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Assessment of PAHs pollution effects on blood metabolic factors of *Periophthalmus waltoni* from northern coast of the Persian Gulf

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ABSTRACT

The present study provides information about the nature of adverse effects on fish and the ecological impact that the PAHs pollutant are having in the northern coast of Hormuz Strait. The glucose and cholesterol levels were higher in fish from the St_3 than in Walton's mudskipper from other stations however St_3 samples had lowest total proteins levels. There were a significant positive correlation between glucose and cholesterol with PAHs concentrations in sediment and tissue samples ($P<0.05$). However total proteins has adverse significant correlation with PAHs concentrations ($P>0.05$).Adverse correlation was seen between length and body weight of fish samples with PAHs concentrations. According to the results of this study the monitoring of contaminants bioaccumulation in the northern part of Hormuz Strait is necessary, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on aquatic organism's health.

Key words: PAHs, blood metabolic factors, *Periophthalmus waltoni*, Hormuz Strait.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, mainly come from incomplete combustion of organic materials and fossil fuel, or from direct petroleum releasing [1]. These contaminants can persist in the environment for a long time. Because of high lipophilicity, they have the potentials to accumulate in marine organisms [2]. Such compounds have adverse effects on health (carcinogenic and/or mutagenic activity) and ecosystem [2]. Also, PAHs are bioavailable to fish and other marine organisms through the food chain, as waterborne compounds and from contaminated sediments [3]. Therefore, there was of great concern over the contaminants during the past years mostly in the Persian Gulf.

Fish have been widely used in toxicological studies as models to evaluate the health of aquatic ecosystems [4]. Mudskippers (Gobiidae: Oxudercinae) live in intertidal habitat of the mudflats and in mangrove ecosystems [5]. At present, Walton's mudskipper, (*Periophthalmus waltoni*) is the dominant fish species in estuaries of the northern part of Hormuz Strait (Persian Gulf). It is a filter and detritus-mud feeder that is in contact with pollutants in the water column and sediments.

The measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination can be used to evaluate the impact of contaminants on aquatic ecosystems, monitoring changes in the physiological condition of fish and water quality [6]. Chemistry of blood plasma offer many vital responses to measure toxicity in fish. Blood is relatively easy to sample and many effect parameters are easy and fast to analyze.

Nevertheless, the use of plasma biochemical parameters as indicators of damage to wild fish resulting from chronic exposure remains of interest.

Because coastal waters in the northern part of Hormuz Strait, receive large inputs of anthropogenic pollutants through industrial and urban discharges, atmospheric deposition and terrestrial drainage [7], the aim of this study was to determine the effects of polycyclic aromatic hydrocarbons (PAHs) concentrations on plasma biochemical parameters of *P. waltoni* by monitoring marine pollution in the northern part of Hormuz Strait (Persian Gulf).

MATERIALS AND METHODS

Fish Sampling

A total of 30 individuals *(P. waltoni*) were collected from three estuaries, including Shour-e-aval (St_1), Souro (St_2) and Bustanoo (St_3) estuaries (3 stations per each), from the northern part of Hormuz Strait in March 2012 (Fig.1). All the samples were used for determining the heavy metals and blood collection.

