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SEASONAL VARIATIONS OF BIOCHEMICAL MARKERS IN TWO SPECIES OF MOLLUSCS AS INDICATION OF HEAVY METALS POLLUTION

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ABSTRACT
This investigation was designed to study the seasonal variation of biochemical markers (Total protein, Vitamin C, Vitamin E, Acetylcholinesterase and Metallothioneins) in two species of Molluscs(*Viviparus bengalensis* and *Corbicula fluminea* from two sites of Al-Hilla river, the results have recorded seasonal variations of totalprotein (17.2,11.9mg/g),Vitamin C (6.8,7.2µg/mg),VitaminE(11.8,12.1µg/mg),Acetylcholinesterase(48.3,50.3nmol/min./mg), and Metallothionein (53.8, 42.3 µg/g) in snail & Clam respectively with highest concentration in digestive gland than gills. The highest values for all biochemical markers have recorded during spring and summer season 2011.

KEY WORDS: Biochemical markers, Seasonal variations, heavy metals , Total protein, Acetylcholinesterase, Vitamins, Metallothioneins

INTRODUCTION
Heavy metals have complex spectra, form colored salts and double salts, and they have been shown to produce marked toxicity at the target sites, and effect on DNA, which is the carrier of inherited information and any gross change in its structure potentially, indicates serious biological changes, Biomarkers 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 with measuring a stress response (Conners , 2004). Vitamin C is a water-soluble vitamin. Like vitamin E, it is an effective antioxidant. Vitamin C can protect essential substances in the body such as proteins, lipids, carbohydrates, DNA and RNA from damage by free radicals (Padayatty etal., 2003). Metallothioneins constitutes an important binding protein with low molecular weight (6-7) KDa and high cysteine content (>30%) which play an important role in detoxification of toxic chemicals and have a role in the maintenance of homeostasis of essential metals and used as biomarkers to assess the toxicity of pollutants (Huang etal., 2007), Acetylcholinesterase is old biomarker may be responding to low level of pollution in the environment (Bouldin etal., 2007) and are considered as unique enzyme whose physiological function to remove acetylcholine from synaptic clefts. Biomarkers can be considered as early defense response for heavy metals impact , So (Bigo etal., 2011) measured the early effects of copper (10 and 50 lg L21), cadmium (2, 10, and 50 lg L21) and mixtures of these metals in the freshwater bivalve Corbicula fluminea exposed for 12 h in laboratory, Measurement of acetylcholinesterase (AChE) is broadly used like present study as biomarker of pollution, AChE activity was monitored in the clam Tapes philippinarum in the Lagoon of Venice (Italy) by (Matozzo *et al*., 2005), no particular correlation between AChE activity and Condition Index (CI) was found, and marked differences in AChE activity were showed when comparing enzyme activity of clams from various sites in the Lagoon of Venice.

MATERIALS & METHODS
Snail and clam samples were collected seasonally 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, Protein concentration was estimated according to method of Bradford (1976), Vitamin C is present in both a reduced form ascorbic acid (AsA) and an oxidized form (dehydroascorbic acid (DHAsA), this method measures total vitamin C (*i.e.*, AsA + DHAsA), and according to (Omaye *et al*., 1979) , (Burtis & Ashwood, 1999). Vitamin E was determined according to (Quaife etal., 1949) which includes Emmerie and Engel color reaction with ferric chloride and α,α--dipyridyl to give a red color, Metallothionein was determined by evaluating the sulphyhydryl (-SH) residue content according to method of Ellman (1958) and (Viarengo etal., 1997). Anew and rapid colorimetric determination was adapted to calculate the Acetylcholinesterase activity in Molluscs according to method descirbed by Ellman *et al*.(1961).

