MEASUREMENTS OF OXIDATIVE STRESS BIOMARKERS LEVELS IN MULLET LIVER CYTOSOL IN THE WEST BLACK SEA REGION OF TURKEY

by
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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality as a thesis for the degree of Master of Science in Biochemistry.

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ABSTRACT

MEASUREMENTS OF OXIDATIVE STRESS BIOMARKERS LEVELS IN MULLET LIVER CYTOSOL IN THE WEST BLACK SEA REGION OF TURKEY

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The Black Sea has been increasingly threatened by pollutants over the past decades due to several factors such as uncontrolled shipping, unmanaged fishing, accidental crude oil spills and dumping of toxic industrial wastes and discharge of domestic wastes from coastal settlements. Industrial areas, rivers and coastal cities have tendency to create local pollution in the Black Sea. The studies concerning biomarker responses to pollutants are relatively limited in the Black Sea. In this study, the degree of pollution was measured and monitored in Zonguldak Harbour, Eregli Harbours, Sakarya River Mouth, Melen and Guluc Stream Mouth and Amasra using glutathione S-transferase (GST), catalase (CAT) and glutathione reductase (GR) activities in the liver cytosol of mullet in August 2005, 2006 and 2007. Mullets caught from Amasra displayed the lowest GST, CAT and GR activities. This region was considered as reference site. Mullets collected from the Zonguldak Harbour, a highly urbanized and industrial city in the West Black Sea Region of Turkey, had 4 times higher GST activities than the reference site. GST activities of the mullet samples caught from Melen and Guluc Stream and Sakarya River Mouth were ranged between 3-4 times higher values compared to reference site. The highly elevated CAT activities were detected in mullets captured from Sakarya River, Melen and Guluc Stream Mouth, Eregli Harbour and Zonguldak Harbour which were ranged between 2-3 times higher values than the reference site in 2005. CAT activities of mullets caught from Sakarya River, Guluc Stream, Melen Stream Mouth and Zonguldak Harbour were about 2 times higher values than the reference site in 2006. CAT activities of mullets from Sakarya River Mouth and Zonguldak Harbour were 1.5 times higher than the reference site in 2007. GR activities of mullets from Zonguldak Harbour, Eregli Harbour, Melen Stream and Guluc Stream Mouth were ranged between 1.4-2 times higher values compared to the reference site.

In this study, the elevated biomarker responses showed the presence of organic pollutants and reactive oxygen species in the Zonguldak Harbour, Eregli Harbour, Sakarya River, Melen Stream and Guluc Stream. These results indicated that these sites have tendency to create local pollution in the Black Sea.

Keywords: Black Sea, GST, Catalase, Glutathione Reductase, Pollution.

ÖZET

TÜRKİYE'NİN BATI KARADENİZ BÖLGESİ'NDE, KEFAL BALIĞI KARACİĞER SİTOZOLÜNDE OKSİDATİF STRES BİYOBELİRTEÇLERİNİN SEVİYELERİNİN ÖLÇÜLMESİ

Aygün, Züleyha

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Karadeniz, kontrolsüz deniz taşımacılığı ve balıkçılık, kaza ile ham petrolün denize dökülmesi, endüstriyel atıkların boşaltılması ve kıyı yerleşkelerinden evsel atıkların bırakılması gibi birçok faktör yüzünden son on yıldır artan bir tehdit altındadır. Endüstriyel alanlar, nehirler ve kıyı şehirleri Karadeniz'de yerel kirlilik yaratma eğilimindedir. Karadeniz'de kirletici maddelerin boyutlarının belirlenmesinde kullanılan biyobelirteçlerle ilgili çalışmalar nispeten azdır. Bu çalışmada, Zonguldak Limanı, Ereğli Limanı, Sakarya Nehir Ağzı, Melen ve Gülüç Deresi Ağzı ve Amasra'da kirliliğin boyutu, kefal karaciğer sitozolünde, glutatyon S-transferaz, katalaz ve glutatyon redüktaz aktivitesi ölçülerek Ağustos 2005, 2006 ve 2007'de belirlendi ve takip edildi. Amasra'dan yakalanan kefaller en düşük GST, CAT ve GR aktivitelerini gösterdi. Bu bölge referans noktası olarak düşünüldü.

Türkiye'nin Batı Karadeniz Bölgesi'nde bulunan, kalabalık nüfusa ve endüstriyel alanlara sahip olan Zonguldak'tan yakalanan kefal balıklarında referans bölgesinden 4 kat fazla GST aktivitesi bulundu. Melen ve Gülüç Deresi Ağzı'ndan ve Sakarya Nehir Ağzı'ndan yakalanan kefal örneklerinin GST aktiviteleri referans bölgesine göre 3-4 kat arasında değişen yüksek değerlerde bulundu. Sakarya Nehri, Melen ve Gülüç Deresi Ağzı, Ereğli Limanı ve Zonguldak Limanı'ndan 2005'te yakalanan kefal balıklarının referans bölgesine göre 2-3 kat arasında değişen oldukça yüksek CAT aktivitelerine sahip olduğu belirlendi. 2006'da, Sakarya Nehri, Gülüç Deresi ve Melen Deresi Ağzı ve Zonguldak Limanı'ndan yakalanan kefallerin CAT aktivitelerinin referans bölgesinden 2 kat daha yüksek olduğu bulundu. 2007'de, Sakarya Nehir Ağzı ve Zonguldak Limanı'ndan yakalanan kefallerin CAT aktiviteleri referans bölgesinden 1.5 kat daha yüksek olduğu bulundu. Zonguldak Limanı, Ereğli Limanı, Melen Deresi ve Gülüç Deresi Ağzı'ndan yakalanan kefallerin GR aktiviteleri referans bölgesine göre 1.4-2 katı arasında değişen değerlerdedir.

Bu çalışmada, yüksek biyobelirteç tepkileri, organik kirleticilerin ve reaktif oksijen radikallerinin Zonguldak Limanı, Ereğli Limanı, Sakarya Nehri, Melen Deresi ve Gülüç Deresi'nde bulunduğunu gösterdi. Bu çalışmanın sonuçları bu bölgelerin Karadeniz'de yerel kirlilik yaratma eğiliminde olduğunu işaret etmektedir.

Anahtar Kelimeler: Karadeniz, GST, Katalaz, Glutatyon Redüktaz, Kirlilik.

DEDICATED TO THE MEMORY OF

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LIST OF ABBREVIATIONS

ε-ACA	ε-Amino caproic acid
BSA	Bovine serum albumin
CAT	Catalase
CDNB	1-chloro-2,4-dinitrobenzene
CYP	Cytochrome P450
DDD	Dichloro diphenyl trichloroethane
DDE	Dichloro diphenyl trichloroethylene
DNB_SG	1-glutathione-2,4-dinitrobenzene
EDTA	Ethylenediaminetetraacetic acid
EROD	7-Ethoxyresorufin O-deethylase
GSSG	oxidized glutathione
GSH	reduced glutathione
GST	Glutathione S-Transferase
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
MFO	Mixed-Function oxidase
MTs	Metallothionenins
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
PAH	Polyaromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PMSF	Phenylmethylsulfonyl fluoride
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

CHAPTER 1

INTRODUCTION

In the 20th century, about one hundred and fifty million tons of synthetic chemicals are produced by chemical industry, in part, released into the environment. These chemicals have a tendency to accumulate in biological tissues. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is eliminated. The degree of accumulation depends on the level of exposure and the mechanisms by which the organism expels, stores, or metabolically inactivates these chemicals. Today, it is well accepted that the aquatic environment has been threatened by an increasing number of chemicals. Contaminating chemicals (pollutants) in the aquatic environment can be harmful not only by their direct effects on the organisms but also indirectly producing some diseases and developmental abnormalities in humans living in a polluted environment. Therefore it is very important to find out the relation between pollutants level and their biological effect.

1.1. Biomarkers

A biomarker is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals (Peakall, 1994; van der Oost et al., 2003). Even though chemical analyses are able to measure a wide range of pollutants quantitatively and accurately, the complex mixture of chemical pollutants cannot be fully assessed. Furthermore, it does not reveal the impact of chemical pollution on the aquatic environment. The use of biochemical markers fulfills this purpose. Biomarker responses are, broadly speaking, of two kinds: those that measure only exposure to a pollutant and those that measure both exposure and toxic effect of environmental chemicals (Arinç et al., 2000). Figure 1.1 shows potential effects and measurable signals induced by environmental chemicals at different levels of biological organization.

1.1.1. Fish Biomarkers

Fish plays an increasingly important role in monitoring studies of water pollution. Many studies demonstrated that it responds changes in the aquatic environment with great sensitivity (Klontz, 1995; Arinç et al., 2000; Bresler et al., 1999; Flammarion et al., 2002). Various biochemical parameters in fish have been tested for their responses to toxic substances and their potential use as biomarkers of exposure or effect. In fish, most extensively investigated biomarkers are metallothioneins, DNA adducts, acetylcholinesterase (AChE) and enzymes involved in the detoxication of xenobiotics and their metabolites (biotransformation enzymes and antioxidant enzymes).

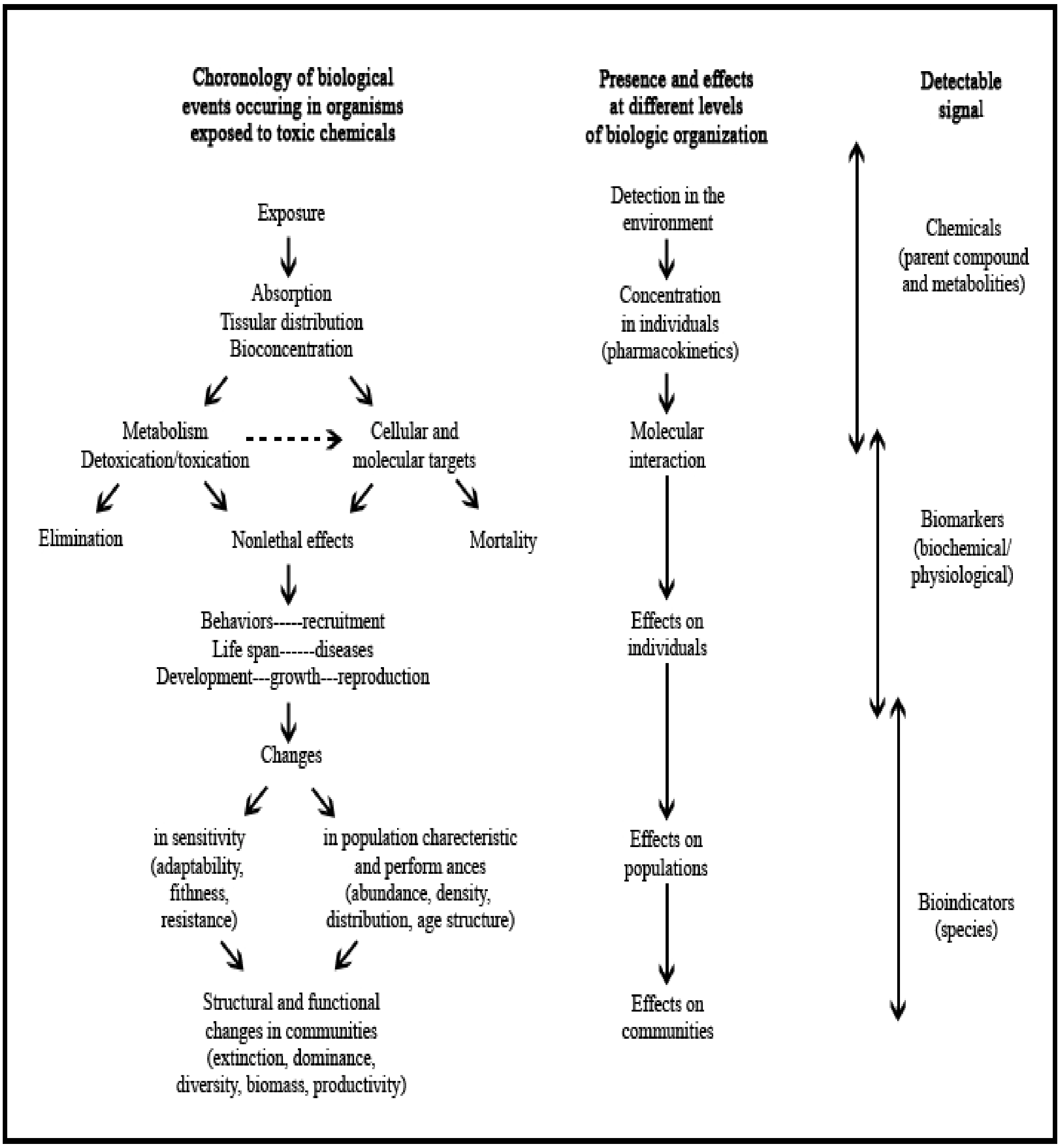

Figure 1.1. Potential effects and related measurable signals induced by environmental chemicals at different levels of biological organizations. Taken from Lagadic and Caquet, 1998.

1.1.1.1. The Metallothioneins

Metallothioneins (MTs) are low molecular weight proteins with a high cysteine content that are inducible by heavy metals and by other conditions of environmental stress. The levels of metallothionein (MT) are used as a biomarker of metal exposure on fish. Some endogeneous and exogeneous factors influence cellular MT and metal content. These factors are suitable for the selection of indicator organs and species, many abiotic and biotic factors, like water pH, temperature, salinity, fish age, size, sex, season, feeding habits and reproductive cycle (Marijić and Raspor, 2006). A group of metals including Cd, Cu, Zn, Co, Ni, Bi and Ag have a potential to induce MTs and correlations between levels of MT and metals in the water or tissues are observed, therefore, MTs are part of the defensive system against metals (Livingstone, 1993).

1.1.1.2. The DNA adducts

DNA adducts are formed when a DNA molecule is exposed to highly reactive radical intermediates. These reactive radical intermediates may bind covalently to cellular macromolecules such as DNA and proteins. The covalent modification of DNA bases by chemicals can alter the structure of DNA. These modifications may eventually lead to mutations and ultimately cancer, especially if the adduct is located in an oncogene or tumor suppressor gene (Lyons et al., 2004).

1.1.1.3. Acetylcholinesterase (AChE)

The primary effects of organophosphates and carbamates are the inhibition of acetylcholinesterase (AChE) enzyme activity (Peña-Llopis et al., 2003) that regulate nerve impulse transmission by hydrolysis of the neurotransmitter acetylcholine in vertebrates and invertebrates. Monitoring studies of neurotoxic chemicals are mostly based on the inhibition of AChE activity. Inhibition of AChE in either fish or crustacea has been used to monitor marine pollution in different studies (Galgani et al., 1992; Burgeot et al., 1996; Fossi et al., 1996; Stien et al., 1998; Sturm et al., 1999; Bresler et al., 1999; Corsi et al., 2003; Lionetto et al., 2004).

1.1.1.4. The Biotransformation Enzymes

Biotransformation can be defined as an enzyme-catalyzed conversion of a xenobiotic compound into a more water-soluble form, which can be excreted from the body more easily than the parent compound (Lech and Vodicnik, 1985; van der Oost et al., 2003). Biotransformation of xenobiotics occurs in two stages. In Phase I, relatively insoluble organic chemicals are catalysed through oxidation, reduction and hydrolysis. Phase II involves conjugation of the chemical or its metabolites with polar compounds such as glucuronic acid, glutathione, sugars, sulfates and phosphates to form water-soluble compounds which are readily excreted.

In mammalian and some nonmammalian groups, cytochrome P450-dependent mixed function oxidase system (monooxygenases, MFO) catalyses most of the oxidative biotransformation of xenobiotics. The cytochrome P450 (CYP)-dependent monooxygenase system is a membrane-bound, multi-component, electron transport system in which the monooxygenation of a wide range of xenobiotics such as organochlorins, dioxins and aromatic hydrocarbons and endogenous molecules such as prostaglandins, steroids and fatty acids are catalysed (Gonzalez, 1988; Guengerich, 1990; Sole´ and Livingstone, 2005).

Glutathione S-transferases, sulfotransferases, UDP-glucuronyl transferase, N-acetyl transferases are the most important Phase II enzymes that have a role in biotransformation of xenobiotic compounds.

1.2. Glutathione S-Transferases

The GSTs (EC.2.5.1.18) are a family of enzymes that participate in cellular detoxification of endogenous as well as foreign electrophilic compounds (Mainwaring et.al., 1996). The glutathione S-transferases (GSTs) are a member of phase II enzyme system. They are cytosolic, mitochondrial, and microsomal proteins with typical molecular masses of around 50 kDa. Cytosolic GSTs are dimeric proteins. GSTs can reduce the toxicity of a number of endogenous and environmental chemicals by conjugating the reactive molecules to the nucleophile scavenging tripeptide glutathione (GSH, γ-glu-cys-gly) (Jemth and Mannervik et.al., 1999). GSH, first described in 1888 as philothion, is the most ubiquitous and abundant non-protein thiol in mammal cells and serves as a necessary nucleophile in a number of detoxification reactions (Tew, 1994). All GST isozymes use reduced GSH as an acceptor species, but they differ in the specificity with which different substrates are transferred to the cysteine thiol of GSH. According to primary amino acid sequences, GSTs have been identified as alpha (α), mu (μ), pi (π), sigma (σ), theta (θ) and zeta (δ).

GSTs detoxify reactive electrophiles (or phase I metabolites) by conjugating them with glutathione. Environmental chemicals such as carcinogens, pesticides, insecticides, herbicides, and reactive intermediates produced by oxidative stress are detoxified by GSTs (Figure 1.2 and 1.3). GSTs catalyze the conjugation of these compounds (often electrophilic and somewhat lipophilic in nature) with reduced glutathione to facilitate excretion from the body (Hayes and Pulford, 1995).

All GSTs have the ability to conjugate GSH with compounds containing an electrophilic center. The electrophilic functional group for conjugation reactions can be provided by a carbon, a nitrogen, or a sulfur atom. Such groups are present in arene oxides, aliphatic and aromatic halides, α-, β- unsaturated carbonyls, organic nitrate esters, organic thiocyanates, olefins, organic peroxides, quinines and sulfate esters (Mannervik et al., 1985; Mannervik and Danielson, 1988). The range of compounds that contain electrophilic center is extremely large and include the parent chemical or metabolite of the carcinogens; benzo[a]pyrene, 5-methylcrysene, aflatoxin B1, 7,12-dimethybenz[a]anthracene, and 4-nitroquinoline-N-oxide. GST detoxifies the pesticides such as alachlor, atrazine, dichloro-diphenyltrichloroethane (DDT), lindane, and methyl parathion, the oxidative-damage products including acrolein, base propenals, cholesterol α-oxide, fatty acid hydroperoxides, and 4-hydroxynonenal, and the anticancer drugs of chlorambucil, melphalan, thiotepa, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and cyclophosphamide, as well as the antibiotic fosfomycin.

