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Eco-Toxicological Impact Of Solid Waste Extract Prepared From Solid Waste Of A Chlor-Alkali Industry On Fresh Water Fish And Its Significance.

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Abstract
The collected solid waste from the dumped site was air dried and powdered. The powder was grayish white with 37% water holding capacity, with a specific gravity of 2.6 and 24% air content. The solid waste contained 106±16.5mg of Hg Kg^{-1} dry weight. Significant decreases in all parameters were observed in the solid waste, might be due regular washing of the solid waste deposits by monsoon rain. The solid waste extract contained 9.75mg of Hg l^{-1}. The exposed fishes appeared lethargic and loss of equilibrium and irregular swimming was observed when compared to control fish. Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. 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. The ATPase activity in SWE exposed fish brain, liver and muscle tissues showed 41.03%, 63.9%, and 49.6% decrease over respective control values, where the SWE exposed fish liver was more damaged than other tissues tested. Significant depression in ATPase enzyme activity may severely affect the ion transport mechanism of the exposed fish body when compared to control fish body. The ions studied showed significant decrease in all tissues of the exposed fish, when compared to the control fish tissues indicating ionic imbalance responsible for the erratic behavior of the SWE exposed fish. Significant differences in ion content of different tissues of the exposed fish when compared to control fish clearly indicated the nature of the toxicant and effects caused by the SWE toxicant on the nervous tissue, synaptic transmission, nerve impulse generation, ionic balance and membrane transport system of the exposed fish.

Key words: Chlor-alkali industry, Solid waste, SWE, Toxicity, Behavioural study, Enzyme, ions

Introduction

Industry is responsible for creating a fantastic range of new chemicals every year all of which eventually find their way into the environment causing environmental pollution. They release large quantities of chemicals in the form of gas, liquid and solid wastes, into the environment. Many of these chemicals are toxic and create pollution problem. The problem of toxic hazard has already reached alarming proportions in this country and is bound to grow with increasing industrialization. Pollution has become an acute problem in some developed and developing countries. Generally the toxic effluents from industry are neutralized before their discharge, but still they contain substantial amount of toxic substances that can cause pollution. At present it is believed that rivers are most severely polluted by industries followed by estuaries, lakes and ocean in declining order (Kumar, 1981). Environmental pollution caused by mercury discharged along with liquid effluent and solid waste generally from Chlor-alkali industry and Aldehyde

industry is well known and a well established fact. Extensive literature on the pollution of surrounding biota through the discharges of effluents and sludges from Chlor-alkali industries is available (Weiss *et al*., 1971; Lodenius, 1980; Suckcharoen & Nourteva, 1982; Lodenius & Tulisalo, 1984; Shaw *et al*., 1986 and Priyadarsan *et al*., 2016 a, b). Studies on loss of mercury into the environment from the chlor-alkali industry were carried-out by Flewelling (1971), Bouveng (1968 & 1972) and Shaw *et al*., (1986). The most significant source of mercury availability in the environment is mostly due to the chlor-alkali industries (Fimreite, 1970; Bothner & Carpenter, 1973; Hartung & Dinman, 1974; and Gonzalez, 1991). Majority of the chlro-alkali industries in India adopt old process of using mercury as cathode in the electrolytic process. This has long since been discarded in Japan (recently in India) and in many other countries after the Minamata Bay mercury poisoning incident. This technology has been banned and by 2010, all mercury cell units are to be converted to membrane technology. M/S Jayashree Chemicals Pvt. Ltd. situated at Ganjam only adopted the membrane technology over mercury cell process in 2012. Prior to 2012, the industry has discharged huge amount of mercury in to the environment along with effluent. The grayish white solid waste (brain mud) which is dumped in pits in the nearby field at Ganjam contained large quantity of mercury. Mishra (2013), Raut (2013) and (2022) reported that mercury content to vary from 480 to 984 µg/g dry weight in the solid waste collected from different areas in and around the industry and mostly at waste dumping sites very nearer to Rushikulya river bed. The river containing the effluent, lechate and sold waste of the industry joins the Rushikulya estuary and Bay of Bengal. The flora and fauna ultimately suffer due to the wastes released in to the environment. The present study aims at understanding the pollution status and impact of contaminated river water on fresh water fish and estuarine fish.

Materials & Methods:

Location of the industry under study: The chlor-alkali industry M/S Jayashree Chemicals Pvt. Ltd., is situated at Ganjam, on the Bank of Rushikulya estuary about 1.5 km. Away from the sea, Bay of Bengal, on the East and 30 km. North of Brahmapur city on the south-eastern side of India at 84^0 53'E longitude and 19^0 16'N latitude. The industry was established in 1962 and started manufacturing caustic soda, liquid chlorine and hydrochloric acid by using a sheet of elemental mercury as a mobile cathode for the electrolysis of brine water (saturated sodium chloride solution) since August 1967.

(GIC map showing location of the industry study sites and Photograph of the industry)

Analysis of solid waste and solid waste extract of the Chlor-alkali industry:

Table-1: Physico-chemical analysis of solid waste collected from the solid waste dumping site and solid waste extract prepared in the laboratory.

