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Variation in Structure and Color of Ostracoda Crustacean Shell as Biomarker for Detection of Water Pollution with Heavy Metals

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ABSTRACT

Variations in structure and color of the shell of freshwater Ostracoda crustacean genus *Cypris* were used as a biomarker to detect the impact of heavy metals, i.e. cadmium in the form of $CdCl_2$ as a case of study. Concentrations of element used in experimental were submitted with their levels that were detected in Iraqi aquatic environment and the expected ranges resulting from the accumulation of these metals in aquatic systems. Therefore, salts of $CdCl_2$ were used to prepare the required concentrations 0.1, 0.1.5, 0.2, and 0.3 mg/L, respectively. Individuals of crustacean were taken at the rate of 10 adults (A) and 10 juveniles (J) at three replicates and the exposure time was recorded, at 6, 12, 24, 48 hours, and the tests were conducted under controlled laboratory conditions of light, pH, and temperature. The results showed that all individuals, A and J, were affected after 12 hours of treatment with $CdCl_2$, and observed slight differences in the level of impact, the activity and resistance of *Cypris* individuals to the impact of element that began with exchanged of color and density of shell, level of the flexibility of shell legator flap muscle within 24 hours, followed by opening and cracking all the shell over 48 hours and exposed the internal structures of the crustacean to direct impact of Cd. When following up on the survival and mortality rate at the level of individuals and comparing the speed of vulnerability of adults and juveniles, differences between both categories of individuals can be noticed, as death rates were recorded after 12 and 24 hours of treatment, amounting to 60 and 70%, respectively, at a concentration of 0.1 mg/L, while with concentrations 0.1.5 and 0.2 mg /L, the percentage increased in J to 80%, but at concentration 0.3, the percentage of mortality rate was equal and became 90% after 24 hours in A and J. The death rate of 100% was recorded with all concentrations used in the experiments after passage 48 hours of treatment with cadmium.

Keywords: biomarker; monitoring, crustacean shell, flexible muscle, adults and juveniles, Cypris, cadmium.

INTRODUCTION

Aquatic ecosystems of various kinds are subject to any pollution that occurs in the environment as water is the recipient of most of the products of natural activities and human activities. Water is a general solvent for many substances, elements and gases, and this medium has the ability to carry, distribute and sediment these components between its three layers; surface, middle and bottom, and thus pollutants are distributed either suspended, dissolved or deposited at the bottom according to their density or composition or the ability to decompose and dissolve (Aldaraji and Al-Salman, 2015, Ahamad et al., 2020, Ali et al., 2020, Al-Saeedi and Al-Salman, 2022, Panel et al., 2023). Studies indicate that aquatic media are exposed to two categories of hazardous pollutants, namely emerging and conventional pollutants; the former disintegrate and decompose continuously between the components of the aquatic medium as well as reduce their concentrations by hydrolysis, evaporation, oxidation, reduction or chemical or physical environmental crash, according to the physicochemical water factors or the nature of the climate and the water system being static or current, as in the case of pesticides, sterilizers, cleaning materials, fats and others (Speight, 2017, Ali et al., 2020, Aljewari and Al-Salman, 2022). In turn, the second type (conventional pollutants) corresponds to the pollutants permanently present and accumulating in the medium that enters it, either because of its great ability to resist the means of decomposition and disintegration that have been referred to or because some of them can transform from one image to another according to the conditions of the aqueous medium, and these pollutants (nitrates NO_3, fluoride Fl, and heavy metals). In biological and environmental terms, the danger of heavy metals is determined in particular by the fact that they can accumulate within the components of the aquatic environment, especially in the sediment layer, or bio-magnify in the cells and bodies of organisms involved in the formation of food chains and networks within the trophic levels of biomass from different aquatic organisms (Al-Salman et al., 2011, Shaker et al., 2018, Iglikowska et al., 2020, Gribof et al., 2018, Ahamad et al., 2020, Padidaa et al., 2021, Aljewari and Al-Salman, 2023). To assess the environmental effect of the danger of environmental toxins on the components and quality of the aquatic system, the possibility of predicting the presence of these pollutants in the water, and determining the degree to which they exceed the environmentally permissible limits as well as reach the level of effect on aquatic organisms, a strict biological control system must be followed that is applied periodically and by various means (Alexander et al., 2022, Kadim and Risjani, 2022).

