

The Role of *Chlorella vulgaris* in Reducing Some Pharmaceutical Wastes Toxicity in Clam *Pseudodontopsis euphraticus*

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Abstract

Applications of microalgae in environmental studies have recently increased. Current uses of immobilized microalga *Chlorella vulgaris* include reducing pharmaceutical substances such as amoxicillin AMX and potassium dichromate $K_2Cr_2O_7$ on freshwater clam *Pseudodontopsis euphraticus* as a biotic model. Recent research pointed out a change in biomarkers of oxidative stress in an evaluation of induced toxicity. Where clams were exposed to different concentrations 100, 200, and 400 mg/L for 7 days and 20, 30, and 50 mg/L for 5 days of amoxicillin and potassium dichromate, respectively. The results showed that exposure to AMX and $K_2Cr_2O_7$ led to a significant change in the activity of antioxidant enzymes, with significant increases ($p < 0.05$) in reactive oxygen species (ROS) production. The highest ROS value was 51.05 $\mu\text{g}/\text{mg}$ under concentrations of 50 mg/L of $K_2Cr_2O_7$, and the highest recorded percentage of Superoxide Dismutase SOD, Catalase CAT, Malondialdehyde MDA, and Glutathione Reductase GSH, as: 33.40 U/m, 33.32 KU/L, 23.22 $\mu\text{mol}/\text{l}$ and 21.30 $\mu\text{g}/\text{g}$ respectively, in concentrations of 50 mg/L of $K_2Cr_2O_7$ non-treated. It was observed in this study that potassium dichromate was more effective than amoxicillin in causing toxicity. According to the current study, immobilized *C. vulgaris* was instrumental in decreasing chemicals toxicity, by relieving oxidative stress on *P. euphraticus* clam, as it recorded a significant decrease $p \leq 0.05$ in ROS values and oxidizing enzymes such as Superoxide Dismutase SOD, Catalase CAT, Malondialdehyde MDA, as well as ascorbic acid. AA, total protein and GPX in treated samples.

Keywords: Biochemical markers, *Chlorella vulgaris*, Freshwater clam, Immobilized algae, Pharmaceutical wastes.

Introduction

The use of microalgae in biotechnology has increased in recent times, frequent uses of immobilized algae are the nutrients, inorganic¹, organic pollutants removal from aquatic systems, culturing for metabolite production, and measurement of toxicity². 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 to

non-target aquatic species^{1,3}. There are probably entering freshwater systems by many pathways including effluents from wastewater treatment plants (WWTPs), chemical industrialization plants, and animal rearing and aquaculture³.

Amoxicillin AMX has been classified as an emerging pollutant it causes great damage to aquatic organisms, such as changes in embryonic development and oxidative stress, and it has been

discovered that AMX is capable of causing DNA damage and cytotoxic effects in common carp blood cells^{4,5}. The most serious issue caused by antibiotic-contaminated water, is the rise of antibacterial drugs and genes for antibiotic resistance, which result in the annual deaths of 700,000 people per year^{2,6}.

Chromium is a highly toxic inorganic pollutant that enters environment from a variety of natural and artificial sources, including medical facilities, textile manufacturers dye, and chrome electroplating. 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, DNA, and cause carcinogenic and mutagenic effects in living beings^{7,8}.

The utilization of biomarkers as early warning tools for contamination in an environment can be toxic and dangerous to aquatic life⁹. 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 organisms¹⁰. Thus, differences in the action of the enzymes that make up the antioxidant protective mechanism

Materials and Methods

Tested Organisms

1-A freshwater clam, *P. euphraticus*, was selected for toxicity testing and collected from the Euphrates River in Al Hindiya District 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, Iraq.

2- The microalgal species used in this study was *Chlorella vulgaris* that belonged to green algae and most commonly used for wastewater treatment which have high growth rates and can grow under a wide range of culture conditions. This microalgal strain was obtained from the Environmental Research and Studies Center, University of Babylon, Iraq.

can be used as an early warning sign of toxic compound contamination¹¹.

Bivalves are considered good bio-indicator organisms for determining the degree of contamination in freshwater and marine ecosystems¹¹⁻¹³. This is due to several significant characteristics, including their wide dispersion, abundance, sedentary behaviour, physical size, and frequently, their ecological and/or economic value. As a result, various authors have studied responses of molluscs reacting to environmental pressures and contaminants¹⁴⁻¹⁷

It has been shown that *C. vulgaris* can adapt to antibiotic stress through its own physiological adaptation and its ability to degrade pollutants, it is therefore a good option for removing antibiotics from aqueous systems¹⁸. 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 metals¹⁹⁻²².

The current study aimed to use immobilized alga as an eco-friendly method to a reduced the toxic effect of amoxicillin and potassium dichromate on some biomarkers in freshwater clam *Pseudodontopsis euphraticus*.

The *C. vulgaris* was identified by microscopic observation and incubated under controlled conditions of light intensity 286 $\mu\text{E}/\text{m}^2/\text{s}$, light/dark period 16:8 hours and temperature 25 ± 2 °C. All equipment and media were sterilized in an autoclave at 121 °C, 1.5 h for 15 min. Modified Chu-10 was used for the algal growth.

The method was followed by taking 50 ml of the algae culture in the stabilization phase and concentrate by centrifugation at 3000 rotation / minute for a period 15 minutes. Afterwards, an equal volume of 2% sodium genes solution was added to it and shaken well to homogenize the mixture (algae and genes) which then placed in a syringe or separating funnel. The contents of the medical syringe or the funnel are gradually distilled in the calcium chloride solution where the algae fall

in the beads form and leave for 5-10 minutes to harden, then wash the beads from the calcium chloride solution with tap water and rinse thoroughly with distilled water by using a tea strainer²³.

Pharmaceutical Substances

Pharmaceutical substances were used in this work, including potassium dichromate $K_2Cr_2O_7$ and pure amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$) was obtained from the General Company for the Manufacture of Medicines and Medical Supplies Samarra, Iraq.

Experimental Design

Cultivation of fresh clams was performed in 24 plastic containers 18 cm x 21 cm x 31 cm, which were selected for the experiment. *P. euphraticus*, which ranged in shell length from 3.5–4.6cm, were collected from the Euphrates River and transported to the laboratory. The stocks were prepared for all macro, and microelements were dissolving the weight of the salt, Table. S1 The components and concentration of modified Chu-10 medium and the concentration of each component (Bleakley and Hayes, 2017).

They were acclimated within standard conditions with dechlorinated water for 5 days and exposed to amoxicillin for 7 days at concentrations of 100, 200, and 300 mg/l, and they were also exposed to $K_2Cr_2O_7$ for 5 days at concentrations of 20, 30, and 50 mg/l. On the other hand, 5–15 beads of

Results

Results recorded the experimental toxicity effects of the amoxicillin (AMX) and potassium dichromate ($K_2Cr_2O_7$) toxicity effects experiments on *P. euphraticus* are shown in Figs.1,2. and Tables 1,2 respectively.

Exposing *P.euphraticus* to amoxicillin showed that the ROS mean values in the exposure experiments without the addition of immobilized alga ranged between 12.99 -22.52 $\mu\text{g}/\text{mg}$ compared to the control which recorded 11.77 $\mu\text{g}/\text{mg}$, while with *C. vulgaris*, ROS mean values decreased and

immobilized *C. vulgaris* were added to all containers of treatments with pharmaceutical substances. At the end of the exposure, the haemolymph was extracted, to study the variation of biochemical biomarkers²⁴.

Measuring Biomarkers

Method of EreI²⁵ was used to determine reactive oxygen species (ROS)activity , and determine super oxide dismutase (SOD) activity using the method described by Marklund&Marklund²⁶. Catalase (CAT) activity was determined according to Goth²⁷. Malondialdehyde (MDA) tested by method of Buege and Aust²⁸. The Glutathione peroxidase GPx activity determined was completed by using the methodology adopted from Hafeman *et al.*,²⁹. GSH was determined according to Moron *et al.*,³⁰. Total protein was determined by Lowry *et al.*,³¹ moreover, ascorbic acid AA was determined by McCormick and Greene³² by three replicates

Statistical Analysis

The results of statistical analyses study and the significance level were considered at $p < 0.05$. Descriptive analyses included means and standard deviations. Variables were tested for normality distribution prior to analysis. To determine the significance of differences, analysis of variance (ANOVA) was used, and *p-values* less than 0.05 were considered significant. SPSS program was also used for the analysis.

ranged from 8.89-16.73g/mg compared to 10.91 $\mu\text{g}/\text{mg}$ in the control group for 100-300mg/L AMX concentrations. In $K_2Cr_2O_7$ experiment, the ROS mean values recorded without *C. vulgaris* 37.50 - 51.05 $\mu\text{g}/\text{mg}$ compared to the control (which recorded 12.58 $\mu\text{g}/\text{mg}$) but with added *C.vulgaris*, ROS mean values were suppressed and ranged from 33.88 -42.66 $\mu\text{g}/\text{mg}$ compared to 11.80 $\mu\text{g}/\text{mg}$ in the control group of 20-50 mg/L concentrations.

