Oxidative stress and genotoxicity assessment of silicon di oxide nanoparticle in snake headed murrel, *Channa punctatus*

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**Abstract**
Consequences of waterborne silicon di oxide nanoparticles (SiO$_2$ NPs) on snake headed fish, *Channa punctatus* were investigated and analyzed for oxidative stress and genotoxicity in a long-term assay. Fish were divided into three groups: Group I (control), Group II and III considered as treated groups. Fish were exposed to 25% and 50% of LC$_{50}$ value (60 and 120 mg/l of SiO$_2$ NPs) at day 7, 15, 21, 28 and 35. Fish exposed to higher concentrations of SiO$_2$ NPs showed major alterations in oxidative stress markers. Significant alterations were noted in SOD, CAT, GST, GSH, GPx and GR in the gills, liver and kidney of fish of both treated groups. A significant increase of SOD, CAT, GST, GPx and GR in the gill, liver and kidney of fish was observed, whereas GSH showed significant decrease in all treated groups in all tissues. LPO was also estimated in gills, liver and kidney and elevation was noted. By standard genotoxicity test such as micronucleus test and comet assay, genetic damage (cytoplasmic, nuclear and DNA damage) was recorded which indicates significant toxicity of SiO$_2$ NPs at higher concentrations. Significant changes in all parameters studied thus reflected an adverse influence of SiO$_2$ NPs exposure on oxidative stress as well as genotoxicity of the fish. SiO$_2$ NPs toxicity appeared to be dose and duration dependent. The presence of SiO$_2$ NPs leads to an activation of the antioxidant defense system and the occurrence of lipid and genetic damage if antioxidant enzymes were unable to overcome oxidative stress.

**Keywords:** *Channa punctatus*, nanoparticle, ecotoxicology, silicon di oxide, oxidative stress, genotoxicity

**1. Introduction**
In numerous branches of science such as nanofabrication, nano-medicine, nano metrology and nano materials, nanoparticles are used to assess health risks of biota. Organisms can be exposed to NPs via inhalation, orally, derm ally, nasal, ocular, respiratory and gastrointestinal routes and distributed through the circulatory and lymphatic systems to major organs. NP in aquaculture is a complex topic that needs to be developed in the near future to ensure human health and environmental safety. Massive amounts of SiO$_2$ NPs are produced annually, and therefore discharged into water bodies [1] possibly causing undesired effects on fish and aquatic invertebrates. SiO$_2$ NPs are one of the most stable among NPs, even in different organic solvents as well as aqueous solutions with high ionic strength [2-5]. Amid innumerable types of nanoparticles, Silicon di oxide (SiO$_2$ Nps) has been favoured as nano structuring, optical imaging, biomedical agent [6, 5]. Biotechnological applications such as gene and siRNA delivery, biosensors, nano medicine and cancer therapy have also been observed [7]. Evaluation of toxicity of SiO$_2$ NPs using human and mammalian models both *in vivo* [8, 9] and *in vitro* [10-12] have been reported. Fishes are most common organisms which are frequently used in ecotoxicological studies as biological indicators. Fish is an important food source for humans and monitoring their Nps levels is therefore important to ensure food safety are also used in the production of fish feeds.

Unfortunately, speedy integration of Nps into everyday life has additionally aggrevated the concern due to release of Nps into the aquatic environment. Despite growing cognizance of Nps bioaccumulation within the aquatic environment in depth studies are required to observe their effects on aquatic organisms. Finite and detailed evaluation of the effects of SiO$_2$ nanoparticle exposure to fish *C. punctatus* will be evaluated in the present study since
Genotoxicity and oxidative stress have not been assessed after chronic exposure (35 days) of fish to sub lethal concentrations of 60 and 120 mg/l.

2. Materials and Methods

2.1. Determination of LC50

The acute toxicity of SiO2 Nps was assessed on the freshwater fish, *Channa punctatus* for 96 h. Nanoparticles with size, 15 nm of SiO2 was evaluated for the median lethal concentration by probit analysis. For LC50 determination fish was exposed with different concentrations (with two replications for each treatment) of SiO2 Nps including control, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 and 300 mg/l for 96 hours, maintaining 10 animals per group. Mortality of fish was observed and recorded throughout the experiment. Median lethal concentrations that was determined by probit analysis was found to be 260 mg/l for SiO2 NPs.

