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# Eco-Toxicological Effects Of Red Mud Waste Lechate On The ATPase Activity Of a Fresh Water Fish, *Oreochromis Mossambicus*, Peters *In Vivo* Under Laboratory Conditions.

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**Highlights:**

- Red mud waste discharged from NALCO, Damonjodi discharged into red mud pond is deadly toxic and contaminates the environment.
- The leached chemicals leaking at the dam site contaminate the nearby crop field &, ponds and finally flow as a small canal and join the river.
- Fish death was reported in the nearby ponds due to excessive leaching of red mud waste.
- The RMWE exposed fishes appeared lethargic. Reddening of eye was noted.
- The ATPase activity of brain, liver, muscle tissues and gill filaments of the RMWE exposed fish were severely affected compared to control fish.
- The lethargicity of fish leading to fish death was due to the RMW discharged from the industry and lechate from the pond entering into neighboring ponds and water bodies.

**Abstract**

Fishes were exposed to graded series of concentrations of the RMWE for acute toxicity studies. The MAC value deduced was 3.15 % of RMWE in 50 liters of water for 30 days. A safety concentration of 3.1 % of RMWE $l^{-50}$ was selected for this study. Inappetence and ataxia was noticed in the contaminated fish on the very first day of introduction of the RMWE. Erratic movement, gradual onset of inactivity and loss of equilibrium was observed in toxicant exposed fish in comparison to non-contaminated fish. Reddening of eye was noted in the RMWE exposed fish. The body weight of the RMWE exposed fish depleted, when compared to control fish. Increase in body weight was marked in the control fish. In the exposed fish 53.2% depletion in body weight was noted on 28d exposure. When the exposed fish was changed to RMWE free medium, no recovery or insignificant partial recovery was noted. Significant depletion of total ATPase activity in the red mud waste extract exposed fish, impacted the metabolic activity, affected the movements of ions across the membrane and severely affected the energy metabolism and disturbed energy budget of the fish. No recovery was noted in the exposed fishes when transferred to toxicant free medium for the same period of exposure indicating irreversible damage caused to the exposed fish.

**Key words:** Toxicology, Red mud, lechate, fish, toxicity, behavioour, growth, ATPase,

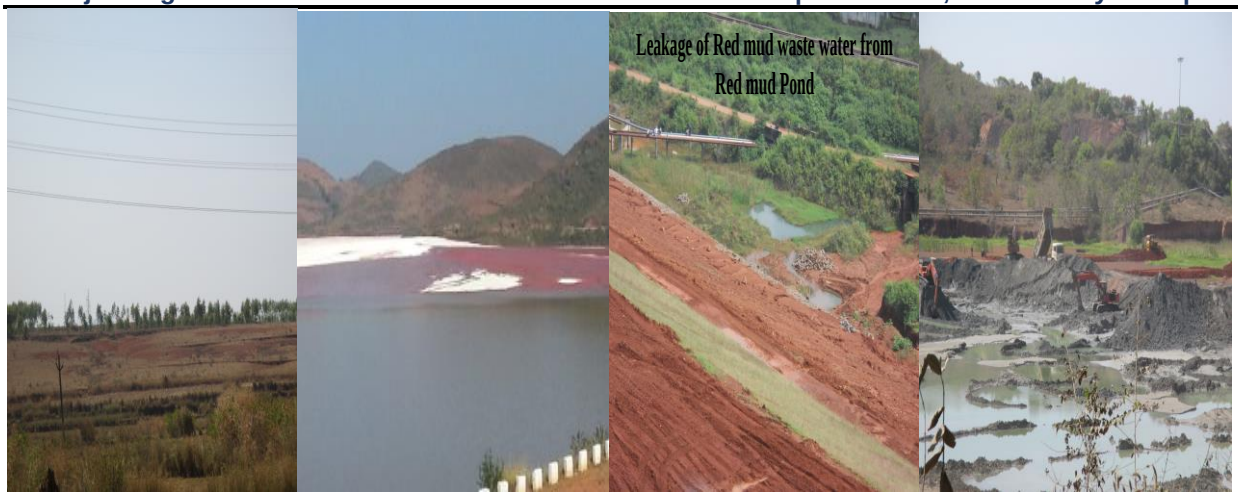
## Introduction

Environmental pollution by industries is well since the last few decades. Alumina industry is a major source of industrial pollution, discharges its highly alkaline red mud waste into a stocking pond nearby. NALCO the industry is located at Damonjodi, Koraput. Chemicals now leach from the red mud pond and contaminate the crop fields and nearby ponds. The authors conducted toxicity testing and behavioral studies on the impact red mud waste lechate on a fresh water fish and reported horrifying results. The same authors also predicted, in future the red mud pond will be a big poisonous pond and may drastically destroy the flora and fauna of Koraput district. Ghorbani *et al*., (2009) studied the biological leaching of aluminium

