IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

Eco-Biochemical Changes Impacted By Red Mud Waste Lechate On Different Tissues Of A Fresh Water Fish, *Oreochromis Mossambicus*, Peters Under Laboratory Conditions.

Dixit*, P. K., Panda*, M. K., and Panigrahi, A. K.,
Laboratory of Environmental Toxicology,
Berhampur University, BERHAMPUR-760 007, Odisha, India.
*Department of Zoology, Berhampur University, Odisha, India

Abstract
Significant depletion in the protein content was noted in red mud waste extract exposed fish brain tissue, when compared to control fish brain tissues. The protein content in liver and muscle tissues of red mud waste extract exposed fishes decreased compared to control fish tissues. The percent decrease of protein content increased with the increase in exposure period and maximum depletion was noted after 28d exposure. It was observed that the exposed brain tissue was least affected compared to liver and muscle tissues of the exposed fish compared to control. The liver tissue was affected most during exposure period compared to muscle. But after 28d of recovery, surprisingly, it was observed that the muscle tissue was more affected compared to other tissues. Brain and muscle tissues could recover insignificantly but the liver tissues were more damaged and the affected tissues could not recover during recovery period. Significant variation in free amino acid content of the tissues of exposed fish brain, Liver and muscle was noted when compared to control fish tissues. The figure indicated significant variation in free amino acid content in the tissues of exposed fish liver tissues, when compared to control fish liver tissues. The brain tissue was least affected than liver and muscle tissues. The data indicates no significant recovery was noted in all the tested fish tissues compared to control fish tissues. The biochemical tests conducted on the exposed fish indicated the interference of the lechate waste on fish tissues. The depletion in DNA & RNA content can be related to cell death. At this stage, we can't infer that the toxicant interfered in DNA synthesis. The decrease in RNA content in exposed fish tissues might be due toxicant interference in protein synthesis mechanism. This view needs further studies. The decrease in protein content might be due to proteolysis or non assemblage of FAA to form proteins. This needs further studies. The decrease in FAA content in exposed fish tissues might be due to non production / synthesis of amino acids. The break down of protein leading to production of FAA was ruled out as the FAA content increase was not marked in the exposed fish. With this work it is premature to say clearly the impact of the toxicant on the biochemical metabolism of the fish. In future molecular level studies can be conducted.

Keywords: NALCO, RMP, RMWE, Lechate, Protein, FAA**.**

Introduction

Man has always been burdened by the products of his activities. Hence, the present situation that exists is different from the historic one, in several important ways. Amongst which, the most important remarkable difference is the way at which man has contributed to the environment, both good and bad .This difference is mostly due to inter related phenomena i.e. growth of population, increasing urbanization and rapid industrialization. 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 quantity thus, pollute the environment. Pollution is caused due to deliberate or unavoidable discharge of industrially produced wastes. These pollutants causing health hazards and definite and undesirable problems to flora and fauna, apart from influencing the ecological balance and brings significant climatic changes. The field under study refers to the Allumina industry specifically to Mining and Refinery complex, NALCO, Damanjodi in the district of Koraput, Orissa, India. Samantra (2002) studied the impact of red mud waste on a crop plant and indicated the adverse effect of red mud waste. Mishra (2002) also studied the direct impact of red mud waste on a fresh water fish and reported that the red mud waste when taken directly affected the physico-chemical properties of aquarium water and suggested that the whole red mud waste should not be taken directly. Keeping in view the discharge of red mud effluents of the industry into red mud pond, leaking of chemicals from the red mud waste dumping pond of the industry, entering into water bodies, during rainy season entry of these chemicals and lechate along with runoff water, over flow of runoff water of the paddy fields and their entry into fresh water bodies like fish ponds, canals, rivers and the water reservoir of the Upper Kolab hydroelectric cum irrigation project, this piece of work was planned. River Karandia is flowing near by the Refinery complex and Damanjodi township. It carries almost all the emissions of the industry, waste, leaked chemicals and waste of the township and at its down stream joins with the Kolab River at its catchments area of Upper Kolab project near Sunabeda. The local report from the local residents about fish kill in ponds nearby dragged our attention and hence, this project was planned to evaluate the eco-biochemical effects of the leached waste of the Red Mud Pond (RMP) / RMW extract of Alumina industry on the macromolecular content of a fresh water fish, *Oreochromis mossambicus*, Peters under laboratory controlled conditions.

Materials & Methods

Location of the industry:

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 60kms from Jeypur, the oldest city of Koraput district. Damonjodi is at a highest of about 1300mts. from sea level, located at latitude 18^0-6'--18^0-58' towards North and longitude 82^0.57'- 83^0.04' East.


(NALCO, Alumina industry and Red mud pond situated near the industry surrounded by natural hills and open earthern dam at Damonjodi.)