\Parameters studied		Solid Waste (SW)		Solid Waste Extract (SWE)
pH	-	8.6±0.4	--	8.3±0.2
Phosphate	-	51.08±7.6 mg Kg^{-1} dry wt.	--	21.6±3.4 mg l^{-1}
Chloride	-	648.4±13.4 mg Kg^{-1} dry wt.	--	18.2±1.4g l^{-1}
Calcium	-	139.24±8.8 mg Kg^{-1} dry wt.	--	88±11 mg l^{-1}
Magnesium	-	28.26±6.4 mg Kg^{-1} dry wt.	--	21.1±3.6 mg l^{-1}
Sodium	-	151±14.6 mg Kg^{-1} dry wt.	--	5.2±0.8 g l^{-1}
Potassium	-	42.7±8.5mg Kg^{-1} dry wt.	--	12.2±16.6 mg l^{-1}
Total nitrogen	-	18.4±2.6 mg Kg^{-1} dry weight	--	4.5±1.3mg l^{-1}
Mercury	-	106.1±16.5 mg Kg^{-1} dry wt.	--	9.75±2.86 mg l^{-1}

(Values are mean of five samples ± standard deviation)

The collected solid waste from the dumped site was air dried and powdered. The powder was grayish white with 37% water holding capacity, with a specific gravity of 2.6 and 24% air content (Table-1). The

solid waste contained 106.0±16.5 mg of Hg Kg^{-1} dry wt.. Significant decreases in all parameters were observed in the solid waste, might be due regular washing of the solid waste deposits by monsoon rain. The SWE prepared from this powder showed the features shown on table-1. The solid waste extract contained 9.75 mg of Hg l^{-1}. This prepared solid waste extract was considered equivalent to the leached chemicals leaching in rainy season and entering into neighboring ponds and crop fields and water bodies like Rushikulya River and estuary. Total ATPase activity was measured with Na^+, K^+ and Mg^{++} ions, in the reaction mixture. 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 UV-Visible Spectrophotometer (Systronics, India). The obtained data was calculated taking the protein value, weight of the tissue and time period. The ATPase activity was expressed as µmoles of inorganic phosphate (ip) liberated mg^{-1} of protein h^{-1}. Both control and toxicant exposed fishes were sacrificed and dissected after 28 days of exposure. Brain, liver, muscle and gill tissues of each fish were dissected out, properly washed, separated and kept in a watch glass. The tissues were soaked and adhered water was removed and weighed. Contamination of these tissues was avoided during autopsy. After soaking with Whatman filter paper, the tissues were then transferred to Kjeldahl flasks continuing concentrated Nitric acid (BDH, Analar grade) and Sulfuric acid (BDH, Analar grade) [HNO_3, H_2SO_4 = 1:1] and digested (Panigrahi, 1980) to a nearly colourless solution on mantle heater. After completion of digestion, the digested solution was cooled and brought to room temperature. The total volume of the solution was diluted to an appropriate volume with double distilled water. The amount Na^+, K^+ and Ca^{++}, were determined by a flame photometer with Polyflex galvanometer and the reading was repeated in flame photometer (Systronics) taking NaCl, KCl and $CaCl_2$ as standards following Systronics User manual. The obtained values were computed from a standard curve. For magnesium determinations, the procedure followed by Orange and Rhei (1970) was adopted. The concentration of Mg^{++} was calculated from the standard curve. The data was analyzed following different statistical tests. Physico-chemical analysis of wastes was carried out following the protocols of APHA (1998). Quantitative determination of mercury in the digested samples was done with the help of Mercury Analyser, a cold vapour atomic absorption spectrophotometer. A suitable aliquot of a blank, standard or sample was stirred for 5 minutes with the help of a magnetic stirrer in acid medium in the presence of 2 ml of 20% $SnCl_2$. Mercury vapour generated after stirring was pumped into the mercury analyser set at 100% transmission. Amount of mercury in the unknown samples was computed from a standard graph (Mercury Analyser, MA5800, ECIL, and India). Results were expressed in mg l^{-1} in and mg Kg^{-1} dry weight or fresh weight and presented as mean of the samples analyzed.

Results

Toxicity testing:

The solid waste extract prepared in the laboratory as mentioned in the material and method chapter was considered equivalent to the lechate leaking from the solid waste dumps located nearer to the industry. The lechate leach to the peripheral area and contaminate ground water and also water bodies like small ponds. The lechate also flow directly into the River Rushikulya mostly in rainy season. In addition, during rainy season, the solid wastes were washed away into the river bed. Many a times during rainy season, the flood water carries all the dumped solid waste deposits into the sea, passing through the estuary. Hence, it was felt necessary to study the lechate of the solid waste. The detailed study indicated the presence of mercury in the lechate. Lechate collected from the dumping site and contaminated area at different time periods indicated significant difference in chemical composition and mercury concentration. Hence, it was planned to prepare the extract by taking the solid waste from the dumping site and preparing the extract in the laboratory, now known as solid waste extract (SWE) for the experimental purpose.

Table-A1: Showing the lethal concentration values of acute toxicity and chronic test.

