

## The Possibility of Using the Fresh Water Bivalve, *Spathopsis Rubens*, in The Nile River, El Mahmoudia Water Stream As Bioindicator For Pollution

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### Abstract

Bivalves are used as bioindicators of heavy metals pollution because they are known to concentrate these elements, providing a time integrated indication of environmental contamination. Trace metals can reach high concentrations in sediments and also in aquatic organisms by bioaccumulation through the food chain. Six heavy metals (Hg, Zn, Pb, Fe, Mg and Cu) were collected and investigated from Abu Hummus, El Behara. The concentration of Hg was high in winter as 2.3µg/g in sediment. The Zn concentration was high in summer in sediment as 8.1µg/g. The Pb concentration was high in winter in water as 3.3µg/l. The concentration of Fe in sediment was high in summer as 492 µg/g. The concentration of Mg was high in sediment as 408µg/g. The concentration of Cu was high in summer in sediment as 301µg/g. The mean concentrations of Fe in the present study are within the permissible limits of law 48/1982 (<1 mg/l) and the guideline of (WHO, 1993) which is <1 mg/l. The mean concentration level of copper is within the permissible limits of law 48/1982 (<1.0 mg/l). The mean levels of the heavy metals (Hg, Zn, Pb, Fe, Mg and Cu) detected in the present study in the water stream are less than the permissible limits recommended by (USEPA, 2005). In the present study there is a significance between all seasons in the protein content in the soft tissue of *Spathopsis rubens* as the mean concentration level in Spring was reported as 102.83mg/g which is higher than that of autumn 100.5mg/g, summer 93.33 mg/g and winter 80.50 mg/g.

In the present study the mean activity level of GPx in spring was higher than the other seasons such as spring 31.33u/g > summer 28.33 u/g >Autumn 26.67 u/g > winter 20.50u/g. The mean activity level of SOD in summer was higher than the other seasons such as summer 38.83 u/g > spring 33.33 U /g >Autumn 28.83U/g > winter 22.83U/g. The mean activity level of CAT in spring was higher than the other seasons such as spring 25.67u/g > summer and autumn19.83u/g > winter 15.17u/g. The mean activity level of MDA in winter was 30.50 U/g > summer 22.50U/g > autumn 18.0 U/g > spring 16.83U/g. In the present study it was found that the mean activity level of MDA increased in winter at the same time the mean activity level of CAT, SOD and GPx were decreased in winter. Negative correlation was reported between CAT and Hg in winter as  $r=-0.88^*$ . A positive correlation coefficient in winter was found between SOD activity level and CAT activity level as  $r=0.838^*$ .

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## Introduction

The Nile River is a source of life to millions of people. Pollution caused by inadequate drainage systems in rural villages, and irrigation wastewater filled with fertilizers and pesticides. Different analytical methods were constructed to monitor the water quality status in freshwater ecosystems [1]. The Nile River water is facing environmental and public health problems of water pollution which affects water quality and influences the balance of the whole ecosystem [2]. The rapid progress in industry led to the release of heavy metals in the ecosystem and especially the fresh water ecosystem. The accumulation of heavy metals in the Nile River water affects the quality of the water. The iron and steel industry releases lead and zinc into the Nile River. Amer and Abdel Gawad [3] monitored the distribution of heavy metals in the Nile River water and studied the impacts of heavy metals on the water quality. Bakhiet [4] and Ayodele and Abubakar [5] suggested that the study of heavy metal contamination in bivalves is important in order to consider them as bioindicators for heavy metal contamination. The pollutants are carried from the source and tend to sink thereby polluting the aquatic environment. Although information on contaminated regions in the tropical areas are lacking, studies on pollution monitoring in fresh water lakes environment have been reported using different indicator species [6, 5]. Freshwater mollusc communities are important in terms of biodiversity and ecosystem health. They play significant roles in the public and veterinary health and thus need to be scientifically more extensively [7].

A lot of researchers studied the ecology and

population dynamics of the gastropods which play an important role in the health of man and his livestock [8]. Ali [9] illustrated that molluscs are suitable candidates to be used in biomonitoring surveys of Lake Qarun in Egypt. Freshwater bivalves provide many ecological services to aquatic systems [10, 11]. Large invertebrates can be considered metabolic reactors because they transfer nutrients and energy from water to sediments by filtering and nutrient mineralization [10, 12]. The study on mollusk as a possible bioindicator of river water quality is because of the fact that they have the ability to concentrate pollutants as they are filter feeders [13]. Industrial effluents contributing to aquatic pollution contain toxic substances which include heavy metals. Indiscriminate discharges of these wastes alter the quality of water and cause hazards to the fauna. Copper is a micro-nutrient and is present as a metal ion in certain enzymes and plays an important role in the transfer of electrons in electron transport chain. It is a component of haemocyanin. There is an increased body of evidence implicating heavy metals as a potential threat to aquatic organism by way of studies on their physiology, biochemistry and ecology. Marine organisms are characterized by a greater spatial ability to accumulate some metals [14]. Marine organisms are characterized by a greater spatial ability to accumulate some metals when compared with bottom sediments [15]. The shellfish represents an important source of protein for coastal communities. Over 90% of human health exposure to several contaminants occurs through diet primarily seafood [16, 17]. In order to evaluate the adverse effect of the pollutants on aquatic organisms, there is a world wide trend to complement physical and chemical parameter with biomarkers in

aquatic pollution monitoring [18, 19].

Since bivalves are filter feeders, they concentrate contaminants to a much higher level than those of the surrounding sea [20]. These contaminants may cause diseases of humans, especially microbial contaminants, because shellfish are often eaten raw or lightly cooked [21, 22]. To reduce the risk, the source of the shellfish should be investigated and better quality would be attained by appropriate treatment following the harvest. The effects of environmental contaminants may result from direct toxic actions on tissues or cells or from alterations of the homeostatic mechanisms including the immune system [23-28]. The protein content in the tissues of animals plays a role in the metabolism of animals [29]. Heavy metals mainly react with proteins and adversely alter the physiological activities hence cause risk of life in different way. Protein acts as enzyme, hormone and basic structural component of the animal. Protein is key substance to show the effect of heavy metal. Proteins respond to stress condition for better survival by altering their levels. The shellfish represents an important source of protein for coastal communities. It has been predictable, for instance, that over 90% of human health exposure to several contaminants occurs through diet primarily seafood [15-17].

