; Roesijadi & Robinson, 1994). It could be expected that Red Pandora will accumulate high level of heavy metals. The high concentrations of Pb, Fe, Ni and Mn in stomach are associated with its food and food habits.

Based on total content of accumulated Hg, tissues could be generally ordered as liver>gonads>stomach>muscle. We observed decrease of Cd concentrations as follow: liver>stomach>gonads>muscle. The trend in Pb level in present study was stomach>liver>gonads>muscle. Chromium mean concentration was found as stomach>liver>muscle. Zinc was mainly accumulated followed by gonads> liver>stomach>muscle. Concerning concentrations of Cu the order were changed as: liver>stomach>gonads>muscle. The distribution pattern of Fe and Mn follows order: stomach>liver>gonads>muscle while nickel follows the sequence: stomach> gonads>liver>muscle.

The gonads showed higher concentrations of metal than those in muscle. Other authors also found higher levels of Cu and Zn in gonads than in muscle other fish species (Papagiannis et al., 2003). High metal levels were then found in gonads that might excrete part of toxic metals (Mormede & Davies, 2001a). Gonads have been reported as a structure likely to present high Zn concentration due to their participation in cellular division and growth processes (Lacerda et al., 1989.).

Higher Hg, Cd, and Cu concentrations were found in liver tissue, while the lowest were detected in muscle tissues. This finding is agreed with those of other studies regarding the differences between heavy metal accumulations in fish tissues (Papagiannis et al., 2003; Mormede & Davies, 2001(a,b); Andres et al., 2000). The highest metal concentrations were found in liver, showing the detoxification and accumulation role of that organ. The low concentrations of Cu and Zn in muscle of examined fish species may reflect the low levels of these binding proteins in the muscle (Papagiannis et al., 2003). However, muscle is commonly analyzed because it is the main fish part consumed by humans and implicated in health risk.

6.2. Comparison with the European dietary standards and guidelines

Dietary standards and guidelines applicable in the UK for heavy metals in fish have been summarized by Ministry of Agriculture, Fisheries and Food (MAFF, 1995). The comparison between mean of metals in muscle tissues to these guidelines is as follows:

Mercury: All the mean levels were below the guideline (500 μg/kg). If these fish were ever to be marked, the consumption of flesh should not be a problem.

Cadmium: The mean concentrations of Cd in during sampling periods were quite below whereas limit of human consumption (200 μg/kg). Even the highest concentrations were found 9.6 μg/kg.

Lead: All the means of Pb were below the guidelines (2000 μg/kg) as a safe limit, the means being about 10-20 times lower.

Copper: During sampling periods all means levels were well under the European limits of 20000 μg/kg wet weight.

Zinc: The muscles of individuals were below human consumption of 50000μg/kg.

Chromium: The Cr levels presented muscle with values above the human consumption limit (100 μg/kg) (Langard & Norseth, 1979).

Nickel: According to Australian legislation, maximum levels of Ni below that is permitted limit (1000 μg/kg) (Sharif et al., 1993).

Table 6.1. Metal concentration (µg/kg wet weight) in muscle from Mediterranean Sea