from red mud bauxite waste by isolated fungi under laboratory conditions. Mohammed *et al*., (2019) reported that heavy metal pollution of Egyptian water was primarily because of agricultural and industrial wastes and fish was the main sufferer in water bodies. Olszewska *et al*., (2016) reported significant amount of arsenic in the red mud waste and also opined that arsenic can bioconcentrate in different trophic levels and can biomagnify in the food chain and food web. The same authors found significant amount of arsenic in few macrophytes and also concluded that inorganic arsenic content was more than organic arsenic in macrophytes. Cuciureanu *et al*., (2020) reported that red mud was the main waste produced during alkaline leaching of bauxite ore and a large quantity of waste was generated in the process and were discharged on large areas year by year needs attention. As these wastes can cause serious environmental and health issues, the author tried to reuse or recycle the red mud wastes for a green future. Patel and Pal (2015) reported that the studied red mud waste was strongly alkaline ranging between 10 to 13 and warned that these wastes were corrosive in nature can cause alarming environmental problems. Murali *et al*., (2018) investigated the toxicological impacts of $Al_2O_3$ nanoparticles on histo-architecture of fresh water fish at sub-lethal concentrations and found loss of cellular architecture because of the toxicant application. Cui *et al*., (2019) reported the leaching behavior of metal elements of red mud was controlled by solubility and not by the concentrations of these metal elements in the red mud waste. This idea can be used while studying the impact of red mud waste on any aquatic animal or plant, as these elements can only be absorbed from the waste, if they are available in the soluble form. Sun *et al*., (2019) studied the geochemical characteristics and presence of toxic elements in alumina refining waste and lechate coming from management facilities and reported the presence of minor elements and trace elements in the waste and these wastes can be toxic to aquatic life due to hyper-alkaline nature of red mud lechate. Zhou *et al*., (2018) reported selective leaching of scandium from red mud and opined that selective leaching is important for reclamation and solid waste treatment while working on red mud waste reclamation. Sarath Chandra and Krishnaiah (2018) conducted a detailed geotechnical analysis of red mud and reported that the waste is iron-oxide rich and commented that the storage and disposal of red mud is the biggest problem and this red mud waste seriously contaminates the aquatic ecosystems. The surrounding areas near industrial establishments may show higher levels of metals via aerial deposition in particulate form. Many environmental pollutants released into the atmosphere eventually settle and reach the soil by direct precipitation or deposition on vegetation and consequently as washings by rain water from vegetation to soil. These activities now require a cost effective, affordable and effective solution to mitigate the pollution problem of the area. Many authors like Authman *et al*., (2015) reported use of fish as bioindicator of the impact of heavy metal pollution. For estimation of pollution level, fishes act as the best tool in toxicological studies of the aquatic systems. The present study was planned to study the impact of lechate of red mud pond on the fish growth & physiology under laboratory controlled conditions.

**Materials & Methods**


(Google maps showing the satellite view of in and around Damonjodi, NALCO- industry**,** location, red mud pond, & extended view to show the flow of lechate waste from the dam.)




**(S**howing the Earthen dam of the industry, red mud pond, leaching of lechate waste from the industry at Damonjodi and reclamation of red mud waste by flyash produced by the industry.)

**Location of the industry**: NALCO produces calcined allumina at refinery complex, Damanjodi, Koraput district, Odisha located at latitude $18^0$-6'-$18^0$-58' towards North and longitude $82^0$.57'-$83^0$.04' East. The industry discharges its red mud waste into a pond surrounded by natural hills from all sides and a small eathern dam was constructed only one at open side.

**Test Organism:** ***Oreochromis mossambicus*, Peters.** [Synonyms: (*Sarotherodon mossambica*, Peters; *Tilapia mossambica*, Peters)]

**Test chemical:** Red mud waste extract (RMWE) equivalent to lechate of the industry was prepared in the laboratory.

The wet red mud waste was air dried under Sun and powdered. The powder was sieved and the thin powder was kept for future use. The 1kg of red mud waste powder was taken mixed with 2L of distilled water and stirred in a stirrer for continuously for 30days with 12hrs of continuous stirring and 12hrs for sedimemntation. After 30days of stirring, the entire content was allowed to sediment and the clear supernatant was decanted and filtered and kept in the refrigerator in a black air tight bottle as red mud waste extract equivalent to leached chemicals leaching from the red pond. We have compared the physico-chemical properties of both leached chemicals coming from the red mud pond and the extract prepared in the laboratory and interestingly we found no significant difference. The fishes were acclimatized in the laboratory aquarium for adaptation for at least 15 days before initiation of the experiment as described by Panda *et al*., (2017) and Dixit *et al*. (2018). Chlorine and contamination free tap water collected from tube wells was used in both control and experimental aquaria. The water of the aquarium was changed daily. Air was bubbled for minimum 18hrs a day in winter & rainy season and 24hrs in summer season, through water of the control and experimental aquaria to maintain the dissolved oxygen at 85 ± 5% air saturation value. The physico-chemical status of both control and exposed aquarium was measured periodically (APHA., 1998; EC, 1976).

