



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

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Highlights:

- The leached chemicals are deadly toxic and interfere with metabolic processes.
- Significant decrease in DNA content was observed in lechate exposed fish at higher exposure periods compared to control fish under controlled conditions.
- Notable changes were noted in RNA content in lechate exposed fish compared to control fish tissues.
- The toxicant, lechate probably interfere in biosynthesis of DNA and consequently impact growth of the fish.

Abstract

The leached chemicals are deadly toxic and interfere with metabolic processes. The toxicity test indicated that 3.1% of the lechate waste is safe for the fish for 30days, however the fish showed signs of toxicity. Significant decrease in DNA content was observed in lechate exposed fish at higher exposure periods compared to control fish under controlled conditions. Notable changes were noted in RNA content in lechate exposed fish compared to control fish tissues. The toxicant, lechate probably interfere in biosynthesis of DNA and consequently impact growth of the fish. The red mud pond of the industry needs attention. The lechate should be collected and recycled back to the pond after dilution. The earthen dam should be checked and cemented to stop leaking and leaching.

Key words: Alumina industry, red mud waste, lechate, fish, Macromolecules.

Introduction

Contamination of the environment is considered a sequel to ecosystem development, and to a great extent, it is purified by the nature itself. It is the excessive contamination that creates environmental hazards. The secondary manifestations of human activity, commonly referred to as contamination or pollution, are the basis for the emergence of a new discipline of science, 'eco-toxicology', which combines a whole group of scientific field relating to the study of environment. The pollutants act in many ways on the living systems. The effect of sub-lethal concentrations of some pollutants is by far the most frequent ecotoxicological problem and it can be far more noxious in the long run for the exposed species resulting in chronic deterioration of the ecosystem. The persistent non-biodegradable pollutants are absorbed by living organisms and are concentrated many times more than the concentration of the surrounding medium. Further these bio-accumulated toxicants pass through the food chain resulting in bio-magnification. Industry is responsible for creating a fantastic array of new chemicals every year, all of which eventually find their way into the environment. NALCO at Damonjodi produces Alumina as the main Product of interest (POI) and this industry discharges its red mud waste in to a natural pond surrounded by mountains from all sides except one, where earthen dam was constructed. This red mud waste pond was our concern and today, the

situation is grim in that area. The leached chemicals of the red mud pond contaminated the surrounding crop fields, ponds and water bodies and they were badly affected. Keeping in view of the discharge of red mud effluents of the industry into red mud pond and leaking of chemicals from the red mud waste dumping pond of the industry entering into water bodies and during rainy season entry of these chemicals and leachate 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 masterminded. 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-toxicological effects of the leached waste of the Red Mud Pond (RMP) of Alumina industry on the growth, behavior and respiration rate of a fresh water fish, *Oreochromis mossambicus*, Peters under laboratory controlled conditions.

Materials & Methods

Location of the industry:

Installation of National Aluminium Co. Limited (NALCO) is a major step towards self sufficiency in good quality of Alumina production. NALCO was set up in 1981, to exploit the large deposit of Bauxite, found in the east coast in 1975. Mishra (2002) and Panda *et al.*, (2017, 2018a, b) indicated that NALCO is producing the best alumina by adopting all modern technologies. The mines and refinery complex of NALCO, Damanjodi is situated at Similigude 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 Similigude junction and further 11kms towards east on project road. It is 60 kms from Jeypore, the oldest city of Koraput district. Damanjodi is at a highest of about 1300 mts. from sea level, located at latitude $18^{\circ}6' - 18^{\circ}58'$ towards North and longitude $82^{\circ}.57' - 83^{\circ}.04'$ East. The area enjoys an annual rainfall of 1723-1855 mm.



(P-1: The industry under study; P-2: Red mud Pond, P-3: Leachate leaking from the pond)

