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Impact of Environmental Mercury on the Physiological and Biochemical Changes of a Freshwater Fish, *Oreochromis Mossambicus*, Peters under Laboratory Controlled Conditions.

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***Abstract*--** **Environmental pollution is considered as one of the major challenges in the modern human society. It is an established fact that pollution is a global problem. It emerged as an environmental issue in the 70s and since then it has drawn attention of the world for a better environmental management and conservation of natural ecosystems and resources. Present study was planned to assess the impact of mercury contained effluent waste on the physiological and biochemical parameters of a fresh water fish. From the analysis of effluent study data it was observed that the effluent is deadly toxic and contained significant amount of mercury. Lethargicity, ataxia and inappetance were observed in toxicant exposed fish compared to control fish. Residual mercury accumulation study indicated that with the increase in exposure period, mercury gets accumulated in different organs of the exposed fish. The whole body respiration of the effluent exposed fishes deceased significantly with the increase in exposure period and effluent concentration showing a positive correlation. The tissue slice respiration study of brain, liver, muscle tissues and gill filaments indicated that the toxicant caused drastic decline in tissue slice respiration compared to control fish tissues. Maximum depletion was noted from 21st day exposure onwards. When the exposed fishes were transferred to toxicant free medium for recovery studies, no significant recovery was observed. This clearly indicated that the toxicant caused irreversible damage to metabolic systems. The ATPase activity study also indicated significant depression in enzyme activity in exposed fish tissues compared to control fish tissues. The biomolecule analysis also indicated significant decrease in protein and FAA content in exposed fish tissues compared to control fish tissues. The analysis of data indicated that residual accumulated mercury was the causative agent of all depressions and damages caused in the toxicant exposed fish compared to control fish.**

***Keywords*—Enzymes, Effluent, Fish, Industry, Mercury, Physiology, Respiration**

I. INTRODUCTION

Pollution is the introduction of contaminants into the natural environments added extraneously by human activity that caused adverse changes in the environmental segments and significantly influenced the biotic component.

Environmental pollution can be caused by natural occurring events and by anthropogenic sources and events too. Pollution can be caused by point sources or non point sources. Pollution has significant impacts on the environment, climate, meteorology, plant and animal life and particularly human health. Earlier pollution was a local phenomenon and now pollution touches all segments of the planet. It has affected the health of all plants, animals and human health. Our health is affected from the food we eat, the water we drink, the land mass where we inhabit and the air we breathe. Approximately 19 million premature deaths are estimated to occur annually as a result of the way we use natural resources to support global production and consumption and which impact the environment for our better and comfortable living. Pollution is an undesirable and unwanted event which impacts drastically our living and living conditions. Pollutants can be of many forms; some are easily noticed and observable such as certain forms of contaminated water, poor air quality, industrial wastes, sewage, coloured effluent, effluent loaded with suspended solids, solid wastes etc. Others are less visible, for example soluble toxicants, heavy metals in plant and animal body and plant and animal products, pesticides in food, excess nutrients in the ponds, lakes, rivers, streams, sea & ocean, fluoride in drinking water and other micro-pollutants in fresh and marine waters. Pollutant sources can be many, natural in origin or artificially manufactured or produced by the industries and many actions of man. Pollutants in the environment remain as a global issue and among the greatest challenges confronted by mankind and a biggest threat for future generations. Among the various kinds of pollutants, heavy metal pollutants have drawn a lot of attention owing to their toxicity and impact on all life forms. Heavy metals exist and available naturally and can be available in the environment by anthropogenic activities. They are mined; isolated and extracted for different use in different processes and purposes. They are thought to be introduced in reasonable quantities in to many environmental compartments due to their varied uses needed by man.

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In different processes to manufacture a product of interest, these heavy metals are discharged in to the environment accidentally, knowingly or unknowingly or carelessly or as a process waste or as an industrial waste. The effluent of the Chlor-alkali industry contains a significant amount of mercury as reported by many authors working on different industries and causing serious threat to the environment. There is no doubt that Chlor-alkali industries are seriously polluting the surrounding environments. But comparatively very few reports are available on the toxicity and toxicological effects of Chlor-alkali industrial wastes on different biotic systems. Shaw *et al*. (1,2,3) and Priyadarsan & Panigrahi (4,5) reported the residual mercury accumulation in different biotic systems available in and around a Chlor-alkali industry. Shaw *et al.* (6) reported the changes in aquatic primary productivity of the estuary contaminated with the effluent of the Chlor-alkali industry. Thorough monitoring and clear assessment of mercury pollution in the vicinity of different Chlor-alkali industries has not been done yet. The present study aims at finding the impact of mercury based effluent and solid waste on a fresh water fish and its ecological implications.

