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Copper and Cadmium Toxicity on Freshwater Snail *Physella acuta* as Biological Indicator.

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Abstract

Biological indicators are essential for detecting contaminants globally because they can inform us about the long-term effects of many pollutants in the environment. About 80 adult freshwater snails, *Physella acuta*, (Gastropod: Physidae) were collected from the Tigris River / Lower Zab near Taqtaq bridge, Erbil Province, Iraq on November 2022. In the laboratory, the animals were placed in tanks with river water for acclimatization. The 24, 48, 72 and 96 hours toxicity of two heavy metals cupper and Cadmium ($CuSO_4 \cdot 5H_2O$ and $CdCl_2.2\ H_2O$) have been investigated using various nominal concentrations, including 0.0, 0.3, 0.5, 0.7 and 0.9 mg $^{-1}$ for Cu and 0.5, 1.0,3.0 ,5.0 and 7.0 mg $^{-1}$ for Cd. The behavioral changes and the snail's mortality were noticed daily and the median lethal concentrations values (LC_{50}) and effects of pH values for 24, 48, 72 and 96 hours were calculated. The LC_{50} values for the 24, 48, 72 and 96 hours exposures to Cu and Cd were 0.911, 0.699, 0.462, 0.209 mg L^{-1} and 2.359, 1.020, 0.094 and 0.040 mg L^{-1}, respectively. Results indicated that Cu showed noticeably greater toxicity than Cd. Also, treated groups showing significant variation in water pH value throughout the studied time. The study concluded that selected snail species can be used as a bioindicator for evaluating the hazards related to environmental pollutants.

1. Introduction:

Biological indicators are essential for detecting contaminants globally because they can inform us about the long-term effects of many pollutants in the environment. As, aquatic ecosystems have suffered from environmental deterioration caused by anthropogenic activity and industrialization [1], [2]. Several sources such as atmospheric, residential and commercial effluents, industrial waste, and other metal-based industries, have brought attention to the issues related to heavy metals contaminating aquatic ecosystems [3]. As a result, the introducing heavy metals into water bodies and environment have significant influence on the food chain, because these metals affect the ecosystem more severely since they persist for long time and possess the ability of bioaccumulation, which harms the quality of the water [4]. In fresh water, naturally existing bioindicators can be used to assess the water quality, which provides a whole picture of the ecosystem [5]. Mollusks have been considered as appropriate bioindicator and biomonitoring topics because of their widespread distribution and enormous number of species [6]. Since they contribute significantly to the biomass at various trophic levels in ecosystems and serve as vital species for ecosystem function and they display a significant accumulation of pollutants, especially heavy metals [1], [6]. Snails, which are a crucial link in the transmission of pollutants, especially heavy metals, are now thought to serve as a crucial biomonitoring tool and early warning indication as the majority of freshwater snail species are widely distributed and constitute an essential source of energy for many consumers, including birds and fish, that are essential members of the food chain [1], [7], [8].

One of the most diverse species of freshwater snails is the hermaphrodite *Physella acuta* (Draparnaud, 1805) [9] which has been used as bioindicators by many researchers, for instance [10] mentioned that freshwater snail *Physella acuta* is a suitable xenobiotic-sensitive organism for toxicity assessment. Also, Al-Warid *et. al*., [11] (2020) concluded that both snails (*Bellamya bengalensis* and *Physella acuta*) are shared good biological indicators characteristics due to their vulnerability to pollution. While recently, [12] in his study shows that *P. insularum* functions well in freshwater habitats biomonitoring for the five metals Cu, Pb, Cd, Ni and Zn exposures.

In this study uses *P. acuta* as test organism to investigate the sub-acute toxicity of these two metals (Cu and Cd) and determine if these species may serve as bioindicators for contamination from heavy metals, also showing effects of heavy metals on water pH values. Hence, this research may offer fundamental knowledge for assessing the level of heavy metal contamination in freshwater ecosystems.

