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Marine pollution by some heavy metals and physiological response of *Ruditapes decussatus*

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Abstract

Bivalves can accumulate toxicants such as heavy metals in their tissues, for this reason, they are considered as good bio-indicators for water quality. The risk is increased due to eating these clams raw or lightly cooked. This study aims to determine the concentration of some selected metals (Cu, Zn, Mn, Cd, and Pb) in the soft tissue of *Ruditapes decussatus* collected in the summer of 2017, from three locations at Alexandrian coasts, Egypt, Abo Quir, (loc.#1), Sedi Beshr (loc.#2) and (loc.#3), El-Max) and to find out whether pollution alters the clam physiological functions or not. The present data showed that the highest mean value of salinity was reported in water samples collected from Abo Quir (loc.#1) and the highest level of dissolved oxygen was reported in water samples collected from Sedi Beshr (loc.#2). The present results showed that the studied heavy metals concentrations are highly significant in samples of water and soft tissue of *Ruditapes decussatus* collocated from Abo Quir bay. From the above-cited results, it is concluded that loc.#1(Abo Quir bay) represents the most polluted site in the present study. Statistical analysis showed a significant increase of MDA and a significant decrease of SOD and GPx in the soft tissue of *Ruditapes decussatus* collected from Abo-Quir Bay (loc.#1). There was a highly significant difference between the concentration of MDA, SOD, and GPx in the tissue of clams collected from the three locations ($p<0.001$). The correlation coefficient of heavy metals in tissue, heavy metals in water, and oxidative stress biomarkers showed that the concentration of MDA in the tissue of calm collected from Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.# 3) were positively correlated with the mean activity level of SOD and the concentration of SOD in the clam's tissue collected from Abo Quir Bay (loc.#1) and Sedi Beshr (loc.#2) were negatively correlated with the activity level of GPx. The present work also showed that the correlations between different heavy metals in the tissues of *R. decussatus* collected from three locations indicated that different rates and mechanisms of metal accumulation were taking place.

Keywords: Environment, Bivalves, Heavy metals, Clam, Protein, Oxidative stress.

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Introduction

Seafood is an important nutritious food source worldwide and appears in all kinds of popular dishes. Since bivalves are filter feeders and tend to bioaccumulate heavy metals at a higher concentration more than those of the surrounding seawater (Cosson, 2000; Fang *et al*., 2003). The risk is enhanced by the fact that these shellfish are eaten raw or relatively lightly cooked (Croci *et al*., 2002; Formiga-Cruz *et al*., 2003). The importance of oxidative stress response as potential biomarkers of environmental pollution has been addressed by different experimental approaches (Orbea *et al.*, 2002; Rodrı´guez-Ariza *et al*., 2003, Ferreira *et al.*, 2005 and Regoli *et al*., 2014). Metal accumulation can cause an increase in Reactive Oxygen Species (ROS) like hydrogen peroxide, superoxide anion radical, hydroxyl radical, leading to oxidative stress (Livingstone, 2001). Antioxidant defense systems that prevent the formation of ROS include antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) that have been extensively used as biomarkers of oxidative stress (Ferreira *et al*., 2005, 2007 and Vrankovic, 2015).

In Egypt, *Ruditapes decussatus* is an abundant benthic bivalve that in direct contact with heavy metals both of natural and anthropogenic origin. This clam is an example of a filter-feeding marine organism satisfying many criteria required for being a biological indicator (Bebianno, 2003). Marine coast is characterized by high fluctuations in chemical and physical parameters and in most cases, there exists a significant occurrence of human activities including industrial, domestic, and agricultural waste discharges (Meherm, 2002). The present work was designed to determine the concentration of some selected metals (Cu, Zn, Mn, Cd, and Pb) in seawater and the soft tissue of *Ruditapes decussatus* collected from Abo– Quir, Sedi Beshr, and EL Max in Alexandria seawater, Egypt. Also the oxidative stress biomarkers (lipid peroxidation (MDA); and antioxidant enzymes; superoxide dismutase (SOD) and Glutathione peroxidase (GPx) were measured to evaluate the physiological response of *Ruditapes decussates*.

Material and methods

The study areas and sample collection: The present investigation was carried out at three locations in the Mediterranean Sea Alexandria, Egypt). The first location is Abo-Quir Bay (loc.#1) which receives different pollutants contributing to various waste source categories discharged through three main openings namely; El-Tabia pumping station, the outlet of Lake Edku (Boughaz El-Maddya), and Rosetta mouth of the Nile River (Shreadah and Tayel, 1992). The second selected area is Sedi Beshr (loc. #2) which does not have any industrial activity like the other two locations Abo-Quir Bay and El-Max (Fig. 1). A water sample is fairly clean. Due to the existence of resorts, it turns to relatively little polluted water (Amin and Galal, 2000).

The third selected area is Max (loc.#3) which is affected by mixed agricultural runoff and industrial wastes from a chloro-alkali plant and receives airborne particles from the fumes of adjacent industrial plants, including an oil refinery and cement factory. *Ruditapes decussatus* clams were collected from the three locations; Abo Quir (loc.#1), Sedi Beshr (loc.#2), and El Max (loc.#3) during Summer 2017. Dead or damaged specimens were eliminated and standardized shell size (3.5-3.8mm) was used. Also, Water samples were collected from a precise depth corresponding to the clam settlements from each location.