II. Materials & Methods

Location of the industry and study sites: The Chlor-alkali industry M/S Jayashree Chemicals Pvt. Ltd. is situated on the side of National Highway-16 at Ganjam. The industry is located very close to Ganjam Township; district Ganjam, Odisha state, India. The industry is located on the Bank of River Rushikulya discharging its effluent into the river directly (initially) and the solid waste collected from the effluent canal was dumped near the banks of Rushikulya river. The sediments collected from the effluent canal containing huge amount of mercury was removed periodically and dumped in and around the industry as huge deposits. During rainy season, flood water carries the dumped solid waste in to river Rushikulya contaminating the river water, Rushikulya estuary and Bay of Bengal. The discharged untreated effluent enters into the River, Rushikulya. The industry is located at 84^0 53'E longitude and 19^0 16'N latitude.

The industry manufactures sodium hydroxide and HCl from raw NaCl following electrolysis procedure, where metallic mercury is used as cathode. Neither mercury is a raw material nor a product. But mercury escapes into the environment from electrolytic cells during washing and cleaning of cells, cell house, compression chamber and hydration chamber. The washings along with mercury escape through liquid waste, which is called as effluent. This effluent waste is discharged into environmental segments via effluent stocking pond.

The discharged effluent contained highly significant amount of inorganic mercury.

(Arc GIS explore expanded photograph showing the area and site map of the industry located near Rushukulya River and Estuary at Ganjam near Bay of Bengal showing effluent storage tank, leaking of effluent from storage tank and effluent discharge in to River Rushikulya)

Test fish: Oreochromis mossambicus, Peters or *Tilapia mossambica*, Peters; (popularly known as *Tilapia* fish)] was collected from the local nursery & acclimatized in the laboratory.

Methodology: Physico-chemical analysis of water and effluent samples were carried out by following the methods illustrated in APHA (10). The dissolved oxygen was measured by modified Winkler's method as described in APHA (10) and Ashby (11) and expressed as mg of O_2 / liter of sample. Residual mercury content was estimated by digestion of the samples in Bethig's apparatus following Wanntorp and Dyfverman, (12) and estimation of mercury in a Mercury Analyser (13). Estimation of amino acids (FAA) by ninhydrin method following the procedure of Lee & Takahasi (14) and analysis of protein precipitated by TCA was estimated by the procedure of Lowry *et al*. (15) and the protocol as explained in Plummer (16) was followed. Manometric reading of oxygen uptake or carbon dioxide evolution and preparation of tissue extracts were carried out by the methods reported by Patel *et al*. (17), Fox *et al* (18) and Panigarhi (19).

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Total ATPase activity, which was determined by measuring the amount of inorganic phosphate produced when ATP was converted to ADP and ip produced as a result of the cleavage of ATP to ADP by molybdenum blue method described by Fiske and Subbarao (20) as modified by Martineck (21) was adopted. All obtained data was subjected to statistical tests.

III. Results

The effluent collected from the discharge point of the industry and the effluent collected from the effluent storage tank was brought to the laboratory and analyzed. Significant variations were noted in all the studied parameters in 2015-16 when compared to the data reported in 2014-2015. The temperature ranged between 28.2±1.2ºC to 30.2±1.5 ºC. The pH of the effluent ranged from 8.9 ± 0.2 to 9.3±0.2 in two sites of the study area. Most of the parameters were higher at the discharge point and by the time it reaches the stocking pond most of the chemicals settle in the effluent canal showing lower value at the tank site. Significant variation was noted pertaining to mercury content of the effluent in all the sites studied. This data clearly showed a significant variation between the sites and the significant depletion in mercury level was due to the new strategy adopted by the company by adopting the membrane system over mercury cell process.

