Metallothionein induction in edible mangrove periwinkles, *Tympanotonus fuscatus var radula* and *Pachymelania aurita* exposed to Oily Drill Cuttings

Anagboso Marykate Ukamaka [1], Chukwu Lucian Obinnaya [1], Otitoloju, Adebayo [2] and Igwo-Ezikpe Miriam[3]

[1] *Aquatic Toxicology and Ecophysiology Laboratory, Dept of Marine Sciences, University of Lagos, Nigeria*
[2] *Dept of Zoology, University of Lagos, Nigeria*
[3] *Dept of Biochemistry, University of Lagos, Nigeria*
obinnalcunilag@yahoo.com

Abstract: Metallothionein levels were monitored over 32 days in two gastropod species *Tympanotonus fuscatus* and *Pachymelania aurita* exposed to oil coated drill cuttings. In *T. fuscatus*, metallothionein levels were enhanced in all treatment groups during the study, with highest level concentrations being observed in organisms exposed 0.01 96h LC_{50} drill cutting concentration. Metallothionein levels in *Pachymelania aurita* on the other hand was reduced in test animals exposed to the drill cuttings in comparison to their background level. The implications of the finding and possible inclusion of metallothionein in biomonitoring programmes involving the evaluation of impact of drill cuttings disposal on aquatic ecosystems are discussed. [Journal of American Science 2010;6(2):89-97]. (ISSN: 1545-1003).

Key words: Metallothionein, induction, mangrove periwinkles, Drill Cuttings

1. Introduction

Several tools are available for measuring impacts from environmental stressors at different levels of biological organisation (Depledge and Hopkin, 1995). Conventionally pollution has been monitored by measurement of levels contaminants in environmental receptors including measurement of bioaccumulation of toxicant in the body tissues of organisms. Currently, emphasis has shifted to measurement of biological responses to complement traditional methods in biomonitoring programmes (Damiens *et al*, 2004). Biological responses otherwise known as biomarkers are sensitive indicators but need to be prognostic to be relevant (Galloway *et al.,* 2002). There proper application according to Handy *et al.,* (2003) is in building a weight of evidence case that pollution is affecting the health of organisms and that certain classes of chemical are responsible. Some commonly measured biomarkers include metallothionein, a common indicator of heavy metal exposure; lysosomal stability, a general indicator of stress; EROD activity, which commonly indicates oxidative stress and acetylcholinesterase inhibition, a marker of organophosphorus and carbamate pesticide exposure.

Biomarker responses are affected by environmental factors (English and Storey, 2003). Times taken to induce the biomarker as well as the persistence of the biomarker are issues to consider in incorporating biomarkers in biomonitoring studies (Howard *et al.,* 2002). Biomarker based approaches has gained immense support and research in developed world (Brown *et al.,* 2004; Ringwood *et al.,* 2004; Handy *et al.,* 2003; Galloway *et al.,* 2002; Viarengo *et al.,* 1999; Moore *et al.,* 1999) but their use in developing world especially in Nigeria is non-existent possible due to lack of local literature and expertise.

Metallothioneins are cysteine rich, low molecular weight proteins of about 7 kDa and apparent molecular weight of 13 kDa. MT pool is made up of different isoforms with certain amino acids substitutions but for the highly conserved cysteine residues (George and Olsson, 1994). The isoforms have different physiological roles and different ways of induction, depending on the cell types and tissues. Metallothioneins are believed to be important in homeostasis of heavy metals such as copper and zinc, and detoxification of heavy metal (Butler and Roesijadi, 2001), thus they are commonly associated with heavy metal pollution. Their induction confers metal tolerance to organisms (Klaasseen *et al.,* 1999) due to their ability to bind some heavy metals such as cadmium and copper (Coucelo *et al*, 2000). This concentration of heavy metals has raised questions on possible trophic transfer especially in edible species (Wang, 2002). In addition accumulation of heavy metals may disturb essential metal homeostasis by causing changes in cellular metal binding and speciation patterns. Because they are additionally synthesis following heavy metals exposure, metallothioneins have been proposed as a potential biomarker for heavy metal exposure (De Smet *et al.,* 2001; Roesijadi, 1994). Evidence of protection against oxidative stress by metallothionein has been

demonstrated in sheep epithelial cell (Pitt *et al.,* 1997) as well as in invertebrates (Viarengo *et al.,* 1999). Metallothionein has also been shown to protect against non metallic chemicals such as carbon tetrachloride (CCl_4). For instance, Klaassen and Liu (1998) reported that MT induction by Zn in the hepatic cells of mice were able to bide ^{14}C from ^{14}C-CCl_4 in the MT-induced animals than in controls, with a concomitant reduction of covalent binding of ^{14}C-CCl_4 to cellular protein and lipid. Furthermore, induction of metallothionein is affected by various biotic and abiotic factors (English and Storey, 2003; Leung *et al*., 2001; Ghoshal *et a.,* 1998). It has been suggested that inclusion of measurement of MT in biomonitoring will of necessity include the quantification of the different isoforms and their induction patterns (Lacorn *et al.,* 2001), major site of induction and the determination of the half-life/persistent (Leung and Furness, 2001; Howard *et al.,* 2002).