Species	Area	Hg	Cd	Pb	Cr	Zn	Cu	Fe	Reference
S. vulgaris	Izmir Bay	5-57	7-277	53-5580	-	3011-9263	-	3336-10694	Demirkurt et al., 1990
P. erythrinus	Israel	53-805	4-80	-	7-120	2200-7590	50-1000	-	Hornung & Kress 1991
D. sargus	Israel	5-1020	12-63	-	-	2050-7370	200-1010	1940-10800	Hornung & Kress 1991
L. mormyrus	Israel	nd-441	nd-60	-	-	3590-8870	170-590	2760-7580	Hornung & Kress 1991
P. ehrenbergi	Israel	187-274	nd-38	-	-	2560-5840	180-520	1720-5270	Hornung & Kress 1991
M. barbatus	Israel	8-313	nd-93	-	-	3010-6140	200-1390	1890-7720	Hornung & Kress 1991
M. barbatus	Greece	-	640-770	--	-	-	2.47-3.37	-	Bei et al., 1992
P. erythrinus	Spain	50-280	10-51	50-350					Pastor et al., 1994
B. boops	Spain	75	312	215					Pastor et al., 1994
M. barbatus	Spain	30-1162	50-510	50-11189					Pastor et al., 1994
D. sargus	Spain	90-130	30-40	50-4390					Pastor et al., 1994
M.merluccius	Italy	129	42	-	-	32500	5610	45000	Focardi et al., 1998
M barbatus	Turkey	16-200	0.57-4.5	40-207	-	-	-	-	Kucuksezgin et al.,2001
P.erythrinus	Adriatic		4.2-19	60-170					Gaspic et al., 2001
M. barbatus	Adriatic	-	7.6-28.9	57-158					Gaspic et al., 2002
P. acarne	Antalya Gulf	-	50	200	-	4000	1500-	-	Yazkan et al., 2002
B. boops	Antalya Gulf	-	30	800	-	4000	1300	-	Yazkan et al., 2002
M. barbatus	Antalya Gulf	-	75	400	-	4000	2000	-	Yazkan et al., 2002
S. solea	Antalya Gulf	-	120	200	-	7000	1100		Yazkan et al., 2002
S. auratus	Antalya Gulf	-	60	300	-	5000	1000	-	Yazkan et al., 2002
E.encrasicolus	Black Sea	-	100-160	<50	330-840	35700-44200	2210-3090	3700-6100	Topcuoglu et al., 2002
M barbatus	Izmir Bay	27-345	0.10-5.7	29-943	-	-	-	-	Kucuksezgin et al.,2002
P. erythrinus	Izmir Bay	144-829	0.21-10	28-376					Kucuksezgin et al.,2002
P. erythrinus	Izmir Bay	144-829	0.21-10	28-376					Kucuksezgin et al.,2002
D. annularis	Izmir Bay	74-370	0.16-4.5	7.1-697					Kucuksezgin et al.,2002
Tmediterraneus	İskenderun			1030	1280	19550	1290	41840	Yılmaz, 2003
M. cephalus	İskenderun			7450	1460	38230	1450	70280	Yılmaz, 2003
P. erythrinus	**Aegean Sea**	**16-716**	**nd-9.6**	**nd-1397**	**nd-1180**	**1352-6693**	**nd-383**	**125-26382**	**This study**

Table 6.2. Metal concentrations (µg/kg wet weight) in liver from Mediterranean Sea

Species	Area	Hg	Cd	Pb	Zn	Cu	Reference
G. melastomus	Italy	nd-660	9-280	nd-650			Storelli et al. 1998
P. erythrinus	Adritic	-	60-408	300-827			Gaspic et al., 2001
M. barbatus	Adriatic		11.2-183	99-970			Gaspic et al., 2002
M.merluccius	Adriatic		6.5-153	49-141			Gaspic et al., 2002
M.barbatus	İzmir Bay	232-504	18-26	380-413			Kucuksezgin et al., 2002
P. acarne	Antalya Gulf	-	100	400	6000	1500	Yazkan et al., 2002
S. auratus	Antalya Gulf		90	410	6000	1100	Yazkan et al., 2002
B. boops	Antalya Gulf		50	1100	6700	1400	Yazkan et al., 2002
M. barbatus	Antalya Gulf		95	450	3500	2000	Yazkan et al., 2002
S. solea	Antalya Gulf		120	220	6500	1100	Yazkan et al., 2002
P.erythrinus	**Aegean Sea**	**125-5451**	**1.4-2245**	**nd-8311**	**2710-78705**	**nd-21986**	**This study**

Table 6.3. Metal concentrations (µg/kg wet weight) in gonads from Mediterranean and other sea

Species	Area	Hg	Cd	Pb	Zn	Cu	Ni	Mn	Reference
X. gladius	Algeria	29-85	-	2380-4010	$(289\text{-}485)\times10^3$	4480-5880	-		Chalabi et al., 1992
G. melastomus	Atlantic	-	-	-	-	110	1690	<2	Vas & Gordon 1993
E. spinax	Atlantic					1660	710	230	Vas & Gordon 1993
D. calceus	Atlantic					<2	3200	340	Vas & Gordon 1993
E. princes	Atlantic					<2	190	<2	Vas & Gordon 1993
L. piscatorius	Atlantic	-	<5	-	-				Mormede et al., 2001
T mediterraneus	İskenderun Bay			710-1400	9000-30000	66-1910	320-1630		Yılmaz, 2003
M. cephalus	İskenderun Bay			4200-11000	21000-51000	920-1980	830-1720		Yılmaz, 2003
P.erythrinus	**Aegean Sea**	**2.0-1858**	**nd-192**	**nd-2927**	**7273-205242**	**nd-20499**	**nd-41513**	**nd-10515**	**This study**

