

CONTENTS

	Page
INTRODUCTION AND AIM OF THE WORK	
Introduction	1
Review of Literature	6
MATERIALS AND METHODS	
Part one	
Heavy metals residues in oyster.....	55
Part two	
Effects of heavy metals on rats.....	60
RESULTS	
Heavy Metals in oyster.....	91
Acute effects of heavy metals on rats.....	93
Effects on growth	93
Effects on organs weight of rats.....	94
Effects on metabolic parameters	97
Effects on enzymatic activities.....	100
Effects on hematological parameters	101
Effects on immune cells.....	104
Chronic effects of heavy metals on rats.....	105
Effects on growth	105
Effects on organs weight of rats.....	107

Effects on metabolic parameters	114
Effects on enzymatic activities.....	120
Effects on hematological parameters	124
Effects on immune cells.....	130
DISCUSSION	
Discussion	134
REFERENCES	
References	158
ENGLISH SUMMARY	
English summary.....	191
ARABIC SUMMARY	
Arabic summary	1

List of Abbreviations

ANOVA	:	Analysis of variance
ACP	:	Acid phosphatase
ALAD	:	Aminolevulinic acid dehydratase
ALP	:	Alkaline phosphatase
ALT/GPT	:	Alanine aminotransferase / Glutamic pyruvic transaminase
AST/GOT	:	Aspartate aminotransferase / Glutamic oxaloacetic transaminase
°C	:	Degree centigrade
Ca-Mg ATPase	:	Calcium magnesium adenosine triphosphatase
Cd	:	Cadmium
CdCl₂	:	Cadmium Chloride
CPK	:	Creatinine-phosphokinase
EP	:	Erythrocytes protoporphyrin
FER	:	Food efficiency ratio
g/dL	:	Gram per deciliter
g/L	:	Gram per liter
gm	:	Gram
gm/L	:	Gram per liter
Hb	:	hemoglobin
Hct	:	Hematocrit
HDL	:	High density lipoprotein
Hg	:	Mercury
HgO	:	Mercuric oxide
i.p.	:	Intraperitoneal
IU/ml	:	International unit per milliliter
LDH	:	Lactate dehydrogenase
LSD	:	Least significant differences
µg/g	:	Microgram per gram
µg/kg	:	Microgram per kilogram
µL	:	Microliter
MCH	:	Mean cell hemoglobin
MCHC	:	Mean cell hemoglobin concentration
MCV	:	Mean cell volume
MeHg	:	Methyl mercury
mg/dL	:	Milligram per deciliter
mg/g	:	Milligram per gram
mg/kg	:	Milligram per kilogram

ml	:	Milliliter
mmol/L	:	Millimole per liter
mol/L	:	Mole per liter
N	:	Normal, Normality
Na – K ATPase	:	Sodium potassium adenosine triphosphatase
NK	:	Natural killer
nm	:	Nanomole
P	:	Probability
Pb	:	Lead
PbB	:	Blood lead
PCV	:	Packed cell volume
PER	:	Protein efficiency ratio
Per Os	:	Per oesophagus
ppb	:	Part per billion
ppm	:	Part per million
r.p.m.	:	Revolution per minute
RBcs	:	Red blood cells
S.C.	:	Subcutaneous
SE	:	Standard Error
SGOT	:	Serum Glutamic oxaloacetic transaminase
SGPT	:	Serum Glutamic Pyruvic transaminase
U/L	:	Unit per liter
WBCs	:	White blood cells

SUMMARY

Water pollution is adding substances that alter the chemical composition, microbial composition, or temperature of water to the degree that harm occurs to resident organisms or humans. Water pollution impacts human health directly, by toxic chemicals in drinking water, and indirectly, by human consumption of organisms in which toxic compounds have been accumulated. Along the Egyptian coast, the water of the Mediterranean sea was subjected to large loads of industrial residues including organic materials, suspended soils and toxic chemicals. Damietta area is a region in Egypt that are heavily exposed to pollution. Domestic and agricultural sewage comes from surrounding village, and industrial wastes from factories that enter the environment. The ship – building industry in the village of Ezbit El-burg, together with the building of Damietta harbor, has led to increased shipping activities that contribute in the water pollution of the area. For these reasons Damietta area was selected for the present study to help to determine the concentration of heavy metals (Pb, Cd and Hg) in oysters collected from Damietta city markets.

