



BIOCHEMICAL CHANGES IN TWO SPECIES OF MOLLUSCS AS ENVIRONMENTAL BIOMARKERS OF POLLUTION IN HILLA RIVER, IRAQ

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ABSTRACT

Biochemical changes as reliable Environmental biomarker have been investigated seasonally from Spring 2011 to Winter 2012 in gills and digestive glands of some Molluscs (*Viviparus bengalensis* & *Corbicula fluminea*) from two sites in Al-Hilla river, the results showed the highest SOD value (5.7, 4.7 U/mg) in gills and (8.16, 6.9 U/mg) in digestive gland during summer 2011, Catalase activity for both species (16.6, 22.8 U/mg), (34.1, 32.2 U/mg) in gills & digestive gland respectively, Glutathione peroxidase (GPX) value (12.4, 10.8 U/mg) (13.1, 14.4 U/mg) in gills & digestive gland for both species respectively, LPx values in snail & Clam as indication of Malondialdehyde (MDA) have value (5.5, 6.13 nmol/mg), (5.3, 7.8 nmol/mg) in gills & digestive gland respectively, according to Canonical correspondence Analysis, close correlations appeared between previous biomarkers, Environmental parameters, and heavy metals.

KEYWORDS: Antioxidants, Snail, Clam, Seasonal variation, Contamination.

INTRODUCTION

The increase in pollution of the aquatic environment by heavy metals has focused attention on the behavior of these elements in the ecosystems and their impact on living beings. Biochemical markers can be defined as any biochemical changes which are produced according to environmental stress or a xenobiotically-induced variation in cellular or biochemical components or processes that is measurable in a biological system or sample (Benford *et al.*, 2000). The use of biomarkers is a trust approach in the assessment of ecosystem purity and increasing the possibility for the detection of early biological changes (Domouhtsidou *et al.*, 2004). Oxidative stress can be defined as a disruption in homeostasis of free radicals and reactive oxygen species, (Livingstone, 2001). SOD are a group of metalloenzymes that play a crucial antioxidant role and constitute a defense system against natural and anthropogenic pollutants, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen on each second, Glutathione peroxidase (GPx) is a family of peroxidase enzymes that reduce lipid hydroperoxides to their corresponding alcohols and reduce free hydrogen peroxide to water, while Lipid peroxidation (LPO) is a self-propagation sequence of chemical reactions which happened in the bulk phase of lipid bilayers and consist of four phases (Initiation, propagation, decomposition, termination) (Connors, 2004, McGevin, 2011, Nadji *et al.*, 2010, Sahan *et al.*, 2010), Biomarkers are beneficial for providing biological information regarding the health status of the organisms. A battery of biochemical parameters was used by (Narbonne *et al.*, 1999) to evaluate the response of mussels to a contaminated environment, Contamination levels were estimated by measurement of heavy metals, and Oxidative stress as a consequence of heavy metals impact was

detected by (Torres *et al.*, 2002) when they found there were strong correlations between antioxidants with Cu and Pb contents with increasing in the activities of catalase and glutathione peroxidase at the polluted sites of study area.

MATERIAL AND METHODS

Samples were collected seasonally from two sites from March 2011 to January 2012 to measure the physicochemical parameters and heavy metals by using clean polyethylene bottle (5L) with three replicates. Snail and clam samples were collected by using plastic bag and when brought to laboratory, they were washed with tap water and Distilled water to remove any residue to make direct and suitable analyses, (Sturgeon *et al.*, 1982), (Otcere, 2003) methods were used to extract and determine heavy metals in Molluscs. Total superoxide dismutase activities was measured by Pyrogallol auto-oxidation according to (Marklund & Marklund, 1974), Briefly, Molluscs's tissue was homogenized by using Pestle motor Mixer provided by Argos Technologies (U.S) Cat.No.A0001 in 50 mM Potassium Phosphate buffer (pH 7.0) and, centrifuge (14000 r.p.m, 4°C, 5 min.), supernatant have been taken and processed according to procedure. This assay was done according to method described by (Clariborn, 1985 and Aebi, 1972), Molluscs's tissue was homogenized in 50 mM Potassium Phosphate buffer (pH 7.0), added 20 µl SDS (5%) to 100 µl (subsample), centrifuge (14000 r.p.m, 4°C, 5 min.), Samples absorbency were monitored spectrophotometrically at 240nm every 30 s for a total of 120 s and activities were expressed as umoleH2O2 consumed per min per mg protein. LPX was estimated by the Thiobarbituric acid assay according to (Aust, 1985 and Burtis, 1999), the absorption was read at the wavelength of 532 nm against blank. Results were expressed as (nmol/g protein).

Statistical Analysis

SPSS 17.0 programs used for least significance differences (LSD ≤ 0.05), Analysis of variance test (ANOVA) between sites and different parameters.

