Mar. Sci. Tech. Bull. (2014) 3(2):27-32
ISSN: 2147-9666

MARINE SCIENCE AND TECHNOLOGY BULLETIN

Use of oxidative stress biomarkers in three Crustacean species for the assessment of water pollution in Kocabas Stream (Canakkale, Turkey).

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ARTICLE INFO

Article history:

Received: 18.11.2014
Received in revised form: 02.12.2014
Accepted : 03.12.2014
Available online : 31.12.2014

Keywords:

Biomarker
Water Pollution
Kocabaş Stream
Can
Biga
Yenice

ABSTRACT

Freshwater invertebrates are commonly used in biological monitoring, but research of biomarkers at biochemical level is still at the early stage of development. Biomarkers in aquatic species are regarded as important for detecting stressor components, such as presence of pollutants and changes in environmental factors (pH, temperature, dissolved oxygen, etc.). In this study, to evaluate the pollution in Kocabaş Stream, we used specific biomarkers (GSH, TBARS and Na^+, K^+-ATPase) in homogenates of whole organisms. Macro invertebrate samples were sampled from three locations receiving increasing levels of urban and industrial waste water discharges along the Kocabaş Stream (Çanakkale-Turkey) in April and July 2013. Locations were selected to include the aquatic communities in poor and good ecological state depending on the measured physico-chemical water parameters and the analysis of benthic macro invertebrate communities. According to the results of the study, statistically important increases in TBARS and GSH level of *Asellus aquaticus*, *Gammarus pulex pulex* and *Potamon ibericum* collected from the stations in Çan and Biga were observed compared to the individuals collected from Yenice. These results indicated that presence of serious pollutants in Kocabaş Stream especially in Çan region also occurred in the physiology of macro invertebrate organisms living in the region.

Introduction

In recent years, issue of riverine ecosystem pollution has attracted the attention of many researchers all over the world. Several anthropogenic processes (industrialization, agricultural applications, domestic waste discharge, etc.) have caused to the contamination of inland water (Farombi et al. 2007). Therefore the concentrations of pollutant such as metals, pesticides, and nutrients in excess can cause negative impacts such as loss of oxygen, fish deaths, loss of biodiversity, as well as various problems on human health (Akbulut et al. 2010).

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Benthic macro-invertebrates are essential components of freshwater environments and play a major role in the food chain as the food resource for fish. Almost all aquatic macro-invertebrates accumulate the contaminant within their bodies in different amounts according to the polluter concentration in waters. Many researches have emphasized the use of observed changes in tissues of these species to define the effects of toxic materials such as heavy metals (Khan and Nugegoda, 2003; Barrento et al. 2009; Selvi et al. 2012). These organisms are often employed for biological monitoring since they are good indicators of the aquatic riverine ecosystems by the use of biomarkers. Biomarkers which are used for determining stress components (pH, dissolved oxygen, salinity, temperature, etc.) in aquatic environments can be described as a variation that measurable in tissues/body fluids of macro-invertebrates caused by exposure to contamination (Depledge and Fossi, 1994; Berra et al. 2003).

Crustaceans are one of the groups of macro-invertebrates exhibiting a wide distribution, inhabiting all types of aquatic habitats. Biomarkers are quite helpful as to form a direct response in the crustaceans to aquatic pollution (Hyne and Maher, 2003). Although very few studies have examined the accumulated metal effects on enzymatic and biochemical activity in macro-invertebrates in the rivers of Çanakkale, or assessed their potential as biomarkers under field conditions (Kaya et al. 2014a; 2014b), none of them were on crustaceans. In this study, biomarkers were used to study the physiological effects on three crustacean organisms (*Gammarus pulex pulex, Asellus aquaticus* and *Potamon ibericum*) due to water pollution in Kocabaş Stream, of which some parts are subjected to domestic, industrial and agricultural pollution.

