Effect of zinc exposure periods on protein and activity of enzymes in the soft tissue of mussel (*Dreissena polymorpha)*

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Abstract

Dreissena polymorpha mussel were collected from Al-Kadesia lake - Haditha /Al-Anbar Governorate-Iraq. The animals were exposed for 2, 4, 6 and 8 days to 16 mg zinc/l of water. The soft tissue was analyzed for the effect of zinc on total protein components and activity of six enzymes (using colorimetric and/or electrophoretic methods).

Zinc was found to cause (a) Change in the intensity of some esterase patterns and increase of total protein by increasing the exposure time to zinc (b) Increase in the activity of the enzymes, alkaline phosphatase (ALP), glutamate oxaloacetate transaminase(GOT) and lactate dehydrogenase (LDH) and decrease in the activity of acid phosphatase (ACP) and glutamate pyruvate transaminase (GPT) with increase of exposure time. These changes may be useful as an earlier indicator for water pollution with zinc.

تأثير فترات التعريض إلى الزنك على البروتينات وفعالية الأنزيمات في النسيج الطري في الحيوان الرخوي *Dreissena polymorpha*

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الخلاصة

جمعت نماذج من الحيوان الرخوي *Dreissena polymorpha* من بحيرة القادسية– حديثة– محافظة الانبار. تم تعريض هذه الحيوانات إلى 16 ملغم زنك/لتر ماء للفترات 2 و4 و6 و8 أيام. استخدمت الكتلة الحية لمعرفة تأثير الزنك على المكونات البروتنية وعلى نشاط ستة أنزيمات (باستخدام الطرق الضوئية أو الترحيل الكهربائي). وجد بأن الزنك قد سبب (أ) زيادة في كمية البروتينات الكلية وتغير في طرز أنزيم الاستريز المرحل كهربائيا (ب) تنشيط في فعالية بعض الأنزيمات (ALP,GOT and LDH) وتثبيط في فعالية أنزيمات أخرى (ACP and GPT). يمكن ان تكون هذه التغيرات كمؤشر على تلوث المياه التي تعيش فيها هذه الحيوانات بالزنك.

Introduction

Mussels have been proposed as sentinel organisms in monitoring programs because of their wide geographical distribution and their high capacity of heavy metals bioaccumulation as zinc (1). Zn is essential in low concentrations to the metabolism of animals being present as constituent of numerous proteins, enzymes and cofactors in many animals and when the concentrations of this essential metal reach a threshold value their presence become first inhibitory and afterwards toxic and even lethal for the organism (2). When *Corbicula fluminea* and *D. polymorpha* exposed to zinc many biochemical changes such were observed as a synthesis of new proteins (metallothionein) (3), also zinc caused changes in activity of enzymes (ALP, ACP, LDH

and SDH) in freshwater crab *Spiralothelphusa hydrodroma* (4) and zinc effect on the activity of ALP, GOT, GPT and LDH in mice (5).

The goal of this study is to follow the biochemical changes in *D. polymorpha* after exposure in the laboratory to zinc and capacity for using these changes as indicator to water contamination with zinc.

Materials and methods

Zebra mussels were collected in November 2008 from Haditha city/Al-Anbar - Iraq . The animals were transferred to the laboratory where they were distributed in a plastic container containing 10 liters of dechlorinated tap water. Seventy five individuals were kept in each container. The water of each container was replaced every days. No food was provided.

Zinc was added in the form $ZnSO_4$, one week after bringing the animals to the laboratory. Animals were exposed to 16mg zinc/l for 2, 4, 6 and 8 days. Control animals were kept under the same conditions and time period without addition of metal.

