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Surveillance ecological study of cellular responses in three marine edible bivalve species to Cd present in their marine habitat, Mediterranean sea in Alexandria, Egypt.

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ABSTRACT

Coastal habitats experience large fluctuations of environmental factors such as salinity; they also serve as the natural sinks for trace metals. Bivalves are exposed to the elevated concentrations of metals in water that can strongly have an effect on their histology. The present work includes a list of three edible bivalve species collected from marine water; Mediterranean sea in Alexandria, Egypt. All the identified species are included in class bivalvia. The present study aims to evaluate the environmental salinity and cadmium during two years 2011-2013 of two locations of the Mediterranean coast of Alexandria, investigate their effect on the histology of three main edible bivalve species. From each station, fifteen bivalve (clams, date mussels and oysters) were collected monthly.

Key words: Habitat; Edible bivalve; Salinity; Cadmium; Marine;

Academic Discipline And Sub-Disciplines

Ecology-marine environment-invertebrate biology-

SUBJECT CLASSIFICATION

Ecology of marine bivalve

TYPE (METHOD/APPROACH)

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INTRODUCTION

Water pollution is a serious problem in the global context. Marine environment is considered to be the ultimate pool of many pollutants (Herrera-Silveira and Morales-Ojeda, 2009). Muduli and Panda (2010) reported that, heavy metal pollution is ubiquitous in our environment and results from diverse activities such as phosphate fertilizers, industrial effluents, foundry wastes, paints, auto-mobiles, mining and rock weathering. The exposure to these pollutants can intensify the production of reactive oxygen species (ROS), which are normally produced in non-stressed cells. However, their excess can lead to oxidative stresses by interacting with and damaging the structure of the DNA molecule causing harmful effects.

Herrera-Silveira and Morales-Ojeda (2009) reported that cadmium (Cd) is a persistent environmental pro-oxidant, which produces a wide variety of detrimental effects in organisms. There is rising demand for economically important edible mollusk species (e.g. oysters, clams, and mussels) for local Egyptian market and for export (FAO, 2004). The date mussel (Lithophaga lithophaga, Linneaus, 1758; Bivalvia: Mytilidae) is a bivalve which bores calcareous substrata by glandular secretion (Morton and Scott, 1980). It is widespread in the shallow depths, of the Mediterranean, of the east Atlantic from Portugal to Moracco and in the Red sea (Fischer et al., 1987). Due to the extremely high price and demand for the mollusk, shallow rocky habitats are heavily threatened by human activity which leads to the desertification of tens of kilometers of the Mediterranean rocky coast each year (Fraschetti et al., 2001).

Grubrelic et al., (2004) ascertained that along the east Adriatic coast date mussels inhabiting rocks which had been in the sea for 24-35 years had characteristics of a healthy population, while in rocks immersed for 51 years, the population showed signs of decay and absence of renewal. Combatant with starting bivalve aquaculture, attention should be paid to monitoring the health status in natural fisheries for: 1) site selection for potential grow-out of hatchery produced bivalves; 2) early detection of health problems that might affect bivalve production such as reproductive failure and mortality; 3) assessment of pathogens that exert great hazards to human health since there is a safety concern regarding the local consumption and export of bivalves from Egyptian fisheries. Mass mortality of bivalves has been recorded to be associated with physical, chemical and biological factors (Sinderman, 1990), water quality and water pollution (Weiss et al., 2007).

Yassien et al., (2000) studied the population structure of the wild stock of *Pinctada radiata* from both the Red and Mediterranean seas, respectively. The present study is a histopathological evaluation of the health of the commercially important date clam *Lithophaga lithophaga*, carpet shell clam, *Tapes deccussatus*, and oyster, *Pinctada radiata*, and in their main fisheries in Egypt ,Alexandria (El Asafra and Eastern Harbor) by histopathological examining monthly to explain the possible impacts of Cd and salinity on the bivalve population. Coastal water has become a major concern because of its values for socio economic development and human health. With the growth of human populations and commercial industries, estuarine water has received large amounts of pollution from a variety of sources such as recreation, fish culture and the assimilation and transport of pollution effluents through river Muduli and Panda (2010). These situations have generated great pressure on the ecosystem, resulting in a decrease of water quality and biodiversity, loss of critical habitats(Herrera-Silveira and Morales-Ojeda, 2009).

Coastal environments are one of the most productive areas in the world that point out the necessity of having a proper knowledge regarding their benthic productivity and the environmental parameters influencing their subsistence (Rodrigues et al., 2001; Ogunwenmo et al., 2004; Yap et al., 2003). All physicochemical parameters are the indicators of the existing water quality of the aquatic water body that would help in enriching the macro benthic community in that habitat (Kröncke, 2006; Sivadas et al., 2011). It is also to be noted that salinity is a main limiting factor determining the diversity and distribution of sensitive macrobenthic community along the coastal environment (Lamptey and Armah, 2008).

Marine habitats are exposed to high levels of abiotic stress including fluctuations in salinity, and pollutants. Estuaries serve as the natural sinks for trace metals from both natural and anthropogenic sources including cadmium (Cd). Concentrations of Cd in estuarine and coastal water are orders of magnitude higher than in the open ocean (Hackney et al., 1998). The changes in seawater chemistry can increase bioavailability of metals by enhancing their solubility (Ardelan et al., 2009; Lopez et al., 2010; Millero and DiTrolio, 2010; Millero et al., 2009). The aim of this study was to investigate the effects of (Cd) and salinity in seawater on the cellular histology in the clam ,oyster and date clam. These are common edible bivalves inhabiting coastal water in Egypt.

Material and Methods:

Sampling locations:

The two stations which were selected from ten locations from Alexandria, Egypt represent the main natural fisheries for the three bivalve species, namely; 1) El Asafra 2) Eastern Harbor in Alexandria. For each speciemen, shell height (dorso-ventral measurement), shell length (anterior-posterior axis), shell width and hinge length were measured to the nearest millimeter by vernier calipers. From each station, fifteen clams (≥ 2.2 cm) that represent the market size clam were collected for histo-pathological changes in digestive tubules, and for taking light microscopic photos. Clams for histo-pathological examination were initially measured for shell parameters using digital Vernier caliper. Bivalves were preserved in Davison solution fixative for further histology processing.

Mollusks have successfully adapted to fresh and marine water. Marine mollusks including rocky shore have been found to be highly responsive indicator species in investigation of pollutant toxicology. Marine mollusks are extensively used in bio-monitoring studies due to their ability to concentrate metallic pollutants. The sensitive aquatic environment is suffering of

pollution that affects both quantity and quality of benthic invertebrate biodiversity. Marine mollusks such as oysters are exposed to multiple stressors which may interactively affect their histology. The factors to be considered in the bivalve selection, are maturity and overall health. Bivalves were collected in sterile plastic bags and were cleaned of fouling organisms and transported to the laboratory within 4-6 hrs. Bivalve dissection was carried out with ultra clean tools.

Five times replicate for analysis of Cd were made. Bivalves paired water samples were collected in acid washed glass bottles. The bottles were rinsed several times in the field with seawater. Healthy bivalves when taken outside seawater close their valves tightly by contracting the adductor muscles. (15 bivalves / month/ species / location) specimen of each studied location were collected during January 2011-December 2013. Specimen of Mollusk was nearly of the same size and weight/species as preferable for human feeding. The following requirements are essential in the selection of sites: sheltered areas offering protection from strong wave with a depth ranging from (3-5 m) All sites contain bivalve beds, as described by Appukuttan and Muthiah (1996). For seawater sample analysis samples were filtered through 0.45 µm Millipore filters to remove any debris particles. Metal extraction was carried out for elemental determinations in samples of seawater. All values are reported as µg/ L for seawater.

Salinity (S‰):

Salinity determination hard glass bottles with tight covers were rinsed twice with sea water before filling with samples, these samples were kept in a shady place till measurement using Inductive Salinometer (Beckman mode). Some parameters were totally or partially measured in the field i.e. as soon as the sample was collected. These steps of the methods would be explained by the term "in situ" in the text. Salinity (‰) was measured in situ using a Salinometer equipment (Thermo Electron Corporation 088749, USA), (Parsons et al., 1985).

Histological studies on digestive glands:

In order to establish the histological state, three individuals from each species and each sampling site were processed for light microscopic study. Specimens were fixed in Davidson, dehydrated and embedded in paraffin. Sections 5 µ thickness were then stained with haematoxylin and eosin (Bigas et al., 2000).

