Republic of Iraq

Ministry of Higher Education & Scientific Research

University of Babylon

College of Science

Department of Biology

Using of Immobolized *Chlorella vulgaris* for Reduction of Amoxicillin and Potasium dichromate against toxicity on two Species of Bivalve

A Thesis

Submitted to the Council of the College of Science, University of Babylon in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Science / Biology

By

Zahraa Hussein Obaid Ali

B.Sc., Degree in Biology / College of Science/ University of Babylon, 2004.
M.Sc. Ecology/ College of Science for Women / University of Baghdad/

Supervised by

Prof.Dr.Jasim Mohammed Salman **Prof. Dr.Nuha F. Kadhim**

2023 A.D **1444 A.H**

بسم الله الرحمن الرحيم

﴿ وَسَخَّرَ لَكُم مَّا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ جَمِيعًا مِّنْه إِنَّ فِي ذَٰلِكَ لَآيَاتٍ لِّقَوْمٍ يَتَفَكَّرُونَ﴾

صدق الله العلي العظيم

الجاثية/ 13

Certification

we certify that this thesis entitled (**Using of Immobolized *Chlorella vulgaris* for Reduction Amoxicillin and Potassium dichromate toxicity against on two Species of Bivalves)** was prepared by (**Zahraa Hussein Obaid Ali**) under our supervision at Department of Biology , College of Science, University of Babylon, as a partial fulfillment of the requirements of Ph.D. degree in the Philosophy of Science in Biology.

Supervisor

Dr. Jasim Mohammed Salman

(Professor)

Supervisor

Dr . Nuha F. Kadhim

(Professor)

Department of Biology \ College of Science \ University of Babylon.

/ / 2023

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Dr. Adi Jassim Abd AL Rezzaq

(Asst. Professor)

Head of Biology Department\ College of Science\ University of Babylon

/ / 2023

Committee Certification

We, the examining committee, certify that we have read this thesis **"Using of Immobolized *Chlorella vulgaris* for Reduction Amoxicillin and Potassium dichromate toxicity against on two Species of Bivalves"** and examined the student " **Zahraa Hussein Obaid Ali**" in its contents and that in our opinion; it meet the standardsof thesis for th Degree of Doctor of Philosophy of Science in Biology/Ecology with "Excellent" degree.

Prof.Dr. Moayed Jassim Yass Alamari
University of Babylon /college of science

Prof.Dr. Atheer Saieb Naji
University of Al-Qasim Green /college of Environmental Sciences
/ /2023

Prof.Dr. Batool Muhammad Hassan
University of Babylon /college of science
/ / **2023**

Assist Prof.Dr. Bassam M. AL-Yaseen
University of Babylon /college of science
/ / **2023**

Assist Prof. Dr. Huda S. Al hasan
University of Babylon /Environmental Research & Study Center
02/10/ **2023**

Prof.Dr Jasim Mohammed Salman
University of Babylon /college of science
/ / **2023**

(Supervisor)

Prof.Dr Nuha F. Kadhim
University of Babylon /college of scienc
/ / **2023**

(Supervisor)

Prof.Dr. Mohammed Hadi Shinen ALshammeri
Dean of College of Science / University of Babylon
/ / **2023**

Acknowledgments

My deep praise and great thanks must be firstly offered to Allah insupporting and patronaging me throughout this work during all difficult times I faced ,Thank Allah for everything

First of all, I am grateful to my supervisors, Prof. Dr. Jassim Muhammad Salman, and Professor Dr. Nuha Faleh Kazem, for their suggestion of the draft thesis and for his advice, assistance, encouragement, and continued great support.

Special thanks are to the Dean of the College of Science and Biology Department-College of Science / University of Babylon, to given me opportunity to complete my study, and allow me utilize laboratories for complete this dissertation and all postgraduate students and Staff in the Department of Biology

Special thanks are to my teachers and employees of Environmental Research and Studies Center, University of Babylon, for their support and encouragement, especially Assistant Professor Dr. Huda S. Alhasan, Assistant Professor Dr. Laila Abdel Karim, Researcher Shaimaa Satae M.Ali , and my thanks and appreciation to the Director of General Company for the manufacture of medicines and medical supplies / Samarra Mr. Khalid Muhyi Alwan, for providing pharmaceutical compound .And thanks are to my generous family, to everyone who gave me advice and helped... You have all the thanks and appreciation.

Zahraa

Dedication

To my mother ....

To Whom he strives to bless comfort and welfare and never stints what he owns to push me on the path of success who taught me to promote life stairs wisely and patiently, to my father.

To Whose love flows in my veins, and my heart always remembers them, brothers,and sisters.

Zahraa

Summary

Aquatic pollution with pharmaceutical compounds is considered one of the most significant environmental challenges worldwide, due to urbanization and industrial waste and the resulting potential risks to human health and aquatic ecosystems.

In this study, two types of pharmaceutical material were used: the antibiotic amoxicillin (AMX) and the disinfectant potassium dichromate ($K_2Cr_2O_7$) to cause poisoning of two selected species of bivalves, *Pseudodontopsis euphraticus* and *Corbicula fluminea*, which were collected from a site on the Euphrates River in the Al-Hindiya District in central Iraq, while, immobilized algae were applied (*Chlorella vulgaris*) to reduce the toxicity of the mentioned pollutants.

Several environmental variables of the riverbank were measured when collecting bivalves including water temperature (12.4 -22.5) °C, air temperature (26.2-33.4) °C, pH (7.3-8.34), and electrical conductivity (1058-1130) µs/cm, salinity (0.55-0.73) ppt, dissolved oxygen (5.9-7.80) mg/L, biological oxygen demand (1.7-3.35) mg/L, and total dissolved solids (430.3-694) mg/L.

The lethal concentration 50 (LC50) of AMX was measured at different concentrations (100, 200, 300) mg/L in a total of 144 clams (*P. P. euphraticus* and *C. fluminea*), and the LC50 values within 96 hours were 410.55 and 441.15 mg/L respectively. While the LC50 values for 96 hours were (73.96 and 76.73) when using the disinfectant potassium dichromate at concentrations of (20, 30, 50, 100, 150) mg/L in (total clams = 216) *P.euphraticus* and *C. fluminea,* respectively.

Selected bivalves showed a variety of behavioral changes after being exposed to pharmaceutical substances, including locking their valves tightly for longer periods than when they are open, and occasionally extending their feet and siphons relatively motionless at high concentrations. Along with the occurrence of irregular swimming bursts, flattening, mucus secretion, and inactivity.

Biochemical indicators of two species of bivalves *(P. euphraticus and C. fluminea)* were studied after exposure to AMX in the absence of immobilized algae (*C. vulgaris*), and the results were as follows: Reactive oxygen species (ROS) levels were measured for both types at (12.99 - 22.52) µg/mg and (30.79 - 42.86) µg/mg respectively. *P. euphraticus* has the following values for (SOD), (CAT), and glutathione peroxidase (GPX): (20.79 - 33.33) U/mL, (25.44 - 30.53) KU/L, and (5.70 - 8.36) U/L, respectively, in a while for *C. fluminea* they were (35.49-50.033) U/mL, (42.51-51.78) KU/L and (16.41-21.12) U/L respectively. Glutathione (GSH) values were recorded for both types at (7.55-10.37) µg /g and (4.25-8.03) µg /g respectively, while the values of ascorbic acid (AA) ranged between (13.41-14.48) µM and (42.92-76.79) µM. The values of total protein (TP) were (9.65-11.14) mg/g and (35.52-19.57) mg/g, while the values of malondialdehyde (MDA) were (17.69-42.43) µmol/L and (9.33-17.21) µmol/L for both types respectively.

When immobilized *C. vulgaris* was applied on *P. euphraticus and C. fluminea* in the presence of AMX, biochemical markers were obtained as shown below: ROS values were (8.89-16.73) µg/mg and (21.33 - 35.49) µg/mg respectively; the SOD values were (12.72-21.62) U/ml and (21.46-35.49) U/ml respectively, and CAT showed values of (17.56-25.15) KU/L and (22.16-35.33) KU/L respectively. While GPX values ranged between (5.02-9.58) U/L and (14.16-19.28) U/L respectively, and GSH values

ranged between (2.83-6.27) µg/g and (6.54-9.46) µg/g respectively. AA values were recorded between (13.41-14.48) µM and (38.11-59.80) µM respectively. The TP values were recorded (14.25-15.60) mg/g and (39.23-41.41) mg/g respectively. Finally, MDA values ranged between (6.77-22.72) µmol/L and (11.60-23.97) µmol/L respectively.

The same conditions as the previous experiment were applied in the absence of immobilized *C. vulgaris* by exposing *P. euphraticus and C. fluminea* to potassium dichromate ($K_2Cr_2O_7$) at different concentrations (20, 30, and 50) mg/L, the biochemical markers were recorded as follows: The ROS values were (37.50-51.05) µg/mg and (37.89-63.66) µg/mg. The SOD values varied from (33.40-28.36) U/ml and (54.04-35.96) U/ml, while CAT values were recorded as (25.76 - 27.16) KU/L and (27.19 - 46.74) KU/L, GPX values varied from (4.35 to 6.34) U/L and (10.99 - 43.35) U/L, and GSH values varied from (8.57 - 16.37) µg/g to (12.79 - 36.86) µg/g, AA values were (20.37-26.94) µM and (35.3 to 50.37) µM, and TP values ranged from (10.86-12.79) mg/g and (18.43-39.11) mg/g. gm respectively, and the MDA values were (29.01-59.88 and 12.68-23.22) µg /L for both clams respectively.

When the immobilized *C. vulgaris* was added to the previous experiment, the biochemical marker showed the following values: ROS from (33.88-42.06) µg/mg to (41.0-51.0) µg/mg, and SOD values varied from (27.19- 46.74) U/ml to (23.36 -31.4) U/ml, while CAT values ranged between (26.88-40.32) KU /L and (24.55-48.99) KU/L, and GPX values were (7.34 -3.87) U/L and (9.37-37.99) U/L. The values of GSH were (9.7 -22.47) µg/mg and (7.58 -30.26) µg/mg, and AA values ranged between (24.83 -27.94) µM and (23.67 - 38.89) µM, while the values of TP were (10.93- 13.56) mg/g and (35.69 - 20.44) mg/g, and the MDA values were (9.81-11.44) µmol/L and (23.06 - 47.92) µmol/L respectively. The

concentrations of 50 g/L $K_2Cr_2O_7$ and 300 g/L AMX are mostly responsible for the high findings.

After long-term exposure to amoxicillin and potassium dichromate, *P. euphraticus* suffered a variety of histological changes including: infiltration of cells and blood cells, cell degeneration, expansion of the lymphatic spaces between tubules, necrosis of digestive gland cells, and narrowing of the tubular lumen, while histological changes appeared in the front gills such as fusion of cilia, cell hyperplasia, blood cell infiltration, necrosis, and rupture of epithelial cells.

Regarding *C. fluminea* changes in the digestive glands have occurred including atrophy and proliferation of digestive tubes, connective tissue infiltration, necrosis of digestive gland cells, narrowing of the tubular lumen, and necrosis of lymph nodes. Histological changes of blood cells in the gills included cell infiltration, epithelial necrosis, epithelial layer rupture, and cilia fusion.

Summary

Aquatic pollution with pharmaceutical compounds is considered one of the most significant environmental challenges worldwide, due to urbanization and industrial waste and the resulting potential risks to human health and aquatic ecosystems.

In this study, two types of pharmaceutical material were used: the antibiotic amoxicillin (AMX) and the disinfectant potassium dichromate ($K_2Cr_2O_7$) to cause poisoning of two selected species of bivalves, *Pseudodontopsis euphraticus* and *Corbicula fluminea*, which were collected from a site on the Euphrates River in the Al-Hindiya District in central Iraq, while, immobilized algae were applied (*Chlorella vulgaris*) to reduce the toxicity of the mentioned pollutants.

Several environmental variables of the river were measured when collecting bivalves including water temperature (12.4-22.5) °C, air temperature (26.2-33.4) °C, pH (7.3-8.34), and electrical conductivity (1058-1130) µs/cm, salinity (0.55-0.73) ppt, dissolved oxygen (5.9-7.80) mg/L, biological oxygen demand (1.7-3.35) mg/L, and total dissolved solids (430.3-694) mg/L.

The lethal concentration 50 (LC50) of AMX was measured at different concentrations (100, 200, 300) mg/L in a total of 144 clams (*P. P. euphraticus* and *C. fluminea*), and the LC50 values within 96 hours were 410.55 and 441.15 mg/L respectively. While the LC50 values for 96 hours were (73.96 and 76.73) when using the disinfectant potassium dichromate at concentrations of (20, 30, 50, 100, 150) mg/L in (total clams = 216) *P.euphraticus* and *C. fluminea,* respectively.

Selected bivalves showed a variety of behavioral changes after being exposed to pharmaceutical substances, including locking their valves tightly for longer periods than when they are open, and occasionally extending their feet and siphons relatively motionless at high concentrations. Along with the occurrence of irregular swimming bursts, flattening, mucus secretion, and inactivity.

Biochemical indicators of two species of bivalves *(P. euphraticus and C. fluminea)* were studied after exposure to AMX in the absence of immobilized algae (*C. vulgaris*), and the results were as follows: Reactive oxygen species (ROS) levels were measured for both types at (12.99 - 22.52) µg/mg and (30.79 - 42.86) µg/mg respectively. *P. euphraticus* has the following values for (SOD), (CAT), and glutathione peroxidase (GPX): (20.79 - 33.33) U/mL, (25.44 - 30.53) KU/L, and (5.70 - 8.36) U/L, respectively, in a while for *C. fluminea* they were (35.49-50.033) U/mL, (42.51-51.78) KU/L and (16.41-21.12) U/L respectively. Glutathione (GSH) values were recorded for both types at (7.55-10.37) µg /g and (4.25-8.03) µg /g respectively, while the values of ascorbic acid (AA) ranged between (13.41-14.48) µM and (42.92-76.79) µM. The values of total protein (TP) were (9.65-11.14) mg/g and (35.52-19.57) mg/g, while the values of malondialdehyde (MDA) were (17.69-42.43) µmol/L and (9.33-17.21) µmol/L for both types respectively.

When immobilized *C. vulgaris* was applied on *P. euphraticus and C. fluminea* in the presence of AMX, biochemical markers were obtained as shown below: ROS values were (8.89-16.73) µg/mg and (21.33 - 35.49) µg/mg respectively; the SOD values were (12.72-21.62) U/ml and (21.46-35.49) U/ml respectively, and CAT showed values of (17.56-25.15) KU/L and (22.16-35.33) KU/L respectively. While GPX values ranged between (5.02-9.58) U/L and (14.16-19.28) U/L respectively, and GSH values

ranged between (2.83-6.27) µg/g and (6.54-9.46) µg/g respectively. AA values were recorded between (13.41-14.48) µM and (38.11-59.80) µM respectively. The TP values were recorded (14.25-15.60) mg/g and (39.23-41.41) mg/g respectively. Finally, MDA values ranged between (6.77-22.72) µmol/L and (11.60-23.97) µmol/L respectively.

The same conditions as the previous experiment were applied in the absence of immobilized *C. vulgaris* by exposing *P. euphraticus and C. fluminea* to potassium dichromate ($K_2Cr_2O_7$) at different concentrations (20, 30, and 50) mg/L, the biochemical markers were recorded as follows: The ROS values were (37.50-51.05) µg/mg and (37.89-63.66) µg/mg. The SOD values varied from (33.40-28.36) U/ml and (54.04-35.96) U/ml, while CAT values were recorded as (25.76 - 27.16) KU/L and (27.19 - 46.74) KU/L, GPX values varied from (4.35 to 6.34) U/L and (10.99 - 43.35) U/L, and GSH values varied from (8.57 - 16.37) µg/g to (12.79 - 36.86) µg/g, AA values were (20.37-26.94) µM and (35.3 to 50.37) µM, and TP values ranged from (10.86-12.79) mg/g and (18.43-39.11) mg/g. gm respectively, and the MDA values were (29.01-59.88 and 12.68-23.22) µg /L for both clams respectively.

When the immobilized *C. vulgaris* was added to the previous experiment, the biochemical marker showed the following values: ROS from (33.88-42.06) µg/mg to (41.0-51.0) µg/mg, and SOD values varied from (27.19- 46.74) U/ml to (23.36 -31.4) U/ml, while CAT values ranged between (26.88-40.32) KU /L and (24.55-48.99) KU/L, and GPX values were (7.34 -3.87) U/L and (9.37-37.99) U/L. The values of GSH were (9.7 -22.47) µg/mg and (7.58 -30.26) µg/mg, and AA values ranged between (24.83 -27.94) µM and (23.67 - 38.89) µM, while the values of TP were (10.93- 13.56) mg/g and (35.69 - 20.44) mg/g, and the MDA values were (9.81-11.44) µmol/L and (23.06 - 47.92) µmol/L respectively. The

concentrations of 50 g/L $K_2Cr_2O_7$ and 300 g/L AMX are mostly responsible for the high findings.

After long-term exposure to amoxicillin and potassium dichromate, *P. euphraticus* suffered a variety of histological changes including: infiltration of cells and blood cells, cell degeneration, expansion of the lymphatic spaces between tubules, necrosis of digestive gland cells, and narrowing of the tubular lumen, while histological changes appeared in the front gills such as fusion of cilia, cell hyperplasia, blood cell infiltration, necrosis, and rupture of epithelial cells.

Regarding *C. fluminea* changes in the digestive glands have occurred including atrophy and proliferation of digestive tubes, connective tissue infiltration, necrosis of digestive gland cells, narrowing of the tubular lumen, and necrosis of lymph nodes. Histological changes of blood cells in the gills included cell infiltration, epithelial necrosis, epithelial layer rupture, and cilia fusion.

Table of Contents

List of Figures

List of plate

	digestive gland , showed 1. degeneration of cell with enlarged vacuoles, 2.anylaysis tubular lumen, 3. large hemolymphac gap between tubules ,4. basophilic triangular shaped cell present in the connective tissue among the tubuleanlysis epithelial cell ,5.diffuse hemocytic infilteration of connective tissue (,6. pyknotic state of cells enlarged vacuoles(6). A &B without *C.vulgaris* and C&D with *C.vulgaris* , $K_2Cr_2O_7$ 30,50 mg/L, (H & E, Scale bar 40X).	
3-7	histological section of gills in clams *P. euphraticus* (control):1. Cilia, Haemocoelic Space, 2.Ventral Epithelia ,3. Dorsal Epithelia ,4. haemocytes, (H & E, Scale bar 40X).	**116**
3-8	A cross section of P. euphraticus gills revealed 1. epithelial lifting , 2. secondary lamellar rupturing ,3. curling of secondary lamellae, 4. hyperplastic interlamellar epithelium , 5. frontal fusion of cilia , 6. hemocytes blocking of hemolymph (H & E, Scale bar 40X). A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris*, $K_2Cr_2O_7$ 30,50 mg/L	**116**
3-9	histological Section of the digestive gland for *C. fluminea* (control) 1. basohilic cell, 2. digestive lume, 3. basement membrane, 4. hemolymph gap between tubules, 5. typical tubules, 6. connective tissue , (H & E, Scale bar 10X)	**118**
3-10	Cross section of the for *C. fluminea* digestive gland , showed 1.degeneration of cell with enlarged vacuoles, 2. narrowing of tubular lumen , 3. basophilic triangular shaped cell present in the connective tissue among the tubule, 4.and dilation in the hemolymphatic spaces between tubules. A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , AMX 200,300 mg/L , (H & E, Scale bar 40x).	**118**
3-11	histological section of gills in clams *C. fluminea*	**120**

	(control): 1.Cilia(1), 2.Haemocoelic Space, 3.haemocytes, 4.Dorsal Epithelia (H & E, Scale bar 40x).	
3-12	Cross section of *C. fluminea* gills ,1. showed epithelial of secondary lamellar rupturing , 2. secondary lamellar sloughing shortening ,3.haemocyte , 4. frontal fusion of cilia(, 5. and epithelial lifting, 6. necrosis , A & B with *Chlorella vulgaris* ,C&D without *Chlorella* (H & E, Scale bar 40x).	**120**
3-13	histological Section of the digestive gland for *C. fluminea* (control) 1. digestive lumen, 2. Tubular epithelium, 3.hemolymphac gap between, 4.typical tubules. (H & E, Scale bar:10X)	**122**
3-14	Cross section of the digestive gland , showed 1. shrinkage epithelial cells, swollen nucleus , 2. pyknotic state of cells enlarged vacuoles (2), enlarged hemolymphe spaces , 3. basophilic triangular shaped cell present in the connective tissue among the tubule 4. Necrosis,5. anylaysis tubular lumen,6. diffuse hemocytic infilteration of connective tissue, 7. lymphocytes(8). A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , $K_2Cr_2O_7$ 30,50 mg/L, (H & E, Scale bar: 40x)	**122**
3-15	histological section of gills in clams *C. fluminea* (control):1. Cilia, 2. Haemocoelic Space ,3. Ventral Epithelia , (H & E, Scale bar: 10X)	**123**
3-16	Cross section of *C. fluminea* gills , showed 1. epithelial of secondary lamellar rupturing , 2. secondary lamellar sloughing shortening , 3. haemocyte ,4. frontal fusion of cilia, 5. epithelial lifting 6. Necrosis A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , $K_2Cr_2O_7$ 30,50 mg/L(H & E, Scale bar: 20x).	**123**

List of Tables

Abbreviations

CAT	Catalase enzyme
SOD	Superoxide Dismutase
GPX	Glutathione peroxidation
PPCPs	Pharmaceuticals and personal care products
LC50	Lethal concentration 50%
GSH	Glutathione
$K_2Cr_2O_7$	Potassium Dichromate
AMX	Amoxicillin
CIP	Ciprofloxacin
MDZ	Metronidazole
CBZ	Carbamazepine
SMX	Sulfamethoxazole
AChE	Acetylcholinesterase
AA	Ascorbic acid
ROS	Reactive Oxygen Species
OTC	Oxytetracycline
Cr	Chromium
TP	Total protein
MDA	Malondialdehyde
EPS	Extracellular Polymeric Substances
GR	Glutathione Reductase
GSSG	Glutathione Disulfide
DO	Dissolved Oxygen
BOD_5	Biological Oxygen Demand
TDS	Total Dissolved Solids

1. Introduction and Literature Review

1-1 Introduction

Water pollution has become one of the most important global environmental issues due to growing urbanization and industrialization (Zhang, 2020a). The issue of the presence of pharmaceuticals in aquatic systems has attracted increasing worldwide interest in the last two decades (Kock *et al*.,2023).It is considered one of the emerging hazardous pollutants due to its potential threats to human populations and aquatic ecosystems, such as medicines, cosmetics, disinfectants, and even their metabolites (Samal *et al*., 2022).

In the last years, growing more attention has been paid to the presence of pharmaceutical substances in aquatic ecosystems, due to their potential to have detrimental impacts on non-target aquatic species(Kayode-Afolayan *et al*., 2022). There are probably entering freshwater systems by many pathways including effluents from wastewater treatment plants (WWTPs), chemical industrialization plants, and animal rearing and aquaculture,hospitals and pharmaceutical plants are the main contributors to the discharge of medications into the ecosystem (Khan *et al*., 2021 ;Samal *et al*., 2022).

Microalgae are eukaryotic organisms that play a significant role in the production of oxygen in the aquatic ecosystem as well as being an important part of the food chain (Leng *et al*.,2022). Microalgae have drawn attention in bioremediation research for their ability to adapt and remove the antibiotics themselves from polluted water, producing valuable biomass. *C. vulgaris*, a unicellular microalga, is one of the most commonly investigated algae in the treatment of wastewater as it is commonly found in freshwater with fast growth rates and short

production times when employed (Sarkheil *et al*.,2022).It has been shown that *Chlorella vulgaris* can adapt to antibiotic stress through its physiological adaptation and its ability to degrade pollutants, it is, therefore, a good option for removing antibiotics from aqueous systems (Ricky *et al*., 2022).

Antibiotics are one of the most extensively utilized pharmaceutical classes in medical and veterinary applications, and they are constantly being discovered in aquatic environments (Felis *et al*., 2020). Amoxicillin(AMX) has been classified as an emerging pollutant because it causes great damage to aquatic non-targed organisms, such as changes in embryonic development , oxidative stress, it is capable of causing DNA damage and also provoking genotoxicity and cytotoxicity (Orozco-Hernández *et al*., 2019; Chowdhury *et al*.,2020).The major problem associated with antibiotic-polluted water is the development of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), which are responsible for 700,000 deaths per year (De Kraker *et al*., 2016).

Chromium is a highly toxic inorganic pollutant that enters the environment from a variety of natural and artificial sources, including medical facilities, textile manufacturers, dye and Oxidizing materials (Awasthi *et al*., 2018). Chromium has been designated as a priority pollutant by numerous environmental and health organizations; when present in excess, it induces toxic effects on the cells such as genotoxicity and oxidative damage and can damage lipids, proteins, and DNA and cause carcinogenic and mutagenic effects in living beings(Laxmi and Kaushik, 2020).

The utilization of biomarkers as early warning tools for contamination in an environment can be toxic and dangerous to aquatic life (Lomartire *et al*.,2021). Chemical compounds can affect biological systems by forming radicals or high-energy molecules, which eventually reflect oxidative stress on organisms and lead to the production of ROS in aquatic organisms(Lee *et al*.,2022). Thus, differences in the action of the enzymes that make up the antioxidant protective mechanism can be used as an early warning sign of toxic compound contamination (Fantón *et al*.,2020).

Bivalves are considered good bio-indicator organisms for determining the degree of contamination in freshwater and marine ecosystems (Baralla *et al.*, 2021). This is due to several significant characteristics, including their wide dispersion, abundance, filter-feeding, sedentary behavior, physical size,and food source. As a result, various authors have studied how molluscs react to environmental pressures and contaminants. It has been used in an increasing number of ecotoxicological applications, well as in environmental risk assessment programs (Chiesa *et al*., 2018).

Algal immobilization technology has received increasing attention and has been used in many applications in the environmental field, such as treating wastewater by removing nutrients, pharmaceutical compounds, hazardous textile dyeing, and heavy metals (Cao *et al*., 2022; Salman *et al*., 2022**).**

1-1-1 Aims of the study:

1-Estimation of the ecotoxicity of medical pollutants as the antibiotic amoxicillin AMX and Potassium dichromate ($K_2Cr_2O_7$) on some biochemical and histological characteristics and bioresponses of two species of bivalve clams *C. fluminea* & *P. euphrates* also calculate LC_{50} in different concentrations**.**

2- Use of the immobilized algae *C. vulgaris* to reduce the toxicity of these pollutants mentioned above by observing the variations in biochemical and histological characteristics in both used clams.

1-2 Literature Review

1-2-1 Ecotoxicology

The word "environmental toxicology", first used by Truhaut (1977), refers to an interdisciplinary scientific field that draws knowledge and techniques from the fields of ecology and toxicology, as well as is sometimes used synonymously with ecotoxicology (Rosner *et al* .,2021).

Environmental toxicology is the study of how hazardous compounds influence living things, whether they are created industrially or organically(Mostafalou and Arab, 2022).It enables us to identify the species and concentrations of pollutants that are harmful to animals, as well as their effects on their health, ecotoxicological data assess environmental risks and suggest potential new strategies for the regulation and control of toxic chemicals in various ecosystems, as well as provide insights into ecosystem health that cannot be solely determined by measuring chemicals in the environment (Altenburger *et al*., 2019).

Ecotoxicological studies have increased, significantly in the last decades following the exponential growth in the production and use of chemicals in agriculture, medicine, and various industrial sectors, leading to an increased release of toxic contaminants into waters globally, particularly, inland waters among the most threatened habitats worldwide by this indiscriminate pollution (Reid *et al.* 2019; Pham *et al*., 2019). Thus, environmental toxicology intended to provide basic knowledge about the environmental effects of pollutants, providing foundations for the development of international guidelines to protect the environment and the species inhabiting it (Sebastiano *et al*.,2022), and from this perspective, ecotoxicological, studies play a clear role in

assessing the effects of pollutants on aquatic habitats ,human health and other lives (Ceschin *et al*., 2021; Mkandawire *et al*. 2014).

Chemical analysis alone cannot properly assess and forecast the impacts of chemicals on aquatic species and ecosystems, making it insufficient for chemical monitoring in the aquatic environment, the environment is experiencing a growing number of (unknown) chemical stressors and mixing effects, In addition, the ecological indices also have a limited capacity to pinpoint the underlying stresses that have a detrimental influence on the ecosystem(Schuijt *et al*.,2021). As a result, further supplementary techniques, such as ecotoxicological testing, which might provide a bridge between chemical monitoring and ecological indices are required that can directly address biological effects and provide a binding to chemical exposure,ecotoxicological tests are described as test systems, that expose biological components (cells, individuals, populations, and communities) to environmental mixtures of chemicals to track biological impacts, ecotoxicological agents may induce immediate and visible effects on organisms, such as viability or reproduction, or produce more subtle effects such as changes in behavioral or physiological traits or immune competence, completely resulting in population decline (Brack *et al*., 2019;Schuijt *et al*., 2021).

Ecotoxicology is used to merge and comprehend the negative impacts of chemicals on organism, communities, and ecosystems, one of the most important steps for ecotoxicological studies is to find a model organism that can provide reproducible information on the acute and chronic effects of environmental pollution. The ideal model organism should be abundant and available, have ecological representativeness within the ecosystem, have a short life cycle, and be easily cultivated and preserved in the laboratory (Świacka *et al*.,2019). The toxicity of

pharmaceuticals relies on the kind and stage of the organism, exposure length, temperature, and concentration of contaminants ecotoxicological studies of pharmatcetal involve ecological, morphological, chemical, physiological, and genetic index (Wang *et al*., 2021a).

1-2-2 Pharmaceutical wastes

It comprises a wide variety of unregulated compounds that are either synthetic or from natural sources. It is classified as an emerging pollutant and has been detected in all water bodies, with concentrations varying from ng/L to g/L (Khasawneh and Palaniandy,2021). Pharmaceutical consumption is estimated at more than 200,000 tons annually in Russia, China, and India (Tijani *et al.*, 2016). They are designed to be active to interact with a living system and produce a drug response at low doses, making them an environmental concern even at environmentally low concentrations, also, they are designed to be stable to reach and interact with target molecules, so they are either very slow to degrade or their ongoing usage results in constant releases into the environment at rates greater than the rate of deterioration (Khan *et al*., 2020). These compounds could pose a serious threat to non-target species in freshwater (Mezzelani *et al*., 2018).

The reasons for the increase in these pollutants in the aquatic environment include population growth, overuse, and the inefficiency of traditional sewage plants in removing these pollutants(Encarnaço *et al*., 2020). According to a United Nations Environment Program report, nearly 80% of the globe's wastewater are discharged directly into water bodies without treatment (Bijekar *et al*., 2022).

1-2-2-1 Antibiotics

Among the pharmaceuticals most commonly found in bodies of water, antibiotics which are chemicals derived from natural, semi-synthetic, and synthetic sources (Baralla *et al*., 2012). It is the most widely used class in human and veterinary applications and is frequently detected in aquatic ecosystems (Bojarski *et al*., 2020). Antibiotic use has increased globally recently, with an estimated 65% increase between the years 2000 and 2015 and a 200% increase projected by 2030 if nothing is done (Klein *et al.,* 2018). Al-Khazrajy and Boxall, (2016) studied the Prevalence of pharmaceuticals the in the natural environment in Iraq, the most used drugs were found to be paracetamol, amoxicillin, and metformin with a total annual consumption exceeding 1000 tons per year.

When antibiotics are not properly eliminated, they enter the environment and cause chronic toxicity to some non-target organisms because they are designed to trigger a biological response in organisms (Liu *etal*.,2018). Some antibiotics disintegrate quickly in the environment, such as penicillin, while others are more stable, such as fluoroquinolones and tetracyclines, allowing them to remain for longer periods in the environment, spread more, and accumulate to higher concentrations (Krzeminski *et al*., 2019).

The presence of antibiotics in the aquatic environment is not without risks, as their permanent presence may lead to the development of antibiotic-resistant microorganisms (Kulik *et al*., 2023). Other associated problems are inhibition of metabolic enzymes, altered mRNA expression (He *et al*., 2021), increased oxidative damage, and oxidative stress (Nunes, 2020) which affect the presence and diversity of living

organisms and may cause genotoxicity, mutations, increased mortality, and a decrease in biodiversity.

In addition, once it enters the environment, it can completely degrade or turn into highly biologically active compounds that are likely to be toxic and can enter and accumulate in aquatic or terrestrial food chains, or both (Larson, 2014).

Antibiotic concentrations in aquatic organisms are related to their habits and position in the food chain, and it is noted that antibiotic levels gradually increased from herbivorous to omnivorous, possibly by food chain enrichment (Tang *et al*., 2021), and there is wide variation in its presence worldwide, with antibiotic concentrations in Asia tending to be higher than those reported in Europe and North America, due to its frequent use in those countries (Tran *et al*., 2018).

1-2-2-2 The Disinfectants

A disinfectant is a chemical or compound that is used to kill or inactivate germs on inert surfaces, disinfectants kill microorganisms by damaging their cell walls or interfering with their metabolism, disinfectants are commonly utilized in hospitals and other healthcare settings for a variety of topical and application-hard surfaces, they are particularly crucial to infection control procedures and aid in the prevention of nosocomial infections (Raeiszadeh and Adeli,,2020; Exner *et al* .,2020).

These chemical compounds can enter sewage systems and contaminate drinking water resources, both direct runoff and indirect sewage will eventually end up in lakes and rivers, endangering aquatic ecosystems, disinfectants threaten aquatic organisms and wildlife in several ways (Luan *et al*.,2020) .

Chemicals in disinfectants can bond with other substances to form harmful compounds. In surface waters, which may allow the synthesis of disinfection by-products, these by-products are very harmful to aquatic life (Zhang *et al*.,2020b), and also Increased disinfection techniques may threaten the environment and public health by hastening the spread of antibiotic resistance (Chen *et al*.,2021).

1-2-3 Pharmaceuticals' Fate in Aquatic Ecosystems

Many processes influence dissipation in the aqueous system, including biodegradation (anaerobic and anaerobic) and abiotic transformation (e.g., UV decomposition, sediment adsorption, and hydrolysis), and depend on the physicochemical properties of drug compounds, such as antibiotic concentrations, half-lives, and environmental factors (Kalyva, 2017).

Pharmaceuticals have three primary probable fates in the aquatic environment: first, pharmaceuticals are mineralized into carbon dioxide and water, for example, aspirin; second, the compounds are metabolized but remain in water-soluble forms of the parent component, so they pass through the wastewater treatment facility and end up in recipient waterways; if the metabolites are bioactive, they may impact aquatic life(Klaminder *et al.*, 2014) Thirdly, the polymer is lipid-soluble and will not degrade fast; some of it will be retained in the sludge(Kayode-Afolayan *et al*., 2022). Many processes for eliminating pharmaceutical compounds from the aqueous ecosystem are summarized as follows,figure (1- 1).

Adsorption is one of the significant ways to remove antibiotics in the aqueous ecosystem, numerous studies on antibiotic absorption in soil and water have been conducted, and sediment adsorption is regarded, as one of the most significant antibiotic fates in aquatic ecosystems (Cheng *et al*., 2022).

Hydrolysis is a key mechanism for the breakdown of various organic compounds, particularly amides, and esters, the key parameters influencing antibiotic hydrolysis rates are temperature, and pH level (Mitchell *et al*.,2014).AMX is a beta-lactam antibiotic that hydrolyzes in this manner, it dissolves rapidly in aqueous circumstances due to lactam ring hydrolysis, generating two components, AMX penilloic acid and AMX diketopiperazine-2'-5' (Hirte *et al*., 2016).

Photolysis is one of the main degradation processes for organic pollutants in aquatic ecosystems and includes direct photolysis, sensitive photolysis, and photooxidation, there are many factors affecting the photodegradation process of pharmaceutical compounds, for example, water properties (e.g., pH and temperature), water content (e.g species inorganic compounds, and contents of dissolved organic), the composition and properties of organic pollutants, and photocatalysts (Cheng *et al*., 2022). For example, oxytetracycline (OTC) undergoes direct photolysis and is considered the main disposal pathway in surface waters (Jin *et al*., 2017) , both direct and indirect Photodegradation,are an important process in the abiotic transformation of pharmaceuticals in waterbodies, Indirect photolysis is brought on by natural photosensitizers, whereas direct photolysis is brought on by sunlight's direct absorption (Nicolaou *et al*., 2007). Its photolysis dissolution in water is affected by several factors, including the intensity of solar radiation, latitudinal, organic matter content, and eutrophication circumstances (Wang *et al*., 2020b).

Antibiotic biodegradation is the elimination of antibiotics from the ecosystem through the utilization of living organism like bacteria, algae, yeast and plant etc , a variety of factors influence this process, including microbial species, anaerobic and aerobic conditions, antibiotic

concentrations, precipitation, and temperature (Liu *et al*., 2021). Anaerobic and aerobic biodegradation are the two most common methods for removing medications from the dissolved phase (Mansouri *et al*., 2021).

Many environmental factors (abiotic and biotic) influence pharmaceuticals'fate in aquatic environments, including pH, temperature, sunlight and light intensity, hydraulic retention time, seasonality, microbial communities, sediment, natural organic matter, suspended particles water body volume, turbidity, the hydraulic regime, weather conditions, etc. (Carpenter *et al*., 2018).

In aquatic environments, salinity has an even greater impact on the distribution and natural degradation of medicinal substances, when freshwater and saltwater meet, the role of salinity becomes more important. For instance, as salinity rises, the coefficient of separation between estrone and sediment rises, resulting in a drop in estrone's aqueous content and a favoring of further adsorption to the sediment (Patel *et al*., 2019). Salinity can change the physical-chemical properties of the drugs and /or the sensitivity of the organisms to them, According to a study by Almeida *et al*. (2022b), salinity changes altered the effects of pharmaceutical drugs antiepileptics and antihistamines in the clam *Ruditapes philippinarum* and caused altered metabolic processes, decreased levels of antioxidants, and biotransformation in the organism.

A further important factor influencing the fate of pharmaceutical preparations is pH, which plays an important role in the biodegradation process,and sorption capacity resulting from its effect on the adsorbent surface charge, the degree of ionization of the materials in the solution, the separation of the active groups in the active sites, and the chemistry

of the solution (Yazdi *et al*.,2018). As a result, it will have an impact on the biological, chemical, and physical features of the medications, such as their toxicity, activity, photosensitivity, and absorption. (Verlicchi *et al.*, 2012; Fernandes *et al*., 2021). It was discovered that the pH of a submerged membrane bioreactor (MBR) had a significant impact on the elimination of antibiotics like ibuprofen, diclofenac, ketoprofen, and sulfamethoxazole (between 5 and 9),at pH 5, the maximum elimination of these antibiotics was achieved (Tiwari *et al*.,2021). According to research by Baena-Nogueras *et al*. (2017), the photodegradation of many pharmaceutical compounds is influenced by pH, Acetaminophen photodegraded faster at pH 4 or 9 than at pH 7, whereas other medications such as diclofenac, ibuprofen, and ketoprofen showed no significant difference.

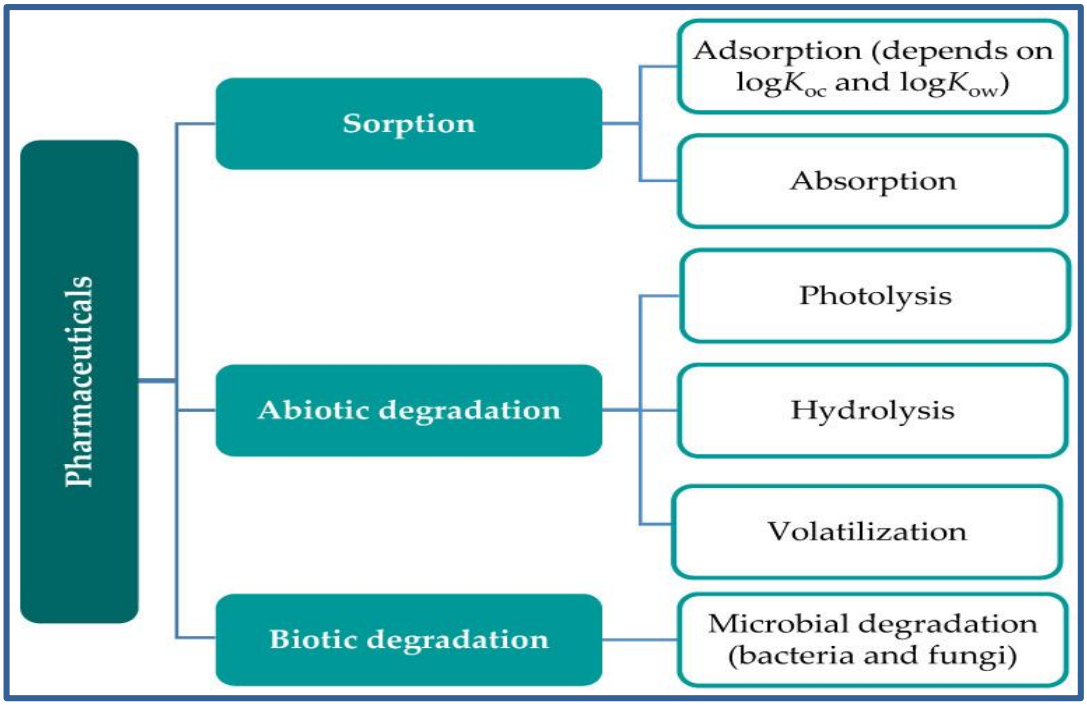

Figure (1- 1): Removal mechanisms of pharmaceuticals in the environment(Bankole *et al*.,2023).

1-2-4 Amoxicillin $C_{16}H_{19}N_3O_5S$ and Potassium Dichromate ($K_2Cr_2O_7$)

1- Amoxicillin $C_{16}H_{19}N_3O_5S$

Amoxicillin (AMX) is an antibiotic in the beta-lactam class. It has broad antibacterial activity against both gram-negative and gram-positive bacteria (Fazelirad *et al*., 2015). Asemi-synthetic antibiotic and consists of D-4-hydroxyphenyl glycine and 6-aminopenicillinic acid, figure (1- 2) ,it works by inhibiting the formation of the bacterial cell wall and causing lysis and cell death by inhibiting a bacterial enzyme called the transpeptidase enzyme, which is necessary for the synthesis of the bacterial cell wall (Hugonnet *et al.*,2016). Average half-life of amoxicillin is about 1.5 hours; it accounts for more than 65% of the antibiotics consumed by many nations and is regarded as one of the major threats to the environment. At higher doses, amoxicillin has been identified as a possible mutagen, carcinogen, and teratogen (Sodhi *et al*., 2021).

Amoxicillin is extremely unstable and rapidly breaks down into several degradation products. The beta-lactam ring can be broken by several biotic and abiotic mechanisms, resulting in the formation of various secondary compounds that may be more resistant to degradation or more toxic than the parent compound (Elizalde-Velázquez *et al*., 2016).

A complete amoxicillin degradation pathway in aqueous media was proposed by Gozlan *et al*.(2013), the four-membered -lactam ring is first opened by hydrolysis or by several metal ions, including mercury, copper, cadmium, cobalt, and zinc, which may catalyze the breakdown of the -lactam ring to produce the intermediate AMX-penicilloic acid, this intermediate chemical might then produce two distinct, more stable compounds, depending on the pH of the medium. At high pH values, the

AMX-penicilloic acid degradation product yields a 6-membered stable diketopiperazine ring and the AMX diketopiperazine degradation product, at low pH values, the AMX-penicilloic acid undergoes a decarboxylation process, yielding the AMX-penicilloic acid. Another amoxicillin metabolite is produced through an indirect photolysis process aided by the existence of natural photo-sensitizers such as humic acids, forming the AMX-S-oxide degradation product, this compound in an aqueous system poses an environmental concern as AMX-S-oxide -lactam is still active and may lead to the development of resistant bacteria(Elizalde-Velázquez *et al*.,2016; Hirte *et al* .,2016).

This antibiotic generates hazardous toxic effects, including alterations in embryonic development and oxidative stress in aquatic creatures, and it has been observed that AMX can cause DNA damage and cytotoxic effects in the common carp's blood cells (Orozco-Hernández *et al*., 2019).

Amoxicillin bioaccumulation in fish tissues, which can be used as food, thus harms humans by triggering immune responses and increasing bacterial resistance genes in fish tissues (Wu *et al*., 2020 a Wu; Elizalde-Velázquez *et al*.,2016).

Amoxicillin was detected in wastewater, marine freshwater, and soil sediments, as (AMX) at the ng/L level in Ghana, Turkey, Italy, and Australia, with the highest concentration of 1.65 mg/L (Lee *et al*., 2021).

In Malaysia's Klang River Estuary, Five substances, namely, (amoxicillin, progesterone, diazinon, bisphenol A, and E1) were detected in every sampling station examined and assessed (Omar *et al*., 2018).

In India, a study by Sodhi *et al*.(2020) showed that amoxicillin and its metabolites (Penicillic acid), were discovered in the River

Yamuna, which is the main supply of drinking water for a population of over 13 million people. Studies by Velpandian *et al* .(2018), also showed the presence of amoxicillin (0.18 µg/L) in aquifers and surface waters AMX concentrations in various Italian cities' sewage effluent ranged from 1.88 to 120 ng/L. (Matozzo *et al* ., 2016 a).

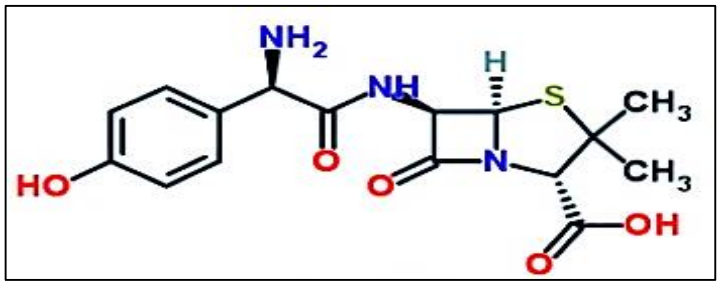

Figure 1-2:Chemical structure of Amoxicillin (Hugonnet *et al.*,2016).

2- Potassium Dichromate ($K_2Cr_2O_7$)

Potassium dichromate ($K_2Cr_2O_7$) was used as a reference toxicant, as recommended by the US EPA (1993). Potassium dichromate is a reddish-orange compound that contains the three elements potassium (26.58%), chromium (35.35%), and oxygen (38.07%), figure (1- 3) , and one gram of chromium is contained in each 2.829g of potassium dichromate (De Freitas *et al*.,2014). According to the World Health Organization (WHO), the maximum allowed quantity of Cr in drinking water is 50 ppb (Sessarego *et al*., 2019).

Chromium is a highly toxic inorganic pollutant that is eliminated into the environment, through various natural and anthropogenic sources, such as the textile industry as mordants and dyes, leather tanning, wood preservation, and chrome electroplating. Potassium dichromate, $K_2Cr_2O_7$, is a common inorganic chemical reagent that is widely used as an oxidizing agent in various laboratories (Bakshi and Panigrahi, 2018).

Numerous environmental and health organizations have designated Chromium as a priority pollutant, and it is estimated that the high level of Cr pollution in the world puts about 16 million individuals in danger (Sharma *et al*., 2022).

When Chrom present in excess Cr(VI) can cause genotoxicity and oxidative damage, damage lipids, proteins, and DNA, and cause oxidative stress, autophagy, an inflammatory response, apoptosis, and excessive generation of (ROS), which results in cell damage (Rahman& Thomas 2021; Laxmi & Kaushik 2020; Sharma *et al*., 2020).

Chrome (VI) is easily bioavailable; it can pass through the cell membrane sulfate/phosphate anion transporter and be reduced to lower oxidation states like pentavalent, tetravalent, and trivalent chromium (Wang *et al*., 2017). Reactive intermediates are formed during this reduction process and react with hydrogen peroxide (H_2O_2) to produce hydroxyl radicals via Haber-Weiss or Fenton-like reactions (De Mattia *et al*., 2004; Chaâbane *et al* .,2021).

Microorganisms have several metal chelating processes and a high metal absorption capability,microorganisms use diverse hazardous substances as a source of energy for growth and development via metabolism, respiration, and fermentation (Yan *et al*.,2023).

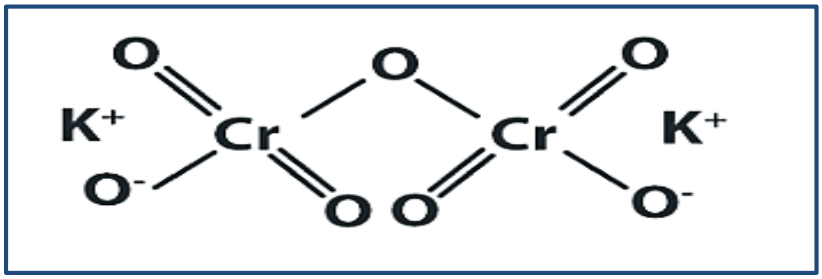

Figure 1-3:Chemical structure Potassium dichromate (Tallarico *et al*.,2014).