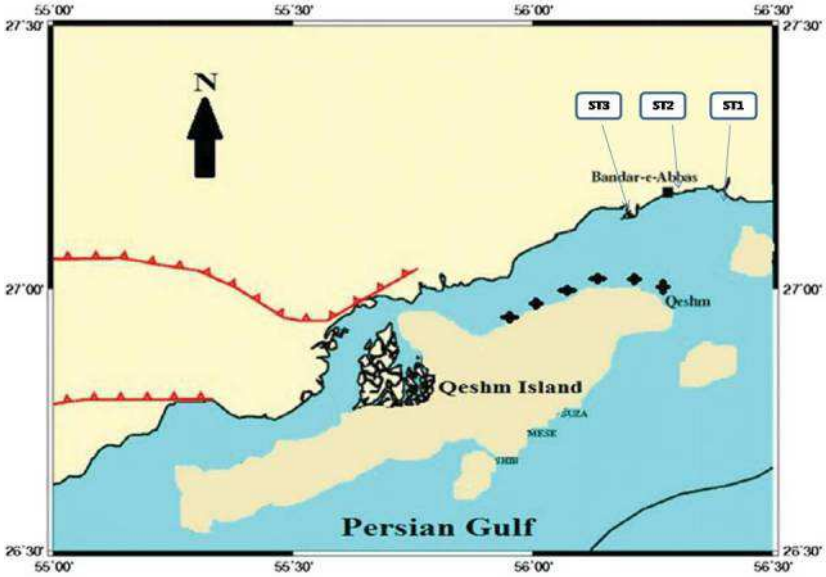


Fig 1. Map of study area and sampling sites
(St_1: 60° 39'18", 25°15'20.40", St_2: 60° 34'23", 25° 18'59", St_3: 60° 28'41", 25° 18'59")

Blood sampling and analysis

Samples were taken quickly caught and while it was physically restrained, blood samples were collected directly from the heart with 2cc plastic syringes coated with sodium heparin. For blood plasma assessment, blood tubes were centrifuged for 5 min at 3,000 rpm. Then, glass tubes were broken, and the resultant blood plasma was emptied into sterile micro tubes for further analysis. Hormonal Data tests include T3, Thyroxin, and THs were made by Vidas Biomerieux System (Electroimmuno Analyzer, France) [8].

Determination Polycyclic Aromatic Hydrocarbons (PAHs)

Water samples

Excavation of organic compounds from water was conducted by the use of the liquid excavation method (LLE) (APHA 1992). To delete sulfuric compounds, specimens were placed for one night in 7 to 8 gram active copper and volume of specimens separated from copper with the help of a rotary machine and decreased to 2ml. To separate and purify oil hydrocarbons, samples were passed through column 1 and 2 of gas chromatography. Column 1 contained inactive gel silica (about 5 percent water) and used to separate polar compounds. Output samples of column 1 decreased up to 1 ml and passed through column 2 containing active gel silica. Compounds PAHs separated by washing column 2 with the help of hexane/dichloromethane solvent (3; 1 v/v). Finally, the volume of specimens decreased with the help of purified nitrogen gas up to dryness and solved in 150 ml isooctane and placed inside special vinalhaye to inject into the GC-MS machine [3].

Sediment and tissue samples

Sediment samples were wrapped in pre-cleaned aluminum foil and kept frozen (-20 ˚C) until analysis. The samples were extracted with a soxhlet extractor using 350 mL dichlomethane (DMC) for more than 8 hours [3]. Tissue was analyzed as described elsewhere [3]. Samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS, Trace, thermo, Bremen, Germany). This instrument was equipped with a 50 m=0.25 mm i.d. HP-5MS capillary column coated with 5% phenyl 95% methylpolysiloxane (film thickness 0.25 mm).Samples were injected

in splitless mode. The oven temperature program started at 90 ˚C (holding time 1 min) and increased to 120˚C at 10˚C/min and then to 310˚C at 4˚C/min (holding Tissue was analysed as described elsewhere [3]. Samples were analysed by gas chromatography coupled to mass spectrometry (GC-MS, Trace, thermo, Bremen, Germany). This instrument was equipped with a 50 m=0.25 mm i.d. HP-5MS capillary column coated with 5% phenyl 95% methylpolysiloxane (film thickness 0.25 mm).Samples were injected in splitless mode. The oven temperature program started at at 90 ˚C (holding time 1 min) and increased to 120˚C at 10˚C/min and then to 310˚C at 4˚C/min (holding time 15 min). Injector, transfer line and ion source temperatures were 280, 280 and 200˚C, respectively. Helium at a flow of 1.1 mly min was used as carrier gas. Data acquisition was in electron impact (70 eV) and selected ion monitoring modes (40-ms dwell time). The ion mass program is reported elsewhere [9].

