Research Article

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ECOTOXICOLOGICAL AND PHYSIOLOGICAL STUDIES ON PINCTADA RADIATA (LEACH, 1814) COLLECTED FROM ALEXANDRIA COASTAL WATER (MEDITERRANEAN SEA, EGYPT)

ABSTRACT:
The present study relates the level of pollution in the coastal water to the physiology of the bivalve Pinctada radiata. Four localities of the Mediterranean Sea, Alexandria, Egypt were chosen for this study (El Asafra, Sidi Beshr, Kait Bay and El Dekhela). Specimens were collected during September to November 2009. Water analysis of selected heavy metals (iron, copper, lead, and cadmium) showed many attributes of the water quality criteria which was below the admissible levels of USEPA. The highest concentration of iron was found in El Dekhela, while that of copper, lead and cadmium was found in Sidi Beshr. Results also revealed fluctuations in the measured biochemical parameters (glucose, triglycerides, creatinine, uric acid and total proteins) in the haemolymph of the oyster. The lowest concentration of glucose in the haemolymph was found in specimens collected from El-Asafra, while that of triglycerides and uric acid was reported in El Dekhela. Kait Bay specimens were found to have the highest level of creatinine, while the maximum level of total proteins was reported in El Dekhela. It was thus concluded that the biochemical parameters in the Pinctada radiata were affected by the low levels of heavy metals in the different localities under investigation of the Mediterranean coastal areas, Alexandria-Egypt.

KEY WORDS:
Bivalve, Pinctada radiata, Heavy metals, Biochemical parameters, Egypt, Alexandria, Mediterranean coast

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INTRODUCTION:
Around the world, the industrial discharges are known to contain heavy metals which may increase trace metal concentrations in the coastal zone, some of which are toxic and can endanger human health (Al-Madfa et al., 1998; Frías-Espericueta et al., 1999). Much concern about the industries and domestic wastes has been generated. These wastes which discharge to the sea constitute a hazard to marine biota (Biney and Ameyibor, 1992) and organisms including pearl oysters and shellfish eventually end up in the food chain (Peerzada et al., 1990). Some groups of chemicals such as carcinogens, mutagens, and neurotoxins are even unaffected by the usual methods of water treatment (Abdel-Shafy and Aly, 2002).

The problem of heavy metal pollution is one of the most important problems and it has gained worldwide attention in recent decades (Soria and Theede, 1990 and Joiris et al., 1998). Many heavy metals occur naturally in marine environments. Some of them were described as pollutants when found in sufficient amounts to produce deleterious effects on some features of the ecological system. Living organisms can be used as more efficient monitors of environmental contamination. Bivalve molluscs such as mussels and oysters are ideal bioindicator organisms for aquatic pollution because of their way of life (Gagnaire et al., 2003). They are well known for their ability to concentrate pollutants in their tissues to several orders of magnitude above ambient levels in sea water. Oysters and mussels can accumulate cadmium in their tissues at levels up to 100,000 times higher than the levels observed in the water in which they live (Avelar et al., 2000). Usually, the levels of pollutants...
accumulated in bivalve tissues have been used for assessing the level of pollution in its habitat (Al-Madfa et al., 1998 and Abd Allah and Moustafa, 2002). Therefore, they have been used extensively as successful bio monitors of aquatic metallic pollutant levels (O’Connor, 2001 and MacFarlane et al., 2005). Avelar et al. (2000); Kavun et al. (2002) and Côksu et al. (2005) reported that Pinctada radiata (Bivalve) could be used as indicator species for heavy metals accumulation studies.

Element concentrations in molluscs at the same location differ between different individuals (Otchere et al., 2003) and different animals in the same community at the same trophic level could accumulate pollutants differently (El-Mosehly and Yassien, 2005). Oysters are bottom dwellers and filter feeders which are prone to land or ships originating trace metals entering the marine environment (Hashim et al., 1994).