1-2-5 Impacts of pharmaceuticals on Aquatic Organisms

The presence of pharmaceuticals in have risk in is not without risk, pharmaceuticals and their metabolites constitute a new and important class of aquatic pollutants and pose a serious threat to the food chain,it can also affect the behavior and reproductive systems of aquatic organisms, with cascading effects on entire ecosystems (Khan and Barros, 2023).

Pharmaceuticals also represent an environmental risk due to the significant effects they can have on a range of non-target aquatic organisms that have similar biological functions and receptors. Pharmaceuticals exhibit not just short-term (acute) toxicity but also long-term (chronic) toxicity. Acute toxicity is caused by single or several exposures in a short period, and it typically presents as a fatal endpoint (mortality or immobilization), while chronic toxicity is described as the onset of negative effects from repeated and prolonged stressor exposure, which frequently manifests as a sub-lethal endpoint or growth inhibition, molecular or biochemical modifications, or behavioral abnormalities (Hejna *et al*.,2022) in aquatic organism as bivalve and fish . It has been observed to cause cytotoxic effects, disrupt lysosomal membrane integrity, and increase hematocytosis in *Clamruditapes philippinarum* (Nicolussi *et al*., 2022). The drugs cause a variety of direct and indirect effects on non-target species in the aquatic environment, such as cytotoxic effects, disruption of lysosomal membrane integrity, and increased spherocytosis in Clam *Ruditapes philippinarum* (Nicolussi *et al*., 2022).

They cause reduced growth of aquatic plants, algae, and environmental microbes and cause oxidative stress and DNA damage

(Mirzaee *et al*., 2023; Liu *et al*., 2023). It may cause changes in metabolism and gene expression and thus lead to negative effects on their growth, development, and survival (Leonard *et al*.,2022) In addition, it showed that the presence of pharmaceuticals in water bodies can lead to the development of antibiotic-resistant bacteria, which poses potential risks to human health (Bereketoglu *et al.*, 2022).

1-2-6 Environmental Biomarkers

A biomarker is a measurement of the interaction between a biological system and an environmental factor, which might be chemical, physical, or biological. Therefore, considered biomarkers are a useful environmental tool to evaluate the exposure and negative effects of stressors on organisms, and environmental biomarkers are described as a change in a biological response happening at the molecular, cellular, or physiological levels that may be linked to exposure to or toxic effects of pollution chemicals (Lionetto *et al*., 2019).

Their joint use in pollution monitoring is strongly recommended. Furthermore, these biomarkers have the benefit of being tested after longer storage durations under freezing conditions, which is superior to other heat-labile and shorter-lived metabolic enzymes(Cao *et al*., 2018; Chahouri *et al*., 2022).

1-2-6-1 Biochemical Markers

The antioxidant system consists of substances that delay and/or prevent oxidation of the cellular substrate at low concentrations and play an important role in the direct removal of free radicals, and can protect cells against free-radical damage by delaying or preventing the oxidation of proteins, carbohydrates, lipids and DNA ,antioxidant defense system

includes both enzymatic and non-enzymatic antioxidants and scavenging processes (Ighodaro and Akinloye, 2018; Martemucci *et al*.,2022).

Differences in the activities of these enzymes represent an early warning indicator to exposure toxic compounds, allowing the identification of changes in biological systems before community-level effects, which has been used to assess oxidative stress in target and non-target organisms for exposure to various pollutants (Oyaneder-Terrazas *et al*.,2022; García *et al*.,2022). (ROS) are chemically reactive molecules containing oxygen that play several beneficial roles for the organism at low or moderate concentrations, they are needed for physiological activities such as intracellular cell signaling and homeostasis, cell death, immune defense against pathogens, and induction of the mitogenic response (Bhagat *et al*., 2016; Juan *et al.*, 2021).

ROS are produced from molecular oxygen as a result of normal cellular metabolism, and ROS can be divided into two groups: free radicals and non-radicals (Birben *et al*., 2012). ROS include reactive oxygen species such as (hydroxy radicals, superoxide anion, hydrogen peroxide, and nitric oxide), stressful environmental stressors rapidly increase cellular ROS concentrations to levels that are higher than antioxidant scavenging capacity (Snezhkina *et al*., 2019; Zandi and Schnug, 2022). Oxidative stress is caused by an imbalance between the generation of ROS and the ability of antioxidant systems to readily detoxify these reactive intermediates. Excessive and unregulated free radical production under oxidative stress may cause damage to DNA, proteins, or lipids, which can severely affect cell health (Pizzino *et al*., 2017; Schuijt *et al*., 2021).

1-2-6-2 Enzymatic Antioxidant

1-2-6-2-1 Superoxide Dismutase (SODs)

It is a group of metalloenzymes that act as important antioxidants and represent the first line of defense against human and natural pollutants. The most important role of this enzyme is to restore cell vitality and slow down the rate of oxidation by dismuting superoxide anion free radical (O_{2-}) into molecular oxygen and hydrogen peroxide (H_2O_2), thereby decreasing the O_{2-} level which damages the cells at excessive concentration,based on the metal cofactors present in the active sites, SODs are classified into four species: copper-zinc-SOD (Cu, Zn-SOD), iron-SOD (Fe-SOD), manganese-SOD (Mn-SOD), and nickel-SOD (Younus,2018; Maurya and Namdeo, 2021).

1-2-6-2-2 Catalase (CAT)

The antioxidant enzyme catalase (CAT) is regarded as the first line of defense. It consists of a tetrapeptide chain and contains heme groups that allow the enzyme to interact with hydrogen peroxide, playing a critical role in preventing oxidative damage to cells by decomposing hydrogen peroxide into water and oxygen, which are the most abundant and prevalent of all enzymes in a cell (Nandi *et al*., 2019). Catalase has the highest turnover numbers of all enzymes, One molecule of catalase can convert over 2.8 million molecules of hydrogen peroxide to water and oxygen per second, and as a result, CAT is an enzyme that gives a clear and early response to pollutants, and it works in conjunction with an enzyme called SODs to reduce oxidative stress in cells (Smejkal and Kakumanu, 2019),figure 1-4.

1-2-6-2-3 Glutathione Peroxidase (GPx)

It is an antioxidant enzyme that belongs to selenocysteine because it binds four atoms of selenium and gives the catalytic activity of GPx, a tripeptide that consists of cysteine, glutamic acid, and glycine (Fundu *et al*., 2019; Peiet *et al*.,2023). Glutathione Peroxidase (GPx) is a cytosolic enzyme that catalyzes the reduction of hydrogen peroxide to water and oxygen as well as catalyzing the reduction of peroxide radicals to alcohols and oxygen (Sarkaya and Doan, 2020).GPX, also known as selenocysteine peroxidase, is more important in inhibiting lipid peroxidation and thus protecting cells from oxidative stress (Ighodaro and Akinloye, 2018). GPX plays a major role in protecting cell membranes from damage via lipid peroxidase (Duran-Alvarez and Jiménez-Cisneros,2021).

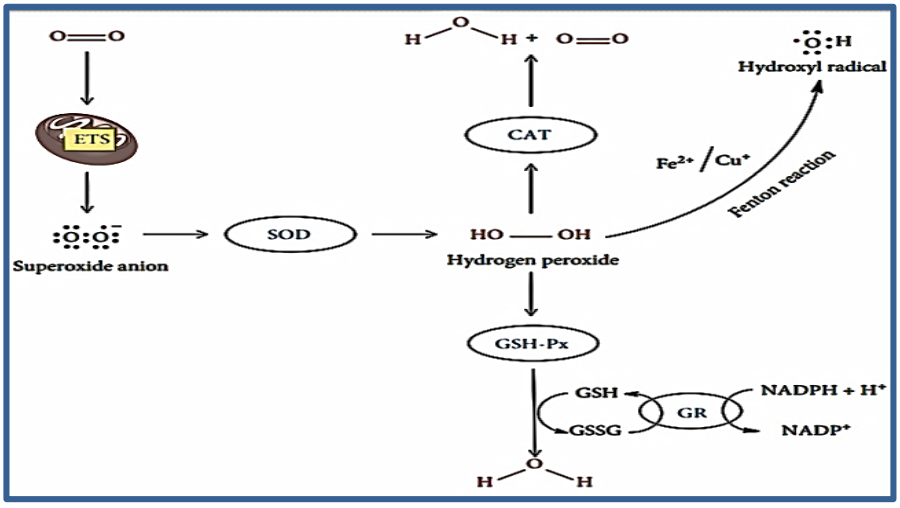

Figure 1- 4:catalase's relationship to other antioxidant enzymes (Nandi et al.,2019).

1-2-6-3 Non-enzymatic Antioxidant

1-2-6-3-1 Glutathione (GSH)

The glutathione system is an important endogenous antioxidative system that can maintain cellular redox balance and protect cells from oxidative damage and death (Tan *et al*.,2023) GSH is made up of three Amino acids (glutamic acid, cysteine, and glycine) and is represented by the symbol GSH when reduced and GSSG when oxidized (Istomina *et al*.,2021). Glutathione is crucial for the integrity of red blood cells, the functioning of proteins, and lipid membranes, and it works as an enzyme co-factor and antioxidant, GSH plays a major role in the elimination of numerous reactive species to protect cells from free radical damage and GSH is also involved in regulation of the cell cycle (Vašková *et al*.,2023).

These enzymes catalyze the reduction of H_2O_2 by GSH into H_2O; GSSG is reduced to GSH under the action of GR and NADPH; GSH reduces H_2O_2 (or lipid-OH) to H_2O (or lipid-oH) in the catalysis of GPX (Tan *et al*.,2023) figure 3. The reduced glutathione/oxidized glutathione ratio (GSH/GSSG) is widely used in clinics for the evaluation of oxidative stress status in biological systems, where increased GSSG-to-GSH ratio is indicative of greater oxidative stress (Bansal and Simon, 2018).

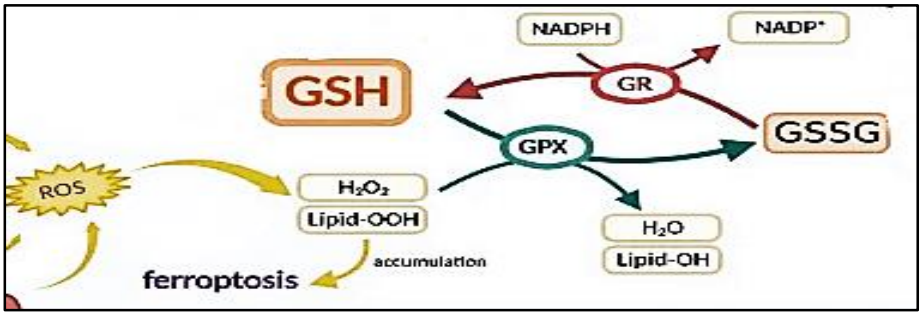

Figure 1-5: Strategies for the GSH system to remove ROS (Tan *et al*.,2023).

1-2-6-3-2 Ascorbic Acid (AA)

Ascorbic acid (AA), also called vitamin C, is a water-soluble vitamin that functions as a powerful antioxidant. Vitamin C can protect vital body substances such as proteins, lipids, carbohydrates, DNA, and RNA from free radical damage (Wu *et al*., 2022a). Ascorbic acid (AA) is known for its antioxidant activity and acts as a reducing agent (Njus *et al*.,2020). Ascorbic acid donates a single reducing equivalent, and the radical it forms, monodehydroascorbate, reacts preferentially with radicals instead of with non-radical compounds, thereby decreasing reactive oxygen species, and reactive nitrogen species by providing electrons to them and preventing the oxidation of other compounds (Fujii *et al*., 2022).

1-2-6-4 Total Protein

Proteins are polypeptide structures consisting of one or more long chains of Amino acid residues Proteins play a variety of crucial roles in cells, including promoting metabolic activity, facilitating DNA replication, giving cells shape, biochemical catalysts, and moving ions from one place to another and they can also be used as an energy source, the Amino acid sequence of different proteins is what sets them apart(LaPelusa and Kaushik, 2022). Many scientists have recently employed proteins as one of the biochemical indicators of the effects of oxidative stress caused by numerous pollutants in aquatic settings (López-Pedrouso *et al*.,2022; Marisa *et al*.,2021).

1-2-6-5 Malondialdehyde (MAD)

Malondialdehyde MDA is a low molecular weight end product of lipid peroxidation and is widely utilized as a biomarker of oxidative stress (Bhagat *et al*.,2016). MDA is one of the final products of

polyunsaturated fatty acids peroxidation in the cellsand it is a highly reactive and toxic byproduct of lipid peroxidation (Juan *et al*.,2021) . MDA is considered a marker of oxidative stress because its accumulation is indicative of increased lipid peroxidation and ROS production Therefore, the presence of MDA in cells and tissues is often used as an indicator of oxidative stress, as it reflects the degree of lipid peroxidation and ROS-mediated damage (Haro Girón *et al*.,2013).

1-2-7 Histological Biomarker

This kind of biomarker involved lesions and alterations in specific tissues species such as(necrosis, inflammation, and degeneration), histopathology is utilized as a reference standard for assessing the sensitivity and specificity of organisms to different species of contamination, and there are important correlations between histological alterations and environmental stress (Ijomah *et al*., 2020).

Histological studies are one of the most appropriate possible biomarkers because they measure response to short and long periods of exposure, use many tissues, are distinguishable, and can be effective (Carvalho *et al*., 2020). Histopathological changes in tissues are identified as reliable biomarkers in distinguishing pollution levels between various, aquatic ecosystems(Abdel-Moneim *et al*.,2012).

Particular attention was paid to the effect of contaminants on gills and digestive glands, which are the main target, The gills and digestive glands of bivalves play a significant role in capturing, absorbing, and digesting food, and they are also directly exposed to environmental pollutants, and the gills filter the water to obtain nutrients and therefore are in constant contact with water contaminants, the mollusk's digestive

gland is the major center of control of metabolic regulation organs (Carballeira *et al*.,2011).

Bivalves are capable of filtering high volumes of water by retaining up to 90% of the particles contained in it, including any contaminant present to concentrate them in their tissues directly from water and through diet, and Use in monitoring programs can give information about pollutants' presence in different areas and marine layers (Varol and Sünbül. 2017).

Many studies have used histological responses in aquatic organisms as biomarkers of pollution damage such as, Joshy *et al*., 2022, employed histopathology of the digestive glands and gills of three bivalves *Mallana bilineata*, *Perna viridis,* and a clam (*Villorita cyprinoid's*) to assess aquatic environmental quality on India's southwest coast.

Studyof Sheir (2020) evaluating and comparing tissue responses as a biomarker for a freshwater clam (*Caelatura nilotica)* as a biomarker for different species of pollution (organic and inorganic) in different environments with different degrees of pollution. According to a study by Shan *et al*. (2020), imidacloprid causes significant histological alterations in adult Asian clams (*Corbicula fluminea*)., including cilia degeneration, decreased lymphocyte adhesion, distension of the epithelial cells in the gills, severe alimentary tube degeneration, and hemolytic infiltration of connective tissue. Marinovi *et al*.(2021) employed histopathological alterations in fish gill histological abnormalities as biomarkers to differentiate between seasonal variations in water quality within the same aquatic ecosystem.

1-3 Algae Immobilization

Algae are autotrophic organisms found in a range of habitats; they are fast-growing and can withstand harsh environmental conditions; they have a wide variety of applications, including food or dietary supplements, pharmaceutical manufacturing, fish feed, fertilizer production, biofuel production, bioremediation, etc. (Palito *et al*., 2021;Chandel *et al*., 2022; Salman *et al*.,2022).

Microalgae-based technology has received widespread attention as a possible alternative to conventional wastewater treatment since it is a solar-powered, environmentally friendly, cost-effective, and sustainable reclamation strategy (Arimbrathod *et al*., 2023).

An immobilized cell is described as a living cell that is prevented from moving independently from its original location to all parts of a system's aqueous phase by natural or artificial carriers (Xiong *et al*., 2021; Hejna *et al*.,2022). The basic idea is that immobilized microalgae in matrices, whether biological or inert, can help produce necessary biotechnological benefits from mass growth, such as the production of a specific metabolite or the removal of contaminants (De-Bashan and Bashan, 2010).

Cell immobilization has several advantages over suspended cells, including making biomass harvesting easier; enhancement of cell growth and morphology; higher cell density; prolonging the cells' metabolic activity; increased resistance to environmental stresses (temperature, acidity, and toxic compounds); and taking up less space, making it easier to handle and use regularly (Eroglu *et al*., 2015). Disabled cells can be reused in successive applications without loss of bioremediation

activities on their own, which reduces production costs; the immobilized cells provide the operational stability of the performance of microorganisms and facilitate continuous cultivation operations (Hejna *et al*., 2022; Chen *et al*., 2023).

Six different species of immobilization techniques have been identified: covalent coupling, affinity immobilization, adsorption, confinement in liquid-liquid emulsions, capture behind semi-permeable membranes, and entrapment in polymers. Organic and inorganic carrier materials are commonly utilized in the immobilization of microorganisms (Kaparapu, 2017; Partovinia and Rasekh, 2018)

In recent years, microalgae-immobilized technology has received increasing attention, and Immobilized systems have been utilized in several applications, including reducing contaminants, energy production, and wastewater bioremediation (Yu *et al.,* 2017; Salman *et al*., 2022; Cao *et al*., 2022).

Immobilized cells are more effective than free cells in removing nutrients, heavy metals, and pigments because they are present in the matrix and are thus protected from the effects of toxic compounds (Revathi *et al*., 2017; Cao *et al*., 2022; Salman *et al*., 2022).

Immobilized microalgae have been used in bioremediation processes to remove many unwanted substances (as nutrients, minerals, and organic pollutants) from various media (Palito *et al*., 2021). Several studies on Immobilized algae indicate their susceptibility to remove pharmaceutical compounds from aquatic environments, such as *Chlorella pyrenoidosa*, which showed the ability to remove amoxicillin from the aqueous media by 91% within 6 hours (Xiao *et al*., 2021) .

Chlorella sorokiniana to clear 100% of ibuprofen within 31 days (De Wilt *et al*., 2016). According to Xie *et al*. (2020b), immobilized *C.vulgaris* demonstrated greater sulfamethoxazole tolerance than the suspended cell, and the removal efficiency of immobilized *C. vulgaris* was more than 12% higher than the suspended cell. *C. vulgaris* was used to remove metronidazole from the culture medium by 100% within 18 days (Hena *et al*., 2020). *Chlorella sp*. removed Florfenicol from the culture medium by 97% within 24 hours (Song *et al*., 2019).

In a study conducted by Encarnaço *et al*. (2020), immobilized microalgae removed Pharmaceuticals and personal care products(PPCPs) more effectively than suspended microalgae(*C. vulgaris)* , assessed the performance and efficiency of free and immobilized microalgae cells in removing four pharmaceuticals, and discovered that immobilized cells removed pharmaceutical substances such as paracetamol and ibuprofen more effectively than free cells.

The ability of immobilization technology can effectively protect *Chlorella vulgaris* from carbamazepine (CBZ) toxicity and improve the removal of CBZ, especially at high concentrations (Liang *et al*., 2022). The results of Kwarciak-Kozowska and Sawik-Dembiczak. (2021)study showed the ability of immobilized algal cells in alginate beads to enhance the uptake of metal Pb(II).

1-3-1 Detoxification of Pharmaceutical Wast by *C. vulgaris*

Microalgae have drawn attention in the bioremediation research community for being non-target organisms for antibiotics and their ability to adapt and remove the antibiotics themselves from polluted water, producing valuable biomass (Li *et al*.,2022).

C. vulgaris is one of the most widely distributed green microalgae, present in most aquatic habitats throughout the world,due to its short life cycle, low cost, ease of cultivation in labs, and sensitivity to a variety of pollutants, it is frequently used in toxicity testing and is one of the most commonly studied algae in wastewater treatment (Ajitha *et al*.,2019;Wong and Roma, 2021).

The presence of antibiotics in wastewater leads to dual responses in *C. vulgaris,* which include either inhibitory or growth-stimulating effects depending on the concentration (Mao *et al*., 2021). *C.vulgaris* can acclimate to antibiotic stress conditions, through spontaneous physiologic adaptation, and can biodegrade contaminants thus making it a promising candidate for antibiotic removal from wastewater (Ricky *et al*., 2022).

Xiong *et al*. (2017) used *C.vulgaris* to remove levofloxacin, a fluoroquinolone, from an initial concentration equal to 5 mg/L. 15% of the antibiotic was removed, after seven days. while Hena *et al*.(2020) investigated the removal of metronidazole (MDZ) from aqueous media by *C. vulgaris.* MDZ stimulated the production of extracellular polymeric substances (EPS), which played a major role in the adsorption of this antibiotic.

Ricky *et al*.(2022) proved *C. vulgaris'* ability to adapt and remove emerging contaminants such as ciprofloxacin (CIP) and AMX, and in this study, CIP removal was higher than AMX removal, specifically about 37% (CIP) versus 25% (AMX). Xiao *et al*. (2021) indicated the ability of *Chlorella pyrenoidosa* to remove the Amoxicillin from the aqueous medium by a removal rate 91%during 6 hours.

Study of Chen *et al*. (2020) showed the effects of antibiotics (sulfonamides and fluoroquinolones) and their potential for removal by

green algae (*C.vulgaris)* and cyanobacteria (*Chrysosporum ovalisporum).*

In Iran, Yazdi *et al.*(2018) studied the removal of penicillin from aqueous solution by *Spirulina platensis* and *Chlorella vulgaris* microalgae from an aqueous solution, these showed that the more cells and biomass of microalgae increased, the greater the efficiency of penicillin removal from a solution.

Several investigators pointed to the ability of *C. vulgaris* to remove chromium, such as the study of Lee *et al*. (2017), was estimated the removal of Cr by 90% and Cu by 89% in the metal removal process because *Chlorella sp*. possessed functional groups such as R-COOH, the hydroxyl group, the sulfate group, and the amine group. Those functional groups play an important role in binding metals to the biosorption process.

According to Elystia *et al*.(2020), *Chlorella sp.* was immobilized in alginate beads and was more effective than empty alginate beads in eliminating Cr (VI) from wastewater. Cr (VI) was largely removed during the biosorption.

Several studies indicated the ability of *Chlorella sp*. algae to remove amoxicillin, such as the Li' *et al*. (2015) study using *C. pyrenoidosa*. In a 48-hour HRT in BG11 medium, the ability of *C. pyrenoidosa* to remove more than 100% of 100–300 mg/L of amoxicillin was observed.

While Yang *et al*. (2017) study indicated that *Scenedesmus obliquus* mixed with UV radiation at 280nm can remove up to 99% of 100 mg/L amoxicillin, *C. pyrenoidosa* paired with Fenton oxidation can remove 93.3% of amoxicillin (1 g/L) at 48 hours.

1-4 Molluscs as Biomonitoring Species

Biomonitoring is a commonly utilized instrument for describing aquatic environment quality which uses several aquatic organisms, including macroinvertebrates, fish and algae, (Świacka *et al*., 2019; Ali *et al.,* 2018; Cunha *et al*., 2017). Many studies have used mollusks as environmental indicators, like Al-Mamoori et al.,(2013), who used snails (*Viviparus bengalensis*) and clams (*Corbicula fluminea*) as heavy metal pollution indicators in the Hilla River in central Iraq.

Ali *et al.* (2015) studied biomarkers (biochemical and molecular) for evaluating water quality in clam (*Unio tigridis*).

Ali and Al-mamoori . (2023) studied biomarkers (Protein profile) in freshwater mussels (*Unio tigridis*) as a bio-indicator for aquatic pollution in the Hilla river, Iraq.

Bivalves, like clams, are frequently utilized as bioindicators for monitoring water pollution (Almeida *et al.,* 2020) . They serve as a good representation of the sample region, due to their stable behavior and being filter feeders clams concentrate chemicals to levels that are substantially higher than those in the surrounding water and reflect an extended period compared to conventional sampling (Cunha *et al*., 2017; Świacka *et al*., 2019).

The majority of bivalve species provide important services in freshwater habitats, such as water filtering, bottom bioturbation, nutrient circulation, habitat creation and modification, and affecting food webs directly i.e., prey and indirectly i.e., movement of nutrients and energy (Vaughn and Hoellein 2018). They have been used in biomonitoring programs for over 40 years and are used in biomonitoring programs for

systematic measurements of the exposure levels of anthropogenic wastes of many pollutants, plus, they are a food source for many other species and can act as a route for transporting marine pollutants through the food chain (Chiesa *et al*., 2018). Using Reyna *et al.* (2019), *Corbicula largillierti* clams as a bioindicator of water quality and as a mirror of aquatic pollution in the central region of Argentina.

Bivalve molluscs have been used as a source of food and bioindicators of environmental pollution. Certain unique attributes of bivalves like sedentary and filter-feeding nature, availability of large numbers in their natural habitats, resistance to variable environmental conditions, ability to uptake and accumulate diverse substances present in their ecosystem, and easy sampling make them potential candidates for environmental bio-monitoring programmes, highly sensitive to environmental changes and responding quickly (Stara *et al.*,2020; Baralla *et al*.,2021).

Recently, concerns that pharmacologically active compounds may have potential ecosystem-threatening effects have prompted several scientists to investigate their presence in bivalves such as Mytilidae bivalves like *M. edulis* and *M. galloprovincialis* are frequently used as indicators in studies of the influence of medicines because their widespread prevalence and well-known physiology (Swiacka *et al*., 2019).

The study by Koagouw *et al*. (2021), biological changes induced by long-term dosed exposure to paracetamol in the blue-lipped mussel *Mytilus edulis*.Wu *et al*. (2020b) studied quinolone residues in farmed bivalves obtained from Taiwan, and they identified flumequine and enrofloxacin at rates of 0.8 ng/g and 0.5 ng/g, respectively.

While Martnez-Morcillo *et al*. (2020) investigated the presence of some macrolides and nitroimidazoles in the bivalve *Ensis Siliqua*, finding ronidazole at levels of 2.26 ng/g dw.

Serra *et al*. (2021) detected the presence of sulfonamides, macrolides, B-lactams, tetracyclines, and quinolones in bivalve hemolymph collected from aquaculture structures on Spain's Mediterranean coast, and levels of enrofloxacin (0.230 g/l) and marbofloxacin (0.435 g/l) respectively.

According to Oliveira *et al*.(2017),long-term exposure to carbamazepine in mussels *Mytilus galloprovincialis* led to adverse physiological and biochemical changes that may have an impact on the ability of the organisms to reproduce, which could have an impact on the sustainability of the population. In southern Iraq, research Mirza and Nashaat,(2019). Molluscan diversity, distribution, and abundance in the Gharaf River.

1-4-1*Corbicula fluminea*

C. fluminea Asian clams called, are frequently employed in the identification and assessment of freshwater ecotoxicological concerns. This species forms an excellent model organism due to its abundance, quick development, ease of collection, early sexual maturity developed in a lab, long life, high fecundity, great tolerance to abiotic changes, well as sensitivity to different pollutants. (Li *et al*., 2018; Seoane *et al*.,2021). Based on their high enrichment capacity and quick responses to contaminants, have been widely used in biomonitoring and other environmental applications(Li *et al*., 2023). These advantages allow *C. fluminea* to be used as a bioindicator for organic pollutants (Wang *et al*.,

2018), metal pollutants (Saidani *et al.*, 2019), and some other emerging pollutants (Guilhermino *et a*l., 2018).

C. fluminea have been used in several ecosystemic studies. For example, the study by Seoane *et al*.(2021) environmental toxicity of two benzophenones (BP-3 and BP-4) and two bisphenols (BPA and BPS) on freshwater bivalves *C. fluminea,* revealed that BP-3 and BPA were the most toxic compounds and revealed differences in All variables studied at the highest levels.

Liu *et al*.(2022) investigated the ecotoxicity of Sulfamethoxazole (SMX) on *C. fluminea* clams, and thier findings revealed that SMX significantly reduced filtration rate and acetylcholinesterase (AChE) activity, and harmed siphon behavior, toxicity, nervousness, oxidative stress, and apoptosis in *C. fluminea.*

While Guo *et al*.(2022) examined the effects of Ciprofloxacin toxicity on biochemical parameters, histological characteristics, and behavioral characteristics of *C. fluminea* and discovered that it may result in significant histopathological abnormalities in the gills and digestive gland coupled with oxidative damage.

Aharchaou *et al.(* 2022) Studied, Impacts of bioaccumulation of Cr (III) and Cr (VI) in the freshwater *C. fluminea*, and note the negative impacts on biomarkers on energy reserves, cellular damage, and mitochondrial efficiency.

According to Wang *et al*.(2021c) study, responses of clams (*C.fluminea*) to low-concentration cadmium Cd stress, low Cd concentrations had an impact on the clam's physiological traits, tissue health, and antioxidant system. In another similar study Pereto *et al*.

(2020) Possibility of using *C. fluminea* as a sentinel species in monitoring gadolinium (Gd) contamination of medicinal origin.

1-4-2 *Pseudodontopsis euphraticus*

P. euphraticus, a freshwater bivalve belonging to the order Unionida, has important ecosystem functions and services (Zieritz *et al*., 2020; Lopes-Lima *et al*., 2021).

P. euphraticus used biomarkers to detect water pollution due to its abundance, capacity for bioaccumulation and bioconcentration of toxins, high sensitivity to environmental changes, and rapid response time, for instance. Salman (2011) employed the Clam *P. euphrates* as a bioaccumulation indicator organism of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) in the Euphrates River/Iraq.

Alkharasani (2022) investigated the morphological, taxonomic, and molecular studies of several bivalvia species, including *P. euphraticus*, *Unio tigridis,* and *C. fluminalis*, based on the number of genera and species documented, the Unionidae family was the most common in the surveyed locations n the Euphrates River/Iraq.

1-5 Global and local studies:

Numerous studies have addressed issues of water pollution with antibiotics, for example, in a study carried out in Tehran (Iran) to look into the antibiotic contamination of drinking water close to animal farms, high levels of tetracycline were discovered in drinking water, and residues of the drug were also discovered in municipal water treatment facilities, ranging from 280 to 540 ng/L (Javid *et al*., 2016).

According to Faleye *et al*. (2018), in their study of antibiotic pollution in different African countries' aquatic environments, it was found that the antibiotic Sulfamethoxazole is most commonly found in surface waters.

According to a study conducted in China (Macao and Guangzhou) and Spain (Madrid), drinking water contains traces of the antibiotics erythromycin, macrolide, clarithromycin, ciprofloxacin, norfloxacin, enrofloxacin, and lomefloxacin (Akhter *et al*., 2023). In Vietnam, antibiotics were found in the aquatic environment, and the study concluded that this is a serious problem since it encourages the formation of bacteria that are resistant to antibiotics(Binh *et al*., 2018).

Bilal *et al.* (2020) investigated the effects of antibiotics in the aquatic environment including their stability, resistance to decomposition, and negative effects on the aquatic environment and biological species where they found that water pollution with antibiotics is currently one of the most dangerous specie of pollution due to its high environmental stability and resistance to biodegradation.

In southern China, thirty different antibiotics at levels as high as 226.8 x 498.1 ng/L were found in drinking water, the antibiotics most commonly found in the water were tetracyclines and quinolones (Liu ,2021).

Many researchers have investigated the presence of pharmaceutical substances in aquatic organisms such as Cunha *et al*.(2017) who studied diclofenac pollution in the Portuguese seaside using blue mussels *((Mytilus galloprovincialis* and *Mytilus edulis)*.

Rodriguez *et al*. (2017) investigated the effects of acute and chronic oxytetracycline (OTC) exposure in trout *Oncorhynchus mykiss* where OTC is widely used in animal treatment and aquaculture around the

world and discovered that OTC is a toxic impact on aquatic organisms.Astudy by Ali *et al.*(2018) investigated the presence of Pharmaceuticals and personal care products PPCPs in aquatic organisms (macroalgae, barnacles, and fish) in the polluted coastal waters of the Saudi Red Sea.

Chiesa *et al*.(2018), study Occurrence of antibiotics in mussels and clams from different FAO zones(Food and Agricultural Organisation marine zones), found evidence of antibiotic water contamination, the study's findings showed that clam tissues had higher-than-acceptable levels of the antibiotic oxytetracycline.

In China, a study by Xie *et al*. (2019) on the distribution of pharmaceuticals and personal care products in the Pearl River Delta regions revealed the presence of trimethoprim, norfloxacin, ofloxacin, and spectinomycin in all environmental matrices and found PPCPs in mussels such as *Mytilus edulis* and *Ostrea gigas* at significantly higher levels than in other species. higher than those in other species.

In Malaysia, Omar *et al*. (2019) investigated the presence of pharmaceuticals(amoxicillin,primidone,dexamethasone,sulfamethoxazol, and diclofenac) in fish and snails in the Klang River estuary, and Progesterones were also found with high levels in fish and mollusk samples, and diclofenac was the most common contaminant.

Kondera *et al*.(2020) researched the Impact of antibiotics oxytetracycline and Gentamicin on *Cyprinus carpio* fish and the results revealed that gentamicin doses increased phagocyte oxidative metabolism, primary hematopoietic cell proliferation, and apoptotic rates.

In Hong Kong, Ruan *et al*. (2020) study the bioaccumulation and spatial distribution of four pharmaceuticals, including (atenolol,

metoprolol, venlafaxine, and chloramphenicol) in a subtropical marine food web, results revealed that mean concentrations of individual pharmaceuticals ranged from 0.03 to 5.88 ng/g wet weight, with invertebrates generally having higher concentrations than fish.

Many researchers have studied environmental toxicity and its effects such as Mezzelani *et al.* (2016) who investigated the ecotoxicity impact and bioaccumulation of non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, acetaminophen, diclofenac ketoprofen, and nimesulide in mussels *Mytilus galloprovincialis* in the Adriatic Sea.

According to the findings of a study conducted by Bojarski *et al*. (2020), antibiotics, even at low environmental concentrations are directly toxic to fish and may cause physiological disorders like oxidative stress, general stress response, histopathological lesions, hematological, metabolic, and reproductive disorders.

In a study by Li *et al*. (2021) on the ecotoxicological effects of the pharmaceutical preparations sulfadiazine (SDZ) and sulfacetamide on zebrafish embryos, the results showed that SDZ exposure had a slight effect on an organism while it had a strong toxic effect when these two substances were mixed as it caused a disturbance in the development of embryos and led to the change of TNF-, and IL-1 genes.

Yuan *et al*. (2022) studied the ecotoxicity of diclofenac on the clam *C.fluminea* and observed that it increased oxidative stress, and MDA levels, induced apoptosis, and produced transcriptional epigenetic changes.

Mezzelani *et al*. (2023) studied on the effect of pharmacological mixtures of carbamazepine and valsartan on the clams (*Mytilus galloprovincialis*), and showed functional alteration in

neurotransmission, cell cycle, gene expression, and immune response stimulation in the clams.

Several studies have indicated the environmental toxicity of amoxicillin according to a study by Umamaheswari *et al*. (2019), prolonged amoxicillin exposure can produce considerable alterations in the hematological, ionic levels, biochemical, and enzymatic parameters of *Labeo rohita*.

Matozzo *et al*. (2016 b) investigated the effect of the antibiotic amoxicillin on the parameters of blood cells in two bivalve species (*Ruditapes philippinarum* and *Mytilus galloprovincialis*). The results showed that AMX had a minor effect on the parameters of blood cells in bivalves.

Another study evaluated by Matozzo *et al* . (2016a) effects of amoxicillin on antioxidant enzyme activities, lipid peroxidation, and protein carbon content in clams and mussels, and the results showed that AMX had a slight effect on mollusks biomarker responses.

Several studies have indicated the environmental toxicity of potassium dichromates, such as a study by De Freitas *et al.* (2014) on evolutionary, acute toxicity, and mutagenicity resulting from exposure to potassium dichromate ($K_2Cr_2O_7$) in the freshwater snail *Biomphalaria glabrata.* Another study examined the applicability of biomarkers of oxidative stress in assessing chromium-induced toxicity in *Labeo rohita* fish (Kumari *et al*., 2014).

Laxmi and Kaushik (2020) studied the effects of hexavalent chromium toxicity on the environment, health risks, bioremediation potential, and the use of an approach biological as an environmentally

friendly alternative to treatment and removal of chromium from wastewater before disposal in the environment.

In Iraq, a study by Mahmood *et al*. (2019) examined the presence of antibiotics in drinking water treatment facilities in the city of Baghdad and concluded that these facilities had relatively high antibiotic concentrations. Ciprofloxacin had the highest concentration (1.270 g/L), followed by levofloxacin (0.177 g/L) and amoxicillin (1.50 g/L).

Ahmed (2022) studied the presence of antibiotic residues such as levofloxacin, ciprofloxacin, and amoxicillin in water, sediment, and *Oreochromis niloticus* and *Planiliza Abuand* fish, as well as the effects of some environmental factors on those antibiotics and their effects on fish.

2- Material &Methods

2-1 Description of the Sampling Area

The samples were collected from a site on Euphrates River/Al Hindiya District, one of the branches of the Euphrates(32°32'29.9"N 44°13'38.7" E), which is about 20 km east of Karbala city and approximately the same distance west of Hilla city where the water level in it is always low, and the flow rate is slow. This site is surrounded by large residential, markets, and farmland , figure (2-1)

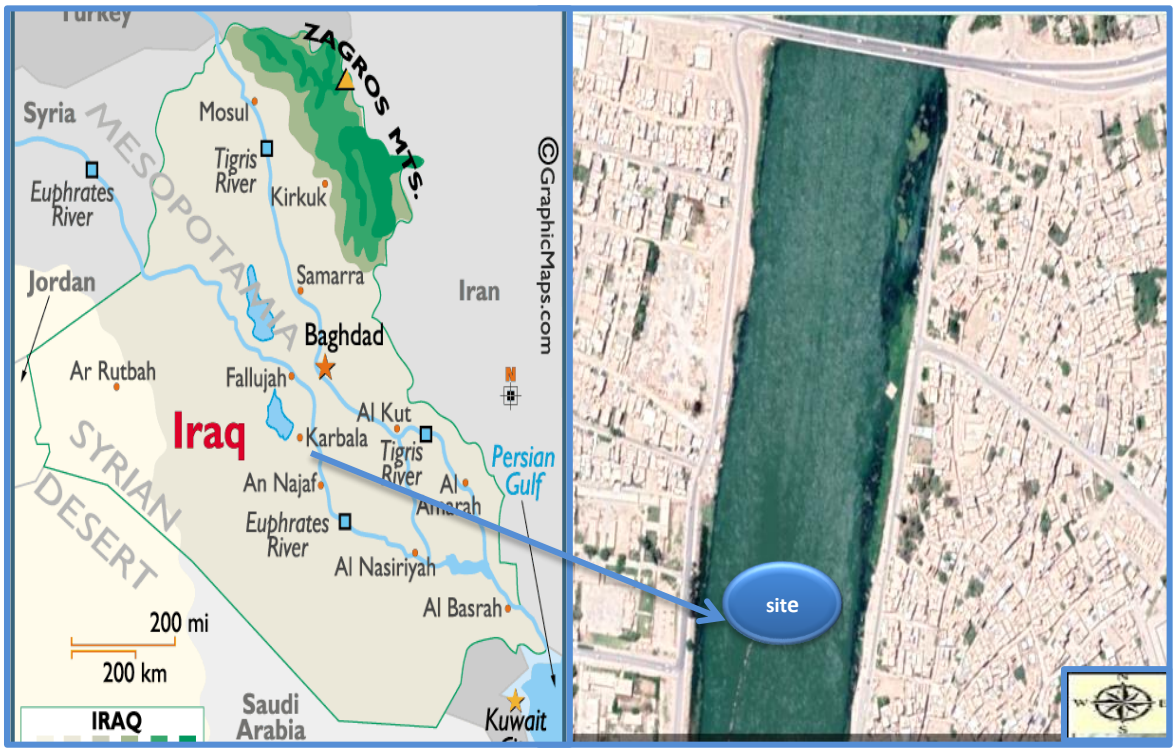

Figure (2-1): Satellite Image of sampling sites(Euphrates River).

2-2-1 The Equipment and Apparatus :

Table (2-1): List of Instruments used in this study

No.	Instruments	Brand	Country
1	Cooling Centrifuge	Hettich	Germany
2	Autoclave	OSAW	India
3	Sensitive High Precision Balance	Denver	Germany
4	UV-Spectrophotometer	EMCLab	Germany
5	Water bath	JuLab SW23	Germany
6	Water Distiller	LabTech	South Korea
7	pH –meter (multi-parameters),	Oakton	U.S.A
8	Incubator	Jiabtech	Germany
9	Olympus Light microscope	Novel	Germany
10	Aquarium air pump	Sebo aquarium	India
11	Anatomical microscope	Hettich	Germany
12	Micropipette(100-1000µl)	Uchen	chain
13	Mercury Thermometer	Uchen	chain
14	Hot plate	Supertek	India
15	Do meter	Milwaukee	chain
16	Multi-Parameters	Oakton	U.S.A
17	digital camera	canon	chain
18	Microtome	Minux® S700	chain

2-2-2 Samples Collection

Water and clams samples were collected from the sites in peried (December 2022- March 2023),using polyethylene containers (5 liters) for physical and chemical analyses, 250 ml glass bottles were used for calculating dissolved oxygen (DO) and Biological oxygen demand (BOD_5) , and bivalve samples were gathered using plastic bags with added little river water . The clam species selected for the current experiment were diagnosed by the Center for Marine Sciences at the University of Basra.

2-3-1 Some Physical and Chemical Parameters of River Water

The water temperature was measured directly at the sampling sites using a graduated mercury thermometer (0°C -100 °C), dissolved oxygen (DO) (mg/L) in water was measured using a DO meter while Biological oxygen demand BOD_5 was calculated after incubation at 20 C˚ for 5 days (APHA,2012). pH , Electrical conductivity (EC) (µS /cm), Salinity (ppt) and total dissolved solids (TDS) (mg/L) were measured pH–meter (multi-parameters), Oakton - U.S.A acalibratinthee the advice.

2-4 Mollusca species used in the experiment

2-4-1 *Pseudodontopsis euphraticus* (Bourguigrat, 1852)

The shape of the organism is relatively large and rhombic, with a length of 7.7 cm, a width of 4.4 cm, and a height of 5.8 cm. The two sides of the shell are equal in size and similar in shape; the apex is flat, the center is concentric, and the lines of the apex are single and densely brown. The shell is characterized by growth lines. Clear and rough The inner side of the shell is light green. The anterior edge is rounded, with pronounced medial convexity. The posterior edge is thin and rounded with a cut edge (Bogan and Alderman, 2008), figure (2-4).

Kingdom: Animalia

Phylum: Mollusca

Class : Bivalvia

Order: Unionoida

Family: Unionidae

Genus : *Pseudodontopsis*

Species: *Pseudodontopsis euphraticus* (bourguigant,1852**)**

2-4-2 Description of *Corbicula fluminea* (O. F. Müller, 1774*)*.

The organism is small in size, semi-triangular to circular, length 2.2 cm, width 3.2 cm, height 1.8 cm, apex protruding upwards, medial location, concentric lines, right and left double bivalves, equal in size and similar in shape, dorsal edge convex upward, ventral only rounded, edge the front and back are rounded (Bogan and Alderman,2008), figure (2-4).

Kindom: Animalia

Phylum: Mollusca

Class : Bivalvia

Order: Venerida

Family: Cyrenidae

Genus: *Corbicula*

Species: *Corbicula fluminea* **(**O. F. Müller, 1774)

Figure (2-2): *1- Pseudodontopsis euphraticus* *2- Corbicula fluminea*

2-5-1 Algae and Culture Conditions

2-5-1-1 Algal Strain

The microalgae strains used in this study were *C. vulgaris* related to Green algae and most commonly used for wastewater treatment which have high growth rates and can grow under a wide range of conditions . These microalgae strains were obtained from the Environmental Research and Studies Center, University of Babylon, Iraq.

Division: Chlorophyta

Class: Trebouxiophyceae

Order: Chlorellales

Family: Chlorellaceae

Genus: *Chlorella*

Species: *Chlorella vulgaris* (Beijerinck ,1890)

2-5-1-2 Media Preparation and Sterilization

Chu-10 modified for algal growth was used the stocks were prepared for all macro and microelements, which are clearly in the table (2-2), 2.5 ml was taken from each stock solution and completed up to one liter of distilled water, then sterilized with an autoclave, except stock solution dipotassium hydrogen phosphate (K_2HPO_4), which was added at the end after sterilization to get one liter of Chu-10. The pH of the Chu-10 was set to 6.4 using (0.01N) of sodium hydroxide or hydrochloric acid, leaving the medium until the next day to use it in the growth of algal (Kassim *et al*., 1999)**.**

Table (2-2): The modified Chu-10 medium's components

The chemical formula of each salt		Con. g/L
1	$MgSO_4$	10
2	$K2HPO_4$	4
3	$NaNO_3$ $CaCl_2$	8 16
4	$FeCl_3$	0.32
5	EDTA-Na	4
6	NaCl	30
7	Na_2CO_3	8

8	Trace metal mix	g\L
1	H_3BO_3	0.288
2	$MnCl_2.4H_2O$	0.02
3	$ZnSO_4.7H_2O$	0.224
4	(NH_4) $Mo_7O_{24}.4H_2O$	0.028
5	$CuSO_4.5H_2O$	0.08
6	Co $Cl_2.6H_2O$	0.004
7	Distilled water	1 L

2-5-1-3 Immobilization of Algae

10 ml of cultured algae were placed in a flask with 100 ml of Chu-10 medium, and it was grown for 14 days under controlled conditions of 286 µE/m²/s, light/dark period of 16:8 hours, and temperature 25±2°C (Chia *et al.*, 2013). then 100 ml of cultured algae were placed in a flask with 1000 ml of Chu-10 medium and incubated for 14 days (Tredici, 2004). each 50 ml of algae sample which was taken in the stationary phase at 12 and 14 days was condensed by centrifuging for 15 minutes at 3000 rpm to prepare the immobilized algae as the following steps:

1- The concentrated algae were taken and added an equal volume of sodium alginate solution (2%) was shaken well to homogenize the components .

2- Then this mixture (algae and alginate) is placed in a medical syringe or a separating funnel. The mixture is dripped drop by drop into a beaker

containing a 3% solution of calcium chloride with constant stirring. It is noted that the drops coming down from the syringe harden in a solution of calcium chloride, and then we use a tea strainer to remove the beads (immobilized organisms)from the calcium chloride solution and then it is kept in distilled water in a cool place. (Adlercreutz and Mattiasson,1982; Al Mosawi *et al*.,2022) , figure 2-3.

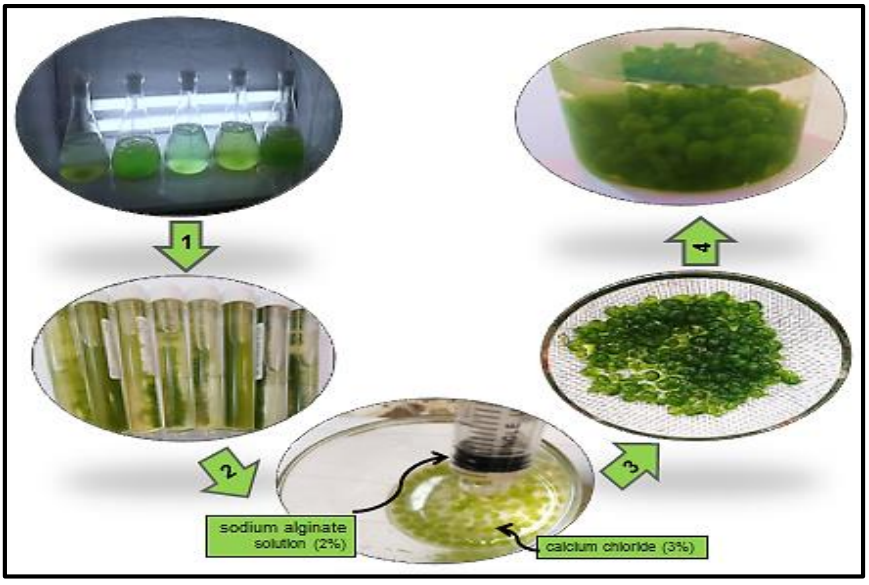

Figure (2-3): Staps of immobilized green algae *(Chlorella vulgaris)*

2-6 Experimental Design

The experiment started with a stage of acclimatization where clams were put in dechlorinated water in plastic tanks 3 for 5 days for purification under conditions controlled (Matozzo *et al*., 2016 a). Then the exposure stage, where clams were exposed to two kinds of medical materials (the antibiotic amoxicillin for 7 days at concentrations of 100,200 and 300 mg/l) figure 2-4 and $K_2Cr_2O_7$ for 5 days at concentrations of 20, 30 and 50 mg/l) (De Freitas *et al*., 2014). Haemolymph tissue was extracted (0.1 mg), and frozen for use in biochemical analysis (Ali *et al*,2015), the immobilization algae (*C.vulgaris*)was added at a rate of 4-5 beads per L of treatment aquaria(Al-Asady,

2014; Porkka, 2021).The ideal bead size is between 3.5 and 4.7 mm (Lee *et al*.,2020).