2.2. Test chemical and toxicity testing

SiO2 Nps (Cat no. 1940323) were obtained from SISCO Research Laboratory (SEL), India. The pre characterized SiO2 nanoparticles of 15 nm size were used for the present study. Nanodispersions were prepared (just before exposure of fish) by ultra-sonication at 100 kHz for 10 min using double distilled water prior to exposure of fish. This was maintained as stock, 50% and 25% of median lethal concentrations (LC50, 96 h) i.e.120 mg/l and 60 mg/l were used as the test concentrations and fish were expose for 35 days duration.

2.3 Fish and exposure conditions

Fish *Channa punctatus* (Bloch) were used as the test animal. Length of the fish was between 18-20 cm and weight was between 65-70 gms. Fish were acclimatized in clean glass aquaria (150 L capacity) for 15 days. Specimens were subjected to a prophylactic treatment of 0.05% potassium permanganate (KMno4) for two mins to avoid any dental infections. During acclimatization period, minced goat liver subjected to a prophylactic treatment of 0.05% potassium to maintain as stock, 50 % and 25 % of median lethal concentrations (LC50, 96 h) i.e.120 mg/l and 60 mg/l were used as the test concentrations and fish were expose for 35 days duration.

Fish were kept in normal tap water, they served as control.

Group II: Fish were treated with 60 mg/l of SiO2 Nps.

Group III: Fish were treated with 120 mg/l of SiO2 Nps.

Five fish were autopsied at intervals of day 7, 15, 21, 28 and 35 and blood was collected immediately from the heart by using insulin syringe (1 ml), 31G X 15/64” (0.25 X 6mm). Blood was used for Micronuclei (MN) and comet assay. Simultaneously Gills, liver and kidney were removed and stored at -80°C for oxidative stress parameters estimated.

2.4 Oxidative stress markers

Antioxidant enzymes were estimated in gills, liver and kidney by homogenizing the tissues using 0.1 M sodium phosphate buffer, pH 6.5 containing 20% (v/v) glycerol, 1 mM EDTA and 1.4 mM diethytheritol (DTE) [13]. Catalase activity (CAT) was determined following the method of [14]. Glutathione peroxidase (GPX) activity was assessed by the method given by [15]. Glutathione S-transferase (GST) was measured by monitoring the formation of adduct between GSH and 1-chloro-2, 4-dinitrobenzene (CDNB) at 340 nm [16]. The activity of Glutathione Reductase (GR) was determined by the method given by [17]. Lipid peroxidation (LPO) was assessed by measuring the formation of thiobarbituric reactive substances (TBARS), according to [18].

2.5 Genotoxicity

2.5.1 Micronucleus test

The micronucleus test was conducted according to the method proposed by [19-20]. On a clean glass slide, a drop of fish blood and a drop of fetal bovine serum (FBS) were mixed thoroughly. The slides were then air-dried and fixed in absolute methanol for 10 minutes. They were stained with 5% Gmansa stain for 10 minutes and allowed to dry. A total of 1,000 red blood cells from the control group and the test group were checked for abnormalities in cytoplasm, nucleus and DNA as suggested by [21] with slight changes.

2.5.2 Comet assay

The alkaline comet analysis (pH> 13) was performed according to the method described by [22], slightly modified. Slides were prepared by pre-coating 1% normal melting point agarose (NMA) in double distilled water and stored at 4°C. To form the second supportive layer, 0.5% low melting point agarose (LMA) was spread uniformly over the first layer of agarose using a coverslip. The slide was further kept at 4°C for 5 min to allow complete polymerization of agarose. After the coverslips were removed, 30 μl of blood sample was gently mixed with 5μl of 1% LMA agarose, pipetted on the supportive layer of 1% NMA agarose and again covered with a coverslip. After keeping coverslips for 5 min on ice, they were removed and the slides were placed into freshly made cold lysis buffer (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, 1.5% Triton X-100 and 1% SDS, pH 10 along with 10% dimethyl sulfoxide) for 2 h. To allow DNA unwinding, slides were then placed in electrophoresis chamber containing cold alkaline electrophoresis buffer (300 mM NaOH and 1 mM Na2EDTA, pH13) for 20 min. Electrophoresis was performed by applying electric current for 20 min. All steps were carried out under minimum light and low temperature. Thereafter, the slides were neutralized and dehydrated with ethanol. After electrophoresis, slides have been washed three times for 15 min in freshly organized neutralization buffer (0.4 M Tris, pH 7.5). Staining was accomplished the usage of 50 μl ethidium bromide (EtBr-10 μg/ml). The stained slides have been determined the usage of fluorescence inverted microscope. Tail length and% Tail DNA are the parameters chosen to evaluate DNA damage.