Table 6.4. Metal concentrations (µg/kg wet weight) in stomach from fresh water and other sea

Species	Area	Hg	Cd	Pb	Zn	Cu	Fe	Mn	Reference
L. limanda	Atlantic	18-87							Dixon & Jones, 1994
P. platessa	Atlantic	nd-108							Dixon & Jones, 1994
R.clavata	Atlantic	nd-83							Dixon & Jones, 1994
M. barbatus	Aegean	17-270	8.6-22	265-537					Kucuksezgin et al., 2002
L. macrolepis	Taiwan		1000-1110	-	40000-50000	8000-9000	$(40\text{-}90)10^3$	1500-2000	Chen & Chen., 1999
C carpio	Japan		33	30	447000	1646	66000	4646	Alam et al., 2002
R. rutilus	France		451		110000				Andres et al., 2000
P. fluviatilis	France		360		25000				Andres etal., 2000
P.erythrinus	**Aegean Sea**	**5.0-948**	**2.3-1139**	**14-18197**	**3200-50000**	**nd-22034**	**$(0.5\text{-}2471)10^3$**	**301-94340**	**This study**

6.3. Concentrations of heavy metals and size relationship in different tissues

Heavy metal content in aquatic organisms can vary with their size. The slope values of the concentration-size correlations depend on several factors, such as availability of the metal in environment, the specific turnover rate of the metal in the animal, tissues type and competition between the opposing effects of ageing and tissue growth on metal accumulation within tissue. In this study, no significant correlation was found between heavy metal levels in muscle, liver, stomach and gonads and length of Red Pandora. The concentrations of most heavy metals in tissues of small-and medium-sized fish species usually decrease or remain unchanged with the fish length increase (Hornung et al., 1987; Philips, 1992; Cossa et al., 1992). The levels of Cu and Zn did not depend on body weight or length. Widianarko et al. (2000) indicated that body concentrations of copper and zinc are regulated and maintained at a certain concentrations. It is well known that one of the most important factors that play a significant role in heavy metal accumulation in marine animals is the metabolic activity. It is also known that the metabolic activity of a young individual is normally higher than that of an older individual. Thus, metal accumulation was shown to be higher in younger individuals than the older ones (Canli & Furness, 1993a, b; Nussey et al., 2000; Widianarko et al., 2000).

6.4. Ratios of Heavy Metals

Zn:Cu ratios suggests that both Zn and Cu, which are essential metals for the organisms, are needed for certain physiological processes. In order to maintain the Zn:Cu ratio, either copper must be absorbed by an active transport process while zinc is being accumulated by organisms, or excess copper or zinc must be excreted from the body by some other process. Considering individuals analysed in muscle, Zn:Cu ratios were seasonally 16±2.8, 13.6±0.84, 19±1.2, 13±0.48 for May/95, September/97, January/98 and July/98, respectively. In liver tissue, Zn:Cu ratios were found as; 9.7±0.71 in January, 5.4±0.53 in July. That is, these animals are able to regulate the concentrations of Zn and Cu in the body in order to keep the ratio constant, despite changes in the availability of these metals in the environment (Bryan, 1971).

The calculated ratios of heavy metals between liver and muscle (L/M) indicated that the overall concentration of metal in tissues and relative distribution between tissues depends on both the fish species and metal properties. Obtained values in Red Pandora are higher than hake and red mullet (Gaspic et al., 2002; GESAMP, 1985; Odzak & Zvonaric, 1995). Generally liver accumulated higher levels of metals than the muscle tissues. Differences between heavy metals concentrations in analysed tissues originate from differences in physiological functions of muscle and liver. Similar mean values were found for L/M_{Cr}=8.5, L/M_{Zn}=11, L/M_{Ni}=8.0 and L/M_{Mn}=16 in this study. Similar results were also obtained for iron and copper (L/M_{Cu}=43, L/M_{Fe}=56). Haeompoietic function of the liver and organs abundant blood supply explains the highest accumulation of Fe (Blasco et al., 1998). The highest enrichment factors Cu can be explained by its relation to low-molecular weight proteins, which are concentrated in hepatic tissues. There were no significant differences related to Cd and Pb distributions the analysed liver and muscles of Red Pandora (L/M_{Cd}=890, L/M_{Pb}=858). The differences between Pb and Cd distributions in liver and muscle originate from different mechanisms involved in cadmium and lead sequestrations in fish. Once absorbed, Cd accumulates in liver due to the formation of soluble metallothioneins. Unfortunately, there is no specific low molecular weight protein in liver cytosol with high affinity for lead. Thus, absorbed lead is quickly distributed to other tissues rather than accumulating in the liver (Phillips & Rainbow, 1992; George & Olsson, 1994; Bond, 1996; Odzak & Zvonaric, 1995).