2-6-1 Determination of the lethal concentration LC50 in bivalve.

The determination of lethal concentrations (LC50) within 96 hours for two species of clams (*P. euphraticus* and *C, fluminea*), were done by exposing a clam to three different concentrations (100, 200, and 300 mg/L) of AMX and five different concentrations (20, 30, 50, 100, and 150 mg/L) of $K_2Cr_2O_7$ mortality is recorded at 24, 48, 72, and 96 hours after exposure. Dead clamss are removed from the experimental contener immediately to avoid organic decomposition and oxygen depletion (Dhara *et al*., 2021; Dhara *et al*., 2022a ; Saha and Saha, 2021; Wu *et al*., 2022b), The number of dead clams in each concentration was recorded and the LC50 were calculated by Probit analysis based on Finney (Finney, 1971) was used**.**

During the bioassay, the behavioral changes of the tested clams exposed to different concentrations of the toxin, such as footwork, clumping tendency, and mucus secretion, were recording to eye observation (Dhara *et al*., 2017; Dhara *et al*., 2022b).

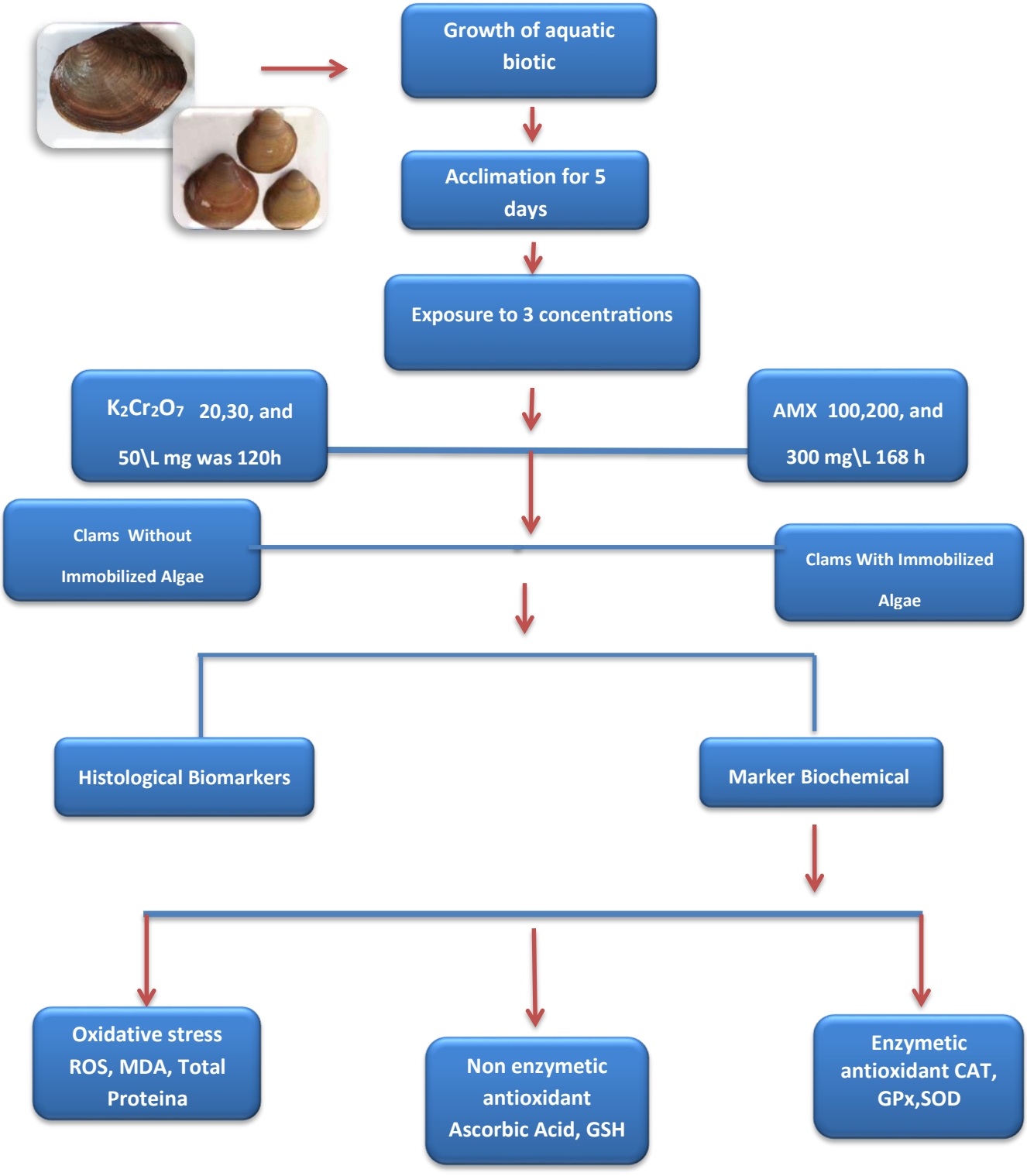

Figure (2-4): A diagram showed the steps and processes used in the experiments

2-7 Biomarkers Determination

2-7-1 Reactive Oxygen Species (ROS)

Reactive Oxygen Species (ROS) were evaluated according to the FOX2 method. The FOX2 test system is based on the oxidation of ferrous ion–*o*dianisidine complex to ferric ion by the various species of oxidants contained in the plasma samples. The Fe(III) ion produced is bound by xylenol orange, forming a complex with an absorption peak at 560 nm **(**Erel,2005).

Reagents preparation

The FOX2 assay works with two reagents that are stable for at least 6 months at 4ºC:

1- reagent 1; contained 150 µM xylenol orange, 140 mM NaCl, and 1.35 M glycerol in 25 mM H_2SO_4.

2-reagent 2; contained 5 mM ferrous ammonium sulfate and 10 mM *o*-dianisidine dihydrochloride in 25 mM H_2SO_4.

Producer

1- 140 µl of the sample was added to 900 µl of reagent 1 and 44 µl of reagent 2.

2- The samples were vortexed and incubated at room temperature for 30 min.

3- Following incubation, absorbance was measured at 560 nm using a Jasco V-550 UV-vis spectrophotometer.

2-7-2 Antioxidant Enzymes

2-7-2-1 Catalase activity assay

This examination was done according to, the technique described by (Goth, 1991).

Producer

1- (0.2 ml) of the sample was incubated in 1 ml of the substrate (65 µmoL per ml H_2O_2 in 60 mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 1 min.

2- The enzyme activity was suspended by adding 1 ml of 32.4 mM ammonium molybdate.

3-The yellow absorption value of the molybdate complex and hydrogen peroxide was measured at 405 nm using a spectrophotometer .

Catalase enzyme activity was calculated according to the following equation:

Catalase activity (KU/l) =(S-B1/B2-B3) * 271

S: Sample reading .

B1: Blank 1 reading contained 1.0 ml substrate, 1.0 mL molybdate, and 0.2 mL sample .

B2: Blank 2 reading contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer, pH 7.4 .

B3: Blank 3 reading contained 1.0 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4, 1.0 mL molybdate, and 0.2 ml of 60 mmol/L sodium-potassium phosphate buffer pH 7.4.

2-7-2-2 Superoxide Dismutase (SOD)

This examination was done according to (Marklund and Marklund,1974).

Principle

(Cu-Zn) SOD activity was determined by using a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA at pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bullet -}$ and the dismutation of this radical by SOD. (Cu-Zn) SOD activities are expressed as units/ml. One unit of (Cu-Zn) SOD activity is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation.

Reagents Preparation

1. Tris- EDTA buffer pH 8.2

A weight of 2.85 g of Tris and 1.11 g of EDTA-Na_2 were dissolved in 1 liter of DW.

2. Pyrogallol Solution (0.2 mM)

A weight of 0.252 g of pyrogallol was dissolved in a solution of 0.6 ml of concentrated hydrochloric acid diluted in 1 liter of DW.

Procedure

Spectrophotometer was adjusted to read zero using a Tris-EDTA buffer. Control and sample test tubes were prepared and then pipetted into test tubes.

Reagents	Test (µl)	Control (µl)
Extract	50	-
Tris buffer	1000	1000
DW	-	50
Pyrogallol	1000	1000

Absorption was read at the wavelength of 420 nm against Tris-EDTA buffer at zero time and after 1 minute of the addition of pyrogallol.

Calculation of SOD Activity

$$\% \text{ Inhibition of pyrogallol autoxidation} = \frac{\Delta A_{test}}{\Delta A_{control}} X \ 100\%$$

$$\text{(Cu-Zn) SOD Activity (U/ml)} = \frac{\% \text{ inhibition of pyrogallol autoxidation}}{50\%}$$

2-7-2-3 Glutathione Peroxidase (GPx)

Glutathione peroxidase activity was determined according to the method of (Hafemann *et al.*,1974).

Principle

The activity of GPx was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3.

$H_2O_2 + 2\ GSH \longrightarrow 2H_2O + 2\ GSSG$

Procedure

1- 0.1 ml of extract was incubated with 0.1 ml of 5mM GSH, 0.1 ml of 1. 25 mM H_2O_2, 0.1ml of 25 mM NaN_3, and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37oC for 10 min.

2-The reaction was stopped by adding 2 ml of 1.65 % HPO_3.

3-The reaction mixture was centrifuged at 1500 rpm for 10 min.

4-2 ml of the supernatant was mixed with 2 ml 0.4 M Na_2HPO_4 and 1 ml of 1mM DTNB.

5-The absorbance of the yellow-colored complex was measured at 412 nm after incubation for 10 min at 37oC against distilled water. A sample without the tissue homogenate processed in the same way was kept as a nonenzymatic reaction.

Calculation:

$$\text{The residue reduced GSH in test tube} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD$$

$$\text{Se - GPX activity (µmol of GSH utilized/m in)} = \frac{\text{Conc. of GSH in STD - Conc. of GSH in test}}{\text{time(3min)}} * D.F.$$

2-7-3 Nonenzymatic Antioxidant

2-7-3-1 Reduced Glutathione (GSH)

Reduced glutathione in the tissue was determined according to the method of (Moron *et al*.,1979).

Principle

With dithionitrobenzene (DTNB), the acid-soluble sulfhydryl groups (non-protein thiols, of which more than 93% is reduced glutathione) form a yellow-colored complex. At a wavelength of 412 nm, the colored complex's absorbance was measured.

Procedure

1- 1000 µl of 25 % TCA mixed with 100 mg of tissue was and kept on ice for a few minutes.

2- This mixture was then centrifuged at 3000 g for a few minutes to settle the precipitate.

3- 300 µl of supernatant, 700 µl of 0.2 M sodium phosphate buffer, pH 8, and 2 ml of DTNB 0.6 mM (made in 0.2 M buffer, pH 8), were mixed.

4- After 10 minutes, the yellow hue was measured at 412 nm against a blank that had the supernatant replaced with 0.1 ml of 5% TCA .

2-7-3-2 Total Vitamin C (Ascorbic Acid)

Total Vitamin C (Ascorbic Acid) was determined by McCormick and Greene (1999).

Principle

In the 2,4-dinitrophenylhydrazine (DNPH) methods, AA is oxidized by Cu^{+2} to DHA and diketogulonic acid.11 When treated with DNPH, the 2,4-dehydrophenylosazon product forms, which, in the presence of sulfuric acid, forms an orange-red complex that absorbs at 520 nm .

Preparation of Reagents

1- Metaphosphoric acid (m-HPO_3) (0.75M (30gm of m-HPO_3 are dissolved in a final volume of 500 ml of DDW. (Stable for 1 week).

2-Sulfuric acid H_2SO_4 (4.5M) Carefully 250 ml of concentrated H_2SO_4 are added to 500 ml of cold DDW. When the solution has cooled to room temperature, DDW is added to 1 liter, with mixing. (Stable for 2 years).

3 -Sulfuric acid H_2SO_4 (12M) Carefully 650 ml of concentrated $H_2SO_4$are added to 300 ml of cold DDW and brings to a final volume of 1 liter (Stable for 2 years).

4- DNPH reagent (0.01M)10 gm of 2,4-DNPH are dissolved in 400 ml of 4.5M H_2SO_4 and brought to a final volume of 500 ml with 4.5M H_2SO_4, then refrigerated overnight and filtered. (Stable for at least 1 week at refrigerated temperature).

5- Thiourea (0.66M) 5 gm of thiourea are dissolved in a final volume of 100 ml of DDW.

6-Copper sulfate (0.027M) 0.6 gm of anhydrous copper sulfate is dissolved in a final volume of 100 ml of DDW. (Stable for 1 year at room temperature).

7- DTCS reagent 5 ml of the thiourea, 5 ml of the copper sulfate, and 100 ml of the 2,4-DNPH reagent are combined. (Store in a bottle at 4C° for a maximum of 1 week).

8- Ascorbic acid standards

Stock standard solution (2.8 mM) is prepared by dissolving 50 mg of ascorbic acid in a final volume of 100 ml of m-HPO_3. Dilutions are made in m-HPO_3 to 2.5, 5, and 20 mg/L (0.014, 0.028, and 0.11 mM) respectively. There are the working standards (All working standards should be prepared daily).

Procedure

The procedure for the determination of total vitamin C in tissue by the 2,4-DNPH method is summarized as follows :

1-Duplicates of each standard and sample test tube are prepared, then pipetted into test tubes. Tubes are mixed in a vortex mixture, then centrifuged at 2500 x g for 10 minutes

2-Tubes are capped and mixed in vortex mixture, then incubated in a water bath at 37°C for 3 hours. The tubes are removed from the water bath and chilled for 10 minutes in an ice bath, slowly mixed.

3-Tubes are mixed in vortex mixture and returned immediately to the ice bath. The spectrophotometer is adjusted with blank to read zero absorbance (A) at 520 nm, and the absorbance of standards and sample is read.

Calculation of Serum Total Vitamin C

The concentration of the samples is obtained from the calibration curve (Figure 2-2) and is multiplied by 5 (to correct for dilution of the serum by m- HPO_3) to give the concentration of vitamin C (ascorbic acid) per liter of serum .

The concentration of ascorbic acid can be determined directly from a standard as follows:

$$\frac{A\ sample}{C\ sample} = \frac{Astd}{C\ std}$$

$$C\ sample = \frac{C\ std * A\ sample}{A\ std} * 5$$

where :

C = concentration (mg/L) or (μM) of AA in sample and standard .

A = absorbance at 520 nm for sample and standard .

5 = factor is added to correct for the dilution of the sample.

2-7-4 Malondialdehyde (MDA)

Malondialdehyhe was determined using the Thiobarbituric acid (TBA) test technique of (Buege and Aust, 1978), on the spectrophotometer.

Principle:

This technique measures the aldehyde breakdown products of lipid peroxidation to quantify lipid peroxides. The basic working concept of the technique is the formation of a red MDA-TBA complex, which can be measured at 535 nm, by the interaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid.

Stock TCA – TBA – HCl Reagent:

Create 100 ml (2.1 ml of concentrated HCl in 100 ml), it was made by dissolving 15% W/V trichloroacetic acid, 0.375% W/V thiobarbituric acid, and 0.25 N HCl. To help TBA dissolve, this solution was slightly heated. Dissolved 15 gm TCA and 0.375 mg of thiobarbituric acid in 0.25 N HCl, and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

1-0.6 ml of TCA-TBA-HCl reagents were added to 0.4 ml of extract.

2- It was well blended, and placed in a boiling water bath for 10 minutes.

3- 1.0 ml of 1N NaOH solution was added after cooling.

This pink absorbance was measured at 535 nm against a blank that included distilled water instead of serum. 0.4 mL of distilled water and 0.6 mL of TCA-TBA-HCl reagent were combined and heated in a blank. Blanks were always filled.

Calculation:

$$\text{Malondialdehyde}(\mu\text{mol/l}) = \frac{\text{Absorbance of sample}}{E_o \times L} \times D$$

Where:

E_o = Extinction coefficient 1.56×10^5 M^{-1} cm^{-1}

L= light path cm.

D = dilution factor = 6.7×10^6

2-7-5 Total Protein

Protein in aquatic organisms under study was estimated by Biuret Method according to (Nowotny and Nowotny, 1979).

Principle:

In an alkaline media, the -CO-NH- bond (peptide) in a polypeptide chain interacts with copper sulfate to produce a purple color that can be detected at 540 nm.

Reagents Required:

1. Biuret Reagent: Dissolve 3 g of copper sulfate ($CuSO_4.5H_2O$) and 9 g of sodium potassium tartrate in 500 ml of 0.2 mol/liter sodium hydroxide; add 5 g of potassium iodide and makeup to 1 liter with 0.2 mol/liter sodium hydroxide.

2. Protein Standard: 5 mg BSA/ml.

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard into the series of labeled test tubes.

2. Pipette out 1 ml of the given sample in another test tube.

3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.

4. Now add 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank and unknown'.

5. Mix the contents of the tubes by vortexing / shaking the tubes and warm them at 37 ºC for 10 min.

6. Now cool the contents to room temperature and record the absorbance at 540 nm against blank.

7. Then plot the standard curve by taking the concentration of protein along X-axis and absorbance at 540 nm along Y-axis.

8. Then from this standard curve calculate the concentration of protein in the given sample.

2-8 Histological Biomarkers

To make histological sections, digestive glands and gills were collected from molluscs after the experiment and preserved in formaldehyde (10%) (Parolini *et al* .,2020). dehydrated using a graded ethanol series (70–100%), and paraffin embedded; 5–8 μm thicknesses were taken using a Leitz 1512 microtome and stained with hematoxylin and eosin (H&E). An Olympus Light microscope (Leica DMi8) provided with a digital camera (Wilson andGamble, 2002; Howard ,2004; Cid *et al.*,2015).

2-9 Statistical Analysis

The current experiment was designed as a factorial experiment it ,included 2 pharmaceutical substances and several concentrations of each substance, 3 and 5 concentrations for each of (AMX and $K_2Cr_2O_7$), respectively, and two types of bivalve *(P. euphraticus and C. fluminea)* .The Statistical Package for SPSS programs version 23.0 was used to analyze the data using variance test (ANOVA) and least significant differences (LSD $\leq$ 0.05), Also Canoco analysis (Canonical correspondence analysis) was usedwith original Version 4.0.

3- Results and Discussion

3-1 The physicochemical Parameters

A difference in environmental parameters was recorded during this study collected area sample, with air temperature values ranging between 26.20 ℃ to 33.40 ℃) and water temperature values ranging between(12.40 ℃ to 22.50 ℃). Throughout the study period (December 2022- March 2023), pH values remained stable and ranged between (7.30-8.34). These results indicated the presence of significant differences($P< 0.05$), Electrical conductivity values ranged from (1058.00 to 1130.00) µs/cm, while salinity had a limited range (0.55-0.73 ppt). Oxygen concentration ranged from (6.90 to 7.80) mg/l, while the Biological Oxygen Demand (BOD_5) ranged from (1.71 to 3.35) mg/l, and the Total Dissolved Solids ranged from (430.30 to 694) mg/l , as shown in table 3-1.

Table 3-1. Some physicochemical parameters of water in collected area

Parameters	Parameters (Min.-Max. Mean±S.D).
Air Temp. (℃)	26.2-33.4 29.36± 3.67
Water Temp.(℃)	12.4 -22.5 16.466 ± 5.32
pH	7.3-8.34 7.800 ± 0.50
E.C (µs/cm)	1058- 1130 1.101 ± 38.17
Salinity (ppt)	0.55 -0.73 0.64 ± 0.090
D.O (mg/l)	6.9 - 7.80 6.35 ± 0.450
BOD_5(mg/l)	1.71 -3.35 2.65 ± 0.85
T.D.S (mg/l)	430.3-694 2.65 ± 0.85

3-2 Water Some Parameters During Experiments

Throughout the experiment, water quality was monitored. Dissolved oxygen (DO), temperature, pH, table (3- 2) shows the water quality parameters that were measured after 24 hours of AMX and $K_2Cr_2O_7$ exposure. Although temperature and pH are important factors in toxicity performance (Mayer *et al.*,1994), water quality parameters in this study remained within allowable levels, and only toxic effects of the AMX and $K_2Cr_2O_7$ were observed.

Table 3-2. Water's physical and chemical characteristics during the experiment

	Conc. (mg/L)	**Tem. (◦C)**	**DO (mg O_2/L)**	**pH**
AMX without Immobilization (*C.vulgaris*) in both clams	100	21.10± 0.43	6.12±0.05	7.0±0.34
	200	21.87±0.53	6.14±0.13	7.3±0.22
	300	21.20±1.20	6.34± 1.23	6.98±0.21
AMX with Immobilization (*C.vulgaris*) in both clams	100	21.23±0.59	6.22±0.01	7.2± 0.31
	`200	21.20± 0.43	6.7± 0.04	7.1±0.17
	300	21.22±0.32	6.25± 1.6	7.5±0.23
$K_2Cr_2O_7$ without Immobilization (*C.vulgaris*) in both clams	20	22.12±0.45	5.56±0.08	6.5±0.03
	30	22.15±0.12	5.24± 0.2	6.3±0.11
	50	22.13±0.65	5.13±0.43	6.1±0.44
$K_2Cr_2O_7$ with Immobilization (*C.vulgaris*) in both clams	20	22.13±0.03	5.06±0.04	6.4±0.45
	30	22.15±0.12	5.87±0.76	6.3±0.32
	50	22.14±0.14	5.23± 0.23	6.0±0.24

3-3 Lethal concentration (LC50)

The most frequent toxicity test is the 96-hour LC50, which assesses the level of a toxicant that results in the death of 50% of the experimental organisms after 96 hours of exposure (Cheng *et al.*,2020). Mortality endpoints in the acute toxicity test on bivalves included immobility, secretion of milky white matter, open shells, feet that extended significantly beyond the shell, and floating on the surface of the solution (Brahma, 2022).

3-3-1 Determination of the LC50 of *P. euphraticus* after Exposure to AMX.

The lethal concentration (LC50) in the experiment was calculated *for P. euphraticus* clams during the 96-hour exposure period. Table (3-3) displays the percentage of test *P. euphraticus* clam mortality at various AMX concentrations. According to log Probit analysis (Finney, 1971), the value of LC_{50} after 96 hours of exposure to AMX was 410.55 mg\l.

Table 3-3. Lethal concentration (LC50) for *P. euphraticus* after 96-h exposure to the AMX

Exposure Conc. (mg/L)	No. of Mortality *P. euphraticus*	% Mortality	LC50 Probit analysis
0	0	0	410.55 mg/l
100	2	11%	
200	5	28%	
300	7	39%	

3-3-2 Determination of the LC50 of *C. fluminea* Exposure to AMX

the lethal concentration (LC50) for C. fluminea clams was determined throughout a 96-hour exposure period. Table (3-4) shows the percentage of test *C. fluminea* mortality at various AMX concentrations. The control level (0 mg/L) of the experiment showed no mortality, and according to Probit analysis(Finney, 1971), which found that the LC50 value was 441.15 mg\l.

Table 3-4. Lethal concentration (LC50) for *C. fluminea* after 96-h exposure to the AMX

Exposure Conc. (mg/L)	No. of Mortality *P. euphraticus*	% Mortality	LC50 Probit analysis
0	0	0%	441. 15 mg\/l
100	2	11%	
200	4	22%	
300	7	39%	

3-3-3 Determination of the LC50 of *P. euphraticus* after exposure to potassium dichromate

Table (3-5) shows the mortality percentage of clam *P. euphraticus* test clams at various $K_2Cr_2O_7$ concentrations. The concentration test revealed no mortality in clams at the control level (0 mg/L) through the exposure period, and the LC50 value for $K_2Cr_2O_7$ according to Probit analysis(Finney, 1971) was 73.69mg\l, which resulted in 50% mortality of the clams after 96h.

Table 3-5. Lethal concentration (LC50) for *P. euphraticus* after 96 h exposure to the $K_2Cr_2O_7$

exposure conc. (mg/l)	No. of mortality *p. euphraticus*	% mortality	lc_{50} probit analysis
0	0	0	73.69 mg/l
20	4	22%	
30	6	33%	
50	8	44%	
100	10	55.55%	
150	17	94%	

3-3-4 Determination of the LC50 of *C. fluminea* after exposure to Potassium dichromate.

Table (3-6) shows the mortality percentage of clam *C. fluminea* test clams at various $K_2Cr_2O_7$ concentration. The concentration test revealed no mortality in clams at the control level (0 mg/L) through the exposure period, and the LC50 value for $K_2Cr_2O_7$ was 76.733 mg\l according to Probit analysis (Finney, 1971), which resulted in 50% mortality of the clams after 96h**.**

Table 3-6. Lethal concentration (LC50) for *C. fluminea* after 96 h exposure to the $K_2Cr_2O_7$

xposure Conc. (mg/L)	No. of Mortality *P. euphraticus*	% Mortality	LC50 Probit analysis
0	**0**	**0**	**76.733 mg/l**
20	**5**	**28%**	
30	**7**	**39%**	
50	**8**	**44%**	
100	**11**	**61%**	
150	**14**	**78%**	

The results of this study indicate that there is a positive relationship between increased concentrations ,mortality rate and that prolonged exposure to AMX and $K_2Cr_2O_7$ caused tissue damage, leading to the death of clams.

This is a result of consistent with Prato *et al*.,(2023), In a 96 h marine copepod *Tigriopus fulvus* exposure, to amoxicillin and carbamazepine, the highest mortality was 44% in *Nauplii* exposed to Carbamazepine CBZ (100 µg/L) and 22% in those exposed to MIX (100 µg/L). For AMX, no effect was demonstrated even at 100 mg/L. while A study by Mhadhbi *et al*. (2022), determined the LC50 of *Dicentrarchus labrax*, after 96 hours of azithromycin exposure, to be 31 mg/L.

According to the study, amoxicillin is the most dangerous antibiotic for fish, with an LC50 value of 35.72 g/L, followed by erythromycin and endosulfan at 89.32 and 242.7 g/L, respectively. Another study by Park and Choi (2008) revealed that amoxicillin is poisonous to the fish *Oryzias latipes*, with a 96-hour LC50 value of 1000 mg/L.

The current study found that the mortality rate increased with increasing exposure duration and chromium concentrations, the found positive link between clams mortality , higher potassium dichromate concentrations and exposure time is in keeping with linear correlations between toxicant concentration and percentage mortality ,Similar to a study by Sadeghi *et al*. (2014), in *Epinephelus stoliczkae*, the LC50 of potassium dichromate for concentrations (0, 62, 66, 70, 74, and 78 mg/L) was 73.09 mg/L after 96 hours. Ghosh and Saha (2022) determined that the median lethal concentrations (LC50-96h) of hexavalent chromium as potassium dichromate in *Oreochromis niloticus* were 93.49 mg/l using probit analysis. Hexavalent chromium has been identified as a serious aquatic toxicant.

Similar observations of LC50-96 h determination in many molluscs upon exposure to heavy metals have also been made by(Dara *et al*., 2022; Ramakritinan *et al*., 2012). pesticide exposure, as studied by Hanna and Sheikha (2023), was determined (LC50-96 h) for Chlorpyrifos on freshwater

bivalves (*Unio Tigridis)* at 157.99 ppm for concentrations (50, 100, 200, 300, and 400 ppm).

3-3-5 Behavioral Responses in Bivalve

Bivalves have significant advantages in controlling and minimizing the effects of toxicity, but mortality occurs under these conditions, when bivalves cannot completely isolate themselves from the effects of toxicity (Hemming and Waller, 2004). The procedure of(Brahma ,2022) was used to study behavioral changes, and the following metrics were used to represent behavioral alterations caused by toxicity: locomotor behavior (number of times of movement in the form of gliding), the tendency to clump during the observation period, duration of complete closure of valves without any foot extension and siphons together, and mucus secretion. Their duration and magnitude of behavioral responses, in addition to their delay in change, are associated with stress and drive ecosystem function (Woodin *et al*., 2020).

As a result of our study, behavioral changes were observed in both species of clams. When exposed to amoxicillin and potassium dichromate, the first and most important indicator of behavioral change was the sudden closing of the valve in the bivalves and the retraction of the body into the shell. Since mollusks are filter feeders, closing the valves and lid may be a strategy to prevent the entry of metals or toxins into the animal's body (Brahma, 2022).

In AMX, it was observed that the clams closed their valves tightly for longer periods and opened them slightly for a short period. And the expansion of the foot and the siphons from time to time, and its relative lack of movement in 96h, No mucus secretion was observed during the behavioral tests in the medium exposed to amoxicillin in both bivalves. Agree with the

study Brahma (2022), which examined behavioral changes in bivalves *Lamellidens corrianus* and *Parreysia gowhattensis* after copper exposure.

In potassium dichromate exposure, it was observed that visible signs of poisoning appeared periodically in bursts of irregular swimming, flattening, and swallowing of air, with excess mucus secretion and decreasing crawling behavior at 96 hours of exposure to higher concentrations. Excessive mucus production can lead to a considerable energy depletion of roughly 70% of the energy consumed by the organism when exposed to contaminants and may contribute to the progressive and final loss of movement and touch reported in affected creatures (Dhara *et al*., 2022).

One of the principal physiological responses of mollusks to chemical stimulation is mucus production, which functions to prevent tissue injury and fluid loss, it also serves to dilute toxins and avoid disastrous interactions between toxins and surface epithelial cells (El-Gendy *et al*., 2019). As a consequence, increased mucus production with increasing concentrations of potassium dichromate highlights responses to severe toxicity and potential damage to clams tissue.

3-4 The Effect of AMX and $K_2Cr_2O_7$ on the Biochemical Markers (with and without immobilized algae)

3-4-1 The Effect of Amoxicillin on Some Biomarkers

3-4-1-1 Reactive Oxygen Species (ROS)

Tables (3-7) and (3-8) show some biochemical markers in *P. euphraticus* and *C.fluminea* clams exposed to amoxicillin (with and without Immobilized *C. vulgaris*) and the study results revealed significant differences in biochemical marker values according to statistical analysis at ($P<0.05$).The results of the current study showed an increase in ROS values in both clams (*P. euphraticus* and *C. fluminea*) in the exposure experiments without the addition of *C.vulgaris*, where the ROS values were (12.99-22.52µg/mg) (30.79-42.86 µg /mg) for both clams, respectively, especially at a concentration of 300 mg/L,compared to the control, which recorded(10.77 and 31.38 µg/mg) respectively, while ROS values were (8.89-16.73 µg / mg)and (21.33-37.47µg/mg) respectively, at a concentration100-300 mg/L with the addition of *C.vulgaris,* compared to the control, which recorded (10.91 and 11.37µg/mg) respectively.

The results are presented as mean ± SD of three replicates, showed The results of the statistical analysis showed significant differences ($p \leq 0.05$) compared to the control group in both species of clams in the exposure experiments with and without the addition of immobilized *C.vulgaris*, LSD levels in *C. fluminea* and *P. euphraticus* clams were 3.32 and 3.44, respectively, figure (3-1). Lyublinskaya *et al*.(2017) indicated the increase in ROS levels due to changes in the intracellular redox balance of cells, and the failure of antioxidant mechanisms to eliminate ROS production can promote this.

In this study, it was found that exposure to AMX enhanced the production of ROS in both clams which are primarily formed in the tissues of aerobic organisms, this can happen either directly as redox cycling molecules or indirectly through the conversion of these compounds during the biotransformation processes (Juan *et al* .,2021).

These medications induce intracellular oxidative stress by increasing respiration and, with it, the release of (ROS) from the respiratory chain, which overwhelms the cellular antioxidant defenses, resulting in DNA damage and, ultimately, cell death (Léger *et al*.,2019).

This is consistent with many studies that indicate that these pharmaceutical substances lead to increased production of ROS in aquatic organisms. According to Matozzo *et al*. (2016), amoxicillin exposure caused the increased produce ROS in both clam *Ruditapes philippinarum* and the mussel *Mytilus galloprovincialis*. Also, a study by Bouzidi *et al*(2023) showed that exposure to triclosan (TCS) produced more ROS in Mediterranean mussels.

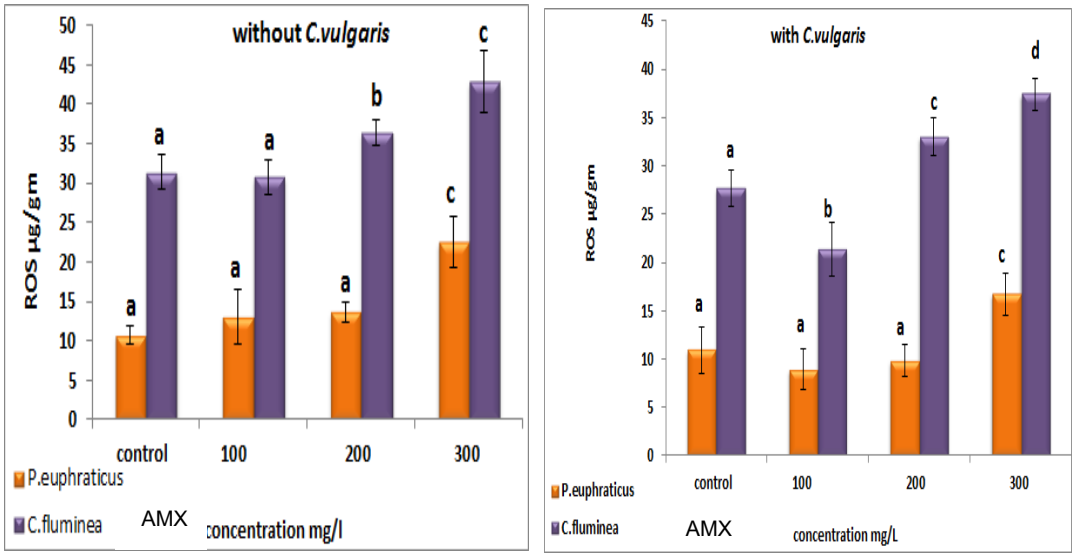

Figure (3-1). Effect of amoxicillin on ROS value in both species of clams

3-4-1-2 Enzymatic Antioxidant: Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX).

The SOD values fluctuated in each of *P. euphraticus* and *C. fluminea* ranging from (20.79 - 33.33 U/ml) and (35.49 - 50.033 U/ml) respectively in the exposure trials without the addition of immobilized *C. vulgaris* for 100-300 mg/L concentrations compared to the control which recorded (17.51 and 15.08 U/ml) respectively. While with *C. vulgaris*, SOD values ranged from (12.72 - 21.62 U/ml) and (21.46 to 35.49 U/ml) compared to the control group which recorded values of (9.80 and 11.37 U/ml) respectively.

Results showed statistically significant differences ($p \leq 0.05$) compared to the control group in both species of clams in the exposure experiments with and without the addition of *C.vulgaris*. LSD levels in *C. fluminea* and *P. euphraticus* clams were 2.678, and 6.674 respectively, figure (3-2)

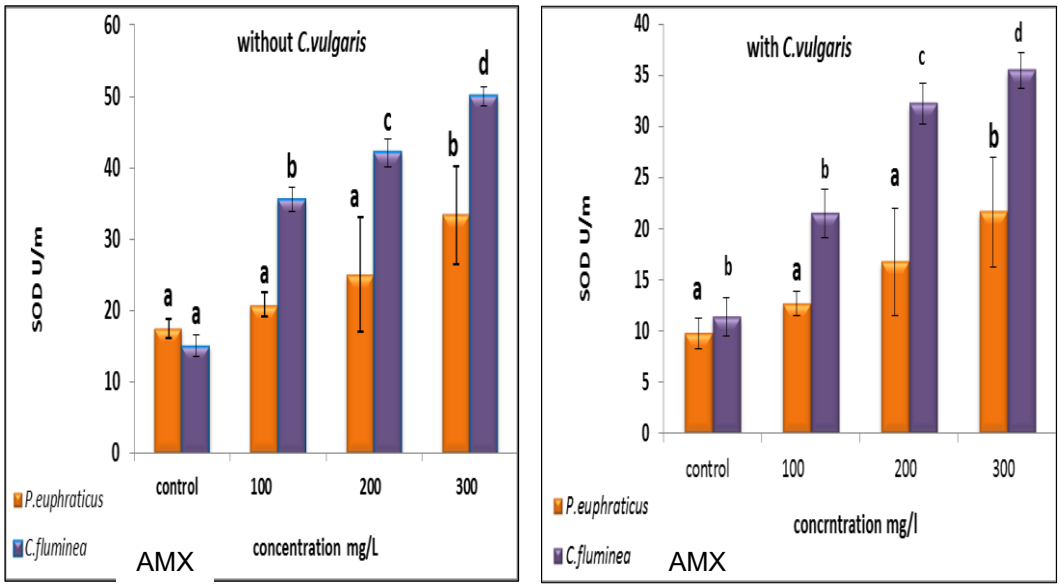

Figure (3-2). Amoxicillin's effect on SOD activity in both species of clams

The CAT values in both clams *P. euphraticus* and *C. fluminea* for 100-300 mg/L concentrations ranged from (25.44 - 30.53 KU/L) and (42.51-

51.78 KU/L) respectively without adding *C .vulgaris* compared to the control group's which recorded (24.14 and 34.57 KU/L) respectively. While with *C. vulgaris*, CAT values ranged from (17.56 - 25.15 KU/L) and (22.16 - 35.33 KU/L) respectively compared to control values which recorded (14.26K U/L and 17.76 KU/L) respectively ,figure (3-3).

The results showed statistically significant differences ($p \leq 0.05$) compared to the control group in both species of clams in the exposure experiments with and without the addition of *C. vulgaris.* LSD levels in *C. fluminea* and *P. euphraticus* clams were 2.262 and 2.981 respectively.

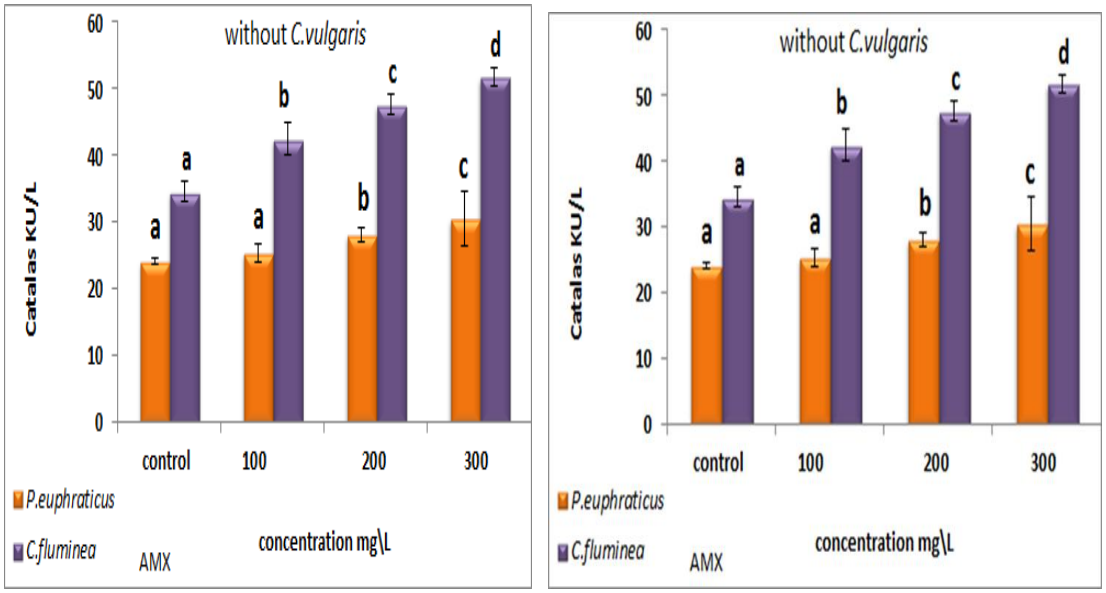

Figure (3-3). Effect of amoxicillin on CAT activity in both species of clams

The Glutathione peroxidase (GPX) values in *both P. euphraticus* and *C. fluminea* ranged between (5.70-8.36KU/L) and (16.41- 21.12 KU/L) respectively for 100-300 mg/L concentrations in the exposure experiments without the addition of immobilized *C .vulgaris* compared to the control which recorded (18.48 and 11.54 KU/L) respectively. But with added immobilized *C .vulgaris*, GPX values ranged from (5.02-9.58 KU/L) and

(14.16 -20.21 KU/L) respectively for 100-300 mg/L concentrations compared to control group values (13.96 and 9.54KU/L).

The results of statistical analysis in both species of clams in the exposure experiments with and without the addition of *C. vulgaris*, revealed statistically significant changes ($p \leq 0.05$) compared to the control group ,figure (3-4). LSD levels in *C. fluminea* and *P. euphraticus* clams were 2.760, and 3.272 respectively

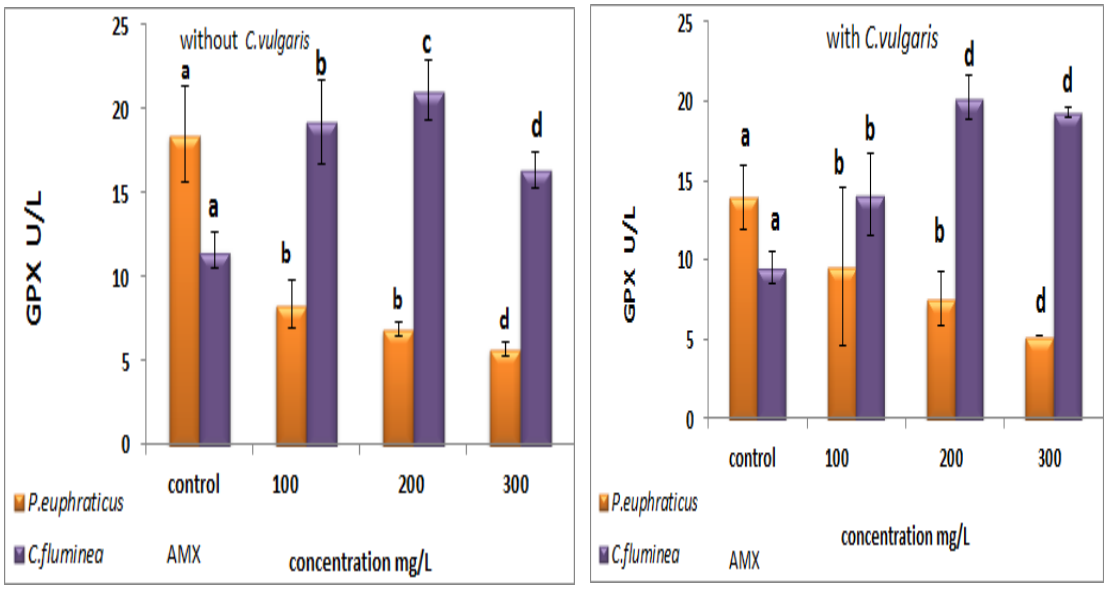

Figure (3-4). Amoxicillin's impact on the activity of GPX in both species of clams

The antioxidant system consists of substances that at low concentrations, stop and/or postpone the oxidation of a cell-substrate It is widely distributed in living things and is critical in the elimination of free radicals, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) is the most important first lines of defense to remove reactive oxygen radicals in antioxidant enzymes (Ighodaro& Akinloye, 2018). Where mostly used as an indicator of oxidative stress to determine pollution stress on organisms(Cabrera *et al*.,2019).

Alteration in the action of the enzymes that comprise the antioxidant defense system can serve as an early warning indicator of toxic compound exposure, allowing changes in biological systems to be recognized before impacts at the community level, like mass mortality of crustaceans or stranding (Oyaneder-Terrazas *et al*.,2022).

In this study, a significant increase of SOD, CAT, and GPX activity in all concentrations of AMX in both organisms compared to the control group indicated that the AMX exposure promoted the generation of ROS. The production of excess ROS increased the SOD and CAT. SOD activity protects tissues and cells from oxidative damage, serves as a defense against oxidative stress, and removes excess ROS by converting it to H_2O_2 (Wu *et al*., 2019; Singh *et al*., 2018).

SOD and CAT work together in antioxidant defense systems, where SOD converts ROS into H_2O_2, and CAT breaks down H_2O_2 into O_2 and H_2O through the redox process (Trchounian *et al.,* 2016; Lee *et al*., 2022).

In this study, the enhanced activity of SOD and CAT demonstrates the defensive mechanism against increased ROS production caused by AMX buildup in both clams. This increase in antioxidant enzymes, however, is insufficient to protect the cell from oxidative stress. Similar results were reported by other researchers, who found that SOD activity in organisms usually increases under stress conditions (Sajjad *et al*., 2012).

GPX is an enzyme in charge of eliminating hydro peroxides H_2O_2 produced in cells and converted O_2 and H_2O (Sarıkaya& Doğan, 2020). GPX is responsible for the transformation of glutathione disulfide (GSSG) to the sulfhydryl form GSH (Yan *et al*., 2017).

In this study, observed increases in level GPX in both clams may result in increases in ROS production, and could indicate that the cell is attempting to defend itself against the scenario of oxidative stress, likely as a protective mechanism for the conversion of excess oxygen and free radicals resulting from exposure to hydrogen peroxide (Ighodaro *et al*., 2018). Cellular biomarkers have a prognostic or diagnostic value for long-term toxicological or ecological effects because they can identify the early onset of biological changes brought on by chemical pollutants (Bio and Nunes, 2020).

The AMX-induced oxidative stress and increased activity of CAT, SOD, and GPX in both clams, indicated an attempt by the organism to detoxify the body, increased activity of both SOD and CAT enzymes represents a necessary adaptive mechanism against cellular damage caused by free radicals (Oyaneder-Terrazas *et al*.,2022).

This study is consistent with those of Elizalde-Velázquez *et al*. (2017), who observed that AMX caused oxidative stress and was also responsible for an increase in enzymatic antioxidant activity (superoxide, catalase, and glutathione peroxidase) in *Cyprinus carpio* gills, kidneys, and brain during acute exposure.

Study of Matozzo *et al.* (2016), AMX had little effect on the activity of the enzyme biomarkers SOD and CAT in two bivalves, the clams *Mytilus galloprovincialis*, and *Ruditapes philippinarum*. Nicolussi *et al.* (2022)study results showed that Amoxicillin (AMX), Trimethoprim (TMP), and Ciprofloxacin (CIP) affected the stability of the lysosomal membrane, in addition to the production of superoxide anion, superoxide phosphatase, and promoted chromosomal aberrations in Clam *Ruditapes Philippinarum*

This study is consistent with several studies indicating that exposure to pharmaceuticals increased the generation of ROS and thus increased antioxidant defenses in bivalves such as. Guo *et al*. (2022) studied the effect of ciprofloxacin on clams *C. fluminea* clams and showed that it boosted ROS generation and SOD enzyme activity in the digestive while decreasing CAT activity. According to Pes *et al*. (2021), exposure of mussels *Mytilus galloprovincialis* to warfarin, dexamethasone, and imidazole increased GPX activity and caused genetic changes.

In the study by Aguirre-Martinez *et al*. (2015) observed that GPX activity was significantly increased compared to control when *C. fluminea* was exposed to tamoxifen. This is not consistent with the findings of (Yuan et al., 2022), who found that *C. fluminea* clams exposure to diclofenac resulted in decreased SOD, CAT, and GPX activities in the gills and digestive glands after 7-day exposure compared with the control group.

Duran-Alvarez and Jiménez-Cisneros (2021) indicated that the increase and depletion of GPX enzyme activity is an indicator of the potential toxic effects of pharmaceutical residues on bivalve species and the ability of these species to prevent oxidative stress, avoiding deeper and irreversible damage.

3-4-1-3 Non Enzymatic Antioxidant: Glutathione (GSH), Ascorbic Acid.

The Glutathione (GSH) values for clam *P. euphraticus* ranged from (7.55 -10.37µg/g) in the exposure experiments without immobilized *C.vulgaris* compared to the control which recorded (5.92 µg/g), while with *C.vulgaris*, GSH values ranged from (6.54 -9.46 µg/g) for 100-300mg/L concentrations compared to 5.14 µg/g in the control group. while In *C. fluminea* clams, the GSH values recorded without *C.vulgaris* were (4.25-

8.03 µg/g) compared to the control which recorded (4.93 µg/g) but with added *C.vulgaris*, GSH values were (2.83-6.72 µg/g) for 100 -300mg/L concentrations compared to 2.77 µg/g in the control group.