Material and methods

Kocabaş Stream and Sampling Stations:

Kocabaş Stream arises from Ida Mountain near Yenice and flows into the south area of Marmara Sea. It passes through Yenice, Çan, Biga and Karabiga counties of Çanakkale. The other known name of this stream is Biga Stream. Its length is 80 km and its discharge is about 15-20 m^3/sn. The recorded maximum discharge was 1345 m^3. This study was carried out at three sampling stations on Kocabaş Stream in April and July 2012 (Figure 1).

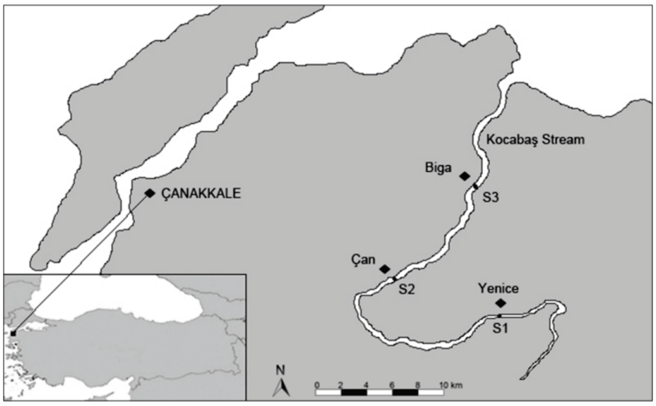

Figure 1. Kocabaş Stream and sampling stations.

First station places near Yenice with height of 266 meters, locating 39º 93´ 86˝ N and 27º 23´ 22˝ E. The water is clean, clear and has got almost constant water regime throughout the year. In the second station, there are settlement, agricultural and industrial areas that passes through Çan town center. The altitude is 36 meter and its coordinates are 40º 22´ 99˝ N and 27º 24´ 30˝ E. The sampling site selected as third station passes through Biga centrum. It is located at 40º 05´ 66˝ N, 27º 12´ 70˝ E and the altitude of the area is 13 meter. There are discharges of domestic, industrial and agricultural wastes into some parts of this area.

Physico-Chemical Analysis of Water

The temperature, pH, electrical conductivity, salinity and dissolved oxygen of each water sample were measured in situ by a pH meter and oxygen meter, respectively (Table 1). In laboratory, the duplicate water samples of about 1000 ml of each group sampled from three sampling sites were filtered through poly carbonate filters (0.45 µm pore size) and the samples were divided into two parts. The first part was used for analysis of physico-chemical parameters, while the other part was used for metal analysis after the addition of 2 ml of concentrated HNO_3 to the water samples.

All water samples were analyzed for different physico-chemical parameters within 48h, COD determined on the same sampling day while BOD have made immediately to avoid no changes in bacterial concentration. The BOD was determined by the Winkler azide method and COD by the dichromate reflux method (Anonymous, 1998). NO_3^- was carried out by Strickland and Parsons (1972).

Metal Analysis (Water and Organisms)

Organism's tissues were dried at the incubator set to 70°C for 24 hours after measuring the wet weight. Afterward, dry weights of samples were weighted. Then, samples were burned over a hot-plate set to 70°C for one hour, following the addition of 5 ml HNO_3. After the samples were burned homogenously and cooled, they were filtered in a 0.45 µm syringe and diluted to 20 ml with distilled water (Smith et al. 2007). The metal analysis in water and organisms were measured with ICP-OES Varian Liberty Sequential.

Total Glutathione (GSH)

Total glutathione (GSH) levels were also carried out according to Owens and Belcher (1965). Their method was started by the addition of 20 µl of NADPH, with changes in absorbance at 412 nm (Thermo Multiscan Go, micro plate reader) recorded over 10 min, and total GSH (µmol g^{-1} wet weight tissue) determined using the standard calibration curve (Smith et al. 2007).

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS assay was performed according to Camejo et al. (1998). Their absorbances were measured (in triplicate) at 530 nm in the microplate reader (Thermo Multiscan Go) against standards (0.5–25 nmol mL- 1,1,3,3-tetraethoxypropane). All data from the trials were computed per mg of cell protein (Bradford, 1976).

