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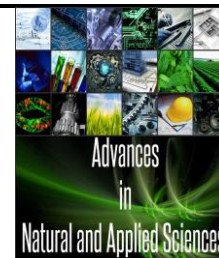
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Assessment of Water Quality by some Environmental Biomarkers in two Fish Species (*Tilapia zilli*, *Aspius vorax*) in Hilla River, Iraq

¹Shaimaa S.Mohamed-Ali, ²Jassim M. Salman, ²Ayad M. J. Almamoori

¹Local Environment researches Center, Babylon University, Iraq.

²Department of Biology, College of Science, University of Babylon, Iraq.

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ABSTRACT

This study was designed to evaluate water quality of Hilla River by using some environmental biochemical markers such as (MT, GPx, CAT, AchE, ROS, SOD, Cytochrome p 450) in two types of freshwater fish (*Tilapia zilli*, *Aspius vorax*) which were collected during the study period (October 2014 to March 2015) from three sites in Hilla river over six month, the concentration of above enzymes showed fast response of two types of fish species according to the water quality parameters fluctuation which effected by pollutants in study area, fish species (*Tilapia zilli*) 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.

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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 (Wang, 2013), these organisms are often exposed to complex mixture of pollutants, which may lead to adverse effects in the these organisms. Aquatic pollution is a major contributor to oxidative stress in fish resulting from the redox cycling of pollution (Arinc, 2000) The pollutants can effect on structural and functional properties in living organisms from the cellular and molecular level to higher biological levels, such as populations or communities (Maria, 2009)

The pollutants enter fish through a number of routes such as skin, gills, oral consumption of water, food and non-food particles and transported in the blood stream to either a storage point, or to the liver for transformation and storage (Nussey, 2000). The cellular responses in the fishes may be happened by chemical biomarkers (enzymatic and non-enzymatic) activities (Nordberg, 2001) This system includes many antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (Van der Oost, 2003), (Yildirim, 2011).

A biomarker is defined as a "biochemical, cellular, physiological or behavioral variation that can

be measured in tissue or body fluid samples or at the level of whole organism, to provide evidence of exposure or effects from one or more contaminant (Depledge, 1994), Biomarker as predictive of advanced toxicity at higher biological levels and using comes from their sensitivity and specificity to pollutants and also for ideal reasons such as the cost and time associated with measuring a stress response (Connors, 2004).

The need for assessment of aquatic ecosystem contamination and of its impact on water dwelling organisms has developed in response to rising aquatic environmental pollution, and lead to widely used to bioindicators for assessment water quality in both marine and fresh water environments (Bamgbose, 1993). This study aimed to evaluate the water quality in Hilla river by some environmental biochemical markers response in two species of fish.

MATERIAL AND 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 were collected over six months with measured some of the water quality parameters such as (pH-Temperature-EC- Salinity- TSS-TDS-D.O.-BOD₅) by multi 350i Germany. Samples of two fish species (*Tilapia zilli*, *Aspius vorax*) 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.

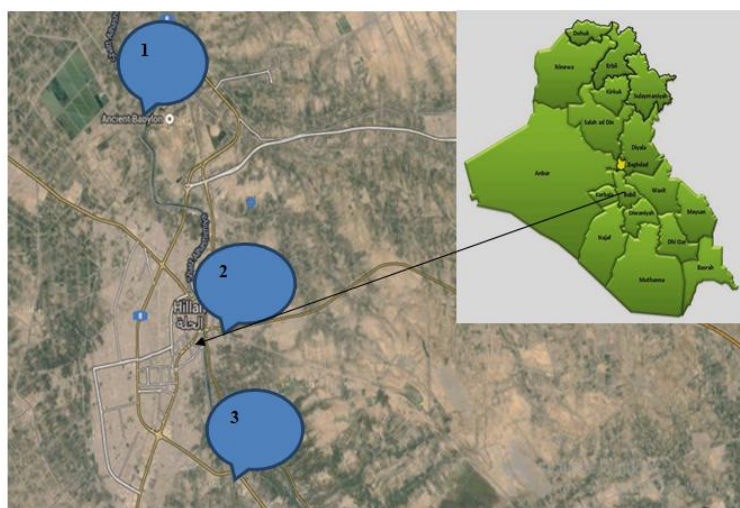


Fig. 1: Satellite Image of study sites on Hilla River, middle of Iraq.

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

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

biochemical (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).

While the results of the concentrations of the enzymes in the three sites revealed that the highest concentration of the enzyme (Metallothionin) in

March in the first station of the second type of fish (*Aspius vorax*), and the highest concentration for the enzyme (MT) in the month of December in the third site of the fish (*Tilapia zilli*) (Table 4).