6.5. Bioaccumulation Factors (BAFs) of Heavy Metals

Generally, BAFs were different when comparisons were made to either metals of same tissues or tissues of same metal. The BAFs of Cd in liver, the BAFs of Zn in gonads and the BAFs of Cr in stomach showed the highest values of all elements. This finding is agreed with Chen & Chen (1999). The BAFs Cd, Zn and Cr were at least 3, 10, 6 times higher than Cu and Pb. The Cu-BAFs in the various fish tissues showed a small range (9-19 %) except muscle tissue would suggest that Cu was regulated to maintain a homeostatic status. The bioavailability of sediment-bound metals to aquatic organisms might be related to the speciation of metal in aqueous and solid phases (Forstner, 1990), nutritional mineral requirement of the organisms (Watanabe et al., 1997), binding site of metals in biological boundary and solubility of metals related to chemical and physical condition.

The present results suggest that the metal levels non-biologically essenstial element, such as Cd, in the fish may increase with prolonged exposure, and thus may pose a threat to human health through consumption of the fish.

6.6. Regional variations

Summarizing evaluations for ten stations in the Aegean Sea, the Hg contaminations of organ tissues could be ordered as Kuşadası>Aliağa> Çeşme>Edremit Bay>İzmir Bay>Gökova>Didim>Marmaris> Göcek>Fethiye. The trend in Cd level in present study was Göcek>Çesme>Marmaris> Kuşadası> Gökova>Didim>İzmirBay>EdremitBay>Aliağa>Fethiye. Concerning concentrations of Pb was Fethiye >Göcek>Didim>İzmir Bay>Çeşme>Aliağa> Gökova>Marmaris> Edremit Bay. We observed decrease of Cr concentrations in this following order: Gökova>Göcek>Marmaris>Fethiye>Edremit Bay=Aliağa =İzmir Bay=Çeşme= Kuşadası=Didim. Zn was mainly accumulated followed by Göcek>Gökova >Aliağa>Çeşme>Kuşadası>Marmaris>Edremit Bay>Didim>Fethiye >İzmir Bay. The mean concentration of copper was found as Fethiye> Marmaris>Göcek >Gökova>Didim>Kuşadası>İzmir Bay>Çeşme>Aliağa> Edremit Bay. The content of Fe, stations could be generally ordered as Göcek>Gökova> Fethiye>Marmaris >Edremit=Aliağa>Kuşadası>Didim>Çeşme>İzmir Bay. The distribution pattern of Nickel follows order: Göcek>Fethiye>Gökova>Marmaris>Aliağa >Ceşme>Didim >Kuşadası>Edremit>İzmir Bay while Mn follows the sequence: Göcek>Fethiye> Gökova>Marmaris>Çeşme>İzmirBay>Kuşadası>Didim>Aliağa>Edremit. These results indicate that (Hg except) highest metal concentrations are generally found in

Red Pandora from the Southern Aegean Sea. This can explain by fact that present environmental problems are due to unmanaged shipping activity, river run-off and untreated sewage discharge by coastal settlements, dumping of toxic, mining activity and industrial wastes.

6.7. Seasonal variations

The natural seasonal variation in heavy metal content can depend on several factors that are not easily differentiated. The biologic activity or metabolism rates an organism often drops when the environment becomes seasonally cooler, whereby the rates of incorporation and release of heavy metal may change. The seasonal difference in fat accumulation could therefore have a considerable influence on the storage capacity for organo-metal compounds, which are known to have a great affinity for lipids. The heavy metal concentration points out a significant variation as a function sampling periods. Samples collected during spring and summer, when their gonads were well developed, generally muscle tissue showed lower metal concentrations. This fact is probably related to the organisms' biological cycle. Total lipid contents decrease in muscle tissue during warm period and lower levels of metal were observed in summer. Thus, the seasonal variations observed in the heavy metal load of bream lead back also to changes in feeding rate, as well as lipid dynamics of tissues and growth of fish (Farkas et al., 2003). Generally, feeding rate decrease in late spring and summer, during spawning periods, stomach tissue lower levels of metal were found in summer and early autumn.