Statistical analysis of the data (Kotz et al., 2006)

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Kirkpatrick and Feeney (2013) reported quantitative data were described using mean and standard deviation .The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test,. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. for normally distributed data, comparison between the two studied groups were done using independent t-test. Correlations between two quantitative variables were assessed using Pearson coefficient. Significance of the obtained results was judged at the 5% level.

RESULTS

Sections of the digestive gland of bivalve collected from Alexandria, El Asafra, the reference site, showing that the epithelial lining is differentiated into absorptive storage cells, fibrellar-cells and secretary cells. Apparently fully connective functional tissue condition epithelium; basement membranes and supporting connective tissue are regular. The digestive gland acinus is separated from one another within the same organ by loose connective tissue.

The specimen collected from Eastern Harbor, showed Cellular lysis of digestive gland epithelium and vaculation of digestive gland cells are shown in digestive gland bivalve collected from Eastern Harbor. Some speciemen from the Eastern Harbor, showed obvious increase in the number of the secretary cell of the epithelial lining of the acini of the digestive gland. The date clam Lithophaga lithophaga digestive acini show intracellular separation, and that the absorptive cells contain many vacuoles. Digestive gland in bivalve showed variation in shape and size in the five locations. digestive gland of oyster collected from Eastern Harbor, showed decrease in the number of secretary cells and increase in the number of the absorptive cells.

Sections of the digestive gland of date clam collected from Eastern Harbor, showed highly granulated acidophilic appears as large granules with obvious secretion. In some cells of the digestive gland of clam Ruditapes decussatus, the nucleus appeared darker in color which might contain heterochromatin. Sections of digestive gland of date clam collected from Eastern Harbor showed degenerated epithelium and that most of the secretary cells (B-cells) disappeared. In the present study, Lithophaga lithophaga collected from the two locations, ranged in length from 8.5 - 5.6 cm, oyster ranged from 5 - 6.3 cm whereas Ruditapes decussatus ranged from 3 -4.3cm.

The normal histological patterns of the digestive gland of date mussels is formed by duct and digestive diverticula consisting of columnar, acidophilic or secretory cells and pyramidal basophilic or generative cells. In the specimen collected from Eastern Harbor, there is a slight separation of connective tissue and haemo lymphal space (sinuses). In the Tapes specimen collected from Eastern Harbor there was an inflammation response and moderate vaculation of digestive cells, the formation of intra cytoplasmic basophilic granules. Extra vaculation of digestive gland cells was reported in specimen of date clam collected from Eastern Harbor.

In oyster collected from El Asafra, the connective tissue of the haemal space surrounding the digestive gland can be considered as moderate. The large standard deviation indicates that some individuals their tissues were in quite poor condition. Inflammatory responses were present in the control connective tissues surrounding particularly the digestive gland tubules. Hepato pancrease tubule degeneration was evident in some individuals of Ruditapes decusatus. Tubules

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and branches were widely separated leading to lake of function of date clam specimen collected from Eastern Harbor. Local degeneration of the tubules was found in the digestive gland of oyster collected from Eastern harbor. The specimen of oyster, collected from El Asafra showed apparently fully functional tissue condition with normal epithelia, basement membrane and surrounding connective tissue are regular in cytology and intact showing no inflammations.

The tissues of Ruditapes decussatus collected from the two locations, are generally regular in cytology and only occasional small-scale anomalies are present. The majority of tissues still function. In oyster, a moderate condition with obvious disruption of the tissues is present, this can be a substantial inflammation. Section of the digestive gland of bivalve collected from El-Asafra, the reference site showing that the epithelial lining is differentiated into absorptive storage cells (Restzellen, R-cells), F-Cells (fibrellar-cells; and secretary cells (Blasezellen, B-cells). Apparently fully connective functional tissue condition epithelium; basement membranes and supporting connective tissue are regular. The digestive gland acinuses are separated from one another within the same organ by loose connective tissue.

Table (1):Comparison between the two studied groups according toCd and S ‰

	Cd		S ‰	
	El asafra (n=5)	**Eastern harbor (n=5)**	**El asafra (n=5)**	**Eastern harbor (n=5)**
January	0.48 ± 0.30	0.98 ± 0.21	38.27 ± 0.31	38.48 ± 0.36
t (p)	3.050* (0.016*)		0.979 (0.356)	
February	0.50 ± 0.04	1.41 ± 0.37	38.29 ± 0.14	38.48 ± 037
t (p)	5.490* (0.005*)		1.083 (0.310)	
March	0.66 ± 0.06	1.24 ± 0.28	38.07 ± 0.67	38.29 ± 0.13
t (p)	4.619* (0.008*)		0.738 (0.499)	
April	0.49 ± 0.05	1.43 ± 0.22	38.26 ± 0.23	38.32 ± 0.34
t (p)	9.428* (<0.001*)		0.340 (0.743)	
May	0.45 ± 0.12	1.47 ± 0.24	38.20 ± 0.12	38.49 ± 0.39
t (p)	8.613* (<0.001*)		1.532 (0.189)	
June	0.62 ± 0.17	0.76 ± 0.73	37.82 ± 0.59	38.14 ± 0.16
t (p)	0.410 (0.701)		1.168 (0.276)	
July	0.36 ± 0.08	1.92 ± 0.09	37.90 ± 0.43	38.39 ± 0.21
t (p)	29.531* (<0.001*)		2.311 (0.050)	
Augusts	0.37 ± 0.04	1.51 ± 0.08	38.06 ± 0.22	38.33 ± 0.25
t (p)	29.531* (<0.001*)		1.840 (0.103)	
September	0.35 ± 0.03	1.46 ± 0.45	37.96 ± 0.15	37.98 ± 0.48
t (p)	5.483* (0.005*)		0.099 (0.924)	
October	0.33 ± 0.02	1.48 ± 0.23	37.57 ± 0.43	38.32 ± 0.24
t (p)	11.229* (<0.001*)		3.395* (0.014*)	
November	0.32 ± 0.05	1.49 ± 0.08	37.70 ± 0.16	38.08 ± 0.08
t (p)	28.032* (<0.001*)		4.750* (0.001*)	
December	0.41 ± 0.06	0.99 ± 0.19	38.37 ± 0.39	38.46 ± 0.33
t (p)	6.441* (<0.001*)		0.440 (0.672)	

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Overall	0.44 ± 0.15	1.34 ± 0.41	38.04 ± 0.41	38.31 ± 0.31
t (p)	15.874* (<0.001*)		4.126* (<0.001*)	

t: Student t-test
*: Statistically significant at $p \leq 0.05$

Table (2):Correlation betweenCd and S ‰ in each group

		Cdvs S‰	
		El asafra (n=5)	**Eastern harbor (n=5)**
January	**r**	-0.538	0.361
	p	0.349	0.551
February	**r**	0.267	0.776
	p	0.664	0.123
March	**r**	-0.167	0.261
	p	0.788	0.671
April	**r**	-0.248	-0.549
	p	0.688	0.338
May	**r**	-0.703	0.519
	p	0.185	0.371
June	**r**	-0.179	0.251
	p	0.773	0.684
July	**r**	-0.836	-0.488
	p	0.078	0.404
Augusts	**r**	-0.935*	-0.060
	p	0.020*	0.924
September	**r**	-0.531	0.518
	p	0.357	0.371
October	**r**	-0.846	0.447
	p	0.071	0.451
November	**r**	0.720	-0.365
	p	0.170	0.546
December	**r**	-0.343	0.115
	p	0.572	0.853
Overall	**r**	-0.009	0.159
	p	0.948	0.224

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r: Pearson coefficient
*: Statistically significant at $p \leq 0.05$