(Red mud powder prepared in the laboratory and lechate leaking from red mud pond of Alumina industry at Damonjodi)

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 with the following specifications. 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*., 2018). The red mud waste is dumped in a pond nearby. The leakage of the waste was marked on the earthen dyke side (see Photo).

Preparation of test solution: 1 Kg dried red mud waste was taken and 2 liters of distilled water was added. The whole content was stirred in a mixer-grinder for 30minutes and then allowed to stand. This was repeated for 30days. After 30days, the whole content was allowed to rest for 2days. The supernatant (lechate) was decanted, filtered and kept in a refrigerator for use.

Test fish: Test fish *Tilapia mossambica*, Peters at present *Oreochromis mossambicus*, Peters was brought from local Nursery and acclimatized for 15days under laboratory controlled conditions. Fishes were fed with goat liver initially and then changed to boiled egg slices. The physico-chemical parameters were recorded daily and constancy was maintained by changing the solution on every 7^{th} day and fresh solution was charged. Experimental fish were sacrificed- Individual fish weight of control and exposed fish was taken and the change in fish weight was recorded. Brain and liver tissues were dissected out carefully and washed thoroughly with distilled water. The sacrificed fish were weighed. Brain, liver and muscle tissues were separated and weighed carefully in a single pan electric balance.

Analysis: The test fishes were sacrificed at 7 days interval (both control and red mud waste effluent exposed). Brain, liver and muscle tissues were removed carefully and kept in watch glasses and weighed separately. Care was taken to avoid contamination during autopsy. The tissues were taken and processed, for studying the change in protein and free amino acid (FAA) content in brain, liver, and muscle of fishes exposed to toxicant at sub-lethal concentration of the red mud waste effluent and control fish tissues.

Fractional separation method was adopted for estimation of biomolecule in tissue sample of both control and red mud waste effluent exposed fish. The protein content in the homogenate was estimated following the method of Lowry *et al*. (1951). The standard graph was plotted taking BSA (Sigma) as standard. Ninhydrin was added and heated for 10 minutes on a hot water bath. The volume was made up to 6 ml and the optical density of the developed color was measured in a visible spectrophotometer at 550nm. The free amino acid content was calculated from a standard graph. The standard curve was prepared by taking glycine (Emerk) as standard.

Results

Fishes were exposed to graded series of concentrations of the lechate effluent for acute toxicity studies. The MAC value deduced was 3.15 % of lechate effluent in 50 liters of water for 30 days. A safety concentration of 3.1 % of LE (Lechate effluent) 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 % / 50L respectively after 30 days of exposure. Changes & percent changes in protein content in brain, liver and muscle tissues of control and RMWE exposed fishes were studied at different days of exposure and recovery (Fig. 1 to 7). The protein content of brain tissues ranged from 156.2 ± 2.62 to 162.8 ± 4.12mg/g dry weight in the control set during the entire period of experimentation (both exposure & recovery). With the increase in exposure period the protein content steadily decreased in RMWE exposed fish brain tissues. On 28d of exposure the protein content of the exposed fish brain tissues decreased from 161.5 ± 3.3mg/g dry weight to 149.4 ± 5.16mg / g dry weight (Fig.1). The protein content of liver tissues ranged from 184.8 ± 8.24 to 188.4 ± 4.74mg/g dry weight in the control set during the entire period of experimentation (both exposure & recovery). With the increase in exposure period the protein content steadily decreased in RMWE exposed fish liver tissues. On 28d of exposure, the protein content of the RMWE contaminated fish liver tissues decreased from 185.6 ± 5.42mg/g dry weight to 105.6 ± 11.83mg / g dry weight (Fig.2).

Fig. 1. Changes in protein content of brain tissues of control and RMWE exposed fish at different exposure periods.

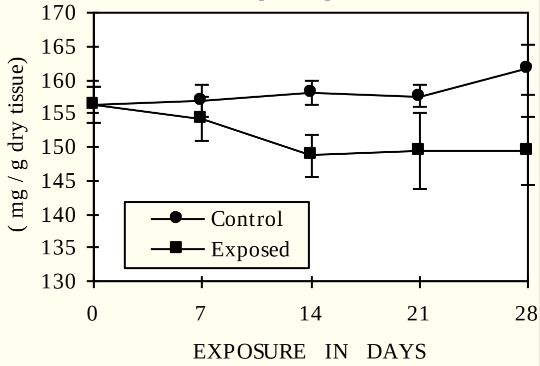


Fig. 2. Changes in protein content of liver tissues of control and RMWE exposed fish at different exposure periods.