The results, the statistical analysis revealed statistically significant changes ($p \leq 0.05$)In both species of clams in the exposure experiments with and without the addition of *C. vulgaris* compared to the control group, LSD levels in *C. fluminea* and *P. euphraticus* clams were 2.033 and 2.721, respectively, figure (3-5)

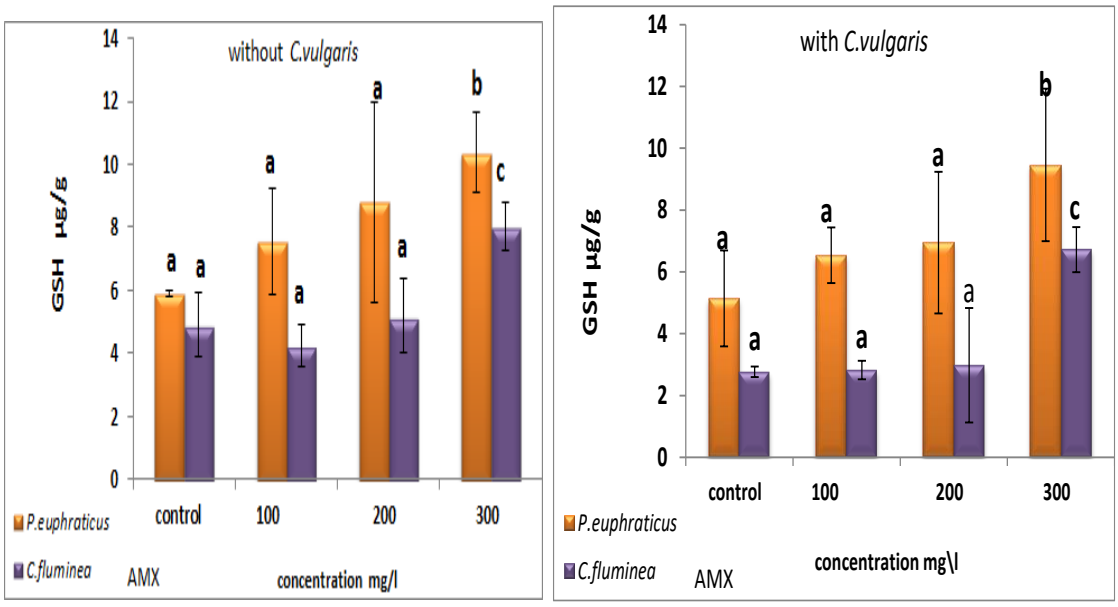

Figure 3-5. Amoxicillin's effects on GSH activity in both species of clams

The Ascorbic acid (AA) values for *P. euphraticus* clams ranged from(13.41 -14.48µM) in the exposure experiments without the addition of immobilized *C.vulgaris* compared to the control which recorded (11.59 µM), while with *C. vulgaris*, The Ascorbic acid values ranged from (10.31 - 13.40 µM) for 100-300 mg/L concentrations compared to (11.47 µM) in the control group.

In *C. fluminea* clams, The AA values were recorded without *C. vulgaris* were (42.92-76.79µM) compared to the control which recorded (32.70 µM),

but with added *C.vulgaris*, AA values ranged from (38.11-59.80µM) compared to (30.77µM) which was recorded in the control group.

The results statistical analysis revealed statistically significant changes ($p \leq 0.05$) in both type clams in the exposure experiments with and without the addition of *C.vulgaris* compared to the control group, LSD levels in *C. fluminea* and *P. euphraticus* clams were 4.51 and 3.128, respectively, figure (3-6).

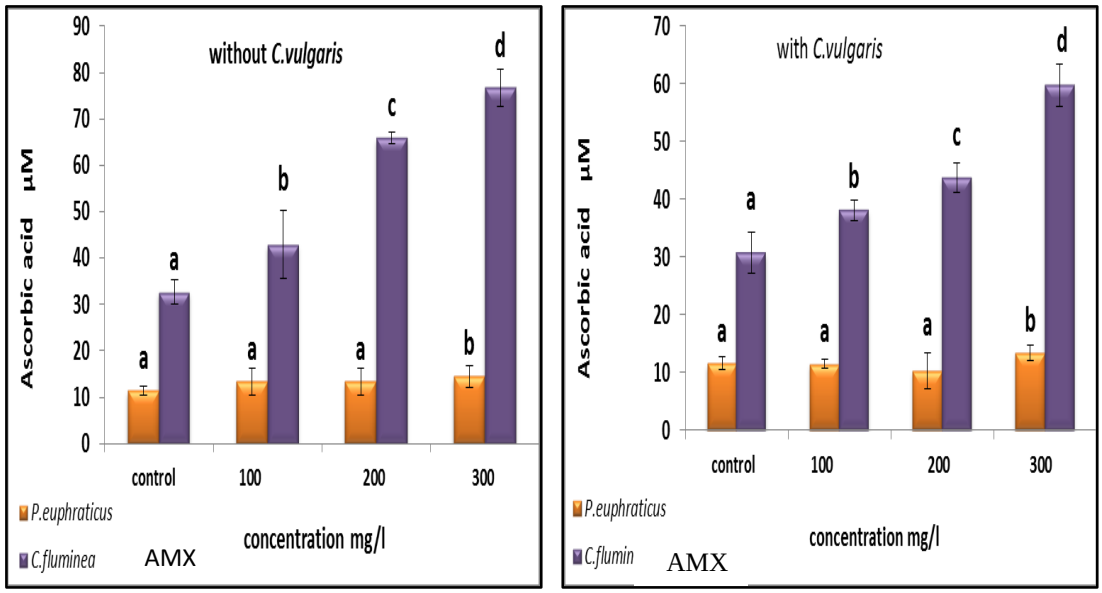

Figure 3-6. Impact of Amoxicillin on Ascorbic acid in both species of clams

Glutathione (GSH) is an important component of the cellular antioxidant defense system (Vacchi-Suzzi *et al*., 2018). GSH serves as the first line of defense for antioxidants, as a radical scavenger, numerous examples demonstrate the role of GSH in antioxidant activity in bivalves (Gnatyshyna *et al*. 2020, and Khoma *et al*. 2021).

GSH plays an important role in the process of removing H_2O_2 and –OH and it is converted into its oxidized form (GSSG) by a reaction catalyzed by GPX; glutathione reductase (GR) then generates GSH from GSSG using NADPH as an electron donor (Regoli and Giuliani, 2014). GSH is crucial

for maintaining redox equilibrium and safeguarding cells from oxidative damage and xenobiotic electro toxicity (Hellou *et al*.,2012).

In the current study, there was an increase in GSH depletion after AMX exposure in both types of clam compared to control, which may be due to excessive ROS production due to loss of redox balance (Yan *et al*., 2017). It is congruent with the findings of Almeida *et al.* (2015), who discovered similar effects in the content of GSH in the clam Ruditapes philippinarum as a result of exposure to the medications carbamazepine (CBZ). And also, with a study by Alkazemi *et al*. (2021), it has already been shown that many drugs can induce cellular changes in the ratio of GSH to GSSG, which may lead to oxidative stress and have an effect on various cellular functions. The enzyme activity of the glutathione system can be induced by pharmaceutical preparations in bivalves, such as *C. fluminea, Mytilus galloprovincialis,* and *Anodonta cygnea* (Martins *et al*.,2014).

The results of a study now show that the ability of AMX to induce cellular changes in the activity of GSH in both types of clams, which may lead to oxidative stress, and an impact on several cellular processes. While contradicting the study by Martyniuk *et al*. (2022), which found that ibuprofen exposure reduced the level of GSH in *Unio tumidus* bivalves.

Ascorbic acid (AA), a low molecular weight antioxidant, protects cells from oxidative damage brought on by ROS by directly interacting with oxidative radicals through rapid electron transfer. Moreover, the ability of Ascorbic acid, to produce new tiny antioxidant molecules like -tocopherol, GSH, and carotene has been connected to the compound's antioxidant activity (Krishnan *et al*. 2009).

Ascorbic acid is an important tool for detecting changes caused by chemicals and pollutants, since ascorbic acid plays a crucial role in cellular metabolism, and the interactions of biomolecules provide a clear picture of toxicant stress and its consequences (Goswami and Bhalla, 2021).

According to the study by Feidantsis *et al.* (2021), ascorbic acid can be used to lessen the effects of oxidative stress and thereby lower the mortality of mussels *(Mytilus galloprovincialis*) in aquaculture settings. The findings of this study agree with those of Trabelsi *et al*. (2020). Ascorbic acid levels in the gills of *Mactra stultorum* clam increased substantially after was exposed to acrylamide. While while disagree with the findings of Goswami and Bhalla's (2021) study, when *Lamellidens marginallis* mussels were exposed to lambda-cylothrin for 21 days, a decrease in the level of ascorbic acid was observed in various mussels.

3-4-2 Total Protein

In clams *P. euphraticus*, the Total protein (TP) values in the exposure experiments without the addition of immobilized *C. vulgaris* ranged from (9.65 - 11.14 mg/g) compared to the control which recorded (13.17 mg/g), while with *C. vulgaris*, TP values ranged from (14.25 - 15.60mg/g) for 100-300mg/L compared to (12.44 mg/g) in the control group.

In *C. fluminea* clams, The TP values were recorded without immobilized *C. vulgaris* were from (35.52 - 19.57 mg/g) compared to the control which recorded (34.64mg/g), but with *C. vulgaris*, TP values recorded ranged from (39.23 -29.41 mg/g) at 100 -300mg/L concentrations compared to (38.78 mg/g) in the control group.

The results statistical analysis revealed statistically significant changes ($p \leq 0.05$) In both species of clams in the exposure experiments with and

without the addition of *C. vulgaris* compared to the control group, and, LSD value in *C. fluminea* and *P. euphraticus* clams were 5.56 and 1.895 respectively ,figure (3-7).

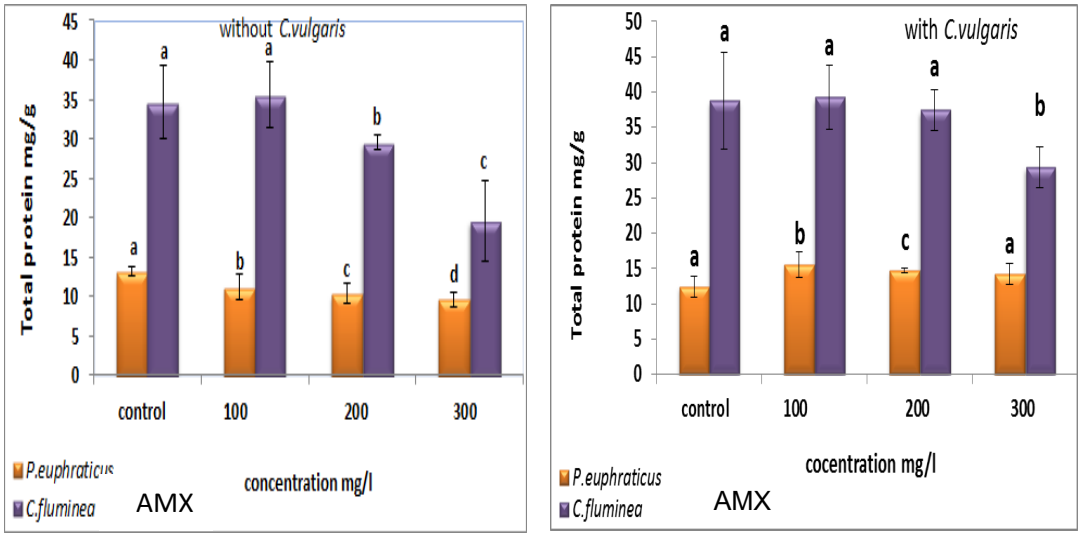

Figure (3-7). Effect of amoxicillin on Total protein in both species of clams

Total proteins (TP) are the most important organic molecules in a living system, and proteins are essential for an organism's physiology and energy mobilization (Topi *et al*., 2021). In a current study, there was a decrease in the level of total protein as a result of excessive ROS generation .As a consequence of excessive ROS production, site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electric charge and increased susceptibility of proteins to proteolysis occur (Neri-Cruz *et al*., 2015; Sharma *et al*., 2012; Juan *et al.*, 2021) .

In general, some researchers have observed that aquatic animals exposed to various contaminants had lower amounts of total protein and albumin (Velisek *et al.*, 2009). This was attributed to stress reduction and xenobiotic detoxification, which increase the metabolic use of structural proteins to meet energy demands(Katz and Orellana, 2012). According to

Jerome *et al*.(2020),a decline in protein levels may be mostly attributable to tissue necrosis or destruction brought on by extensive toxicant exposure oxidative stress has been linked to such occurrences

3-4-3 Malondialdehyde (MDA)

The (MDA) values for *P. euphraticus* clams ranged from (17.69 - 42.43µmol/l) in the exposure experiments without addition of immobilized *C. vulgaris* compared to the control which recorded (13.28µmol/l), while with *C. vulgaris*, values recorded ranged from (11.60 -23.97µmol/l) for 100-300mg/L concentrations compared to(8.89 µmol/l) in the control group.

While in *C. fluminea* clams recorded without immobilized *C.vulgaris* the value of MDA ranged between (9.33 to 17.21µmol/l) compared to the control which recorded (8.81µmol/l) but with added *C .vulgaris*, MDA values ranged from (6.77-22.72µmol/I) compared to (4.75µmol/l) in the control group for 100-300mg/L concentrations.

The results of statistical analysis revealed statistically significant changes ($p \leq 0.05$) in both types of clams in the exposure experiments with and without the addition of *C.vulgaris* compared to the control group. LSD values in *C. fluminea and P. euphraticus* clams were 3.10 and 3.546 respectively, figure (3-8).

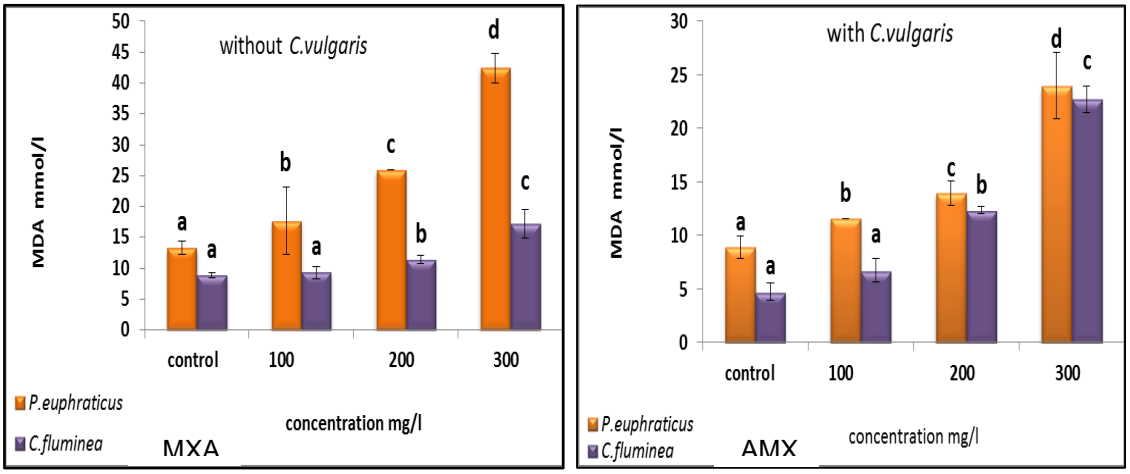

Figure 3-8. Amoxicillin's effects on MDA in both species of clams

Enzymes and non-enzyme in the antioxidant defenses can reflect the condition of the free radical scavenging system, while lipid peroxidation (LPO) is often used to reflect the degree of free radical reactions in organisms and to indirectly reflect the degree of cell damage (Guo *et al*., 2022). Malondialdehyde (MDA) is widely used as a biomarker of oxidative stress, and an increased level of oxidative damage in terms of lipid oxidation has been reported in different species of snails exposed in vitro to environmental pollutants (Chen *et al*.,2015).

The results of the current study showed an increase in the level of MDA activity compared to the control groups for both species of clamss, which may be due to the increased production of ROS (Yan *et al*., 2017). Reactive oxygen species produced inside the cell react with cell membrane lipids, forming lipid peroxides that further degrade to malondialdehyde, and their formation is an indicator of cell damage and, in extreme cases, leads to the death of the organism (Chen *et al*., 2014; Chen *et al*., 2015; Shabani *et al*., 2020).

This could be a sign of increased oxidative stress and impaired antioxidant function Drozdz-Afelt *et al*. (2022). The results of this study are

consistent with several studies such as Martinez-Morcillo *et al*.(2020), which indicated that the presence of pharmaceutical compounds in seafood tissues increased levels of catalase, glutathione peroxidase, and MDA. And a study by Brando *et al*.(2014) found that short-term exposure to paracetamol increased the content of lipid peroxidation and glutathione S-transferase in *Corbicula fluminea*.

In another similar study, Aguirre-Martnez *et al*.(2015), exposed *Corbicula fluminea* to Ibuprofen, Carbamazepine, Novobiocin, and Tamoxifen significantly increased LPO levels. According to Guo *et al*.(2022), exposing *Corbicula fluminea* to Ciprofloxacin resulted in significant increases in MDA content in the gills and digestive glands at a 2.5 g/g CIP concentration. In the study by (Chen *et al*. 2015), exposing *Corbicula fluminea* to Fluoxetine, resulted in a significant increase in Malondialdehyde levels.

Studied by Matozzo *et al*. (2016a), effects of amoxicillin on clams (*Ruditapes philippinarum)* and Mussels (*Mytilus galloprovincialis*) It was noted that there was a slight increase in the levels of lipid peroxidation and carbon protein content.

On the other hand, the study by Pidad *et al*. (2020) showed no significant changes in oxidative stress parameters were observed in lipid oxidation (LPO) levels upon paracetamol and acetylsalicylic acid exposure in mussels Mytilus spp. It may have caused a limited change in antioxidant defenses and damage, which was reversed by the activation of this type's adaptive mechanisms.

3-4-4 The Role Of Immobilized Algae *C. Vulgaris* In Reducing Amoxicillin Toxicity

The present study indicated that *C. vulgaris* has a distinct role in relieving oxidative stress in clams through its pronounced effect on biochemical biomarkers during the study period. A gradual decrease in the values of ROS, SOD, CAT, GSH, TP, and MDA was observed when adding immobilized algae *C. vulgaris* compared to untreated samples.

C. vulgaris has been widely used in bioremediation to remove many environmental contaminants, such as heavy metals, organic compounds, or pharmaceuticals, due to its wide distribution in aquatic habitats, rapid growth rates, and tolerance to harsh environmental conditions (López-Pedrouso et al., 2022).

When microalgae are exposed to pharmaceutical compounds, they exhibit a variety of responses and use a variety of biotic and abiotic methodologies to detoxify and survive. Biosorption, bioaccumulation, and biodegradation are major ways for algae to remove antibiotics from media. The process of uptake of the antibiotic amoxicillin begins with the cell wall upon binding to an active group (Leng *et al.,* 2020; Liu *et al*., 2021). Then the absorbed antibiotics accumulate inside the cells through active diffusion,as a result of the amoxicillin accumulation inside the *C. vulgaris* algae, free radicals are formed inside the cell by increasing the photosynthetic activity of the cell, which aids in the process of biodegradation of the antibiotic (Gayosso-Morales *et al*.,2023).

According to Ricky *et al.* (2022), biodegradation was the predominant method of removal, followed by biosorption and bioaccumulation in the CIP

and AMX assays, the removal of CIP was higher than that of AMX, at about 25% (CIP) vs. 37% (AMX).

Several studies have shown that *C. vulgar* contributes to the removal of many antibiotics by many processes, for example, the removal of Norfloxacin by Photodegradation by 36.8% (Zhang *et al*., 2012), Levofloxacin by 82.34% by bioaccumulation and biodegradation, and azithromycin by 92.77% biodegradation (Kiki *et al*., 2020), metronidazole by 100% via bio adsorption (Hena *et al*.,2020). Antibiotic levofloxacin through subsequent intracellular biodegradation and accumulation (Xiong *et al*.,2018). Additionally, (Xiong *et al*.,2017) used *C. vulgaris* to remove some antibiotics such as levofloxacin and fluoroquinolones; with an initial concentration of 5 mg/L, after 7 days, about 15% of the antibiotic had been eliminated.

In the current study, chlorella used a biodegradation pathway to lessen the toxicity of amoxicillin, acting to degrade amoxicillin as a carbon source for cellular growth. According to Ricky et al.(2022), *C. vulgaris* was able to remove antibiotics at rates of 76% for CIP and 46% for AMX, respectively, using a biodegradable method. According to Xie *et al.* (2019) there are two main ways that biodegradation antibiotics can be broken down by microalgae: first, through metabolic degradation, where the antibiotics act as an electron donor or acceptor and a carbon source for the microalgae; and second, through co-metabolism, where the antibiotics are reduced by the enzymes and turn into non-toxic product compounds.

According to (Vo *et al.,* 2020), antibiotics are biodegraded into various metabolic intermediates or mineralized into H_2O and CO_2. Microalgae contain a sophisticated enzyme system made up of families of phase I and

phase II enzyme inside and outside the algae cells; such as glutathione-S-transferase and cytochrome P450 (Xiong *et al*.,2018).

In a similar study performed by Zhao *et al.(* 2021), AMX was removed by *C. vulgaris* with an efficiency of 25% 58 ,as well as the study of Xiao et al.,2021 who used *Chlorella pyrenoidosa* to remove amoxicillin which achieved about 91% clearance, within 6 hours.

Many investigators reported that cell immobilization of biomass protects cells from compound toxicity, and maintains metabolic cell activity for a longer period, giving higher cell density; improved operational stability, increased resistance to environmental stresses (temperature and acidity), and taking up less space, making it easier to handle and use regularly (Eroglu *et al.,* 2015; Pang *et al*., 2020; Cao *et al.,* 2021).

This was confirmed in this study, where the *C.vulgaris* immobilization process played an important environmental role in reducing oxidative stress caused by amoxicillin toxicity in both species of clamss. This makes it more effective at removing these compounds from the medium and thus reducing their toxic effects on the organism.

This is because microalgae have a high ability to adapt to various and harsh environmental conditions and can act as a good biological absorbent, and provide a high absorption capacity for minerals and nutrients (Hejna *et al.,* 2022). According to Xie *et al*. (2020a), immobilized C.*vulgaris* demonstrated greater sulfamethoxazole tolerance than the suspended Thus, compared to a suspended reactor, the removal efficiency of living immobilized *C. vulgaris* was 12% greater.

This study agrees with the results of Xie *et al*.,(b2020), who reported that Immobilized *C.vulgaris* disrupted the toxicity of sulfamethoxazole SMX

and increased the removal efficiency by 85.1% and 86.2% SMX, respectively from the medium.

Immobilization of microalga is recently been used to remove many pollutants, such as heavy metals, nitrogen, and phosphorus (Salman *et al.*,2022; Sarkheil *et al.*,2022) and pharmaceuticals, such as antibiotics and PPCPs (Chandel *et al*., 2022). This is because microalgae have a high ability to adapt to various and harsh environmental conditions and can act as a good biological absorbent, and provide a high absorption capacity for minerals and nutrients (Hejna *et al*., 2022).

Table 3-7.The biochemical markers *in P.euphraticus* during acute exposure period to antibiotic amoxicillin (Min., Max., Mean±SD of three replicates).

Biochemical markers	Without Immobilization of Algae (*C.vulgaris*)				Immobilization with Algae *(C.vulgaris*)			
	Control	100 mg/l	200 mg/l	300 mg/l	Control	100 mg/l	200 mg/l	300 mg/l
ROS (µg/mg)	9.63-11.92 10.77 ± 1.14 a	10.5–6 17.00 12.99 ± 3.49 a	14.83-12.38 13.66 ± 1.22 a	19.35-25.93 22.52 ± 3.29 b	8.54-13.54 10.91 ± 2.37 a	7.22-11.26 8.89 ± 2.10 a	11.72 - 8.54 9.80 ± 1.68 a	14.46 – 18.75 16.73 ± 2.15 b
SOD(U/mL)	16.22-18.92 17.51 ± 1.35 a	18.92-22.14 20.79 ±1.67 a	16.49 -32.43 25.04 ± 8.03 a	27.03-40.54 33.33 ± 6.80 b	8.11– 10.81 9.80 ± 1.47 a	11.62-13.92 12.72 ± 1. 15 a	10. 81 20.54-16.75 ± 5.21 a	16.22 – 27.03 21.62 ± 5.40 b
CAT (KU/L)	24.14-24.9 24.14 ± 0.39 a	23.93-26.77 25.44 ±1.42 a	27.08 -29.15 28.21 ± 1.04 b	27.39-35.28 30.53 ± 4.18 c	11.58 – 17.49 14.26 ± 2.99 a	15.86-18.42 17.56 ±1.47 b	22.42 - 24. 56 23.41 ± 1.07 c	23.59 - 26.24 25.15 ± 1.38 d
GPX (U/L)	16.82 - 21.82 18.48 ± 2.88 a	6.94 – 9.78 8.36 ± 1.42 b	6.50 - 7.38 6.91 ± 0.44 c	5.26 - 6.02 5.70 ± 0.39 d	11.90 - 15.90 13.96 ± 2.00 a	5.62 – 15.14 9.58 ± 4.95 b	6.14 - 9.43 7.50 ± 1.71 c	4.98 - 5.06 5.02 ± 0.04 d
GSH(µg/g)	5.82 - 6.00 5.92 ± 0.09 a	5.64 - 8.81 7.55 ±1 .68 a	5.46 - 11.81 8.79 ± 3.18 a	9.11 - 17.32 10.37 ± 1.25 b	3.96 - 6.90 5.14 ± 1.55 a	5.55 - 7.31 6.54±0 .90 a	4.86 - 9.40 6.95 ± 2.29 a	7.25-12.13 9.46± 2.47 b
Ascorbic acid (AA) µM	10.95-12.28 11.59 ± 0.91 a	10.08-15.65 13.41 ± 2.94 a	11.66-16.66 13..37 ± 2.91 a	12.49-17.14 14.48 ± 2.39 b	10.34-12.65 11.47 ± 1.13 a	10.47-11.99 11.31 ± 0.77 a	7.11-13.51 10.31± 3.19 a	11.80-14.27 13.40± 1.39 b
Total protein (mg/g)	12.74-14.38 13.17 ± 0.58 a	9.37-12.58 11.14 ± 1.63 b	9.04-11.39 10.38 ± 1.20 c	8.67-10.45 9 .65 ± 0.905 d	11.09-13.95 12.44 ± 1.43 a	13.59-17.18 15.60 ±1.83 b	14.43-15.06 14.74 ± 0.31 c	12.66-15.45 14.25 ± 1.43 a
MDA (µm/l)	12.19-14.36 13.28 ± 1.06 a	12.18-23.21 17.69 ± 5.51 b	25.90-25.90 25.9± 0.00 c	40.01-44.87 42.43 ± 2.43 d	7.69-9.62 8.89 ± 1.04 a	11.60-11.69 11.60 ± 0.00 a	12.79-15.13 13.96 ± 1.17 b	20.51-26.47 23.97± 3.09 c

*similar letters mean that there were no significant differences between treatments.
** Different letters mean that there were significant differences between treatments

Table 3- 8. The biochemical markers *in C. fluminea* acute exposure period to antibiotic amoxicillin (Min., Max., Mean±SD of three replicates).

	Without Immobilization of Algae (*C.vulgaris*)				Immobilization with of Algae (*C.vulgaris*)			
Biochemical markers	Control	100 mg/l	200mg /l	300mg/ l	Control	100 mg/l	200mg /l	300mg /l
ROS Microgram/gm	28.88-33.12 31.38 ± 2.21 a	28.43 - 32.74 30.79 ± 2.18 a	35.17-38.54 36.97± 1.69 b	42.86 - 50.80 42.86 ± 3.98 c	25.5-29.15 27.67± 1.92 a	18.98-24.46 21.33± 2.8 b	30.76-34.24 33.00± 1.94 c	36.65-39.32 37.41± 1.66 d
SOD ACTIVITY U/ml	13.51-16.50 15.08± 1.500 a	33.88-37.84 35.49± 1.72 b	40.12-44.05 42.08± 1.96 c	48.65-51 50.033 ±1.35 d	9.80-13.51 11.37± 1.91 a	19.00-23.77 21.46± 2.388 b	30.1-32.43 32.21± 2.01 c	33.77-37.22 35.49± 1.72 d
Catalase KU/L	36.10 -33.05 34.57± 1.57 a	45.11 -41.92 42.51 ±2.35 b	46.36 -49.31 47.68 ± 1.50 c	50.46-53.10 51.78 ± 1.32 d	16.119 -19.42 17.76 ± 1.65 a	21.09-23.24 22.16± 1.0 b	32.2-35.37 34.0±1.6 c	35.48 -38.28 35.33± 3.0 d
GPX U/L	10.12 – 12.22 11.54 ± 1.08 a	17.44-22.10 19.25 ± 2.49 b	19.23-22.58 21.21 ± 1.75 c	17.34-29.23 16.41 ± 1.07 d	8.70-10.71 9.54±1.04 a	11.21-16.06 14.16± 2.59 b	19.23-21.82 20.21± 1.40 c	19.12-20.54 19.28± 0.30 d
GSH (µg/g)	4.06-6.06 4.93 ± 1.02 a	3.21-5.02 4.25 ±0.67 a	4.08-6.45 5.19± 1.19 a	8.81-7.25 8.03 ± 0.77 b	2.58-2.94 2.77±0.17 a	3.12-2.50 2.83±0.3 a	1.78-5.117 2.98±1.85 a	6.120 -7.557 6.72±0.73 b
Ascorbic acid µM	30.63 -35.70 32.70± 2.65 a	35.23-49.62 42.92± 7.24 b	64.81 -67.34 65.90± 1.29 c	73.67-81.2 76.79± 3.97 d	27.4-34.45 30.77± 3.50 a	36.78-40.23 38.11± 1.85 b	41.1-46.34 43.76± 2.60 c	55.56-62.20 59.80± 3.68 d
Total protein (mg/g)	30.23-39.597 34.64± 4.70 a	32.56 - 40.330 35.52 ± 4.20 a	29.78-30.65 29.5 ± 0.97 b	15.4 -15.15 19.57± 5.01 c	25.43-31.736 38.78. ±6.8 a	35.6-44.35 39.23± 4.55 a	36.56-38. 59 37.41± 2.90 a	28.56-30.359 29.41± 2.90 b
MDA mmol/l)	8.51 – 9.27 8.81±0.40 a	8.23 – 10.29 9.33± 1.03 a	10.87-12.10 11.39± 0.63 b	19.92-15.50 17.21 ± 2.36 c	5.41-3.87 4.75±0.79 a	7.89-5.61 6.77±1.1 a	8.11-6.91 12.36± 0.36 b	9.11-6.65 22.72± 1.25 C

*similar letters mean that there were no significant differences between treatments.
** Different letters mean that there were significant differences between treatments

3-4-6 The Effect of the Potassium Dichromate on Some Biomarkers (with and without immobilized algae).

3-4-7 Reactive Oxygen Species (ROS)

The result showed in Tables (3-9) and (3-10) the biochemical markers in *P. euphraticus and C. fluminea* clams exposed to disinfectants Potassium dichromate ($K_2Cr_2O_7$) with and without immobilized of Algae *C. vulgaris* and the study results revealed differences in biochemical marker values appear significant changes in concentration of biochemical markers according to statistical analysis at ($P<0.05$).

The results of the current study showed an increase in ROS values in both clams(*P. euphraticus and C. fluminea*) in the exposure experimentes without the addition of *C .vulgaris* especially at a concentration of 50 mg/L, where the ROS values were (37.50-51.05 µg/mg) (37.89-63.66 µg/mg) for the both clams respectively, compared to (12,58 and 16.71) in the control group respectively, while with immobilized *C. vulgaris*, ROS values were (33.88-42.06 µg / mg) (41.03-51.0 µg / mg), especially at a concentration of 50 mg/L, compared to(11.80 and14.71) respectively figure (3-9).

The results of the statistical analysis showed a significant difference between the control and treatment group in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$), the results of LSD values in *C. fluminea* and *P. euphraticus* clams were 3.143 and 5.717 respectively

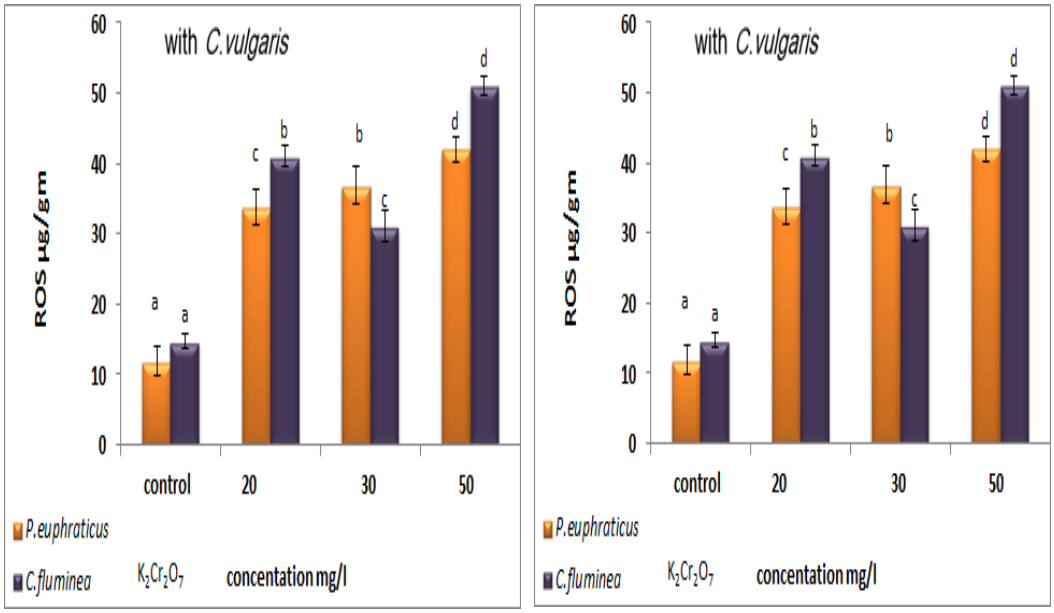

Figure 3-9: Effect of $K_2Cr_2O_7$ on ROS in both species of clams.

In this study, it was found that exposure to $K_2Cr_2O_7$ enhanced the production of ROS in both species of clams. Metal exposure induces oxidative stress in aquatic organisms by forming ROS like hydroxyl radicals, superoxide, and peroxide, and chromium is considered one of the most important metals for producing ROS (Kim and Kang, 2023). The main species of ROS produced by Cr (VI) in the cell are hydroxyl radicals and superoxide (Lushchak *et al.*, 2008).

Cr (VI) is structurally similar to sulfate, which allows its entrance into the cell through sulfate channels. Once Cr (VI) is inside the cell, it is promptly reduced to Cr (III), which causes the generation of reactive oxygen species (ROS). This reduction results in a massive generation of ROS, and consequently, it is one of the most important damages induced by Cr (VI) exposure (Machado *et al.*,2019). and production of ROS causes biomolecule oxidation, which destroys cellular DNA, proteins, and lipids, leading to cell damage and the death of the organism (Saleh *et al.*,2022).This is consistent with many studies that show that exposure to $K_2Cr_2O_7$ leads to increased

ROS production in organisms, such as (Ahmed *et al.* ,2013; Awasthi *et al.*,2018; Chaâbane *et al.*,2020;Chakraborty *et al.*, 2022), found that exposure to $K_2Cr_2O_7$ affects antioxidant responses and causes increased ROS activity, DNA damage, and apoptosis in fish *Channapunctatu* . According to Li *et al.* (2011) exposure to hexavalent chromium increases ROS production, which negatively affects cell function and viability by damaging proteins and lipids**.** Studies suggest that Cr (VI) toxicity is mainly due to an increase in ROS production, which are produced by the Fenton reaction (Mohamed *et al*.,2020; Li *et al*.,2022)

3-4-7 Enzymatic Antioxidant: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX).

The results of the current study showed an increase in the values of SOD activity in both clams (*P. euphraticus* and *C. fluminea*), in exposure experiments without adding immobilized *C. vulgaris*. It was higher for the values of (33.40 U/ml) and(54.04 U/ml) for both clams, respectively, when the concentration was 50 mg/L and the lowest SOD values were (28.36 U/ml) and (35.96 U/ml) respectively at a concentration of 20 mg/L, compared to (15.02 and 14.02) in the control group. while with adding immobilized *C .vulgaris* it was higher for the values of (46.74 U/ml) (31.4 U/ml) for both clams, respectively, when the concentration was 50 mg/L, and the lowest SOD values were (27.19 U/ml) (23.36. U/ml))at a concentration of 20 mg/L, respectively compared to 10.91 and 20.35 in the control group figure (3-10).

The results of the statistical analysis showed a significant difference between the control and treatment group in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$),

the results of LSD levels in *C. fluminea* and *P. euphraticus* clams were 2.332, and 2.785 respectively

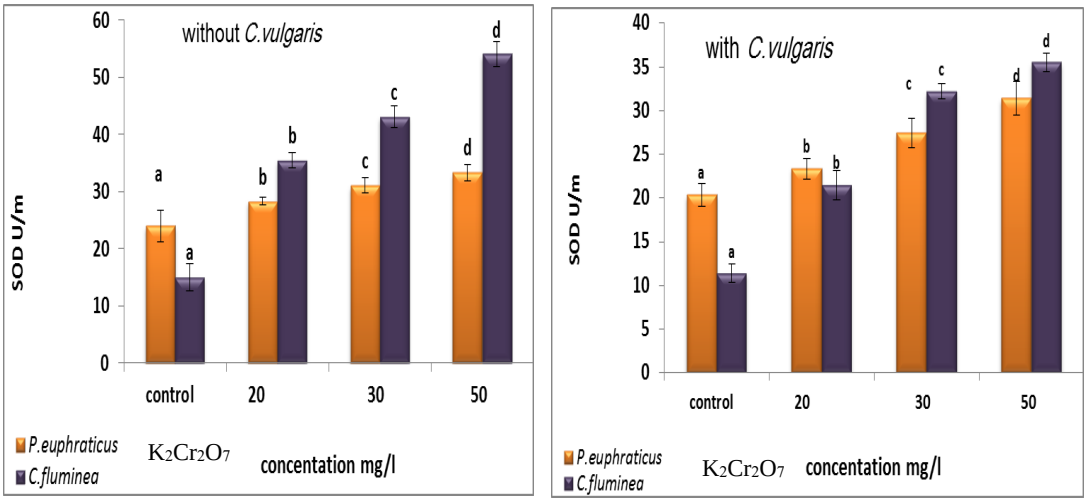

Figure 3-10: Effect of $K_2Cr_2O_7$ on SOD activity in both species of clams.

Without adding *C. vulgaris*, the CAT values in both clams, *P. euphraticus and C. fluminea* ranged from (26.88 - 40.32 KU/L) and (24.55 to 48.99 KU/L) respectively compared to the control group's which recorded (23.60 and 9.71 KU/L) respectively. While with *C. vulgaris*, CAT values ranged from (25.76 - 27.16 KU/L) and (27.19 - 46.74 KU/L) respectively compared to (22.82 and 10.91 KU/L) in the control group for 20-50 mg/L concentrations figure (3-11).

The results of the statistical analysis showed a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of immobilized *C. vulgaris* ($p \leq 0.05$), LSD values in *C. fluminea* and *P. euphraticus* clams were 3.934, and 2.252 respectively.

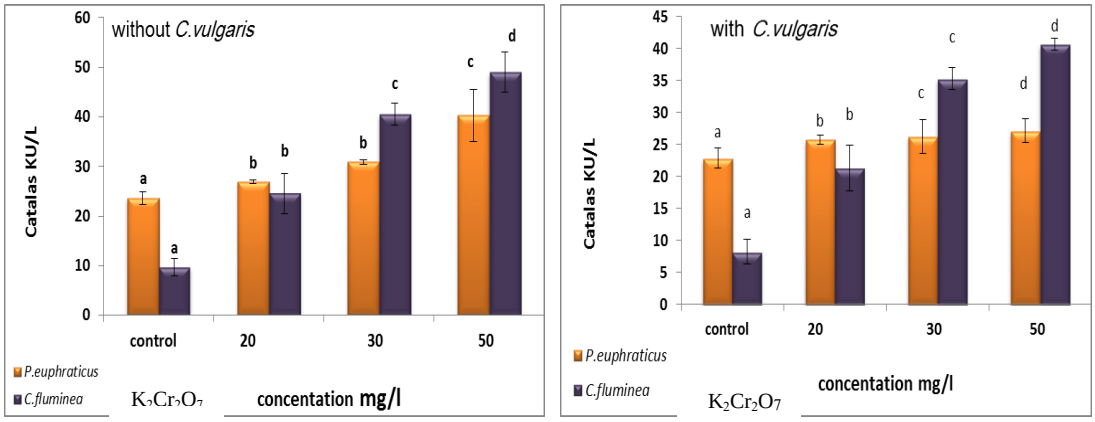

Figure 3-11: Effect of $K_2Cr_2O_7$ on CAT activity in both species of clams

Glutathione peroxidase (GPX) values in both clams, *P. euphraticus* and *C. fluminea,* without the addition of immobilized *C. vulgaris,* ranged from (4.35 to 6.34 U/L) and (10.99 to 43.35 U/L) compared to the control group's (7.99 and 19.05 U/L) respectively, while with *C. vulgaris*, GPX values ranged from(7.34-3.87U/L) and (9.37-37.99 U/L) compared to (6.28 and 18.11U/L) in the control group for 50-20 mg/L concentrations respectively figure (3-12). The results of the statistical analysis showed a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of immobilized *C. vulgaris* ($p \leq 0.05$), and LSD levels in *C. fluminea* and *P. euphraticus* clams were 3.699 and 2.030respectively

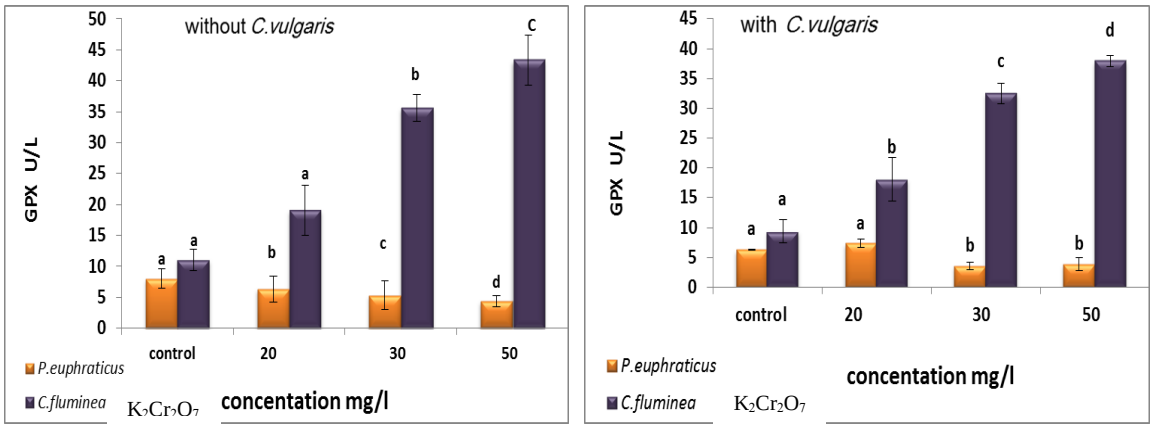

Figure 3-12. Effect of $K_2Cr_2O_7$ on the activity of Glutathione Peroxidase (GPX) in both species of clams

In this study, an increase in CAT and SOD activity was observed along with an increase in the concentration of potassium dichromate during the experimental periods in both species of clams. This increase is due to the toxic stress response caused by chromium. ROS are highly reactive, which damages cell organelles, and these Cr (VI)-mediated ROS can be neutralized by the antioxidant system. Antioxidants of the cell minimize Cr (VI) toxicity and protect the cell organelles from Cr (VI)-mediated oxidative damage . Thus, the antioxidant activity of the animal cell actively participates in the detoxification of Cr (VI) toxicity(Singh *et al*.,2022).

Mandal (2022), suggesting that increased SOD activity and Catalase is a defense mechanism to prevent tissue damage by eliminating excess ROS induced by hexavalent chromium. Asimilar observation was made by Kumari *et al*. (2014) in *Labeo rohita* trout after chromium treatment. Chaâbane *et al*. (2020) reported a significant increase in SOD activity in the gills and digestive glands of bivalve molluscs *(Venus verrucose*) after hexavalent exposure to chromium. In another similar study, Lee *et al*. (2022) found SOD activity in the liver and gills of *Paralichthys. olivaceus* was significantly increased after hexavalent chromium.

In this study, a fluctuation in GPX activity was observed in both species of clams. A decrease in GPX activity was observed during the experimental periods in *P. euphraticus,* while an increase in GPX activity in *C. fluminea* may be due to the ability of Cr (VI) to induce oxidative stress. According to Iztleuov *et al*., 2017, overexpressing glutathione peroxidase shields cells against the toxicity of Cr^{+6} by lowering the number of o-hydroxyl radicals. In a similar study, Kumar *et al*. (2013) observed an increased level of GPX in fish *Cyprinus carpio* after Cr (VI) exposure, and this was attributed to the formation of ROS.

The current study's findings clearly show that $K_2Cr_2O_7$ produces oxidative stress in both species of clams. Increased CAT, SOD, and GPX activity may imply that these enzymes play a vital role in shielding cells from the detrimental effects of chromium as well as in creating an adaptive response to chromium toxicity, in agreement with other studies such as Kumari *et al.* (2014). Also note similar results in the study of Patlolla *et al.* (2009), Increased activities of antioxidant enzymes SOD and CAT after chromium treatment, because these enzymes have a protective role against damage caused by oxygen free radicals, their induction can be an adaptive response to oxidative stress.

3-4-8 Non-Enzymatic Antioxidant: Glutathione (GSH) Ascorbic Acid

The values of Glutathione (GSH) in the current experiment in both *P. euphraticus* and *C. fluminea* clams without adding immobilized *C. vulgaris* ranged from (8.57 - 18..01 to µg/g and 12.79 - 36.86 to µg/g) for concentrations of 20–50 mg/L compared to the control group (21.3and 49.14 µg/g) respectively. While with immobilized *C. vulgaris*, the Glutathione (GSH) values ranged from (9.7 - 22.47 µg/g) and (7.58 -30.26 µg/g) compared to the control group which recorded (18.87 µg/g and 37.58 µg/g) respectively figure 3-13.

The results showed a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$). LSD levels in *C. fluminea* and *P. euphraticus* clams were 3.849, and 4.769 respectively.

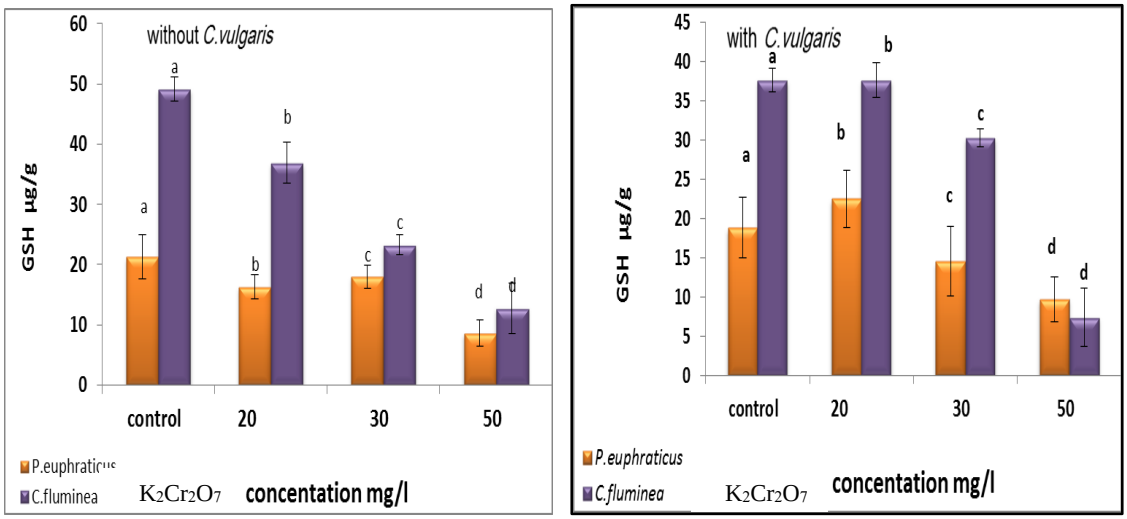

Figure 3-13. The effect of $K_2Cr_2O_7$ on the activity of Glutathione in both species of clams

The values of ascorbic acid in the current experiment in both *P. euphraticus* and *C. fluminea* clams without the addition of immobilized *C. vulgaris* ranged from (26.37 - 20.37 µM) and (35.37 - 50.37 µM) respectively compared to the control group of (25.36 and 15.06 µM) While with *C. vulgaris*, the ascorbic acid values ranged from (24.83 -27.94 µM) and (23.67 to 38.89 µM) respectively for concentrations of 20–50 mg/L compared to (23.92 µM and 11.45 µM) in the control group figure (3-14).