In amoxicillin, without adding immobilized alga, in the exposure experiments' CAT, mean values

ranged from 25.44-30.53 KU/L, compared the control group of 24.14 KU/L, while with *C. vulgaris*, a pronounced elevation of CAT values was recorded which ranged from 17.56 -25.15 KU/L compared to 14.26 KU/L in the control group for 100-300mg/L AMX concentrations. In $K_2Cr_2O_7$, without *C. vulgaris*, higher CAT value was recorded (26.88 -33.32KU/L) compared to the control which recorded 23.60 KU/L ,but with adding immobilized *C. vulgaris*, CAT mean values were decreased and ranged from 27.16–25.76 KU/L compared to 22.82 KU/L in the control group of 20 -50 mg/L concentrations.

Superoxide Dismutase (SOD) mean values for AMX varied from 20.79 to 33.33 U/m in the exposure trials without the addition of immobilized *C. vulgaris*, compared to the control which recorded 17.51 U/m. while with *C. vulgaris*, SOD mean values were significantly decreased and ranged from 12.72 -21.62 U/m compared to 9.80 U/min the control group of 100-300 mg/L concentrations. In $K_2Cr_2O_7$, the SOD mean values without *C. vulgaris* were 28.36 -33.40 U/m as opposed to the control group 24.02 U/m values, but with the addition of *C. vulgaris*, the SOD mean values ranged from 23.36 - 31.41U/m while that of control group's was 20.35 of 20-50 mg/L concentrations.

In AMX, the GPX mean values in the exposure experiments without the addition of immobilized *C. vulgaris* ranged between 5.70 to 8.36 U/L compared to the control which recorded 18.48 U/L, While with *C. vulgaris*, GPX mean values was apparently not affected and ranged from 5.02-9.58 U/L compared to 13.96 U/L in the control group for 100-300 mg/L concentrations. However in case of $K_2Cr_2O_7$, The GPX mean values recorded without *C. vulgaris* were 4.35 to 6.34 U/L compared to the control which recorded 7.99 U/L but with added *C. vulgaris*, GPX mean values ranged from 3.57-7.34 U/L compared in to 6.28 U/L in the control group of 20-30mg/L concentrations.

The GSH values for AMX mean ranged from 7.55 - 10.37 μ g/g in the exposure experiments without adding immobilized *C. vulgaris* compared to the control which recorded 5.92 μ g/g, while with *C. vulgaris*, GSH values slightly decreased and ranged

from 6.54 -9.46 μ g/g compared to 5.14 μ g/g in the control group for 100 -300 mg/L concentrations. In $K_2Cr_2O_7$, The GSH mean values recorded (without *C. vulgaris*) 21.30 - 16.30 μ g/g compared to the control which recorded 8.57 μ g/g but on the addition of *C. vulgaris*, GSH mean values ranged from 14.52-22.47 μ g/g compared to 9.7 μ g/g in the control group 20- 30mg/L concentrations.

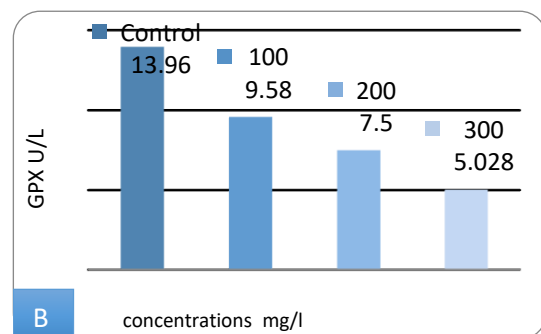
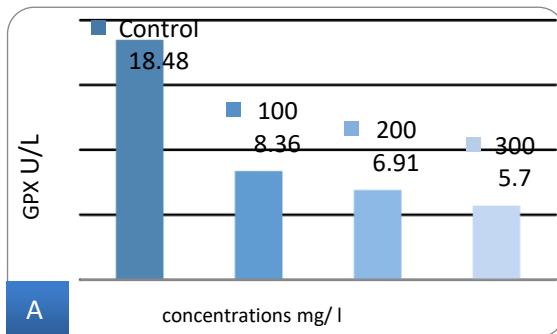
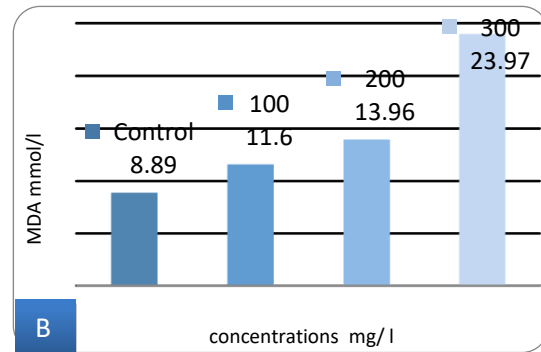
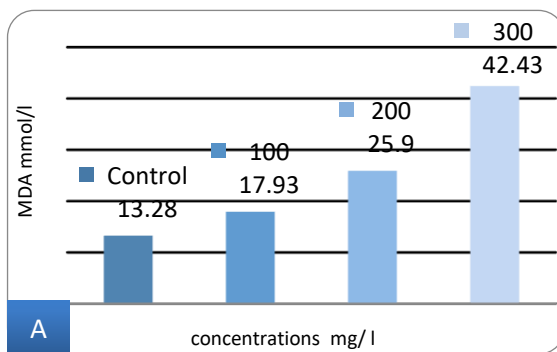
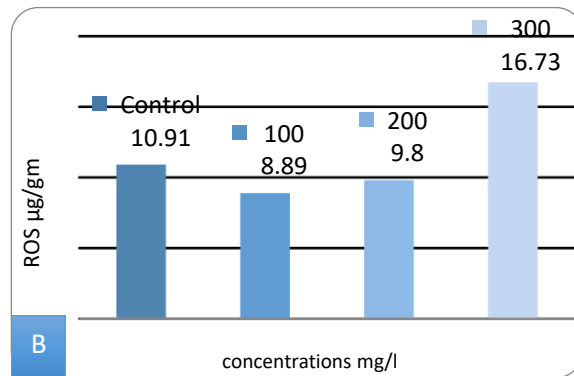
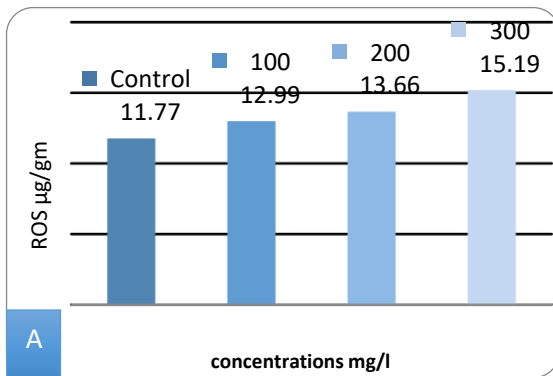
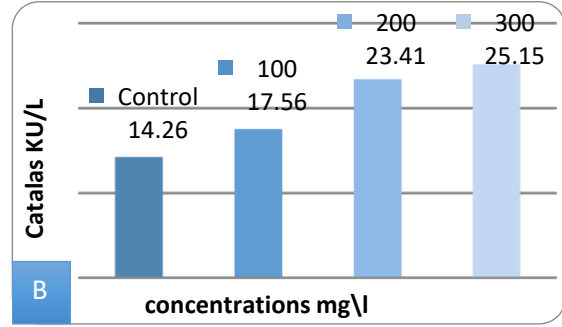
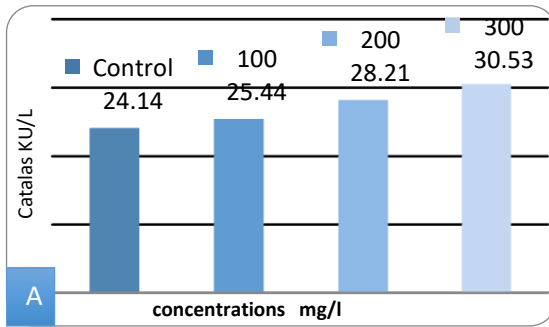
The malondialdehyde (MDA) mean values produced during the effect of AMX ranged from 17.69 -42.43 μ mol/L in the exposure experiments without addition of immobilized *C. vulgaris*, compared to the control which recorded 13.28 μ mol/l, while *C. vulgaris*, MDA mean values were highly decreased and recorded from 11.60 -23.97 μ mol/l compared to 8.89 μ mol/l in the control group for 100-300 mg/L concentrations. While in $K_2Cr_2O_7$ experiment, the MDA mean values recorded (without *C. vulgaris*) from 12.68 to 23.22 μ mol/l compared to the control which showed 7.99 μ mol/l but with added *C.vulgaris*, MDA mean values were largely decreased and ranged from 9.81- 11.44 μ mol/l compared to 8.86 μ mol/l in the control group of 20-50 mg/L concentrations.

