Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60360-8

Metallothionein induction and antioxidative responses in the estuarine polychaeta *Capitella capitata* (Capitellidae)

Suriya J*, S Bharathiraja, V Sekar, R Rajasekaran

CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Porto Novo-608 502, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 16 June 2012
Received in revised from 9 July 2012
Accepted 7 August 2012
Available online 28 August 2012

Keywords:
Copper
Bioaccumulation
Capitella capitata
Metallothionein
Biomarker
Antioxidant

ABSTRACT

Objective: To evaluate the antioxidant response induced by copper (Cu) exposure in *Capitella capitata*. The capacity of Cu bioaccumulation was also evaluated through the metal quantification. **Methods:** Worms were exposed to different concentrations of Cu 50, 100, and 200 μg/L for 7, 14 and 21 days respectively. In all the assays, control groups were run in parallel, employing only saline water (10%). The concentrations of Cu in the digested acidic solutions of worms were determined by using the inductively coupled plasma-Optical emission spectrophotometry. The total protein content and other anti oxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) were recorded for the experiment period. **Results:** Bioaccumulation of Cu metals in polychaetes was increased parallel to exposure time. A peak concentration (197.30±12.32 μg/g) of metallothioneins (MTs) was registered at 14th day of 100 μg/L group compared to control group (37.29 μg/g). Catalase activities (CAT) in Cu treated-worms were increased significantly ($P<0.05$) after 7 and 14 days of exposure. The concentration of Cu had significant ($P<0.05$) influences on the activity of SOD. The same physiological activity was recorded in GST evaluation. **Conclusions:** These results of biochemical variables in *C. capitata* suggest us a useful model species for monitoring of environmental disturbance by heavy metal pollution. Antioxidant defenses were confirmed as sensitive biomarkers for metal stress in polychaete worms.

1. Introduction

Heavy metal contamination in marine ecosystems is of global concern. Metals generally enter the aquatic environment via atmospheric deposition, geological matrix erosion or due to anthropogenic impacts caused by industrial effluents, domestic sewage and mining wastes[1]. It will be toxic to physiological and behavioral effects on the aquatic biota which results in adverse effects on humans. Many investigations are going on to detect and reduce the effects of heavy metals on ecosystem, aquaculture and humans and to quantify its presence in coastal waters and tissues of aquatic animals[2, 3].

Some studies have been developed to assess certain biological parameters as specific markers or indicators which help to examine the existence or quantity of heavy metals[4]. Biomarkers offer a qualitative measure of exposure to toxic chemicals or environmental stresses[5]. In this aspect, polychaetes are of considerable interest as they are abundant, widely distributed and have proven ecological significance in benthic communities. Many species of this group have the ability of surviving in metal-contaminated environments, as they possess metallothioneins (MTs) or other similar metal-binding proteins.

Metallothionein is a family of cysteine-rich, heat stable,

*Corresponding author: Suriya J, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Porto Novo-608 502, Tamil Nadu, India.
Tel.: + 91-4144-243223; + 91-4144-243533
Fax: + 91-4144 -243555
E-mail: jsuriyamb08@gmail.com
Mobile: 9629427888
Foundation Project: This work is financially support by the funding agency MoEn&F, Govt of India (grant No. G4/3535/2010).

low molecular weight (MW ranging from 3 500 to 14 000 Da) proteins which have the capacity to bind both essential (such as zinc, copper, selenium) and non-essential (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which consist around 30% of its amino acidic residues[6]. These MTs play important roles in the homeostasis of the essential metals like copper (Cu) and zinc (Zn) as well as being involved in the detoxification of non-essential metals, such as cadmium (Cd)[7]. MTs as well as metallothionein-like proteins (MTLPs) induction in aquatic organisms have been recognized as a potential biomarker of heavy metal toxicity and bioaccumulation[8].

Cu is one of the most common contaminants found at high concentrations in aquatic environment. That is to say, aquatic organisms are being exposed to elevated levels of Cu. In contrast to Cd, Cu as an essential element for the subsistence of many animals, is a component of many metalloenzymes and respiratory pigments and plays an important role in the activities of cellular metabolism[9]. Both deficient and excessive amounts of Cu can cause adverse effects in all species.

Hence aerobic respiration, using oxygen as a nutrient, come to predominate in biological systems. Organisms generally adapt several antioxidant mechanisms in their biological systems as defense processes, and these antioxidant systems can provide suitable biomarkers for the assessment of environmental stress. One representative biomarker is a phase II enzyme that plays a crucial role in mitigating oxidative stress in all life forms. Response of animals to heavy metal stress conditions were demonstrated in marine invertebrate such as the mollusks *Monodonta lineata*, *Nucella lapillus*[10], the Mediterranean clam *Ruditapes decussates*[11], the polychaetes *Perenereis nuntia* and *Laeonereis acuta*[12,13].

