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A study of some environmental biomarkers in fresh water clam (*Unio tigridis*) and fresh water crab (*Sesarma boulengeri*) in Hilla river

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Abstract

This study was designed to study some environmental biochemical markers such as (MT ,GPx , CAT, AchE, ROS, SOD, Cytochrome p 450) in two species of freshwater organisms (Clam :*Unio tigrids* and Crab: *Sesarma boulengeri*) which were collected during the study period (October 2014 to March 2015) from three sites in Hilla river, the concentration of above enzymes showed fast response of two species according to the water quality parameters fluctuation which effected by pollutants in study area .The species *Sesarma boulengeri* has recorded more response to the changing in water quality than another species. This study indicates that the biochemical markers different in response in aquatic organism according to water quality parameters fluctuation affected by pollution.

Key words: Environmental biomarker , aquatic organism ,clam , crab ,Hilla river.

Introduction

The rapid development of industry and agriculture leads to bring more pollutant in the environment either heavy metal or organic compounds which undergo transformation or degradation by aquatic organisms [1].

In balance exist between the amount of free radical generated and antioxidant advires in normal condition to disposal them, thereby protecting the organism from effects of pollutants, oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted as a result of the depletion of antioxidants or excessive accumulation of the reactive oxygen species ROS ,or both leading to damage to macromolecular components [2]. *Sesarma boulengeri* has five pairs of legs used to walk and swim .. Including a pair is a huge hooks called the Gulf Edaed used in eating and hunting, and defend itself, and can through these claws to destroyed the shells easily to get what's inside of molluscs[3]. In the studies considered other species of crustaceans vital as an indicator of pollution or considered the existence of indicators of pollution inside their bodies , A study has been on the assessment and the monitoring of contamination in the sediments using a type of crustacean *Ruditapes philippinarum* and test biological accumulation and biological response index which was rated heavy influence and control pollution at the site of sediment transport from Lake Shihwa to operation of tidal power plant Was exposing these crustaceans in sediment pollutants hours and different levels of nonylphenol of heavy metals and after measuring the level of contaminants in water, sediment and crustacean through the level MT, proteins and effectiveness of enzymes oxidative stress found that heavy elements moved to the Clam through the high efficiency of the enzymes oxidative stress and the physiological response because of sediments [4] .

Material & Methods

The study was included three sites on Al-Hilla river from north of city which is the first site (Sinjar region), this site is far about (7 Km) from the center of Hilla city , second sites (Al- farsi region) is far at south of the city, and third site (Hashemia region) which far about (35 Km) of the center of Hilla city , and the variability of the sites are according to specification in component and diversity of living organisms.(Figure 1).

Water samples water collected over six months with measured some of the water quality parametes such as (pH-Temperature-EC- Salinity- TSS-TDS-D.O.-BOD-) by multi 350i Germany. Samples of two fish species (*Sesarma boulengeri* , *Uniotigrids*) were collected from study sites, from October 2014 to March 2015 and placed in cool Box until extraction of enzymes by homogenization by using Pestle motor Mixer Provided by Argos Technologies (U.S) Cat.No.A0001 in 50 mM Potassium Phosphate buffer (pH 7.0) with centrifugation (14000 r.p.m, 4°C, 15 min.) , finally, each enzyme was measured according to procedure clarified by ELISA Kit (Elabescience Company, China).

Statistical Analysis

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



Fig. (1): Satellite Image of study sites on Hilla River, middle of Iraq.

Results

This study was included some of physical and chemical properties of the Al-Hilla river in the three study site and determine the water quality through biochemica (biomarker) through measuring the concentrations of enzymes for the two fish species.

The results of the temperatures in the study stations between (12- 22.8 c°) in the first station and (13- 22) in the second station and (11- 25) in the third station and the highest temperature ever recorded in October in the first station and ranged values of the (pH) between (8.70-12) in the first station (8.10 – 11.0) in the second station (6.70- 12.3) in the third station, where the highest value recorded from pH . and higher values of (TDS) recorded in the third station and the highest value recorded for the (TSS) in the second station and the highest value of (DO) recorded in the third station in March so were the values of the (BOD5) in the third station is the highest value (0.01- 3.5) in March and the month higher the values of salinity in the first and the third station in most months.(Table 1).