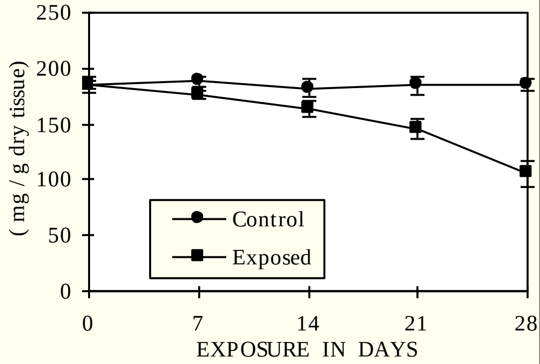


Fig.3. Changes in protein content of muscle tissues of control and RMWE exposed fish at different exposure periods.

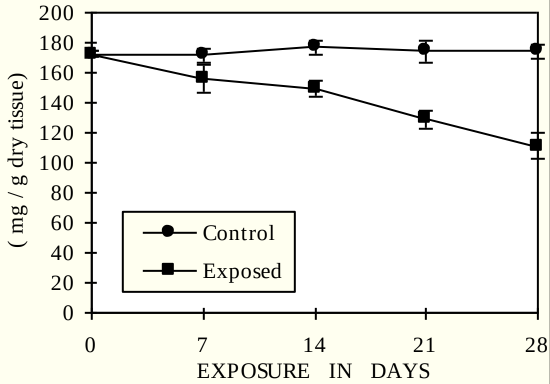


Fig. 4. Percent changes in protein content in exposed fish tissues at different days of exposure.

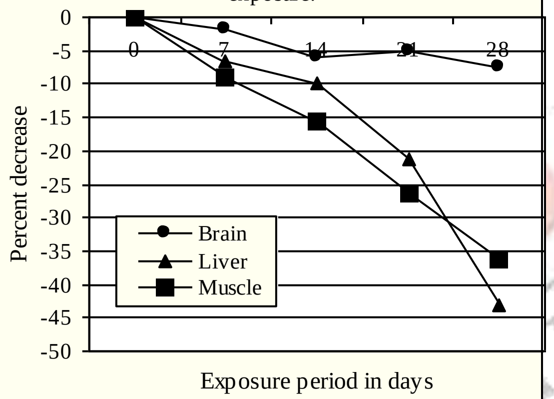


Fig. 5. Changes in protein content in control and RMWE exposed fish tissues after 28d exposure and 28d recovery.

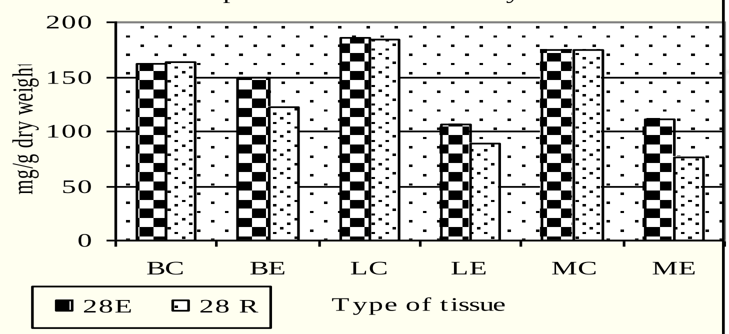


Fig. 6. Percent change of protein content in different tissues after 28 of exposure and 28d of recovery.

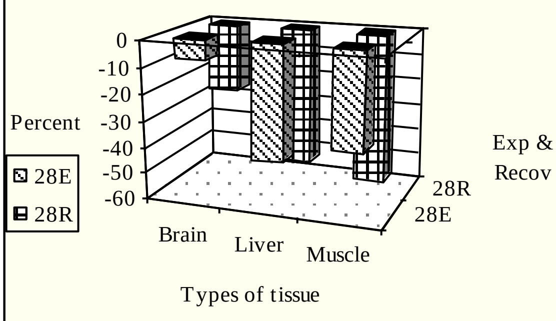
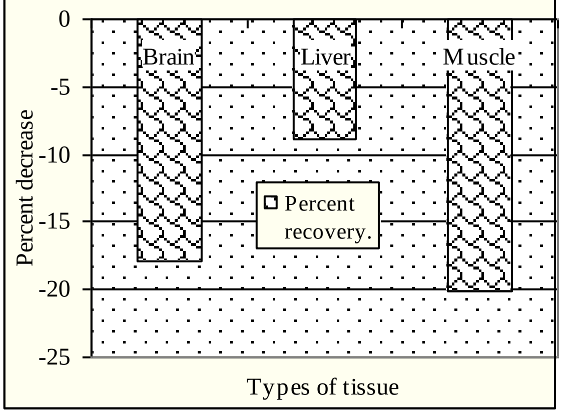


Fig. 7. Percent recovery of protein content after 28d of recovery.



