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MOLECULAR BIOMARKERS IN THE CLAM (*CORBICULA FLUMINEA*) AND SNAIL (*VIVIPARUS BENGALENSIS)* INDUCED BY ACUTE EXPOSURE TO Zn AND Pb

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ABSTRACT
Two Molluscs species (*Viviparus bengalensis* and *Corbicula fluminea*) induced by acute exposure of Zn and Pb for different concentrations (20, 50, 100 µg/l) to monitor Molecular biomarkers response (DNA damage) , this monitoring adapted to evaluate the heavy metals stress in these species to get the early signal warning, DNA fragmentation test showed the highest DNA lysis level (9200) have recorded for snail after Zn exposure (100µg/l) at 96hr , as supporting for these results , comet assay index revealed the highest comet index after after Zn exposure for both Snail & Clam and according to according to Tail length , DNA tail moment and Classes of damage .

KEY WORDS: DNA damage, Molluscs, Heavy metals, acute exposure, Zn, Pb.

INTRODUCTION

Although some heavy metals are necessary for human health at low concentration, they may become harmful at higher concentration and causes wide spectrum of toxicological effect on aquatic organisms, Heavy metals can effect on structural and functional properties in living organisms from the cellular and molecular level to higher biological levels, such as populations or communities (Marie *et al*., 2006). Biomarkers as predictive of advanced toxicity at higher biological levels and using comes from their sensitivity and specificity to pollutants and also for ideal reasons such as the cost and time associated with measuring a stress response (Conners, 2004). A toxic effect of Heavy metals on aquatic organisms can be measured in terms of Molecular responses of the organisms, so molecular biomarkers can serve as early warning indicators of contaminants exposure effect (Valavanidis *et al.*, 2006). Injuries to DNA from environmental stress introduce deviations from its normal, intact structure which may, if left unrepaired, result in a mutation or a block of DNA replication. The aim of the Molecular biomarker is to improve a sequence of sensitive molecular assay that will lead to the rapid detection and make progress for the properties of the pollutants to which the organism is reaction. Molecular Biomarkers such as DNA strand breakage accomplished by many researchers like (Black *et al*., 1996), who studied the sensitivity of a freshwater mussel, *Anodonta grandis*, to DNA damage following lead (Pb) exposure, the results showed no evidence of strand breakage was observed in any of the analyzed tissues from the mussels exposed to higher Pb concentrations (500 and 5000 mg/L), They suggested a threshold effect for DNA damage and repair resulting from low-level Pb exposure. Comet assay (Single cell gel electrophoresis) has been used as reliable tool to detect important biomarker (DNA damage) which used by (Frenzilli *et al*., 2001) who was depended on Comet assay index with using the total oxyradical scavenging capacity to detect DNA integrity in Mussel (*Mytilus galloprovincialis*) and they illustrate the seasonal variability of DNA damage according to environmental stress in highly eutrophicated orbetello lagoon in Italy.

MATERIALS &METHODS

Heavy metals Exposure protocol

Molluscs are Corbicula fluminea and Viviparus bengalensis were brought live to laboratory quickly and left in dechlorinated water aquarium for 2 days as acclimation and depuration stage with fixation all environmental factors. After that a group of 6 to 10 of each Mollusca species was exposed to 24, 48, 72, 96 hr. as Acute exposure in plastic container to different concentration of Pb (20,50,100)µg/L which prepared from Stock solution 1g/l (CH3COO)2pb.3H2O) and Zn (20 , 50, 100) µg/L which prepared from Stock solution 1g/l (Znso4.7H2o) , with photoperiod 12:12 light & dark cycle ,these concentrations verified after preparation by Atomic absorption Spectrophotometer type 6300(Shimadzu, Japan), All samples collected after each exposure to determine all previous Biomarkers as soon as possible to detect the early warning signal (different biomarkers) in Molluscs.