Scientific research confirms that among the most effective ways to monitor the environmental pollution of water as well as evaluate its quality and suitability for various uses is the use of organisms from various organic levels, starting from some types of bacteria, algae, invertebrates, and aquatic plants, such as biosensors, biomonitors or biomarkers that can deal with pollutants and environmental toxins as well as sensitivity to their presence in the aquatic medium. They exhibit reflex reactions that can be measurable or predictable to the extent of the effect of the polluted material and its implications for the future of the aquatic system as well as its food chains, the composition, distribution and diversity of organisms and the natural balance within this medium, or monitoring the level of internal biochemical heterogeneity of their biological components (Al-Salman, 2012, Al-Salman et al., 2013,Thanomsit, 2016, Lemos, 2021, Efrain et al., 2023, Schmitz et al., 2022). Among the aquatic organisms that have attracted the attention of researchers in the field of environmental control is the Crustacea group, which includes a special group called Ostracoda (Mitra et al., 2012, Marco and Lemos, 2021). Ostracods are a class of crustaceans called Ostracoda, sometimes known as seed shrimp. About 70,000 species have been identified and, at present, there are (only 13,000), distributed in several orders. They are small crustaceans, usually ranging in size from 0.2 to 30 mm, and these crustaceans are similar to bivalved arthropod. Biogeological studies and paleontology indicate that they were recorded in the Cambrian and Paleozoic periods, either living in marine or fresh water, including (Darwinulacea and Carbonita) have been known in the Carboniferous and by the Jurassic period (Zaharoff et al., 2013, Matthew, 2002, Schierwate and Desalle, 2021).

These crustaceans can sense the variables of the surrounding environmental environment from the presence of a group of sensilla (hairs or bristles) implemented through the channels located in different locations of the body, and the two shells are linked by a flexible cytoplasmic link which linked to a group of fibers and internal structures that control the movement of opening and closing the shell and protecting the organisms from external influences (Joanes et al., 2020, Padidaa et al., 2021) the distribution of appendages and their number, the shape of the shell and its color are diagnostic characteristics to identify genera and species (Schierwate and Desalle, 2021). The following figure shows these different shapes and structures.

GENUS OF CYPRIS (STUDIED OBJECT)

The genus *Cypris*, considered the oldest ostracod generic name erected using the Linnean system, comprises a reduced number of large-bodied species, mostly found in Africa and Asia. Only six of them are known to occur in Europe (Joanes *et al*., 2020). *Cypris* is related to mussels and shrimp, small, about 0.5–2 mm long, with a hard-outer shell, its individuals are found at shallow water bodies, stream, canals, biological ponds. *Cypris* it is found either wandering or at the bottom or climbing on aquatic plants in search of food and shelter according to the intensity of lighting, the water temperature, and the availability of the food source. The appendages that protrude outside the carapace, are used for swimming and movement, or for pushing food particles into the body, or for crawling on the surface

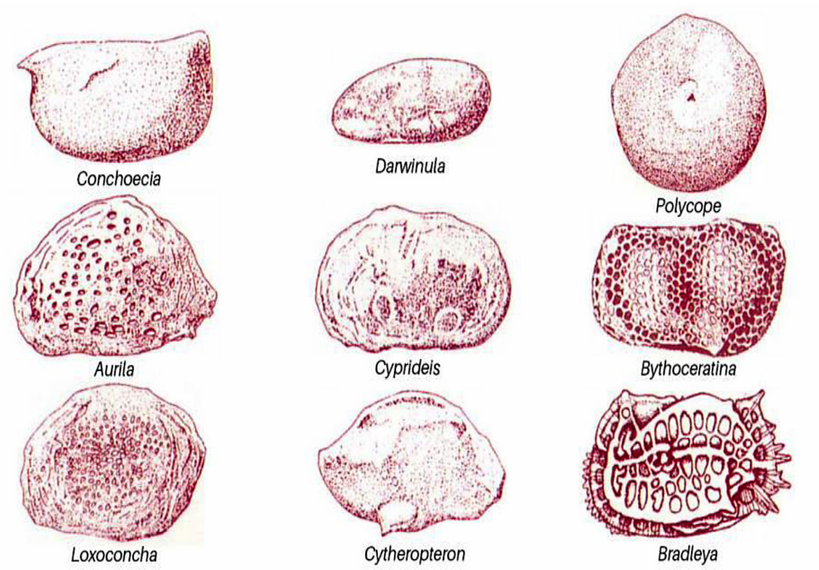


Fig. 1. Except for Conchoecia (a myodocopid), all the ostracods on this diagram are podocopids; lengths vary from 0.7 to 1 mm. Chris Wardle, BGS ©UKR

of the bottom (Myers et al., 2023). Classification of *Cypris* is a type of crustacean (Ostracods) as mentioned by (Joanes et al., 2020, Matthew, 2022): *Kingdom: Animalia, Phylum: Arthropoda, Subphylum: Crustacea, Class: Ostracoda, Order: Podocopida, Superfamily: Cypridoidea, Family: Cyprididae, Genus: Cypris sp.*