The excess metal ions have the potential to exert toxic effects including cellular toxicity in bivalves (Giguere et al., 2003 and Sokolova et al., 2004). In the field, organisms are generally exposed to mixture of contaminants (Amiard, Triguet and Amiard, 1998). Metal bioaccumulation in aquatic organisms has received extensive attention over the last several decades because metal toxicity is directly dependent on metal accumulation (Luoma, 1989).

The aim of the present study is to evaluate the concentration of some heavy metals (iron, copper, lead and cadmium) in seawater in four locations along the coast of Alexandria, Egypt. Also, the study investigates the impact of these metals on the physiology and health status of the pearl oyster, Pinctada radiata (Leach, 1814).

MATERIAL AND METHODS:

Study area:

Four localities exposed to different levels of pollution, El Asafra, Sidi Beshr, Kait Bay and El Dekhela (Fig. 1) were chosen for this study. Water and mollusc samples were collected from these stations during the period September-November, 2009.

Sampling:

Water samples:

Coastal water samples were collected from the four stations on the coast of Alexandria at 3 - 5 m depth for the determination of iron, copper, lead and (2000); Kavun et al. (2002) and Côksu et al. (2005) could be used as indicator species for heavy metals accumulation studies.

Element concentrations in molluscs at the same location differ between different individuals (Otchere et al., 2003) and different cadmium. Seawater samples were filtered through 0.45 µm millipore filters to remove any debris particles then stored at 20°C until analysis. All values are reported as µg/L for seawater. All the precautions recommended by Kremling (1983) to minimize risks of sample contamination were followed during collection and treatment of samples.

Molluscan samples:

The factors to be considered in the selection are maturity and overall health. Pinctada radiata (Leach, 1814) of 20-25 gm weight and above 1.5 to 2.0 year old are ideal and even smaller specimens are also suitable for this study (Fig. 2). Oysters were collected in sterile plastic bags, cleaned from attached organisms and rinsed with seawater from their sampling locations and transported to the laboratory within 4 & 6 h. Oyster shells were surface–cleaned with ethanol and notched along the ventral edge enough to insert a needle. Haemolymph from 25 oysters from each location was withdrawn from the adductor muscle of the oyster using a 21, gauge hypodermic syringe (Sokolova et al., 2004).

Analytical methods:

Biochemical parameters:

The haemolymph was used for the estimation of triglycerides contents according to the enzymatic colorimetric procedure of Fossati and Prencipe (1982), where the triglycerides are enzymatically hydrolyzed to glycerol and free fatty acids. The liberated glycerol reacts with glycerol kinase and glycerol, 3, phosphate oxidase yielding H$_2$O$_2$. The H$_2$O$_2$ concentration is determined through the Trinder’s reaction. Total proteins were estimated according to the method described
by Lowery et al. (1951) depending on the phenolic group of tyrosine and tryptophan residues in the protein that form a blue purple color complex with Folin,Ciocalteau reagent. The intensity of color depends on the amount of these amino acids present and will thus vary for different proteins. Creatinine was measured using the kinetic method described by Henry (1974). Uric acid was determined enzymatically based on the method of Cunningham and Keaveny (1978). Glucose level was measured according to the enzymatic colorimetric method described by Trinder (1969). Glucose in the sample is oxidized by the enzyme glucose oxidase to gluconic acid with the liberation of hydrogen peroxide, which is degraded by the enzyme peroxidase and together with 4, aminophenazone - phenol forms a pink colored chromogen which is measurable using Trinder indicator at 515 mm.

Heavy metals:

The concentration of iron, copper, lead and cadmium were determined in the collected seawater samples using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer model 2380) under the recommended conditions and the detection limits in the manual for each metal (Bernhard, 1976).

Statistical analysis:

Statistical analysis was performed using two-way ANOVA using SPSS computer program (version 14.0) and significant difference between metal concentrations in the different localities was expressed at P < 0.05. The analytical method was checked by 5 replicate measurements for the studied metals in a sample of marine water.