In AMX, the total protein (TP) mean values in the exposure experiments without the addition of immobilized *C. vulgaris* ranged from 9.65 to 11.14 mg/g compared to the control, which recorded 13.17 mg/g, while with *C. vulgaris*. TP mean values significantly increased and recorded ranged from 14.25 to 15.60mg/g, compared to 12.44 mg/g in the control group for 100-300mg/L. In $K_2Cr_2O_7$. The TP mean values recorded without *C. vulgaris* were from 10.86 to17.25mg/g compared to the control, which recorded 12.12mg/g but with *C. vulgaris*, TP mean values recorded ranged from 10.93 to 15.42 mg/g compared to 29.21 mg/g in the control group at a 20-30mg/L concentrations.

The Ascorbic acid AA mean values for AMX ranged from 13.41 to 14.48 μ M in the exposure experiments without the addition of immobilized *C. vulgaris* compared to the control which recorded 11.59 μ M, while with *C. vulgaris*, AA mean values were apparently unaffected and ranged from 10.31 - 13.40 μ M compared to 11.47 μ M in the control group for 100-300 mg/L concentrations. In

$K_2Cr_2O_7$. The AA mean values recorded without *C. vulgaris* were 25.27-27.93 μM compared to the control which recorded 25.36 μM but with adding *C. vulgaris*. AA mean values were apparently

unaffected and ranged from 24.83-27.94 μM compared to 23.92 μM in the control group in a 20-50 mg/L concentrations.



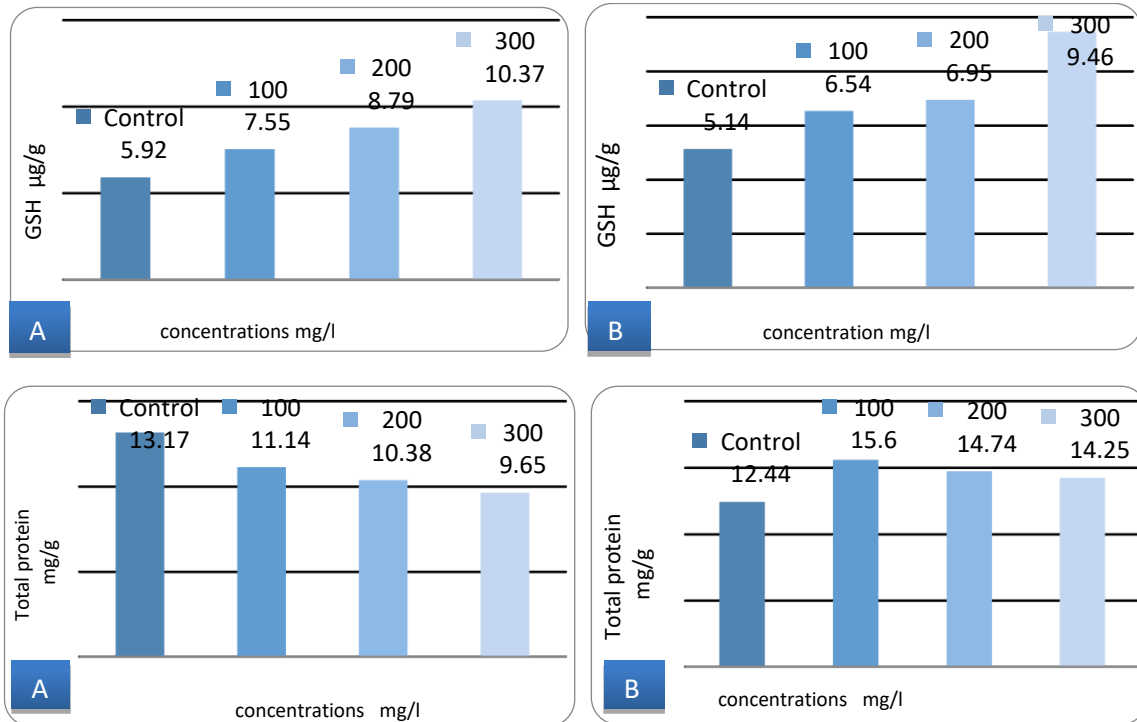
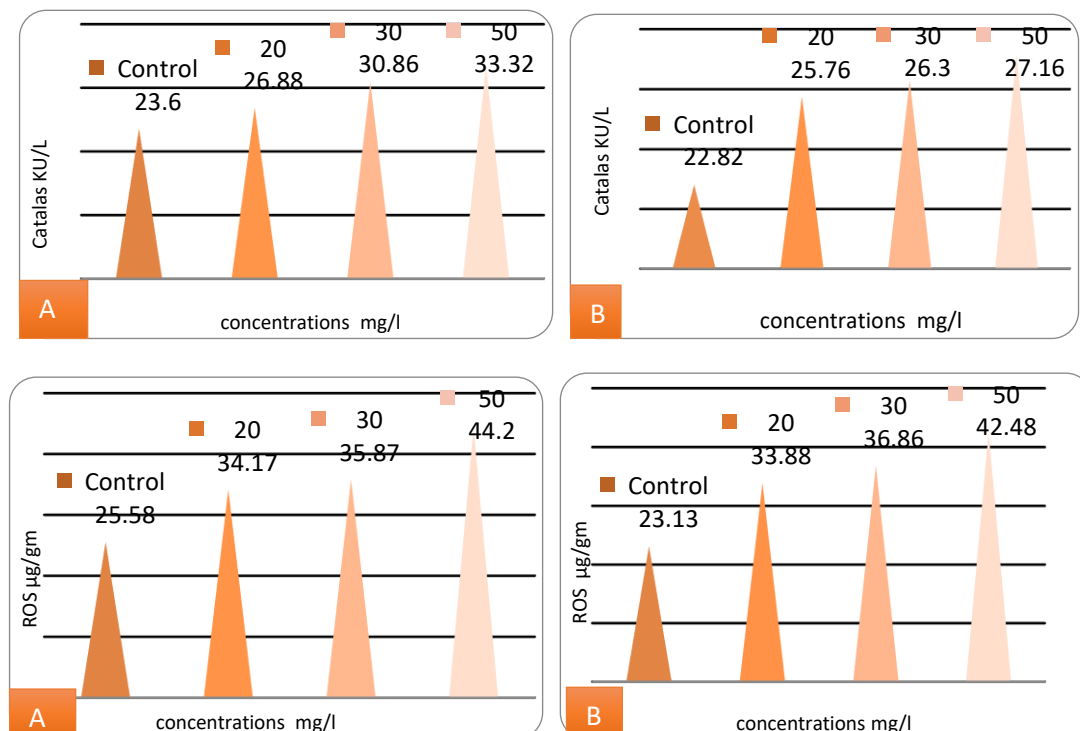
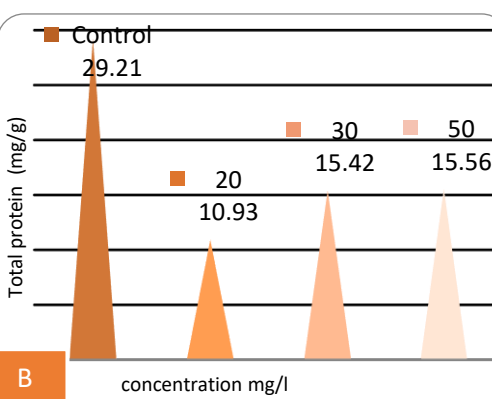
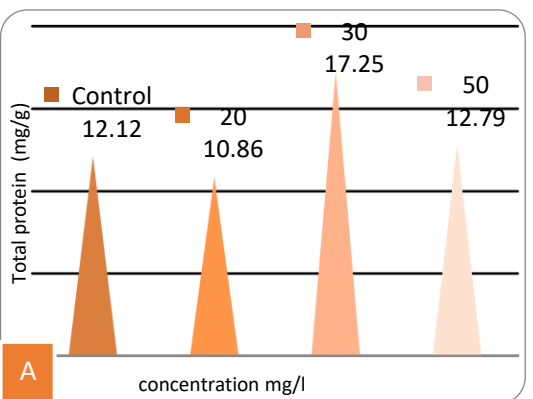
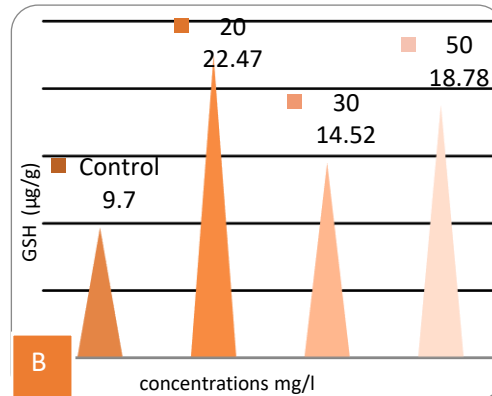
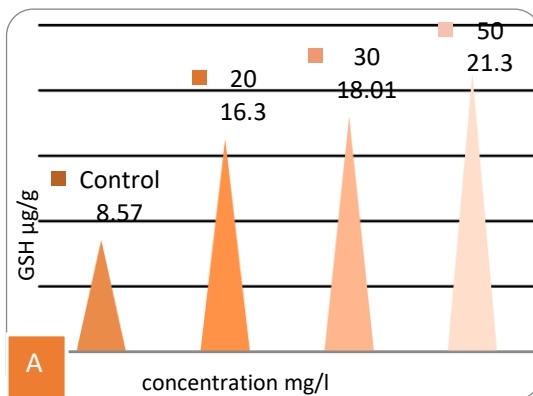
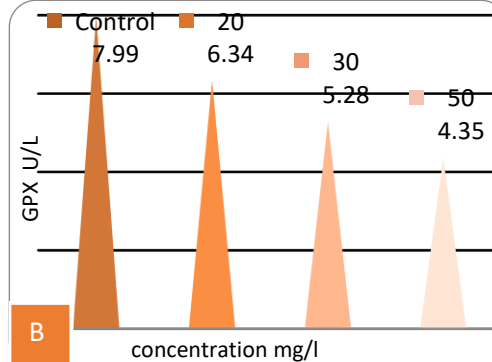
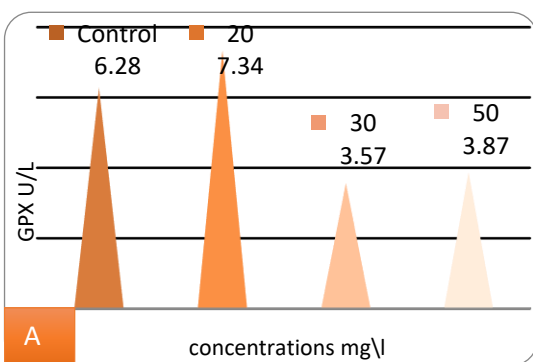
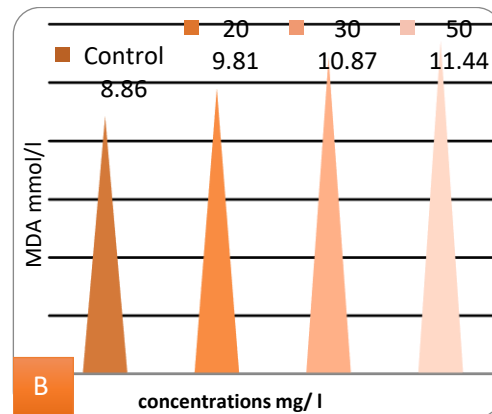
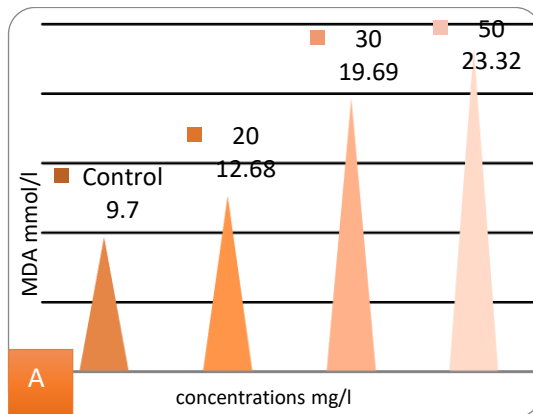


