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Toxicological Effects Of Red Mud Waste Lechate On The Ionic Content Of Different Tissues Of a Fresh Water Fish, *Oreochromis Mossambicus*, Peters Under Laboratory Controlled Conditions.

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Highlights:

- The leached chemicals leaking from the red mud dam site at Damonjodi contaminates the nearby crop fields & ponds.
- Fish death was reported by the fisherman and inhabitants of the area in the nearby ponds due to excessive mixing of leached red mud waste as lechate.
- The RMWE exposed fishes appeared lethargic and imbalance in movement was observed. Reddening of eye of exposed fish was noted.
- Sodium, Potassium, Calcium and Magnesium ion contents significantly decreased in red mud waste extract exposed fish tissues compared to control fishes after 28days of exposure.
- Depletion of ion content in different tissues of the waste extract (WE) exposed fishes indicate the impact of the toxicant and the cause of lethargicity and imbalance in the movement of the waste extract exposed fishes compared to control fish.

Abstract

Fishes were exposed to the maximum allowable concentration value of red mud waste extract for a period of 30days to study the impact of the lechate waste. The MAC value deduced from the toxicity test was 3.1 % of waste extract (WE) in 50 liters of water for 30 days. Lethargicity was noticed in the contaminated fish on the first day of introduction of the toxicant, waste extract. Gradual onset of inactivity and loss of equilibrium was marked in WE exposed fish compared to control fish. Reddening of eye and whole body was noted in the WE exposed fish. The sodium ion and potassium ion content significantly decreased in brain, liver and muscle tissues of the red mud waste extract exposed fishes. The muscle showed the highest percent decrease in sodium ion content, when compared to liver and brain tissues of the waste extract exposed fish. The muscle tissue of the exposed fish was significantly affected than brain and liver tissues of the RMWE exposed fishes except in magnesium ion where it was least affected. Significant depletion was noted in potassium ion content of the exposed fish tissues. The red mud waste extract which is equivalent to the lechate leaking from the red mud pond is highly toxic and affects the fish. Decrease in ion content in different tissues of the waste extract (Red Mud Waste Extract / lechate from red mud pond) exposed fishes indicated the intensity of the toxicant, waste extract. The cause of lethargicity and imbalance in the movement of the waste extract exposed fishes compared to control fish was due to the toxicant used for the study.