Catalase (CAT) is an oxidative stress indicator in many species including aquatic organisms. CAT catalyzes the decomposition of H_2O_2 to water and oxygen in cells. Particularly CAT and glutathione S-transferase (GST) seem to be suitable tools to detect oxidative stresses that were generated during exposure to contaminants. MT and MTPs induction is also considered as an indicator of antioxidant processes and scavenger of free radicals[14].

Among several strong oxidant inducers, the toxic effect of Cu has been well established as a potential trigger for reactive oxygen species (ROS) generation during the redox cycle. Suzuki *et al*[12] reported that Cu (Cu^+ and Cu^{2+})-induced oxidation can be perform by reduction of H_2O_2 by the Harber-Weiss and Fenton reaction of Cu cations, resulting in cellular damages due to hydroxyl radicals ($HO^{\bullet}$). For this reason, Cu was selected as a definite inducer of oxidative stress in the polychaete *Capitella capitata (C. capitata)* in this study.

Polychaete species can exhibit a marked capability to survive in disturbed environments and may have an elevated tolerance to chemical stress. There were only few studies on *C. capitata*. The deposit-feeding polychaete *C. capitata* is a cosmopolitan opportunistic species that inhabits estuarine and coastal environments. Due to its rapid population growth and high tolerance to natural (*e.g* salinity) as well as anthropogenic (*e.g* chemical contaminants) stresses, this *Polychaete* species is often abundant in polluted estuaries. *C. capitata* is, therefore, a useful test specimen to investigate the impacts of pollutants, including metals[15].

Uppanar estuary is one of the polluted estuaries in the south east coast of India with particular reference to SIPCOT industrial complex which is located on the bank of Uppanar estuary covering an area of about 520 acres with 59 industries. It is specifically established for chemical, petrochemical, fertilizers, pharmaceutical, dyes, soap detergent, packing materials resins, pesticides, drugs, antibiotics *etc* manufacturing industries. Most of the industries are wet process industries and they consume large quantity of water for their manufacturing process. There is no report on this kind of biomarker in this estuary.

In the present research, Cu toxicity of metal polluted sediment and laboratory cultured polychaete *C. capitata* was tested. We also investigated the induction of metallothionein as a biomarker.

2. Materials and methods

2.1. Test organisms

C. capitata weighing (1.5 ± 0.5) g was collected from Uppanar estuary (11° 43′ N, 79° 46′ E) Cuddalore, Tamil Nadu in May, 2011. Sediment samples containing *C. capitata* were brought to the laboratory using cool box. The worms were carefully extracted and transferred to a plastic dishes which containing filtered seawater with artificial soil sediment for seven days (salinity 10‰, pH 8) for acclimatization[13]. During this period worms were fed with *Artemia salina* and the water was changed every three days. Photoperiod and temperature were applied at 12L:12D and 20 ℃ respectively. The continuous aeration was given throughout the culturing process.

2.2. Metal exposure and accumulation

Initially organisms were transferred to dishes without sand, being maintained for another 4 days before the beginning of the assays. To assess the effects of the copper, the worms were exposed to various concentrations of copper as $CuSO_4 \cdot 5H_2O$ (Sigma). Then every ten healthy worms with the similar fresh weight 1.5 g were placed in different glass beakers at

20 ℃ with 1 L seawater with addition of $CuSO_4$ and the final concentration was 50, 100, and 200 μg/L. The worms were exposed for 7, 14 and 21 days. In all the assays, control groups were run in parallel, employing only saline water (10%) with the same characteristics cited above. All glassware used in these experiments had been earlier soaked using 5% HNO_3 to eliminate any adsorption of metals onto the beakers and roofed with glass utensils in order to reduce evaporation. Triplicates were prepared for each concentration. Before frozen at −20 ℃ worms were weighed for further analysis. Worms dried at 60 ℃ and the dried worms were taken at constant weight then digested in the concentrated HNO_3 and $HClO_4$ at 100 ℃. The digested solutions were made up to the known volumes with distilled water. The concentrations of Cu in the digested acidic solutions were determined by using the inductively coupled plasma-optical emission spectrophotometry (ICP-OES-Perkin-Elmer)[16]. The content of Cu was expressed as μg/g dry weight.

2.3. Metallothionein analysis

One gram of each copper treated polychaete tissues were placed in 3 mL ice-cold Tris-HCl 25 mmol buffer (pH 7.2 at 20 ℃), 0.1 mmol phenylmethylsulfonyl fluoride (PMSF) as antiprotease and 0.5 mmol dithiothreitol (DTT) as the sulfydryl -protecting agent to prevent the MT from oxidation, then homogenized with distilled water and the homogenate was centrifuged at 10 000×*g* for 20 min at 4 ℃ in individual 1.5 mL micro tubes for taking apart of MT from total protein. To precipitate non MT proteins, 1 mL of supernatant aliquot was heated at 100 ℃ for 10 min. Then the heat-treated homogenates were cooled on ice for 5 min and centrifuged at 10 000×*g* for 20 min at 4 ℃. Concentration of MT in the tissue samples were estimated with the mercury-saturation assay with small modifications[17]. The blanks were prepared concurrently by adding 200 μL Tris-HCl (20 mmol, pH 7.2) to replace heat-treated sample.