In various fish species, weight of liver is positively associated with seasonally fluctuating actual content of water and glycogen, with maximum in late autumn and winter and minimum in summer (Svobodova et al., 1999). According to our results, generally liver tissue showed higher metal concentrations in winter. The maximum hepatosomatik index was measured in January in this study.

CHAPTER SEVEN
CONCLUSION

7.1 Conclusion

This study fills a gap by providing in formation on heavy metal concentrations in Red Pandora from Aegean Sea. In muscle tissue Hg, Pb and Zn are similar to those reported in fish from Mediterranean countries. The Cd and Cu levels are lower than the results in fish muscle tissue reported from Mediterranean region. On the contrary, the concentrations of Hg, Pb, Cd, Zn and Cu are higher in liver from Aegean Sea than the other areas. For gonads, the observed concentrations of Hg, Cd, Ni, Zn and Mn are higher than other results from Mediterranean and Atlantic. Although Pb level were found lower than the other area, Cu values are similar to those detected in the previous studies. The heavy metal levels (except Zn) in this study tended to be higher than these reported in the current literature.

The concentrations were the lowest in muscle and observed metal levels are below the proposed limit for human consumption of toxic compounds such as mercury and cadmium. The Hg, Cd, Cu levels were usually several times higher in the liver, which is known to be important detoxification center in fish. The gonads showed higher concentrations of metal than those in muscle that might excrete part of toxic metal. The high concentrations of Pb, Fe, Ni and Mn in stomach are associated with its food and food habits.

In this study, no significant correlation was found between heavy metal levels in muscle, liver, stomach and gonads and length of Red Pandora. The comparison of muscle concentrations of Hg, Cd, Pb, Cr, Zn, Cu, Ni and Mn demonstrate significant seasonal variation. The biologic activity or metabolism rates in organism often drops when the environment becomes seasonally cooler, whereby the rates of incorporation and release of heavy metal may change. The seasonal difference in fat accumulation could therefore have a considerable influence on the storage capacity for organo-metal compounds, which are known to have a great affinity for lipids. According to our results, generally liver tissue showed higher metal concentrations in winter and there are significant seasonal variations of Cd, Cu and Ni. These can be explained by weight of liver is positively associated with seasonally fluctuating actual content of water and glycogen, with maximum in late autumn and winter and minimum in summer. The significant seasonal variations of Zn and Ni were found in ovaries tissues while seasonal variations of Cu and Fe were detected in testis. Seasonal variations for Cd, Pb and Cr concentrations were observed in stomach. Generally, feeding rate decrease in late spring and summer, during spawning periods, stomach tissue lower levels of metal were found in summer and early autumn.

Analysis of covariance was performed to compare the regional differences in the metal concentrations of tissues. The covariance analysis show that there are regional differences of Hg, Pb, Cu, Fe, Ni and Mn in muscle tissues. The covariance analysis of liver concentrations of Hg, Pb, Cr, Cu, and Ni demonstrated significant variations. The significant regional variations of Cd and Pb were found in ovaries tissues while regional variations were not detected in testis. Significant station-season interactions were observed for Hg, Cu, Fe, Ni, Mn concentrations in stomach. The results presented above clearly demonstrated that the Southern Aegean coast is facing metal pollution due to unmanaged shipping activity, river run-off and untreated sewage discharge by coastal settlements, dumping of toxic, mining activity and industrial wastes.

Fish tissues (muscle, liver, stomach, etc.) are commonly used as indicators of degree of contamination of marine environment with trace metals. Liver analysis is used to determine recent metal accumulations, while gonad analyses are assessing possible transfer and/or influence of metals through the reproductive processes and also human consumption. Due to consumption of food are primary routes of metal uptake by fish; metal analysis is carried out in stomach. Most researchers advocated the use of bioindicators for assessing the effects of pollutant stress in fish. For future research it may be fruitful to integrate studies of heavy metal concentrations in fish tissue with investigations of bioindicators like fish pathology and histology, as well as extensive ecological surveillance both at the population and community level.

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