

**Genotoxic Potency Of Mercuric Chloride In Gill Cells Of Marine Gastropod
Planaxis sulcatus Using Comet Assay**

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Abstract

In vivo and *in vitro* exposures were used to investigate the genotoxicity of mercuric chloride (HgCl₂) to the marine snail, *Planaxis sulcatus*. The comet assay protocol was validated on gill cells exposed *in vitro* to hydrogen peroxide (H₂O₂, 0–50 µM). Snails were exposed *in vivo* for 96 h to HgCl₂ (10, 20, 50 and 100 µg/l). Our results showed significant concentration-dependent increases in the tail DNA (TDNA) and olive tail moment (OTM) in exposed snails for all doses compared with controls. *In vitro* exposure to HgCl₂ (10-100 µg/l) resulted in significantly higher values for TDNA at all concentrations. Our results showed that DNA damage increased in the gill cell with increasing exposure time. This study demonstrates the usefulness of comet assay for detection of DNA damage after exposure to HgCl₂ and the sensitivity of marine snail *P. sulcatus* as a good candidate species for metal pollution.

Keywords: Comet assay; *Planaxis sulcatus*; mercuric chloride; genotoxicity; DNA damage; *in vivo*; *in vitro*

Capsule: We have reported concentration-dependent increase in tail DNA and olive tail moment measure by comet assay in marine gastropod *Planaxis sulcatus* exposed to *in vivo* and *in vitro* to HgCl₂

Highlights

- An approach to evaluate genotoxicity of HgCl₂ in marine gastropod was presented
- *In vivo* and *in vitro* effects of HgCl₂ in gill cells of gastropod was evaluated using comet assay
- Concentration dependent increase in tail DNA and olive tail moment is reported in exposed gastropods
- HgCl₂ was found to be genotoxic to marine gastropod *Planaxis sulcatus*

Introduction

The increase in the discharge of genotoxic chemical from either industrial or municipal waste waters into the aquatic ecosystem has become a great concern to environmentalists around the world. Heavy metals are of great ecological concern due to their toxic and persistent nature. They are widely spread in the biosphere, from both natural and man-made activities. In recent decades, the amount of potentially toxic heavy metals has risen steadily in both marine and freshwater ecosystems due to anthropogenic emissions (Pacyna et al., 2006; Sunderland et al., 2009). Among the toxic metals, mercury is ubiquitous in the environmental (Goldman & Shannon, 2001). In the last few decades, there has been many reports on mercury pollution in various places in India (Krishnakumar and Bhat, 1998; Kaladharan et al., 1999). Marine organisms from Mumbai have been found to be heavily contaminated with mercury (Pandit et al., 1997; Mishra et al., 2007). Rajathy, 1997 has reported high level of Hg (0.013–0.40 µg/g, wet wt) in fish from Ennore estuary, Tamilnadu. Menon & Mahajan, 2013 has surveyed five villages along Ulhas river estuary and thane creek and found high Hg levels in gills, kidney and skin in fish *Mugil cephalus*. Studies have been reported Hg pollution in Amba estuary (Ram et al., 2009a), Hooghly River (Sarkar et al., 2004), Rushikulya estuary (Panda et al., 1990; Shaw et al., 1988; Das & Sahu, 2002), Tamiraparani estuary (Magesh et al., 2011). The concentrations of mercury reported in sediments and coastal waters of India are presented in table 1. Karunasagar et al., 2006 has reported mercury pollution in Kodaikanal lake due to a thermometer factory. Aquatic mercury pollution of the Ulhas estuary, India have been reported by Ram et al., 2009b. UNEP report on global study on mercury, predicted India to be one of the hotspot for mercury pollution due to increase in gold mining activities (Mago, 2003).