2.6 Statistical analysis

All data was expressed as mean ± standard deviation. For statistical comparisons of data among treatments, ANOVA was used. All statistical analysis was performed by the Sigma Plot 14.5 software (Systat software Inc).

3. Results

3.1. Oxidative stress Biomarkers

Antioxidant enzyme activities are depicted in table 1,2,3,4,5 and 6.SOD,CAT,GST, GPx and LPO were showing non-significant differences at day 7 in gills but in liver and
kidney, they exhibit significant (p<0.05) increase from day 7 onwards in both the treated groups (table 1,2,3,4,7). In table 5, GR showed statistically significant increase from day 7 in all treated groups in all three tissues. GSH level was however, significantly decreased in all tissues in both treated groups till end of the experiment (table 6). All results are dose and duration dependent.

3.2. Micronucleus test

Table 8 exhibits statistically significant increase in Micro nuclei (MN), irregular nucleus (IR), sticky cells (SC) and vacuolated cytoplasm (VC) from day 7 onwards in both treated groups till end of the experiment as compared to control (Group I).

3.3. Comet assay

Tail length and% of tail DNA showed similar results as shown by micronucleus test. They showed statistically significant increase from day 7 onward in both treated groups (Table 9).

4. Discussion

According to [23-25] oxidative stress has been suggested to play an important role in the mechanisms of toxicity of a number of nanoparticles whether by excessive generation of ROS or by depletion of cellular antioxidant capacity. According to [25, 26] ROS generation and cellular oxidative stress have been cited as possible mechanisms of NPs’ toxicity. Present results show alteration of antioxidant (SOD, CAT, GST, GR, GPx and GSH) enzymes in fish during exposure to SiO2 NPs for 35 days, indicating that oxidative stress was induced in fish tissues due to the exposure to SiO2 NPs. ROS such as superoxide anion (O2−), hydroxyl radical (HO•) and hydrogen peroxide (H2O2) elicited a variety of physiological and cellular evirations including inflammation, DNA damage and apoptosis [25-27]. In agreement to above study DNA damage was also observed in the present study.

Antioxidant enzymes SOD and CAT levels were significantly higher in the SiO2 NPs exposed liver, gills and kidney. In a recent study, it is proposed that NP’s can convert endogenous hydrogen peroxide (H2O2) into highly reactive hydroxyl radical (HO•) by a Fenton-like mechanism (NP’s + H2O2 + H+ → NP’s + HO• + H2O), and hence, free radicals are generated in the tissues of NP’s treated organisms [28]. Similarly, there may be production of free radicals in SiO2-treated fish, and hence, increased levels of SOD and CAT enzymes were observed in the liver, gills and kidney of fish. Elevated level of SOD involves in the removal of anion radicals, and an increased level of CAT enzyme removes the hydrogen peroxides in treated fish. Similar to report by [29] that xanthine oxidase, NAD (P) H oxidase etc, are operative in production of superoxide in normal cells, there is great probability that in the present study also a similar mechanism may be operative.