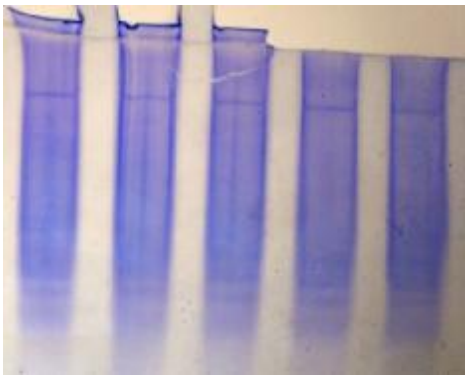
The flesh of the control and exposed animals to the zinc were removed from the shell and weighted. The flesh was homogenized with 5 volumes of Tris -HCl buffer pH 7.2 in a homogenizer to study enzymes activity and protein content, and homogenized with 2 volumes for electrophoretic study. Pools at least of 10 mussels were used for each homogenization. the homogenates were centrifuged in eppendorf centrifuge for 5 min. at speed of 15000 RPM.

Electrophoresis was carried out in Hsi slab gel electrophoresis unit connected to Consort E 815 power supply. The slab of polyacrylamide gels for protein and esterase (7.5%) were prepared in the laboratory (6). Assay of protein content, six enzymes (ALP, GOT, GPT, ACP and LDH) were conducted (7, 8, 9,10).

Statistical analysis: Results were expressed as mean ± standard error of mean (SEM).The data were subjected to one way analysis of variance (ANOVA), by using the Statistical Analysis System Genstat Discovery program (No.3). P values at 5% and were regarded as significant.

Results

The results of the experiment on the effects of zinc on the electrophoretic patterns of proteins (on polyacrylamide slab gel) didn't reveal any changes in the number or intensity of bands (Fig.1). Zymogram of esterase on polyacrylamide showed changes under the effect of zinc, the intensity of esterase stain increased by increasing zinc concentration (Fig. 2).



Control 2 days 4 days 6 days 8 days

Fig. (1) zinc effect on protein patterns with different exposure periods

Control 2 days 4 days 6 day 8 days

Fig. (2) zinc effect on estrase patterns with different exposure periods

Total protein increased significantly by increasing exposure period to zinc (Fig.3). Mean of control was 0.816mg/dl, in second days was 0.851 mg/dl, fourth days was 0.918 mg/dl, sixth days was 0.908 mg/dl and in eight days was 0.927mg/dl.

The activity of ALP changed significantly. Enzyme activity mean of control animals was 6.083K.A.U/dl, exposed animals for 2 days was 6.716 K.A.U/dl, for 4 days was 6.883K.A.U/dl, 6 days was 6.133K.A.U/dl and exposed for 8 days was 5.767K.A.U/dl .(Fig.4). The ACP activity decreased significantly when the animals exposed to zinc in different periods. Control animals mean was 5.398U/l, the mean in exposed animals to 2 days was 5.255U/l, 4 days was 4.921U/l, 6 days was 4.686U/l and 8 days was 3.928U/l (Fig.5). The LDH activity differed significantly. Control animals mean was 26.52U/l, mean of exposed animals to 2 days was 30.57U/l, 4 days was 28.32U/l, 6 days was 26.53U/l and 8 days was 26.08U/l (Fig.6).