Contamination of fresh water with a wide range of pollutants has become a matter of concern over last few decades. The defence mechanisms against free radical-induced oxidative damage include the following catalytic removal of free radicals and reactive species by factors such as CAT, SOD, GPx. Animal CAT are heme-containing enzymes that convert hydrogen peroxide ( $H_2O_2$ ) to water and  $O_2$ , and they are largely localized in subcellular organelles such as peroxisomes. Mitochondria and the endoplasmic reticulum contain little CAT. The intracellular  $H_2O_2$  cannot be eliminated unless it diffuses to the peroxisomes [30]. GSH-Px removes  $H_2O_2$  by coupling its reduction with the oxidation of GSH. GSH-Px can also reduce other peroxides. Most animal tissues contain both CAT and GSH-Px activity. SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation [31]. SOD is the antioxidant enzyme that catalysed the dismutation

of the highly reactive superoxide anion to  $O_2$  and to the less reactive species  $H_2O_2$ . Peroxide can be destroyed by CAT or GPx reactions [32]. Among the biomarker of stress, the primary key events in oxidative damage are lipid peroxidation (MDA) [33-36].

Aim of the work: *Spathopsis rubens* had been chosen as example of bivalve which lives in El Behara governorate fresh water, to study the levels of heavy metals such as Zn, Cu, Pb, Mg, Fe and Hg in water, sediment and flesh of *Spathopsis rubens* collected from El Mahmodia stream, River Nile. The aim of the present study is to establish its suitability as bio-indicator that could be used to monitor heavy metals pollution in Nile River and to determine CAT, SOD, GPx and the potential of lipid peroxidation. To know the effect of pollutants on Biochemicals (protein, lipid and Carbohydrates) in *Spathopsis rubens*.

#### Material and Methods

In December 2016 to July 2017 the selected bivalves were collected from Abu Hummus, River Nile, El Beheira Egypt (Figure.1). The shell sizes of the detected samples were ranging from (10–15 cm) in length, from (6–9 cm) in width and from (2.6 to 4.5 cm) in height. The sediment and water samples were collected in corresponding to the clam settlements to determine the initial level of heavy metals.

Samples of *Spathopsis rubens* were collected from Abu Hummus El Beheira, Egypt. Abu hummus lies between the Cairo-Alexandria Agricultural road and the El Mahmodea stream at; 31.10063°N-30.310063°E. The water samples, sediment and flesh of *Spathopsis rubens* were collected from the river water side. Water samples were collected in plastic bottles, pre-rinsed with distilled water. The bivalves were chosen by harvesting only large but with similar sizes and healthy. A total of [15-20] samples were collected/location/season then were kept in plastic containers filled with water.

The biochemical analysis includes the determination of metal analysis, organic pollutants, protein, lipids, carbohydrates and antioxidant enzymes (CAT, SOD, GPx and MDA). The analysis of heavy metals (Cu, Fe, Mg, Zn, Pb and Hg) of fresh water was done according to Ayodele and Abubakar [5]. The heavy metals in sediment and in soft tissues were measured

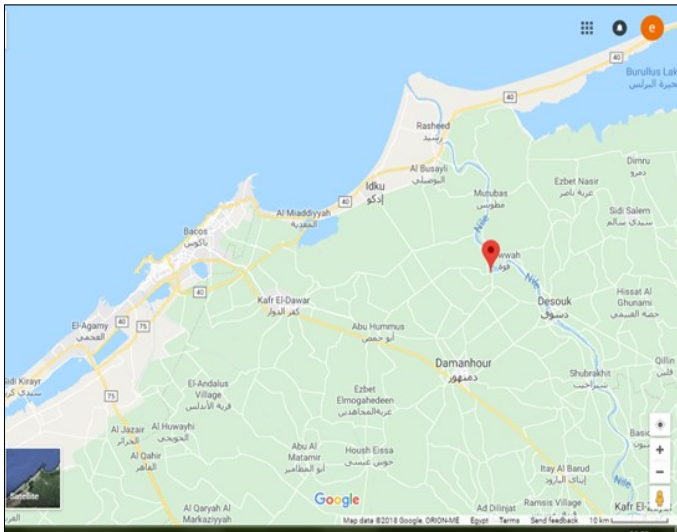


Figure 1. The map of Abu Hummus, ElBehara (Google map).



Figure 2. *Spathopsis rubens*

according to [37]. The results are presented as mean  $\pm$  S.D. values. One-way analysis of variance (ANOVA) was used to test the significance of depuration in each metal concentration and TPHs. Post hoc test was used to analyse the multiple comparisons among water, sediment and soft parts. All statistical analyses were performed using the SPSS 15.0 software [38]. Determination of carbohydrate and lipids were according to [39]. Determination of protein was estimated by Lowry's method [40]. Determination of Catalase activity (CAT) was measured according to Aebigh [41]. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured using the procedure of Beauchamp and Fridovich [42, 43]. Glutathione peroxidase activity levels were determined by the method of Pagtia and Valentine [44]. Lipid peroxidation (Malondialdehyde) was determined by the method of OhKawa [45].

## Results

Bivalve samples were collected from their natural beds from Abu Hummus, El Behirea, Egypt. The survey in the present study was reported as the following: *Spathopsis wahlbergi hartmanni* (Martens, 1866), *Spathopsis rubens arcuata* (Cailliaud, 1823), *Lanistes carinates* (Olivier, 1804) and *Melanoides tuberculata* (Müller, 1774), *Melanoides tuberculata* (Müller, 1774), *Lanistes carinates* (Olivier, 1804), *Mutela singularis* (Pallary, 1924). The *Spathopsis rubens* had

been chosen (Figure. 2) in the present study.

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The mean concentration level of Hg was higher in winter in sediment as 2.3 $\mu$ g/g than in water and in tissue.

The mean concentration level of Zn was higher in summer in sediment as 8.1 than in water and tissue. The mean concentration level of Pb was higher in winter in water as 3.3 $\mu$ g/g than in sediment and tissue. The mean concentration level of Fe was higher in summer as 492 $\mu$ g/g than in winter and tissue. The Mg concentrations were higher in sediment as 408 $\mu$ g/g than in water and tissue. The Cu concentrations were higher in summer in sediment as 301 $\mu$ g/g than in water and tissue.

