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Seasonal variation of catalase enzyme and heavy metal (Pb, Cd, Ni) in *Pinctada radiata* in Persian Gulf, Iran

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Abstract

We have different possibilities and tools to assess the impact of pollution on marine ecosystems. The ecotoxicological approaches are based on the use of biomonitors and biomarkers. The purpose of our study was to evaluate the antioxidant enzyme catalase (CAT) as biomarkers in an edible species, *Pinctada radiata* (Mollusca, Bivalvia) associated with the environmental pollution(heavy metal, Cd, Pb, Ni) in the Persian gulf. Samples were collected seasonly from march 2012 till march 2013 at three station in the Persian gulf (Nakhilo, Hendorabi and lavan Islnad). Mollusc bivalves (*Pinctada radiata*) with the same shell length (40-50mm) were collected from the sampling sites, transferred to the laboratory and dissected the same day. The mantle was dissected and samples were prepared for biomarker analyses and measuring the heavy metal in soft tissue. The enzyme concentration were determined by using standard methods. Biochemical data showed a no significant The results concluded from the amount of heavy metals in the sediment and soft tissue of pearl oyster(*pinctada radiata)* show no significant correlation with the level of catalase enzyme. The present study strongly suggests that monitoring programs should compare sites with similar physicochemical characteristics when using a complementary biomarker approach.

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Introduction

Biologically relevant indicators are required to monitor ecosystem status in relation to diffuse anthropogenic pollutants or acute accidental discharges. Responses and tolerances of individual taxa to various anthropogenic stressors have been extensively investigated in temperate systems (Collier and Varanasi, 1991; Aas and Klungsoyr, 1998; Baussant *et al.*, 2009; Brooks *et al.*, 2009) but few data exist for marine ecosystem. Natural and anthropogenic factors produce oxidative stress in the aquatic biota (Winston and Di Giulio, 1991; Kelly *et al.*, 1998). Xenobiotics may stimulate the generation of reactive oxygen species (ROS): O2_, H2O2, _ OH, etc. (Halliwell and Gutteridge, 1999) and increased levels of ROS often enhance antioxidant defenses. Aquatic organisms are exposed to significant amount of heavy metals from industrial, agricultural and anthropogenic activities. Metal accumulation causes an increase in ROS leading to oxidative stress in fish (Roche and Boge´, 1993; Dautremepuits *et al.*, 2002). Heavy metals can promote oxidative damage by directly increasing the cellular concentration of ROS and by reducing the cellular antioxidant capacity (Pinto *et al.*, 2003). Mussels and oysters are filter-feeding bio-accumulators that have been used as sentinel organisms in numerous monitoring programs (NOAA, 1995). The analyses of biomarkers in these bivalves have been also incorporated into biomonitoring studies to evaluate the effects of pollutants (Viarengo *et al.*, 2000) in areas contaminated with heavy metals (Najimi *et al.*, 1997; Regoli and Principato, 1992), domestic sewage (Radenac *et al.*, 1998), pesticide residues (Narbonne *et al.*, 1991; Mora *et al.*, 1999) and organic compounds (Akcha *et al.*, 2000). Biomarkers of oxidative stress, such as catalase (CAT), glutathione peroxidase (GPX) activities, and lipid per oxidation have been used for both bivalve and fish species, although their interpretation is usually difficult due to their implication in a wide range of biological functions (Van der Oost *et al.*, 2003).

Despite the considerable understanding of their links with contaminant exposure, the use of biomarkers is often limited by their strong variability due to natural biological and environmental cycles (Nahrgang *et al.*, 2010a). Bivalve mollusks, such as mussels and oysters are characterized by their aptitude to concentrate both metals and organic contaminants, their immobility, their limited ability to metabolize accumulated contaminants, their abundance, their persistence, and their ease of collection They have become recognized biomonitors of pollutants in coastal waters and have been used in different international monitoring programs such as the Mussel Watch (USA) and the RNO (France) (Stellio *et al.*, 2006; Yungkul *et al* 2008; Rocher *et al.*, 2006).