Lethal concentration values	Toxicant (SWE) concentration, Dilution percentage (%)					MAC value SWE	Used conc. of SWE
	Time period in hours				Days	30days	28days
	24h	48h	72h	96h	28days		
LC_0	5.4	5.4	5.1	4.5	2.85	2.85%	2.8%
LC_{10}	6.2	6.1	5.9	5.6	3.11		
LC_{50}	8.4	6.6	6.4	6.3	3.95		
LC_{90}	9.1	8.8	8.5	8.1	4.68		
LC_{100}	9.9	9.7	9.6	9.4	4.95		

MAC	6.5	6.3	5.9	6.1	2.8		

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 from the jars and solution of the jar was immediately changed to avoid any infection and release of decomposition wastes from the dead fishes. 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 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. 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. Control fish remained clinically healthy throughout the experimental period without showing any signs of toxicity or contamination. No death of fishes was recorded in the control set jars. No of fishes died in each jar and death time period was recorded in each jar for 24, 48, 72, and 96hours. After this acute toxicity period, experimental fishes were allowed for 30days further exposure for studying impact of chronic exposure of the toxicant on the freshwater fish, *Tilapia*. Acute toxicity testing revealed the following information. After 24h of exposure the lethal concentration values were, LC_0- 5.4% SWE, LC_{10}- 6.2% SWE, LC_{50}- 8.4% SWE, LC_{90}- 9.1% SWE and LC_{100}- 9.9% SWE; after 48hrs, the LC values were LC_0- 5.4% SWE, LC_{10}-6.1% SWE, LC_{50}- 6.6% SWE, LC_{90}- 8.8% SWE and LC_{100}- 9.7% SWE; after 72hrs, the LC values were LC_0- 5.1%SWE, LC_{10}-5.9%SWE, LC_{50}- 6.4% SWE, LC_{90}- 8.5% SWE and LC_{100}- 9.6% SWE; after 96hrs, the LC values were LC_0- 4.5%SWE, LC_{10}-5.6%SWE, LC_{50}- 6.3% SWE, LC_{90}- 8.1% SWE and LC_{100}- 9.4% SWE. 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 deduced MAC values were 5.6%SWE after 24h, 5.3% SWE after 48h, 5.1%SWE after 72h, 4.9%SWE after 96hours and the MAC value after 28days was 2.8% SWE). The figure clearly indicated the existence of significant positive correlation ($r=0.989$; $p\leq0.01$) with lethal concentration values at all exposure periods. The solid waste concentration decreased with the increase in exposure period showing a significant negative correlation ($r= -0.987$; $p\leq0.01$). The maximum allowable concentration (MAC) value of the of the solid waste extract (SWE) of the Chlor-alkali industry was found to be 2.85% SWE concentration for 30 days and to be on the safer side 2.8% SWE containing 0.195µg l^{-1} of mercury (Hg) was considered for 28 days of the exposure, for future studies. No mortality was observed in the control set, during the entire period of experimentation.

Behavioral studies: All the exposed fish appeared lethargic after exposure to the solid waste extract. The major clinical symptoms such as inappetance and ataxia appeared immediately after exposure. At higher concentration of the SWE prepared from the solid waste dumped by the industry, the exposed fish showed erratic movements. The other signs of toxicity such as loss of equilibrium, gradual onset of inactivity, erratic swimming with irregular collision to the inner glass walls of the aquarium were observed. Infection of eyes, exophthalmia and involutions of test fish were observed. Autopsy studies revealed that the liver and brain of exposed fish were congested, pale and tender. The brain somatic index (BSI) in the control fish remained in between 0.821 to 0.826 both in exposure periods and recovery periods. The changes in total ATPase activity in brain, liver and muscle of control and SWE exposed fish at different days of exposure and recovery and it's percent changes were shown in Table-E-2. The ATPase enzyme activity ranged between 30.6 ± 2.1 to 31.6 ± 1.2 µmole of ip liberated mg^{-1} of protein hr^{-1} during the entire period of experimentation in the control brain tissue. In the SWE exposed fish brain, the enzyme activity decreased from 31.6 ± 1.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 26.4 ± 1.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 7days of exposure; from 31.2 ± 2.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 25.5 ± 2.4µmole of ip liberated mg^{-1} of protein hr^{-1}after 14days of exposure; from 30.9 ± 1.6µmole of ip liberated mg^{-1} of protein hr^{-1} to 21.8 ± 1.3µmole of ip liberated mg^{-1} of protein hr^{-1}after 21days of exposure and from 31.2 ± 2.3µmole of ip liberated mg^{-1} of protein hr^{-1} to 18.4 ± 3.1µmole of ip liberated mg^{-1} of protein hr^{-1}after 28days of exposure (Table-E-2). The enzyme activity in SWE exposed fish brain tissue declined by 16.5% on 7th day of exposure, by 18.3% on 14th day of exposure, by 29.4%% on 21st day of exposure and by 41.03% on 28th day of exposure (Table-E-1). When the SWE exposed fish was transferred to toxicant free medium, the ATPase activity in exposed fish brain instead of showing any signs of recovery further declined from 32.1 ± 4.5µmole of ip liberated mg^{-1} of protein hr^{-1} to 14.6±3.8µmole of ip liberated mg^{-1} of protein hr^{-1} on 14th day of recovery & from 32.2± 3.6µmole of ip liberated mg^{-1} of protein hr^{-1} to 11.5 ± 2.2µmole of ip liberated mg^{-1} of protein hr^{-1} on 28th day of