Heavy metals accumulate in the environment due to their slow decay rate (Migliore et al., 1999). Although the concentration of mercury in surface water is reported to be low, molluscs are most affected due to its capacity to accumulate toxicants. Among molluscs, bivalves and gastropods are an excellent sentinel organism due to their sedentary life style. Mercury has been shown to bioaccumulate in gastropods (Tessier et al., 1994), oysters (Bigas et al., 2001; Blackmore & Wang, 2004; Liu & Wang, 2014), clams (Chin & Chen, 1993; Boisson et al., 1998; Giani et al., 2012) and mussels (Soto et al., 2011; Kraemer et al., 2013). Higher bioaccumulation of mercury has been reported in digestive gland cells compared to gills and whole soft tissue in mussel (Kljakovic-Gaspic et al., 2006). Table 2 shows the concentration of mercury reported in gastropods from different parts of the world. Mercury has become a public threat due to biomagnifications through the food web (Pickhardt et al., 2005; Campbell et al., 2008; Kidd et al., 2012; Kwon et al., 2012). Bioaccumulations of mercury in molluscs can cause several biological effects, including genotoxic and immunotoxic effects (Gagnair et al., 2004; Guidietal et al., 2010; Sheir et al., 2010). Neurotoxic effects of mercury chloride have been investigated in zebra fish, *Danio rerio* (Senger et al., 2006). There are few studies on immunotoxic effects of mercury chloride *in vitro* in bivalves (Brousseau et

al., 1997; Sauve et al., 2002; Gagnaire et al., 2004) and fishes (Vocchia et al., 1994). Genotoxic effects of mercury has been reported in molluscs, particularly in mussel (Chatziargyriou & Dailianis, 2010). Fournier et al., 2001 has studied the chronic exposure of bivalves (*Mya arenaria*) exposed to HgCl₂.

Gastropods are omnipresent in the aquatic environment and are identified as a suitable bioindicator for heavy metal pollutions (Selgrade, 1999, 2005; Gundacker, 2000; Galloway and Depledge, 2001; Galloway & Handy, 2003; Auffret, 2005; Woolhiser et al., 2005; Itziou & Dimitriadis, 2011; Abdel-Halim et al., 2013). Gastropods have been reported to have higher bioaccumulation capacity for Hg than bivalves (Bhattacharya & Sarkar, 1996; Wang et al., 2005).

The integrity of DNA can be greatly affected by genotoxic agents due to DNA strand breaks, loss of methylation and formation of DNA adducts (Pisoni et al., 2004). The relationship between DNA damage and the exposure of gastropods to environmental contaminants is well documented (Sarkar et al., 2008, 2011; An et al., 2012; Bhagat et al., 2012). Currently, the comet (or single cell gel electrophoresis) assay, is widely used in both the research field and laboratory tests because of its versatile and reliable nature (Jha, 2008; Frenzilli et al., 2009; Navarro & Martinez, 2014; Pellegrini et al., 2014; Vignardi et al., 2015). Multiple classes of DNA damage can be investigated in single cells using this technique. One of major advantage of using this assay is requirement of small number (<10,000) of cells for detecting DNA damage (Lee and Steinert, 2003).

In view of the current trend of increasing mercury pollution, it has become necessary to monitor the ecotoxicological impact of the heavy metal on marine organisms. In our previous studies we have used comet assay to study DNA damage on gill cells from marine snails (Sarkar et al., 2013; Sarkar et al., 2014). In this study, the genotoxic effect of mercury chloride were examined in the gastropod, *P. sulcatus* using alkaline comet assay.

Materials and Methods

Chemical reagents

Mercuric chloride (HgCl₂, analytical grade) was obtained from Shivprasad Enterprise, (SD Fine Chemicals, Goa, India); low melting agarose (LMA), normal melting agarose (NMA), ethylenediaminetetraacetic acid disodium salt (Na₂EDTA), ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), ethidium bromide (EtBr), gluaiacol glycerol ether, trypan blue were purchased from Sigma Aldrich Pvt. Ltd. (Mumbai, India); dimethyl sulfoxide was obtained from Scitech Scientific (Qualigens, Goa, India); tris buffer and triton X-100 were procured from Sadhale Enterprise (Merck, Goa, India).

Animal Collection

In this study marine gastropod, *P. sulcatus* were used to study of genotoxicity of HgCl₂. Arambol is situated extreme north of Goa and was chosen because of its unpolluted environment. There are no known industries close to the site and is exempt from any direct sewage outputs or harbour activities. A total of around 300 snails were collected during low tide from intertidal rocks scattered along the Arambol, Goa, India. Organisms were transferred to the laboratory using plastic boxes. Identification was carried out by the experts from Zoological Survey of India; Kolkata India using the certified reference samples (Subba Rao, et al., 1992). The collected snails were distribute in five groups (roughly 50 snails in each group) and were acclimatized in 4 liters plastic aquaria for 48 h in aerated seawater at room temperature before the beginning of the experiment. Snails were not fed during mercury exposure.