The dissolved oxygen was significantly less and 3.1±0.6mg $l^{-1}$was observed. The effluent contained 1.12±0.11µg of mercury l^{-1}. Fig.1 indicted the residual mercury accumulation in toxicant exposed fish brain; liver; muscle and gill tissues compared to control fish tissues under laboratory controlled conditions. Maximum accumulation was observed in exposed fish gill tissues compared to exposed brain, liver and muscle tissues. Interestingly steady increase (positive correlation, $r=0.938$; $P \geq 0.05$) in mercury accumulation was noticed in all exposed fish tissues with the increase in exposure period showing a positive correlation. Exposed brain tissues accumulated the least when compared to liver, muscle and gill tissues. A maximum of 7.5µg of mercury /g gill dry tissue; 5.6µg of mercury /g liver dry tissue; 5.7 µg of mercury /g muscle dry tissue and 2.8 µg of mercury /g brain dry tissue was noted after 28days of exposure (Fig.1). When the toxicant exposed fishes were transferred to toxicant free fresh water for recovery studies, no significant recovery was observed, rather during recovery period brain and liver showed higher accumulation of mercury compared to muscle and gill tissues. Recovery brain tissues accumulated the least when compared to liver, muscle and gill tissues.

A maximum of 6.9µg of mercury /g gill dry tissue; 6.8µg of mercury /g liver dry tissue; 5.1 µg of mercury /g muscle dry tissue and 5.4µg of mercury /g brain dry tissue was noted after 28days of recovery (Fig.1). When the exposed fishes were transferred to toxicant free medium brain and liver tissues showed higher accumulation due to internal transportation of accumulated mercury in different tissues. Gill and muscle tissues were in direct contact with fresh water hence decrease in mercury accumulation was noticed. Accumulation of higher amount of mercury in brain and liver probably led to death of the fishes during recovery period. With the increase in effluent concentration the residual accumulation increased at all exposure periods and at all effluent concentrations. The residual mercury level showed a positive trend ($r = 0.973$, $P \leq 0.01$) with the increase in exposure period and with the increase in effluent concentration.

Table-1:
Physico-Chemical Properties of the effluent collected from the effluent canal immediately living the industry and from storage tank in 2015-2016. Data mean of 3 estimations ± Standard deviation.

Sl. No	Parameter	Discharge Effluent
1	Temperature (ºC)	30.2±1.5
2	pH	9.3±0.2
3	Alkalinity($CaCO_3$ mg l^{-1})	258.2±12.4
4	Hardness (as $CaCO_3$ in mg l^{-1})	381.8±17.4
5	Chlorinity (in mg l^{-1})	727.6±48.4
6	Dissolved oxygen(mg l^{-1})	3.1±0.6
7	BOD(mg l^{-1})	31.3±1.8
8	COD (mg l^{-1})	321.4±9.2
9	Suspended solids (mg l^{-1})	146.3±34.5
10	Total nitrogen (in mg l^{-1})	2.4±0.5
11	Total phosphorus (mg l^{-1})	0.21±0.06
12	Total mercury (µg l^{-1})	1.12±0.11

Fig.1: Residual mercury accumulation in exposed fish tissues at different days of exposure and recovery.

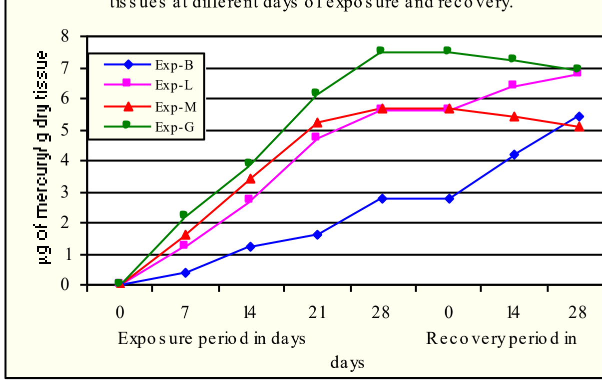


Fig. 2. Changes in whole body respiration in exposed ishes compared to control fishes.