2. Experimental Program:

2.1 Sample Collection:

Snail samples were collected from the Tigris River / Lower Zab near Taqtaq bridge, Erbil Province, Iraq on November 2022. Eighty snails (*Physella acuta*) were collected. Snails had been kept in suitable polyethylene bags containing water from the same environment (stones and some vegetation). In the laboratory, snails they were kept in a glass aquarium using dechlorinated tap water which was exchanged every 24 hours' interval for one month with continuously aerated for acclimatization and feed with algae. Acclimatized snails were placed in to glass aquaria container that holds 500 ml of a specific concentration. For well aeration, stone diffusers and air pumps were provided. The toxicity tests were conducted under fluorescent lighting at ambient temperature (25–28 °C) and 12-hour day, 12-hour night. In each experiment, snails were almost the same size and received the same amount of aeration and no food was provided. The tests were conducted in a static environment without solution renewal until the test's end.

2.2 Experimental Design and Toxicity Test:

The standard metal stock solution (100 mg L^{-1}) of both Cu and Cd prepared created using analytical metallic salts of CuSO4·5H2O and CdCl2·2H2O respectively by dilution with deionized water in one liter (1 L) volumetric flask. Different nominal concentrations of heavy metals, 0.0, 0.3, 0.5, 0.7 and 0.9 mg L-1 for Cu and 0.5, 1.0,3.0 ,5.0 and 7.0 mg L^{-1} for Cd had been made from stock solutions. The experimental design consisted of 10 glass aquaria including three replications. Sub-acute Cu and Cd toxicity tests was conducted for a four-day (24, 48, 72 and 96-hours) period. The behavioral and morphological changes as well as daily snail's mortality was observed and the LC_{50} values for 24, 48, 72 and 96 h were determined. At each interval, the dead snails were removed out of the test containers and after being placed in pure freshwater, the snails were declared dead and immediately removed if they did not regain their health. The snails undergo all tests did not experience any stress, as shown by their complete survival throughout the duration of the experiment in the control water. The toxicity test procedures was modified from [13].

2.3 Statistical Analysis:

In order to analyze data and median lethal concentration (LC_{50}) for the toxicity test, Probit Analysis (SPSS version 25) was used. Two-way analysis of variance (ANOVA) and Tukey's post hoc test was used to check the significance of a treatment effects by Graph Pad Prism. The p-value limit for statistical significance was 0.05.

3. Results and Descussion:

3.1 Behavioral and Morphological Changes:

This experiment involved exposing snails to varied concentrations of Cu and Cd, and behavioural and morphological changes were noticed. Their use in toxicity experiments are due to it's easy adaptation and manipulation at the laboratory conditions and it can be treated with the desired amount of contaminants and their rapidly response to pollutants in the sublethal doses [14]. The snails had vertical movement, extend their bodies out of the shell, and attach their bodies to the glass container wall when they were not exposed to these metals [12]. Whereas; the snails released white mucus with a slower movement rate at moderate amounts of these metals [12], [15]. Along treatment periods, snails showed a steady decrease in the rate of crawling and clumping, and none of the treatments showed a touch response. Also, they were incapable to retract their bodies within shells. Snails expelled bubbles, slowed down their metabolism, and sunk to the tank bottom with closed their operculum. One significant drawback in this static toxicity test was the snails′ movement or crawling during the experiment in an attempt to escape out of the experimental aquaria. However, snails possess operculum that may protect them against contaminated environment [12], [16].

The treated snails couldn't stick to the tank walls because these metals may be interfered with cell mechanics and harmed the snails′ cells and tissues. The metals enter the cells, resulting in necrosis of the cells and causes death. Alternatively, the high metabolic rates during that period might have resulted in a significant absorption of toxins, or the activity of the snails during starving could have diminished their resistance to toxins [16]. Utilizing snails′ responsive behavior, can observe the effects of environmental change [8].