There were significant positive links between concentrations of all enzymes (GPx, CAT, MT, AChE) and temperatures in the first station of the type of fish (*T.zilli*) and by a factor of correlation ($r = 0.585, 0.559, 0.546, 0.471$) at respectively, and it found a significant correlation positive between the concentration of the two enzyme (ROS) ($r = 0.484$) for the type of fish (*A.vorax*) with an enzyme (SOD) in the first site and the presence of significant correlation positive also between the concentration of enzyme (CAT) to the type of fish (*A. vorax*) with (Table 2)

Table 2: Biochemical markers in fish species (*Tilapia zilli*- *Aspius vorax*) 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 U/mg
<i>Tilapia zilli</i>	Oct. (2014)	3120 ± 0.00100	1.9107 ± 0.0100	1.0650 ± 0.0010	2.7440 ± 0.100	4,7300 ± 0.0100	22.700 ± 0.100	0.3790 ± 0.001
	Nov. (2014)	0.3000 ± 0.100	1.9313 ± 0.0100	0.7550 ± 0.0010	99.11 ± 0.010	13.970 ± 0.0100	1.8660 ± 0.100	3.0400 ± 0.0100
	Dec. (2014)	0.6300 ± 0.0100	3.0530 ± 0.1000	3.0009 ± 0.0001	6.6790 ± 0.100	1.4076 ± 0.0100	2.6260 ± 0.100	0.5800 ± 0.0100
	Jan. (2015)	0.3120 ± 0.001	6.3340 ± 0.1000	0.5250 ± 0.0010	2.860 ± 0.100	10.970 ± 0.0100	1.780 ± 0.100	0.660 ± 0.0100
	Feb. (2015)	2.6500 ± 0.0100	3.4570 ± 0.1000	1.1200 ± 0.0100	1.314 ± 0.01	47.200 ± 0.100	8.5710 ± 0.100	4.7200 ± 0.0100
	Mar. (2015)	2.3600 ± 0.0100	1.9050 ± 0.1000	0.4500 ± 0.01000	1.417 ± 0.100	27.060 ± 0.0100	4.425 ± 0.100	8.9660 ± 0.001
<i>Aspius Vorax</i>	Oct. (2014)	0.1590 ± 0.0100	1.8570 ± 0.1000	2.0950 ± 0.00100	5.4430 ± 0.100	1.4340 ± 0.100	1.7390 ± 0.100	2.4500 ± 0.0100
	Nov. (2014)	0.3000 ± 0.1000	1.9360 ± 0.1000	0.7050 ± 0.00100	93.200 ± 0.100	11.76 ± 0.0100	1.1830 ± 0.100	0.330 ± 0.0100
	Dec. (2014)	1.2300 ± 0.0100	1.5807 ± 0.01000	9.9700 ± 0.0100	1.0259 ± 0.100	2.6970 ± 0.100	1.7430 ± 1.00	9.7100 ± 0.0100
	Jan. (2015)	1.2240 ± 0.0100	1.8460 ± 0.1000	7.7340 ± 0.00100	9.4840 ± 0.100	1.4180 ± 0.100	1.2830 ± 1.00	8.2400 ± 0.0100

	Feb. (2015)	0.6300 ± 0.0100	6.4370 ± 0.1000	5.2300 ± 0.0100	5.5480 ± 0.100	2.665 ± 0.100	3.3170 ± 0.100	2.1500 ± 0.0100
	Mar. (2015)	0.600 ± 0.100	1.5430 ± 0.1000	3.1660 ± 4.8167	2.2253 ± 0.100	2.2840 ± 0.100	4.268 ± 0.100	19.9400 ± 0.0100

P < 0.05

The concentration of the enzyme (AChE) in the (*T.zilli*) of fish when the correlation coefficient (r = 0.470), in the same time Significant correlation was found between the positive correlation coefficient (r

= 0.579) between the values of enzyme (GPx) in the (*A. vorax*) of fish with the concentration of the enzyme (CAT) in the second site (Table 3)