The results of a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$), and LSD levels in *C. fluminea* and *P. euphraticus* clams were 4.576 and 1.286 respectively.

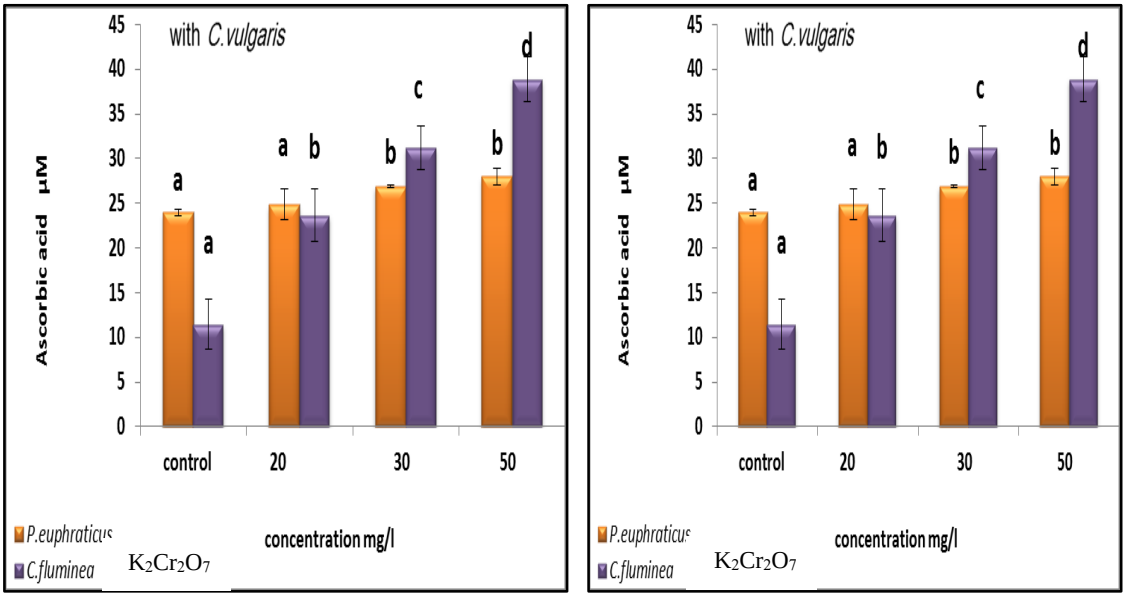

Figure 3-14. $K_2Cr_2O_7$'s impact on the activity of ascorbic acid in both species of clams.

Glutathione (GSH) is the most abundant intracellular antioxidant and thiol-dependent Glutathione (GSH) is often found in molar concentrations within cells, helps maintain the cellular redox state, and protects the cellular system from the harmful and toxic effects of lipid peroxidation (Kim and Kang, 2023). Intracellular depletion is a marker of oxidative stress, GSH immediately scavenges reactive oxygen species (ROS), including HO•, RO•, RO_2•, HOCl, and ONOO, from a cell to reduce the damage caused by it (Vairetti *et al*,.2021).

In a current study, clams *P. euphraticus* and *C. fluminea* had lower levels of GSH after exposure to different concentrations of $K_2Cr_2O_7$ in vitro compared to the control. It was similar to Chen *et al*., 2018 study reporting significant depletion of GSH in Japanese medaka (*Oryzias latipes*) after hexavalent chromium exposure.

A decrease in GSH was attributed to the increased activation of enzymes that catalyze hydrogen peroxide reduction. In another study, a

significant decrease in GSH levels was reported after an initial increase upon exposure to hexavalent chromium (Yuan *et al*., 2017)

In a similar study by Lee *et al*. (2022),decreased levels of GSH were observed in *Paralichthys olivaceus*, which were significantly depleted after exposure to chromium, it may be due to excessive production of free radicals after exposure to chromium.

While this study is not consistent with a study by Lushchak *et al*. (2008) on the effect of potassium dichromate on the antioxidant defenses of goldfish, a high increase in GSH enzyme level was observed after exposure to the glutathione system due to oxidative stress induced by potassium dichromate.

Ascorbic acid, a vital nutrient for aquatic organisms to promote healthy growth and development, is used by the organism to reduce oxidative stress. Moreover, as a powerful antioxidant that protects cells from (ROS), it is essential for processing toxins and detoxification as well as alleviating oxidative stress (Lin *et al.,* 2018).

According to a study by Pehlivan. (2017) Vitamin C is a potent reducing agent and free radical scavenger in biological systems. Vitamin C is an important component of the first line of defense against free radicals, preventing oxidative damage to proteins and lipid membranes and serving as an effective source of antioxidants.

Poljak *et al*. (2005) indicated, ascorbic acid serves to reduce the toxicity of Cr (VI) by acting as a reducing agent, reducing the stability of Cr (V), acting as an antioxidant, reducing the formation of supramolecular anion and hydrogen peroxide, and quenching free radicals formed through Cr (VI) conversion to Cr.

In the current study, a fluctuation was observed in the level of ascorbic acid in both species of clamss, with an increase in the level of this enzyme with increasing concentrations during the experiment that was exposed to potassium dichromate compared to the control group. This is because exposure to Cr (VI) significantly increases levels of oxidative stress by increasing ROS production (Zhao *et al*., 2020).

Several studies showed that ascorbic acid has a role as an antioxidant and works to reduce oxidative stress by reducing the generation of ROS and also showing protective actions, such as According to Gegotek and Skrzydlewska (2022), ascorbic acid plays a role in the cell as an anti-inflammatory and antioxidant. (Kim and Kang, 2023) studied the effects of ascorbic acid on the toxicity of chromium in rockfish *Sebastes schlegeliion*, the presence of ascorbic acid was observed to play an effective role in alleviating the toxicity of chromium and reducing its effect on antioxidant responses, MT gene expression, and neurotoxicity.

3-4-9 Total Protein

Without immobilized *C. vulgaris*, the total protein values in both clams, *P. euphraticus and C. fluminea* in the experment ranged from (10.86 - 12.79 mg/g) and (18.43 -39.11 mg/g) compared to the control group's (16.31 and 47.76 mg/g) respectively. While with immobilized *C. vulgaris*, total protein values ranged from (10.93-13.56 mg/g) and 20.44 to35.69 mg/g) for 20-50 mg/L concentrations compared to (29.21 and 42.54 mg/g) in the control group respectively.

The results of the statistical analysis showed a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$) figure

(3-15), and LSD value in *C. fluminea* and *P. euphraticus* clams were 4.698 and 2.368 respectively.

In the current study, a decrease in the level of total protein was observed in both species of clams when exposed to potassium dichromate compared to the control group. The reduction in protein level might be attributed to the dispersal of biomolecules and raised catabolism to counter the induction of stress by toxins(Dhara *et al*.,2022).

It may be due to potassium dichromate stimulating ROS production. The excessive generation of ROS leads to protein degradation due to oxidation of the side chains of amino acids, leading to a change in the function of the protein and ultimately the integrity of the organism(Juan *et al*., 2021). The primary reason for the decline in protein concentration is the increased consumption of protein to fulfill metabolic processes' increased energy needs, which increases proteolysis to meet the high energy demands during heavy metal stress(Patil, 2011). Many different species of molluscs exposed to heavy metals showed similar signs of lower protein content (Gulbhile and Zambare, 2013; Dhara *et al*.,2022).

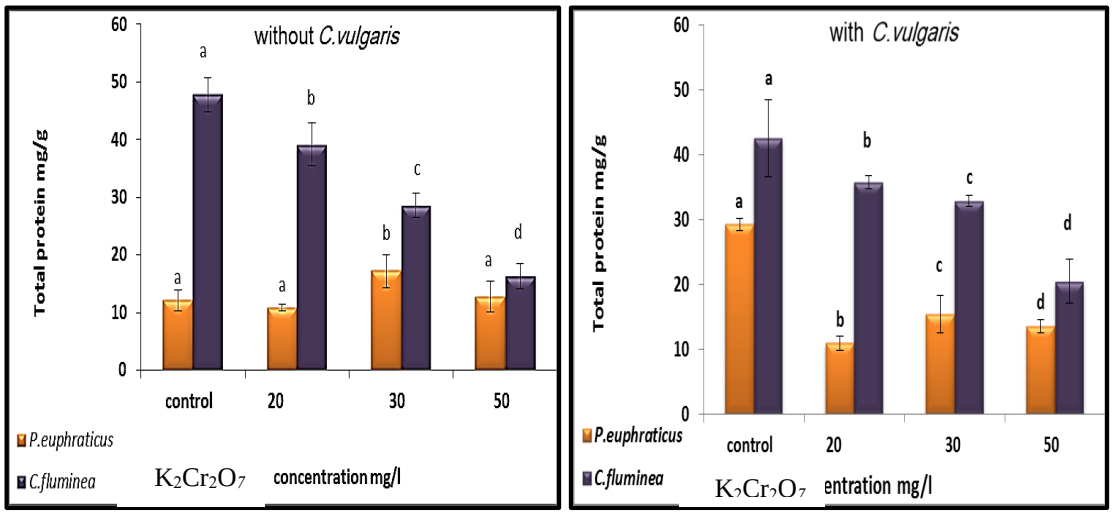

Figure 3-15:Impact of $K_2Cr_2O_7$ on Total protein in both species of clams..

3-4-10 Malondialdehyde (MDA)

Malondialdehyde (MDA) values in both species, *P. euphraticus* and *C. fluminea*, without the added immobilized (*C. vulgaris*), ranged from (12.68 - 23.22 and 29.01 to 59.88 µmol/I) compared to the control group's(9.7 and 8.033 µmol/I) respectively, while with immobilized algae *(C. vulgaris*), MDA values ranged from (9.81 -11.44 and 23.06 to 47.92 µmol/I) for 50-20 mg/L concentrations compared to (8.86 and 9.67 µmol/I) respectively in the control group figure (3-16).

The results of the statistical analysis showed a significant difference between control and treatment in both species of clams in the exposure experiments with and without the addition of *C. vulgaris* ($p \leq 0.05$), and LSD levels in *C. fluminea* and *P. euphraticus* clams were 3.659 and 1.286 respectively

In the current study, an increase in MDA activity was seen in both species calm compared to the control group, MDA is widely used as a biomarker of oxidative stress, and an increased level of oxidative damage in terms of lipid oxidation has been reported in different species of bivalve exposed in vitro to environmental pollutants (Chen *et al*.,2015).

Excessive ROS levels are harmful and can cause damage to the cell membrane, leading to lipid peroxidation (Bejaoui *et al*., 2019; Rusdi *et al*., 2021). Rusdi *et al*. (2021) suggest that the elevated MDA level in mussels (*Pernaviridis*) indicates that an organism has experienced oxidative stress. Sarica et al.,(2019) indicated the role of $k_2Cr_2O_7$ in increasing lipid peroxide production.

According to Chaâbane *et al*. (2020) Chromium (VI) toxicity generates ROS that may lead to adverse effects on bivalves *Venus verrucosa* by modifying their fatty acid levels due to oxidative damage.

In the current study, *P. euphraticus and C. fluminea*, two species of clams, were used to test the viability of utilizing *Chlorella vulgaris* to lessen potassium dichromate toxicity.

Several studies indicated a high ability of *C.vulgaris* to remove Cr (VI) from aqueous media, such as, Wang *et al.* (2021b) reported the ability of *C. vulgaris* to remove chromium at a rate of 59.91%, and the resilience of Cr-treated algae was confirmed by the recovery of antioxidant activities, especially SOD and MDA content, within 72 hours.

Zhou *et al*. (2020) reported a high ability of *C. vulgaris* to remove chromium; it had a removal rate of chromium (III) nitrate (89.0 ± 3.2%) and chromium (VI) dichromate (88.1 ± 1.3%) over 72 h. while Shen *et al*.(2019), recorded that *C. vulgaris* was able to remove varying concentrations of Cr (VI) from water with a removal efficiency that ranged from 40 to 100%.

Yen *et al*. (2017) investigated chromium (VI) removal using live and dead cells of microalgae, and they found that removal of Cr by living algal cells occurred not only through uptake but also via bioreduction, such as the enzymatic reduction of Cr (VI) to Cr (III). Pagnanelli *et al*. (2013) showed that biosorption and bioreduction mechanisms were contributors to Cr (VI) removal by *C. vulgaris.*

Daneshvar *et al*.(2019) indicated that microalgae can reduce the toxic effects of Cr (VI) by bio-absorbing it on the microalgae cell wall by binding to functional groups such as hydroxyl and carboxyl groups and converting Cr (VI) into non-toxic Cr (III). Consistent with the study by Luo *et al*. (2023), *C.*

vulgaris is a typical green alga often used to remove Cr from aquatic environments because of its high efficiency, economy, and ability to absorb Cr.

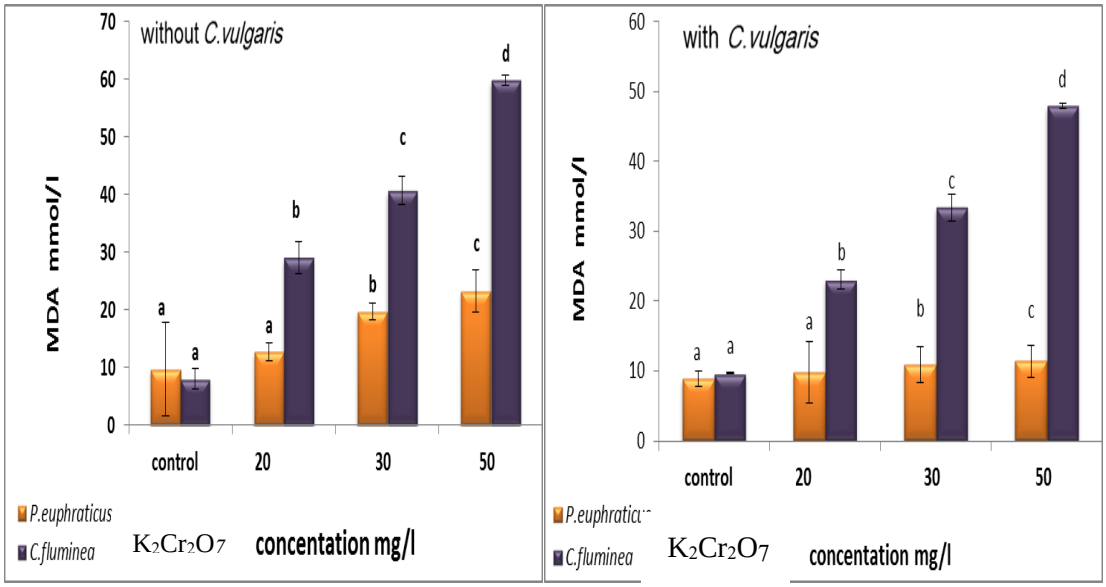

Figure 3-16: Effect of $K_2Cr_2O_7$ on MDA activity in both species of clams.

Table 3-9.The biochemical markers *in P.euphraticus* during acute exposure period to potassium dichromate. (Min., Max., Mean±SD of three replicates).

Biochemical markers	without Immobilization of Algae (*C. vulgaris*)				Immobilization with Algae (*C. vulgaris*)			
	control	20 mg/	30 mg/l	50 mg/l	Control	20 mg/l	30 mg/l	50 mg/l
ROS (µg/mg)	10.74–14.77 12.58 ± 1.76 a	30.70-44.17 37.50± 6. 73 b	36.31-45.94 40.97 ±4.82 c	45.08 – 57.20 51.05 ± 6.06 d	9.53 – 13.54 11.80 ± 2.05 a	31.62 –36.55 33.88± 2.48 b	34.03 – 38.69 36.86± 2.66 c	40.06 – 43.64 42.06 ± 1.82 d
SOD(U/ml)	21.62– 27.03 24.02 ± 2.75 a	27.62-29.05 28.36 ±0.71 b	29.73-32.43 31.10 ± 1.35 b	33.55 - 36.04 33.40 ± 1.44 c	19.11– 21.62 20.35 ± 1.255 a	24.32-29.73. 23.36 ±1.18 b	25.62 - 28.92 27.43 ± 1.67 b	33.25 – 29.34 31.41 ± 1.96 c
CAT (KU/L)	22.67 – 25.13 23.60 ± 1.33 a	26.61 - 27.16 26.88 ±0.38 b	30.52 - 31. 20 30.86 ± 0.48 c	45.99-35.41 40.32 ± 5.30 d	21.59– 24.59 22.82 ± 1.57 a	25.08-26.45 25.76 ± 0.68 b	24.11 - 29.25 26.30 ± 2.65 c	25.27-28.92 27.16 ± 1.82 d
GPX (U/L)	6.18-9.06 7.99 ± 1.57 a	4.26 – 8.42 6.34 ± 2.08 a	2.78 – 7.42 5.28 ± 2.34 b	3.70 - 5.34 4.35 ± 0.86 c	6.14 - 6.42 6.28 ± 0.14 a	6.66– 8.02 7. 34 ± 0.68 a	3.10 – 4.34 3.57± 0.67 b	3.02 – 5.10 3.87 ± 1.08 a
GSH(µg/ g)	18.99 - 25.56 21.30 ± 3.69 a	14.38 - 18.46 16.30 ±2.05 b	18.15-16.06 18.01 ± 1.88 c	6.42 - 10.79 8.57 ± 2.18 d	15.00 - 22.77 18.78 ± 3..88 a	18.46-25.70 22.47 ±3.68 b	11.03-18.01 14.52 ± 4.39 c	6.42 - 10.79 9.7 ± 2.89 d
Ascorbic acid (AA) µM	24.52 - 26.54 25.36 ± 1.05 a	26.35 - 27.11 26.37 ± 0.38 a	24.14 - 26.29 25.27± 1.08 a	27.68-19.12 20.37± 1.28 b	23.57 - 24.27 23.92 ± 0.34 a	22.86 - 26.10 24.83 ± 1.73 a	26.73-26.99 26.88 ± 0.13 b	27.03 - 28.86 27.94 ± 0.91 b
Total protein (mg/g)	10.29 – 13.95 12.12 ± 1.83 a	10.16 - 11.39 10.86 ± 0.62 a	15.42 - 17.25 17.25 ± 1.85 b	11.03-14.43 12.79 ± 1.70 a	28.24 – 30.07 29.21 ± 0.92 a	9.93 - 12.22 10.93 ± 1.17 b	12.49 - 18.35 15.42± 2.93 c	14.40-15.50 13.56 ± 1.06 d
MDA (µmol/l)	8.00 - 11.70 9.70± 1.86 a	10.77-14.6 12.68 ± 2.70 a	17.38-12.38 19.69 ± 2.52 b	22.38-24.13 23.22 ± 0.87 c	5.77 – 5.86 8.86 ± 0.09 a	8.40– 11.22 9.81 ± 1.41 a	8.97-12.82 10.87± 1.92 b	11.5-11.73 11.44 ± 0.41 c

*similar letters mean that there were no significant differences between treatments.

** Different letters mean that there were significant differences between treatments

Table 3-10. The biochemical markers *in C. fluminea during* acute exposure period to Potassium dichromate. (Min., Max., Mean±SD of three replicates).

Biochemical markers	without Immobilization of Algae (*C. vulgaris*)				Immobilization with Algae (*C. vulgaris*)			
	control	20 mg/l	30 mg/l	50 mg/l	Control	20mg/ l	30 mg/l	50 mg/l
ROS (µg/mg)	14.43-18.13 16.71± 1.99 a	27.82-29.65 27.89± 1.5 b	49.86-56.52 53 .0± 3.86 c	63.41-66.99 63.66 ±3.21 d	13.8-15.78 14.71± 0.99 a	39.78-42.64 41.03 ±1,46 b	29.45-30.43 31.14± 2.14 c	49.78 - 52.59 51.0± 1.41 d
SOD(U/ml)	13.03-17.62 15.02± 2.35 a	34.42-37.05 35.69± 1.31 b	41.43-45.14 43.10± 1.88 c	52.43-56.55 54.04± 2.2 d	9.67-11.45 10.91± 1.08 a	28.81 -25.45 27.19 ±1.68 b	40.33-38.68 39.59± 0.84 c	45.89-47.89 46.74± 1.03 d
CAT (KU/L)	8.13-11.57 9.71 ±1.73 a	21.67-29.18 24.55± 4.04 b	38.27-42.66 40.51± 2.19 c	44.41-50.58 48.99±4 .01 d	6.10-9.78 8.26±1. 92 a	18.5-25.4 21.36 ±3.59 b	33.4-36.75 35.31± 1.72 c	39.78-41.67 40.67± 0.92 d
GPX (U/L)	9.74 -13.06 10.99± 1.80 a	15.26-22.52 19.05± 3.53 b	33.42-37.78 35.62± 2.18 c	40.70-45.34 43.35± 2.39 d	8.67-10.67 9.37±1 .12 a	15.67 -19.68 18.11 ±2.14 b	28.67 -34.95 32.50± 3.36 c	34.56 -40.67 37.99± 3.12 d
GSH (µg/g)	44.56-52.78 49.14± 4.19 d	35.06-38.52 36.86± 1.73 c	20.46-27.06 23.30± 3.39 b	10.53-14.51 12.79± 2.04 a	35.23-40.47 37.58 3.70 d	29.78-31.46 30.26 ±1.18 c	13.56-16.67 15.11± 2.19 b	6.56-8.53 7.35±1 .50 a
Ascorbic acid (AA) µM	14.2-16.35 15.6±1 .52 a	33.56-37.22 35.3±2 .58 b	40.40-43.13 42.5±1 .93 c	48.22-53.14 50.37± 3.45. d	9.45-13.45 11.45± 2.82 a	21.35-25.56 23.67 ±2.97 d	29.24-32.65 31.24± 2.41 c	37.11-40.67 38.89± 2.51 a
Total protein (mg/g)	45.91-51.25 47.76± 3.01 a	35.81-43.16 39.11± 3.73 b	26.25-30.08 28.58± 2.04 c	14.13-18.43 16.31± 2.28 d	36.78-48.67 42.54± 5.96 a	34.65-36.67 35.69 ±1.01 b	31.89-33.67 32.83± 0.89 c	16.89-23.67 20.44± 3.40 d
MDA (µmol/l)	6.70-9.40 8.033± 1.35 a	27.68-30.68 29.01± 1.52 b	39.42-42.33 40.69± 1.50 c	57.38-60.13 59.88± 3.69 d	8.55-10.8 9.67±1 .12 a	19.06-27.78 23.06 ±4.40 b	30.67-35.78 33.40± 2.57 c	45.9-50.45 47.92± 2.31 d

*similar letters mean that there were no significant differences between treatments.
** Different letters mean that there were significant differences between treatments.

3-5 Histological Biomarkers

3-5-1 Digestive Gland and Gills in clams (*P. euphraticus*) exposure to Amoxicillin

3-5-1-1 Digestive Gland in clams *P. euphraticus* exposure to Amoxicillin

plate (3-1)shows the digestive gland of the clams *P. euphraticus* in control groups, which included regular digestive cells, a digestive gland lumen, normal tubules, hemolymphatic between tubules, basohilic cells, and tubular epithelium.

In contrast, several histological modifications were seen in concentrations of 200–300 mg/L of amoxicillin, including shrinkage of epithelial cells, an expanded nucleus, a pyknotic condition of cells, enlarged vacuoles, and increased hemolympheal gaps,immobilized *C. vulgaris* was present or not .A & B with *C. vulgaris* ,C&D without *C. vulgaris*, AMX 200,300 mg/L, in plate 3-2.

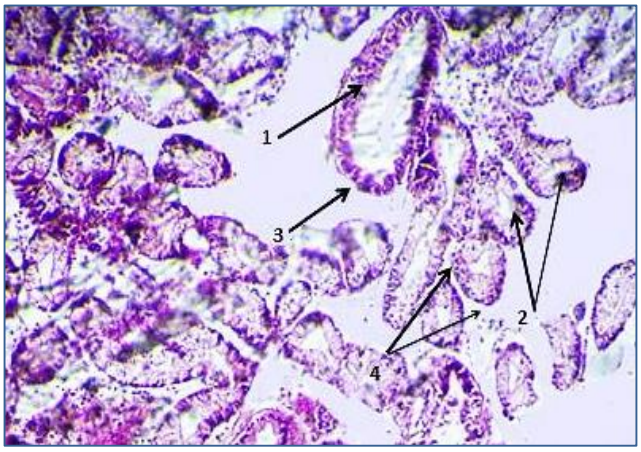

Plate (3-1): histological Section of the digestive gland for *P. euphraticus* (control) 1. basohilic cell, 2. digestive lume, 3. basement membrane, 4. hemolymphac gap between tubules, 5. typical tubules, 6. connective tissue , (H & E, Scale bar; 10X).

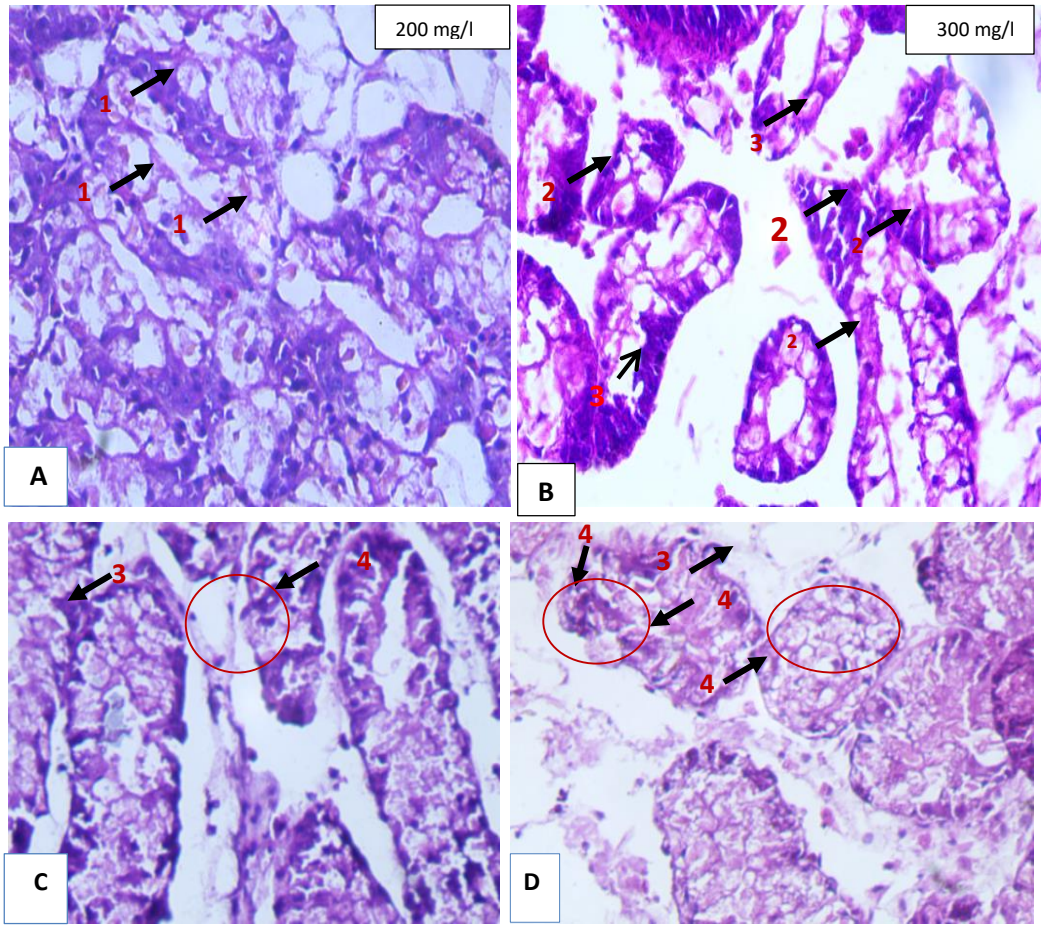

plate (3-2) .Cross section of the *P. euphraticus* clams digestive gland , showed 1. shrinkage epithelial cells, swollen nucleus, 2. pyknotic state of cells enlarged vacuoles ,3. enlarged hemolymphe spaces ,4. basophilic triangular shaped cell present in the connective tissue among the tubule((H & E, Scale bar 40x) .A & B with *Chlorella vulgaris* ,C&D without Chlorella vulgaris, AMX 200,300 mg/L

3-5-1-2 Gills in clams *P. euphraticus* exposure to Amoxicillin experiment.

Histological changes of the gills were seen following AMX treatment at 200–300 mg/l, as illustrated in plate (3-4), which included epithelial lifting, secondary lamellar rupturing, secondary lamellar curling, and hyperplastic interlamellar epithelium. In contrast, typical clams gills contain cilia, dorsal epithelia, hemocoelic space, and ventral epithelia, as shown in figure 3- 3.

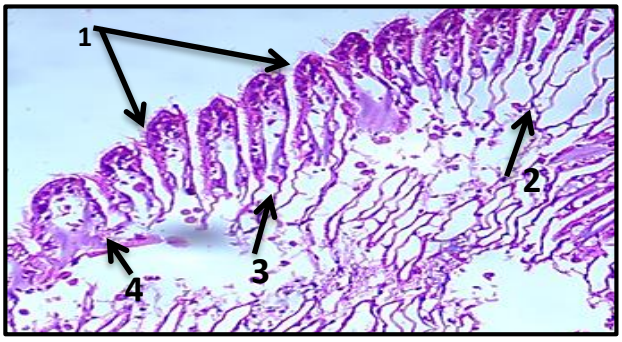

plate 3- 3: histological section of gills in clams *P. euphraticus* (control): 1. Cilia, 2. Haemocoelic Space, 3. Ventral Epithelia, 4. Dorsal Epithelia, (H & E, Scale bar 10X).

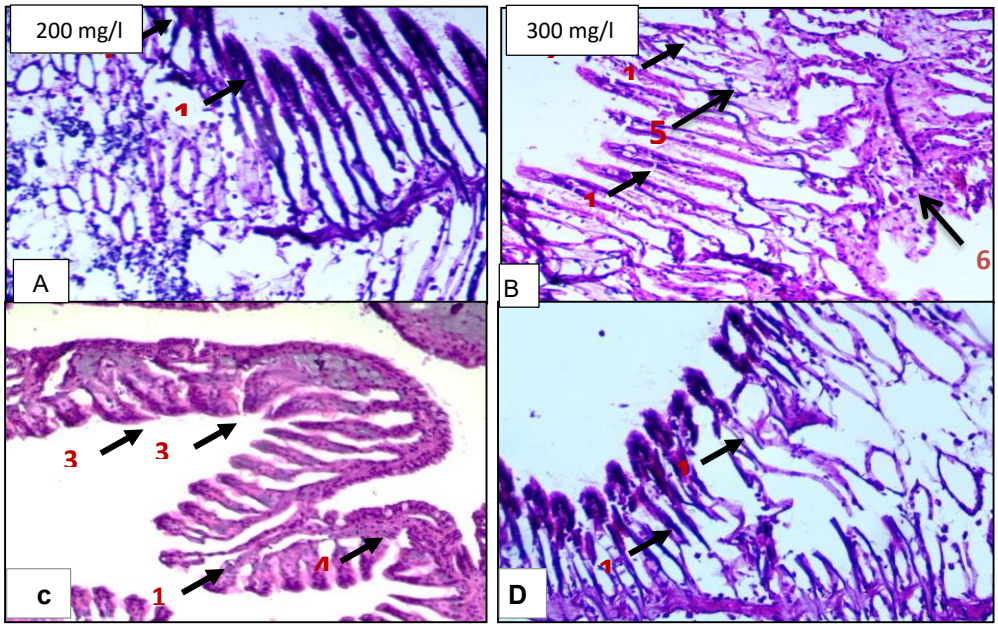

plate 3-4 . Cross section of clams *P. euphraticus*, showed 1. epithelial lifting,secondary lamellar rupturing ,3. curling of secondary lamellae ,4. hyperplastic interlamellar epithelium,5. Necrosis 6, haemocytes (H & E, Scale bar 10X) , A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris*, AMX 200,300 mg/L

3-5-2 The Digestive Gland and Gills in clams (*P. euphraticus*) exposure to Potassium Dichromate experiment.

3-5-2-1 Digestive Gland in clams *P. euphraticus* exposure to Potassium Dichromate

plate 3-6 shows the histological changes in the digestive gland of *P. euphraticus*. After being exposed to 30 and 50 mg/l of potassium dichromate. In contrast, plate 3-5 shows the digestive gland of clams *P. euphraticus* typical histological structure in control groups, which included normal digestive cells and lumen, normal tubules, intertubular tubules, basal cells, and tubular epithelium, immobilized *C. vulgaris* was present or not .

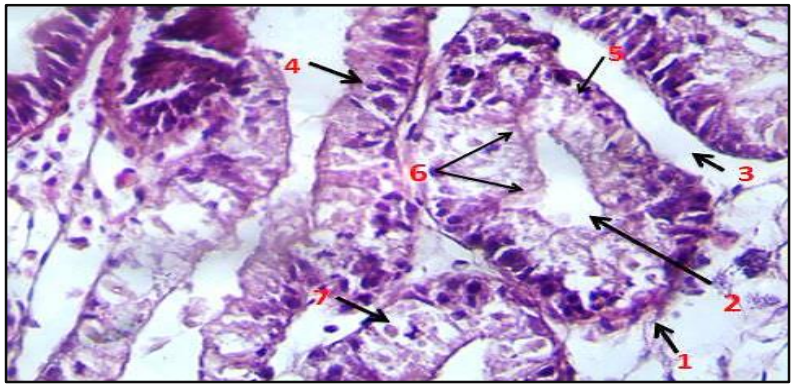

plate (3-5). histological Section of the digestive gland for *P. euphraticus* (control) 1. digestive tubule, 2. digestive lume ,3. hemolymphac gap between tubules,4. basohilic cell ,5. epithelial cell, 6. digestive cell, 7. Vacuole . (H & E, Scale bar 40X).

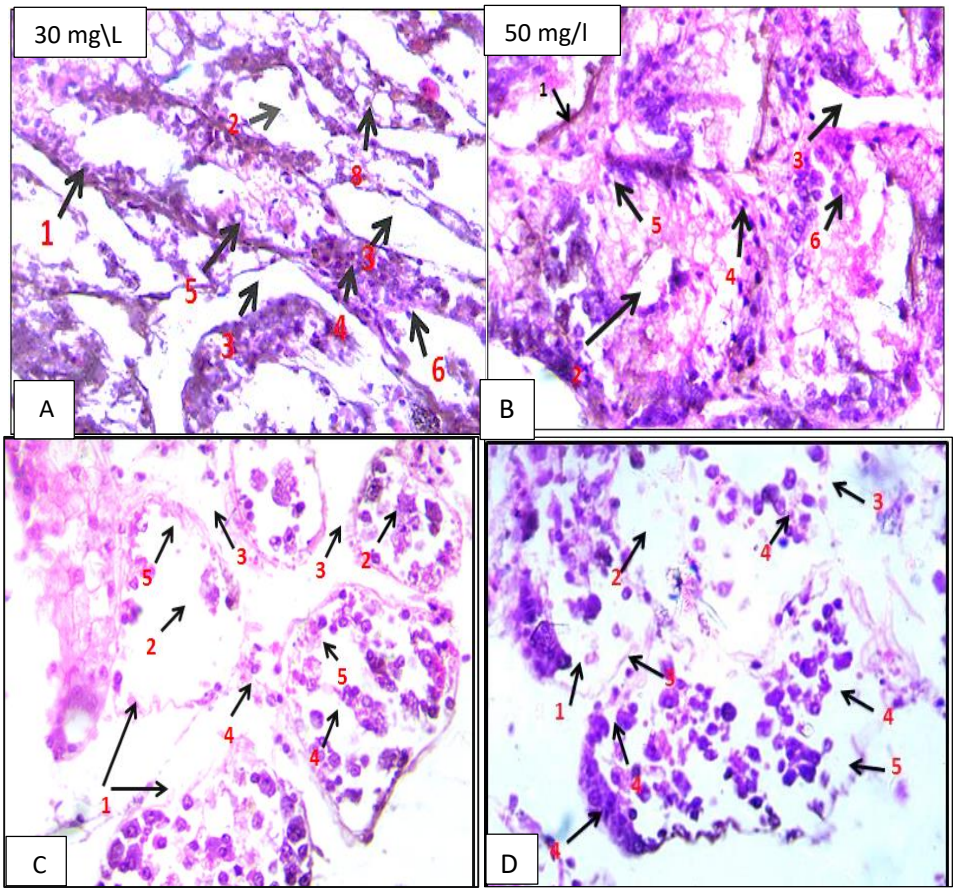

plate (3-6) . Cross section of the *P. euphraticus* clams digestive gland , showed 1. degeneration of cell with enlarged vacuoles, 2.anylaysis tubular lumen, 3. large hemolymphac gap between tubules ,4. basophilic triangular shaped cell present in the connective tissue among the tubuleanlysis epithelial cell ,5.diffuse hemocytic infilteration of connective tissue (,6. pyknotic state of cells enlarged vacuoles(6). A &B without *C.vulgaris* and C&D with *C.vulgaris* , $K_2Cr_2O_7$ 30,50 mg/L, (H & E, Scale bar 40X).

3-5-2-2 Gills in clams *P. euphraticus* exposure to Potassium Dichromate.

Histological sections of clams *P. euphraticus* showed many histological changes after acute exposure to 20 and 50 mg/l of potassium dichromate plate (3-8). In contrast to the control group plate (3-7), which showed normal gill histological that contained cilia, dorsal epithelium, squamous space, and ventral epithelium.

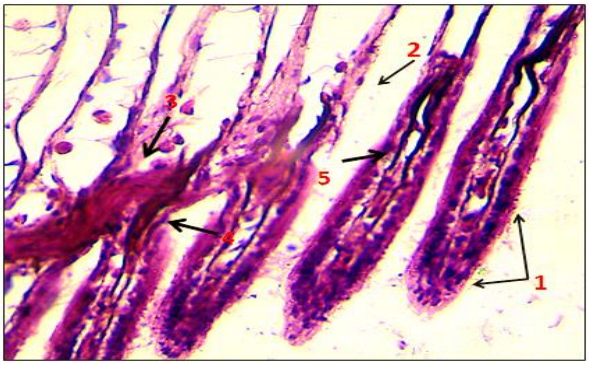

plate (3-7)*:* histological section of gills in clams *P. euphraticus* (control):1. Cilia, Haemocoelic Space, 2.Ventral Epithelia ,3. Dorsal Epithelia ,4. haemocytes, (H & E, Scale bar 40X).

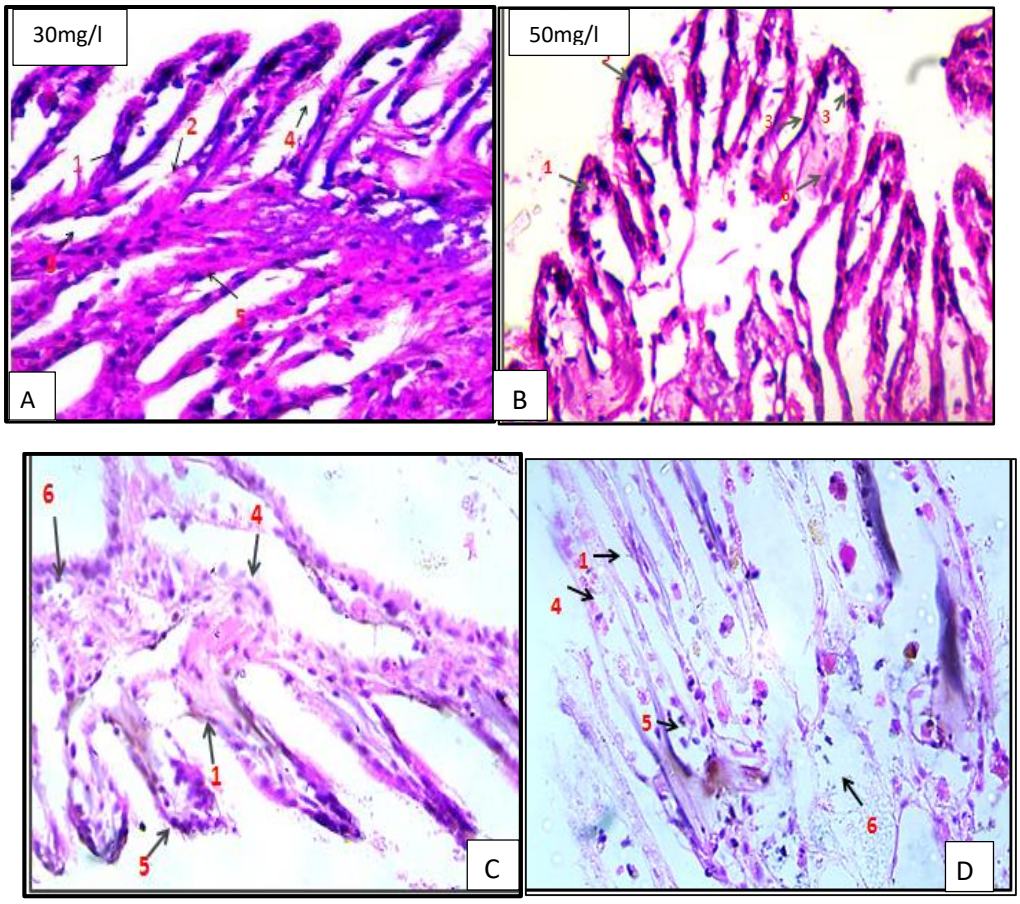

plate (3-8). Cross section of clams *P. euphraticus* of gills, revealed 1. epithelial lifting , 2. secondary lamellar rupturing ,3. curling of secondary lamellae, 4. hyperplastic interlamellar epithelium , 5. frontal fusion of cilia , 6. hemocytes blocking of hemolymph (H & E, Scale bar 40X). A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris*, $K_2Cr_2O_7$ 30,50 mg/L

3-5-3 Digestive Gland and Gills in clams (*C. fluminea*) exposure to Amoxicillin

3-5-3-1 Digestive Gland in clams *C. fluminea* exposure to Amoxicillin experiment

The digestive gland of clams *C. fluminea* exhibited typical histological structure in control groups, which contained basohilic cells, digestive lume, basement membrane, hemolymph gap between tubules, typical tubules, and connective tissue plate (3-9). In contrast, many histological alterations were seen after exposure to 200 and300 mg/L of amoxicillin in plate (3-10), including degeneration of cells with enlarged vacuoles, narrowing of the tubular lumen, basophilic triangular-shaped cells present in the connective tissue among the tubules, and dilation in the hemolymphatic spaces between tubules. In the presence and absence of immobilized *C. vulgaris.*

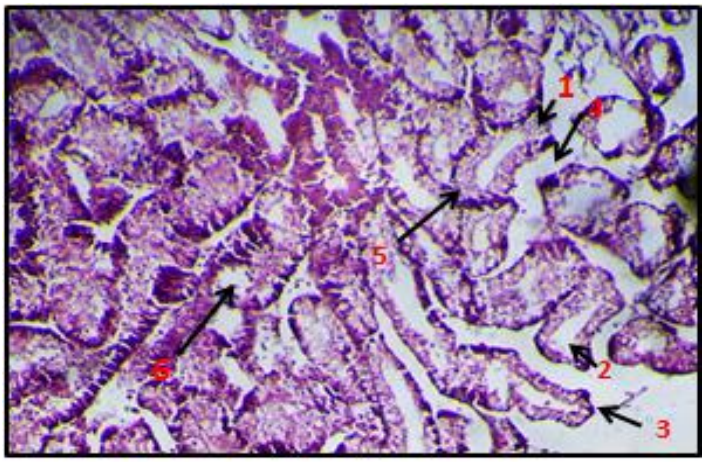

plate (3-9). histological Section of the digestive gland for *C. fluminea* (control) 1. basohilic cell, 2. digestive lume, 3. basement membrane, 4. hemolymph gap between tubules; 5. typical tubules;6. connective tissue ,(H & E, Scale bar:10X.)

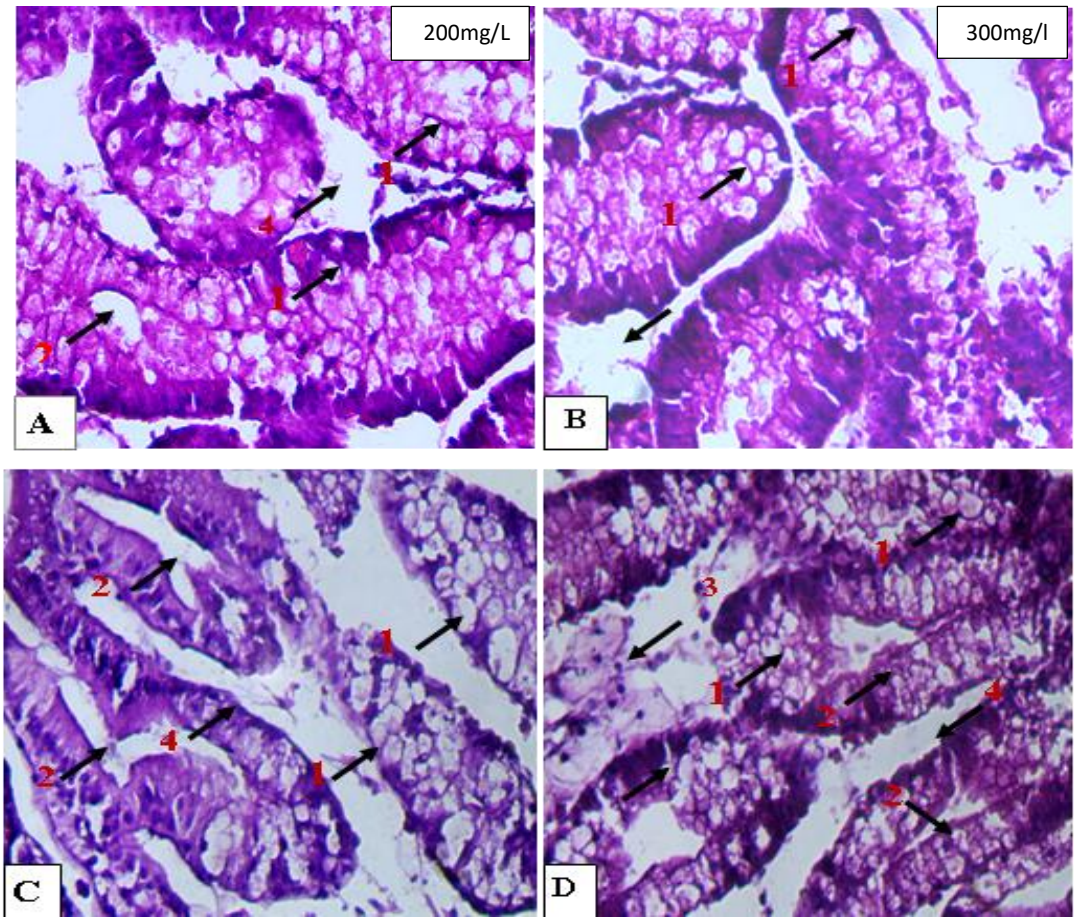

plate (3-10). Cross section of the for *C. fluminea* digestive gland , showed 1.degeneration of cell with enlarged vacuoles, 2. narrowing of tubular lumen , 3. basophilic triangular shaped cell present in the connective tissue among the tubule, 4.and dilation in the hemolymphatic spaces between tubules. A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , AMX 200,300 mg/L , (H & E, Scale bar 40x).

3-5-3-2 Gills in clams *C. fluminea* exposure to Amoxicillin experiment

The gills of *C. fluminea* clamss showed a typical histological structure in the control groups, which contained cilia, dorsal epithelium, hemocoely space, and ventral epithelium plate 3-11.

In contrast, plate 3-12 shows several histological changes after exposure to 200–300 mg/L amoxicillin for 7 days, including secondary lamellar rupture, secondary laminar wrinkling, hyperplastic interstitial epithelium, and frontal fusion of the cilia. In the presence and absence of *Chlorella vulgaris*.

