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Genotoxicity Studies of Heavy Metals by Single Cell Gel Electrophoresis (SCGE) in Two Fish Species – *Puntius Narayani* and *Rasbora Daniconius*

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ABSTRACT Mine waste can deteriorate water quality by disturbing its physicochemical parameters as well as through the release of heavy metals. Metals leached into the water ultimately affect the humans often through the food chain transfer. The response of aquatic organisms to the metals in the environment may vary from species to species. The present study was carried out to investigate the response of two fish species *Puntius narayani* and *Rasbora daniconius* to mining effluents. The two species were evaluated for the body growth and genotoxicity by single cell gel electrophoresis. The study indicates that both species respond differently to the metal pollution as evidenced by reduction of body size and body weight and also genetic damage in the fishes. *Puntius narayani* was seen to be more sensitive to metal pollution and can, therefore, be considered as a good animal model for evaluation of metal pollution in aquatic ecosystems.

INTRODUCTION

Large-scale as well as small-scale mining operations, are inherently disruptive to the environment, producing enormous quantities of waste that may pollute the air, land and water (Mensah et al. 2015). Aquatic pollutants may have multiple consequences at the organismic population, and community and ecosystem levels, affecting organ function, reproductive status, population size, species survival and thereby biodiversity. Besides deterioration of water quality, mining effluents also release large amount of heavy metals in the aquatic ecosystem. The mine wastes constitute a potential source of contamination in the environment, as heavy metals and acid are released in large amounts (Ledin and Peterson 1996). Therefore, assessment of the heavy metals in the water around mining areas is essential as all aquatic organisms are directly or indirectly affected by the degradation of water quality. Heavy metals in the aquatic ecosystems are of significance, due to their toxicity, bioaccumulation potential, and their ability to induce damage in DNA (Szefer et al. 1990). All these measurable changes if evaluated and quantified can serve as indicators of pollutant stress on the aquatic organisms. Fish, being an important resident group of the aquatic ecosystem are considered as bio-indicators for providing early warnings of environment induced damage. Metals toxicity can be evaluated by assessing the genetic damage caused in the fishes. Fishes, in fact, are exposed to multi-pollution states and are susceptible to acute and chronic environmental changes. Fishes are therefore used as a test organism in which it is possible to detect DNA damage induced by direct mutagens and pro-mutagens in both fresh and marine water (Mitchelmore and Chipman 1998; Lemos et al. 2005). Fish tissues are commonly analyzed to determine contaminant concentration and to assess the health risk because it is consumed by humans (Begüm et al. 2005). The analysis of DNA alterations in aquatic organisms is widely accepted as a suitable method for evaluating genotoxic contamination of the environment and can be used to detect exposure in a wide range of species (Frenzilli et al. 2009). The alkaline comet assay was approved in fish as more sensitive to the genotoxicity of river contaminants than the micronucleus test (de Andrade et al. 2004). Unlike classical chromosome breakage studies, such as micronucleus formation or cell survival studies, comet assay allows scanning complete genomes in all cell cycles, rather than just during mitosis (Sunjog et al. 2012). Considering this, fish was chosen as model organisms in the present study for assessing genotoxicity by alkaline comet assay or a single cell gel electrophoresis in the two species of fishes. Every species may respond differently to environmental stress.

Objective

The present study was therefore undertaken to assess the DNA damage observed in two different species of fishes *Puntius narayani* and *Rasbora daniconius* due to metal pollution. The study was designed to look at the morphometric changes and genetic damage by single cell gel electrophoresis.

