

Hussein AB, Alaaeldeen A O Abbas, N Hussein and Noreldaim Elkhidir*

Imam Abdulrahmen Bin Faisal University, Kingdom of Saudi Arabia

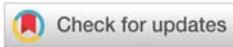
Received: 03 October, 2019

Accepted: 11 November, 2019

Published: 12 November, 2019

***Corresponding author:** Noreldaim Elkhidir, Imam Abdulrahmen Bin Faisal University, Kingdom of Saudi Arabia, E-mail: nhelkhidir@iau.edu.sa

<https://www.peertechz.com>



Research Article

Investigation of physiological responses of *Procambarus clarkii* and some enzyme activities to selected heavy metals

Abstract

Mean enzyme activities of Aspartate Transaminase (ALT), Aka Alanine Transaminase (AST) and alkaline phosphatases of the digestive gland of freshwater *Procambarus clarkii* were subjected to different sublethal concentrations of lead, mercury and cadmium were measured. The results obtained showed that the investigated enzymes were affected by the sublethal concentrations of the heavy metals. The mean activity values of ALT, AST and Alkaline phosphatase decreased under all heavy metals. Mercury showed the highest effect followed by cadmium and lead. On the other hand, the mean activity values of acid phosphatase increased with the increase in heavy metals concentrations. Lead gave the highest effect followed by mercury and cadmium. There is a linear relationship between the sublethal concentrations and the decrease or increase of the mean activity values of the enzymes measured. The ALT, AST and alkaline phosphatase would be used as biomarkers for Hg pollution whereas acid phosphatase would be used as an indicator for Pb pollution in the presence for other heavy metals.

Introduction

Immense research has been undertaken to evaluate the effects of environmental pollution on the genetics and physiology of living organisms at large. Global urbanization has vastly modified landscapes and increased the density of impervious land cover, including parking lots and paved roads [1]. Roads reduce habitat area, fragment habitats, change soil hydrology, reduce water quality, and introduce chemical pollutants [2-4].

It is now generally accepted by population biologists that living organisms must either get adapted to new environmental changes and stresses or suffer depression in fitness, sometimes serious enough to cause local species extinction. Several biochemical methods such as colourimetric and electrophoretic, have been introduced as suitable tools for examining the responses of living organisms to environmental stresses. The most commonly used of these is the colourimetric technique.

Heavy metals are considered to be toxic to living organisms at high concentrations, and their accumulation threatens the health of organisms living along roads [5,6]. They can as well contaminate ecosystems and groundwater used for human

consumption if heavy metals become disassociated from soil materials [7].

Sufficient evidence has been proven that heavy metals such as mercury, lead, cadmium, and copper can reduce the diversity of macroinvertebrates, alter food-web structures, and reduce ecosystem services in streams [8-11].

Cadmium is found in the environment as part of several, mainly zinc-rich, ores. In mammalian biology, cadmium exposure jeopardizes health and mechanisms of cadmium toxicity are multifarious [12]. Mainly because bioavailable cadmium mimics other metals that are essential to diverse biological functions, cadmium follows a Trojan horse strategy to get assimilated. Metals susceptible to cadmium deceit include calcium, zinc, and iron. The wealth of data addressing cadmium toxicity in animal cells is briefly reviewed with special emphasis on disturbance of the homeostasis of calcium, zinc, and iron. Cadmium has been shown to inhibit enzymic activities and protein synthesis *in vivo*, however, it is not yet as clear the extent to which cadmium would readily express the same effects *in vivo* [13].

Mercury, an environmental contaminant, is a risk factor for health in whole living organisms. A study by Altunkaynak et



al., [16], revealed HgO inhalation resulted in reduction of the total number of primordial, primary and Graaf follicles. Also, mean volume of ovary, medulla and cortex, corpus luteum (c. luteum) and Graaf follicles was decreased in the Hg group. Such structural alterations could be attributed to the toxic influence of HgO on rat ovary.