DNA Extraction

DNA extraction Kit (CAT# A1120) used to identify DNA fragment and we followed the protocol clarified by Promega Corporation, Madison, WI, U.S.A, and after extraction, DNA samples visualized by Electrophoresis (UV transilluminator) type (CS-Cleaver scientific Ltd., U.K)

Comet Assay

This assay has been done according to (Sing *et al*., 1988; steinert, 1996) with some modification clarified by

(Conners, 2004). It involves the encapsulation of cells in a low-melting-point agarose (LMA) and normal melting point agarose (NMA) suspension, lysis of the cells in neutral or alkaline (pH>13) conditions, and electrophoresis of the suspended lysedcells. Pictures have viewed by Fluorescence microscope type Optika B-300. DNA damage was expressed as a tail extent moment value (product of the tail length by the tail DNA content)

Statistical Analysis

SPSS 17.0 programs used for least significance differences (LSD ≤ 0.05), Analysis of variance test (ANOVA).

RESULTS

Lane 2 showed control sample of Snail Sp. with no lysis level and 10000 bp, lane 3 with lysis level (9200) and 10000-800bp for Snail Sp. which exposed to Zn 100 µg/l at 96 hr., lane 4showed damage with lysis level 7700 and 8500-800bp which for pb 100 µg/l at 96 hr, lane 5 represented Interaction exposure (Zn+pb) 100 µg/l at 96 hr of Snail Sp. which have 8000-10000bp and lysis level(7000) in comparison to DNA ladder (lane1) (Figure 1, Table 1).

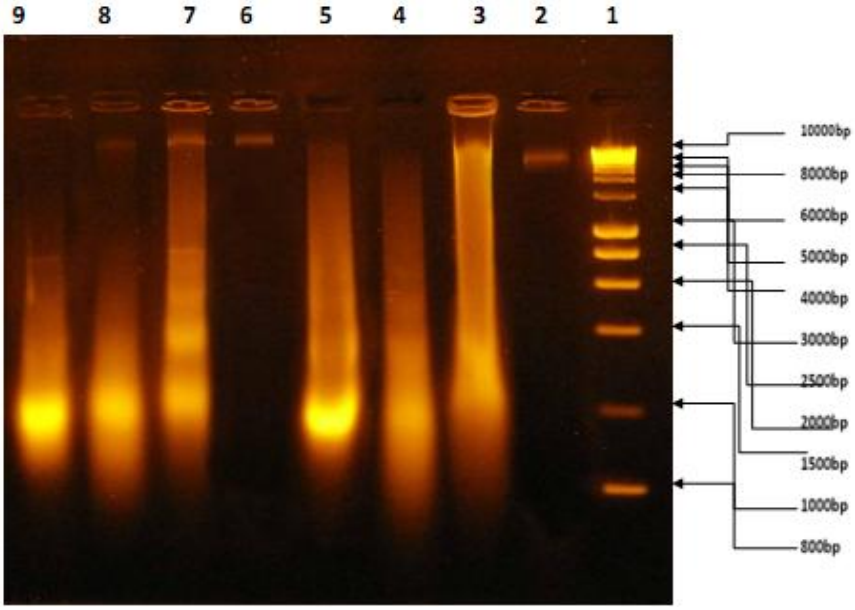

FIGURE 1: DNA damage in snail & clam induced by acute heavy metal exposure

TABLE 1: The Quantitative variations of DNA damage in snail & clam induced by acute heavy metal exposure

Lane number	Treatment	M.V(bp) Approx.	DNA lyses level
1	DNA Ladder	10000 bp	-
2	Control Snail Sp.(non-treated)	10000-10000 bp	0
3	Zn 100 (µg/l) 96 hr. (Snail Sp.)	10000-800bp	9200
4	Pb 100(µg/l) 96hr. (Snail Sp.)	8500-800 bp	7700
5	Interaction (Zn+pb) 100 (µg/l) 96 hr. (Snail Sp.)	8000-1000bp	7000
6	Control Clam Sp.(non-treated)	10000-10000 bp	0
7	Zn 100 (µg/l) 96 hr. (Clam Sp.)	10000-900 bp	9100
8	Pb 100(µg/l) 96hr. (Clam Sp.)	8000-800 bp	7200
9	Interaction (Zn+pb) 100 (µg/l) 96 hr. (Clam Sp.)	6000-950 bp	5050