Table 1: Physicochemical parameters in study area sites during study period.
 *Rang (first line) and Mean±SD (second line).

Sites	Site 1	Site 2	Site 3
Parameters			
W.Temp. C°	(12-22.8) (16.9667+ - 4.04063)	(13- 22) (17.3333 +- 3.82971)	(11-25) (17.1667 +- 4.833391)
pH	(8.70- 12.00) (9.6667 +- 1.20941)	(8.10-11.0) (10.3333+ - 1.14833)	(6.70-12.30) (9.3833 +- 1.87554)
EC(µc/cm)	(1088-1236.0) (1.1937+ - 61.28513)	(1088-1236.0) (1.1927 +- 59.46315)	(1042-.1255) (1.1820 +- 58.92)
Salinity ppt	(0.50- 6.00) (1.5667+- 2.18785)	(0.50 – 0.60) (0.5500+- 0.05477)	(0.30- 6.00) (0.5167 +-0.050)
T.D.S.(mg/l)	728.9-828.1 (1.1952 +- 64.16048)	728.9-828.1 (1.1957+- 60.06219)	698.1- 840.8 (1.1855+- 59.54829)
T.S.S.(mg/g)	(20.2 – 29.1) (23.7550 +- 3.00009)	(22.63 – 50.20) (35.4717 +- 4.4790)	(24.20-34.20) (29.1783+ - 3.99740)
DO(mg/l)	(1-6.80) (5.6267 +- 2.27338)	(1.50- 6.70) (5.7167 +- 2.07115)	(6.00- 9.00) (7.3833 +- 0.97245)
BOD ₅ (mg/l)	(0.1-2.30) (0.8967+-0.90778)	(0.1-2.5) (1.3000 +-0.98184)	(0.01- 3.5) (1.1850 +- 1.315)

P < 0.05

The results in (Figure 2-3-4) and table (2,3,4) refers to variation in value and according to the statistical analysis results showed significant changes in the concentrations of enzymes in this species , the concentrations of the enzyme SOD within the significant differences between the three sites and the ranged of (1.163-10.5 U/mg) in the st.1 (0.63-12.8 U/mg) in the st.2 and (0.30-2.7 U/mg) in the st.3 highest value 12.8 U/mg recorded in the st.2 during January 2014, And the lowest value(0.15 U/mg) recorded in the st.1 during October 2014, While varied concentrations of the ROS within the ranges varying (60.5 -673.2 U/mg) in the st.1 (76.4- 352.3 U/mg) in the st.2 and (78.8-323.7 U/mg) in the st.3 and its highest value 673.2 U/mg recorded in the st.1 during October 2014 and the lowest value 60.5 U/mg recorded in the st.1 during March 2015. Varied concentrations of the enzyme AChE in this species within the significant differences between

the sites and the ranged of (0.7- 34.4 U/mg) in the st.1 and (0.69- 4.53 U/mg) in the st.2 (0.60 – 40.07U/mg) in the st.3 while highest value 40.07U/mg recorded in the st.3 in February 2015 and the lowest value 0.60U/mg recorded in the st.3 during March 2015. The study results showed that the concentrations of the MT were among the ranges (83.2 -5834.7U/mg) in the st.1 (83.2-4719U/mg) in the st.2 and (130.2-5425U/mg) in the st.3 and the highest value 5834.7U/mg found in the st.1 during February 2015 and the lowest value 83.2U/mg recorded in the first and second site during December 2014. concentrations of the enzyme CAT were within the significant differences between the three site (28.9-1174.2U/mg) in the st.1 (10.73-291.7U/mg) in the st.2 (20.5-1174U/mg) in the st.3 and highest value (1174.2U/mg) recorded in the st.1 during February 2015 and the lowest value 10.73U/mg recorded during October 2014. In contrast concentration of enzyme GPx within the significant differences and within the ranges (63.9-4902U/mg) in the st.1 (199.2-6604U/mg) in the st.2 and (462.8-4002.8U/mg) in the st.3 and the highest value recorded was 6604.8U/mg in the st.2 during February 2015 and the lowest value recorded was 63.9U/mg in the st.1 through November 2014.