Metallothioneins (MTs): Most scientists agree that biochemical and physiological mechanisms allowing mollusk bivalve species to accumulate and tolerate high amounts of heavy metals are based on their metal handling by metallothioneins (MTs). These are low molecular weight, cysteine rich, cytosolic proteins of ubiquitous occurrence which are suggested to inactivate toxic metal ions by binding them to sulfur atoms of the peptide cysteine residues (Amiard *et al.*, 1997). In fact, it has repeatedly been shown that concentrations levels of MT can be correlated to accumulated fractions of toxic metal ions such as copper or cadmium in animals' tissues (Roesijadi *et al.*, 2000). In addition, metallothionein concentrations in molluscs may also vary due to influence by nonmetallic pollutants. Hence, the idea was raised that MTs might be used as biomarkers for environmental pollution by measuring their concentrations in bivalves from contaminated habitats.

Catalase is widely used as a marker involved in the primary defense against oxidative damage (Borgeraa *et al.*, 2006; Abel *et al.*, 1998). Oxidative stress results in the formation of many Reactive oxygen species (ROS) (Semadi *et al.*, 1993) causing cell and tissue damage (Rodier *et al.*, 1996). The CAT activity tells us about the degree of alteration of the cell (Torreilles *et al.*, 2006). Thus, the CAT activity was reported in fish and bivalves exposed to organic pollutants (Boening *et al.*, 1999; Blanchette *et al.*, 1999).

In this work, antioxidant enzyme such as catalase (CAT) has been studied. Field experiments are usually performed to determine whether this enzyme can be used as biomarkers of heavy metal. Thus, antioxidant enzymes play a crucial role in maintaining cell homeostasis. This enzyme has been proposed as biomarkers of contaminant-mediated oxidative stress in a variety of marine organisms, and their induction and/ or inhibition reflects a specific response to pollutants (Jee and Kang, 2005). The main of this work is the study of the relationships between heavy metals (Pb, Cd, Ni) and oxidative stress enzyme.

Materials and methods

Samples Collection

Persian Gulf is in the subtropical zone, lying almost completely between the latitudes 24°and 30°N and longitudes of 48° and 52°30′E. Maximum depth of the Persian Gulf is100m, with an average 36m depth and most of the basin is less than 60m depth. The study area is located on the Nakhilo, Hendorabi and lavan island.

The geographical location of the island was the sample is collected, shown in Table 1. Due to the availability of different habitats of pearl oyster on the islands of Lavan and Hendorabi and Nakhilo, random starting point sampling was started. About 360 *Pinctada radiata* samples of silimalar size were collected in four seasons from March 2012till march 2013.

Sampling of pearl oyster was carried out for two purposes, the determinations of heavy metals (Ni, Cd, Pb) and concentration of catalase in soft tissue. The samples were transferred to the laboratory in ice-cold container in order to reduce their metabolism. They were dissected in the laboratory the same time for preventing enzyme destruction (Ferreira-Cravo *et al.*, 2009).

Catalase determination

Catalase is an antioxidant enzyme acting against the toxicity of oxygen radicals (Régoli *et al.*, 2004). Catalase measurement was began in accordance with the instruction of catalase kit(Eastbiofarm Company)using washing the tissue with Phosphate Buffered Saline (PBS), pH=7.4. To measure the level of CAT enzyme, Enzyme-Linked Immunosorbent Assay (ELISA) reader (Model: BioTac El-x800) was used. Measuring absorption of samples was performed at 450 nm.

Heavy metal determination

After being sampled, the individuals were immediately stored in plastic bags and transported separately to the laboratory, where the calcareous shells were removed, and the visceral masses of all specimens were rinsed with abundant distilled water to remove sediments or surface debris. Because of the variability in the ability of mollusc tissues to accumulate pollutants (Bargagli *et al.*, 1986; Catsiki, 1986), heavy metal contents were analyzed in tissues of *P. radiata*. The samples obtained were there after placed, separately, in an oven at 105°C for 24 hours. A total of 180 samples were obtained. The metal content of these samples was determined using Atomic absorption flame emission spectroscopy (AAS).