The objective of this study was to study the metallothionein responses of two common gastropod species of Lagos lagoon on exposure to drill cuttings. Drill cuttings are produced during exploitation of crude oil, and in Nigeria their discharges are allowed after treatment in offshore water according to guidelines of the Department of Petroleum Resources (DPR, 2002). Accidental discharges however in inland waters and swamps areas are not ruled out and may affect the natural biota in the influence of exploitation areas. This study is therefore a step in studying a common biomarker response of local organisms exposed to sublethal concentration of drill cuttings.

Tympanotonus fuscatus and *Pachymelania aurita* were chosen for the study because of their abundance in the Niger Delta region and around the mangrove swamps and sandy shores of the Lagos lagoon complex. Two subspecies of *T. fuscatus* are found in West Africa, *T. fuscatus fuscatus* (Linne) with tubercular spines and *T. fuscatus* (var radula) with smooth or granular shell ornamentations. Both types are found at the inter-tidal zone usually one variety predominates in a particular creek or shore. However there is increasing evidence to suggest that both varieties could be ecotypes of the same species (Oronsaye, 2002). *P. aurita* is regarded as a dominant member of the faunal community of the Lagos lagoon with a mean annual production rate that varied from -1.59 g and 0.79 g/0.5 m^2/yr and a production: biomass ratio that varies between 0.02 and 0.12 (Brown, 1991). Both gastropod species are edible and very rich in protein (Egonmwan, 1980, Adebayo-tayo *et al.,* 2005), and thus serve as important source of protein to local inhabitants, but their high microorganisms content is a health issue which raises concern over their consumption (Adebayo-tayo *et al.,* 2005).

2. Materials and Methods

Animals

The animal species used in the study were adult stages of the following:

1. *Tympanotonus fuscatus* var *radula L* (periwinkle) (Mollusca, Gastropoda, Megagastropoda, Melanidae), (length 32-35 mm , diameter of aperture 0.7 – 9.0 mm)
2. *Pachymelania aurita* (periwinkle) (Mollusca, Gastropoda, Megagastropoda, Potamidae), (length 35 – 40 mm, diameter of aperture 0.8 – 1.0 mm).

P. aurita and *T. fuscatus* species were collected from field by hand picking into a holding bucket from the edge of the Lagos Lagoon and adjacent mangrove swamps at low tide. They were taken to the laboratory and left in holding tanks with a thin layer of sediment serving as substrate and food source to organism, and some water to allow them to acclimatise to laboratory conditions. Lagoon water and sediments were also simultaneously collected for use in the bioassay procedures.

Test Chemicals

The toxicant used for the bioassays is drill cuttings which were collected from Shell Development Petroleum Corporation. The cuttings were coated with the oil based mud used during the drilling process.

General Bioassay procedure

Biomarker studies were carried out using plastic tanks, which measured 30 cm x 25 cm x 16 cm. The collected sediments were sun dried to standardise moisture content, and thereafter passed through a sieve (0.25mm) to obtain uniform substrate particles. 100 g of sediment was introduced into the experimental chamber and lagoon water was used as the diluents for the entire bioassay test conducted.. Sediments were first spread to form a uniform layer in the bioassay container. Thereafter, pre-determined concentrations of drill cuttings were added followed by the addition of 1 liter of water less the volume of the drill cuttings. The contents of the container were then gently mixed to achieve even distribution of cuttings on the sediment before the introduction of test species. This procedure was adopted in all cases of bioassay.

Sublethal Toxicity Test

Static renewal method was adopted during exposure period. Test solutions and sediment were renewed on day 4, 8, 16 and 32. For the series of bioassays, one hundred and twenty (120) specimens of each test species were exposed to drill cuttings including control in two replicates (20 animals per treatment per replicate). The concentrations of drill cuttings used were derived from the $96hLC_{50}$ value

obtained for the toxicant in a previous study with the organisms, and are as follows:

Drill cuttings was tested against *T. fuscatus* as follows:

- 10.24ml (i.e. 0.01 of the 96hLC_{50}) plus 989.76 ml lagoon water,
- 1.024ml (i.e. 0.1 of the 96hLC_{50}) plus 998.976 ml lagoon water, and
- Untreated control.

The test concentrations for *P. aurita* were as follows:

- 12.2ml/l (i.e. 0.01 of the 96hLC_{50}) plus 987.78 ml lagoon water,
- 1.22 ml/l (i.e. 0.1 of the 96hLC_{50}) plus 998.78 ml lagoon water, and
- Untreated control.

Tissue preparation

For each treatment, specimens of each species were randomly collected from bioassay chambers on day 4, 8, 16 and 32 for analysis. Samples from different individuals of the same species were pooled to minimise individual variability. The digestive glands of the organisms were chosen for metallothionein studies. In removing the digestive gland of the periwinkles, the anterior shell was gently broken and the digestive gland removed.

Metallothionein Determination

Metallothionein levels were assessed on the digestive gland homogenates content of the samples by evaluating the sulphyhydryl (-SH) residue according to Ellman (1959); Viarengo *et al* (1997). The amount of MT was defined assuming a cysteine content of 23. The MT concentrations of 3 replicates for each measurement were calculated using reduced glutathione (GSH) as a standard and expressed as (n mol MT).g^{-1}. Chemicals were obtained from Ikzus Environment, Italy.