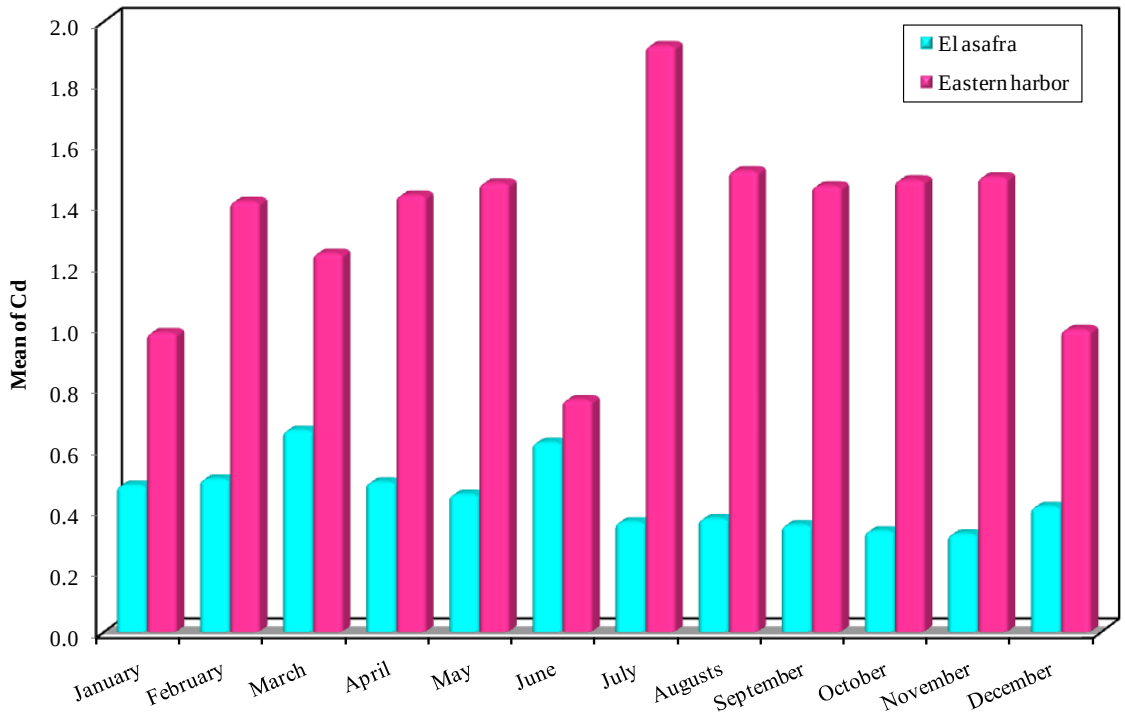

Figure (1): Comparison between the two studied groups according to Cd

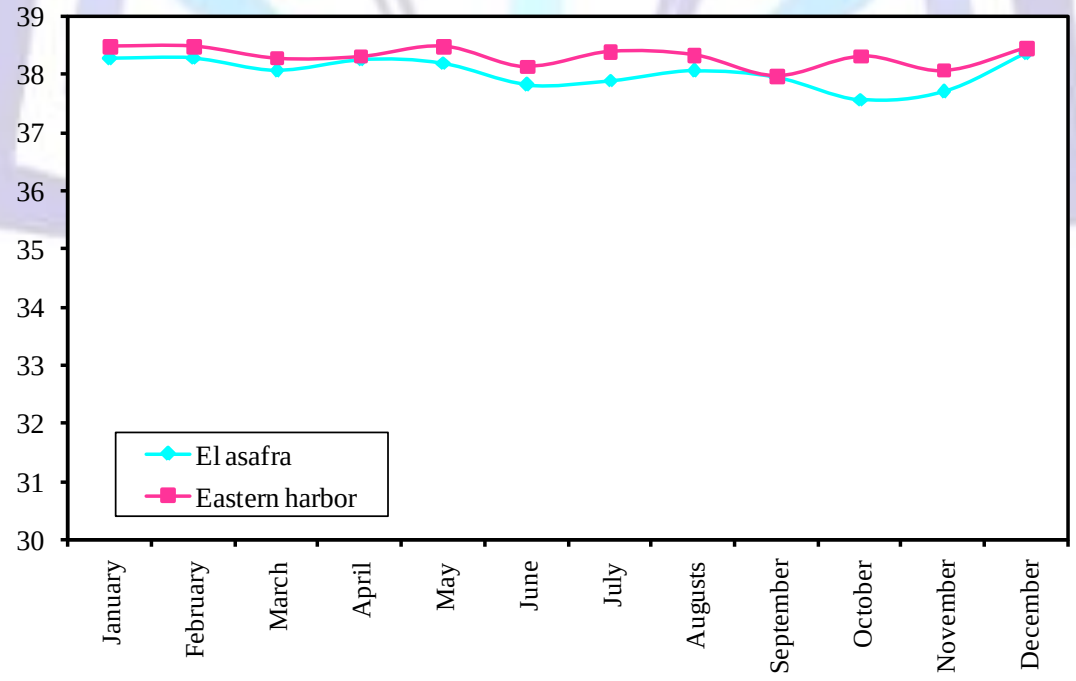

Figure (2): Comparison between the two studied groups according to S ‰

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Figures

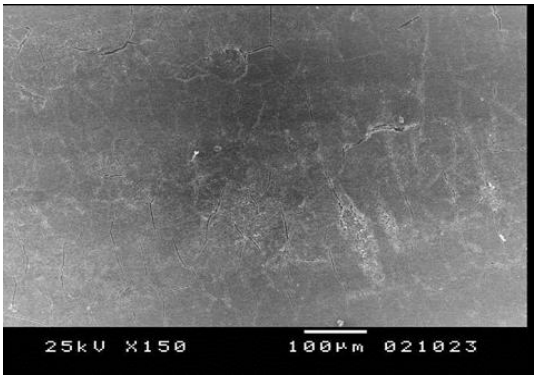

Fig. 3 : Scanning electron micrograph of the outer shell of *lithophaga lithophaga*

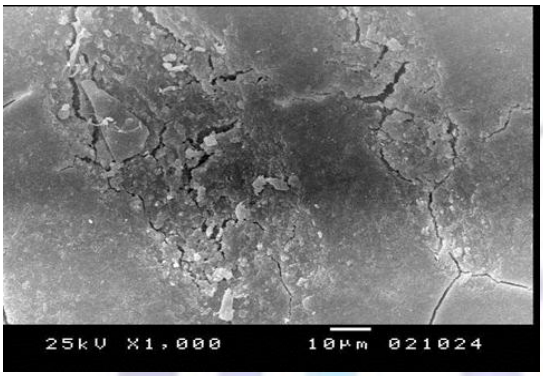

Fig. 4 : Scanning electron micrograph of the outer shell of *lithophaga lithophaga*

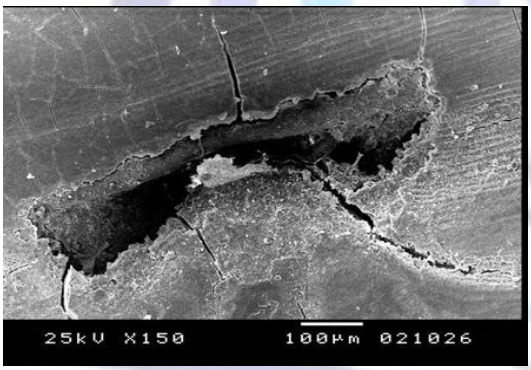

Fig. 5 : Scanning electron micrograph of the outer shell of *lithophaga lithophaga*

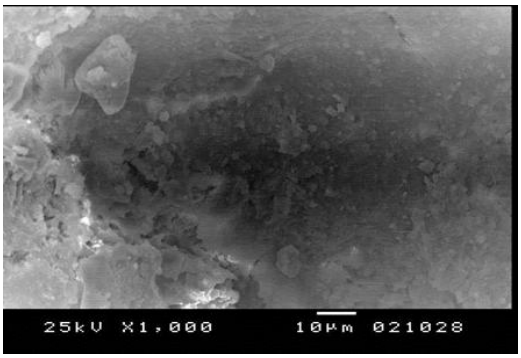

Fig. 6 : Scanning electron micrograph of the outer shell of *lithophaga lithophaga*

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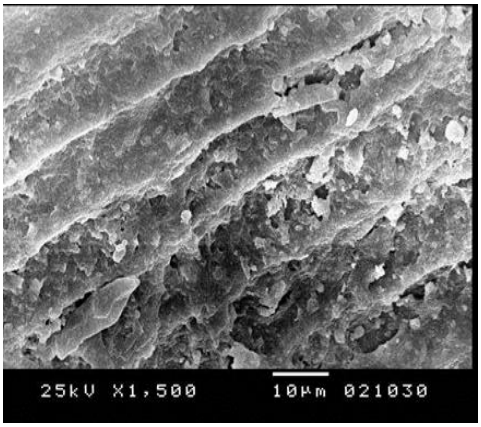

Fig. 7 : Scanning electron micrograph of the outer shell of *lithophaga lithophaga*