The laboratory apparatus were acid soaked (nitric acid) before the analysis. After acid soaked, it is rinsed thoroughly with tap water and distilled water to ensure any traces of cleaning reagents were removed. Finally, it is dried and stored in a clean place (Radojevic and Bashkin, 1999). The sediments were kept cool in icebox during the transportation to the laboratory (Al-Shiwafi *et al*l, 2005; Jung *et al*l, 2005). The surface sediments air-dried and after homogenization using pestle and mortar, it is passed through a 2-mm mesh screen and stored in polyethylene bags based on method used by Romic and Romic (2003) for further analysis. Before the determination of these heavy metals was conducted, the samples are digested using aqua regia digestion. Approximately 2g of each sample digested with 15 mL of aqua-regia (1: 3 HCl: HNO_3) for 3-4h at 90°C. After cooling, the digested samples were filtered and kept in plastic bottles before the analysis. Radojevic and Bashkin (1999) stated that aqua regia has ability to extract all the metals in soil sample and widely used in most of the soil analysis. The samples were then analyzed for heavy metals and base cations using AAS with specific flame and wavelength (Atomic Absorption Spectrometer Model AA 200).

Statistics analysis

Statistical analyses were performed with Spss 18. When requirements of normality and homogeneity of variances were met one-way ANOVA (season) was used for bivalve data, respectively. A Tukey HSD post hoc test was used to test differences among the sampling season with $p \leq 0.05$ as the significance level applied for all analyses. Correlations between variables were determined by the Pearson product–moment correlation.

Results

The results concluded from the amount of heavy metals in the sediment and soft tissue of pearl oyster (*pinctada radiata)* show no significant correlation with the level of catalase enzyme due to the values which are almost identical (Table 2). The results of catalase measurement are given in Table 2. Results of concentration of catalase in pearl oyster (*pinctada radiata)* soft tissue showed that the highest and lowest CAT concentration belong to the stations Hendorabi on spring and Nakhilo on autumn respectively.

CAT concentration at different stations: Nakhilo<Lavan<Hendorabi. .

Table 1. Location, number of samples analyzed (N), Shell length (mm) of oysters, and descriptions of sampling *Pinctada radiata* collected from Lavan and Hendorabi Island, Persian Gulf.

No	Location	Latitude (N)	Longitude (E)	N	Shell length mean (mm)
1	Lavan	$26^{\circ} 47'$	$53^{\circ} 20'$	120	45.4
2	Nakhilo	$26^{\circ} 50'$	$53^{\circ} 29'$	120	46.2
3	Hendorabi	$26^{\circ} 40'$	$53^{\circ} 35'$	120	48.6

Considering a general trend, the concentration of the catalase as a biomarker shows an inverse relationship with heavy metals pollution level. While it has a variable relationship with contamination level of investigated metals in sediment. Assessment results of correlation coefficient between metals, nickel, Cadmium and lead with catalase show no significant correlation between the mentioned parameters (Figure 2). The accumulation of heavy metals in tissues of organisms depends on environmental conditions and physicochemical parameters. Since the measuring of these parameters can be Effective for assaying of catalase as a biomarker.

Table 2. Seasonal variation of trace metal concentrations ((µg g^{-1} dw) in *Pinctada radiata* and catalase concentration in ng/ml.

	Station	Pb((µg g^{-1} dw)	Cd((µg g^{-1} dw)	Ni((µg g^{-1} dw)	(CAT) ng/ml
Spring	Hendorabi	17.5±6.24 a	16.75±2.04a	5.17±1.69a	66.89±19.48 b
	Lavan	17.5±7.26a	25.1±0.74b	5.62±1.57a	57.08±15.52b
	Nakhilo	20.17±5.51a	8.7±0.54a	6.50±2.33a	62.12±18.50a
Summer	Hendorabi	20.0±6 06 a	26.72±2.57b	5.37±2.44a	65.0±20.26 b
	Lavan	18.33±5.51a	18.33±5.51a	6.22±1.71a	64.23±19.2a
	Nakhilo	18.33±5.79a	32.47±4.72b	6.67±1.71a	51.02±14.56a
Autumn	Hendorabi	26.83±3.01a	18.28±1.81a	7.13±1.58a	35.33±9.64a
	Lavan	20.5±5.27a	20.05±5.27a	6.8±2.03a	32.98±8.54a
	Nakhilo	18.33±6.93a	15.15±5.8a	8.08±1.28a	31.85±9.5a
Winter	Hendorabi	21.83±5.01a	15.68±2.79a	7.67±1.32a	42.55±13.82a
	Lavan	18.82±5.85a	18.83±5.85a	6.87±1.96a	43.5±14.5a
	Nakhilo	23.83±2.56a	34.25±1.49b	7.22±2.34a	41.2±13.78a