Key words: Red mud waste, lechate, fish, Sodium, Potassium, Calcium, Magnesium

Introduction

Industry is responsible for creating a wide spectrum of new chemicals every year all of which eventually find their way into the environment. For most of these chemicals, not even the chemical formulae are known and much less are known about their acute, chronic or genetic impact on plants and animals. At present, the industry is the focus of attention, the world-over, as strongest polluter of the environment. Every underdeveloped or developing country aiming to be highly developed, explore nature and tried to find out methods and adopted industrial revolution, leaving behind the consequence of such trend. When the problems of environmental status and quality was taken into consideration it became apparent that, it was not enough to alleviate undesirable consequences of man's activities by technological means or to prevent them forbidding certain practices of the are of certain chemicals. Rapid industrialization and exploitation of natural resources on massive scale, accumulated undesirable substances in huge quantities in the environmental segments, thus, pollute the environment significantly affecting all microbial flora and fauna, plants, animals including man. The field under study refers to the Alumina industry specifically to Mining and Refinery complex, NALCO, Damanjodi in the district of Koraput, Orissa, India. For analysis of various factors of pollution in alumina industry it becomes imperative for us to discuss the manufacturing process from ore mining to refining in brief with reference to NALCO. The alumina is extracted in two important stages i.e. one during mining operation & the second from the ore refinery. Installation of National Aluminium Co. Limited (NALCO) is a major step towards self sufficiency in good quality of Alumina production. After all the stages of reactions of bauxite refining process the red mud is finally generated. For 8 lakhs MT alumina production 10 lakh MT of red mud is generated and is being canalized in to red mud pond by 2nd stage pump in slurry form at the rate of $370m^3$/ hr with 150gps solid consistency and 2.3gpl Na_2O the dry red mud flow in to the Red Mud Pond is 110MT/hr. For storing red mud waste a pond was constructed near refining complex towards the west side of the industry surrounded by natural hills on all sides. At the opening side a dam is constructed by the company to store the red mud waste. The mines and refinery complex of NALCO, Damonjodi is situated at Similiguda block, under Potangi tahasil in the district of Koraput, Odisha state, India. From the district head quarters Koraput, it is 38 kilometers towards south-east on road, i.e. 27kms towards south in NH-43 up to Similiguda junction and further 11kms towards east on project road. It is 60 kms from Jeypore, the oldest city of Koraput district. Damonjodi is at a highest of about 1300 mts. from sea level, located at latitude 18^0-6'--18^0-58' towards North and longitude 82^0.57'- 83^0.04' East. The area enjoys an annual rainfall of 1723-1855 mm. The area enjoys a modest climate with little high rainfall when compared to other areas of Koraput district (Panda, 2022). Panda *et al*., (2017 and 2018[a], b) reported that the red mud waste is deadly toxic with following composition: Chemical properties: Typical = Al_2O_3(%)- 98.7; Na_2O(%)- 0.38 ; Fe_2O_3(%)- 0.01; SiO_2(%)- 0.012; and CaO(%)-0.042. Alumina hydrate: Physical properties: Typical: LOI (110-1000°C)%- 34-36, Moisture-3-6; GRANULOMETRY: Typical- 45Micron(%)-3-6. Chemical properties: Typical = Al_2O_3 (%) - 65±0.5, Na_2O (%) Total-0.23-0.30, Na_2O (%) Soluble- 0.015-0.025, SiO_2(%)-0.007-0.010, Fe_2O_3(%)-0.006-0.008 and Hydrate Content- 99.0%. Panda *et al*., (2017) working on red mud waste and RMW lechate on fresh water fish reported that the lechate leaking from the red mud pond on the dam side and the extract which was prepared in the laboratory are almost equivalent as both the toxicants showed severe impact on fish, on economically and non-economically important plants and animals.

Keeping in view; the discharge of red mud effluents of the industry into red mud pond and leaking of lechate chemicals from the red mud pond of the industry in to the environment and its entry into nearby water bodies (ponds) drew our attention. During rainy season entry of these chemicals and lechate along with runoff water and over flow of runoff water of the paddy fields, their entry into fresh water bodies like fish ponds, canals, rivers and the water reservoir of the Upper Kolab hydroelectric cum irrigation project, this project was planned. The reports published by Panda *et al*., (2017, 2018a,b, 2023) pertaining to the impact of red mud lechate / red mud waste extract on growth, physiological activity and enzyme activity indicated the acute nature of the wastes where the impact is severe and non reversible. The authors also indicated the lethargicity, loss of equilibrium and reddening of the body was the observed effects of red mud waste. In addition, the local report from the local residents about fish kill in ponds nearby dragged our attention and hence, this project was aimed to evaluate the impact of the leached waste of Red Mud Pond / red mud waste extract (prepared in the laboratory on the behavior and ionic balance of a fresh water fish, *Oreochromis mossambicus*, Peters under laboratory controlled conditions.