Table 1. The mean concentration levels of heavy metals in water, sediment and bivalve tissue collected in autumn (2016-2017)

Heavy metals	Water (no 1), µg/L	Sediment µg/g (no 1)	Tissue (µg/g) Mean (no 6)
Hg	1.1	2.1	0.93
Zn	3.1	4.2	2.08
Pb	2.7	2.2	1.73
Fe	300	417	299.7
Mg	2.44	3.7	2.61
Cu	1.95	2.2	1.35

Table 2. The mean concentration levels of heavy metals in water, sediment and bivalve tissue collected in Winter (2016-2017)

Heavy metals	Water µg/L	Sediment µg/g	Mean of tissue µg/g
Hg	1.8	2.3	1.46
Zn	3.9	4.5	2.17
Pb	3.3	2.5	1.65
Fe	292	392	322
Mg	2.6	3.5	1.73
Cu	2.1	2	1.66

Table 3. The mean concentration levels of heavy metals in water, sediment and bivalve tissue collected in Spring (2016-2017)

Heavy metals	Water µg/L (no 1)	Sediment µg/g (no 1)	Average (no 6) tissue µg/g
Hg	1.5	1.9	1.26
Zn	4.1	5.2	2.83
Pb	2.2	2.6	1.32
Fe	235	400	252.33
Mg	3.2	4.1	2.38
Cu	1.98	2.3	1.41

Table 4. The mean concentration levels of heavy metals in water, sediment and bivalve tissue collected in Summer (2016-2017)

Heavy metals	Water µg/L(no 1)	Sediment µg/g (No 1)	tissue µg/L(no 6)
Hg	1.6	2.2	1.10
Zn	5.6	8.1	4.23
Pb	2.8	2.9	0.89
Fe	321	492	274.2
Mg	4.1	4.8	2.21
Cu	2.1	3.1	1.14

Table 5. The mean activity level of GPx during (2016-2017).

GPx	Mean ± SD.
Autumn (n = 6)	26.67 ± 6.35
Winter (n = 6)	20.50 ± 4.85
Spring (n = 6)	31.33 ± 6.35
Summer (n = 6)	28.33 ± 9.09
F (p)	2.681 (0.074)

Means with different letters are significant; F, p: F and p values for ANOVA test. Significance between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table (6): The mean activity level of SOD during (2016-2017).

SOD	Mean ± SD.
Autumn (n= 6)	28.83 <sup>bc</sup> ± 5.67
Winter (n = 6)	22.83 <sup>c</sup> ± 4.36
Spring (n = 6)	33.33 <sup>ab</sup> ± 7.81
Summer (n = 6)	38.83 <sup>a</sup> ± 8.64
F (p)	5.919* (0.005*)

Means with different letters are significant; F, p: F and p values for ANOVA test. Significance between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table 7. The mean activity level of CAT during (2016-2017).

CAT	Mean ± SD.
Autumn (n = 6)	19.83 ± 5.46
Winter (n = 6)	15.17 ± 5.38
Spring (n = 6)	25.67 ± 8.80
Summer (n = 6)	19.83 ± 3.76
F (p)	2.951 (0.057)

Means with different letters are significant; F, p: F and p values for ANOVA test. Significance between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table 8. The mean activity level of MDA during (2016-2017).

MDA	Mean ± SD.
Autumn (n = 6)	18.0 <sup>b</sup> ± 2.83
Winter (n = 6)	30.50 <sup>a</sup> ± 8.26
Spring (n = 6)	16.83 <sup>b</sup> ± 6.94
Summer (n = 6)	22.50 <sup>b</sup> ± 6.28
F (p)	5.620* (0.006*)

Means with different letters are significant; F, p: F and p values for ANOVA test. Significance between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table 9. The total protein content in

Protein	Mean ± SD.
Autumn (n = 6)	100.5 ± 15.04
Winter (n = 6)	80.50 ± 12.10
Spring (n = 6)	102.83 ± 18.67
Summer (n = 6)	93.33 ± 13.7
F (p)	2.657 (0.076)

Means with different letters are significant. F, p: F and p values for ANOVA test, Significant between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table 10. The total lipid content in

Lipid	Mean ± SD.
Autumn (n = 6)	12.78 ± 2.34
Winter (n = 6)	10.60 ± 2.90
Spring (n = 6)	10.25 ± 1.20
Summer (n = 6)	9.38 ± 1.54
F (p)	2.837 (0.064)

Means with different letters are significant. F, p: F and p values for ANOVA test, Significant between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

Table 11. The carbohydrates content in

Carbohydrates	Mean ± SD.
Autumn (n = 6)	12.05 ± 1.91
Winter (n = 6)	10.62 ± 2.50
Spring (n = 6)	13.40 ± 2.72
Summer (n = 6)	11.78 ± 3.63
F(p)	1.029(0.401)

Means with different letters are significant. F, p: F and p values for ANOVA test, Significant between groups was done using Post Hoc Test (LSD). \*: Statistically significant at  $p \leq 0.05$

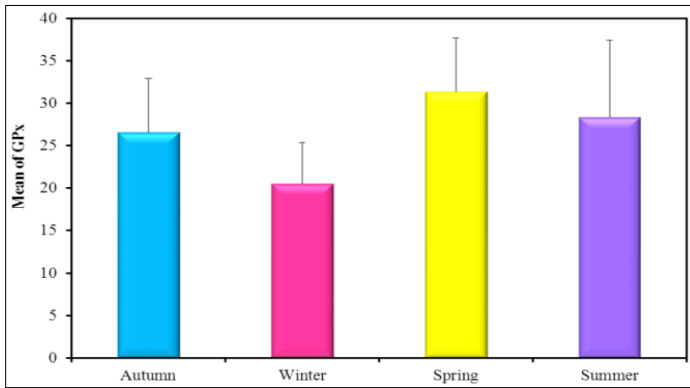


Figure 3. The mean concentration of the activity levels of GPx (mU/mg.protein) in different seasons (2016-2017).

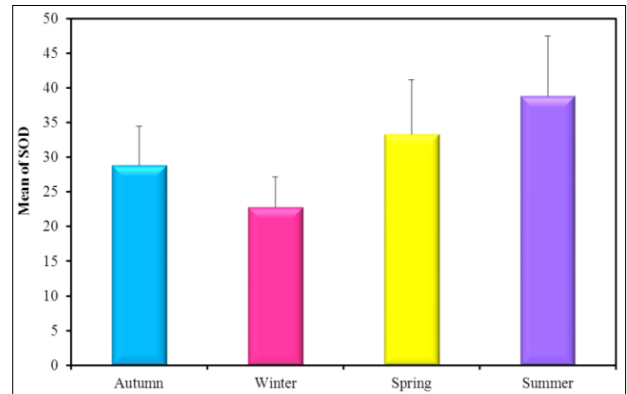


Figure 4. The mean concentration of the activity levels of SOD (U/g.tissue) in different seasons (2016-2017).

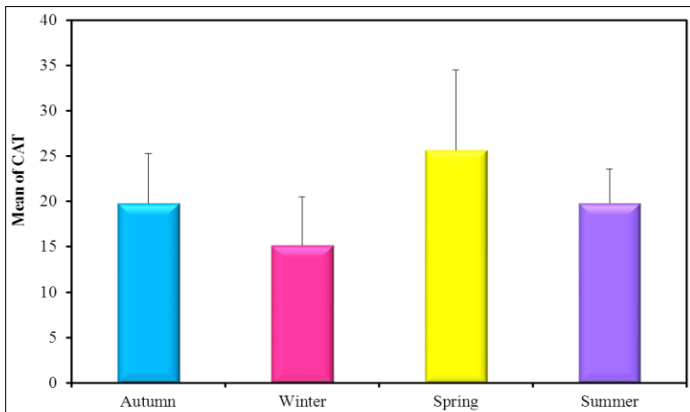


Figure 5. The mean concentration levels of activity of CAT(U/g) in different seasons during (2016-2017).

