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Research Article

Fly ash induced lipid peroxidation in the fish *Cirrhinus mrigala*

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Abstract
Coal fly ash (CFA), is a byproduct of coal combustion in a thermal power plant, as well as industrial solid waste. Its improper disposal causes environmental, ecological, and human health risks. In aquatic systems, fly ash contamination leads to the bio-accumulation of metals, oxidative stress, tissue damage and threats to biodiversity. The consumption of contaminated food poses a hazard to the food chain. Therefore, the present study aimed to evaluate fly ash induced lipid peroxidation (LPO) in the fish *Cirrhinus mrigala*. To assess the toxicity of CFA, collected from the dumping station of Solapur Super Thermal Power Station, located at village Fatatewadi and Aherwadi, District: Solapur, fish were exposed to three concentrations (1 g/L, 3 g/L, and 5 g/L) for both acute (4 days) and chronic (30 days) durations, along with a control group. The MDA level was measured in the liver, gills, intestine, and muscles for both acute and chronic time. The MDA levels were increased significantly with increasing CFA concentrations. In all the organs, MDA level significantly increased in both acute and chronic durations. The highest MDA level was observed in the gills and the lowest activity was noticed in the intestine for both acute and chronic exposure. This concludes that CFA contamination induces oxidative stress and toxicity in freshwater fish, highlighting its potential impact on aquatic life.

Keywords: Coal fly ash (CFA), heavy metals, LPO, Malondialdehyde (MDA), Oxidative stress.

INTRODUCTION

Fly ash is a waste byproduct generated from the burning of coal in electric power plants (Rebeiz *et al.,* 1996; Sharma *et al.,* 2010), producing approximately a billion tons annually (Sultana *et al.,* 2021; Zierold *et al.,* 2020; Feuerborn *et al.,* 2019). It is a powdery microparticle ranging in size from 0.1 µm to 100 µm, with diverse shapes (Qi *et al.,* 2019), and contains various toxic compounds, including metals. Fly ash is typically disposed of in the surrounding area and enters the ecosystem through rainwater and air (Yi *et al.,* 2024), eventually passing through the food chain, which ultimately affects flora and fauna. Fly ash pollution in the aquatic system has an adverse effect on the biota and the ecosystem (Adriano and Weber., 2001; Ghio *et al.,* 2002; Jaunjal *et al.,* 2024). It also alters the physical, chemical, and biological components of the aquatic ecosystems (Borm., 1997; Manz *et al.,* 1999; Adriano and Weber., 2001; Ghio *et al.,* 2002).

Fly ash not only affects aquatic animals but may also be destructive to terrestrial animals. The air particles of fly ash affect human health causing asthma, lung damage, inflammation, and immunological reactions (Lockwood and Evans, The Earth Justice). It also causes heart diseases, cancer, respiratory disease, and stroke. Fly ash enters the ecosystem during construction and affects organisms (Srivastava *et al.,* 2012). The heavy metals leach out from fly ash and enter the groundwater and aquatic systems. It affects the quality of aquatic life and disrupts ecosystems (Souza *et al.,* 2013; Meyer *et al.,* 2015; Tuberville *et al.,* 2016; Finger *et al.,* 2016; Van Dyk *et al.,* 2017). Therefore, fly ash may be a major source of pollution in both terrestrial and aquatic ecosystems (Ghio *et al.,* 2002).

Recently, the effect of fly ash has been studied in mammals indicating that it has the potential to promote the production of reactive oxygen species (Rozhina *et*