The area enjoys a modest climate with little high rainfall when compared to other areas of Koraput district. Southeast side is covered by Deomali hill range. NALCO produces calcined allumina at refinery complex, Damanjodi, Koraput district, Odisha located at latitude $18^{\circ}6' - 18^{\circ}58'$ towards North and longitude $82^{\circ}.57' - 83^{\circ}.04'$ East with the following specifications. Chemical properties: Typical = $\text{Al}_2\text{O}_3(\%)$ - 98.7; $\text{Na}_2\text{O}(\%)$ - 0.38 ; $\text{Fe}_2\text{O}_3(\%)$ - 0.01; $\text{SiO}_2(\%)$ - 0.012; and $\text{CaO}(\%)$ -0.042. Alumina hydrate: Physical properties: Typical: LOI (110-1000°C)%- 34-36, Moisture-3-6; Granulometry: Typical- 45Micron(%)-3-6. Chemical properties: Typical = $\text{Al}_2\text{O}_3(\%)$ - 65 ± 0.5 , $\text{Na}_2\text{O}(\%)$ Total-0.23-0.30, $\text{Na}_2\text{O}(\%)$ Soluble- 0.015-0.025, $\text{SiO}_2(\%)$ -0.007-0.010, $\text{Fe}_2\text{O}_3(\%)$ -0.006-0.008 and Hydrate Content- 99.0% (Samantray, 2000 and Panda *et al.*, 2018a). The red mud waste is dumped in a pond nearby. The leakage of the waste was marked on the earthen bandha (earthen dyke) side (see Photo). The area enjoys an annual rainfall of 1723 mm in 1998. The relative humidity of area ranges from a minimum of 47% and maximum of 88% in 1998. The temperature of the area varied from a minimum of 15°C to maximum 35°C in 1997-98 and from a minimum of 9°C to maximum of 42°C in 1998-99. South east side is covered by Deomali hill range. The slope of the area is towards the northwest. A small river Karandia is flowing near by the Refinery complex and the Damanjodi township. It carries almost all the emissions of the plant and township. At its down stream, the river joins with the Kolab River at its catchment area of Upper Kolab project near Sunabeda.

MAINTENANCE OF FISHES IN LABORATORY AQUARIUM

Oreochromis mossambicus, Peters (*Sarotherodon mossambica*, Peters or *Tilapia mossambica*, Peters) of medium size (12-18 g) was collected from a local nursery of the Fisheries Department located at Berhampur (Ganjam), Orissa and nursery of the Fisheries Department of Parlakhemundi (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) 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 on summer season, through water of the control and experimental aquaria to maintain the dissolved oxygen at $85 \pm 5\%$ air saturation values. The physico-chemical status of both control and exposed aquarium was measured periodically (APHA, 1998) 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 ± 200 lux, intensity. The hardness of water was maintained at 76.5 ± 4.2 mg l⁻¹ in control aquarium and at 85.1 ± 6.6 mg l⁻¹ in exposed aquarium. The specific conductivity was maintained at $3.52 \times 100 \mu\text{mho}$ and at $3.61 \times 100 \mu\text{mho}$ 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 (KMnO₄) 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. Again the fish was allowed to settle for 2-3 days before starting a fresh experiment. 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 DNA and RNA 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. DNA and RNA content of the tissues was measured following the protocol of Burton (1956) and Volkin and Cohn (1954). The DNA, RNA, Protein content was calculated from the standard graph plotted, taking DNA, and RNA (Sigma) as the standard. All obtained data were analyzed using student's "t" test as per requirement. Independent data represents mean $\pm$ standard deviation. Correlation coefficient (r) analysis was carried out between days of exposure vs. exposure period. ANOVA test was carried out for all the data of all the tables to find out levels of significance.

Results

Toxicity study: The observations showed LC₀₀ values at 24 hours to be 5.8% and 5.6% for 48 hours. The observations showed LC₁₀ values at 24 hours to be 10.4% and 10.1% for 48 hours. The results showed that the LC₅₀ values for test fish were 19.5% at 24 hours, 19.0% at 48 hours of exposure. The results showed that the LC₉₀ values for test fish were 22.5% at 24 hours, 22.1% at 48 hours of exposure. The results showed that the LC₁₀₀ values for test fish were 24.8% at 24 hours, 24.2% at 48 hours of exposure. The LC₁₀, LC₅₀, LC₉₀ and LC₁₀₀, 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 (Table-1). No mortality was observed in the control set, during the entire period of experimentation.