RESULTS
Total protein values in gills of snail ranged between (2.9-6.2mg/g) (14.7-16.2 mg/g) in site 1 during autumn2011&winter 2012 and winter & spring in both gills and digestive gland respectively.non enzymatic antioxidants (Vitamin C&Vitamin E) had been measured seasonally , vitamin C has values ranged(1.6-4.5µg/mg) in site 1 during winter & summer in gills , Vitamin E values have a highest value (10.6 µg/mg) in gills during winter in site 2 and the lowest value in gills (5.6 µg/mg) in site 1

during winter while for digestive gland, Metallthionein values in gills of snail Sp. ranged (26.8-42.1 µg/g), (25.6-51.2 µg/g) in site 1& site2 during winter&summer respectively. Acetylcholinesteras activity concentration in gills represented with a highest value (nmol/min./mg) in site 2 during winter, and lowest value (20.1 nmol/min./mg) in site1 during summer(Table 1, Fig.1.2.5).

TABLE 1: Seasonal variations of biochemical markers Concentrations in Snail *Viviaparus bengalensis* [gills &digestive gland (d.g.)] in site 1 & site 2 of study area (Mean±S.D).

Sites	S.1				S.2				
Season	SP.	Sum.	Aut.	Wint.	SP.	Sum.	Aut.	Wint.	Control
	2011		2012		2011		2012		
T. protein (mg/g) gill	7.8 ± 0.1	4.1 ± 0.06	2.9 ± 0.01	6.2 ± 0.02	6.1 ± 0.1	4.3 ± 0.01	2.9 ± 0.02	9.3 ± 0.01	15 ± 0.01
Vit. C (µg/mg)gill	2.6 ± 0.1	4.5 ± 0.1	3.6 ± 0.3	1.6 ± 0.16	3.8 ± 0.152	3.7 ± 0.01	2.1 ± 0.01	6.7 ± 0.1	7.2 ± 0.1
Vit. E (µg/mg)gill	6.6 ± 0.1	8.4 ± 0.1	7.5 ± 0.02	5.6 ± 0.1	7.8 ± 0.01	7.7 ± 0.10	6.13 ± 0.06	10.6 ± 0.1	11.6 ± 0.1
MT. (µg/g) gill	38.1 ± 1.01	42.1 ± 0.01	32.6 ± 1.05	26.8 ± 0.01	30.3 ± 0.1	51.2 ± 0.11	27.9 ± 0.01	25.6 ± 0.1	22.5 ± 0.1
ACH.gill (nmol/min./mg)	40.3 ± 0.1	20.1 ± 0.01	30.4 ± 0.01	20.8 ± 0.1	20.9 ± 0.01	20.6 ± 0.1	40.1 ± 0.01	± 0.11	55.1 ± 0.01
T. protein (mg/g) d.g.	16.2 ± 0.1	15.8 ± 0.01	15.4 ± 0.06	14.7 ± 0.1	11.8 ± 0.1	12.8 ± 0.10	17.2 ± 0.2	11.7 ± 0.1	25.3 ± 0.1
Vit. C (µg/mg) d.g	4.4 ± 0.2	4.2 ± 0.01	2.5 ± 0.1	6.8 ± 0.11	3.9 ± 0.01	5.5 ± 0.1	5.8 ± 0.1	6.2 ± 0.21	6.9 ± 0.01
Vit. E (µg/mg) d.g	9.4 ± 0.1	9.3 ± 0.01	7.5 ± 0.1	11.8 ± 0.2	8.9 ± 0.01	11.5 ± 0.1	10.8 ± 0.1	11.6 ± 0.11	12.5 ± 0.1
MT. (µg/g) d.g.	48.9 ± 0.01	53.8 ± 0.1	44.8 ± 0.1	42.5 ± 0.001	46.1 ± 0. 06	48.8 ± 0.1	36.8 ± 0.1	37.8 ± 0.3	31.5 ± 0.1

$p \leq 0.05$

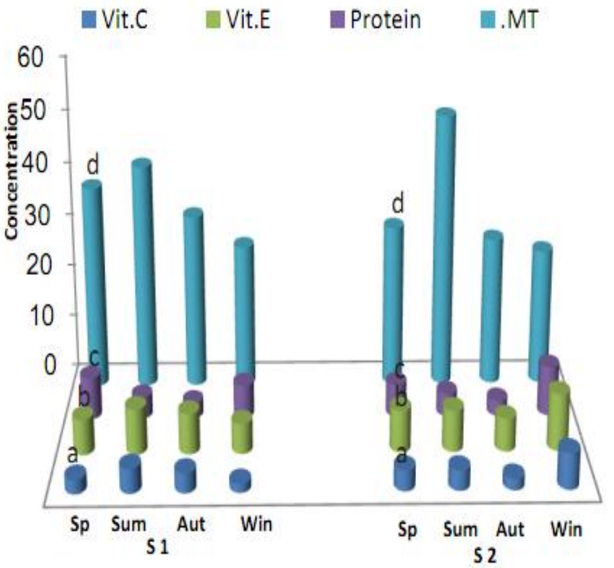

FIGURE 1: Seasonal variations of Biochemical markers in Snail (gill) in site 1&site2 of study area