Physicochemical analysis of seawater: Determination of physicochemical parameters (salinity, dissolved oxygen, and pH) was measured in seawater samples. Salinity was determined using an Inductive Salinometer

(Beckman mode) according to (Grasshoff, 1976). The pH-value of the water sample was measured using Bench type (JENWAY, 3410 Electrochemistry Analyzer pH-meter). dissolved oxygen determination was done according to a modified Winkler´s method (Grasshoff, 1976).

Fig.1. location map of the study sites.

Analysis of heavy metals: The concentrations of heavy metals: Copper (Cu), Zinc (Zn), Manganese (Mn), Cadmium (Cd), and lead (Pb), in water samples and soft tissues of *Ruditapes decussatus* were measured using the atomic absorption spectrophotometer by the method described by UNEP/FAO/IAEA/IOC, (1984); El-Sikaily *et al*. (2004). The results of metal concentrations in water samples and soft tissues were expressed as (µg/l) and (µg/g) of dry weight respectively.

Physiological and biochemical analysis: The determination of biochemical markers of oxidative stress in the soft tissue of *Ruditapes decussatus* occurred by the measurement of lipid peroxidation (MDA); according to the method of Yoshioka *et al*. (1979), and antioxidant enzymes; superoxide dismutase (SOD) and Glutathione peroxidase (GPx); were determined according to the method of Beauchamp and Fridovich, (1971) and Rao (1996); respectively. Also, total protein content (T.p.) was measured according to the method described by Lowry *et al*. (1951).

Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov, Shapiro, and D'agstino tests were used to verify the normality of distribution of variables, F - test was used to compare two groups for normally distributed quantitative variables. Pearson coefficient was used to correlate between quantitative variables. The significance of the obtained results was judged at the 5% level. The minimum level of statistical significance was set at $P < 0.05$.

Results

The physico-chemical parameters obtained from an analysis of seawater samples collected from the three studied locations; Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.#3) collected in Summer 2017 are presented in Table 1 and Figs. 2 & 3. The present data showed that the highest mean value of salinity was reported in loc.#1 as 36.74% while the highest mean value of dissolved oxygen was; 10.0 ± 0.19 mg/l in (loc.#1). The temperature ranged between (34.6- 37 .5) in the studied location.

Table (1): The mean and standard deviation (Mean±S.D.) of Physico-chemical parameters of water samples collected from; Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.#3).

Physico-chemical parameters	Abo Quir	Sedi Beshr	El Max	F	p
Salinity‰	$36.74^{a}\pm0.98$	$34.01^{b}\pm0.79$	$34.37^{b}\pm0.72$	18.841^{*}	$<0.001^{*}$
pH	7.84 ± 0.10	7.75 ± 0.07	7.81 ± 0.09	1.544	0.246
Dissolved Oxygen (mg/L)	$9.02^{c} \pm 0.24$	$10.0^{a} \pm 0.19$	$9.56^{b} \pm 0.19$	33.476^{*}	$<0.001^{*}$
Temperature	$37.50^{a}\pm1.52$	$34.67^{b}\pm1.21$	$35.83^{b}\pm4.02$	1.831	0.194

F, p: F and p values for ANOVA test, significant between groups was done using Post Hoc Test (LSD).
*: Statistically significant at $p \leq 0.05$. Data were expressed using Mean ± SD.
F ratio: Frequency, P-Value: Probability.
Means with Common letters are not significant (Means with Different letters are significant).

The heavy metal concentrations of water collected from the studied locations are presented in Table (2) and fig. (4) during summer 2017. The results showed that the heavy metals concentrations of Zn, Mn, Pb, Cu, and Cd is highly significant in samples of water collocated from Abo Quir bay; its concentrations were 5.22 ± 0.37, 4.50±0.49, 5.43±0.55, 3.48± 0.43 and 3.59a ± 0.41(μg/l) respectively. The pattern of metals occurrence in the water samples of selected locations can be summarized as follows in descending order Abo Quir Bay (loc.#1) >El-Max (loc.#3)> Sedi Beshr (loc.#2). From the above-cited results, it is indicated that; loc.#1 (Abo Quir Bay) represents the most polluted site in the present study.

Table (2): Mean and standard deviation (Mean±S.D.) of heavy metals concentration in water (μg/l) collected from Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.#3).

Heavy metals in water (μg/l)	Abo Quir(loc.#1)	Sedi Beshr(loc.#2)	El Max(loc.#3)	F	p
Zn	$5.22^{a} \pm 0.37$	$2.70^{c} \pm 0.63$	$3.67^{b} \pm 0.53$	35.891^{*}	$<0.001^{*}$
Mn	$4.50^{a} \pm 0.49$	$2.53^{b} \pm 0.40$	$2.92^{b} \pm 0.41$	34.669^{*}	$<0.001^{*}$
Pb	$5.43^{a} \pm 0.55$	$3.73^{b} \pm 0.44$	$4.13^{b} \pm 0.28$	24.860^{*}	$<0.001^{*}$
Cu	$3.48^{a} \pm 0.43$	$2.03^{c} \pm 0.20$	$2.87^{b} \pm 0.31$	29.510^{*}	$<0.001^{*}$
Cd	$3.59^{a} \pm 0.41$	$1.89^{b} \pm 0.33$	$2.04^{b} \pm 0.28$	45.343^{*}	$<0.001^{*}$

Means with Common letters are not significant (Means with Different letters are significant)
F, p: F and p values for ANOVA test, significant between groups.*: Statistically significant at $p \leq 0.05$
Data were expressed using Mean ± SD. F ratio: Frequency, P-Value: Probability.