Materials & Methods

Experimental fish: *Oreochromis Mossambicus*, Peters
Toxicant used: Red mud waste extract prepared from Red Mud waste collected from the red mud pond. The red mud waste was brought to the laboratory, air dried and powdered. One kg of dried red mud powder was mixed with one liter of water. The mixture was stirred with a blender for one month and then allowed to stand for two days. The supernatant was decanted and filtered. The filtered supernatant is known as RMWE (Red mud waste extract) used for the experiment. The RMWE is almost equivalent to the leached effluent coming out from the red mud pond (Panda *et al*., 2017). 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. The LC_{10}, LC_{50}, LC_{90} and LC_{100}, determined for *Tilapia* fish was found to be 3.45, 8.15, 12.6 and 15.5 % RMWE l^{-50} respectively after 30 days of exposure. Contaminated fishes looked lethargic in comparison to the control fish (Panda *et al*., 2018 a, b).
Maintenance of fishes in laboratory aquarium: Oreochromis mossambicus, Peters of medium size (12-18 g) was collected from a local nursery of the Fisheries Department located at Berhampur (Ganjam), Orissa. The fishes were acclimatized in the laboratory aquarium for adaptation for at least 15 days before initiation of the experiments as described by Panda *et al.,* (2017). 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 on summer season, through water of the control and experimental aquaria to maintain the dissolved oxygen at 85 ± 5% air saturation values. The physico-chemical status of both control and exposed aquarium was measured periodically and care was taken to maintain at the same level during the entire period of experimentation. The pH was maintained within 7.2±0.5 range in the control aquarium and because of addition of RMWE, the pH increased to 7.9±0.4 and the medium was little alkaline. The illumination of the aquarium area was maintained at 2200±200lux, intensity. The hardness of water was maintained at 76.5±4.2mg l^{-1} in control aquarium and at 85.1±6.6mg l^{-1} in exposed aquarium. The specific conductivity was maintained at 3.52x100µmho and at 3.61x 100µmho in control and exposed aquarium. The transparency was maintained at 0.01 to 0.025 in control and at 0.06 to 0.075, measured at 540nm. The food habit was slowly changes from live earthworm pieces to sliced goat liver and finally to sliced / minced boiled egg white during holding and experimental period. To prevent infection before experiment, the fish were washed with 1% dilute Potassium permanganate solution in the aquarium. The potassium permanganate solution was slowly flushed out from the aquarium and the aquaria were freshly recharged with fresh water without causing any stress to the fish.

Both control and red mud waste effluent exposed fishes were sacrificed and dissected after 28 days of exposure. Different tissues of each fish were dissected out, properly washed, separated and kept in a watch glass. The tissues were soaked and adhered water was removed and weighed. Contamination of these tissues was avoided during autopsy. After soaking with Whatman filter paper, the tissues were then transferred to Kjeldahl flasks continuing concentrated Nitric acid (BDH, Analar grade) and Sulfuric acid (BDH, Analar grade) [HNO_3, H_2SO_4 = 1:1] and digested (Panigrahi, 1980) to a nearly colourless solution on mantle heater. After completion of digestion, the digested solution was cooled. The volume of the solution was made up with double distilled water. The amount Na^+, K^+ and Ca^{++}, were determined by a flame photometer with Polyflex galvanometer and the reading was repeated in flame photometer (Systronics) taking NaCl, KCl and $CaCl_2$ as standards following Systronics User manual. The obtained values were computed from a standard curve. For determination of magnesium ion, the procedure suggested by Orange and Rhei (1970) was adopted. The concentration of Mg^{++} was calculated from the standard curve.

Results

The control fish remained clinically healthy through out the experimental period of 30days. The red mud waste extract exposed fishes showed lethargicity in the initial periods of exposure. With the increase in exposure period imbalance in the fish movement was noted. Slowly the colour of the fish changed to light brick red and at the end the fishes turned to light brown. The loss of weight, decrease in food intake, depletion in respiratory physiology followed by imbalance in the movement indicated us to study the changes in ionic content of the exposed fish tissues and compare with control fish tissues.

Fig.1-8 indicated the changes in sodium ion, potassium ion, calcium ion & magnesium ion content of control and RMWE exposed fish brain, liver, and muscle tissues after 28 day of exposure and percent changes in the ion contents of different fish tissues after 28d of exposure to red mud waste extract. The sodium ion content declined from 336.4 ± 7.2µg g^{-1} dry weight to 241.2 ± 5.6µg g^{-1} dry weight in brain, from

322.2 ± 9.7µg g^{-1} dry weight to 196.8 ± 11.4µg g^{-1} dry weight in liver & from 302.4 ± 7.9µg g^{-1} dry weight to 154.2 ± 11.7µg g^{-1} dry weight in muscle (Fig. 1) after 28 days of exposure showing maximum decrease by 28.3%, 38.9% and 49% in brain, liver, and muscle tissues, respectively (Fig.2). The muscle showed the highest percent decrease in sodium ion content, when compared to liver and brain tissues of the waste extract exposed fish.

