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Eco-physiological Impact of Industrial SWE Prepared from A Caustic-Chlorine Industry on A Fresh Water Fish and Its Eco-Toxicological Significance

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Abstract

Industrial solid waste cause significant damage to the environment and inhabiting fauna.The solid waste was dumped near the river bank. During rainy season, the washings of the solid waste enter in to the river. The leached chemicals contaminate the surrounding areas and the lechate also enter in to water bodies. In the present study we have selected this lechate as the test material. As the chemical concentration varied in each and every collection, it was planned to prepare the lechate waste in the laboratory which we call as SWE. In the present study, the SWE exposed fish appeared lethargic after exposure to the SWE compared to control fish. The major clinical symptoms such as inappetance and ataxia appeared after 2 to 3 days exposure. At higher concentrations of the SWE, the exposed fish showed erratic movement leading to collision to inner side of the aquarium. Fish death started in exposed aquarium after 20days of exposure. The whole body oxygen uptake decreased with the increase in exposure period. When the fish was transferred to toxicant free medium, no recovery was noted. It seems 28days of exposure to the SWE was enough for the fish not to recover 100%, showing a damage of 99.9% when compared to 28d control value and finally all the 28d exposed fish died after 22d of recovery onwards indicating permanent damage to the metabolic and biochemical systems caused by mercury. The exposed fishes also showed depression in active metabolism and significant decrease in whole body respiration rate. Further cellular respiration also indicated that SWE exposed fish tissues showed significant depression in respiratory activity at all exposure period. No significant recovery was noted indicating severe damage to the metabolic system. The enzyme activity also showed similar depletion in tissue slice respiration rate. Significant depletion of total ATPase activity, affected the movements of ions across the membrane and severely affected the energy metabolism and disturbed energy budget of the fish. The SWE is toxic and can kill all the inhabiting plants and animals including fish and man in long run. Care should be taken, the effluent and solid waste of the industry should be treated before disposal into aquatic ecosystems to protect the environmental segments from mercury contained toxicants.

Keywords: Industry Toxicology SWE fish *Tilapia* WB respiration tissue slices respiration ATPase activity.

Introduction

Pollution of the natural water resources has been a subject of much discussion since the subject pollution came into lime light. Though man and its subjects of interest are in more physical with air and soil than water, pollution of the water resources at the site close to man's activities is much more hazardous than pollution of the air and soil. This is because of the fact that pollutants introduced into the soil phase of the terrestrial environment do not get easily dispersed so as to cover a greater area. It is agreed that out of the three phase of the environment, subject of the environment are in most close contact with the air phase and any pollution of the same may be proved disastrous as it is evident from the Bhophal gas tragedy. However, pollution of the air is a temporary phenomenon until and unless the pollutants are released continuously. Besides, to bring an aerial environment under serious stress, a large quantity of the pollutants is required to be introduced into the environment .This is because the vast and open aerial environment favors rapid dispersion of the pollutants and hence dilution of the same. In contrast, once a pollutant is introduced in an aquatic system for a long time, seriously threatening the survivability of the existing flora and fauna. The case is more serious with a close system such as ponds and lakes. However, in flowing ecosystems or in ecosystems connected with ocean the pollutants get somewhat diluted. Nevertheless, quite elevated levels of pollutants have been reported from semi enclosed type of aquatic systems in which the pollutants were discharged some years before. In Minamata Bay Hg contamination of fish and sediments still continues though discharge of Hg from the acetaldehyde plant was completely stopped in 1971 (Nishimura and Kumagaii, 1983**).** Powell (1983**)** reported presence of mercury in river system which had received mercurial discharge from a chlor-alkali plant some years before. Thus pollution of an aquatic system gives a more persistent effect than pollution of a terrestrial system. The Chlor-alkali industry was dumping its solid wastes on the riverside (bank of the river) and effluents initially directly for around 60years and later indirectly for around 20years, contaminating both aquatic and terrestrial ecosystems. Heavy metal contamination caused by either natural processes or by human activities is one of the most serious eco-toxicological problems (Reedy and Prasad, 1990). Chlor-alkali industries adopting mercury as cathode in the mercury cell process discharge significant amount of mercury in to the environment in the effluent. The effluent collected from the cell house, washings of the cell house, electrolysis cells, hydration chamber and compression chamber are canalized as effluent and stores in treatment chamber-1 for treatment. The effluent is generally never treated except pH neutralization and sedimentation. The sediments were collected periodically from treatment point-1 & 2 and the effluent canal and dumped nearby. These dumped sediments known as solid waste contained metallic mercury. The surface Mercury when gets exposed evaporates and joins air. During rain the same evaporated mercury returns to water bodies and land masses. The industry discharges its effluents through effluent canal. The sediments collected from effluent canal, treatment pond-1 and treatment pond-2, were dumped nearer to the Rushikulya River bank. Lechate chemicals and rain washed chemicals from solid waste dumps enter into the river and finally reach to the estuary and ultimately enter into the Sea (Sahu, 1987; Mishra, 2013, Raut, 2013 and Priyadarsan & Panigrahi, 2024a, b). Panigrahi, (1980**)** and Sahu (1987) reported presence of huge amount of mercury in the solid wastes and sediments dumped by the industry people. The photographs clearly indicated that the solid waste dumping site is not a safer place, as most of the domesticated animals graze in that area (Priyadarsan, 2024). All grazing plants contained mercury absorbed from the dumping site. These grazing animals also drink the effluent water as no fresh water was available in the vicinity. Earlier Panigrahi (1980) reported presence of mercury in the milk of the cows roaming in the contaminated site. This mercury ultimately