EXPERIMENTAL AND METHODS

Field work

The *Cypris* samples used in the study were collected from water bodies in the suburbs of the agricultural city of Baghdad from some ponds and waterways that contain organic residues from environments preferred by various crustaceans, including Ostracods, and they were collected during the early morning hours between 7–8 in the spring of 2023 by a type of manual nets (bottom scraping nets) and placed with a quantity of bottom soil in plastic containers prepared for this purpose; samples of water were also taken. The same collection area in clean ethylene urine bottles were added to the clay samples containing crustaceans in equal amounts to maintain their vitality, and then the samples were transferred to the Environment and Pollution Research Laboratory in the Department of Biology at the College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad, until the completion of the experiments.

Laboratory work

Purification and preparation of culture medium for crustacean individuals

The containers brought from the field were left for three days for the crustaceans to adapt to the laboratory atmosphere. The samples were placed under controlled conditions of 24°C and illuminated by 8:16 hours of light and darkness, with a W36 luminance intensity. Afterwards, the crustaceans were caught and transported by small plankton nets with fine-pores. After collection they were distributed to permanent farm tanks in several glass tanks size 60×30×30 cm prepared for permanent breeding equipped with pure water (liquefied water). It was detected that it was free of concentrations of heavy elements studied and the samples were left for a month in the laboratory inside a laboratory prepared medium for the purpose of adapting organisms to laboratory conditions and to gradually remove contaminated materials by replacing water every three days as a method of quarantine health and access to adapted generations of tested crustaceans, and left until they started throwing the juveniles, after which the juveniles were isolated from the adults in different containers to complete the experiments.

Processing of Culture media

This work was divided as follows:

a) Clean and sterile glass beaker (500 ml) was prepared using the sterilizer (Heraus-5042) for an hour under a temperature of 250 °C and a pressure of (1 atmosphere) to prepare the culture media required for the growth of these crustaceans as well as following the stages of effect of different concentrations of cadmium chloride. Then, the culture medium was prepared in these sterile beakers by adding 200 ml^3 distilled water that was subjected to detection to ensure that it is free of any concentrations of heavy elements or other toxins; then, 4 ml was added to it from a pure algae culture for *Chlorococcum humicola*, a solution of yeast with a concentration of 0.5 g/L added by 1 ml per week, and organic fertilizer with a concentration of 0.5 g/L added by 1.5 ml per week, as well as a number of straw sticks, as stated in Al-Salman (2010). The cultures were left for 5 days so that bacteria and algae could grow and then become a suitable environment for the development of crustaceans.

b) Preparation of chemical concentrations. Pure powder of cadmium chloride salts $CdCl_2$ was used, where the required concentrations 0.3, 0.2, 0.1.5, 0.1 mg/L were prepared respectively, and the materials were approved by BDH. The experimental part and application of the stages of exposure to cadmium included the following.

1. Ten individuals of equal age (adult and juvenile crustaceans) were selected after transferring them from development tanks to Petri dishes to identify the existing species and genera of them, classify and isolate them into independent groups as well as select the individuals of the crustaceans targeted by the study, based on several taxonomic keys (Zaharoff, et al., 2013, Ali et val., 2020, Schierwate and DeSalle, 2021). They were also left for several hours to adapt to the new environment by observing the movement and effectiveness of the crustacean individuals in the new medium.
2. Concentrations of Cd prepared from $CdCl_2$ were added simultaneously and under the same conditions in proportions calculated for the relationship between culture size and added concentration volume/volume.
3. The results were recorded starting from the appearance of the effect during the first hours of the first day for a period of 24 and then for a period of 96, 72 and 48 hours until 100% of the deaths were recorded. The temperature and pH of the culture media were monitored and recorded with each reading.
4. The crustaceans were examined during the stages of treatment with cadmium using light microscopy to follow the stages of influence and identify the reactions of organisms under the influence of the element. Then, the dead individuals were removed, permanent slides were prepared and photographed directly using a ZEISS camera microscope. Three repetitions were taken for each sample and the general rates of these repetitions were calculated.

The results were analyzed statistically by applying several tests, including, the χ^2 test was used to find out the relationship between both the concentration of $CdCl_2$ and the extent of its effect on adult individuals and juvenile crustacean individuals. Correlation analysis (R) was used to measure the relationship between different concentrations of $CdCl_2$ and the percentage of adult and juveniles' mortality, as well as the Determination Coefficient (R^2) as this factor refers to the percentage of the total change of the dependent (crustacean mortality) and the independent environmental variable represented by cadmium concentrations.