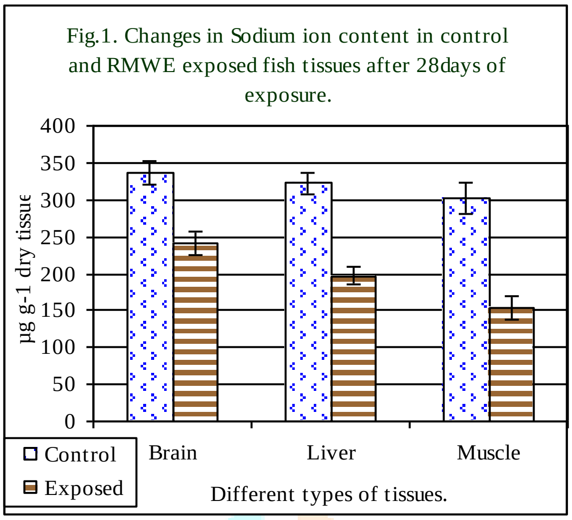


Fig.1. Changes in Sodium ion content in control and RMWE exposed fish tissues after 28days of exposure.

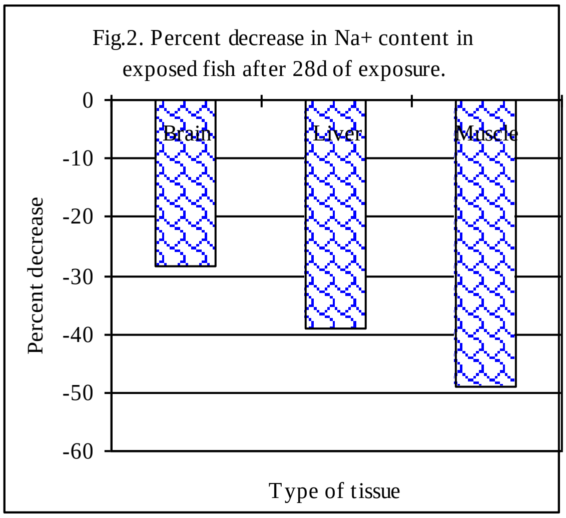


Fig.2. Percent decrease in Na+ content in exposed fish after 28d of exposure.

The potassium ion content declined from 319.4 ± 3.8µg g^{-1} dry weight to 212.6 ± 11.2µg g^{-1} dry weight in brain, from 385.6 ± 17.2µg g^{-1} dry weight to 236.4 ± 14.6µg g^{-1} dry weight in liver and from 392.6 ± 26.4µg g^{-1} dry weight to 204.5 ± 16.4µg g^{-1} dry weight in muscles respectively after 28days of exposure (Fig. 3). Brain, liver and muscle of the exposed fish showed 33.4%, 45.3% and 49.9% decrease in potassium ion content, when compared to the control value, where muscle was highly affected showing the maximum depletion (Fig. 4). Significant ($P \leq 0.01$) decrease in potassium ion content in brain, liver and muscle of exposed fish, when compared to the control fish tissues were marked.

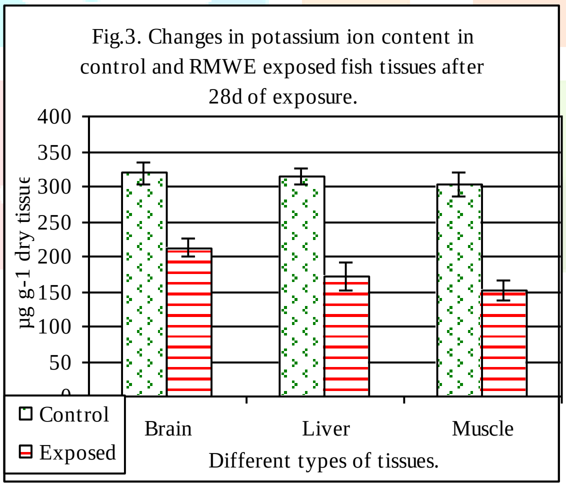


Fig.3. Changes in potassium ion content in control and RMWE exposed fish tissues after 28d of exposure.