Table-1. Acute toxicity values of the lechate of the Alumina industry on a fresh water fish under laboratory controlled conditions. (Values obtained from toxicity statistical graph)

Lethal concentration (LC)	Lechate concentration (LC in %)		Lechate concentration (LC in %) after 30 days of exposure.	
	24 h	48 h	30days	Used concentration
LC ₀₀	5.8%	5.6%	3.15%	3.1%
LC ₁₀	10.4%	10.1%	3.45%	
LC ₅₀	19.5%	19.0%	8.15%	
LC ₉₀	22.5%	22.1%	12.6%	
LC ₁₀₀	24.8%	24.2%	15.5%	

Behavioral study: 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. Basing on these observations, it was felt necessary to study the whole body oxygen uptake / respiration rate of the exposed fishes when compared to control fish. Uncontaminated fish remained clinically normal and healthy for the entire experimental period without showing any signs of toxicity or contamination. The fish lost its equilibrium after 25th 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. The body weight significantly decreased in the effluent exposed fish. The whole body respiration, tissue slice respiration depleted significantly. Basing on these observations, it was felt necessary to study the impact of lechate effluent on the biomolecular of exposed fish tissues.

Fresh set of experiment was planned to study the effect of RMWE on the DNA content in fish tissues (brain, liver and muscle) at different exposure periods and 28d of recovery (Fig.33 to 39). Non-significant variations in DNA content were noted between different tissues of the RMWE exposed fishes, when compared to its respective control values at lower exposure periods and significant variations were noted at higher exposure periods. Exposed fish brain tissue was least affected than muscle and liver (Fig. 1 to 6). The impact was significant and severe on liver tissues of the exposed fishes when compared to control fish. In case of exposed brain tissues of the fish, no significant change was noted. Whatever changes that was observed during early period of poisoning, the values came within the standard deviation range of the control fish up to 14days of exposure. Then DNA content of the exposed fish brain tissue decreased by 2.9%, 5.98%, 8.6% and 19% on 7, 14, 21, and 28 day of exposure respectively (Fig. 1 and 4). The difference was visible after 21days of exposure but the difference was not significant. On 28th day of exposure, the impact was noticeable, where 19.01% decrease in DNA content was noted. The fish was allowed to recover for 28days and it was observed that instead of showing any signs of recovery the DNA content further depleted to 41.3% when compared to its respective control value (Fig. 4 & 5). Due to depletion in the value on 28d of recovery, it was found that the exposed fish brain tissue showed further depletion of DNA content by 22.3% (Fig. 6) instead of showing any recovery in the values.

Fig.1. Changes in DNA content in brain tissue of control and RMWE exposed Tilapia fish at different exposure periods.

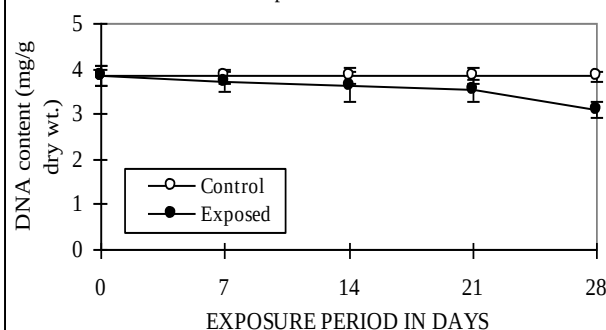


Fig.2. Changes in DNA content in liver tissue of control and RMWE exposed Tilapia fish at different exposure periods.

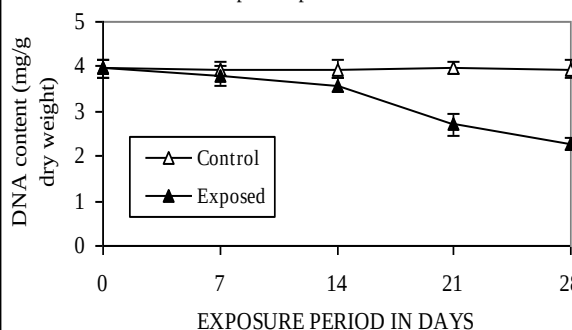


Fig.3.Changes in DNA content of muscle tissue of control and RMWE exposed Tilapia fish at different exposure periods.