Figure 1. Effect of the antibiotic amoxicillin (AMX) on mean of biochemical markers SOD,CAT,ROS,MDA,GPX, GSH, Total Protein and Ascorbic Acid in mussel in *P.euphraticus*. A- without Immobilization of (*C. vulgaris*), B- with using Immobilized *C. vulgaris*





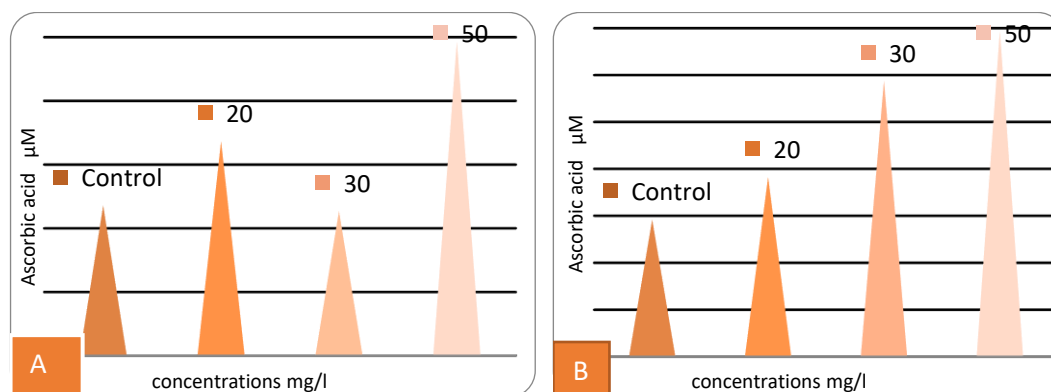


Figure 2. Effect of the Potassium Dichromate ($K_2Cr_2O_7$) on mean of biochemical markers SOD,CAT,ROS,MDA,GPX, GSH, Total Protein and Ascorbic Acid in mussel in *P.euphraticus*. : c- without Immobilization of (*C. vulgaris*), D- with Immobilization of the alga.

Table 1. The mean of biochemical markers in *P. euphraticus* during acute exposure period to antibiotic amoxicillin (Min., Max., Mean±SDof three replicates).

Biochemical markers	Without Immobilization of (<i>C. vulgaris</i>)				Immobilization with (<i>C. vulgaris</i>)			
	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 11.77±0.94	10.56-17.00 12.99±3.49	14.83-12.38 13.66±1.22	19.35-25.93 22.52±3.29	8.54-13.54 10.91±2.37	7.22-11.26 8.89±2.10	11.72-8.54 9.80±1.68	14.46-18.75 16.73±2.15
SOD(U/m)	16.22-18.92 17.51±1.35	18.92-22.14 20.79±1.67	16.49-32.43 25.04±8.03	27.03-40.54 33.33±6.80	8.11-10.81 9.80±1.47	11.62-13.92 12.72±1.15	10.81-20.54 16.75±5.21	16.22-27.03 21.62±5.40
CAT (KU/L)	24.14-24.9 24.14±0.39	23.93-26.77 25.44±1.42	27.08-29.15 28.21±1.04	27.39-35.28 30.53±4.18	11.58-17.49 14.26±2.99	15.86-18.42 17.56±1.47	22.42-24.56 23.41±1.07	23.59-26.24 25.15±1.38
MDA (µm/l)	12.19-14.36 13.28±1.06	12.18-23.21 17.69±5.51	25.90-25.90 25.9±0.00	40.01-44.87 42.43±2.43	7.69-9.62 8.89±1.04	11.60-11.69 11.60±0.00	12.79-15.13 13.96±1.17	20.51-26.47 23.97±3.09
GPX (U/L)	16.82-21.82 18.48±2.88	6.94-9.78 8.36±1.42	6.50-7.38 6.91±0.44	5.26-6.02 5.70±0.39	11.90-15.90 13.96±2.00	5.62-15.14 9.58±4.95	6.14-9.43 7.50±1.71	4.98-5.06 5.02±

									0.04
GSH($\mu\text{g/g}$)	5.82 - 6.00 5.92 \pm 0.09	5.64 - 8.81 7.55 \pm 1.68	5.46 - 11.81 8.79 \pm 3.18	9.11 - 17.32 10.37 \pm 1.25	3.96 - 6.90 5.14 \pm 1.55	5.55 - 7.31 6.54 \pm 0.90	4.86 - 9.40 6.95 \pm 2.29	7.25 - 12.13 9.46 \pm 2.47	
Total protein (mg/g)	12.74-14.38 13.17 \pm 0.58	9.37-12.58 11.14 \pm 1.63	9.04-11.39 10.38 \pm 1.20	8.67-10.45 9.65 \pm 0.905	11.09-13.95 12.44 \pm 1.43	13.59-17.18 15.60 \pm 1.83	14.43-15.06 14.74 \pm 0.31	12.66 - 15.45 14.25 \pm 1.43	
Ascorbic acid (AA) μM	10.95-12.28 11.59 \pm 0.91	10.08-15.65 13.41 \pm 2.94	11.66-16.66 13.37 \pm 2.91	12.49-17.14 14.48 \pm 2.39	10.34-12.65 11.47 \pm 1.13	10.47-11.99 11.31 \pm 0.77	7.11-13.51 10.31 \pm 3.19	11.80 - 14.27 13.40 \pm 1.39	