recovery. The enzyme activity decreased by 54.5% on 14th day recovery and decreased by 64.2% over the 28th day exposure value, after 28 days of recovery, indicating no recovery at all (Table-E-1). The exposed fish brain enzyme activity could not recover even partly during recovery period but showed 13.5% and 23.3% further depletion on 14th and 28th day of recovery. No recovery in the exposed fish brain enzyme activity as marked on 14d and 28d of recovery indicated the acute toxic nature of the toxicant. The ATPase enzyme activity ranged between 28.2±2.1 to 28.7±4.1µmole of ip liberated mg^{-1} of protein hr^{-1} during the entire period of experimentation in the control liver tissue. In the SWE exposed fish liver, the enzyme activity decreased from 28.4 ± 2.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 23.6±3.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 7days of exposure; from 28.7±4.1µmole of ip liberated mg^{-1} of protein hr^{-1} to 14.8±1.5µmole of ip liberated mg^{-1} of protein hr^{-1}after 14days of exposure; from 28.2±2.1µmole of ip liberated mg^{-1} of protein hr^{-1} to 12.8±2.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 21days of exposure and from 28.3 ± 3.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 10.2±3.4µmole of ip liberated mg^{-1} of protein hr^{-1}after 28days of exposure (Table-E-1). The enzyme activity in SWE exposed fish liver tissue declined by 16.9% on 7th day of exposure, by 48.4% on 14th day of exposure, by 54.6% on 21st day of exposure and by 63.9% on 28th day of exposure (Table-E-2). The data indicated that the liver of the SWE exposed fish was damaged maximum when compared to SWE exposed fish muscle and brain tissues of the SWE exposed fish. When the SWE exposed fish was transferred to toxicant free medium, the ATPase activity in exposed fish liver instead of showing any signs of recovery further declined from 28.4±3.6µmole of ip liberated mg^{-1} of protein hr^{-1} to 9.9 ± 2.1µmole of ip liberated mg^{-1} of protein hr^{-1} on 14th day of recovery and from 28.6 ± 1.8µmole of ip liberated mg^{-1} of protein hr^{-1} to 10.2 ± 1.6µmole of ip liberated mg^{-1} of protein hr^{-1} on 28th day of recovery (Table-E-1). The enzyme activity decreased by 65.1% on 14th day recovery and decreased by 64.3% over the 28th day exposure value, after 28 days of recovery, indicating no recovery at all (Table-E-2). The exposed fish liver enzyme activity could not recover even partly during recovery period but showed 1.2% and 0.37% further depletion on 14th and 28th day of recovery. No recovery in the exposed fish liver enzyme activity as marked on 14d and 28d of recovery indicated the acute toxic nature of the toxicant. The ATPase enzyme activity ranged between 22.6 ± 1.2 to 24.6± 1.1µmole of ip liberated mg^{-1} of protein hr^{-1} during the entire period of experimentation in the control muscle tissue. In the SWE exposed fish muscle, the enzyme activity decreased from 24.2 ± 1.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 21.6 ± 2.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 7days of exposure; from 23.6 ± 2.1µmole of ip liberated mg^{-1} of protein hr^{-1} to 17.4 ± 4.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 14days of exposure; from 23.2 ± 1.5µmole of ip liberated mg^{-1} of protein hr^{-1} to 15.6 ± 2.5µmole of ip liberated mg^{-1} of protein hr^{-1}after 21days of exposure and from 22.6 ± 1.2µmole of ip liberated mg^{-1} of protein hr^{-1} to 11.4 ± 3.2µmole of ip liberated mg^{-1} of protein hr^{-1}after 28days of exposure in exposed muscle tissues. The enzyme activity in SWE exposed fish muscle tissue declined by 10.7% on 7th day of exposure, by 26.3% on 14th day of exposure, by 32.8%% on 21st day of exposure and by 49.6% on 28th day of exposure. The data indicated that the muscle of the SWE exposed fish was damaged more when compared to SWE exposed fish brain tissue. The exposed fish brain was least affected when compared to fish liver and muscle. When the SWE exposed fish was transferred to toxicant free medium, the ATPase activity in exposed fish muscle instead of showing any signs of recovery further declined from 22.4 ± 3.4µmole of ip liberated mg^{-1} of protein hr^{-1} to 11.6 ± 2.6µmole of ip liberated mg^{-1} of protein hr^{-1} on 14th day of recovery and from 22.6 ± 3.8µmole of ip liberated mg^{-1} of protein hr^{-1} to 10.4 ± 2.8µmole of ip liberated mg^{-1} of protein hr^{-1} on 28th day of recovery. The enzyme activity decreased by 65.1% on 14th day recovery and decreased by 64.3% over the 28th day exposure value, after 28 days of recovery, indicating no recovery at all (Table-E-1). The exposed fish muscle enzyme activity could not recover even partly during recovery period but showed 1.4% and 4.4% further depletion on 14th and 28th day of recovery. No recovery in the exposed fish muscle enzyme activity as marked on 14d and 28d of recovery indicated the acute toxic nature of the toxicant.

Table-E-1: Showing percent changes (decrease) in ATPase activity in exposed fish tissues when compared to respective control values. Data calculated from the mean values.