Reagent and working solutions preparation:

Sulfhydryl reference (reduced glutathione) standard was prepared by adding 0.725 Sol C_2 (resuspension buffer Component 2) to a microtube. The reaction buffer (sol D) was obtained by mixing 200ml stabilised Ellman's buffer and 3.4ml concentrated Ellman's reagent and equilibrated at 23^{o}C. Absolute ethanol was also equilibrated at –20^{o}c. The homogeniting buffer was obtained by adding 1 part of sol B (protease inhibitor) to 99 parts of sol A extraction buffer. The resuspension buffer was prepared by mixing equal amounts of sol C_1 (resuspension buffer, Component 1) and sol C_2 (resuspension buffer, Component 2). The blank solution was obtained by mixing 50µl Sol C (resuspension buffer, Component 1) and 1.950 µl Sol D (reaction buffer).

Procedure for the extraction and evaluation of metallothionein content

0.5 g of tissue was homogenised at 0-4^{o}C using the already prepared homogenating buffer and centrifuged at 30,000g at 4^{o}c for 20min. The protein content was then determined from the supernatant by means of Bradford assay. The supernatant was collected in a 2 ml tube. 1.5 ml of cold absolute ethanol was added to the tube and incubated at –20^{o}c for 30-60min. the mixture was then centrifuge at 12-16,000g at 4^{o}c for 5 minutes. 1.95 ml of the previously prepared reaction buffer, which was equilibrated at room temperature (RT) was then added. The resulting mixture was mixed, incubated for 2 minutes at room temperature and then centrifuge at 12-16,000g. Absorbance was thereafter read at 412nm against the blank solution, and a standard curve was obtained by plotting absorbance against the concentration. Absorbance was also read for samples at 412. To calculate metallothionein concentration, the $ABS^{MT}{}_{412}$ value of sample was interpolated over the standard curve to obtain the concentration (nmol) of sulfhydryl groups, i.e. cysteine residues (nmol Cys^{MT}), due to metallothionein present in the sample. To obtain the concentration of metallothionein (nmol MT) per gram of tissue, the following was applied:

$$(\text{nmol MT})g^{-1} = \frac{(\text{nmol } Cys^{MT})}{0.1\text{g. } n^{\circ cys}}$$

where: 0.1g is the amount of tissue equivalent to 0.3ml of supernatant subjected to precipitation.

$n^{\circ cys}$ is the number of cysteine residue present in the investigated metallothionein.

To express metallothionein concentration per mg of the total protein present in the supernatant, the following was applied:

$$(\text{nmolMT}).mg^{-1} = \frac{(\text{nmol } Cys^{MT})}{mg.n^{\circ cys}}$$

where: mg is the amount of protein present in the 0.3ml supernatant subjected to ethanol precipitation.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the means of results obtained, and where a significant difference ($P < 0.05$) was obtained, Duncan test was used to detect the source of difference. Unpaired Sample t-test was also used to test for significant difference between means of biomarker results obtained between *T. fuscatus* and *P. aurita*. All statistics were first determined at $P < 0.05$ significant level, and where high significant difference was observed, a significant level of $P < 0.01$ or $P < 0.001$ is applied.

3. Results

For *T. fuscatus* MT values ranged from 0.7568 nmol g^{-1} to 1.5510 nmol g^{-1} (mean = 0.9742 nmol g^{-1})

in control organisms, 0.7677 nmol g^{-1} to 4.3571 nmol g^{-1} (mean = 2.3361 nmol g^{-1}) in organisms exposed to 1.024 ml/l, and 0.7677 nmol g^{-1} to 2.0959 nmol g^{-1} (mean = 1.3417 nmol g^{-1}) in 10.24 ml/l exposed organisms **(Table 1).** Metallothionein level was enhanced in all treatment groups including control during the experiment. The concentrations were significant for all treatment groups (ANOVA, $P < 0.05$). Post hoc test with Duncan indicated that for all treatment groups there was significant difference ($P < 0.01$) in MT concentration on all sampling days. MT was however greater induced in organisms exposed to drill cuttings than in control organisms, with higher values in *T. fuscatus* exposed to 0.01 96h LC_{50} than those exposed to 0.1 96h LC_{50} drill cuttings concentration. Concurrently with the observed enhanced production of MT was increase in total protein in the organism during the study (**Table 1**). The observed increase in protein production was higher in control and in 0.01 96h LC_{50} drill cutting concentration exposed *T. fuscatus* than in *T. fuscatus* exposed to 0.1 96h LC_{50} drill cutting concentration, but a higher percentage of the new protein was converted to MT in 0.01 96h LC_{50} drill cutting concentration exposed *T. fuscatus,* but not in control and in 0.1 96h LC_{50} drill cutting exposed *T. fuscatus* (**Figure 1).**

In *P. aurita,* the observed background MT level was higher than that of *T. fuscatus.* The values of MT recorded during the experiment ranged from 3.4430 $nmol^{MT}$ g^{-1} to 3.8865 $nmol^{MT}$ g^{-1} in control organisms (mean = 3.4998 $nmol^{MT}g^{-1}$), 0.1902 $nmol^{MT}g^{-1}$ to 3.8807^{MT} nmol g^{-1} (mean = 2.2543 $nmol^{MT}g^{-1}$) in organisms exposed to 1.22 ml/l, and 0.3301 $nmol^{MT}g^{-1}$ (mean = 1.4554 $nmol^{MT}g^{-1}$) to 3.3865^{MT} nmol g^{-1} in 12.22 ml/l exposed organisms **(Table 2)**. In the control, the concentration of MT was comparatively unchanged with time, and the values were always higher than those of exposed groups on all sampling days but for day 32 when the level of the group exposed 1.22 ml/l was essentially equal to the control value. Both drill cuttings exposed groups exhibited similar trend of MT fluctuation. In both groups, MT was severely depressed on day 4, and thereafter started to rise.