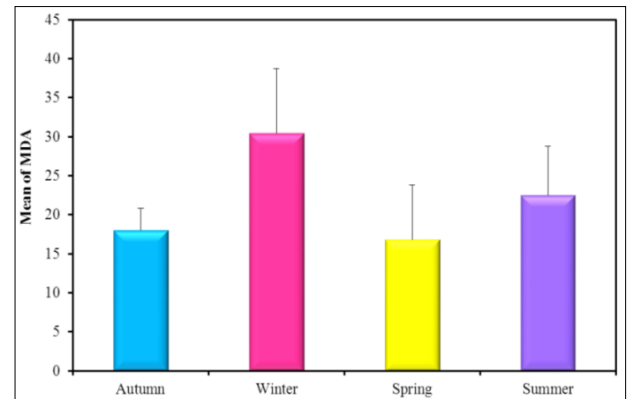


Figure 6. The mean concentration levels of activity of MDA (nmol/mg tissue) in different seasons (2016-2017).

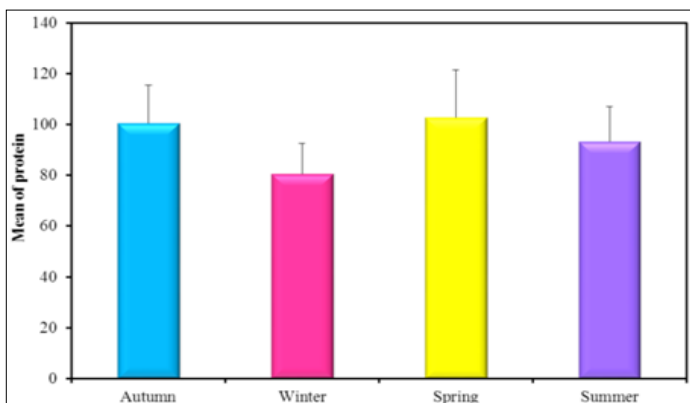


Figure 7. The mean concentration levels of total Protein (g/dl) in different seasons during (2016-2017).

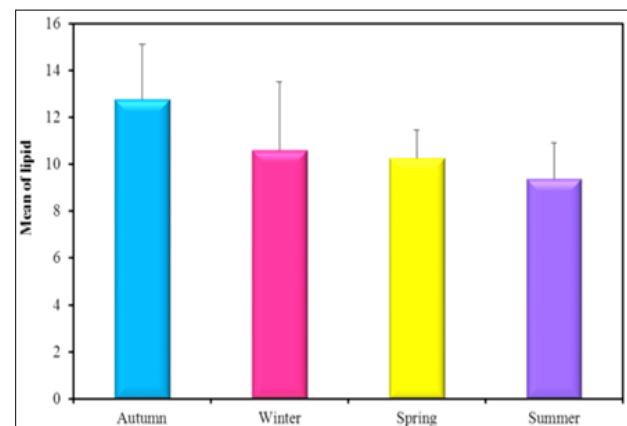


Figure 8. The mean concentration levels of lipid (mg/dl) in different seasons during (2016-2017).



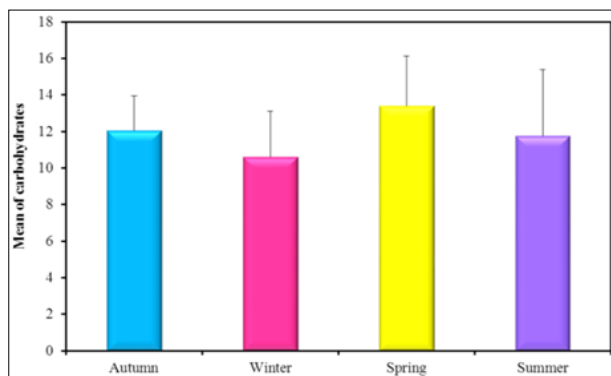


Figure 9. The mean concentration levels of carbohydrates in different seasons during (2016-2017).

A Histogram of the mean activity level of different enzymes in the bivalve (2016-2017) (Figures 3 - 6)

A histogram representing the selected biochemical parameters in the bivalve (2016-2017) (Figures 7 - 9) (Tables 5 - 11)

A histogram of the mean concentration levels of the selected heavy metals in water ( $\mu\text{g/g}$ ) in different seasons during the year (2016-2017) (Figures 10 - 15) (Tables 12 - 16)

In Autumn the activity of GPx and of MDA were positively correlated with the carbohydrate contents in the bivalve as  $r=0.956^*$  and  $r=0.865^*$ ; respectively. The activity level of SOD is negatively correlated with MDA, Hg, Fe as  $r=-0.873^*$ ,  $r=-0.998^*$ ,  $r=-0.925^*$ ; respectively. The activity of CAT is negatively correlated with the lipid content, Pb as  $r=-0.922^*$ ,  $r=-0.87^*$ ; respectively.

The total protein content is negatively correlated with Fe concentration level in tissues as  $r=-0.908^*$ . The lipid contents is positively correlated with the carbohydrate contents as  $r=0.877^*$  and  $r=0.910^*$ ; respectively. Both Hg and Pb are positively correlated with Fe concentration level in tissues of the bivalve as  $r=0.932^*$  and  $r=0.856^*$ ; respectively.

The correlation coefficient in winter was only between SOD mean activity inhibition level and CAT mean activity level as  $r=0.838^*$ . Negative correlation was found between CAT and Hg as  $r=0.88^*$ . The mean

concentration level of Fe and Mg in tissues showed a negative correlation as  $r=-0.835^*$ .

In spring only the mean concentration level of Pb and Cu in tissue showed a high significant correlation as  $r=0.978^*$ .

In summer there were positive correlation between the mean activity level of GPx and lipid content and Hg in tissues as  $r=0.837^*$  and  $r=0.865^*$ ; respectively. The mean activity level of MDA was positively correlated with the mean concentration level of Fe in tissues as  $r=0.821^*$ . The mean level of the total protein content was positively correlated with Pb mean concentration level as  $r=0.893^*$  and negatively correlated with Cu mean concentration level as  $r=-0.912^*$ . Whereas the mean concentration level of the lipid content was negatively correlated with the mean concentration level of the carbohydrate contents as  $r=-0.828^*$  and Pb in tissue was also negatively correlated with Cu in tissues as  $r=-0.985$ .