finds a way to enter into human body. Hence, this project was planned to study the impact of SWE prepared from the solid waste which is equivalent to the solid waste lechate of a Chlor-alkali industry on the changes on the physiological parameters of the exposed fish tissues and to find out the impact of mercury on the analyzed parameters of the SWE exposed fishes.

Materials & method

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

(Arc GIS explore expanded photograph showing the area and site map of M/S. Jayashree Chemicals Pvt. Ltd, located near Rushukulya River and Rushikulya estuary at Ganjam near Bay of Bengal, photographs of solid waste dumping sites near the industry.)

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

Maintenance of fish in the aquarium:

Oreochromis mossambicus, Peters of medium size (12-18 g) were collected from the local nursery of the Fisheries Department of Berhampur (Ganjam), Orissa. The fish were allowed to grow in the laboratory reservoirs for acclimatization at least for 15 days before starting the experiments. The fish were maintained in aquarium of 60x60cm containing 50litres of water. Chlorine-free tap water was used in both control and experimental aquarium. The water was changed daily. Air was bubbled through water of the aquarium to maintain the dissolved oxygen at 85±5% air saturation value. The physico-chemical quality of the water of both control and exposed aquarium were measured during the experimental period (APHA, 1995) and maintained at the same level during the entire period of experimentation. Living earthworms from garden showing no contamination by any toxicant were collected and fed daily to both control and exposed fish initially and slowly the diet was changed to toxicant-free chopped goat liver and then to small slices of boiled eggs during holding and through out the experimental exposure

period. After acclimatization, the fish were washed thoroughly with 1% dilute Potassium permanganate ($KMnO_4$) solution, so as to prevent any infection (Priyadarsan & Panigrahi, 2024a; Priyadarsan & Panigrahi, 2018a).

Test solution: The solid waste was collected from the solid waste dumping sites and also from the dried sediments collected from the effluent canal and brought to the laboratory in glass jars and kept in the refrigerator for experimental use. The solid waste was air dried in shade, powdered and sieved. One kg of solid waste powder was mixed with 2liters of distilled water and stirred for 12hrs and allowed to rest for 12hrs. This process alternate stirring and rest was repeated for 15 days. After 15days the mixture was allowed to rest and the supernatant extract was carefully decanted and the extract was preserved in the fridge for use. This decanted extract is known as SWE (solid waste extract). The control and SWE exposed fishes were not disturbed or stimulated. They were allowed to remain undisturbed. All the obtained values were statistically analyzed (Priyadarsan, 2024).

Table-1: Water quality of control and solid waste extract (SWE) exposed aquarium during holding and experiments in the laboratory controlled conditions. (Transparency was measured in terms of optical density (OD) at 540 nm taking double glass distilled water as standard). (Priyadarsan, 2024)

Water Quality	Control	Exposed (SWE)
Colour	Transparent	Grayish
pH	7.1 ± 0.3	7.8 ± 0.4
Temperature, °C	26 ± 2	26 ± 2
Illumination, Lux	2200 ± 200	2200 ± 200
Total hardness, mg l^{-1}	76.5 ± 3.4	115.1 ± 8.5
Specific conductivity,	2.54 x 100µmho	4.52 x 100µmho
Transparency, at 540nm	0.01 - 0.025 (OD)	0.07 - 0.105 (OD)
Hg in the medium, mg l^{-1}	00	0.195±0.024

The mortality rate of test-fish was studied following the method described by Panigrahi (1980). Graded series of concentrations (micro range, middle range and mega range) of solid waste extract (SWE) was prepared in small 2liter rectangular jars. Ten healthy acclimatized *Tilapia* fishes were allowed to live in those jars. Over crowding of fishes were avoided. The control and exposed fishes were fed daily and timely. Water was changed daily to maintain the constancy of the medium in each jar. Observation on the toxicity of the industrial effluent was made at 24, 48, 72, 96 hours and 28 days after the experimental animals were first exposed to the solid waste extract prepared in the laboratory. Individuals showing no respiratory movements, no opercular movements and no response to a tactile stimulus were recorded as dead, and were immediately removed. The test fishes were exposed to maximum allowable concentration (MAC) of the solid waste extract, where no mortality was noticed during experimentation and this was expressed in percentage (%).