al., 2021; Lewis *et al.,* 2003; Voelkel *et al.,* 2003; Marra et *al.,* 2018). It may induce oxidative stress, neurodegeneration, cardiovascular diseases, as well as inflammatory injuries (Aruoma *et al.,*1998). In mammals such as rats, guinea pigs, and mice, fly ash induces ROS production in tracheal cells (Jiang *et al.,* 2000; Diabate *et al.,* 2002). Furthermore, fly ash leachate induces ROS production and stimulates LPO in fish cells (Nacci *et al.,* 2002; Xiang and Shao, 2003; Sarmento *et al.,* 2004). In the fish *Channa punctata,* fly ash leachate induces oxidative stress, elevates the antioxidant system, and increases LPO (Ali *et al.,* 2004) and lactate dehydrogenase activity (Ali *et al.,* 2007). Fly ash leachate also induces hepatocyte toxicity in the fish *Channa punctata* (Ali *et al.,* 2007) due to oxidative stress. It has been suggested that fly ash can affect signal transduction, gene expression, cell proliferation, and the response of target cells (Yin *et al.,* 2011). Free radicals' attacks on lipids and induce LPO as a marker of oxidative stress. Therefore, the present study aimed to understand the toxicity level of fly ash on LPO in fish thereby promoting public awareness regarding the disposal and management of industrial waste. Furthermore, fish serve as pollution bioindicators for water pollution and are crucial for indicating the health of an ecosystem. This study may highlight the concern about fly ash and its impact on biodiversity deterioration.

MATERIALS AND METHODS

Experimental setup

The fish *Cirrhinus mrigala* fingerlings were procured from a fish breeding center Nalawade fish center Kale, Tal: Panhala, Dist.: Kolhapur Maharashtra. The weight of the supplied fish was 3.18±0.21 g, and mean length was 4.72±0.44 cm. Fish were brought to the laboratory, reared, acclimatized in the aquarium, and fed daily with fish feed. During rearing water was changed frequently to remove faecal matter and ammonia from the water. The water parameter was checked to ensure normal conditions. After acclimatization, which took approximately 8 days, the fish were used for the experiment.

The fly ash was collected from the dumping station of Solapur Super Thermal Power Station, located at village Fatatewadi and Aherwadi, District: Solapur. The fish were divided into four groups: 1) Control (water without fly ash) 2) Treated with 1g/L, 3) Treated with 3 g/L, and 4) Treated with 5 g/L. The treated fish were reared in water containing fly ash. Prior to the experiment, feeding was restricted, but it resumed 24 hours after exposure to the test substance. Fish were exposed to fly ash at specified concentrations (1g/L, 3g/L, and 5g/L) for both acute and chronic durations, along with a control group. The fly ash concentrations were selected based on preliminary studies and in accordance with Ali *et al.* (2004, 2007). There were 10 fish in each experimental and control group, and the experiment was replicated three times.

Methodology

The fish were anesthetized, and tissues from the gills, liver, muscle, and intestine were dissected for LPO analysis following the method of Wills (1966). In present study, lipid peroxidation was measured using a spectrophotometer at 532 nm against a blank. Briefly, the tissues were homogenized in a potassium phosphate buffer (75 mM, pH 7.04), 1 mM $FeCl_3$, and 1 mM ascorbic acid. The tissue extracts were then transferred into tubes and mixed. The mixture was centrifuged for 20 min at 3000 rpm. Then, 0.2 mL of supernatant was transferred to another glass tube, followed by the addition of 1.8 mL of distilled water, 1 mL of 20% TCA, and 2 of 0.67% thiobarbaturic acid (TBA). In the blank tube, 2 mL of distilled water, 1mL of 20% TCA, and 2mL of 0.67% TBA were added. The tubes were placed in a boiling water bath for 15 minutes and then cooled to room temperature. Then the absorbance was recorded at 532 nm, with the end product of total lipid peroxidation being malondialdehyde (MDA). The MDA level was expressed as nanomoles of MDA/mg tissues. The control group was reared in water without fly ash, alongside the experimental groups.

Animal ethical approval

The experiments were performed as per Animal ethical approval from Department of Forest (Wildlife), Nagpur (No: Desk-22(8) /Research/CR-03/885/23-24.Nagpur, Dated 23th June 2023).