Roychoudhury et al., [15], tested the effects of Mercury on ovarian granulosa cells which were incubated with mercuric chloride [mercury (II) chloride or HgCl₂] at the doses 50–250µg mL⁻¹ for 18 h and compared with control group without Hg addition. Release of progesterone and insulin-like growth factor by ovarian granulosa cells was assessed by RIA and apoptosis by TUNEL assay. Their observations show that progesterone release by granulosa cells was significantly ($P < 0.05$) inhibited at all the doses, while insulin-like growth factor release was not affected at any of the doses used, although a decreasing trend in the release of IGF-I was noted in comparison to control.

The implications of mercury and lead concentrations on breeding physiology and phenology of Arctic birds showed significant relationships with breeding onset and condition in female eider ducks, factors that could influence reproductive success in this species [16]. On a study by [17], on bluegill sunfish they conclude that exposure to wastewater effluent invokes a metabolic cost that leads to compensatory respiratory improvements in O₂ uptake, delivery, and utilization.

The red swamp *P. clarkia* is a freshwater macro-invertebrate that has been introduced to freshwater in Egypt in 1980. The species rapidly expanded in all aquatic ecosystems including clear and polluted water, and became more adapted to the local ecosystems and became an important component of the local aquatic fauna [18].

The present study focuses on the effect of freshwater quality and the mode of action and role on enzyme activities of cadmium, mercury and lead on *P. clarkia*. The enzymes covered by the study included alanine transaminase (ALT), aspartate transaminase (AST), acid and alkaline phosphatases.

Materials and Methods

Collection of juvenile *P. clarkia*

Samples of juvenile *P. clarkia* were collected from El-Kased at Tanta. The collected specimens were then carried to the laboratory and stored in plastic containers filled with dechlorinated distilled water. Juveniles were fed on lettuce leaves and left for 48 h under laboratory conditions.

Test Solutions

Test solutions of Pb, Hg and Cd were prepared from their salts (Hg and Cd as chlorides and Pb as acetate) in dechlorinated tap-water and sublethal doses of 0.1, 0.2 and 0.5ppm concentrations of the three metals were prepared. Twenty juveniles of *P. clarkia* about 4cm long were exposed to the sub-lethal concentrations of the metals as described above for a period of 4 weeks.

Colourimetric determinations

After 4 weeks, digestive glands of *P. clarkia* were taken out and dried on filter paper, weighed, homogenized in a known volume of 0.25M cold sucrose solution and then centrifuged (3000ppm) for 20 minutes. The supernatant extractions were stored at 4°C until analysis which was conducted 5 days later. Colourimetric determinations of ALT, AST were carried out according to Reitman and [19], whereas acid and alkaline phosphatases were determined according to [20].

Data was analyzed using Excel 2010 to calculate the means, standard deviations and assess the variation between treatments as detailed in [Appendix 1](#).

Results

Effects of cadmium

Tables 1,4 shows the mean enzyme activity of ALT, AST, acid and alkaline phosphatase of the digestive gland of *P. clarkia*. It is evident that the mean activities levels of ALT, AST and alkaline phosphatase decrease with the increase of concentrations, for ALT, 0.511, 0.498, 0.382µmole/g tissue/min of the digestive gland; for AST, 0.212, 0.201, 0.198µmole/g tissue/min of the digestive gland and for alkaline phosphatase, 0.313, 0.300, 0.281 King and King units/tissue/min at 0.1, 0.2 and 0.5ppm Cd, respectively (Table 1). Acid phosphatase showed an increase with the increase in Cd concentrations, i.e., 3.012, 3.233 and 3.444 King and King units/tissue/min at 0.1, 0.2 and 0.5ppm Cd, respectively (Table 4). As compared to the control mean enzyme activity of all enzymes, it can be noticed that there is a significant difference between the mean activities values ($P < 0.001$).

Table 1: Mean activity levels of ALT, AST and alkaline phosphatase of the digestive gland of *P. clarkia* kept in different cadmium concentrations for 4 weeks replicated three times (R1, R2, R3). ALPh: Alkaline phosphatase.