Statistical Analysis

The data obtained from biomarker and metal analysis of Crustaceans and water quality parameters were analyzed with One-way ANOVA by using the Minitab-User Guide program. Duncan multiple comparison test was used to determine differences of stations. A value of $P < 0.05$ (95% confidence interval) was considered to be significant in these statistical comparisons (Logan, 2010).

Results

Water quality parameters were measured in April and July 2012 at the sampling areas were presented in Table 2. The results of water quality parameters in Kocabaş Stream

Table 1. Water quality parameters, their units and methods of analysis.

Data	Abbreviations	Units	Analytical Methods
Temperature	T	°C	Oxygen meter (YSI 100), in situ
pH	pH	Ph unit	pH meter (YSI), in situ
Dissolved Oxygen	DO	mg L^{-1}	Oxygen meter (YSI 100), in situ
Phosphate	PO_4^{-3}	mg L^{-1}	Strickland and Parsons (1972)
Nitrate	NO_3^-	mg L^{-1}	Strickland and Parsons (1972)
Metal Analysis (Water and Organisms)			ICP-OES Varian Liberty Sequential
Chemical Oxygen Demand	COD	mg L^{-1}	Anonymous (1998)
Biological Oxygen Demand	BOD	mg L^{-1}	Anonymous (1998)
Total Suspended Solids	TSS	mg L^{-1}	Gravimetric analysis
Glutathione	GSH	nmol/mg	Owens and Belcher (1965)
Thiobarbituric Acid Reactive Substances (TBARS)	TBARS	nM/mg	Camejo et al. (1998)

showed no significant differences among the stations, in temperature and salinity, while there were significant differences in terms of pH, EC, BOD, COD, phosphate, nitrate and TSS levels ($P > 0.05$).

It was concluded that Biga station was the second polluted water after Çan station. We estimated that the resource of pollution can be domestic water of Biga city, villages and agricultural runoff. Therefore, besides continual monitoring of the study area additional measures have to be taken for preventing pollution.

According to the results of the some heavy metal concentrations (Fe, Cu, Zn, Pb, Cd) in on the samples there were no statistical differences among the mean values of Cd and Mn (April-2012) of three stations from Kocabaş Stream (Table 3). On the other hand, there were significant differences among the means of Fe, Cu, Zn and Pb (April and July 2012) ($P < 0.05$).

The metal concentrations of *Gammarus pulex pulex* which were collected from Kocabaş Stream are given in Table 5. The concentrations of Zn, Fe, Cu, and Pb in *G. pulex pulex* were observed to be different in all stations and reached the maximum level in station Çan ($P < 0.05$).

The metal concentrations of *Potamon ibericum* which were sampled from Kocabaş Stream are shown in Table 6.

Table 2. Water quality parameters (mean±SE) of three stations in Kocabaş Stream.