Aliquots of the heat-treated homogenate were fractionated by gel-permeation chromatography [Sephadex G 75 (26 mm ×60 cm), sigma]. The elution was made with Tris-HCl 25 mmol, pH 7.2 containing 0.5 mmol DTT. For each fraction, the absorbance at 254 nm was measured and copper concentration analyzed by ICP-OES (Perkin-Elmer). The total protein was determined from the above supernatant without heat treatment by Bradford[18] method using bovine serum albumin as a standard. MT and protein content determination of each sample and blank was performed in triplicate.

2.4. Antioxidant assays

For antioxidant assays, frozen samples were homogenized with distilled water and then the homogenate was centrifuged at 10000×*g* for 20 min at 4 ℃. The supernatant was kept under 4 ℃ until being measured for superoxide dismutase (SOD), CAT and GSH activities.

SOD assay was based on the ability of SOD to inhibit the auto-oxidation of pyrogallol (50 mmol) in a 50 mmol Tris-HCl buffer (pH 8.3). The reaction mixture contained 4.5 mL Tris-HCl buffer, 100 μL supernatant and 10 μL pyrogallol. Oxidation of pyrogallol absorbance was measured at 325 nm. One unit of SOD activity was defined as 50% inhibition of the oxidation process (mmol/mg protein/min)[19].

CAT activity was determined according to a modified method of Greanwald[20]. It was based on the decomposition rate of H_2O_2 by the enzyme, which can be measured as absorbance decrease per minutes at 240 nm. The substrate was prepared by adding 0.05 mL of supernatant with 1 mL H_2O_2 (30 %) and 1.95 mL 0.67 mol phosphate buffer (pH 7.0). The concentration of H_2O_2 was determined 3-4 min after the initiation of the reaction by the addition of supernatant. One unit of catalase activity was defined as 50% H_2O_2 consumption at 1 min (mmol/mg protein/min).

GST activity was determined as described by Regoli *et al*[21] by increase of absorbency at 340 nm due to the conjugation of glutathione (GSH) to 1-chloro-2, 4-dinitrobenzene (CDNB; Sigma). Enzyme activity values were expressed in GST units, where one unit was the enzyme amount necessary to conjugate 1 μ mol of CDNB per minute and per milligram of total protein present in the homogenate, at 25 ℃ and pH 7. GST activity was measured in supernatant from worms exposed to copper.

2.5. Statistical analysis

In all cases a minimum of three independent experiments was conducted and each sample was triplicated. Data presented are means, standard deviations. Data were analyzed with two way analyses of variance (ANOVA), and pair wise multiple comparisons were illustrated using origin statistical tool to compare Cu treatments at $P<0.05$.

3. Results

3.1. Copper concentration in polychaete C. capitata

During all the experimental periods, there was no mortality observed. The worms, exposed to different copper concentrations, exhibited a significant ($P<0.05$) accumulation of this element after 7, 14 and 21 days exposure (Figure 1). The control values remained stable over period of testing time. Maximum concentration of copper was observed at 21 days with 200 μg/L [(67.89±2.47) μg/g dry weight].

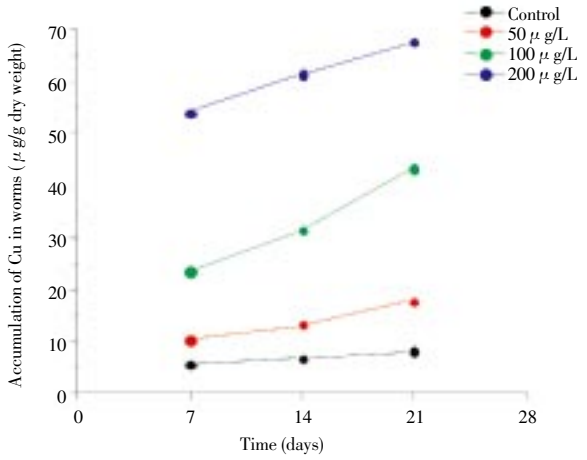

Figure 1. Accumulation of copper (μ g/g dry weight) in *C. capitata* exposed to various concentrations of Cu after 7, 14 and 21 days.