Statistical analysis

In the present study, the data were statistically analysed, and differences between the experimental groups were examined using two-way ANOVA. The Tukey post-hoc test was used to determine which groups were different. Statistical significance was accepted as $p < 0.05$. Data are expressed as arithmetic mean ± S.D. of three observations (S.D., standard deviation). Error bars indicate standard deviation. A probability level of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The present study showed significant increases in MDA levels in both the acute (4 days) and chronic (30 days) duration of fly ash-treated groups compared to the control group of the fish *Cirrhinus mrigala*. The acute and chronic fly ash-treated groups showed a significant increase in MDA levels compared to the control group. In the fish, acute and chronic exposure to fly ash showed significant increase in MDA levels in gills, liver, muscles, and intestine compared to the control (Fig. 1, 2, 3, 4). In the fish, acute exposure to fly ash showed a sig-

nificant increase in MDA levels at different concentrations in the gills (Fig. 1), liver (Fig. 2), muscles (Fig. 3), and intestine (Fig. 4) compared to the control. Similarly, in the chronic exposure at the same concentration, significant elevations of MDA levels were observed in the gills (Fig.1), liver (Fig. 2), muscles (Fig. 3), and intestine (Fig. 4). The MDA level was observed in the acute and chronic exposure in the gills>liver>muscle>intestine. The high MDA level was reported in the gills (Fig.1) and was lowest in the intestine (Fig.4) in both the acute and chronic treatments. The results illustrate the significant levels of MDA that are directly proportional to LPO activity in vital tissues of fish after exposure to fly ash. During the chronic period, the group showed a significant increase in MDA level compared to the acute period and the control group. Fish were exposed to chronic concentrations of fly ash resulting in a highly significant impact on MDA levels in tissue, while acute concentrations had a moderately significant impact on MDA levels. The MDA level increases with the increases in concentration of fly ash in all the organs studied in acute and chronic studies. The LPO in gills, liver, muscle and intestinal were significantly different ($p<0.05$).

Fish are frequently utilized as indicator organisms in ecotoxicological research due to their various roles in the food chain, their ability to accumulate harmful substances, and their sensitivity to low levels of mutagens. Consequently, the application of fish biomarkers as measures of pollution impacts is becoming increasingly significant, enabling the early identification of aquatic environmental issues (van der Oost *et al.* 2003). The free radical activity has caused LPO (Parihar *et al.,* 1996; Cheeseman, 1982). The available information on the effect of coal fly ash on lipid peroxidation is limited in fishes. Earlier, it has been reported that fly ash contains various metals and toxic compounds that may induce oxidative stress and LPO production in fish (Grabowski *et al.,* 1999; Van Maanen *et al.,* 1999; Ghio *et al.,* 2002; Lewis *et al.,* 2003). In *Channa punctatus,* contamination in water enhances the production of reactive oxygen species (ROS) and leads to toxicity (Javed *et al.,* 2017). Fly ash also induces ROS generation in the lung epithelium, inflammation in mammals (Diabate *et al.,* 2011), and LPO has been reported earlier (Huang *et al.,* 2003; Safari *et al.,* 2015). In *Hetropneustens fossilis* (Bloch), fly ash exposure alters blood composition and induces oxidative stress toxicity in the kidney of fish (Pradhan *et al.,* 2022). In the present study noticeably higher LPO levels were observed in the gills, liver, muscle, and intestine (Fig. 1, 2, 3, & 4). Oxidative stress induces the generation of malondialdehyde (MDA), which leads to alterations in the structure and function of cells (Oakes *et al.,* 2003; Wong *et al.,* 2007).

In the gills, a significant increase in LPO was observed as compared to the other organs (Fig.1). The gills are the most sensitive organ that is directly in contact with the pollutants and heavy metals in the water (Santos *et al.,* 2022). The *Channa punctata* (Bloch) gill showed higher oxidative stress, a response biomarker of LPO (Ali *et al.,* 2004). In the in vitro and animal models previously noted, copper, iron, and vanadium from fly ash also induce the production of ROS (Ghio *et al.,* 2002). In the present study it has been observed that LPO increases with an increase in fly ash concentration and duration in gills. Similarly, in the gills of fish *Sparus aurata*, MDA contents increased upon exposure to *Pelagia noctiluca* crude venom (Ensibi *et al.* 2013). The increase in MDA level in the gill tissues reported after etoxazole treatment in *Oreochromis niloticus* (Uner *et al.* 2006). In fish, Ibuprofen induces oxidative stress in the gills of *Cyprinus Carpio* (Islas-Flores et al. 2014). The heavy metals, pesticides, and other pollutants also induce lipid peroxidation in fish (Ensibi *et al.,* 2013). Additionally, the induction of oxidative stress has been reported in zebrafish exposed to copper and microplastics (Santos *et al.,* 2022). In mammals, fly ash particles precipitate in the lungs upregulating ROS, various antioxidant enzymes, and inflammation (Kumar *et al.,* 2004; Borm, 1997; Ghio *et al.,* 2002).