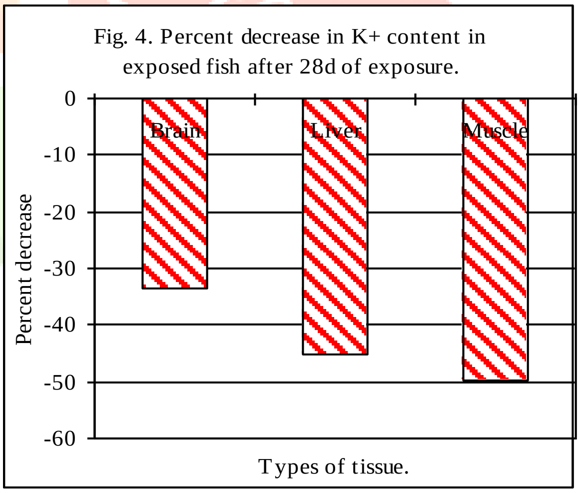


Fig. 4. Percent decrease in K+ content in exposed fish after 28d of exposure.

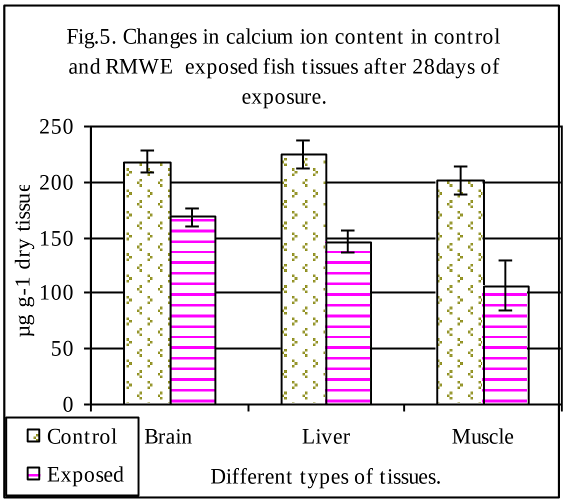


Fig.5. Changes in calcium ion content in control and RMWE exposed fish tissues after 28days of exposure.

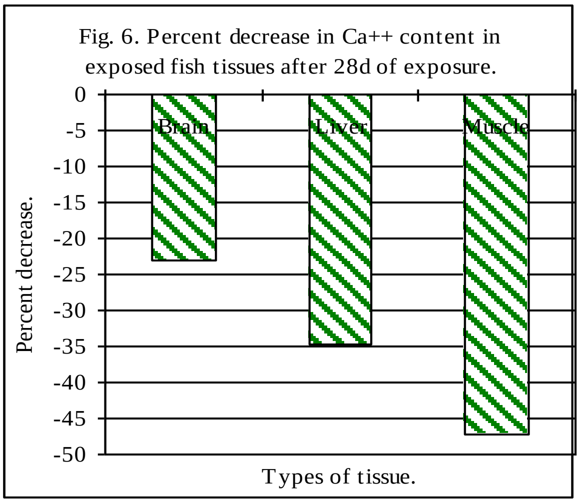
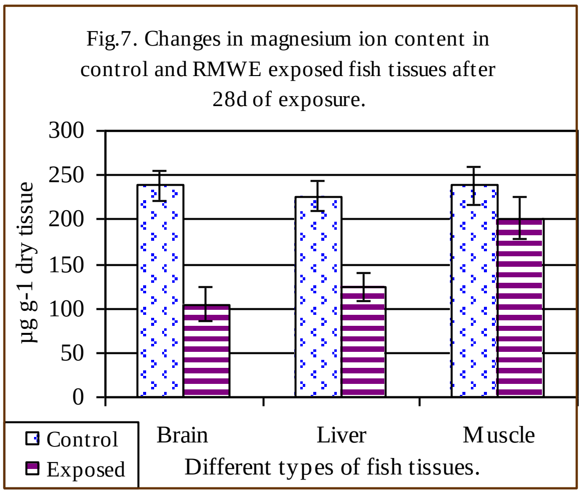
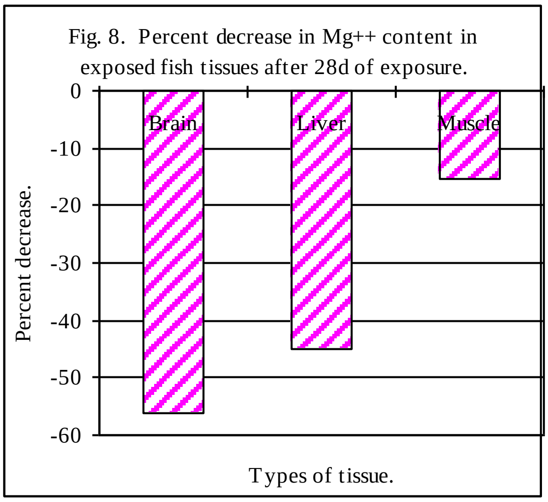


Fig. 6. Percent decrease in Ca++ content in exposed fish tissues after 28d of exposure.