3.2. Metallothionein induction

Through the experimental period, the amount of MTs appearing in the control worms were steady at [(37.15±0.15), (41.29±1.37), (42.47±1.93) μ g/g] fresh weight on 7th, 14th and 21st day respectively. The highest concentration of MTs was obtained with worm exposed 14 days to 100 μ g/L [(197.3± 12.32) μ g/g fresh weight]. Elevation of MT concentrations was dependent on the duration of exposure. Longer exposure to Cu (21 days) induced a decrease of the MT content which remained clearly higher than control levels. MTs were reported higher (P<0.05) in worms-exposed to different concentrations of Cu than in control worms (Figure 2).

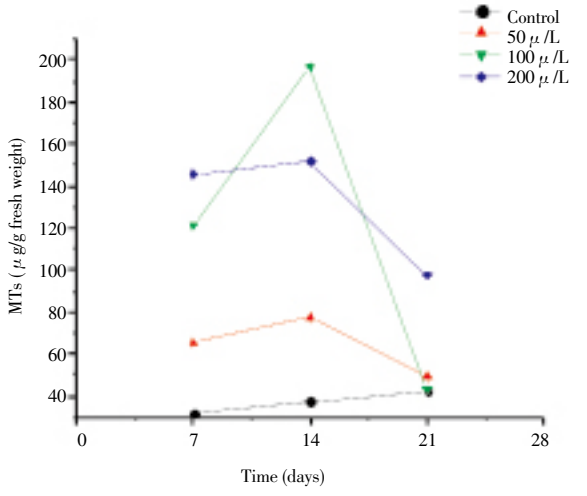

Figure 2. Metallothionein content in *C. capitata* after 7, 14, 21 days of Cu treatment.

3.3. Total soluble protein content

In untreated group of *C. capitata* protein content ranged between [(1458±63), (1475±89), (1493±48) μ g/g] fresh weight after 7, 14 and 21 days respectively, which indicated that they remained stable during the experiment. Figure 3 indicates the MTS contain high amount of copper compared to zinc and cadmium. In the worms exposed to Cu for 7, 14 and 21 days, the protein content was significantly higher than control worms (Figure 4). The higher concentrations of total soluble protein content were obtained with worm exposed to 200 μ g/L after 14 days shown in Figure 4. After 14 days of exposure the protein content increased gradually.

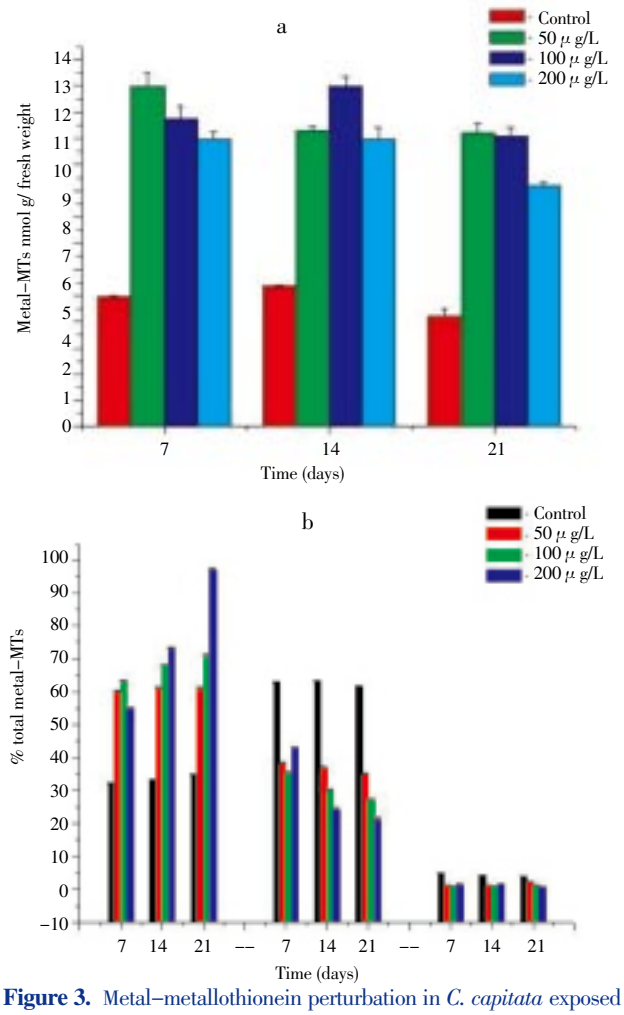

Figure 3. Metal-metallothionein perturbation in *C. capitata* exposed 7, 14 and 21 days to various concentrations of Cu.