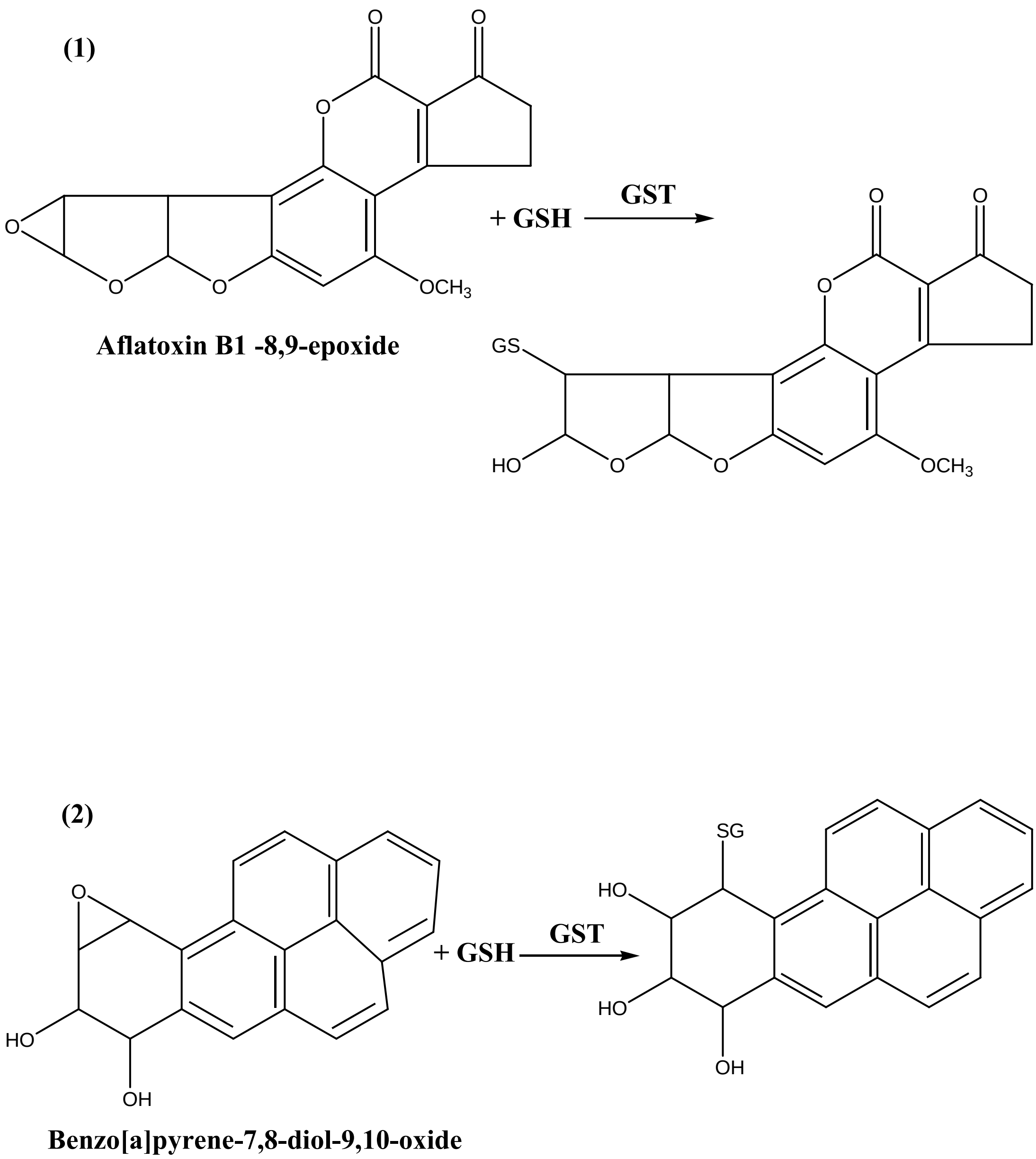

Figure 1.2. Detoxification of carcinogens by GSTs (taken from Hayes and Pulford, 1995).

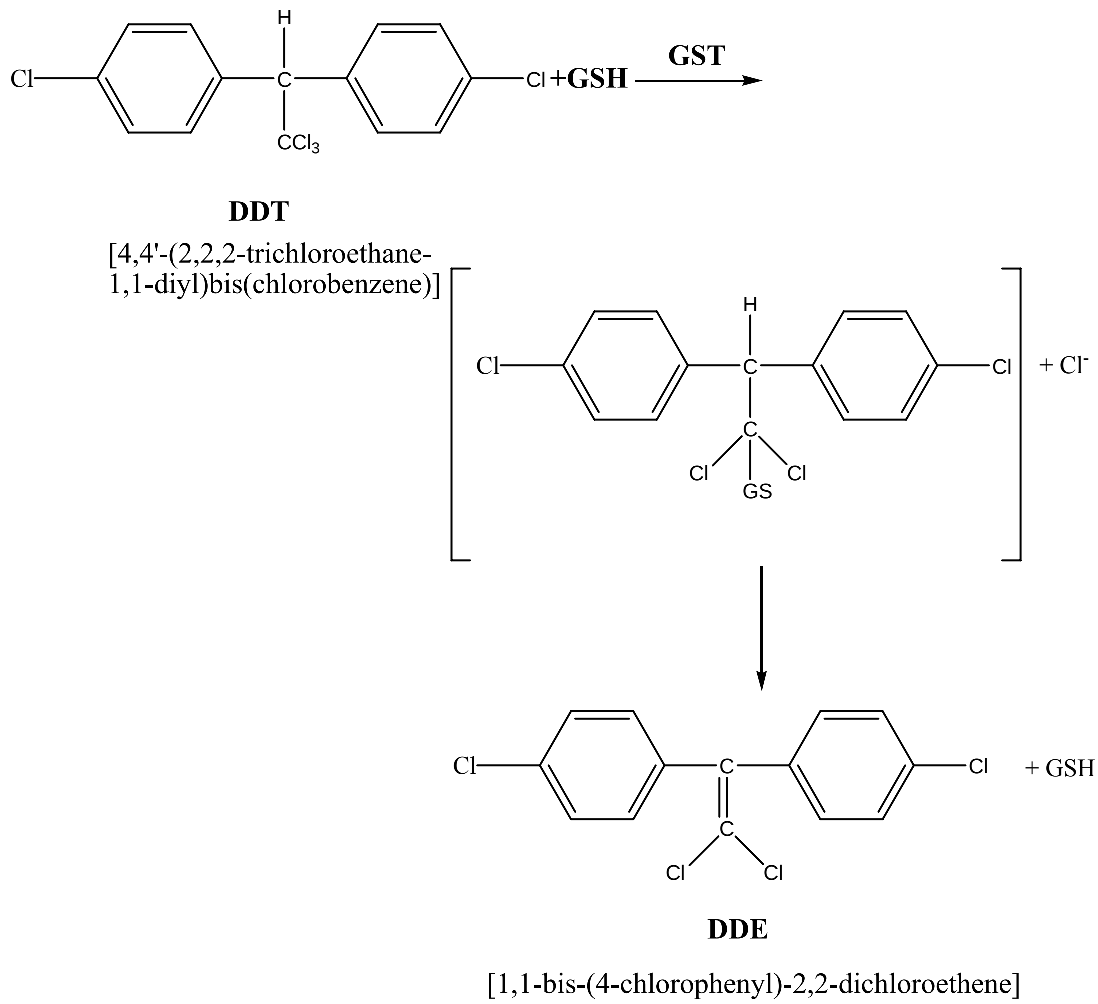

Figure 1.3. Detoxification of a pesticite by GSTs (taken from Hayes and Pulford, 1995).

PAHs and halogenated xenobiotics significantly alter fish hepatic GST activity (Vigano et al., 1995; van der Oost et al., 2003; Martı´nez-Go´mez et al., 2006). The detoxification of several xenobiotics to a more polar compound is connected with a consumption of the cellular glutathione pool. The GST activity has been used as an environmental biomarker for water pollution (Şen and Kirikbakan, 2004; Sureda et al., 2006).

1.3. The Oxidative Stress

Xenobiotics increase the intracellular formation of reactive oxygen species (ROS) and may damage the macromolecules and reduce the levels of oxidant defenses in living organisms. Most reactive oxygen species are produced from the endogenous molecules as by-products of normal and essential metabolic reactions, such as energy generation from mitochondria. They may be produced by the detoxification reactions involving the liver cytochrome P-450 enzyme system. Besides endogenous molecules, exogenous molecules such as environmental pollutants may also produce reactive oxygen species.

ROS are a kind of free radicals. Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons. Table 1.1 shows some examples of free radicals. Free radicals may produce damage when they react with important cellular components such as DNA, or the cell membrane. ROS such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical ($^{\bullet}OH$) can produce lipid peroxidation, enzyme inactivation and DNA adducts (Davies, 1995; Jifa et al., 2006). Cells may function poorly or die if this occurs. However, organisms have antioxidant defense systems to prevent free radical damage. Antioxidant defense systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) and numerous low-molecular weight antioxidants such as glutathione, β-carotene (vitamin A), ascorbate (vitamin C), and a tocopherol (vitamin E) (Halliwell and Gutteridge, 1999). An imbalance between the oxidizing processes and the antioxidant defence systems of an organism causes oxidative stress.

Most of the studies on oxidative stress in fish have mainly focused on toxicological aspects of xenobiotics, induction of biotransformation enzymes such as glutathione S-transferase and biomarkers of antioxidant enzyme system (Di Giulio et al., 1989; Winston and Di Giulio, 1991).

1.3.1. The Antioxidant Enzymes

Cells produce a variety of defense mechanisms to fight with the harmful effects of free radicals. ROS generated from both exogeneous and endogeneous sources are detoxified by a set of antioxidant enzymes which protect proteins, lipids and DNA against damage. The antioxidant defence system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and other low molecular weight scavengers such as glutathione (GSH).

Table 1.1. Examples of radicals and nonradicals

Radicals	**Non radicals**
Reactive oxygen species (ROS)	
Superoxide, $O_2^{\bullet-}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Hypobromous acid, HOBr
Hydroperoxyl, $HO_2^{\bullet}$	Ozone, O_3
Hypochlorite ion (OCl^-)	
Reactive chlorine species (RCS)	
Atomic chlorine, $Cl^{\bullet}$	Hypochlorous acid, HOCl
	Nitryl (nitronium) chloride NO_2Cl
	Chloramines
Reactive nitrogen species (RNS)	
Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2
Nitrogen dioxide, $NO_2^{\bullet}$	Nitrosyl cation, NO^+
	Nitroxyl anion, NO^-
	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynitrite, $ONOO^-$
	Peroxynitrous acid, ONOOH
	Nitronium (nitryl) cation, NO_2^+
	Alkyl peroxynitrites, ROONO
	Nitryl (nitronium) chloride, NO_2Cl

1.3.1.1. The Superoxide Dismutase

Superoxide dismutase (EC 1.15.1.1) metabolizes superoxide anion radical ($O_2^{\bullet -}$) into a less reactive species, molecular oxygen and hydrogen peroxide (H_2O_2) (Nordberg and Arnér, 2001). H_2O_2 in turn may be inactivated by catalase. Thus SOD is an important antioxidant defense enzyme in nearly all cells exposed to oxygen. SOD catalysed reaction may be written with the following half-reactions at which the oxidation state of the metal cation oscillates between n and n+1:

$$M^{(n+1)+} - SOD + O_2^{\bullet -} \rightarrow M^{n+} - SOD + O_2$$

$$M^{n+} - SOD + O_2^{\bullet -} + 2H^{+} \rightarrow M^{(n+1)+} - SOD + H_2O_2$$

where M = Cu (n=1); Mn (n=2); Fe (n=2); Ni (n=2).

1.3.1.2. The Catalase

Catalase (EC 1.11.1.6) is a heme protein belonging to the class of oxidoreductases with ferriprotoporphyrin-IX at the redox center. It detoxifies hydrogen peroxide to water without forming free radicals. It is present in mammalian and non-mammalian aerobic cells and many anaerobic organisms (Halliwell and Gutteridge, 1999; Dama´sio et al., 2007). Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell.

CAT is a tetrameric enzyme containing 4 ferrihemoprotein groups per molecule and each 500 amino acids long. It has a molecular mass of 240 kDa.

Catalase activity varies greatly between tissues. The activity is the highest in the liver and kidney, and the lowest in connective tissues. Catalase catalyzed reactions take place in two steps: with the first hydrogen peroxide molecule oxidizing the heme to an oxyferryl species in which one oxidation equivalent is removed from the iron and one from the porphyrin ring to generate a porphyrin cation radical (1). The second hydrogen peroxide is then used as a reductant of compound I to regenerate the resting state enzyme, water, and oxygen (2) (Switala and Loewen, 2002).

$$\text{Enz(Por-Fe}^{3+}) + H_2O_2 \longrightarrow \text{Cpd I (Por}^{+}\text{ - Fe}^{4+}{=}O) + H_2O \qquad (1)$$

$$\text{Cpd I (Por}^{+}\text{ - Fe}^{4+}{=}O) + H_2O_2 \longrightarrow \text{Enz(Por-Fe}^{3+}) + H_2O + O_2 \qquad (2)$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

CATs are the most extensively used bioindicator to assess the induction of antioxidant defenses by PCBs and heavy metal exposure.

1.3.1.3. Glutathione Redox Cycle

Cellular glutathione (L-γ-glutamyl- cysteinyl-glycine) is a tripeptide that plays a critical role in oxidative stress. It is ubiquitous non-protein thiol that is mainly produced intracellularly. GSH synthesis takes place in the cytosol through two consecutive ATP-dependent enzymatic reactions: the formation of γ-glutamylcysteine from glutamate and cysteine catalyzed by glutamate–cysteine ligase (GCL), and formation of GSH from γ-glutamylcysteine and glycine catalyzed by GSH synthetase (GSS) (Figure 1.4) (Lu, 1999).

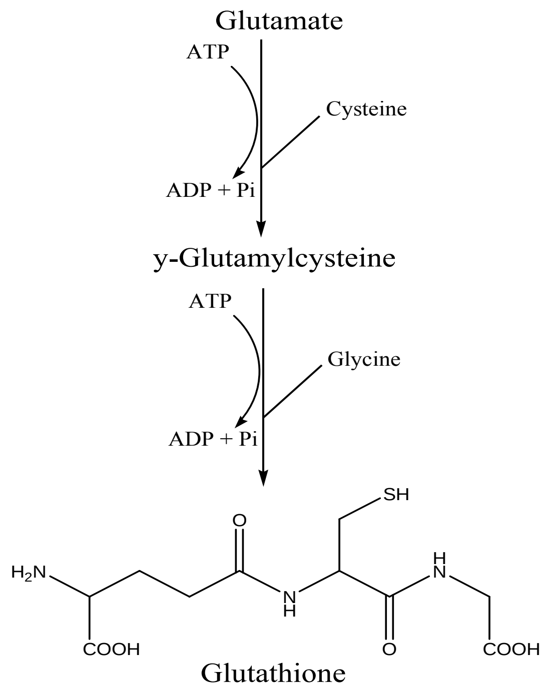

Figure 1.4. Synthesis of glutathione (γ-glutamyl- cysteinyl-glycine).

Reduced glutathione (GSH) acts as an intracellular reductant and nucleophile that is involved in important physiological functions such as the synthesis of proteins, amino acid transport, maintenance of the thiol disulfide status (acting as a redox buffer), free radical scavenging, detoxification of electrophiles, act as a non-toxic storage form of a cysteine, maintenance of thiol-disulfide status, signal transduction and as a defence system against oxidizing molecules and potentially harmful xenobiotics such as metals (Ritola et al., 2000; Peña-Llopis et al., 2001).

Antioxidant enzymes have very important roles in glutathione redox cycle (Figure 1.5). These enzymes include Glutathione Peroxidase (GPx), Glutathione S-Transferase (GST) and Glutathione Reductase (GR). Glutathione S-transferases are a member of phase II enzymes. GSTs catalyze the conjugation of the sulphydryl group of GSH and an electrophilic atom of a xenobiotic compound resulting in the formation of less toxic products and allowing their elimination (Peña-Llopis et al., 2001). Glutathione peroxidase (GPx) oxidizes GSH to glutathione disulfide (GSSG) by reducing H_2O_2 to H_2O as well other peroxides and GSSG is reduced back to GSH by glutathione reductase (GR) using NADPH as a cofactor (Leggatt et al., 2007).

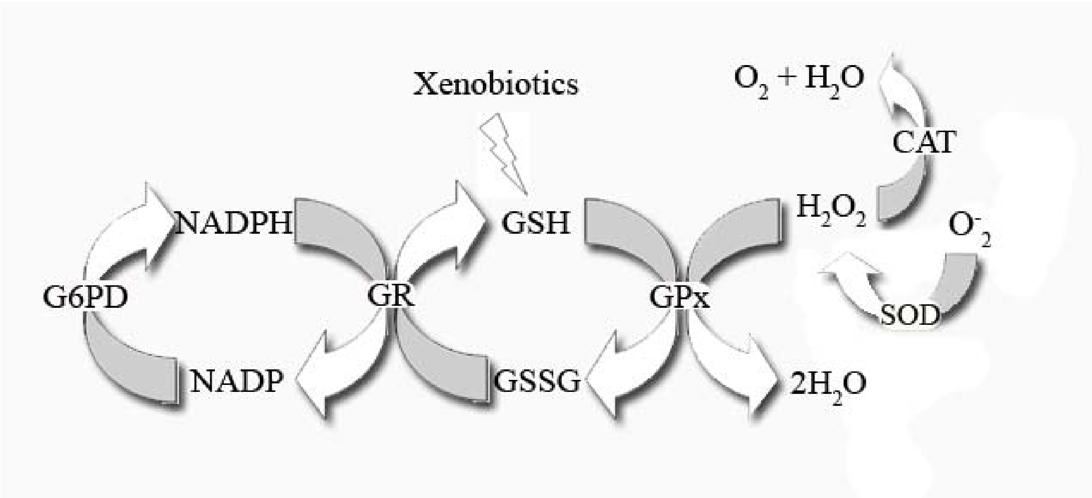

Figure 1.5. Glutathione Redox Cycle (taken and modified from Grosicka-Maciag et al., 2007)

1.3.1.4. Glutathione Reductase

Glutathione reductase (GR) (EC 1.6.4.2), is a flavoprotein, which reduces oxidized glutathione (GSSG) to the sulfhydryl form glutathione (GSH) by NADPH. Glutathione reductase (GR) is known to play a key role in response to oxidative stress, heavy metals and herbicides in both plants and animals. The native enzyme of most GRs is a homodimer of 100–120 kDa. Glutathione reductase has been mainly localized in chloroplasts, mitochondria, cytosol and peroxisomes.

GR is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH (Figure 1.4). Oxidized glutathione is reduced by a multi-step reaction in which GR is initially reduced by NADPH. The reduced GR (GRred) reacts with a molecule of GSSG, resulting in a disulfide interchange, which produces a molecule of GSH and the GRred-SG complex. An electron rearrangement in GRred-SG results in a second disulfide interchange, splitting off the second molecule of GSH and restoring the GR to the oxidized form.

General reaction catalyzed by glutathione reductase:

$$ROH + GSSG + NADPH + H^+ \xrightarrow{GR} ROOH + 2GSH + NADP^+$$

1.4. Antioxidant Enzyme Activities in Fish

Most of the research on oxidative stress in fish focuses on toxicological aspects, such as the effects of different xenobiotics on antioxidant–enzyme activities, induction of biotransformation processes and other biomarkers of oxidative damage (Trenzado et al., 2006). Like all aerobic organisms, fish are also susceptible to the attack of reactive oxygen species and, as a consequence, have an antioxidant defence system, including non-enzymatic scavengers (GSH) and enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and phase II enzyme, glutathione S-transferase.

It has been demonstrated that hepatic GST activity increases after exposure of fish to PAHs, PCBs, OCPs and PCDDs (Chen et al., 1998; Bello et al., 2001; van der Oost et al., 2003). Increases in hepatic CAT activity have been observed in some experiments with fish exposed to PCBs, domestic wastes or PAH-containing sediments (Di Giulio et al., 1989; Rudneva-Titova and Zherko, 1994; van der Oost et al., 2003). In other laboratories and field studies aimed at evaluating the effects of PAHs and PCBs in antioxidant defences have showed that SOD, CAT and GST levels increases with these pollutants (Burgeot et al., 1996; Rudneva-Titova and Zherko 1994; Monteiro et al., 2006) which indicate an elevated antioxidant status attempting to neutralize the impact of the ROS. In addition, it has been shown that oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene) in tilapia (Oreochromis niloticus) increases some antioxidant enzymes; CAT, GR and GST, while decreasing SOD activity (Peixoto et al., 2006). An increased GR activity has been reported in laboratory experiments with fish exposed to PCBs, PAHs and DDE contaminated food (Otto and Moon, 1995; van der Oost et al., 2003). Besides these pollutants, compounds released from the rubber industry may affect not only phase I detoxication enzymes but also antioxidant defense components such as total glutathione (tGSH), hepatic GR and glutathione S-transferase (GST) as well, and increased hepatic ethoxyresorufin-O-deethylase (EROD) activity, and glutathione reductase (GR) activity have been reported in exposed fish (Stephensen et al., 2005).

Rodr´ıguez-Ariza and coworkers (1993) have reported significantly high antioxidant enzyme activities in mullet samples from a contaminated site with PCBs, PAHs and pesticides, and proposed that antioxidant enzyme activities in fish might be useful tools for biomonitoring of environmental pollution.