The liver acts as a biological indicator of water contamination in fish, surpassing other organs (Gul *et al.*

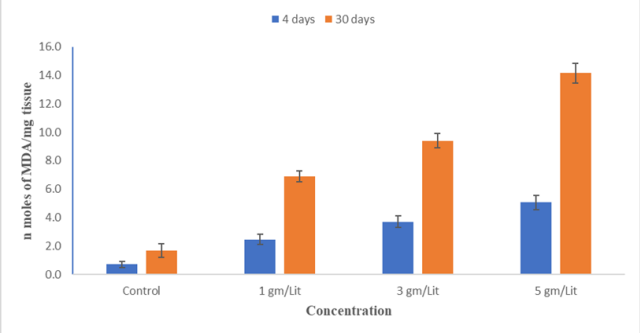

Fig. 1. *Gills MDA (Melanoaldehyde) content in Cirrhinus mrigala exposed to fly ash in acute (4 days) and chronic (30 days) exposure, Data represent the mean ± SD*

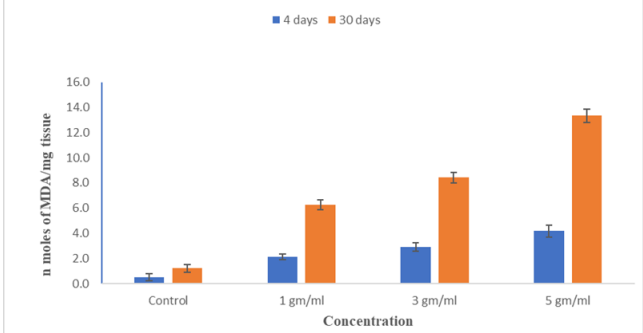

Fig. 2. *Liver MDA (Melanoaldehyde) content in Cirrhinus mrigala exposed to fly ash in acute (4 days) and chronic (30 days) exposure, Data represent the mean ± SD*

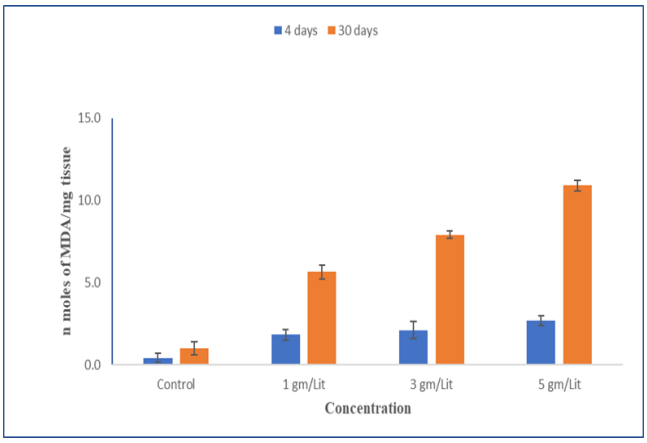

Fig. 3. *Muscle MDA (Melanoaldehyde) content in Cirrhinus mrigala exposed to fly ash in acute (4 days) and chronic (30 days) exposure, Data represent the mean ± SD*

