

**Genotoxicity of two heavy metal compounds: lead nitrate and cobalt chloride in Polychaete *Perinereis cultrifera***

***Nisha Singh, Jacky Bhagat\*, Baban S Ingole***

Biological Oceanographic Division, CSIR-National Institute of Oceanography

Dona Paula, Goa – 403004, India

\*Corresponding author: Jacky Bhagat, e-mail: bhagatjack@gmail.com

Present Address: Biological Oceanographic Division, CSIR-National Institute of Oceanography

Dona Paula, Goa-403004, India

Tel: +91(0)832-2450242, Fax: +91(0)832-2450602

**Abstract:**

The present study explores the *in vivo* and *in vitro* genotoxic effects of lead nitrate, [Pb(NO<sub>3</sub>)<sub>2</sub>] a recognized environmental pollutant and cobalt chloride (CoCl<sub>2</sub>), an emerging environmental pollutant in polychaete *Perinereis cultrifera* using comet assay. Despite widespread occurrence and extensive industrial applications, no previous published reports on genotoxicity of these compounds are available in polychaete as detected by comet assay. Polychaetes were exposed *in vivo* to Pb(NO<sub>3</sub>)<sub>2</sub> (0, 100, 500, and 1000 µg/l) and CoCl<sub>2</sub> (0, 100, 300, and 500 µg/l) for 5 days. At 100 µg/l Pb(NO<sub>3</sub>)<sub>2</sub> concentration, tail DNA (TDNA) values in coelomocytes were increased by 1.16, 1.43 and 1.55 fold after day 1, day 3 and day 5, whereas, OTM showed 1.12, 2.33 and 2.10 fold increase *in vivo*. Pb(NO<sub>3</sub>)<sub>2</sub> showed a concentration and time-dependent genotoxicity whereas CoCl<sub>2</sub> showed a concentration-dependent genotoxicity *in vivo*. A concentration-dependent increase in DNA damage was observed in *in vitro* studies for Pb(NO<sub>3</sub>)<sub>2</sub> and CoCl<sub>2</sub>. DNA damage at 500 µg/L showed almost three-fold increase in TDNA and approximately four-fold increase in OTM as compared to control *in vitro*. Our studies suggest that Pb(NO<sub>3</sub>)<sub>2</sub> and CoCl<sub>2</sub> have potential to cause genotoxic damage, with Pb(NO<sub>3</sub>)<sub>2</sub> being more genotoxic in polychaete and should be used more carefully in industrial and other activities.

**Keywords:** DNA damage; invertebrate; single cell gel electrophoresis; environmental pollution

## Introduction

Heavy metal pollution in soil has drastically increased, resulting in deterioration of the environment and posing a potential risk to human and animal health. Heavy metals with no established biological functions such as cadmium, mercury, lead etc. are extensively studied because of their toxicity and carcinogenicity in human or animals. Whereas, toxicological studies of heavy metals with bio-importance such as cobalt, copper, chromium, nickel etc. are scarce. Lead pollution has been an important global environmental issue affecting organisms through contaminated soil, water or air. Lead is everywhere and has a very wide range of application from smelting, mining, refineries, and battery manufacturing etc. (Mohammadi et al., 2008). The marine environment forms a sink for lead by adsorbing it onto sediment, suspended particles and its availability to benthic organisms. Anthropogenic emissions, especially over the last two hundred years, have resulted in an increase in lead concentrations up to several orders of magnitude higher than estimated natural background levels in soil and water (Hoffman et al., 1995). Higher pollution of lead has been reported in estuaries as compared to marine areas around India (Chakraborty et al., 2015). Cobalt is widespread in the environment and commonly used in petroleum, plastic and chemical industries. Cobalt has not gained much attention from ecotoxicologists until recently when reports of its extensive use and discharge started appearing in sediment and water from several parts of the world viz. Macedonia (Stafilov et al., 2009), democratic republic of Congo (De Putter et al., 2011), Ghana (Cynthia et al., 2011), Sweden (Strom, 2012), Turkey (Durmaz et al., 2016), Nigeria (Attahiru et al., 2015), Uganda (Mwongyera et al., 2014), Mexico (Shumilin et al., 2013), and India (Pol et al., 2015). The anthropogenic addition of cobalt has been reported in Swarna estuary, India (Fernandes and Nayak, 2015). High values of cobalt (above apparent effect threshold) have been reported in Swarna Estuary and Gurpur estuary in Karnataka, India (Fernandes and Nayak, 2015). Significant enrichment of cobalt and lead were reported in Rameswaram coast-Gulf of Mannar (Koigoora et al., 2013). A recent study by Squadrone et al., (2016) evaluated the human exposure of cobalt and other metals through consumption of heavily contaminated fish.

Cobalt is categorized as Group 2B (possible carcinogen to human) elements by International Agency for Research on Cancer (IARC) (Lison et al., 2001). In the same evaluation, Cobalt (II) compounds were reported to induce DNA damage, DNA-protein cross-links, sister chromatid exchanges, gene mutations, and aneuploidy *in vivo* and *in vitro* studies. In recent years several studies have been conducted on cobalt pollution and its impact on plants, corals, and silkworms (Begovića et al., 2016; Reichelt-Brushetta & Hudspith, 2016; Biscere et al., 2015; Ashfaq et al., 2009).