Table 3: Biochemical markers in fish species (*Tilapia zilli- Aspius vorax*) in site 2 in Hilla river . (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 U/mg
<i>Tilapia zilli</i>	Oct. (2014)	3120 ± 0.00100	1.9220 ± 0.100	8.5990 ± 0.00100	2.5822 ± 0.100	1.0806 ± 0.100	4.9786 ± 0.10	22.200 ± 0.100
	Nov. (2014)	0.3000 ± 0.100	1.8860 ± 0.1000	0.5300 ± 0.01000	68.610 ± 0.0100	4.690 ± 0.0100	1.6690 ± 0.100	2.5700 ± 0.0100
	Dec. (2014)	0.6300 ± 0.0100	1.9080 ± 0.1000	7.6510 ± 0.00100	1.3024 ± 0.100	2.9820 ± 0.100	5.166 ± 0.100	4.336 ± 0.001
	Jan. (2015)	0.3120 ± 0.001	3.3080 ± 0.1000	0.4200 ± 0.01000	1.352 ± 0.100	2.7190 ± 0.100	2.626 ± 0.100	1.166 ± 0.001
	Feb. (2015)	2.6500 ± 0.0100	1.7100 ± 1.000	1.2300 ± 0.01000	1.421 ± 0.100	56.310 ± 0.0100	4.011 ± 0.100	35.6400 ± 0.0100
	Mar. (2015)	2.3600 ± 0.0100	1.555 ± 0.0707	40.5150 ± 0.00707	5.9135 ± 0.070	5.325 ± 0.070	1.699 ± 0.007	0.2750 ± 0.007
<i>Aspius Vorax</i>	Oct. (2014)	0.1590 ± 0.0100	1.9780 ± 0.1000	0.6050 ± 0.00100	3.5710 ± 0.100	54.380 ± 0.010	1.544 ± 0.100	9.400 ± 0.100
	Nov. (2014)	0.3000 ± 0.1000	1.8160 ± 0.1000	0.3590 ± 0.00100	2.8190 ± 0.100	2.8800 ± 1.00	1.288 ± 0.100	4.0300 ± 0.0100
	Dec. (2014)	1.2300 ± 0.0100	1.6307 ± 0.11269	0.6000 ± 0.1000	97.900 ± 0.100	44.530 ± 0.0100	66.900 ± 0.100	36.800 ± 0.100
	Jan. (2015)	1.2240 ± 0.0100	1.9220 ± 0.1000	0.3360 ± 0.00100	1.440 ± 0.100	57.860 ± 0.0100	1.225 ± 0.100	2.1200 ± 0.0100

	Feb. (2015)	0.6300+ ± 0.0100	7.2440 ± 0.1000	0.6000 ± 0.1000	97.300 ± 0.100	31.00 ± 1.00	2.7740 ± 0.100	0.6900 ± 0.0100
	Mar. (2015)	0.600 ± 0.100	1.7793 ± 19.2547	29.8100 ± 9.28393	1.9549 ± 0.100	7.517 ± 189.6	1.346 ± 0.070	14.1967 ± 12.04

P< 0.05

There is a significant correlation between positive ($r = 0.538$) between the enzyme (ROS) in kind (*A. vorax*) with the focus (MT) in the third station. Amore were high concentrations in terms of

secretion of enzymes by the fish in them and the third station is more the type of fish with a high level of enzyme secretion in type enzyme is the type (*T.zilli*).

Table 4: Biochemical markers in fish species (*Tilapia zilli*- *Aspius vorax*) in site 3 in Hilla river. (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 U/mg
<i>Tilapia zilli</i>	Oct. (2014)	3120 ± 0.00100	1.9530 ± 0.1000	7.7700 ± 0.01000	1.360 ± 0.100	16.150 ± 0.010	1.0640 ± 1.00	0.322 ± 0.0001
	Nov. (2014)	0.3000 ± 0.100	1.8020 ± 0.1000	4.3800 ± 0.01000	75.650 ± 0.0100	12.63 ± 0.010	1.4780 ± 0.100	0.5600 ± 0.010
	Dec. (2014)	0.6300 ± 0.0100	1.9290 ± 0.1000	34.7000 ± 0.1000	5.612 ± 0.100	1.1909 ± 0.100	1.3290 ± 1.00	4.5700 ± 0.0100
	Jan. (2015)	0.3120 ± 0.001	1.8950 ± 0.1000	18.0100 ± 0.01000	2.4427 ± 0.100	7.5310 ± 0.100	1.0280 ± 0.100	0.6400 ± 0.0100
	Feb. (2015)	2.6500 ± 0.0100	77.900 ± 0.1000	1.4450 ± 0.00100	1.0322 ± 0.100	1.571 ± 0.0100	4.199 ± 0.100	34.500 ± 0.100
	Mar. (2015)	2.3600 ± 0.0100	1.8415 ± 0.0707	9.4535 ± 0.00071	4.522 ± 0.070	41.32 ± 0.007	1.258 ± 0.0707	2.2435 ± 0.0071
	<i>Aspius Vorax</i>	Oct. (2014)	0.1590 ± 0.0100	3.0210 ± 0.1000	2.5100 ± 0.01000	1.248 ± 0.100	2.280 ± 0.0100	1.889 ± 0.100
Nov. (2014)		0.3000 ± 0.1000	1.8640 ± 0.1000	0.7150 ± 0.00100	2.819 ± 0.100	37.150 ± 0.0100	1.1610 ± 0.100	0.6400 ± 0.0100
Dec. (2014)		1.2300 ± 0.0100	3.4770 ± 0.1000	1.2550 ± 0.00100	92.070 ± 0.100	37.400 ± 0.100	1.4150 ± 0.100	0.3600 ± 0.0100
Jan. (2015)		1.2240 ± 0.0100	49.4000 ± 0.1000	1.1700 ± 0.01000	1.342 ± 0.100	48.700 ± 0.100	62.900 ± 0.100	4.600 ± 0.100