Date	**Parameters**	**Yenice (S1)**	**Çan (S2)**	**Biga (S3)**
April	T (°C)	11.46 ± 0.09	13.38 ±0.09	12.50 ± 0.13
July		23.14 ± 0.07	26.72 ±0.20	25.63 ± 0.20
April	Ph	7.91 ± 0.03	8.07 ± 0.03*	8.03 ± 0.04
July		8.05 ± 0.02*	7.85 ± 0.05	8.15 ± 0.03*
April	EC (µS cm^{-1})	217 ± 6.12	569 ± 24.11*	544 ± 24.86*
July		483 ± 7.54	980 ± 14.64*	762 ± 7.00*
April	S (‰)	0.11 ± 0.01	0.26 ± 0.01*	0.17 ± 0.01
July		0.17 ± 0.01	0.33 ± 0.01*	0.21 ± 0.01
April	DO (mg L^{-1})	9.78 ± 0.08	8.18 ± 0.03	9.25 ± 0.07
July		8.50 ± 0.20*	7.59 ± 0.24	8.34 ± 0.16*
April	PO_4^- (mg L^{-1})	0.04 ± 0.01	0.58 ± 0.04*	0.09 ± 0.01
July		0.07 ± 0.01	0.84 ± 0.05*	0.13 ± 0.01
April	NO_3^- (mg L^{-1})	0.16 ± 0.02	4.45 ± 0.16*	2.54 ± 0.13
July		0.24 ± 0.03	7.27 ± 0.10*	5.84 ± 0.19
April	COD (mg L^{-1})	3.16 ± 0.05	15.28 ± 1.35*	6.41 ± 0.13
July		5.65 ± 0.20	16.59 ± 1.96*	8.52 ± 0.19
April	BOD (mg L^{-1})	2.55 ± 0.25	19.29 ± 0.33*	12.22 ± 0.50
July		4.73 ± 0.10	25.93 ± 0.33*	17.04 ± 0.74
April	TSS (mg L^{-1})	14.98 ± 1.23*	10.30 ± 0.29	6.80 ± 0.18
July		8.65 ± 1.12	7.30 ± 0.25	6.13 ± 0.55

Table 3. Heavy metal concentrations in water (µg L^{-1}) in Kocabaş Stream.

Date	Heavy Metals	Yenice (S1)	Çan (S2)	Biga (S3)
April	Fe	28.56 ± 0.67	83.62 ± 3.15*	18.49 ± 0.43
July		30.79 ± 0.12	87.97 ± 3.15*	19.75 ± 0.43
April	Cu	1.37 ± 0.09	4.10 ± 0.22*	1.66 ± 0.07
July		3.57 ± 0.05	8.44 ± 0.14*	2.90 ± 0.03
April	Zn	10.68 ± 0.41	91.47 ± 1.09*	35.85 ± 2.47*
July		12.89 ± 0.32	96.03 ± 0.78*	37.09 ± 1.85*
April	Pb	n.d.	3.42 ± 0.14*	n.d.
July		n.d.	7.74 ± 0.22*	n.d.
April	Cd	0.09 ± 0.01	0.17 ± 0.01	0.11 ± 0.01
July		2.31 ± 0.01	4.50 ± 0.01*	1.37 ± 0.05
April	Mn	2.04 ± 0.03	5.88 ± 0.11	3.44 ± 0.15
July		4.24 ± 0.08	9.99 ± 0.17*	4.65 ± 0.06

nd: not detected

Table 4. Heavy metal concentrations (µg g^{-1}) in *Asellus aquaticus*

Date	Metal	Yenice	Çan	Biga
April	Fe	18.46 ± 0.27	41.38 ± 0.44*	22.18 ± 0.32
July		20.31 ± 0.69	42.34 ± 0.37*	23.72 ± 0.67
April	Cu	1.95 ± 0.06	9.19 ± 0.12*	4.05 ± 0.06
July		2.05 ± 0.06	12.91 ± 0.23*	5.21 ± 0.12
April	Zn	11.11 ± 0.45	33.47 ± 0.88*	19.87 ± 0.31*
July		12.31 ± 0.51	34.17 ± 0.87*	21.18 ± 0.51*
April	Pb	0.14 ± 0.02	1.07 ± 0.04*	0.52 ± 0.04
July		0.38 ± 0.08	1.23 ± 0.06*	0.85 ± 0.03
April	Cd	0.02 ± 0.01	0.23 ± 0.03*	0.04 ± 0.01
July		0.04 ± 0.01	0.27 ± 0.06*	0.07 ± 0.01