While for Clam Sp. and after the same acute exposure for Zn and Pb, lane 6 showed no lysis of DNA for Control sample, lane 7 showed highly DNA damge after Zn 100 µg/l at 96 hr. with lysis level 9100 and 10000-900bp , lane 8 was detected with lysis level 7200 and 8000-800bp which for pb 100 µg/l at 96 hr while for Interaction exposure, lane 9 have lysis level 5050 and 6000-950bp(Figure 1, Table 1) After acute exposure of metals in snail, the highest tail length was recorded in 100 µg/l at 96hr.of both Zn, pb, and Interaction but the highest one for Zn 100 µg/l in comparison to control (Figure 2), and the same trend adapted from Clam with highest tail length in Zn 100 µg/l at 96 hr. than pb 100 µg/l and interaction 100 µg/l(Figure 3) After acute exposure in snail Sp., the highest tail moment (15.8%) for Zn 100 µg/l at 96hr., and the lowest value (12.6 %) in Interaction 20 µg/l at 96hr.(Figure 4) while in clam Sp., the highest value of DNA tail moment (14.8%) for Zn 100 µg/l at 96hr., and the lowest value (11.6 µg/l) for Interaction 20 µg/l at 96hr(Figure 5).

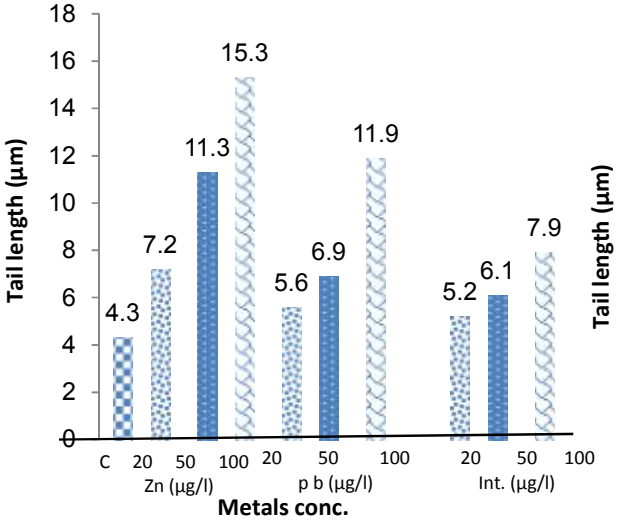

FIGURE 2: Tail length (µm) in snail sp. after 96hr. acute exposure of Zn, Pb, and Interaction (Zn+pb) for different Concentration.

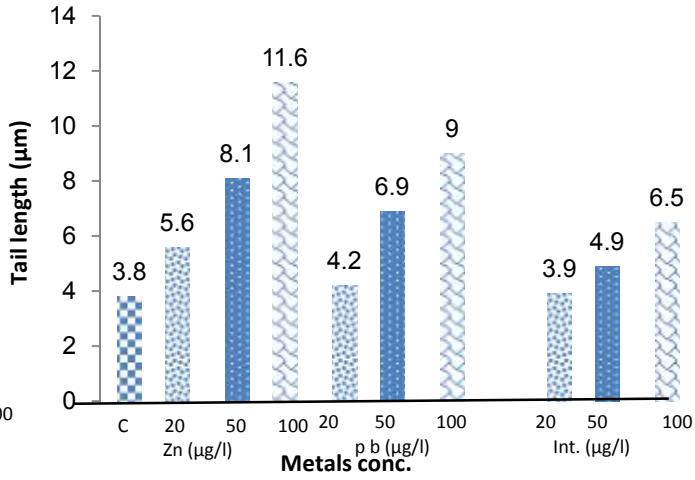

FIGURE 3: Tail length (µm) in Clam sp. after 96hr. acute exposure of Zn, Pb, and Interaction (Zn+pb) for different Concentration.