1.5. The Black Sea

The Black Sea is an inland sea between southeastern Europe and the Anatolian peninsula (Turkey) and is ultimately connected to the Atlantic Ocean via the Mediterranean and Aegean Seas and various straits. The Bosporus strait connects it to the Sea of Marmara, then the long island-bound strait of the Dardenelles to the Aegean Sea region of the Mediterranean. These waters separate Eastern Europe and Western Asia. The Black Sea is also connected to the Sea of Azov by the Strait of Kerch (Tuncer et al., 1998).

The Black Sea has an area of 436,400 km^2 and a maximum depth of 2200 m. Like the Mediterranean Sea, there is a net inflow of seawater through the Bosporus into the oceanic region that is lost to evaporation or into geologic faults and so gradually raises the salinity. Freshwater flows in from the surrounding areas, especially central and middle-eastern Europe, 320 km^3 per year. The northwestern sector is subject to discharges from the Danube, Dnieper and Dniester rivers.

The Black Sea has been increasingly threatened by pollutants over the past decades due to several factors such as uncontrolled shipping, unmanaged fishing, accidental crude oil spills and dumping of toxic industrial wastes and discharge of domestic wastes from coastal settlements. The most significant process degrading the Black Sea has been the massive over-fertilisation of the sea by compounds of nitrogen and phosphorus, largely as a result of agricultural, domestic and industrial sources. This over-fertilisation produces a phenomenon called eutrophication, which has changed the structure of the Black Sea ecosystem. The nitrogen and phosphorus compounds (nutrients) enter the Black Sea through rivers. Another problem is the discharge of insufficiently treated sewage into the Black Sea or into downstream stretches of rivers and from there to the sea. Oil pollution continues to threaten Black Sea coastal ecosystems. Currently levels of oil pollution are not high in the open Black Sea but are unacceptable in many coastal areas and river mouths. According to Black Sea scientists, every year about 30,000 tons of oil enter the sea from domestic sewage plants, 15,500 tons from industry (including the oil industry), and 53,000 tons flows down the Danube River. The total annual discharge is arround 98,500 tons. Heavy metals, such as cadmium, copper, chromium and lead, are usually associated with waste from heavy metal industry and the ash remaining from burning coal for generating electricity. Pesticides are mostly introduced through rivers and streams discharging from agriculture. Radioactive substances have been introduced to the Black Sea in small quantities from nuclear power generation and in more significant amounts as a result of the Chernobyl accident in 1986. Reservoirs in the Dnieper River however, still contain huge amounts of radioactive material locked up in their sediments (Mee, 1992).

In April 1992, the six Black Sea countries signed the Convention for the Protection of the Black Sea against Pollution (the Bucharest Convention). In order to provide a practical programme of actions for cleaning up and protecting the Black Sea, the Black Sea Environmental Programme (BSEP) was founded in 1993. The BSEP was responsible for assisting countries to prepare the Black Sea Action Plan. In 1996, the Istanbul Commission agreed to implement the Action Plan through its Secretariat, which was to be functioning by January 1997. The overall aim of the Black Sea Action Plan is to “enable the population of the Black Sea region to enjoy a healthy living environment in both urban and rural areas, and to attain a biologically diverse Black Sea ecosystem with viable natural populations of higher organisms, including marine mammals and sturgeons, and which will support livelihoods based on sustainable activities such as fishing, aquaculture and tourism in all Black Sea countries" (Mee, 1992).

After the Bucharest convention, the numbers of studies on the environmental pollution have increased in the participant countries. The studies on the Turkish Coasts of the Black Sea mainly focused on the chemical analyses. These studies have demonstrated that the Kizilirmak, Yesilirmak, Sakarya, Filyos and Guluc rivers are important sources for Cd, Cu, Pb and Zn (Tuncer et al., 1998; Barlas, 1999). In addition to inorganic pollutants, the presence of pesticides and PCBs, including lindane, heptachlor, heptachlor epoxy, aldrin, dieldrin, endrin, DDE, DDD and DDT have been shown in different studies (Tuncer et al., 1998; Bakan and Ariman; 2004).

1.6. Test Species

In this study, three kinds of mullet species used are so-iuy mullet (*Mugil soiuy*), golden grey mullet (*Liza aurata*), and flathead mullet (*Mugil cephalus*). The mullets are economically important marine fish belong to the family of Mugilidae. They live predominantly inshore in the protected waters of whole Mediterranean, Black sea, Sea of Azov and along the Atlantic coast. They are resistant and tolerant to pollutants and to various environmental conditions. Mullets are herbivorous scavengers, feeding primarily on algae and detrital (dead) plant material.

So-iuy mullet also called Russian grey mullet live predominantly in Russia, Korea, China, Black Sea and Mediterranean Sea. Length of so-iuy mullet in the Black Sea is usually 20-30 cm. Golden grey mullet live in Eastern Atlantic, Mediterranean and Black Sea. The flathead mullet live in California, USA, Nova Scotia, Canada to Brazil, Cape Cod to southern Gulf of Mexico, most of West Indies and Caribbean, Mediterranean Sea and Black Sea.

Mullets are very important for our economy due to the marketing of their meat and eggs. There is a large commercial and sport fishing demand for this organism and consumed in large quantities in Turkey, which increase the importance of this organism from the ecological and toxicological standpoints.

1.7. The Aim of the Study

After the Bucharest convention, the numbers of studies on the environmental pollution have increased in the participant countries. The studies on the Turkish Coasts of the Black Sea mainly focused on the chemical analyses. Even though chemical analyses are able to measure a wide range of pollutants quantitatively and accurately, the complex mixture of chemical pollutants cannot be fully assessed. Furthermore, it does not reveal the impact of chemical pollution on the aquatic environment. The use of biomarkers fulfills this purpose. The studies concerning biomarker response to pollutants are limited in the Black Sea. It has been reported that the main sources of pollution in the Black Sea are the rivers which flow into region and coastal cities.

In this study our aim was to measure the levels of oxidative stress biomarkers, catalase and glutathione reductase and phase II enzyme, glutathione S-transferase in the liver cytosol of mullet in different sites of the West Black Sea Region of Turkey and to find the areas that create local pollution in this region. In addition, the relation between the results of monitoring study and ecological consequences was discussed in this study.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

Hydrogen peroxide (H_2O_2: 19128700), isopropanol (C_3H_8O:), potassium dihydrogen phosphate (KH_2PO_4: 104871), dipotassium hydrogen phosphate (K_2HPO_4:105101), sodium carbonate (Na_2CO_3: 106392), sodium hydroxide (NaOH: 106462), Triton X-100 (11869) were purchased from Merck, Darmstadt, Germany.

ε-Amino caproic acid (ε-ACA: A2504), bovine serum albumin (BSA: A7511), 1-chloro-2,4-dinitrobenzene (CDNB: 24438), cupper (II) sulfate-pentahydrate ($CuSO_4.5H_2O$: 12849), ethylene diamine tetra acetic acid disodium salt (EDTA: E5134), phenylmethane sulfonyl fluoride (PMSF: P7626), follin phenol reagent (F9252), β-nicotinamide adenine dinucleotide, reduced form (NADPH; A1395), oxidized glutathione (GSSG: G4626), potassium chloride (KCl: 12636), reduced glutathione (GSH: G6013), sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$:S2377) were purchased from Sigma Chemical, Company, Saint Louis, Missouri, USA.

All the other chemicals were of analytical grade and were obtained from commercial sources at the highest grade of purity available.

2.1.2 Fish Collection

In this study, the fish samples were collected in August 2005, August 2006 and August 2007 from different sites of the West Black Sea Region of Turkey. Three different species of mullet were captured by fish net. These are so-iuy mullet (*Mugil Soiuy*), flathead mullet (*Mugil Cephalus*) and golden grey mullet (*Liza Aurata*). The fish sampling sites were shown in Figure 2.1.

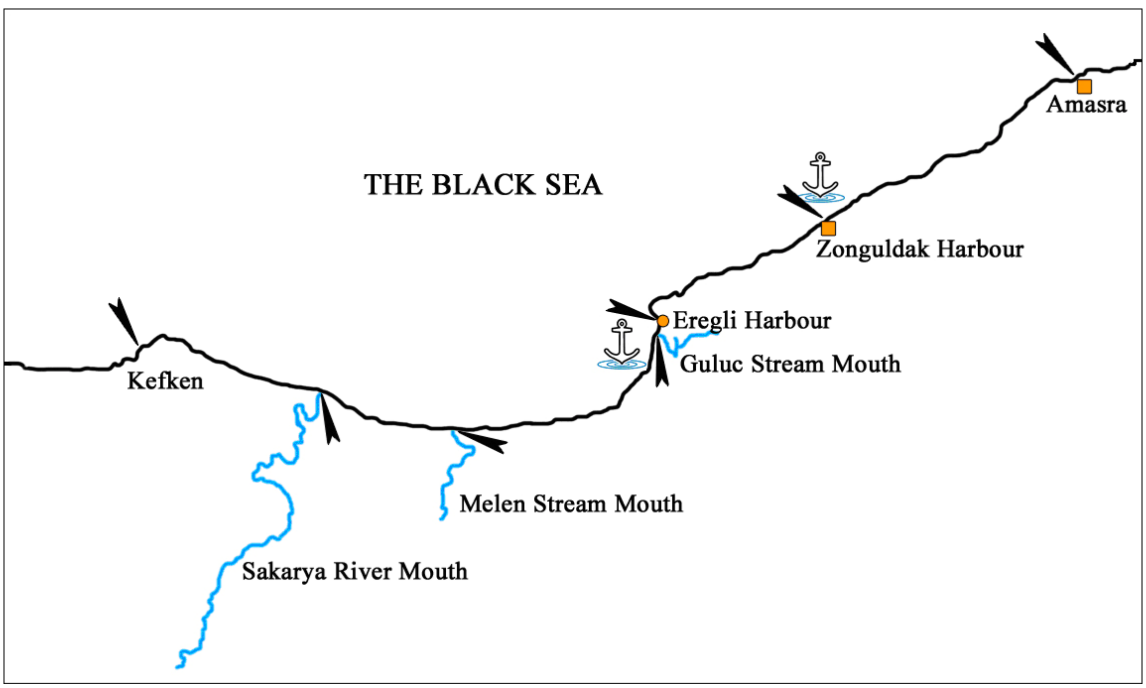

Figure 2.1. The sampling sites in the West Black Sea Region of Turkey

2.2 Methods

2.2.1 Preparation of Fish Liver Cytosols

The method described for fish microsome preparation by Arinç and Şen (1993) was used for the preparation of fish liver cytosols. Fish were killed by decapitation and the livers, weighing approximately 9-12 grams, were removed immediately. The livers were first wrapped by freezing bags and covered by aluminum foil and frozen by putting into liquid nitrogen. Freshly frozen fish livers were transported in liquid nitrogen to our laboratory in Bolu. Cytosols from fish liver samples were prepared at Middle East Technical University, Biology Department and Gülhane Military Medical Academy, Biochemistry Department in Ankara. The liver samples were transported to laboratory in Ankara in liquid nitrogen.

Each fish liver cytosol was prepared separately. In the laboratory, the livers were taken from liquid nitrogen and thawed on ice. All subsequent steps were carried out in 0-4°C ice bath. Livers were washed first with cold distilled water, and then with cold 1.15% KCl solution to remove as much blood as possible. After draining on a paper towel, tissues were weighed and cut into small pieces with scissors. The resulting tissue mince was homogenized in 1.15% KCl solution containing 10 mM EDTA pH 7.7, 0.25 mM ε-ACA, 0.1 mM PMSF at a volume equal to 2.5 times the weight of liver tissue using Potter-Elvehjem glass homogenizer packed in crushed ice, coupled motor (Black & Decker, V850 multispeed drill)-driven Teflon pestle at 2400 rpm, twelve passes were made for homogenization.

The homogenate was centrifuged at 13300×g (Sigma 3K30 Refrigerated Centrifuge, Saint Louis, Missouri, USA) by using 12156 rotor for 40 minutes to remove cell debris, nuclei and mitochondria. The supernatant fraction containing endoplasmic reticulum and other fractions of the cell was filtered through two layers of cheesecloth. The supernatant (cytosol of liver cells) was separated from microsomes by centrifugation at 45000 rpm (70000×g) for 60 minutes using Sorvall-Kombi ultracentrifuge, Ivan Sorvall Inc., Newton, Connecticut, 06740 USA with T-880 rotor. The supernatant fraction (cytosol) was sampled into small aliquots and put into eppendorf tubes, stored in liquid nitrogen after gassing with nitrogen.

2.2.2 Protein Determination

Concentrations of cytosolic proteins were determined by method of Lowry *et al.* (1951). Bovine serum albumin was used as standard. Before protein determination, liver cytosols were recentrifuged at 20000 rpm for 30 min. The supernatant fractions were tkaen and stored at – 80 °C. The supernatant fractions were diluted to 1:200 with distilled water. An aliquot of 0.1, 0.25 and 0.5 mL of cytosols was mixed with distilled water in order to complete the volume to 0.5 mL in test tubes. After that, they were mixed with 2.5 mL of alkaline copper reagent which was prepared freshly by mixing 2% copper sulfate, 2% sodium potassium tartrate and 2% Na_2CO_3 in 0.1 N NaOH in the written order and incubated at room temperature for 10 minutes. Then, 0.25 mL of 1.0 N Folin-Phenol reagent was added to the tubes, mixed within 8 seconds and incubated at room temperature for 30 minutes. The resulting color intensity was measured at 660 nm.

The standard curve of BSA from 20 to 200 μg was plotted and used for the determination of protein concentration of samples.

2.2.3. Determination of Total Glutathione S-Transferase (GST) Activity

Total Glutathione S-Transferase (GST) activity was determined according to the method of Habig *et al* (1974). 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate. CDNB is a common substrate for all isozymes of GSTs. The principle of the method depends on the formation of 1-glutathione-2,4-dinitrobenzene (DNB_SG) from 1-chloro-2,4-dinitrobenzene by conjugation reaction catalyzed by GSTs. Figure 2.2. shows the reactants and products of GSTs catalyzed reaction. 1-glutathione-2,4-dinitrobenzene (DNB_SG) has absorbance at 340 nm. Glutathione S-transferase catalyzed conjugation reaction of 1-chloro-2,4-dinitrobenzene is monitored by the increase in the absorbance at 340 nm due to the 1-glutathione-2,4-dinitrobenzene (DNB_SG) formation.

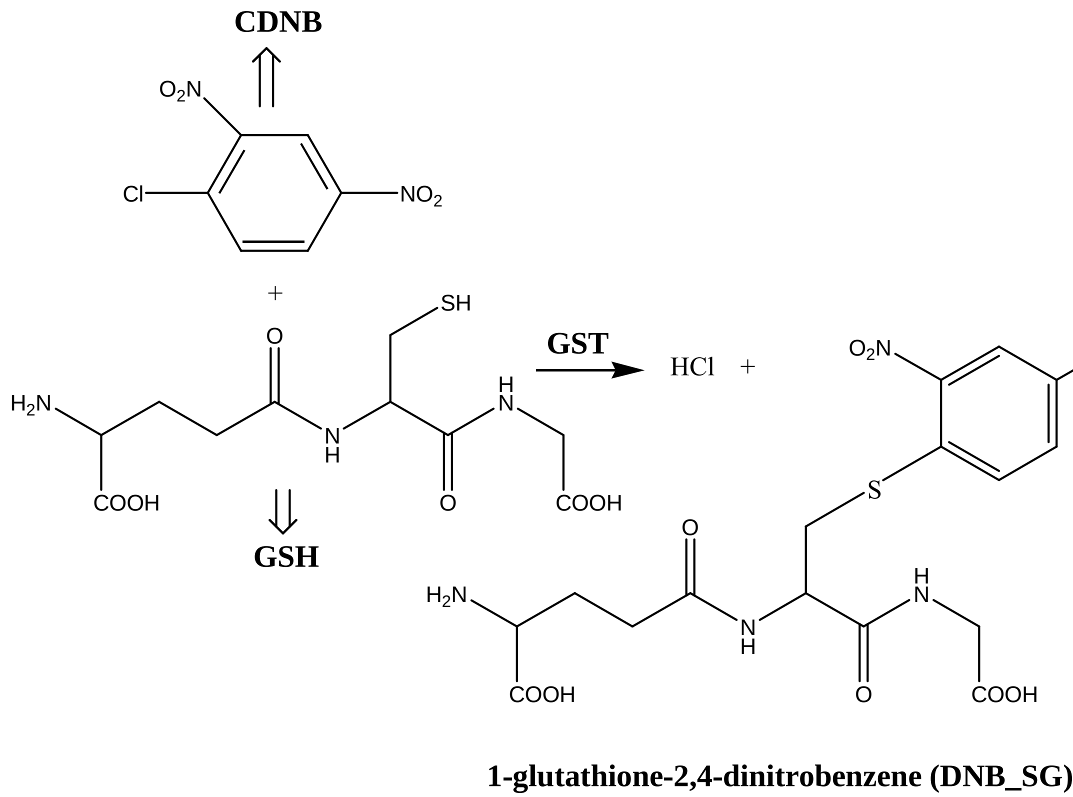

Figure 2.2. 1-glutathione-2,4-dinitrobenzene formation by GST catalyzed reaction

A typical reaction mixture contained 2500 μL of 50 mM potassium phosphate buffer, pH 7.5, 200 μL of 20 mM GSH, 150 μL enzyme source diluted to 100-250 fold with 10 mM pH 7.5 phosphate buffer. The reaction was initiated by the addition of 150 μL of 20 mM CDNB into 3 mL quartz cuvette (1.0 cm light path). Then the thioether formation is followed at 340 nm for 2 minutes. Each time, there should be blank reading (reaction with no enzyme source) for making substraction of non-enzymatic product formation from the GST assay.

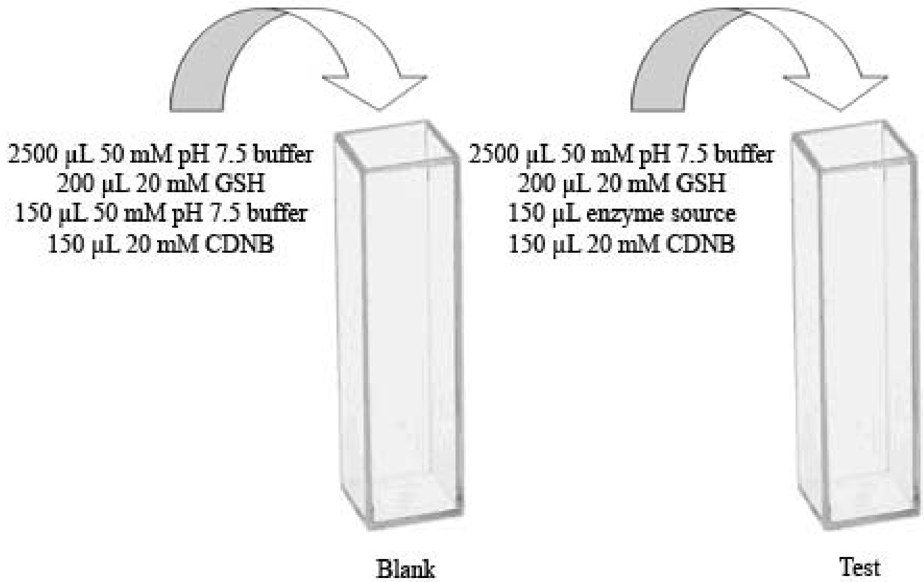

Figure 2.3. Reaction constituents for the determination of total GST activity in fish liver cytosol.

The enzyme activity was calculated using 0.0096 (μM^{-1} cm^{-1}) as an extinction coefficient (ε_{340}) of thioether formed by GST. The following equation was used for the calculation of enzyme specific activity.

$$[E]= \frac{OD_{Test} - OD_{Blank}}{\varepsilon_{340} \times b\ (\text{light path})} \times \text{Dilution Factor} \times \text{Tube Dilution} \times \frac{1}{\text{mg protein}}$$

2.2.4. Determination of Catalase Activity

Catalase activity of fish liver cytosols was determined according to the method of Aebi (1984). In the UV range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm ($\varepsilon_{240} = 0.0364\ \mu M^{-1}cm^{-1}$).