A progressive increase of CAT indicates the emergence of an increasing source of hydrogen peroxide during a 35 day period after SiO2 NPs treatment. It is well established that H2O2 is produced through two-electron reduction of O2 by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme A oxidase, or uric acid oxidase [30]. Additionally, Kupffer cells, which are fixed to the endothelial cells, lining the hepatic sinusoids have a great capacity to endocytose exogenous particles and secrete large amounts of ROS [31]. Elevated CAT level in the present study may be due to the production of H2O2 and ROS after toxicity of SiO2 NPs. Since the amount SiO2 NPs in the liver accumulates gradually and is at a maximum after 35 days, it is suggested that the substrate for CAT must be generated by the SiO2 NPs directly or indirectly. It is possible that the activation of CAT may be due to an increased production of H2O2 by a mechanism different from ·O2−-dismutation. Indeed, the fact that H2O2 generation may be central to silica nanoparticle toxicity has recently been deduced, since catalase treatment decreases the nanotoxic effects of SiO2 nanoparticles [32]. Higher activity of GPx as observed in liver, kidney and gills of C. punctatus suggests a rapid peroxide-mediated GSH oxidation.

GSH has a significant role in the cellular redox status. It functions both as a free radical scavenger and as a substrate for GST and GPx. GSH depletion along with increased CAT and SOD activities in SiO2-exposed tissues suggests oxidative stress to be the primary mechanism for toxicity of SiO2 NPs in fish tissues. Studies have shown that hepatic GSH depletion impairs the cell's defense and is known to result in liver injury [33]. Similar results have been reported by [34, 25] following 7 days exposure to silicon-based quantum dots and SiO2 NPs in gibel carp and zebra fish respectively. Present results are consistent with earlier studies and suggest that SiO2 NPs toxicity is mediated via oxidative stress and free radicals [35-36]. Glutathione (GSH) and glutathione-dependent enzymes are both important in removal of intermediate toxins formed by xenobiotics during their bio activation. When there is a drop in GSH below a critical level, it is enabled to bind with xenobiotics and they covalently bind with DNA, RNA and proteins resulting in cellular damage [37]. Therefore, DNA damaging potential may be due to a reduction in GSH level or due to the formation of reactive oxygen species (ROS). In the present study histological damage was also observed in gills, liver and kidney.

GR is essential for the recycling of GSSG to GSH with NADPH as co-substrate. Hence, NADPH depletion may delay GR up regulation to counter GSH oxidation. This observation is supported by other studies that showed no significant alteration in the level of GR in human epithelial cells in the presence of pure silica nanoparticles [38].

LPO is one of the major outcomes of free radical mediated injury that directly damages membranes and contributes to DNA damage [39-40]. Increased ROS production indicate a failure of the antioxidant defense system ROS can act on the plasma membrane, resulting in LPO, or directly interact with DNA molecule, causing DNA damage [41]. GSH reduction by xenobiotics below a certain level allows LPO enhancement evoked by endogenous substances. The present study thus indicated that although the glutathione levels in C. punctatus were elevated due to NP exposure, they probably were not enough to get rid of oxidative stress since it leads to a concomitant increase in LPO in all tissues. In the present study, SiO2 NPs induced oxidative stress was further evidenced by depletion of GSH and induction of CAT, SOD, GST, GPx and GR with dose and duration of nanoparticles. It is possible that the loss of GSH may compromise cellular anti-oxidant defences and lead to the accumulation of ROS that are generated as byproducts of normal cellular function. Cell integrity is affected by oxidative stress when the production of active oxidants overwhelms the antioxidant defence mechanisms. There is a balance between free radical production and free radical...
scavenging, repair of damage caused by free radicals. Exposure of SiO₂ NPs in fish can upset this balance by depletion or inhibition of antioxidant systems and/or increasing the formation of ROS through mitochondrial dysfunction. The results of the present study supported the earlier studies conducted by [42].