The protein content of muscle tissues ranged from 171.5 ± 4.21 to 176.8 ± 4.64mg/g dry weight in the control set during the entire period of experimentation (both exposure & recovery). With the increase in exposure period the protein content steadily decreased significantly with the increase in exposure period in RMWE exposed fish muscle tissues. On 28d of exposure the protein content of the exposed fish muscle tissues decreased from 174.2 ± 5.13mg/g dry weight to 111.2 ± 9.12mg / g dry weight (Fig.3). The percent decrease in protein content in brain tissues decreased showing a positive correlation. The protein content of the RMWE exposed fish brain tissues decreased by 1.7%, 5.9%, 5.2% and 7.5% on 7, 14, 21, and 28 day respectively (Fig.4). The percent decrease in protein content in liver tissues decreased showing a highly significant positive correlation. The protein content of the RMWE exposed fish liver tissues decreased by 6.5%, 9.8%, 21.3% and 43.1% on 7, 14, 21, and 28 day respectively (Fig.4). The percent decrease in protein content in muscle tissues decreased showing a highly significant positive correlation. The protein content of the RMWE exposed fish muscle tissues decreased by 8.9%, 15.7%, 26.3% and 36.2% on 7, 14, 21, and 28 day of exposure respectively (Fig.4). On 28d of exposure the protein content of exposed fish brain tissue decreased from 161.5 ± 3.3mg/g dry weight to 149.4 ± 5.16mg / g dry weight and the protein content of 28d recovery fish brain tissue decreased from 162.8 to 121.5 mg/g dry weight showing further depletion (Fig.5). When compared to the 28d exposure value, the protein content of 28d recovery fish brain protein content value decreased significantly indicating no recovery in the biomolecular content. On 28d of exposure the protein content of exposed fish liver tissue decreased from 185.6mg/g dry tissue weight to 105.6mg/g dry tissue weight and the protein content of 28d recovery fish liver tissue decreased from 184.2 to 88.4mg/g dry weight showing further depletion (Fig.5). When compared to the 28d exposure value, the protein content of 28d recovery fish liver protein content value decreased significantly indicating no recovery in the biomolecular content. On 28d of exposure the protein content of exposed fish muscle tissue decreased from 174.2mg / g dry tissue weight to 111.2mg / g dry tissue weight and the protein content of 28d recovery fish liver tissue decreased from 175.1 to 76.5mg/g dry weight showing further depletion (Fig.5). When compared to the 28d exposure value, the protein content of 28d recovery fish muscle protein content value decreased significantly indicating no recovery in the biomolecule content. Out of the three types tissue studied, the protein content of muscle tissue was drastically affected compared to liver and brain tissues of the RMWE exposed fishes. On 28th day of exposure, the liver tissue showed the highest damage caused by the toxicant to the tune of 43.1%, where exposed fish muscle showed 36.2% decrease and exposed fish brain showed 7.5% decrease only. It was expected that during recovery period, there will be some improvement in the protein content of the exposed fish tissues but to our surprise, the protein content further depleted in all the three tissues studied. On 28th day of recovery 25.4%, 52.01% and 56.3% depletion was seen in brain. Liver and muscle of 28d recovery fish (Fig. 6). No recovery was noted in all the three fish tissues studied during recovery period. Instead of showing any recovery, further depletion by 17.9% in exposed brain, 8.91% in exposed liver and 20.1% in exposed fish muscle was observed (Fig. 7). It seems the impact was more on muscle tissues which come in direct contact with the toxicant medium than liver and brain tissues, where the toxicant is to be absorbed and then translocated to different organs of the exposed fish. The Free amino acid content of the RMWE exposed fish tissues showed significant depletion at higher exposure periods, when compared to their respective control fish tissue values. However, at lower exposure period significant differences were not marked in all types of tissues studied. Decrease in FAA content was noted on 7th day of exposure in brain and liver tissues of the exposed fish but these values were within the standard deviation range of the control fish brain and liver tissues. Only in case of muscle observable difference was noted (Fig. 8, & 11). In case of exposed fish brain tissues, significant decrease was noted after 21days of exposure in comparison to control fish brain tissue. The FAA content of RMWE exposed fish brain tissues decreased by 1.9%, 2.9%, 16.3% and 31.4% after 7, 14, 21 & 28 day of exposure respectively, in comparison to their respective control values (Fig.11). The FAA content of RMWE exposed fish liver tissues decreased by 0.2%, 17.4%, 35.6% and 54.3% after 7, 14, 21 & 28 day of exposure respectively, in comparison to their respective control values (Fig.11). The FAA content of RMWE exposed fish muscle tissues decreased by 12.99%, 21.6%, 35.8% and 51.6% after 7, 14, 21 & 28 day of exposure respectively, in comparison to their respective control values (Fig.11). Fig. 12 shows the comparison of FAA content in brain, liver and muscle tissues of control fishes and 28d exposed fishes and 28d recovery of exposed fishes. Fig. 12 showed the changes in FAA content in control, 28d exposed and 28d recovery fish tissues. Fig. 13 clearly indicated that during recovery period, no recovery was observed in all the three types of fish tissues studied when compared to their respective 28d exposure value. On 28d of recovery, the FAA content decreased by 49.9% in brain tissues, 72.2% in liver tissues and 72.4% in muscle tissues of the recovery fish in comparison to their respective control values. It was expected that the FAA content of the 28d exposed fishes would recover, when the RMWE contaminated fishes were transferred to toxicant free control medium and the recovery period was made identical with the exposure period (28days). But to our surprise further depletion in FAA content in all the tissues studied were marked. After 28d of recovery instead of any recovery in the macromolecular content, the FAA content further decreased by 18.5% in brain tissue, 17.9% in liver tissues and 20.8% in muscle tissues when compared to their respective 28d exposure values.