Significant variation was marked in the calcium ion content in different tissues of the exposed fish, when compared to control fish tissues. The calcium ion content decreased from 218.5 ± 12.4µg g^{-1} dry weight to 168.2 ± 7.4µg g^{-1} dry weight in brain, from 224.6 ± 3.4µg g^{-1} dry weight to 146.4 ± 5.4µg g^{-1} dry weight in liver and from 201.5 ± 11.6µg g^{-1} dry weight to 106.5 ± 14.8µg g^{-1} dry weight in muscle (Fig.5) after 28 days of exposure. Maximum depletion by 47.1% in muscle tissues, 34.8% in liver tissue and least depletion by 23% in brain tissues of the RMWE exposed fish, when compared to control fish tissues were recorded (Fig.6). Significant ($P \leq 0.01$) decrease in calcium ion content in brain, liver and muscle of exposed fish, when compared to the control fish tissues were marked. The magnesium ion content decreased from 238.5 ± 17.2µg g^{-1} dry weight to 104.8 ± 19.3µg g^{-1} dry weight in brain, from 226.5 ± 16.5µg g^{-1} dry weight to 124.2 ± 15.2µg g^{-1} dry weight in liver and from 238.1 ± 21.4µg g^{-1} dry weight to 201.5 ± 24.1µg g^{-1} dry weight in muscle (Fig. 7) after 28 days of exposure. Maximum depletion by 56.1% in brain tissue, 45.2% in liver tissue and least depletion by 15.4% in muscle tissues of the RMWE exposed fish, when compared to control fish tissues were recorded (Fig.8). The decrease in ion content in different tissues was statistically significant. Out of the four ions studied in the exposed fish tissues, significant decrease was recorded in sodium ion and calcium ion content and highly significant differences were observed in potassium ion content in all the tissues studied. Out of the three tissues studied the muscle tissue was the most affected than the least affected was on brain tissues. The analysis of variance ratio test for ions like Na^+, K^+, Mg^{++} and Ca^{++} in brain, liver and muscle of the control and RMWE exposed fish showed significant difference between rows and significant difference between columns. Significant differences in ion content of different tissues of the exposed fish, when compared to control fish clearly indicated the nature of the toxicant and effects caused by the toxicant (RMWE) on the ion transport system consequent impact on ionic balance, membrane transport system, synaptic transmission across nerve tissue and nerve impulse generation, of the red mud waste extract exposed fish.