Cobalt chloride ( $\text{CoCl}_2$ ) has been reported to cause oxidative stress in cells by production of reactive oxygen species (ROS) or altering antioxidant mechanism, leading to DNA damage in the cell (Akinrinde et al., 2016). Over the past decade, comet assay has become one of the standard methods for assessing DNA damage with applications spreading in different fields of research. The Comet assay has applications in testing novel chemicals for genotoxicity, monitoring environmental contamination with genotoxins, human biomonitoring, molecular epidemiology, and fundamental research in DNA damage and repair (Frenzillet et al., 2009).

It is essential to include aquatic invertebrates as test organisms under relevant exposure conditions for environmental pollution studies because they represent key species in the transformation, detoxification, and cycling of environmentally relevant contaminants discharged into aqueous environments. Further, they are known to be good indicators of species richness, pollution and benthic community patterns with ecosystem condition (Włodarska-Kowalczyk and Kedra, 2007). Polychaetes are one of the most abundant groups of macrofauna in benthic community, and play a vital role in ecosystem functioning, not only because of their dominance, but also their different feeding modes and reproductive strategies (Lewis and Galloway, 2008). *Perinereis cultrifera* (Grube, 1840) has been studied as a biomonitor for marine pollution (Snani et al., 2015; Guemouda et al., 2014). Several studies have reported *P. cultrifera* as a suitable bioindicator for heavy metal contamination in sediment (Dean, 2008; Ghirardini et al., 1999). In view of the current trend of increasing lead and cobalt pollution, this study was carried out to evaluate the *in vivo* and *in vitro* genotoxicity of  $\text{CoCl}_2$  and  $\text{Pb}(\text{NO}_3)_2$  in polychaete *P. cultrifera*.

## **Materials and Methods:**

### *Chemicals and Instruments*

Ethidium bromide, ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), gluaiacol glycerol ether, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were purchased from Sigma-Aldrich Pvt. Ltd. (Mumbai, India); Frosted slides and cover slips were supplied by Yogeshwari Agencies (Himedia, Goa, India); dimethyl sulfoxide was obtained from Scitech Scientific (Qualigens, Goa, India); Tris buffer and triton X-100 were obtained from Sadhale Enterprise (Merck, Goa, India). Electrophoresis apparatus (Technosource, Mumbai), Comet workstation (Comet 6.0, Andor technology, UK), fluorescent microscope (DM1000, Leica, Germany) were used for comet assay.

### *Collection of organisms*

*P. cultrifera* [Animalia (Kingdom), Annelida (Phylum), Polychaeta (Class), Phyllodoceida (Order), Nereididae (Family), *Perinereis* (Genus), *Perinereis cultrifera* (Species)] were collected from the

rocks scattered along Palolem, Goa (15° 0'44.79"N, 74° 1'0.13"E) during low tide in the month of December, 2015. They were brought to the laboratory in a plastic container within 3 hours of collection. Palolem was selected as reference site because of its pristine environment (Gaitonde et al., 2006). Organisms were identified in a stereo microscope using the key from Fauvel, (1932). The temperature of the pool-water was recorded as 28°C at the time of collection. Seawater and sediment from the collection site were used to eliminate disturbance factors that might affect uptake rate of heavy metal and hence potential toxicity or physiological responses (Sun and Zhou, 2007). Prior to use, the sediment was rinsed using filtered seawater (0.2µm), sieved through 450µm mesh and allowed to settle in tap water to kill the meiofauna. The worms were maintained in sediments overlain by seawater (35‰ salinity) in an aerated glass aquarium and acclimatized for 48 hrs in the laboratory.

#### *In vivo exposures*

The experiment was performed using glass aquaria of 2.5 liters containing 3cm of bottom layer sediment and 1.5 L of filtered (0.2µm) seawater. During the whole experiment, the water temperature was 26±2°C with 35‰ salinity. Lead and cobalt toxicity was tested in the form of lead nitrate, Pb(NO<sub>3</sub>)<sub>2</sub> and cobalt chloride (CoCl<sub>2</sub>). Pb(NO<sub>3</sub>)<sub>2</sub> and CoCl<sub>2</sub> stock solution of 100mg/l was prepared with ultra pure water and stored at 4°C in 250 ml volumetric flask. Appropriate volumes of stock solution were added in the seawater in different glass jars and stirred to mix well along with sediment to achieve different exposure concentrations (100, 300 and 500 µg/l for Pb(NO<sub>3</sub>)<sub>2</sub> and 100, 500 and 1000 µg/l for CoCl<sub>2</sub>). The water and sediment were allowed to stabilize before the introduction of polychaetes. Worms were rinsed using filtered seawater (0.2µm) and were transferred equally (12 polychaetes) in each experimental jar. Control groups were not given any treatment.

The experimental units were aerated by air bubbling in the superficial layers of the water column to provide an oxygen-saturated environment and exposed to 12-12 hr of the light-to-dark regime during the exposure period of five days. All beakers used in the experiments were covered with glass utensils in order to reduce evaporation and heat dissipation. During the acclimation and exposure periods, worms were fed natural sieved sediment without additional food supply. All exposure sediment and solution was renewed on the third day of exposure period to get rid of worm waste products.