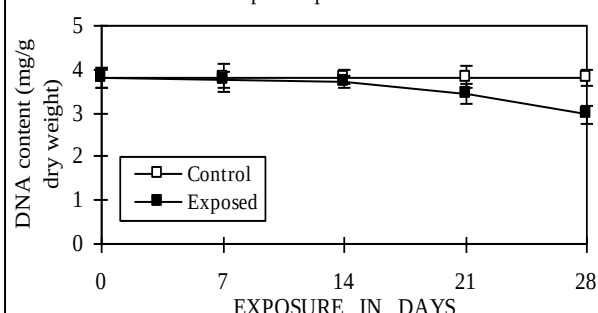
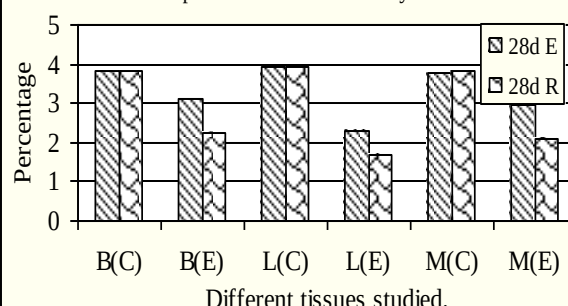
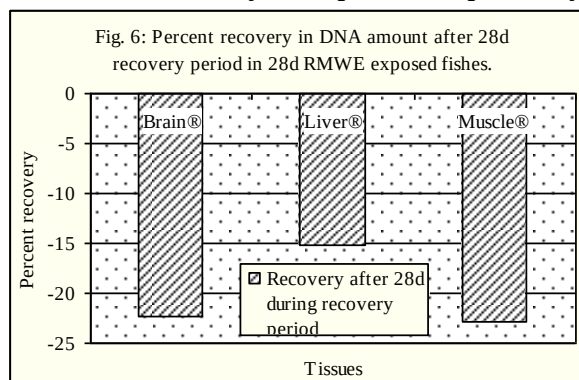
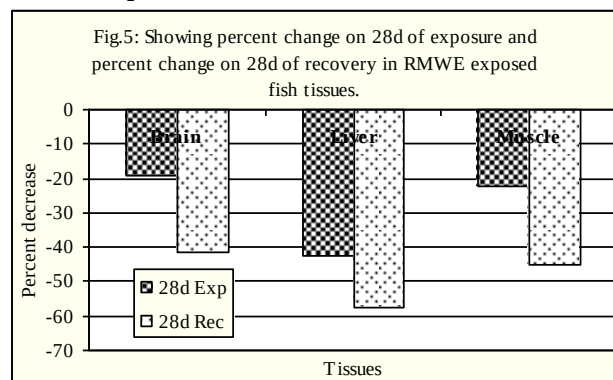


Fig. 4: Percent changes in DNA content in different tissues of control and RMWE exposed fishes on 28d of exposure and 28d of recovery.

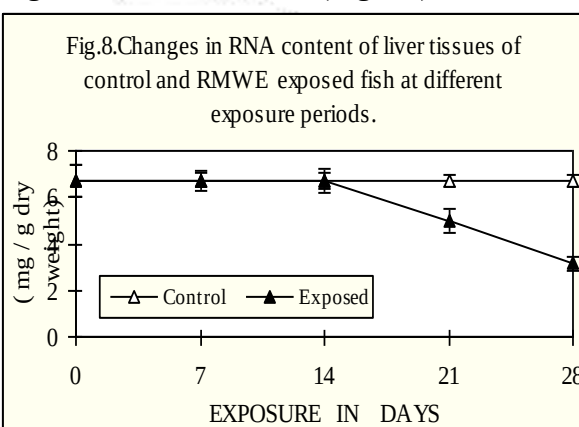
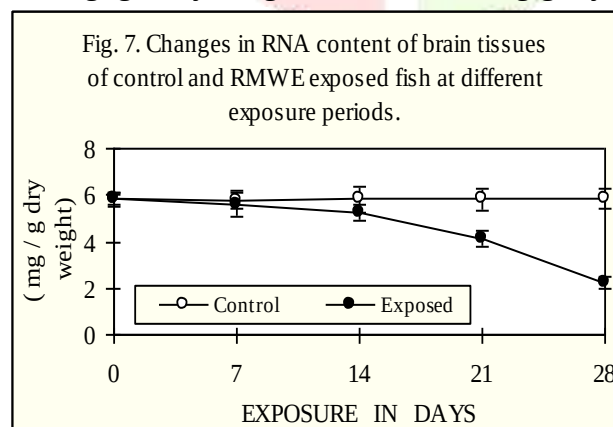


In case of exposed liver tissues of the fish, significant changes were noted. Whatever changes that was observed, the values were significant at all exposure periods. Then DNA content of the exposed fish liver tissues depleted 3.5%, 8.9%, 31.6% and 42.3% on 7, 14, 21 and 28 day of exposure respectively (Fig. 2 & 4). The difference was visible after 14days of exposure but the difference was not significant. On 21st & 28th day of exposure, the impact was noticeable, where 31.6% & 42.3% decrease in DNA content was noted, respectively. The fish was allowed to recover for 28days and it was observed that instead of showing any signs of recovery the DNA content of the exposed liver tissues further depleted to 57.4% on 28d of recovery when compared to its respective control value (Fig. 4 & 5). Due to depletion in the value on 28d of recovery, it was found that the exposed fish liver tissue showed further depletion of DNA content by 15.1% (Fig. 6) instead of showing any recovery in the values. Then DNA content of the exposed fish muscle tissues depleted 1.6%, 2.9%, 9.7% and 22.1% on 7, 14, 21 and 28 day of exposure respectively (Fig. 3 & 4).