Discussion

In preliminary study of the present investigation, the animals showed all regular features of toxic waste poisoning; such as excitation, irritation and restlessness followed by lethargicity and irregular movements. Towards the end of the experimental period i.e. prior to death the locomotion of fish almost ceased and remained suspended vertically in the water medium, indicated loss of equilibrium. 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 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 the earlier reports of Panda *et al.*, (2017, 2018a,b and 2023). Panigrahi (1980) documented the detail behavioral changes in relation to mercuric nitrate intoxication. Autopsy study revealed the shrinked brain, pale and tender liver, corroded gill filaments covered with RMWE in the toxicant exposed fishes. The same author had observed swelling of the eyes at the initial stage followed by reddening of eyes and at higher exposure period subsequent blindness in the contaminated fish, but such type of symptom was not observed in alumina industrial effluent exposed fish, probably due to absence of mercury as a base in the toxicant. During autopsy studies, it was observed that the gill filaments were heavily coated with brown colored mucilage. This colored mucilage layer coated over the gill filaments acted as a barrier for gaseous exchange during oxygen absorption. In the lechate of red mud waste the major elements and ions were Al, alkaline metals like Na, K, Ca, Mg, and other metals like Si and Cl^-, F^-, SO_4^{2-}, and NO_3^{2-}. The observed values were much higher than the prescribed limits enlisted by PCBs and Environmental Act and regulations of GOI (Government of India) and also by USEPA for USA. The aluminium concentration in red mud lechate ranges from 1115.6 to 1485.2mg/liter and sodium ranges between 1182.5 to 7356.5mg/liter which is very high when compared to regulatory standards. The presence of minor elements like Cr, Cu, As, B, Ba, Fe, Mo, Mn, Ni in the lechate of the red mud pond warrants attention. Presence of these chemicals is not very important if available at lower quantities but their presence in huge quantities might cause deleterious affects, needs attention. Sun *et al*., (2019) also reported the above metals in very high quantities in red mud waste lechate in China and the same author also compared their data with other information collected from many countries where very high values were obtained. The reported values were much higher than the prescribed limits of those countries. Once these chemicals were discharged and available in the open environment can cause serious detrimental health conditions. Fishes are highly affected by the discharged toxicants when compared to rest of the animals available in the environment from human beings stand point. Murali *et al*., (2018) correctly opined that this can be one of the reasons why fishes were selected and used to assess the toxic effects and the general health of aquatic systems and we agree and affirm with the statement given by Murali *et al*., (2018), as observed from our observations observed both in the field and laboratory controlled conditions. 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. 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. 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 response of ATPase to RMWE (Panda *et al*., 2023) 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. This was probably due to the total effect of all chemicals present in the leachate acted as a whole to produce such a higher toxic effect. Panigrahi (1980) reported reduction in Na^+, K^+ Mg^{++} and Ca^{++} ion concentration in different tissues of the animal and gill tissues of mercury contaminated fish, when compared to control fish. These ions are very important because they control the permeability of the cell membrane. The change in ion concentration leads to disruption of neurotransmission. The decrease in the concentration of minerals tend to decrease the ionic regulations of the different tissues and also added that the depletion in

Na^+ and K^+ level tends to decrease in the active membrane transport. No significant variation in case of exposed liver and brain was marked when compared to control fish, so far as Ca^{++} studies are taken into account. However, a significant depletion in Ca^{++} content was observed in gill tissues. It is feasible that the requirement of calcium in liver and brain tissues increased in mercury intoxicated fishes and the decrease of calcium content of gills did not promote a homeostatic adaptation. These ions play a crucial role in the circulatory system of the animals. The plasma ion concentration plays a significant role in different metabolism. Any change in ion concentration will definitely reflect in the metabolic activity and physiological activity of the organism. It can be concluded that the changes in different enzymes activities and ion concentrations were related to nervous over excitation, which lead to impaired swimming and erratic movements of the exposed fish. Once a toxicant is available in the aquatic environment, the toxicant can enter into fish body either through gills or through surface body skin of the fish or by both. When the toxicant is absorbed by gills, the chemical passes to blood vascular system directly and the same chemical is transported by blood vascular system and circulated to all organs of the fish body by circulatory system. By circulation, the toxicant reach to different organs / tissues of the fish body and caused damage to organs and functions of the organs, thereby, depression in active metabolism was noted No recovery was marked in the exposed fish either by behavioral change or in respiratory physiology, when the contaminated fish environment was changed to RMWE free medium. This indicated the drastic effect of the toxicant on the organism. The main causative agent, who affected the exposed fish, was probably due to the soluble chemicals of red mud waste of the NALCO plant. With the increase in exposure period, the parameters decreased significantly showing an inverse relationship. This also indicated the toxic nature of the toxicant, red mud waste extract prepared from red mud waste of NALCO industry. The fast increase in the rate contamination of aquatic environments with industrial pollutants in recent years has resulted in an escalation of scientific interests in the study of biological impacts of pollution. Because of the importance of fishes in the aquatic environment, any deleterious effect of the toxicant on this is likely to be reflected on the entire ecosystem affecting all microbial life, plant and animals.

Conclusion

The change in behavior and imbalance of red mud waste extract exposed experimental fish followed by 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 where fish death was noticed. 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. The imbalance and irregular movement of the exposed fish due to disturbance in ionic imbalance as observed in the fish. 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 aquatic flora and fauna including human beings inhabiting in the contaminated area.

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References:

Cuciureanu, A., Cernica, G., Stanescu, B.A., and Ionescu, I.A. (2020): Approaches regarding red mud valorization for a green future and sustainable environment. Rev. Chi., (Bucharest),71(1): 51-56.