## Discussion

In the present study *Spathopsis rubens* was collected from El Beheira, Egypt, Abu Hummus. These species was already detected in previous reports [46]. In the present study the heavy metals (Hg, Zn, Pb, Fe, Cu and Mg) were detected in the four seasons from autumn (2016) to summer (2017). In fresh water, in sediment and in the soft tissues of *Spathopsis rubens*. Some enzyme activities were detected as; CAT, SOD, GPx and MDA. The total protein, Lipid and carbohydrates in the soft tissues of *Spathopsis rubens* were detected. There

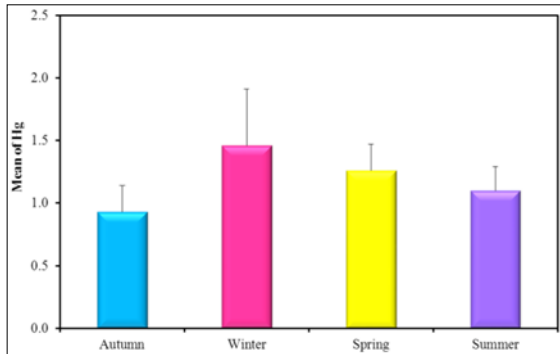


Figure 10. the mean concentration level of Hg in the different seasons during the year (2016-2017).

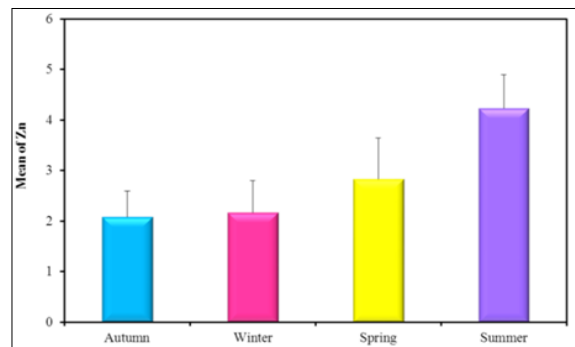


Figure 11. the mean concentration level of Zn in the different seasons during the year (2016-2017).

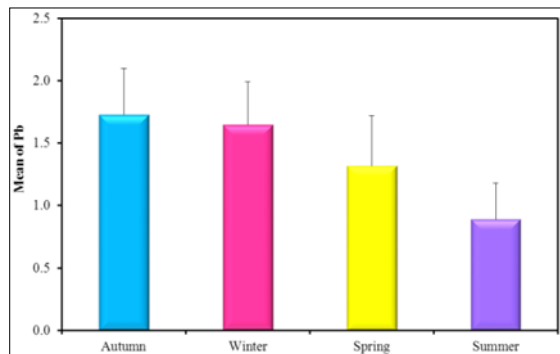


Figure 12. the mean concentration level of Pb in different seasons during the year (2016-2017).

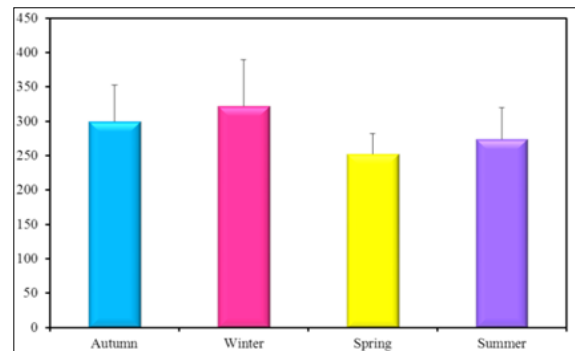


Figure 13. the mean concentration level of Fe in different seasons during the year (2016-2017).

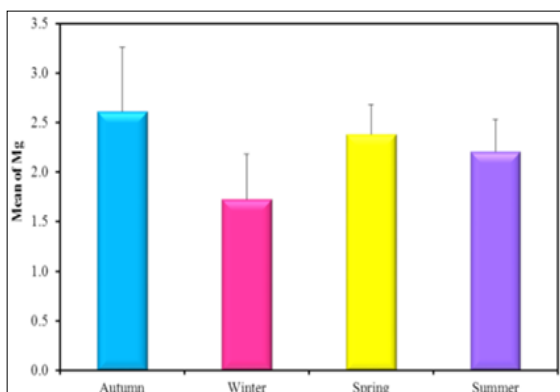


Figure 14. the mean concentration level of Mg in different seasons during the year (2016-2017).

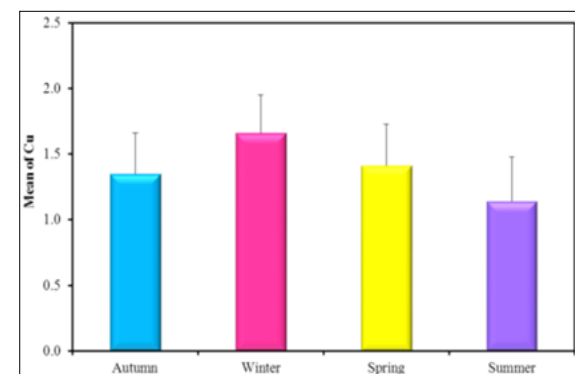


Figure 15. the mean concentration level of Cu in different seasons during the year (2016-2017).

Table 12. The mean concentration levels of heavy metals in the tissue (Zn, Pb, Fe, Mg, Cu and Hg) in different seasons during the year ( 2016-2017):

Zn	Mean ± S.D.
Autumn (n = 6)	2.08 <sup>b</sup> ± 0.52
Winter (n = 6)	2.17 <sup>b</sup> ± 0.63
Spring (n = 6)	2.83 <sup>b</sup> ± 0.82
Summer (n = 6)	4.23 <sup>a</sup> ± 0.67
F (p)	13.300* (<0.001*)

Pb	Mean ± S.D.
Autumn (n = 6)	1.73 <sup>a</sup> ± 0.37
Winter (n = 6)	1.65 <sup>a</sup> ± 0.34
Spring (n = 6)	1.32 <sup>a</sup> ± 0.40
Summer (n = 6)	0.89 <sup>b</sup> ± 0.29
F (p)	7.032* (0.002*)

Fe	Mean ± S.D.
Autumn (n = 6)	299.67 ± 52.82
Winter (n = 6)	322.33 ± 67.30
Spring (n = 6)	252.33 ± 29.97
Summer (n = 6)	274.17 ± 45.59
F (p)	2.157 (0.12)

Mg	Mean ± S.D.
Autumn (n = 6)	2.61 <sup>a</sup> ± 0.65
Winter (n = 6)	1.73 <sup>b</sup> ± 0.45
Spring (n = 6)	2.38 <sup>a</sup> ± 0.30
Summer (n = 6)	2.21 <sup>ab</sup> ± 0.32
F(p)	4.043* (0.021*)

Cu	Mean ± SD.
Autumn (n = 6)	1.35 ± 0.31
Winter (n = 6)	1.66 ± 0.29
Spring (n = 6)	1.41 ± 0.32
Summer (n = 6)	1.14 ± 0.34
F (p)	2.7 (0.07)

Hg	Mean ± SD.
Autumn (n = 6)	0.93 <sup>b</sup> ± 0.21
Winter (n = 6)	1.46 <sup>a</sup> ± 0.45
Spring (n = 6)	1.26 <sup>ab</sup> ± 0.21
Summer (n = 6)	1.10 <sup>b</sup> ± 0.19
F (p)	3.756* (0.027*)

F, p: F and p values for ANOVA test, Significant between groups was done using Post Hoc Test (LSD).