Experimental study of SWE:

The physico-chemical analysis of solid waste extract was carried out following the methods as described in Analytical Methods manual (APHA, 1995). The sediment and solid waste collected from the site were air dried and ground to powder in a grinder. The dried solid waste samples were weighed in a monopan electric balance and digested in a Bethge's apparatus with an acid digestion mixture (Wanntorp and

Dyfverman, 1955). One kg of solid waste powder was taken in a mixer and 2liters of distilled water was added. The mixture was homogenized in the mixer for 15days continuously at 12hrs interval. The mixture was stirred for 1 hr and the content was allowed to rest for 1hr. The process was repeated for everyday for 15days. After 15th day of stirring, the whole content was allowed to settle and the supernatant was decanted carefully. The decanted supernatant was the prepared solid waste extract (SWE) used for the experimental purpose. Effluent, SWE and sediment samples were also digested following the same technique. Residual mercury concentration measurements were made in a Mercury Analyser (ECIL, MA5800A). Fish tissue weights were measured in a single pan electric balance and the whole fish body weight was measured on a top pan balance. The whole animal oxygen uptake rates of the test and control fish were measured using five wide mouth 2 liters capacity flasks. Each flask containing the test solution and a test fish was hermetically sealed. A reference flask was kept without fish to check any change of oxygen concentration during the experiments, due to the presence of microorganisms. Any change of oxygen concentration, caused by the microorganism was computed with the final data. After 30 minutes, the dissolved oxygen of all experimental flasks was determined according to the modified Winkler's method (Ashby, 1973 and Panigrahi, 1980). The same procedure was repeated for the control fish. The reduction of the dissolved oxygen concentration equals the amount of dissolved oxygen consumed by the fish in 30 minutes. The oxygen uptake was expressed as mg O_2 g^{-1} h^{-1} (Panigrahi, 1980). The control and SWE exposed fish was dissected and brain, liver and muscles without bones were carefully removed, cleaned and properly washed in distilled water and suspended in 0.25% sucrose solution for future analysis. These tissues were removed the adhered solutions were drained and soaked and weighed. Tissue slices were prepared approximately 0.35 mm thick using a razor blade and recessed guide and floated into Kreb's ringer phosphate medium pre-gassed with oxygen. One or two slices were picked up on a bent wire, drained of excess fluid and the surface was soaked with the help of Whatman filter paper and weighed in a single pan balance (Dhona) and transferred immediately to the medium. The weighted slices were subsequently transferred to the incubation medium in the Warburg flask. Manometric reading of oxygen uptake or carbon dioxide evolution and preparation of tissue extracts were carried out by the methods reported by Patel *et al*. (1973) and Fox *et al* (1975). The tissues were grinded in a micro-tissue homogenizer and the tissue homogenate was processed for analysis of total ATPase activity. Inorganic phosphate produced as a result of the cleavage of ATP to ADP was measured by the method of Fiske and Subbarao (1925) as modified by Martinek (1970). Colour development proceeded at room temperature for 30 minutes. Protein was determined by the procedure developed by Lowry *et al*. (1951), using a spectrophotometer. The ATPase activity was expressed as µmoles of inorganic phosphate liberated mg^{-1} of protein, h^{-1}. All calculations were made on the basis of wet and dry weight of slices. Under the laboratory experimental conditions, dry weight of the tissues were calculated and presented below: Brain tissue- 11.45% of the wet weight; Liver tissue - 12.76% of the wet weight; Muscle tissue - 16.37% of the wet tissue; Gill tissue - 18.41% of the wet tissue. All the obtained data was statistically analyzed to verify the validity of data in biological analysis.

Results

All the SWE exposed fish appeared lethargic after exposure to the SWE compared to control fish. The major clinical symptoms such as inappetance and ataxia appeared after 2 to 3 days exposure. At higher concentrations of the SWE, the exposed fish showed erratic movement leading to collision to inner side

of the aquarium. At higher exposure periods, the exposed fish appeared lethargic and irregular swimming activity was observed when compared to control fish. Fish death started in exposed aquarium after 20days of exposure. Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. It seems 28days of exposure to the SWE was enough for the fish not to recover 100%, showing a damage of 99.98% when compared to 28d control value and finally all the 28d exposed fish died after 22d of recovery onwards indicating permanent damage to the metabolic and biochemical systems caused by mercury. The SWE exposed fish showed paralytic movements and the body showed banding with lateral movement. The exposed fishes also showed depression in active metabolism, depletion in ventilation rate and significant decrease in whole body respiration rate.