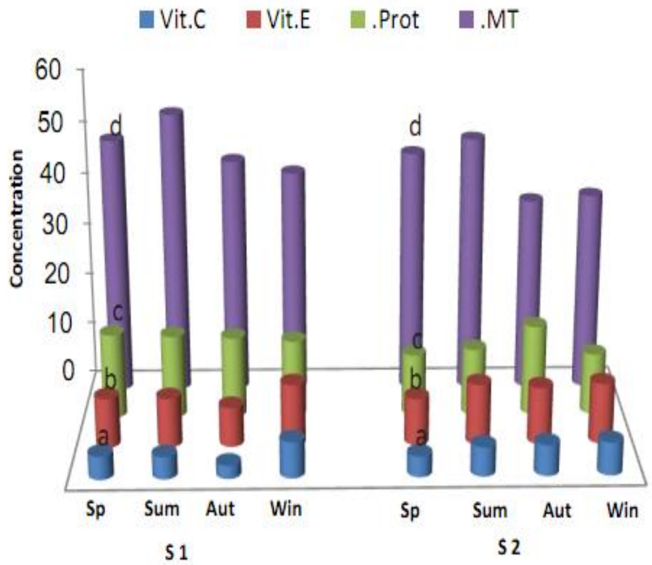

FIGURE 2: Seasonal variations of Biochemical markers in Snail (d.g) in site 1&site2 of study area

In clam Sp., additional biochemical markers exhibited different seasonal variations. Total protein values in gills ranged between (5.8-9.5mg/g)(7.6-10.2 mg/g) in site 1 both gills and digestive gland respectively. Vitamin C has values ranged(1.9-4µg/mg) in site 1 during autumn&summer in gills , and (1.6-4.7 µg/mg) in site 2 during autumn&spring in gills. Vitamin E values had a highest value (8.7 µg/mg) in gills during spring in site

2.Metallthionein values in gills ranged from (26.8-41.9 µg/g), (20.1-32.6 µg/g) in site 1& site2 during spring,summer,.Acetylcholinesteras activity concentration in gills represented with a highest value (0. nmol/min./mg) in site 1 during spring, and lowest value (20.8 nmol/min./mg) in site1 during summer(Table 2, Fig.3,4,6)

TABLE 2: Seasonal variations of biochemical markers Concentrations in Clam *Corbicula fluminea* [gills &digestive gland (d.g.)] in site 1 & site 2 of study area (Mean±S.D).