Table 2. The mean of biochemical markers in *P. euphraticus* during acute exposure period to Potassium dichromate. (Min., Max., Mean \pm SD of three replicates)

Biochemical markers	without Immobilization of (<i>C. vulgaris</i>)				Immobilization with (<i>C. vulgaris</i>)			
	control	20(mg/l)	30 (mg/l)	50(mg/l)	Control	20(mg/l)	30(mg/l)	50(mg/l)
ROS ($\mu\text{g/mg}$)	10.74-14.77 12.58 \pm 1.76	30.70-44.17 37.50 \pm 6.73	36.31-45.94 40.97 \pm 4.82	45.08-57.20 51.05 \pm 6.06	9.53-13.54 11.80 \pm 2.05	31.62-36.55 33.88 \pm 2.48	34.03-38.69 36.86 \pm 2.48	40.06-43.64 42.06 \pm 1.82
SOD(U/m)	21.62-27.03 24.02 \pm 2.75	27.62-29.05 28.36 \pm 0.71	29.73-32.43 31.10 \pm 1.35	33.55-36.04 33.40 \pm 1.44	19.11-21.62 20.35 \pm 1.255	24.32-29.73 23.36 \pm 1.18	25.62-28.92 27.43 \pm 1.67	33.25-29.34 31.41 \pm 1.96
CAT (KU/L)	22.67-25.13 23.60 \pm 1.33	26.61-27.16 26.88 \pm 0.38	30.52-31.20 30.86 \pm 0.48	30.99-35.41 33.32 \pm 2.22	21.59-24.59 22.82 \pm 1.57	25.08-26.45 25.76 \pm 0.68	24.11-29.25 26.30 \pm 2.65	25.27-28.92 27.16 \pm 1.82
MDA($\mu\text{mol/l}$)	8.00-11.70 9.70 \pm 1.86	10.77-14.6 12.68 \pm 2.70	17.38-12.38 19.69 \pm 2.52	22.38-24.13 23.22 \pm 0.87	5.77-8.86 8.86 \pm 0.09	8.40-11.22 9.81 \pm 1.41	8.97-12.82 10.87 \pm 1.92	11.5-11.73 11.44 \pm 0.41
GPX (U/L)	6.18-9.06 7.99 \pm 1.57	4.26-8.42 6.34 \pm 2.08	2.78-7.42 5.28 \pm 2.34	3.70-5.34 4.35 \pm 0.86	6.14-6.14 6.28 \pm 0.14	6.66-8.02 7.34 \pm 0.68	3.10-4.34 3.57 \pm 0.67	3.02-5.10 3.87 \pm 1.08
GSH($\mu\text{g/g}$)	6.42-10.79 8.57 \pm 2.18	14.38-18.46 16.30 \pm 2.05	18.15-16.06 18.01 \pm 1.88	18.99-25.56 21.30 \pm 3.69	6.42-10.79 9.7 \pm 2.89	18.46-25.70 22.47 \pm 3.68	11.03-18.01 14.52 \pm 4.39	15.00-22.77 18.78 \pm 3.88

Total protein (mg/g)	10.29	-	10.16	-	15.42	-	11.03	-	28.24	-	9.93	-	12.49	-	14.40
	13.95		11.39		17.25		14.43		30.07		12.22		18.35		15.50
	12.12 ±		10.86 ±		17.25 ±		12.79 ±		29.21 ±		10.93 ±		15.42 ±		15.56 ±
	1.83		0.62		1.83		1.70		0.92		1.17		2.93		1.06
Ascorbic acid (AA) μM	24.52	-	26.35	-	24.14	-	27.68	-	23.57	-	22.86	-	26.73	-	27.03
	26.54		27.11		26.29		28.32		24.27		26.10		26.99		28.86
	25.36 ±		26.37 ±		25.27 ±		27.93 ±		23.92 ±		24.83 ±		26.88 ±		27.94 ±
	1.05		0.38		1.08		0.33		0.34		1.73		0.13		0.91

Discussion

In this study, it was found that exposure to AMX and $K_2Cr_2O_7$ enhanced the production of ROS, this is consistent with many studies which indicated that these pharmaceutical substances led to an increased production of ROS in aquatic organisms³³⁻³⁵

Previous studies showed that AMX and $K_2Cr_2O_7$ caused significant changes in the activity of antioxidant enzymes and induced oxidative stress^{36,37}.

In this study, increased levels of the antioxidant enzymes CAT, SOD, GSH, and MDA were observed in oysters' *P. euphraticus* after exposure to the pharmaceuticals AMX and $K_2Cr_2O_7$ than the control group, which possibly due to increased oxidative stress on the clams (Tables 1,2 and Figs.1,2. respectively).

The Super oxide dismutase SOD and CAT are the most important first lines of defence to remove Reactive oxygen radicals in antioxidant enzymes. They are mostly used as an indicator of oxidative stress to determine pollution stress on organisms^{38,39}, and could indicate that the cells are attempting to defend itself against the scenario of oxidative stress. It is a protective mechanism for the conversion of excess oxygen and free radicals resulting from exposure to hydrogen peroxide^{10,40}. Cellular biomarkers have a prognostic or diagnostic value for long-term toxicological or ecological effects by early identifying the onset of biological changes induced by chemical pollutants⁴¹. Our results are consistent with Elizaldi-Velasquez who reported that the AMX induced oxidative stress, and it was also responsible for raising the activity of the enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) in the gills,

kidneys, and brain of *C. carpio* during acute exposure⁴². Also the study of⁴³, showed that Cr^{6+} affects antioxidant responses and causes increased SOD-CAT activity, DNA damage and apoptosis in fish *Channapunctatus*. In this study, GPx levels in *P. euphraticus* decreased considerably following chromium and AMX exposure, this may be due to the fact that Cr(VI) compounds cause a decrease in glutathione concentrations due to an increase in glutathione disulphide (GSSG), which is an important marker of oxidative stress in cells as reported by^{44,45}.

The enzyme activity of the glutathione system can be induced by pharmaceutical preparations in bivalves, as in *M. galloprovincialis* and *Curbicula fluminea*⁴⁶, hexavalent chromium increased GSH in *Venus verrucosa* soft tissues, due to oxidative stress⁴⁵.

The enzyme activity of the glutathione system can be induced by pharmaceutical preparations in bivalves, as in *M. galloprovincialis* and *Carbicula fluminea*⁴⁶. According to a study carried out by Shaaban et al. ,Hexavalent chromium increased GSH in *Venus verrucosa* soft tissues, due to oxidative stress⁴⁵. MDA is widely used as a biomarker of oxidative stress, and increased level of oxidative damage in terms of lipid oxidation has been reported in different species of snails exposed *in vitro* to environmental pollutants⁴⁷. Excess ROS produced within the organism's body may react with the lipid of the cell membrane, forming lipid peroxides that are further degraded into malondialdehyde. The MDA formation is an indicator of cell damage, which leads to tissue damage and, in extreme cases, death of the organism⁴⁸. Rusdi et al. suggested that the elevated

MDA level in green-lipped mussels (*Pernaviridis*) indicated that an organism has experienced oxidative stress⁴⁹. Proteins are the most important organic molecules in a living system. Proteins play an essential role in an organism's physiology and providing an information on an animal's general energy mobilization. Proteins are broken down into amino acids under stress conditions by organisms to meet their metabolic needs⁵⁰. In clams, an environment with high levels of pollution leads to a rise in protein breakdown and a decrease in cell protective proteins⁴⁹.

The study carried out by Ahmad et al. showed that potassium dichromate caused a considerable reduction in renal tissue proteins, albumin levels and hepatic tissue proteins when compared the control group of mollusks⁵⁰. Ascorbic acid or vitamin C is a primary nutrient that, acts as a reducing factor and a non-enzymatic antioxidant in the cell. Ascorbic acid is used as a reducing agent for potassium dichromate from Cr (VI) to Cr (III). According to Chaâbane et al. chromium (VI) causes a significant increase in the levels of both GSH and vitamin C in soft tissues of *Venus verrucosa*⁵¹. Increased Catalase, SOD enzyme activity and MDA level as well as reduced GPx activity significantly indicated that *P. euphraticus* clams had experienced oxidative stress.