METHODOLOGY

Fish sampling involved the collection of two species *viz. Puntius narayani* and *Rasbora daniconius* from the upstream and downstream sites of the river in the vicinity of mines. The downstream site showed an influx of mining effluents as was visible through the runoffs and was considered as the experimental site. The upstream site was away from the mine and was considered as the control site. The fishes were subjected to morphometric analysis with respect to their length, breadth, and weight and heavy metal analysis. Gills, liver, and muscles were analyzed for presence of heavy metals. Tissue samples obtained from *Puntius narayani* and *Rasbora daniconius* were dried, powdered, digested according to standard procedure of Sreedevi et al. (1992) and analyzed using atomic absorption spectrophotometer. Standardized procedures used for heavy metal detection in the tissues was as per IS 3025 Part 53: 2003, SOP/ITG/AAS/INST-01. Values of heavy metals were recorded in mg/ltr. Genetic damage evaluation was done using gill cells as per the standard procedure. Gill cells were immediately collected and processed for genetic damage studies. Single cell gel electrophoresis (SCGE) assay, commonly called the comet assay was performed to assess the genotoxicity. The extent of DNA damage in the fishes was analyzed using "Comet Assay Software Project Lab" (CaspLab), which is a cross-platform image analysis software to measure the level of DNA damage in SCGE. Images of 25 cells were obtained from each of the two replicate slides per sample, and, among the parameters available for analysis, the tail moment, tail length, and tail intensity were used to assess DNA damage. Comparison of DNA damage was made in both species of fishes caught from polluted (downstream) as well as unpolluted reference site (upstream). The data was tabulated and analyzed statistically. The statistical significance of associations between various qualitative parameters was evaluated through Fisher's exact test (two tailed). Online calculators of statistic were used for standard deviation at www. easycalculation.com and fisher's test at www. graphpad.com.

RESULTS

Puntius narayani and *Rasbora daniconius* collected from the upstream and downstream sites were compared for its morphometric variations and extent of damage caused to the DNA. A total of 50 fishes were evaluated from each site (Fig. 1).

1) Morphometric Analysis

a) Puntius narayani

The average body length of the fishes from the downstream site was observed to be 3.83 ± 0.47 cms, which was comparatively smaller than the fishes from the upstream site (P = 0.0313) as indicated in Table 1. There was significant reduction in body breadth of fishes from downstream site to 1.13±0.06 (P = 0.0004*)* as compared to upstream fishes which had average body breadth of 1.95±0.07. Body weight of the fishes from downstream region was also found to be reduced to an average of 0.71±0.20 gms as compared to fishes from upstream which had average weight of 1.23±0.33 gms *(*P= 0.0056*)*. Ob-

Table 1: Comparison of the body dimensions and weight of the two species of fishes studies from the upstream and downstream sites

Factors	*Puntius narayani*		*Rasbora daniconius*	
	Upstream	*Downstream*	*Upstream*	*Downstream*
Body length (Cm)	4.65 ± 0.36	3.83 ± 0.47	6.65 ± 1.63	6.45 ± 0.21
Body breadth (cm)	1.95 ± 0.07	1.13 ± 0.06	1.75 ± 0.92	1.55 ± 0.21
Body weight (gms)	1.23 ± 0.33	0.71 ± 0.20	2.81 ± 2.43	2.30 ± 0.13