In a typical enzyme activity measurement, enzyme solution (cytosol) was pretreated with 1% Triton-X-100 for 10 minutes (1:10 dilution). Then the mixture diluted further to make a total dilution of 1000, 2000 or 4000 with 50 mM phosphate buffer, pH 7.5. After that 2 mL of diluted sample is added into quartz cuvettes (Figure 2.4). The reaction was started by the addition of 1 mL H_2O_2. The rate of reaction was followed by the decrease in absorbance at 240 nm for about 1 min. The decrease in absorbance was recorded against a blank tube containing 1 mL of 50 mM potassium phosphate buffer, pH 7.5 instead of substrate H_2O_2.

The specific enzyme activity was calculated using 0.0364 ($\mu M^{-1}\ cm^{-1}$) as an extinction coefficient (ε_{240}). Specific enzyme activity was calculated from the difference in the absorbance at 240 nm per unit time using the following equation.

$$[E]= \frac{OD_{Test} - OD_{Blank}}{\varepsilon_{240} \times b \text{ (light path)}} \times \text{Dilution Factor} \times \text{Tube Dilution} \times \frac{1}{\text{mg protein}}$$

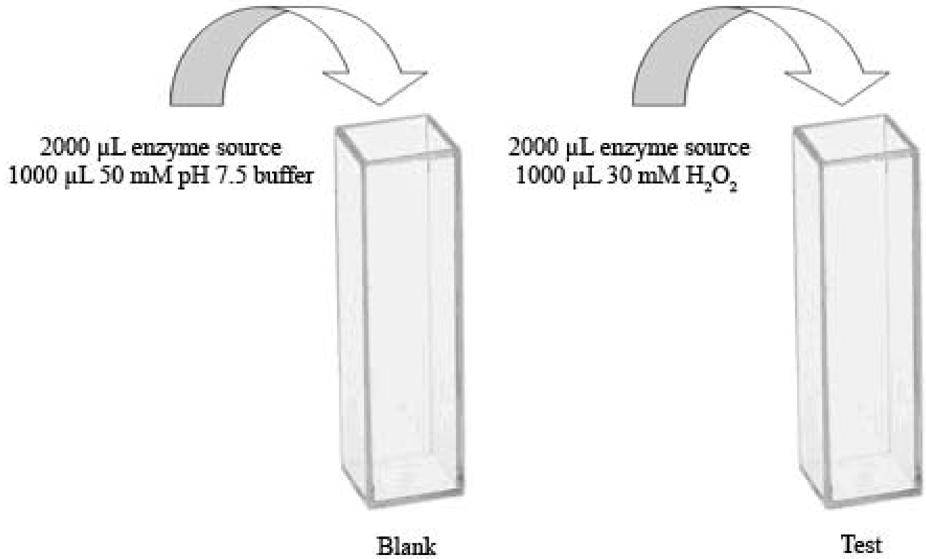

Figure 2.4. Reaction constituents for the determination of CAT activity

2.2.5. Determination of Glutathione Reductase Activity

Glutathione reductase (GR) activities of fish liver cytosols were determined according to the method of Carlberg and Mannervick (1985). Glutathione reductase catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH). One GR activity unit is defined as the amount of enzyme catalyzing the reduction of one micromole of GSSG per minute at pH 7.6 and 25°C. As shown in the reaction, one molecule of NADPH is consumed for each molecule of GSSG reduced. Therefore, the reduction of GSSG is determined indirectly by the measurement of the consumption of NADPH. The rate of decrease at 340 nm is directly proportional to the GR activity in the sample.

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

Figure 2.5. Decomposition GSSG to GSH by GR

A typical reaction medium for the determination of glutathione reductase activity contained 400 µL of 0,1 M potassium phosphate buffer, pH 7.6, 20 µL of 0,5 mM EDTA, pH 7.6, 100 µL 0,1 mM NADPH, 20 µL enzyme source and distilled water at final volume of 2 mL in 3 mL quartz cuvette (1.0 cm light path) (Figure 6). The reaction is started by adding 70 µL of 1 mM GSSG. Then the GSH formation is followed by measuring the NADPH utilization spectrophotometrically at 340 nm for 5 minutes.

The enzyme activity was calculated using 6,22 (μM^{-1} cm^{-1}) as an extinction coefficient (ε_{340}). Specific enzyme activity was calculated from the difference in the absorbance at 340 nm per unit time using the following equation.

$$[E]= \frac{OD_{Test} - OD_{Blank}}{\varepsilon_{340}} \times \text{Dilution Factor} \times \text{Tube Dilution} \times \frac{1}{\text{mg protein}} \times 1000$$

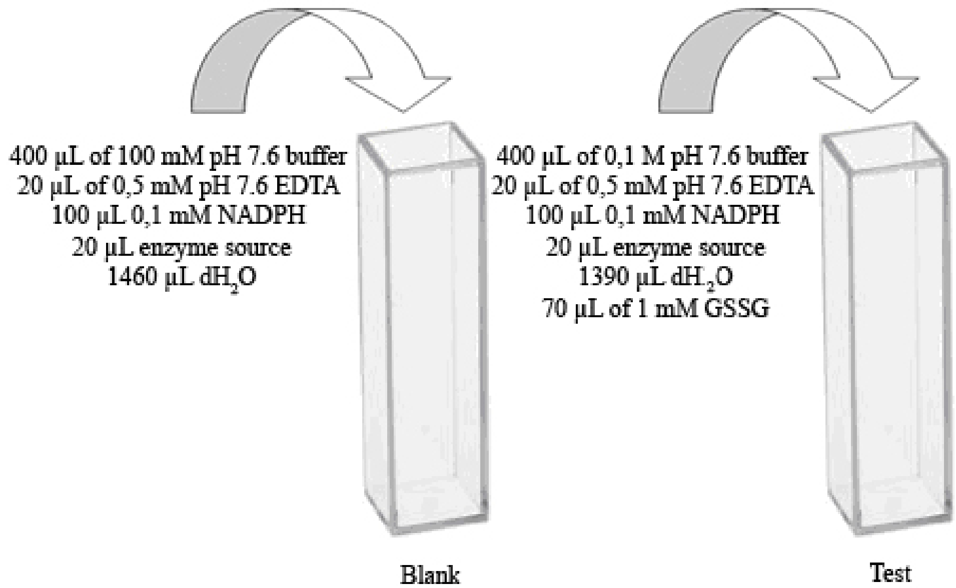

Figure 2.6. Reaction constituents for the determination of GR activity

2.2.6. Statistical Analysis

The results were expressed as means ± standard error of mean (SEM). All data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's Multiple Comparison Post Hoc Test.

CHAPTER 3

RESULTS

Exposure to certain pollutants can enhance reactive oxygen species production in marine animals. In highly polluted areas, the rate of generation of reactive oxygen species exceeds that of their removal, inducing oxidative stress and increasing biomarkers levels. In this study, phase II biotransformation enzyme, glutathione S-transferases and antioxidant enzyme activities, catalase and glutathione reductase were measured in the liver cytosols of mullets. The fish samples were collected in August 2005, August 2006 and August 2007 from different sites of the West Black Sea Region of Turkey. Three different mullet species used throughout this study are so-iuy mullet (*Mugil soiuy*), flathead mullet (*Mugil cephalus*) and golden grey mullet (*Liza aurata*).

3.1. Liver cytosolic Total GST Activities of mullets caught from different sites of the West Black Sea Region of Turkey.

For this study, 150 mullets were caught from seven different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth, Kefken and Amasra) of the West Black Sea in August 2005, August 2006 and August 2007. Cytosols were prepared from one fish liver at a time separately and a sample number was given to each of the cystosol preparation.

Total GST activities in the cytosolic fractions were determined spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate by monitoring the thioether formation at 340 nm as described by Habig and co-workers (Habig *et al*., 1974).

In 2005, 60 mullets were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken) of the West Black Sea Region of Turkey. Table 3.1, 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7 show cytosolic total GST activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average total GST activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from six different sites along a pollution gradient in the West Black Sea Region of Turkey in 2005 were summarized in Table 3.8. Total GST activities were represented as mean ± standard error of the mean.

Figure 3.1 illustrates the graphical representation of total GST activities fish samples with standard error of the mean. Mullets that caught from Zonguldak Harbour and Guluc Stream Mouth had highly elevated total GST activities among the other sampling sites along the West Black Sea Region of Turkey.

Table 3.1. Total GST activities of the so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	832
2	1237
3	1848
4	1081
5	2317
6	999
7	507
Average activity± standard error of the mean n = number of fish liver	1260±207 n=7

Table 3.2. Total GST activities of flathead mullets (*Mugil cephalus*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	1523
2	771
Average activity± standard error of the mean n = number of fish liver	1147±376 n=2

Table 3.3. Total GST activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	794
2	530
3	525
4	2605
5	1360
6	629
7	992
8	715
9	846
10	1230
11	769
12	955
13	465
14	688
15	536
16	1275
Average activity± standard error of the mean n = number of fish liver	932±131 n=16

Table 3.4. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Guluc Stream Mouth (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	2629
2	1314
3	1099
4	1052
5	1516
6	1129
7	1637
8	1864
9	581
10	1439
11	1439
12	1447
13	428
Average activity± standard error of the mean n = number of fish liver	1352±154 n=13

Table 3.5. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	1259
2	481
3	559
4	671
5	467
6	469
7	401
8	1563
9	502
10	1723
Average activity± standard error of the mean n = number of fish liver	810±159 n=10

Table 3.6. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	787
2	250
3	779
4	521
5	649
6	1354
7	834
8	1162
9	412
10	885
11	1729
Average activity± standard error of the mean n = number of fish liver	851±129 n=11

Table 3.7. Total GST activities of flathead mullet (*Mugil cephalus*) caught from Kefken (August 2005).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	455
Average activity± standard error of the mean n = number of fish liver	455±0 n=1

Table 3.8. The average total GST activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2005.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	Total GST Activities (nmol CDNB conjugate formed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	7	1260±207
Zonguldak Harbour	*Mugil cephalus*	2	1147±376
Eregli Harbour	*Liza aurata*	16	932±131
Guluc Stream Mouth	*Mugil soiuy*	13	1352±154
Sakarya River Mouth	*Mugil soiuy*	10	810±159
Melen Stream Mouth	*Mugil soiuy*	11	851±129
Kefken	*Mugil cephalus*	1	455±0

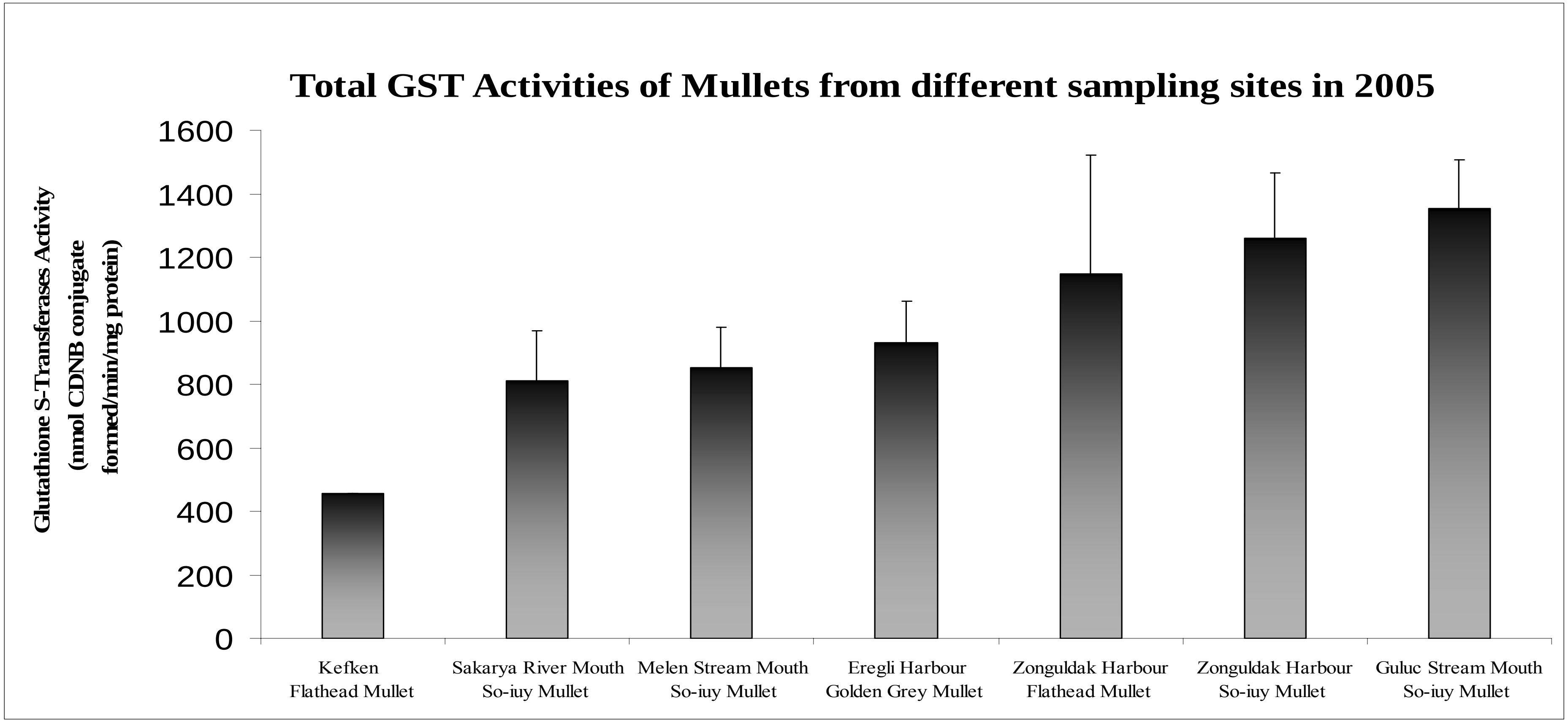

Figure 3.1. Total GST activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken. Fish were collected in August 2005.

So-iuy Mullets that caught from Guluc Stream Mouth showed the highest total GST activities (1352±154 nmol CDNB conjugate formed/min/mg protein, n=13) among the other sampling sites (see Table 3.4). So-iuy mullets and flathead mullets caught from Zonguldak Harbour had also very high total GST activities (1260±207 nmol CDNB conjugate formed/min/mg protein, n=9; 1147±376 nmol CDNB conjugate formed/min/mg protein, n=2, respectively) compared to other sampling sites (see Tables 3.1 and 3.2). Total GST activity of golden grey mullets from Eregli Harbour was 932±131 nmol CDNB conjugate formed/min/mg protein, n=16 (see Table 3.3). Total GST activities of so-iuy mullets from Sakarya River Mouth and Melen Stream Mouth were 810±159 nmol CDNB conjugate formed/min/mg protein, n=10 and 851±129 nmol CDNB conjugate formed/min/mg protein, n=11, respectively (see Tables 3.5 and 3.6).

In 2006, 71 mullets were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.9, 3.10, 3.11, 3.12, 3.13, 3.14, 3.15 and 3.16 show cytosolic total GST activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average total GST activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from seven different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2006 were summarized in Table 3.17. Total GST activities were represented as mean ± standard error of the mean.

Figure 3.2 illustrates the graphical representation of total GST activities with standard error of the mean. Fish samples captured from Melen Stream Mouth, Zonguldak Harbour and Guluc Stream Mouth had highly elevated total GST activities than the other sampling sites along the West Black Sea Region of Turkey.

Table 3.9. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	770
2	1006
3	823
4	724
5	946
6	1387
7	398
8	1057
9	1017
10	228
11	1007
12	747
13	2234
Average activity± standard error of the mean n = number of fish liver	950±135 n=13

Table 3.10. Total GST activities of golden grey mullets (*Liza aurata*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	2458
2	657
3	1136
4	825
5	404
6	722
7	634
8	1310
9	1461
Average activity± standard error of the mean n = number of fish liver	1067±208 n=9

Table 3.11. Total GST activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	529
2	306
3	454
4	581
5	764
6	1115
7	417
8	575
9	799
10	473
11	392
12	614
Average activity± standard error of the mean n = number of fish liver	585±64 n=12

Table 3.12. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Guluc Stream Mouth (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	672
2	1014
3	2166
4	663
5	415
6	512
7	959
8	499
9	634
10	417
11	1174
Average activity± standard error of the mean n = number of fish liver	830±154 n=11

Table 3.13. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	582
2	609
3	1318
4	1016
5	1291
6	127
7	1068
8	185
9	662
10	490
Average activity± standard error of the mean n = number of fish liver	735±134 n=10

Table 3.14. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	547
2	891
3	921
4	1054
5	1031
6	1808
7	1188
8	1697
9	1590
Average activity± standard error of the mean n = number of fish liver	1192±140 n=9

Table 3.15. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	114
2	133
3	617
Average activity± standard error of the mean n = number of fish liver	288±165 n=3

Table 3.16. Total GST activities of flathead mullets (*Mugil cephalus*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	194
2	449
3	204
4	203
Average activity± standard error of the mean n = number of fish liver	263±62 n=4

Table 3.17. The average total GST activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2006.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	Total GST Activities (nmol CDNB conjugate formed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	13	950±135
Zonguldak Harbour	*Liza aurata*	9	1067±208
Eregli Harbour	*Liza aurata*	12	585±64
Guluc Stream Mouth	*Mugil soiuy*	11	830±154
Sakarya River Mouth	*Mugil soiuy*	10	735±134
Melen Stream Mouth	*Mugil soiuy*	9	1192±140
Amasra	*Mugil soiuy*	3	288±165
Amasra	*Mugil cephalus*	4	263±62

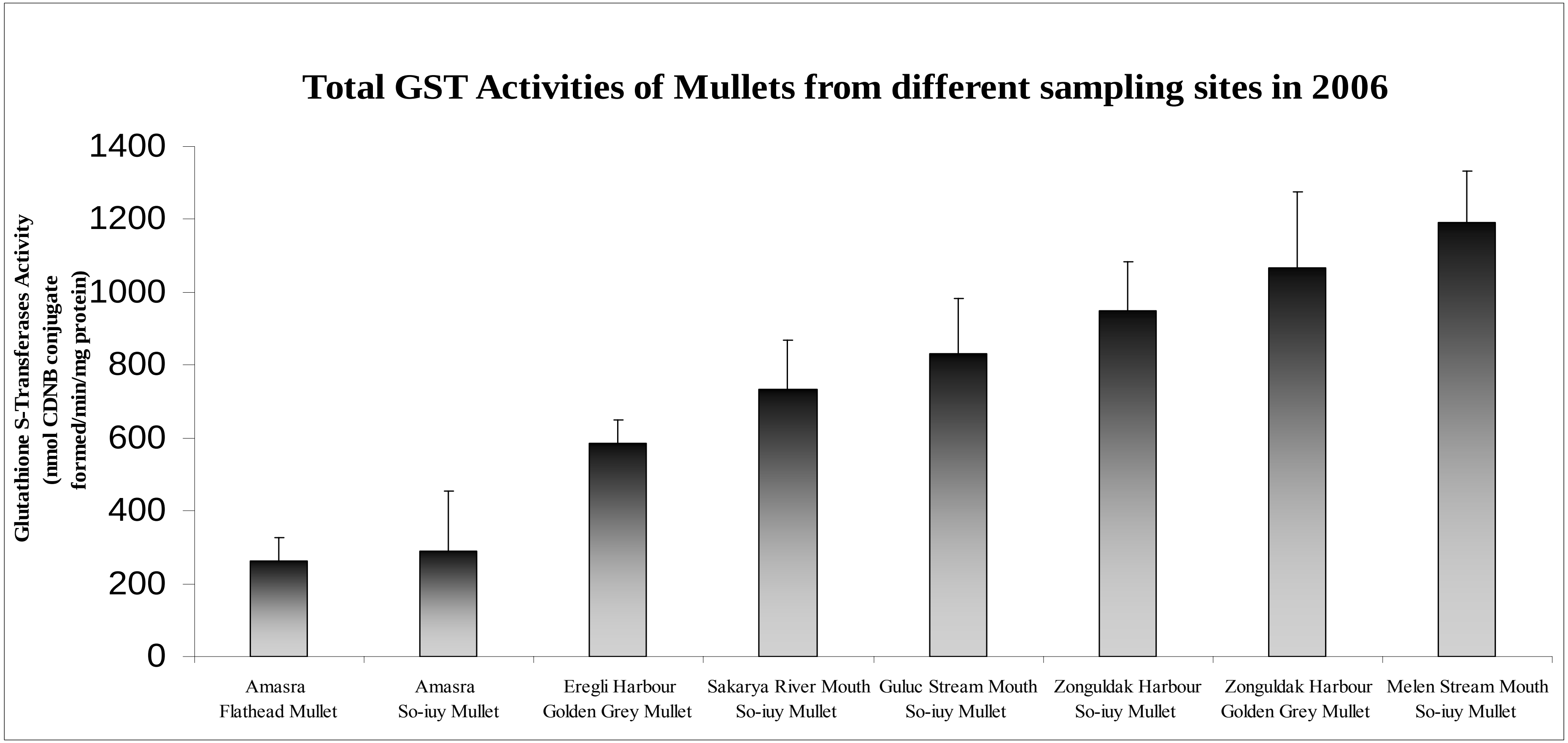

Figure 3.2. Total GST activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra. Fish were collected in August 2006.