	Feb. (2015)	0.6300 ± 0.0100	1.4610 ± 0.1000	0.8750 ± 0.00100	2.860 ± 0.100	15.07 ± 0.0100	2.859 ± 0.100	0.6300 ± 0.0100
	Mar. (2015)	0.600 ± 0.100	4.6253 ± 240.95	17.3550 ± 6.84160	1.258 ± 698.0	3.6808 ± 282.9	3.648 ± 3050.3	7.0450 ± 4.1569

P < 0.05

T.zilli has more response in secretion to these enzymes than another type *A. vorax* (Fig.2-7).

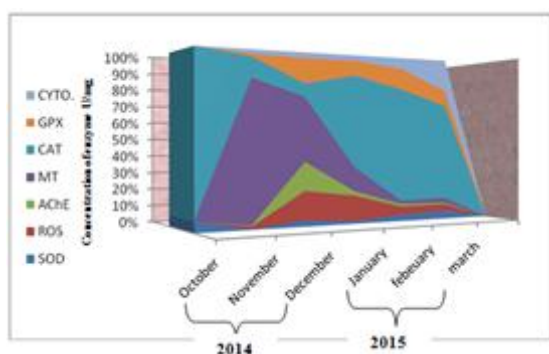


Fig. 2: Concentration of enzyme in *T.zilli* in site (1).

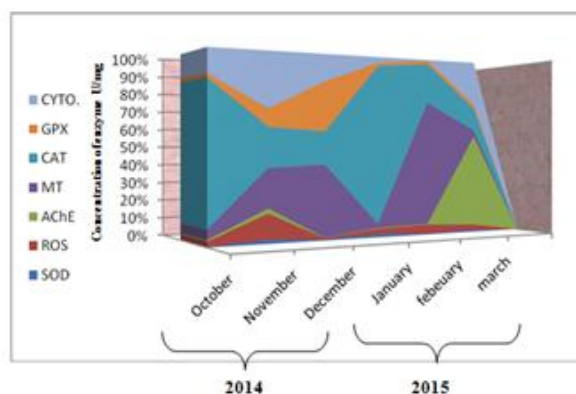


Fig. 5: Concentration of enzyme in *A.vorox* in Site (2)

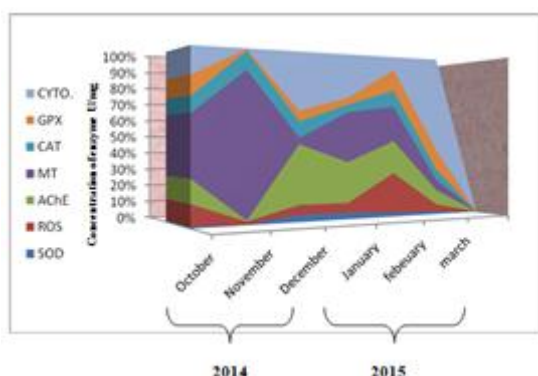


Fig. 3: Concentration of enzyme in *A.vorox* in site (1).

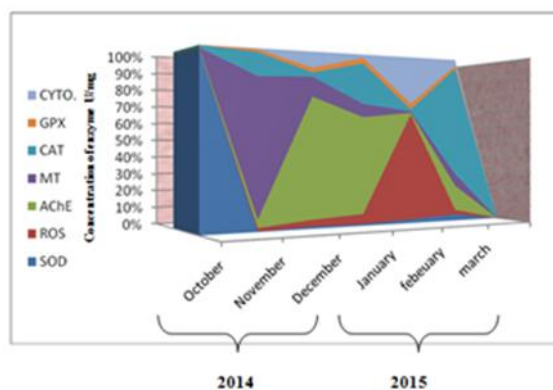


Fig. 6: Concentration of enzyme in *T.zilli* in site (3)