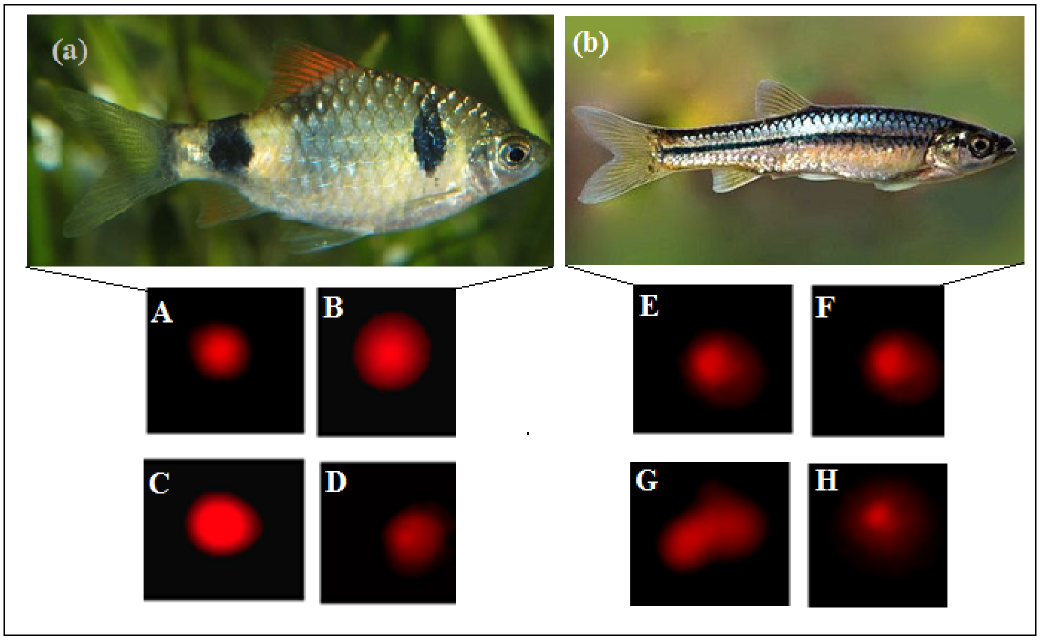

Fig. 1. Fishes studied for genetic damage on account of mining effluents (a) *Puntius narayani* (b) *Rasbora daniconius*. Genetic damage as indicated by comet assay in both species of fishes: *Rasbora daniconius* (A, B, C, D) and *Puntius narayani* (E, F, G, H)

servations indicate that *Puntius* species is more sensitive to change in the water quality.

b) Rasbora daniconius

This species belongs to the Cyprinidae family. It is characterized by an oblong and compressed body. The average body length of the fishes in the upstream site was 6.65±1.6 cms. Fishes from the downstream site were slightly smaller than the fishes from the upstream site with length of 6.65±0.21cms (P = 0.709). Fishes from the downstream site also showed decrease in the body breadth and weight as compared to the fishes from upstream site. However, the decrease was found to be statistically insignificant (P = 0.4569 and P = 0.1038 respectively). Results suggest that *Rasbora* species are more tolerant to the water quality change on account of mining effluents.

2) Genotoxicity Studies

The genetic analysis was done using SCGE to assess the amount of genetic damage caused due to mining effluents. The DNA damage analysis through the software CASPLAB showed significant damage in the fishes from the downstream as compared to the upstream, in both the species. Various parameters with respect to the genetic damage were calculated by using the software "CASP-LAB". Of the various parameters, the head and tail DNA along with their percentage, tail length, tail moment and olive tail moment were quantified. DNA analysis revealed that the head region, head radius and head DNA of the fishes from the upstream sample was significantly higher than the fishes from the downstream sample. The tail length, tail moment and the olive tail moment, showed a significant increase in the fishes from the downstream site. Increase in the tail length is indicative of genetic damage (Fig. 1).

a) Puntius narayani

The head DNA percentage was found to be significantly higher in the upstream as compared to the downstream fishes *Puntius narayani*. This indicates that the DNA of the fishes in the upstream region was more intact as compared to that in the fishes of downstream site. There was a significantly higher tail DNA percentage in the downstream sample as compared to the upstream sample (Table 2). The tail length was found to be significantly higher in the downstream as compared to the upstream. This indicates that genetic damage was observed in the fishes from the downstream as compared to the upstream. The damaged DNA was reflected as tail DNA. The tail DNA percentage showed genetic damage of 11.1 ± 6 percent in the fishes from the downstream site. This indicates that there is 11.1 ± 6 percent damage in the fishes on account of heavy metal pollution due to mining effluents.

b) Rasbora daniconius

A similar trend was observed in the case of *Rasbora daniconius*. Fishes from the downstream site showed more genetic damage as compared to *Rasboras* from upstream site. The head DNA percentage was found to be significantly greater in the fishes of the upstream as compared to the downstream. The tail DNA percentage showed genetic damage of 6.5 ± 7 in the fishes from the downstream site. From this it can be concluded that compared to the *Puntius* species, *Rasbora* is more resistant to DNA damage.