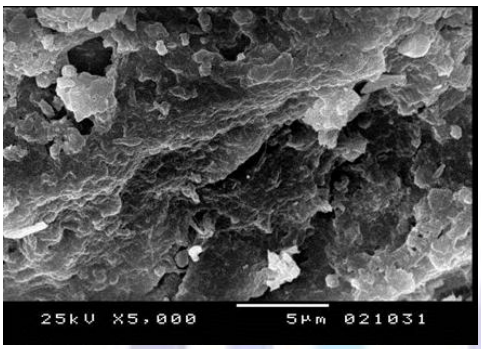

Fig. 8 : Scanning electron micrograph of the outer shell of *Lithophaga lithophaga*

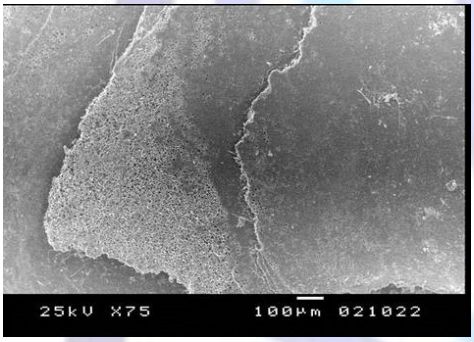

Fig. 9 : Scanning electron micrograph of the outer shell of *Pinctada radiata*

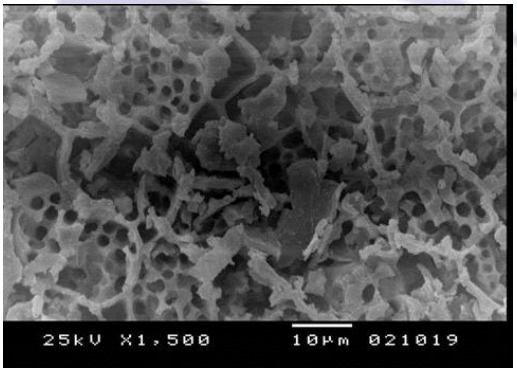

Fig. 10 : Scanning electron micrograph of the outer shell of *Pinctada radiata*

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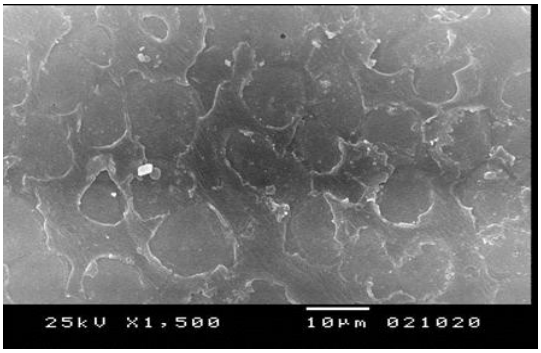

Fig. 11 : Scanning electron micrograph of the outer shell of *Pinctada radiata*

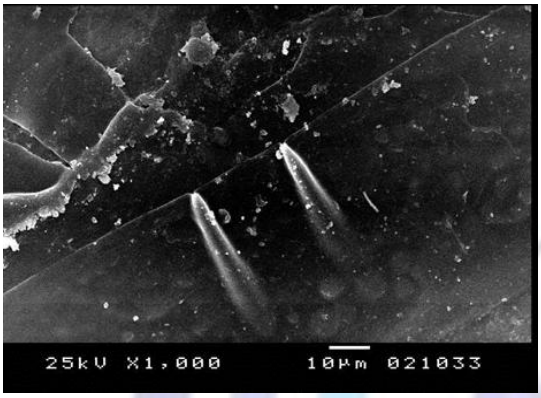

Fig. 12 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

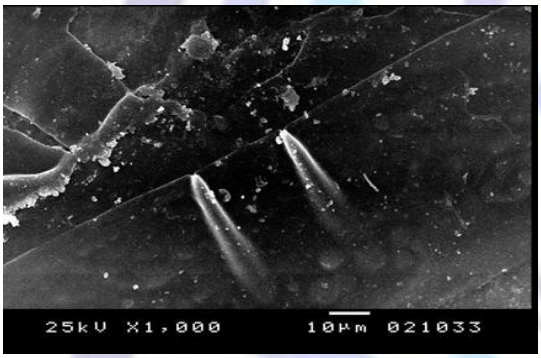

Fig. 13 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

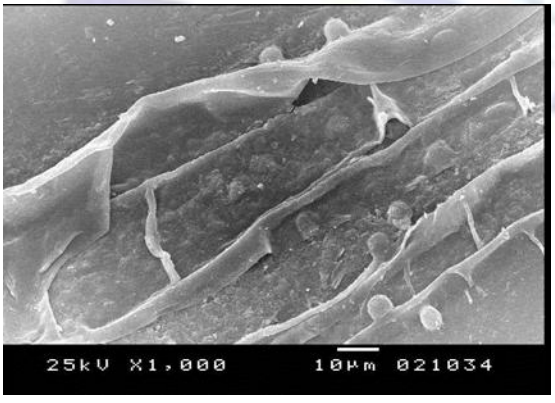

Fig. 14 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

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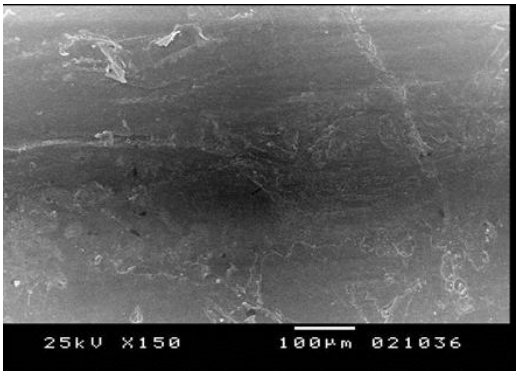

Fig. 15 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

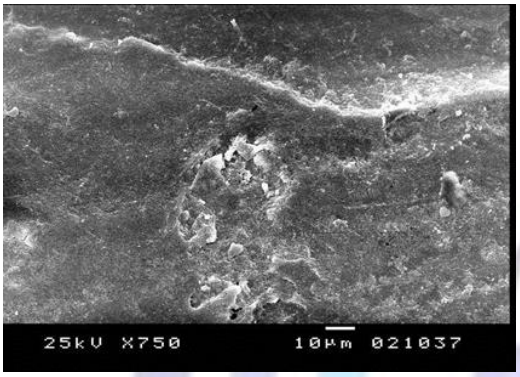

Fig. 16 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

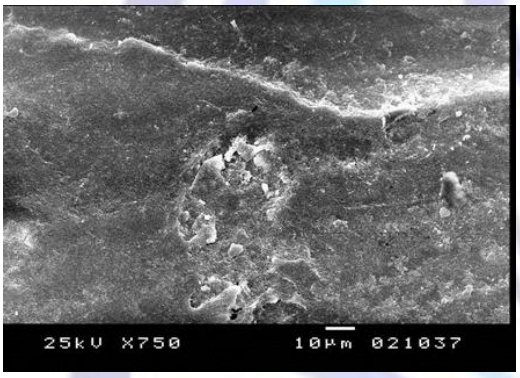

Fig. 17 : Scanning electron micrograph of the outer shell of *Ruditapes decussatus*

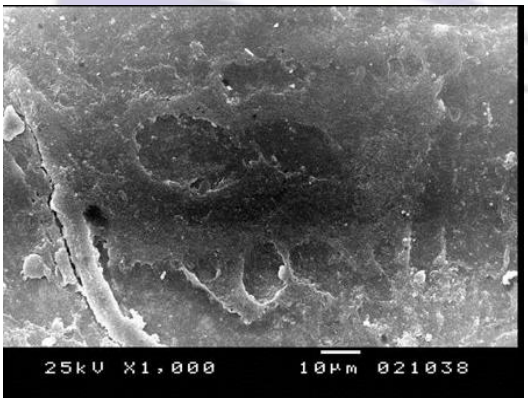

Fig. 18 : Scanning electron micrograph of the outer shell of *Ruditapes decussauts*

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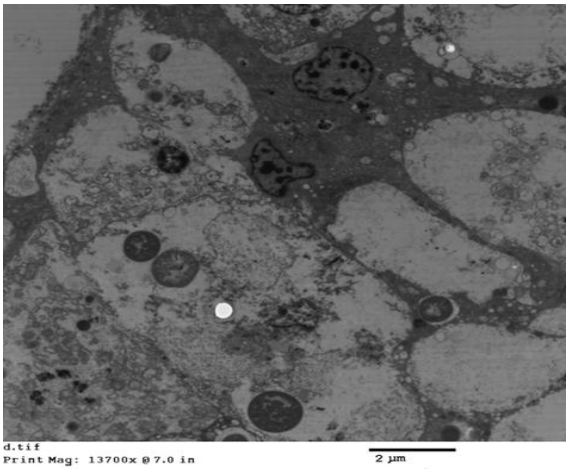

Fig.19 : Photomicrograph of ultrastructre of digestive gland of *lithophaga lithophaga* showing electron dense cytoplasm and digestive vacuoles containing residual bodies.