The lowest total GST activities were measured in the fish samples captured from Amasra. Mullets captured from Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth and Melen Stream Mouth had highly elevated total GST activities.

The total GST activities of so-iuy mullets caught from Zonguldak Harbour and Guluc Stream Mouth were 950±135 nmol CDNB conjugate formed/min/mg protein, n=13 and 830±154 nmol CDNB conjugate formed/min/mg protein, n=11, respectively (for detail see Tables 3.9 and 3.12). The Total GST activity of so-iuy mullets caught from Sakarya River Mouth was 735±134 nmol CDNB conjugate formed/min/mg protein, n=10 (see Table 3.13).

So-iuy mullets caught from Melen Stream Mouth had the highest total GST activity (1192±140 nmol CDNB conjugate formed/min/mg protein, n=9) among the other sampling sites (see Table 3.14).

The results of total GST activities indicate that GST inducer pollutants levels were higher in the fish samples caught from Melen Stream Mouth, Zonguldak Harbour and Guluc Stream Mouth than the other sampling sites along the West Black Sea Region of Turkey.

In 2007, 19 mullets were caught from four different sites (Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.18, 3.19, 3.20 and 3.21 show cytosolic total GST activities obtained from the livers of individual fish caught from the four different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average total GST activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from four different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2007 were summarized in Table 3.22. Total GST activities were represented as mean ± standard error of the mean.

Figure 3.3 illustrates the graphical representation of total GST activities with standard error of the mean. It is apparent that the average activity of the total GST from Zonguldak Harbour and Sakarya River Mouth were higher compared to the other sampling sites along the West Black Sea Region of Turkey.

Table 3.18. Total GST activities of flathead mullets (*Mugil cephalus*) caught from Zonguldak Harbour (August 2007).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	376
2	555
3	1550
4	1322
5	1281
Average activity± standard error of the mean n = number of fish liver	1017±231 n=5

Table 3.19. Total GST activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2007).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	464
2	329
3	357
4	398
5	480
Average activity± standard error of the mean n = number of fish liver	406±29 n=5

Table 3.20. Total GST activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2007).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	308
2	1894
3	1226
4	636
Average activity± standard error of the mean n = number of fish liver	1016±349 n=4

Table 3.21. Total GST activities of golden grey mullets (*Liza aurata*) caught from Amasra (August 2007).

Sample No	Average Activity (nmol CDNB conjugate formed/min/mg protein)
1	270
2	188
3	362
4	214
5	288
Average activity± standard error of the mean n = number of fish liver	285±37 n=5

Table 3.22. The average total GST activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2007.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	Total GST Activities (nmol CDNB conjugate formed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil cephalus*	5	1017±231
Eregli Harbour	*Liza aurata*	5	406±29
Sakarya River Mouth	*Mugil soiuy*	4	1016±349
Amasra	*Liza aurata*	5	285±37

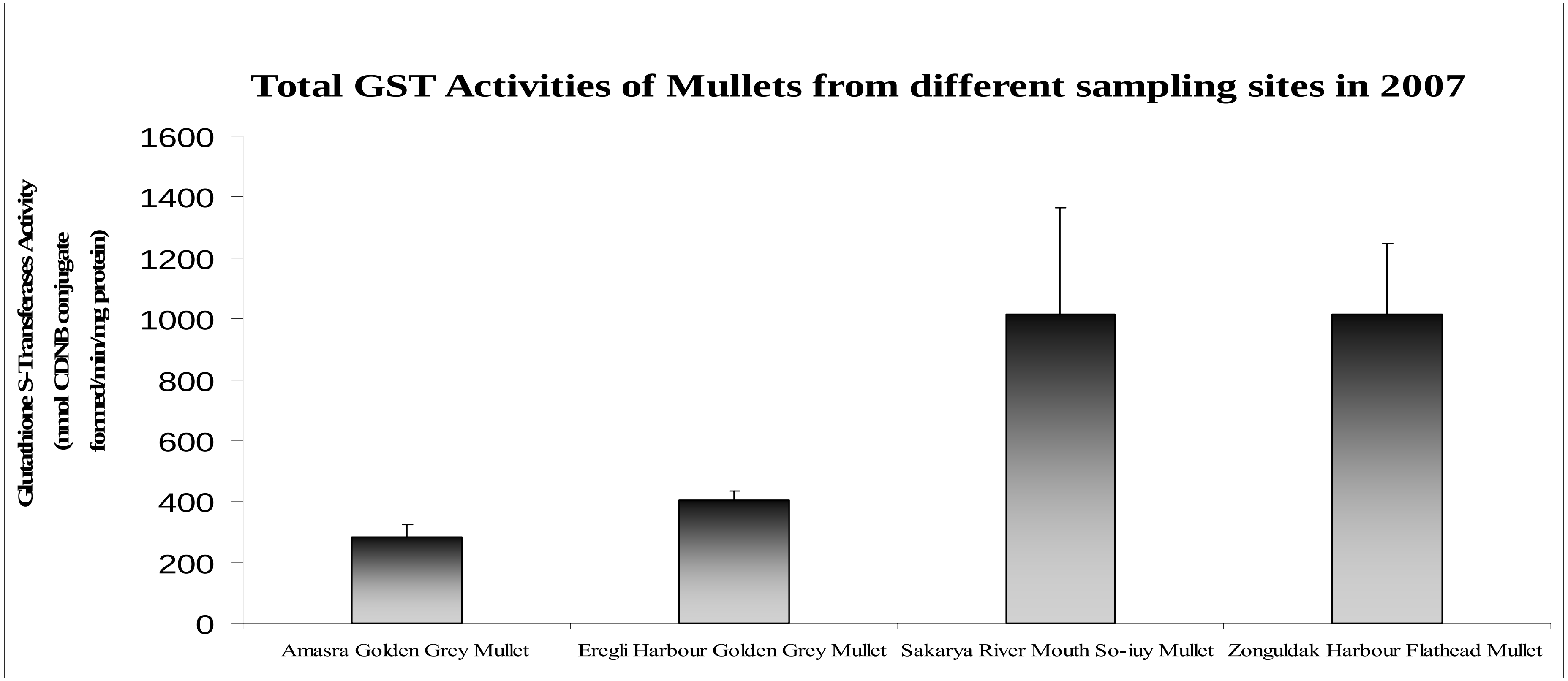

Figure 3.3. Total GST activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra. Fish were collected in August 2007.

The lowest liver cytosolic total GST activity of golden grey mullets (288±165 nmol CDNB conjugate formed/min/mg protein, n=3) was obtained from Amasra in August 2007.

Flathead mullets captured from Zonguldak Harbour had the highest total GST activity (1017±231 nmol CDNB conjugate formed/min/mg protein, n=5) among the other sampling sites along the West Black Sea Region of Turkey (see Table 3.18).

The average total GST activity of So-iuy mullets captured from Sakarya River Mouth was 1016±349 nmol CDNB conjugate formed/min/mg protein, n=4 (see Table 3.20).

3.2. Liver cytosolic Catalase (CAT) Activities of Mullets caught from different sites of the West Black Sea Region of Turkey.

Catalase activities were determined according to the method of Aebi (1984). The CAT assay is based on the decomposition of H_2O_2 which can be followed directly by the decrease in absorbance at 240 nm in the UV range (ε_{240}=0.0364 $\mu M^{-1}cm^{-1}$).

In 2005, 60 mullet were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken) of the West Black Sea Region of Turkey. Table 3.23, 3.24, 3.25, 3.26, 3.27, 3.28 and 3.29 show cytosolic CAT activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average CAT activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from six different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2005 were summarized in Table 3.30. CAT activities were represented as mean ± standard error of the mean.

Figure 3.4 illustrates the graphical representation of CAT activities with standard error of the mean. It is apparent that the average activity of the CAT from Zonguldak Harbour, Sakarya River Mouth, Melen Stream Mouth and Guluc Stream Mouth were higher compared to the other sites of the West Black Sea Region of Turkey.

Table 3.23. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	292
2	153
3	432
4	322
5	252
6	360
7	443
Average activity± standard error of the mean n = number of fish liver	322±34 n=7

Table 3.24. CAT activities of flathead mullets (*Mugil cephalus*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	438
2	285
Average activity± standard error of the mean n = number of fish liver	362±77 n=2

Table 3.25. CAT activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2005).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	173
2	105
3	334
4	353
5	168
6	201
7	188
8	122
9	213
10	116
11	146
12	229
13	130
14	204
15	244
16	197
Average activity± standard error of the mean n = number of fish liver	195±18 n=16

Table 3.26. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Guluc Stream Mouth (August 2005).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	495
2	211
3	169
4	150
5	356
6	243
7	174
8	265
9	278
10	487
11	385
12	238
13	200
Average activity± standard error of the mean n = number of fish liver	281±32 n=13

Table 3.27. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2005).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	350
2	355
3	373
4	356
5	341
6	353
7	238
8	317
9	403
10	437
Average activity± standard error of the mean n = number of fish liver	352±17 n=10

Table 3.28. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2005).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	404
2	421
3	346
4	180
5	349
6	221
7	430
8	308
9	326
10	284
11	569
Average activity± standard error of the mean n = number of fish liver	349±32 n=11

Table 3.29. CAT activities of flathead mullet (*Mugil cephalus*) caught from Kefken (August 2005).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	78
Average activity± standard error of the mean n = number of fish liver	78±0 n=1

Table 3.30. The average CAT activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2005.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	CAT Activities (nmol H_2O_2 consumed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	7	322±34
Zonguldak Harbour	*Mugil cephalus*	2	362±77
Eregli Harbour	*Liza aurata*	16	195±18
Guluc Stream Mouth	*Mugil soiuy*	13	281±32
Sakarya River Mouth	*Mugil soiuy*	10	352±17
Melen Stream Mouth	*Mugil soiuy*	11	349±32
Kefken	*Mugil cephalus*	1	78±0

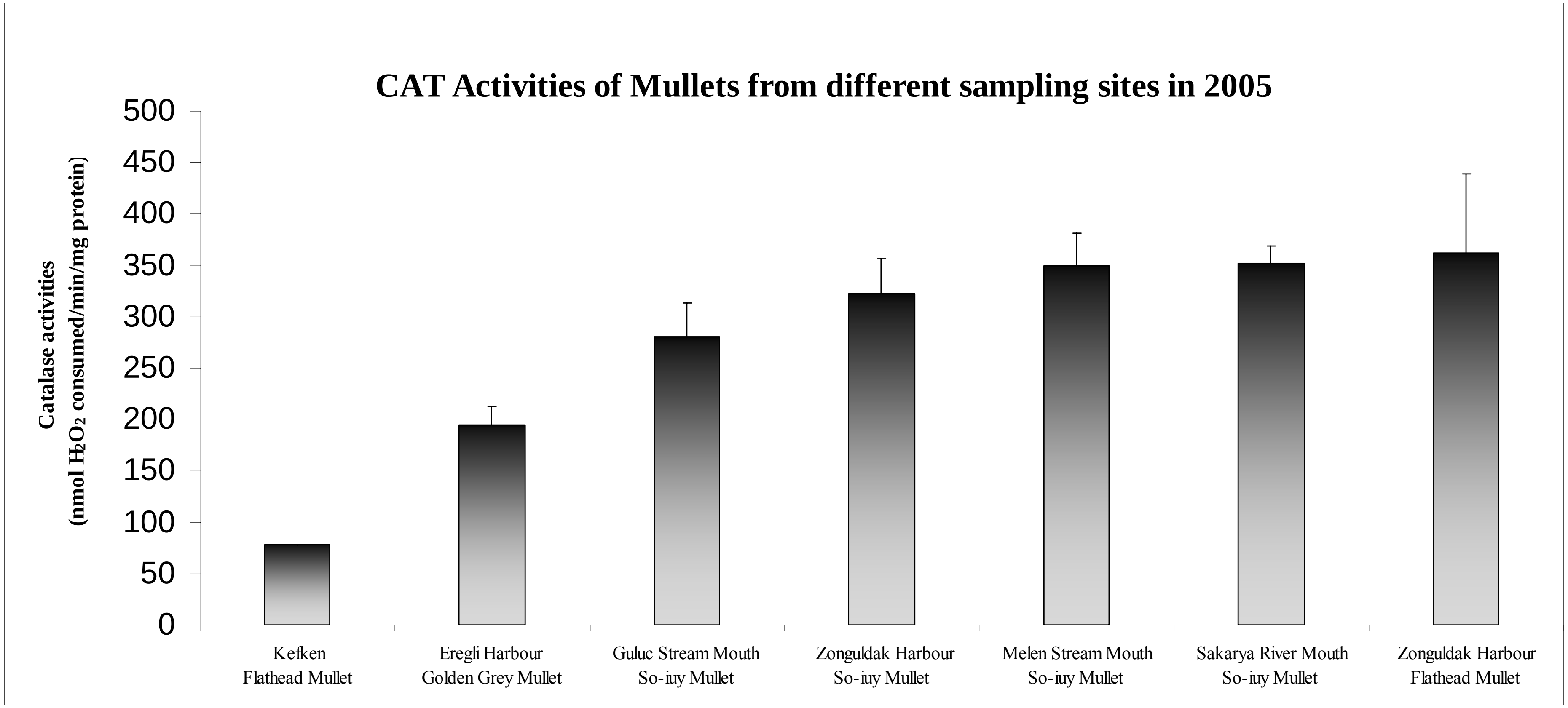

Figure 3.4. CAT activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken. Fish were collected in August 2005.

Flathead mullets captured from Zonguldak Harbour had the highest CAT activity (362±77 nmol H_2O_2 consumed/min/mg protein, n=2) among the other sampling sites (see Table 3.24).

So-iuy mullets captured from Sakarya River Mouth and Melen Stream Mouth had also highly elevated CAT activities (352±17 nmol H_2O_2 consumed/min/mg protein, n=10 and 349±32 nmol H_2O_2 consumed/min/mg protein, n=11, respectively) (see Tables 3.27 and 3.28). The CAT activity of so-iuy mullets captured from Zonguldak Harbour was also highly elevated CAT activities (322±34 nmol H_2O_2 consumed/min/mg protein, n=7 (see Table 3.23). The CAT activity of so-iuy mullets captured from Guluc Stream Mouth was 281±32 nmol H_2O_2 consumed/min/mg protein, n=13 (see Table 3.26). The CAT activity of golden grey mullets captured from Eregli Harbour was 195±18 nmol H_2O_2 consumed/min/mg protein, n=16 (see Table 3.25).

In 2006, 71 mullets were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.31, 3.32, 3.33, 3.34, 3.35, 3.36, 3.37 and 3.38 show cytosolic CAT activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average CAT activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from six different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2006 were summarized in Table 3.39. CAT activities were represented as mean ± standard error of the mean.

Figure 3.5 illustrates the graphical representation of CAT activities with standard error of the mean. It is apparent that the average activity of the CAT from Zonguldak Harbour, Sakarya River Mouth and Guluc Stream Mouth were higher compared to the other sites of the West Black Sea Region of Turkey.

Table 3.31. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	459
2	659
3	239
4	73
5	161
6	460
7	139
8	209
9	272
10	96
11	322
12	252
13	338
Average activity± standard error of the mean n = number of fish liver	283±46 n=13

Table 3.32. CAT activities of golden grey mullets (*Liza aurata*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	136
2	225
3	112
4	241
5	170
6	90
7	140
8	172
9	179
Average activity± standard error of the mean n = number of fish liver	163±16 n=9

Table 3.33. CAT activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2006).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	119
2	83
3	92
4	125
5	124
6	157
7	131
8	156
9	134
10	241
11	142
12	147
Average activity± standard error of the mean n = number of fish liver	138±12 n=12

Table 3.34. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Guluc Stream Mouth (August 2006).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	256
2	425
3	291
4	158
5	139
6	321
7	158
8	101
9	210
10	137
11	267
Average activity± standard error of the mean n = number of fish liver	224±30 n=11

Table 3.35. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2006).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	250
2	228
3	98
4	341
5	181
6	356
7	234
8	367
9	378
10	141
Average activity± standard error of the mean n = number of fish liver	257±32 n=10

Table 3.36. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2006).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	145
2	203
3	159
4	210
5	162
6	293
7	264
8	244
9	117
Average activity± standard error of the mean n = number of fish liver	200±20 n=9

Table 3.37. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	162
2	100
3	106
Average activity± standard error of the mean n = number of fish liver	123±20 n=3

Table 3.38. CAT activities of flathead mullets (*Mugil cephalus*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	179
2	103
3	97
4	89
Average activity± standard error of the mean n = number of fish liver	117±21 n=4

Table 3.39. The average CAT activities of liver cytosols of mullet caught from different sites of the West Black Sea Region of Turkey in August 2006.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	CAT Activities (nmol H_2O_2 consumed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	13	283±46
Zonguldak Harbour	*Liza aurata*	9	163±16
Eregli Harbour	*Liza aurata*	12	138±12
Guluc Stream Mouth	*Mugil soiuy*	11	224±30
Sakarya River Mouth	*Mugil soiuy*	10	257±32
Melen Stream Mouth	*Mugil soiuy*	9	200±20
Amasra	*Mugil soiuy*	3	123±20
Amasra	*Mugil cephalus*	4	117±21

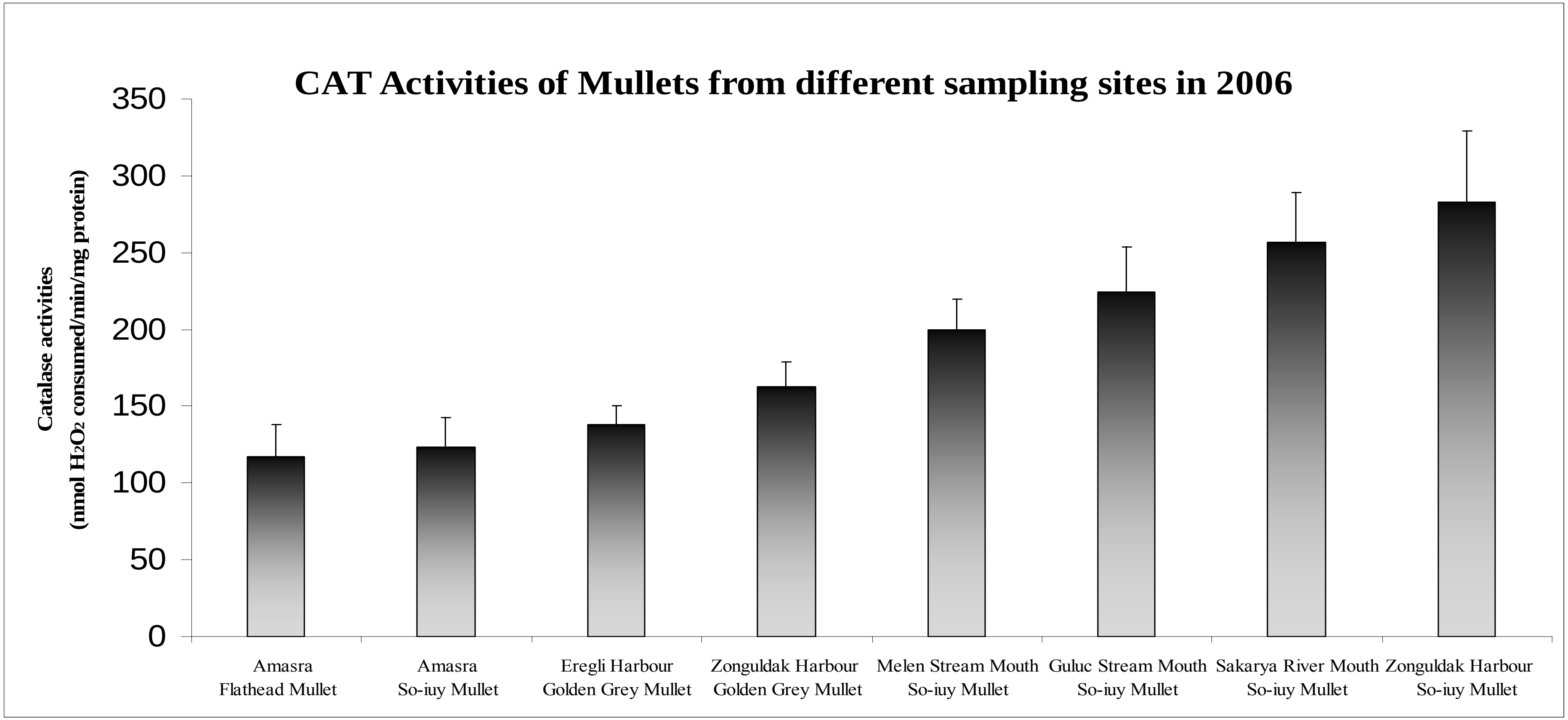

Figure 3.5. CAT activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra. Fish were collected in August 2006.