Table (3) and fig. 5 showed that; the concentrations of Zn, Mn, Pb, Cu, and Cd in the soft tissue of *Ruditapes decussates* were accumulated in the samples collected from Abo Quir Bay (loc.#1) more than the concentration of metals in the samples collected from Sedi Beshr (loc.#2) and El-Max (loc.#3). It showed significant differences between the samples taken from the three studied locations ($p \leq 0.001$) and its pattern of accumulation was Abo Quir Bay (loc.#1) >El-Max (loc.#3)> Sedi Beshr (loc.#2). The mean concentrations of Mn, Pb, and Cd in the soft tissue of the studied clam at Abo Quir Bay exceeded the allowable limit of (WHO, 1989).

Table (3): Mean and standard deviation (Mean±S.D.) of heavy metals concentration (µg/g) in the soft tissue of *Ruditapes decussatus* collected from Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.#3)

Heavy metals In tissue xof the clam (µg/g)	Number of replicants	Abo Quir	Sedi Beshr	El Max	WHO (1989) (µg/g)	F	p
Zn	5	$6.35^{a} \pm 0.59$	$4.73^{b} \pm 0.48$	$5.32^{b} \pm 0.56$	100	13.597^{*}	$<0.001^{*}$
Mn	5	$5.95^{a} \pm 0.82$	$2.63^{c} \pm 0.53$	$4.08^{b} \pm 0.48$	5.4	42.338^{*}	$<0.001^{*}$
Pb	5	$5.50^{a} \pm 0.66$	$2.93^{c} \pm 0.41$	$4.47^{b} \pm 0.53$	2	33.756^{*}	$<0.001^{*}$
Cu	5	$5.03^{a} \pm 0.42$	$2.92^{c} \pm 0.40$	$3.92^{b} \pm 0.44$	30	38.079^{*}	$<0.001^{*}$
Cd	5	$5.25^{a} \pm 0.61$	$1.83^{c} \pm 0.33$	$3.33^{b} \pm 0.68$	1	56.099^{*}	$<0.001^{*}$

Means with Common letters are not significant (Means with Different letters are significant)
F, p: F and p values for ANOVA test, significant between groups *: Statistically significant at $p \leq 0.05$
Data were expressed using Mean ± SD. F ratio: Frequency, P-Value: Probability.

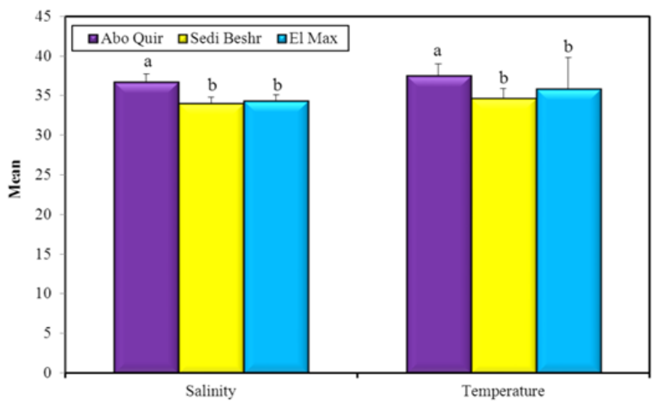

Figure: (2) Mean and standard deviation (Mean± SD) of salinity and temperature in Summer (mg/l) between the three studied locations. Data in the same column with different superscript letters (a, b, and c) are significantly different, $p<0.05$ (one-way ANOVA).

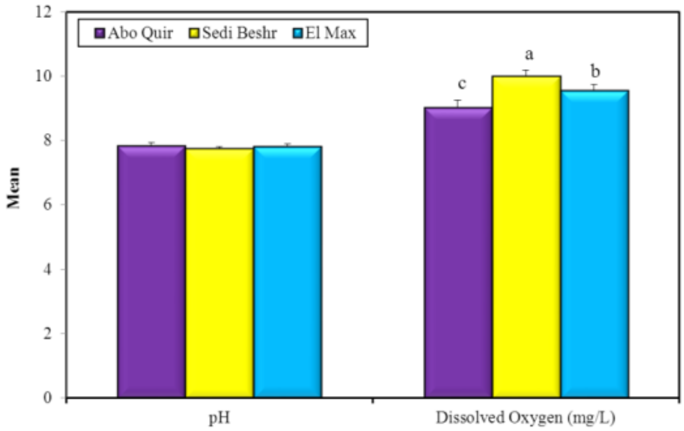

Figure (3): Mean and standard deviation (Mean± SD) of pH and dissolved oxygen (mg/l) in Summer between the three studied locations. Data in the same column with different superscript letters (a,b, and c) are significantly different, p<0.05(one-way ANOVA.