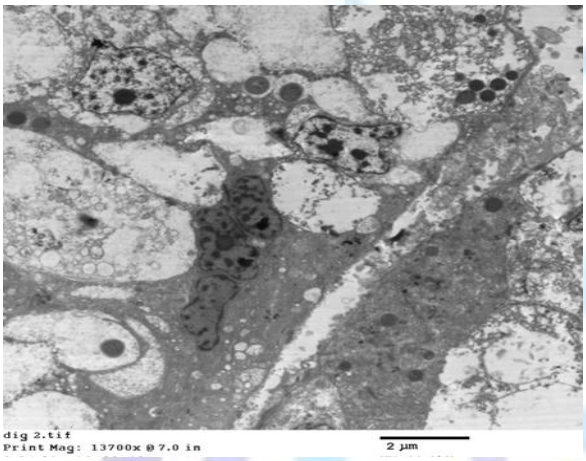

Fig.20 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing a number of residual bodies , Lysis of cytoplasm and Irregularly-shaped nuclei; some of which show karyolysis.

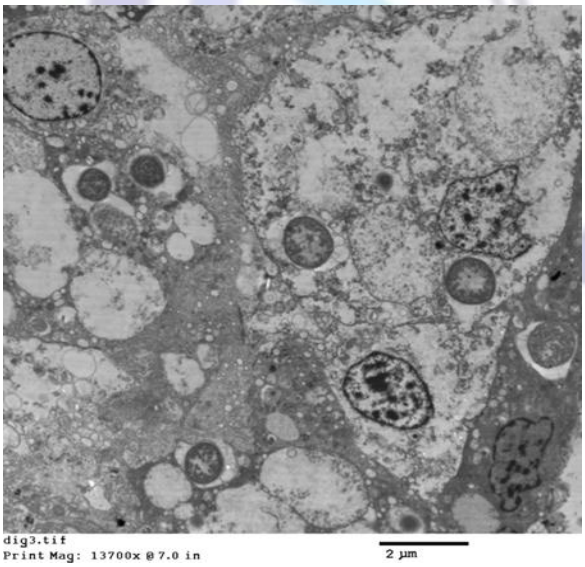

Fig.21 : Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing Irregularly-shaped nuclei.

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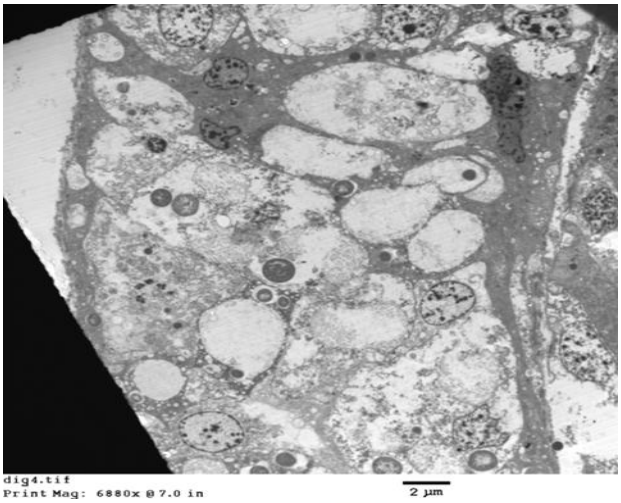

Fig.22 :Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing lysis of cytoplasm and Irregularly-shaped nuclei.

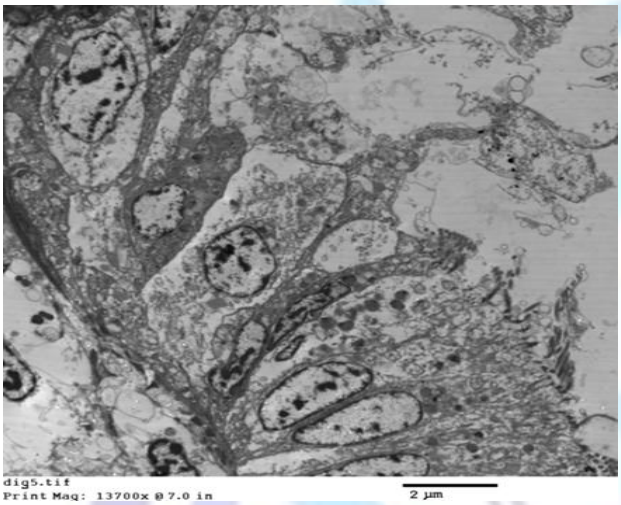

Fig.23 : Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing a number of residual bodies , Lysis of cytoplasm and disrupted and ruptured apical border

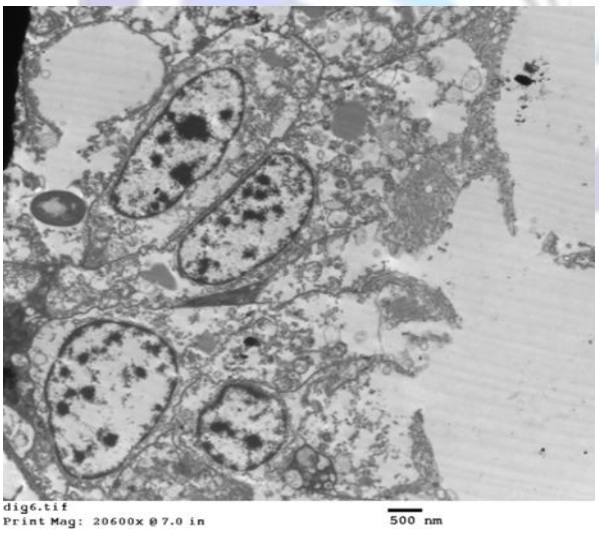

Fig.24 : Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing Vacuolization and disintegration of cytoplasm and with highly disrupted apical surface.

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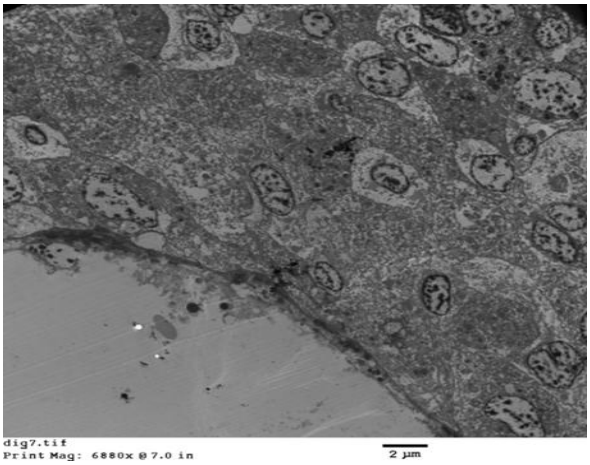

Fig.25 :Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing Lysis of cytoplasm and show karyolysis.

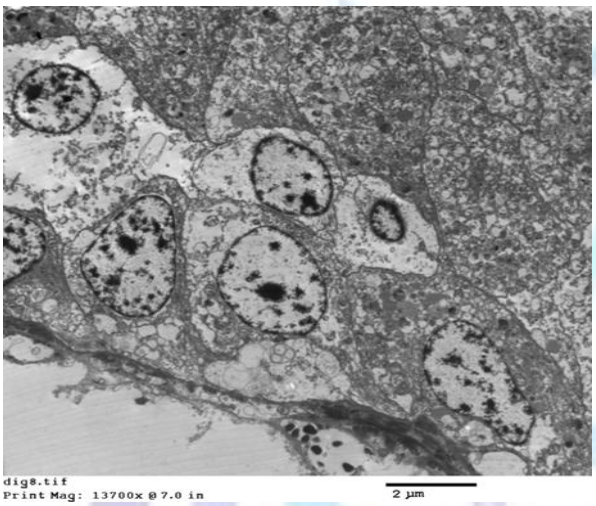

Fig.26 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing a number of residual bodies , Lysis of cytoplasm and Irregularly-shaped nuclei.