Tissues	Exposure period in days					Recovery in days	
	0	7	14	21	28	14	28
Brain	0	16.45	18.27	29.4	41.03	54.52	64.28
Liver	0	16.9	48.43	54.61	63.96	65.14	64.33
Muscle	0	10.74	26.27	32.76	49.56	48.21	53.98
Percent recovery during recovery period. Data calculated from 28d exposure value					Brain	13.47	23.25
					Liver	1.18	0.37
					Muscle	1.35	4.42

Significant depression in ATPase enzyme activity may severely affect the ion transport mechanism of the exposed fish body when compared to control fish body. The correlation coefficient analysis between days of exposure and total ATPase activity in control fish brain showed a positive but non-significant correlation, whereas, the exposed fish brain showed a significant negative correlation ($r = -0.976$, $P \leq 0.01$). The control set showed positive but insignificant correlation. The percent change in the enzyme activity in the exposed fish brain when compared to control fish brain showed the existence of a positive significant correlation ($r = 0.974$, $P \leq 0.01$) with the exposure period. The analysis of variance ratio test indicated the existence significant difference between rows and non-significant difference between columns. The correlation coefficient analysis between days of exposure and the total ATPase of liver slices of the control fish did not show any significant correlation, whereas the exposed fish liver showed a negative and significant correlation ($r = -0.971$, $P \leq 0.01$) with the exposure period. The control muscle did not show any significant correlation between exposure period and enzyme activity. However, the muscle of the exposed fish showed negative and significant correlation ($r = -0.988$, $P \leq 0.01$) with the exposure period. The percent change in the enzyme activity showed negative and significant correlation with the exposure period. The analysis of variance ratio test for muscle showed significant difference between rows and significant difference between columns. The exposed fishes were when transferred to SWE free medium the enzyme activity showed no recovery at all rather further depletion was noted. During recovery period the exposed brain tissue was drastically affected, might be due to mobilization and transport of more amount of residual mercury from the body tissues to the brain. The changes observed in exposed liver and muscle tissues were interesting and the observed changes were not statistically significant. Liver and muscle showed the highest virtual recovery not by further increase but by lower range depletion when compared to brain tissues. The table indicate that the changes in sodium ion, potassium ion, calcium ion & magnesium ion of both control and solid waste extract (SWE) exposed fish brain, liver, and muscle tissues after 28 day of exposure and percent changes in different ion concentration in different tissues of the SWE exposed fish, when compared to control fish. The sodium ion content in the brain tissue of the SWE exposed fish decreased from $332.8 \pm 18.6 \mu g\ g^{-1}$ tissue to $231.2 \pm 22.6 \mu g\ g^{-1}$ of control fish brain tissue; in the liver tissue of the SWE exposed fish decreased from $318.4 \pm 15.4 \mu g\ g^{-1}$ tissue to $194.5 \pm 21.2 \mu g\ g^{-1}$ of control fish liver tissue and in the muscle tissue of the SWE exposed fish decreased from $272.2 \pm 11.6 \mu g\ g^{-1}$ tissue to $154.6 \pm 17.8 \mu g\ g^{-1}$ of control fish muscle tissues after 28days of exposure (Table-E2). The ion content depleted by 30.53%, 38.91% and 43.2% in SWE exposed fish brain, liver and muscle tissues when compared to respective control fish tissues. The exposed fish muscle tissue showed the highest percent decrease, when compared to liver and brain tissues of the exposed fish. Significant ($P \leq 0.01$) decrease in potassium ion content in brain, liver and muscle of exposed fish, compared to the control fish, was marked. The potassium ion content in the brain tissue of the SWE exposed fish decreased from $324.6 \pm 21.6 \mu g\ g^{-1}$ tissue to $206.2 \pm 19.4 \mu g\ g^{-1}$ of control fish brain tissue; in the liver tissue of the SWE exposed fish decreased from $302.8 \pm 18.5 \mu g\ g^{-1}$ tissue to $181.4 \pm 15.4 \mu g\ g^{-1}$ of control fish liver tissue and in the muscle tissue of the SWE exposed fish decreased from $284.1 \pm 19.4 \mu g\ g^{-1}$ tissue to $121.6 \pm 14.8 \mu g\ g^{-1}$ of control fish muscle tissues after 28days of exposure (Table- E2). The ion content depleted by 36.5%, 40.1% and 57.2% in SWE exposed fish brain, liver and muscle tissues when compared to respective control fish tissues. The exposed fish muscle tissue showed the highest percent decrease, when compared to liver and brain tissues of the exposed fish. Significant ($P \leq 0.01$) decrease in potassium ion content in brain, liver and muscle of exposed fish, compared to the control fish, was marked.

Table-E2: Showing changes in ion (Na^+, K^+, Ca^{++} and Mg^{++}) concentrations in control and SWE exposed fish tissues (brain, liver and muscle) exposed for 28days under laboratory controlled conditions. Data were the mean of samples ± standard deviation. The percent change was calculated from the mean of samples.

Ions studied	Tissues Studied	Changes in ion content in µg g^{-1} dry weight		Percent Change
		Control	Exposed	
Na^+	Brain	332.8±18.6	231.2±22.6	30.5
	Liver	318.4±15.4	194.5±21.2	38.9
	Muscle	272.2±11.6	154.6±17.8	43.2
K^+	Brain	324.6±21.6	206.2±19.4	36.5
	Liver	302.8±18.5	181.4±15.4	40.1
	Muscle	284.1±19.4	121.6±14.8	57.2
Ca^{++}	Brain	206.5±11.6	181.4±23.7	12.2
	Liver	211.2±17.8	146.5±25.4	30.6
	Muscle	194.2±19.2	116.4±21.8	40.1
Mg^{++}	Brain	232.5±26.2	184.6±17.4	20.6
	Liver	210.2±14.8	146.2±18.6	30.5
	Muscle	218.6±22.2	187.4±19.2	14.3