Statistics analysis

Analyses were performed using the SPSS software (Version 16). The data were tested to check normality using the Kolmogorov-Smirnov test, which showed they have a normal distribution. The correlation between heavy metals and plasma enzymes parameter concentrations were evaluated by analysis of variance followed by Tukey's test. A 5% significance level was employed throughout**.**

RESULTS AND DISCUSSION

Table 1 summarizes the biometry results of fish in different stations. The St_3 samples have minimum mean weight and length (9.88±2.63cm, 6.8±0.27g) continued with St_1 and St_2.

Table 1: Biometry results (Mean± sd) of fish samples in different stations

Species	Station	Total length(cm)	Weight(g)
P. waltoni	St_1	10.06±1.5	8.01±0.11
	St_2	10.03±2.06	7.9±0.2
	St_3	9.88±2.63	6.8±0.27

[1]*St_1: Shoure-e- aval estuary, St_2: Souro estuary, St_3: Bustano estuary*

The result of PAHs mean concentrations in fish, water and sediment samples from various locations were shown in Table 2. The highest ∑PAHs were observed in St_3 fish tissues (284.14 µg/kg). For all tested samples, the lowest concentration of total PAHs were obtained from St_1 station water samples (3.09µg/kg). The comparison of biochemical blood parameters between Walton's Mudskipper sampled at different stations is presented in Figure 2. The glucose and cholesterol levels were higher in fish from the St_3 than in walton's mudskipper from other stations however St_3 samples had lowest total proteins levels. Whereas significant differences were found for glucose, cholesterol and total protein ($P<0.01$).There were a significant positive correlation between glucose and cholesterol with PAHs concentrations in sediment and tissue samples ($P<0.05$). However total proteins has adverse significant correlation with PAHs concentrations ($P>0.05$) (Table 3).

Our PAHs concentration data in *P. waltoni* ranged from 138 to 284.14 µg kg^{-1} dry weight with an average of 206.37 µg kg^{-1} dry weight in different stations, which were lower than those reported in *Liza kluzignery* in a previous study from the same area [3] (the ∑PAH was 195.06 µg kg^{-1} dry weight in that study).To our knowledge, the present study was the second with the data of PAHs in fish from the northern coasts of Hormuz strait being available. PAHs can be readily metabolized and eliminated from fish. The length of a food chain is one of potential factors to influence biomagnification of hydrophobic organic compounds [10]. The results of this study showed that the accumulation of PAHs in the fish were higher than sediments and water (Table 2). Because of their lipophylic nature, PAHs tend to accumulate more in marine organisms than in other matrices, such as sediment [11]. The PAHs concentrations found in the sediment and fish at the (St_3) were higher than those of the other stations; therefore, it reveals that is an area requiring special concern to avoid future environmental problems. Industrial and vehicle atmospheric emissions, a great deal of sewage and waste water from the city of Bandar Abbas, diesel oil leakage/contamination from frequent cargo and fishing ships contributed to PAH inputs In this area.

Table 2: Concentrations mean of PAHs (µg kg^{-1} dry weight) in fish tissue, sediment and water samples from three stations of north coast of Hormuz Strait (Persian Gulf)

PAHs	Water			Sediment			Fish Tissue		
Mean ±SD	St_1	St_2	St_3	St_1	St_2	St_3	St_1	St_2	St_3
	3.09^a	4.28^b	5.49^c	41.29^a	124.38^b	226.05^c	138.00^a	196.97^b	284.14^c
	0.43	0.20	0.38	0.47	2.90	1.39	1.45	3.13	2.45

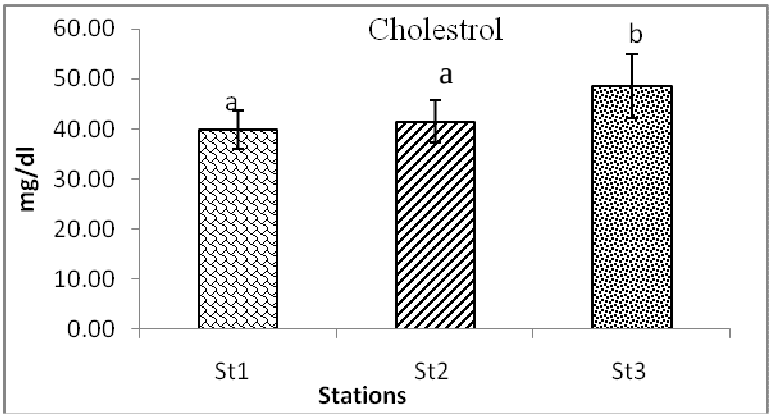
Adverse correlation was seen between length and body weight of fish samples with PAHs concentrations (Fig 3, 4). Marine organisms can rapidly convert up to 99% of the PAHs to metabolites within 24 h of uptake. In addition, the