Table 5. Heavy metal concentrations (µg g^{-1}) in *Gammarus pulex pulex*

Date	Metal	Yenice	Çan	Biga
April	Fe	25.64 ± 0.36	44.81 ± 0.68*	35.85 ± 0.24*
July		32.75 ± 0.34	57.98 ± 0.89*	40.41 ± 0.56
April	Cu	2.97 ± 0.21	9.69 ± 0.32*	5.91 ± 0.14
July		3.61 ± 0.04	11.77 ± 0.22*	7.65 ± 0.09
April	Zn	16.38 ± 0.43	41.04 ± 0.65*	26.22 ± 0.38
July		26.01 ± 0.19	60.81 ± 0.31*	41.94 ± 1.37*
April	Pb	0.75 ± 0.05	2.58 ± 0.041*	1.12 ± 0.11
July		1.29 ± 0.02	5.78 ± 0.11*	2.96 ± 0.09
April	Cd	0.08 ± 0.01	0.37 ± 0.05*	0.25 ± 0.05*
July		1.19 ± 0.11	0.64 ± 0.04*	0.31 ± 0.02*

According to these results, metal accumulation (except Cd) in the whole body of living organisms individuals from the polluted area (Çan and Biga) were higher than Yenice station, and the difference was statistically significant ($P < 0.05$; Table 4, 5, 6).

The TBARS levels of the three macro invertebrates are given in Table 7. The TBARS values measured at all stations were statistically different ($P < 0.05$) and found to be higher in the *Potamon ibericum* and *Asellus aquaticus* that were collected from station Çan when compared to Yenice station.

GSH values measured in the three stations were statistically different ($P < 0.05$), and the highest GSH level was detected in the living organisms collected from the polluted area (Çan and Biga) compared to relatively clean area (Yenice).

Table 6. Heavy metal concentrations (μg g^{-1}) in *Potamon ibericum*.

Date	Metal	Yenice	Çan	Biga
April	Fe	39.14 ± 0.24	73.05 ± 0.87*	67.62 ± 1.25*
July		48.24 ± 0.63	84.24 ± 1.51*	72.81 ± 0.96*
April	Cu	8.05 ± 0.06	15.51 ± 0.41*	11.93 ± 0.35
July		9.96 ± 0.18	17.24 ± 0.17*	14.81 ± 0.31
April	Zn	37.45 ± 0.31	68.95 ± 0.49*	53.24 ± 0.64*
July		42.46 ± 0.58	81.67 ± 1.47*	63.41 ± 1.25*
April	Pb	1.94 ± 0.02	3.03 ± 0.08*	3.58 ± 0.22*
July		2.49 ± 0.07	4.44 ± 0.22*	5.29 ± 0.14*
April	Cd	0.12 ± 0.02	0.51 ± 0.04	0.29 ± 0.03
July		0.21 ± 0.02	0.96 ± 0.04	0.65 ± 0.08

Table 7. TBARS (nm/mg protein) in three organisms sampled from Kocabaş Stream

	Asellus aquaticus (w.b.)	*Gammarus pulex pulex* (w.b.)	*Potamon ibericum* (hepatopancreas)
Yenice	1.56 ± 0.12[b]	1.29 ± 0.10[b]	3.56 ± 0.51[b]
Çan	2.48 ± 0.34[a]	n.d.	5.88 ± 0.83[a]
Biga	1.74 ± 0.26[b]	1.87 ± 0.32[a]	n.d.

n.d.: enough individuals can not found w.b.: whole body

Discussion

Agricultural processes, domestic sewage and industrial effluents are listed among the sources of anthropogenic metal inputs. Thus, heavy metals are released as a result of these activities. All metals essential or not can be highly toxic to aquatic macro invertebrates due to their oxidative potential. These pollutants accumulating in the tissues of aquatic organisms may lead to oxidative damage of basic biological molecules, such as lipid peroxidation, protein and DNA damage (Vlahogianni, 2007; Farombi et al. 2007).