The mortality rate of test-fish was studied following the method described by Panigrahi (1980) and Finney (1971). Observation on the toxicity of the industrial leached effluent was made at 24, 48, 72, 96 hours and 28 days after the experimental animals were first exposed. The test fishes were exposed to maximum allowable concentration (MAC) of the RMW lechate which was almost equivalent to the leached chemicals leaking near the red mud pond. Mortality of fish was counted at 24h interval. Individuals showing no respiratory movements, no opercular movements and no response to a tactile stimulus were recorded as dead, and were immediately removed, to avoid contamination. Number of dead fishes in each aquarium was counted and expressed in percentage of death. In the control aquarium, no death was observed and was presented as survival percentage (PS-%). Different mortality rate (LC-%) values were computed. Percentage of death at different concentrations was noted down such as $LC_0$, $LC_{10}$, $LC_{50}$, and $LC_{100}$ of the effluent of the Alumina industry and the values were deduced from the standard graphical interpolations. The fishes were sacrificed at 7 days interval (both control and RMWE exposed). Brain, liver and muscle tissues were removed, kept in watch glasses, and weighed separately. The tissues were kept in ice-cold 0.25M sucrose. Known weight of the tissues were homogenized, centrifuged and the supernatant was taken for testing. Inorganic phosphate produced in the reaction was measured by the method of Fiske and Subbarow (1925) as modified by Martinek (1970). Protein was estimated by the procedure developed by Lowry *et al*. (1951), using a spectrophotometer. The enzyme activity was presented as μmoles of ip liberated $mg^{-1}$ of protein $h^{-1}$. The obtained data was statistically analyzed.

**Results**

Graded series of concentrations of the red mud effluent waste extract was prepared in rectangular jars with chlorine free tap water. Fish was introduced to each jar. A standard control was maintained, where no toxicant was applied. The fish were observed for behavioral changes and mortality of fish was recorded at 24h interval. After 96hours of exposure daily mortality rate observation was discontinued and the exposed fishes were allowed to survive up to 30days of exposure. Mortality was noted on 28$^{th}$ day of exposure for long term experiments. The result indicated that the maximum allowable concentration value of red mud waste extract (RMWE) of the alumina industry was found to be 3.15ml $l^{-50}$ for 30 days. As a precautionary measure 3.10ml RMWE $l^{-50}$ was considered safe for future sub-lethal toxicological studies. The observations showed $LC_{00}$ values at 24 hours to be 5.8% and 5.6% for 48hours. The observations showed $LC_{10}$ values at 24 hours to be 10.4% and 10.1% for 48hours. The results showed that the $LC_{50}$ values for test fish were 19.5% at 24 hours, 19.0% at 48 hours of exposure. The results showed that the $LC_{90}$ values for test fish were 22.5% at 24 hours, 22.1% at 48 hours of exposure. The results showed that the $LC_{100}$ values for test fish were 24.8% at 24 hours, 24.2% at 48 hours of exposure. The $LC_{10}$, $LC_{50}$, $LC_{90}$ and $LC_{100}$, values of the waste of the industry after 28 days of exposure were recorded to be 3.45, 8.15, 12.6 and 15.5% of the effluent. No mortality was observed in the control set, during the entire period of experimentation. No mortality was noticed in the control set, during the entire period of experimentation. All the exposed fish appeared sluggish when exposed to the red mud waste extract. The major symptoms such as lack of appetite appeared after appeared after 2 to 5 days exposure. At higher doses / concentrations of the toxicant, the contaminated fish exhibited unpredictable movements leading to collision to inner side of the aquarium. It was observed that all the exposed fishes looked colored because of the extract waste. On examination of the gills, it was found that the whole gill surface was coated with a slimy layer which was the cause of irritation of the fishes as the gaseous exchange was hampered in the exposed fishes, when compared to control fishes. The contaminated fishes lost their equilibrium as the exposed fishes were hitting to the inner lining of the glass walls of the aquarium. Uncontaminated fish remained clinically normal and healthy for the entire experimental period without showing any signs of toxicity or contamination. Reddening of the eye was noted in the earlier period of exposure. After 25$^{th}$ day of exposure the reddening of the eye disappeared and the entire eye became opaque white leading to blindness. Photograph-1 is the control fish showing normal non infected eye. Photograph-2 showed the waste exposed fish after 25$^{th}$ day of exposure, where the color of the eye is white and opaque. Probably the ophthalmic nerve was affected by the toxicant. Initial swelling and browning of the exposed fish eye was due to external deposition of this color waste and irritation caused by the waste extract or waste effluent. The fish lost its equilibrium after 25$^{th}$ day of exposure onwards. This might be due to impact of the waste on the fish. The eye of the 82% exposed fishes became white and opaque and these fishes lost direction of movement. Hence they were swimming irregularly inside the exposed aquarium. Interestingly, the color of the fish changed to reddish brown as per the color of the effluent. During recovery studies, this color disappeared and fish could regain its pre-test color. In recovery period, except the color no other parameter returned to normalcy. Basing on these change i9n behavior, it was felt necessary to study the whole body oxygen uptake / respiration rate and ATPase activity of the exposed fishes when compared to control fish.