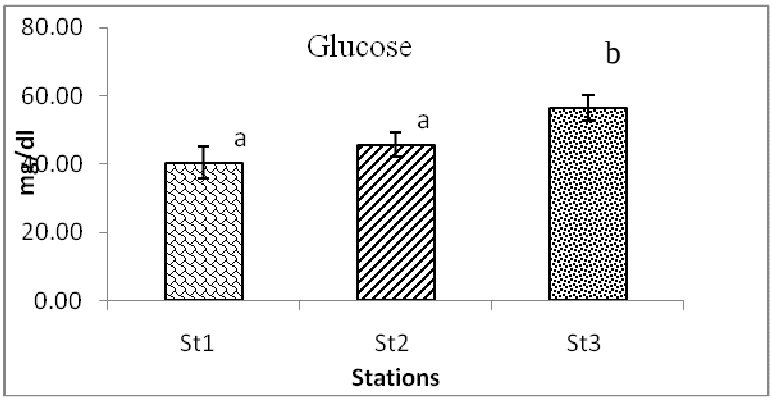
half-life of PAHs was generally very short. It is from 6 to 9 days for fluorene, phenanthrene, anthracene and fluoranthene which is contrary to any other persistent organic pollutants class, as polychlorinated biphenyls [12]. Our results are in agreement with Mohammadizadeh et al. [3] finding that reported in *Liza kluzignery* in a previous study from the same area.

Table 3: Correlation of biochemical blood parameters of *P. waltoni* with PAHs

Correlation	Glucose	cholesterol	Total Protein
Water PAHs	.729**	.323	-.588**
Sediment PAHs	.859**	.590**	-.715**
Tissue PAHs	.861**	.598**	-.713**

***. Correlation is significant at the 0.05 level (2-tailed).*





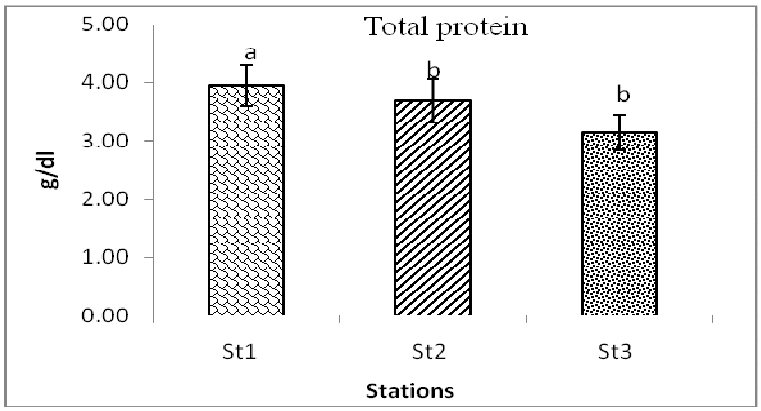


Fig 2: The comparison of biochemical blood parameters between Walton's Mudskipper sampled at different stations (Mean ± SD)

According to Barton [13], stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic state. If severe or long lasting, the response then becomes maladaptive and threatens the fish health and wellbeing. Therefore, in the presence of stressors (contaminants /pollutants), blood parameters and blood chemistry can be employed as standard laboratory test to determine

metabolic disturbances in fish [14]. The use of a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish [7].