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 compared to untreated samples ($p < 0.05$). Because *C. vulgaris* has been used to remove many environmental pollutants, (such as (heavy metals, organic compounds or pharmaceuticals), due to its widespread occurrence in aquatic habitats, rapid growth rates, and tolerance to harsh environmental conditions^{52, 53}. Algae can at the same time utilize many methods or mechanisms that supplement each other to remove medicines and other toxic compounds from the environment. These mechanisms included intracellular and extracellular biodegradation, adsorption, bioaccumulation photolysis and hydrolysis⁵⁴. Additionally, Xiong et al. used *C. vulgaris* to

eliminate some antibiotics such as levofloxacin and fluoroquinolones; with an initial concentration was of 5 mg/L, after 7 days, about 15% of the antibiotic had been eliminated⁵⁵. There are several studies indicating the ability of *C. vulgaris* to clear the amoxicillin antibiotic, such as the study by Rickey et al. who indicated the susceptibility of *C. vulgaris* to removal of AMX (by 37%) from the medium by a biodegradation mechanism⁵⁶. In a similar the study of Xiao et al.⁵⁷ who used *Chlorella pyrenoidosa* to remove amoxicillin which achieved about 91% clearance, within 6 hours. As well as study performed by Zhao et al., AMX was removed by *C. vulgaris* with an efficiency of 25%⁵⁸

Many investigators reported that cell immobilization could protect the organism's growth against the toxicity of both heavy metals at LC50 as compared to lethal concentrations and maintain metabolic cell activity for a longer period^{58, 59}. This makes it more effective in removing these compounds from the medium and thus reducing toxic effects on the organism. Immobilization of microalgal cells are recently used to remove many pollutants such as heavy metals, nitrogen, phosphorus, and pharmaceutical materials from polluted wastewater, because microalgae have a high ability to adapt to various and harsh environmental conditions⁶⁰ and can act as a good biological absorbent, and provide a high absorption capacity for minerals and nutrients. This study agrees with the results of Xie et al. who reported that immobilized *C. vulgaris* disrupted the toxicity of SMX and increased the removal efficiency by 85.1% and 86.2% SMX, respectively from the medium⁶¹.

The possibility of using *C. vulgaris* as a cheap and effective sorbent material to remove chromium ions from wastewater without the need for pretreatment. The maximum chromium ion removal (99.75%) was under the following conditions; pH, 60 min., contact time, 60 mg/50 mL at a concentration of 100 ppm⁶². The alga removed chromate by adsorption, the alga contain functional groups, such as carboxyl (COO⁻), amino (NH₂⁻), sulfate (SO₄²⁻), and hydroxyl (OH⁻), which acted as binding sites for metals⁶³. Also, the possibility of using green algae as a good bio absorbent for

removing Cr(VI) from aqueous solutions in the environment, and showed that *C. glomerata* dry

biomass is a suitable candidate to remove Cr(VI) from aqueous solutions⁶⁴.

Conclusion

According to the obtained results of the present study, the pharmaceutical substances; AMX and $K_2Cr_2O_7$ cause oxidative stress in *P. euphraticus* due to increased ROS formation and CAT, SOD, MDA, GSH, and Ascorbic Acid activities in clams. So it might suggest the critical role of these enzymes in cell protection against the deleterious effects of pharmaceutical compound. Also, it showed that $K_2Cr_2O_7$ is the most harmful and effective toxin in clams. A recent study confirmed

the high adaptability of immobilized *C. vulgaris* to environments containing pharmaceuticals, as well as its ability to reduce the toxic effects of these substances and thus reduce oxidative stress on non-target aquatic organisms. More researches are needed to determine whether immobilized *C. vulgaris* can reduce the toxic impacts of other pharmaceuticals on aquatic creatures that are not targets.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Babylon.

Authors' Contribution Statement

This work was carried out in collaboration between all authors. Z.H.O. diagnosed the cases then collected the samples and doing the tests. J M S,

wrote and edited the manuscript with revisions idea. N.F.K ., wrote and analyzed the data with revisions idea . All authors approved the final manuscript.

References

1. Touliabah HES, El-Sheekh MM, Ismail MM, El-Kassas H. A Review of Microalgae- and Cyanobacteria-Based Biodegradation of Organic Pollutants. *Molecules*. 2022 Feb 8; 27(3): 1141. <https://doi.org/10.3390/molecules27031141>
2. Sarkheil M , Ameri M ,Safari O. Application of alginate-immobilized microalgae beads as biosorbent for removal of total ammonia and phosphorus from water of African cichlid (*Labidochromis*) recirculating aquaculture system. *Environ Sci Pollut Res Int*. 2022; 29(8) :11432-11444. <https://doi.org/10.1007/s11356-021-16564-w>
3. Kovalakova P, Cizmas L, McDonald TJ, Marsalek B, Feng M, Sharma VK. Occurrence and toxicity of antibiotics in the aquatic environment: A review. *Chemosphere*. 2020 Jul; 251: 126351. <https://doi.org/10.1016/j.chemosphere.2020.126351>
4. Orozco-Hernández JM, Gómez Oliván LM, Heredia-García G, Luja-Mondragón M, Islas-Flores H, SanJuan-Reyes N, et al. Genotoxic and cytotoxic alterations induced by environmentally-relevant concentrations of amoxicillin in blood cells of *Cyprinus carpio*. *Chemosphere*. 2019 Dec; 236: 124323. <https://doi.org/10.1016/j.chemosphere.2019.07.054>.

5. Barbooti MM, Zahraw SH. Removal of amoxicillin from water by adsorption on water treatment residues. *Baghdad Sci J.* 2020 Sep 8; 17(3): 1071–9. [http://dx.doi.org/10.21123/bsj.2020.17.3\(Suppl.\).1071](http://dx.doi.org/10.21123/bsj.2020.17.3(Suppl.).1071)
6. Stanton IC, Bethel A, Leonard AFC, Gaze WH, Garside R. Existing evidence on antibiotic resistance exposure and transmission to humans from the environment: a systematic map. *Environ Evid.* 2022 Mar 12; 11(1): 8. <https://doi.org/10.1186/s13750-022-00262-2>.
7. Awasthi Y, Ratn A, Prasad R, Kumar M, Trivedi SP. 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, *Channapunctatus* (Bloch, 1793). *Aquat Toxicol.* 2018 Jul; 200: 158–67. <https://doi.org/10.1016/j.aquatox.2018.05.001>.
8. Laxmi V, Kaushik G. Toxicity of Hexavalent Chromium in Environment, Health Threats, and Its Bioremediation and Detoxification from Tannery Wastewater for Environmental Safety. In *Bioremediat. J Singapore: Springer Singapore*; 2020: 223–43. https://doi.org/10.1007/978-981-13-1891-7_11
9. Cavbera J, Mariela González P, Puntarulo S. Oxidative effects of the harmful algal blooms on primary organisms of the food web. *Biocell.* 2019; 43(2): 41–50. <https://doi.org/10.32604/biocell.2019.06163>.
10. Lee JW, Kim JH, Lee DC, Lim HJ, Kang JC. Toxic Effects on Oxidative Stress, Neurotoxicity, Stress, and Immune Responses in Juvenile Olive Flounder, *Paralichthys olivaceus*, Exposed to Waterborne Hexavalent Chromium. *Biology.* 2022 May 17; 11(5): 766. <https://doi.org/10.3390/biology11050766>
11. Fantón N, Bacchetta C, Rossi A, Gutierrez MF. Effects of a glyphosate-based herbicide on the development and biochemical biomarkers of the freshwater copepod *Notodiaptomu scarteri* (Lowndes, 1934). *Ecotoxicol Environ Saf.* 2020, 196 Jun; 196:110501. <https://doi.org/10.1016/j.ecoenv.2020.110501>
12. Esposito G, Pastorino P, Prearo M. Environmental Stressors and Pathology of Marine Molluscs. *J Mar Sci Eng.* 2022 Feb 23; 10(3): 313. <https://doi.org/10.3390/jmse10030313>
13. El-Gendy KS, Gad AF, Radwan MA. Physiological and behavioral responses of land molluscs as biomarkers for pollution impact assessment: A review. *Environ Res.* 2021 Feb; 193: 110558. <https://doi.org/10.1016/j.envres.2020.110558>
14. Primost MA, Averbuj A, Bigatti G, Márquez F. Embryonic shell shape as an early indicator of pollution in marine gastropods. *Mar Environ Res.* 2021 May; 167: 105283. <https://doi.org/10.1016/j.marenvres.2021.105283>
15. Ajala M, Ameer W ben, Annabi A. First evidence of the utility of cephalopods for biomonitoring program in the field: case of *Sepia officinalis* south west of Mediterranean Sea (Gulf of Gabes, Tunisia). *Environ. Sci Pollut Res.* 2022 Apr 6; 29(19): 28675–87. <https://doi.org/10.1007/s11356-021-17804-9>
16. Chahouri A, Agnaou M, el Hanaoui M, Yacoubi B, Moukrim A, Banaoui A. Assessment of seasonal and spatial variation responses of integrated biomarkers in two marine sentinel bivalve species: Agadir Bay (Southern of Morocco). *Mar Pollut Bull.* 2022 Jan; 174: 113179. <https://doi.org/10.1016/j.marpolbul.2021.113179>
17. Zaidi M, Athmouni K, Metais I, Ayadi H, Leignel V. The Mediterranean limpet *Patella caerulea* (Gastropoda, Mollusca) to assess marine ecotoxicological risk: a case study of Tunisian coasts contaminated by metals. *Environ Sci Pollut Res.* 2022 Apr 6; 29(19): 28339–58. <https://doi.org/10.1007/s11356-021-18490>
18. Salem OMA, Abdelsalam A, Boroujerdi A. Bioremediation potential of *Chlorella vulgaris* and *Nostocpaludosum* on azo dyes with analysis of metabolite changes. *Baghdad Sci J.* 2021; 18(3): 454–64. <http://dx.doi.org/10.21123/bsj.2021.18.3.0445>
19. Salman JM, Kaduem NF, Juda SA. Algal immobilization as a green technology for domestic wastewater treatment. *IOP Conf Ser: Earth Environ Sci.*; 2022. <https://doi.org/10.1088/1755-1315/1088/1/012005>
20. El-Sheekh MM, Metwally MA, Allam N, Hemdan HE. Simulation Treatment of Industrial Wastewater Using Microbiological Cell Immobilization Technique. *Iran J Sci Technol Trans Sci* 2020 Jun 19; 44(3): 595–604. <https://doi.org/10.1007/s40995-020-00866-8>
21. Alhumairi A, Hamouda R, Saddiq A. Comparative study between immobilized and suspended *Chlorella* sp in treatment of pollutant sites in Dhiba port Kingdom of Saudi Arabia. *Heliyon.* 2022 Sep; 8(9): e10766. <https://doi.org/10.1016/j.heliyon.2022.e10766>
22. Haider Z, al Mosawi A, Omran A, Ajanabi H, Mohammed A, al Mamoori J. Immobilize Algae To Removal Copper And Lead From Aquatic Ecosystem. *Nat Volatiles Essent Oils.* 2022; 9(1): 850-860.