\*: Statistically significant at  $p \leq 0.05$ .

Table 13. Correlation between different studied parameters in Autumn (2016-2017)

		SOD	CAT	MDA	Protein	Lipid	Carbohy- drates	Hg	Zn	Pb	Fe	Mg	Cu
GPx	r	0.070	-0.655	-0.033	0.189	0.714	0.956*	-0.117	-0.009	0.444	0.050	0.691	0.764
	p	0.895	0.158	0.950	0.721	0.111	0.003	0.825	0.987	0.378	0.925	0.128	0.077
SOD	r		0.303	-0.873*	0.744	-0.544	-0.167	-0.998*	0.200	-0.639	-0.925*	-0.534	0.003
	p		0.560	0.023	0.090	0.265	0.751	<0.001	0.704	0.172	0.008	0.275	0.996
CAT	r			-0.052	0.481	-0.922*	-0.805	-0.296	-0.579	-0.870*	-0.536	-0.432	-0.581
	p			0.922	0.334	0.009	0.054	0.570	0.228	0.024	0.273	0.393	0.227
MDA	r				-0.588	0.374	0.148	0.865*	-0.503	0.483	0.796	0.521	0.157
	p				0.220	0.465	0.779	0.026	0.309	0.332	0.058	0.289	0.766
Protein	r					-0.499	-0.106	-0.775	-0.364	-0.793	-0.908*	-0.020	-0.189
	p					0.314	0.841	0.070	0.479	0.060	0.012	0.970	0.719
Lipid	r						0.877*	0.522	0.234	0.910*	0.692	0.714	0.619
	p						0.022	0.289	0.655	0.012	0.128	0.111	0.190
Carbo- hydrate s	r							0.128	0.084	0.685	0.329	0.724	0.821
	p							0.809	0.874	0.133	0.524	0.104	0.045
Hg	r								-0.161	0.639	0.932*	0.482	-0.028
	p								0.761	0.172	0.007	0.333	0.958
Zn	r									0.365	0.072	-0.453	0.069
	p									0.477	0.892	0.367	0.897
Pb	r										0.856*	0.449	0.606
	p										0.029	0.371	0.202
Fe	r											0.426	0.262
	p											0.400	0.616
Mg	r												0.365
	p												0.477
Cu	r												
	p												

r: Pearson coefficient

\*: Statistically significant at  $p \leq 0.05$

Table 14. Correlation between different studied parameters in Winter (2016-2017)

		SOD	CAT	MDA	Protein	Lipid	Carbohy- drates	Hg	Zn	Pb	Fe	Mg	Cu
GPx	r	0.800	0.632	-0.542	0.421	0.169	0.217	-0.714	-0.079	0.055	-0.564	0.284	-0.227
	p	0.056	0.178	0.267	0.405	0.748	0.680	0.111	0.882	0.918	0.244	0.585	0.665
SOD	r		0.838*	-0.570	0.290	-0.035	-0.404	-0.664	0.202	-0.142	-0.108	0.075	-0.605
	p		0.037	0.238	0.577	0.948	0.427	0.150	0.701	0.788	0.839	0.888	0.203
CAT	r			-0.456	0.032	0.190	-0.314	-0.880*	-0.146	0.236	0.159	-0.160	-0.318
	p			0.363	0.952	0.719	0.545	0.021	0.783	0.653	0.763	0.763	0.538
MDA	r				-0.339	-0.606	0.136	0.604	-0.408	0.517	0.211	-0.226	0.479
	p				0.511	0.202	0.797	0.205	0.422	0.293	0.688	0.667	0.337
Protein	r					-0.286	0.116	-0.027	0.608	-0.017	-0.035	-0.393	0.185
	p					0.583	0.827	0.960	0.200	0.974	0.947	0.441	0.725
Lipid	r						0.345	-0.570	-0.309	-0.145	-0.311	0.408	-0.029
	p						0.503	0.238	0.552	0.785	0.548	0.422	0.956
Carbo- hydrate s	r							-0.090	-0.550	0.440	-0.614	0.238	0.705
	p							0.865	0.258	0.383	0.195	0.650	0.118
Hg	r								0.329	-0.258	0.134	-0.046	0.122
	p								0.525	0.622	0.801	0.931	0.818
Zn	r									-0.657	0.277	-0.243	-0.406
	p									0.156	0.595	0.643	0.425
Pb	r										0.212	-0.511	0.768
	p										0.687	0.300	0.074
Fe	r											-0.835*	0.105
	p											0.039	0.844
Mg	r												-0.474
	p												0.343
Cu	r												
	p												

Table 15. Correlation between different studied parameters in Spring (2016-2017):

		SOD	CAT	MDA	Protein	Lipid	Carbohy- drates	Hg	Zn	Pb	Fe	Mg	Cu
GPx	r	0.235	0.407	0.138	-0.754	-0.274	-0.765	0.069	-0.590	0.649	0.293	-0.324	0.529
	P	0.654	0.423	0.795	0.083	0.600	0.076	0.897	0.218	0.163	0.573	0.530	0.281
SOD	R		0.357	0.709	-0.274	0.248	0.011	0.090	0.023	-0.288	-0.079	0.165	-0.457
	P		0.488	0.115	0.600	0.636	0.983	0.866	0.965	0.580	0.882	0.754	0.363
CAT	R			0.778	-0.779	0.594	0.030	0.774	-0.513	0.316	0.392	0.258	0.224
	P			0.068	0.068	0.214	0.955	0.071	0.298	0.542	0.442	0.621	0.670
MDA	R				-0.621	0.790	0.242	0.391	-0.317	0.096	0.119	0.236	-0.028
	P				0.188	0.061	0.644	0.443	0.541	0.857	0.823	0.653	0.958
Protein	R					-0.406	0.431	-0.265	0.700	-0.773	-0.434	0.240	-0.652
	P					0.425	0.393	0.612	0.122	0.071	0.390	0.647	0.160
Lipid	R						0.372	0.291	-0.056	0.108	0.320	0.048	0.060
	P						0.468	0.576	0.917	0.838	0.536	0.929	0.911
Carbohy- drates	R							0.316	0.050	-0.523	-0.584	0.804	-0.413
	P							0.541	0.926	0.287	0.223	0.054	0.415
Hg	R								-0.243	-0.129	0.208	0.616	-0.130
	P								0.643	0.808	0.692	0.192	0.805
Zn	R									-0.727	0.225	-0.226	-0.731
	P									0.101	0.668	0.667	0.099
Pb	R										0.296	-0.471	0.978*
	P										0.569	0.346	0.001
Fe	R											-0.622	0.201
	P											0.187	0.702
Mg	R												-0.395
	P												0.438
Cu	R												
	P												