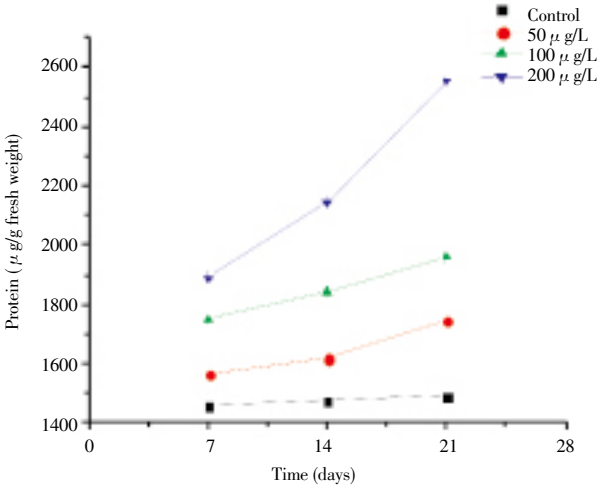

Figure 4. Effects of various concentrations of Cu on total soluble protein content of *C. capitata* after 7, 14 and 21 days of exposure.

3.4. Enzymatic activities

MTs are proteins that act not only as metal stores, but also as antioxidants[34]. SOD is a group of metalloenzymes that catalyze the conversion of reactive O_2 to produce H_2O_2, which in itself is an important ROS. H_2O_2 is subsequently detoxified by two types of enzymes: CAT and POD. Therefore, the three enzymes can cooperate to neutralize ROS. SOD activity in copper-exposed worms was higher ($P<0.05$) compared to the control group (Figure 5). The highest SOD activity was recorded at 14th day of 100 μg Cu (II) exposed worms. CAT activity was significantly increased in Cu treated worms compare to control worms. These increases are more important for higher Cu concentration on 14th day (Figure 6). GST activity was increased only in worms exposed to the higher copper concentration when compared to the other groups tested (Figure 7).

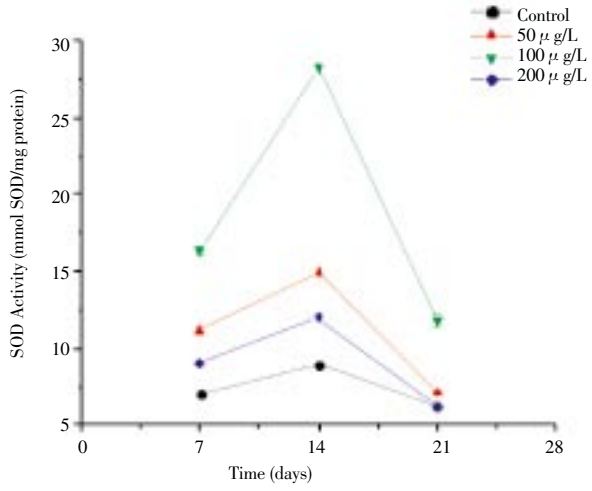

Figure 5. Effects of various concentrations of Cu on Superoxide Dismutase activity of *C. capitata* after 7, 14 and 21 days of exposure.

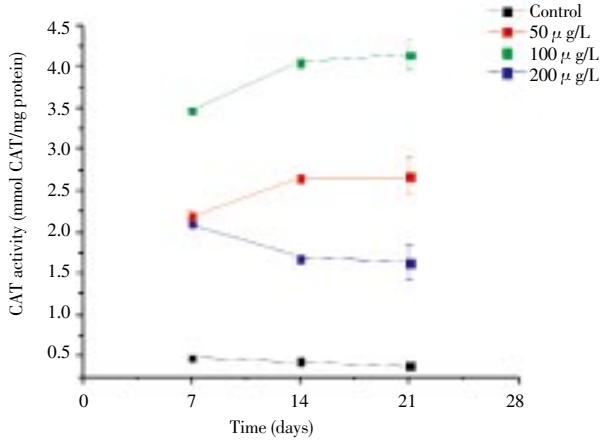

Figure 6. Effects of various concentrations of Cu on catalase activity of *C. capitata* after 7, 14 and 21 days of exposure.

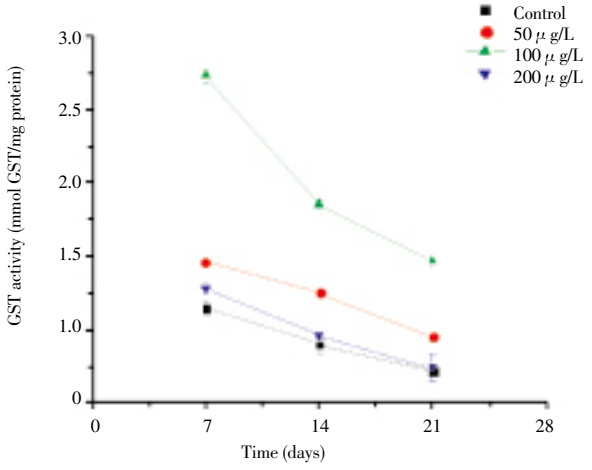

Figure 7. Effects of various concentrations of Cu on GST activity of *C. capitata* after 7, 14 and 21 days of exposure.