The changes in total ATPase activity in brain, liver and muscle of uncontaminated and RMWE exposed fish at different exposure and recovery periods and it's percent changes were shown in Fig. 1. The enzyme activity ranged 31.45 – 31.94µmole of ip liberated/ mg of protein/ hr during the entire period of experimentation in the control brain tissue (Fig. 1). The brain tissue showed a maximum decrease by 6.96% and the enzyme activity declined from 31.88 ± 3.16 to 29.66 ± 4.12µmole of ip liberated/ mg of protein/ hr on 7$^{th}$ day of exposure to RMWE (Fig.1). The brain tissue showed a maximum decrease by 23.12% and the enzyme activity declined from 31.66 ± 2.65 to 24.34 ± 3.85µmole of ip liberated/ mg of protein/ hr on 14$^{th}$ day of exposure. The brain tissue showed a maximum decrease by 39.09% and the enzyme activity declined from 31.75 ± 2.95 to 19.34 ± 2.14µmole of ip liberated/ mg of protein/ hr on 21$^{st}$ day of exposure. The brain tissue showed a maximum decrease by 48.81% (Fig.2) and the enzyme activity declined from 31.9 ± 3.19 to 16.33 ± 3.65µmole of ip liberated/ mg of protein/ hr on 28$^{th}$ day of exposure (Fig.1). After the exposed fish was transferred to toxicant free medium, the ATPase activity declined on 28$^{th}$ day of recovery. No recovery in the brain enzyme activity was marked on 14d of recovery rather further depletion in the enzyme activity was noted. This enzyme activity decreased by 49.5% on 14$^{th}$ day recovery and decreased by 61.5% over the 28$^{th}$ day exposure value, after 28 days of recovery, indicating no recovery, rather further damage was noted. The exposed fish brain enzyme activity could not recover even partly but further depletion in the enzyme activity on 28$^{th}$ day of recovery was noted.

**Fig.1: Changes in total ATPase activity in brain, liver, muscle and gill of control and RMWaste lechate exposed fish, at different days of exposure.**

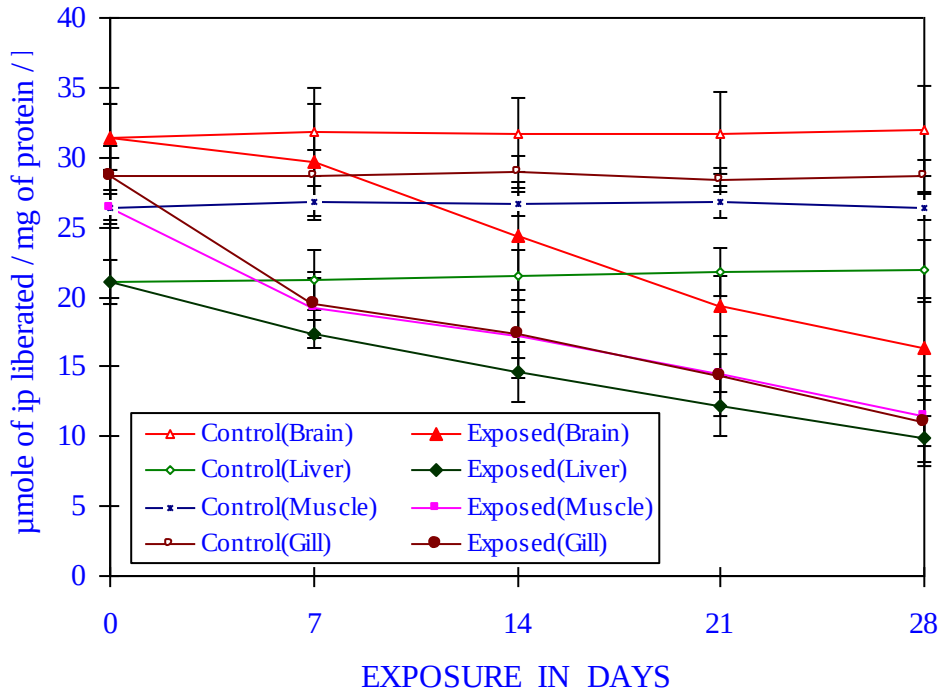
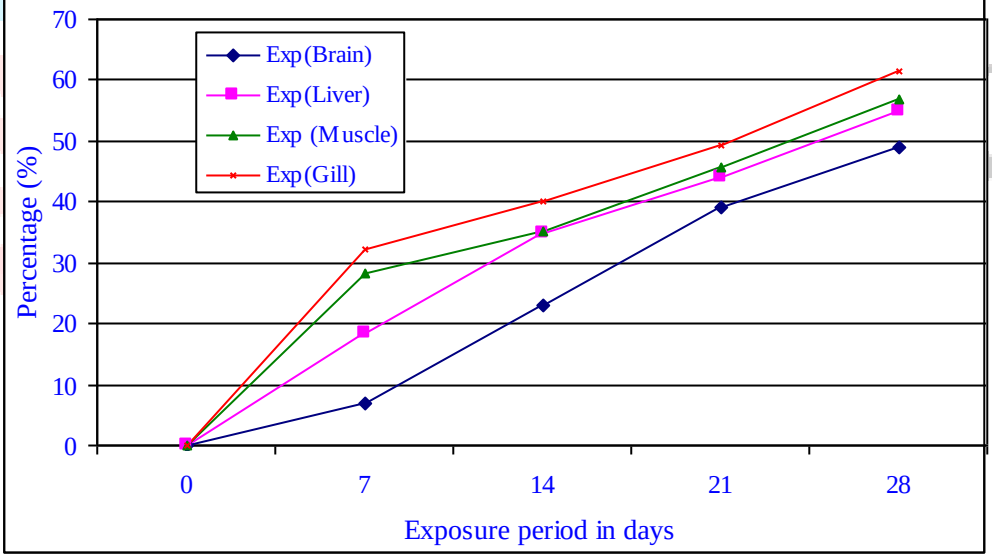



Fig.2: Percent changes in ATPase activity in RMWE exposed fish compared to its respective control values at different days of exposure. Data calculated from the mean of the samples.