DISCUSSION

Mining effluents degrades the water quality of aquatic ecosystems, by altering the physicochemical parameters, and by releasing metals which harm the aquatic organisms as a result of biomagnifications and bioaccumulation. The drastic change in the water quality and increase in the heavy metal concentration in the downstream region on account of mining was reported in study of Fernandes (2015) in the same study site. Some studies (Giesy et al. 2000; Schoenfuss et al. 2008) also revealed that exposure to mining effluents can lead to abnormal physiological responses and cause adverse effects on the development, growth, behavior, and reproduction of fish. Therefore, the change in the morphometric characters observed in the downstream fishes can be associated with the change in water quality on account of heavy metals and degradation of some physicochemical parameters. Change in water quality as possible factor for reduction in the body length and weight of the fishes is also indicated in the studies of Zhao

Table 2: CASP-LAB analysis of the cells of both species of fishes studied

Factor analyzed	*SITES*	*Puntius narayani*	*Rasbora daniconius*
Head Area	Upstream	691.6 ± 693	2953.1 ± 316
	Downstream	381.7 ± 253	1691.3 ± 1139
Tail Area	Upstream	25.2 ± 16	92.2 ± 87
	Downstream	61.0 ± 41	413.4 ± 410
Head DNA	Upstream	114.2 ± 121	357.8 ± 89
	Downstream	59.5 ± 40	164.6 ± 100
Tail DNA	Upstream	2.0 ± 2	2.2 ± 2
	Downstream	7.5 ± 6	4.3 ± 13
Head DNA%	Upstream	97.7 ± 1	99.2 ± 1
	Downstream	89.0 ± 6	93.8 ± 7
Tail DNA%(Intensity)	Upstream	2.3 ± 1	0.6 ± 0.5
	Downstream	11.1 ± 6	6.5 ± 7
Head Radius	Upstream	13.3 ± 6	29.7 ± 2
	Downstream	10.4 ± 4	22.1 ± 7
Tail Length	Upstream	3.0 ± 0	2.6 ± 0.1
	Downstream	4.3 ± 2	3.5 ± 6
Comet Length	Upstream	30.5 ± 12	28.8 ± 3
	Downstream	36.1 ± 8.5	30.7 ± 18
Head Mean X	Upstream	50.1 ± 12	78.4 ± 1
	Downstream	42.6 ± 8	59.7 ± 4
Tail Mean X	Upstream	54.2 ± 15	55.3 ± 2
	Downstream	63.9 ± 8	58.7 ± 8
Tail Movement	Upstream	0.07 ± 0.04	0.02 ± 0.01
	Downstream	0.6 ± 0.49	0.13 ± 1
Olive TailMovement	Upstream	0.28 ± 0.15	0.15 ± 0.1
	Downstream	1.27 ± 0.8	0.81 ± 1.3