Oysters have been considered to be potential bioindicator for monitoring metal pollution in marine environment that it is a sessile organism. The levels of these metals in water may not be lethal to these organisms but they concentrate such metals in their tissues create hazards when used as food for human consumption. On the other hand, oysters have a great distribution and are, commercially valuable seafood species in all costal and non coastal areas that it has a highly nutritional

value and it is a desirable food due to its contribution of high quality animal's protein, richness in calcium and phosphorus and generous supply of vitamins. Study of heavy metals concentration in oyster samples is necessary to identify either oyster is safe from heavy metals pollution or not.

The continual production and release of pollutants into the environment has resulted in the need for a sensitive study that will effectively monitor the environment for harmful contaminants. Many pollutants released into the environment that affect the human health, the physiological, biochemical and haematological parameters shows dangerous effect on animals that may has a similar effect on the human health...So the study of the effect of heavy metals on the selected parameters in albino rats is necessary to reflect the dangerous effect on human health.

This work was designed to accomplish the following objectives :

I – Field studies :

- 1- Selection of study sites : Collection of the oyster samples from different markets in many cities of the Damietta governorate, these cities are subjected to different level of heavy metals pollution, so their source were different polluted areas.
- 2- Measurement of the levels of heavy metals in the tissue homogenate of the oyster samples at the selected sites.

II – Laboratory studies :

1- Albino rats (*Rattus norvegicus*) were exposed to the same dosage of heavy metals (Pb, Cd and Hg) concentrations that was found in the oyster samples for the acute (Pb = 0.581, Cd = 0.8 and Hg = 0.876) (for 48 hours) and chronic (Pb =2.169, Cd = 0.2380 and Hg = 0.2919) (for 6 weeks and followed by 2 withdrawal weeks) treatments.

2- Assessment of growth rate (performance parameters) of rats included the body weights, body weights gain, absolute and relative organs weight

3- Quantification of enzymes and metabolites from rats plasma. Enzymes assayed were Aspartate aminotransferase (AST/GOT), Alanin aminotransferase (ALT/GPT), alkaline phosphatase (ALP)and acid phosphatase (ACP). Metabolites assayed were glucose, total lipids, cholesterol, total proteins, albumin, globulin and urea.

4- Assessment of hematological parameters (red blood cells count, hemoglobin content and hematocrit value) of the rats blood.

5 – Assessment of some immunological parameters including white blood cell count and differential leucocytes count.

Data obtained in the present study could be summarized as follow:

I – Field studies :

Heavy metal concentrations in oyster samples :

A) The three heavy metals concentration that are measured in the examined oyster tend to bioaccumulate in oyster body at various levels. Metal concentration in oyster can be arranged in a descending order as follow : Pb, Cd and Hg.

B) The safety levels of analyzed oysters according to the permissible limits that recommended by (FAO/WHO, 1992) for Hg and (GESAMP,

1991) for Pb and Cd which showed that oyster samples were safe from the cadmium pointed of view but they were subjected to various degree of pollution with lead (41 % over the permissible limit) and mercury (19% over the permissible limits).

C) In examined oyster samples metal interrelationships showed negative correlation for Pb :Cd and Hg:Cd, in contrast a significant positive correlation ($P \leq 0.05$) was estimated between Hg : Pb.

II – Laboratory studies :

1- Body weight and body weight gain :

- **In the acute treatment**, neither body weight nor body weight gain showed a significant differences among treatments when compared to the control.

- **In the chronic treatment**, a significant decrease in mercury treated group and insignificant decrease in lead and cadmium treated groups were recorded for body weight and very high significant decrease in body weight gain in all heavy metal treatments . The accumulative body weight gain show a significant decrease in all heavy metal treatments when compared to the control.

2 - Organ weights:

- **In the acute treatment**, a very high significant increase were recorded for absolute and relative liver weights in all heavy metals treatment, a very high significant increase for absolute and relative heart weights in lead and cadmium groups and insignificant increase in mercury group, absolute and relative spleen weights showed a decrease in all treatments and this decrease was significant in cadmium and mercury groups, all treatments increase absolute and relative kidneys weights and this

increase was high significant and very high significant respectively, in lead and cadmium group only and significant increase for absolute and relative testicles weights in all heavy metal treatments when compared to the control.