(Table 2).: Biochemical markers in (*Sesarma bouleengeri*) in site 1 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CYPs s U/mg
<i>Sesarma Boulengeri</i>	October(2014)	10.56	6.73	8.91	1.39	2.42	8.17	10.50
		+ -	±	±	±	±	±	±
		0.01000	0.10	0.001	0.10	0.10	0.01	0.10
	November(2014)	2.50	1.59	34.46	5.79	1.15	63.97	2.42
		+ -	±	±	±	±	±	±
		0.10	0.01	0.01	0.10	0.10	0.01	0.01
	December(2014)	3.00	1.55	0.77	83.27	28.91	5.004	36.30
		+ -	±	±	±	±	±	±
		0.00	0.10	0.01	0.01	0.010	0.10	0.10
	January(2015)	3.01	3.68	4.90	1.50	2.21	2.31	0.68
		+ -	±	±	±	±	±	±
		0.01	0.01	0.10	0.10	0.01	0.10	0.01
	February(2015)	2.76	1.932	25.25	5.83	1.17	4.14	36.59
		+ -	±	±	±	±	±	±
		0.01	0.10	0.01	0.10	0.10	0.10	0.01
	March(2015)	1.26	60.50	22.80	5.55	8.51	4.90	8.34
		+ -	±	±	±	±	±	±
		0.001	0.10	0.10	0.10	0.10	0.10	0.01

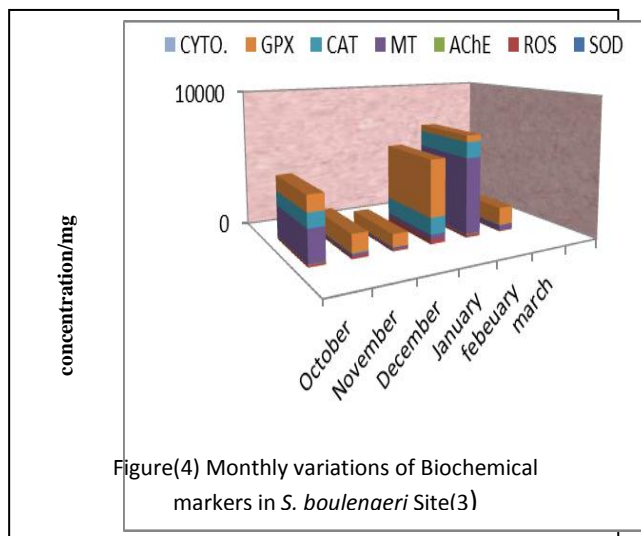
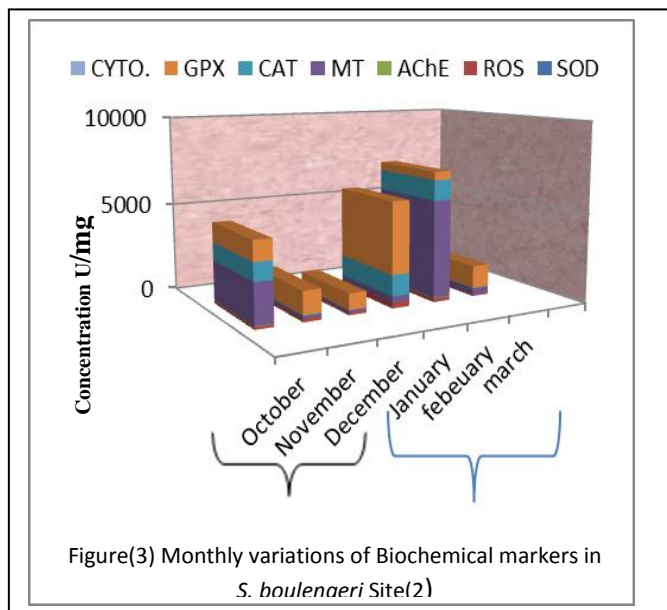
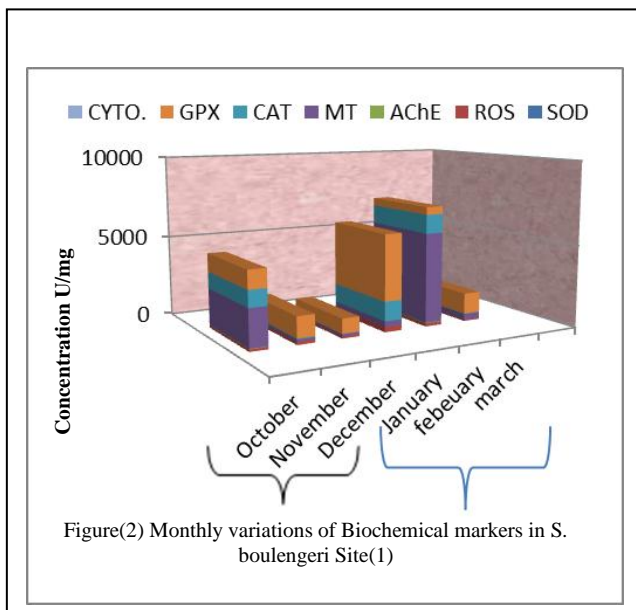
Table 3: Biochemical markers in (*Sesarma bouleleri*) in site 2 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CYPs s U/mg
<i>Sesarma Bouleleri</i>	October(2014)	10.83	1.92	0.85	98.52	10.73	1.99	0.36
		±	±	±	±	±	±	±
		0.010	0.10	0.001	0.010	0.010	0.100	0.010
	November(2014)	10.60	1.77	0.96	1.02	35.76	5.07	4.23
		±	±	±	±	±	±	±
		0.10	0.01	0.01	0.010	0.010	0.100	0.010
	December(2014)	12.80	1.85	1.49	83.27	11.52	4.85	0.99
		±	±	±	±	±	±	±
		0.10	0.01	0.001	0.010	0.010	1.00	0.010
	January2015	0.63	3.52	4.53	1.279	2.917	1.89	10.50
		±	±	±	±	±	±	±
		0.010	0.100	0.10	0.10	0.100	0.100	0.10
	Feberuary2015	3.36	76.40	7.970	4.71	2.300	6.60	26.44
		±	±	±	±	±	±	±
		2.48	0.100	0.010	0.10	1.00	0.100	0.010
	March2015	1.23	3.28	0.690	1.26	26.94	2.03	7.06
		±	±	±	±	±	±	±
		0.001	0.100	0.01	0.100	0.010	0.100	0.010