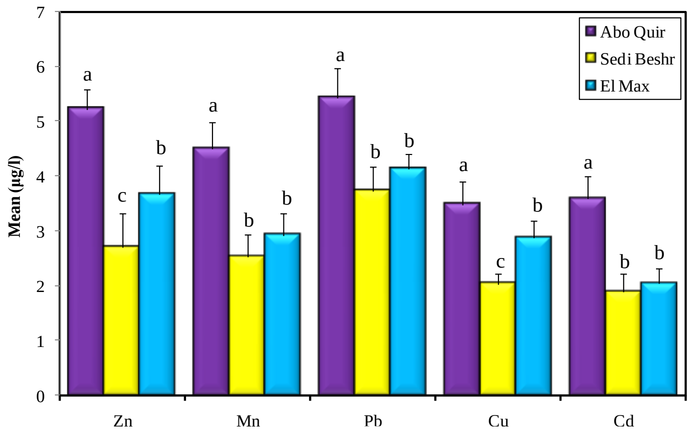

Figure (4): Mean and standard deviation (Mean± SD) of heavy metals in water (µg/l) in the three studied locations in Summer. Data in the same column with different superscript letters (a, b, and c) are significantly different, p<0.05(one-way ANOVA).

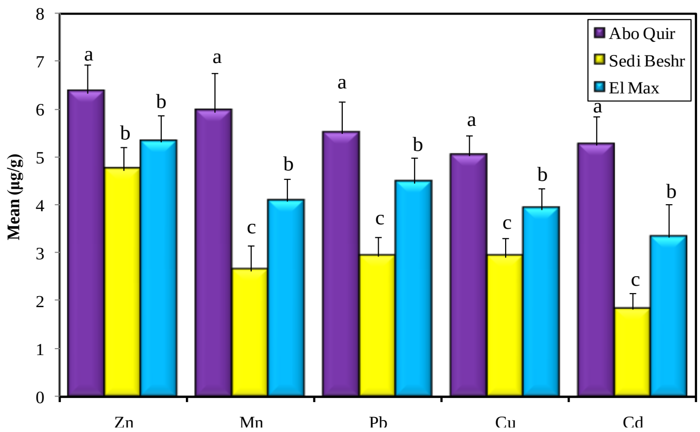

Figure (5): Mean and standard deviation (Mean± SD) of heavy metals in tissue (µg/g) in the three studied locations in summer. Data in the same column with different superscript letters (a, b, and c) are significantly different, p<0.05(one-way ANOVA).

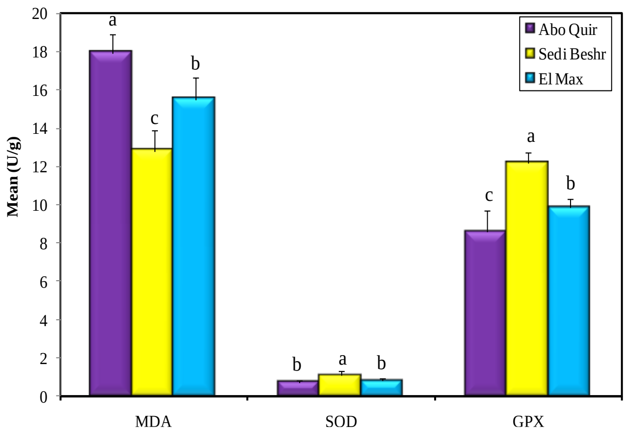

Figure (6): Mean and standard deviation (Mean± SD) of oxidative stress biomarkers in the tissue of the clam (U/g) from the three locations in Summer. Data in the same column with different superscript letters (a, b, and c) are significantly different, p<0.05(one-way ANOVA).

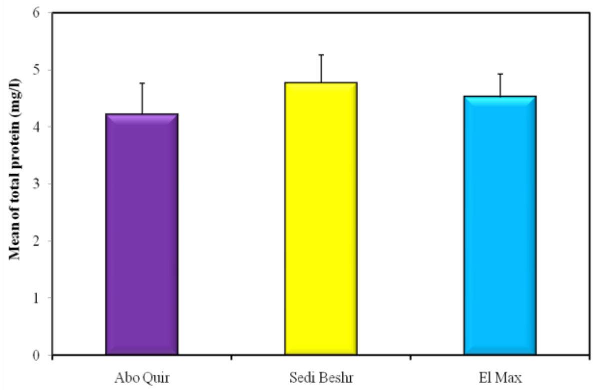

Figure (7): Mean and standard deviation (Mean± SD) of total protein in the tissue of the clam (mg/l) from the three locations in summer. Data in the same column with different superscript letters (a, b, and c) are significantly different, p<0.05(one-way ANOVA).

Oxidative stress biomarker in the tissue of *Ruditapes decussatus* was showed a significant difference in the three studied locations (Table 4, fig 6 and 7). The mean activity level of MDA at Abo Quir Bay recorded the highest value with a value of 17.93±0.98 (U/g). Statistical analysis showed a significant decrease of SOD and GPx in the soft tissue of *Ruditapes decussatus* collected from Abo-Quir Bay (loc.#1). On the other side, total protein showed no significant differences in the three studied locations.