Fig.8. Changes in FAA content of brain tissues of control and RMWE exposed fishes at different exposure periods.

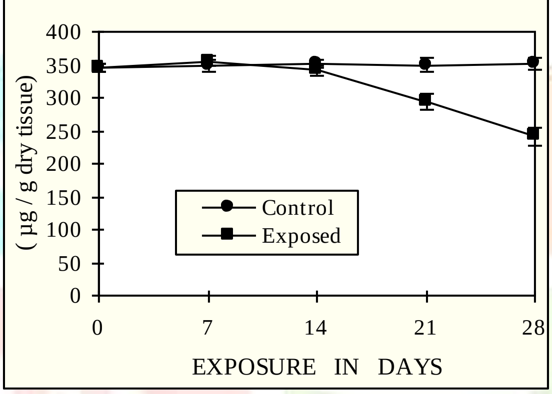


Fig.9. Changes in FAA content of liver tissues of control and RMWE exposed fishes at different exposure periods.

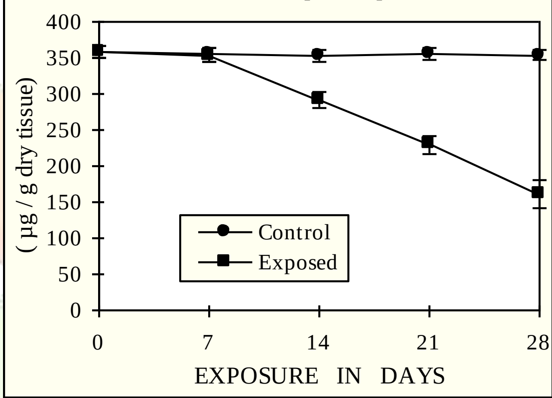


Fig. 10. Changes in FAA content of muscle tissues of control and RMWE exposed fishes at different exposure periods.

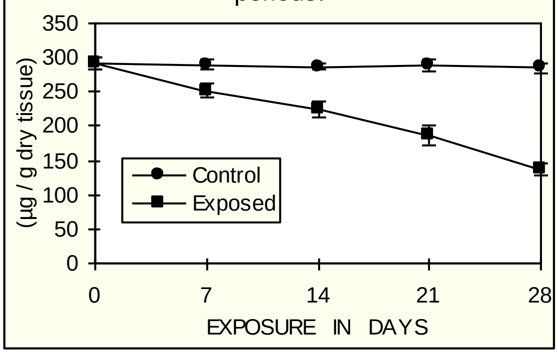


Fig. 11. Percent decrease in FAA content in exposeed fish tissues at different days of excposure.

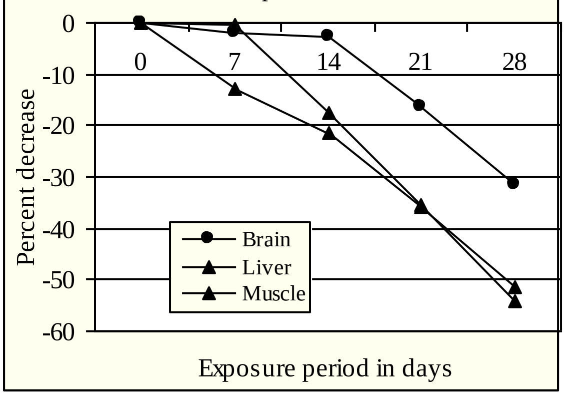


Fig. 12. Changes in FAA content in control and RMWE exposed fish tissues after 28d of exposure and 28d recovery.