Table 4: Biochemical markers in (*Sesarma bouleleri*) in site 3 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CYPss 450 U/mg
<i>Sesarma Bouleleri</i>	October	0.38	1.46	31.34	2.25	9.98	1.10	0.350
		±	±	±	±	±	±	±
		0.010	0.100	0.010	0.100	0.100	0.100	0.010
	November	0.62	1.92	2.89	1.30	57.98	1.26	0.77
		±	±	±	±	±	±	±
		0.001	0.10	0.001	0.100	0.010	1.00	0.010
	December	1.32	1.22	0.89	1.35	20.56	8.94	1.38
		±	±	±	±	±	±	±
		0.010	0.100	0.010	0.100	0.010	0.100	0.010
	January	1.162	3.23	0.84	3.37	1.17	4.00	10.08
		±	±	±	±	±	±	±
		0.001	0.100	0.010	0.010	0.100	0.100	0.010
	Feberuary	3.21	1.89	40.07	5.42	1.13	4.62	36.60
		±	±	±	±	±	±	±
		0.01	0.010	0.010	1.00	0.100	0.100	0.100
	March	0.30	78.8	0.60	3.63	30.50	1.25	8.39
		±	±	±	±	±	±	±
		0.10	0.01	0.10	0.10	0.10	0.10	0.01

P< 0.05



The results in Table 5-6-7 and Figure (5-6-7) The study results showed with respect to species *U.tigridis* a discrepancy and significant differences in concentrations enzymes in the three sites of the study SOD concentration within the ranges and 0.610 U/mg in the st.1 (2.82-5.76U/mg) in the st.2 (0.63-5.84 U/mg) in the st.3, the highest value 5.84U/mg recorded in the st.3 during february 2015 and the lowest value was 0.63 U/mg in the st.1 during march 2015, While the concentrations of the ROS in the three sites within the ranges (147.4- 1066 U/mg) in the st.1 (76.9-1238 U/mg) in the st.2 and (141-1077 U/mg) in the st.3, highest value 1238 U/mg recorded in the st.2 during December 2014 and the lowest value 76.9U/mg in