ALT	C	0.1Cd	0.2Cd	0.5Cd
R1	0.531	0.511	0.488	0.366
R2	0.531	0.510	0.508	0.361
R3	0.529	0.513	0.498	0.360
Mean	0.530	0.511	0.498	0.362
StDev	0.0006667	0.000882	0.005774	0.001856
AST	C	0.1Cd	0.2Cd	0.5Cd
R1	0.319	0.217	0.195	0.201
R2	0.306	0.207	0.207	0.191
R3	0.312	0.213	0.202	0.202
Mean	0.312	0.212	0.201	0.198
StDev	0.0065064	0.005033	0.006028	0.006083
ALPh	C	0.1Cd	0.2Cd	0.5Cd
R1	0.320	0.308	0.298	0.279
R2	0.323	0.301	0.282	0.283
R3	0.325	0.330	0.319	0.282
Mean	0.323	0.313	0.300	0.281
StDev	0.003	0.015	0.019	0.002



Effects of mercury

Tables 2,4 show the mean enzyme activity at different concentrations of Hg. It can be observed that the mean enzymes activities of ALT, AST and alkaline phosphatase decreased with the increase in Hg concentrations i.e. for ALT, 0.501, 0.488. 0.362µmole/g tissue/min of the digestive gland; for AST, 0.282, 0.240, 0.188µmole/g tissue/min of the digestive gland and for alkaline phosphatase, 0.300, 0.298, 0.272 King and King units/tissue/min at 0.1, 0.2 and 0.5ppm Hg, respectively (Table 2). Acid phosphatase increased with the increase in Hg concentrations, i.e., 2.981, 3.213 and 3.541 King and King units/tissue/min at 0.1, 0.2 and 0.5ppm Hg, respectively (Table 4). When comparing the control mean activities of all enzymes with that of the control, it could be observed that there are significant differences ($P < 0.001$).

Effects of lead

Tables 3,4 show the mean enzyme activities of ALT, AST, acid and alkaline phosphatase. It could be observed that the mean enzyme activities of ALT, AST and alkaline phosphatase decrease with the increase in Pb concentrations, i.e., for ALT, 0.521, 0.474, 0.402µmole/g tissue/min of the digestive gland; for AST, 0.243, 0.239, 0.221µmole/g tissue/min of the digestive gland and for alkaline phosphatase, 0.310, 0.290, 0.273 King and King units/tissue/min at 0.1, 0.2 and 0.5 ppm Pb, respectively (Table 3). On the other hand, acid phosphatase increased with the increase of Pb concentrations, i.e. 3.412, 3.524 and 3.714 King and King units/tissue/min at 0.1, 0.2 and 0.5ppm Pb, respectively (Table 4). Comparing the mean enzyme activities of all enzymes with the control treatment, it could be observed that there is significant differences between the mean activity values and that of the control ($P < 0.001$).

Table 2: Mean activity levels of ALT, AST and alkaline phosphatase of the digestive gland of *P. clarkia* kept in different mercury concentrations for 4 weeks replicated three times (R1, R2, R3). AIPh: Alkaline phosphatase.

ALT	C	0.1Cd	0.2Cd	0.5Cd
R1	0.529	0.499	0.476	0.346
R2	0.541	0.501	0.498	0.375
R3	0.519	0.504	0.489	0.366
Mean	0.530	0.501	0.488	0.362
StDev	0.011	0.003	0.011	0.015
AST	C	0.1Cd	0.2Cd	0.5Cd
R1	0.310	0.272	0.234	0.179
R2	0.309	0.291	0.239	0.197
R3	0.318	0.283	0.247	0.188
Mean	0.312	0.282	0.240	0.188
StDev	0.005	0.010	0.007	0.009
AIPh	C	0.1Cd	0.2Cd	0.5Cd
R1	0.320	0.299	0.299	0.267
R2	0.329	0.291	0.306	0.279
R3	0.319	0.309	0.288	0.269
Mean	0.323	0.300	0.298	0.272
StDev	0.006	0.009	0.009	0.006

Table 3: Mean activity levels of ALT, AST and alkaline phosphatase of the digestive gland of *P. clarkia* kept in different lead concentrations for 4 weeks, replicated three times (R1, R2, R3). AIPh: Alkaline phosphatase.