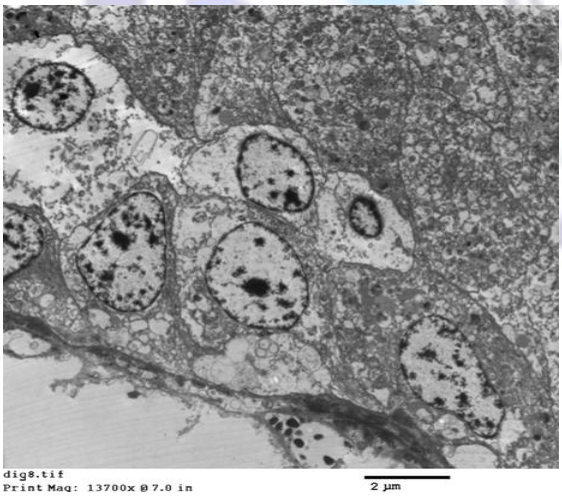

Fig.27 :Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing Lightening of the karyoplasms and vacuolization of the cytoplasm.

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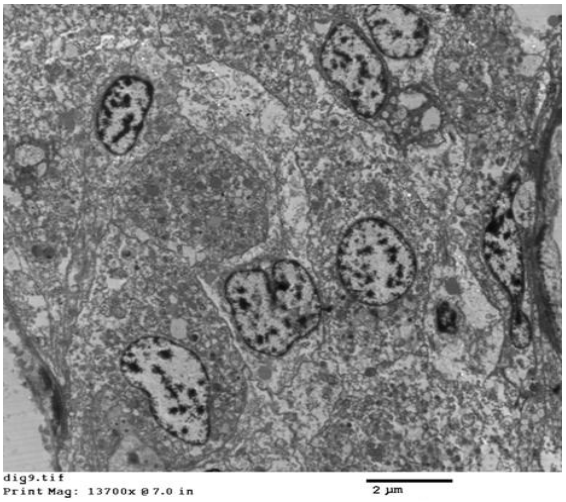

Fig.28 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing a number of residual bodies , Lysis of cytoplasm and Irregularly-shaped nuclei.

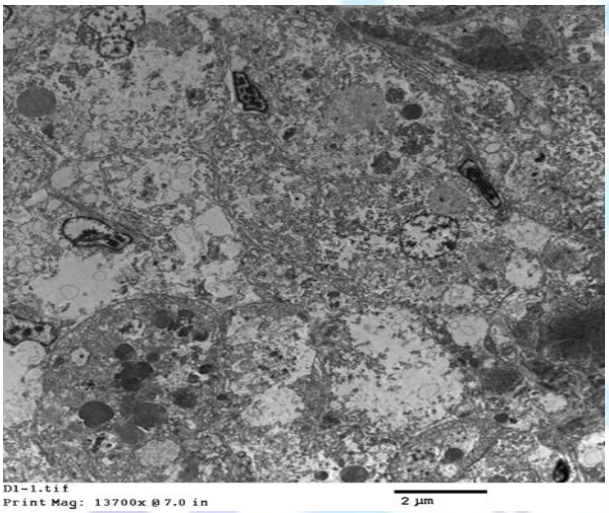

Fig.29 :Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing karyolysis and extensive degeneration of cytoplasm.

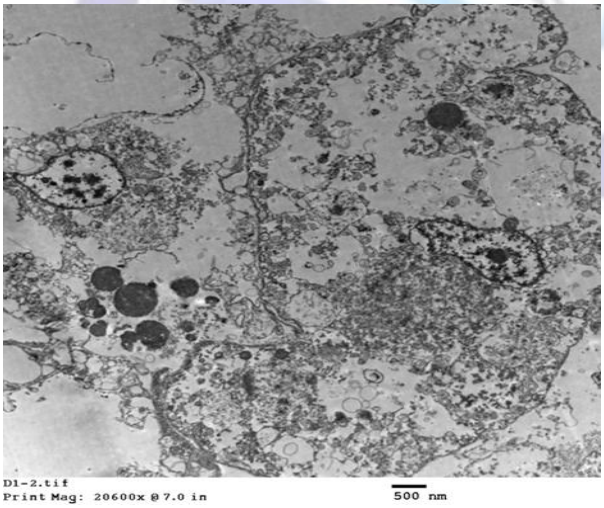

Fig.30 :Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing formation of residual bodies.

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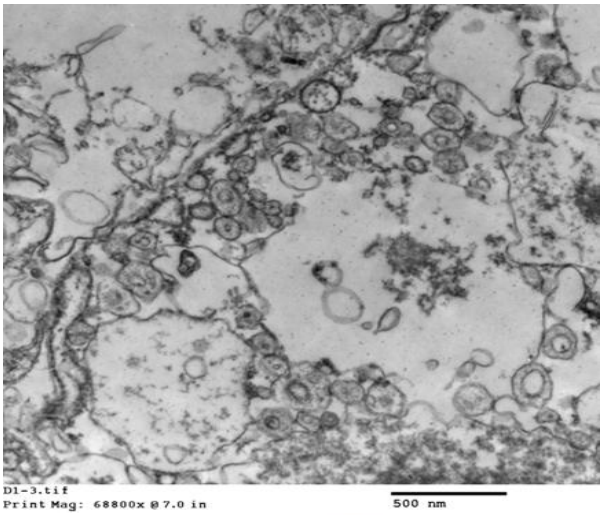

Fig.31 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing a number of residual bodies , Lysis of cytoplasm.

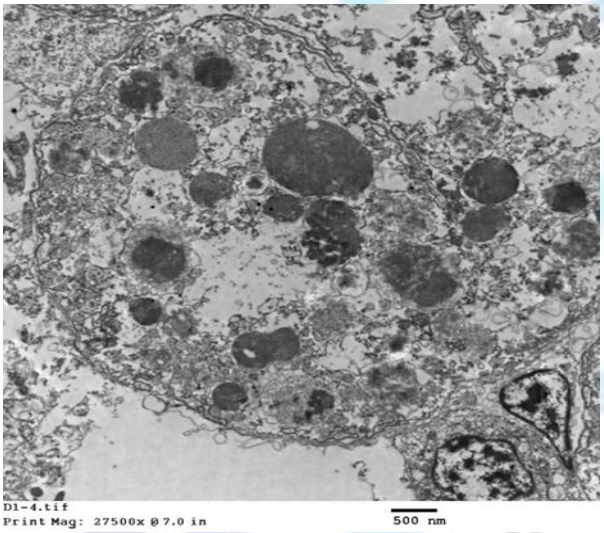

Fig.32 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing Degenerated cytoplasm occupied by large number of residual bodies.

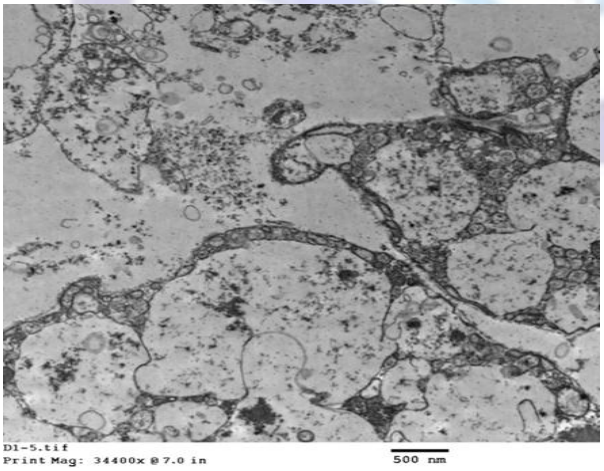

Fig.33 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing Lysis and Degenerated cytoplasm.

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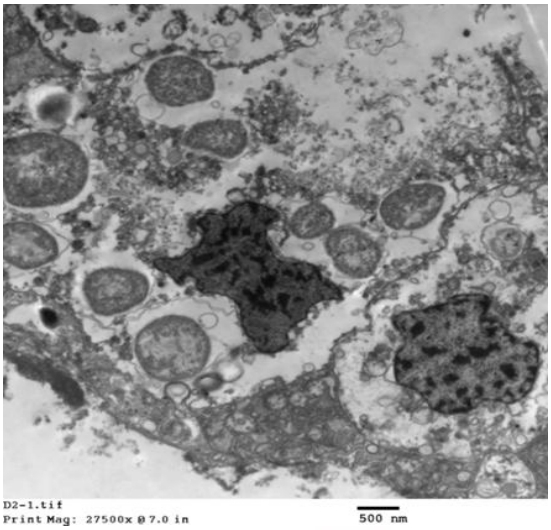

Fig.34 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* showing Irregularly-shaped nuclei and Degenerated cytoplasm.