In this study, we investigated the levels of heavy metals and certain biomarkers of oxidative stress in three macro invertebrates collected from the Kocabaş Stream (Çanakkale-Turkey) in April and July 2012. Various studies have shown that exposure to metal ions usually being together with the induction of oxidative stress (Valko et al. 2005), in turn triggers a series of defense mechanisms (Winston and Di Giulio, 1991). Metals can contribute to oxidative damage by both augmenting the cellular concentration of reactive oxygen substances (ROS) and decreasing the cellular antioxidant capacity (Zhang et al. 2010). An important component of antioxidant defense, glutathione (GSH) is a non-enzymatic, oxyradical scavenger. Glutathione reacts with free radicals and peroxides and protects cells against oxidative damage (Meister and Anderson, 1983). In this study, it was observed that the GSH level rises depending on the increasing level of pollution. The high metal levels were measured in the water and tissues at Çan and Biga stations have caused an increase in the ROS production and according to this moderate oxidative stress conditions, it was also observed that, the GSH levels in the living tissues increased. In a similar study, Kaya et al. (2014a) considered that heavy metal polluted areas of the Sarıçay Creek have caused oxidative stress on *Asellus aquaticus* species compared to clean areas and it was also observed that, antioxidant enzyme activities have changed in critical amounts.

Lipid peroxidation for example is a well known mechanism of cellular injury and is an indicator of oxidative damage in cells and tissues. Therefore, the measurement of TBARS is widely used as an indicator of oxidative stress in invertebrates (Wheatley, 2000). Structural and functional changes in proteins and variation on enzymatic activities are also other stress-related effects (Risso-de Faverney et al. 2000). Therefore, at the biochemical level, chemical changes in lipids and alterations in the activity of antioxidant enzymes may confirm the presence of metal-induced oxidative stress in organisms. Failure of antioxidant defenses to remove exogenous ROS produced by pollutants either as consequence of inhibition by those compounds or overwhelming by ROS excess, will throw into confusion the balance of the antioxidant/prooxidant system within the organisms leading to oxidative damage. This is a toxicity phenomenon widely displayed in field conditions, which implies the increase in the levels of lipid peroxidation of the affected organisms (Regoli, 2000; Livingstone, 2001; Regoli et al. 2004). In the present study three crustacean species exposed to of heavy metals and organic pollutants had increased levels of lipid peroxidation. These results clearly evidenced that in those sites being contaminated by pollutants, pro-oxidant conditions defeated antioxidant defenses leading to oxidative stress.

References

Akbulut, M., Kaya, H., Çelik, E.Ş., Odabaşı, D.A., Sağır, O.S. and K. Selvi. 2010. Assessment of surface water quality in the Atikhisar Reservoir and Sarıçay Creek (Çanakkale, Turkey). Ekoloji, **19(74)**:139-149.

Anonymous. 1998. Standard methods for the examination of water and wastewater. In: American Public Health Association (ed) Washington, DC.

Barrento, S., Marques, A., Teixeira, B., Carvalho, M. L., Vaz-Pires, P. and M. L. Nunes. 2009. Accumulation of elements (S, As, Br , Sr , Cd, Hg, Pb) in two populations of *Cancer pagurus*: Ecological implications to human consumption. Food and Chemical Toxicology, **47**:150–156.

Berra, E., Forcella, M., Giacchini, R., Marziali, L., Rossaro, B., and P. Parenti. 2003. Evaluation of enzyme biomarkers in freshwater invertebrates from Taro and Ticino River, Italy. Annales de Limnologie - International Journal of Limnology, **40(3)**:169–180.

Bradford, M.M. 1976. A rapid and sensitive method for the quantization of protein utilizing the principle of dye-protein binding. Analytical Biochemistry, **72**: 248-254.

Camejo, G., Wallin, B., and M. Enojarvi. 1998. Analysis of oxidation and antioxidants using microtiter plates. In: Free Radical and Antioxidant Protocols. (ed. D. Armstrong) Methods in Molecular Biology, **108**: 377-386.

Depledge, M.H. and M.C. Fossi. 1994. The role of biomarkers in environmental assessment (2). Invertebrates. Ecotoxicology, **3**:161–172.