Induction of MT was higher in organisms exposed to 1.22 ml/l compared to organisms exposed to 12.22 ml/l, such that by the end to the end of the exposure period, MT concentration in organisms exposed to 1.22 ml/l was higher than the background value. Induction of MT in the 12.22 ml/l exposed organisms continued to be depressed as the concentration remained lower than the background value **(Table 2, Figure 2)**. Correlation between MT concentration in the digestive gland of *P. aurita* and exposure time was positive for control (b = 0.965) and for *P. aurita* exposed to 0.01 96hLC_{50} drill cuttings (b = 0.523) and weakly negative for *P. aurita* exposed to 0.1 96hLC_{50} drill cuttings concentration (b = -0.096).

Table 1: Protein and metallothionein concentrations in *T. fuscatus* on sublethal exposure to drill cuttings (mean ± SD)

Incubation Time (Day)	n^o Cys	Wt of tissue (g)	Protein (mg/l)	nmol Cys^{MT}	nmol MT g^{-1}
0	23	0.1	5.483 ± 0.014^{d}	1.766 ± 0.00^{d}	0.7677 ± 0.00^{c}
4	23	0.1	4.958 ± 0.014^{e}	1.741 ± 0.00^{d}	0.7568 ± 0.00^{d}
8	23	0.1	9.299 ± 0.000^{c}	1.567 ± 0.00^{c}	0.6812 ± 0.00^{e}
16	23	0.1	21.790 ± 0.014^{b}	2.564 ± 0.00^{b}	1.1146 ± 0.00^{b}
32	23	0.1	22.574 ± 0.108^{a}	3.567 ± 0.01^{a}	1.551 ± 0.00^{a}
Incubation Time (Day)	**n^o Cys**	**Wt of tissue (g)**	**Protein (mg/l)**	**nmol Cys^{MT}**	**nmolMT g^{-1}**
0	23	0.1	5.483 ± 0.014^{c}	1.766 ± 0.00^{e}	0.7677 ± 0.00^{e}
4	23	0.1	4.237 ± 0.020^{e}	2.894 ± 0.00^{c}	1.2581 ± 0.00^{c}
8	23	0.1	4.508 ± 0.014^{d}	2.471 ± 0.00^{d}	1.0745 ± 0.00^{d}
16	23	0.1	20.761 ± 0.012^{b}	9.713 ± 0.00^{b}	4.2232 ± 0.00^{b}
32	23	0.1	22.269 ± 0.026^{a}	10.021 ± 0.00^{a}	4.3571 ± 0.00^{a}
Incubation Time (Day)	**n^o Cys**	**Wt of tissue (g)**	**Protein (mg/l)**	**nmol Cys^{MT}**	**nmolMT g^{-1}**
0	23	0.1 ± 0.014	5.483^{e}	1.766 ± 0.00^{e}	0.7677 ± 0.00^{e}
4	23	0.1 ± 0.013	7.112^{d}	1.898 ± 0.00^{d}	0.8254 ± 0.00^{d}
8	23	0.1 ± 009	9.23^{c}	2.486 ± 0.00^{c}	1.0809 ± 0.00^{c}
16	23	0.1 ± 0.014	16.516^{b}	4.459 ± 0.00^{b}	1.9387 ± 0.00^{b}
32	23	0.1 ± 0.063	16.738^{a}	4.821 ± 0.00^{a}	2.0959 ± 0.00^{a}

Mean ± SD; *n* =3 (mean and SD are calculated from three measurements, considering three pooled samples at each concentration. Means not sharing the same superscript (a, b, c, d or e) in each column are significantly different ($P < 0.001$, Duncan).