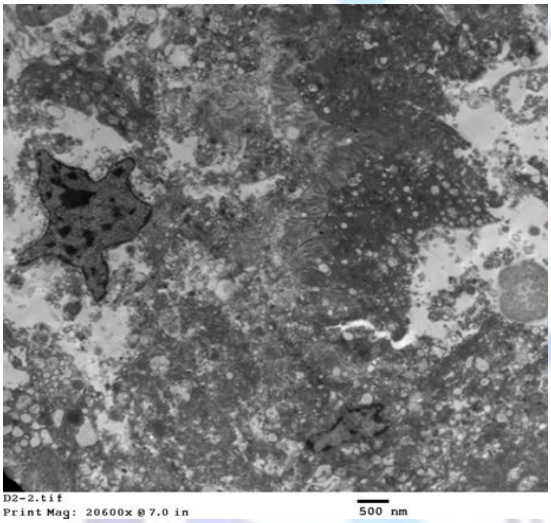

Fig.35 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* collected from Eastern Harbor , showing Severe degeneration of cytoplasm and Irregularly-shaped nucleus.

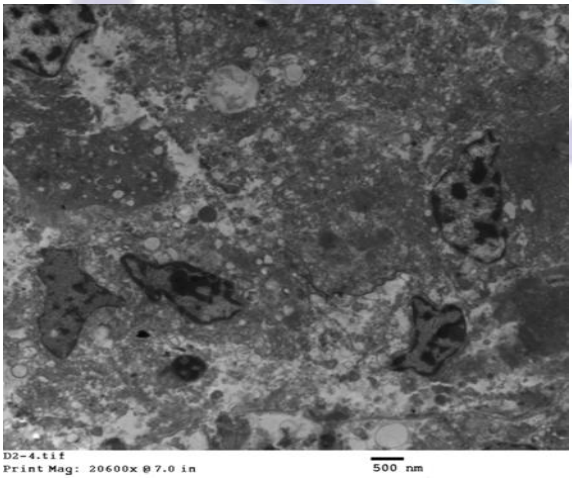

Fig.36 : Photomicrograph of ultrastructure of digestive gland of *Lithophaga lithophaga* collected from the Eastern Harbor, showing Severe degeneration of cytoplasm and Irregularly-shaped nucleus. Some cells show necrotic electron-dense cytoplasm; others with irregularly shaped nuclei and degenerated cytoplasm

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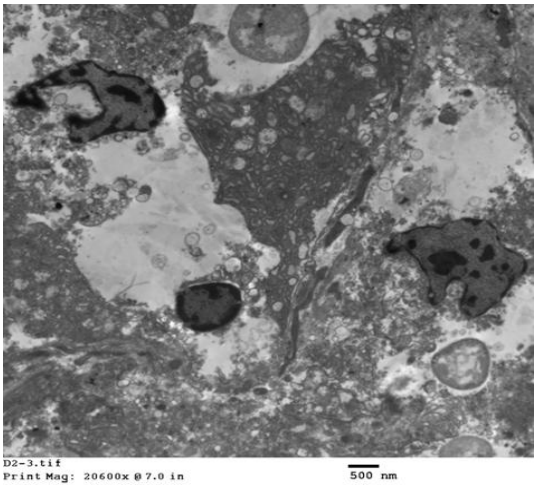

Fig.37 : Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing Severe degeneration of cytoplasm and Irregularly-shaped nucleus.

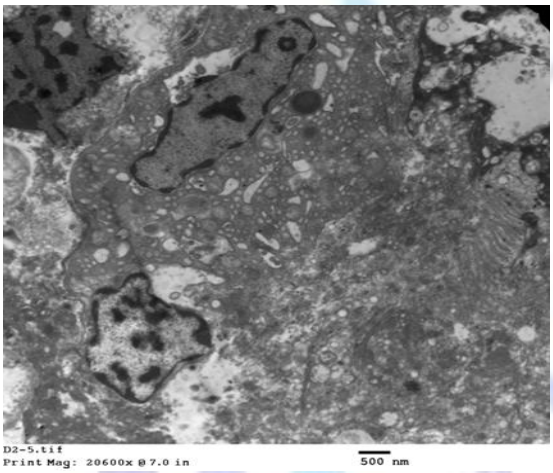

Fig.38:Photomicrograph of ultrastructure of digestive gland of Lithophaga lithophaga showing margination of heterochromatin, abnormal nucleolus and large number of smooth endoplasmic reticulm.

Discussion

Various a biotic and biotic factors can affect date mussels growth rate, among them particularly important are: 1-The composition of the substratum,2- hydrodynamic conditions, 3- habitat physical features, 4- food concentration and 5- intra-spaces competition for food and space (Galinou-Mitsoudi and Sinis, 1997).The most important factor in determining growth rate of mollusks is probably the food supply, since if food is scare, growth will be retarded regardless of all other conditions (Devescovi, 2009). Owing to the substantial difference in date mussels total biomass, the intensity of intraspecies compition for food and space could also play an important role. In mollusks, population density influnences both growth and morphometry of the shell through either food regulation, physical interference, or their interaction (Alunno-Bruscia et al., 2001). The width of large date mussels growing in artificial structures is lower than that of individuals of similar length samples in natural structures, where competion was propabably greater. It seems that, under optimal growth conditions, the endolithic date mussel tends to monopolizethe substratum in depth leading to an elongated shape of the shell (Devescovi,2009).

The main goal of the present study was to evaluate the survival of the three bivalve species; Pinctada radiata, Ruditapes decussatus and *Lithophaga lithophaga*. Photomicrographs of section of the digestive gland of Pinctada radiata, Ruditapes decussatus and lithophaga lithophaga; collected from El-Asafra, the reference site showing that the epithelial linning is differentiated into absorptive storage cells (Restzellen, R- cells) , F-cells and secretory cells (Blasezellen, B-cells). Many pale (storage cells, R-cells) and few dark secretory cells (B-cells) and small number of (connective tissue, F-cells) are representing in the epithelial lining. Apparently fully functional tissue condition epithelium; basement membranes and supporting connective tissue are regular in cytology.

This study extended the geographic distribution of the boring bivalve within Egypt and presented findings on the distribution and density of this relation to types of substrate, depth and wave exposure in areas under different anthropogenic pressures. This study on the Egyptian coast contributes to filling some gapes in limited regional knowledge on marine invasions by providing basic information and also some ecological aspects about a poorly studied introduced species with considerations for future studies. The results indicate no major risk to public health from trace elements in these species collected from Egypt. Histopathological and electron microscopical studies in the digestive glands in the three species found; Lithophaga lithopgaga, Pinctada radiata, and Ruditapes decussates. The present study indicates that the Lithophaga lithophaga is affected by Cd more than *Pinctada radiata* and Ruditapes decussatus and this might be one of the reasons of the declined population in Egyptian fisheries. The Mediterranean Sea is one of the Atlantic Ocean adjacent seas. It is connected to Atlantic Ocean through the narrow Gibraltar Strait, (Kamel and Maiyza, 2000). This Atlantic Water occupying the upper 200 m layer is likely to flow into Mediterranean Sea with its general salinity36.0–36.5 (Millot , (2007) and Said et al. , 2011).

Low salinity in the present study in Eastern harbor ; could be investigated on the bases of mixing process of surface Atlantic Water characterized by low salinity <38.6 following into the Mediterranean water and mixing with the underlying water causing decrease of salinity. These observations may be due to inter annual variability in vertical convection during winter season, (Said et al., 2011). The previous data showed that, the maximum depth of vertical salinity mixing depends on the winter season and reach 100–150 m during 1989 and 250 m during 1990 while the vertical averages of temperature and salinity increased reaching their maximum values at 50 m depth then decreased with increasing water depths (Said et al. , 2007).

Monitoring surveys of European freshwaters show that aquatic organisms are often exposed to a mixture of hazardous substances in their natural environment. Under current chemical legislation, however, hazardous substances are tested and risk assessed as single entities and not in the mixture in which they occur in the environment. As a consequence, the actual risks to aquatic environments could well be underestimated (Kortenkamp et al., 2009). Understanding how seafood will be influenced by coming environmental changes is a research priority. The aim of this experiment was to compare the Cd contamination and salinity from different coastal areas in Egypt. Mussels are filter-feeding animals that depend on natural primary productivity for their growth and development, competing for the capture of phytoplankton, microbes, and detritus in the water column (Theodorou et al., 2014; Dupont et al., 2014).