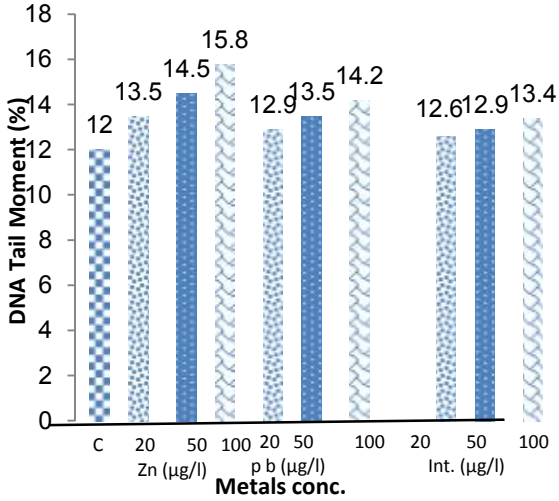

FIGURE 4: percentage of DNA tail Moment in snail sp. after 96hr. acute exposure of Zn, Pb, and Interaction (Zn+pb) for different Concentration.

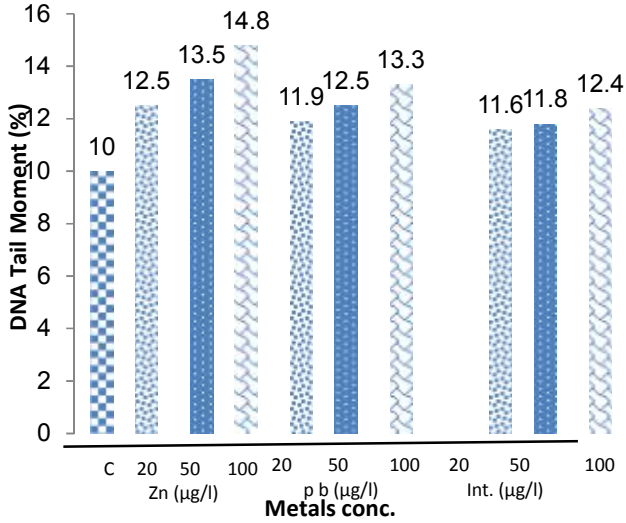

FIGURE 5: percentage of DNA tail Moment in Clam sp. after 96hr. acute exposure of Zn, Pb, and Interaction (Zn+pb) for different Concentration**.**

DISCUSSION

The relationships between different biomarkers as multiple biomarkers and as rapid indicators of environmental quality have been shown by many studies as (Wedderburn *et al*., 2000 & Rie *et al*., 2000). Heavy metals can produce chemical or physical modification to DNA which measured by means of DNA fragmentation as revealed clearly in this study, and Double strand breakage , the efficiency of uptake/bioaccumulation/removal and the metabolic competence for Phase I/II reactions play important roles in DNA adducts formation and DNA damage (Dolcetti *et al*., 2002). The redox activity of some metal ions like Cu (II) and Fe (III) catalyses the oxidation of GSH resulting in thyil and hydroxyl radicals increase the DNA damage in studied Molluscs species concerned with action of highly reactive heavy metals metabolites, feed behavior and habitat, and the continuous increasing of heavy metals lead to generation of ROS which causes a lot of Damage and Induction of DNA damage (An *et al*., 2012). DNA damage produced via alkali labile sites as result of damage in repair mechanism or by defect in cell redox status leads to destruction of hydrogen bonds between two strands of the double helix crosslinks (Mitchelmore & chipman, 1998), Heavy metals can hinder multiple physiological and specific biochemical processes such as enzyme and or membrane specific reactions, and lipid peroxyl radicals can damage the cells by changing the fluidity and permeability of the membrane or destruction directly DNA and other intracellular molecules(Rajkumar *et al*., 2011) and the DNA damage may be related with organic xenobiotic transformation(Maria *et al*., 2009). The positive relationship between DNA damage and heavy metals pollution in this approved by (Jebali *et al*., 2007) In addition , Oxidative stress which result from heavy metals stress after acute exposure produced by reactive oxygen

species, or other non-oxygen containing free radicals , can cause DNA fragmentation (Zapata *et al*., 2012).

CONCLUSION

We concluded that molecular biomarker represented by DNA damage is highly sensitive to heavy metals stress and can adapt as a suitable biomarker to evaluate pollution by heavy metals.

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