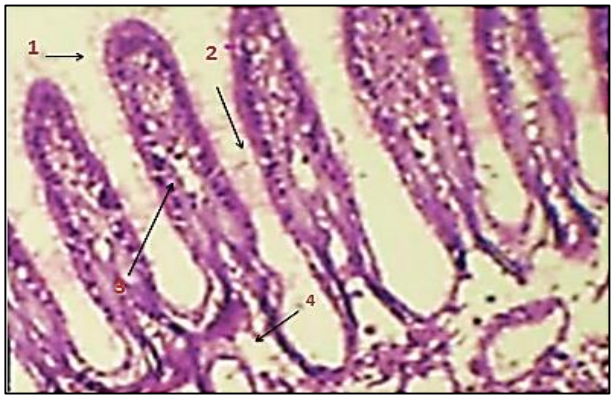

plate (3- 11) *:* histological section of gills in clams *C. fluminea* (control): 1.Cilia(1), 2.Haemocoelic Space, 3.haemocytes, 4.Dorsal Epithelia ((H & E, Scale bar 40x)

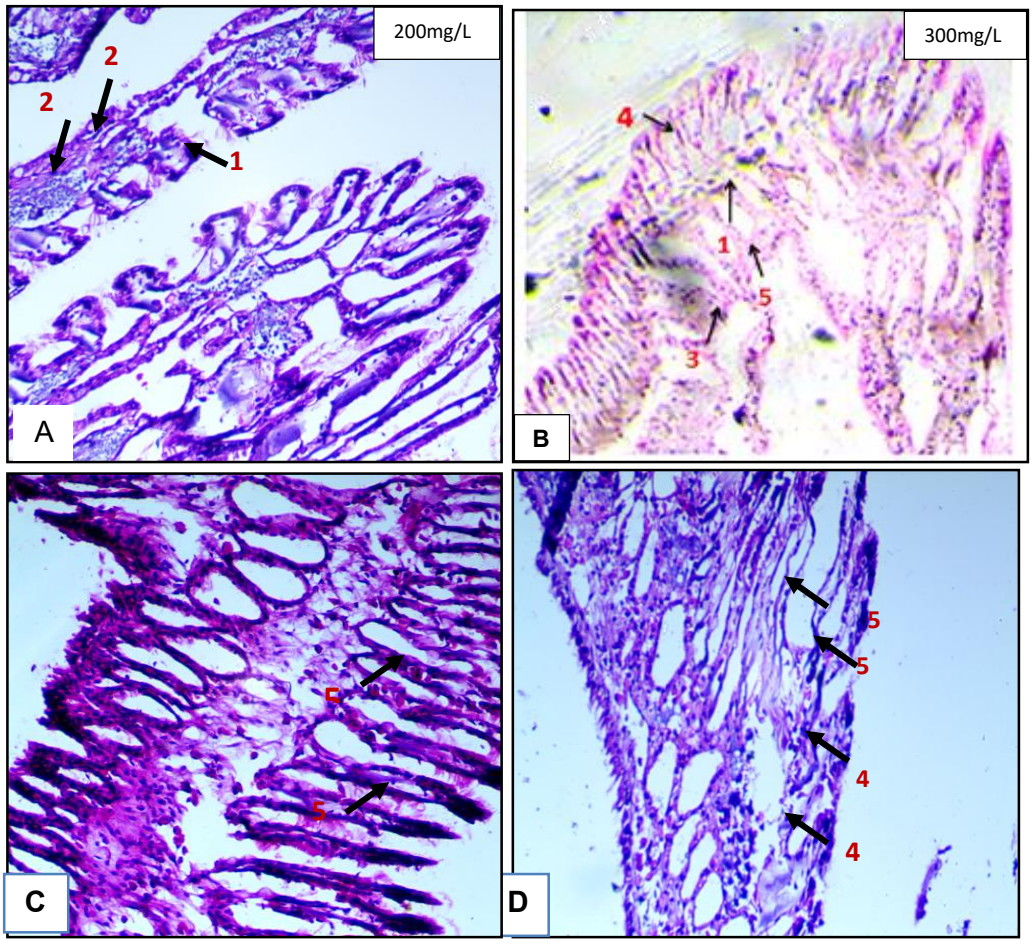

plate (3-12). Cross section of *C. fluminea* gills ,1. showed epithelial of secondary lamellar rupturing , 2. secondary lamellar sloughing shortening ,3.haemocyte , 4. frontal fusion of cilia(, 5. and epithelial lifting, 6. necrosis , A & B with *Chlorella vulgaris* ,C&D without *Chlorella* ((H & E, Scale bar 40x).

3-5-4 Digestive Gland in clams *C. fluminea* exposure to potassium dichromate experiment

3-5-4-1 Digestive Gland in clams *C. fluminea* exposure to potassium dichromate experiment

The digestive gland of clams *C. fluminea* exhibited typical histological structure in control groups, which contained digestive gland lum, hemolymph gap between tubules, typical tubules, and Tubular epithelium plate(3-14). In contrast, many histological alterations were seen after exposure to 20 and 50 mg/L of potassium dichromate plate (3-13), including degeneration of cells with enlarged vacuoles, narrowing of the tubular lumen, basophilic triangular-shaped cells present in the connective tissue among the tubules, and dilation in the hemolymphatic spaces between tubules. In the presence and absence of immobilized *C. vulgaris.*

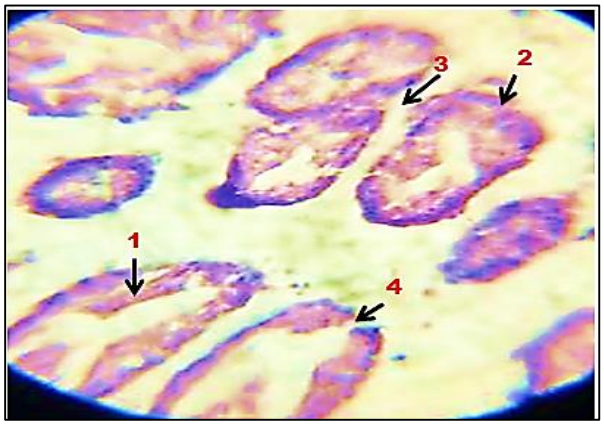

plate (3-13). histological Section of the digestive gland for *C. fluminea* (control) 1. digestive lumen, 2. Tubular epithelium, 3.hemolymphac gap between, 4.typical tubules. (H & E, Scale bar:10X)

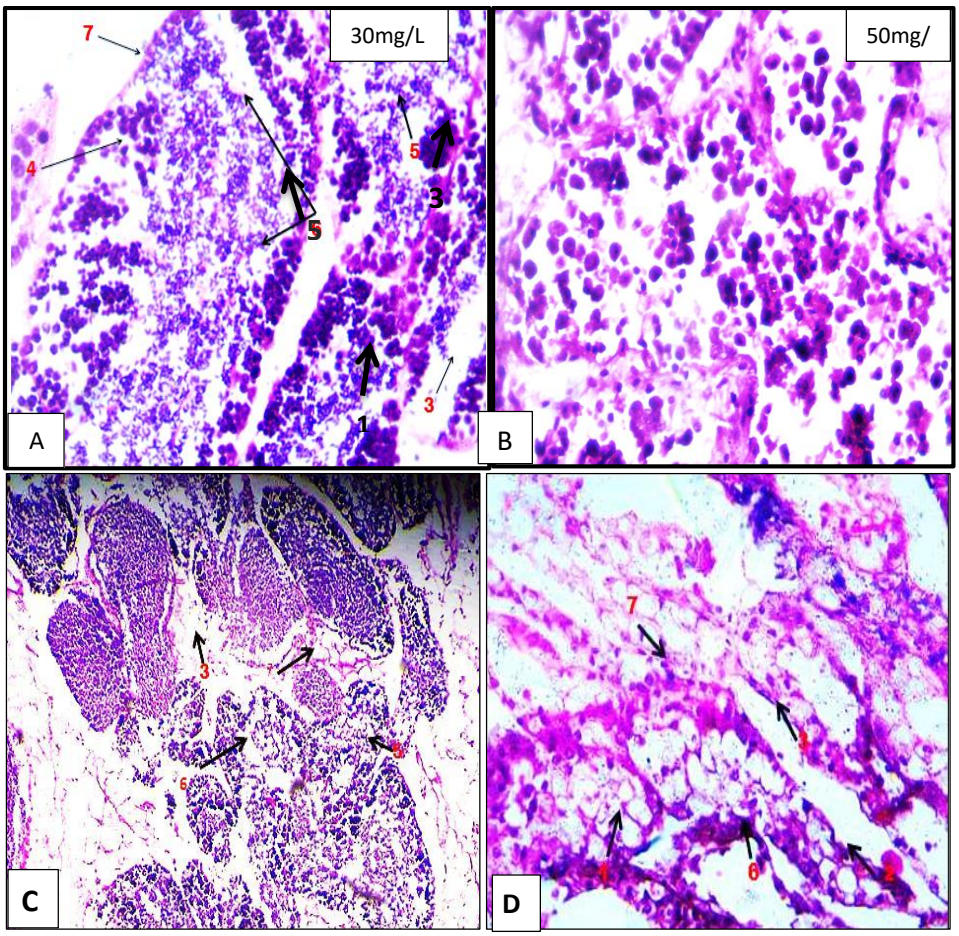

plate (3-14). Cross section of the *C. fluminea* digestive gland , showed 1. shrinkage epithelial cells, swollen nucleus , 2. pyknotic state of cells enlarged vacuoles (2), enlarged hemolymphe spaces , 3. basophilic triangular shaped cell present in the connective tissue among the tubule 4. Necrosis,5. anylaysis tubular lumen,6. diffuse hemocytic infilteration of connective tissue, 7. lymphocytes(8). A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , $K_2Cr_2O_7$ 30,50 mg/L, (H & E, Scale bar: 40x).

3-5-4-2 Gills in clams *C. fluminea* exposure to potassium dichromate experiment

plate 3-15 showed normal gill histological that contained cilia, squamous space, and ventral epithelium in control groups ,in contrast, histological sections of clams *C. fluminea* showed many histological changes after acute exposure to 30 and 50 mg/l of potassium dichromate plate 3-16.

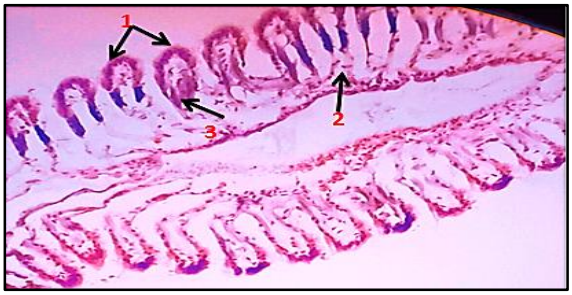

plate 3-15: histological section of gills in clams *C. fluminea* (control):1. Cilia, 2. Haemocoelic Space ,3. Ventral Epithelia , (H & E, Scale bar: 10X).

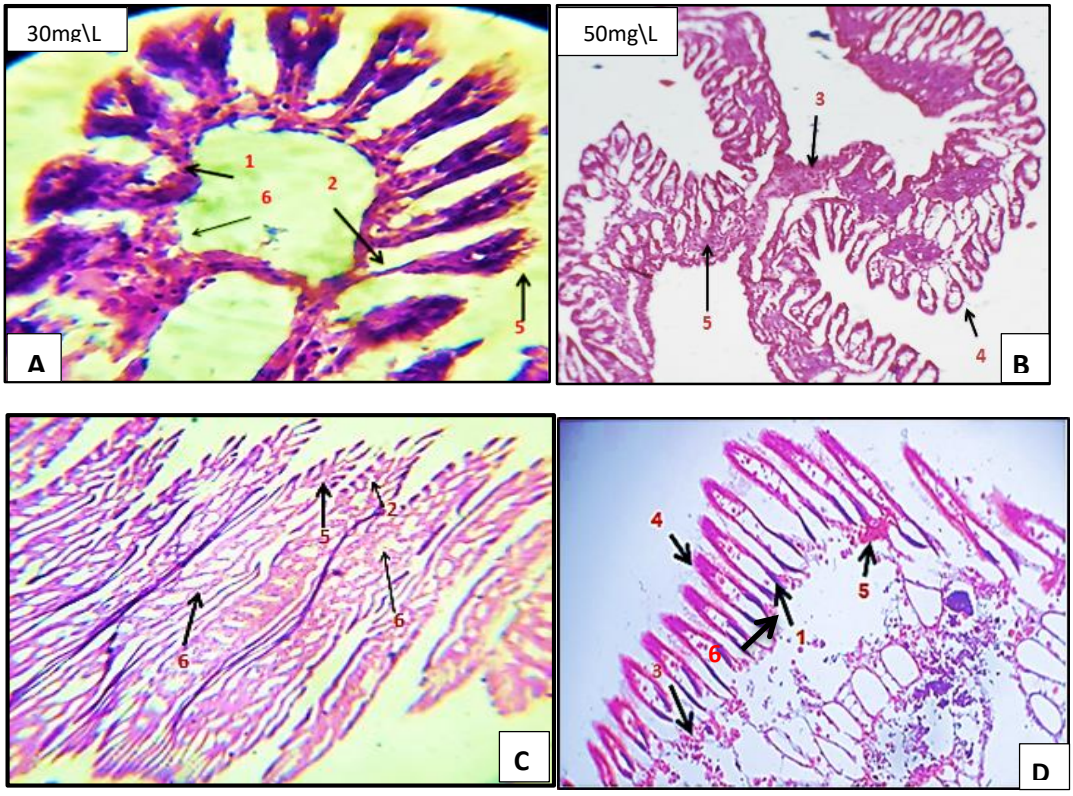

plate (3-16). Cross section of *C. fluminea* gills , showed 1. epithelial of secondary lamellar rupturing , 2. secondary lamellar sloughing shortening , 3. haemocyte ,4. frontal fusion of cilia, 5. epithelial lifting 6. Necrosis A & B with *Chlorella vulgaris* ,C&D without *Chlorella vulgaris* , $K_2Cr_2O_7$30,50 mg/L(H & E, Scale bar: 20x).

Histological investigation of a group of untreated bivalves, *P. euphraticus* and *C. fluminea* revealed the histological structure of normal digestive glands as plate (3-1), (3-5), (3-9) and (3-13). In contrast, Histological changes in the digestive glands, which appear as biomarkers of environmental stress, are shown in plate (3-2), (3-6), (3-8) and (3-13) (C, D), which include cell degeneration, amoebic cell infiltration, tubular lumen narrowing, dilation of intertubular spaces, cell degeneration, and cell necrosis, and it has been observed that these histological changes in the digestive glands were less in the experiment with immobilized algae, as shown in plate (3-2), (3-6), (3-8) and (3-13) (A, B).

Histological investigation of a group of untreated bivalves, *P. euphraticus* and *C. fluminea* revealed the histological structure of normal gills as shown in plate (3-3), (3-7), (3-11) and (3-15).In contrast, gill tissues showed a variety of morphological changes as biomarkers of oxidative stress, including moderate epithelial uplift.

As a result of hypertrophy, adjacent secondary lamellae have fused, The secondary lamellae showed interlaminar hyperplasia, desquamation, necrosis, and cellular hyperplasia, which are characteristic of gills affected contamination Bassey (2019), as shown in plate (3-4), (3-8),(3-12) and (3-16), but it was observed that these histological changes in the gills were a lesser degree in an experiment with deactivated algae, as shown in plate (3-4), (3-8),(3-12) and (3-16) (A, B).

Histopathological studies of target organs along with the studies of oxidative stress would give the complete picture of pharmacetical hazards and their toxic potential in aquatic animals .Analysis of histological malformations is a widely used method to assess the effects of toxicants and the environment (Hussain *et al*., 2022). The utilization of gills and digestive

glands in this study is currently due to their importance in the metabolization of contaminants such as medicines and detoxification processes. Because the gills and digestive glands are target tissues for many contaminants, they were chosen to evaluate the drug's effects; the digestive glands are primarily responsible for the accumulation and metabolism of organic contaminants (Trombini *et al.*, 2019).The gill is an essential organ in bivalve molluscs that aids in respiration and the sifting of food, and analysis of histological abnormalities is a popular technique for determining how toxicants and the environment affect organisms (Hossain *et al*., 2023). According to Joshy *et al.* (2022), increased concentrations of toxicants, such as pharmaceuticals, metals, pesticides, etc., impede the metabolic functions of cells inside tissues, which results in cell death, hyperplasia, or cellular inequality.

The most commonly observed histological alterations in these study included necrosis, vacuolation, haemocyte infiltration and granulocytomas, in the digestive gland, as well as necrosis, edema and epithelial hyperplasia in the gills.Necrotic cell death and excessive vacuole formation are common diseases of muscle tissue due to exposure to toxins (Alonso *et al*., 2019).

Cell morphology observation is the most essential method in toxicological investigations (Wu *et al*., 2019). Histopathological abnormalities are often used as biomarkers in assessing the health of organisms exposed to contaminants and have been regarded as the most effective technique for evaluating toxic effects in both laboratory and field research (Shah and Parveen, 2022). The current data show that there is a link between acute exposure to amoxicillin antibiotics and potassium dichromate and the histological changes observed in various tissues of the clams. The histological changes observed in this study agree with many studies, such as those studied by Rodrigues *et al*. (2019) and the histopathological impact of

anti-erythromycin on Oncorhynchus, which caused gradual gill histological changes such as mucosal hyperplasia, hyperplasia of the epithelial cells, and hypertrophy of mucous cells.

The biochemical tests demonstrated that the balance of the antioxidant system was disrupted as a result of the increase in the production of ROS, leading to oxidative damage to the digestive gland, and with an increase in the concentration of amoxicillin and potassium dichromate. As a result, severe histopathological changes with oxidative damage appeared. It is consistent with the findings of the study by Guo *et al*., 2022 toxic effects of ciprofloxacin on the histological properties of *C. fluminea* in various substrates: high CIP concentrations caused an expansion of the tubule lumen and thinning of the epithelium in the digestive gland.

studied Awoke and Nkwuda (2019),chloramphenicol-induced histopathological changes in the gills of juvenile *Clarias gariepinus*, Histopathological changes included tubular lumen obstruction, blood vessel epithelial congestion degeneration, lamellar fusion, and primary and secondary gill lamellae degeneration.

Sheir (2020) investigated the histology responses of the clam *Caelatura nilotica* in contaminated areas. It was noted that the histological composition of the gills and digestive glands changed, the appearance of cilia between the gill threads., the epithelium lost its integrity, and the digestive gland showed infiltration of blood cells and necrosis in some digestive tubes.

Many studies that are consistent with a current study showing that exposure to hexavalent chromium causes histopathological changes in the gills and digestive glands, such as the study of Mishra and Mohanty. (2008) Histological changes were observed in the gills of *Channa punctatus* after

acute exposure to hexavalent chromium as epithelial hyperplasia, edema, lamellar fusion, epithelial necrosis and desquamation, epithelial elevation, and secondary lamellar wrinkling.

Wang *et al*., 2020, discovered that Cr(VI) exposure caused histological alterations in *Geloina erosa* gills, including swelling of epithelial cells and cilia destruction.

In this experiment, it was found that amoxicillin and potassium dichromate cause histological abnormalities in the digestive gland and gills of these two species of clams, perhaps due to their ability to cause oxidative stress, and potassium dichromate was the most responsible for causing histological changes in both species, perhaps because of its toxicity. Whereas in an experiment treated with immobilized *C. vulgaris* inhibition, histological changes may have been less severe, possibly due to the ability of this type of algae to degrade these chemicals and convert them into less dangerous compounds, thus reducing the oxidative stress of the clams (Ricky *et al*.,2022; Ndlela ***et al***.,2023).

3-6 Principal Component Analysis (PCA)

3-6-1 Correlation between Amoxicillin and biochemical markers in Clam *C. flumine*

In a current study, there is a correlation between amoxicillin concentrations and a biochemical marker in *C. fluminia*. In the case of without immobilization algae (*C. vulgaris)*, TP showed a positive correlation with CAT, SOD, and while GPX showed a positive inverse correlation, while GSH, AA, ROS, and MDA showed a negative inverse correlation. In the case of immobilization algae (*C. vulgaris)*, CAT, SOD, and GPX showed a positive correlation, whereas GSH, AA, ROS, MDA, and TP showed a negative relationship. Figure 3-17

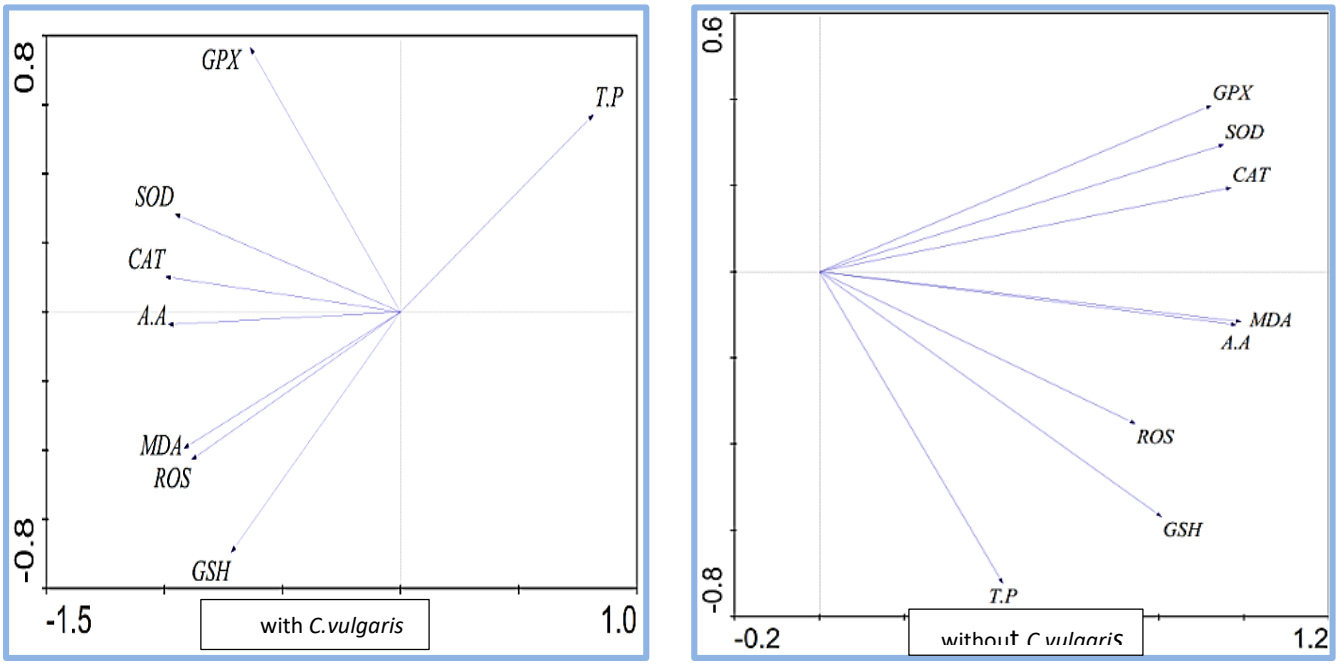

Figure 3-17: Correlations between the antibiotic amoxicillin and biochemical markers in Clam *C. fluminea*

3-6-2 Correlation between Amoxicillin and Biochemical Markers in Clam *P.euphraticus*

A current study found a correlation between amoxicillin concentrations and a biochemical marker in Clam *P. euphraticus.* In the absence of immobilization (*C. vulgaris*), TP and GPX showed negative correlation, AA and GPH showed positive inverse correlation, while SOD, CAT, ROS, and MDA showed negative inverse correlation. In the case of immobilized algae(*C. vulgaris*), CAT, TP, SOD, and GSH showed correlation results. Positive correlation, AA and ROS negative direct correlation, while GPX showed a negative inverse correlation. Figure 3-18

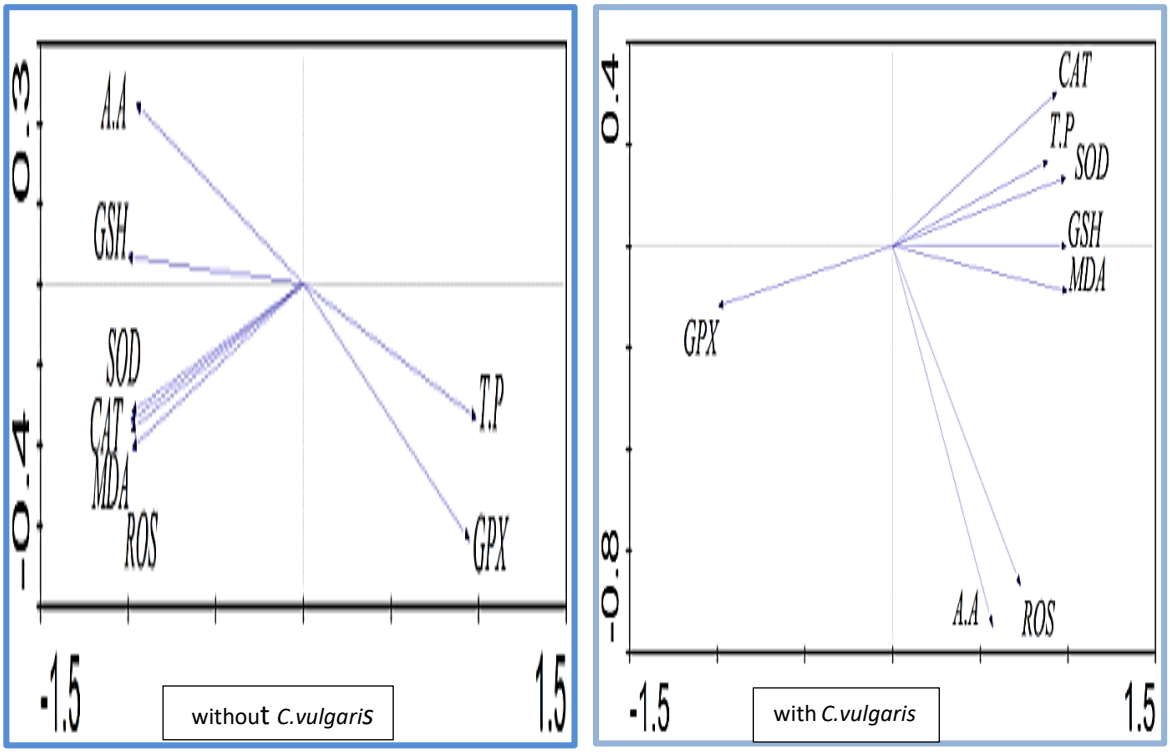

Figure 3-18: Correlations between the antibiotic amoxicillin and biochemical markers in Clam *P.euphraticus*

3-6-3 Correlation between Potassium Dichromate and Biochemical Markers in Clam *C. flumine*

Observed a correlation between potassium dichromate concentration and a biochemical markers in Clam *C. flumine*. In the absence of immobilization algae (*C. vulgaris*), TP and GSH showed a positive connection. AA, SOD, MDA, ROS and CAT demonstrated positive inverse correlations, while GPX and ROS demonstrated negative inverse correlations. In the case of immobilized algae (*C. vulgaris),* GSH and TP had positive correlations, while ROS, SOD, AA,CAT and MDA had negative correlations, and GPX had a negative inverse correlation figure 3-19.

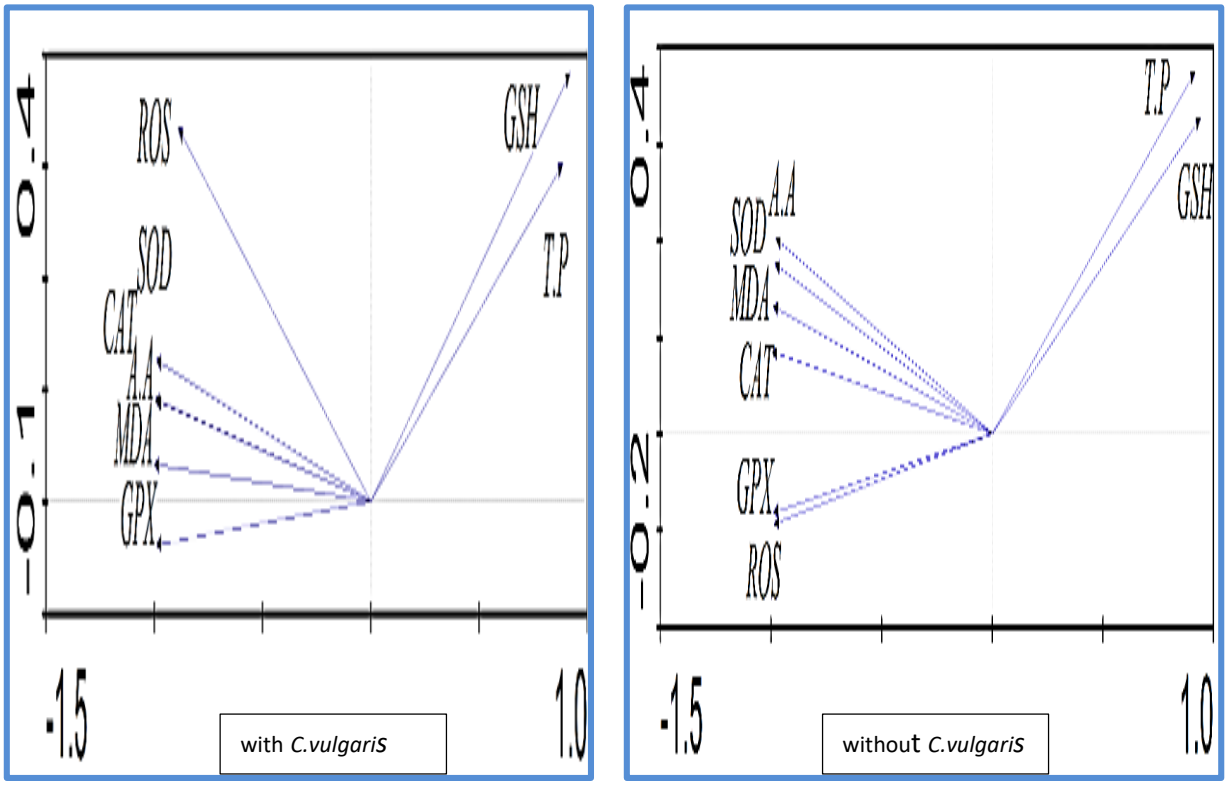

Figure 3-19: Correlations between the potassium dichromate and biochemical markers in Clam *C. flumine*

3-6-4 Correlation between Potassium Dichromate and Biochemical Marke Clam *P.euphraticus*

A current study found a correlation between potassium dichromate concentrations and a biochemical marker in Clam *P. euphraticus*. In the absence of immobilization (*C. vulgaris)*, GPX, GSH and AA showed negative correlation, MDA, and CAT showed positive inverse correlation GPX and ROS showed negative inverse correlation while SOD and ROS showed negative inverse correlation. In the case of immobilization algae (*C. vulgaris*), GSH and TP showed positive correlation correlations, ROS, SOD, AA , CAT and MDA showed negative correlations while GPX negative inverse correlation in Clam *P.euphraticus*. Figure 3-20

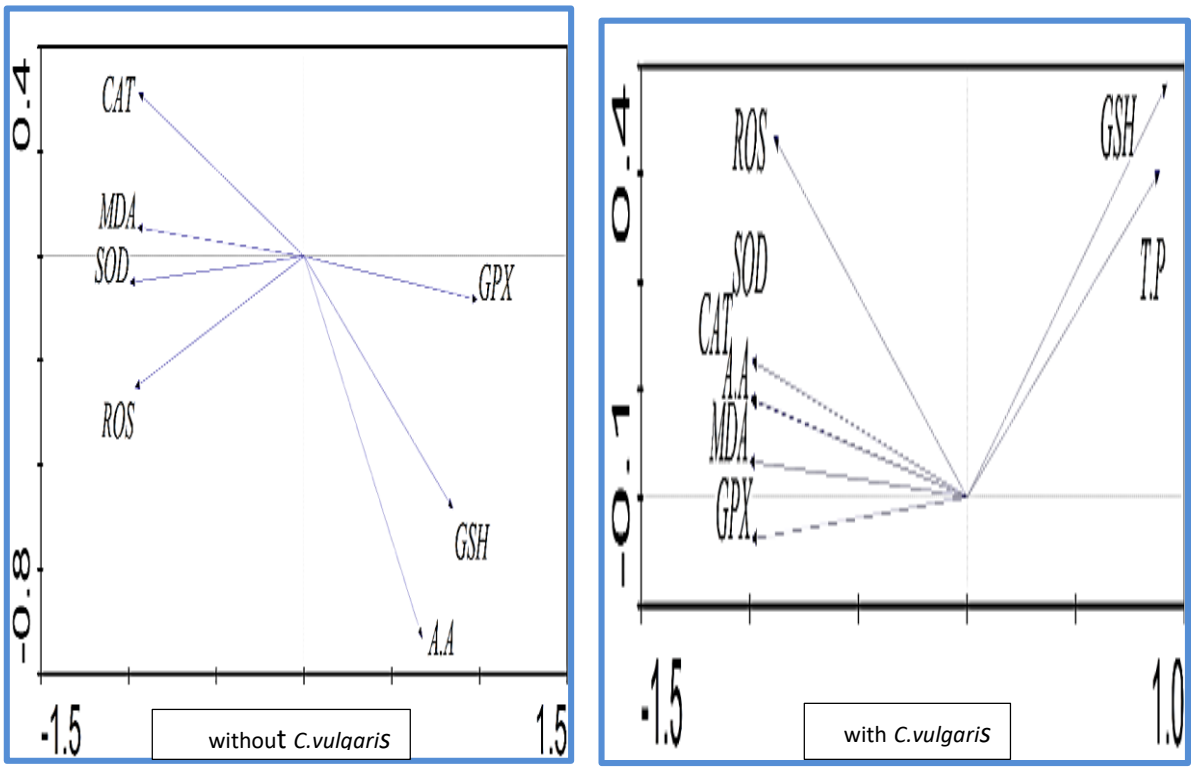

Figure 3-20: Correlations between the potassium dichromate and biochemical markers in Clam *P.euphraticus*

Conclusions

1- Mortality rate increased with high concentrations of AMX and $K_2Cr_2O_7$

2- Many behavioral changes, such as valve shutting, body retraction into the shell, stretching effect on the foot, and siphoning, have been observed in the clam species under study when exposed to varying concentration of AMX and $K_2Cr_2O_7$, and no mucus secretion was observed upon exposure to amoxicillin,while mucus secretion was observed upon exposure to potassium dichromate.

3- The causes of AMX and $K_2Cr_2O_7$ Increased levels of CAT, SOD, GSH, and MDA as a result of excessive ROS generation.

4- High exposure to AMX and $K_2Cr_2O_7$ causes a decrease in total protein and an increase in concentrations of ascorbic acid and MDA, which may be due to the increased production of ROS as a tool for detecting changes caused by different chemical pollutants.

5- High concentrations of AMX and $K_2Cr_2O_7$ lead to histological damage in digestive glands and gills of *P. euphraticus* and *C. fluminea.*

6- Used Application of immobilized algae, *C. vulgaris,* had an important role in reducing the toxicity of AMX and $K_2Cr_2O_7$ and minimizing oxidative stress in the two species of clam under study.

7- Potassium dichromate was the most toxic and harmful in both species of studied clams; the species *C. fluminea* suffered more from oxidative stress when exposed to pharmaceuticals from the species *P. euphraticus* clams.

Recommendations

1- Study the fate and transport of pharmaceutical waste in different aquatic systems.

2- Study the mechanization of the bioremediation of pharmaceutical waste by mollusca species.

3- Study the bioaccumulation and bioconcentration of medicend waste in different aquatic organisms, especially mollusca,

4-Application of immobilized algae in biomonitoring and treatment of medical waste water

Reference

Abdel-Moneim, A. M., Al-Kahtani, M. A., & Elmenshawy, O. M. (2012). Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. Chemosphere, 88(8), 1028-1035.

Adlercreutz ،P.k and Mattiasson ،Bo. (1982). Oxygen supply immobilized cells: 3. Oxygen supply by hemoglobin or emulsions of perfluoro chemicals. European Journal of applied microbiology and biotechnology. Vol 16, Issue 4, pp 165—170.

Aharchaou, I., Maul, A., Pons, M. N., Pauly, D., Poirot, H., Flayac, J., ... & Vignati, D. A. (2022). Effects and bioaccumulation of Cr (III), Cr (VI) and their mixture in the freshwater mussel *Corbicula fluminea*. Chemosphere, *297*, 134090.

Ahmed, M. K., Kundu, G. K., Al-Mamun, M. H., Sarkar, S. K., Akter, M. S., & Khan, M. S. (2013). Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis*. Ecotoxicology and environmental safety, *92*, 64-70.

Ahmed,Mayada Hussein (2022). Effect of the interaction of some environmental factors with antibiotics on the health of some fish species in Shatt AlArab, Basrah - Iraq.Thesis ,The College of Agriculture, University of Basrah.

Ajibola, A.S., Amoniyan, O.A., Ekoja, F.O., Ajibola, F.O., 2021. QuEChERS approach for the analysis of three fluoroquinolone antibiotics in wastewater: concentration profiles and ecological risk in two nigerian hospital wastewater treatment plants. Arch. Environ. Contam. Toxicol. 80, 389–401.

Ajitha, V., Sreevidya, C. P., Kim, J. H., Singh, I. B., Mohandas, A., Lee, J. S., & Puthumana, J. (2019). Effect of metals of treated electroplating industrial effluents on antioxidant defense system in the microalga *Chlorella vulgaris*. Aquatic Toxicology, 217, 105317.

Akhter, S., Bhat, M. A., Ahmed, S., Siddiqi, W. A., Ahmad, S., & Shrimal, H. (2023). Profiling of Antibiotic Residues in Surface Water of River Yamuna Stretch Passing through Delhi, India. Water, 15(3), 527.

Al Mosawi, Z. H. A., Ajanabi, A. O. H., & Al Mamoori, A. M. J. (2022). Immobilize Algae To Removal Copper And Lead From Aquatic Ecosystem. nveo-natural volatiles & essential oils journal| nveo, 850-860.

Al Mosawi, Z. H. A., Ajanabi, A. O. H., & Al Mamoori, A. M. J. (2022). Immobilize algae to removal copper and lead from aquatic ecosystem. *nveo-natural volatiles & essential oils journal| nveo*, 850-860 .

Al-Asady, Raid Kadhim Abed .(2014). Using of some algae and aquatic plants in bioremediation of waste water from wastewater plants in Al-DewaniyaCity,Iraq. A Thesis, Science in Biology, University of Al-Qadisiyah

Alemu, K., Assefa, B., Kifle, D., Kloos, H., 2018. Removal of organic pollutants from municipalwastewater by applying high-rate algal pond in Addis Ababa,Ethiopia. Earth Syst. Environ.2, 50.

Ali, A.M., Rønning, H.T., Sydnes, L.K., Alarif, W.M., Kallenborn, R., Al-Lihaibi, S.S., 2018. Detection of 904 PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. Sci. Total 905 Environ. 621, 654–662.

Ali, S. S. M., & Al-mamoori, A. M. (2023). Protein profile biomarker as bio indicator for aquatic pollution in Hila river, Iraq. *Mesopotamia Environmental Journal (mesop. environ. j) ISSN: 2410-2598*, *7*(1), 36-45.

Ali, Shaimaa Satyi Mohammed,. Salman, Jasim M., Jebur, Ayad.(2015). Using some environmental factors and biochemical indicators of selected aquatic organisms evaluate water quality in Hilla river,iraq. Thesis. Babylon University.

Alkazemi, D., Rahman, A., & Habra, B. (2021). Alterations in glutathione redox homeostasis among adolescents with obesity and anemia. Scientific Reports, 11(1), 3034.

Alkharasani,Ali A. N., and Aldammy,Hanan Z. M. H. (2022) Morphological, taxonomic, and molecular study of some species of class Bivalvia (Mollusca: Bivalvia) from Middle Region of Iraq. A Thesis. College of Education for Pure Science.University of Karbal.

Al-Khazrajy, O. S., & Boxall, A. B. (2016). Risk-based prioritization of pharmaceuticals in the natural environment in Iraq. Environmental science and pollution research, 23, 15712-15726.

Almamoori ,. A. M,. Salman, J. M, Hughes,. A. Randall.(2013). Environmental Biomarkers in Selected Molluscs as a Tool for Biomonitoring of Pollution by Heavy Metals. A Thesis.Babylon University.

Almamoori, A. M., & Salman, J. M. (2022). Molecular Markers in Molluscs as Biomonitoring Species. Research Aspects in Biological Science *Vol. 3*, 118-123.

Almeida, A., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M., & Freitas, R. (2022a). Responses of *Ruditapes philippinarum* to contamination by pharmaceutical drugs under ocean acidification scenario. Science of The Total Environment, *824*, 153591.

Almeida, Â., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M., & Freitas, R. (2022b). Salinity-dependent impacts on the effects of antiepileptic and antihistaminic drugs in *Ruditapes philippinarum.* Science of the Total Environment, 806, 150369.

Almeida, Â., Freitas, R., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M., & Figueira, E. (2015). Chronic toxicity of the antiepileptic carbamazepine on the clam *Ruditapes philippinarum. Comparative Biochemistry and Physiology Part C:* Toxicology & Pharmacology, 172, 26-35.

Almeida, Â., Silva, M. G., Soares, A. M., & Freitas, R. (2020). Concentrations levels and effects of 17alpha-Ethinylestradiol in freshwater and marine waters and bivalves: A review. Environmental research, *185*, 109316.

Almeida, A.C., Gomes, T., Habuda-Stanić, M., Lomba, J.A.B., Romić, Ž., Turkalj, J.V., Lillicrap,A., (2019). Characterization of multiple biomarker responses using flow cytometry to improve environmental hazard assessment with the green microalgae *Raphidocelis subcapitata.* Sci. Total Environ. 687, 827–838.

Alonso Truhaut, R. (1977). Ecotoxicology: objectives, principles and perspectives. Ecotoxicology and environmental safety, *1*(2), 151-173.

Alonso, A., Suárez, P., Ruiz, Y., Dobal, V., & San Juan, F. (2019). Gonadal histopathological disorders in *Mytilus galloprovincialis* male exposed to tars used in mussel farms. Frontiers in Marine Science, 6, 577.

Al-Subiai, S. N., Jha, A. N., & Moody, A. J. (2009). Contamination of bivalve haemolymph samples by adductor muscle components: implications for biomarker studies. Ecotoxicology, 18(3), 334-342.

Altenburger, R., Brack, W., Burgess, R. M., Busch, W., Escher, B. I., Focks, A., ... & Krauss, M. (2019). Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. Environmental Sciences Europe, 31(1), 1-17.

Amenorfenyo, D. K., Huang, X., Zhang, Y., Zeng, Q., Zhang, N., Ren, J., & Huang, Q. (2019). Microalgae brewery wastewater treatment: potentials, benefits and the challenges. International journal of environmental research and public health, 16(11), 1910.

Aranda, F. L., & Rivas, B. L. (2022). removal of amoxicillin through different methods, emphasizing removal by biopolymers and its derivatives. an overview. Journal of the Chilean Chemical Society, 67(3), 5643-5655.

Arimbrathodi, S. P., Javed, M. A., Hamouda, M. A., Aly Hassan, A., & Ahmed, M. E. (2023). BioH2 production using microalgae: highlights on recent advancements from a bibliometric analysis. *Water*, *15*(1), 185.

Atil, P., & Pathan, T. (2019) . toxicity evaluation and behavioral studies of freshwater bivalve lamellidens corrianus exposed to dimethoate. Journal of Universal Science and Technology

Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., & Trivedi, S. P. (2018). An in vivo analysis of Cr6+ induced biochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (Bloch, 1793). Aquatic toxicology, *200*, 158-167.

Awoke, J. S., & Nkwuda, P. J. (2019). Evaluation of Histopathological Effects of Sub-lethal Concentrations of Chloramphenicol Antibiotic on the Gill of *Clarias gariepinus* Juvenile (Burchell, 1822). *Aquaculture Studies*, *19*(1), 01-07.

Ayele, A., & Godeto, Y. G. (2021). Bioremediation of chromium by microorganisms and its mechanisms related to functional groups. Journal of Chemistry, 2021, 1-21B) p. 113082.

Baena-Nogueras, R. M., González-Mazo, E., & Lara-Martín, P. A. (2017). Degradation kinetics of pharmaceuticals and personal care products in surface waters: photolysis vs biodegradation. Science of the total environment, *590*, 643-654.

Bakshi, A., & Panigrahi, A. K. (2018). A comprehensive review on chromium induced alterations in fresh water fishes. Toxicology reports, 5, 440-447.

Bankole, D. T., Oluyori, A. P., & Inyinbor, A. A. (2023). The removal of pharmaceutical pollutants from aqueous solution by Agro-waste. *Arabian Journal of Chemistry*, *16*(5), 104699.

Bansal, A., & Simon, M. C. (2018). Glutathione metabolism in cancer progression and treatment resistance. Journal of Cell Biology, 217(7), 2291-2298.

Baralla, E., Demontis, M. P., Dessì, F., & Varoni, M. V. (2021). An Overview of antibiotics as emerging contaminants: occurrence in bivalves as biomonitoring organisms. Animals, 11(11), 3239.

Bassey, B. O. (2019). Histopathological and biochemical response of *Chrysichthys nigrodigitatus* to environmental stressors from two polluted lagoons, South-west Nigeria. *J Toxicol Risk Assess, 5*(025), 1852-1858.

Bebianno, M. J., Mello, A. C. P., Serrano, M. A. S., Flores-Nunes, F., Mattos, J. J., Zacchi, F. L., Bainy, A.C.D. (2017). Transcriptional and cellular effects of paracetamol in the oyster Crassostrea gigas. Ecotoxicology and environmental safety, 144, 258–267.

Beijerinck, M. W. (1890). Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. Bot. Ztg., 48, 781-788.

Bejaoui, S., Fouzai, C., Trabelsi, W., Rabeh, I., Chetoui, I., Telahigue, K., El Cafsi, M., Soudani, N., 2019b. Evaluation of lead chloride toxicity on lipid profile in *Venus verrucosa* gills. Int. J. Environ. Res. 13, 793–800.

Bereketoglu, C., Pradhan, A., & Olsson, P. E. (2020). Nonsteroidal anti-inflammatory drugs (NSAIDs) cause male-biased sex differentiation in zebrafish. *Aquatic Toxicology*, *223*, 105476.

Bhagat, J., Ingole, B. S., & Singh, N. (2016). Glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation as biomarkers of oxidative stress in snails: A review. Invertebrate Survival Journal, 13(1), 336-349.

Bhattacharya, T., Dey, P. S., Akter, R., Kabir, M. T., Rahman, M. H., & Rauf, A. (2021). Effect of natural leaf extracts as phytomedicine in curing geriatrics. Experimental Gerontology, 150, 111352.

Bijekar, S., Padariya, H. D., Yadav, V. K., Gacem, A., Hasan, M. A., Awwad, N. S., ... & Jeon, B. H. (2022). The state of the art and emerging trends in the wastewater treatment in developing nations. *Water*, *14*(16), 2537.

Bilal, M., Mehmood, S., Rasheed, T., & Iqbal, H. M. (2020). Antibiotics traces in the aquatic environment: persistence and adverse environmental impact. Current opinion in environmental *science & health*, 13, 68-74.

Binh, V. N., Dang, N., Anh, N. T. K., & Thai, P. K. (2018). Antibiotics in the aquatic environment of Vietnam: sources, concentrations, risk and control strategy. *Chemosphere*, *197*, 438-450.

Bio, S., & Nunes, B. (2020). Acute effects of diclofenac on zebrafish: indications of oxidative effects and damages at environmentally realistic levels of exposure. *Environmental Toxicology and Pharmacology*, *78*, 103394

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. World allergy organization journal, *5*(1), 9-19.

Bogan, A. E. and Alderman, J. M. (2008). Workbook and key to the freshwater bivalves of South Carolina. South Carolina State Documents Depository.

Bojarski, B., Kot, B., & Witeska, M. (2020). Antibacterials in aquatic environment and their toxicity to fish. Pharmaceuticals, *13*(8), 189.

Bouzidi, I., Mougin, K., Beyrem, H., Alghonaim, M. I., Alsalamah, S. A., Qurtam, A. A., ... & Sellami, B. (2023). Physiological Impairment and Biochemical Modifications Induced by Triclosan in Mediterranean Mussels. Animals, 13(4), 583.

Brack, W., Aissa, S. A., Backhaus, T., Dulio, V., Escher, B. I., Faust, M., ... & Altenburger, R. (2019). Effect-based methods are key. The European Collaborative Project solutions recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environmental Sciences Europe, *31*(1), 1-6.

Brahma, N. (2022). Behavioral changes in *Lamellidens corrianus, Parreysia (Parreysia) gowhattensis,* and *Melania hainesiana* due to coppe.

Branchet, P., Arpin-Pont, L., Piram, A., Boissery, P., Wong-Wah-Chung, P., & Doumenq, P. (2021). Pharmaceuticals in the marine environment: What are the present challenges in their monitoring?. Science of the Total Environment, *766*, 142644.

Brandão, F. P., Pereira, J. L., Gonçalves, F., & Nunes, B. (2014). The impact of paracetamol on selected biomarkers of the mollusc species *Corbicula fluminea*. Environmental toxicology, 29(1), 74-83.

Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in enzymology* (Vol. 52, pp. 302-310). Academic press.

Cabrera, J., González, P. M., & Puntarulo, S. Á. (2019). Oxidative effects of the harmful algal blooms on primary organisms of the food web.

Cao, S., Teng, F., Lv, J., Zhang, Q., Wang, T., Zhu, C., ... & Tao, Y. (2022). Performance of an immobilized microalgae-based process for wastewater treatment and biomass production: nutrients removal, lipid induction, microalgae harvesting and dewatering. Bioresource Technology, 127298.