23. Adlercreutz P, Mattiasson B. Oxygen supply to immobilized cells. *Appl Microbiol Biotechnol.* 1982; 16(4): 165–70. <https://doi.org/10.1007/BF00505826>
24. Eggermont M, Cornillie P, Dierick M, Adriaens D, Nevejan N, Bossier P, et al. The blue mussel inside: 3D visualization and description of the vascular-related anatomy of *Mytilus edulis* to unravel hemolymph extraction. *Sci Rep.* 2020 Dec 21; 10(1): 6773. <https://doi.org/10.1038/s41598-020-62933-9>
25. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem.* 2005 Dec; 38(12): 1103–11. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>
26. Buege JA, Aust SD. Aust SD Microsomal Lipid peroxidation. *Methods Enzymol* 1978; 52: 302–10. [https://doi.org/10.1016/s0076-6879\(78\)52032-6](https://doi.org/10.1016/s0076-6879(78)52032-6)
27. Hafeman DG, Sunde RA, Hoekstra WG. Effect of Dietary Selenium on Erythrocyte and Liver Glutathione Peroxidase in the Rat. *J Nutr.* 1974 May 1; 104(5): 580–7. <https://doi.org/10.1093/jn/104.5.580>
28. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta Gen Subj.* 1979 Jan 4; 582(1): 67–78. [https://doi.org/10.1016/0304-4165\(79\)90289-7](https://doi.org/10.1016/0304-4165(79)90289-7)
29. Lowry DH. Protein measurements with the Folin phenol reagent. *J Biol Chem.* 1951; 193: 265–275.
30. McCormick DB and GHL. Vitamins. In Burtis, C.A. Ashwood E.R. *Tietz Textbook of Clinical Chemistry.* WB Saunders Co, Philadelphia. 1999: 999–1028.
31. Orozco-Hernández JM, Oliván LMG, Heredia-García G, Luja-Mondragón M, Islas-Flores H, San Juan-Reyes N, Dublán-García O. Genotoxic and cytotoxic alterations induced by environmentally-relevant concentrations of amoxicillin in blood cells of *Cyprinus carpio*. *Chemosphere.* 2019; 236: 12432. <https://doi.org/10.1016/j.chemosphere.2019.07.054>
32. Elizalde-Velázquez A, Martínez-Rodríguez H, Galar-Martínez M, Dublán-García O, Islas-Flores H, Rodríguez-Flores J, et al. Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (*Cyprinus carpio*): The role of amoxicilloic acid. *Environ Toxicol.* 2017 Apr; 32(4): 1102–20. <https://doi.org/10.1002/tox.22307>
33. Sodhi KK, Kumar M, Singh DK. Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies. *J Water Process Eng.* 2021 Feb; 39: 101858. <https://doi.org/10.1016/j.jwpe.2020.101858>
34. Marouani N, Tebourbi O, Mokni M, Yacoubi MT, Sakly M, Benkhalifa M, et al. Hexavalent Chromium-Induced Apoptosis in Rat Uterus: Involvement of Oxidative Stress. *Arch Environ Occup Health.* 2015 Jul 4; 70(4): 189–95. <https://doi.org/10.1080/19338244.2013.828673>
35. González-González ED, Gómez-Oliván LM, Islas-Flores H, Galar-Martínez M. Developmental Effects of Amoxicillin at Environmentally Relevant Concentration Using Zebrafish Embryotoxicity Test (ZET). *Water Air Soil Pollut.* 2021 May 1; 232(5): 196. <https://doi.org/10.1007/s11270-021-05148-6>
36. Singh, V., Pandey, B., & Suthar, S, Surindra. Phytotoxicity of amoxicillin to the duckweed *Spirodela polyrrhiza*: Growth, oxidative stress, biochemical traits and antibiotic degradation. *Chemosphere.* 2018, 201: 492–502. <https://doi.org/10.1016/j.chemosphere.2018.03.010>
37. Drozd-Afelt JM, Koim-Puchowska BB, Kaminski P. Analysis of oxidative stress indicators in Polish patients with prostate cancer. *Environ Sci Pollut Res Int.* 2022 Jan 19; 29(3): 4632–40. <https://doi.org/10.1007/s11356-021-15922-y>
38. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med.* 2018 Dec 1; 54(4): 287–93. <https://doi.org/10.1016/j.ajme.2017.09.001>
39. Bio S, Nunes B. Acute effects of diclofenac on zebrafish: Indications of oxidative effects and damages at environmentally realistic levels of exposure. *Environ Toxicol Pharmacol.* 2020 Aug; 78: 103394. <https://doi.org/10.1016/j.etap.2020.103394>
40. Sanajou S, Şahin G. Mechanistic Biomarkers in Toxicology. *Turk J Pharm Sci.* 18(3): 376–384, Jun. 2021. <https://doi.org/10.4274/tjps.galenos.2020.10270>
41. Elizalde-Velázquez A, Martínez-Rodríguez H, Galar-Martínez M, Dublán-García O, Islas-Flores H, Rodríguez-Flores J, et al. Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (*Cyprinus carpio*): The role of amoxicilloic acid. *Environ Toxicol.* 2017 Apr; 32(4): 1102–20. <https://doi.org/10.1002/tox.22307>
42. Awasthi Y, Ratn A, Prasad R, Kumar M, Trivedi SP. 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). *Aquat Toxicol* 2018 Jul; 200: 158–67. <https://doi.org/10.1016/j.aquatox.2018.05.001>
43. Osioma E, Ezugworie TT. Comparative evaluation of antioxidant enzymes, lipid peroxidation, nitric oxide, and non – microsomal oxidases in *Galatea paradoxa*