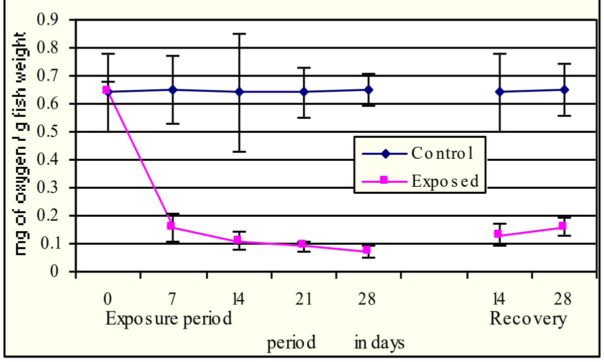
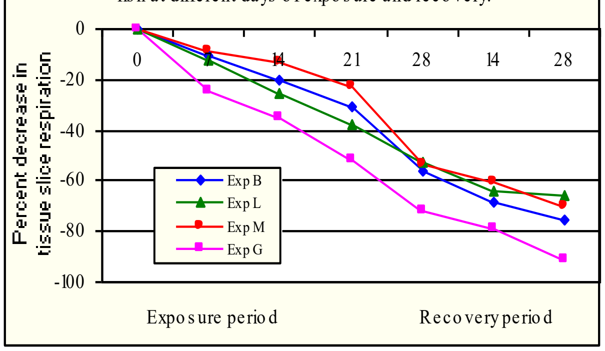


Fig. 3. Pwercent decrease in tissue slice respiration of brain, liver, muscle tissues and gill filaments in exposed fish at different days of exposure and recovery.



The whole body oxygen uptake significantly decreased with the increase in exposure period (Fig.2). The whole body oxygen uptake in control fish was maintained through out the experimental period, no significant variation was noted. On 7th day of exposure, the respiration rate significantly decreased. During this period, the exposed fish appeared lethargic, inappetance, ataxia and loss of equilibrium. After 28d exposure blindness was also observed in the exposed fish. Control fish remained clinically healthy throughout the experimental period.

Fig.4. Changes in ATPase activity in exposed fish tissues compared to control fish tissues at different exposure and recovery periods.

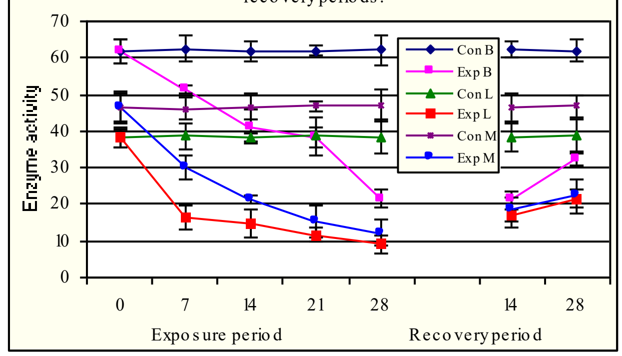
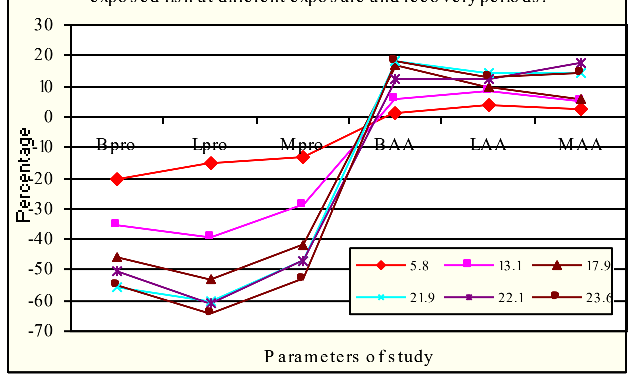


Fig. 5. Interaction &changes in protein and FAA with the changes in residual mercury level in diffrent tissues of the exposed fish at different exposure and recovery periods.



The exposed fishes were transferred to toxicant free medium for recovery studies. No recovery was marked in the exposed fish rather further depletion of respiration rate was noted. After 10days of recovery, fish death was observed. All the exposed fishes could not survive the full recovery period (Fig.2). After observing the fishes in exposure and recovery periods, it was planned to study the tissue slice respiration by Warburg's apparatus. Fig.3 showed the changes in tissue slice respiration in brain, liver and muscle tissues and gill filaments.