RESULTS:

Variations in the concentration of some heavy metals (iron, copper, lead and cadmium) in seawater samples are tabulated in table 1 and shown in figure 3. Sidi Beshr was found to have the highest concentrations of copper, lead and cadmium compared to the levels at the other localities. The lowest and highest concentrations of iron in seawater were found in Kait Bay (6.62 µg/L) and El Dekhela (19.78 µg/L), respectively. The average concentration of iron in seawater was reported to be 10.0 µg/L, 18.22 µg/L, 6.62 µg/L and 19.78 µg/L at El Asafra, Sidi Beshr, Kait Bay and El Dekhela, respectively. The average concentration of cadmium in seawater ranged between 0.37 µg/L at El Asafra to 1.33 µg/L at Sidi Beshr. The cadmium concentrations in seawater from different localities were found to be 0.37 µg/L, 1.33 µg/L, 0.59 µg/L and 0.83 µg/L at El Asafra, Sidi Beshr, Kait Bay and El Dekhela, respectively.

Table 1. Levels of some heavy metals in seawater samples in the study areas

<table>
<thead>
<tr>
<th></th>
<th>El Asafra</th>
<th>Sidi Beshr</th>
<th>Kait Bay</th>
<th>El Dekhela</th>
<th>Admissible levels of heavy metals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (µg/L)</td>
<td>10.00 ± 0.47</td>
<td>18.22 ± 0.44</td>
<td>6.62 ± 0.51</td>
<td>19.78 ± 0.99</td>
<td>3.1 µg/L (USEPA, 2005)</td>
</tr>
<tr>
<td>Copper (µg/L)</td>
<td>0.82 ± 0.05</td>
<td>1.28 ± 0.35</td>
<td>1.00 ± 0.31</td>
<td>0.61 ± 0.02</td>
<td>210 µg/L (USEPA, 2005)</td>
</tr>
<tr>
<td>Lead (µg/L)</td>
<td>0.31 ± 0.02</td>
<td>2.14 ± 0.10</td>
<td>0.24 ± 0.03</td>
<td>1.92 ± 0.16</td>
<td>210,8.1 µg/L (USEPA, 2005)</td>
</tr>
<tr>
<td>Cadmium (µg/L)</td>
<td>0.37 ± 0.04</td>
<td>1.33 ± 0.59</td>
<td>0.59 ± 0.02</td>
<td>0.83 ± 0.03</td>
<td>40 µg/L (USEPA, 2005)</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± Standard deviation (SD). F: F test (ANOVA)
*: Statistically significant at p ≤ 0.05.
Fig. 3. Concentrations of iron (a), copper (b), lead (c) and cadmium (d) in seawater samples from different localities along Alexandria coast.

The biochemical parameters measured in the haemolymph collected from the oyster showed fluctuations at the different studied areas as shown in table 2 and figure 4. Concerning the glucose concentrations, they were 175.4 mg/dl, 381.2 mg/dl, 402.4 mg/dl and 274.0 mg/dl at El Asafra, Sidi Beshr, Kait Bay and El Dekhela, respectively. The highest concentration of triglyceride was found in El Dekhela, 147.2 mg/dl, 161.0 mg/dl, 134.8 mg/dl and 178.2 mg/dl were the concentrations of triglycerides at El Asafra, Sidi Beshr, Kait Bay and El Dekhela, respectively. The concentration of creatinine ranged from 2.86 mg/dl at Sidi Beshr to 4.78 mg/dl at Kait Bay. The creatinine concentrations at El Asafra, Sidi Beshr, Kait Bay and El-Dekhela were 3.62 mg/dl, 2.86 mg/dl, 4.78 mg/dl and 4.48 mg/dl, respectively. The concentration of uric acid was found in El-Dekhila (3.2 mg/dl), while in the other localities the concentrations were found to be 2.54 mg/dl, 2.86 mg/dl, 4.78 mg/dl and 4.48 mg/dl at El Asafra, Sidi Beshr, Kait Bay and El-Dekhela, respectively. The highest concentration of total proteins (4.20 mg/dl) and the lowest concentration (2.42 mg/dl) were found at El Dekhela and Sidi Beshr, respectively. The concentrations of protein at the other localities were 3.2 mg/dl and 2.48 mg/dl at El Asafra and Kait Bay, respectively.