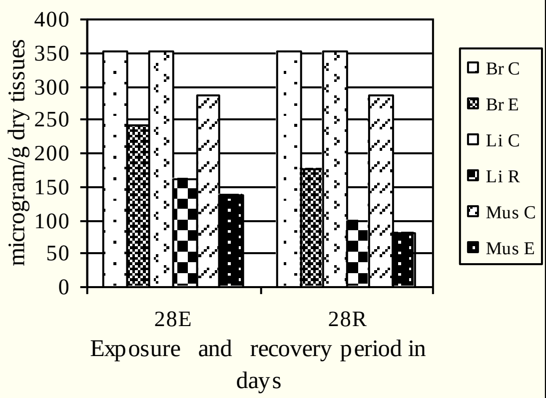
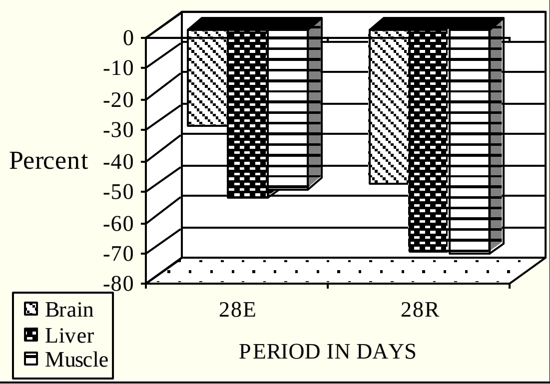


Fig. 13. Percent decrease in FAA content in different fish tissues of 28d of Exposure and 28d of Recovery period



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 biomolecule, non production of energy rich biomolecule, 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. 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.