Significant variation was marked in the calcium ion content in different tissues of the SWE exposed fish, when compared to control fish tissues. The calcium ion content in the brain tissue of the SWE exposed fish decreased from 206.5±11.6µg g^{-1} tissue to 181.4±23.7µg g^{-1} of control fish brain tissue; in the liver tissue of the SWE exposed fish decreased from 211.2 ±17.8µg g^{-1} tissue to 146.5±25.4µg g^{-1} of control fish liver tissue and in the muscle tissue of the SWE exposed fish decreased from 194.2±19.2µg g^{-1} tissue to 116.4±21.8µg g^{-1} of control fish muscle tissues after 28days of exposure (Table- E2). The ion content decreased by 12.2%, 30.6% and 40.1% in SWE exposed fish brain, liver and muscle tissues when compared to respective control fish tissues. The exposed fish muscle tissue showed the highest percent decrease, when compared to liver and brain tissues of the exposed fish. Significant ($P \leq 0.05$) decrease in calcium ion content in brain, liver and muscle tissue of the SWE exposed fish was marked when compared to the control fish. The magnesium ion content in the brain tissue of the SWE exposed fish decreased from 232.5 ±26.2µg g^{-1} tissue to 184.6±17.4µg g^{-1} of control fish brain tissue; in the liver tissue of the SWE exposed fish decreased from 210.2±14.8µg g^{-1} tissue to 146.2±18.6µg g^{-1} of control fish liver tissue and in the muscle tissue of the SWE exposed fish decreased from 218.6± 22.2µg g^{-1} tissue to 187.4 ± 19.2µg g^{-1} of control fish muscle tissues after 28days of exposure (Table- E2). The ion content depleted by 20.6%, 30.5% and 14.3% in SWE exposed fish brain, liver and muscle tissues when compared to respective control fish tissues. The exposed fish liver tissue showed the highest percent decrease in magnesium ion content, when compared to brain and liver tissues of the SWE exposed fish. Significant ($P \leq 0.05$) decrease in magnesium ion content in brain, liver and muscle tissues of the exposed fish, when compared to the control fish tissues was observed. Out of the four ions studied in the exposed fish tissues, significant decrease was recorded in potassium ion and sodium ion content and highly less significant differences were observed in potassium ion and magnesium ion content in all the tissues studied. Out of the three tissues studied the liver was the most affected and the least affected was brain for some ion and for the rest of the ions the muscle was least affected. The analysis of variance ratio test for ions like Na^+, K^+, Ca^{++} and Mg^{++} in brain, liver and muscle of the control and SWE exposed fish showed significant difference between rows and significant difference between columns. The analysis of variance ratio test for percent decrease in ions like Na^+, K^+, Ca^{++} and Mg^{++} in brain, liver and muscle tissues of the control and SWE exposed fish showed significant difference between rows and non-significant difference between columns. Significant differences in ion content of different tissues of the exposed fish when compared to control fish clearly indicated the nature of the toxicant and effects caused by the SWE toxicant on the nervous tissue, synaptic transmission, nerve impulse generation, ionic balance and membrane transport system of the exposed fish. The exposed fishes did not recover when transferred to SWE free medium. The enzyme activity showed no recovery in liver, brain and muscle tissues but little variation during recovery was not statistically significant. Exposed liver tissues showed the highest damage when compared to brain and muscle tissues of the exposed fish, when compared to control fish tissues.