Analysis of solid waste extract: The temperature of the SWE prepared in the laboratory was 28.2^0C. The pH of the SWE was alkaline and the value recorded was 8.3±0.2. The alkalinity was 241.8±18.5 as $CaCO_3$ in mg l^{-1}. The hardness of the SWE sample was 392.2±12.6 as $CaCO_3$ in mg l^{-1}. The chlorinity was 1018.6±24.6mg l^{-1}; Phosphate-21.6±3.4mg l^{-1}; Chloride-18.2±1.4mg l^{-1}; Calcium- 88±11mg l^{-1}; Magnesium -21.1±3.6mg l^{-1}; Sodium -5.2±0.8mg l^{-1}; Potassium -12.2±16.6mg l^{-1}; Total nitrogen -4.5±1.3mg l^{-1} and Mercury -9.75 mg/liter. The dissolved oxygen content was low and ranged within 2.2±0.4mg l^{-1}. The suspended solids were low but significant and the value was 102.5±6.4mg l^{-1} (Priyadarsan, 2024).

Toxicity testing of the SWE revealed the following information. After 24h of exposure the lethal concentration values were, LC_0- 5.4%SWE; LC_{100}-9.7%SWE and after 28days of exposure in chronic poisoning, the LC values were LC_0- 2.85%SWE, LC_{10}-3.11%SWE, LC_{50}- 3.95%SWE, LC_{90}-4.68%SWE and LC_{100}-4.95%SWE. The MAC (maximum allowable concentration) value after 28days was 2.8% SWE.

Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. The rudimentary thickened gill filament with a lot of slime coating was seen in the exposed fish gills after 28d exposure to SWE. All the exposed fish appeared lethargic after exposure to the SWE. The major clinical symptoms such as inappetance and ataxia appeared after 2 to 3 days exposure. At higher concentrations of the SWE, the exposed fish showed imbalance and erratic movement leading to collision to inner side of the aquarium. At higher exposure periods, the exposed fish appeared lethargic and irregular swimming activity was observed when compared to control fish. We have observed fish body bending and fish death started in exposed aquarium after 24days of exposure (Priyadarsan, 2024).

Fig.1 showed the changes in whole body oxygen uptake in control and SWE exposed fish at different exposure and recovery period. The data indicated the whole body respiration rate of the control fish and SWE exposed fish at 7d interval for a period of 28days. The respiration rate decreased when the exposure period was enhanced showing a negative correlation. When the SWE exposed fishes were transferred to SWE free medium, no recovery was noted. Rather the whole body oxygen uptake rate (respiration rate) of the exposed fishes further declined significantly and after 21^{st} day of recovery, death of the exposed fishes were noticed The figure clearly indicated that the respiration rate of the exposed fish gradually decreased significantly with the increase in exposure period. The exposed fishes were transferred to SWE free tap water for recovery studies. It was observed that the exposed fish could not recover to its pre-test activity and the respiration rate further depleted. Fig.2 indicated the percent change in the respiration in SWE exposed fish, where a maximum of 50% decrease was observed. After 7d exposure 27.3%, 14d exposure 31.3%, at 21d exposure 38.7% and at 28d exposure 50% depletion in respiration rate was observed. When the exposed fish was transferred to SWE free medium no recovery

in respiration rate was noted at 14d and 28d recovery (Fig.2). An experiment was conducted to test the possibility of recovery at different days of exposure. The 7d exposed fishes were transferred to toxicant free medium for 7days recovery. The 14d exposed fishes were transferred to toxicant free medium for 14 days, where the exposure period equals the recovery period.

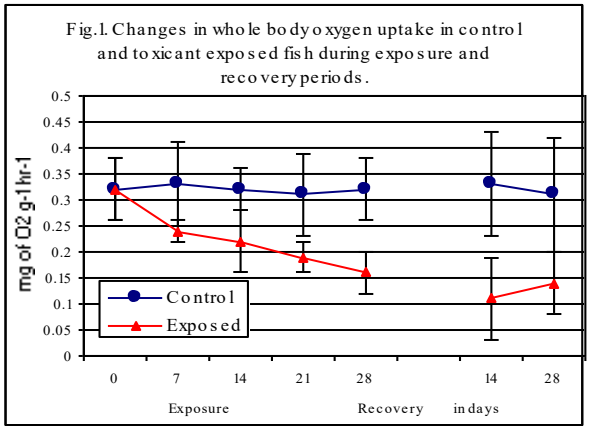


Fig.1. Changes in whole body oxygen uptake in control and toxicant exposed fish during exposure and recovery periods.