Mercury Exposure

The HgCl₂ stock solution was dissolved in ultra pure water (100 mg/L) and stored at 4°C. Working solutions ranged from 10-100 mg/L, in accordance with previous studies by Tran et al., 2007. Snails were exposed to different concentrations of HgCl₂ (10, 20, 50, 100 µg/l) for 24, 48, 72, 96 h. Every 24 h, 1/3 of the total volume of water was changed and replaced with fresh seawater from the sampling site with respective concentrations of HgCl₂. Snails kept in water from Arambol were used as controls.

Cell harvesting

The shells of the snails were gently broken and the gills were excised out carefully. Gills were chopped into small pieces in 1 ml of cold extrusion buffer (71.2 mM NaCl, 5 mM EGTA, 50.4 mM guaiacol glycerol ether, pH 7.5). It was then centrifuged at 5000 rpm for 3 min. The pellet was then washed with phosphate buffer saline (1.2 M NaCl, 0.027 M KCl, 11.5 mM K₂HPO₄, 0.08 M Na₂HPO₄, pH 7.3) to remove the cell debris. It was suspended in the same buffer. For *in vivo* and *in vitro* exposures, gill cells from five to six snails were pooled together and was used for each slide in performing comet assay. Cell viability was tested using trypan-blue exclusion (Absolom, 1986). Cells were treated with 0.04% trypan-blue and percentage of stained cells in each sample was determined using a hemocytometer under Leica microscope (Leica Microsystems, Germany).

In vitro exposure

To validate the comet assay protocol, freshly dissociated gill cells were treated with H₂O₂ (1; 10; 25 and 50 µM) in PBS at room temperature (25°C) for 30 min in dark. For in vitro mercury exposure, gill cell suspensions were exposed to four different concentrations (10, 20, 50 and 100 µg/l) of mercury

chloride in PBS at room temperature (25°C) for 1h in dark. The control cells were incubated in PBS without H₂O₂ or HgCl₂. Three replicates per condition were performed.

Measurement of water quality parameters and mercury concentration in gastropods

The physicochemical parameters of water samples at Arambol were determined using the methods of Grasshoff et al., 1983. The mean temperature and pH value were 27.25°C and 8.21 respectively. Conductivity and turbidity were found to be 48 mS/cm and 9.2 NTU respectively. Nitrite, nitrate and total phosphate in water samples were 1.00±0.07 µmol/L, 1.07±0.09 µmol/L and 1.04±0.10 µmol/L respectively. Dissolve oxygen and biological oxygen demand were 3.26±0.05 mg/L and 1.44±0.05 mg/L respectively. The concentration of mercury in the tissues of control snails was measured using Direct Mercury Analyzer (DMA 80, Milestone, N. America) and was found to be 9.92±1.54 µg/kg wet weight.

Comet assay

DNA damage in gills of *P. sulcatus* was evaluated by comet assay using the method of Singh et al., 1988 with slight modifications. Briefly, 20 µl of control or Hg-treated cells suspension were added to 100 µl of LMA (5.5 mg/ml in PBS) and placed on a frosted microscope slide which had previously been coated with a 100 µl layer of NMA (10 mg/ml in ultra pure water). The agarose layer was allowed to solidified at 4°C before addition of the final protecting layer of 100 µl of 0.5% LMA (50 mg per 10 ml Tris Buffer).

After the agarose had solidified, the covers slips were removed and the slides placed in lysing solution (2.5 M NaCl, 0.1 M di-sodium EDTA, 0.01 M Tris Buffer, 0.2 M NaOH, pH 10.0) at 4°C in dark for 1 h. Alkaline DNA unwinding was carried out in a gel electrophoresis chamber containing a freshly prepared ice-cold electrophoretic buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) for 15 min and electrophoresis was performed in the same buffer at 20V (cc. 1V/cm) and 300 mA for 15 min in accordance with our previous studies (Sarkar et al., 2013). After electrophoresis, the slides were neutralized four times at an interval of 5 min with 0.4 M Tris buffer (pH 7.5) and dehydrated overnight in 100% cold ethanol.

Scoring of DNA damage using image analysis

The DNA in the nuclei was stained with ethidium bromide (20 µg/ml). The slides were analyzed, in a dark room, at 40X magnification by use of a fluorescent microscope (DM100 Leica, Leica Microsystems, Germany) equipped with a 515–560 nm wide-band excitation filter and a 590 nm barrier filter. On each slide, 50 randomly cell nuclei were chosen and the extent of DNA migration was determined using Komet 6.0 from Kinetic Imaging, UK. All experiments were carried out in triplicate to take into account possible variations between different cell preparations. Results are

expressed in terms of the percentage of DNA migrated from the comet head to the tail region (TDNA) (Anderson et al., 1994) and olive tail moment (OTM) which is the product of the tail length and the fraction of total DNA in the tail.