The lowest CAT activities were measured in the fish samples caught from Amasra. The average CAT activities of so-iuy mullets and flathead mullets were 123±20 nmol H_2O_2 consumed/min/mg protein, n=3 and 117±21 nmol H_2O_2 consumed/min/mg protein, n=4, repectively.

So-iuy mullets captured from Zonguldak Harbour had the highest CAT activity (283±46 nmol H_2O_2 consumed/min/mg protein, n=13) among the other sampling sites. (see Table 3.31).

The CAT activities of so-iuy mullets captured from Guluc Stream Mouth and Sakarya River Mouth were 224±30 nmol H_2O_2 consumed/min/mg protein, n=11 and 257±32 nmol H_2O_2 consumed/min/mg protein, n=10, respectively (see Tables 3.34 and 3.35). The CAT activity of So-iuy Mullets captured from Melen Stream Mouth was 200±20 nmol H_2O_2 consumed/min/mg protein, n=9 (see Table 3.36).

In 2007, 19 mullets were caught from four different sites (Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.40, 3.41, 3.42 and 3.43 show cytosolic total CAT activities obtained from the livers of individual fish caught from the four different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average CAT activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from four different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2007 were summarized in Table 3.44. CAT activities were represented as mean ± standard error of the mean.

Figure 3.6 illustrates the graphical representation of CAT activities with standard error of the mean. The average activities of the CAT from Sakarya River Mouth and Zonguldak Harbour were higher compared to the other sites along the West Black Sea Region of Turkey.

Table 3.40 CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2007).

Sample No	Average Activity ($nmol\ H_2O_2$ consumed/min/mg protein)
1	160
2	140
3	165
4	184
5	208
Average activity± standard error of the mean n = number of fish liver	171±12 n=5

Table 3.41. CAT activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2007).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	130
2	80
3	156
4	112
5	127
Average activity± standard error of the mean n = number of fish liver	121±12 n=5

Table 3.42. CAT activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2007).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	197
2	236
3	157
4	156
Average activity± standard error of the mean n = number of fish liver	187±19 n=4

Table 3.43. CAT activities of golden grey mullets (*Liza aurata*) caught from Amasra (August 2007).

Sample No	Average Activity (nmol H_2O_2 consumed/min/mg protein)
1	134
2	87
3	76
4	116
5	88
Average activity± standard error of the mean n = number of fish liver	100±11 n=5

Table 3.44. The average CAT activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2007.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	CAT Activities (nmol H_2O_2 consumed/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil cephalus*	5	171±12
Eregli Harbour	*Liza aurata*	5	121±12
Sakarya River Mouth	*Mugil soiuy*	4	187±19
Amasra	*Liza aurata*	5	100±11

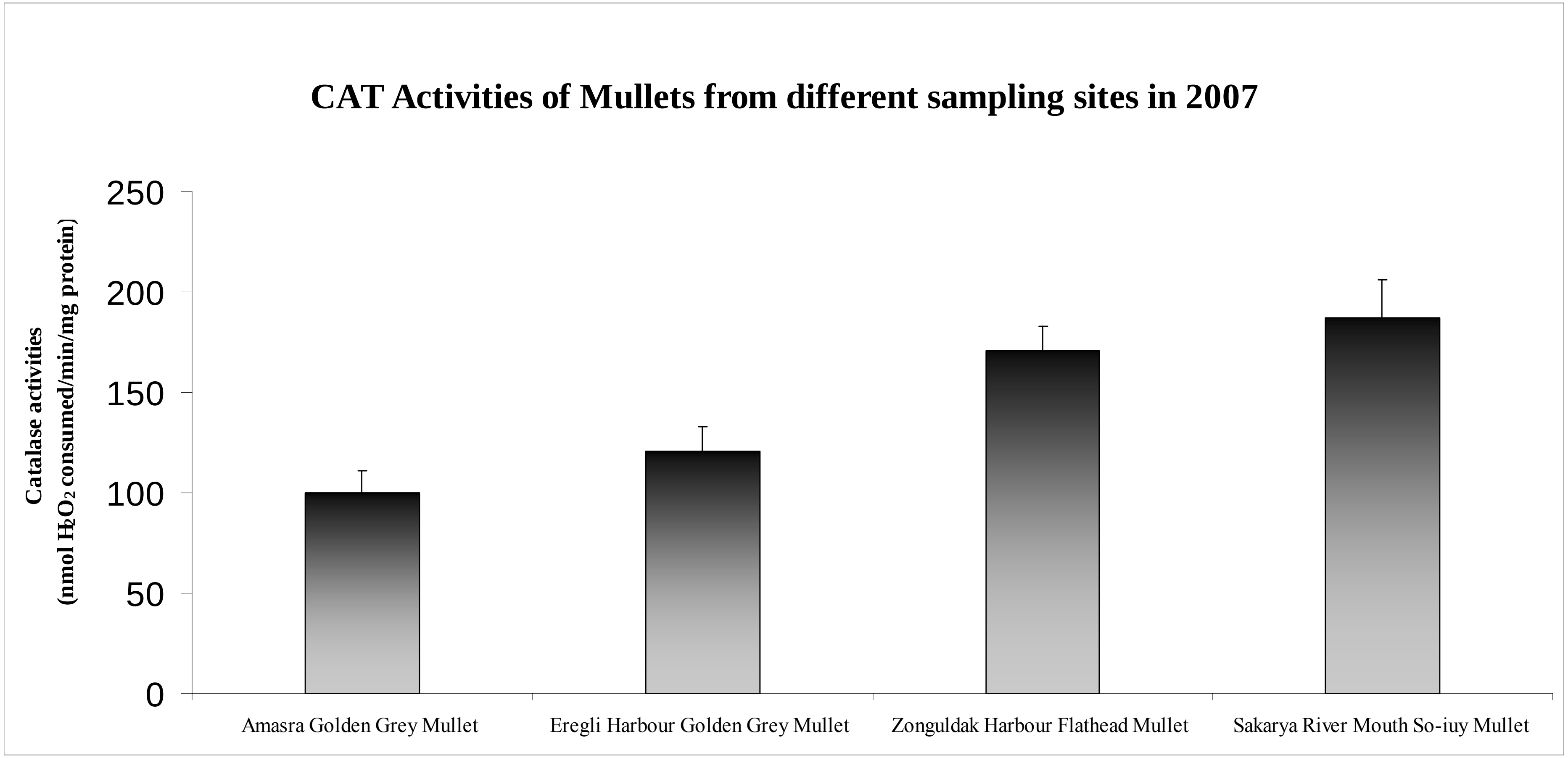

Figure 3.6. CAT activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra. Fish were collected in August 2007.

The lowest liver cytosolic CAT activities were measured in golden grey mullets caught from Amasra. So-iuy mullets captured from Sakarya River Mouth had the highest CAT activity (187±19 nmol H_2O_2 consumed/min/mg protein, n=4) among the other sampling sites (see Table 3.42). The CAT activity of flathead mullets captured from Zonguldak Harbour was 171±12 nmol H_2O_2 consumed/min/mg protein, n=5 (see Table 3.40).

3.3. Liver cytosolic Glutathione Reductase (GR) Activities of Mullets caught from different sites of the West Black Sea Region of Turkey.

The GR activities were determined spectrophotometrically by measuring the rate of NADPH oxidation. The oxidation of NADPH to $NADP^+$ is followed by a decrease in absorbance at 340 nm (Carlberg and Mannervick, 1985).

In 2005, 60 mullets were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken) of the West Black Sea Region of Turkey. Table 3.45, 3.46, 3.47, 3.48, 3.49, 3.50 and 3.51 show cytosolic GR activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average GR activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from six different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2005 were summarized in Table 3.52. GR activities were represented as mean ± standard error of the mean.

Figure 3.7 illustrates the graphical representation of GR activities with standard error of the mean among different collection sites. It is apparent that the average activity of the GR from Melen Stream Mouth, Guluc Stream Mouth and Sakarya River Mouth were higher compared to the other sites along West Black Sea Region of Turkey.

Table 3.45. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	11.4
2	5.7
3	17.6
4	16.9
5	14.4
6	17.5
7	20.5
Average activity± standard error of the mean n = number of fish liver	14.9±1.9 n=7

Table 3.46. GR activities of flathead mullets (*Mugil cephalus*) caught from Zonguldak Harbour (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	13.0
2	13.6
Average activity± standard error of the mean n = number of fish liver	13.3±0.3 n=2

Table 3.47. GR activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	21.4
2	14.6
3	21.7
4	16.1
5	19.6
6	12.1
7	19.3
8	16.1
9	15.4
10	19.3
11	17.2
12	20.2
13	18.1
14	13.9
15	15.5
16	17.8
Average activity± standard error of the mean n = number of fish liver	17.4±0.7 n=16

Table 3.48. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Guluc Stream Mouth (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	20.7
2	21.4
3	18.5
4	14.1
5	21.9
6	19.1
7	24.7
8	20.3
9	26.2
10	18.4
11	19.1
12	22.3
13	23.7
Average activity± standard error of the mean n = number of fish liver	20.8±0.9 n=13

Table 3.49. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	20.8
2	22.9
3	27.1
4	21.9
5	15.5
6	28.5
7	15.5
8	15.5
9	13.7
10	15.5
Average activity± standard error of the mean n = number of fish liver	19.7±1.7 n=10

Table 3.50. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	24.5
2	30.5
3	29.9
4	24.1
5	16.8
6	18.6
7	29.1
8	22.6
9	29.6
10	12.1
11	16.1
Average activity± standard error of the mean n = number of fish liver	23.1±1.9 n=11

Table 3.51. GR activities of flathead mullet (*Mugil cephalus*) caught from Kefken (August 2005).

Sample No	Average Activity (nmol/min/mg protein)
1	8.8
Average activity± standard error of the mean n = number of fish liver	8.8±0 n=1

Table 3.52. The average GR activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2005.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	GR Activities (nmol/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	7	14.9±1.9
Zonguldak Harbour	*Mugil cephalus*	2	13.3±0.3
Eregli Harbour	*Liza aurata*	16	17.4±0.7
Guluc Stream Mouth	*Mugil soiuy*	13	20.8±0.9
Sakarya River Mouth	*Mugil soiuy*	10	19.7±1.7
Melen Stream Mouth	*Mugil soiuy*	11	23.1±1.9
Kefken	*Mugil cephalus*	1	8.8±0

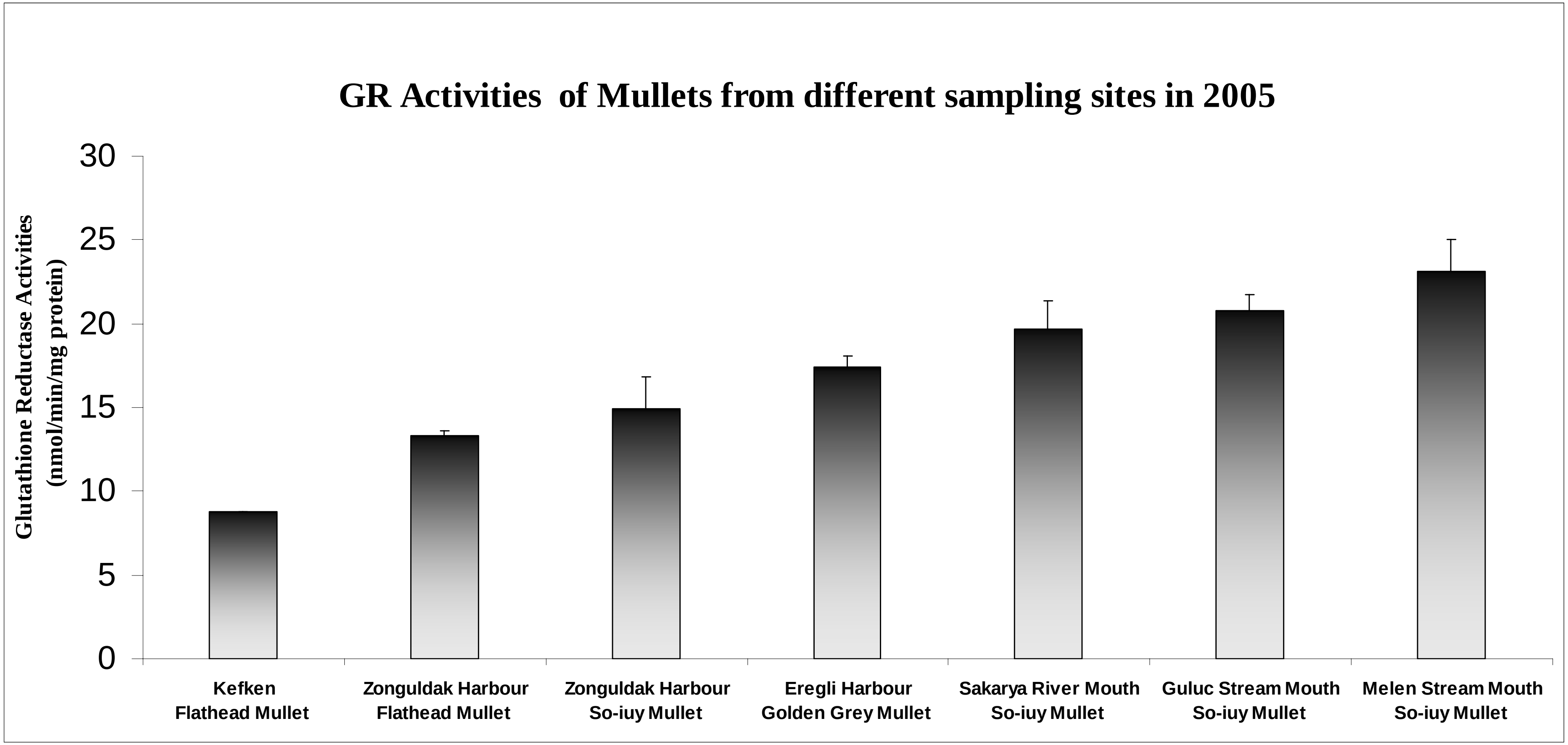

Figure 3.7. GR activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Kefken. Fish were collected in August 2005.

So-iuy mullets captured from Melen Stream Mouth had the highest GR activity (23,1±1,9 nmol/min/mg protein, n=11) among the other sampling sites (see Table 3.50). So-iuy mullets from the other stream and river had also highly elevated GR activities. The GR activity of so-iuy mullets captured from Guluc Stream Mouth was 20,8±0,9 nmol/min/mg protein, n=13 (see Table 3.48). The GR activities of So-iuy Mullets captured from Sakarya River Mouth was 19,7±1,7 nmol/min/mg protein, n=10 (see Table 3.49). The GR activity of Golden Grey Mullets captured from Eregli Harbour was 17,4±0,7 nmol/min/mg protein, n=16 (see Table 3.47). Flathead Mullets captured from Zonguldak Harbour had the GR activity as 13,3±0,3 nmol/min/mg protein, n=2 (see Table 3.45).

In 2006, 71 mullets were caught from six different sites (Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.53, 3.54, 3.55, 3.56, 3.57, 3.58, 3.59 and 3.60 show cytosolic GR activities obtained from the livers of individual fish caught from the six different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average GR activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from six different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2006 were summarized in Table 3.61. GR activities were represented as mean ± standard error of the mean.

Figure 3.8 illustrates the graphical representation of GR activities with standard error of the mean. It is apparent that the average activity of the GR activities from Guluc Stream Mouth, Zonguldak Harbour, Eregli Harbour and Melen Stream Mouth were higher compared to the other sites of the West Black Sea Region of Turkey.

Table 3.53. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	18.3
2	21.9
3	22.0
4	18.5
5	22.0
6	19.6
7	22.7
8	21.8
9	23.2
10	17.6
11	26.2
12	16.1
13	16.1
Average activity± standard error of the mean n = number of fish liver	20.4±0.8 n=13

Table 3.54. GR activities of golden grey mullets (*Liza aurata*) caught from Zonguldak Harbour (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	22.3
2	10.1
3	24.4
4	16.7
5	13.4
6	11.7
7	16.8
8	16.1
9	14.1
Average activity± standard error of the mean n = number of fish liver	16.2±1.6 n=9

Table 3.55. GR activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	18.8
2	18.8
3	22.7
4	22.5
5	16.1
6	16.7
7	17.3
8	14.9
9	17.2
10	20.6
11	14.0
12	17.2
Average activity± standard error of the mean n = number of fish liver	18.1±0.8 n=12

Table 3.56. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Gülüç Stream Mouth (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	22.8
2	16.1
3	23.7
4	24.8
5	19.7
6	23.2
7	14.6
8	21.0
9	20.1
10	27.6
11	14.0
Average activity± standard error of the mean n = number of fish liver	20.7±1.3 n=11

Table 3.57. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	13.9
2	7.4
3	7.8
4	10.2
5	7.8
6	7.4
7	11.4
8	8.3
9	10.4
10	11.3
Average activity± standard error of the mean n = number of fish liver	9.6±0.7 n=10

Table 3.58. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Melen Stream Mouth (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	26.5
2	16.7
3	14.4
4	29.2
5	16.7
6	16.1
7	18.0
8	12.7
9	17.8
Average activity± standard error of the mean n = number of fish liver	18.7±1.8 n=9

Table 3.59. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	14.7
2	12.2
3	13.4
Average activity± standard error of the mean n = number of fish liver	13.4±0.8 n=3

Table 3.60. GR activities of flathead mullets (*Mugil cephalus*) caught from Amasra (August 2006).