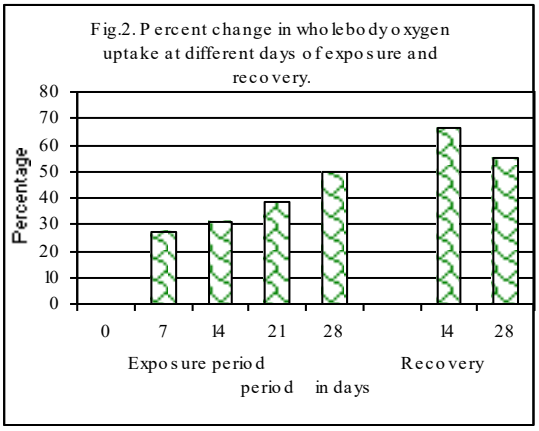


Fig.2. Percent change in whole body oxygen uptake at different days of exposure and recovery.

The 21d exposed fishes were transferred to toxicant free medium for 21 days and the 28d exposed fishes were transferred to toxicant free medium for 28 days to observe percentage of recovery. It was observed that the 7d exposed fishes could recover to its pre-test activity and 14d exposed fishes could recover to its pre-test activity fully. But the 21d exposed fishes could not recover significantly. The 28d exposed fishes could not recover at all. We may conclude that if the fishes were exposed for a shorter duration the possibility of recovery is possible but prolonged exposure probably led to death where no recovery was noticed (Fig.3). The residual mercury accumulation in fish body increased with the increase in exposure period showing a positive correlation ($P \leq 0.01$). A maximum of 21.9µg of Hg g^{-1} tissue was noted (Fig.3a) was observed in 28d exposed fishes. The exposed fish accumulated 5.89µg of Hg g^{-1}, 13.09µg of Hg g^{-1}, 17.99µg of Hg g^{-1} at 7d, 14d and 21d exposure period. In the control fish no residual mercury was observed (Fig.3a). The exposed fishes also showed depression in active metabolism and significant decrease in whole body respiration rate. Further cellular respiration also indicated that SWE exposed fish tissues showed significant depression in respiratory activity at all exposure period. No significant recovery was noted indicating severe damage to the metabolic system (Table-2).

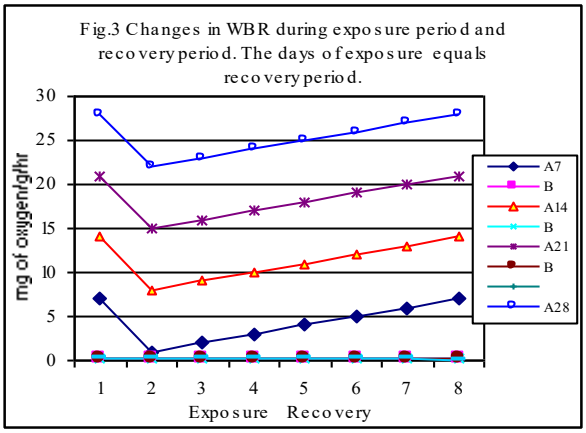


Fig.3 Changes in WBR during exposure period and recovery period. The days of exposure equals recovery period.

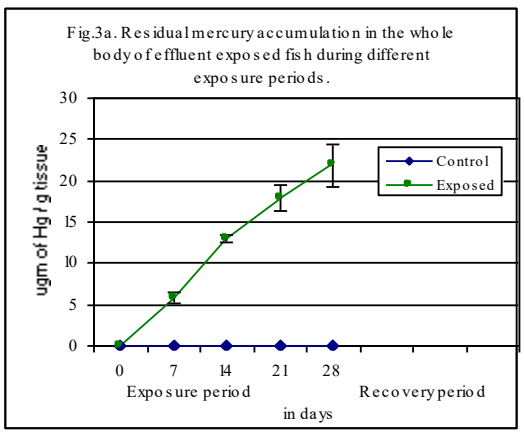


Fig.3a. Residual mercury accumulation in the whole body of effluent exposed fish during different exposure periods.

Table-2 showed the changes in respiration rate in different tissues of the fishes exposed to SWE and similar depletion was also noted in different tissues of the recovery fishes. The exposed fishes were

transferred to toxicant free water for recovery studies. Changes in oxygen uptake (µl of O_2 g^{-1} hr^{-1}) by brain, liver, muscle tissues and gill filaments of 28d control fish, 28d SWE exposed fish and 28d recovery of exposed fish during recovery period. The tissue slice respiration decreased in all the types of tissues like brain, liver, muscle tissues and gill filaments of SWE exposed fishes. During recovery studies, it was observed that the exposed fish tissues could not recover at all indicating permanent damage to the exposed system.

Table-2: Changes in oxygen uptake (µl of O_2 $g^{-1}hr^{-1}$) by brain, liver, muscle tissues and gill filaments of 28d control and 28d exposed fish, *Tilapia mossambica*. Data are the mean of samples ± standard deviation. Percent change calculated from the mean of the samples.