Statistical analysis

The data were expressed as mean of 150 cells (three slides of 50 cells each) \pm SD. Genotoxic results from the control and Hg treated animals were analyzed by analysis of variance (ANOVA) followed by Tukey HSD post-test. Kolmogorov–Smirnov test for normality of distribution was used prior to ANOVA. Three levels of significance are reported: (a) $p < 0.05$, (b) $p < 0.01$, and (c) $p < 0.001$. All statistical comparisons were performed using OriginPro 8.5.0 SR1.

Results

Cell viability

Cell viability in the gill cells of snail exposed *in vivo* and *in vitro* to HgCl_2 remained above 85% for the duration of exposure for *P. sulcatus*.

In vitro hydrogen peroxide

Gill cells suspension of *P. sulcatus* was used for the *in vitro* exposure to hydrogen peroxide, H_2O_2 . The concentration range 1-50 μM was used as a positive control. The genotoxicity of mercury was evaluated using comet assay, and data were reported as percentage of tail DNA (TDNA) and olive tail moment (OTM). As shown in Fig. 1, *in vitro* exposure H_2O_2 displayed significant increases in both TDNA and OTM when compared to the control group. The TDNA ranged from 12.43 ± 0.08 to 37.85 ± 0.82 and OTM from 1.77 ± 0.17 to 12.13 ± 2.85 . The *in vitro* H_2O_2 exposure produced statistically significant levels of DNA damage in TDNA as compared to control ($p < 0.001$). Almost seven fold increase in OTM was observed at 50 μM compared with control ($p < 0.001$).

In vitro exposure

Fig. 1 shows statistically significant increase ($p < 0.001$) in TDNA in the concentration range of 0-100 $\mu\text{g/l}$ of HgCl_2 . The lowest TDNA (14.34 ± 0.25) and OTM (1.92 ± 0.40) was observed in *P. sulcatus* exposed to 10 $\mu\text{g/l}$ of HgCl_2 . After 60 min of exposure with 100 $\mu\text{g/l}$ of HgCl_2 , TDNA increased by 2.6 fold as compared to control. There was no significant differences observed in OTM for samples exposed to 10 and 20 $\mu\text{g/l}$ of HgCl_2 . Further the DNA damage was found to be concentration dependent with the highest TDNA and OTM recorded at 100 $\mu\text{g/l}$ in *P. sulcatus*.

In vivo exposure

Fig. 2 shows the extent of DNA damage measured using comet assay in snails exposed *in vivo* to HgCl₂. There is no statistical differences in TDNA and OTM was observed in control DNA during the exposure time. The TDNA values of gills increased significantly after treatment with 10 µg/l of HgCl₂ compared with control (TDNA= 20.26%, p<0.001). At 24 h, statistically significant differences were observed in TDNA at all concentration of HgCl₂ as compared to control. At 48 h, the TDNA of 10 µg/l was 19.46± 0.61, 20 µg/l 22.79±0.71, 50 µg/l 28.92±0.95 and 100 µg/l was 32.57± 0.19. Mean values for 50 µg/l were statistically higher (p < 0.001) than that of 10 µg/l. Decrease in TDNA was also observed at gastropods exposed to 50 µg/l from 48 h to 72 h. After 72 h, 10 µg/l differed statistically from that of 50 and 100 µg/l (p < 0.05), whereas 20 µg/l did not differed statistically from 10 and 50 µg/l. At 72 h, 50 µg/l showed a slight decrease in OTM values from 48 h. At 50 µg/l HgCl₂ exposure, there was significant increase in OTM values from 4.74±0.82 on 24 h to 7.14±1.0 on 96 h.

Discussion

Eighty percent of mollusc are represented by gastropods. Recently, gastropods has attracted attention because of their potential as sentinel organisms. Intersex development in gastropods due to tributyltin pollution have been reported by several authors. Spada et al., 2012 has reported very high level of total mercury (224–1867 µg/kg d.w.) in gastropod *Hexaplex trunculus* from Taranto Gulf, exceeding the maximum level fixed by the European Commission (0.5 mg/kg w.w.). However the concentration of mercury in control snails in this study was found to be 9.92±1.54 µg/kg wet weight.