Table (4): Mean and standard deviation (Mean±SD) of oxidative stress biomarkers in the tissue of the clam (U/g) and total protein (mg/l) in Summer collected from the three locations

Oxidative Stress Biomarkers (in tissue)	Abo-Quir Bay	Sedi Beshr	El-Max	F	p
MDA (U/g)	$17.93^{a} \pm 0.98$	$12.80^{c} \pm 1.06$	$15.50^{b} \pm 1.13$	35.295^{*}	$<0.001^{*}$
SOD (U/g)	$0.72^{b} \pm 0.09$	$1.07^{a} \pm 0.22$	$0.80^{b} \pm 0.10$	9.665^{*}	0.002^{*}
GPx (U/g)	$8.57^{c} \pm 1.12$	$12.15^{a} \pm 0.60$	$9.85^{b} \pm 0.47$	32.444^{*}	$<0.001^{*}$
Total protein (mg/l)	4.21 ± 0.55	4.76 ± 0.50	4.52 ± 0.40	1.957	0.176

The correlation coefficient of concentrations of heavy metals in seawater, heavy metals in tissue, and oxidative stress biomarkers of R. decussates collected from the studied locations were summarized in Tables 5, 6, and 7. The concentration of MDA in the tissue of calm collected from Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.# 3) were positively correlated with the mean activity level of SOD at r= 0.283, 0.564 and o.625 respectively. In contrast, the concentration of SOD in the tissue of R. decussates collected from Abo Quir Bay (loc.#1) and sedi Beshr (loc.#2) were negatively correlated with the activity level of GPx. While, in El-Max (loc.# 3) concentration of SOD in the clam tissue was a significant correlation (p = 0.03) with GPx (r= 0.854), a negative significant correlation with the concentrations of Zn and Pb, and a negative correlation with Mn and Cd.

Table (6) showed a high negative significant correlation (P= 0.001) between the concentrations of Zn with Pb (r= -.976) and Cu (r= -0.970) in the tissue of *R. decussates* collected from Sedi Beshr (loc.#2). A negative significant correlation was also demonstrated between the concentration of Mn and Cd (p= 0.02). In contrast, a positive significant correlation was detected between the concentration of Pb with Cu (r= 0.996) and concentration of Mn with Cd (r= 0.873) in the tissue of *R. decussatus* collected from Sedi Beshr (loc.#2) and El-Max (loc.# 3) respectively. Moreover, a positive significant correlation was shown between Zn in the tissue of clams with Pb (r= 0.826) and Cu (r= 0.947) in seawater collected from Abo Quir Bay (loc.# 1). The correlation among the concentration of heavy metals in seawater collected from the studied locations showed only a positive significant correlation between the concentrations of Pb with Cu collected from Abo Quir Bay (loc.#1) and El-Max (loc.# 3) (r= 0.889 and 0.832 respectively).

Discussion

In the present study, marine bivalves *Ruditapes decussatus* were collected from three areas of the Alexandrian coast, Egypt, Abo Quir Bay (loc.#1), Sedi Beshr (loc.#2), and El-Max (loc.#3). These species were identified in prior reports (Matias *et al*., 2009). Developmental processes in marine bivalves are recognized to be affected by different types of physical and natural factors involving nourishment quality temperature, salinity, photoperiod, sea hydrodynamics, and biomechanics (Osman and Whitlatch, 2004; Bates, 2005; Lambert, 2005). Salinity and temperature and are two of the most important factors impacting the distribution of marine living forms (Epelbaum *et al*., 2009). In this study, water temperature and pH showed non-significant differences in the three examined locations. Yet, there was a significant difference in the other physico-parameters e.g. dissolved oxygen level and salinity.PH ratio is an important limiting chemical factor for aquatic life that may influence the biochemical reactions of the aquatic organism. The severe alteration in pH of the water may lead to a harmful or fatal effect on aquatic organisms and as a result, affect human and animal health. In general, water flows have a pH range between 6 to 9 and any alteration in this range in pH can affect life forms in aquatic systems. If the pH value increases higher than this range, minor amounts of ammonia are required to reach a level that is toxic to fish, whereas when pH declines, the acidity of the water increases affecting the fish (Murdoch, 1991). In the present study, the pH value of water samples collected from the studied locations ranged between 7.75–7.84.

. The highest salinity value is documented through summer may be described to the higher degree of evaporation (Damotharan *et al*., 2010). Higher salinity may probably be due to the deposition of organic wastes, and inputs of different pollutant materials in this reach (Francis *et al*., 2014). Similarly, in the present study, the highest mean salinity (S‰) value of the water sample collected from the three studied locations recorded in Abo-Quir Bay with a value of 36.74a±0.98.

The dissolved oxygen (DO) is one of the more important parameters available in the field of water pollution control, as it allows the assessment of the aerobic conditions of a water-course, that receives a discharge of pollutants (Parvez *et al*., 2006). Benthic organisms have behavioral and physiological adaptations for low dissolved oxygen. Organisms respond by decreasing metabolism and thus O2 consumption (Wu, 2002). This can result in diminished growth and reproduction (Grove and Breitburg, 2005).). Even though it does not operate directly on growth as many toxins do, but limits the extent of aerobic metabolism. The most important natural physical factors affecting oxygen concentration in the aquatic environment are salinity and temperature. Dissolved O2 solubility is reduced with increasing temperature and salinity (Ospar, 2005). These findings confirmed the present results.