Farombi, E.O., Adelowo, O.A. and Y.R. Ajimoko. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. International Journal of Environmental Research and Public Health, **4(2)**:158-165.

Francesco, R. 2000. Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: A predictive biomarker of oxidative stress. Aquatic Toxicology, **50(4)**: 351–361.

Francesco R., Frenzilli, G., Bocchetti, R., Annarumma, F., Scarcelli, V., Fattorini, D. and M. Nigro. 2004. Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a field translocation experiment. Aquatic Toxicology, **68(2)**: 167–178.

Hyne, R. V. and W. Maher. 2003. Invertebrate biomarkers: Links to toxicosis that predict population decline. Ecotoxicology and Environmental Safety, **54(3)**: 366–374.

Kaya, H., Akbulut, M., Selvi, K., Ileri, B. and M. Duysak. 2014a. Heavy metal accumulation, biomarker responses and sensitivity to oxidative stress in isopoda *Asellus aquaticus* from Sarıçay Creek (Canakkale-Turkey). Ekoloji, **23(91)**: 8-15.

Kaya, H., Selvi, K., Akbulut, M. and A. Tulgar. 2014b. The use of biomarkers to determine the effects of the water pollution on the Odonata larvae, *Aeshna affinis* in Sarıçay Creek (Çanakkale-Turkey). Fresenius Environmental Bulletin, **23(1)**:57-63.

Khan, S. and D. Nugegoda. 2003. Australian freshwater crayfish *Cherax destructor* accumulates and depurates nickel. Bulletin of Environmental Contamination and Toxicology, **70**:308–314.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin, **42**:656–666.

Logan, M. 2010. Biostatistical design and analysis using a practical guide. Wiley-Blackwell, London.

Meister, A. and M.E. Anderson. 1983. Glutathione. Annual Review of Biochemistry. **52**:711-760.

Owens, C.W.I. and R.V. Belcher. 1965. A colorometric micro-method for determination of glutathione. Biochemical Journal, **94**: 705-711.

Risso-de Faverney, C., Lafaurie, M., Girard, J.P. and R. Rahmani. 2000. Effect of heavy metals and 3-methylcholanthrene on expression and induction of CYP1A1 and metallothionein levels in trout (*Oncorhynchus mykiss*) hepatocyte cultures. Environmental Toxicology and Chemistry, **19**:2239–2248.

Selvi, K., Akbulut, M., Kaya, H. and A. Tulgar. 2012. Lead bioaccumulation in gill, muscle and hepatopancreas tissues of Mediterranean green crab, *Carcinus aestuarii* (Nardo, 1847). Marine Science and Technology Bulletin, **1**:13-16.

Smith, C., Shaw, B. and R.D. Handy. 2007. Toxicity of single walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): Respiratory toxicity, organ pathologies and other physiological effects. Aquatic Toxicology, **82(2)**:94-109.

Strickland, J.U.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. 2nd edition, Bulletin of the Fisheries Research Board of Canada (SAUS), 167-310.

Valko, M., Morris, H. and M.T.D. Cronin. 2005. Metals, toxicity and oxidative stress. Current Medicinal Chemistry, **12**:1161–1208.

Vlahogianni, T., Dassenakis, M., Scoullos, M.J. and A. Valavanidis. 2007. Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels *Mytilus galloprovincialis* for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. Marine Pollution Bulletin, **54**:1361–1371.

Wheatley, R. A. 2000. Some recent trends in the analytical chemistry of lipid peroxidation. Trends in Analytical Chemistry, **19**:617–628.

Winston, G.W. and R.T. Di Giulio. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology, **19**:137-161.

Zhang, Y., Songa, Y., Yuana, H., Xua, Y., Hec, Z. and L. Duana. 2010. Biomarker responses in the bivalve (*Chlamys farreri*) to exposure of the environmentally relevant concentration so flead, mercury, copper. Environmental Toxicology and Pharmacology, **30**:19–25.