Genotoxicity of nanoparticles needs to be carefully assessed since it is associated with the risk of inherited genetic damage and several other diseases. Hence, studies are required to get an insight and probe into DNA damaging effect of NP and the risks associated with it. Studies have shown that small particles, less than 30 nm in size enter the cell nucleus either by passing through the nuclear pore or by direct interaction with DNA during synthesis, thereby representing a nano-specific mechanism of toxicity [43]. Recent toxicity guidelines have emphasized the use of at least two assays for in vitro and in vivo studies [44]. Therefore, the present study has used comet and micronucleus assay for assessing SiO₂ NPs induced DNA damage. Micronucleus assay typically detect ROS-induced chromosome breakage and aneuploidogenic effects that can be due to physical disturbance of spindle or mitotic apparatus [45], typically appears at the end of mitotic telophase due spindle defects during segregation in anaphase. Thus, misrepair of DNA double strand breaks occur leading to the formation ofacentric chromosomes or chromatid fragments, or whole chromosomes that fail to be incorporated in the daughter nuclei [46]. Cytoplasmic anomalies such as sticky cells and vacuolated cytoplasm were evident in treatment groups. Vacuolated cytoplasm was also prominent in erythrocytes of fishes exposed to nanoparticles. This could be attributed to unequal distribution of haemoglobin [47]. Present study results coincides with another study of [48], who found similar results in Oreochromis mossambicus after exposure to NPs of silicon dioxide, aluminum oxide, titanium dioxide and iron oxide for short-term (24, 72 and 96 h) and long-term durations (15, 30 and 60 days). ROS are formed when xenobiotics get bio-transformed leading to the formation of reactive intermediates. Although organism has an antioxidative stress, which is induced by long-term exposure to clastogenic xenobiotics get a defensive mechanism against genotoxicants [65]. Consequently, oxidative stress and oxidative damage is believed to have significant effect on nano-toxicity. The DNA damage in fish evidenced that SiO₂ nanoparticles not only lead to cellular damage through ROS production but also cause genetic damage.

Table 1: SOD (units/min/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

|                      | Gills I   | Gills II   | Gills III  | Liver I   | Liver II   | Liver III  | Kidney I   | Kidney II  | Kidney III |
|----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                       | Day 7     | Day 15    | Day 21    | Day 28    | Day 35    |           |           |           |           |
|                       | 8.55±0.19 | 8.65±0.08 | 8.90±0.14 | 8.74±0.04 | 7.11±0.06 | 7.47±0.07 | 22.75±0.12 | 23.15±0.04 | 23.35±0.06 |
|                       | 8.5±0.08  | 8.84±0.06 | 9.8±0.08  | 6.75±0.08 | 7.58±0.03 | 9.27±0.06 | 22.75±0.09 | 23.56±0.05 | 23.95±0.11 |
|                       | 8.60±0.09 | 9.47±0.05 | 11.58±0.07 | 6.67±0.06 | 8.29±0.08 | 11.23±0.05 | 22.75±0.05 | 23.96±0.10 | 24.15±0.09 |
|                       | 8.63±0.10 | 10.48±0.07 | 14.48±0.04 | 6.78±0.04 | 9.2±0.05  | 13.85±0.04 | 22.76±0.10 | 24.31±0.03 | 24.78±0.12 |
|                       | 8.66±0.05 | 12.65±0.06 | 18.45±0.08 | 8.68±0.06 | 10.42±0.04 | 16.90±0.05 | 22.76±0.08 | 24.87±0.06 | 26.56±0.07 |

Table 2: CAT (µM H2O2 decomposed/min/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

|                      | Gills I   | Gills II   | Gills III  | Liver I   | Liver II   | Liver III  | Kidney I   | Kidney II  | Kidney III |
|----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                       | Day 7     | Day 15    | Day 21    | Day 28    | Day 35    |           |           |           |           |
|                       | 12.39±0.13 | 13.46±0.16 | 13.79±0.07 | 17.84±0.04 | 18.25±0.02 | 18.59±0.03 | 3.51±0.09 | 3.86±0.09 | 4.15±0.07 |
|                       | 13.41±0.05 | 14.60±0.03 | 17.91±0.05 | 17.84±0.10 | 19.18±0.07 | 19.91±0.05 | 3.51±0.09 | 4.20±0.07 | 4.30±0.06 |
|                       | 13.47±0.05 | 16.47±0.06 | 18.91±0.07 | 17.85±0.07 | 22.23±0.06 | 27.55±0.05 | 3.52±0.09 | 4.92±0.07 | 6.12±0.11 |
|                       | 13.48±0.03 | 19.24±0.07 | 21.42±0.08 | 17.85±0.08 | 23.44±0.03 | 35.34±0.07 | 3.52±0.05 | 5.25±0.13 | 7.01±0.08 |
Table 3: GST (nM CDNB conjugates/min/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

| Day | Group I | Group II | Group III |
|-----|---------|---------|-----------|
| 7   | 175.58±0.05 | 176.45±0.03 | 177.45±0.01 |
| 15  | 175.60±0.04 | 178.52±0.02 | 180.18±0.04 |
| 21  | 175.61±0.05 | 181.26±0.04 | 185.95±0.01 |
| 28  | 175.63±0.01 | 186.71±0.05 | 192.45±0.02 |
| 35  | 175.65±0.02 | 191.77±0.03 | 202.49±0.04 |