R. decussatus is among the benthic invertebrates which are indirect contact with heavy metals both of anthropogenic and natural origin. The areas under investigation exposed to large amounts of an industrial zone, which may reflect the reason Abo-Quir Bay and El-Max have a high concentration of heavy metals in the collected species. Utilization of heavy metals in industry, marine transport, and agriculture waste from the El-Mahmoudia canal that flow off in El-Max leads to a wide distribution of these components in a marine environment. Their toxicity, potential for human exposure, and environmental occurrence is dependant on several factors such as their concentration and source (Wu and Zhang, 2010, Radwan *et al*., 2012; Radwan *et al*., 2014; Radwan, 2016; Radwan *et al*., 2016; Radwan *et al*., 2017; Radwan *et al*., 2018).

The heavy metals were accumulated within the clam soft tissues at high concentration levels amounting to thousands of times those accumulating in water due to filter-feeding of these aquatic organisms (Salman, 2011). The gained results demonstrate the process of bio-magnification across the tropic levels. This phenomenon was detected in other bivalves when put in comparison to water (El-Shenawy, 2002; El-Gamal, 2011). Filter feeder oyster uptake dissolved metal ions in solution or particular form, via food by filtering large volumes of water each day (Sajwan *et al*., 2008). There are multiple ways to preserve homeostasis of essential metals to detoxify non-essential metals in the clam's tissues which include binding metals to low-molecular-weight proteins, such as storing them within metal-containing granules or lysosomes, so that they can accumulate metals within their tissues in higher levels than their ambient water (Marigomez, 2002 and Jitar *et al*., 2015). Consequently, *R. decussatus* was considered as a possible bio-monitor of contamination with heavy metals.

In the present study, a high accumulation of the selected heavy metals during summer 2017 was noticed in the tissues of *R. decussatus* collected from the three studied locations. The metabolism is sensitive to water temperature. Thus, any seasonal changes in the temperature could affect the metabolism with subsequent affection on the detoxification rate and accumulation of toxic materials. The metabolic acceleration owing to heat may lead to an acceleration of metal accumulation within bivalve tissues, whereas, the reduction in the metabolic rates of bivalves once the environment becomes colder, may diminish the rate of assimilation of metals (Lannig *et al*., 2006).

In the present work, a positive significant correlation was showed only between Zn in the tissue of clams with Pb (r= 0.826) and Cu (r= 0.947) in seawater collected from Abo Quir Bay (loc.# 1). Like in other studies (Tessier et al., 1984; Ahn et al. 2001), few correlations between metal concentrations in A. umbonella digestive glands and the environment were detected. The present work also showed that the correlations between different heavy metals in the tissues of *R. decussates* collected from three locations indicated that different rates and mechanisms of metal accumulation were taking place. This might be due to differences in toxicokinetic properties and efficient pathways of detoxification found in bivalves (Yap *et al.,* 2002). Metallothioneins (MTs) are low molecular weight non-enzymatic proteins that are heat stable and rich in cysteine, free of aromatic amino acids. The thiol groups of cysteine residues allow MTs to bind essential and non-essential metals with high affinity (Amiard et al., 2006). MTs play a role in the homeostatic control of essential metals (Cu, Zn) to accomplish metabolic and enzymatic demands (Roesijadi, 1996). They also have an important role in the detoxification of non-essential trace metals such as Ag, Cd, and Hg, which protects organisms against oxidative stress by scavenging the intracellular free radicals (Langston *et al*., 1998 and Li *et al*., 2013). Recent studies have shown that Cd can accumulate in marine invertebrates, and this can result in an elevation of the intracellular level of Metallothioneins (Amiard et al., 2006). Furthermore, Duffus, (1980) indicated that Zn reduced the Cd concentration as it increases the rates of formation of the metallothionein protein. This may show that the negative significant correlation (P= 0.001) between the concentrations of Zn with Pb and Cu and negative correlation between Zn with Cd in the tissue of R. decussates collected from Sedi Beshr (loc.#2) and El-Max (loc.#3).

The pollution toxicity in aquatic organisms may be connected to increased production of 'reactive oxygen species' (ROS) leading to great oxidative damage (Livingstone, 2001). Under normal conditions, cells possess antioxidant defenses that prevent the generation of ROS, and repair or degrade oxidatively modified molecules (Halliwell and Gutteridge, 1999). Alterations in the levels of antioxidants have been considered as biomarkers of contaminant-mediated pro-oxidant challenge in a variety of aquatic organisms, including mussels (Regoli *et al*., 2004). ROS can be extremely toxic to aquatic organisms such as mussels, often leading to lipids oxidation in membranes (MDA is an end product of lipid peroxidation LPX), protein oxidation, and DNA damage (Almeida *et al*., 2007). Ferreira *et al*., 2005 stated that this oxidative damage could occur when detoxifying and antioxidant systems are unable to neutralize the active intermediates produced by xenobiotic and their metabolites. In the current study, the mean activity level of MDA in the tissues of *R. decussates* collected from Abo Quir Bay and El-Max (loc.#3) were significantly higher than those found in the tissues of clams collected from Sedi Beshr (loc.#2). Pampanin *et al*. (2005) showed that lipid peroxidation is a familiar mechanism of cellular injury in vertebrates and invertebrates, and is considered as an indicator of oxidative damage in tissues. Consequently, the mean activity of MDA has recorded a significant increase at Abo-Quir Bay, where pollution toxicity increased.