Various studies on mercury compounds and their genotoxic effects have been performed on mussel (Scarpato et al., 1992; Verlecar et al., 2008). The mussel, *Mytilus edulis* exposed to 50 µg/l of HgCl₂ for 8 days had reported to accumulate significantly higher amount of Hg in gills as compared to the control mussel (Sheir et al., 2010). The author also reported that gill cell accumulated the highest amount of mercury in comparison to digestive gland, adductor muscle or hemolymph. Yap et al., 2004 has studied genetic abnormalities in mussel *Perna viridis* exposed to mercury. Numerous studies have stressed the deleterious effects of mercury on mussels (Rajalekshmi & Mohandas, 1993; Duchemin et al., 2008; Pytharopoulou et al., 2013), crab (Fossi et al., 1996), shrimp (Lee et al., 2000; Jose et al., 2011), fish (Yadav et al., 2009). Although there are many reports on Hg pollution, few researchers have studied the effect of metals on gastropods (Conti & Cecchetti, 2003; Noel et al., 2011; Cardoso et al., 2013). Studies suggests that gastropods are a good bioindicator of metal pollution (Pandewara & Yallapragada, 2000; Yawei et al., 2005).

The DNA damage was measure using comet assay which is a widely used assay in environmental monitoring for evaluation of DNA damage and repair. It has proved its applicability in monitoring DNA damage in mussels (Lain et al., 2008; Rank and Jensen, 2003; Shaw et al., 2000; Siu et al., 2004), clams (Almeida et al., 2013; dos Santos & Martinez, 2014), fishes (Nogueira et al., 2006),

shrimps (Hook and Lee, 2004), crabs (Gouveia et al., 2005). The choice of tissue plays an important role in studying the DNA damage in an organism due to its ability to accumulate and metabolize xenobiotics. Various cells (hepatocytes, gills, lymphocytes), isolated from fish, molluscs, marine mammals after exposure to different genotoxicants have been used for single cell gel electrophoresis for the detection of DNA damage. In this study, we have used gill cells as they are exposed to a large volume of seawater and are important tissue for uptake of contaminants. They accumulate higher concentrations of metal (Dious & Kasinathan, 1992) and are the sites for metabolic activities which convert toxicants into reactive products (Mitchelmore et al., 1998; Wilson et al., 1998).

The DNA damage occurred in this study could be due to various reasons such as DNA single strand breaks, DNA double strand breaks, DNA adduct formations, DNA–DNA, and DNA–protein cross-links (Mitchelmore et al., 1998) resulting from the interaction of mercury or their metabolites (methylmercury) with DNA. There are several mechanisms through which mercury can cause DNA damage. One of them are production of reactive oxygen species such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\bullet$) (Tsuzuki et al., 1994). The antioxidant mechanism in cell detoxifies these ROS and helps to maintain the oxidative balance. Any failure of these antioxidant mechanisms due to the overproduction of ROS can cause oxidative stress in the cell. The ROS produced can also act on plasma membrane and induce lipid peroxidation. LPO results in production of MDA which can react with DNA and form DNA adduct. Geret et al., 2002 has also reported increased level of MDA in *Mytilus edulis* exposed to Hg. Verlecar et al., 2007 has studied the oxidative stress in *Perna viridis* exposed to mercury and observed an increase in thiobarbituric acid reactive substances (TBA-RS) in gills and digestive gland. Oxidative stress is directly or indirectly related to DNA damage. The DNA damage occurred in this study might be due to oxidative stress caused by accumulation of mercury in the tissues of snails. The other mechanism though which mercury can cause DNA damage is by inhibition of DNA repair system.

In vivo and *in vitro* studies with the comet assay have well been documented in aquatic animals (Maffei et al., 2009; Kolarević et al., 2013; Gačić et al., 2014; Pellegrini et al., 2014; Valerio et al., 2014). Monteiro et al., 2011 has conducted *in vivo* and *in vitro* exposure to assess the genotoxic effect of lead on fish *Prochilodus lineatus*. The author demonstrated the usefulness of comet assay to detect lead toxicity in gill and liver cells of the fish. Present *in vivo* and *in vitro* study clearly indicated increase in TDNA and OTM at higher concentrations of $HgCl_2$. Tran et al. (2007) by performing *in vivo* studies with *Mytilus edulis* exposed to $HgCl_2$ (20 $\mu g/l$), has reported similar findings that $HgCl_2$ increases TDNA in exposed mussel. Geret et al., 2002 has reported an increase in metallothionein (MT) levels in mussel (*Mytilus edulis*) after 21 days of exposure to mercury. Toxicity of mercuric chloride has been evaluated *in vivo* on different aquatic organisms such as