The changes in total ATPase activity in liver of control and RMWE exposed fish at different exposure and recovery periods and it's percent changes were shown in Fig. 1 and 2. The liver tissue showed a maximum decrease by 18.4% after exposure to RMWE (Fig.2) and the enzyme activity declined from 21.24 ± 2.14 to 17.33 ± 0.95µmole of ip liberated $mg^{-1}$ of protein $hr^{-1}$ on $7^{th}$ day of exposure (Fig.1). The liver tissue showed a maximum decrease by 34.81% after exposure to RMWE (Fig.2) and the enzyme activity declined from 21.57 ± 1.85 to 14.61 ± 2.18µmole of ip liberated $mg^{-1}$ of protein $hr^{-1}$ on $14^{th}$ day of exposure (Fig.1). The liver tissue showed a maximum decrease by 44% after exposure to RMWE (Fig.2) and the enzyme activity declined from 21.84 ± 1.72 to 12.23 ± 2.14µmole of ip liberated $mg^{-1}$ of protein $hr^{-1}$ on $21^{st}$ day of exposure (Fig.1). The liver tissue showed a maximum decrease by 54.98% (Fig.2) and the enzyme activity declined from 21.88 ± 2.22 to 9.85 ± 1.64µmole of ip liberated $mg^{-1}$ of protein $hr^{-1}$ on $28^{th}$ day of exposure (Fig.1). After transfer to pollutant free medium, the ATPase activity for the exposed fish declined on both $14^{th}$ day of recovery and on $28^{th}$ day of recovery. This enzyme activity decreased by 59.4% on $14^{th}$ day recovery and decreased by 71.5% over the $28^{th}$ day exposure value, after 28 days of recovery, indicating no recovery. The exposed fish liver enzyme activity could not recover even partially on $28^{th}$ day of recovery. Non recovery

but further depletion of the enzyme activity during recovery period indicated acute toxic nature of the toxicant, RMWE. The changes in total ATPase activity in muscle of uncontaminated and RMWE exposed fish at different exposure and recovery periods and it's percent changes were shown in Fig. 1 & 2. The muscle tissue showed a maximum decrease by 28.2% of the enzyme activity (Fig.2) on 7$^{th}$ day of exposure, when compared to control and the enzyme activity declined from 26.84 ± 1.1 to 19.28 ± 2.14µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 7$^{th}$ day of exposure (Fig.1). The muscle tissue showed a maximum decrease by 35.3% of the enzyme activity (Fig.2) on 14$^{th}$ day of exposure, when compared to control and the enzyme activity declined from 26.68 ± 0.91 to 17.27 ± 1.65µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 14$^{th}$ day of exposure (Fig.1). The muscle tissue showed a maximum decrease by 45.6% of the enzyme activity (Fig.2) on 28$^{th}$ day of exposure in comparison to control fish enzyme activity declined from 26.76 ± 1.14 to 14.55 ± 1.35µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 21$^{st}$ day of exposure (Fig.1). The muscle tissue showed a maximum decrease by 56.8% of the enzyme activity (Fig.2) on 28$^{th}$ day of exposure, in comparison to the control and the enzyme activity declined from 26.45 ± 0.95 to 11.43 ± 2.18µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 28$^{th}$ day of exposure (Fig.1). The changes in total ATPase ( $Na^+$, $K^+$, $Mg^{++}$ dependent), activity in gill filaments of uncontaminated and RMWE exposed fish at different exposure and recovery periods and it's percent changes were shown in Fig. 1 & 2. The muscle tissue showed a maximum decrease by 32.1% of the enzyme activity (Fig.2) on 7$^{th}$ day of exposure, when compared to control and the enzyme activity declined from 28.62 ± 1.95 to 19.44 ± 2.31µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 7$^{th}$ day of exposure (Fig.1). The gill filaments showed a maximum decrease by 40.1% of the enzyme activity (Fig.2) on 14$^{th}$ day of exposure, when compared to control and the enzyme activity declined from 28.94 ± 1.15 to 17.35 ± 1.65µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 14$^{th}$ day of exposure (Fig.1). The gill filaments showed a maximum decrease by 49.24% of the enzyme activity (Fig.2) on 28$^{th}$ day of exposure in comparison to control fish enzyme activity declined from 28.33 ± 0.85 to 14.38 ± 2.85µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 21$^{st}$ day of exposure (Fig.1). The gill filaments showed a maximum decrease by 61.4% of the enzyme activity (Fig.2) on 28$^{th}$ day of exposure, in comparison to the control and the enzyme activity declined from 28.65 ± 1.14 to 11.06 ± 3.21µmole of ip liberated mg$^{-1}$ of protein hr$^{-1}$ on 28$^{th}$ day of exposure (Fig.1). Significant depression in ATPase enzyme activity may severely affect the ion transport mechanism of the exposed fish body when compared to control fish body. The correlation coefficient analysis between days of exposure and $Na^+$, $K^+$, $Mg^{++}$ dependent ATPase activity in control fish brain showed a positive but non-significant correlation, whereas, the exposed fish brain showed a negative correlation ($r = -0.977$, $P \leq 0.01$). The control set showed positive but insignificant correlation. The percent change in the enzyme activity in the exposed fish brain when compared to control fish brain showed the existence of a negative correlation ($r = -0.969$, $P \leq 0.01$) with the increase in exposure period. The analysis of variance ratio test indicated the existence significant difference between rows and non-significant difference between columns. The correlation coefficient analysis between days of exposure and the $Na^+$, $K^+$, $Mg^{++}$ dependent ATPase of liver slices of the control fish did not show any significant correlation, whereas the exposed fish liver showed a negative and significant correlation ($r = -0.945$, $P \leq 0.05$) with the increase in exposure period. The percent change in ATPase activity showed a negative correlation with the exposure period which was significant. The analysis of variance ratio test indicated the presence of significant differences between rows and insignificant difference between columns. The severe impact of RMWE on the ATPase activity of different tissues of the exposed fish indicated probable effect on the ion transport system of the tissues of the exposed fish. This unit was planned to test and observe any change in sodium ion, potassium ion and calcium ion content in brain, liver and muscle tissues of the RMWE exposed fish and compare with control fish tissues.