Discussion

Once a toxicant is available inn 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 (Mac Leod and Pessah, 1973). These chemicals once available in tissues cause deposition and induce changes in the tissues finally may cause pathological disorders. Similar observations were noted by Mathur (1969), while working with different insecticides. Leaching of chemicals from the dumping site and its entry into fish ponds affects the water quality. With time, the concentration of the toxicant increases and these discharged chemicals react with the environmentally available chemicals and form complexes. In different reaction processes the dissolved oxygen is generally used up and the ionic concentration of the pond water increases significantly due to continuous addition of extraneous chemicals. The deficiency in dissolved oxygen and non availability of essential chemicals might lead to serious effects on fish and ultimately became the causative reason for fish death. Ray & David (1962) reported fish kill in pond where algal growth lead to decomposition of algae and bottom deposits caused fish death due to utilization of dissolved oxygen by the decomposed waste of the pond. 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. Physiological and biochemical impacts are the fundamental causes of measurable whole organism impacts (Mehrle and Mayer, 1980). A decline in liver somatic index and brain somatic index in exposed fish could be correlated with degeneration of cells and decrease in other biochemical variables. Stone *et al*., (1977) reported no significant effect on body weight or size of kidneys, liver as related to body weight in lead exposed Japanese quail. The leached effluent of the alumina industry collected from the site showed higher toxicity when compared to the reports of Mishra (2002) and the leached effluent were much diluted when compared to the RMWE prepared in the laboratory. Panda *et al*. (2017) rightly reported the death percentage for any fixed exposure period increased with the increasing dose / concentration of any type of toxicant. Exposed fishes appeared lethargic when compared to the control fish. Inappetence and ataxia was observed in the exposed fish after first day of introduction of the effluent. Effluent exposed fishes showed erratic movements, loss of equilibrium, gradual onset of inactivity when compared to the control fish. The Body weight, BSI and LSI significantly declined in the leached effluent exposed fish when compared to the control fish. The whole body respiration decreased with the increase in exposure period. Maximum depletion was noted on 28d of exposure. No recovery was marked, when the exposed fish was transferred to toxicant free medium for the same period of exposure. Instead of recovery, fish death was noted during recovery period. The biochemical tests conducted on the exposed fish indicated the interference of the lechate waste on fish tissues. The depletion in DNA content can be related to cell death. At this stage, we can’t infer that the toxicant interfered in DNA synthesis. The decrease in RNA content in exposed fish tissues might be due toxicant interference in protein synthesis mechanism. This view needs further studies. The decrease in protein content might be due to proteolysis or non assemblage of FAA to form proteins. This needs further studies. The decrease in FAA content in exposed fish tissues might be due to non production / synthesis of amino acids. The break down of protein leading to production of FAA was ruled out as the FAA content increase was not marked in the exposed fish. With this work it is premature to say clearly the impact of the toxicant on the biochemical metabolism of the fish. In future molecular level studies can be conducted. From the above experiments and obtained data, it is understood that the leached chemicals coming out from the red mud pond is deadly toxic. The existence of a close quantitative relationship between RNA content and protein synthesis was pointed out by Caldwell *et al*.,(1950) in cultures of microorganisms. Price (1952) showed the existence of an excellent correlation between the synthesis of RNA and the synthesis of proteins. Gale and Folkes (1953) showed the synthesis of proteins in the presence of glucose and amino acids and also pointed out that if purines and pyrimidines were added to the said medium, nucleic acids were synthesized. The same authors also pointed out that in absence of amino acids in the medium, instead of nucleic acids synthesis, the presence of purine and pyrimidines will enhance protein synthesis. Toxicant effect on macromolecular content is often due to an indirect action on nucleic acid and protein synthesis, since a toxicant that interfere with energy yielding reactions is directly an inhibitor of the synthesis of RNA, DNA and protein (Holbrook, 1980 and Barron and Adelman, 1984). The presence of variety of metal elements like Al, Ca, Cr, Ba, Mg, Fe, Cu, Pb, Mn, etc and solubility helps the rise in concentration of these metals in leached chemicals of the red mud pond. Cui *et al*., (2019) reported similar findings and also reported that the leaching of these metals elements is controlled by solubility. The same author also opined that the leaching of the above cited metal elements strongly dependent on the precipitation / dissolution of their oxides, surface solids and dissolution. The reports of Sun *et al*., (2019) is informative and the report indicated that the red mud lechate was hyper-alkaline (pH more than 12) and the pH of lechate coming out of red mud pond from NALCO red mud pond is above 12.8 in the field and more than 13 in the red mud waste coming from the industry.. The lechate coming out of the red mud pond had higher Aluminium chloride, fluoride, nitrate, sodium and sulphate. These high values observed in our study are frustrating as these values were much higher than the recommended ground water standards. There have been two suggestions in the literature as the mechanism of the stimulation. One involves an actual increase in the rate of chain elongation (Coupar and Chesterton, 1977 and Green *et al*., 1975), the other, activation of arrested transcription complexes (Benecke *et al*., 1977 and Green *et al*., 1975). Both mechanisms have been hypothesized to result, the removal of protein from the endogenous chromatic template (Benecke *et al*., 1977, Coupar and Chesterton, 1977, Green *et al*., 1975 and Gariglio *et al*., 1974). This probably suggests that at very high concentrations of the toxicant significant bimolecular changes can be marked. But at very low concentrations of the toxicant, the observed insignificant inhibition might be a transitional short stayed phase. Here, it can be stressed that, as the data of nucleic acids, and protein contents of individual parts or tissues were found to be decreasing and very low insignificant amount of FAA content increasing in the experimental fish system at initial period of exposure is totally confusing, it would be very difficult to give any confirmative conclusion. The fish death in ponds and water bodies of the site was only due to the leached chemicals leaking from the red mud pond and entering into ponds. The lechate chemicals cause irreversible damage to the metabolic system of the exposed fish, as they could not recover even after 28days of recovery- a period similar to exposure period, affecting respiration rate of the fish. This waste can kill fish and all types of plants and other animals inhabiting in a pond, where this waste created havoc after entry. Further work is necessary on all aspects of the fish exposed to pollutants, particularly red mud waste or lechate of red mud pond. The results obtained in this investigation does not agree with the findings of Saakyan and Petrosyan (1964); Nieman (1965);

Ben-zioni *et al*., (1967), Kahane and Poljakoff-Mayber (1968); Nieman and Poulsen (1971); Tsenov *et al*. (1973) and Dungey and Davis (1982), as they showed decrease in (loss of DNA and RNA) polynucleotides and protein and a concomitant increase in total free amino acid content inside the seedlings. The above possibilities were confirmed by the previous workers of this laboratory and similar results were also observed by us in animal system. The study of Sarath Chandra and Krishnaiah (2018) studied the lechate of red mud wastes and their impact on their environment. Now the most headaches of the alumina industry owners and the biggest burden for the government is storage and safe disposal of red mud wastes. We agree with the above report. We have also raised our concern on two aspects- one is increase the storage capacity of the red mud pond by raising the height of the pond, yes, if increased to what extent? Second, what will be the fate of the lechate? If the storage tank is not strong and thick plastic lined from all sides, leaching is bound to take place. We can not escape by saying that these lechate will not harm any one, as it is located far away from the township. This statement is not acceptable under any circumstances, as we understand leaching of chemicals from the earthen storage tanks is a must and the lechate will flow to long distances and will meet any water body or get stored in small tanks at places. In rainy season all these small tanks will be joined by rain run off water and flow like a small stream to join any other big flowing water body. In some industries these lechate have heavy metals in huge quantities and low level of radioactive materials. This report raises concern of all environmentalists.

Acknowledgement

Authors wish to thank the Head, Department of Zoology and Botany, Berhampur University for providing the working facilities for conducting the research work.