RESULTS

Seasonal variations of antioxidants in Molluscs Sp. (gills & digestive gland) Biochemical markers in Molluscs gills and digestive gland represented by antioxidants revealed seasonal variation as response for heavy metals stress, for

snail Sp. & Clam Sp. the highest SOD value (5.7, 4.7 U/mg) in gills and (8.16,6.9 U/mg) in d.g during summer, Catalase activity for both species (16.6, 22.8 U/mg), (34.1, 32.2 U/mg) in gills& digestive gland respectively, GPX value (12.4,10.8 U/mg) (13.1, 14.4 U/mg) in gills& digestive gland for both species respectively, LPx values in snail Sp.& Clam Sp. as indication of MDA have value (5.5,6.13 nmol/mg), (5.3,7.8 nmol/mg) in gills& digestive gland respectively(Table 1 &Table 2) and Figure 1&2.

TABLE 1: Seasonal variations of Antioxidant enzymes concentrations in Snail *Viviparus bengalensis* (Lamarck, 1822) [gills &digestive gland (d.g.)] in site 1 & site 2 of study area (Mean ± S.D).

Sites		S.1				S.2				
Enzy.Conc. ↓	SP.	Sum.	Aut.	Wint.	SP.	Sum.	Aut.	Wint.	Control	
SOD (U/mg) gill	2.1±0.001	4.7±0.02	1.6±0.011	2.24±0.01	.22±0.01	3.3±0.01	2.2±0.01	2.1±0.001	1.5±0.001	
SOD(U/mg) d.g.	4.8±0.0001	8.16±0.01	2.92±0.01	4.6±0.02	4.05±0.001	5.97±0.01	2.8±0.015	3.9±0.01	2.1±0.001	
Catalase(U/mg)gill	11.5±0.10	16.6±0.001	15.6±0.015	15.1±0.001	13.6±0.10	16.1±0.005	11.4±0.1	13.5±0.001	10.3±0.06	
Catalase (U/mg) d.g.	28.2±0.1	32.1±0.05	24.9±0.001	26.3±0.1	32.3±0.001	34.1±0.06	28.1±0.001	28.5±0.1	19.6±0.05	
GPX.(U/mg) gill	8.1±0.001	10.3±0.1	9.3±0.1	10.8±0.1	10.5±0.100	6.9±0.001	10.7±0.1	12.4±0.001	5.9±0.001	
GPX.(U/mg) d.g	12.1±0.000	10.9±0.001	8.6±0.43	12.03±0.004	11.6±0.010	13.8±0.06	12.6±0.1	13.1±0.001	9.8±0.1	
LPX. nmol/mg) gill	2.2±0.001	4.5±0.1	2.9±0.001	3.8±0.001	3.6±0.060	5.5±0.10	3.7±0.001	2.2±0.10	1.9±0.001	
LPX. (nmol/mg) d.g.	4.8±0.06	4.7±0.12	2.4±0.06	2.9±0.001	4.4±0.001	5.3±0.10	4.8±0.001	2.7±0.001	2.5±0.10	

p≤0.05

TABLE 2. Seasonal variations of Antioxidant enzymes concentrations in Clam Sp. *Corbicula fluminea* (Müller, 1774)[gills &digestive gland(d.g.)] in site 1 & site 2 of study area (Mean ± S.D).

Sites		S.1				S.2				
Enzy.Conc. ↓	SP.	Sum.	Aut.	Wint.	SP.	Sum.	Aut.	Wint.	Control	
SOD (U/mg) gill	2.9±0.01	3.1±0.001	1.6±0.01	1.3±0.01	3.1±0.001	5.7±0.1	2.9±0.01	2.4±0.01	2.1±0.0001	
SOD (U/mg) d.g.	3.8±0.01	4.8±0.01	2.9±0.01	2.8±0.01	4.9±0.001	6.9±0.01	3.9±0.01	3.6±0.01	3.5±0.1	
Catalase (U/mg)gill	17.8±0.1	17.11±0.001	12.9±0.001	10.8±0.01	20.3±0.001	22.8±0.1	21.03±0.001	15.2±0.001	11.6±0.1	
Catalase (U/mg) d.g.	26.3±0.1	26.7±0.2	22.1±0.001	23.2±0.10	31.7±0.1	32.2±0.1	28.5±0.10	26.1±0.001	20.3±0.1	
GPX. (U/mg) gill	10.8±0.1	7.9±0.001	6.5±0.1	8.4±0.10	10.8±0.1	10.1±0.001	6.8±0.1	7.2±0.1	6.1±0.1	
GPX. (U/mg) d.g	12.4±0.001	12.8±0.1	12.2±0.001	12.1±0.001	14.1±0.06	13.7±0.2	14.4±0.1	12.6±0.10	11.9±0.001	
LPX. (nmol/mg) gill	3.8±0.1	6.13±0.06	2.6±1.01	2.3±0.001	3.8±0.1	4.4±0.1	1.7±0.01	1.8±0.06	1.6±0.2	
LPX. (nmol/mg) d.g.	4.5±0.1	7.8±0.001	6.5±0.06	6.5±0.1	5.3±0.06	7.7±0.001	4.2±0.1	6.7±0.1	3.9±0.001	