Table 4: GR (nM NADPH oxidized/min/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

| Day | Group I | Group II | Group III |
|-----|---------|---------|-----------|
| 7   | 175.65±0.02 | 175.59±0.03 | 194.63±0.02 |
| 15  | 175.67±0.04 | 178.52±0.03 | 180.18±0.04 |
| 21  | 175.68±0.05 | 181.26±0.04 | 185.95±0.01 |
| 28  | 175.70±0.01 | 186.71±0.05 | 192.45±0.02 |
| 35  | 175.72±0.02 | 191.77±0.03 | 202.49±0.04 |

Table 5: GSH (nM GSH/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

| Day | Group I | Group II | Group III |
|-----|---------|---------|-----------|
| 7   | 175.65±0.02 | 175.59±0.03 | 194.63±0.02 |
| 15  | 175.67±0.04 | 178.52±0.03 | 180.18±0.04 |
| 21  | 175.68±0.05 | 181.26±0.04 | 185.95±0.01 |
| 28  | 175.70±0.01 | 186.71±0.05 | 192.45±0.02 |
| 35  | 175.72±0.02 | 191.77±0.03 | 202.49±0.04 |

Table 6: LPO (nM TBRS formed/h/mg protein) changes in the gills, liver and kidney of fish Channa punctatus after exposure to SiO₂ NPs

| Day | Group I | Group II | Group III |
|-----|---------|---------|-----------|
| 7   | 175.65±0.02 | 175.59±0.03 | 194.63±0.02 |
| 15  | 175.67±0.04 | 178.52±0.03 | 180.18±0.04 |
| 21  | 175.68±0.05 | 181.26±0.04 | 185.95±0.01 |
| 28  | 175.70±0.01 | 186.71±0.05 | 192.45±0.02 |
| 35  | 175.72±0.02 | 191.77±0.03 | 202.49±0.04 |

Table 7: Changes in the Tail Length and Tail DNA% in the blood of fish Channa punctatus after exposure to SiO₂ NPs

| Group | T L | T DNA% |
|-------|-----|--------|
| I     |     |        |
| II    |     |        |
| III   |     |        |
| IV    |     |        |
| V     |     |        |

Table 8: Changes in the Micronucleus (MN), Irregular Nucleus (IR), Sticky Cells (SC) and Vacolated cytoplasm (VC) in the blood of fish Channa punctatus after exposure to SiO₂ NPs

| Group | MN | IN | SC | VC |
|-------|----|----|----|----|
| I     |    |    |    |    |
| II    |    |    |    |    |
| III   |    |    |    |    |
| IV    |    |    |    |    |
| V     |    |    |    |    |

Table 9: Changes in the Tail Length and Tail DNA% in the blood of fish Channa punctatus after exposure to SiO₂ NPs

| Day  | TL | T DNA% |
|------|----|--------|
| I    |     |        |
| II   |     |        |
| III  |     |        |
| IV   |     |        |
| V    |     |        |

n.s. = Non- Significant; * = P < 0.05; Values are mean± SD
5. Conclusions
In conclusion, the present study demonstrates that SiO\textsubscript{2} nanoparticle-mediated and SiO\textsubscript{2} nanoparticle-induced antioxidiant enzymes and DNA damage is clearly observed in animal model \textit{Channa punctatus}. Further, they can provide an indication of the sub-lethal impacts of stressors such as SiO\textsubscript{2} and act as a biomarker for early detection of hazardous impacts at the population level. Additionally, present study also increases public awareness regarding toxicity levels of water bodies and suggests that stringent regulations of government and monitoring of water bodies ought to be observed.

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7. Declaration of interest
Authors have no conflicts of interest.

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