RESULTS AND DISCUSSION

Through the results obtained and statistical analysis, it is clear that cadmium chloride at concentrations 0.1, 0.15, 0.2, 0.3 mg/L, as well as the duration of treatment and the exposure time and the physiological age of the organism factors had a major role in explaining the results of the effect intensity and presence of cadmium in the aqueous medium on the biological, composition and fertility of the genus *Cypris* individuals that were subjected to treatment and testing, when tracking the results, the following was found:

Impact of $CdCl_2$ on shell, external morphology, and body appendages in adult individuals

When following up the effect on the level of changes in the outer shell, the biological structure of the body and the body appendages, it was found that the levels of effect were successive with a number of behaviors and biochemical variables according to the duration of exposure, cadmium chloride concentration in the culture medium as well as the physiological age of adult and juveniles individuals compared to the standard development medium, (the control). From the Figure 2, which represents the treatment with the standard medium free of any

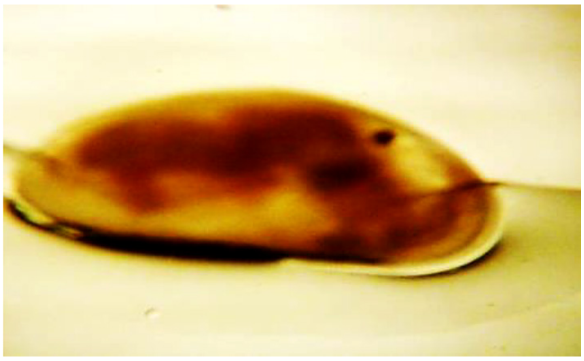


Figure 2. External view of crusty body and shape and color of the shell in controlled medium (mg/L $CdCl_2$)

concentration of cadmium, it was found that the general shape of the animal shows all the details of *Cypris* genus that have been referred to in the paragraph of the animal studied or tested, from the color of the greenish-brown shell, body appendages, convexity from above and other morphological characteristics that characterize this genus, in addition to the activity of individuals in movement and nutrition. Moreover, the individuals in the standard medium, which were a month old, were able to give new embryos after a period of presence in the medium and continued their normal biological activity.

Figures 4 to 7 show the stages of treatment effect with culture media containing different concentrations of cadmium (0.1, 0.15, 0.2, 0.3 mg/L) respectively. It was found that there were many variables obtained on the general shape, structure, and color of the shell, in addition to the effect of internal structures and the effectiveness of the ligament muscle in adult individuals A and juveniles J of the genus *Cypris* at exposure for 24, 48 hours.

Treatment with concentration 0.1 mg/L

When treated with this concentration of the element, reduced movement with the beginning of contact with the contaminated medium was noticed, but after successive periods individuals of *Cypris* tried to restore activity, and after 12 hours, the activity slowed again and small spots began to appear on the shell at the end of 24 hours with a slight divergence of the shell shutters on one side of the body with the attempt of the crustacean individuals to withdraw the appendages as well as the gradual cessation of feeding and movement were noted. When entering the second day of the cadmium treatment, an increase in the effect was observed in the shape, composition, and color of

Figure 3. External view of crusty adult body and shape and color of the shell) in the treated medium at 0.1 mg/L of $CdCl_2$

the shell with an increase in the spacing of the shell shutters and death some individuals in successive hours, which indicates that the effect has moved from the coverings of the body to the connective muscle, and then the biomass of the cortex. Figure 2 shows the stages of this effect.

Treatment with concentration 0.15 mg/L

When increasing the concentration of $CdCl_2$ to 0.1.5 mg / L in the culture medium, it was found that the effect became faster and more intense; as it can be seen from the Figure 4, it was found that the animal tried to withdraw most of the limbs and introduce a larger amount of water as a kind of volume amplification, but after 48 hours, it was found that the shell has become relatively lighter color with increased curvature and concealment of appendages inside with a gradual opening of the shell shutters and the animal gradually stops moving and feeding with the emergence of a gradual opening of the shell shutters from the ventral side, which shows that the effect has reached the components of the ligament muscle that controls the closure of the cortex shutters.

Treatment with concentration 0.2 mg/L

When treated with concentration (0.2) mg / L, it was found that during the first half of 24 hours, symptoms appeared on the shell, presented by formation of some white spots with the gradual emergence of the openness of the shell and slow movement of the animal with an attempt to curvature the body as a behavioral reaction by withdrawing the largest amount of water into the body to envisage the effect of the toxic substance. This effect was multiplied by eroding the surface of the shell and increasing the area of white spots with wide openness of the shell shutters at the end of the second treatment time, which were followed by the death of most individuals, and as it appears from Figure 5.