3.2 Assessments of Toxicity:

Environmental pollution monitoring worldwide depends heavily on bio-indicator systems [8]. Biological indicators can be used to identify the presence of contaminants and quantify their harmful effects. Snails have been shown to provide an environmentally friendly method for environmental biomonitoring [14]. Short-term toxicity test was used to investigate the toxicity and tolerance of heavy metals (Cu and Cd) in *P. acuta*, with mortality standing as the endpoint. A comparison of the LC_{50} values for two different metals is shown in Table 1. The LC_{50} concentrations (mg L^{-1}) of 24, 48, 72 and 96 hours Cu were 0.911, 0.699, 0.462, and 0.209 mg L^{-1}, respectively, while LC_{50} concentrations (mg L^{-1}) were 2.359, 1.020, 0.094, and 0.040 for 24, 48, 72 and 96 hours Cd for exposure time respectively. The results of LC_{50} indicated that Cu was more toxic to *P. acuta.* than Cd for all measured exposure time Table 1. The snail showed a significant sensitivity ($P \leq 0.05$) to Cu, which greatly raised the acute toxicity to *P. acuta.*, resulting in a low LC_{50} value these results came in accordance with results obtained by [17], [18]. Figures 1 and 2 showing comparison of number of deaths after

Table 1. Median lethal concentrations (LC_{50}) values (mg L^{-1}) for *P. acuta* at different exposure times for Cu and Cd.

Time (hour)	LC_{50} (mg L^{-1})	Time (hour)	LC_{50} (mg $^{-1}$)
Cu		Cd	
24	0.911	24	2.359
48	0.699	48	1.020
72	0.462	72	0.094
96	0.209	96	0.040

exposure to different doses of heavy metals (Cu and Cd) at different exposure time (24, 48, 72 and 96) hours. There were significant differences between Cu treated groups as well as within groups Figure 1. As shown in the Figure 1 as exposure time increase, mortality of individuals increased. Different Cu concentrations caused death to snails at all studied exposure time especially 96-hour that having more effects than others (24, 48 and 72-hrs) because according to Balistrieri and his coworkers [19] stated that in experimental water, the concentrations of dissolved metals were increased with time. Whereas; regarding to dosages, 0.9 mg L^{-1} severely affected snails and causing higher number of deaths compared to other treated doses (0.0, 0.3, 0.5 and 0.7 mg L^{-1}) this might be due to presence high concentrations of Cu ions in aqueous media and recently [19] discovered that metal concentration is time dependent and short-term Cu exposure may lead to oxidative stress and extended exposure resulted in oxidative damage [20]. In addition, Brix *et. al.*, 2011 [21] noticed that high doses of Cu exposure can cause hemolymph acidity, decreased ammonia excretion, and an increase in titratable acid excretion.

This means increasing Cu concentrations can affect severely to aquatic snails. Figure 2 illustrated differences between Cd treatments, as shown that snails exposed to (3 mg L^{-1}) Cd significantly more affected than other treated groups at different

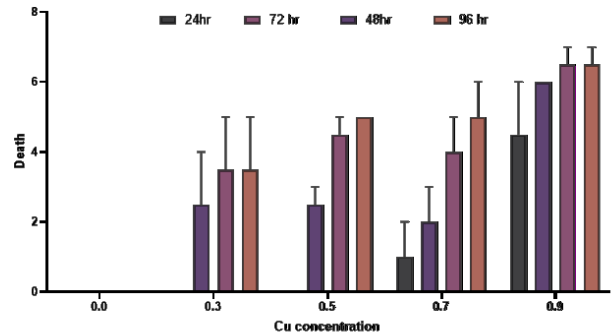

Figure 1. The relationship between Cu concentrations (mg L^{-1}), exposure time and number of animal death.

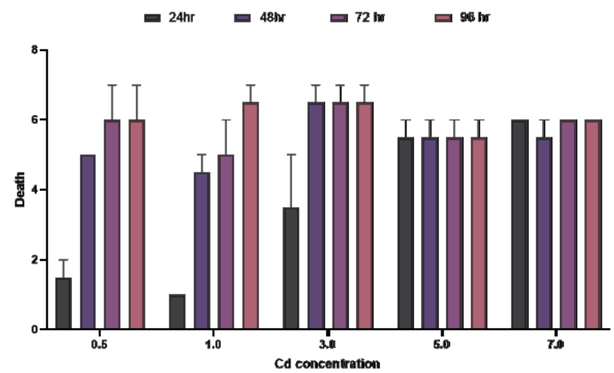

Figure 2. The relationship between Cd concentrations (mg L^{-1}), exposure time and number of animal death.

times (48, 72 and 96) hours as compared to 24 hours exposure, however, in Figure 2 demonstrated that time exposure affect's on snail's mortality especially between first three doses of Cd (0.5, 1 and 3 mg L^{-1}) at 48, 72 and 96- hours of treatment.