Sites		S.1				S.2			
Season	SP.	Sum.	Aut.	Wint.	SP.	Sum.	Aut.	Wint.	Control
	2011		2012		2011		2012		
T. protein (mg/g) gill	8.5 ± 0.1	7.2 ± 0.01	9.5 ± 0.1	5.8 ± 0.2	5.2 ± 0.10	5.3 ± 0.01	3.1 ± 0.1	2.9 ± 0.01	13.8 ± 0.2
Vit. C (µg/mg)gill	2.1 ± 0.01	4 ± 1.1	1.9 ± 0.1	3.5 ± 0.02	4.7 ± 0.1	4.2 ± 0.01	1.6 ± 0.1	1.8 ± 0.1	5.6 ± 0.01
Vit. E (µg/mg)gill	6.1 ± 0.1	7.2 ± 0.01	4.8 ± 0.1	8.2 ± 0.2	8.7 ± 0.1	8.2 ± 0.01	5.6 ± 0.2	5.8 ± 0.01	9.4 ± 0.11
MT. (µg/g) gill	26.8 ± 0.1	41.9 ± 0.01	39.5 ± 0.1	34.9 ± 0.02	27.9 ± 0.01	32.6 ± 0.1	21.3 ± 0.1	20.1 ± 0.001	18.1 ± 0.2
ACH.gill (nmol/min./mg)	40.3 ± 0.1	20.8 ± 0.01	30.2 ± 0.1	30.6 ± 0.33	30.4 ± 0.2	20.9 ± 0.001	50.3 ± 0.1	50.2 ± 0.01	59.1 ± 0.01
T. protein (mg/g) d.g.	11.8 ± 0.1	9.1 ± 0.01	11.9 ± 0.1	10.8 ± 0.11	9.6 ± 0.1	5.8 ± 0.01	5.4 ± 0.1	10.8 ± 0.11	16.5 ± 0.1
Vit. C (µg/mg) d.g	2.6 ± 0.1	6.8 ± 0.12	5.4 ± 0.2	5.2 ± 0.01	6.8 ± 0.1	6.5 ± 0.1	7.2 ± 0.001	5.7 ± 0.12	7.3 ± 0.2
Vit. E (µg/mg) d.g	7.6 ± 0.1	10.8 ± 0.1	10.3 ± 0.22	10.2 ± 0.1	11.8 ± 0.10	11.4 ± 0.001	12.1 ± 0.1	10.7 ± 0.1	12.9 ± 0.01
MT. (µg/g) d.g.	26.9 ± 0.01	42.3 ± 0.01	26.8 ± 0.1	30.3 ± 0.11	33.5 ± 0.1	36.8 ± 0.1	26.6 ± 0.001	27.9 ± 0.01	22.3 ± 0.01

$p \leq 0.05$

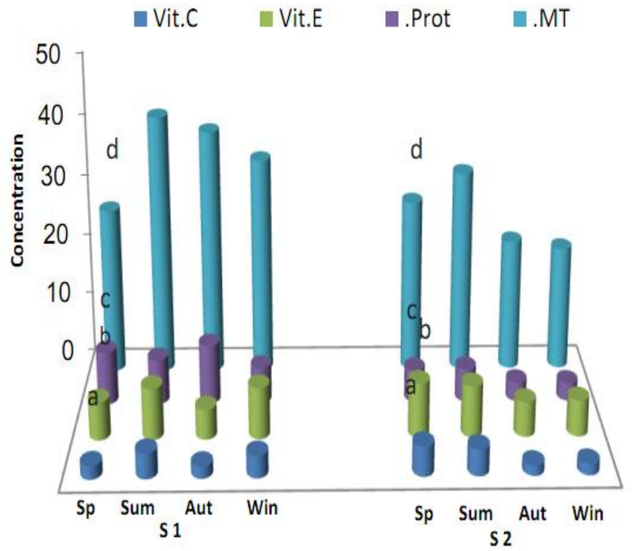

FIGURE 3: Seasonal variations of Biochemical markers in Clam (gill) in site 1&site2 of study area

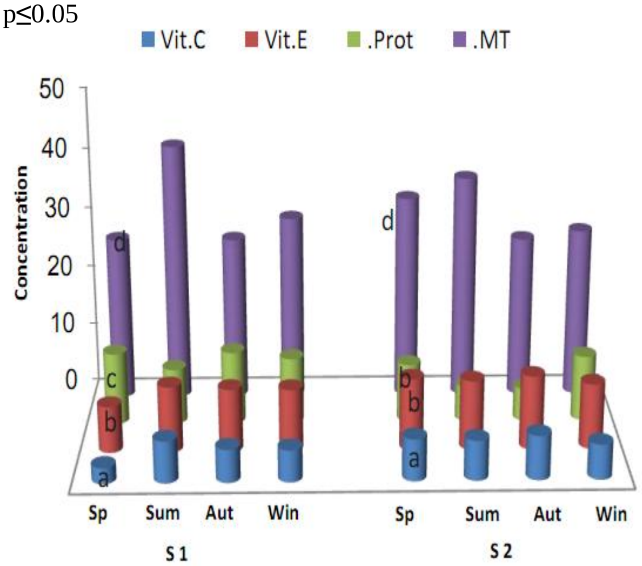

FIGURE 4: Seasonal variations of Biochemical markers in Clam (d.g) in site 1&site2 of study area