Treatment with concentration 0.3 mg/L

When the concentration increased to the level of 0.3 mg/L, it was found that the crustacean individuals have shown symptoms of effect from the first 6–12 hours of treatment, as the crustaceans stopped moving and feeding with the withdrawal of all body appendages with elongation of the body and the openness of the shell shutters appeared more than the previous two concentrations and the shell became more transparent, which means the erosion of the calcareous material, but when entering the second day of treatment, it was found that the shell shutters are completely away from each other, which shows the complete cessation of the ligament muscle working and exposure of the entire body to the impact of the heavy element as well as biomass crashes at the end of the treatment Figure 6.

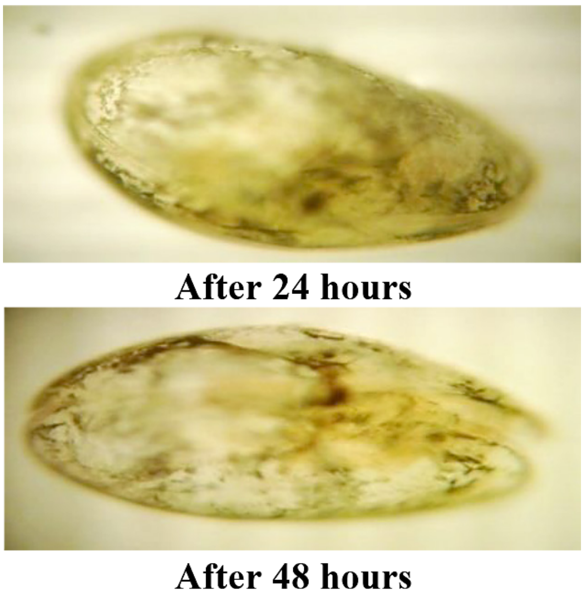


Figure 4. External view of crusty adult body and shape and color of the shell in the treated medium at 0.15 mg/L of $CdCl_2$

Impact of $CdCl_2$ on shell, morphology, and body appendages in juveniles' individuals

Three days after the excretion of the juveniles and their adult-like appearance, crustacean juveniles were exposed to the same concentrations of cadmium (0.1, 0.1.5, 0.2, 0.3 mg/L). It was found that there is a difference in the levels of impact worse at the level of the shell, the time

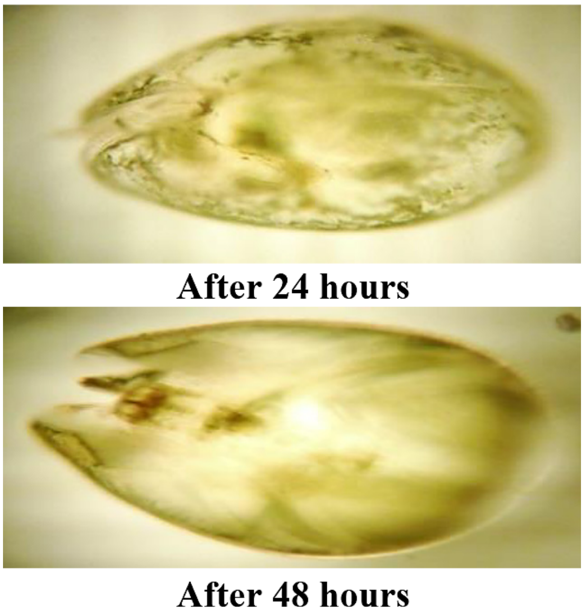


Figure 5. External view of crusty adult body and shape and color of the shell in the treated medium at 0.2 mg/L of $CdCl_2$

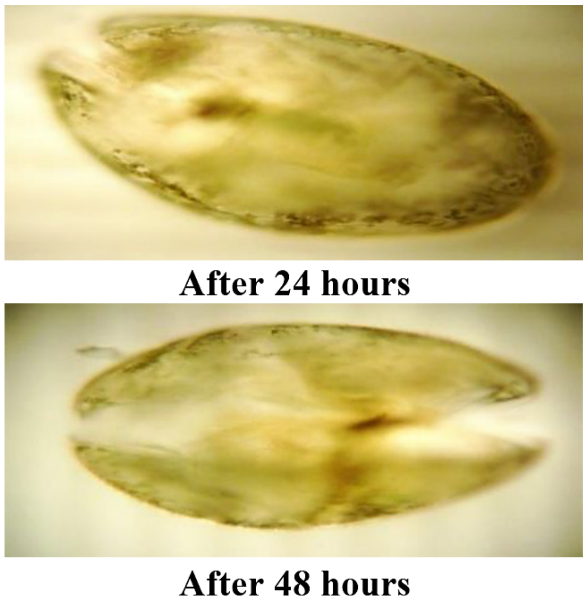


Figure 6. External view of crusty adult body and shape and color of the shell in the treated medium at 0.3 mg/L of $CdCl_2$

of mortality and its rates, the speed of response and endurance. It is believed that the shell and the strength of the muscle ligament of their shutters had the largest role in the endurance of the intensity of the impact of cadmium. Figure 7 shows that the shell was transparent; the size was relatively smaller than that of adults with body appendages appearing without any effect in the standard medium. These individuals gave populations of new embryos after 50–55 days of experiments.