the st.2 during January 2015, concentrations of the enzyme AChE showed significant differences between the three sites where varied range (0.30-34.5 U/mg) in the first site (0.35-33.61 U/mg) . in the st.2 and (0.52-41.3U/mg) in the st.3 and its highest value(41.3U/mg) recorded in the st.2 during February 2015 and the lowest value 0.30 U/mg recorded in st.1 . While varied concentrations of the enzyme MT have differences among the three sites and within the ranges (95.0-355 U/mg) in the st.1 (61.5-5400.7 U/mg) in the st.2 and (1510.9-5834.7 U/mg) in the st.3, highest value 5834.7 U/mg in the st.3 during February 2015 and the lowest value 61.5 U/mg in the st2 during January 2015. The concentrations of the enzyme CAT in this species varied within the significant differences between the three sites and within the ranges (155.8-1169 U/mg) in the st.1 (10.2 -1038U/mg) in the st.2 (50.6- 1174 U/mg) in the st.3 and its highest value 1174U/mg recorded in the st.3 during February 2015 and the lowest value 10.2 U/mg recorded in the st.2 during November 2014. concentrations of the enzyme GPx has significant differences and ranged 408.7-4887.3 U/mg) in the st.1 (134.5 -6099.8 U/mg) in the st.2 (22.31- 1142U/mg) in the st.3 and the highest valu 6099.8U/mg recorded in the st.2 in March 2015 and the lowest value (22.31 U/mg) recorded in the st.3 in January 2015. The results of the study indicates a variation in the concentration of the (Cytochrom P450) within the ranges (0.34735.30 U/mg) in the st.1 (0.531- 35.41 U/mg) in the st.2 and (0.35- 36.28 U/mg) in the st.3and the highest value (36.28 U/mg) recorded in the st.2 during December 2014 and the lowest value (0.35 U/mg) appeared in the st.3 during January

Table 5: Biochemical markers in (*Unio tigridis*) in site 1 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CYP s U/mg
<i>Unio tigridis</i>	October(2014)	5.08	1.24	0.30	1.39	1.55	1.78	2.89
		+ -	±	±	±	±	±	±
		0.01	0.10	0.001	0.10	0.01	0.01	0.01
	November (2014)	3.15	7.06	1.21	5.79	3.08	4.25	0.34
		+ -	±	±	±	±	±	±
		0.01	0.01	0.0	0.10	0.01	1.00	0.00
	December (2014)	0.63	1.68	34.58	83.27	8.40	4.08	30.36
		+ -	±	±	±	±	±	±
		0.00	0.010	0.01	0.010	0.10	0.10	0.01
	January (2015)	3.97	1.47	27.82	1.50	7.32	1.59	32.80
		+ -	±	±	±	±	±	±
		0.01	0.10	0.010	0.10	0.10	0.10	0.10
	February (2015)	2.92	3.29	2.38	5.83	2.92	4.88	34.83
		+ -	±	±	±	±	±	±
		0.010	0.10	0.001	0.10	0.10	0.100	0.01
	March (2015)	0.61	1.06	20.96	5.55	1.16	4.64	35.03
		+ -	±	±	±	±	±	±
		0.010	0.100	0.01	0.10	0.49	0.10	0.01