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The tissue slice respiration decreased significantly in all the exposed fish tissues like brain, liver, muscle tissues and gill filaments when compared to control fish tissues. The gill filaments of the exposed fish suffered the most compared to liver, muscle and brain tissues. On 28d of exposure 72.3% decrease was noted in exposed gills, 56.4% decrease in exposed brain, 53.7% decrease in exposed muscle tissues and 52.7% decrease was observed in liver tissues after 28d of exposure, when compared to respective control fish tissues (Fig. 3). The gill filaments of the exposed fish suffered the most compared to liver, muscle and brain tissues. On 28d of recovery instead of any recovery further depletion was seen. After 28d of recovery, 90.8% decrease was noted in exposed gills, 75.3% decrease in exposed brain, 70.2% decrease in exposed muscle tissues. And 66.2% decrease was observed in liver tissues when compared to respective control fish tissues (Fig. 3). Fig.4 showed the changes in total ATPase activity of control and effluent treated fish tissues. This study was conducted just to understand the impact of mercury based effluent on fish and the change in behavior of the exposed fishes compared to control fishes. It was observed that the ATPase activity decreased with the increase in exposure period showing a negative correlation. The analysis of variance ratio test indicated the existence of significant difference between columns and significant difference between rows. With the increase in exposure period the enzyme activity depleted in exposed fish brain, liver and muscle tissues. When the exposed fishes were transferred to toxicant free medium, no significant recovery was noted. The data indicated that mercury contained effluent is dangerously toxic and impacts the physiological activity. Fig.5 indicates the percent changes in protein and FAA content in toxicant exposed fish tissues. The protein content significantly decreased in exposed fish brain, liver and muscle tissues compared to control fish tissues. But interestingly the FAA content percent changes increased in all the exposed fish tissues. Increase in FAA content and decrease in protein content in exposed fish tissues indicated either the toxicant induced protein breakdown or the toxicant did not allow the produced amino acids to synthesize proteins. In both the cases the picture is very clear, mercury is the only causative agent to impact badly on the biomolecular content of the exposed fish. The figure also indicated that the depletion in protein level was due to increase in residual mercury content and increase in FAA content was due to induction and interference of mercury in protein biosynthesis and amino-acid condensation to form proteins. The analysis of variance ratio test indicated the existence of non significant difference between columns and non significant difference between rows.

These exposed fishes died gradually after 12days of recovery onwards. It can be concluded that if fish is exposed for a shorter period (less than 7days), the exposed fishes can recover. But when the exposure period was extended further, the toxicant caused / induced irreversible damage to the physiological activity leading to metabolic collapse and finally the exposed fishes died because of the toxicant.

IV. Discussion

Reports that all forms of mercury are toxic to plant and animal life, hence its use and discharge in to the environment is alarming. The effluent collected (2014-2015) from the effluent canal and effluent storage tank indicated significant differences from the data of 2015-2016. The effluent collected from effluent canal had higher values in different parameters tested. The reasons were mostly due to dilutions of other washing waters. Generally the effluent water is discharged in night time and in the day time the other industrial washings were discharged. This mechanism of discharging the effluent in the night time which contained significant amount of elemental mercury is our only concern. This manipulation was to manage the data for outsiders and government machinery and the local people to know the real facts. Presence of mercury to the tune of 3.58±0.62µg / liter in the effluent collected from the effluent canal and 4.85±0.0.58µg / liter in the effluent collected from the storage tank constructed by the industry was the major concern. Significant decrease in dissolved content in the effluent indicated that the effluent was not suitable for any plant or animal life. The low BOD was also indicative of non-existence of life in the effluent. The effluent collected from the storage tank was more toxic and contained significant amount of mercury compared to the effluent collected from the effluent canal. The suspended solids were more in the effluent canal compared to the suspended solid content of the storage tank. This was mostly due to settling/sedimentation of suspended solids in the storage tank. Little decrease in dissolved content in the effluent of the storage tank showed the possibility of life in the surround areas of the effluent tank. The amount of total nitrogen & phosphorus present in both the types of effluent was normal. The effluent of the storage tank contained high amount of chloride and sodium content compared to the effluent collected from the effluent canal. The fresh solid waste collected from the solid waste dumping pit in 2014-2015 from the solid waste dumps contained huge amount of mercury to the tune of 345.6±28.4µg of Hg / Kg dry weight of the solid waste.