#### *Isolation of Coelomocytes*

The worms were carefully picked up and 20-30µl coelomic fluid was extracted by gently inserting 1ml syringe fitted with 21G needle (chilled prior to use) into the posterior part of worm avoiding the

guts. Coelomic fluid was mixed with phosphate buffer saline (PBS, 1.2 M NaCl, 0.027 M KCl, 11.5 mM  $K_2HPO_4$ , 0.08 M  $Na_2HPO_4$ , pH 7.3) and stirred at 3000 rpm for 2 minutes. The cell pellet was used for further study.

#### *In vitro exposures*

Coelomocytes from 6-8 polychaetes were isolated and mixed with PBS solution. Appropriate volumes of  $Pb(NO_3)_2$  and  $CoCl_2$  stock solution of 1000  $\mu g/l$  were added in the solution to achieve different exposure concentrations. Coelomocytes were exposed to different concentrations of  $Pb(NO_3)_2$  (100, 300 and 500  $\mu g/l$ ), and  $CoCl_2$  (100, 300 and 500  $\mu g/l$ ) for 1 hour while kept on ice in microcentrifuge tubes. 50  $\mu g/l$  of  $H_2O_2$  was used as a positive control. Cells were washed thrice with PBS and later used for comet assay.

#### *Comet assay*

Comet assay was performed in coelomocytes according to the methods of Singhet al., (1988) with slight modifications. Cell concentrate was mixed with 200  $\mu l$  of 0.55% low melting agarose (LMA) and 100  $\mu l$  of the mixture was dropped on to a slide previously coated with 1% normal melting agarose (NMA). Once the cell layer solidified, 100  $\mu l$  of 0.5% LMA was poured to make the protective third layer, followed by cell lysis in cold lysis 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 for 2 hours. Further steps were performed in yellow/dimmed light to prevent DNA damage that may arise from direct exposure to light. The slides were placed in electrophoretic buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) for 40 minutes for alkali unwinding of DNA strands. Electrophoresis was carried out in same buffer at 25 volts ( $\sim 0.74$  V/cm between electrodes) and 300 mA for 20 minutes under cold condition to avoid DNA damage due to heat generated during flow of current. The slides were neutralized with Tris buffer (0.4 M Tris, pH 7.4) for 2-3 times with an interval of 5 minutes each. DNA was stained with 100  $\mu l$  of ethidium bromide (20  $\mu g/ml$ ) and visualized at 40X magnification under Leica DM 1000 LED fluorescent microscope equipped with an excitation filter of 515-560 nm, barrier filter of 590 nm. Images were analyzed using Komet<sup>TM</sup> 6.0 image analysis software linked with ANDOR Camera. All the measurements are expressed in terms of percentage of DNA migrated from the comet head to the tail region (mean percentage tail DNA, TDNA), and mean olive tail moment (OTM).

#### *Statistical Analysis*

Worms were collected once from the sampling sites and three technical replicates were used for comet assay. In each replicate, fifty randomly selected cells per slide were analyzed for *in vivo* and *in vitro* comet assay with 2 slides per incubation (total 100 cells) and experiments were performed in

triplicate (total of n=300 cells/treatment). Results were expressed as mean  $\pm$  standard deviation. Kolmogorov–Smirnov test was used for normality distribution. Data were analyzed by analysis of variance (ANOVA) followed by Tukey HSD post-test. All statistical comparisons were performed using OriginPro 8.5.0.

## Results

### *In vivo Exposure*

Induction of DNA strand breaks measured in terms of TDNA and OTM was found to be minimum as well as consistent in case of control group exposed to only seawater throughout 5 days. The increase in DNA damage was observed with increase in concentration along with the lengthening of time duration. At 100 $\mu$ g/l Pb(NO<sub>3</sub>)<sub>2</sub> concentration, TDNA values in coelomocytes were increased by 1.16, 1.43 and 1.55 fold after day1, day3 and day5, whereas, OTM showed 1.12, 2.33 and 2.10 fold increase (Fig. 1). Significant increase in TDNA was observed at day1, day3 and day5 for 300 $\mu$ g/l and 500 $\mu$ g/l as compared to control (p<0.005). When TDNA values for 100 $\mu$ g/l and control at day1 and day3 were compared, it was not found to be significant. OTM values for 500 $\mu$ g/l were found to be significantly different as compared to control on day1, day3 and day5 (p<0.005). Hence the DNA damage response was both time-dependent as well as concentration-dependent.

A concentration-dependent increase in DNA damage response was observed in polychaetes coelomocytes to different concentrations of CoCl<sub>2</sub> (Fig. 2). Compared to control, the DNA damage (TDNA and OTM) was higher at all concentrations of CoCl<sub>2</sub>. On day1, a significant increase in TDNA and OTM was observed at 1000  $\mu$ g/l as compared to all the other exposures tested (p<0.005). The DNA damage decreased on day3, compared to first-day (Fig. 2). However, there is an increase in DNA damage observed on day5 after a decrease on day3 of exposure. At an exposure concentration of 1000  $\mu$ g/l, 2.7, 2.4 and 4.5 fold increase in OTM were observed in coelomocytes of polychaetes after day1, day3, and day5 respectively as compared to control. On day5, all the exposure concentration showed a significant increase in TDNA and OTM as compared to control (p<0.005).

### *In vitro exposure*

DNA integrity in *P. cultrifera* exposed to various concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> (0, 100, 300, 500  $\mu$ g/L) were in the range from 27.66 $\pm$ 0.98 to  $\pm$ 28.95 for TDNA and 7.53 $\pm$ 0.04 to 28.95 $\pm$ 2.02 for OTM. There is a gradual increase in DNA damage with concentration, hence showing a clear concentration-dependent response for both the comet parameters assessed (Fig. 3). Significant increase in TDNA was observed at all concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> as compared to control (p<0.005).