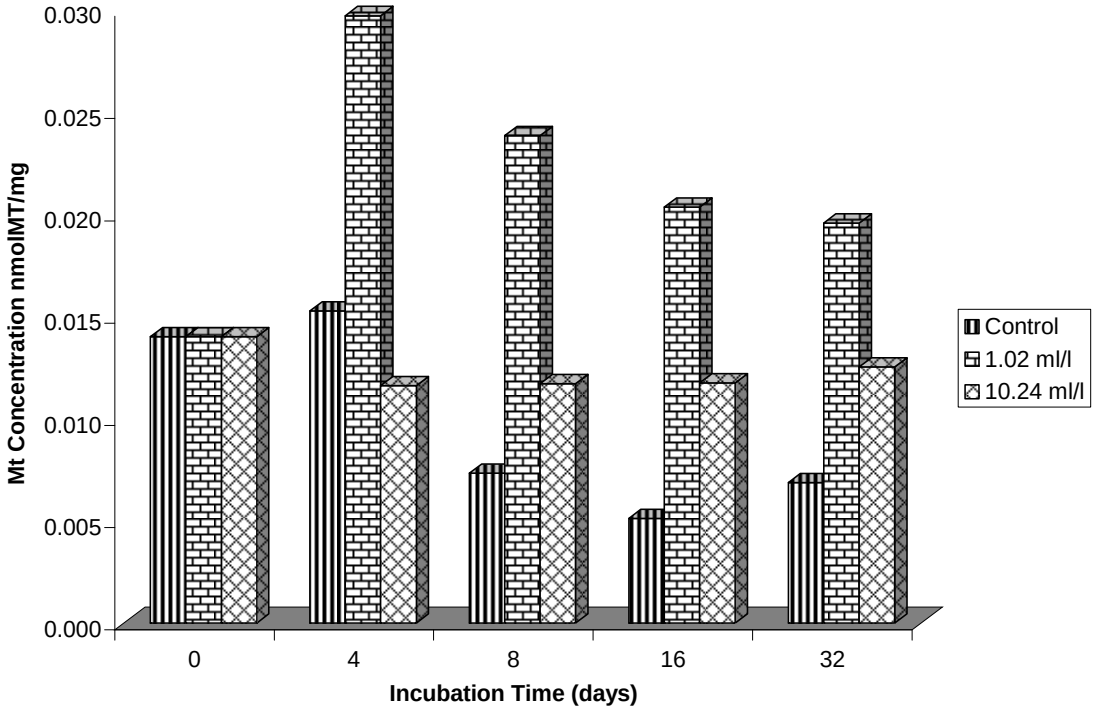

Figure 1: Metallothionein per total protein levels in the digestive gland of *T. fuscatus*

Table 2: Induction of metallothionein in *P. aurita* on sublethal exposure to drill cuttings (mean ± SD)

Incubation Time (Day)	n° Cys	Wt of tissue (g)	Protein (mg/l)	nmol Cys^{MT}	nmol MT g^{-1}
0	23	0.1	11.147 ± 0.025^{d}	7.789 ± 0.00^{e}	3.3865 ± 0.00^{a}
4	23	0.1	10.915 ± 0.013^{e}	7.809 ± 0.00^{d}	3.3952 ± 0.00^{b}
8	23	0.1	20.906 ± 0.012^{c}	7.919 ± 0.00^{c}	3.443 ± 0.00^{c}
16	23	0.1	38.688 ± 0.028^{b}	8.01 ± 0.00^{b}	3.4828 ± 0.00^{d}
32	23	0.1	42.666 ± 0.145^{a}	8.72 ± 0.00^{a}	3.7914 ± 0.00^{e}
Incubation Time (Day)	**n° Cys**	**Wt of tissue (g)**	**Protein (mg/l)**	**nmol Cys^{MT}**	**nmol MT g^{-1}**
0	23	0.1	11.147 ± 0.025^{e}	7.789 ± 0.00^{b}	3.3865 ± 0.00^{d}
4	23	0.1	13.246 ± 0.025^{d}	0.438 ± 0.00^{e}	0.1902 ± 0.00^{a}
8	23	0.1	21.931 ± 0.054^{c}	1.478 ± 0.00^{d}	0.6426 ± 0.00^{b}
16	23	0.1	23.266 ± 0.029^{b}	7.294 ± 0.00^{c}	3.1714 ± 0.00^{c}
32	23	0.1	27.940 ± 0.014^{a}	8.926 ± 0.00^{a}	3.8807 ± 0.00^{e}
Incubation Time (Day)	**n° Cys**	**Wt of tissue (g)**	**Protein (mg/l)**	**nmol Cys^{MT}**	**nmol MT g^{-1}**
0	23	0.1	11.147 ± 0.025^{e}	7.789 ± 0.00^{a}	3.3865 ± 0.00^{e}
4	23	0.1	24.431 ± 0.0356^{a}	0.759 ± 0.00^{e}	0.3301 ± 0.00^{a}
8	23	0.1	21.323 ± 0.064^{b}	1.284 ± 0.00^{d}	0.5583 ± 0.00^{b}
16	23	0.1	16.106 ± 0.052^{d}	2.939 ± 0.00^{c}	1.2778 ± 0.00^{c}
32	23	0.1	16.766 ± 0.029^{c}	3.965 ± 0.02^{b}	1.724 ± 0.00^{d}

Mean ± SD; *n* =3 (mean and SD are calculated from three measurements, considering three pooled samples at each concentration. Means not sharing the same superscript (a, b, c, d or e) in each column are significantly different (Duncan, $P < 0.001$).