The difference was visible after 21days of exposure but the difference was not significant. On 21st & 28th day of exposure, the effect was noticeable, where 9.7% & 22.1% decrease in DNA content was noted, respectively. The fish was allowed to recover for 28days and it was observed that instead of showing any signs of recovery the DNA content of the exposed muscle tissues further depleted to 44.9% on 28d of recovery when compared to its respective control value (Fig. 4 & 5). Due to depletion in the value on 28d of recovery, it was found that the exposed fish muscle tissue showed further depletion of DNA content by 22.8% (Fig. 6) instead of showing any recovery in the values.

The RNA content of control and RMWE exposed fish tissues were estimated at different exposure and recovery periods to observe the changes induced by the toxicant (Fig. 7 to 10). In case of brain tissues, the RNA content insignificantly decreased from the respective control values till 14th day of exposure (Fig.7). After 21days of exposure, the RNA content drastically declined and maximum decrease was noted on 28d of exposure. The RNA content depleted from 5.82 ± 0.43 mg/ gm dry weight to 2.24 ± 0.26 mg/g dry weight after 28d of exposure (Fig.7). The 28d contaminated fish was allowed to recover in RMWE free medium for another period of 28days. After 28days of recovery, the RNA content decreased from 5.83 ± 0.28 mg/ gm dry weight to 1.64 ± 0.34 mg/g dry weight in fish brain tissue (Fig. 10).



Significant variations in RNA content were noted between different tissues of the RMWE exposed fishes, in comparison to its respective control values. RMWE exposed fish brain tissue was least affected than muscle and liver (Fig. 7 to 12). The impact was significant and severe on muscle tissues of the exposed fishes when compared to control fish. In case of exposed brain tissues of the fish, non-significant change was noted at lower concentrations.. Whatever changes that was observed during early period of poisoning, the values came within the standard deviation range of the control fish up to 14days of exposure. Then RNA content of the exposed fish brain tissue decreased by 3.3%, 9.9%, 29.3% and 61.5% on 7, 14, 21, and 28 day of exposure respectively (Fig. 7 and 10).

Fig.9. Changes in RNA content of muscle tissues of control and RMWE exposed fish at different exposure periods.

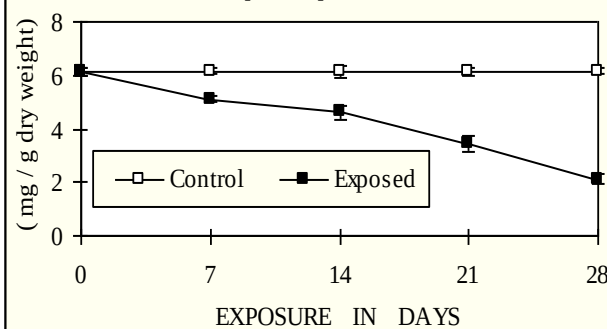


Fig.10. Changes in RNA content in control and RMWE exposed tissues after 28d exposure and 28d recovery.

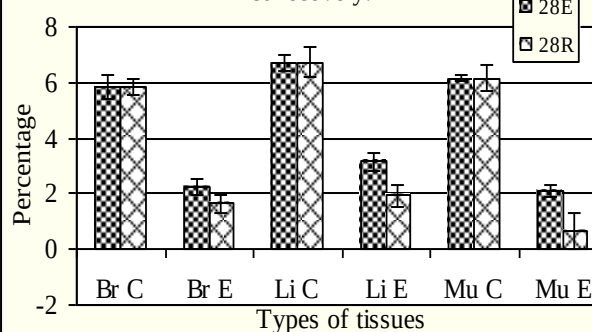


Fig. 11. Percent decrease in RNA content in RMWE exposed fish tissues after 28E and 28R.