- **In the chronic treatment**, absolute and relative liver weight show a very high significant increase followed by significant decrease among treatment. A very high significant increase for absolute heart weight and followed by significant decrease among treatment. Relative heart weight showed a very high significant increase followed by a high significant decrease in mercury treatment and showed a very high significant decrease in lead and cadmium treated groups. Absolute and relative spleen weight showed a very high significant increase in all treatments followed by a very high significant decrease. Absolute and relative kidneys weight shows a very high significant increase in mercury group and a high significant decrease in lead and cadmium treated groups. The absolute and relative testicles weight showed a high significant increase in mercury group followed by a very high significant decrease at the end of the treatment, while showed a high significant decrease in cadmium and lead treated groups.

3 – Biochemical parameters :

- **In the acute treatment**, the glucose level showed a very high significant increased in lead, cadmium and mercury treated groups. Total protein in this study recorded a significant increase in all heavy metal treatments when compared to the control. Albumin and globulin shows a significant increase in all treatments. No significant differences were recorded in total lipids and cholesterol among treatments. Urea shows a very high significant increase in all heavy metals treatments.

- **In the Chronic treatment**, the glucose level showed a significant increase in all heavy metal treatments during all weeks. Total protein recorded a significant increase in all heavy metal treatments. Albumin showed a very high significant decrease in all heavy metals treatment during the time of the experiment. Globulin level showed a very high significant increase in all heavy metals treatments at all durations. Total lipids and cholesterol level showed a significant decrease at all treatments at all durations comparing to the control. A very high significant increase were recorded for urea level in all heavy metal treatments at all durations.

4 – Enzymatic activities :

- **In the acute treatment**, the data recorded that AST and ALT activities show a high significant and a very high significant increase respectively in all heavy metal treatments when compared to the control. ALP and ACP activities show a significant increase at all heavy metals treatment.

-**In the chronic treatment**, the data showed that AST and ALT activities very high significantly decreased in all treated group (except lead group which elevate them) and then increase at the end of the treatment when compared to the controls. ALP activity recorded a significant decrease in all treatments at all durations. ACP show a very high significant increase in all heavy metals treatments during the time of the treatment.

5 – Haematological study :

- **In the acute treatment**, there is a significant decrease in red blood cell count, a very high significant decrease in hemoglobin content, a significant decrease in packed cell volume, a significant increase in mean

cell volume and mean cell hemoglobin in all heavy metals treatments. A significant decrease and insignificant change were recorded for mean cell hemoglobin concentration in lead treated group and cadmium and mercury groups respectively comparing to the control.

- **In the chronic treatment**, a very high significant decrease in red blood cell count and hemoglobin content in lead and mercury treated group in all durations, while showed a very high significant increase in cadmium group at the time of the treatment. PCV show a significant decrease in all heavy metals treatments at all duration. No significant change were recorded in MCV in all treatments and followed by a significant increase in lead and mercury treated group and a significant decrease were recorded for MCV in cadmium treated group at all durations. MCH show a very high significant decrease in cadmium treated group at all durations, significant increase in lead and mercury treated groups at all durations. A very high significant decrease were recorded for MCHC in mercury treated group which showed a very high significant increase in lead treated group at all durations, while showed a significant decrease followed by increase in the cadmium treated group when compared to the control.

5 – Immunological study :

- **In the acute treatment**, WBCs showed a decrease in all treatments and this decrease is significant in lead and cadmium treated groups. An insignificant increase were shown in granular leucocytes count in all treatments. A high significant decrease were recorded for lymphocyte count in all treatments. Macrophage count show a very high significant increase in all treatments.

- **In the chronic treatments**, a very high significant increase were recorded for WBCs count in all treatments and followed by insignificant change at the end of the experiment. A granular leucocytes count show a very high significant decrease, followed by increase and then decrease in all treatments. Lymphocyte count showed a very high significant increase, followed by decrease and then increase at the end of treatment in all treatments. A very high significant increase were recorded for macrophage count in all treatments when compared to the control.

The withdrawal of heavy metals for 2 weeks after 6 weeks exposure in the chronic treatment resulted in the gradual and partially recovery the normal values of the studied parameters.