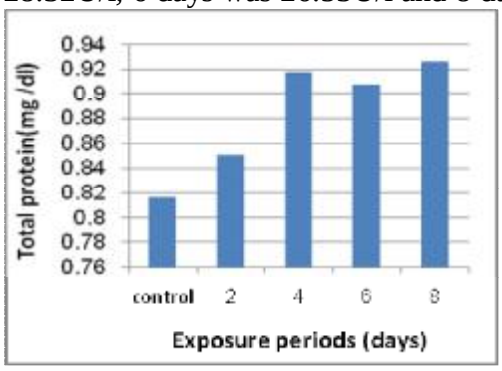


Fig. (3) zinc effect on total protein with different exposure periods

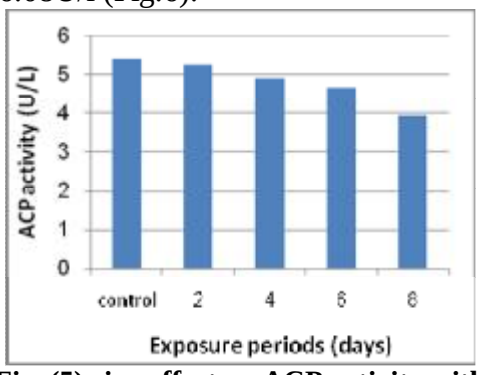


Fig. (5) zinc effect on ACP activity with different exposure periods

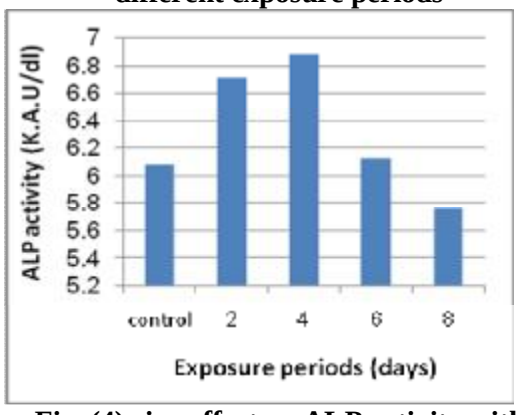


Fig. (4) zinc effect on ALP activity with different exposure periods

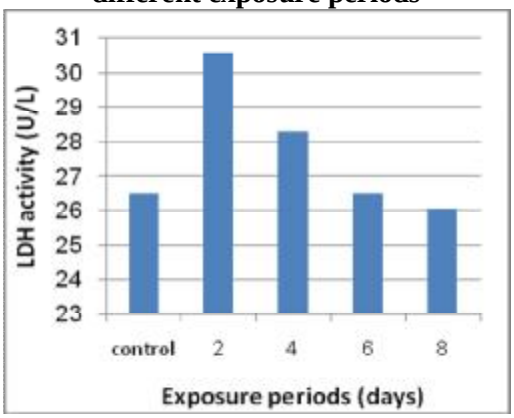


Fig. (6) zinc effect on LDH activity with different exposure periods

Significant increase GOT activity in the exposed animals in comparison with control animals. Mean of control animal was 59.5U/ml, in exposed animals to zinc for 2 days was 68.67U/ml, 4 days was 72.5U/ml, 6 days was 79U/ml and 8 days was 82.67U/ml (Fig.7).

Significant reduction of GPT activity in the exposed animals. Mean of control animal was 87.66U/ml, in exposed animals to zinc for 2 days was 73.166U/ml, 4 days was 65.166U/ml, 6 days was 63.166U/ml and 8 days was 56.166U/ml (Fig.8).