p≤0.05

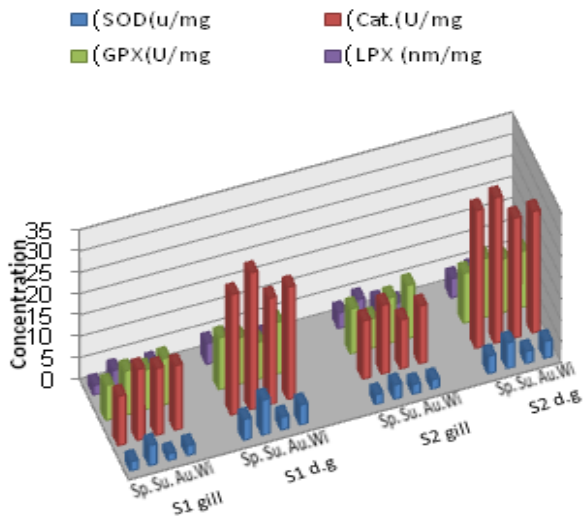


Figure1: seasonal variations of antioxidants in snail Sp. for site 1 & site 2 of study area in gill&digestive

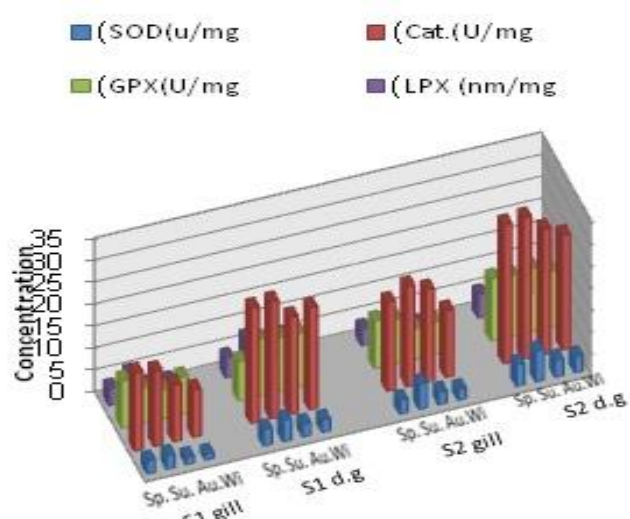


Figure 2: seasonal variations of antioxidants in Clam Sp. for site 1 & site 2 of study area in

DISCUSSION

Biomarkers reflects environmental impacts at an early phase and are considered as prognostic tools for increased levels of pollution and modulated to provide a quantitative estimation of the probability of adverse effect in aquatic organisms (Frenzilli *et al.*, 2001), fluctuation in different biomarkers in this study is compatible with many studies such as (Cravo *et al.*, 2012) in Portugal. The fluctuation in biomarker responses in this study because these responses are transient and variable according to the different species and pollutants (An *et al.*, 2012), This role of antioxidant enzymes may be enhanced or inhibited under stress depending on the intensity and the period of the stress, and the susceptibility of exposed species (Otitolaju & Olagoke, 2011), The activity of antioxidant enzymes, among other defense mechanism have vital role in preventing cellular damage to cellular constituents (Orbea *et al.*, 2002), The seasonal variation of antioxidants in this study (Figure 1&2) coincided with many studies such as

(Regoli & principato, 1995) and the increasing of antioxidants with highest values in summer due to the production of reactive oxygen species has been linked to temperature increase. The Possible reason for decreasing in antioxidant enzymes concentration as indicated during study period was returned to negative feedback mechanism, low internal pollutants concentrations, low metabolic generation of ROS (reactive oxygen species), and enzyme damage or destruction from apoptotic or necrotic cell death and tissue atrophy (Conners, 2004). Increased intracellular ROS (reactive oxygen species) activities due to heavy metal contamination leads to decreased concentration of SOD (Olakolu *et al.*, 2012) and activity of SOD in this study because it's first line defense against oxidative stress and participation in scavenge ROS and preserve the redox balance (Fang *et al.*, 2012). The lowest value of antioxidants and other biochemical markers in winter through study period are coincided with many studied such as study of (Dspotovic' *et al.*, 2012) when they recorded that the lowest values of GSH content in the digestive gland of the mussel *Mytilus galloprovincialis* in winter, when there is less food and the gonads are in a state of rest while increasing of antioxidants activity could rather be related to a higher metabolic activity of organism during the warmer season (Vidal *et al.*, 2002), the increased of antioxidants concentration in this study as shown in results are probably temporarily correlated to the increased metabolic activity as consequences of seasonal temperature elevations in addition to reproductive cycle (Filho *et al.*, 2001).

CONCLUSION

We concluded that Seasons have an important role in different biomarker responses against heavy metals, and Increasing and decreasing of biomarkers in this study due to oxidative stress induced by pollutants which lead to increase ROS (Reactive oxygen species).

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