Various a biotic and biotic factors can affect date mussels growth rate, among them particularly important are: 1-The composition of the substratum, 2- hydrodynamic conditions, 3- habitat physical features, 4- food concentration and 5- intra-spaces competition for food and space (Galinou-Mitsoudi and Sinis, 1997). The most important factor in determining growth rate of mollusks is probably the food supply, since if food is scare; growth will be retarded regardless of all other conditions (Devescovi, 2009). Owing to the substantial difference in date mussels total biomass, the intensity of interspecies competing for food and space could also play an important role. In mollusks, populationdensity influnences both growth and morphometry of the shell through either food regulation, physical interference, or their interaction (Alunno-Bruscia et al., 2001). The width of large date mussels growing in artificial structures is lower than that of individuals of similar length samples in natural structures, where competing was probably greater. It seems that, under optimal growth conditions, the date mussel tends to monopolize the substratum in depth leading to an elongated shape of the shell (Devescovi, 2009).

The main goal of the present study was to evaluate the survival of the three bivalve species. Hydrological parameters showed no significant (ANOVA $p<0.01$) difference in hydrological conditions were noted between the two locations. Along the Mediterranean coast of Alexandria city, there are many areas with high activities of shipping and pleasure boating activities, incorporating numerous Harbours and marina [Shreadah et al., 2006; Shobier et al., 2011; Shredah, et al., 2012) The previous study revealed that, during the last three decades, many touristic cities were constructed along the western coastal area exhibiting signs of stress, population pressure which cause an impact on the area, (Hemiada et al., 2008).

REFERENCES

1. Alunno-Bruscia,M.; Edwin Bourget, Marcel réchette (2001): Shell allometry and length-mass-density relationship for Mytilus edulis in an experimental food-regulated situation. Mar. Ecol. Prog. Ser. Vol. 219: 177–188.
2. Appukuttan, K K and Muthiah, P (1996) Technology of edible oyster culture. CMFRI Bulletin-Artificial reefs and Seafarming technologies, 48 . pp. 64-69.
3. Ardelan,M.V.; Holm-Hansen, O.; Hewes, C.D.; Reiss, C.S.; Silva,, N.S.;Dulaiova, H., Steinnes,E. and Sakshang, E.(2009): Natural iron enrichment around the Antarctic Peninula in the Southern Ocean BiogeoScience,7,11-25.
4. **Devescovi, M. (2009):**Biometric differences between date mussels Lithophaga lithophaga colonizing artificial and natural structures ACTA ADRIAT., 50(2): 129 – 138. UDC: 594.124(262.3): 57.087.1
5. Yoko L. Dupont, Kristian Trøjelsgaard, Melanie Hagen, Marie V. Henriksen, Jens M. Olesen, Nanna M. E. Pedersen and W. Daniel Kissling (2014):Spatial structure of an individual-based plant–pollinator network . Oikos 000: 001–010, 2014.doi: 10.1111/oik.01426

6. FAO/WHO(FoodAdditiveOrganizationoftheUnitedNationsandWorldHealthOrganization), 2004.SummaryEvaluationsPerformedbytheJointFAO/WHO-Expert CommitteeonFoodAdditives(JECFA1956–2003).InternetEdition. ILSI- Press, Washington,DC ⟨http://jecfa.ilsi.org⟩.
7. Fischer W, Wictorin K, Bjo¨rklund A, Williams LR, Varon S, Gage FH(1987) Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by NGF. Nature 329:53– 61.
8. Fraschetti S, Bianchi CN, Terlizzi A, Fanelli G, Morri C, Boero F (2001) Spatial variability and human disturbance in shallow subtidal hard substrate assemblages: a regional approach. Mar Ecol Prog Ser 212:1–12
9. Galinou-Mitsoudi, S. & A. I. Sinis, 1997. Population dynamics of the date mussel Lithophaga lithophaga, (L. 1758) (Bivalvia: Mytilidae), in Evoikos gulf (Greece). Helgolander, 51: 137-154
10. Herrera-Silveira, J.A. and Morales-Ojeda, S.M, (2009): "Evaluation of the health status of a coastal ecosystem in southeast Mexico: assessment of water quality, phytoplankton and submerged aquatic vegetation," Marine Pollution Bulletin, 59. 72-86.
11. Kamel, M.S., Maiyza, I.A., 2000. On the formation of intermediate and deep water masses in the Eastern Mediterranean Sea. Qatar Univ. Sci. J. 20, 197–204.
12. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.
13. Kortenkamp, A., Backhaus, T., Faust, M., 2009, State of the art report on mixture toxicity, Report to the European Commission.
14. Kotz S, Balakrishnan N, Read CB, Vidakovic B. Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J.: Wiley-Interscience; 2006.
15. Kröncke, I. (2006). Structure and function of macrofaunal communities influenced by hydrodynamically controlled food availability in the Wadden Sea, the open North Sea and the deep-sea. A synopsis. Senckenbergiana maritima 36(2): 123-164.
16. Emmanuel Lamptey & Ayaa Kojo Armah,2008: Factors Affecting Macrobenthic Fauna in a TropicalHypersaline Coastal Lagoon in Ghana, West Africa. Estuaries and Coasts (2008) 31:1006
    – 1019DOI 10.1007/s12237-008-9079-
17. Intestinal Stem Cell Replacement Follows a Pattern of Neutral Drift Carlos Lopez-Garcia1,*, Allon M. Klein2,3,*, Benjamin D. Simons3,†, Douglas J. Winton1,†(2010):Science 5 November 2010: Vol. 330 no. 6005 pp. 822-825.DOI: 10.1126/science.1196236.
18. Millero, F.J., F. Huang, N. Williams, J. Waters, and R. Woosley. 2009. The effect of composition on the density of South Pacific Ocean waters.Marine Chemistry 114:56–62.Millero and Ditrolio, 2010
19. Morton, B., Scott, P. J. B. (1980). Morphological and functional specializations of the shell, musculature and pallial glands in the Lithophaginae (Mollusca: Bivalvia). J. Zool. Lond. 192: 179-203.
20. Millot, C., 2007. Interannual salinification of the Mediterranean inflow. Geophys. Res. Lett. 34 (21), November.
21. Muduli, B.P. and Panda, C.R, (2010):"Physico chemical properties of water collected from Dhamra Estuary," International Journal of Environmental Science, 1(3). 334-342.
22. Ogunwenmo, Christiana, A., & Osuala, I. A.,2004 Physico-chemical parameters and macrobenthos of an estuarine creek and an artificial pond in Lagos, southwestern Nigeria.acta SATECH, 1(2): 128-132 (2004).
23. Parsons, T. R.; Y. Matia and G. M. Malli, "Determination of Petroleum Hydrocarbons, A Manual of Chemical and Biological Method for Seawater Analysis," Pergamon Press, Oxford, 1985.
24. Rodr´ıguez, S., Querol, X., Alastuey, A., Kallos, G., and Kakaliagou,O.: Saharan dust contributions to PM10 and TSP levels in Southern and Eastern Spain, Atmos. Environ., 35, 2433–2447, 2001.
25. Sindermann, C.J. 1990. Principal Diseases of Marine Fish and Shellfish, Vol. 2, 2nd edition. Academic Press, New York.
26. Sivadas S. Baban Ingole and Mandar Nanajkar, (2011), Temporal variability of macro fauna from a disturbed habitat in Zuari estuary, west coast of India, Environmental Monitoring and Assessment 173, pp 65–78.
27. Said, M.A., Gerges, M.A., Maiyza, I.A., Hussein, M.A., Radwan, A.A., 2011. Changes in Atlantic Water characteristics in the southeastern Mediterranean Sea as a result of natural and anthropogenic activities. Oceanologia 53 (1), 81–95.
28. Said, M.A., Maiyza, I.A., Hussein, M.A., Radwan, A., 2007. Characteristics of the Egyptian Mediterranean water masses durum cold and warm winters. Acta Adriat. 48 (2), 145–159.
29. -Yassien, M.H., Abdel-Razek, F.A. and Kilada, R.W. 2000. Growth estimates of the pearl oyster, Pinctada radiata, from the Eastern Mediterranean. Egypt. J. Aquat. Biol. & Fish., 4: 105-118.