The degree of DNA damage in *P. cultrifer* showed a concentration-dependent increase with an increase in  $\text{CoCl}_2$  concentration. The level of DNA damage was highest (TDNA=  $89.80 \pm 1.05$ , OTM=  $57.69 \pm 1.68$ ) in positive control (50  $\mu\text{g/l}$   $\text{H}_2\text{O}_2$  concentration) and lowest in control (TDNA=  $27.66 \pm 0.98$ , OTM=  $7.53 \pm 0.04$ ). Significant increase in TDNA and OTM was observed at all the  $\text{CoCl}_2$  concentration as compared to control value ( $p < 0.005$ ). DNA damage at 500  $\mu\text{g/L}$  showed an almost three-fold increase in TDNA and an approximately four-fold increase in OTM as compared to control (Fig. 4).

## Discussion

Comet assay has been applied to study the genotoxic effect of persistent and emerging environmental pollutants such as oil contamination (Morales-Caselles et al., 2009), fluoranthene (Palmqvist et al., 2006), silver nanoparticle (Conget et al., 2014), and nano-titanium dioxide (Galloway et al., 2010) in polychaete. In the present study, we observed concentration and time-dependent increase in TDNA and OTM in polychaete exposed *in vivo* to  $\text{Pb}(\text{NO}_3)_2$ . Our study showed 2.4 and 3.7 fold increase in TDNA and OTM respectively in coelomocytes after 5 days of exposure to 500  $\mu\text{g/l}$   $\text{Pb}(\text{NO}_3)_2$ . Our results are in accordance with those of Osman et al., (2008) where *in vivo* exposure to 100-500  $\mu\text{g/l}$  of  $\text{Pb}(\text{NO}_3)_2$  for 168h has shown dose and time-dependent increase in DNA damage as measured by comet assay in catfish *Clarias gariepinus* (Burchell, 1822).  $\text{Pb}(\text{NO}_3)_2$  is known for its toxicity and can induce DNA damage, oxidative stress, necrosis and cell death (Yedjou et al., 2015). Our *in vitro* study showed 2.5 and 3.8 fold increase in TDNA and OTM after exposure to 500  $\mu\text{g/l}$  of  $\text{Pb}(\text{NO}_3)_2$ . A wide range of biological response has been reported on exposure of humans to lead. The effects produced are at sub cellular level or on the overall functioning of the body.  $\text{Pb}(\text{NO}_3)_2$  induced DNA damage is widely studied in human cells (Yedjou et al., 2010; Zhang et al., 2009) and rat blood and liver cells (Bas et al., 2015; Rabbani-Chadegani et al., 2009). *In vitro* exposure to  $\text{Pb}(\text{NO}_3)_2$  has shown to induce single strand DNA breaks in lymphocytes from human peripheral blood (Pasha Shaik et al., 2006).

Genotoxicity of Pb is also related with oxidative stress that occurs when there is an imbalance between the formation and removal of ROS (Hermes-Lima et al., 1991). The ability to detoxify the ROS and its intermediates are crucial in an organism as it leads to damage in the DNA, lipid or proteins. Toxic effect of lead was examined in different life stages of polychaete *Hydroides elegans* (Haswell, 1883) (Gopalakrishnan et al., 2008). Activation of antioxidant system has been observed in polychaete *Perinereis aibuhitensis* (Grube, 1878) exposed to  $\text{Pb}^{2+}$  (Tian et al., 2014). Exposure to lead has shown to cause oxidative stress by elevating the malondialdehyde levels, intracellular ROS, caspase-3 activity and depleting intracellular glutathione (GSH) levels (Liu et al.,

2004). In another study Flora et al., (2007) also reported a significant reduction in superoxide dismutase, catalase, glutathione (GSH), along with significant increase in ROS and thiobarbituric acid reactive substances (TBARS) levels in wistar rats. Pb inhibits antioxidant enzymes CAT, SOD, GPx by forming complex with its substrate (Ercal et al., 2001). In another study, Yedjou et al.,(2010) also reported a dose-dependent increase in cytotoxic and oxidative stress in human liver carcinoma (HepG2) exposed to  $Pb(NO_3)_2$ . The DNA damage can be in the form of single- and double-strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations, and structural and numerical chromosomal aberrations. Increase in frequency of chromosomal aberrations was also reported in fish *Hoplias malabaricus* (Bloch, 1794) (Ramsdorf et al., 2008) and in ICR Swiss Webster mice (Nayak et al., 1989) exposed to  $Pb(NO_3)_2$ . Inorganic salts of lead such as lead chromate and lead acetate also showed significant increase in DNA damage in human lymphocyte and lung fibroblasts respectively (Xie et al., 2005; Wozniak and Blasiak, 2003).