Discussion

The development of human factors in coastal areas causes an increasing deterioration of the quality of the marine environment, including increased intake of toxic elements through domestic and industrial wastes. This anthropogenic pollution affects adaptation and maintenance of aquatic populations such as bivalve mollusks (Bocquené *et al.*, 1997). As molluscs are thermo conforming organisms, they must constantly counteract environmental temperature fluctuations and change their metabolic rate, which results in oscillation in the levels of ROS (Wilhelm *et al.*, 1993). In those animals, ROS generation, oxidation rates, and antioxidant status are most likely related to ambient temperature and metabolic activity (Wilhelm *et al.*, 2000).

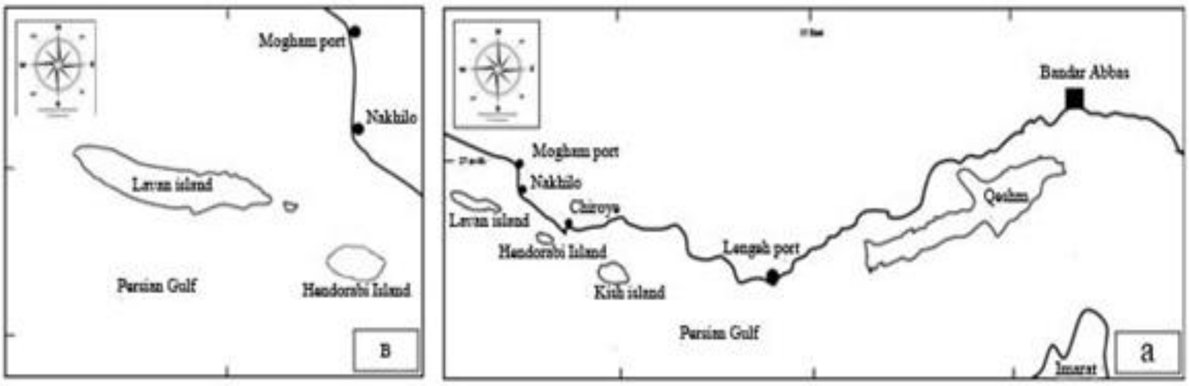

Fig. 1. Geographical location where sample collected (Nakhilo, Lavan, Hendorabi Island.)

Changes in individual antioxidant parameters are difficult to predict and interpret since their increase or decrease can occur depending on the capacity of a given organism to cope with the present stressor. This is why the use of antioxidant enzyme activities in biomonitoring studies is complicated. Another reason is that the levels and composition of chemical pollutants in the environment often display wide seasonal variations in response to the climate and other factors (Pavlović *et al.*, 2004). It is also known that several classes of pollutants are capable of enhancing the formation of ROS and thereby provoking oxidative stress. Some of these pollutants include PCBs, PAHs, phenols, and heavy metals (Van der Oost R, *et al.*, 2003). According to a study on pearl oyster, Assessment results of correlation coefficient between metals, nickel, Cadmium and lead with catalase show no significant correlation between the mentioned parameters. Sea currents can cause lack of opportunities for sedimentation and deposition of pollutants at the stations away from the beach (Mortazavi *et al.*, 2006). Thus, pollutants are transferred in to the coastal and marginalized areas and deposited there. The amount of heavy metals in sediment and pearl oyster (*pinctada radiata)* soft tissue show no significant differences at all stations. Among the factors affecting the metabolism power of a compound in living body, parameters like environmental, biological and physiological characteristics, types of pollutants, its biological availability and the biological accumulation potentiality of the pollutant can be named (Yin *et al.*, 2006).