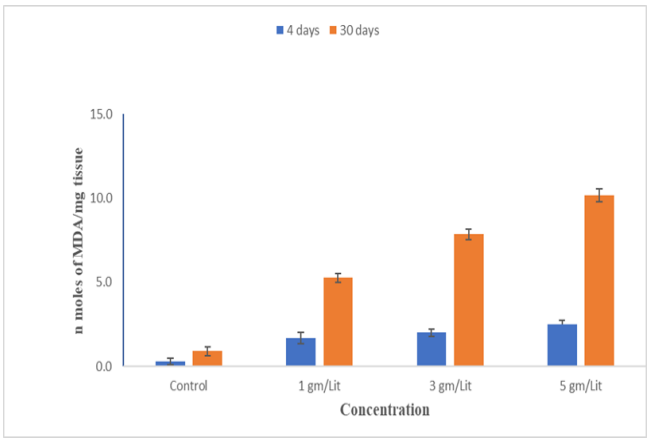

Fig. 4. *Intestine MDA (Melanoaldehyde) content in Cirrhinus mrigala exposed to fly ash in acute (4 days) and chronic (30 days) exposure, Data represent the mean ± SD*

2004). LPO is correlated with mitochondrial dysfunction, ROS, and liver diseases (Shi *et al.,* 2021). Thus, the oxidative degradation of lipids leads to the development of various liver disorders, as well as inflammation (Spahis *et al.,* 2017). Abnormal peroxidation leads to lipotoxicity in the liver (Almeda-Valdes *et al.,* 2015; Marra *et al.,* 2018; Sies and Cadenas, 1985). In the current study *Cirrhinus mrigala*, LPO was significantly increased in the liver in a dose- and time-dependent manner compared to the control. Similar to the present finding, TBARS (Thiobarbituric acid reactive substances) are increased in the liver tissue of *Clarias gariepinus* collected from polluted rivers (Osagie and Morayo, 2023). Fly ash leachate induces pro-apoptosis in hepatocytes of fish *Channa punctata Bloch* (Ali *et al.,* 2007). The toxicity of fly ash and the formation of ROS triggers an inflammatory response during phagocytosis and inflammation in rats, leading to LPO, DNA damage, and protein oxidation (Gilmour *et al.,* 2004). In liver cancer high LPO levels are caused by excessive ROS production (Marnett, 2002; Niedernhofer *et al.,* 2003). Similarly, heavy metal-polluted water leads to the accumulation of metals in the gills, liver, kidneys, skin, muscles, and other tissues of fish (Emon *et al.,* 2023). ROS also damages muscle structure and integrity (Jimenez *et al.,* 2022). Therefore, toxicity alters membrane integrity, fluidity and permeability (Mello *et al.,* 2015), and induces cell death in *Clarias gariepinus* Juveniles (Ogueji *et al.,* 2017). In muscles and intestines, LPO was observed to be increased compared to the control (Fig. 3, 4). Similarly, in mice, CFA generates ROS and induces lipid peroxidation, protein oxidation, and DNA damage (Putri *et al.,* 2020), leading to cardiovascular diseases, various types of cancer, neurodegeneration, and aging (Diplock, 1998). Therefore, the present findings suggest that fly ash induces the generation of ROS and oxidative stress in all tissues, leading to metabolic abnormalities in the liver, gills, and other tissues.

Conclusion

The present study suggests that coal fly ash can have a significant impact on fish, as it elevates the levels of lipid peroxidation in vital organs. The exposure of *C. mrigala* to coal fly ash may be linked to changes in tissue enzymes, leading to stress. The present findings indicate that the liver, gills, and muscles could serve as potential indicators for detecting residual fly ash in aquatic systems in fish. The gills may be more susceptible to water contamination, suggesting that fly ash induces oxidative stress in the fish's gills, which may lead to hypoxia. However, further investigation is required to establish specific biomarkers for monitoring the aquatic environment. LPO levels increase and the ability to maintain homeostasis is reduced, resulting in poor nutritional quality of fish meat and fish health issues that can lead to death. Therefore, the fly ash may harm aquatic habitats, their diversity, and the livelihood of humans.

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AUTHORS' CONTRIBUTIONS

Both the authors contributed equally in experimental work and manuscript preparation.

Conflict of interest

The authors declare that they have no conflict of interest.

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