**Discussion**

In the present investigation, reddening of eyes was marked. This might be due to other toxicants present in the red mud waste. Swelling of eyes and reddening of eye can be attributable to mercury poisoning and consequent early blindness might be due to the combined effect of the chemicals present in the lechate of the solid waste (Pattanaik, 2002). Higgins (1974), Mac Leod and Pessah (1973) reported that loss of appetite, loss of weight, nervousness, dizziness, imbalance, irregular swimming and gradual introduction of inactivity were the sub-clinical effects of inorganic organic mercury intoxication. Identical observations were marked here in exposed *Tilapia* fish when exposed to RMWE prepared from dry red mud waste collected from the industrial site. Panigrahi (1980) also reported similar symptoms relating to mercury based fungicide. Mac Leod and Pessah (1973) reported neurological damage caused by mercury on fish, basing on the behavioral studies. In the present investigation though we found similar symptoms & results, but it will be difficult to say, what really caused the changes or the effects were due to the combined effect of the variety of chemicals present in the RMWE. In preliminary study of the present investigation, the

animals showed all regular features of mercury poisoning; such as excitation, irritation and restlessness. Towards the end of the experimental period i.e. prior to death the locomotion of fish almost ceased and remains suspended vertically in the water medium, indicated loss of equilibrium. Samant (1989) reported an identical trend but in the present investigation the observed changes were more significant. The early signs of poisoning were probably due to the effect of combined chemicals of the extract, which showed probably synergistic effects on fish.

The observed depression in active metabolism in red mud waste extract exposed fish indicated probable damage to the nervous tissues, inhibition of enzymes or inhibition of vital metabolic system was in agreement with Mac Leod and Pessah (1973) and Panigrahi (1980). It is possible that even an insignificant depletion in the normal active metabolic rate may be indicative of pollutant stress in the animals (MacLeod & Pessah,1973). Loss in weight due to starvation was noted by Larson and Lewander (12973). But the loss due to starvation was far less. Panigrahi and Misra (1978,1979,1980) reported the loss of body weight due to mercury intoxication and confirmed that this loss in body weight was only due to mercury stress. In addition, exposed fish maintained their feeding habit only after a short span of time. Hence the weight loss can not be correlated with starvation but can be related with the red mud waste intoxication. A significant correlation exists between the changes in body weight and days of the exposure. Blindness and exophthalmia in mercury exposed fish were probably caused by the effects of mercury deposition on the brain and optic nerves. Magos *et al*., (1985) reported very insignificant difference in the neuron-toxicities of methyl mercury and ethyl mercury. A significant increase in residual mercury content in the brain is hazardous. Different types of toxic chemicals absorption and deposition in the brain of any animal is dangerous and hardous. Brain tissue should therefore be carefully examined for carcinogenic or histo-pathological signs. Gopalkrishnan (1961) reported the different stages of eye infection, the second stage of infection of eye being the reddening of the eye ball and whitening of the corneas. Similar eye infection was observed with a prior swelling of the eyes. This was probably being due to the destruction of brain cells and nerve cells which causes loss in consistency in the whole tissues impacted by the toxicant. Stevenson *et al*., (1977) reported no change in body weight gain between control and lead exposed rats up to 8 weeks exposure. Growth is generally used to analyse the impact of toxicants on fish (Buikema *et al*., 1982). However, biochemical and biomolecular changes during toxicosis should precede reductions in fish growth, as growth is the culmination of several biochemical reactions and processes. Physiological and biochemical impacts are the fundamental causes of measurable whole organism impacts (Mehrle and Mayer, 1980).