P < 0.05

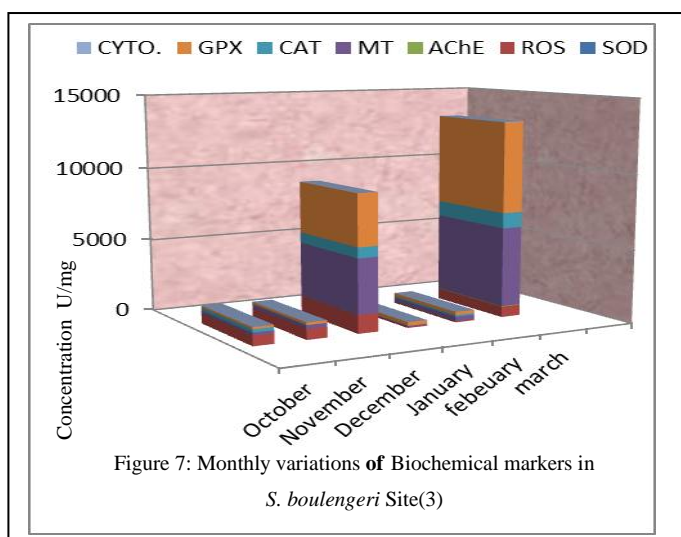
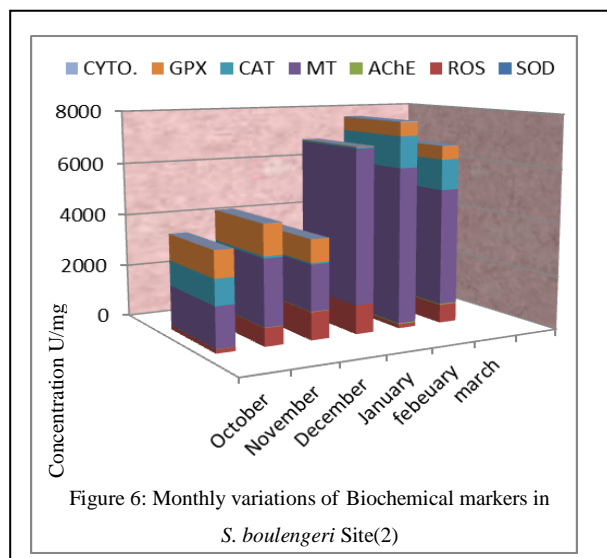
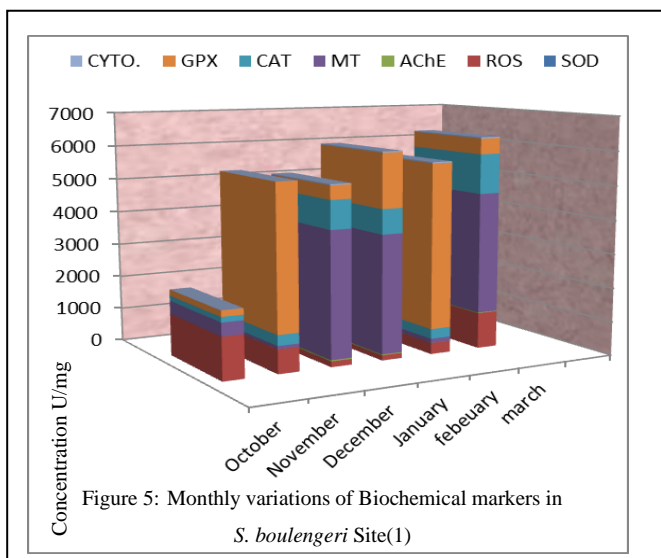
Table 6 : Biochemical markers in (*Unio tigridis*) in site 2 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CY s U/mg
<i>Unio tigridis</i>	October(2014)	5.50 ± 0.100	6.87 ± 0.10	0.35 ± 0.001	1.87 ± 0.010	2.12 ± 0.100	1.34 ± 0.100	4.4770 ± 0.001
	November(2014)	3.92 ± 0.010	7.130 ± 1.00	0.79 ± 0.001	2.50 ± 0.100	10.26 ± 0.010	1.64 ± 0.010	0.5870 ± 00.01
	December(2014)	5.76 ± 0.01	1.23 ± 1.00	5.37 ± 0.001	3.72 ± 0.100	7.12 ± 0.100	3.49 ± 0.100	4.560 ± 0.010
	January(2015)	3.14 ± 0.010	76.90 ± 0.100	0.63 ± 0.01	61.57 ± 0.010	15.50 ± 0.100	2.35 ± 0.100	1.2960 ± 0.001
	Feberuary(2015)	4.09 ± 0.01	78.09 ± 0.010	1.180 ± 0.01	3.08 ± 0.010	1.127 ± 0.100	2.18 ± 0.100	0.5310 ± 0.001
	March(2015)	2.82 ± 0.010	7.23 ± 0.100	33.61 ± 0.01	5.40 ± 0.100	1.038 ± 1.00	6.099 ± 0.100	35.41 ± 0.010