- exposed to varying concentrations of 'uproot' a glyphosate-based herbicide. *Egypt J Aquat Biol Fish.* 2022 May 1; 26(3): 213–28. <https://doi.org/10.21608/EJABF.2022.239713>
44. Chen H, Zha J, Yuan L, Wang Z. Effects of fluoxetine on behavior, antioxidant enzyme systems, and multixenobiotic resistance in the Asian clam *Corbiculafuminea*. *Chemosphere.* 2015 Jan; 119: 856–62. <https://doi.org/10.1016/j.chemosphere.2014.08.062>
45. Chaâbane M, Bejaoui S, Trabelsi W, Telahigue K, Chetoui I, Chalghaf M, et al. The potential toxic effects of hexavalent chromium on oxidative stress biomarkers and fatty acids profile in soft tissues of *Venus verrucosa*. *Ecotoxicol Environ Saf.* 2020; 195: 110562. <https://doi.org/10.1016/j.ecoenv.2020.110562>
46. TopićPopović N, Krbavčić M, Barišić J, Beer Ljubić B, Strunjak-Perović I, Babić S, et al. Comparative Tissue Responses of Marine Mollusks on Seasonal Changes in the Northern Adriatic Sea. *Appl Sci.* 2021 Mar 23; 11(6): 2874. <https://doi.org/10.3390/app11062874>
47. Péden R, Rocher B, Chan P, Vaudry D, Poret A, Olivier S, et al. Highly polluted life history and acute heat stress, a hazardous mix for blue mussels. *Mar Pollut Bull.* 2018 Oct; 135: 594–606. <https://doi.org/10.1016/j.marpolbul.2018.07.066>
48. López-Pedrouso M, Lorenzo JM, Varela Z, Fernández JÁ, Franco D. Finding Biomarkers in Antioxidant Molecular Mechanisms for Ensuring Food Safety of Bivalves Threatened by Marine Pollution. *Antioxidants.* 2022 Feb 11; 11(2): 369. <https://doi.org/10.3390/antiox11020369>
49. Ahmad MK, Syma S, Mahmood R. Cr(VI) induces lipid peroxidation, protein oxidation and alters the activities of antioxidant enzymes in human erythrocytes. *Biol Trace Elem Res.* 2011 Dec; 144(1–3): 426–35. <https://doi.org/10.1007/s12011-011-9119-5>
50. Wirth R, Pap B, Böjti T, Shetty P, Lakatos G, Bagi Z, et al. *Chlorella vulgaris* and Its Phycosphere in Wastewater: Microalgae-Bacteria Interactions During Nutrient Removal. *Front Bioeng Biotechnol.* 2020 Sep 22; 8. <https://doi.org/10.3389/fbioe.2020.557572>
51. Leong YK, Huang CY, Chang JS. Pollution prevention and waste phycoremediation by algal-based wastewater treatment technologies: The applications of high-rate algal ponds (HRAPs) and algal turf scrubber (ATS). *J Environ Manage.* 2021; 296: 113193. <https://doi.org/10.1016/j.jenvman.2021.113193>
52. Xiao G, Chen J, Show PL, Yang Q, Ke J, Zhao Q, Liu Y. Evaluating the Application of Antibiotic Treatment Using Algae-Algae/Activated Sludge System. *Chemosphere.* 2021; 282: 130966. <https://doi.org/10.1016/j.chemosphere.2021.130966>
53. Xiong JQ, KMB, & JBH. Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris*. *ChemEng J.* 2017; 313: 1251–1257. <https://doi.org/10.1016/j.cej.2016.11.017>
54. Zhao Z, Xue R, Fu L, Chen C, Ndayisenga F, Zhou D. Carbon dots enhance the recovery of microalgae bioresources from wastewater containing amoxicillin. *Bioresour Technol.* 2021 Sep; 335: 125258. <https://doi.org/10.1016/j.biortech.2021.125258>
55. Ricky R, Chiampo F, Shanthakumar S. Efficacy of Ciprofloxacin and Amoxicillin Removal and the Effect on the Biochemical Composition of *Chlorella vulgaris*. *Bioengineering.* 2022 Mar 24; 9(4): 134. <https://doi.org/10.3390/bioengineering9040134>
56. Cao S, Teng F, Lv J, Zhang Q, Wang T, Zhu C, et al. Performance of an immobilized microalgae-based process for wastewater treatment and biomass production: Nutrients removal, lipid induction, microalgae harvesting and dewatering. *Bioresour Technol.* 2022 Jul; 356: 127298. <https://doi.org/10.1016/j.biortech.2022.127298>
57. Hejna M, Kapuścińska D, Aksmann A. Pharmaceuticals in the Aquatic Environment: A Review on Eco-Toxicology and the Remediation Potential of Algae. *Int J Environ Res Public Health.* 2022 Jun 23; 19(13): 7717. <https://doi.org/10.3390/ijerph19137717>
58. Ayele A, Godeto YG. Bioremediation of Chromium by Microorganisms and Its Mechanisms Related to Functional Groups. *J Chem.* 2021. <https://doi.org/10.1155/2021/7694157>
59. Al-Homaidan AA, Al-Qahtani HS, Al-Ghanayem AA, Ameen F, Ibraheem IBM. Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. *Saudi J Biol Sci.* 2018 Dec; 25(8): 1733–8. <https://doi.org/10.1016/j.sjbs.2018.07.011>
60. Ukhurebor KE, Aigbe UO, Onyancha RB, Nwankwo W, Osibote OA, Paumo HK, et al. Effect of hexavalent chromium on the environment and removal techniques: A review. *J Environ Manage.* 2021 Feb; 280: 111809. <https://doi.org/10.1016/j.jenvman.2020.111809>
61. Hejna M, Kapuścińska D, Aksmann A. Pharmaceuticals in the Aquatic Environment: A Review on Eco-Toxicology and the Remediation Potential of Algae. *Int J Environ Res Public Health.* 2022; 19(13): 7717. <https://doi.org/10.3390/ijerph19137717>

62. Ayele A , Godeto YG. Bioremediation of Chromium by Microorganisms and Its Mechanisms Related to Functional Groups. J Chem. 2021; 2021. <https://doi.org/10.1155/2021/7694157>
63. Al-Homaidan AA, Al-Qahtani HS, Al-Ghanayem AA, Ameen F, Ibraheem IBM. Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. Saudi J Biol Sci. 2018;25(8):1733–8. <https://doi.org/10.1016/j.sjbs.2018.07.011>
64. Ukhurebor KE, Aigbe UO, Onyancha RB, Nwankwo W, Osibote OA, Paumo HK, et al. Effect of hexavalent chromium on the environment and removal techniques: A review. J Environ Manage. 2021;280:111809. <https://doi.org/10.1016/j.sjbs.2018.07.011>

دورطحلب *Chlorella vulgaris* المقيد في تقليل سمية بعض المخلفات الصيدلانية في محار *Pseudodontopsis euphraticus*

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الخلاصة

تم في الأونة الأخيرة زيادة تطبيقات الطحالب الدقيقة المقيدة في الدراسات البيئية. تشمل الاستخدامات الحالية للطحالب الدقيقة المقيدة *Chlorella vulgaris* تقليل المواد الصيدلانية مثل الأموكسيسيلين AMX وثنائي كرومات البوتاسيوم $K_2Cr_2O_7$ على محار المياه العذبة *Pseudodontopsis euphraticus* كنموذج حيوي. أشار البحث الحالي إلى حدوث تغيير في المؤشرات الحيوية للإجهاد التأكسدي في تقييم السمية المستحدثة. حيث تم تعريض المحار لتركيزات مختلفة 100 ، 200 ، 400 ملغم / لتر لمدة 7 أيام و 20 ، 30 ، 50 ملغم / لتر لمدة 5 أيام من أموكسيسيلين وثنائي كرومات البوتاسيوم على التوالي. وأظهرت النتائج أن التعرض لـ AMX و $K_2Cr_2O_7$ أدى إلى حدوث تغيير كبير في نشاط الإنزيمات المضادة للأكسدة ، مع زيادات كبيرة في إنتاج ROS أعلى قيمة مسجلة لـ ROS كانت 51.05 ميكروغرام / ملغم بتركيزات 50 ملغم / لتر من $K_2Cr_2O_7$ ، وأعلى نسبة مسجلة كانت SOD و CAT و MDA و GSH ، على النحو التالي: 33.40 وحدة/مل ، 33.32 كيلو وحدة/لتر ، 23.22 ميكرو مول / لتر و 21.30 ميكروغرام / لتر من $K_2Cr_2O_7$ بتركيزات 50 ملغم / لتر من $K_2Cr_2O_7$ غير المعالجة. لوحظ في هذه الدراسة أن ثنائي كرومات البوتاسيوم كان أكثر فعالية من الأموكسيسيلين في إحداث السمية. وفقاً للدراسة الحالية ، كان لنوعطحلب المقيدة *C. vulgaris* دوراً أساسياً في تقليل سمية المواد الكيميائية ، من خلال تخفيف الإجهاد التأكسدي على محار *P. euphraticus* ، حيث سجل انخفاضاً كبيراً في قيم ROS والإنزيمات المؤكسدة مثل GSH و SOD و CAT و MDA ، وكذلك حامض الاسكوربيك AA ، البروتين الكلي و GPX في العينات المعالجة.

الكلمات المفتاحية: المؤشرات الكيموحيوية، *Chlorella vulgaris*، محار المياه العذبة، الطحالب المقيدة، المخلفات الصيدلانية.