ALT	C	0.1Cd	0.2Cd	0.5Cd
R1	0.544	0.511	0.499	0.370
R2	0.523	0.515	0.475	0.371
R3	0.523	0.477	0.489	0.344
Mean	0.530	0.501	0.488	0.362
StDev	0.012	0.021	0.012	0.015
AST	C	0.1Cd	0.2Cd	0.5Cd
R1	0.317	0.290	0.255	0.198
R2	0.319	0.271	0.221	0.178
R3	0.301	0.284	0.244	0.188
Mean	0.312	0.282	0.240	0.188
StDev	0.010	0.010	0.017	0.010
AIPh	C	0.1Cd	0.2Cd	0.5Cd
R1	0.337	0.306	0.299	0.286
R2	0.310	0.301	0.302	0.270
R3	0.322	0.293	0.294	0.259
Mean	0.323	0.300	0.298	0.272
StDev	0.014	0.007	0.004	0.014

Table 4: Mean activity levels acid phosphatase of the digestive gland of *P. clarkia* kept in different cadmium, mercury and lead concentrations for 4 weeks, replicated three times (R1, R2, R3).

Cd	C	0.1Cd	0.2Cd	0.5Cd
R1	3.032	3.112	3.389	3.541
R2	2.799	3.019	3.022	3.391
R3	2.908	2.998	3.289	3.401
Mean	2.913	3.043	3.233	3.444
StDev	0.1165804	0.060671	0.189727	0.083865
Hg	C	0.1Cd	0.2Cd	0.5Cd
R1	2.921	2.990	3.265	3.660
R2	2.940	2.971	3.221	3.461
R3	2.878	2.982	3.153	3.503
Mean	2.913	2.981	3.213	3.541
StDev	0.0259358	0.007789	0.046072	0.085644
Pb	C	0.1Cd	0.2Cd	0.5Cd
R1	2.880	3.431	3.530	3.730
R2	2.987	3.402	3.513	3.702
R3	2.871	3.401	3.529	3.711
Mean	2.913	3.412	3.524	3.714
StDev	0.0645316	0.017073	0.009539	0.014295

Discussion

The data obtained in the presents study suggests enzymic activities of the digestive gland of *P. clarkia* has been significantly reduced due to the increase in sublethal concentrations of Cd, Hg and Pb, with respect to ALT, AST and alkaline phosphate. Mercury's high affinity for the sulfhydryl moieties of enzyme catalytic sites is a common motif for enzyme inactivation. These permanent covalent modifications



inactivate the enzyme, thereby inducing devastating effects on an organism's metabolic functions [21]. There are many suggestions for the mechanisms that caused such inhibitory effects. For example, Mercury (MeHg) and perhaps Hg⁺² exerts its toxic effects through numerous mechanisms. In neurons MeHg disrupts Ca homeostasis by affecting both voltage-gated calcium channels as well as disruption of intracellular channels. MeHg like cadmium, binds to sulfhydryl groups on cysteine, which may compromise the function of enzymes and ion channels. MeHg also interacts with DNA and RNA resulting in reductions in protein synthesis and structural disruptions in the neurons. On plants DNA alterations due to Hg heavy metal exposure included the disappearance of normal DNA bands and the appearance of new bands compared to the untreated controls were observed [22]. Atchison and Hare [23], observed disruption of electron transport in the mitochondria through inhibition of enzymes.

Interestingly, it was evident that the sublethal doses of the heavy metals applied in this study did not express any influence on acid phosphatase. This could be attributed to acid phosphatase as a temporary enzyme [24].

Conclusions

It is evident that sublethal concentrations of Cadmium (Cd), Mercury (Hg) and Lead (Pb) significantly affected enzymic activity in *P. clarkia* digestive system as expressed by the decline in enzymic activity expressed by aspartate transaminase (ALT), alanine transaminase (AST) and alkaline phosphatase. The highest effects are expressed by mercury doses compared to control treatments.

The present results demonstrate the potential of *P. clarkia* to act as bioindicator for important toxic heavy metals which may help as a methodological basis for ecological risk assessment of heavy metals in freshwater systems. To eliminate heavy metal contaminations and possible risks on freshwater fauna, plants are being used as removal agents of pollutants/toxic chemicals from the environment.