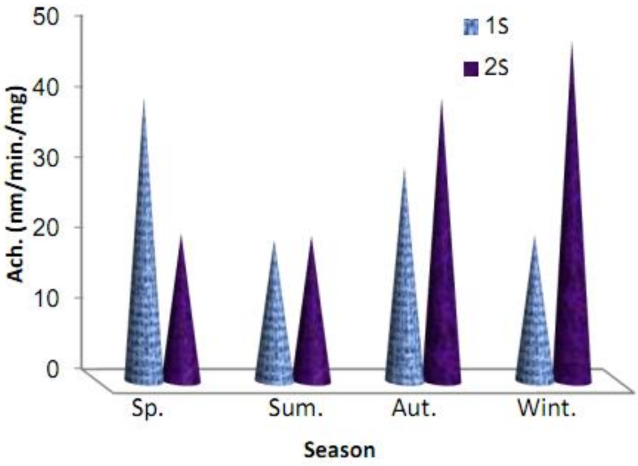

FIGURE 5: Seasonal variations of Acetylcholinesterase in Snail (gill) in site 1&site2 of study area

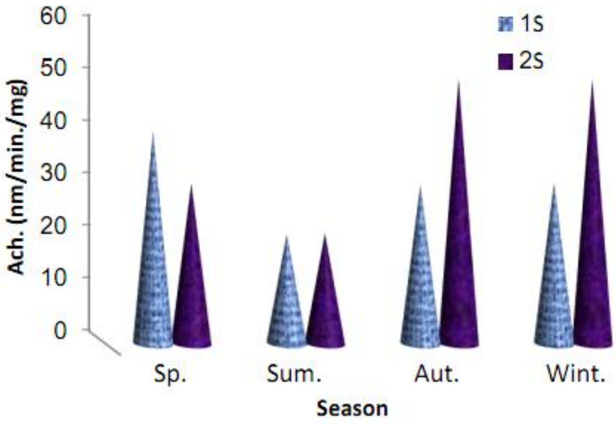

FIGURE 6: Seasonal variations of Acetylcholinesterase in Clam (gill) in site 1&site2 of study area

DISCUSSION

Biochemical responses as an important tool for detection the impact of heavy metals pollutants were taken into account by many studies such as (Regoli etal., 1998), The fluctuation in biomarker responses in this study because these responses are transient and variable according to the different species and pollutants (An etal., 2012). The highest concentration of different biomarkers in warm season and summer have been agreed with (Barreira etal., 2006) due to Increases in water temperature may enhance accumulation and toxicity of contaminants, the inhibition of acetylcholinesterase activity with increased level of Metallothionein in molluscs during study period in comparison to control in coincided with many studies such as (Lionetto etal., 2003) , all results have indicated the high response of metallothionein according to heavy metals concentration like (Lafontaine etal., 2000) study on zebra mussels (Dreissena polymorpha) which their results showed that MT. exhibited the highest correlation coefficients with heavy metals, elevation of metallothioneins concentration during summer in this study have been agreed with many research such as (Zorita etal., 2007). The reduction in acetylcholinestrase activity through study period seasonally due to sediment mobilization caused by clam harvesting plays an important role in resuspension of persistent contaminants having neurotoxic activity (Matozzo etal., 2005), also the inhibition of acetylcholinesterase activity as response of heavy metals aligned with many study such as (Monserrat etal., 2002), moreover, Because of its participation in neurotransmission, AChE is adapted highly sensitive to biotic and abiotic parameters (Banni etal., 2010), Non significance correlation in some times in this study between heavy metals and biomarkers may be due to the fact that metals didn't approach threshold to induce these biomarkers, or the biomarkers didn't response specifically to these heavy metals, or even that other factors can be affecting the biomarkers variability, Previous studies confirmed that biomarkers can be influenced by seasonal parameters as in this study which including temperature, gametogenesis, salinity, nutrition, starvation, and light penetration in addition to seasonal effects (Shaw etal., 2004), the highest values of biochemical recorded in digestive gland due to their importance in metals detoxification and metabolism which considered as long-term storage tissue

CONCLUSION

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

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