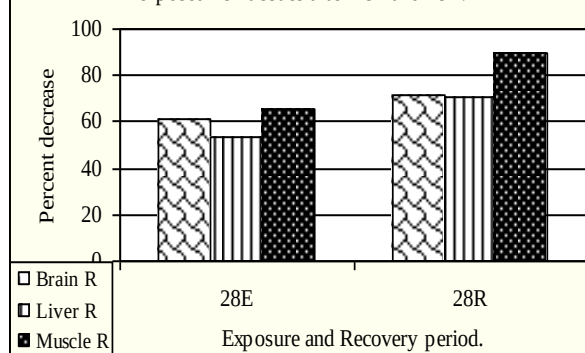
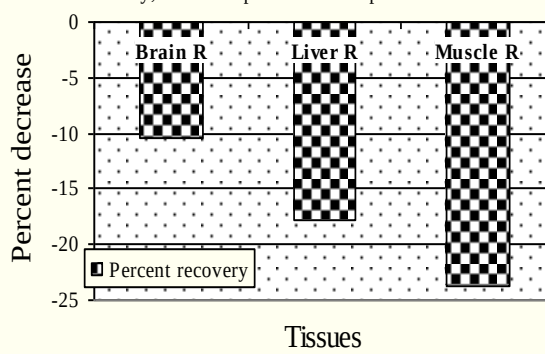


Fig. 12. Showing Percent recovery on 28th day of recovery, when compared to 28d exposure value.



The difference was visible after 21 days of exposure but the difference was not significant. On 28th day of exposure, the impact was noticeable, where 61.5% decrease in RNA content was noted. The fish was allowed to recover for 28 days and it was observed that instead of showing any signs of recovery the RNA content further depleted to 71.9% when compared to its respective control value (Fig. 11). Due to depletion in the value on 28d of recovery, it was found that the exposed fish brain tissue showed further depletion of RNA content by 10.4% (Fig. 12) instead of showing any recovery in the values. In case of liver tissues, the RNA content insignificantly decreased from the respective control values till 14th day of exposure (Fig. 8). After 21 days of exposure, the RNA content drastically declined and maximum decrease was noted on 28d of exposure. The RNA content depleted from 6.71 ± 0.29 mg/g dry weight to 3.15 ± 0.32 mg/g dry weight after 28d of exposure (Fig. 8). The contaminated fish was allowed to recover in RMWE free medium for another period of 28 days. After 28 days of recovery, the RNA content decreased from 6.72 ± 0.54 mg/g dry weight to 1.95 ± 0.39 mg/g dry weight in fish brain tissue (Fig. 10). In case of exposed liver tissues of the fish, significant changes were noted. Whatever changes that was observed, the values were significant at all exposure periods. Then RNA content of the exposed fish liver tissues depleted 0.5%, 0.45%, 26.1% and 53.1% on 7, 14, 21 and 28 day of exposure respectively (Fig. 8 & 10). The difference was visible after 14 days of exposure but the difference was not significant. On 21st & 28th day of exposure, the impact was noticeable, where 26.1% & 53.1% decrease in RNA content was noted, respectively. The fish was allowed to recover for 28 days and it was observed that instead of showing any signs of recovery the RNA content of the exposed liver tissues further depleted to 70.9% on 28d of recovery when compared to its respective control value (Fig. 10 & 11). Due to depletion in the value on 28d of recovery, it was found that the exposed fish liver tissue showed further depletion of RNA content by 17.8% (Fig. 12) instead of showing any recovery in the values. In case of muscle tissues, the RNA content significantly decreased from the respective control values in all exposure periods (Fig. 9). But the depletion after 21 days of exposure was highly significant. After 21 days of exposure, the RNA content drastically declined and maximum decrease was noted on 28d of exposure. The RNA content depleted from 6.14 ± 0.11 mg/g dry weight to 2.11 ± 0.19 mg/g dry weight after 28d of exposure (Fig. 9 & 10). The RMWE contaminated fish was allowed to recover in pollutant free medium for another period of 28 days. After 28 days of recovery, the RNA content of recovery fish muscle decreased from 6.16 ± 0.45 mg/g dry weight to 0.65 ± 0.46 mg/g dry weight in fish brain tissue (Fig. 10). In case of exposed muscle tissues of the fish, significant changes were noted. The changes that were observed, the values were significant at all exposure periods. Then RNA content of the exposed fish liver tissues depleted 16.8%, 24.8%, 43.6% and 65.6% on 7, 14, 21 and 28 day of exposure respectively. The difference was visible after 7 days of exposure but the difference was not

significant. On 14, 21 & 28 day of exposure, the impact was noticeable, where 24.8, 43.6 & 65.6% decrease in RNA content in muscle tissue was noted, respectively. The fish was allowed to recover for 28 days and it was observed that instead of showing any signs of recovery the RNA content of the exposed muscle tissues further depleted to 70.9% on 28d of recovery when compared to its respective control value (Fig.10 & 11). Due to depletion in the value on 28d of recovery, it was found that the recovery fish liver tissue showed further depletion of RNA content by 23.8% (Fig. 12) instead of showing any recovery in the values, when compared to 28d exposure value.