4. Discussion

The present study deals with enzymatic and protein variation in *C. capitata* for metal exposure. The maximum concentration of copper observed at 21 day (200.00 μg/L) was higher than that reported for the polychaete *Hediste japonica* after 6 days exposure to Cu concentration of 150.00 μg/L (22.41 μg/g)[22], and this result was in agreement with the previous observation for the polychaetes *Nereis diversicolor* exposed to various concentrations of Cd, Cu and Zn for 21 days[23]. Our results also indicated that Cu accumulation increased with exposure time. Marcano *et al*[24] also indicated the accumulation rate of Cu [(29.1±23.6) μg/g dry weight] during 8 days exposure. Generally metals are bind with -SH compounds present in polychaete worms[25]. MTs has high metal affinity and can be easily induced by metal treatment which supports to use it as biomarkers of metal exposure, especially in the marine environment. Metal response elements (MREs) of the MT gene expresion elevated when it comes in contact with metals. Metal-binding regulatory factors (MRFs) are responders of MREs which activates MT gene transcription[26]. MT synthesis can be controlled both at the level of transcription, and of translation and there is evidence of high levels of MT mRNA accompanied by low levels of MT protein. Marcano *et al*[24] reported that MT like proteins (10-20 kDa) from the polychaete *Eurythoe complanata* have a higher affinity for zinc than for copper. Some of the polychaetes have been used in metal toxicity assessment using MT or MTLP as biomarker. This is the first report on *C. capitata* like these studies. Preliminary studies of the use of MT as a biomarker for metal contamination were performed under laboratory conditions using unrealistically high levels of contaminants, and under conditions that were often very remote from field conditions.

Cu toxicity may be increase ROS which activate antioxidant response element (ARE) for MT induction like an immune response in polychaetes. Increase of MT concentrations in animals exposed to Cu is clearly due to an activation of their transcription but can be also related to a decrease of MTs

degradative process. Additionally, Cu is known to induce severe oxidative stress and a toxic effect in the polychaetes[27].

It is well known that the protein degradation of MTs depends on its metal content. In trout MTs half life changed from 12 to 30 days after Cu contamination[28]. In *C. capitata* exposed to Cu an increase of metal-MTs was observed which can lead to some modification of MT degenerative process. A decreased of MT concentrations was observed in *C. capitata* exposed to higher concentration of copper 200 μg/L after 14 days.

The perturbation varied according to the duration of exposure to different concentration was illustrated in Fig 3. In the literature, results on protein content modification due to copper exposure were inconsistent. The increase level of protein content with metallothionein act as defensive mechanisms during exposure to metal contamination. In untreated worms MTs were mainly bound to Zn [(65.00±5.90)%] as it has been previously demonstrated for other animal species (Fig 3). In untreated worms Zn bound MTs used to transport information through nervous system. In Cu contaminated worms the amount of Zn is gradually replaced by Cu in metal bound MTs. Fig 3 shows the percentage of Zn-MTs decreased to (37.00±1.40)% in worm exposed 7 days to 100 μg/L. After 7 days of exposure MTs were in mostly linked to Cu. In the same time the quantity of Cd-MTs was not disturbed and remained stable. More-over there was no significant perturbation of Cd-MTs content was observed in *C. capitata* until the end of experiment (21 days). Cadmium has higher affinity for MTs but it was not seen in case of Zn.

The decrease of MT content observed in worm exposed 21 days to copper is partly due to a decrease of Cu-MTs and Zn-MTs content. In worm exposed 14 days to 200 μg/L MTs were quite saturated by copper (95.90±9.95)% of Cu-MTs. The induced alterations can inhibit the different cellular defense and lead to an increase of pathology severity[29]. We can conclude that Cu has displaced Zn from MTs. The function of MTs includes intracellular metal metabolism and storage: MTs act as a reservoir of Zn which rescues their enzymatic activities by rapid exchange with apo-metalloproteins. It is possible that, in Cu contaminated worms, high levels of MTs captured metals from other metalloproteins and so lead to their inactivation.

The biotransformation of Cu accounts for significant increase of total soluble protein content which could be related to the development of defensive mechanism induced to overcome the stress situation by Cu. The effect of stress induction may reflect in enzyme degradation and tissue necrosis. These effects can be overcome by increasing amount of protein. Such hypothesis has been advanced to explain changes of protein content observed in the polychaete *Laeonereis acuta* which exposed to arsenic and in the prawn M. malcolmsonii exposed to endosulfan[30,31]. Cd stress increase ROS generation by inhibit the electron transfer chain in the mitochondria. The antioxidant enzymes play an important role in protecting cellular systems from oxidative damages induced by ROS. The enhanced activity of SOD was reported in many polychaetes such as in *N. diversicolor*[32].

CAT is an enzyme, which dismute superoxide anions (O_2) and decompose H_2O_2. The catalase activity helps the worms to overcome the stress induced by Cu. The Cu treated worms physiological conditions were changed by free radicals. Catalase activity response acts as defensive mechanism with MTs to limit Cu toxic impact at the cellular system. Similar elevations in CAT level have been reported in polychaetes Perinereis nuntia exposed to copper[11].