Cui, Y., Chen, J., Zhang, Y., Peng, D., Huang, T and Sun, C. (2019): pH- dependent leaching characteristics of major and toxic elements from red mud. Int. J. Environ. Public Health, 16: 2046; doi:10.3390 / ijerph16112046.

Ghorbani .Y., Oliazadeh, M., and Shahverdi, A. R. (2009): Microbiological leaching of Al from the waste of Bayer Process by some selective fungi. Iran. J. Chem. Eng., 28(1): 109-115.

Mohammed, A. T., Magdy, F., Abo-El fotoh, Mayada, R. F., Yasmina, M., Abd El-Hakim and Walla M. El-Hady (2019): Sources and toxicological impacts of surface water pollution on fish in Egypt. Zagazig Veterinary Journal, 47(1): 103-119.

Murali, M., Athif, P., Suganthi, P.,Bukhari, A. S., Mohmed, H. E. Syed, Basu, H. and Singhal, R. K.(2018): Toxicological effects of Al_2O_3 nanoparticles on histo-architecture of the fresh water fish, *Oreochromis mossambicus*. Environ. Toxcicol. & Pharmacol., 59,74-81.

Olszewska, J. P., Meharg, A. A., Heal, K. V., Carey, M., Gunn, D. M., Searle, K. R., Winfield, I. J. and Spears, B. M. (2016): Assessing the legacy of red mud pollution in a shallow Freshwater Lake: Arsenic accumulation and Speciation in macrophytes. Environ. Sci. Technol., 50, 9044-9052.

Orange and Rhein (1970): In manual of clinical laboratory procedures (Eds. Faulkner, W. R. and King, J. W.): The Chemical Rubber Co. U. S. A. 59.

Panda, M. K., Dixit, P. K. and Panigrahi, A. K. (2017): Toxicological effects of leached chemicals of red mud waste of NALCO on a fresh water fish and its ecological implications. National J. of Life Sciences,14(2):119-124.

Panda, Manasi K., Dixit, P. K. and Panigrahi, A. K. (2018a): Toxicological effects of lechate of red mud pond of NALCO on the tissue slice respiration rate of different organs of a fresh water fish, *Oreochromis mossambicus*, Peters under laboratory conditions. National J. of Life Sciences,15(2):149-152.

Panda, Manasi K., Dixit, P. K. and Panigrahi, A. K. (2018b): Impact of leached chemicals of red mud waste on respiration rate of a fresh water fish, *Oreochromis mossambicus*, Peters and its ecological implications. Life Science Bulletin, 15(1):89-93.

Panda, M. K. (2022): Environmental contamination caused by the leached chemicals of red mud waste and its impact on a fresh water fish and its ecological implications. Ph.D. thesis, Berhampur University, Odisha, India.

Panda, Manasi K., Dixit, P. K., and Panigrahi, A. K. (2023): Eco-toxicological effects of Red Mud Waste lechate on the ATPase activity af a fresh water fish, *Oreochromis mossambicus*, Peters *in vivo* under laboratory conditions. IJCRT 2307713 July 2023, 11(7).

Panigrahi, A. K. (1980): Eco-physiological effects of mercurial compounds on some fresh water fish (Toxicological effects of inorganic mercury [$Hg(NO_3)_2$]on two fresh water fishes, *Tilapia mossambica*, Peters and *Anabas scandens*. Cuv. and Val. and its ecological implications. Ph. D. Thesis, Berhampur University.

Patel, S. and Pal, B. K. (2015): Current status of an industrial waste: Red mud an overview.IJLTEMAS,4(8): 1-16.

Sarath Chandra, K. and Krishnaiah, S. (2018): A detailed geotechnical investigation on red mud and chemical analysis of it's leachate. IGC-2018, 1-5.

Sun, C., Chen, J., Tian, K., Peng, D., Liao, X. and Wu, X. (2019): Geochemical characteristics and toxic elements in Alumina refining wastes and leachates from Management facilities. Int. J. Environ. Res. Public Health, 16, 1297; doi:10.3390/ijerph16071297.

Zhou, K., Teng, C., Zhang, X., Peng, C. and Chen, W.(2018): Enhanced selective leaching of scandium from red mud. Hydrometallurgy, 182:57-63.

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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