More research needs to be conducted to explore the dynamic of enzymic activities under normal favorable ecological conditions and their behavior throughout the life cycle of *P. clarkia* and related freshwater species to reach a comprehensive understanding of long terms and cumulative effects on species physiology. As some toxic doses of heavy metals are less species-specific, further investigation are recommended to explore likely impacts on higher trophic levels in freshwater ecosystems.

Research on new alternative natural adsorbents is required as biological treatments are more environmentally friendly. Strict and continued ecological monitoring is required to assess whether prevalent heavy metals concentrations exceed legal allowed values established by international legislation authorities including the UN Environment Programme.

References

1. Boving TB, Stolt MH, Augenstern J, Brosnan B (2008) Potential for localized groundwater contamination in a porous pavement parking lot setting in Rhode Island. *Environmental Geology* 55: 571-582. [Link: http://bit.ly/33Nj8a0](http://bit.ly/33Nj8a0)
2. Forman RT, Alexander LE (1998) Roads and their major ecological effects. *Annual review of ecology and systematics* 29: 207-231. [Link: http://bit.ly/2NBqVCs](http://bit.ly/2NBqVCs)
3. Spellerberg IAN (1998) Ecological effects of roads and traffic: a literature review. *Global Ecology and Biogeography Letters* 7: 317-333. [Link: http://bit.ly/2K8D53y](http://bit.ly/2K8D53y)
4. Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation biology* 14: 18-30. [Link: http://bit.ly/36UIExq](http://bit.ly/36UIExq)
5. Li FR, Kang LF, Gao XQ, Hua W, Yang FW, et al. (2007) Traffic-related heavy metal accumulation in soils and plants in Northwest China. *Soil and Sediment Contamination* 16: 473-484. [Link: http://bit.ly/32xyiPh](http://bit.ly/32xyiPh)
6. Green SM, Machin R, Cresser MS (2008) Effect of long-term changes in soil chemistry induced by road salt applications on N-transformations in roadside soils. *Environ Pollut* 152: 20-31. [Link: http://bit.ly/2X071nJ](http://bit.ly/2X071nJ)
7. Norrström AC, Jacks G (1998) Concentration and fractionation of heavy metals in roadside soils receiving de-icing salts. *Science of the Total Environment* 218: 161-174. [Link: http://bit.ly/34POxJi](http://bit.ly/34POxJi)
8. Maltby L, Forrow DM, Boxall AB, Calow P, Betton CI (1995) The effects of motorway runoff on freshwater ecosystems: 1. Field study. *Environmental Toxicology and Chemistry: An International Journal* 14: 1079-1092. [Link: http://bit.ly/2NA5Sjp](http://bit.ly/2NA5Sjp)
9. Clements WH, Carlisle DM, Lazorchak JM, Johnson PC (2000) Heavy metals structure benthic communities in Colorado mountain streams. *Ecological applications* 10: 626-638. [Link: http://bit.ly/2qK0gdm](http://bit.ly/2qK0gdm)
10. Hirst H, Jüttner I, Ormerod SJ (2002) Comparing the responses of diatoms and macro-invertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology* 47: 1752-1765. [Link: http://bit.ly/2pRjPAK](http://bit.ly/2pRjPAK)
11. Carlisle DM, Clements WH (2005) Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. *Freshwater biology* 50: 380-390. [Link: http://bit.ly/200oDLZ](http://bit.ly/200oDLZ)
12. Martelli A, Rousselet E, Dycke C, Bouron A, Moulis JM (2006) Cadmium toxicity in animal cells by interference with essential metals. *Biochimie* 88: 1807-1814. [Link: http://bit.ly/2X6OQN1](http://bit.ly/2X6OQN1)
13. Müller T, Schuckelt R, Jaenicke L (1994) Evidence for radical species as intermediates in Cadmium/Zinc-Metallothionein-dependant DNA damage in vitro. *Environ Health Perspect* 102: 27-29. [Link: http://bit.ly/2NzLn6p](http://bit.ly/2NzLn6p)
14. Altunkaynak BZ, Akgül N, Yahyazadeh A, Altunkaynak ME, Turkmen AP, et al. (2016) Effect of mercury vapor inhalation on rat ovary: Stereology and histopathology. *J Obstet Gynaecol Res* 42: 410-416. [Link: http://bit.ly/2ruU0qp](http://bit.ly/2ruU0qp)
15. Roychoudhury S, Massanyi P, Slivkova J, Formicki G, Lukac N, et al. (2015) Effect of mercury on porcine ovarian granulosa cells in vitro. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 50: 839-845. [Link: http://bit.ly/2CuMs9h](http://bit.ly/2CuMs9h)
16. Provencher JF, Forbes MR, Hennin HL, Love OP, Braune BM, et al. (2016) Implications of mercury and lead concentrations on breeding physiology and phenology in an Arctic bird. *Environ Pollut* 218: 1014-1022. [Link: http://bit.ly/2K8JziQ](http://bit.ly/2K8JziQ)
17. Du SNN, McCallum ES, Vaseghi-Shanjani M, Choi JA, Warriner TR, et al. (2018) Metabolic costs of exposure to wastewater effluent lead to compensatory adjustments in respiratory physiology in bluegill sunfish. *Environ Sci Technol* 52: 801-811. [Link: http://bit.ly/2Q1ZuDo](http://bit.ly/2Q1ZuDo)
18. Ibrahim MA, Khalil MT, Mobarak FM (1995) On the feeding behavior of the exotic crayfish *Procambarus clarkia* in Egypt and its prospect in the biocontrol of the local vector snails. *J Union Arab Biol* 4: 321-340. [Link: http://bit.ly/2K9W5id](http://bit.ly/2K9W5id)