GST activity was reported as a biomarker of stress exposure in the population of clamworms (*Perinereis gualpensis*)[33]. A time- and dose-dependent increase of GST was found in *P. nuntia* exposed to Cd[14]. Enzymes of the GST family are composed of many cytosolic, mitochondrial, and microsomal proteins which is the most abundant non protein thiol in the living organisms and it plays a crucial role in intracellular protection against toxic compounds such as Cu. *N. succinea* (Polychaeta, Nereididae) used as a good test organism for examining the effect of heavy metals along with the MTLP content accompanied by the increased expression of glutathione S-transferase theta (GST-T) gene as an antioxidant defense upon exposure of copper. The highest activity was observed on 7th day of 100 μg exposed worm. Cu contamination of fish has been shown to produce firstly an increase of GST activities which is understood as a defensive mechanism to counteract Cu toxicity.

The present study was carried out to identify the copper exposure on physiological and enzymatic changes in *C. capitata*. The apparent modifications of MTs-metal content confirmed their importance in metal in cellular metabolism. The increase of SOD, CAT, GST and total protein content level suggested that an oxidative stress may be associated with increase of MTs level in the worms. The high induction of MTs observed in *C. capitata* during exposure to Cu make them potentially useful biomarker to monitor heavy metal contamination.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

The authors are gratefully acknowledge to the Director, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, India for providing all support during the study period and to the funding agency MoEn&F, Govt of India (G4/3535/2010) for the financial support.

References

[1] Alam MGM, Tanaka A, Allinson G, Laurenson LJB, Stagnitti F, Snow ET. A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of Lake Kasumigaura. *Jpn Ecotoxicol Environ Safety* 2002; **53**(3): 348-354.

[2] Mantelatto FLM, Avelar WEP, Silva DML, Tomazelli AC, Lopez JLC, Shuhama T. Heavy metals in the shrimp *Xiphopenaeus kroyeri* from Ubatuba Bay Saint Paulo. *Brazil Bull Environ Contam Toxicol* 1999; **62**: 152-159.

[3] Turoczy NJ, Mitchell BD, Levings AH, Rajendram VS. Cadmium, copper, mercury and zinc concentrations in tissues of the king crab

Pseudocarcinus gigas from southeast Australian waters. *Environ Int* 2001; **27**: 327-334.

[4] Pederson SN, Lundebye AK, Depledge MH. Field application of metallothionein and stress protein biomarkers in the shore crab Carcinus maenas exposed to trace metals. *Aquat Toxicol* 1997; **37**: 183-200.

[5] Sarkar A, Ray D, Shrivastava AN, Sarker S. Molecular biomarkers: their significance and application in marine pollution monitoring. *Ecotoxicol* 2006; **15**: 333-340.

[6] Alam MA, Gomes A, Sarkar S K, Shuvaeva OV, Vishnevetskaya NS, Gustaytis MA, et al. Trace metal bioaccumulation by Soft-bottom Polychaetes (Annelida) of sundarban mangrove wetland, India and their potential use as contamination indicator. *Bull Environ Contam Toxicol* 2010; **85**: 492-496.

[7] Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. *Aquat Toxicol* 2006; **76**: 160-202.

[8] Rhee JS, Won EJ, Kim RO, Choi BS, Choi IY, Park GS, et al. The polychaete, *Perinereis nuntia* ESTs and its use to uncover potential biomarker genes for molecular ecotoxicological studies. *Environ Res* 2012; **112**: 48-57.

[9] Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev* 1985; **65** (2): 238-309.

[10] Cunha I, Mangas-Ramirez E, Guilhermino L. Effects of copper and cadmium on cholinesterase and glutathione S-transferase activities of two marine gastropods (*Monodonta lineata* and *Nucella lapillus*). *Comp Biochem Physiol C Toxicol Pharmacol* 2007; **145**(4): 648-657.

[11] Hoarau P, Garello G, Gnassia-Barelli M, Roméo M, Girard JP. Purification and partial characterization of glutathione S-transferases from the clam *Ruditapes decussatus*. *Eur J Biochem* 2002; **269**(17): 4359-4366.

[12] Won EJ, Kim RO, Rhee JS, Park GS, Lee J, Shin KH, et al. Response of glutathione S-transferase (GST) genes to cadmium exposure in the marine pollution indicator worm, *Perinereis nuntia*. *Comp Biochem Physiol C Toxicol Pharmacol* 2011; **154**: 82-92.

[13] Geracitano LA, Bocchetti R, Monserrat JM, Regoli F, Bianchini A. Oxidative stress responses in two populations of *Laeonereis acuta* (Polychaeta, Nereididae) after acute and chronic exposure to copper. *Mar Environ Res* 2004 a; **58**: 1-17.