When increasing the concentration of $CdCl_2$ to 0.15 mg/L in the culture medium, it was found that the effect became faster and more intense, as evidenced by the Figure 9, it was found that the crustaceans tried to withdraw most of the limbs and introduce a larger amount of water as a kind of volume amplification, but after 48 hours, it was found that the shell has become relatively dull in color with increased curvature and concealment of suffixes inside with the gradual opening of the shell shutters and the animal gradually stopped moving due to the inability to pull and control the appendages.

When following up in the images of the Figure 10, which represents the containment of the culture medium at a concentration of 0.2 mg/L from $CdCl_2$, it was found that the effect on the components of the shell and the ligament muscle has begun faster and was greater during the first 24 hours of the treatment, as the opening of the shell shutters was recorded faster and larger with a contraction in the components of biomass and poor movement and feeding. At the passage of 48 hours of the treatment, the deterioration of the state of the shell and the fragmentation of most of the calcareous material as well as the gradual breakdown of the components of the body, especially the appendages were observed; the recorded mortality ratio was greater than at the previous concentration.

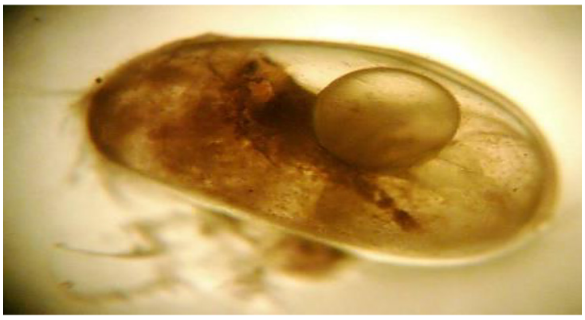
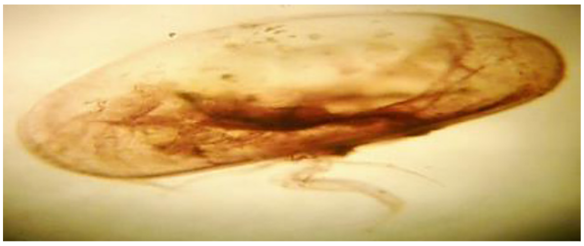


Figure 7. External view of the body of the crusty juveniles and the shape and color of the shell in the standard medium (concentration of 0 mg/L $CdCl_2$

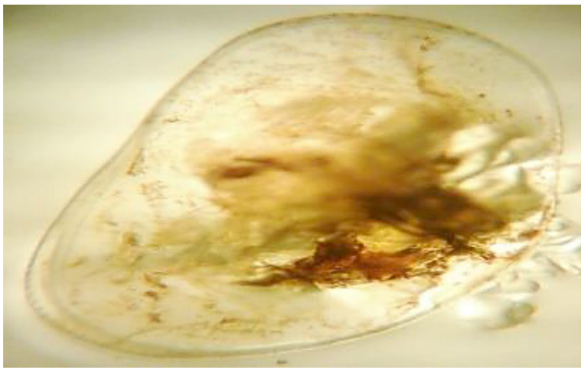


After 24 hours

After 48 hours

Figure 8. External view of the body of crusty juveniles and shape and color of the shell in the treated medium at a concentration of 0.1 mg/L of $CdCl_2$

After 24 hours



After 48 hours

Figure 9. External view of the body of the crusty juveniles and the shape and color of the shell in the treated medium at a concentration of 0.15 mg/L of $CdCl_2$

Figure 10. External view of the body of the crusty juveniles and shape and color of the shell in the treated medium at 0.2 mg/L of $CdCl_2$