Hinton *et al*., (1973) reported that mercury is the causative agent in fish tissue enzyme alteration even at very low concentration and prolonged exposure and higher accumulation of metals finally leads to structural alterations (Hossain and Dutta, 1986). The maintenance of certain concentration gradient in different tissues as a matter of fact depends on the integrity of the $Na^+$, $K^+$-ATPase activity. ATPase is pointedly required for the transport of ions against concentration gradient as well as across the membranes. Yagi *et al*., (1976) reported the existence of a strong inter relationship between total ionic dependent ATPase activity and Na , K , Ca and Mg ion content of the tissues. Ahuja and Subrahmanyam (1978) concluded that ATPase plays a central role in synaptic transmission and nerve impulse generation. The decrease in brain ATPase activity may be due to over excitation of the fish to the stress (Ahuja and Subrahamanyam, 1978). The same author reported decrease in brain $Na^+$, $K^+$-ATPase activity in the trained rats may be due to their nervous over excitation during training. A drastic depletion in this enzyme activity in liver, gill and muscle was observed in the exposed fish. Panigrahi (1984) reported a similar trend in mercury based fungicide exposed fishes. The data of this investigation coincides with the findings of Rath and Misra (1980) in different vital organs of a freshwater fish exposed to dichlorovos. Since liver is the active site of all metabolisms, depletion in ATPase activity in the liver caused diminishing of enzymes activity at 10 mg/kg and 20 mg/kg MMC in rats. The same author reported a slight activation of the enzymes activity with an injection of Hg. Verma *et al*., (1978) reported depression in ATPase activity by chronic Chlordane intoxication in freshwater fishes and suggested that the mechanism of the action of the pesticides on ATPase activity system may be probably due to the uncoupling of oxidative phosphorylation which presumably caused a depletion in the phosphorylation product -ATP. Panigrahi (1980) also showed similar trend as suggested by Verma *et al*., (1978). Panigrahi (1980) noted that the reduction in available free phosphate would be directly proportional to the reduction of total ATP produced. Panigrahi (1980) reported a significant difference in the response of the ATPase activity to inorganic mercury both in *in vivo* and *in vitro* conditions. In the exposed fish a gradual decrease of $Na^+$, $K^+$, $Mg^{++}$-dependent ATPase activity with the exposure period was marked in all the tissues. In contrast, in *in vitro* studies, mercury contained in the solid waste at low concentrations stimulated the total ATPase activity. However, on further increase in solid waste concentration, the total ATPase activity decreased significantly. In the present investigation, the

difference in action of the enzyme activity was due to the red mud waste extract. Stimulation occurs at low concentration of the stress and the fish behavior was more erratic. The extreme behavior of the exposed fish, exposed to red mud waste, now can be correlated with the enzyme activity .There exists a strong correlation among the ions concentration in the tissue, ATPase activity , AChE activity and metabolic rate (Panigrahi,1980 & Webb,1966) with the behavior of the fish. Any change in the above said parameters due to the toxicant, will change the behavior of the fish directly or indirectly. The above investigation reported by Pattaniak (2002) strongly indicated that the enzyme activities were related to residual mercury concentration in the tissues. We can conclude here that probably the causative agent for all such observed changes in this study is the soluble chemicals present in the red mud waste extract of the NALCO plant at Damanjodi.

The decrease in activity of the fish, the decrease in oxygen uptake by the fish, the decrease in tissue slices respiration, the lethargic movement by the fish may be well correlated to the decrease in ATPase activity. Differences were well marked between *in vivo* and *in vitro* results. At lower concentrations, there appears to be sufficient evidence from those studies to indicate that ATPase inhibition was probably the site of attack of inorganic mercury in fish tissues. The decrease in the enzyme activity in the present investigation might be only due to the soluble chemicals of the red mud waste. Similar results were observed by Panigrahi (1980), and Pattanaik (2002). Hence, basing on the present data, we cannot assume that the observed changes were only due to mercury contained in the toxicant, as the present toxicant, red mud waste extract does not contain mercury even at very low concentrations. Hence, the observed depletion was probably due to the combined effect all the chemicals present in the waste extract rather than a single chemical. We are not inclined to criticize the obtained data earlier by other workers in the field but a clear message can be given by saying that the enzyme activity can be influenced by any of the toxicant(s) available in the environment. Hence, the measurement of this enzyme can be a single parameter indicator for measuring and monitoring pollution of the aquatic environments. Excess discharge of any industrial wastes, leakage of red mud wastes from red mud ponds as in the present case might cause severe damage to the ecological system. The discharge of waste chemicals (RMW) particularly inorganic ions restrict oxygen uptake of fish by causing circulatory and respiratory failures, which in turn significantly affect smooth osmotic exchange of gases. There are reports that a thick film of red mud waste is deposited on the gill filaments of fish causing severe disruption in gaseous exchange. The leached chemicals of the red mud waste increased the turbidity of the water and transparency decreased significantly. This might have played an important role to decrease the dissolved oxygen present in the exposed aquarium. The MAC value of the toxicant is generally altered or influenced by water quality, temperature, size of the fish and by any environmental parameter. Smaller fishes can tolerate lower concentrations. Bigger fishes can resist up to a concentration where all small fishes die. With the increase in exposure period toxic tests becomes more pronounced in small fishes, when compared to bigger fishes. Probably the toxicity varies and corresponds to age of the fish. In this piece of investigation it was presumed that all types of chemical compounds with other soluble chemicals would be discharged out as lechate. The extract prepared in the laboratory contains the lechate of the red mud pond, which was observed in the spot at Damonjodi, Koraput. In the present investigation, the medium of the experimental aquarium was changed periodically to ensure the maintenance of toxicant concentration.