[14] Won EJ, Rhee JS, Kim RO, Ra K, Kim KT, Shin KH, et al. Susceptibility to oxidative stress and modulated expression of antioxidant genes in the copper-exposed polychaete Perinereis nuntia. *Comp Biochem Physiol C Toxicol Pharmacol* 2012; **155**(2): 344-351.

[15] Selck H, Decho AW, Forbes VE. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. *Environ Toxicol Chem* 2009; **18**(6): 1289-1297.

[16] Baumann Z, Fisher NS. Modeling metal bioaccumulation in a deposit-feeding polychaete from labile sediment fractions and from pore water. *Sci Total Environ* 2011; **409**(13): 2607-2615.

[17] Ng TY, Rainbow PS, Amiard-Triquet C, Amiard JC, Wang WX. Decoupling of cadmium biokinetics and metallothionein turnover in a marine polychaete after metal exposure. *Aquat Toxicol* 2008; **89**(1): 47-54.

[18] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1976; **72:** 248-254.

[19] Rhee JS, Won EJ, Kim RO, Lee J, Shin KH, Lee JS. Expression of superoxide dismutase (SOD) genes from the copper-exposed polychaete, *Neanthes succinea*. *Mar Pollut Bull* 2011; **63**(5-12): 277-286.

[20] Greanwald RA. *Handbook of methods for oxygen radical research*. Boca Raton: CRC Press; 1985.

[21] Regoli F, Principato GB, Bertoli E, Nigro M, Orlando E. Biochemical characterization of the antioxidant system in the scallop *Adamussium colbecki*, a sentinel organism for monitoring the Antarctic environment. *Polar Biol* 1997; **17**: 251-258.

[22] Sun F, Zhou Q. Metal accumulation in the polychaete *Hediste japonica* with emphasis on interaction between heavy metals and petroleum hydrocarbons. *Environ Pollut* 2007; **149**: 92-98.

[23] Zhou QX, Rainbow PS, Smith BD. Tolerance and accumulation of the trace metals zinc, copper and cadmium in three populations of the polychaete *Nereis diversicolor*. *J Mar Biol Assoc* 2003; **83**: 65-72.

[24] Marcano L, Nusetti J, Rodriguez-Gruuf J, Vilas J. Uptake and depuration of copper relation to metal- binding protein in *Eurythoe complanata*. *Comp Biochem Physiol* 1996; **114**(3): 179-184.

[25] Casado-Martinez MC, Smith BD, Luoma SN, Rainbow PS. Bioaccumulation of arsenic from water and sediment by a deposit feeding polychaetes (*Arenicola marina*): a biodynamic modeling approach. *Aquat Toxicol* 2010; **98**: 34-43.

[26] Davies SR, Cousins R. Metallothionein expression in animal: a physiological perspective on function. *J Nutr* 2000; **130**(5): 1085-1088.

[27] Bonnard M, Roméo M, Triquet CA. Effects of Copper on the burrowing behavior of estuarine and coastal invertebrates, the Polychaete *Nereis diversicolor* and the Bivalve *Scrobicularia plana*. *Hum Ecol Risk Assess* 2009; **15**(1): 11-26.

[28] Olsson PE. Metallothionein gene expression and regulation in fish. In: Hochachka PW, Mommsem TP editors. Biochemistry and molecular biology of fishes. Elsevier Science Publishers; 1993, p. 259-278.

[29] Fattorini D, Notti A, Nigro M, Regoli F. Hyperaccumulation of vanadium in the Antarctic polychaete *Perkinsiana littoralis* as a natural chemical defense against predation. *Environ Sci Pollut Res Int* 2011; **17**(1): 220-228.

[30] Ventura-Lima J, Sandrini JZ, Cravo MF, Piedras FR, Moraes TB, Fattorini D, et al. Toxicological responses in *Laeonereis acuta* (Annelida, Polychaeta) after arsenic exposure. *Environ Int* 2007; **33**(4): 559-564.

[31] Bhavan PS, Geraldine P. Aberrations in various parameters of bioenergetics in the prawn *Macrobrachium malcomsonii* following exposure to endosulfan. *Aquaculture* 2000; **1**: 15.

[32] Sun F, Zhou Q. Oxidative stress biomarkers of the polychaete *Nereis diversicolor* exposed to cadmium and petroleum hydrocarbons. *Ecotoxicol Environ Saf* 2008; **70**(1): 106-114.

[33] Ayoola JA, Garcia-Alonso J, Hardege JD. Glutathione-S-transferase in *Nereis succinea* (Polychaeta) and its induction by xeno-estrogen. *Environ Toxicol* 2011; **26**(5): 559-565.

[34] Klaassen CD, Liu J, Choudhuri S. Metallothionein: An intracellular protein to protect against cadmium toxicity. Ann Rev *Pharmacol Toxicol* 1999; 39: 267-274.