The present study shows the mean level of SOD and GPX in the tissue of *R. decussatus* collected from Sedi Beshr (loc.#2) were higher than those collected from Abo Quir Bay and El-Max (loc.#3). Moreover, in El-Max (loc.# 3) concentration of SOD in the clam tissue shows a negative significant correlation with the concentrations of Zn and Pb and a negative correlation with the concentration of Mn and Cd. Enzymes with antioxidant properties such as SOD & GPx (Géret *et al*., 2003) are defense biomarkers. The most important role of the antioxidant defenses is to stop the action of excess oxyradicals produced from exposure to xenobiotics (Valavanidis *et al*., 2006). The highly significant correlation between the mean activity of GPx with SOD in the tissue of *R. decussates* collected from El-Max (loc.#3) and negative correlation in tissues of clams collected from Abo Quir and Sedi Beshr (loc.#2) Bay. This may be due to that GPx activity increases more rapidly than other defenses during aerobic recovery in marine bivalves that are suggestive of their task as first responders to oxyradical detoxification (Pannunzio and Storey, 1998). Finally, it is important to accept that, although the accumulation of metals in *R.*

decussatus is in response to antioxidant defense components, it cannot be considered to be due to the effect of heavy metals alone, but also due to other physical or chemical contaminants that may be present in these sites (El-Raey *et al.,* 2006). We can conclude that the levels of the recorded metals exceed the maximum permissible levels except for zinc and copper as mentioned by WHO (1989). This finding showed that *R. decussatus* collected from Abo Quir Bay and El-Max are not safe for human consumption. Also, the presence of heavy metals induces biological stress and oxidative damage to *R. decussatus*.

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Table (5): Correlation coefficient of heavy metals in tissue, heavy metals in water, and oxidative Stress biomarkers:

Heavy metals in water															
				Heavy Metals in tissue						Oxidative Stress Biomarkers			Summer /Abo Quir Bay		
Cd	Cu	Pb	Mn	Zn	Cd	Cu	Pb	Mn	Zn	T. P.	GPx	SOD			
-0.293	-0.240	-0.432	0.340	-0.019	0.633	-0.545	0.750	-0.150	-0.017	0.800	-0.373	0.283	r	MDA	Oxidative Stress Biomarkers
0.573	0.647	0.392	0.510	0.972	0.178	0.264	0.086	0.777	0.974	0.056	0.466	0.586	p		
0.414	0.734	0.353	-0.206	0.310	0.440	-0.357	0.531	-0.311	0.743	0.132	-0.222		r	SOD	
0.414	0.097	0.492	0.696	0.550	0.382	0.487	0.278	0.548	0.090	0.803	0.673		p		
-0.168	0.169	0.416	-0.465	-0.821*	-0.265	0.941*	0.100	0.491	-0.049	0.238			r	GPx	
0.750	0.749	0.412	0.353	0.045	0.612	0.005	0.850	0.322	0.927	0.650			p		
-0.366	-0.121	-0.144	0.185	-0.557	0.596	0.025	0.864*	0.249	-0.006				r	T. p.	
0.476	0.820	0.786	0.726	0.251	0.212	0.962	0.026	0.634	0.990				p		
0.193	0.947*	0.826*	0.155	-0.014	0.502	-0.245	0.228	0.324					r	Zn	Heavy Metals in Tissue
0.714	0.004	0.043	0.770	0.979	0.310	0.640	0.665	0.531					p		
-0.417	0.264	0.628	0.464	-0.767	0.283	0.328	-0.026						r	Mn	
0.411	0.613	0.182	0.354	0.075	0.586	0.525	0.961						p		
0.100	0.155	-0.066	0.056	-0.232	0.721	-0.065							r	Pb	
0.850	0.770	0.901	0.916	0.658	0.106	0.902							p		
0.032	0.015	0.255	-0.512	-0.633	-0.416								r	Cu	
0.953	0.978	0.626	0.299	0.178	0.411								p		
0.171	0.286	0.149	0.669	-0.040									r	Cd	
0.746	0.583	0.778	0.146	0.939									p		
0.586	-0.061	-0.381	0.034										r	Zn	Heavy Metals in Water
0.221	0.908	0.456	0.949										p		
-0.118	-0.124	-0.015											r	Mn	
0.824	0.815	0.978											p		
0.037	0.889*												r	Pb	
0.944	0.018												p		
0.296													r	Cu	
0.568													p		
													r	Cd	
													p		

Table (6): Correlation coefficient of heavy metals in tissue, heavy metals in water, and oxidative stress biomarkers in Sedi Beshr (loc.#2).