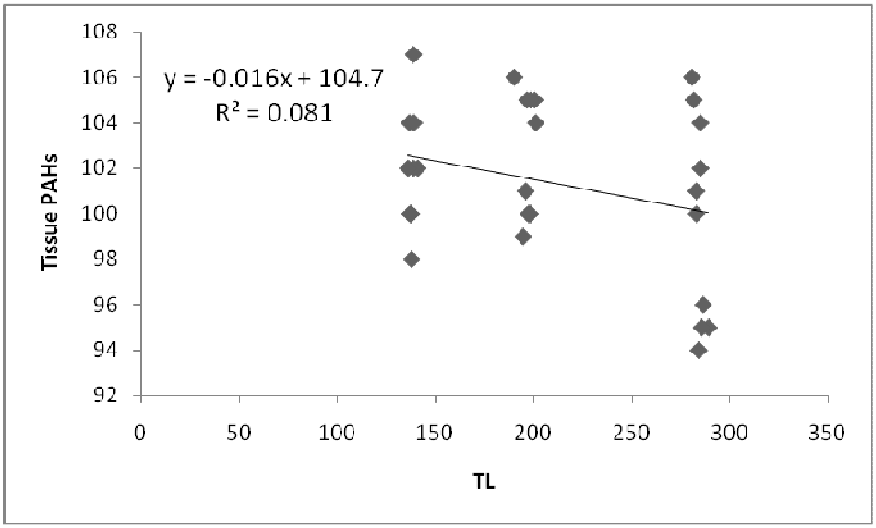


Fig 3: Correlation of total length (TL) of *P. waltoni* with PAHs concentrations

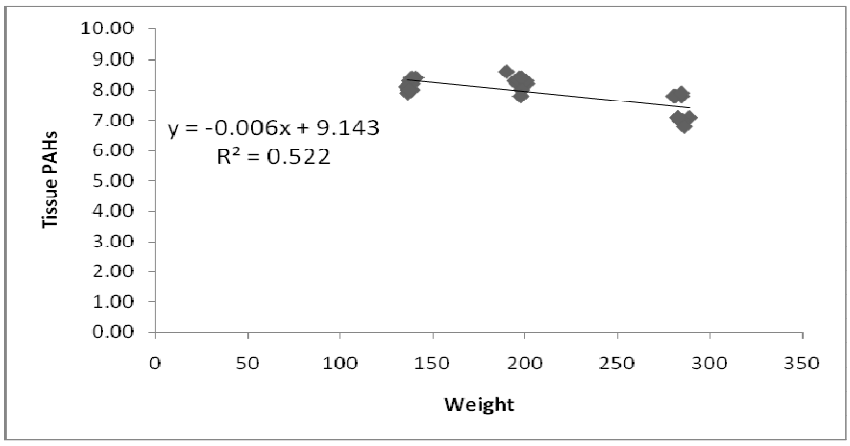


Fig 4: Correlation of weight (W) of *P. waltoni* with PAHs concentrations

The higher concentrations of cholesterol and glucose found in St_3 mudskippers can be due to contaminants, since changes in carbohydrate metabolism measured as plasma glucose and cholesterol (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) may be used as general stress indicators in fish. In a stress situation, glucose production provides energy substrates to tissues, in order to cope with the increased energy demand. Cholesterol is major degradation products and indicators of carbohydrate, lipid and protein metabolism [15].

Some studies concluded that contaminants can induce hyperglycaemia and hypercholesteremia in different fish species [16]. Our data is compatible with a toxicant stress inducing higher glycaemia and cholesteremia.

In turns, plasma total protein was used as a general index of fish health. Although this parameter may be influenced by the diet, high values may reflect haemoconcentration, impaired water balance [17]. Under pollutant conditions such as St_3 station in our case study, fish species may constitute a providing energy to cope with the stress situation. Physiological mechanism with an important role in depletion of total protein (hypoproteinemia) content might also be attributed to the destruction or necrosis of cellular function and impairment in protein synthetic [18]. Based on Shakoori et al. [19], the depletion of total protein content also may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period. Some other studies indicate a decrease in total protein content during heavy metal exposure.

CONCLUSION

According to the results of this study and regional conditions such as high evaporation, semi-closed, waste water discharge, etc., in the Persian Gulf, the monitoring of contaminants bioaccumulation in the northern part of Hormuz Strait is necessary, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on aquatic organisms health.

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