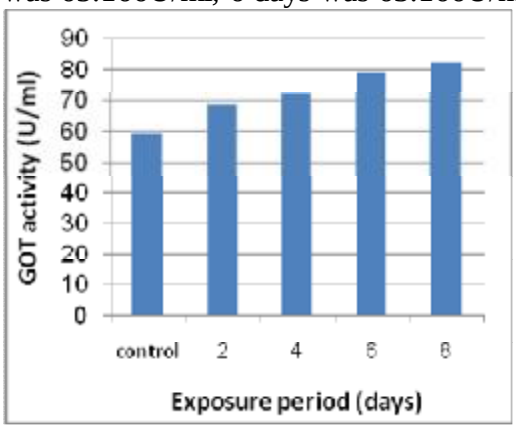


Fig. (7) zinc effect on GOT activity with different exposure periods

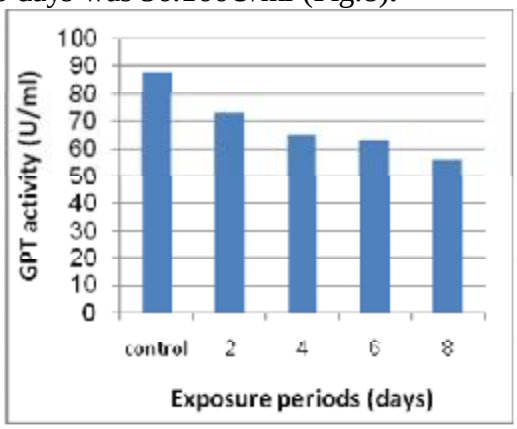


Fig. (8) zinc effect on GPT activity with different exposure periods

Discussion

The accumulation of heavy metals is a characteristic feature of aquatic invertebrates(1,2,3,11,12), so it is expected that mussel *Dreissena polymorpha* accumulated an effective quantities of the zinc. Although the heavy metals known to induce changes in electrophoretic profile of protein (metallothionein or metal binding protein) on polyacrylamid gel (12), zinc didn't cause such changes in *Dreissena polymorpha* after 8 days of exposure to 16mg/l in this study. A similar result was found in the *Dreissena polymorpha* taken from southwest France, and this was explained because the insufficient accumulated zinc to trigger gene metallothionein expression (3). Total protein increased in tissues of *D. polymorpha* with increasing the exposure time to zinc , and this may be to face the stress caused by zinc. Similar results were found in the gills of clam *Pseudontopsis euphraticus* exposed to 0.4 mgHg/l for 21 days(13). On the other hand a decrease in the total proteins was found in the kidney of clam *Pseudontopsis euphraticus* exposed to 0.4 mgHg/l for 21 days (14).

The electrophoretic profile of esterase in *D. polymorpha* (this study) revealed an increase in the intensity of bands with an increase of the exposure time to the zinc.

A similar electrophoretic pattern of esterase in shrimp *Callianassa tyrrhena* was observed after the exposure to cadmium and mercury (6,15), it is suggested that the above biochemical responses are not specific for zinc but possibly represent a common response to heavy metals.

Alkaline phosphatase is a metalloenzyme with an active zinc containing center and may be replaced by other metals in polluted environment (16) .This enzyme is a brush border enzymes and it was reported that this enzyme split various phosphorus esters and mediated transport (17), involves in active transport (18), and glycogen metabolism(19).Thus any alteration in the activity of alkaline phosphatase affect the organism activity (4). The initial activation of the enzyme (present study) followed by a reduction was identical to what have been observed in shrimp *Callianassa tyrrhena* exposed to 0.1 to 0.4 µg Hg/l for 6 days (6). Zinc caused a reduction in the activity of enzyme in different organs of fish *Mugil cephalus* (20) and *Lepomis macrochius* (21).

It has been reported that generally the increased activity of acid phosphatase attributed to the activation of the enzyme, which is kept (ACP) in latent state inside the membrane of lysosomes (22). It was found that the manganese increased the acid phosphatase activity in the cerebellum of rabbit (23). In this study the activity of acid phosphatase decreased in the presence of zinc in the ambient environment, and this may inferred as a response to altered metabolism due to zinc stress (4). This is consistent with that reported in fish *Channa punctatus* (24) and *Fundulus heteroclitus* (25). On the other hand an increase in the activity of acid phosphatase has been shown in the field crab *Spiralothelphusa hydrodroma* exposed to 81 mg/l for 30 days (4), and in *cavia porcellus* exposed to pesticide chlorpyrifos (26).

LDH activity increased in fish *chana punctatus* exposed to pesticides ,quinalphos, dichlorvos and suquin (27), and in the the freshwater fish *cyprinus carpio* exposed to the pestiside cypermethrin after 8 and 12 days of treatment (28). In the present study the LDH activity of the freshwater mussel *Dreissena polymorpha* increased with the increasing of the exposure time to 16mg zinc/l . A similar result was observed in the crab *Spiralothelphusa hydrodroma* exposed to zinc, which might have depending on anaerobic carbohydrates metabolism, cumulative effect or possibly to meet the increased energy demands under toxic effect of zinc (4).

GOT activity increased while GPT activity decreased with increase zinc duration of exposure in the mussel *Dreissena polymorpha* (present study). These changes are probably due to a function response to zinc as in the fish *Mugil cephalus* (20) or in freshwater snail *Melanopsis nodusa* (29). Transaminases in mammalian are indicators of liver, heart, and other organs function and they are also reported to be indicator of degradation(30).

In conclusion, zinc was found to cause changes of protein components in the mussel *Dreissena polymorpha*. Therefore this mussel model is suitable to be use as an indicator for water contamination with zinc**.**

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