Lead accumulates in sediments and can pose a hazard to sediment-dwelling organisms. *Ruditapes philippinarum* (Adams & Reeve, 1850), when exposed to 700  $\mu\text{g/L}$  of  $PbCl_2$ , has shown to accumulate Pb reaching 170 and 160  $\mu\text{g/g}$  (dw) in soft body and gill, respectively (Blasco and Puppo, 1999). Rainbow et al., (2011) study on *Hediste diversicolor* (O.F. Muller, 1776) shows the bioaccumulation capacity of Pb, where Pb body burden reached 89  $\mu\text{g/g}$  dry weight in sediment containing 336  $\mu\text{g/g}$  of Pb dry weight. Polychaete *Laeonereis acuta* (Treadwell, 1923) were reported to accumulate cadmium after short term exposure for 4-24 h (Sandrini et al., 2006). The author also reported increase in oxidative stress parameters after 8 h of exposure to polychaete. Lead nitrate is also reported to be phytotoxic by affecting seedling growth plant *Sesbania grandiflora* (Malar et al., 2014). A recent study by Venkatachalam et al., (2017) also reported that lead exposure caused amplification of new DNA bands in medicinal plant *Acalypha indica* L.

We observed concentration-dependent increase in TDNA and OTM in  $CoCl_2$  exposed polychaetes. A dose-dependent increase in DNA damage and chromosome aberrations were also reported in *Eisenia hortensis* (Machaelson, 1890) earthworm coelomocytes exposed to  $CoCl_2$  (Ciğerci et al., 2016). In our study, a slight decrease in TDNA and OTM was observed at Day3 when compared to Day1. The decrease in DNA damage can be attributed to induction of one or more repair mechanism (Palmqvist et al., 2003). An increase of 1.2, 1.6 and 1.4 fold were observed for TDNA at 100, 500 and 1000  $\mu\text{g/l}$  respectively at Day5 as compared to Day1. However, at 1000  $\mu\text{g/l}$ , no significant changes in both of these comet parameters were observed for Day1 and Day5. The genetic toxicology of cobalt was widely studied in plants and mammalian models (Yildiz et al., 2009; Kirkland et al., 2015). There are several publications describing induction of direct DNA damage in mammalian cells, mainly using



the comet assay as detection method. Induction of DNA damage by  $\text{CoCl}_2$  in human Jurkat T lymphocytes (Caicedo et al., 2008) and in isolated human lymphocytes (Ponti et al., 2009) was reported using the comet assay. De Boeck et al., (1998) and Van Goethem et al., (1997) also reported induction of direct DNA damage with low concentrations of ultrafine cobalt metal in human leukocytes and blood lymphocytes from three different donors using the alkaline version of the comet assay, although in a later paper, De Boeck et al., (2003) were unable to reproduce the DNA damage responses. Multiple toxicities of cobalt to the nematode *Caenorhabditis elegans* and its progeny have been reported by Wang et al., (2007). The elevated level of DNA damage as measured by comet assay may be due to the formation of ROS, which react with DNA and forms DNA adducts leading to single strand breaks (Misra et al., 1993). Our *in vitro* study demonstrates a significant increase in TDNA and OTM in coelomocytes of polychaetes exposed to cobalt chloride. *In vitro* studies suggest that Co(II) ions exert genotoxicity via fenton like mechanism or by affecting the DNA repair pathways (Lison, 2001). Significant increase in TDNA was observed in Balb/3T3 mice fibroblasts exposed to  $\text{CoCl}_2$  (Ponti et al., 2009).

The DNA damage reported in this study may be due to accumulations of metal ions and its interaction with cellular components DNA, protein or lipids. The increase in the concentration of genotoxic pollutants is reflected by an increase in the proportion of DNA damage via interfering with DNA repair mechanism, forming DNA adducts (Sarkar et al., 2010). Higher concentration of genotoxic pollutants produces more profound effect on genetic material producing more fragmented or damaged DNA. The high amount of fragmented DNA is reported in our study with higher exposure of  $\text{Pb}(\text{NO}_3)_2$  and  $\text{CoCl}_2$ .

## **Conclusion**

Marine polychaetes, one of the most dominant benthic macrofauna communities, have been studied as an indicator organism to study the toxicity of heavy metals (Dean, 2008). Their deposit or filter feeding nature and maximizing exposure to contaminants make them suitable indicator organism for environmental biomonitoring. *P. cultrifera* experimental exposure to  $\text{Pb}(\text{NO}_3)_2$  shows a consistent concentration and time-dependent response hence establishing itself as a good candidate for monitoring lead concentration in the marine ecosystem. The exposure of coelomocytes from polychaetes to  $\text{CoCl}_2$  establishes that the organism doesn't have a consistent response but shows a higher level of DNA damage when exposed to cobalt, suggesting that it can be used for biomonitoring of increasing level of cobalt in marine environment. The results of genotoxicity exposure tests through *in vitro* and *in vivo* experiment are very similar thus clearly validating the data of the exposure experiments. This study was limited to evaluation of DNA damage in polychaetes *P.*

*cultriferato* single heavy metal. For a better understanding of genotoxic substance and its effect on marine organisms, further study using multiple biomarkers and exposures in combinations need to be conducted to understand the effect at the ecosystem level and also accumulation of genotoxic substance in lower organisms and its transfer to higher trophic level.

### **Acknowledgements**

The authors are thankful to the Director, Council of Scientific & Industrial Research (CSIR)-NIO for all the facilities and encouragement and CSIR New Delhi for their financial support (Project No. PSC0206). Department of Biotechnology (Govt. of India), New Delhi is acknowledged for Senior Research Fellowship (SRF) to Jacky Bhagat. They also like to thank Dr. Sanitha Sivadas and Mr. Periasamy for their help in sampling and identification of polychaete. They are grateful to Mr. D P Rao for providing facilities to conduct comet assay. This is contribution No..... of CSIR-NIO, Goa.