crabs (Botton, 2000), mussel (Sheir et al., 2010), fishes (Low & Sin, 1996; Pereira et al., 2009). Arabi, 2004 has detected occurrence of mercury induced DNA damage in carp gill cells using *in vitro* comet assay. Voccia et al., 1994 has studied *in vitro* mercury-related cytotoxicity in rainbow trout (*Oncorhynchus mykiss*). Taddei et al., 2001 has used comet assay to evaluate *in vitro* genotoxicity of methyl mercury chloride in isolated bottlenose dolphin leukocytes and showed dose dependent increase in DNA strand breakage.

In our work, we have demonstrated concentration as well as time dependent increase in the TDNA and OTM as measured using comet assay in *P. sulcatus* exposed to HgCl₂. The accumulation of mercury in gill cells indicate high concentration of Hg in the environment. Berto et al., 2006 has reported positive correlation between total mercury concentration in sediments and in gastropod, *N. reticulatus*. Several studies has shown dose-dependent response of Hg on marine organisms (Lalancette et al., 2003; Pellisso et al., 2008). In this study, control snail showed statistically significant difference when compared with exposed snails, indicating the effectiveness of the method. Results indicate that all the tested concentrations including 10 µg/l are affecting the DNA. The TDNA and OTM has increased with increase in the concentration of HgCl₂ showing that the DNA damage increases with increase in Hg concentration. After 96 h of exposure, OTM value of 10 µg/l were not statistically different from 24 h and 48 h exposure. 20 µg/l had OTM value of 4.03± 0.41 at 48 h, which was significantly higher ($p < 0.001$) than that of control, i.e. 1.52± 0.78. HgCl₂ exposure induced a significant ($p < 0.05$ – 0.001) and concentration-dependent increase in DNA damage in the exposed snails.

In conclusion our study demonstrated, (1) the sensitivity of marine snail *P. sulcatus* as a good candidate species for metal pollution; (2) the usefulness of comet assay for detection of DNA damage after exposure to HgCl₂; (3) suggests the monitoring of Hg level in sea food to protect the human and environmental health and (4) recommends the integrative use of comet assay with other biomarkers.

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Figure captions:

Fig 1 Comet parameters (TDNA and OTM) in gill cells of *P. sulcatus* exposed to hydrogen peroxide (H₂O₂) and mercuric chloride (HgCl₂) *in vitro*. Values are means ± standard deviation (ns) non significant, (a) p<005, (b) p<001, (c) p < 0001 significantly different from the control (ANOVA, Tukey HSD post-test)

Fig 2 Percentage tail DNA and OTM in gill cells of *P. sulcatus* exposed to HgCl₂ *in vivo*. Data represented as means ± standard deviation. T0 refers to time zero or zero day of exposure (ns) non significant, (a) p<005, (b) p<001, (c) p < 0001 significantly different from the control (ANOVA, Tukey HSD post-test)

Tables :

Table 1 Concentrations of Hg reported in the coastal sediments and waters of India

Table 2 Concentrations of Hg reported in terms of dry weight (µg/g) and wet weight (µg/g) in gastropods from different parts of the world