Carballeira, C., Espinosa, J., & Carballeira, A. (2011). Linking δ15N and histopathological effects in molluscs exposed in situ to effluents from land-based marine fish farms. Marine pollution bulletin, *62*(12), 2633-2641.

Carpenter, C. M., & Helbling, D. E. (2018). Widespread micropollutant monitoring in the Hudson River estuary reveals spatiotemporal micropollutant clusters and their sources. Environmental science & technology, 52(11), 6187-6196.

Carvalho, T. L. A. D. B., Nascimento, A. A. D., Gonçalves, C. F. D. S., Santos, M. A. J. D., & Sales, A. (2020). Assessing the histological changes in fish gills as environmental bioindicators in Paraty and Sepetiba bays in Rio de Janeiro, Brazil. *Latin american journal of aquatic research*, *48*(4), 590-601

Ceschin, S., Bellini, A., & Scalici, M. (2021). Aquatic plants and ecotoxicological assessment in freshwater ecosystems: A review. Environmental Science and Pollution Research, 28(5), 4975-4988.

Chaâbane, M., Bejaoui, S., Trabelsi, W., Telahigue, K., Chetoui, I., Chalghaf, M., ... & Soudani, N. (2020). The potential toxic effects of hexavalent chromium on oxidative stress biomarkers and fatty acids profile in soft tissues of *Venus verrucosa.* Ecotoxicology and Environmental Safety, 196, 110562

Chahouri, A., Agnaou, M., El Hanaoui, M., Yacoubi, B., Moukrim, A., & Banaoui, A. (2022). Assessment of seasonal and spatial variation responses of integrated biomarkers in two marine sentinel bivalve species: Agadir Bay (Southern of Morocco*). Marine Pollution Bulletin, 174*, 113179.

Chakraborty, R., Renu, K., Eladl, M. A., El-Sherbiny, M., Elsherbini, D. M. A., Mirza, A. K., ... & Gopalakrishnan, A. V. (2022). Mechanism of chromium-induced toxicity in lungs, liver, and kidney and their ameliorative agents. Biomedicine & Pharmacotherapy, 151, 11311.

Chandel, N., Ahuja, V., Gurav, R., Kumar, V., Tyagi, V. K., Pugazhendhi, A., ... & Bhatia, S. K. (2022). Progress in microalgal mediated bioremediation systems for the removal of antibiotics and pharmaceuticals from wastewater. Science of The Total Environment, 825, 153895.

Chen, H., Zha, J., Yuan, L., Wang, Z. (2015). Effects of fuoxetine on behavior, antioxidant enzyme systems, and multixenobiotic resistance in the Asian clam Corbicula fuminea. Chemosphere 119, 856-862Page 14/21.

Chen, K., Zhou, J.L. (2014). Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95, 604-612.

Chen, S., Zhang, W., Li, J., Yuan, M., Zhang, J., Xu, F., ... & Wang, L. (2020). Ecotoxicological effects of sulfonamides and fluoroquinolones and their removal by a green alga (*Chlorella vulgaris*) and a cyanobacterium (*Chrysosporum ovalisporum*). Environmental Pollution, *263*, 114554.

Chen, Z., Guo, J., Jiang, Y., & Shao, Y. (2021). High concentration and high dose of disinfectants and antibiotics used during the COVID-19 pandemic threaten human health. Environmental Sciences Europe, *33*(1), 1-4.

Chen, Z., Osman, A. I., Rooney, D. W., Oh, W. D., & Yap, P. S. (2023). Remediation of heavy metals in polluted water by immobilized algae: current applications and future perspectives. Sustainability, 15(6), 5128.

Cheng, P., Zhou, C., Chu, R., Chang, T., Xu, J., Ruan, R., ... & Yan, X. (2020). Effect of microalgae diet and culture system on the rearing of bivalve mollusks: Nutritional properties and potential cost improvements. Algal Research, *51*, 102076.

Cheng, Z., Dong, Q., Liu, Y., Yuan, Z., Huang, X. (2022). Fate characteristics, exposure risk, and control strategy of typical antibiotics in a Chinese sewerage system: A review. Environment International, 107396.

Chia, M. A.; Lombardi, A. T. and Melao, M. G. G. (2013) Growth and biochemical composition of *Chlorella vulgaris* in different growth media. Anais da Academia Brasileira de Ciencias, 85(4): 1427-1438.

Chiesa, L. M., Nobile, M., Malandra, R., Panseri, S. and Arioli, F. (2018). Occurrence of antibiotics in mussels and clams from various FAO areas. Food Chem. 240: 16–23.

Chowdhury, J., Mandal, T. K., & Mondal, S. (2020). Genotoxic impact of emerging contaminant amoxicillin residue on zebra fish (*Danio rerio)* embryos. Heliyon, 6(11), e05379 .

Cid, A., Picado, A., Correia, J.B., Chaves, R., Silva, H., Caldeira, J., de Matos, A.P.A., Diniz, M.S. (2015) Oxidative stress and histological changes following exposure to diamond nanoparticles in the freshwater Asian clam Corbicula §uminea (Müller, 1774). Journal of Hazardous Materials 284, 27-34.

Cunha, M., Silva, M. G., De Marchi, L., Morgado, R. G., Esteves, V. I., Meucci, V., ... & Freitas, R. (2023). Toxic effects of a mixture of pharmaceuticals in *Mytilus galloprovincialis*: The case of 17α-ethinyl estradiol and salicylic acid. *Environmental Pollution*, 121070.

Cunha, S.C., Pena, A., Fernandes, J.O.,(2017). Mussels as bioindicators of diclofenac contamination in 1065 coastal environments. Environ. Pollut. 225, 354–360. 1066 .

Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. Frontiers in microbiology, 10, 338.

De Freitas Tallarico, L., Borrely, S. I., Hamada, N., Grazeffe, V. S., Ohlweiler, F. P., Okazaki, K., ... & Nakano, E. (2014). Developmental toxicity, acute toxicity and mutagenicity testing in freshwater snails *Biomphalaria glabrata* (Mollusca: Gastropoda) exposed to chromium and water samples. Ecotoxicology and Environmental Safety, 110, 208-21.

De Kraker, M. E., Stewardson, A. J., & Harbarth, S. (2016). Will 10 million people die a year due to antimicrobial resistance by 2050?. PLoS medicine, 13(11), e1002184.

De Mattia, G., Bravi, M.C., Laurenti, O., De Luca, O., Palmeri, A., Sabatucci, A., Mendico, G., Ghiselli, A., (2004). Impairment of cell and plasma redox state in subjects professionally exposed to chromium. Am. J. Ind. Med. 46, 120–125.

De Wilt, A., Butkovskyi, A., Tuantet, K., Leal, L. H., Fernandes, T. V., Langenhoff, A., & Zeeman, G. (2016). Micropollutant removal in an algal treatment system fed with source separated wastewater streams. Journal of hazardous materials, *304*, 84-92.

Dhara, K., Guhathakurta, H., (2021). Toxicity of neem (Azadirachta indica a, juss) Leaf extracts on fresh water snail, Bellamya bengalensis (Lamarck, 1882). Bioinfolet A Q. J. Life Sci. 18 (1a), 35–39.

Dhara, K., Saha, S., Chukwuka, A. V., Pal, P., Saha, N. C., & Faggio, C. (2022b). Fluoride sensitivity in freshwater snail, Bellamya bengalensis (Lamarck, 1882): An integrative biomarker response assessment of behavioral indices, oxygen consumption, haemocyte and tissue protein levels under environmentally relevant exposure concentrations. Environmental Toxicology and Pharmacology, 89, 103789.

Dhara, K., Saha, S., Pal, P., Chukwuka, A. V., Panigrahi, A. K., Saha, N. C., & Faggio, C. (2022a). Biochemical, physiological (haematological, oxygen-consumption rate) and behavioural effects of mercury exposures on the freshwater snail, *Bellamya bengalensis*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 251, 109195.

Drozdz-Afelt, J. M., Koim-Puchowska, B. B., & Kaminski, P. (2022). Analysis of oxidative stress indicators in Polish patients with prostate cancer. Environmental Science and Pollution Research, 29, 4632-4640.

Duran-Alvarez, J. C., & Jiménez-Cisneros, B. (Eds.). (2021). Pharmaceuticals in Marine and Coastal Environments: Occurrence, Effects, and Challenges in a Changing World. Elsevie.

El-Gendy, K.S., Radwan, M.A., Gad, A.F., Khamis, A.E., Eshra, E.-S.H., (2019).Physiological traits of land snails Theba pisana as simple endpoints to assess the exposure to some pollutants. Environ. Sci. Pollut. Res. 26 (7), 6922–6930.

Elizalde-Velázquez, A., Gómez-Oliván, L. M., Galar-Martínez, M., Islas-Flores, H., Dublán-García, O., & SanJuan-Reyes, N. (2016). Amoxicillin in the aquatic environment, its fate and environmental risk. Environmental health risk-hazardous factors to living species, 1, 247-267

Elizalde-Velázquez, A., Martínez-Rodríguez, H., Galar-Martínez, M., Dublán-García, O., Islas-Flores, H., Rodríguez-Flores, J., ... & Gómez-Oliván, L. M. (2017). Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (*Cyprinus carpio*): the role of amoxicilloic acid. Environmental toxicology, 32(4), 1102-1120.

El-Nahhal, Y., & El-Dahdouh, N. (2015). Toxicity of amoxicillin and erythromycin to fish and mosquito. Ecotoxicology and Environmental Contamination, 10(1), 13-21.

Elystia, S., Edward, H. S., & Putri, A. E. (2020). Removal of Chromium (VI) and Chromium (III) by using Chlorella sp Immobilized at Electroplating Wastewater. In IOP Conference Series: Earth and Environmental Science (Vol. 515, No. 1, p. 012078).

Encarnação, T., Palito, C., Pais, A. A., Valente, A. J., & Burrows, H. D. (2020). Removal of pharmaceuticals from water by free and immobilised microalgae. Molecules, 25(16), 3639.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry, 38(12), 1103-1111.

Exner, M., Bhattacharya, S., Gebel, J., Goroncy-Bermes, P., Hartemann, P., Heeg, P., ... & Trautmann, M. (2020). Chemical disinfection in healthcare settings: critical aspects for the development of global strategies. *GMS Hygiene and Infection Control*, *15*

Faleye, A. C., Adegoke, A. A., Ramluckan, K., Bux, F., & Stenström, T. A. (2018). Antibiotic residue in the aquatic environment: status in Africa. *Open* Chemistry, *16*(1), 890-903.

Fazelirad, H., Ranjbar, M., Taher, M. A., & Sargazi, G. (2015). Preparation of magnetic multi-walled carbon nanotubes for an efficient adsorption and spectrophotometric determination of amoxicillin. Journal of industrial and engineering chemistry, *21*, 889-892.

Feidantsis, K., Georgoulis, I., Giantsis, I. A., & Michaelidis, B. (2021). Treatment with ascorbic acid normalizes the aerobic capacity, antioxidant defence, and cell death pathways in thermally stressed *Mytilus galloprovincialis*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 255, 110611.

Felis, E., Kalka, J., Sochacki, A., Kowalska, K., Bajkacz, S., Harnisz, M., & Korzeniewska, E. (2020). Antimicrobial pharmaceuticals in the aquatic environment-occurrence and environmental implications. European Journal of Pharmacology, *866*, 172813.

Fernandes, J. P., Almeida, C. M. R., Salgado, M. A., Carvalho, M. F., & Mucha, A. P. (2021). Pharmaceutical compounds in aquatic environments—Occurrence, fate and bioremediation prospective. Toxics, *9*(10), 257.

Ferrando, L., & Matamoros, V. (2020). Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. *Science of the Total Environment, 703*, 134740.

Finney**,** D. J. (1971). Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of Information in Medicine, 10(04), 237–245.

Fujii, J., Osaki, T., & Bo, T. (2022). Ascorbate is a primary antioxidant in mammals. Molecules, 27(19), 6187.

Fundu, T. M., Kapepula, P. M., Esimo, J. M., Remacle, J., & Ngombe, N. K. (2019). Subcellular Localization of glutathione peroxidase, change in glutathione system during ageing and effects on cardiometabolic risks and associated diseases. Glutathione System and Oxidative Stress in Health and Disease.

Gab-Man,p.&Ee-Yung,Ch.(2004) Histological studies on hermaphroditism,gametogenesis and cyclic changes in the structures of marsupial gills of theintroduced asiatic clam, *corbicula fluminea*, and the Korean clam, corbiculaleana. Journal of Shellfish Research.23(1):179184.

Garbowski, T., Pietryka, M., Pulikowski, K., & Richter, D. (2020). The use of a natural substrate for immobilization of microalgae cultivated in wastewater. Scientific Reports, 10(1), 1-9.

García, C., Villalobos, T., Figueroa, D., & Araneda, O. F. (2022). Paralytic Shellfish Toxins Accumulation Induces Antioxidant Responses in Tissues of Mytilus *chilensis, Ameghinomya antiqua,* and *Concholepas concholepas* during a Bloom of Alexandrium pacificum.

Gayosso-Morales, M. A., Rivas-Castillo, A. M., Lucas-Gómez, I., López-Fernández, A., Calderón, A. V., Fernández-Martínez, E., ... & González-Pérez, B. K. (2023). Microalgae, a current option for the bioremediation of pharmaceuticals: A review. *Folia Microbiologica*, *68*(2), 167-179

Gegotek, A., & Skrzydlewska, E. (2022). Antioxidative and Anti-Inflammatory Activity of Ascorbic Acid. Antioxidants 2022, 11, 1993.

Ghosh, D., & Saha, S. K. (2022). Determination of the Lethal Concentration 50%(LC50) of Hexavalent Chromium in *Nile Tilapia (Oreochromis niloticus*) . Zoology and Botany 10(4): 123-131, 2022.

Gnatyshyna, L., Khoma, V., Mishchuk, O., Martinyuk, V., Spriņģe, G., & Stoliar, O. (2020). Multi-marker study of the responses of the Unio tumidus from the areas of small and micro hydropower plants at the Dniester River Basin, Ukraine. Environmental Science and Pollution Research, *27*, 11038-11049

Goswami, D. B., & Bhalla, R. (2021). Ameliorating effect of L-Ascorbate on protein and ascorbic acid content in different tissues of freshwater bivalve *Lamellidens marginallis* on exposure to *lambda-cyalothrin. Environment Conservation Journal, 22*(1&2), 159-166.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica chimica acta, 196(2-3), 143-151.

Gozlan, I., Rotstein, A., Avisar, D., (2013). Amoxicillin-degradation products formed under controlled environmental conditions: identification and determination in the aquatic environment. Chemosphere 91, 985e992.

Grinchenko, A. V., Sokolnikova, Y. N., Ilyaskina, D. V., & Kumeiko, V. V. (2021). Seasonal Changes in Hemolymph Parameters of the Bivalve *Modiolus kurilensis Bernard*, 1983 from Vostok Bay, Sea of Japan. Russian Journal of Marine Biology, 47(4), 300-311.

Guilhermino, L., Vieira, L.R., Ribeiro, D., Tavares, A.S., Cardoso, V., Alves, A., Almeida, J.M. (2018) Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*. Science of the total environment 622-623, 1131-1142.

Gulbhile, S., Zambare, S.,(2013). Role of caffeine (1, 3, 7-Trimethylxanthine) on arsenic induced alterations of DNA level in the freshwater bivalve, *lamellidens corrianus* (Lea). Int. J. Curr. Microbiol. App. Sci. 2 (11), 194–201

Guo X, Feng C (2018) Biological toxicity response of Asian Clam (*Corbicula fluminea*) to pollutants in surface water and sediment. Sci Total Environ 631-632:56–70

Guo, X., Feng, C., Bi, Z., Islam, A., & Cai, Y. (2022). Toxicity effects of ciprofloxacin on biochemical parameters, histological characteristics, and behaviors of *Corbicula fluminea* in different substrates. Environmental Science and Pollution Research, *29*(16), 23700-23711.

H.Regoli, F., & Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Marine environmental research, 93, 106-117.

Hafeman, D. G., Sunde, R. A., & Hoekstra, W. G. (1974). Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. The Journal of nutrition, 104(5), 580-587.

Han, M., Zhang, C., & Ho, S. H. (2022). Immobilized microalgal system: An achievable idea for upgrading current microalgal wastewater treatment. Environmental Science and Ecotechnology, 100227.

Hanna, N. S., & Shekha, Y. A. (2023). Acute Toxicity of Chlorpyrifos on the Freshwater Bivalves (Unio Tigridis) and Effects on Bioindicators. *Baghdad Science Journal*.

Haro Girón, S., Monserrat Sanz, J., Ortega, M. A., Garcia-Montero, C., Fraile-Martínez, O., Gómez-Lahoz, A. M., ... & Álvarez-Mon, M. (2023). Prognostic value of malondialdehyde (MDA) in the temporal progression of chronic spinal cord injury. *Journal of Personalized Medicine, 13*(4), 626

He, X. Q., Cui, Y. Y., Lin, X. H., & Yang, C. X. (2021). Fabrication of polyethyleneimine modified magnetic microporous organic network nanosphere for efficient enrichment of non-steroidal anti-inflammatory drugs from wastewater samples prior to HPLC-UV analysis. *Talanta, 233*, 122471.

Hemming, J. M., & Waller, W. T. (2004). Diazinon and chlorpyrifos toxicity to the freshwater Asiatic clam, Corbicula fluminea Muller, and the estuarine hooked mussel, Ischadium recurvum Rafinesque. Florida Scientist, 1–8

Hejna, M., Kapuścińska, D., & Aksmann, A. (2022). Pharmaceuticals in the Aquatic Environment: A Review on Eco-Toxicology and the Remediation Potential of *Algae. International Journal of Environmental Research and Public Health, 19(13),*

Hellou, J., Ross, N. W., & Moon, T. W. (2012). Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environmental Science and Pollution Research, 19, 2007-2023..

Hena, S., Gutierrez, L., & Croué, J. P. (2020). Removal of metronidazole from aqueous media by *C. vulgaris*. Journal of hazardous materials, 384, 121400.

Hirte, K., Seiwert, B., Schüürmann, G., & Reemtsma, T. (2016). New hydrolysis products of the beta-lactam antibiotic amoxicillin, their pH-dependent formation and search in municipal wastewater. Water research, *88*, 880-888.

Hossain, M. A., Sarker, T. R., Sutradhar, L., Hussain, M., & Iqbal, M. M. (2023). Toxic effects of chlorpyrifos on the growth, hemocytes counts, and vital organ's histopathology of freshwater mussel, *Lamellidens marginalis*. Journal of King Saud University-Science, *35*(2), 102482.

Howard, D. W. (2004). Histological techniques for marine bivalve mollusks and crustaceans (Vol. 5). NOAA, National Ocean Service, National Centers for Coastal Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Cooperative Oxford Laboratory.

Hugonnet, J. E., Mengin-Lecreulx, D., Monton, A., den Blaauwen, T., Carbonnelle, E., Veckerle, C., ... & Arthur, M. (2016). Factors essential for L, D-transpeptidase-mediated peptidoglycan cross-linking and β-lactam resistance in Escherichia coli. *Elife*, *5*, e19469.

Hussain, M., Hossain, M. A., Begum, M., & Roy, N. C. (2022). Freshwater mussel (Lamelliedens marginalis) to reduce the lead (Pb) bioaccumulation in aquaculture of stinging catfish, Heteropneustes fossilis. Journal of Applied Aquaculture, 1-17

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine, 54(4), 287-293.

Ijomah, O. O., Adesuyi, A. A., Njoku, K. L., Ojokuku, S. A., Moses, U. D., & Adesuyi, O. O. (2020). Histopathological effects and biomarker response of earthworms, *Eisenia fetida,* after exposure to crude oil contaminated *soils. Environmental analysis, health and toxicology, 35*(4) Impact. Nova Science Publishers Inc.pp542.

Istomina, A., Yelovskaya, O., Chelomin, V., Karpenko, A., & Zvyagintsev, A. (2021). Antioxidant activity of Far Eastern bivalves in their natural habitat. Marine Environmental Research, 169, 105383.

Iztleuov, M., Umirzakova, Z., Iztleuov, E., Sambaeva, S., Iztleuova, G., Yesmukhanova, D., ... & Kolishbaeva, I. (2017). The effect of chromium and boron on the lipid peroxidation and antioxidant status (in experiment). Journal of Environmental Management & Tourism, 8(3 (19)), 529-536.

Jaouani, R., Dellali, M., Mouneyrac, C., Hassine, S. B., Ali, M. B., Hedfi, A., ... & Boufahja, F. (2021). Assessment of carbamazepine acute toxicity in the cockle *Cerastoderma edule* through chemical, physiological and biochemical tools. Brazilian Journal of Biology, *82*

Javid, A., Mesdaghinia, A., Nasseri, S., Mahvi, A. H., Alimohammadi, M., & Gharibi, H. (2016). Assessment of tetracycline contamination in surface and groundwaterresources proximal to animal farming houses in Tehran, Iran. Journal of Environmental Health Science and Engineering, 14(1), 4, 5 Pages

Jerome, F. C., Hassan, A., & Chukwuka, A. V. (2020). Metalloestrogen uptake, antioxidant modulation and ovotestes development in *Callinectes amnicola (blue crab*): a first report of crustacea intersex in the *Lagos lagoon (Nigeria*). The Science of the total environment, 704, 135235.

Jin, X., Xu, H., Qiu, S., Jia, M., Wang, F., Zhang, A., & Jiang, X. (2017). Direct photolysis of oxytetracycline: Influence of initial concentration, pH and temperature. Journal of Photochemistry and Photobiology A: Chemistry, 332, 224-231.

Joshy, A., Sharma, S. K., Mini, K. G., Gangadharan, S., & Pranav, P. (2022). Histopathological evaluation of bivalves from the southwest coast of India as an indicator of environmental quality. Aquatic Toxicology, *243*, 106076.

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. International Journal of Molecular Sciences, 22(9), 4642.

Junter, G. A., & Jouenne, T. (2017). Immobilized viable cell biocatalysts: a paradoxical development.

Kalyva, M. 2017. Fate of pharmaceuticals in the environment-A review. id: diva2:1085088.

Kaparapu, J. (2017). Micro algal immobilization techniques. J Algal Biomass Utln, 8, 64-70.

Kaparapu, J., & Geddada, M. N. R. (2016). Applications of immobilized algae. Journal of Algal Biomass Utilization, 7(2), 122-128.

Kassim, T.I.; Al-Saadi, H. and Salman, N.A. (1999) Production of somphyto-and zooplankton and their use as live food for fish larva. Iraq. J. Agric. Prod., 4(5):188-201.

Katz, A., Orellana, O.,(2012). Protein synthesis and the stress response. Cell-free Protein Synthesis. Intech, pp. p111–p134

Kayhan, F. E. (2013). Insecticides and defence mechanisms of aquatic organisms. Review of Hydrobiology, *6*(1).

Kayode-Afolayan, S. D., Ahuekwe, E. F., & Nwinyi, O. C. (2022). Impacts of pharmaceutical effluents on aquatic ecosystems. *Scientific African*, e01288.

Kayode-Afolayan, S.D., Ahuekwe, E.F., Nwinyi, O.C.(2022). Impacts of pharmaceutical efuents on aquatic ecosystems. Scientifc African, e01288.

Khan, A. H., Aziz, H. A., Khan, N. A., Hasan, M. A., Ahmed, S., Farooqi, I. H., ... & Mahtab, M. S. (2021). Impact, disease outbreak and the eco-hazards associated with pharmaceutical residues: a critical review. International Journal of Environmental Science and Technology, 1-12.

Khan, H. K., Rehman, M. Y. A., & Malik, R. N. (2020). Fate and toxicity of pharmaceuticals in water environment: An insight on their occurrence in South Asia. Journal of environmental management, 271, 111030.

Khasawneh, O. F. S., & Palaniandy, P. (2021). Occurrence and removal of pharmaceuticals in wastewater treatment plants. *Process Safety and Environmental Protection, 150*, 532-556.

Khoma V, Gnatyshyna L, Martinyuk V, Mackiv T, Mishchenko L, Manusadžianas L, Stoliar O (2021) Common and particular biochemical responses of *Unio tumidus* to herbicide, pharmaceuticals and their combined exposure with heating. Ecotoxicol Environ Saf 208:111695.

Kiki, C., Rashid, A., Wang, Y., Li, Y., Zeng, Q., Yu, C. P.,and a Sun, Q.(2020). Dissipation of antibiotics by microalgae: kinetics, identification of transformation products and pathways. Journal of hazardous materials, *387*, 121985. doi.org/10.1016/j.jhazmat.2019.121985.

Kikuchi, M., Syudo, A., Hukumori, M., Naito, C., & Sawai, J. (2017). Changes in aquatic toxicity of potassium dichromate as a function of water quality parameters. Chemosphere, *170*, 113-11.

Kim, J. H., & Kang, J. C. (2023). Detoxification effects of ascorbic acid on the oxidative stress, neurotoxicity, and metallothionein (MT) gene expression in juvenile *rockfish, Sebastes schlegelii* by the dietary chromium exposure. Fish & Shellfish Immunology, 132, 108464.

Klaminder, J., Jonsson, M., Fick, J., Sundelin, A., & Brodin, T. (2014). The conceptual imperfection of aquatic risk assessment tests: highlighting the need for tests designed to detect therapeutic effects of pharmaceutical contaminants. Environmental Research Letters, 9(8), 084003.

Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., ... & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National Academy of Sciences, 115*(15), E3463-E3470.

Koagouw, W., Stewart, N. A., & Ciocan, C. (2021). Long-term exposure of marine mussels to paracetamol: is time a healer or a killer?. Environmental Science and Pollution Research, 28(35).

Kock, A., Glanville, H. C., Law, A. C., Stanton, T., Carter, L. J., & Taylor, J. C. (2023). Emerging challenges of the impacts of pharmaceuticals on aquatic ecosystems: A diatom perspective. Science of the Total Environment, 878, 162939.

Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A., & Collins, J. J. (2007). A common mechanism of cellular death induced by bactericidal antibiotics. *Cell, 130*(5), 797-810.

Kondera, E., Bojarski, B., Ługowska, K., Kot, B., & Witeska, M. (2020). Effects of oxytetracycline and gentamicin therapeutic doses on hematological, biochemical and hematopoietic parameters in *Cyprinus carpio* juveniles. *Animals*, *10*(12), 2278.

Kovalakova, P., Cizmas, L., McDonald, T. J., Marsalek, B., Feng, M., & Sharma, V. K. (2020). Occurrence and toxicity of antibiotics in the aquatic environment: A review. Chemosphere, *251*, 126351.

Krzeminski, P., Tomei, M. C., Karaolia, P., Langenhoff, A., Almeida, C. M. R., Felis, E., ... & Fatta-Kassinos, D. (2019). Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review. Science of the Total Environment, 648, 1052-1081.

Kulbacka J, Saczko J, Chwiłkowska A (2009) Oxidative stress in cells damage processes. Pol *Polski merkuriusz lekarski* : organ *Polskiego Towarzystwa Lekarskiego* 27:44–47 (in Polish).

Kulik, K., Lenart-Boroń, A., & Wyrzykowska, K. (2023). Impact of Antibiotic Pollution on the Bacterial Population within Surface Water with Special Focus on Mountain Rivers. *Water*, *15*(5), 975.

Kumar, M., Jaiswal, S., Sodhi, K. K., Shree, P., Singh, D. K., Agrawal, P. K., & Shukla, P. (2019). Antibiotics bioremediation: perspectives on its ecotoxicity and resistance. Environment international, 124, 448-461.

Kumar, P., Kumar, R., Nagpure, N. S., Nautiyal, P., Kushwaha, B., & Dabas, A. (2013). Genotoxicity and antioxidant enzyme activity induced by hexavalent chromium in Cyprinus carpio after in vivo exposure. Drug and Chemical Toxicology, *36*(4), 451-460.

Kumari, K., Khare, A., & Dange, S. (2014). The applicability of oxidative stress biomarkers in assessing chromium induced toxicity in the fish *Labeo rohita*. BioMed research international, 2014.

Kwarciak-Kozłowska, A., & Sławik-Dembiczak, L. (2021). Biosorption of lead from municipal wastewater by alginate beads, free and alginate-immobilized Chlorella vulgaris. Desalination and Water Treatment, 218, 303-308.

LaPelusa, A., & Kaushik, R. (2022). Physiology, proteins. In StatPearls [Internet]. StatPearls Publishing.

Larsson, D. J. (2014). Antibiotics in the environment. Upsala journal of medical sciences, 119(2), 108-112.

Laxmi, V., & Kaushik, G. (2020). Toxicity of hexavalent chromium in environment, health threats, and its bioremediation and detoxification from tannery wastewater for environmental safety. In Bioremediation of industrial waste for environmental safety (pp. 223-243). Springer, Singapore.

Lee H., Jeong D., Im S.J., and Jang A. (2020). Optimization of alginate bead size immobilizedwith *Chlorella vulgaris* and *Chlamydomonas reinhardtii* for nutrient removal. Bioresource Technology 302: 1-5.

Lee, J. W., Kim, J. H., Lee, D. C., Lim, H. J., & Kang, J. C. (2022). Toxic Effects on Oxidative Stress, Neurotoxicity, Stress, and Immune Responses in Juvenile *Olive Flounder, Paralichthys Olivaceus,* Exposed to Waterborne Hexavalent Chromium. Biology, 11(5), 766.

Lee, L., Hsu, C. Y., & Yen, H. W. (2017). The Effect of Hydraulic Time Retention (HRT) on Chromium (VI) Reduction Using Autotrophic Cultivation of. *Chlorella vulgaris*. Bioprocess Biosystem engineering.

Lee, S., Kim, C., Liu, X., Lee, S., Kho, Y., Kim, W. K., … & Choi, K. (2021). Ecological risk assessment of amoxicillin, enrofloxacin, and neomycin: Are their current levels in the freshwater environment safe?. Toxics, 9(8), 196.

Léger, L., Budin-Verneuil, A., Cacaci, M., Benachour, A., Hartke, A., & Verneuil, N. (2019). β-lactam exposure triggers reactive oxygen species formation in enterococcus faecalis via the respiratory chain component DMK. Cell Reports, 29(8), 2184-2191.

Leng, L., Wei, L., Xiong, Q., Xu, S., Li, W., Lv, S., … & Zhou, W. (2020). Use of microalgae based technology for the removal of antibiotics from wastewater: a review. Chemosphere, 238, 124680.

Leonard, A. F., Morris, D., Schmitt, H., & Gaze, W. H. (2022). Natural recreational waters and the risk that exposure to antibiotic resistant bacteria poses to human health. Current opinion in microbiology, *65*, 40-46.

Li, D., Wang, P., Wang, C., Fan, X., Hu, B. (2018) Combined toxicity of organophosphate flame retardants and cadmium to *Corbicula fluminea* in aquatic sediments. Environmental pollution 243, S597672460.

Li, H., Pan, Y., Wang, Z., Chen, S., Guo, R., & Chen, J. (2015). An algal process treatment combined with the Fenton reaction for high concentrations of amoxicillin and cefradine. *RSC advances, 5*(122), 100775-100782

Li, H., Shi, J., Gao, H., Yang, X., Fu, Y., Peng, Y., ... & Zhou, D. (2022). Hexavalent chromium causes apoptosis and autophagy by inducing mitochondrial dysfunction and oxidative stress in broiler cardiomyocytes. *Biological Trace Element Research, 200*(6), 2866-2875.

Li, R., Wang, B., Niu, A., Cheng, N., Chen, M., Zhang, X., … & Wang, S. (2022). Application of biochar immobilized microorganisms for pollutants removal from wastewater: A review. Science of The Total Environment, 155563.

Li, S., Show, P. L., Ngo, H. H., & Ho, S. H. (2022). Algae-mediated antibiotic wastewater treatment: A critical review. Environmental Science and Ecotechnology, *9*, 100145.

Li, Y., Yang, W., Zheng, X. Q., Yao, C., & Wu, Y. (2021). Combining passive sampling with toxicity testing to evaluate potential ecotoxicological effects of pharmaceuticals in wastewater-impacted rivers. Water Science and Engineering, *14*(3), 201-209.

Li, Z. H., Li, P., & Randak, T. (2011). Evaluating the toxicity of environmental concentrations of waterborne chromium (VI) to a model teleost, Oncorhynchus mykiss: a comparative study of in vivo and in vitro. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 153(4), 402-407.

Li, Z., He, X., & Feng, C. (2023). A review of freshwater benthic clams *(Corbicula fluminea)*: Accumulation capacity, underlying physiological mechanisms and environmental applications. Science of The Total Environment, 857, 159431.

Liang, L., Bai, X., & Hua, Z. (2022). Enhancement of the immobilization on microalgae protective effects and carbamazepine removal by *Chlorella vulgaris*. Environmental Science and Pollution Research, 1-12.

Lin, T. J., Huang, Y. L., Chang, J. S., Liu, K. T., Yen, M. C., Chen, F. W., … & Yeh, I. J. (2018). Optimal dosage and early intervention of L-ascorbic acid inhibiting $K_2Cr_2O_7$-induced renal tubular cell damage. Journal of Trace Elements in Medicine and Biology, 48, 1-7.

Lionetto, M. G., Caricato, R., & Giordano, M. E. (2019). Pollution biomarkers in environmental and human biomonitoring. The Open Biomarkers Journal, *9*(1).

Liu, C., Tan, L., Zhang, L., Tian, W., & Ma, L. (2021). A review of the distribution of antibiotics in water in different regions of China and current antibiotic degradation pathways. Frontiers in Environmental Science, 221.

Liu, L. (2021). Occurrence and Health Risk Assessment of Antibiotics in Drinking Water of a City in Southern China. *In IOP* Conference Series: Earth and Environmental Science (Vol. 657, No. 1, p. 012048). IOP Publishing.

Liu, L., Wu, W., Zhang, J., Lv, P., Xu, L., & Yan, Y. (2018). Progress of research on the toxicology of antibiotic pollution in aquatic organisms. *Acta Ecologica Sinica*, *38*(1), 36-41.

Liu, R., Li, S., Tu, Y., & Hao, X. (2021). Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. Journal of Environmental Management, 285, 112149.

Liu, S., Zhao, H., Zheng, M., Wang, H., Jing, C., Zhang, W., & Hu, F. (2022). The physiological, biochemical and transcriptional responses to sulfamethoxazole in the Asian clam, *Corbicula fluminea* (OF Müller, 1774). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 260, 109406.

Liu, Y., Cai, D., Li, X., Wu, Q., Ding, P., Shen, L., … & Zhang, L. (2023). Occurrence, fate, and risk assessment of antibiotics in typical pharmaceutical manufactories and receiving water bodies from different regions. *PloS one, 18*(1), e0270945.

Lomartire, S., Marques, J. C., & Gonçalves, A. M. (2021). Biomarkers based tools to assess environmental and chemical stressors in aquatic systems. Ecological Indicators, 122, 10720.

Lopes-Lima, M., Gürlek, M. E., Kebapçı, Ü., Şereflişan, H., Yanık, T., Mirzajani, A., ... & Froufe, E. (2021). Diversity, biogeography, evolutionary relationships, and conservation of Eastern Mediterranean freshwater mussels (Bivalvia: Unionidae). Molecular Phylogenetics and Evolution, 163, 10726

López-Pedrouso, M., Lorenzo, J. M., Varela, Z., Fernández, J. Á., & Franco, D. (2022). Finding biomarkers in antioxidant molecular mechanisms for ensuring food safety of bivalves threatened by marine pollution. Antioxidants, 11(2), 369.

Lowry, O. H., Rose Brough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193, 265-275.

Luan, X., Liu, X., Fang, C., Chu, W., & Xu, Z. (2020). Ecotoxicological effects of disinfected wastewater effluents: a short review of in vivo toxicity bioassays on aquatic organisms. *Environmental Science: Water Research & Technology*, *6*(9), 2275-2286.

Luo, L., Yang, C., Jiang, X., Guo, W., Ngo, H. H., & Wang, X. C. (2023). Impacts of fulvic acid and Cr (VI) on metabolism and chromium removal pathways of green microalgae. *Journal of Hazardous Materials*, 132171

Lushchak, V., Kubrak, O. I., Nykorak, M. Z., Storey, K. B., & Lushchak, V. I. (2008). The effect of potassium dichromate on free radical processes in goldfish: possible protective role of glutathione. Aquatic Toxicology, *87*(2), 108-114.

Lyublinskaya, O. G., Ivanova, J. S., Pugovkina, N. A., Kozhukharova, I. V., Kovaleva, Z. V., Shatrova, A. N., ... & Nikolsky, N. N. (2017). Redox environment in stem and differentiated cells: a quantitative approach. *Redox biology*, *12*, 758-769

Machado, A. B., Caprara, J. F., de Franceschi, I. D., Linden, R., Berlese, D. B., & Feksa, L. R. (2019). Effects of chronic exposure to hexavalent chromium in water on oxidative stress parameters in Wistar rats. Acta Scientiarum. Biological Sciences, 41, 43771.

Mahmood, A. R., Al-Haideri, H. H., & Hassan, F. M. (2019). Detection of antibiotics in drinking water treatment plants in Baghdad City, Iraq. Advances in Public Health, 2019.

Mallick, N., (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review. Biometals, 15: 377–390.

Mandal, A. K. (2022). Chromium Induced Developments of Diseases and their Inhibitions by Cargos. Asian Journal of Biochemistry, Genetics and Molecular Biology, 12(4), 108-119

Mansouri, F., Chouchene, K., Roche, N., & Ksibi, M. (2021). Removal of Pharmaceuticals from water by adsorption and advanced oxidation processes: State of the art and trends. Applied Sciences, 11(14), 6659.

Mansouri, F., Chouchene, K., Roche, N., and Ksibi, M. 2021. Removal of Pharmaceuticals from water by adsorption and advanced oxidation processes: State of the art and trends.Applied Sciences, 11(14),6659.

Mao, Y., Yu, Y., Ma, Z., Li, H., Yu, W., Cao, L., & He, Q. (2021). Azithromycin induces dual effects on microalgae: Roles of photosynthetic damage and oxidative stress. Ecotoxicology and Environmental Safety, 222, 112496.

Marinović, Z., Miljanović, B., Urbányi, B., & Lujić, J. (2021). Gill histopathology as a biomarker for discriminating seasonal variations in water quality. Applied Sciences, 11(20), 9504.

Marisa, I., Asnicar, D., Matozzo, V., Martucci, A., Finos, L., & Marin, M. G. (2021). Toxicological effects and bioaccumulation of fullerene C60 (FC60) in the marine bivalve *Ruditapes philippinarum*. Ecotoxicology and environmental safety, 207, 111560.

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47(3), 469-474.

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, *2*(2), 48-78.

Martínez, J. L. (2008). Antibiotics and antibiotic resistance genes in natural environments. Science, 321(5887), 365-367.

Martinez-Morcillo, S., Rodríguez-Gil, J. L., Fernández-Rubio, J., Rodriguez-Mozaz, S., Míguez-Santiyán, M. P., Valdes, M. E., ... & Valcárcel, Y. (2020). Presence of pharmaceutical compounds, levels of biochemical biomarkers in seafood tissues and risk assessment for human health: results from a case study in North-Western Spain. International Journal of Hygiene and Environmental Health, *223*(1), 10-21.

Martins, J. C., Campos, A., Osório, H., Da Fonseca, R., & Vasconcelos, V. (2014). Proteomic profiling of cytosolic glutathione transferases from three bivalve species: Corbicula fluminea, Mytilus galloprovincialis and Anodonta cygnea. International Journal of Molecular Sciences, *15*(2), 1887-1900

Martyniuk, V., Khoma, V., Matskiv, T., Baranovsky, V., Orlova-Hudim, K., Gylytė, B., ... & Stoliar, O. (2022). Indication of the impact of environmental stress on the responses of the bivalve mollusk *Unio tumidus* to ibuprofen and microplastics based on

biomarkers of reductive stress and apoptosis. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 261, 109425.

Matozzo, V., Battistara, M., Marisa, I., Bertin, V., & Orsetti, A. (2016 a). Assessing the effects of amoxicillin on antioxidant enzyme activities, lipid peroxidation and protein carbonyl content in the clam *Ruditapes philippinarum* and the mussel *Mytilus galloprovincialis*. Bulletin of environmental contamination and toxicology, *97*(4), 521-527.

Mayer Jr, F. L., Marking, L. L., Bills, T. D., & Howe, G. E. (1994). Physicochemical factors affecting toxicity in freshwater: Hardness, pH and Temperature. JL Hamelink, PF Landrum, HL Bergman et WH Benson (responsables de la publication), Bioavailability: Physical, Chemical and Biological Interactions. Lewis Publishers, Boca Raton, 5-22

Matozzo, V., Bertin, V., Battistara, M., Guidolin, A., Masiero, L., Marisa, I., & Orsetti, A. (2016 b). Does the antibiotic amoxicillin affect haemocyte parameters in non-target aquatic invertebrates? The clam *Ruditapes philippinarum* and *the mussel Mytilus galloprovincialis* as model organisms. Marine Environmental Research, *119*, 51-58.

Maulood, B.K. ; Hassan, F. M. ; Al- Lami, A. A. ; Toma, J. J. and Ismail, A. M. (2013) Checklist of Algal Flora in Iraq. Ministry of Environment, Iraq, pp: 94 .

Maurya, R., & Namdeo, M. (2021). Superoxide dismutase: A key enzyme for the survival of intracellular pathogens in host. Reactive Oxygen Species.

McCormick, D.B. and Greene, H.L. (1999). Vitamins. In Burtis, C.A. and Ashwood E.R. (eds). Tietz Textbook of Clinical Chemistry. 3rd edn, W.B. Saunders Co., Philadelphia, pp. 999-1028.

Mezzelani, M., Fattorini, D., Gorbi, S., Nigro, M., & Regoli, F. (2020). Human pharmaceuticals in marine mussels: Evidence of sneaky environmental hazard along Italian coasts. Marine Environmental Research, 162, 105137.

Mezzelani, M., Gorbi, S., Da Ros, Z., Fattorini, D., d'Errico, G., Milan, M., ... & Regoli, F. (2016). Ecotoxicological potential of non-steroidal anti-inflammatory drugs (NSAIDs) in marine organisms: bioavailability, biomarkers and natural occurrence in *Mytilus galloprovincialis*. Marine environmental research, *121*, 31-39.

Mezzelani, M., Gorbi, S., Regoli, F., (2018). Pharmaceuticals in the aquatic environments: evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60.

Mezzelani, M., Peruzza, L., d'Errico, G., Milan, M., Gorbi, S., & Regoli, F. (2023). Mixtures of environmental pharmaceuticals in marine organisms: Mechanistic evidence of carbamazepine and valsartan effects on *Mytilus galloprovincialis. Science of The* Total Environment, 860, 160465.

Mhadhbi, L., El Ayari, T., Tir, M., & Kadri, D. (2022). Azithromycin effects on the European sea bass (Dicentrarchus labrax) early life stages following acute and chronic exposure: Laboratory bioassays. Drug and Chemical Toxicology, 45(3), 1295-1301.

Mirza, N. N., & Nashaat, M. R. (2019). Abundance, diversity and distribution of Mollusca in the Gharaf River, Southern Iraq. Iraqi Journal of Science, 469-485.

Mirzaee, S. A., Noorimotlagh, Z., Ahmadi, M., Rahim, F., Martinez, S. S., Nourmohammadi, A., & Jaafarzadeh, N. (2021). The possible oxidative stress and DNA damage induced in Diclofenac-exposed Non-target organisms in the aquatic environment: A systematic review. *Ecological Indicators, 131*, 108172.

Mishra, A. K., & Mohanty, B. (2008). Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, Channa punctatus (Bloch). Environmental Toxicology and Pharmacology, 26(2), 136-141.

Mitchell, S. M., Ullman, J. L., Teel, A. L., & Watts, R. J. (2014). pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin. Science of the total environment, 466, 547-555.

Mizhir, A. H., & Jasim, N. A. (2022, March). Biodiversity of Mollusca in Bahr AL-Najaf Depression in winter. In *IOP* Conference Series: Earth and Environmental Science (Vol. 1002, No. 1, p. 012001). IOP Publishing.

Mkandawire M, Jaime A, da Silva T, Dudel EG (2014) The *Lemna* bioassay: contemporary issues as the most standardized plant bioassay for aquatic ecotoxicology. Crit Rev Environ Sci Technol 44(2):154–197.

Mohamed, A. A. R., El-Houseiny, W., Abd Elhakeem, E. M., Ebraheim, L. L., Ahmed, A. I., & Abd El-Hakim, Y. M. (2020). Effect of hexavalent chromium exposure on the liver and kidney tissues related to the expression of CYP450 and GST genes of Oreochromis niloticus fish: Role of curcumin supplemented diet. *Ecotoxicology and* environmental safety, 188, 109890.

Moreno-Garrido, I. (2008). Microalgae immobilization: current techniques and uses. Bioresource technology, *99*(10), 3949-3964.

Moron, M.S.; Depierre, J.W. and Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta., 582: 67-78.

Morris, J. J., Rose, A. L., & Lu, Z. (2022). Reactive oxygen species in the world ocean and their impacts on marine ecosystems. Redox Biology, 102285.

Mostafalou, S., & Arab, A. (2022). Environmental toxicology and developing countries

Nandi, A., Yan, L. J., Jana, C. K., & Das, N. (2019). Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative medicine and cellular longevity, 2019 .

Ndlela, L. L., Schroeder, P., Genthe, B., & Cruzeiro, C. (2023). Removal of Antibiotics Using an Algae-Algae Consortium (Chlorella protothecoides and Chlorella vulgaris). Toxics, 11(7), 588

Neri-Cruz, N., Gómez-Oliván, L. M., Galar-Martínez, M., del Socorro Romero-Figueroa, M., Islas-Flores, H., García-Medina, S., ... & SanJuan-Reyes, N. (2015). Oxidative stress in Cyprinus carpio induced by hospital wastewater in *Mexico. Ecotoxicology*, *24*, 181-193.

Nicolussi, G., Fabrello, J., Asnicar, D., Ciscato, M., Matozzo, V., & Marin, M. G. (2022). Effects of Three Widely Used Antibiotics and Their Mixture on the Haemocytes of the Clam *Ruditapes philippinarum.* Frontiers in Environmental Science

Nikolaou, A., Meric, S. and Fatta, D.,(2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. Analytical and bioanalytical chemistry, 387(4), pp.1225-1234.

Njus, D., Kelley, P. M., Tu, Y. J., & Schlegel, H. B. (2020). Ascorbic acid: The chemistry underlying its antioxidant properties. Free Radical Biology and Medicine, 159, 37-43.

Nowotny, A., & Nowotny, A. (1979). Protein determination by the Biuret method.

Nunes, B. (2020). Ecotoxicological effects of the drug paracetamol: a critical review of past ecotoxicity assessments and future perspectives. Non-Steroidal Anti-Inflammatory Drugs in Water, 131-145.

Nunes, B., Nunes, J., Soares, A. M., Figueira, E., & Freitas, R. (2017). Toxicological effects of paracetamol on the clam Ruditapes philippinarum: exposure vs recovery. *Aquatic Toxicology*, *192*, 198-206.

Olakolu, F.C., Chukwuka, A.V., (2014). Trace metal concentrations and antioxidant activity in ovarian tissue of blue crab *Callinectes amnicola* from Lagos lagoon and implications for reproductive success. Zool. Ecol. 24 (3), 278–284.

Oliveira, P., Almeida, Â., Calisto, V., Esteves, V. I., Schneider, R. J., Wrona, F. J., ... & Freitas, R. (2017). Physiological and biochemical alterations induced in the mussel *Mytilus galloprovincialis* after short and long-term exposure to carbamazepine. Water *research*, *117*, 102-114.

Omar, T. F. T., Aris, A. Z., Yusoff, F. M., & Mustafa, S. (2018). Occurrence, distribution, and sources of emerging organic contaminants in tropical coastal sediments of anthropogenically impacted Klang River estuary, *Malaysia. Marine pollution bulletin, 131, 284-293*

Omar, T.F.T., Aris, A.Z., Yusoff, F.M., Mustafa, S., (2019). Occurrence and level of emerging organic contaminant in fish and mollusk from Klang River estuary, Malaysia and assessment on human health risk. Environ. Pollut. 248, 763–773.

Orozco-Hernández, J. M., Oliván, L. M. G., Heredia-García, G., Luja-Mondragón, M., Islas-Flores, H., SanJuan-Reyes, N., ... & Dublán-García, O. (2019). Genotoxic and cytotoxic alterations induced by environmentally-relevant concentrations of amoxicillin in blood cells of *Cyprinus carpio.* Chemosphere, *236*, 124323

Oyaneder-Terrazas, J., Figueroa, D., Araneda, O. F., & García, C. (2022). Saxitoxin Group Toxins Accumulation Induces Antioxidant Responses in Tissues of *Mytilus chilensis, Ameghinomya antiqua,* and *Concholepas concholepas* during a Bloom of Alexandrium pacificum. Antioxidants, *11*(2), 392.