Considering the above-mentioned results indicated that, dose-response relationships and time of exposure affected value of both Cu and Cd, as (Walker *et. al.* 2006) stated that the heavy metal doses, the age and size of the utilized snails in the test may be additional variable that affected the values of LC_{50} in this studied. According to number of deaths in various exposure time, Cupper was significantly more toxic at low concentration than cadmium, causing death at the same levels of response as Zhang *et. al.* (2020) [18] stated that snails were found to be very sensitive to Cu^{2+}, while Cd^{2+} and Hg^{2+} demonstrated a moderate level of acute toxicity this may be because of specific differences of metals or increase in these metals' bioavailability may be linked to their increased sensitivity at higher temperatures. Bioavailability is an important aspect to take into account in metal toxicity evaluations because, rather than total metal concentrations, the bioavailable metal fraction indicates the dangerous metal fraction [22]. The results also showed that *P. acuta* represents a good sensitivity range for the sublethal assessment during their exposure to metals such as Cu and Cd. Recently, [17] also determined that *Physa acuta* exhibit moderate to severe copper and cadmium sensitivity, indicating that they are a good test organism, especially for long-term ecotoxicological evaluation. These results may be due to those different concentrations of heavy metals especially copper and cadmium alter the mollusks' soft tissues and hemolymph's enzyme levels at varying exposure times or may lead to cell damage and a significant drop in oxygen absorption rates might result cell death and lead to death of the snails as mentioned by [8], [16], [23] this indicated that freshwater snails are sensitive to environmental changes especially the presence of heavy metals can alter their habitat. On the other hand, may affects on their operculum that protect them from their toxic water environment [12]. Findings indicated that a freshwater snail, Physa acuta, is a useful bioindicator to highlight the detrimental effects of heavy metals in aquatic environments due to their sensitivity to pollution as indicated previously by [11], [24].

4. Effects on Water pH:

Significant pH value differences seen in Figure 3. Groups treated with (0.5, 0.7 and 0.9 mg L^{-1}) Cu showing highly significant ($p \leq 0.05$) water pH values (moderately alkaline) at 96- hours exposure when compared to other exposure times. This means that increasing dosage and exposure time may affect significantly on water pH and this might severely affect freshwater snails. Likewise, 96- hour Cd treated groups showing higher value of pH as shown in Figure 4. Moreover; 3 mg $^{-1}$ of Cd having distinct higher pH level because at high pH value cadmium accumulation increased and also its toxicity increased [25]. Nevertheless, 1, 5 and 7 mg L^{-1} Cd treated tanks significantly differing in water hydrogen ion values especially comparing to lower Cd treated tank (0.5 mg L^{-1}) at various time of exposure. The results demonstrated that increasing metal concentration in water bodies may affects on water pH values and this could change snail's habitat and affecting their normal life. This might be due to stress that faced snails during experiment. As recent study done by [26] determined that aquatic species may be subjected to acute or chronic stress due to pH changes, which can impact their immune systems and daily activities. Also, [27] predicted that polluted environment by heavy metals would lead to physiological stressed and impaired snails defense system. As a result, pH variations may endanger aquatic life, mostly due to potential interactions with abiotic or chemical stressors [28]. The findings of this investigation suggest that the pH may influence the diversity of snail populations and serve as a reliable indication of various chemical environments as determined previously by [29].

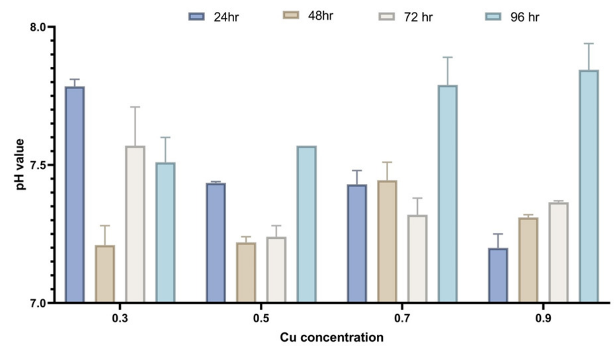

Figure 3. The relationship between Cu concentrations (mg L^{-1}), exposure time and water pH value.