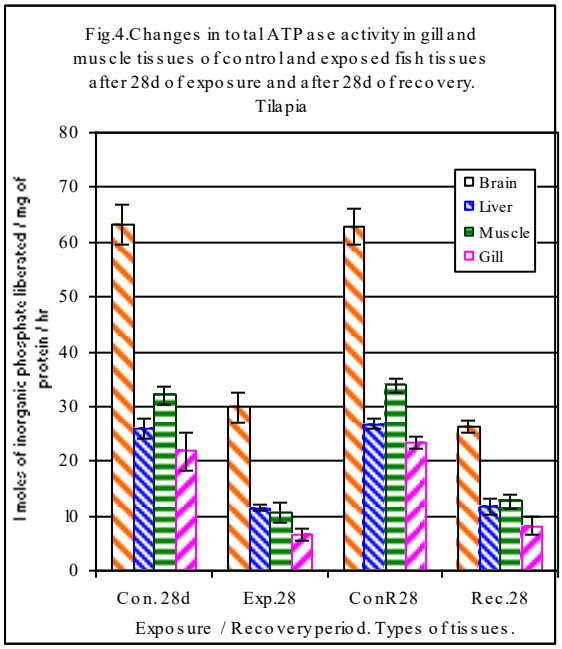
Types of tissue	Exposure period (µl of O_2 $g^{-1}hr^{-1}$)		Recovery period(µl of O_2 $g^{-1}hr^{-1}$)	
	Con. 28d	Exp.28	ConR28	Rec.28
Brain	552.6± 18.6	154.4±19.2	553.5±14.5	131.9±13.5
Liver	498.2±9.8	112.6±16.4	492.1±13.7	136.2±22.4
Muscle	488.9±12.3	183.2±21.1	484.3±18.8	289.5±14.5
Gill	465.8±19.4	109.5±26.4	467.8±21.4	294.7±19.5
Types of tissue	Percent change in 28d exposure		Percent change in 28d recovery Percent recovery in parentheses.	
Brain	-72.05		-76.2 (-4.15)	
Liver	-77.4		-72.3 (+5.1)	
Muscle	-62.5		-40.2 (+22.3)	
Gill	-76.5		-37.0 (+39.5)	

The data shown in table-2 is indicative of drastic damage to the exposed metabolic system. The brain tissues showed 72% decrease after 28d exposure and 76% depletion after 28d recovery indicating further damage by 4.2%. The liver tissues showed 77.4% decrease after 28d exposure and 72.3% depletion after 28d recovery indicating insignificant partial recovery by 5.1%. The muscle tissues showed 62.5% decrease after 28d exposure and 40.2% depletion after 28d recovery indicating significant partial recovery by 22.3%. The gill filament showed 76.5% decrease after 28d exposure and 37% depletion after 28d recovery indicating significant recovery by 39.5% (Table-2). Table-3 is a statistical table indicating the regression analysis values and the level of significance of the obtained data.

Table- 3.Correlation coefficient (r) values between days of exposure and change in parameters in control and SWE exposed fish. P = Levels of significance. WBR=Whole body Respiration.

Status	WBR	Changes in O_2 uptake by tissue slices				Changes in ATPase activity			
		Brain	Liver	Gill	Muscle	Brain	Liver	Gill	Muscle
Control	0.000	-0.484	0.000	-0.260	-0.378	0.402	-0.446	-0.665	-0.560
P ≤	N S	N S	N S	N S	N S	N S	N S	N S	N S
Exposed	-0.974	-0.979	-0.990	-0.941	-0.982	-0.942	-0.930	-0.992	-0.981
P ≤	0.001	0.001	0.001	0.01	0.001	0.01	0.01	0.001	0.01

The data presented in the present investigation clearly indicated the existence of strong correlation values of mercury poisoning accumulation and its impact on different parameters. Highly significant correlations were noted between exposure period and different parameters studied.