Sample No	Average Activity (nmol/min/mg protein)
1	10.7
2	12.2
3	10.3
4	12.1
Average activity± standard error of the mean n = number of fish liver	11.3±0.5 n=4

Table 3.61. The average GR activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2006.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	GR Activities (nmol/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil soiuy*	13	20.4±0.8
Zonguldak Harbour	*Liza aurata*	9	16.2±1.6
Eregli Harbour	*Liza aurata*	12	18.1±0.8
Guluc Stream Mouth	*Mugil soiuy*	11	20.7±1.3
Sakarya River Mouth	*Mugil soiuy*	10	9.6±0.7
Melen Stream Mouth	*Mugil soiuy*	9	18.7±1.8
Amasra	*Mugil soiuy*	3	13.4±0.8
Amasra	*Mugil cephalus*	4	11.3±0.5

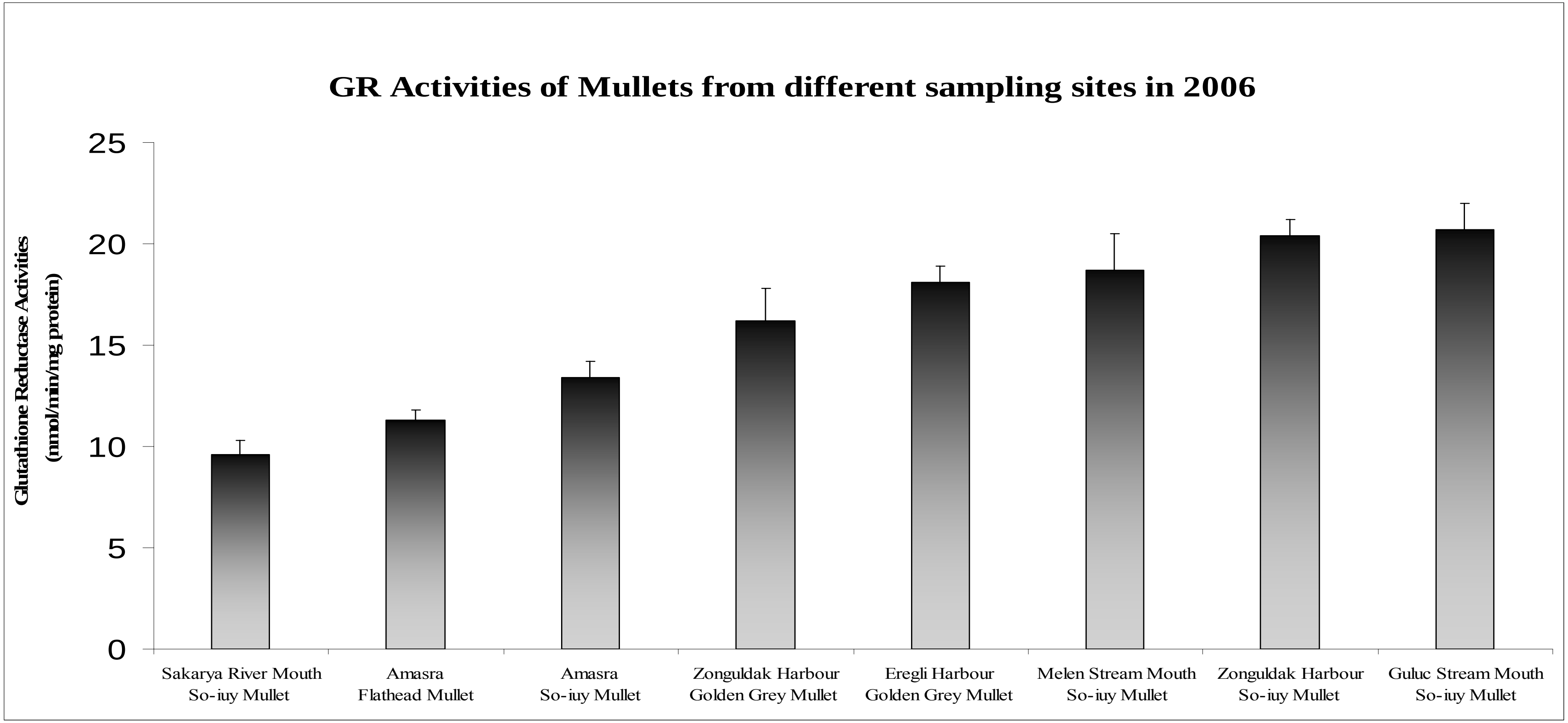

Figure 3.8. GR activities of so-iuy mullet, flathead mullet and golden grey mulet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Guluc Stream Mouth, Sakarya River Mouth, Melen Stream Mouth and Amasra. Fish were collected in August 2006.

The lowest GR activities were measured in so-iuy mullets and flathead mullets captured from Amasra. So-iuy mullets displayed 13.4±0.8 nmol/min/mg protein, n=3 activity. Flathead mullets displayed 11.3±0.5 nmol/min/mg protein, n=4 activity.

So-iuy mullets captured from Guluc Stream Mouth and Melen Stream Mouth had highly elevated GR activities which were 20,7±1,3 nmol/min/mg protein, n=11 and 18.7±1.8 nmol/min/mg protein, n=9, respectively (see Table 3.56 and 3.58). However, GR activities of so-iuy mullets caught from Sakarya River Mouth exhibited very low GR activity in this year which was 9.6±0.7 nmol/min/mg protein, n=10.

In 2006, so-iuy mullets captured from Zonguldak Harbour displayed highly elevated GR activities which was 20,4±0,8 nmol/min/mg protein, n=13 (see Table 3.53). The GR activities of golden grey mullets captured from Zonguldak Harbour was 16,2±1,6 nmol/min/mg protein, n=9 (see Table 3.54). There was no statistically significant difference between GR activities of different mullet species in Zonguldak Harbour.

In 2007, 19 mullets were caught from four different sites (Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra) of the West Black Sea Region of Turkey. Table 3.62, 3.63, 3.64 and 3.65 show cytosolic total GR activities obtained from the livers of individual fish caught from the four different sites along the West Black Sea Region of Turkey. All activities were measured in duplicates and sometimes in triplicates.

The average GR activities in the cytosolic fractions of liver of so-iuy mullet, flathead mullet and golden grey mullet caught from four different sites along a pollution gradient in the West Black Sea Region of Turkey in August 2007 were summarized in Table 3.66. GR activities were represented as mean ± standard error of the mean.

Figure 3.9 illustrates the graphical representation of GR activities with standard error of the mean. In 2007, the average GR activity from Eregli Harbour was higher compared to the other sites of the West Black Sea Region of Turkey.

Table 3.62. GR activities of flathead mullets (*Mugil cephalus*) caught from Zonguldak Harbour (August 2007).

Sample No	Average Activity (nmol/min/mg protein)
1	19.6
2	11.9
3	8.0
4	10.5
5	7.7
Average activity ± standard error of the mean n=number of fish liver	11.5±2.1 n=5

Table 3.63. GR activities of golden grey mullets (*Liza aurata*) caught from Eregli Harbour (August 2007).

Sample No	Average Activity (nmol/min/mg protein)
1	17.7
2	22.0
3	16.9
4	13.9
5	18.9
Average activity ± standard error of the mean n=number of fish liver	17.9±1.3 n=5

Table 3.64. GR activities of so-iuy mullets (*Mugil soiuy*) caught from Sakarya River Mouth (August 2007).

Sample No	Average Activity (nmol/min/mg protein)
1	12.4
2	12.4
3	10.9
4	9.3
Average activity ± standard error of the mean n=number of fish liver	11.3±0.8 n=4

Table 3.65. GR activities of golden grey mullets (*Liza aurata*) caught from Amasra (August 2007).

Sample No	Average Activity (nmol/min/mg protein)
1	17.0
2	9.5
3	9.2
4	13.0
5	7.3
Average activity ± standard error of the mean n=number of fish liver	11.2±1.7 n=5

Table 3.66. The average GR activities of liver cytosols of mullets caught from different sites of the West Black Sea Region of Turkey in August 2007.

Sampling Sites & Type of Mullet	Fish Species	Number of fish (n)	GR Activities (nmol/min/mg protein) Average activity± standard error of the mean
Zonguldak Harbour	*Mugil cephalus*	5	11.5±2.1
Eregli Harbour	*Liza aurata*	5	17.9±1.3
Sakarya River Mouth	*Mugil soiuy*	4	11.3±0.8
Amasra	*Liza aurata*	5	11.2±1.7

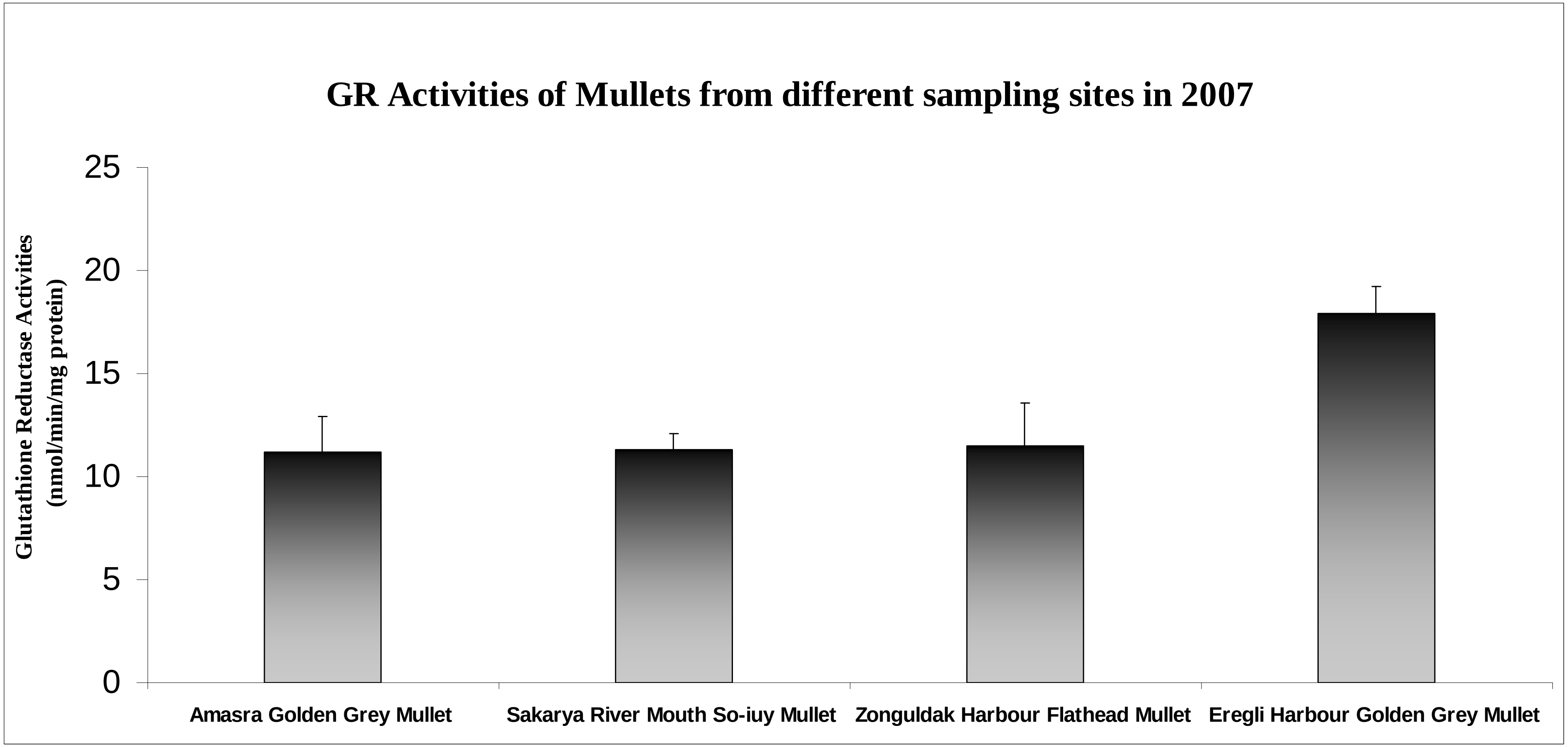

Figure 3.9. GR activities of so-iuy mullet, flathead mullet and golden grey mullet sampled from different sites: Zonguldak Harbour, Eregli Harbour, Sakarya River Mouth and Amasra. Fish were collected in August 2007.

The lowest liver cytosolic GR activity of golden grey mullets was obtained from Amasra (see Table 3.65). Golden grey mullets captured from Eregli Harbour had the highest GR activity (17,9±1,3 nmol/min/mg protein, n=5) (see Table 3.63). The GR activity of flathead mullets captured from Zonguldak Harbour was 11.5±2 nmol/min/mg protein, n=5 (see Table 3.62). The GR activity of so-iuy mullets caught from Sakarya River Mouth was 11.3±0.8 nmol/min/mg protein, n=4 (see Table 3.64).

CHAPTER 4

DISCUSSION

The number of chemicals produced and released into the environment has been continuously increasing in industrialized countries. The ultimate sink for many of the chemical pollutants is the marine environment (Hahn and Stegeman, 1994). The marine environment can neutralize some chemicals. However, many of the chemicals introduced into the environment are highly persistent compounds such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), pesticides, alkyltin compounds and heavy metals (i.e. Pb, Hg, Cd etc.). Because of their chemical stability and persistency, they tend to accumulate in the different compartment of the marine environment. Pollutants bioaccumulated in the organism, first cause changes at the molecular and cellular level. This may lead to adverse effect in the organism which in turn may cause changes at the population and the community level in the years to come.

Contaminants usually appear in the environment as very complex mixtures that can cause interactive effects on the biota impossible to evaluate only by means of chemical analyses. In this sense, biomarkers offer an integrated measure on effects of pollutant exposure. Changes in the levels of phase II enzymes glutathione S-transferease and antioxidant enzymes have been proposed as biomarkers of contaminant in a variety of marine organisms, including invertebrates, mussels, and fish (Regoli and Principato, 1995; Livingstone, 2001; Regoli et al., 2004; Orbea et al., 2002; Regoli et al., 2002; Şen and Kirikbakan, 2004). Glutathione-S-transferases (GSTs) catalyse the conjugation of electrophilic compounds (or phase I metabolites) with glutathione (GSH) and are one of the main enzymes involved in xenobiotic phase II metabolism. GSTs detoxify a number of environmental carcinogens and epoxide intermediates (Gallagher et al., 1996). Significant alterations in fish GST activities have been reported in some field studies as a result of PAHs and halogenated xenobiotic exposure (Vigano et al., 1995; van der Oost et al., 2003). In addition, GST assay may provide a useful tool for biomonitoring of oxidative stress (Di Giulio et al., 1993). Detoxification enzymes, especially GST helps in eliminating reactive compounds by forming their conjugates with glutathione and subsequently eliminating them as mercapturic acid, thereby protecting cells against ROS induced damage (Rodriguez-Ariza et al., 1991). Since the induction of antioxidants represents a cellular defense mechanism to counteract toxicity of reactive oxygen species (ROS), they have been extensively used in several field studies to assess the extent of pollution in rivers, lakes and coastal waters (Goksoyr, 1995). Catalases (CATs) are hematin-containing enzymes that facilitate the removal of hydrogen peroxide (H_2O_2), which is metabolised to molecular oxygen (O_2) and water. Glutathione reductase (GR) activity maintains the homeostasis of reduced (GSH) and oxidized (GSSG) glutathione under oxidative stress conditions (Winston and Di Giulio, 1991). It has been shown that in laboratory experiments with molluscs and fish, benzo(a)pyrene (B(a)P) as well as other polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) cause significant alterations in the activities of antioxidant enzymes (Livingstone, 1998).

The Black Sea is under the risk of chemical pollution as a result of industrial, anthropogenic, and agricultural activities. It has been reported that the main sources of pollution in the Black Sea are the rivers which flow into region and coastal cities (Mee, 1992; Tuncer et al., 1998). The major rivers flowing into the Black Sea and their discharges are Danube, Dnepr, Dniesta, Don and Kuban. In addition to these, a large number of smaller rivers and streams flow into the Black Sea along the coast of Turkey. The Sakarya River, from its source in the West Anatolia, flows through many industrial and agricultural areas including Eskişehir and Adapazarı (Sakarya) and drains to the Black Sea. It has been well known that the Sakarya River carries many pollutants to the Black Sea from inner regions of Turkey (Tuncer et al., 1998; Barlas, 1999). Industrial areas found in Duzce and Adapazarı drain their discharges into Efteni Lake. The Melen Stream originates from this lake and flows through many industrial and agricultural areas. The Guluc Stream is very close the Eregli Harbour and Eregli Iron and Steel Factory. It has been reported that this small stream was highly polluted primarily by domestic waste discharges from Eregli and villages around the stream. It also flows through many agricultural areas. Tuncer and coworkers (1998) have reported for this stream that if no action is taken to stop domestic waste discharges into these small streams, they will soon become open sewers. Zonguldak and Eregli, with increasing population, shipping activities, uncontrolled discharges from industries and coastal settlements, are among these polluted local regions. Zonguldak is an important coal mining area in Turkey. Zonguldak suffers from particulate discharges from the thermal power plant and coal processing wastes from the streams, resulting in permanently elevated turbidity levels along the coast of the city (Tuncer et al., 1998).

In this study our aim was to determine and biomonitor the level of pollution by measuring phase II enzyme; glutathione S-transferase and antioxidant enzymes; catalase and glutathione reductase in the liver cytosol of mullet in different sites of the West Black Sea Region of Turkey. Mullet is chosen as a test organism. Because it is resistant and tolerant to pollutants and various environmental conditions. It can live in polluted areas (Balık et al., 1992; Arınç and Şen, 1999). It is also consumed in large quantities in Turkey. Liver plays an important role in vital functions of basic metabolism and it is the major organ of accumulation, biotransformation, and excretion of contaminants in fish. It is commonly used for monitoring environmental exposure of fish to contaminants (Arınç and Şen, 1999; Arinç et al., 2000; Ferreira et al., 2004).

In this study 150 fish samples were collected from the different sites of the West Black Sea Coast of Turkey. In all samples, glutathione S-transferase, catalase and glutathione reductase activities were measured. Figure 4.1 illustrates total GST activities of mullets caught from different sites of the West Black Sea Coast of Turkey in 2005, 2006 and 2007. The lowest total GST activities were measured in fish samples (so-iuy mullet, flathead mullet and golden grey mullet) captured from Amasra. There was no statistical difference between the mullet species caught from Amasra. Amasra is the touristic place in the West Black Sea Region of Turkey. Since there were no industrial settlements and agricultural activities in this small town, this place appeared to be good reference site. Kefken is another touristic place in this region of Turkey. Kefken was considered as reference site at the begining of this study. However, only one fish sample could be caught in this region and this place was not considered in the statistical analysis.

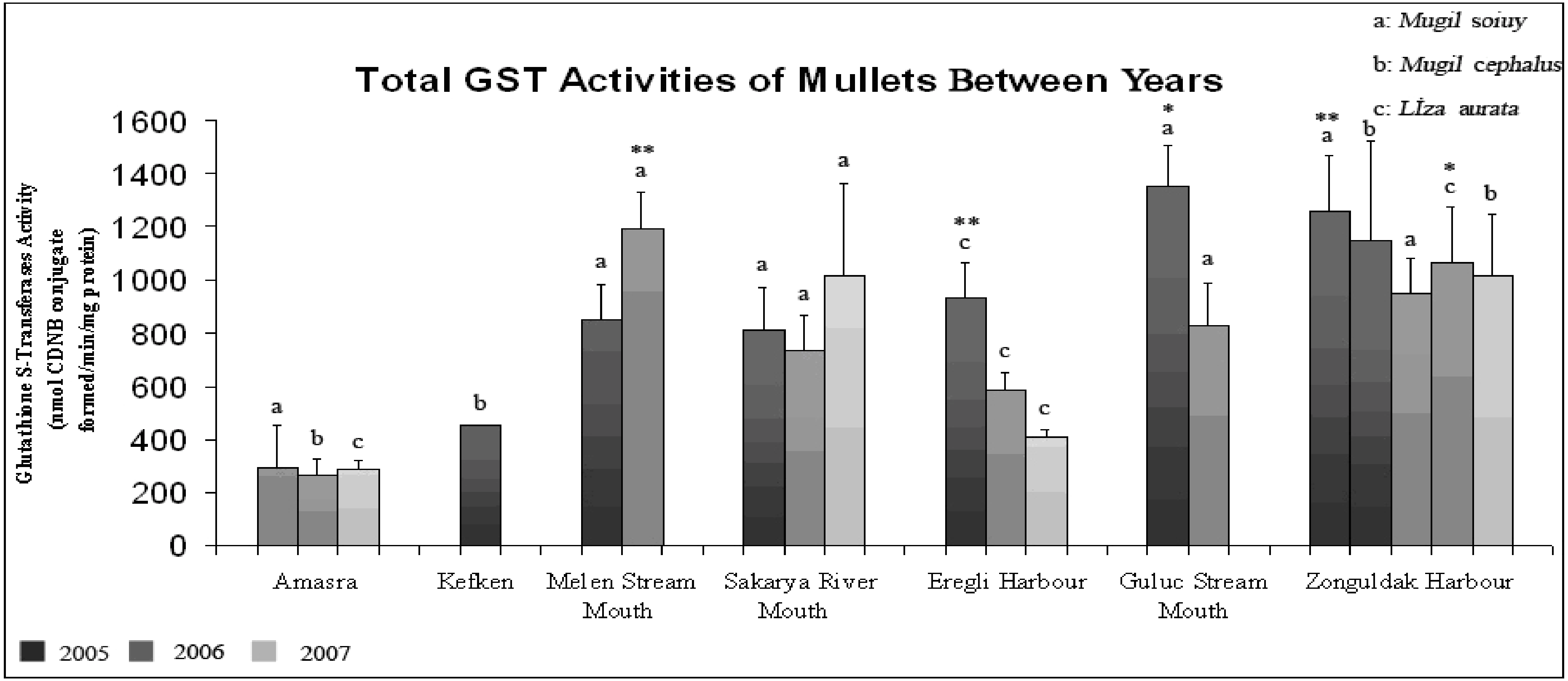

Figure 4.1. The total GST activities of mullets sampled from different sites sites of the West Black Sea Region of Turkey in 2005, 2006 and 2007.