Pagnanelli, F., Jbari, N., Trabucco, F., Martínez, M. E., Sánchez, S., & Toro, L. (2013). Biosorption-mediated reduction of Cr (VI) using heterotrophically-grown *Chlorella vulgaris:* Active sites and ionic strength effect. *Chemical engineering journal*, *231*, 94-102.

Palito, C., Encarnação, T., Singh, P., Valente, A. J., & Pais, A. A. (2021). Microalgae Immobilization and Use in Bioremediation. In Marine Microbial Bioremediation (pp. 122-141). CRC Press.

Park, S., & Choi, K. (2008). Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology, 17, 526-538.

Parolini, M., De Felice, B., Gazzotti, S., Annunziata, L., Sugni, M., Bacchetta, R., & Ortenzi, M. A. (2020). Oxidative stress-related effects induced by micronized polyethylene terephthalate microparticles in the *Manila* clam. Journal of Toxicology and Environmental Health, Part A, 83(4), 168-179.

Partovinia, A., Rasekh, B. (2018). Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments. Critical Reviews in Environmental Science and Technology, 48(1), 1–38.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chemical reviews, *119*(6), 3510-3673.

Patil, A.G.(2011) Protein Changes in Different Tissues of Freshwater Bivalve Parreysia cylindrica after Exposed to Indoxacarb. Recent Research in Science and Technology, 3(3): 140-142

Patlolla, A. K., Barnes, C., Yedjou, C., Velma, V. R., & Tchounwou, P. B. (2009). Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental Toxicology: An International Journal*, *24*(1), 66-73.

Pehlivan, F. E. (2017). Vitamin C: An antioxidant agent. Vitamin C, *2*, 23-35.

Pei, J., Pan, X., Wei, G., & Hua, Y. (2023). Research progress of glutathione peroxidase family (GPX) in redoxidation. Frontiers in Pharmacology, *14*, 1147414

Peng, F.Q. et al. (2014) Biotransformation of progesterone and norgestrel by two fresh water microalgae(*Scenedesmus obliquus* and *Chlorella pyrenoidosa*): transformation kinetics and products identification. Chemosphere 95, 581–588.

Pereto, C., Coynel, A., Lerat-Hardy, A., Gourves, P. Y., Schäfer, J., & Baudrimont, M. (2020). *Corbicula fluminea:* a sentinel species for urban Rare Earth Element origin. Science of the Total Environment, *732*, 138552.

Pes, K., Friese, A., Cox, C. J., Laize, V., & Fernández, I. (2021). Biochemical and molecular responses of the Mediterranean mussel *(Mytilus galloprovincialis*) to short-term exposure to three commonly prescribed drugs. *Marine* Environmental Research, *168*, 105309.

Pham HV, Torresan S, Critto A, Marcomini A (2019) Alteration of freshwater ecosystem services under global change–a review focusing on the Po River basin (Italy) and the Red River basin (Vietnam). Sci Total Environ 652:1347–1365.

Piedade, F., Bio, S., & Nunes, B. (2020). Effects of common pharmaceutical drugs (paracetamol and acetylsalicylic acid) short term exposure on biomarkers of the mussel Mytilus spp. Environmental toxicology and pharmacology, *73*, 103276.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity, 2017.

Poljšak, B., Gazdag, Z., Jenko-Brinovec, Š., Fujs, Š., Pesti, M., Bélagyi, J., ... & Raspor, P. (2005). Pro-oxidative vs antioxidative properties of ascorbic acid in chromium (VI)-induced damage: an in vivo and in vitro approach. Journal of Applied Toxicology: An International Journal, 25(6), 535-548.

Porkka, T. (2021). Optimization of Microalgal Immobilization for Cultivation in Aquaculture Wastewater (Master's thesis, Itä-Suomen yliopisto).

Prato, E., Biandolino, F., Grattagliano, A., Ruscito, A., Lofrano, G., Libralato, G., ... & Parlapiano, I. (2023). Individual and combined effects of amoxicillin and carbamazepine to the marine copepod *Tigriopus fulvus. Environmental Science and Pollution Research*, 1-10.

Raeiszadeh, M., & Adeli, B. (2020). A critical review on ultraviolet disinfection systems against COVID-19 outbreak: applicability, validation, and safety considerations. Acs Photonics, *7*(11), 2941-2951.

Rahman, Z., & Thomas, L. (2021). Chemical-assisted microbially mediated chromium (Cr)(VI) reduction under the influence of various electron donors, redox mediators, and other additives: an outlook on enhanced Cr (VI) removal. Frontiers in Microbiology, 11, 619766.

Ramakritinan, C. M., Chandurvelan, R., & Kumaraguru, A. K. (2012). Acute Toxicity of Metals: Cu, Pb, Cd, Hg and Zn on Marine Molluscs, *Cerithedia cingulata G.,* and *Modiolus philippinarum H.*

Ranatunga, M., Kellar, C., & Pettigrove, V. (2023). Toxicological impacts of synthetic pyrethroids on non-target aquatic organisms: a review. Environmental Advances, 100388.

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., ... & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews, *94*(3), 849-873.

Revathi S, Kumar SM, Santhanam P, Kumar SD, Son D, Kim M-K (2017). Bioremoval of the indigo blue dye by immobilized microalga *Chlorella vulgaris* (PSBDU06). J Sci Ind Res 76(1):50–56

Reyna, P. B., Ballesteros, M. L., Albá, M. L., Bertrand, L., González, M., Miglioranza, K. S. B., ... & Hued, A. C. (2019). A multilevel response approach reveals the Asian clam *Corbicula largillierti* as a mirror of aquatic pollution. Science of the Total Environment, 692, 175-187.

Rice, E. W., Bridgewater, L., & American Public Health Association (Eds.). (2012). Standard methods for the examination of water and wastewater (Vol. 10). Washington, DC: American public health association.

Ricky, R., Chiampo, F., & Shanthakumar, S. (2022). Efficacy of Ciprofloxacin and Amoxicillin Removal and the Effect on th Biochemical Composition of *Chlorella vulgaris*. Bioengineering, 9(4), 134.

Rodrigues, S., Antunes, S. C., Correia, A. T., & Nunes, B. (2017). Rainbow trout (Oncorhynchus mykiss) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. *Ecotoxicology*, *26*, 104-117.

Rodrigues, S., Antunes, S. C., Nunes, B., & Correia, A. T. (2019). Histopathological effects of the antibiotic erythromycin on the freshwater fish species *Oncorhynchus mykiss*. Ecotoxicology and Environmental Safety, *181*, 1-10.

Rosner, A., Armengaud, J., Ballarin, L., Barnay-Verdier, S., Cima, F., Coelho, A. V., ... & Cambier, S. (2021). Stem cells of aquatic invertebrates as an advanced tool for assessing ecotoxicological impacts. Science of the Total Environment, 771, 144565.

Ruan, Y., Lin, H., Zhang, X., Wu, R., Zhang, K., Leung, K.M., Lam, J.C.W., Lam, P.K., (2020). Enantiomer-specific bioaccumulation and distribution of chiral pharmaceuticals in a subtropical marine food web. J. Hazard. Mater. 394, 122589.

Rusdi, Komala, R., & Utami, T. P. (2021). Antioxidant enzyme activities and malondialdehyde level in green mussel (*Perna viridis L.)* at Jakarta Bay, Indonesia. In *AIP* Conference Proceedings (Vol. 2331, No. 1, p. 050007). AIP Publishing LLC.

Sadeghi, P., Savari, A., Movahedinia, A., Safahieh, A., & Azhdari, D. (2014). Determination the lethal concentration (LC50) of potassium dichromate and behavioral responses in epaulet grouper (*Epinephelus stoliczkae*). Journal of Oceanography, 5(17), 1-9.

Saha, S. S. N. C., & Saha, N. C. (2021). Study on acute toxicity of bifenthrin to (*Clarias batrachus Linn.*). Indian J. Ecol, 48, 545-548.

Saidani, W., Sellami, B., Khazri, A., Mezni, A., Dellali, M., Joubert, O., ... & Beyrem, H. (2019). Metal accumulation, biochemical and behavioral responses on the Mediterranean clams *Ruditapes decussatus* exposed to two photocatalyst nanocomposites (TiO2 NPs and AuTiO2NPs). Aquatic toxicology, *208*, 71-79.

Sajjad. Zare, H.P. (2012) Changes activities of antioxidant enzymes in oilseed rape in response to salinity stress. International Journal of Agriculture and Crop Sciences 7, 398-403

Saleh, E. M., Hamdy, G. M., & Hassan, R. E. (2022). Neuroprotective effect of sodium alginate against chromium-induced brain damage in rats. Plos one, 17(4), e0266898.

Salman, J. M. (2011). The Clam *Pseudodontpsis euphraticus* (Bourguignat, 1852) as a Bioaccumulation Indicator Organism of Heavy Metals in Euphrates River-Iraq. Journal of Babylon University, Pure and Applied Sciences, 13(3).

Salman, J. M., Kaduem, N. F., and Juda, S. A.(2022). Algal immobilization as a green technology for domestic wastewater treatment. In IOP Conference Series: Earth and Environmental Science (Vol. 1088, No. 1, p. 012005).

Samal, K., Mahapatra, S., & Ali, M. H. (2022). Pharmaceutical wastewater as Emerging Contaminants (EC): treatment technologies, impact on environment and human health. Energy Nexus, 100076.

Santos, C. E., de Coimbra, R. N., Bermejo, S. P., Pérez, A. I. G., & Cabero, M. O. (2017). Comparative assessment of pharmaceutical removal from wastewater by the microalgae *Chlorella sorokiniana, Chlorella vulgaris and Scenedesmus obliquu*s. Biological Wastewater Treatment and Resource Recovery, 99.

Sarica, Z. S., Eren, M., & Senturk, M. (2019). Effect of Boron on the Potassium Dichromate Induced Oxidative Damage in Brain Tissue of Sprague Dawley Rats. Pakistan Journal of Zoology, 51(5), 1905-191.

Sarıkaya, E., & Doğan, S. (2020). Glutathione peroxidase in health and diseases. Glutathione system and oxidative stress in health and disease, 49.

Sarkheil, M., Ameri, M., & Safari, O. (2022). Application of alginate-immobilized microalgae beads as biosorbent for removal of total ammonia and phosphorus from water of African cichlid (Labidochromis lividus) recirculating aquaculture system. *Environmental Science and Pollution Research*, 1-13.

Schuijt, L. M., Peng, F. J., van den Berg, S. J., Dingemans, M. M., & Van den Brink, P. J. (2021). (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. Science of the total environment, 795, 148776.

Sebastiano, M., Messina, S., Marasco, V., & Costantini, D. (2022). Hormesis in ecotoxicological studies: a critical evolutionary perspective. Current Opinion in Toxicology.

Seoane, M., Cid, Á., Herrero, C., & Esperanza, M. (2021). Comparative acute toxicity of benzophenone derivatives and bisphenol analogues in the Asian clam Corbicula fluminea. *Ecotoxicology*, *30*, 142-153

Serra-Compte, A., Pikkemaat, M. G., Elferink, A., Almeida, D., Diogène, J., Campillo, J. A., ... & Rodríguez-Mozaz, S. (2021). Combining an effect-based methodology with chemical analysis for antibiotics determination in wastewater and receiving freshwater and marine environment. Environmental Pollution, *271*, 116313.

Sessarego, S., Rodrigues, S.C., Xiao, Y., Lu, Q., Hill, J.M.,(2019). Phosphonium-enhanced chitosan for Cr (VI) adsorption in wastewater treatment. Carbohydr. Polym. 211, 249–256.

Shah, Z. U., & Parveen, S. (2022). Oxidative, biochemical and histopathological alterations in fishes from pesticide contaminated river Ganga, India. Scientific Reports, 12(1), 3628

Shan, Y., Yan, S., Hong, X., Zha, J., & Qin, J. (2020). Effect of imidacloprid on the behavior, antioxidant system, multixenobiotic resistance, and histopathology of Asian freshwater clams (Corbicula fluminea). Aquatic Toxicology, 218, 105333

Sharma, A., Kapoor, D., Wang, J., Shahzad, B., Kumar, V., Bali, A. S., et al. (2020). Chromium bioaccumulation and its impacts on plants: An overview. Plants (Basel) 9 (1), 100. Epub 2020/01/17.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany, 2012.

Sharma, P., Singh, S. P., Parakh, S. K., & Tong, Y. W. (2022). Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction. Bioengineered, 13(3), 4923-4938.

Sheir, S. K. (2020). Histological Responses of The Freshwater Clam, Caelatura nilotica (Cailliaud, 1827) to Different Environmental Degrees Of Mixed Pollution. Egyptian Academic Journal of Biological Sciences, B. Zoology, 12(2), 49-66.

Singh, V., Pandey, B., & Suthar, S, Surindra. Phytotoxicity of amoxicillin to the duckweed *Spirodela polyrhiza*: Growth, oxidative stress, biochemical traits and antibiotic degradation. Chemosphere. 2018; 201: 492-502.

Singh, V., Singh, N., Verma, M., Kamal, R., Tiwari, R., Sanjay Chivate, M., ... & Mishra, V. (2022). Hexavalent-chromium-induced oxidative stress and the protective role of antioxidants against cellular toxicity. *Antioxidants*, *11*(12), 2375

Smejkal, G. B., & Kakumanu, S. (2019). Enzymes and their turnover numbers. *Expert Review of Proteomics*, *16*(7), 543-544.

Snezhkina, A. V., Kudryavtseva, A. V., Kardymon, O. L., Savvateeva, M. V., Melnikova, N. V., Krasnov, G. S., & Dmitriev, A. A. (2019). ROS generation and antioxidant defense systems in normal and malignant cells. Oxidative medicine and cellular longevity, 2019.

Sodhi, K. K., Kumar, M., & Singh, D. K. (2021). Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies. Journal of Water Process Engineering, 39, 101858.

Sodhi, K. K., Kumar, M., Balan, B., Dhaulaniya, A. S., & Singh, D. K. (2020). Isolation and characterization of amoxicillin-resistant bacteria and amoxicillin-induced alteration in its protein profiling and RNA yield. Archives of microbiology, 202(2), 225-232.

Song, C.F., Wei, Y.L., Qiu, Y.T., Qi, Y., Li, Y., Kitamura, Y., (2019). Biodegradability and mechanism of florfenicol via Chlorella sp. UTEX1602 and L38: Experimental study.Bioresour. Technol. 272, 529–534

Stara, A., Pagano, M., Capillo, G., Fabrello, J., Sandova, M., Albano, M., ... & Faggio, C. (2020). Acute effects of neonicotinoid insecticides on *Mytilus galloprovincialis*: A case study with the active compound thiacloprid and the commercial formulation calypso 480 SC. Ecotoxicology and environmental safety, 203, 110980.

Sutherland, D. L., and Ralph, P. J. (2019). Microalgal bioremediation of emergingcontaminants - Opportunities and challenges. Water Res. 164:114921. doi: 10. 1016/j.watres.2019.11492.

Świacka, K., Maculewicz, J., Smolarz, K., Szaniawska, A., & Caban, M. (2019). *Mytilidae* as model organisms in the marine ecotoxicology of pharmaceuticals-a review. Environmental Pollution, *254*, 113082.

Tan, M., Yin, Y., Ma, X., Zhang, J., Pan, W., Tan, M., ... & Li, H. (2023). Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death & Disease, 14(2), 131.

Tang, J., Wang, S., Tai, Y., Tam, N. F., Su, L., Shi, Y., et al. (2020). Evaluation of Factors Influencing Annual Occurrence, Bioaccumulation, and Biomagnification of Antibiotics in Planktonic Food Webs of a Large Subtropical River in South China. Water Res.

Tijani JO, Fatoba OO, Babajide OO, Petrik LF (2016) Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review. Environ Chem Lett 14(1):27–49

Tiwari, B., Ouarda, Y., Drogui, P., Tyagi, R. D., Vaudreuil, M. A., Sauvé, S., ... & Dubé, R. (2021). Fate of pharmaceuticals in a submerged membrane bioreactor treating hospital wastewater. Frontiers in Water, 3, 730479.

Topić Popović, N., Krbavčić, M., Barišić, J., Beer Ljubić, B., Strunjak-Perović, I., Babić, S., ... & Čož-Rakovac, R. (2021). Comparative tissue responses of marine mollusks on seasonal changes in the Northern Adriatic Sea. Applied Sciences, *11*(6), 2874.

Touliabah, H. E. S., El-Sheekh, M. M., Ismail, M. M., & El-Kassas, H. (2022). A review of microalgae-and cyanobacteria-based biodegradation of organic pollutants. Molecules, 27(3), 1141.

Trabelsi, W., Fouzai, C., Chetoui, I., Bejaoui, S., Telahigue, K., Rabeh, I., ... & Soudani, N. (2020). Oxidative stress biomarkers in the gills of the bivalve *Mactra stultorum* exposed to acrylamide. Scientia Marina, *84*(2), 143-150.

Tran, N.H., Reinhard, M., Gin, K.Y.H.,(2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182e207.

Trchounian, A., Petrosyan, M., Sahakyan, N. (2016) Plant Cell Redox Homeostasis and Reactive Oxygen Species. In: Gupta, D.K., Palma, J.M., Corpas, F.J. (Eds.). Redox State as a Central Regulator of Plant-Cell Stress Responses. Springer International Publishing, Cham, pp. 25-50.

Tredici, M.R. (2004) Mass production of microalgae: photobioreactors. In: Richmond A. [Eds.] Handbook of Microalgal Culture, Biotechnology and Applied Phycology. Oxford: Blackwell Science, pp: 588.

Umamaheswari, S., Renuka, S. S., Ramesh, M., & Poopal, R. K. (2019). Chronic amoxicillin exposure affects *Labeo rohita*: assessment of hematological, ionic compounds, biochemical, and enzymological activities. Heliyon, *5*(4), e01434.

US EPA Environmental Protection Agency (EPA).(1993). In: Weber, C.I., (Ed.), Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms, fourth ed., EPA-600/4-90-027.

Vacchi-Suzzi, C., Viens, L., Harrington, J. M., Levine, K., Karimi, R., & Meliker, J. R. (2018). Low levels of lead and glutathione markers of redox status in human blood. Environmental geochemistry and health, *40, 1175-1185.*

Vairetti, M., Di Pasqua, L. G., Cagna, M., Richelmi, P., Ferrigno, A., & Berardo, C. (2021). Changes in glutathione content in liver diseases: an update. Antioxidants, 10(3), 364.

Varol, M., & Sünbül, M. R. (2017). Organochlorine pesticide, antibiotic and heavy metal residues in mussel, crayfish and fish species from a reservoir on the Euphrates River, Turkey. Environmental pollution, *230*, 311-31.

Vašková, J., Kočan, L., Vaško, L., & Perjési, P. (2023). Glutathione-Related Enzymes and Proteins: A Review. Molecules, 28(3), 1447.

Vaughn, C. C., & Hoellein, T. J. (2018). Bivalve impacts in freshwater and marine ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 183-208

Velisek, J., Svobodova, Z., Piackova, V., (2009). Effects of acute exposure to bifenthrin onsome haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). Vet. Med. 54 (3), 131–137.

Velpandian, T., Halder, N., Nath, M., Das, U., Moksha, L., Gowtham, L., & Batta, S. P. (2018). Un-segregated waste disposal: an alarming threat of antimicrobials in surface and ground water sources in Delhi. Environmental Science and Pollution Research, 25, 29518-29528.

Verlicchi, P., Al Aukidy, M., & Zambello, E. (2012). Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review. Science of the total environment, 429, 123-155.

Vo, H. N. P., Ngo, H. H., Guo, W., Nguyen, K. H., Chang, S. W., Nguyen, D. D., ... and Bui, X. T. (2020). Micropollutants cometabolism of microalgae for wastewater remediation: effect of carbon sources to cometabolism and degradation products. Water research, 183, 115974. doi.org/10.1016/j.watres.2020.115974.

Wang, G., Zhang, C., & Huang, B. (2020). Transcriptome analysis and histopathological observations of Geloina erosa gills upon Cr (VI) exposure. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 231, 108706.

Wang, H., Xi, H., Xu, L., Jin, M., Zhao, W., & Liu, H. (2021 a). Ecotoxicological effects, environmental fate and risks of pharmaceutical and personal care products in the water environment: a review. Science of The Total Environment, 788, 147819.

Wang, Q., Hong, X., Chen, H., Yuan, L., Zha, J. (2018) The neuropeptides of Asian freshwater clam (*Corbicula fluminea*) as new molecular biomarker basing on the responses of organophosphate chemicals exposure. Ecotoxicology and Environmental Safety 160, 52-59.

Wang, W., Zheng, B., Jiang, X., Chen, J., & Wang, S. (2020b). Characteristics and source of dissolved organic matter in Lake Hulun, A Large shallow eutrophic steppe lake in northern China. *Water*, *12*(4), 953.

Wang, Y., Su, H., Gu, Y., Zhao, J., (2017). Carcinogenicity of chromium and chemoprevention: a brief update. OncoTargets Ther. 10, 4065–4079.

Wang, Y., Wang, Z. J., Huang, J. C., Zhou, C., Zou, H., He, S., & Chen, V. Y. C. (2021 b). Feasibility of using *Chlorella vulgaris* for the removal of selenium and chromium in water: Competitive interactions with sulfur, physiological effects on algal cells and its resilience after treatment. Journal of Cleaner Production, 313, 127939.

Wang, Z., Kong, F., Fu, L., Li, Y., Li, M., & Yu, Z. (2021c). Responses of Asian clams (*Corbicula fluminea*) to low concentration cadmium stress: Whether the depuration phase restores physiological characteristics. Environmental Pollution, *284*, 117182.

Wilson, I., Gamble, M., (2002) The hematoxylins and eosin. In: Bancroft, J.D., Gamble, M. (Eds.), Theory, Practice of Histological Techniques. Churchill Livingston e- Elsevier Science Ltd., London, UK, p. 796.

Wirth, R., Pap, B., Böjti, T., Shetty, P., Lakatos, G., Bagi, Z., ... & Maróti, G. (2020). *Chlorella vulgaris* and its phycosphere in wastewater: Microalgae-bacteria interactions during nutrient removal. Frontiers in bioengineering and biotechnology, 8, 557572.

Wong, Y. C., & Roma, D. N. (2021). Potential of the Biodegradability and Characteristics of Bioplastic From Microalgae Residues. International Journal on Algae, 23(1).

Woodin, S. A., Wethey, D. S., Olabarria, C., Vázquez, E., Domínguez, R., Macho, G., & Peteiro, L. (2020). Behavioral responses of three venerid bivalves to fluctuating salinity stress. Journal of Experimental Marine Biology and Ecology, 522, 151256

Wu, C. F., Chen, C. H., Wu, C. Y., Lin, C. S., Su, Y. C., Wu, C. F., ... & Chang, G. R. (2020b). Quinolone and organophosphorus insecticide residues in bivalves and their associated risks in Taiwan. *Molecules*, *25*(16), 3636.

Wu, L., Xu, W., Li, H., Dong, B., Geng, H., Jin, J., ... & Xie, S. (2022a). Vitamin C Attenuates Oxidative Stress, Inflammation, and Apoptosis Induced by Acute Hypoxia through the Nrf2/Keap1 Signaling Pathway in Gibel Carp (Carassius gibelio). Antioxidants, *11*(5), 935.

Wu, M., Miao, J., Li, Y., Wu, J., Wang, G., Zhang, D., & Pan, L. (2022b). Impact of P-Chloroaniline on Oxidative Stress and Biomacromolecules Damage in the Clam *Ruditapes philippinarums:* A Simulate Toxicity Test of Spill Incident. International Journal of Environmental Research and Public Health, 19(9), 5092

Wu, Y., Gu, E., Li, H., Tian, C., Feng, C. (2019) Oxidative stress and histological changes in *Corbicula fluminea* exposed to nano-Al13 and monomeric Al coagulants. Environmental Science: Nano 6, 2736-2748.

Wu, Y., Wan, L., Zhang, W., Ding, H., & Yang, W. (2020). Resistance of cyanobacteria *Microcystis aeruginosa* to erythromycin with multiple exposure. Chemosphere, 249, 126147.

Xiao, G., Chen, J., Show, P. L., Yang, Q., Ke, J., Zhao, Q., ... & Liu, Y. (2021). Evaluating the application of antibiotic treatment using algae-algae/activated sludge system. Chemosphere, *282*, 130966.

Xie, B., Tang, X., Ng, H. Y., Deng, S., Shi, X., Song, W., ... & Liang, H. (2020b). Biological sulfamethoxazole degradation along with anaerobically digested centrate treatment by immobilized microalgal-bacterial consortium: performance, mechanism and shifts in bacterial and microalgal communities. Chemical Engineering Journal, 388, 124217.

Xie, P., Chen, C., Zhang, C., Su, G., Ren, N., and Ho, S. H.(2020 a). Revealing the role of adsorption in ciprofloxacin and sulfadiazine elimination routes in microalgae. Water research, 172, 115475. doi.org/10.1016/j.watres.2020.115475.

Xie, P., Ho, S. H., Peng, J., Xu, X. J., Chen, C., Zhang, Z. F., ... & Ren, N. Q. (2019). Dual purpose microalgae-based biorefinery for treating pharmaceuticals and personal care products (PPCPs) residues and biodiesel production. Science of the total environment, *688*, 253-261..

Xiong, J. Q., Govindwar, S., Kurade, M. B., Paeng, K. J., Roh, H. S., Khan, M. A., & Jeon, B. H. (2019). Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, *Scenedesmus obliquus*. Chemosphere, 218, 551-558.

Xiong, J. Q., Kurade, M. B., & Jeon, B. H. (2017). Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris*. Chemical Engineering Journal, *313*, 1251-1257.

Xiong, J. Q., Kurade, M. B., & Jeon, B. H. (2018). Can microalgae remove pharmaceutical contaminants from water?. Trends in biotechnology, 36(1), 30-44. doi.org/10.1016/j.tibtech.2017.09.003

Xiong, Q., Hu, L. X., Liu, Y. S., Zhao, J. L., He, L. Y., and Ying, G. G. (2021). Microalgae-based technology for antibiotics removal: From mechanisms to application of innovational hybrid systems. Environment International, 155, 106594. doi.org/10.1016/j.envint.2021.106594.

Xiong, Q., Liu, Y. S., Hu, L. X., Shi, Z. Q., Cai, W. W., He, L. Y., & Ying, G. G. (2020). Co-metabolism of sulfamethoxazole by a freshwater microalga *Chlorella pyrenoidosa*. Water Research, 175, 115656.

Yan, G., Gao, Y., Xue, K., Qi, Y., Fan, Y., Tian, X., ... & Liu, J. (2023). Toxicity mechanisms and remediation strategies for chromium exposure in the environment. Frontiers in Environmental Science, 11, 161.

Yan, S., Wu, H., Qin, J., Zha, J., & Wang, Z. (2017). Halogen-free organophosphorus flame retardants caused oxidative stress and multixenobiotic resistance in Asian freshwater clams (Corbicula fluminea). Environmental pollution, 225, 559-568.

Yazdi, M., Sayadi, M. H., & Farsad, F. (2018). Removal of penicillin in aqueous solution using *chlorella vulgaris* and *spirulina platensis* from hospital wastewater. *Desalin.* Water Treat, 123, 315-320.

Yen, H. W., Chen, P. W., Hsu, C. Y., & Lee, L. (2017). The use of autotrophic *Chlorella vulgaris* in chromium (VI) reduction under different reduction conditions. Journal of the Taiwan Institute of Chemical Engineers, *74*, 1-6.

Younus, H. (2018). Therapeutic potentials of superoxidn dismutase. International journal of health sciences, 12(3), 88.

Yu, Y., Zhou, Yangyang, Wang, Zhiliang, Lopez Torres, Oscar, Guo, Ruixin, Chen, Jianqiu, (2017). Investigation of the removal mechanism of antibiotic ceftazidime by green algae and subsequent microbic impact assessment. Sci. Rep.

Yuan, N., Wu, J., Zheng, M., Wang, H., Bao, E., Chu, Y., & Hu, F. (2022). A multibiomarker approach to assess the ecotoxicological effects of diclofenac on Asian clam *Corbicula fluminea* (OF Müller, 1774).

Zandi, P., & Schnug, E. (2022). Reactive oxygen species, antioxidant responses and implications from a microbial modulation perspective. Biology, 11(2), 155.

Zhang, H., Hong, X., Yan, S., Zha, J., Qin, J. (2020a) Environmentally relevant concentrations of bifenthrin induce changes in behaviour, biomarkers, histological characteristics, and the transcriptome in *Corbicula fluminea*. Science of the total environment 728, 138821.

Zhang, H., Tang, W., Chen, Y., & Yin, W. (2020b). Disinfection threatens aquatic *ecosystems. Science, 368(6487),* 146-147.

Zhang, J. (2020). Environmental Problems of human settlements and countermeasures based on ecological engineering. In Study *of* Ecological Engineering of Human Settlements (pp. 1-39). Springer, Singapore.

Zhang, J., Fu, D.,and Wu, J. (2012). Photodegradation of Norfloxacin in aqueous solution containing algae. Journal of Environmental Sciences, *24*(4),

Zhang, S. Q., Li, P., Zhao, X. L., He, S. W., Xing, S. Y., Cao, Z. H., ... & Li, Z. H. (2021). Hepatotoxicity in carp (*Cyprinus carpio*) exposed to environmental levels of norfloxacin (NOR): some latest evidences from transcriptomics analysis, biochemical parameters and histopathological changes. Chemosphere, *283*, 131210.

Zhao, L., Yuan, B. D., Zhao, J. L., Jiang, N., Zhang, A. Z., Wang, G. Q., & Li, M. Y. (2020). Amelioration of hexavalent chromium-induced bioaccumulation, oxidative stress, tight junction proteins and immune-related signaling factors by Allium mongolicum Regel flavonoids in *Ctenopharyngodon idella.* Fish & Shellfish Immunology,

Zhao, Z., Xue, R., Fu, L., Chen, C., Ndayisenga, F., & Zhou, D. (2021). Carbon dots enhance the recovery of microalgae bioresources from wastewater containing amoxicillin. Bioresource Technology, 335, 125258 .

Zhong, X., Zeng, M., Bian, H., Zhong, C., & Xiao, F. (2017). An evaluation of the protective role of vitamin C in reactive oxygen species-induced hepatotoxicity due to hexavalent chromium in vitro and in vivo. Journal of Occupational Medicine and Toxicology, 12, 1-12.

Zieritz, A., Froufe, E., Bolotov, I., Gonçalves, D. V., Aldridge, D. C., Bogan, A. E., ... & Lopes-Lima, M. (2021). Mitogenomic phylogeny and fossil-calibrated mutation rates for all F-and M-type mtDNA genes of the largest freshwater mussel family, the Unionidae (Bivalvia). Zoological Journal of the Linnean Society, *193*(3), 1088-1107.

Zou, H., Huang, J. C., Zhou, C., He, S., & Zhou, W. (2020). Mutual effects of selenium and chromium on their removal by *Chlorella vulgari*s and associated toxicity. Science of The Total Environment, *724*, 138219.

Appendix 1. Lethal concentration (LC_{50}) for *C. fluminea* after 96-h exposure to the AM

Confidence Limits							
	Probability	95% Confidence Limits for concentration			95% Confidence Limits for log(concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	28.998	.	.	1.462	.	.
	.020	39.893	.	.	1.601	.	.
	.030	48.842	.	.	1.689	.	.
	.040	56.874	.	.	1.755	.	.
	.050	64.372	.	.	1.809	.	.
	.060	71.527	.	.	1.854	.	.
	.070	78.453	.	.	1.895	.	.
	.080	85.222	.	.	1.931	.	.
	.090	91.883	.	.	1.963	.	.
	.100	98.474	.	.	1.993	.	.
	.150	131.186	.	.	2.118	.	.
	.200	164.775	.	.	2.217	.	.
	.250	200.366	.	.	2.302	.	.
	.300	238.836	.	.	2.378	.	.
	.350	281.047	.	.	2.449	.	.
	.400	327.979	.	.	2.516	.	.
	.450	380.833	.	.	2.581	.	.
	.500	441.159	.	.	2.645	.	.
	.550	511.040	.	.	2.708	.	.
	.600	593.394	.	.	2.773	.	.
	.650	692.486	.	.	2.840	.	.
	.700	814.874	.	.	2.911	.	.
	.750	971.324	.	.	2.987	.	.
	.800	1181.134	.	.	3.072	.	.
	.850	1483.545	.	.	3.171	.	.
	.900	1976.368	.	.	3.296	.	.
	.910	2118.139	.	.	3.326	.	.
	.920	2283.702	.	.	3.359	.	.
	.930	2480.724	.	.	3.395	.	.
	.940	2720.926	.	.	3.435	.	.
	.950	3023.393	.	.	3.480	.	.
	.960	3421.982	.	.	3.534	.	.

a. Logarithm base = 10.

Appendex 2 Lethal concentration (LC50) for *P. euphraticus* after 96-h exposure to the AMX

Confidence Limits							
	Probability	95% Confidence Limits for concentration			95% Confidence Limits for log(concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
PROBIT	.010	26.961	.	.	1.431	.	.
	.020	37.095	.	.	1.569	.	.
	.030	45.419	.	.	1.657	.	.
	.040	52.891	.	.	1.723	.	.
	.050	59.866	.	.	1.777	.	.
	.060	66.523	.	.	1.823	.	.
	.070	72.967	.	.	1.863	.	.
	.080	79.264	.	.	1.899	.	.
	.090	85.462	.	.	1.932	.	.
	.100	91.595	.	.	1.962	.	.
	.150	122.034	.	.	2.086	.	.
	.200	153.292	.	.	2.186	.	.
	.250	186.416	.	.	2.270	.	.
	.300	222.220	.	.	2.347	.	.
	.350	261.509	.	.	2.417	.	.
	.400	305.195	.	.	2.485	.	.
	.450	354.396	.	.	2.549	.	.
	.500	410.555	.	.	2.613	.	.
	.550	475.613	.	.	2.677	.	.
	.600	552.287	.	.	2.742	.	.
	.650	644.549	.	.	2.809	.	.
	.700	758.507	.	.	2.880	.	.
	.750	904.191	.	.	2.956	.	.
	.800	1099.574	.	.	3.041	.	.
	.850	1381.213	.	.	3.140	.	.
	.900	1840.224	.	.	3.265	.	.
	.910	1972.276	.	.	3.295	.	.
	.920	2126.494	.	.	3.328	.	.
	.930	2310.020	.	.	3.364	.	.
	.940	2533.774	.	.	3.404	.	.
	.950	2815.540	.	.	3.450	.	.

3-Lethal concentration (LC_{50}) for *P. euphraticus* after 96 h exposure to the $K_2Cr_2O_7$

Confidence Limits				
	Probability	95% Confidence Limits for concentration		
		Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	-65.493-	-315.379-	-10.819-
	.020	-49.183-	-267.512-	-.225-
	.030	-38.834-	-237.271-	6.627
	.040	-31.049-	-214.610-	11.869
	.050	-24.717-	-196.249-	16.205
	.060	-19.328-	-180.681-	19.955
	.070	-14.602-	-167.086-	23.299
	.080	-10.370-	-154.964-	26.344
	.090	-6.522-	-143.988-	29.161
	.100	-2.980-	-133.931-	31.801
	.150	11.686	-92.931-	43.369
	.200	23.342	-61.456-	53.674
	.250	33.342	-35.764-	63.826
	.300	42.322	-14.287-	74.537
	.350	50.644	3.724	86.353
	.400	58.540	18.733	99.647
	.450	66.180	31.199	114.565
	.500	73.699	41.654	131.059
	.550	81.217	50.641	149.022
	.600	88.857	58.639	168.407
	.650	96.753	66.039	189.310
	.700	105.075	73.165	212.011
	.750	114.055	80.315	237.051
	.800	124.055	87.817	265.391
	.850	135.711	96.146	298.842
	.900	150.377	106.200	341.357
	.910	153.920	108.577	351.677
	.920	157.768	111.140	362.907
	.930	161.999	113.939	375.274
	.940	166.725	117.043	389.109
	.950	172.115	120.558	404.913
	.960	178.447	124.658	423.510

a. A heterogeneity factor is used.

a. Logarithm base = 10.

4-Lethal concentration (LC50) for *C. fluminea* after 96 h exposure to the $K_2Cr_2O_7$

Confidence Limits				
	Probability	95% Confidence Limits for concentration		
		Estimate	Lower Bound	Upper Bound
PROBIT[a]	.010	-105.865-	-487.598-	-32.975-
	.020	-84.468-	-418.290-	-19.635-
	.030	-70.893-	-374.432-	-11.056-
	.040	-60.681-	-341.518-	-4.523-
	.050	-52.374-	-314.810-	.855
	.060	-45.303-	-292.131-	5.487
	.070	-39.104-	-272.296-	9.598
	.080	-33.553-	-254.582-	13.326
	.090	-28.505-	-238.517-	16.760
	.100	-23.858-	-223.772-	19.964
	.150	-4.618-	-163.324-	33.832
	.200	10.673	-116.373-	45.945
	.250	23.791	-77.488-	57.732
	.300	35.572	-44.472-	70.221
	.350	46.489	-16.499-	84.414
	.400	56.847	6.662	101.266
	.450	66.870	25.294	121.346
	.500	76.733	40.146	144.592
	.550	86.596	52.275	170.561
	.600	96.618	62.660	198.888
	.650	106.977	72.046	229.515
	.700	117.894	80.976	262.751
	.750	129.674	89.897	299.335
	.800	142.793	99.261	340.643
	.850	158.084	109.683	389.284
	.900	177.323	122.317	450.966
	.910	181.970	125.312	465.920
	.920	187.019	128.546	482.186
	.930	192.569	132.080	500.093
	.940	198.769	136.005	520.115
	.950	205.839	140.454	542.977
	.960	214.146	145.650	569.867
	.970	224.359	151.999	602.965

a. A heterogeneity factor is used.

5-principal component analysis(PCA) in *C.fluminea* clams

C.fluminea+AMX with *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.889	0.086	0.025	0.000	1.000
Cumulative percentage variance					
of species data	88.9	97.5	100.0	0.00	
Sum of all eigenvalues					1.000
C.fluminea+AMX without *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.817	0.171	0.013	0.000	1.000
Cumulative percentage variance					
of species data	81.7	98.7	100.0	0.00	
Sum of all eigenvalues					1.000
C.fluminae+K2Cr2O7 with *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.935	0.045	0.020	0.000	1.000
of species data	93.5	98.0	100.0	0.00	
Sum of all eigenvalues					1.000
C.fluminae+K2Cr2O7 without *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.957	0.035	0.008	0.000	1.000
of species data	95.7	99.2	100.0	0.00	
Sum of all eigenvalues					1.000

6-principal component analysis(PCA) in *P.euphraticus* clams

P.euphraticus+AMX with *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.908	0.077	0.015	0.000	1.000
Cumulative percentage variance					
of species data	90.8	98.5	100.0	0.00	
Sum of all eigenvalues					1.000
P.euphraticus+AMX without *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.945	0.053	0.001	0.000	1.000
Cumulative percentage variance					
of species data	94.5	99.9	100.0	0.00	
Sum of all eigenvalues					1.000
P.euphraticus +K2Cr2O7 without *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.879	0.088	0.033	0.000	1.000
of species data	87.9	96.7	100.0	0.00	
Sum of all eigenvalues					1.000
P.euphraticus +K2Cr2O7 with *C.vulgaris*					
Axes	1	2	3	4	Total inertia
Eigenvalues	0.740	0.237	0.023	0.000	1.000
of species data	74.0	97.7	100.0	0.00	
Sum of all eigenvalues					1.000

الخلاصة:

يعتبر التلوث المائي بالمركبات الدوائية من أهم التحديات البيئية على مستوى العالم، وذلك بسبب التوسع الحضري المخلفات الصناعية وما ينتج عن ذلك من مخاطر محتملة على صحة الإنسان والنظم البيئية المائية.

تم في هذه الدراسة استخدام نوعين من المواد الصيدلانية: المضاد الحيوي أموكسيسيلين (AMX) والمطهر ثنائي كرومات البوتاسيوم ($K_2Cr_2O_7$) لإحداث التسمم لنوعين مختارين من ثنائية المصراع، *P. euphraticus and C. fluminea* ، والتي تم جمعها من موقع على نهر الفرات في قضاء الهندية وسط العراق، بينما يتم إضافة الطحالب المقيدة (*Chlorella vulgaris*) لتقليل سمية الملوثات المشار اليها.

تم قياس العديد من المتغيرات البيئية لضفة النهر عند جمع ثنائية المصراع بما في ذلك درجة حرارة الماء (12.4-22.5) درجة مئوية، ودرجة حرارة الهواء (26.2-33.4) درجة مئوية ودرجة الحموضة (7.3-8.34) والتوصيل الكهربائي (1058-1130) ميكروسمنز/سم والملوحة (0.55-0.73) جزء بالالف و الأكسجين المذاب (5.9-7.80) ملغم/لتر والطلب الحيوي للأوكسجين(1.7-) 3.35ملغم/لتر وإجمالي المواد الصلبة الذائبة (430.3-694) ملغم/لتر .

تم قياس التركيز المميت 50 (LC50) لتراكيز مختلفة من الأموكسيسيلين (100، 200، 300 ملغم/لتر) في إجمالي 144 محارة من (*P. euphraticus* و*C. fluminea*)، حيث كانت قيم LC50 خلال 96 ساعة هي 410.55 و 441.15 ملغم/لتر على التوالي، بينما بلغت قيم التركيز LC50 لمدة 96 ساعة (73.96 و 76.73) عند استخدام المطهر ثنائي كرومات البوتاسيوم بتراكيز (20، 30، 50، 100، 150) ملغم/لتر في (إجمالي المحار=216) *P. euphraticus* و*C.fluminea* على التوالى.

أظهرت ذوات المصراع المختارة مجموعة من التغيرات السلوكية بعد تعرضها للمواد الصيدلانية، بما في ذلك قفل صماماتها بإحكام لفترات زمنية أطول مما كانت عليه عندما تكون مفتوحة، وفي بعض الأحيان تكون الأقدام والسيفونات ممتدة بلا حراك عند التراكيز العالية. بالاضافة الى حدوث حركات سباحة غير منتظمة، والتسطيح، وإفراز المخاط،وابتلاع الهواء، وقلة الحركة.

تمت دراسة المؤشرات البيوكيميائية لنوعين من المحار ذوات الصدفتين *P. euphraticus* و *C. fluminea* بعد التعرض للأموكسيسيلين في غياب الطحالب المقيدة (*C. vulgaris*) وكانت النتائج كما يلي: كانت قيم أنواع الأوكسجين التفاعلية (ROS) لـ كلا النوعين (12.99 - 22.52) µg/mg و (30.79 - 42.86) µg/mg على التوالي. يحتوي *P. P. euphraticus* على القيم التالية لـ (SOD)، (CAT)، والجلوتاثيون بيروكسيديز (GPX): (20.79 - 33.33)وحدة/مل ، (25.44- 30.5) KU /L و (5.70 - 8.36) U/L, وعلى التوالي، بينما في *C. fluminea* كانت (35.49-50.033) وحدة/مل و (42.51-51.78) KU /L و(16.41-21.12) U/L ، على التوالي. سجلت قيم الجلوتاثيون (GSH) لكلا النوعين (7.55-10.37)غم/ملغم و(4.25-8.03)غم/غم على التوالي، بينما تراوحت قيم حامض الاسكوربيك (AA) بين (13.41-14.48)ميكرومول و(42.92-76.79)ميكرومتر. وكانت قيم البروتين الكلي (TP) (9.65-11.14)ملغم/غم و(35.52-19.57)ملغم/غم، في حين كانت قيم المالونديالدهيد (MDA) (17.69-42.43)ميكرومول/لتر و(9.33-17.21)ميكرومول/لترلكلا النوعين على التوالي.

عندما تم تطبيق *C.vulgaris* المقيدة على *C.fluminea* و *P. euphraticus*في وجود ألاموكسيلين، كانت المؤشرات البيوكيميائية كما هو موضح أدناه: كانت قيم ROS (8.89 - 16.73) µg/mg و(21.33 - 35.49) µg/mg على التوالي; وكانت قيم SOD (12.72 -21.62) وحدة/ مل و (21.46 - 35.49) وحدة / مل على التوالي، وأظهرت CAT قيم (17.56 - 25.15) KU/L و (22.16 - 35.33)KU/L على التوالي. بينما تراوحت قيم GPX بين (5.02-9.58) U/Lو(14.16-19.28) U/L على التوالي، وتراوحت قيم GSH بين (2.83-6.27)ميكروغرام/غم و(6.54-9.46) ميكروغرام/غم على التوالي. سجلت قيم AA بين (13.41-14.48)ميكرومول و (38.11-59.80)ميكرومول على التوالي. وكانت قيم TP المسجلة (14.25 - 15.60) ملغم / غم و (39.23 - 41.41) ملغم / غم على التوالي. وأخيرا،تراوحت قيم MDA بين(6.77-22.72) ميكرومول/لترو(11.60-23.97) ميكرومول/لتر على التوالي.

تم تطبيق نفس شروط التجربة السابقة في غياب *C. vulgaris* المقيد عن طريق تعريض *P. euphraticus* و*C.fluminea* إلى ثنائي كرومات البوتاسيوم ($K_2Cr_2O_7$) بتراكيزمختلفة (20، 30، 50) ملغم / لتر، سجلت المؤشرات البيوكيميائية على النحو التالي: كانت قيم ROS (37.50-51.05) µg/mg و (37.89-63.66)µg/mg . تراوحت قيم SOD من (33.40-28.36) وحدة/مل و(54.04-35.96) وحدة/مل، بينما تم تسجيل قيم CAT (27.16 - 25.76) KU/Lو(27.19 - 46.74) KU/L، وتباينت قيم GPX من (4.35 إلى 6.34)U/L و (10.99

U/L(43.35 - ، وتراوحت قيم GSH من (8.57 - 16.37) ميكروغرام / غم إلى (12.79 - 36.86) ميكروغرام / غم، وكانت قيم AA (20.37 - 26.94) ميكرومول و(35.3 إلى 50.37) ميكرومول، وتراوحت قيم TP من (10.86-12.79) ملغم/غم و(18.43-39.11) ملغم/غم. غم على التوالي، وكانت قيم MDA (29.01-59.88 و12.68-23.22) ميكروغرام/لتر لكلا الكائنين على التوالي.

عند إضافة الطحالب المقيدة *C. vulgaris* إلى التجربة السابقة، أظهرت المؤشرات البيوكيميائية القيم التالية: ROS من (33.88-42.06) µg/mg إلى (41.0-51.0) µg/mg ، وتراوحت قيم SOD من (27.19- 46.74) وحدة/مل إلى (23.36 -31.4) وحدة/مل، بينما تراوحت قيم CAT بين (26.88- 40.32) و(24.55-48.99) KU/L، وكانت قيم GPX(-7.34 3.87) U/Lو (9.37-37.99) U/L. وكانت قيم GSH (22.47- 9.7) ميكروغرام/ملغم و(7.58 -30.26) ميكروغرام/ملغم، وتراوحت قيم AA بين (24.83 -27.94) ميكرومول و(23.67 - 38.89) ميكرومول، بينما كانت قيم TP (13.56 - 10.93) ملغم/غم و (35.69 - 20.44) ملغم/غم، وكانت قيم MDA (11.44 - 9.81) ميكرومول/لتر و (23.06 - 47.92) ميكرومول/لتر، على التوالي. إن تركيز 50 غم/لتر $K_2Cr_2O_7$ و 300 غم/ لتر أموكسيلين هي المسؤولة عن النتائج العالية.

بعد تعرض *P. euphraticus* لفترة طويلة لمادتي AMX و$K_2Cr_2O_7$ عانى العديد من التغيرات النسيجية بما في ذلك: ارتشاح الخلايا وخلايا الدم، وتنكس الخلايا، وتوسيع المساحات اللمفاوية بين الأنابيب، ونخر خلايا الغدة الهضمية، وتضيق التجويف الأنبوبي، بينما ظهرت تغيرات نسجية في الخياشيم الأمامية مثل اندماج الأهداب و تضخم الخلايا و ارتشاح خلايا الدم و النخر وتمزق الخلايا الظهارية.

فيما يتعلق بـ *C. fluminea* فقد حدثت تغيرات في الغدد الهضمية بما في ذلك ضمور وانتشارالأنابيب الهضمية وارتشاح الأنسجة الضامة ونخر خلايا الغدة الهضمية وتضييق التجويف الأنبوبي ونخر الغدد الليمفاوية. فيما شملت التغيرات النسيجية لخلايا الدم في الخياشيم تسلل الخلايا ونخر الظهارة وتمزق الطبقة الظهارية وانصهار الأهداب.