<table>
<thead>
<tr>
<th></th>
<th>El Asafra</th>
<th>Sidi Beshr</th>
<th>Kait bay</th>
<th>El Dekhela</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>175.40 ± 19.89</td>
<td>381.20 ± 26.17</td>
<td>402.40 ± 12.20</td>
<td>274.00 ± 17.46</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>147.20 ± 7.85</td>
<td>161.00 ± 6.25</td>
<td>134.80 ± 7.60</td>
<td>178.20 ± 6.18</td>
</tr>
<tr>
<td>Creatinine</td>
<td>3.62 ± 0.27</td>
<td>2.86 ± 0.36</td>
<td>4.78 ± 0.35</td>
<td>4.48 ± 0.16</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2.54 ± 0.81</td>
<td>2.70 ± 0.27</td>
<td>1.86 ± 0.25</td>
<td>3.20 ± 0.25</td>
</tr>
<tr>
<td>Total proteins</td>
<td>3.20 ± 0.25</td>
<td>2.42 ± 0.23</td>
<td>2.48 ± 0.30</td>
<td>4.20 ± 0.25</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± Standard deviation (SD). F: F test (ANOVA); *: Statistically significant at p ≤ 0.05.
DISCUSSION:

Alexandria coast is recognized as a highly contaminated site with trace metals due to heavy industrial, agricultural and sewage disposals (El-Deeb and Aboul–Naga, 2002). Al-Madfa et al. (1998) reported that, around the world the coastal zones are subjected to the direct release of urban and industrial discharges, such inputs are known to contain heavy metals which may increase trace metal concentrations in these zones, some of which are toxic and can endanger human health.

The discharge of industrial wastes enriched with chemical fertilizers in addition to drainage waters greatly affect the hydrography and chemistry of seawater as well as the fish production, which is greatly affected by such great quantities of pollutants (Shriadah, 1991).

Alexandria coastal water is considered as one of the metal polluted areas among the Mediterranean Sea (Abdel,Moati, 1991; Atta, 1991). El,Max receives different types and amounts of effluents, agricultural, domestic and industrial effluents (Nessim et al., 2005). El,Rayis and Abd Allah (2005) reported that Omoum Drain in Egypt after the construction of the Aswan High Dam and controlling the Nile River water flow becomes one of the main land based sources regularly discharging its water directly to the Mediterranean Sea at El Max Bay west of Alexandria.

In the present study, the highest value of iron concentration was found in El Dekhela station west to El Max Bay. This could be attributed to the external inputs from land based sources, biological activities, sewage, industrial and agricultural discharges which are considered as the major sources of iron into El Dekhela beach. Similar results were reported by Younes (1997) and Shakweer et al. (2006). El Dekhela area is located at the western site of Alexandria beaches and is considered as one of the most important fishing grounds at Alexandria coastline. The high concentration of Fe of the coastal area of the Mediterranean could be attributed to the highest rate of fresh water discharge reaching this area. Aboul-Dahab (1985) reported the concentration of Fe to be 74 µg/L at El Max Bay which could be due to the release of iron from the bottom sediments. Kremling (1983) found that the enrichment of dissolved Fe in the anoxic bottom layer is well documented in areas characterized by the coexistence of oxygen and hydrogen sulphide. The concentration of Fe in El Dekhela indicates the tendency of iron to increase in less oxygenated water and this may indicate the release of iron from the bottom sediments in poorly oxygenated water, which is characterized by the presence of hydrogen sulphide ions and their adsorption in oxygenated water (Emara and Shriadah, 1991).