Discussion

The industrial wastes once thrown into the environment, these wastes spread all over and affect the flora and fauna. For example, the red mud waste discharged from the NALCO plant was stored in a natural pond surrounded by hills from all sides except one side, where the industry people have constructed three dams. Unfortunately, they have never visualized probably that the leached chemicals from the red mud pond, will create future problems. The data obtained in this piece of investigation revealed that the extract of the red mud is deadly toxic and can seriously affect the animals, particularly fish. In the leachate 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 alumina concentration in red mud leachate 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 leachate also warrants attention. Presence of these chemicals is not very important if available at lower quantities but their presence in huge quantities needs attention. Sun *et al.*, (2019) also reported the above metals in very high quantities in red mud waste leachate 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. The disturbances in growth and growth substances are always related with metabolic changes inside the plant body (Levitt, 1972; Poljakoff - Mayber & Gale, 1975) and animal body. Growth of an animal like fish depends on the culmination of physiological and biochemical processes. Any change or interference of any toxic absorbed chemicals will have a serious impact on the physiological processes. The impacts will be more pronounced, when changes in biochemical processes will be observed. The interference of the extraneous chemicals might be by way of alteration / inhibition / stimulation of processes. This is the reason why, fishes were used to evaluate the toxic effects and health of the aquatic system (Murali *et al.*, 2018). Acute lethal toxicity tests are usually done to assess the numerical value of toxicity and to compare potencies of toxicant. When the animals were exposed to toxicants, at higher concentrations death of animals were observed. At lower concentration of the toxicant animal death was not observed but the animal definitely suffers from the impact of the toxicant. The impact may be on physiological processes or biochemical metabolism. At higher concentration of the toxicant animal death was noted. The death might be due to inhibition of the processes or interference in metabolic processes. At lower concentration fish death was not observed but the animal suffered because of stress. With the increase in toxicant concentration fish mortality was noted. In addition, fish death depended on exposure period and the type of toxicant applied. It was observed that with the increase in exposure period to a particular toxicant, fish death increased. From our data, we can conclude that fish death is related to exposure period and toxicant concentration. The fish death was probably due to non tolerance of the animal to toxicant concentration. 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 (MacLeod and Pessah, 1973). The depression in active metabolism and interferences in different metabolic processes reduced the scope of activity of the animal. The absorption and entry of RMWE into the blood vascular system finally the chemicals enter into the tissues of different organs of the fish body. These chemicals once available in tissues cause deposition and induce changes in the tissues finally may cause pathological disorders. 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. It was reported that mercurials can react with uracil, uridine, or thymidine in four different ways: 1. coordination to carbonyl oxygen of the neutral legand 2. electrophillic attack on the ring with displacement of a protein 3. formation of a mercury-carbon bond 4. electrophilic attack with displacement of a protein and 5. formation of a mercury nitrogen bond (Steele and Johannesson, 1975; Peters, 1977, and Love, 1980) reported toxicant induced stimulation of protein and nucleic acid content indicated an acceleration of cellular metabolism and growth that might be responsible for the observed stimulation of juvenile growth. Such type of behavior was not studied in the present study. In the present investigation decrease in DNA & RNA content was noted in RMWE fish tissues. The decrease in DNA and RNA content in RMWE exposed fish tissues indicated deadly nature of the toxicant. 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). Yamane and Davidson (1961) and Katz (1963) have found that the Hg(II) ions are bound predominantly to the pyrimidine and purine bases in pure DNA, preference being shown to AT-base pair (Wong *et al.*, 1979) even though reaction with phosphate group cannot be excluded. Passow *et al.*, (1961) reported that “mercury, as a protein denaturant, binds to membranes, altering ionic distribution and osmo-regulatory activity of the animal”. The observation indicated that with the increase in exposure period the DNA and RNA content decreased significantly. Free amino acid content indicated a negative correlation with exposure period. Negative correlation was also observed with exposure period versus DNA and RNA content. The data are totally in agreement with the findings of Panigrahi (1984). Liver being the active site of all metabolic functions, it plays a crucial role in biotransformation of toxic agents (Bruin, 1976). The situation that exhibited heavy metal induced stimulation of the incorporation of thymidine into tissue DNA is not particular to lead but of particular interest is nuclear pyknosis in view of mercury binding to nucleic acids (Vallee and Ulmer, 1972) and reported DNA damage induced by mercury (Cantoni *et al.*, 1982). 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 are 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. These soluble metal elements will affect and increase the ionic strength in the outer environment of the aquatic animals and will enrich the water bodies. Consequently this type enrichment of metal ions in water bodies will impact the ionic transport across membranes and disturb the osmo-regulation system of the aquatic animals and plants. The reports of Sun *et al.*, (2019) is informative and the report indicated that the red mud lechate was hyperalkaline (pH more than 12) and the pH of leachate 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. If sub cellular structures are the actual site of toxic action of mercury it would lend credence to our hypothesis proposed earlier (Sharma *et al.*, 1981 and Sharma, 1985).that mercury toxicity is at least partly due to its combination with co-enzyme-A and resultant interference in CoA functions, the co enzyme A exists in free and ester forms in every tissue in different intracellular compartments (Robishaw and Neely, 1985 and Sharma *et al.*, 1988). The decrease in DNA content in liver, brain and muscle tissue was most probably due to loss of cells. The decline in brain DNA content may also be due to decreased glial cell number as a consequence of toxicity. The decrease in RNA content in liver, brain and muscle of exposed fish was most probably due to an increased RNA breakdown. Ahuja and Subrahmanyam (1978) reported a decline in RNA content in the brain cells due to nervous over excitation induced by prolonged training. A striking decrease in protein content was observed in treated fish exposed to leached chemicals of the red mud waste when compared to control fish. The decrease in free amino acid content may be due to decrease in peptidase activity or might be due to proteolysis or might be due to inhibition in amino acid synthesis as consequence of toxicant poisoning. Since structural RNA constitutes more than 80% of the total RNA content (Ahuja and Subrahmanyam, 1978) the decline in RNA content could be most probably due to decrease in structural RNA, which will not reflect the change in protein content. It was reported that the effect of MMC, EMC,