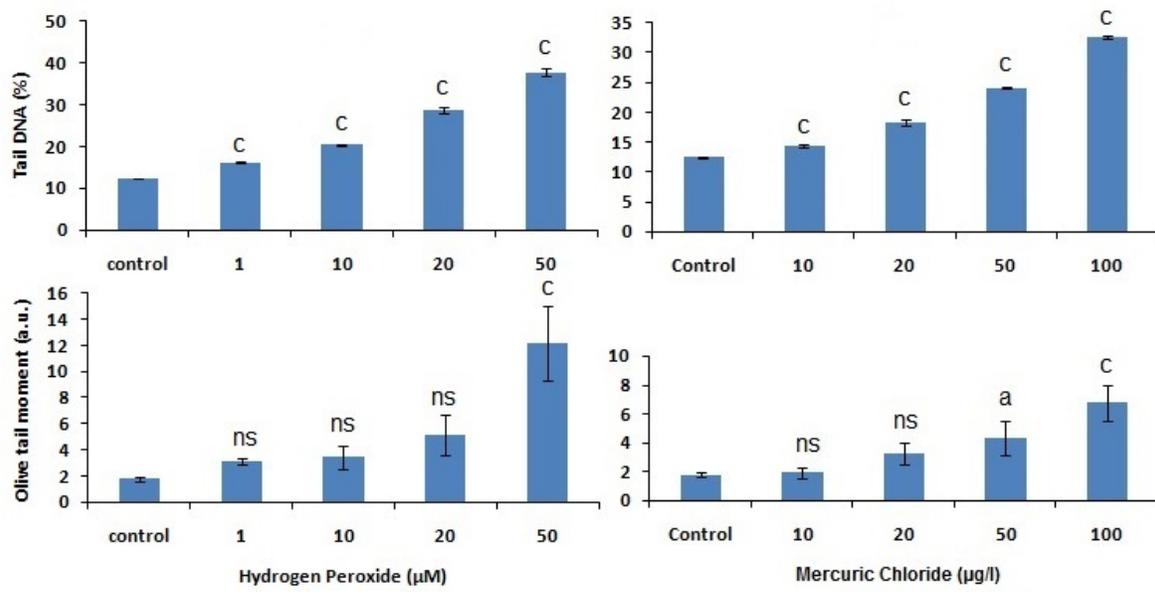


Fig. 1

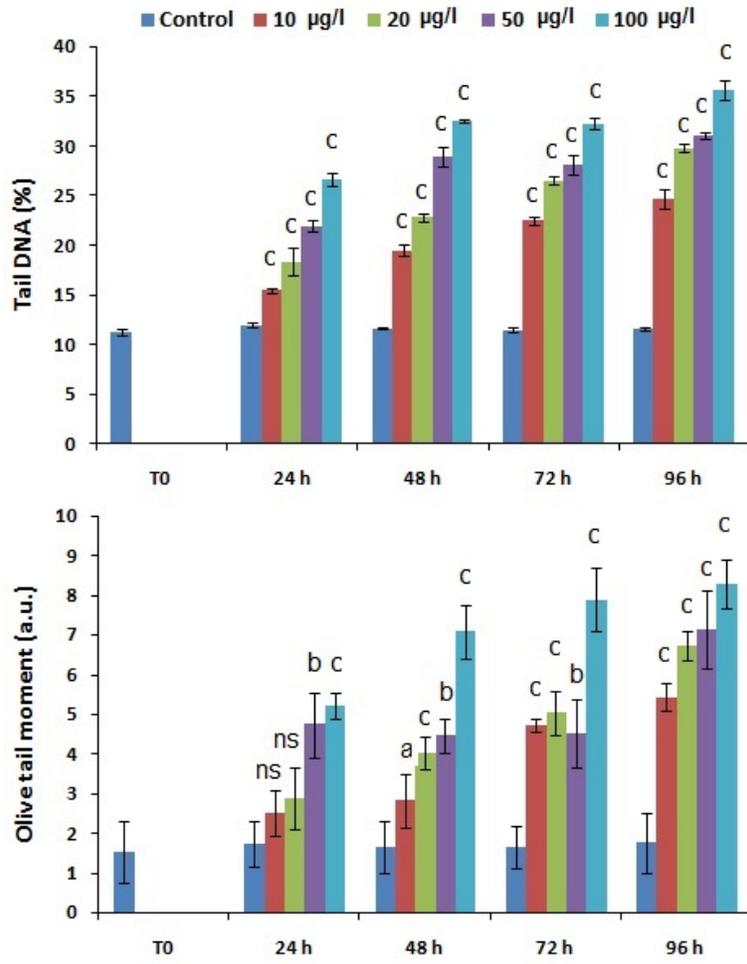


Fig. 2

Table 1. Concentrations of Hg reported in the coastal sediments and waters of India