Discussion

Environmental pollution by mercury is considered to be one of the most important problems faced by all plants, animal and mankind and studied extensively. Mercury as an element has multiple uses but when it is present in the environment in excess, it can be considered harmful and equally dangerous at higher level. Mercury finds its way in many different ways into the environment. The most important source of mercury in the environment was the burning of fossil fuel, Chlor-alkali industry waste and Aldehyde industry waste. Chlor-alkali industry is accepted as the bigger source of mercury pollution, where mercury is used as a cathode in the electrolytic process. This metallic mercury travels from electrolysis cell to hydration chamber, compression chamber and ultimately mercury is recycled back into the electrolytic cell. In the process of manufacture of caustic soda (NaOH) mercury evaporates in the cell house during electrolysis due to high heat generated during electrolysis process and moves along with chlorine gas released during electrolysis to compression chamber. Mercury reacts with sodium ion released during electrolysis to form sodium-mercury amalgam. The amalgam is transported to the hydration chamber by a pipe line. In the hydration chamber the amalgam is hydrated with water. In the process caustic soda is produced and metallic mercury is released free. The produced NaOH contains mercury as impurities. The metallic mercury so released is then transported back (recycled) to the cells of the cell house for future electrolysis. The hydrogen gas thus produced in the hydration chamber also contains mercury vapor which is canalized to compression chamber for further reaction with chlorine gas to produce HCl acid, which also contains residual mercury. During transport, spillage of mercury from pipe lines, during washing of the cells, cell house, hydration chamber and compression chamber, mercury escapes into the environment along with washings called effluent. This escaped mercury settles in the settling effluent tank and effluent canal along with sediment. This sediment is periodically removed and dumped outside as solid waste in the environment which contains mercury. The operation of industries dealing with mercury allows mercury availability in the environment. Mercury is discharged into the environment in elemental form, which under goes natural oxidation and reduction along with environmental chemicals under different climatic conditions and seasonal variations. Elemental mercury is converted to inorganic mercury and organic mercury mediated by microbes and other flora and fauna. Mercury is absorbed by plants and animals from the environment. The live biotic systems absorb, accumulate and mercury bioconcentrate and the same chemical gets biomagnified in the food chain and food web and this becomes a threat to human health and mankind (Wu and Wang, 2011). The same authors also pointed out that the toxic responses are specific to different types of metals and different types of species, but the sub-cellular basis underlying such inter-species and inter-metal differences was not very clear and needs a detailed study. We agree with the views of above authors. Chlor-alkali industries discharge mercury in elemental form in to the environment or more appropriately mercury escapes into the environment in elemental form along with effluent coming from four sources and the whole effluent accumulates in the effluent stocking pond for treatment, where the pH was adjusted and the effluent is discharged through the effluent canal into the environment. The mercury present in the effluent or in the sediments collected from the effluent canal and settling tank or treatment tank contained elemental mercury only. This elemental form of mercury which has escaped from the industry under goes transformation naturally and also by biotransformation, to form other forms of mercury either in the inorganic form or in the organic form, after absorption and conversion by biological agents (Raut, 2013, Mishra, 2013). Hence it was felt necessary to study and understand movement of mercury in the environment and absorption, mobilization of mercury and impact of mercury on animals particularly fish available in river and the estuary. The absorption of mercury (contained in the effluent) through the skin, gill and gastro-intestinal tract of fish is well evident (Raut, 2013). The toxicity of effluent becomes more apparent in a very shorter period in aquatic animals. Panigrahi (1980) documented the detail behavioral changes in relation to mercury intoxication. The effluent which was found to contain very high amount of mercury, when released from the factory finds its way finally into the Rushikulya river estuary. Out of twelve analyses carried out in twelve months, only once, in the month of March, a lower concentration of mercury (0.0268mg 1^{-1}) was observed. Though the concentration in March was low, the value was in itself much higher than the permissible limit. Higher concentration of mercury, as recorded in the month of January, was to the tune of 1.549mg 1^{-1}. Concentration of mercury in the effluent was found to be fluctuating having a mean value of 0.4474 ± 0.4466 mg 1^{-1}. Elevated levels of alkalinity at station I and III might be because of the effluent discharge. In polluted system OH^- ions released from chemical factories play on important role in increasing measured is not the true alkalinity. Variance ratio test analysis revealed a significant difference in the alkalinity between seasons as well as between stations. It was observed that the residual mercury accumulations in fishes are mostly weight dependent and indirectly length dependent. The changes in body length and body weight were time dependent. It depends on the

habitational time period of the fish in the estuary and the residual mercury increases with time. The size of the fish also depends on environmental parameters and totally genetic. Hence uniform biological rule of growth cannot be adopted as a standard protocol for estimations, measurements and interpretation to understand a biological mechanism might be related to environmental stress. The residual accumulation of mercury in fish is definitely time dependent. The figures however indicated that residual accumulation occurred in fish body and the correlation is positive for both the parameters but not significant. Fishes with higher weight accumulated less mercury and fishes with lower body weight accumulated more amount of mercury. Hence, the accumulation of mercury cannot be only either due to weight of the fish or length of the fish. It may be due to the metabolic activity and time of exposure of the fish to the toxicant. The fishes having higher weight and length might have reached to the contaminated site late when compared to other fishes where higher accumulation was marked. In field conditions when the area is open and free movement of fishes occur, it is really difficult to assess the residual accumulation and interpret with either weight or length of the fish. It may so happen during fish catch, large fishes might have reached the site from sea without getting contaminated and the fisherman catches those fishes for us for analysis. Residual accumulation in static ecosystems is easy to assess but in dynamic ecosystems, difficult to assess. Similar interpretations are also equally valid when we correlate fish length with residual accumulation. Long-term exposure of animals to toxicants might cause pathological changes in addition to physiological and biochemical changes. The rapid absorption of the toxicant (industrial effluent) through the gill, skin and gastro-intestinal tract of fish was well evident in the observed exposed fish. Similar findings and trends were also reported earlier in mercury intoxication (Panigrahi, 1980). The observed depletion in metabolic activity in exposed fish indicate probable damage caused to respiratory system, and inhibition of enzymes or an important system, was totally acceptable and agree with the findings of Panigrahi (1980). Panda *et al*., (2017) reported the effect of red mud waste and red mud waste extract on fresh water fishes whole body oxygen uptake separately and also indicated that these wastes depress active metabolism and the exposed fish intake of oxygen decreases significantly. Panigrahi & Misra (1978, 1980) and Panigrahi (1980) reported depletion in oxygen uptake in mercury exposed fishes under laboratory experimental conditions. Our findings agree with the findings of above authors. Considerable information are available pertaining to residual toxicity levels in fresh water, estuarine and marine fishes but relatively very little work has been done on the mechanism of toxic action of mercurial compounds especially on studies concerning active transport across cellular membranes. Jackim (1974) reported significant depression of (Na^+, K^+) ATPase activity to be associated with higher absorption and accumulation of mercury. Na^+, K^+-ATPase is well known to play an important role in nerve impulse generation and synaptic transmission (Ahuja and Subramanyam, 1978). The same author also reported the indication of suppressed or stimulated enzyme activity was caused when organisms exposed to minor doses of metals. Studies have shown that cadmium ion has damaging effects on respiration and ATPase activity of the pulmonary alveolar microphage (Cross *et al*., 1970). Panigrahi (1980) reported depression of ATPase activity in vivo and in vitro in freshwater fishes following inorganic mercury intoxication. Panigrahi (1984) reported a similar trend in freshwater fishes exposed to mercury based fungicide. Metals can combine with enzymes in many ways among which are sulfahydryl binding, chelation and salt formation. A good number of references are available pertaining to the inhibitions of ATPase activity in fish by polychloride biphenyls (Desaiah *et al*., 1972), Toxaphene (Desaiah and Koch, 1975); DDT (Desaiah *et al*., 1975) and by Kepone and Mirex (Desaiah *et al*., 1975 and 1977). Earlier it was thought that the ATPases might be involved in the transport of ions in the nerve and interfere with a variety of membrane linked functions (Holan, 1969). This earlier thought is now a reality, where ATPase, AChE and ions play an important role in nerve impulse generation and synaptic transmission from PNS to CNS and vice versa to respond to external stimulus. This stimulus can be physical or chemical impact on the animal system. The absorption of mercury (contained in the effluent) through the skin, gill and gastro-intestinal tract of fish is well evident (Raut, 2013). The toxicity of effluent becomes more apparent in a very shorter period in aquatic animals. Panigrahi (1980) documented the detail behavioral changes in relation to mercury intoxication. Macleod and Passah (1973) reported that loss of appetite, loss of weight, nervousness, dizziness, loss of equilibrium, erratic swimming and gradual onset of inactivity were the sub clinical effects of inorganic mercury intoxication. Neurological damage in inorgano-mercury intoxicated fish relating to behavioral studies was also reported (Panigrahi 1980), and he opined that the damage was caused only by inorganic mercury. Panigrahi and Misra (1978, 1980) reported the loss of body weight due to mercury intoxication and confirmed that this loss in body weight was only due to mercury stress. In addition, expose fish maintained their feeding habit only after a short span of time. Hence the weight loss cannot be correlated with starvation but can only be related with mercury based effluent intoxication, whereas the control fish showed increase in weight. The differences observed between control fish and