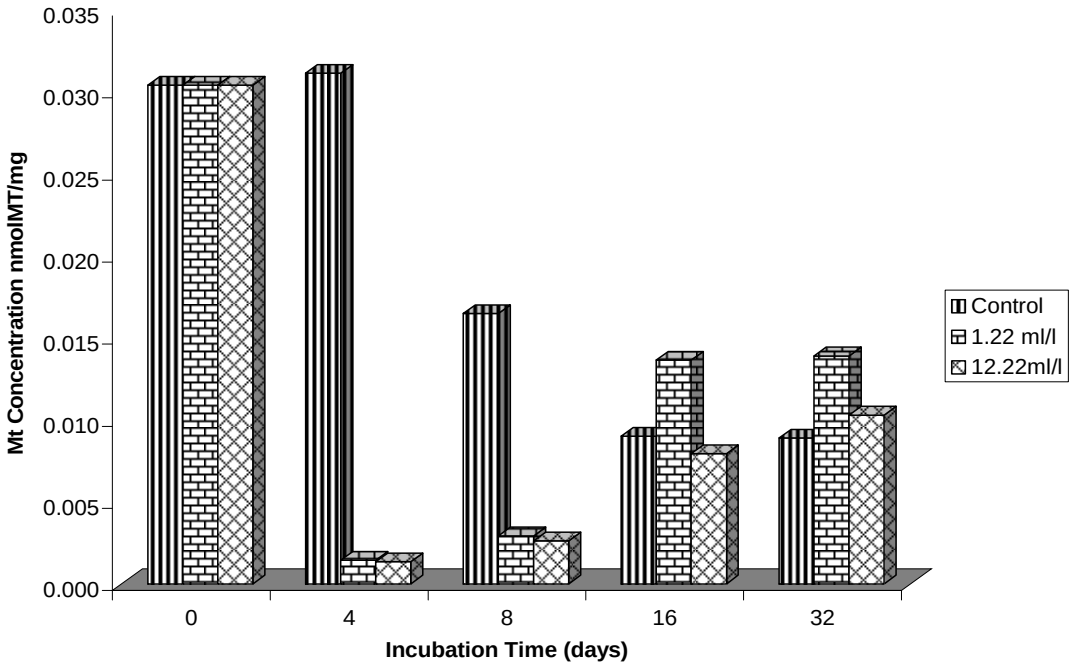

Figure 2: Varying levels of Metallothionein as fraction of total protein in the digestive gland of *P. aurita*

ANOVA revealed that there was statistical difference ($P < 0.001$) in MT concentration for each treatment in the digestive gland of *P. aurita* during the study. Post hoc analysis with Duncan test showed that MT concentration was significantly different ($P < 0.001$) on each sampling day for all treatment. Comparison of MT levels in control *P. aurita* with that of the drill cuttings exposed groups showed that there was significant difference (ANOVA, $P < 0.001$) among them.

As was observed in *T. fuscatus* the average protein concentration increased above the background level during the study. The observed increase in protein concentration was higher in control organisms relative to drill cuttings exposed groups. A further examination of the results show that in control, but for the observed increase on day 4, the relative concentration of MT relative to total proteins steadily reduced on all sampling days during the study. In drill cuttings exposed organisms, there was a fall in the concentration of MT relative to total protein on day 4, after which the ratio started to increase, but the basal ratio was not achieved in both groups during the study.

4. Discussion

In this study, metallothionein level in *T. fuscatus* increased in all treatment groups including control. However, over time MT synthesis was greatest in species exposed to 0.01 96h LC_{50} drill cutting concentration compared to control and species exposed to 0.1 96h LC_{50} rill cutting concentration. At low exposure concentration, the organism initially favoured MT production to other proteins but with time, the production of enzymes and proteins was enhanced and the ability to produce more MT increased. At higher concentration of drill cuttings, the organism responded by mobilisation of enzymes (proteins) necessary for MT induction, but the ability to produce proteins was inhibited with the result that the organism production of MT was suppressed. Increase in MT production was observed only after day 16 following substantial increase in protein production during this period. A study of relative concentration of MT to total protein showed that in *T. fuscatus*, low exposure to drill cuttings promoted proteins synthesis including metallothionein. On the other hand enhanced protein synthesis was not proportionate to MT production in control and at higher exposure to drill cuttings, hence MT compared to total proteins concentration was reducing in these groups. The findings suggest that on exposure of *T. fuscatus* to sublethal concentration of drill cuttings, the organisms channelled energy for growth and reproduction into production of enzymes and metallothionein to fight the stressed environmental condition.

In *P. aurita*, exposure to drill cuttings elicited different responses in each treatment group during the study. In control, though the change in MT levels during the study was significant, the difference in the average mean value observed on any sampling was very low with high levels of protein synthesis. In drill cuttings exposed *P. aurita* on the other hand MT production was suppressed though increased concentration of proteins was observed during the

period. Consequently, the ratio of MT to total protein decreased at the end of the study period.

Therefore the exposure to drill cuttings suppressed the production of MT, while at the same time enhanced the production of other proteins in *P. aurita.* At very low (0.01 96hLC_{50}) concentration exposure, the organism tended to recover with time and normal MT production rate resumed. It may be that exposure of *P. aurita* to very low concentration of drill cuttings resulted in the inhibition of endogenous MT, and the organism in response increased protein synthesis of enzymes required for metallothionein production as was observed as time of exposure increased. At higher exposure concentration (0.1 96hLC_{50}), MT production was severely suppressed. The organism in response elevated protein production necessary for metallothionein production. Over time the synthesis of MT increased but at a rate lower than that observed in low concentration exposed organism as the amount of other proteins decreased. Thus exposure of *P. aurita* to higher concentration of drill cuttings resulted in the inhibition of MT production. The organism tried to compensate by increasing the production of enzymes and proteins for production of MT, but this compensatory mechanism was severely inadequate as the organism's ability to produce endogenous MT remained suppressed and the organism remained stressed.