Locations	Sediments (µg/kg)	Coastal waters (ng/l)	References
Bheemili, Central east coast of India	5.6–66.3	-	Chakraborty et al., 2014
Visakhapatnam, Andhra pradesh	7.7–9.4	-	Chakraborty et al. 2014
Kakinada, Central east coast of India	35.3–65.2	-	Chakraborty et al. 2014
Gangavaram, Central east coast of India	10.4–38.4	-	Chakraborty et al. 2014
Goutami Godavari Estuary, Central east coast of India	24.1–49.8	-	Chakraborty et al. 2014
Mandovi estuary, Goa	140	90	Verlecar et al., 2012
Ulhas Estuary, Maharashtra	460-6,400	-	Ram et al., 2009b
Amba Estuary, Maharashtra	50-2660	-	Ram et al., 2009a
Thane creek, Maharashtra	170 - 8210	-	Zingde & Desai, 1981
Chennai Harbour, Tamilnadu	237-338	100-2100	Jaysankar et al. 2003
Ulhas estuary, Maharashtra	0.5-55.28	-	Ram et al., 2003
Mangalore, Karnataka	133–172	45–1088	Ramaiah & De, 2003
Mumbai, Maharashtra	210–1390	180–440	Ramaiah & De, 2003
Mormugao, Goa	53–194	152–456	Ramaiah & De, 2003
Kodai Lake, Tamilnadu	276-350*	356-465	Karunasagar et al., 2006
Hooghly river, West Bengal	-	200-270	Sansgiry et al 1988
Arabian sea off Indian coast	-	26-130	Singbal et al., 1978

*units in mg/kg

Table 2. Concentrations of Hg reported in terms of dry weight ($\mu\text{g/g}$) and wet weight ($\mu\text{g/g}$) in gastropods from different parts of the world

Gastropods	Locations	dry weight ($\mu\text{g/g}$)	References
<i>Monodonta turbinata</i>	Tel Shikmona, Israel	0.012-0.123	Hornung et al., 1981
	Acre, Israel	0.959 to 1.939	Hornung et al., 1981
<i>Monodonta turbiformis</i>	Tel Shikmona, Israel	0.098-0.127	Hornung et al., 1981
<i>Patella caerulea</i>	Tel Shikmona, Israel	0.157	Hornung et al., 1981
	Acre, Israel	0.462-1.035	Hornung et al., 1981
<i>Cerithidea cingulata</i>	Hooghly Estuary, India	0.15	Bhattacharya & Sarkar, 1996
<i>Littoraria undulata</i>	Hooghly Estuary, India	0.29-0.43	Bhattacharya & Sarkar, 1996
<i>Nerita articulata</i>	Hooghly Estuary, India	0.22-0.27	Bhattacharya & Sarkar, 1996
<i>Cerithidea obtusa</i>	Hooghly Estuary, India	0.34-0.52	Bhattacharya & Sarkar, 1996
<i>Arcularia circumcinta</i>	Haifa Bay, Israel	38.7	Hornung et al 1984
<i>Arcularia gibbosula</i>	Haifa Bay, Israel	18.2	Hornung et al 1984
<i>Nassarius reticulatus</i>	Venice Lagoon, Italy	0.3-1.3	Berto et al., 2006
<i>Hexaplex trunculus</i>	Gulf of Taranto, Italy	0.224-1.867	Spada et al., 2012
	La Spezia, Italy	0.558	Giordano et al., 1991
	Gulf of Manfredonia, Italy	0.266	Giordano et al., 1991

	Croatian Coasts– Adriatic Sea	0.293	Buzina et al., 1989
wet weight (µg/g)			
<i>Bolinus brandaris</i>	Adriatic Sea, Italy	0.03-0.63	Bille et al., 2015
<i>Nassarius mutabilis</i>	Adriatic Sea, Italy	0.03-0.46	Bille et al., 2015
<i>Gibbula adriatica</i>	Grado and Marano Lagoons, Italy	0.480–1.220	Brambati,1997
<i>Rapana venosa</i>	Bohai Sea, China	0.038-0.199	Yawei et al., 2005
<i>Neverita didyma</i>	Bohai Sea, China	0.027-0.096	Yawei et al., 2005
<i>Peringia ulvae</i>	Ria de Aveiro, Portugal	0.030-0.250	Cardoso et al., 2013
<i>Littorina littorea</i>	France	0.040-0.088	Noel et al., 2011
<i>Haliotis tuberculata</i>	France	0.040-0.185	Noel et al., 2011
<i>Aplexa hypnorum</i>	Wash, East England	0.20-0.34	Moriarty & French, 1977
<i>Potamopyrgu jenkinsi</i>	Wash, East England	0.29-0.38	Moriarty & French, 1977
<i>Hydrobia neglecta</i>	Wash, East England	<0.01-0.07	Moriarty & French, 1977
<i>Planaxis sulcatus</i>	Arambol, Goa, India	0.009-0.01	This study