Table 7: Biochemical markers in (*Uniotigridis*) in site 3 during study period. Mean±SD

Species	Months	Biochemical biomarker						
		SOD U/mg	ROS U/mg	AchE U/mg	MT U/mg	CAT U/mg	GPx U/mg	CYP 450 U/mg
<i>Unio tigridis</i>	October(2014)	0.63 ± 0.01	1.50 ± 0.100	7.65 ± 0.010	1.51 ± 0.10	9.59 ± 0.100	9.95 ± 0.100	8.03 ± 0.001
	November(2014)	0.630 ± 0.010	6.79 ± 0.100	19.0 ± 0.001	2.46 ± 0.100	79.01 ± 0.010	1.14 ± 0.100	10.78 ± 0.010
	December(2014)	2.99 ± 0.01	1.03 ± 0.10	20.99 ± 0.01	1.72 ± 0.10	56.57 ± 0.01	8.57 ± 0.10	4.72 ± 0.001
	January(2014)	3.85 ± 0.010	1.07 ± 1.00	0.52 ± 0.010	5.74 ± 1.00	50.60 ± 0.10	22.31 ± 0.010	0.35 ± 0.010
	Feberuary(2014)	5.840 ± 0.010	1.41 ± 1.00	41.33 ± 0.010	5.83 ± 0.100	1.17 ± 0.100	4.99 ± 0.100	36.28 ± 0.010
	March(2014)	0.900 ± 0.100	6.89 ± 0.100	41.14 ± 0.010	4.38 ± 0.100	1.14 ± 0.100	5.03 ± 0.100	11.59 ± 0.19

P < 0.05



Discussion :

The rapid development of industry and agriculture all bring more pollutant in the environment either heavy metal or organic compounds which undergo transformation or degradation by aquatic organisms[1].

the impact of physical and chemical factors on the concentrations of biochemical markers in species *Unio tigridis* in the st.3 was to due to the nature of the response of this species of aquatic organisms to pollutants Reactive oxygen species ROS liberated with a high rate due to contaminant and thus it have limited ability to excrete contaminants via their excretion orange metabolism organic chemical and inactivate toxic heavy

metal therapy causing higher bioaccumulation of many toxicants This corresponds to with the many studies with [5,6].

Species *Sesarma boulengeri* showed different concentrations of enzymes in the three sites this species which is characterized by its tissue and shell help to deposition of heavy metals and minerals from the water and sediment and helps to deposition of iron, zinc, manganese, copper, according to a study [7,8]. It is worthy that this species in the three sites have varied enzymatic activities the first site is characterized by an increase in the secretion of the Cytochrom p450 which showed correlation negative with the value of the BOD5 that measure the organic oxygen demand, which is a measure of the presence of organic pollution through rate oxygen consumption. When consumed by organisms for their analysis to less complexity vehicles [9].

Therefore, and as a result of increased the biochemical markers secretion rate for MT and response of ROS, and then gradually low rate for ROS with increase MT and counter-productive because the MT excretion increases in Mollusca because of the metallothionin are involved in both homeostasis and detoxification and their accumulation is more obvious, hence reflection this has a significant role in accumulation, storage and excretion of metal in tissues [10].

This species of Molluscs in the st.1 was associated with significant correlation with the value of Electrical conductivity also increased secretion of the MT This means that the increase in electrical conductivity means an increase of pollutants and contaminated elements have activities and in aquatic environment because the increase EC means increased movement elements and thereby affected by responses the organism to the pollutants [11]. Since the MT is a very rich with Cystein, which is clearly associated with contaminated elements Cu, Cd, Zn so it responds to the movement of heavy metals contaminated and that means increased secretion of this enzyme in cellular response [12] But in terms of the concentration of the enzyme MT, GPx for species *Unio tigridis* the negative of having links with an enzyme SOD in the second site and to the fact that some of the enzymes is increasing its concentration, or at least according to the nature of the work of each enzyme affect the cellular response towards pollutant, So the response MT, GPx is different from the time and the rate of response to the SOD and so the one enzymes never work the second enzyme action and thus concentrations vary because of specialization in the enzymatic action determines the concentration of the enzyme that responds to the type of pollutant from the other enzymes We found that the first cellular biochemical markers appeared in response when exposed to contaminated is Cytochrom p450, followed by MT, SOD, CAT and GSH respectively. (Verlecar *et al.*, 2006). The fastest cellular response to pollutant come from concentrations MT since the natural product for the presence of heavy metal is the presence of availability of ROS SOD after the role of MT to remove of ROS. [13] At a time when at least the concentration MT increases the concentration SOD after it depending on the studying [14]. which pointer to GPx that the concentration decreases its focus during the winter and that the function protection of the cells from free radical particularly lipid peroxidation and this gives the inversion in the secretion of the enzyme with SOD.