r: Pearson coefficient

\*: Statistically significant at  $p \leq 0.05$

Table 16. Correlation between different studied parameters in Summer (2016-2017)

		SOD	CAT	MDA	Protein	Lipid	Carbo- hydrates	Hg	Zn	Pb	Fe	Mg	Cu
GPx	r	-0.106	-0.395	-0.185	-0.095	0.837*	-0.667	0.865*	-0.113	-0.022	-0.338	0.390	0.038
	P	0.841	0.438	0.725	0.858	0.038	0.148	0.026	0.831	0.968	0.512	0.445	0.942
SOD	r		-0.309	0.437	0.693	-0.198	0.544	0.108	0.637	0.363	0.574	0.204	-0.371
	P		0.552	0.387	0.127	0.706	0.264	0.839	0.174	0.479	0.234	0.699	0.469
CAT	r			0.579	-0.419	-0.662	0.274	-0.350	0.192	-0.583	0.584	-0.386	0.528
	P			0.228	0.408	0.152	0.600	0.497	0.716	0.224	0.223	0.450	0.281
MDA	r				0.030	-0.451	0.251	0.061	0.363	-0.310	0.821*	0.216	0.331
	P				0.955	0.370	0.631	0.909	0.479	0.549	0.045	0.681	0.521
Protein	r					-0.239	0.482	-0.267	0.268	0.893*	0.176	0.367	-0.912*
	P					0.649	0.333	0.609	0.608	0.016	0.739	0.474	0.011
Lipid	r						-0.823*	0.806	-0.403	-0.021	-0.673	0.393	0.093
	P						0.044	0.053	0.428	0.969	0.143	0.441	0.861
Carbohydrates	r							-0.592	0.752	0.181	0.697	-0.520	-0.294
	P							0.216	0.085	0.732	0.124	0.290	0.572
Hg	r								0.065	-0.316	-0.112	0.296	0.353
	P								0.902	0.542	0.833	0.569	0.492
Zn	r									-0.145	0.797	-0.508	0.017
	P									0.785	0.057	0.304	0.974
Pb	r										-0.265	0.473	-0.985*
	P										0.612	0.344	<0.001
Iron	r											-0.255	0.191
	P											0.625	0.717
Mg	r												-0.339
	P												0.511
Cu	r												
	P												

r: Pearson coefficient.

Statistically significant at  $p \leq 0.05$

is a general acceptance that fresh water ecosystems undergo little ecological stress when subjected to salinities up to 1000 mgL<sup>-1</sup>. Much of the knowledge of the impacts of salinity on aquatic ecosystems comes from field sampling a long a gradient of salinity from which it is difficult attribute cause of ecological change [47].

Bivalves have been used as bioindicators of pollution because they have the ability to concentrate heavy metals to several other magnitudes [48]. The mean concentrations of Fe in the present study are within the permissible limits of law 48/1982 (<1 mg/l) and the guideline of [49] which is <1 mg/l. The mean concentration level of copper is within the permissible limits of law 48/1982 (<1.0 mg/l), the values of the measured metal. The mean levels of the heavy metals in water are less than that of the permissible limits recommended by [50]. The changes in metabolic rates of bivalves within the seasons and the variation in bioavailability of metals in the surrounding environment with time might be responsible for the health status of the molluscs [51]. The present study is in agreement with Cossa and Rondeau [51] in that the higher metal burden and concentration in the wet season (such as for Fe and Zn). Lower levels for Cd and Hg could be attributed to wash out of the lagoons during the rainy period. Biological variables such as changes in the tissue composition as well as the season of sampling and the hydrodynamics of the lagoons have to be considered. Seasonal variations are related to a great extent to seasonal changes in flesh weight during development of gonadic tissues [52].

The present observation showed that the mean concentration levels of Cu, Hg and Fe in tissues are higher in winter than the other seasons as; the mean concentration level of Hg in winter 1.46 µg/g > spring 1.26µg/g > summer 1.1 µg/g> autumn 0.93µg/g. The mean concentration level of Cu in winter 1.6 µg/g> spring 1.4µg/g > summer 1.14µg/g> Autumn 1.35µg/g and the mean concentration level of Fe in winter 322.33 µg/g> Autumn 299.67µg/g > summer 274.17 µg/g> spring 252.33µg/g .The mean concentration levels of Pb and Mg are higher in autumn than the other seasons as Pb in Autumn 1.73 µg/g> winter 1.65µg/g > spring

1.32µg/g> summer 0.89µg/g, Mg in autumn 2.6 µg/g> spring 2.38µg/g > summer 2.4µg/g> Autumn 1.73µg/g. The mean concentration level of Zn is higher in summer than the other seasons as, Zn in summer 4.23 µg/g> spring 2.83µg/g > winter 2.17µg/g> autumn 2.08µg/g.

The present observation showed that the mean concentrations level of heavy metal in the sediment were high when compared with standard values [48]. The mean concentration level of Hg in sediment is higher in winter than the other seasons as, winter 2.3 µg/g > summer 2.2 µg/g >Autumn 2.1 µg/g > spring 1.9 µg/g.The mean concentration level of Zn, Cu, Pb, Fe and Mg were higher in summer than the other seasons as; Zn in summer 8.1 µg/g > spring 5.2 µg/g winter 4.5 µg/g > Autumn 4.2 µg/g, Cu in summer 3.1 µg/g > spring 2.3 µg/g > Autumn 2.2 µg/g > winter 2 µg/g, Pb in summer 2.9 µg/g > spring 2.6 µg/g > winter 2.5 µg/g>Autumn 2.2 µg/g. Fe in summer 492 µg/g > Autumn 417 µg/gram > spring 400 µg/gram > winter 395 µg/g, Mg in summer 4.8 µg/gram > spring 4.1 µg/gram > Autumn 3.7 µg/gram > winter 3.5 µg/g.The variations in metal concentration of the shellfish tissues in the present study could be related to the concentration of heavy metals in the fresh water.