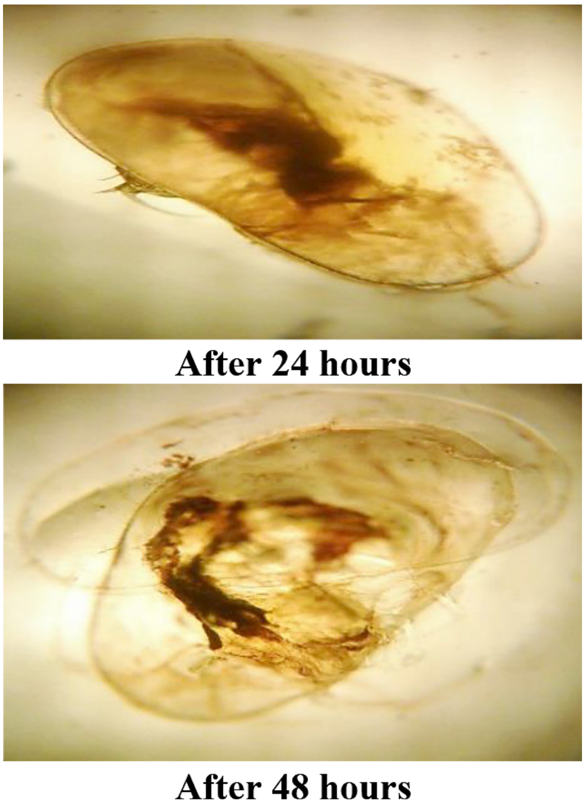


Figure 11. External view of the body of the crusty juveniles and shape and color of the shell in the treated medium at 0.3 mg/L of $CdCl_2$

The most influential stage was when treating with the medium containing a concentration of 0.3 mg /L from $CdCl_2$, when it was found that the animal's reaction was rapid by trying to shrink and withdraw all the appendages and refrain from feeding and movement during the first hours of treatment, but the individuals could not withstand much as the gradual opening of the shell shutters, and the pallor of the color of the shell occurred. Upon entering the second day the 48-hour stage, a complete paralysis of the work of the ligament muscle and the opening of the shell was almost complete with the biomass crash and distortion of the general view of the body and its various appendages (Figure 11).

From the results obtained from the applied experiments of the study and follow-up of the stages of the impact of $CdCl_2$ in the structure and construction of the shell and its color variation shown in figures (2 to 11), noted a major ability of Cd to interact with the building material of this shell and work on a gradual fragmentation of the calcareous substance, that appears in the form of spots which are pale in color, gradually expanding with increasing concentration and exposure time to the element, as well as the age of the tested crustacean. Therefore, these variables in shape and color can be considered as a biomarker to monitor the stages of the impact of heavy metals on the biology of various crustaceans, including *Cypris sp*. These conclusions are consistent with the opinions of researchers (Mitra et al., 2012, Shashkova and Grigoriev, 2013, Iglikowska, 2021) who followed the effect of heavy metals on various crustaceans.

Applied studies in the field of biomonitoring confirm that the body shell first carries out a process of adsorption of pollutants including metals and withdrawing them from the medium. This depends on the physiochemical characteristics of each element and the ability of association with the components of this shell. Through either changing the medium to become more acidic, or performing a gradual substitution process instead of calcium, or the formation of gradual soluble compounds in the aqueous medium, the shells gradually lose hardness (Shashkova and Grigoriev, 2013, Goud and Lanka, 2021, Marco and Lemos, 2021). Adornado, (2018) pointed there were studies indicating that some crustacean shells could be an effective biosorption of heavy metals in surface water, as example a Philippine mud crab (*Scylla serrata*) and mussel (*Perna viridis*) shells which are used for lead and chromium adsorption from polluted medium. During her research she discovered that these crustaceans have chitin, which permits the metal to be removed from solutions. Komi and Hamblin (2016), Esguerra et al. (2018) pointed out that the non-toxic qualities of crustacean shells make them a good bio-sorbent and both chitin and chitosan, which are organic compounds, may be acetylated to produce metal complexes.

Impact of $CdCl_2$ in survival rate, biology and activity of adults and juveniles of Cypris

When following up the level of effect by comparing the resistance of juveniles and adult of *Cypris* for the intensity of the effect of cadmium in the aqueous medium shown in Table 2 during times of exposure and the interpretation of the relationship between the correlation and determination coefficients, associated with the type of organism (adults and juveniles of *Cypris*) – see Table 3 – it was found that all tested individuals were able to stay in the standard solutions until the end of the experiment and put its embryos after several days for adults and after 50–55 days for juveniles. These findings are consistent with the studies of Al-Salman (2011) Iglikowska (2021) and Marco and Lemos (2021). In the media treated with cadmium chloride, it was found that there is a difference in response time and intensity of the effect as well as its relationship to the structure of the shell, the completion of the body synthesis and the ligament muscle, and the ability of individuals to control nutritional behavior and protect the body from external influences. It was found that the effect of the element has started since the treatment at a concentration of 0.1 mg /L, where the effects appeared on adults within 24 hours and recorded a percentage of 60, 60, 80, 90%, while in juveniles the effect appeared after 12 hours and recorded 50, 30, 20, 10%. After 24 hours the mortality rates of 70, 80, 90, 100%, in concentrations of 0.1, 0.1.5, 0.2 mg/L, respectively were recorded, while in adults, the mortality rate was 100% after 48 hours when treated at concentration 0.3 mg/L.