exposed fish in terms of body weight was only due to the administration of mercury based effluent in the exposed aquarium. On autopsy, he found muscular atrophy, which can be correlated with the weight loss in the goshawks. Hanku *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. A decline in LSI and BSI in exposed fish could be correlated with the degeneration of cells, cellular atrophy and decrease in other macromolecular variables caused due to the effect of mercurial compounds present in the effluent. The observed changes are in agreement with Panigrahi (1980) and Panigrahi & Misra (1978, 80). The same authors opined and confirmed that the observed changes were solely due to the mercurial compound. Hence, here the observed changes were only due to mercury present in the effluent. Concisely, at this stage it is not possible to establish the cause of loss in body weight and decrease in liver and brain somatic index, except a generalized comment on the behavior of the exposed fish to toxicant stress that the observed symptoms were most probably be due to mercury intoxication. 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 (2018). The reports of Panigrahi and Mishra (1978) 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 (1978), the observed effects reported were the impact of mercury as mercuric nitrate, a single chemical on fresh water fishes. 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 both the years under study in this project, fishes were collected from estuarine area, particularly from stations-nearer to Bay of Bengal and inside the estuarine belt. Out of total 17 different fish species collected, only 10 varieties were found common in the fish catches of both the years. Absence or presence of fish in a particular year did not indicate any environmental variation. But in 2015, more varieties of fishes were available. The availability of good number of fishes might be due to the decrease in environmental mercury load. No residual mercury in the body of some fishes in 2015 may be either due to less availability of mercury in the environment or due to fresh fish coming from the sea. The estuary was contaminated as the effluent of the industry was discharged directly into the river Rushikulya and (LC) leached chemicals from the SW (solid waste) dump enter into Rushikulya River and consequently into the estuary leading to Bay of Bengal. Mercury is discharged into the environment through effluent and solid waste routes, contaminating the adjacent aquatic and terrestrial ecosystems, respectively. This addition of mercury is the primary contamination. The secondary contamination occurs through the chimney into the atmosphere and its fall out by the process of precipitation. All these discharges collectively seem to cause a major environmental threat. Long-term exposure of animals to toxicants might cause pathological changes in addition to physiological and biochemical changes. The rapid absorption of the toxicant (industrial effluent) through the gill, skin and gastro-intestinal tract of fish was well evident in the observed exposed fish. The observed depletion in metabolic activity in exposed fish indicate probable damage caused to respiratory system, and inhibition of enzymes or an important system, was totally acceptable and agree with the findings of Panigrahi (1980). Mishra (2002) and Panda *et al*., (2017) reported the effect of red mud waste and red mud waste extract on fresh water fishes whole body oxygen uptake separately and also indicated that these wastes depress active metabolism and the exposed fish intake of oxygen decreases significantly. Considerable information are available pertaining to residual toxicity levels in fresh water, estuarine and marine fishes but relatively very little work has been done on the mechanism of toxic action of mercurial compounds especially on studies concerning active transport across cellular membranes. 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 high and the behavioral changes were more acute and drastic confirming mercury poisoning in the affected areas at Ganjam, Odisha.

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