The positive correlation between the concentration of the enzyme AChE and concentrations of enzymes type GPx, CAT, MT, Cytochrom p450 in the st.2, due to this enzyme reflects his work toward Specialist

materials Organic phosphate , Carbamate , Short half –live also pointed [15] ,depending on substrate hydrolysis and sensitivity to inhibition [16] , Because this type of mollusca has the ability to store materials and its accumulation and thus is an indication that the st.2 contains a type above by concentration the enzyme and specialty at its work, and follow the work that the enzyme action of enzymes MT,CAT,Cytochrom p450 correlation positive and it shows that the site contains organic compounds ,the action of cytochrom p450 can result in the production of $O_2 \bullet$, which in turn can be metabolized by superoxide dismutase SOD to H_2O_2 This hydrogen peroxide molecules can then be reduced to H_2O_2 and O_2 by catalase CAT [17]. The positive correlation between enzymes CAT, GPx may be due to the response comes linked positively to same site due to the stimulation of the two enzymes due to the competition between enzymes because of ROS which results either because lipids peroxid or heavy elements thus of the presence of the work GPx is not enough to get rid of contaminants so it was the work CAT complementary to get rid of the heavy elements, respectively [18] This reflects the activities of enzyme in the second site .

The inverse relationship between the enzymes in the third site, which has been associated with relationship counterproductive between SOD and MT,GPx does not only reflect the nature of the work, but rather reflects the rate and the concentration of pollutants and its kind, we found the first and third site contained a high concentration of SOD at the same time vary correlation negative with values GPx,CAT,MT While in the second site increase in the concentration of the MT,GPx,CAT compared with the another sites and lower SOD This gives idea that the second site contained high levels of heavy metals and compounds lipids peroxid and organic compounds and this is what was agreed with the studying [19] and which indicated that the species *Unio tigris* may discriminate in the second site secretion enzymes CAT,MT,GPx,AChE at a higher rate of SOD more than its activity in the first and third site.

Species *Sesarma bouleengeri* showed different concentrations of enzymes in the three sites this species which is characterized by its tissue and shell help to deposition of heavy metals and minerals from the water and sediment and helps to deposition of iron, zinc, manganese, copper, according to a study [7, 8]. it is worthy that this species in the three sites have varied enzymatic activities the first site is characterized by an increase in the secretion of the Cytochrom p450 which showed correlation negative with the value of the BOD5 that measure the organic oxygen demand, which is a measure of the presence of organic pollution through rate oxygen consumption. When consumed by organisms for their analysis to less complexity vehicles[9]. If the biological oxygen demand means the decomposition of organic matter and thus lower the rate of secretion enzyme and the surrounding where they become the DO in anaerobic conditions, whether in the water or sediment and the lack of oxygen is reflected on the aquatic organism [20] and correlation of biochemical markers MT,Cytochrom p450,CAT in the first and second site with value BOD5 which relationship with DO and it follows the decomposition of organic matter or not decomposition , especially the presence of Cytochrom p450 with O_2 which is important in the work of the of biochemical markers through its role in the binding Cytochrom p450 with Arylhydrocarbon receptor that are found in xenobiotic can not get rid of them (Halliwell,1999), The correlation concentration of enzyme

Cytochrom p with the value EC that are related to the situation ion heavy elements and rapid the response to organic pollutant[19].

All of this suggests that the second site More sites excretion of biochemical marker and this means that the proportion of exposure to pollutants .

Conclusion

We have concluded that biochemical marker in this study have important role in the evaluation of water quality and pollution degree according to response from aquatic organism to pollutants and fluctuation in water quality.

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