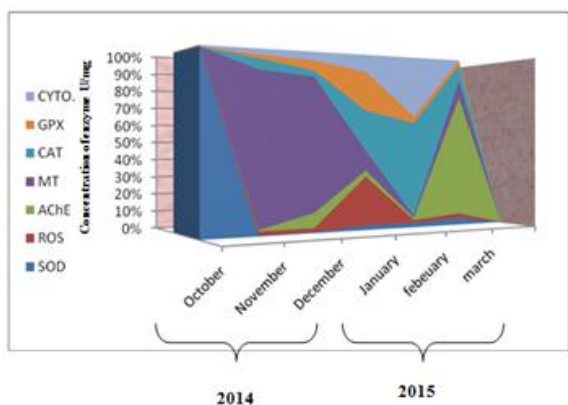


Fig. 4: Concentration of enzyme in *T.zilli* in site (2).

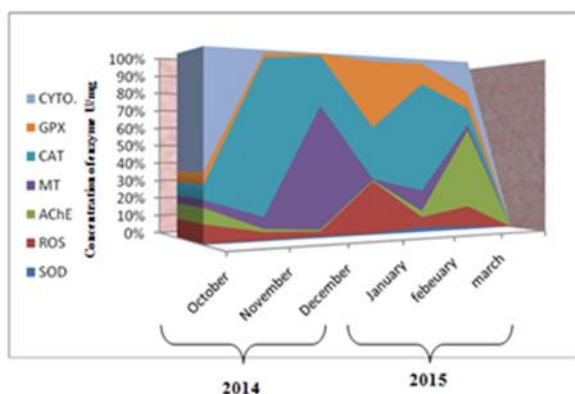


Fig. 7: Concentration of enzyme in *A.vorox* in site (3)

Discussion:

According to the changes of physical and chemical environment of the three stations there were changes in the level of concentration of enzymes values the highest temperature, which was in the month of October in the first site corresponding concentrations high enzymes (GPx,CAT, MT,AchE,) in (*T. zilli*) and (MT) in (*A. vorax*) this is due to high temperature in the summer months leads to stimulate and increase the accumulation of pollutants, especially (Zn) which in turn leads to increase in toxicity (Everall,1987),thus for the cellular response to the secretion of enzymatic tissue where this type of fish (*T. zilli*) accumulate in the interweave of pollutants and thus give the fastest response (Ibrahim, 2013), It was noted in the third site has higher values in (pH, DO,BOD₅,Salinity) in the temperate months for temperature (in March) and (October) and this highest concentration increase pollutants may be of heavy elements because this station accompanied in the same month by increasing in the concentration of (MT, ROS,) in the two species of fish and this as indicated by some studies (Wenjuan, 2012) and that the increase in the presence of those values of moderation in temperature confirmed by this study.

The increase in salinity in the third site and the first site accompanied by a rise in the concentration of enzymes (ROS, SOD) and (ROS, MT) respectively in both types from fish, and this goes back to the level of environmental pressures that are found in plants may be caused by vegetation or and as confirmed by a study (Ross, 2006) which indicated that the increase of exposure to salinity lead to an increase in the level of enzymes (ROS,SOD, CAT) with including the level of secretion variation, Or may be due to the sources of domestic waste put on the edges of the river and this increases the accumulation of heavy metal have led to the production of these enzymes (Kadar, 2005)and other added to the flow of that region periods because of the open gate to the bridge leading to

recession salts and therefore this leads to environmental pressures on the fish life.

The results indicate that the highest values of enzymes were in the third site and concentrated in (*T. zilli*),This means that this type of fish has the ability to accumulate biological heavy metals and toxins, according to concentrations in (gills – Intestine - muscle) respectively (Enjie,2011).

The high concentration of the enzyme (CAT) in the second site of the (*A.vorax*) that guide and index to the presence of persistent organic pollutants because it is excreted from the cells in the fish cellular response towards the kind of contaminated such as Catalase, it is beneficial to eliminate the excess H₂O₂ to protect the cells from oxidative damages (Dizdaroglu,2002)

Through the concentration of enzymes in the three sites, it found that more factors affecting the concentrations of enzymes are the temperatures, (BOD₅), (TSS) and (TDS) respectively, and more sites has a highest concentration in the level of enzymes is the second site and this indicates the level of contamination based on the response to the secretion of enzymes level . At the same site (second) was seen that high concentration of the enzyme (GPx) has not been seen in the first and the third site in both types of fish, and this indicates that the type of contaminated differed from what exists in the other sites, especially in the case of a increasing concentrations of chromium or organic concentrated fertilizer (Velma,2000)

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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