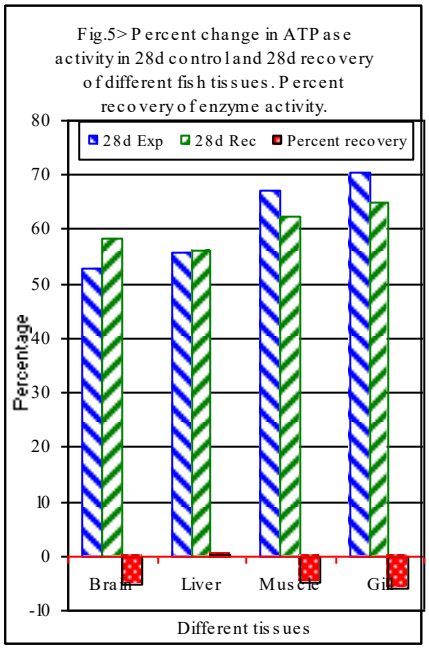


Fig.4 indicated the changes in ATPase activity of control and SWE exposed fish tissues like brain, liver, muscle tissues and gill filaments. Significant depletion of total ATPase activity affected the movements of ions across the membrane and severely affected the energy metabolism and disturbed energy budget of the fish. The ATPase activity depleted from 63.3 ±3.6□moles of ip (inorganic phosphate) liberated / mg of protein / hr to 29.8±2.8□moles of ip liberated / mg of protein / hr after 28d of exposure (Fig.4) showing 59% depletion Fig.5) in the enzyme activity of exposed brain tissues. The ATPase activity depleted from 25.9 ±1.8□moles of ip (inorganic phosphate) liberated / mg of protein / hr to 11.4±0.5□moles of ip liberated / mg of protein / hr after 28d of exposure (Fig.4) showing 55.9% depletion Fig.5) in the enzyme activity of exposed liver tissues. The ATPase activity depleted from 32.1 ±1.6□moles of ip (inorganic phosphate) liberated / mg of protein / hr to 10.5±1.8□moles of ip liberated / mg of protein / hr after 28d of exposure (Fig.4) showing 67.2% depletion Fig.5) in the enzyme activity of exposed muscle tissues. The ATPase activity depleted from 21.8 ±3.4□moles of ip (inorganic phosphate) liberated / mg of protein / hr to 6.4±1.1□moles of ip liberated / mg of protein / hr after 28d of exposure (Fig.4) showing 70.6% depletion Fig.5) in the enzyme activity of exposed gill tissues. The statistical analysis indicated the importance and validity of data. No recovery was marked in 21d and 28d exposed fishes. These fishes survived for few days during recovery period. These exposed fishes died gradually after 12days of recovery onwards. It can be concluded that if fish is exposed for a shorter period (less than 7days), the exposed fishes can recover. But when the exposure period was extended further, the toxicant caused / induced irreversible damage to the physiological activity leading to metabolic collapse and finally the exposed fishes died because of the toxicant. The SWE / lechate of the solid waste is deadly toxic where once exposed irreversible damage is caused to the exposed system.

Discussion

It was ascertained from our study (Panigrahi, 1980; Misra, 2013 and Priyadarsan, 2024) that the situation at Rushikulya estuary and Ganjam area is grim because of the Chlor-alkali industry located at Ganjam. The industrial wastes contained significant amount of mercury, which were discharged into the environment carelessly and at times intentionally. The industry was discharging mercury vapour generated during electrolysis, which was escaping into the Cell house. This mercury contained cell house air was discharged through the vent by high speed exhausts into the atmosphere, which moves to distances and settle either on aquatic or terrestrial ecosystems and many a times gets deposited on plant body. The second route of discharge as discussed earlier was the effluent coming from the industry. This effluent is nothing but the cell house washings, washings of the cell chambers, washings of the compression chamber and washings of the hydration chamber. When washings were carried out in all those spots, the adhered mercury and spilled mercury escape from these areas along with washings. Now this metal, mercury becomes an important component of the effluent, which was discharged into the environment. As per law, these effluents are to be treated before discharge into the environment. The industry people only adjusted the pH of the effluent by adding an acid or alkali for the purpose. This type pseudo treatment was done inside the premises of the industry in a settling tank. Afterwards, the effluent was discharged initially directly into River Rushikulya and later after complains the effluent was stocked in a soaking pond constructed near the river and effluent was directed into the pond. The spilled or over flooded effluent joins the river directly and the chemicals present in the effluent were leaching from the soaking pond into the river and nearby areas and contaminating the ground water also. Then third one type of discharge is more important. The sediment of the settling and treatment tanks and the effluent canal were removed periodically manually and dumped near the industry protected by temporary fencing and very nearer to the river bank, which contained highly significant amount of mercury. Surface evaporation of mercury is as expected during day time due to high air temperature, which escapes into the air from solid waste dumps. Mercury along with other chemicals was leaching to nearby areas and most importantly into the river. Interestingly during rainy season the washings containing mercury along with rain run off water escape into the river and during flood the whole solid waste dumps of the area gets cleaned by flood water and the solid waste containing mercury enter into the river then into the estuary and ultimately into the Sea. All the above events occurring in this hot spot contaminated area for long 70years without being questioned by any one like governments both at the centre and state, PCBs, district or state administrators. It seems nobody was involved or no bodies was interested to safe guard the environment and protect the natural environments and most importantly the flora and fauna, on which man depends. This was intentional because the industry people never probably wanted to adopt any recycling technology recommended to protect the natural environment. Mercury was available in plants and animals of the area. Many eyebrows were raised in the past on the issue but no action was taken. Some local NGO's raised their voice but a futile exercise, only for money. Ultimately, it was our laboratory which took the challenge and worked on this industrial issue since 1978 till today. Our laboratory has seen ups and down on this issue and published more then 100 research papers and more than 35 doctoral scholars worked on different aspects of the mercury pollution problem. Many suggestions were given for recycling techniques and reclamation procedures to the industry. They adopted few in reality and some they adopted only to show but not to implement. Ultimately in 2010 as per decision the Mercury cell process was phased out and the Membrane technology was adopted. Afterwards the administration of the industry removed all the solid waste