References

Barron, M. G. and I. R. Adelman (1984): Can. J. Fish. Aquat. Sci., 41:141.

Benecke, R. K. Takano, J. Schmidt & H-D Henatsch (1977): Tetanus toxin induced actions on spinal Renshaw cells and Ia-inhibitory interneurones during development of local tetanus in the cat. Experimental Brain Research, 27:.271–286.

Ben-Zioni, A., Itai, C. and Vaadia, Y. (1967): Water and Salt Stresses, Kinetin and Protein Synthesis in Tobacco Leaves. Plant Physiol., 42, 361-365.

Caldwell, P. C., Mackor, E. L. and Hinshelwood, C. (1950): J. Chem. Soc: 3151.

Coupar, B. E. H. and Chesterton, C. J. (1977): The Mechanism by which Heparin Stimulates Transcription in Isolated Rat Liver Nuclei Polyribonucleotide Elongation Rates and the Number of Transcribing RNA Polymerase Molecules Present. European Journal of Biochemistry, 79 (2): 525-533.

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.

Dungey, N. O. and Davis, D.D. (1982): Protein turnover in isolated barley leaf segments and the effect of stress. J. Exp. Bot., 33:12–20.

Gale, E. F. and Folkes, J. P. (1953): The assimilation of amino acids by bacteria. 18. The incorporation of glutamic acid into the protein fraction of *Staphylococcus aureus*. Biochem. J., 55(5): 721-729.

Garoglio, P., Buss, J. and Green. M. H. (1974): Sarkosyl activation of RNA polymerase activity in mitotic mouse cells. Pesticide Biochemistry and Physiology, 4 (1): 01-11.

Green, C. E., and Phillips, R. L. (1975): Plant regeneration from tissue cultures of Maize. Crop Sci., 15(3): 417-421.

Holbrook, D. J. Jr. (1980): Effects of toxicants on nucleic acid and protein metabolism. p.261-284. In: E. Hodgson and F. E. Guthrie (ed.). Introduction to biochemical toxicology. Elsevier, New York.

Kahane, I. and A. Poljakoff-Mayber (1968): Effect of substrate salinity on the ability for protein synthesis in Pea roots. Plant Physiol., 43(7): 1115-1119.

Lowry, C. H.; Rosebrough, J. N.; Farr, A. L. and Randall, R. J. (1951): Protein measurement with the folin-phenol reagent. J. Biol. Chem., 193:265.

MacLeod, J. C. and Passah, E. (1973): Temperature effects on mercury accumulation, toxicity and metabolic rates on rainbow trout (*Salmo gairdeneri*). J. Fish. Res. Bd. Canada, 30, 485.

Mathur, D. S. (1969): Toxicity of Aldrin to certain fish. Sci. and Cult. 35:1.

Mehrle, P. M. and F. L. Mayer (1980): Clinical tests in aquatic toxicology: state of the art. Environ. Health. Perspectives, 34: 139-143.

Mishra, M. (2002): Physiological changes induced by red mud waste of NALCO, Damonjodi on a fresh water fish and its ecological implications. Ph. D. thesis. Berhampur University. Orissa, India.

Nieman, R. H. (1965): Expansion of Bean Leaves and its Suppression by Salinity. Plant Physiol., 40(1):156–161.

Nieman, R. H. and L. L. Poulsen (1971): Plant growth suppression on Saline media: Interactions with Light. Bot. Gaz., Univ. Chicago Press, 132(1): 14-19.

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. (2018): 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.

Price, W. H. (1952): J. Gen. Physiol., 35: 741.

Ray, P. and David, A. (1962): A cases of fish mortality caused by precipitation of ferric ion in the DAHA at WIWAN (North Bihar): Ind. Jr. Fish., 9: 117.

Saakyan, R.G. and G.P. Petrosyan (1964): Effect of soil salinity on the level of nucleic acids and nitrogenous substances in grape leaves. FIZIOL RAST, 11: 681-688.

Samantray, A. C. (2000): Eco-physiological effects of waste of an Aluminium industry on a crop plant. Ph. D. thesis, Berhampur University, Orissa. India.

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.

Stone, C. L.; Foy, M. R. S.; Jones, A. L. and Mahaffey, K. R. (1977): D-ALADase-A sensitive indicator of lead exposure in Japanese Quail. Poultry Science, 56:174-181.

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.

Tsenov, E. I., Strogonov, B. P. and Kabanov,V. V. (1973): Effect of NaC1 on content and synthesis of nucleic acids in tomato tissues. Soviet Plant Physiol,- agris.fao.org (40-46).

Volkin, E. and Cohn, W. E. (1954): Methods of biochemical analysis (Ed. Glic. D), Wiley (Interscience), New York: p. 1-287.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.