dimethyl mercury, PMA, p-HMB, p-HMBS, HgCl_2 , HgSO_4 , $\text{Hg}(\text{ClO}_4)_2$ on He La cell viability and DNA and RNA synthesis in intact cells and in isolated nuclei. Frenkel and Randles (1982); though obtained similar results as found by Gruenwedel and Cruikshank (1979) in HeLa cells, but they have observed some minor differences such as: DNA synthesis is more sensitive especially to Me-Hg specially, when the synthesis is measured immediately after the addition of Methyl Hg to the culture. But according Gruenwedel and Cruikshank (1979) there was no significant differences in the sensitivity of RNA and DNA synthesis to the Me-Hg cells. But the results of Frenkel and Randles (1982) with nuclei, however, demonstrated that Me-Hg dose have a direct inhibitory effect on DNA synthesis. The trend obtained in this study was probably a result of interaction of the compound with chromatin or with an essential enzyme or both remains to be established. Direct interaction of Me-Hg with double stranded DNA has been demonstrated by physical method (Chrisman *et al.*, 1977 and Anderson *et al.*, (1980) and currently investigations on this aspect by different bio-chemical approaches by different workers are in progress. The simplest explanation for the observations as made by Frenkel and Randles (1982) was that Me-Hg restricts initiation of RNA chain by all the 3 polymerases, as well as elongation polymerases I and III. The same authors reported in contrast, the elongation by polymerase II was not restricted by Me.Hg and was even stimulated by it at some concentrations. The effect of Me-Hg on transcription in isolated nuclei i.e. specific stimulation of polymerase II - catalyzed synthesis may be similar to the effect of poly anions such as heparin (Novello and Stripe, 1969; Coupar and Chesterton, 1977) as well as the anionic detergent Sarkosyl (Green *et al.*, 1975). 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). The lechate is a complex waste containing many toxic chemicals. There is every possibility that these waste chemicals might be interacting with macromolecules and macromolecular biosynthesis, thereby impacting different metabolic processes.

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