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This amount of mercury was found to be much higher compared to any other solid waste collected from this locality earlier and reported earlier from any of the Chlor-alkali industries of India. Significant amount of phosphate, chlorides, calcium, sodium, potassium and nitrogen content was noticed in the solid waste, which were much more than the stipulated guidelines. The mercury speciation study revealed that the effluent collected from the effluent canal was having more amounts of inorganic mercury (less than 98.4%) and less of organic mercury (little more than 1%). The inorganic mercury estimated was the combined value of elemental mercury and inorganic mercury. The mercury speciation study revealed that the effluent collected from the effluent canal nearer to the effluent storage tank was having more amounts of inorganic mercury (less than 96.5%) and less of organic mercury. The inorganic mercury estimated was the combined value of elemental mercury and inorganic mercury. The mercury speciation study revealed that the effluent collected from the effluent storage pond was having more amounts of inorganic mercury (less than 89.4%) and less of organic mercury (little more than 9.4%). The inorganic mercury estimated was the combined value of elemental mercury and inorganic mercury. The mercury speciation study revealed that the effluent collected from the solid waste deposit site on the surface was having more amounts of inorganic mercury (less than 95.8%) and less of organic mercury (little more than 5.1%). The inorganic mercury estimated was the combined value of elemental mercury and inorganic mercury. The mercury speciation study revealed that the effluent collected from the solid waste deposit site at one foot depth was having more amount of inorganic mercury (less than 91.2%) and less of organic mercury (little more than 8.4%). The inorganic mercury estimated was the combined value of elemental mercury and inorganic mercury. Mercury is a persistent chemical and can remain in the environment for a pretty long time in some form or other. The temperature of the effluent is important when discharged in to aquatic water bodies. Maintenance of pH to a particular range is important since all the biochemical activities depend on pH of the environment. Suspended materials play the role of scavenger for the particulars metals in aquatic systems. The suspended particles in water bodies reduce solar energy penetration and reduce photosynthetic activity in case phytoplankton and all aquatic plants. They cause ecological imbalance by mechanical abrasive actions, blanketing action and sedimentation, reduction of light penetration, availability as a surface for growth of bacteria and fungi, reduction of temperature fluctuation and absorption of various chemicals, specifically the heavy metals.

Higher loads of suspended solids cause a significant deterioration in the survival conditions for aquatic organisms. Salinity and hardness, which may together be represented as dissolved solids, play an important role in distribution and maintenance of many organisms in the estuary. Mercury gets into the environment and gets circulated in all environmental segments through mercury cycle.

The reports of Panigrahi & Misra (22,23,24); Nishimura and Kumagai (25); Powell (26); Mishra (27); Rauta (28); Priyadarsan & Panigrahi (4,5) and Priyadarsan (9) clearly indicated that environmental mercury is toxic and if available in the environmental segments can significantly affect the live biota inhabiting in the contaminated site. These authors also opined that environmental mercury can easily be absorbed by the fishes and can accumulate significant amount of mercury in their tissues, organs and whole body. These exposed fishes showed the symptoms similar to mercury poisoning. The observed lethargicity, ataxia, loss of equilibrium, blindness in effluent exposed fish was only due to the mercury present in their body / tissues. The death of the exposed fishes during recovery period was only due residual mercury in brain, liver and muscle tissues. The loss of body weight and decrease in BSI and LSI in exposed fish compared to control fish was reported by Panigrahi & Misra (22,23) and Priyadarsan & Panigrahi (4,5) earlier in mercury exposure. The observed data in the present study agrees with the reports of above authors. From the analysis of effluent study data it was observed that the effluent is deadly toxic and contained significant amount of mercury. Residual mercury accumulation study indicated that with the increase in exposure period, mercury gets accumulated in different organs of the exposed fish. The whole body respiration of the effluent exposed fishes deceased significantly with the increase in exposure period and effluent concentration showing a positive correlation. The tissue slice respiration study of brain, liver, muscle tissues and gill filaments indicated that the toxicant caused drastic decline in tissue slice respiration compared to control fish tissues. Maximum depletion was noted from 21st day exposure onwards. When the exposed fishes were transferred to toxicant free medium for recovery studies, no significant recovery was observed. This clearly indicated that the toxicant caused irreversible damage to metabolic systems. The ATPase activity study also indicated significant depression in enzyme activity in exposed fish tissues compared to control fish tissues. The biomolecule analysis also indicated significant decrease in protein and FAA in exposed fish tissues compared to control fish tissues.

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The analysis of data indicated that residual accumulated mercury was the causative agent of all depressions and damages caused in the exposed fish compared to control fish. The effluent of the Chlor-alkali industry is deadly toxic and should not be discharged in to the environment. The discharged elemental mercury transforms in to inorganic mercury and organic mercury by natural oxidation and reduction followed by bio-methylation mediated by biological agents. The effluent can be used at sub-lethal concentration by diluting with fresh water and should be biologically treated before discharge in to the natural environment.

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Conflict Of Interest

The authors declare that they have no conflict of interest for this publication.

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