et al. (2012). Although heavy metals occur naturally in trace quantities in the aquatic environment, leaching of heavy metals through mining and other activities is considered to be among important pollutants as they do not degrade or decompose. Metals released through effluents in the aquatic environment enter the food chain and consequently build up in the organisms up the chain (Rajeshkumar and Li 2018). With regard to metal pollution, it is established that gills and kidney are the first organs exposed to heavy metals. Gill and digestive system are the regions where heavy metals are received and accumulated and stored (Yildiz et al. 2010). These metals may then disrupt functioning of the immune, reproductive, nervous and endocrine system in animals and these effects can be at the level of organ, tissue or cell (Geeraerts and Belpaire 2010). Examination of the two fish species *Puntius narayani* and *Rasbora daniconius* collected from the upstream and downstream region revealed a significant decrease in the body weight in the downstream site as compared to the upstream site. The reduction may be due to environmental stress experienced by the fish on account of metals such as iron and zinc as well as some physicochemical parameters of the water such as turbidity and suspended solids. Even if these metals are in small concentrations they can be acutely toxic and injurious through chronic exposure in organism. The genetic damage seen in the present study may therefore be associated with accumulation of these heavy metals in the body of the fishes. Studies of Mensah et al. (2015) and Hilson (2002) have shown that illegal small scale mining operations carried out in the open air without conforming to environmental standards are the ones which release contaminated water into the surrounding environment, thereby polluting nearby rivers. Therefore, from the observations of the present study it can be postulated that the synergistic effect of altered physicochemical parameters and heavy metal contamination from mining effluents may result in hampered growth in the fishes and also result in genetic damage in both the species of fishes.

Another important observation from this study is that *Puntius narayani* can be considered as a biological indicator of heavy metal toxicity through comet assay evaluation. When the genetic damage in downstream fishes of *Puntius narayani* and *Rasbora daniconius* was compared, the *Puntius* species was found to be more susceptible to the effect of pollutants as compared to *Rasbora* species. *Rasbora* species was found to be more tolerant to the pollutants in water especially the heavy metals. The heavy metals detected in the study site were manganese, zinc and iron. Cadmium was seen in traces (Fernandes 2015). Low concentrations of some heavy metals are essential for aquatic animals. However, at high concentration levels, they accumulate in different organs, damage tissues and interfere with the normal growth and proliferation (Alkarkhi et al. 2009). The metals found in the water were iron, manganese, zinc, and chromium. Iron can be deleterious as physiological evidence indicates that iron preferentially crosses the apical membrane of both the gills and intestine in the ferrous (Fe^{2+}) state. In mammals, prenatal and postnatal exposure to manganese is associated with embryo-toxicity, fetal-toxicity, and decreased postnatal growth. Bioaccumulation of heavy metals throughout the food chain may finally affect human beings (Bawuro et al. 2018). Manganese excess may cause a Ca^{2+} pump dysfunction, affecting neuro-muscular transmission in benthic marine invertebrates. This neurodegenerative disorder is due to the accumulation of manganese inside intracellular compartments, such as the Golgi apparatus and mitochondria. In mammals, prenatal and postnatal exposure to manganese is associated with embryo-toxicity, fetal-toxicity, and decreased postnatal growth (Sánchez et al. 1993; Colomina et al. 1996). Therefore, increase in manganese concentration is a matter of concern. Heavy metals can be taken up into fish either from ingestion of contaminated food via the alimentary tract or through the gills and skin (Elliott et al. 1988; Drevnick et al. 2006; Sfakianakis et al. 2015). Effectively, after the absorption, metals in fish are then transported through the blood stream to the organs and tissues where they are accumulated (Adeyemo et al. 2010; Fazio et al. 2014). The present study suggests that the metals from the surrounding water taken up by the fishes, has caused the genetic damage on account of bioaccumulation. The sensitivity of *Puntius narayani* and *Rasbora daniconius* in uptake of metals from the surrounding may be different and therefore the study revealed greater impact on the body growth of *Puntius narayani* as well as susceptibility to DNA degradation as compared to *Rasbora daniconius*.

CONCLUSION

It is abundantly clear that metals induce the response in the fish as evidenced by reduction of body size and body weight and also genetic damage. Of the two species of fishes studied, *Puntius narayani* was seen to be more susceptible to the metal pollutants in the aquatic environment and can thus represent a good model system for evaluation of pollution in aquatic ecosystems. *Rasbora daniconius*, on the other hand, was found to be more tolerant to aquatic pollution.

RECOMMENDATIONS

Puntius narayani being more sensitive to metal pollution can, therefore, be considered as a good model system or bio indicator for evaluation of metal pollution in aquatic ecosystems by comet assay.

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