Induction of metallothionein is usually a primary response to heavy metal pollution both in fish (Killie *et al.*, (1992); Hogstrand *et al.,* (1996)) and in invertebrates (Viarengo and Nott, (1993); Roesijadi (1994)) It has also been associated with acclimation of fish (Dixon and Sprague, 1981) and invertebrate (Sathyanathan, 1996) to metal. Induction of MT however is not restricted by heavy metals alone. For instance, Ghoshal *et al* (1998) have demonstrated MT induction in response to restraint stress. Its induction has also been associated with non-mettallic elements as well with endogenous biological processes. MT induction has been linked to reduced growth in laboratory experiments in fish (Roch and McCarter, 1984) and in field experiment with invertebrates (Leung *et al.,* 2001). In the present study, additional synthesis of MT by *T. fuscatus* could be attributed to a physiological response of the organism on exposure to the chemical and suggested that energy for growth and reproduction was concentrated to increase the production of this protein. The implication is that organisms chronically exposed to low levels of drill cuttings are more likely to have stunted growth and lower reproductive rate and population density than reference organisms. In *P. aurita,* exposure to drill cuttings suppressed metallothionein production in a concentration dependent manner. The implication was that the organism's ability to cope with stress may have been compromised, making the organism more vulnerable to external stressors. Our findings demonstrated the presence of MT in the two gastropod species studied and the variations in their induction on exposure to sublethal concentrations of the toxicant.

Ours is a preliminary study on the response of MT in these gastropod species and our findings lend credence to the inclusion of metallothionein as a general biomarker during biomonitoring programmes during evaluation of impacts of drill cuttings on aquatic lives. However, its application in this regard demand further study on characterization and quantification of the isoforms, confounding factors and most importantly the responses of the organisms in their natural environments.

Acknowledgements:

Authors are grateful to the School of Postgraduate Studies, University of Lagos, Nigeria for financial support to carry out this work.

Corresponding Author:
Chukwu Lucian Obinnaya
Aquatic Toxicology and Ecophysiology Laboratory, Dept of Marine Sciences,
University of Lagos, Nigeria
obinnalcunilag@yahoo.com

References

1. Adebayo-Tayo BC, Onilude, Ogunjobi AA and Adejoye DO. Bacteriological and Proximate Analysis of Periwinkles from Two Different Creeks in Nigeria. *World Applied Sciences Journal* 2005; **1 (2):** 87-91.
2. Brown CA. Community structure and secondary production of benthic macrofauna of Lagos Lagoon and Harbour. . M.Phil Thesis University of Lagos, Nigeria, 1991; 359 pp.
3. Brown RJ, Galloway TS, Lowe D, Browne MA, Dissanayake A, Jones MB and Depledge MH. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. *Aquatic Toxicology* 2004; **66:**267-278.
4. Damiens G, His E, Gnassia-Barelli M, Quiniou F and Roméo M. Evaluation of biomarkers in oyster larvae in natural and polluted conditions. *Comparative Biochemistry and Physiology*, Part C 2004; **138:** 121–128.
5. De Smet H, De Wachter B, Lobinski R, and Blust R. Dynamics of (Cd, Zn)-metallothioneins in gills, liver and kidney of common carp Cyprinus carpio during cadmium exposure. *Aquatic Toxicology* 2001b; **52**: 269–281.

6. Depledge MH and Hopkins SP. Methods to assess the effects on brackish, estuarine and near-coastal water organism in: Linthurst, R.S. Bourdeau, P. and Tardiff, R.G. (eds). *Methods to assess the effects of chemicals on ecosystems,* John Wiley & Sons, Chichester, UK 1995; 125-149 pp.

7. Dixon DG and Sprague JB. Copper bioaccumulation and hepatoprotein synthesis during acclimation to Copper by juvenile rainbow trout. *Aquatic Toxicology* 1981; **1:**69-81.

8. Domouhtsidou GP, Dailianis S, Kaloyianni M and Dimitriadis VK. Lysosomal membrane stability and metallothionein content in *Mytilus galloprovincialis* (L), as biomarkers combinations with trace metals concentrations. *Marine Pollution Bulletin* 2004; **48:** 572-586.

9. DPR. *Environmental Guidelines and Standards for the Petroleum Industry in Nigeria* (EGASPIN), revised edition. Department of Petroleum Resources, Lagos, Nigeria, 2002; 314 pp.

10. Egonmwan RIE. The biology of *Tympanotonus fuscatus var radula* (gastropoda; prosobranchis, potamidae). M.Sc Thesis, University of Lagos, Nigeria, 1980; 145 pp.

11. Ellman GL. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* 1959; **82:**70-77.

12. English TE and Storey KB. Freezing and anoxia stresses induce expression of metallothionein in the foot muscles and hepatopancreas of the marine gastropod *Littorina littorea*. *The Journal of Experimental Biology* 2003; **206:** 2517-2524.