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### Figure Captions:

**Fig. 1:** Olive tail moment (OTM) and percentage tail DNA (TDNA) in coelomocytes of polychaetes *P. cultrifera* exposed to different concentrations of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>](in vivo). Values are represented as means± standard deviation. Experiments were performed in triplicate (total of n=300 cells/treatment).

**Fig. 2:** Olive tail moment (OTM) and percentage tail DNA (TDNA) in coelomocytes of polychaetes *P. cultrifera* exposed to different concentrations of cobalt chloride (CoCl<sub>2</sub>) (in vivo). Values are represented as means± standard deviation. Experiments were performed in triplicate (total of n=300 cells/treatment).

**Fig. 3:** Olive tail moment (OTM) and percentage tail DNA (TDNA) in coelomocytes of polychaetes *P. cultrifera* exposed to different concentrations of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>](in vitro). Values are represented as means± standard deviation. Experiments were performed in triplicate (total of n=300 cells/treatment).

**Fig. 4:** Olive tail moment (OTM) and percentage tail DNA (TDNA) in coelomocytes of polychaetes *P. cultrifera* exposed to different concentrations of cobalt chloride (CoCl<sub>2</sub>) (in vitro). Values are represented as means± standard deviation. Experiments were performed in triplicate (total of n=300 cells/treatment).