Lead levels in inland waters are usually higher than in open oceans, through of course large variations from place to place (Fergusson, 1975). Coastal water can receive significant inputs of lead from industry and/or sewage of the water sheds and lead concentrations in them can reflect these inputs (Shakweer et al., 2006). Attia et al. (1987) reported high value of lead (45µg/l) in water samples from El-Temsah shipping company–such area has a variety of industrial activity and receives industrial effluents, and 30µg/L in water samples from Abu Quir beach. Abou-Taleb et al. (2004) found that lead concentrations in the Abu-Quir beach was 4.6 µg/L. Sidi Beshr is located southwest of Abu Quir city, and is protected from the westward currents by a rocky platform and from the northward currents by a relatively large island. It is widely opened to the sea from the northeast area. Predominant currents flow into Miami Beach northwesterly over the rocky platform which constitutes the main inlet of marine currents into Sidi Beshr. The northwest winds generally cause the formation of shallow currents inducing transportation of coastal sediments to the east (Samir and Badr-El-Din, 2001). Miami has no industrial activity on the adjacent
coast. Only domestic wastes contribute through an outfall to Miami Beach, which does not carry significant amount of heavy metals (Samir and Badr-El-Din, 2001).

El Dekhela was reported to have the second highest concentration of lead in the present study. El Max district is an industrial zone and as a consequence of growing petrochemical industries, metal plating, industrial dyes and the uncontrolled disposal of the resulting wastes, coastal water of El Max Bay receives huge amount of industrial wastes as lead. Water mass is highly polluted by agrochemical, industrial and domestic wastes discharges into the bay through the Omoum drain from lake Maruit (Samir, 2000). He added that the eastern part of the bay receives brackish water from the Nubaria canal loaded with tanneries wastes. Aboul-Dahab (1985) reported the variations of lead in the seawater in the near shore water of El Max Bay may be due to the release of the atmospheric fumes evolved from a cement factory. The transport of lead to the surface waters of the inshore zone is probably through aerosol fallout, especially in highly industrialized area. The sea transport activity by a large number of medium and giant vessels can be a source of lead to the Egyptian sea water (Shakweer et al., 2006).

Tomazelli et al. (2003) observed large concentration of lead in less impacted areas of Piraciaba and Mogi Guacu basins. This fact suggested that it was not possible to infer about concentrations of these heavy metals, based only on a broad evaluation of human impacts. Bioavailability or specific sources may be responsible for high concentrations in apparently less impacted environments. It is believed that another source for lead may existed along the Mediterranean coast of Alexandria.

The sources of cadmium (Cd) exposure are air and water. Other sources of Cd arise from the recycling and incineration of municipal solid waste and hazardous wastes and pipe galvanization which are also abundant in the vicinity of polluted sites (APHA, 1986). Abou-Taleb et al. (2004) recorded the concentration of cadmium in the coastal water in Abu Quir beach as 1.8 µg/L. Aboul-Dahab (1985) reported the concentration of dissolved cadmium in El Max Bay as 0.6 µg/L. In the present study, the concentrations of heavy metals are considered less than that reported by USEPA (2005). Thus, it is concluded that the coastal area of the Mediterranean Sea in Egypt, might be considered relatively unpolluted with heavy metals, which is in agreement with El,Sikaily et al. (2003).

The physiology of animals might be affected by the intake of metals by the organisms (Tomazelli et al., 2003). Metal intake may differ in relation to the ecological needs, metabolism of animals and concentrations of the heavy metals in the water and food, as well as to other factors such as salinity and temperature (Roesijadi and Robinson, 1994). Environmental stress may cause changes in the cellular function that alter the physiology of the organisms. The present study used a set of biomarkers to assess the state of health of Pinctada radiata in the inspected locations. Khair-Allah et al. (2006) reported that a single biomarker cannot provide the basic answers as to what is the actual state of health in the natural populations. Morris et al. (1982) suggested that pollutants might influence the metabolism of the whole organism. Marine molluscs such as oysters are exposed to multiple stressors in estuaries, including varying environmental temperature and levels of trace metals which may affect their physiology (Sokolova, 2004).

In the present study, the mean value of the triglycerides for the different groups showed a little change. This may be related to the conversion of muscle fat into carbohydrates through metabolic pathways (gluconeogenesis). El-Said (1993) reported that gluconeogenesis leads to the maintenance of an adequate glucose level necessitated by a greater muscular activity. Also, lipids are influenced by the changes in the surrounding. Salinity and heavy metals had various impacts on lipid of marine organisms (Tort et al., 1987). Prakash and Rao (1995) reported that lipid peroxidation induced by metals at sub-lethal levels alter the physiological and biochemical characteristics of the biological systems. They also reported that antioxidant enzyme activity levels were significantly higher in the gills of Perna viridis during heavy metal exposure.