dumps from the area and recharged with fresh soil and trying to develop a good greenery of the area. Mercury pollution problem of the area was solved. But nature suffered as usual. These solid wastes were either dumped in some other area or they must have thrown into the sea to escape from the law and order authorities of the state. Priyadasan and Panigrahi, (2018a, b) reported presence of huge amount of mercury in the solid wastes and sediments dumped by the industry people. Borg *et al*. (1970) reported inappetance, muscular weakness, ataxia and loss of body weight as the main clinical symptoms of mercury poisoning. On autopsy, he found muscular atrophy, which can be correlated with the weight loss in the goshawks. Hanko *et al*. (1970) reported loss of appetite, weakness of the extremities, excitation in the animal and loss of body weight due to mercury poisoning in chickens. Significant depletion in protein content in brain, liver and muscle tissues were observed in SWE exposed fish compared to control fish tissues. Interestingly the free amino-acid content increased with the increase in exposure period in different tissues of the SWE exposed fish. The protein decreased was probably due to proteolysis of protein molecules induced by mercury. The increase in FAA content either due to breakdown of protein or non-synthesis of protein induced by mercury. The present report agrees with the observations of Mishra (2013) and Priyadarsan (2024). Panigrahi (1980) reported significant change in the behaviour of the exposed fish in mercurial toxicity, when compared to control fish. Residual mercury in exposed fish tissues depleted the respiration rate of tissues, which ultimately reflected on the whole body oxygen uptake and ventilation rate when compared to control fish tissues as reported by Priyadasan and Panigrahi (2018a,b). The reports of Panigrahi and Mishra (1978a&b) agree with our findings except that we conducted the experiment by taking the solid waste extract containing mercury for our experiment where the impact was synergistic effect of all chemicals present in the extract in addition to mercury. If we compare the impact of our study with the findings of Panigrahi and Misra (1978a&b), the observed effects reported were the impact of mercury as mercuric nitrate, a single chemical on fresh water fishes. The same authors also reported residual mercury accumulation in fish tissues and depletion of tissue slice respiration in different tissues (Panigrahi, 1980) and correlated the impact to be an effect of mercury poisoning. We got similar results and agree with the findings of Panigrahi and Misra (1978a&b) and Panigrahi (1980). Maximum residual mercury was recorded in exposed fish gill filaments. Exposed fish brain accumulated slowly when compared to other fish tissues but higher accumulation during recovery period might be due to transportation from other tissues. Significant depletion in respiration rate in exposed fish brain, liver, muscle and gill tissues when compared to control fish tissues was due to residual mercury accumulation in different tissues and its impact on respiratory metabolism. Different aspects concerning the biochemical assessment of functional impairment in vital organs were explored in toxicological studies. Untoward effects can usually be traced back in the functional incompetence of organs. Whenever dysfunction occurs in a tissue it has its origin in biochemical abnormality may become apparent before the onset of morphological changes and precedes the development of chronic degenerative disease. Explanation of biochemical tests facilitates diagnosis of pre-toxic condition in man and it may be valuable in the routine screening of chemicals in animal experimentation. Biochemical studies are the useful tools in aiding diagnostic pathology which covers a wide range embracing various organs and systems. Pesticides act either as a selective toxicant or may display rather a broad spectrum of adverse biological activities. Toxicant effects on growth may be studied by examining macromolecules involved in growth, such as DNA, RNA and protein. However, toxicant-induced changes in macromolecular content (RNA, DNA and protein) and RNA / DNA, RNA / protein, and protein / DNA ratios have received very little attention in fish. The residual

mercury accumulation in fish tissues increased the body burden and impacted severely the respiratory metabolism which was reflected in depletion of whole body oxygen uptake and residual mercury accumulation in brain affected the nervous system leading to erratic swimming, nervous disorders and paralytic movements. In the present case the impact was very severe drastic impact was noticed in the changes in protein content of the exposed fish tissues and increment in FAA content can be correlated to proteolysis of the protein present in fish tissues or non-synthesis of protein after amino acid synthesis and the behavioral changes were more acute and drastic confirming mercury poisoning in the affected areas at Ganjam, Odisha.

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