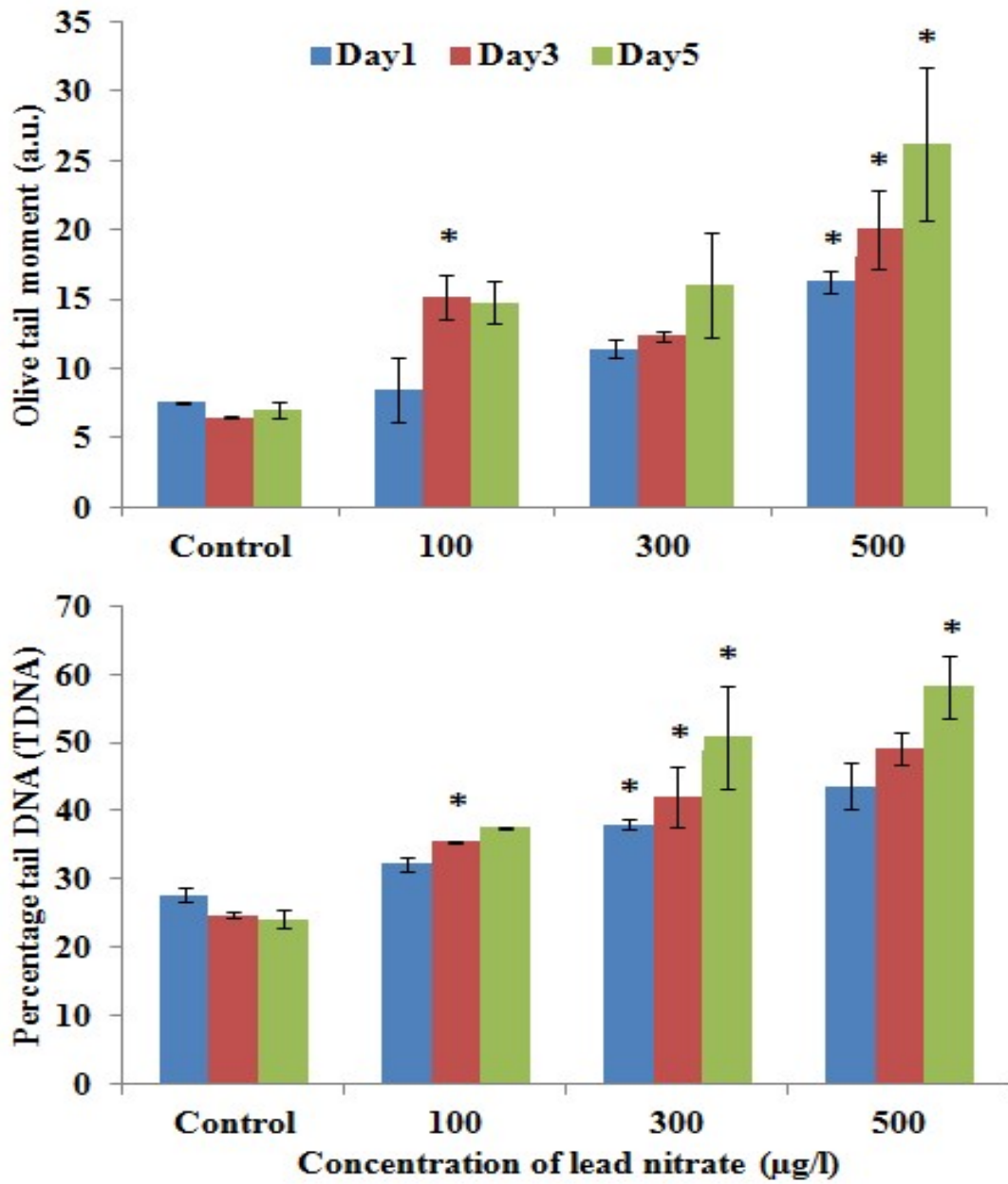


Fig.1

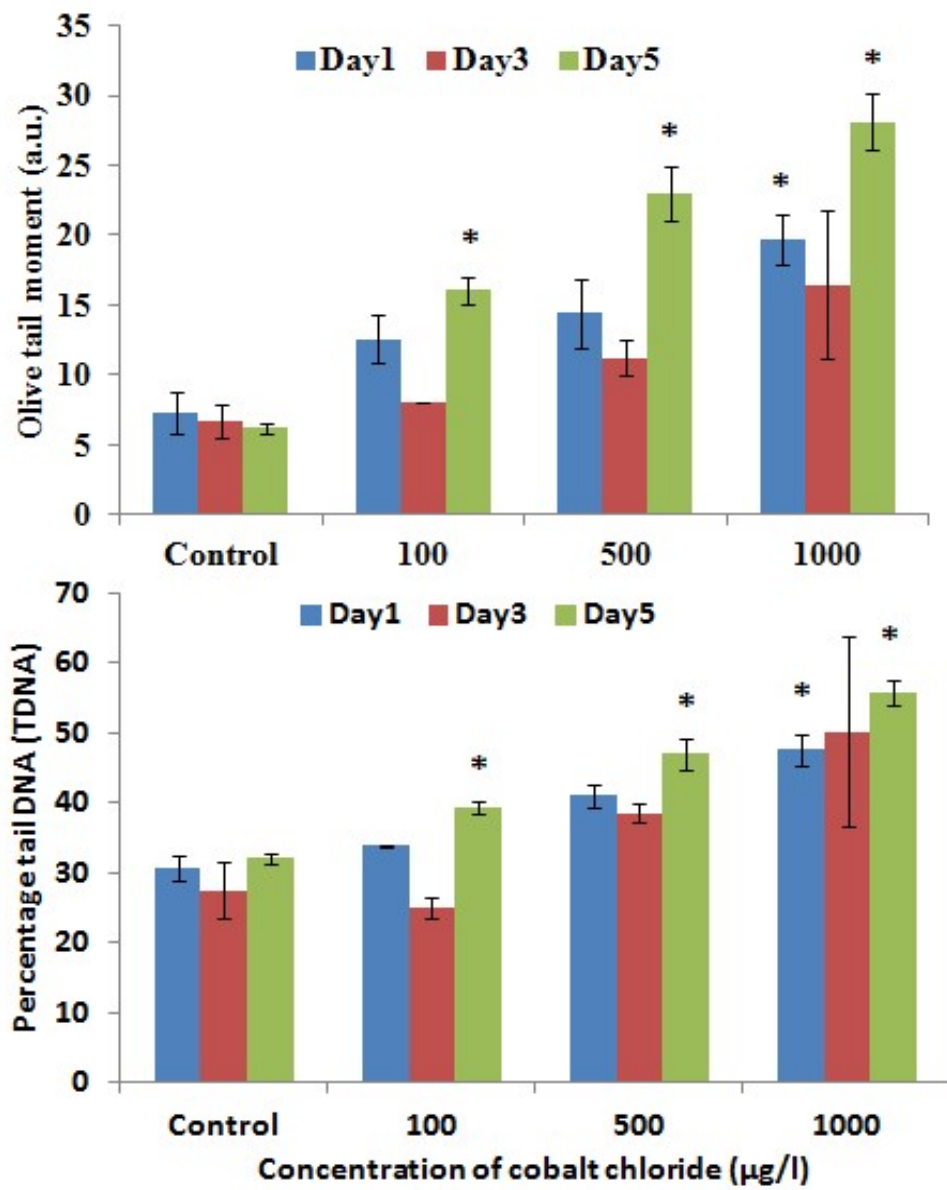


Fig.2

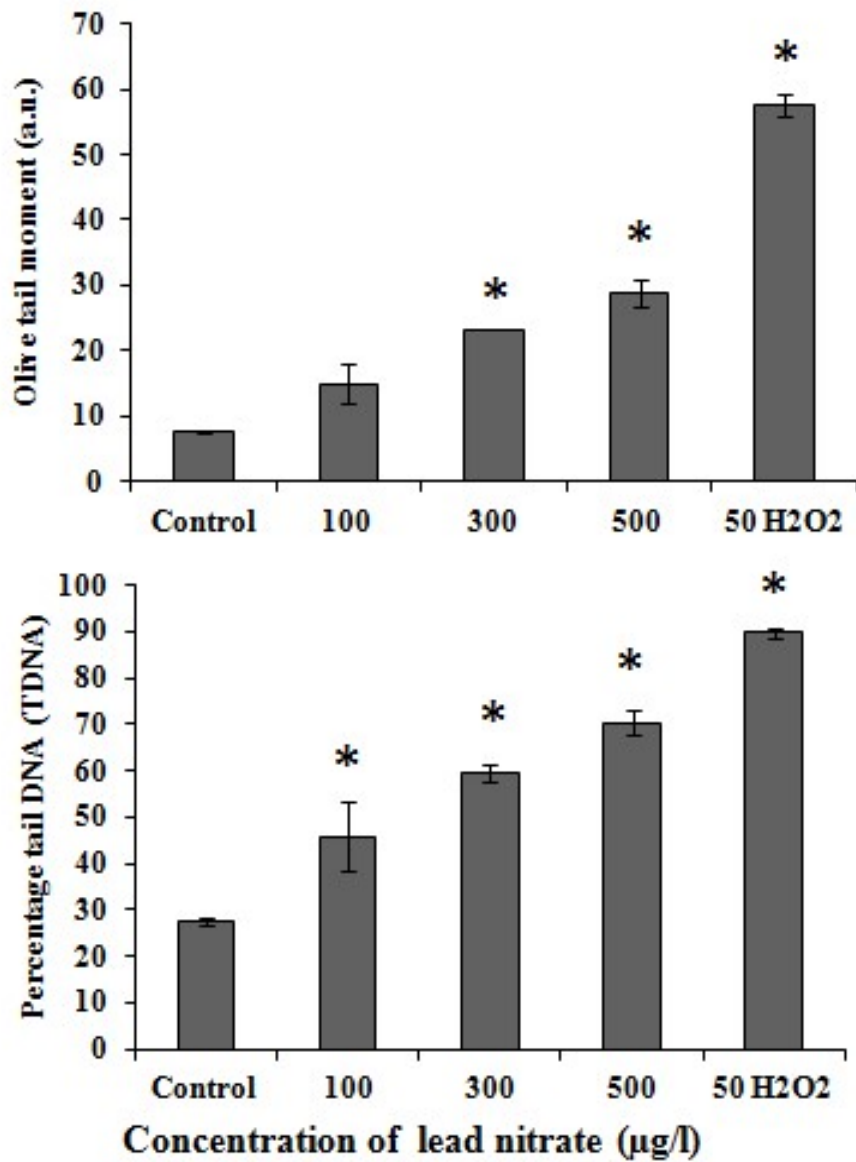