Abdulah [53] reported that the high level of heavy metals in the lake may be related to their concentration in the stream and rivers discharging into the lake. The high level of Zn, Cu and Pb in the river indicates the quality of the water prevailing at the period of sampling. Trace metal concentrations in clams depend on numerous environmental and biological factors [54]. Earlier studies by Chouba et al. [55] in Tunisia demonstrated higher concentrations of heavy metals in clams during high rainfall periods. These findings are in agreement with that of the present study. Studies have shown that during the spawning period, proteins and carbohydrate contents, which have a high affinity for heavy metals, are accumulated for gonad tissue production, energetic storage and consumption [56].There is no access waste water treatment in Abo Hummus rural areas as 20% of Egyptian villages have inadequate potable water [57-58].

Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing



a serious threat to the survival of aquatic organisms [59]. The aquatic environment is subjected to various types of pollutants which enter water bodies [60]. It is estimated that the total amount of reused treated wastewater in Egypt was about 1.4 billion m<sup>3</sup> in 2000 [61]. Industrial waste water is considered the second of the main sources of Nile water pollution. Effluent wastewater is often partially treated [62]. Major pollutants in agricultural drains are salts, nutrients, pesticide residues, pathogens and toxic organic and inorganic pollutants [63, 64]. At high pollution stress however, protein synthesis can be suppressed indicating disturbance of normal metabolic processes [65-67]. The fall in protein level during pollutant exposure may be due to increased catabolism and decrease in protein synthesis [68]. The digestive gland is the main site of degradation and detoxification of toxicants and hence resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein is the tool to access the extent of toxicity [69].

In the present study there is a significance between all seasons in the protein content in the soft tissue of *Spathopsis rubens* as the mean concentration level in Spring was reported as 102.83mg/g which is higher than that of autumn 100.5 mg/g, summer 93.33 mg/g and winter 80.50 mg/g. Kharat et al. [70] studied depletion in protein content in the tissues of *Macrobrachium kistnensis* exposed to different concentrations of tributyltin chloride stress on protein metabolism similar results were obtained by Sole and Porte [71]. Inhibition in the protein synthesis was reported to be due to non-selective blocking of phosphorylation process in the central nervous system [72]. Bivalves generally store carbohydrates in large amounts during their growing seasons and use them over the rest of the year although proteins may be an energy reserve in some bivalve species. Lipids have been reported to function most importantly as energy storage substances and physical properties of biological membranes. In the present study the higher concentration of lipid in autumn 12.78 mg/g than in winter 10.60 mg/g, spring 10.25 mg/g and in summer 9.38mg/g. Fall in carbohydrates level may be due to the prolonged exposure of the metabolism to the heavy

metals and this may be the reason for inactivation of the enzyme, involved in the carbohydrate metabolism [73]. In the present study the higher concentration of carbohydrates was found in spring 13.40 mg/gm than in autumn 12.05 mg/gm, summer 11.78 mg/gm and in winter 10.62mg/gm.

Free radicals are able to react with biological macromolecules and produce enzyme activation, lipid peroxidation [74]. Antioxidant enzymes activity levels of marine bivalve *Perna viridis* during heavy metals exposure were significantly higher in tissues. The mantle was observed to significantly contribute to the organismal response to lipid peroxidation as indicated by high activity levels of antioxidant enzymes [75]. Cu strongly stimulates the lipid peroxidation damage of the gill plasma membranes [76, 77]. Pannunzio and Storey [78] observed a suppression of GPx activity during anoxia exposure in the hepatopancreas of the marine gastropods *Littorina littorea*. Main enzymes involved in detoxification from reactive oxygen species. SOD and GPx have been shown to contribute to antioxidant defense in the mussels [79]. Glutathione is considered a scavenger able to protect cells from oxidative damage [80, 81]. Aerobic organisms are protected against oxidative stress by antioxidant systems which mobilize enzymes such as the (Cu-Zn superoxide dismutase) which transfers O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub> [82].

Oxidative stress induced by copper exposure, evidenced by increased lipid peroxidation products such as malondialdehyde has also been demonstrated for the mussels *Mytilus galloprovincialis* [83], *Perna perna* [84], *Ruditapes decussatus* [85], and for the oyster *Crassostrea virginica* [86]. Antioxidant defenses may be increased or inhibited by chemical stressors. The occurrence of one kind of response or the other depends on the intensity and duration of the applied stress and the susceptibility of the species that are exposed [87]. There are several reports on increased SOD and CAT activities in bivalves in the presence of excess free radicals [88]. Dietary copper appears to be innocuous to the digestive system at low concentrations as copper is a cofactor of enzymes such as cytosolic SOD (Cu-SOD iso-enzyme) [89] and is also part of the hemocyanin molecule. The excess of this metal could be

sequestered into vacuoles or immobilized by biological compounds for a possible excretion [90, 91]. Metals can induce oxyradical production leading to lipid peroxidation [92]. In the present study the mean activity level of GPx in Spring was higher than the other seasons such as spring 31.33U/g > summer 28.33 U/g > autumn 26.67 U/g > winter 20.50U/g. The mean activity level of SOD in summer was higher than the other seasons such as summer 38.83 U/g > spring 33.33 U/g > autumn 28.83U/g > winter 22.83U/g. The mean activity level of CAT in spring was higher than the other seasons such as spring 25.67U/g > summer and autumn 19.83U/g > winter 15.17U/g. The mean activity level of MDA in winter was 30.50 U/g > summer 22.50U/g > autumn 18.0 U/g > spring 16.83U/g. In the present study it was found that the mean activity level of MDA increased in winter at the same time the mean activity level of CAT, SOD and GPx were decreased in winter.

### Conclusion

Fe, Hg and Cu are higher in winter season while Pb, Zn and Mg are higher in summer in tissue of *Spathopsis rubens*. Pb and Hg are higher in winter season, Zn, Fe and Mg are higher in summer. While Cu is higher in summer and winter in fresh water. Fe, Zn, Cu, Pb and Mg are higher in summer while Hg is higher in winter in sediment.

The high ratio of protein and carbohydrates in spring while the higher ratio of lipids in autumn. CAT, GPx are higher in spring, SOD is higher in summer while MDA is higher in winter. By the effect of aquaculture activities, irrigation, mechanized farming and future increased loading of agro-industrial effluents and domestic waste, The pollution increase in winter due to rain water (winter) which move pollutants to river Nile and the effect of pollutants appear in *Spathopsis rubens* on the following season, this effect has a disturbance of ecosystem and food chains in the aquatic environment.

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