A significant depression of $Na^+$, $K^+$-ATPase activity was observed with the excessive absorption of the toxicant from the environment. The decrease in total ATPase activity in gill *in vivo* of the exposed fishes lead to the decrease in ion exchange and also, in addition, gaseous exchange. The depletion in total ATPase activity with the exposure period in liver, brain and muscle tissues caused the depression in the activity of exposed fish was most probably due to mercury present in the waste, as reported by Pattanaik (2002). In the present investigation, it was found that this toxicant (red mud waste extract, RMWE) can depress the enzyme activity and hence the active metabolism. It may be quite possible that mercury and metabolites in the tissues, jointly responsible for the observed toxic action of mercury *in vivo* and at higher concentration *in vitro*. Further more, Yagi *et al*.,(1976) correlated the decrease in $Na^+$, $K^+$, $Mg^+$ content in different tissues of PCB poisoned rat with total ATPase activity. No clear evidence is available to correlate ATPase inhibition with fish mortality. The depression in ATPase activity can be related to fish respiration and fish metabolism deserves attention. However, the depletion in respiration rate, ATPase activity in all vital tissues, non availability of oxygen due to non functional gill filements due to brown layering on the gill surfaces etc can be linked to fish death. Desaiah *et al*. (1975) suggested that "such a case is possible due to detoxifying mechanism working *in vivo* preventing development of a graded dosage inhibition type of response even though such graded response has been reported *in vitro"*. But in contrast, in our recovery experiments, no significant recovery of total ATPase activity in brain, liver and muscle was noticed. Hence, the hypothesis that detoxifying mechanism decrease the dosage inhibition type of response, was not

agreeable, as no significant recovery was noticed in the fishes. The amount of recovery may be mostly due to the excretion of toxicants from the body. The cation exchange system has direct relation with the ATPase enzyme activity. The activity of this particular enzyme depends on several factors such as concentration of substrate (ATP), products (inorganic phosphate, ADP) and metal accelerators ($Na^+$, $K^+$ and $Mg^{++}$). Hence any sort of change in one of these above mentioned factors would lead to the inhibition of enzyme activity or it might be due to the resultant of all the above factors. The decrease in $Na^+$-ATPase, $K^+$-ATPase and $Mg^{++}$-ATPase might be due to the disturbance of $Na^+$, $K^+$ and $Mg^{++}$ ion concentration in different tissues of the fish.

The response of ATPase to RMWE varied from fish to fish, tissue to tissue and also within the same tissue. The toxicant caused permanent damage to cell and other vital systems. Due to decline in enzyme activity the metabolic activity declined significantly. The decline in enzymatic activity, periodic failure of nerve impulse generation, depletion in metabolic activity can be correlated to red mud waste toxicity which gets reflected in the behavior of the organism in the form of impaired swimming, periodic outburst, imbalance, ataxia, inappetence, lethargicity etc. Although quantifying these effects and changes, will be a very difficult task in the present setup of conditions, still then an approximation can be made from the structural and behavioral changes. The change in behavior in exposed organisms was always compared with that of control organisms. Both the sets were kept under identical conditions and the only difference being the exposed set gets the pollutant stress. So the difference in both the sets might be due to the toxicant stress. In both control and exposed *Tilapia* fish, a great variation in total ATPase was marked among brain, liver, and muscle tissues. It has been observed that at very low concentrations of the waste no activation of the enzyme activity was marked in all the three types of tissues. This was probably due to the total effect of all chemicals present in the lechate acted as a whole to produce such a higher toxic effect. It was observed from the data that the RMWE prepared from the red mud waste coming out from the Alumina Industry is dangerously toxic and caused irreversible damage and caused significant behavioral changes. Decrease in body weight was the consequences of tissue hydration, depression of metabolism, decrease in respiration rate and ventilation rate, decrease in somatic indices, destruction and none functioning or inhibition of key enzymes, destruction of energy rich biomolecules, non production of energy rich biomolecules, ionic imbalances leading to non transport of essential chemicals across membranes. When all the parameters studied showed higher inhibition or higher depletion, the exposed fish became more and more lethargic, ultimately loss of movement and fish death was observed.

**Conclusion**

The experimental fish death with pollutant exposure confirms the report and complains of the local people pertaining to fish death in the contaminated area because of entry of leached chemicals from the red mud pond of the industry (NALCO) into fish ponds nearby or water bodies nearby where fish death was noticed. Hence care should be taken to restrict leaching of chemicals from the red mud pond and also restrict their entry into water bodies for safety of human beings inhabiting in and around the area at Damonjodi where NALCO industry is located.

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**Declarations**

**Author contribution statement**

Prof. A.K. Panigrahi: Conceptualization, planning, Original draft preparation, supervision, reviewing and editing; Dr. P. K. Dixit: execution of the project, draft preparation, editing, reviewing of the script; Research work conducted by scholars – Manasi K. Panda- red mud waste collection, analysis of samples, and related field work, experimental work, data calculation, preparation of first draft and editing. Ms. Panda contributed reagents & glassware for laboratory experiment related work and other expenses related to field visits.

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