13. Galloway TS, Sanger RC, Smith KI, Fillman G, Readman JW, Ford TE and Depledge MH. Rapid assessment of marine pollution using multiple biomarkers and chemical immunoassays. *Environmental Science Technology* 2002; **36:** 2219-2226.

14. George SG and Olsson PE. Metallothionein assay methods. In: Kramer, KJM (ed). *Biomonitoring of Coastal Waters and Estuaries.* CRC, Boca Raton, Fl, USA, 1994; 151-171 pp.

15. Ghoshal K, Wang Y, Sheridan JF and Jacob ST. Metallothionein induction in response to restraint stress. *J. Biol Chem.* 1998; **273 (43):**27904-27910.

16. Handy RD, Galloway TS and Depledge MH. A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. *Ecotoxicology* 2003; **12:**331-343.

17. Hogstrand C, Galvez F, and Wood C. Toxicity, silver accumulation and metallothionein induction in freshwater rainbow trout during exposure to different silver salts. *Environmental Toxicology and Chemistry* 1996; **15:**1102–1108.

18. Howard CL, Dingman CF, Berninger JP, Jahan-Tigh RR and Miller CM. Heavy metal exposure on metallothionein induction and metal binding in grass shrimp. 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 19, 2002.

19. Killie P, Kay J, Leaver M and George S. Induction of piscine metallothionein as a primary response to heavy metal pollutants: applicability of new sensitive molecular probes. *Aquatic Toxicology* 1992; **22:** 279-286.

20. Klaassen CD and Liu J. Induction of metallothionein as an adaptive mechanism affecting the magnitude and progression of toxicological injury. *Environmental Health Perspectivesss* 1998; **106** Suppl 1.

21. Klaassen CD, Liu J and Choudhuri. Metallothionein: An intracellular protein to protect against cadmium toxicity. *Annual Review of Pharmacology and Toxicology* 1999; **39:** 267-294.

22. Lacorn M, Lahrssen A, Rotzoll N, Simat TJ and Steinhart H. Quantification of metallothionein isoforms in fish liver and its implications for biomonitoring. *Environmental Toxicology and Chemistry* 2001; **20 (1):** 140–145.

23. Leung KMY, Morgan IJ, Wu RSS, Lau JS and Furness RW. Growth rate as a factor confounding the use of the dogwhelk *Nucella lapillus* as biomonitor of heavy metal contamination. *Marine Ecology Progress Series* 2001; **221:**145-159.

24. Moore MN, Wade T, Wedderburn RJ, Lowe DM, Balashov G, Buyukgun-gor H, Daurova Y, Denga Y, Kostylev E, Mihnea PE, Moncheva S, Tabagari S, Ciocan C, Ozkoc H and Depledge MH. The UNESCO/IOC "Black Sea Mussel Watch Pilot Study": Biological effects and contaminant residues. In: *Black Sea Pollution Assessment, Black Sea Environmental Series* Vol. 10 United Nations Publications, New York, 1999; 279-292pp.

25. Oronsaye CG. The effect of transplantation experiment on the shell morph of the shell fish *Tympanotonus fuscatus fuscatus Tropical Ecology* 2002; **43(2):** 351-354.

26. Pitt BR, Schwarz M, Woo ES, Yee E, Wasserloos K, Tran S, Weng W, Mannix RJ, Watkins SA, Tyurina YY, Tyurin VA, Kagan VE and Lazo JS. Overexpression of metallothionein decreases

sensitivity of pulmonary endothelial cells to oxidant injury. *The Am J Physiol* (*Lung Cell Mol* Physio) 1997; **17:** L856-L865.

27. Ringwood AH, Hoquet J, Keppler CJ and Gielazyn M. Linkages between cellular biomarker responses and reproductive success in oysters (*Crassostrea virginica*). *Marine Environmental Research* 2004; **58:**151-155.
28. Roch M, and McCarter JA. Metallothionein induction, growth, and survival of chinook salmon exposed to zinc, copper, and cadmium. *Bulletin of Environmental Contamination and Toxicology* 1984; **32:**478-485.
29. Roesijadi G. Metallothionein induction as a measure of response to metal exposure in aquatic animals. *Environmental Health Perspective* 1994; 102(supplement 12):91-96.
30. Sathyanathan B. Kinetics and mechanism of tolerance induction on acclimation of *Villorita cyprinoides* (Hanley) to copper and zinc. *Journal of Bioscience* 1996; **21(6):** 809-818.
31. Viarengo A and Nott JA. Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comparative biochemistry and physiology. C. Comparative pharmacology and toxicology* 1993; **104 (3):**355-372.
32. Viarengo A, Burlando B, Cavaletto M, Marachi B, Ponzano E, Blasco J. Role of metallothionein against oxidative stress in the mussel *Mytius galloprovincialis Am J Physiol* (*Regul Integr Comp Physiol*) 1999; **277:** R1612-R1619.
33. Viarengo A, Ponzano E, Dondero F and Fabbri R. A simple spectrophotometric method fot metallothionein evaluation in marine organisms: an application to to Mediterranean and Antarctic milluscs. *Marine Environmental Research* 1997; **277:** 69-84.
34. Wang WX. Interaction of trace metals and different marine food chains. *Marine Ecology Progress Series* 2002; **243:** 295-309.

14/09/2009