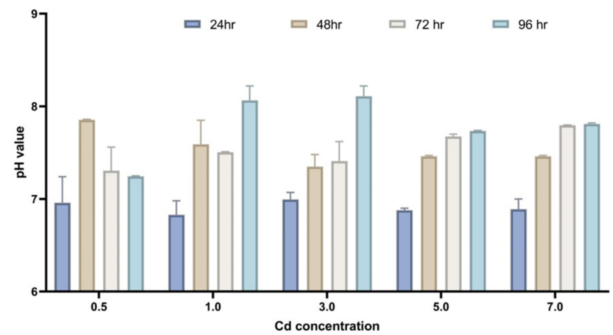

Figure 4. The relationship between Cd concentrations (mg L^{-1}), exposure time and water pH value.

5. Conclusion:

The results showed that *Physella acuta* could be used as a biological indicator for the subacute toxicity test and can serve as the basis for evaluating the risks and hazards related to environmental pollutants. Cupper (Cu) was more toxic at low concentrations compared to cadmium (Cd). The most effective biological response to identify the effects of metal toxicity will vary depending on the target organisms, metals of concern, routes of exposure, and kind of exposure (long or short term). The presence of heavy metals (Cu and Cd) in water body may affect water pH value and can affect on aquatic invertebrate life. So, further studies are needed to evaluate other water physico- chemical properties in relation to heavy metal toxicity.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

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سمية النحاس والكادميوم على حلزون *Physella acuta* المياه العذبة كمؤشر بيولوجي

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الخلاصة

تُعتبر المؤشرات البيولوجية ضرورية للكشف عن الملوثات عالميًا، لأنها تُمكننا من فهم الآثار طويلة المدى للعديد من الملوثات في البيئة. تم جمع حوالي 80 حلزونًا بالغًا من نوع (*Gastropod* : *Physidae*) *Physella acuta* ، وهي حلزونات تعيش في المياه العذبة، من نهر دجلة / الزاب الأسفل بالقرب من جسر طق طق في محافظة أربيل، العراق في شهرتشرين الثاني 2022 . في المختبر، تم وضع الحيوانات في أحواض مملوءة بماء النهر لتتأقلم. تمت دراسة السمية لمدة 24 ، 48 ، 72 ، و 96 ساعة لعنصرين ثقيلين هما النحاس والكادميوم ($CuSO_45H_2O$ *and* $CdCl_2.2H_2O$) باستخدام تراكيز سمية مختلفة، تشمل 0.0 ، 0.3 ، 0.5 ، 0.7 ، و 0.9 ملغم لتر $^{-1}$ للنحاس، و 0.5 ، 1.0 ، 3.0 ، 5.0 ، و 7.0 ملغم لتر $^{-1}$ للكادميوم. تمت ملاحظة التغيرات السلوكية وموت الحلزونات يوميًا، وتم حساب قيم التركيزات القاتلة المتوسطة (LC_{50}) لمدة 24 ، 48 ، 72 و 96 ساعة. كانت قيم LC_{50} للتعرض لمدة 24 ، 48 ، 72 ، و 96 ساعة للنحاس والكادميوم على التوالي 0.911 ، 0.699 ، 0.462 ، 0.209 ملغم لتر $^{-1}$ ، و 2.359 ، 1.020 ، 0.094 ، و 0.040 ملغم لتر $^{-1}$. أظهرت النتائج أن النحاس كان أكثر سمية بشكل ملحوظ من الكادميوم. كما أظهرت المجموعات المعالجة تغيرًا ملحوظًا في قيمة الرقم الهيدروجيني للماء طوال فترة الدراسة. خلصت الدراسة إلى أن الأنواع المختارة من الحلزونات يمكن استخدامها كمؤشر بيولوجي لتقييم المخاطر المتعلقة بالملوثات البيئية.

الكلمات الدالة: المؤشر البيولوجي، النحاس، الكادميوم، حلزون المياه العذبة، السمية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.