** Significantly different from the lowest values (reference site) with P<0.05
* Significantly different from the lowest values (reference site) with P<0.01

When total GST activities were considered, the highest activities were detected in so-iuy mullets captured from Zonguldak Harbour which was 1260±207 nmol CDNB conjugate formed/min/mg protein (n=7) in 2005. The highest total GST activity of flathead mullets was detected in Zonguldak Harbour which was 1147±376 nmol CDNB conjugate formed/min/mg protein (n=2) in 2005. These value was 4.4 times higher than those obtained from the reference site. There were no statistical differences between the mullet species in total GST activities. The highest GST activity of golden grey mullets was detected in Zonguldak Harbour which was 1067±208 nmol CDNB conjugate formed/min/mg protein (n=9) in 2006. This was 4 times higher than those obtained from the reference site. In 2007, the highest total GST activities were measured in flathead mullets caught from Zonguldak Harbour (1017±231 CDNB conjugate formed/min/mg protein (n=5)) which was 4 times higher than total GST activities of flathead mullets from reference site. There was no statistical difference in total GST activities in the fish captured from Zonguldak Habor between the years. The highly elevated GST activities in Zonguldak Harbour indicate the presence of organic pollutant chemicals and ROS in this Region of Turkey.

So-iuy mullets caught from Guluc Stream Mouth displayed significantly high total GST activities in 2005 and 2006 which were 4.7 and 3 times higher than total GST activities of so-iuy mullets from the reference site, respectively. Similar to Guluc Stream Mouth, so-iuy mullets caught from Melen Stream Mouth displayed highly elevated total GST activities in 2005 and 2006 which were 3 and 4 times higher than total GST activities of reference site, respectively. However, mullet samples couldn’t be caught both of these sites in 2007.

So-iuy mullets caught from Sakarya River Mouth displayed highly elevated total GST activities in 2005, 2006, and 2007 which were 2.8, 2.6 and 3.5 times higher than the reference site, respectively, indicating the presence of total GST activity inducer chemicals in this site.

There were no statistical differences between the sampling years in total GST activities in the fish samples caught from Zonguldak Harbour, Sakarya River Mouth, Melen Stream Mouth and Guluc Stream Mouth. However, golden grey mullets caught from Eregli Harbour displayed highly elevated GST activity in 2005 which was 3 times higher than the total GST activities of golden grey mullets caught from reference site. Total GST activity in fish caught from Eregli Harbour was 2 times higher in 2006 and 1.4 times higher in 2007 than the reference site. Thus, total GST activities in fish caught from Eregli Harbour decreases in the following sampling years, indicating a decrease in the levels of organic pollutants that cause an induction in GST activity in this region of Turkey.

Figure 4.2 illustrates CAT activities of mullets caught from different sites of the West Black Sea Coast of Turkey in 2005, 2006 and 2007. The lowest liver cytosolic CAT activities of so-iuy mullets, flathead mullets and golden grey mullets were obtained from Amasra. This region was considered as reference site.

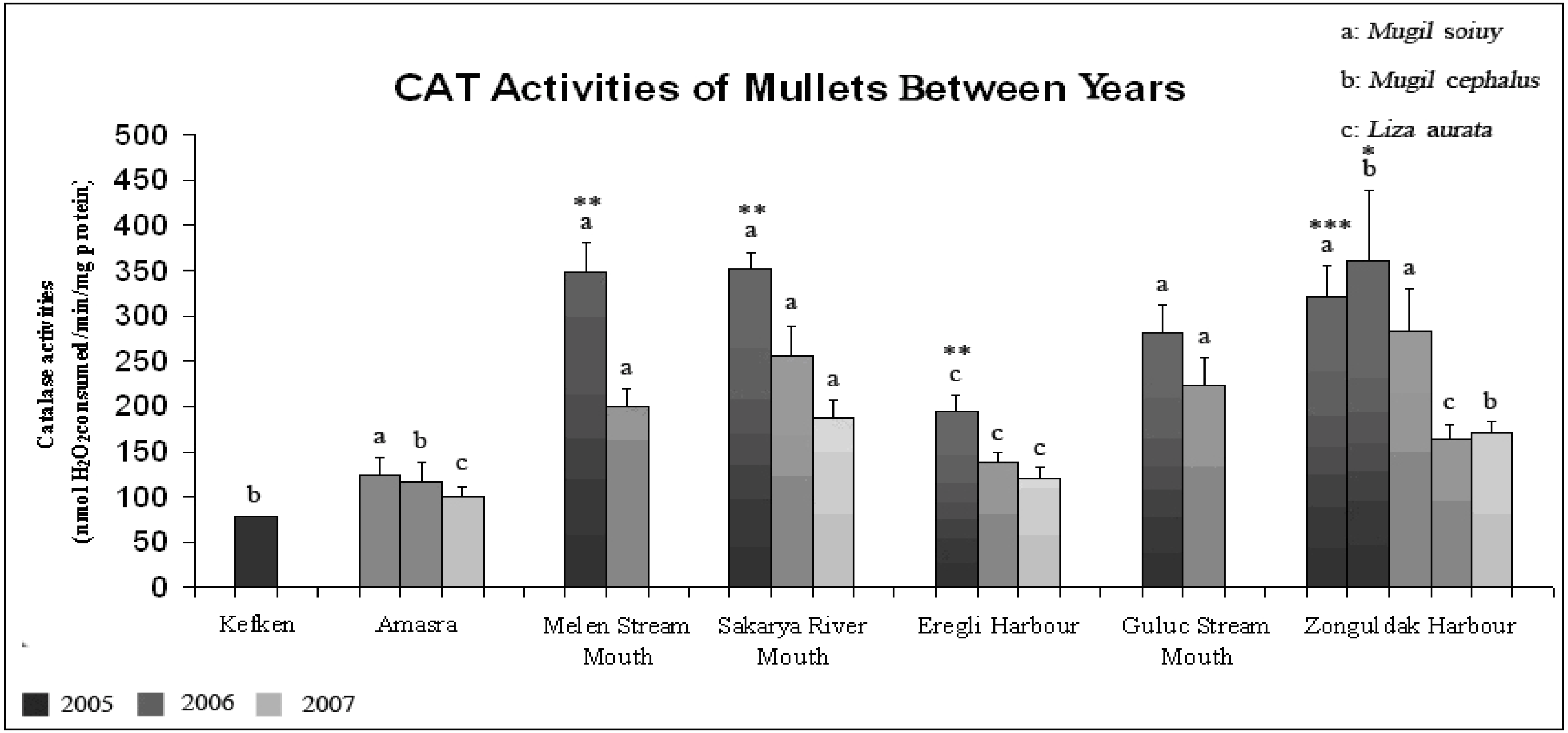

Figure 4.2. The CAT activities of mullets sampled from different sites sites of the West Black Sea Region of Turkey in 2005, 2006 and 2007.

*** Significantly different from the lowest values (reference site) with P<0.05
** Significantly different from the lowest values (reference site) with P<0.01
* Significantly different from the lowest values (reference site) with P<0.001

In 2005, so-iuy mullets sampled from Zonguldak Harbour, Sakarya River Mouth, Guluc Stream Mouth and Melen Stream Mouth displayed highly elevated CAT activities with respect to reference site. The highest CAT activities were detected in so-iuy mullets captured from Sakarya River Mouth, Melen Stream Mouth and Zonguldak Harbour which were 352±17 nmol H_2O_2 consumed/min/mg protein (n=10), 349±32 nmol H_2O_2 consumed/min/mg protein (n=11) and 322±34 nmol H_2O_2 consumed/min/mg protein (n=7) in 2005. These results were about 3 times higher than those obtained from the reference site. So-iuy mullets sampled from Guluc Stream Mouth also displayed highly elevated CAT activity which was 2 times higher than the reference site. Similarly, the highest CAT activity of flathead mullets was detected in Zonguldak Harbour which was 362±77 nmol H_2O_2 consumed/min/mg protein (n=2) in 2005. This result was 3 times higher than those obtained from the reference site. In addition, there was no statistical difference in CAT activities of different mullet species captured from Zonguldak Harbour. These higher CAT activities in the fish samples from Zonguldak Harbour, Sakarya River Mouth, Guluc Stream Mouth and Melen Stream Mouth indicate that these sites were highly contaminated with CAT inducing chemicals in 2005. Although fish samples caught in 2005 displayed highly elevated CAT activities at different sites of the West Black Sea Coast of Turkey, CAT activities in mullets decreased in the following sampling years. In 2006, CAT activities of so-iuy mullets from Sakarya River Mouth, Guluc Stream Mouth, Melen Stream Mouth and Zonguldak Harbour were about 2 times higher than those obtained from the reference site. In 2007, CAT activity of so-iuy mullets from Sakarya River Mouth was 1.5 times higher than the reference site. CAT activity of flathead mullets from Zonguldak Harbour was 1.4 times higher than the reference site. The highest CAT activities of golden grey mullets were detected in Eregli Harbour in 2005 (195±18 nmol H_2O_2 consumed/min/mg protein, n=16) which was 2 times higher than the reference site. The CAT activity golden grey mullets caught from Eregli Harbour was 1.4 times higher than the reference site in 2006. CAT activities of golden grey mullets from Eregli Harbour were 1.2 times higher than CAT activities of golden grey mullets from reference site in 2007. CAT activity results clearly indicate that the amount of chemical pollutants that trigger CAT induction gradually decreases in these regions.

Figure 4.3 illustrates GR activities of mullets caught from different sites of the West Black Sea Coast of Turkey in 2005, 2006 and 2007. The lowest liver cytosolic GR activities of so-iuy mullets, flathead mullets and golden grey mullets were obtained from Amasra. This region was considered as reference site. In 2005, so-iuy mullets captured from Melen Stream Mouth had the highest GR activity (23.1±1.9 nmol/min/mg protein, n=11) among the other sampling sites which was 1.7 times higher than the GR activities of so-iuy mullets from the reference site. The GR activity of so-iuy mullets captured from Guluc Stream Mouth was 20.8±0.9 nmol/min/mg protein, n=13 which was 1.6 times higher than the reference site. The GR activities of so-iuy mullets captured from Sakarya River was 19.7±1.7 nmol/min/mg protein, n=10 which was 1.5 times higher than the reference site. This result clearly indicates that the rivers and streams in the West Black Sea Region of Turkey bring some GR inducer pollutant chemicals from the inner sites of Anatolia and West Black Sea Region.

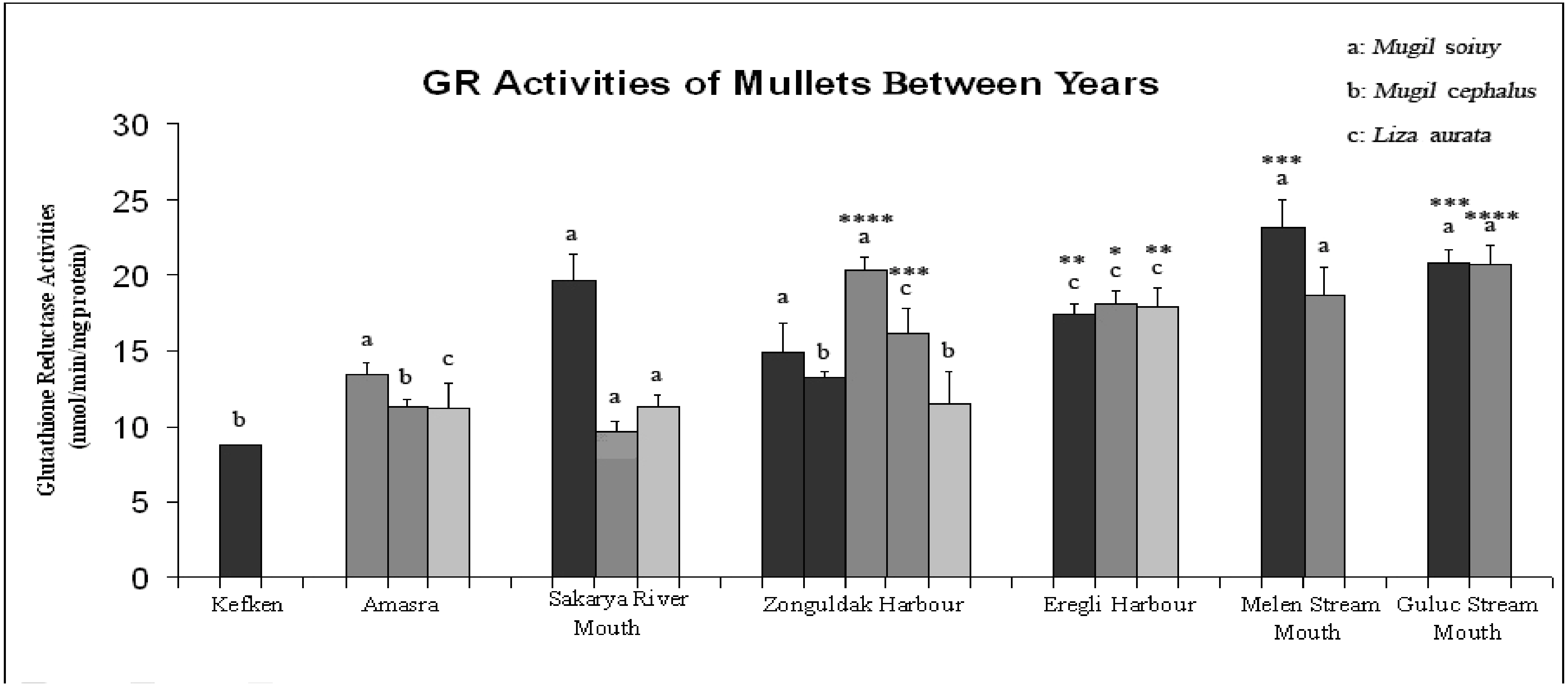

Figure 4.3. The GR activities of mullets sampled from different sites sites of the West Black Sea Region of Turkey in 2005, 2006 and 2007.

**** Significantly different from the lowest values (reference site) with P<0.06
*** Significantly different from the lowest values (reference site) with P<0.05
** Significantly different from the lowest values (reference site) with P<0.01
* Significantly different from the lowest values (reference site) with P<0.001

The GR activity of golden grey mullets captured from Eregli Harbour was 17.4±0.7 nmol/min/mg protein, n=16 which was 1.6 times higher than the reference site. Flathead mullets and so-iuy mullets captured from Zonguldak Harbour had the GR activity of 13.3±0.3 nmol/min/mg protein, n=2 and 14.9±1.9 nmol/min/mg protein, n=7, respectively. In 2006, so-iuy mullets captured from Guluc Stream Mouth had the highest GR activities (20.7±1.3 nmol/min/mg protein, n=11) among the other sampling sites which was 1.5 times higher than the reference site. The GR activities of so-iuy mullets and golden grey mullets captured from Zonguldak Harbour was 20.4±0.8 nmol/min/mg protein, n=13 and 16.2±1.6 nmol/min/mg protein, n=9, respectively which were 1.5 and 1.4 times higher than the reference site. The GR activity results of 2006 indicated that Zonguldak harbour was more polluted with GR inducer chemicals in 2006 than 2005. There were no statistical differences in the GR activities of fish captured from Eregli Harbour between 2005 and 2006. In 2006, golden grey mullets captured from Eregli Harbour had elevated GR activity (18.1±0.8 nmol/min/mg protein, n=12) which was 1.6 times higher than those obtained from the reference site. So-iuy mullets captured from Melen Stream Mouth had also high GR activity compared to other sampling sites (18.7±1.8 nmol/min/mg protein, n=9) which was 1.4 times higher than the reference site. The GR activities of fish samples caught from Guluc Stream Mouth and Melen Stream Mouth were not significantly changed between 2005 and 2006. However, we couldn't find fish samples in 2007 from these sites.

In 2007, golden grey mullets captured from Eregli Harbour had the highest GR activity (17.9±1.3 nmol /min/mg protein, n=5) which was 1.6 times higher than the reference site. The GR activity of flathead mullets captured from Zonguldak

Harbour was 11.5±2.1 nmol/min/mg protein, n=5 and so-iuy mullets captured from Sakarya River Mouth was 11.3±1.7 nmol/min/mg protein, n=4. These values were not significantly different than the reference site.

When the GR activities of fish samples caught in different years were compared, there was no statistically significant difference in the fish samples caught from Eregli Harbour. This result indicates that the level of GR inducing chemicals didn't change in this region. Although, GR activities of fish caught from Sakarya River Mouth was 1.5 times higher than the reference site in 2005, GR activities decreased in the following years indicating a decrease in the GR inducing pollutants in this region.

The presence of chemical pollutants in the West Black Sea Region of Turkey has been also reported in different studies. In these studies, chemical analysis were performed to measure the levels of the specific pollutants. Tuncer and coworkers (1998) have reported that the Sakarya River and Guluc Stream are important source for Cd, Cu, Pb and Zn in the Black Sea. In addition to inorganic pollutants, concentrations of pesticides and PCBs, including lindane, heptachlor, epoxy, aldrin, dieldrin, endrin, DDE, DDD and DDT have been also measured in different studies (Tuncer et al., 1998; Bakan and Ariman, 2004) in the Black Sea. The use of DDT in Turkey has been banned for the last 30 years. Despite this restriction, Tuncer and coworkers have shown that DDT is present in Turkish rivers, streams and domestic and industrial discharges, which indicates their illegal use. However, the studies clearly demonstrated that chronic exposure of fish to pollutants impairs growth and alters lipid metabolism in fish (Levesque et al., 2002). The gonads size and hormone levels are also altered in the fish from polluted waters (Munkittrick et al., 1998; Levesque et al., 2002). Pollution of the sea threats both the health status of human and existence of the economically important fish species. The Black Sea is the important source of many fish species. However, the numbers of fish species has decreased in the Black Sea. In this study, we also observed this decrease, too. We hardly found fish samples in 2007. Overfishing may be considered as one of the reasons of this decrease. However, the other important reason of this decrease must be the presence of these pollutants in the Black Sea.

The results of this study clearly indicated the presence of organic pollutants and reactive oxygen species in some sites of the West Black Sea Coast of Turkey. It is clear that Zonguldak Harbour, Eregli Harbour, Guluc Stream, Sakarya River and Melen Stream have tendency to create local pollution in the West Black Sea Region of Turkey. It is well known that millions of litres of untreated sewage run into the rivers and harbour in the West Black Sea Region of Turkey. Recently, the surface sediment of the Zonguldak Harbour has been collected and the excavated sediment has been dumped into the open sea in the Black Sea. It is clear that this sediment will create pollution in the open sea in the years to come. As a result of sea pollution, economically important fish species are under the risk. Furthermore, the consumption of fish from contaminated areas may produce serious health effects in humans.

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