When following the relationship between the pH variables of the culture medium as well as the percentage of adult and juveniles mortality of *Cypris* under the influence of cadmium chloride, it was found that the pH factor has not changed much, and it was believed that this was due to the

Table 1. Mortality% (adults A and juveniles J of *Cypris*) under the influence of $CdCl_2$

Medium type	No. of individual	6 hours	12 hours	24 hours	48 hours	pH value
Control	A+J	0	0	0	0	7.6
0.1	A	0	0	60%	100%	7.6
	J	0	0	70%	100%	7.5
0.15	A	0	0	60%	100%	7.5
	J	0	0	80%	100%	7.4
0.2	A	0	0	80%	100%	7.3
	J	0	0	80%	100%	7.2
0.3	A	0	0	90%	100%	7.1
	J	0	0	90%	100%	7.1

Table 2. Correlation and determination coefficient values (A and J of *Cypris*) in the treated media at different concentrations of $CdCl_2$

Correlation coefficient and determination measurement	Correlation coefficient value	Determination coefficient value
Correlation coefficient and determination adults of cypris under the influence of $cdcl_2$	93%	86%
Correlation coefficient and determination juveniles of cypris under the influence of $cdcl_2$	86%	74%
Relationship between adults of cypris mortality and ph values under $cdcl_2$ influence	10%	08%
Relationship between adults of cypris mortality and ph values under $cdcl_2$ influence	7%	04%

Note: A – refers to adults and J – juveniles.

presence of algae, which create a state of equilibrium similar to the state of main puffer solution in most aquatic media (Gribof et al., 2018, Al-Hussani and Al-Salman, 2020, Al-Saeedi and Al-Salman, 2022). This is confirmed by the results of the statistical analysis, which shows that the relationship was very weak, as the value of the correlation coefficient for adults and juveniles recorded values of 10% and 9%, and the coefficient of determination also has a low value of 0.08% 3% and 8%, respectively.

The results of the statistical analysis also confirm that the increase in cadmium concentration and exposure time has important effects on the life and composition of genus *Cypris,* also the correlation coefficient and determination in different cases, observed a strong relationship between the concentration value and the percentage of mortality, whether in adults or juveniles, as the correlation coefficient recorded in them had very high values of 93%, 86%, 86%, 74% respectively. This means that 86% of the mortality rate is due to high concentration and 14% due to other environmental factors, as shown in Table 2. The conclusions of the study are consistent with (Mitra et al., 2012, Bonsignor et al., 2018 and Ahamad et al., 2020).

Cadmium also tends to bind with muscles and accumulate in the cells of different organisms depending on the so-called biological discrimination for the heavy element in the selection of organs and tissues in living bodies, the physicochemical nature and the location of the element in the periodic table and chemical groups in terms of the strength of the electric charge as well as the similarity of the element to the elements necessary for life of calcium, iron, potassium and others in chemical behavior and dealing with living matter, and these conclusions are consistent with to the studies by Al-Salman et al. (2011), Adornado (2018) and Padidaa et al. (2021) and Yap et al. (2021).

CONCLUSIONS

From the results of the current study, the following conclusions can be drawn. The presence of Cd in aquatic systems, even at low concentrations as 0.1 mg/liter, has a significant effect on the vitality of Ostracoda; these organisms sense its presence in the medium and make several rapid responses, so they can be adopted as an environmental monitor and the variation of some of its characteristics as a good biomarker for monitoring water pollution with heavy metals, especially cadmium are the focus of the research. The shell and connecting muscle are synchronized in the degree of response and sensitivity to the presence of heavy metals in the aqueous medium, so the change color, transparency, appearance of white spots on it and opening of its shutters to any degree is evidence of the responding to the impact of cadmium element on the effectiveness and behavior of the tested individuals.The results showed that the level of Cd impact was faster and more severe on juveniles than on adult individuals, which shows the importance of shell and degree of its integration in protecting of individuals from pollutants that reach the water in general or in limited breeding circles of crustacean.

Environmental media factors (temperature, pH, water purity, conductivity, presence of algae and some aquatic plants) play an influential role in mitigating or increasing the severity of the biological effect of the heavy metal on crustaceans and other aquatic organisms.

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