19. Reitman S, Frankel S (1957) A colourimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminase. *Am J Clin Pathol* 28:56-63. [Link: http://bit.ly/2Q1ZUcW](http://bit.ly/2Q1ZUcW)
20. Kind PR, King EJ (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with aminoatipyrina. *J Clin Pathol* 7: 322-326. [Link: http://bit.ly/32wkEj](http://bit.ly/32wkEj)
21. Ynalvez R, Gutierrez J, Gonzalez-Cantu H (2016) Mini-review: toxicity of mercury as a consequence of enzyme alteration. *Biometals* 29: 781-788. [Link: http://bit.ly/2X81AD9](http://bit.ly/2X81AD9)
22. Malar S, Sahi SV, Favas PJC, Venkatachalam P (2014) Assessment of mercury heavy metal toxicity-induced physiochemical and molecular changes in *Sesbania grandiflora* L. *International journal of environmental science and technology* 12: 3273-3282. [Link: http://bit.ly/32B5aa2](http://bit.ly/32B5aa2)
23. Atchison WD, Hare MF (1994) Mechanisms of methylmercury induced neurotoxicology. *FASEB J* 8: 622-629. [Link: http://bit.ly/34NdSDv](http://bit.ly/34NdSDv)
24. Tolba MR (1999) The red swamp crayfish *Procamparus clarki* (Decapoda: Cambaridae) as bioindicator for total water quality including Cu and Cd pollution. *Egypt J Aquat Biol Fish* 3: 59-71.

Discover a bigger Impact and Visibility of your article publication with Peertechz Publications

Highlights

- ❖ Signatory publisher of ORCID
- ❖ Signatory Publisher of DORA (San Francisco Declaration on Research Assessment)
- ❖ Articles archived in worlds' renowned service providers such as Portico, CNKI, AGRIS, TDNet, Base (Bielefeld University Library), CrossRef, Scilit, J-Gate etc.
- ❖ Journals indexed in ICMJE, SHERPA/ROMEO, Google Scholar etc.
- ❖ OAI-PMH (Open Archives Initiative Protocol for Metadata Harvesting)
- ❖ Dedicated Editorial Board for every journal
- ❖ Accurate and rapid peer-review process
- ❖ Increased citations of published articles through promotions
- ❖ Reduced timeline for article publication

Submit your articles and experience a new surge in publication services
(<https://www.peertechz.com/submission>).

Peertechz journals wishes everlasting success in your every endeavours.

Copyright: © 2019 Hussein AB, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Hussein AB, Abbas AAO, Hussein N, Elkhidir N (2019) Investigation of physiological responses of *Procambarus clarkii* and some enzyme activities to selected heavy metals. *Open J Environ Biol* 4(1): 023-027. DOI: <https://dx.doi.org/10.17352/ojeb.000013>