Uma Devi (1996) indicated that bivalves prefer to depend on carbohydrates rather than on lipids on exposure to either sub-lethal or lethal stress of Cd. She added that bivalves might shift to an aerobic metabolism in order to encounter the heavy metals stress in the environment. Higher metabolism in individuals can cause a reduction in metal concentrations in soft tissues because the tissues grow more quickly than the metal can be absorbed. This mechanism can explain the variations recorded in Cd and Cu concentrations (Otchere, 2003) in the present study.

Total proteins comprise the major part of solids in plasma. Measurement of serum proteins is a good rapid mean for studying the impact of the entire environment, since they are essential for the biochemical processes. Analyses of the total proteins of the haemolymph are widely used for diagnostic purposes since the change in serum proteins is an indication of stress.

The lowest total protein concentration in Sedi Beshr was found to have higher levels of heavy metals than the other localities. The
depletion of the haemolymph protein in response to heavy metals levels could be interpreted as a disturbance in the dynamic equilibrium between anabolic and catabolic processes in protein metabolism. Changes in environmental factors as anoxia induces a rapid increase in transcript and protein levels of ferritin, an iron-binding protein as well as changes in the activities of some antioxidant enzymes and a strong increase in reducing glutathione content (English and Storey, 2003). Sreedevi et al. (1992) observed reduction in the concentrations of the total proteins in the different organs of the Mussel Lamellibrachia marginalis, exposed to sub-lethal dose of nickel. Viarengo (1989) reported that the rate of protein synthesis decreased significantly in various tissues of mussels gathered from a coastal area characterized by a high level of pollution, when compared with those in unpolluted area.

The anhydride of creatinine is formed largely in muscle by irreversible non-enzymatic dehydration of creatine phosphate (Murray et al., 1990). The increase of serum creatinine level is accompanied by the decrease in serum protein, reflecting an increased catabolic rate of protein. This increase of catabolic rate pours an added amount of nitrogen into the blood stream to be excreted, this amount of nitrogen appearing as creatinine reflects increase in tissue catabolism (Hilmy et al., 1978).

Thus, considering the previous results it is recommended that Pinctada radiata could be used as a model for biomonitoring marine pollution. It is strongly recommended that the discharges of waste products of many industrial factories and the wastes of the summer season be prevented from being poured directly or indirectly into Alexandria coast before being subjected to several treatments and refinement procedures to reduce their harmful effects on the marine biota and the ecosystem. It may be suggested that the disturbances in the functions of the internal organs of Pinctada radiata is a consequence of protein synthesis. It is concluded from the present study that the toxic effects of heavy metals are the result of the interaction between the low level of metals and the biota in the environment.

REFERENCES:


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Drasataseen wa fiqisilologicah no al-majhar biytikata radian (1814) min nashaiti
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Ahman Hasmirwaela, Ihasah Redahwoel, Heddaysa Hasmir Rudyal 3

1. Qasem el-alujwom, Kelaa el-alujwom, Jonmiuriyya Msr al-arbiyya
2. Qasem el-alujwom, Kelaa el-alujwom, Jonmiuriyya al-askenyadiyya, Jonmiuriyya Msr al-arbiyya.

Fi Sayedi Shin, ototh at-taaydzi daa al-foosiharat

To oth al-taayzali min al-dehun al-qubsi al-biun, al-injil al-qubsi al-duriyya.

Tdoort al-durah: Pinctada radiata

Dairinwa: 2009

Nahd al-ansariyya al-wadnih al-salwah al-saltibi, li al-duriyya

Tsolamwi: 2009

Hyde: 2009

Ataamad anh al-ansariyya al-saltibi, li al-duriyya

Ahl jadidin bi al-bahr al-asby al-mustawatu, Jonmiuriyya al-askenyadiyya, li al-doura

Tdairinwa: 2009

Ku lab fikisilologicah no al-majhar biytikata radian (1814) min nashaiti
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