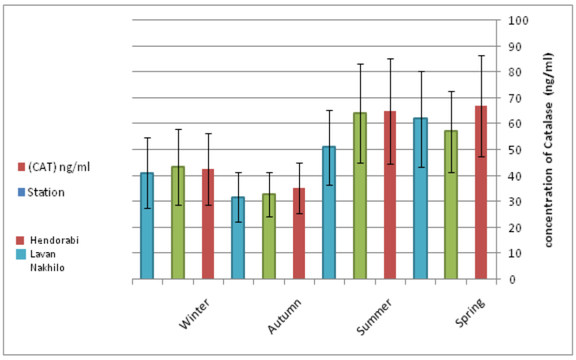

Fig. 2. Heavy metal (Ni, Cd, Pb) measured seasonally in the sampling sites, there was no significant Difference between the sites and season.

In case of relation of heavy metals including Cadmium, lead, Nickel with concentration of catalase on a type of oyster. The results showed that the antioxidant activity and environmental parameters are also high at stations where contain high concentration of these elements. In this research, it was expressed that changes in environmental parameters as a result of the reproductive cycle of the oyster, water temperature(seasonal conditions) and food access are including among factors affected the its activity(Vlahogianni *et al.*, 2007).

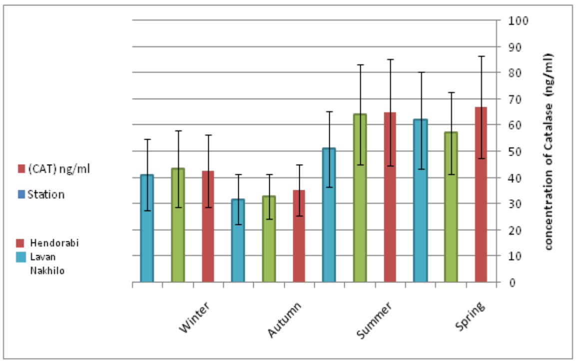

Fig. 3. Catalse enzyme measured seasonally in the sampling sites.

Results of the study suggest that the pollution load of the mentioned heavy metals in areas near the coast is higher than the offshore areas (Angel *et al.*, 1999). This finding is matched with the studies conducted by Angel and his colleagues. Bivalves are scientifically advocated as suitable bioindicators for biomonitoring water pollution. An assessment of HM pollution in the Saronikos Gulf of Greece was conducted through evaluation of oxidative stress in *Mytilus galloprovincialis* mussels. Gills and mantle of mussels from the polluted sites (Elefsis Bay present in the Saronikos Gulf) recorded two to three times concentrations of most metals involving Cd, Cr, Ni, Pb, Cu and Fe than the unpolluted reference site (Stylida in Malaikos Gulf). The investigation clearly highlighted the role of metals in causing oxidative damage and also demonstrated that presence of metals at elevated concentrations overcome the actions of antioxidant enzymes like CAT(Vlahogianni *et al.*, 2007).

Conclusion

Aquatic ecosystems are under constant pressure of anthropogenic pollutants originating from various sources. In the recent decades, there have increasing concerns about pollutants entering the aquatic environment. A need to discover simple and reliable ways to monitor the level of particular chemicals such as heavy metals or other pollutants in the aquatic environment, and to elucidate the mechanisms of pollutants uptake and storage in organisms, has resulted in a proliferation of studies into the use of biomonitor organisms. The success of some Mollusc Bivalves is due to some factors including their wide geographical distribution, abundance, sedentary, tolerance to environmental changes, high bioconcentration factors, population stability, and size, adaptability for field, cage and laboratory experiments. In general, the amounts of heavy elements in the environment of the organism are not equivalent to those stored in their tissue. Although the purpose of this paper was not found a significant relationship between metals in sediment and soft tissue of pearl oyster and catalase enzyme, but more research must be done in different enzyme and other kind of pollution.

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