						Heavy metals in tissue					heavy metals in water				
Summer / Sedi Beshr location			**Oxidative Stress Biomarkers**												
			SOD	**GPx**	**T. P.**	**Zn**	**Mn**	**Pb**	**Cu**	**Cd**	**Zn**	**Mn**	**Pb**	**Cu**	**Cd**
Oxidative Stress Biomarkers	**MDA**	**r**	0.564	0.170	0.597	-0.710	-0.365	0.636	0.681	0.442	0.106	-0.314	-0.108	-0.778	-0.616
		p	0.243	0.747	0.211	0.114	0.477	0.175	0.137	0.381	0.842	0.545	0.838	0.068	0.193
	SOD	**r**		-0.235	-0.150	-0.300	-0.594	0.133	0.173	0.824*	0.716	-0.268	0.543	-0.568	-0.198
		p		0.654	0.777	0.564	0.214	0.802	0.743	0.044	0.110	0.608	0.265	0.240	0.707
	GPx	**r**			0.466	0.034	0.147	-0.084	-0.117	-0.406	-0.197	0.415	0.290	0.212	0.087
		p			0.352	0.949	0.781	0.874	0.826	0.425	0.708	0.414	0.577	0.687	0.869
	T. p.	**r**				-0.428	0.403	0.503	0.530	-0.324	-0.665	0.368	-0.439	-0.567	-0.509
		p				0.397	0.429	0.309	0.280	0.531	0.150	0.473	0.384	0.241	0.302
Heavy Metals in Tissue	**Zn**	**r**					-0.060	-0.976*	-0.970*	-0.011	-0.158	0.280	0.325	0.742	0.064
		p					0.910	0.001	0.001	0.984	0.765	0.591	0.530	0.091	0.904
	Mn	**r**						0.205	0.169	-0.881*	-0.623	0.764	-0.344	-0.051	0.381
		p						0.697	0.750	0.020	0.186	0.077	0.504	0.923	0.456
	Pb	**r**							0.996*	-0.121	-0.031	-0.224	-0.506	-0.730	-0.100
		p							<0.001	0.820	0.954	0.670	0.305	0.099	0.851
	Cu	**r**								-0.060	-0.048	-0.241	-0.523	-0.777	-0.183
		p								0.909	0.928	0.645	0.287	0.069	0.729
	Cd	**r**									0.604	-0.603	0.340	-0.284	-0.476
		p									0.204	0.205	0.509	0.585	0.340
Heavy Metals in Water	**Zn**	**r**										-0.451	0.680	0.000	0.372
		p										0.369	0.137	1.000	0.468
	Mn	**r**											0.208	0.008	0.289
		p											0.692	0.987	0.578
	Pb	**r**												0.217	0.394
		p												0.679	0.440
	Cu	**r**													0.432
		p													0.393

Table (7): Correlation coefficient of heavy metals in tissue, heavy metals in water, and oxidative stress biomarkers in El-Max (loc.#3).

Summary / El Max location			Oxidative Stress Biomarkers			Heavy Metals in tissue					Heavy metals in water				
			SOD	**GPx**	**T. P.**	**Zn**	**Mn**	**Pb**	**Cu**	**Cd**	**Zn**	**Mn**	**Pb**	**Cu**	**Cd**
Oxidative Stress Biomarkers	**MDA**	**r**	0.625	0.308	0.110	-0.673	-0.199	-0.589	-0.270	-0.094	0.143	-0.174	0.145	0.253	0.388
		p	0.185	0.553	0.836	0.143	0.705	0.219	0.604	0.860	0.787	0.742	0.784	0.628	0.448
	SOD	**r**		0.854*	0.408	-0.844*	-0.447	-0.857*	0.063	-0.132	-0.283	0.285	-0.089	0.200	0.812*
		p		0.030	0.422	0.035	0.374	0.029	0.905	0.803	0.587	0.584	0.866	0.704	0.050
	GPx	**r**			0.670	-0.700	-0.790	-0.817*	0.442	-0.474	-0.433	0.392	-0.090	0.239	0.526
		p			0.145	0.122	0.062	0.047	0.381	0.342	0.391	0.442	0.866	0.648	0.284
	T. p.	**r**				-0.591	-0.683	-0.689	0.848*	-0.295	-0.072	0.670	-0.467	0.005	-0.122
		p				0.216	0.135	0.130	0.033	0.570	0.893	0.145	0.350	0.992	0.819
Heavy Metals in Tissue	**Zn**	**r**					0.346	0.759	-0.106	-0.076	0.313	-0.575	0.073	-0.397	-0.525
		p					0.501	0.080	0.841	0.887	0.546	0.232	0.891	0.436	0.285
	Mn	**r**						0.602	-0.618	0.873*	0.241	-0.080	-0.040	-0.217	0.026
		p						0.206	0.191	0.023	0.646	0.880	0.941	0.680	0.961
	Pb	**r**							-0.471	0.275	-0.104	-0.283	0.452	0.136	-0.474
		p							0.346	0.598	0.845	0.586	0.369	0.798	0.342
	Cu	**r**								-0.399	0.189	0.429	-0.647	-0.382	-0.358
		p								0.433	0.720	0.396	0.165	0.455	0.486
	Cd	**r**									0.098	0.373	-0.248	-0.144	0.194
		p									0.854	0.466	0.635	0.786	0.713
Heavy Metals in Water	**Zn**	**r**										-0.413	-0.621	-0.822*	-0.323
		p										0.416	0.188	0.045	0.532
	Mn	**r**											-0.339	0.193	0.100
		p											0.511	0.714	0.851
	Pb	**r**												0.832*	0.034
		p												0.040	0.949
	Cu	**r**													0.115
		p													0.828