Fig.3

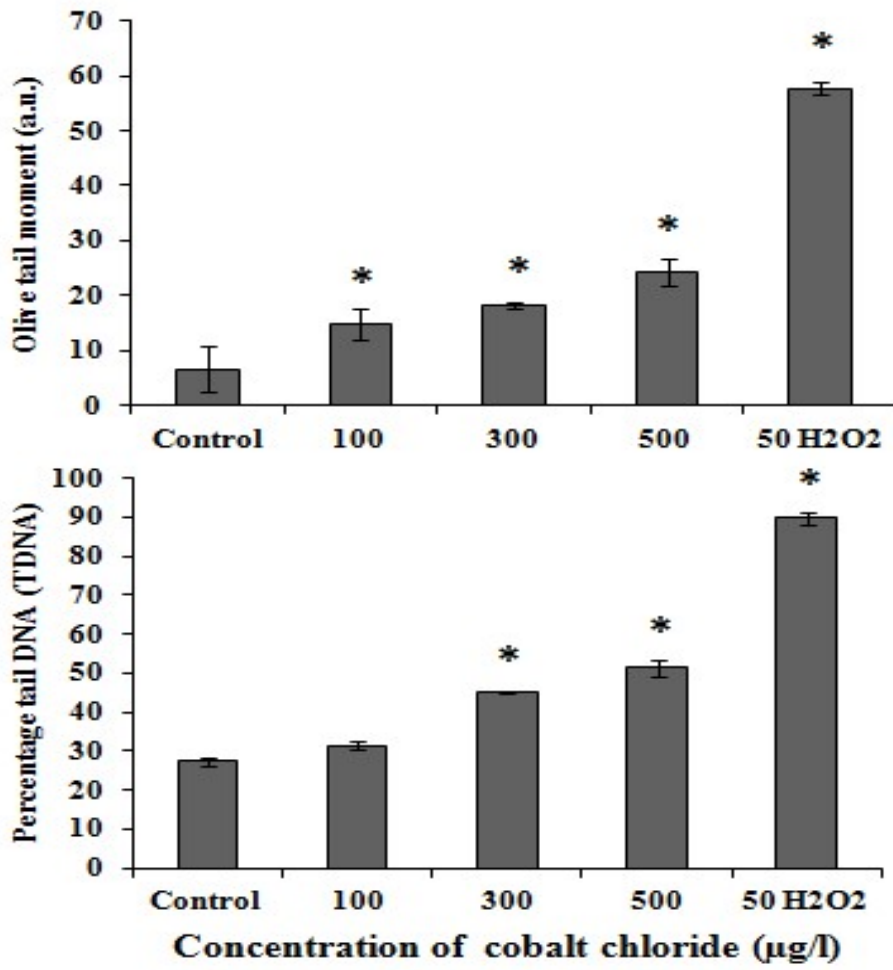
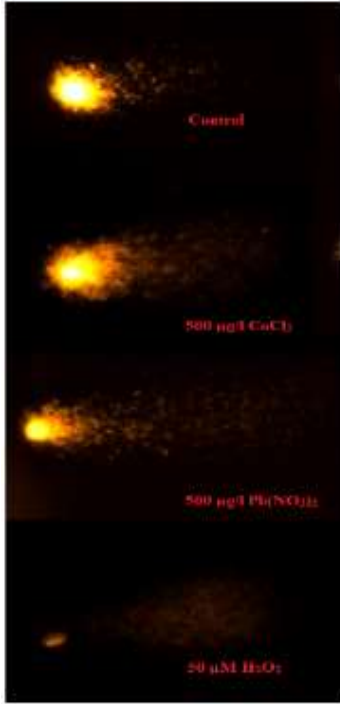


Fig.4.



DNA Damage

