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Toxicological Studies of Pulp and Paper Mill Effluent on Fresh Water Fishes Inhabiting Crop Fields

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Abstract: The aim of this study was to assess the effect of effluent contained river water on the crop field inhabiting fresh water fishes particularly during pre-harvest time. The polluted river water contained significant amount of heavy metals and this river water was used to irrigate the surround crop fields. Significant amount of Cd, Hg and Pb was found in the crop field soil and water. Residual cadmium, mercury and lead were noted in the fish body residing in the contaminated crop fields. The respiration rate of the exposed crop field fishes carefully collected from the contaminated crop fields significantly decreased compared to control fishes (collected from a non contaminated area). The observed effects were only due to heavy metal accumulation in the fish body absorbed from the effluent contaminated crop fields. Two carnivorous fishes adopting bimodal gaseous exchange and mud dwelling are recommended for their effective utilization in reclamation process of paper mill effluent waste along with potential micro and macrophytes for a safer environment as these two fishes are tolerant and resistant to effluent stress.

Keywords: Paper Mill, Effluent, Heavy metal, Toxicology, Fish, Whole body respiration

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Introduction

Pulp and Paper mills are the major polluters of aquatic environments. These industries add toxic heavy metals like mercury, cadmium, lead and other heavy metals into the environment, polluting nearby water bodies and affecting aquatic flora and fauna. The reports of Tripathy and Panigrahi (2023a) indicated the presence of heavy metals in all crop and non-crop plants available in the paper mill effluent contaminated crop fields. The paper mill effluents are discharged from the industry into the nearby water body after physical and chemical treatments (Tripathy *et al.*, 2022) indicating the need of proper biological treatment. Dixit *et al.* (2018) reported presence of heavy metals in the final discharged Paper mill effluent even after biological treatment. Tripathy

Fig. 1: Google photomap showing JK Papers Ltd., Effluent canal, effluent joining Nagavalli River, S1 and S2 effluent collection sites and nearby crop fields contaminated by effluent mixed river water.

et al. (2022) reported that the effluent of the paper mill severely affected the flora in and around the effluent canal and impacted the water quality of the river. These authors also indicated that usage of this contaminated river water to irrigate the crop fields would have far reaching consequences (Tripathy and Panigrahi, 2023a). The present authors during the visit to collect water, soil and plant samples from the crop fields found the presence of variety of fresh water fishes in the crop fields. These fishes collected during cultivation time are the only protein rich diet for tribal people and people residing in the neighboring villages. The residents of the area are socio-economically backward and eat rice cultivated in the contaminated area along with these dried fish in their regular diet. These fishes are generally sun dried mixed with common salt preserved for the whole year. Based on our observation of the contaminated area and feeding habit of the inhabitants, the present study was planned to assess the physico-chemical status of the effluent for a longer period of time and analyze the impact of effluent on nearby crop fields and crop field inhabiting animals, mostly fishes, where this contaminated river water is being used for irrigation.

Materials and Methods

JK Pulp and Paper Mills, Jaykaypur is located at latitude 19.247ºN and longitude 83.409 º E and its effluent waste drains into the River Nagavalli (Fig. 1) at 19.252ºN and 83.420º E. The industry collects river water from the upstream for all its requirements and discharges its effluent into the river at downstream. The effluent samples were collected and physico-chemical analysis was carried out at the site periodically. Samples from the mid effluent canal [S] were collected in plastic containers and brought to the laboratory for analyses.

Physico-chemical analyses of effluent and crop field soil samples of nearby crop fields were conducted periodically by following the procedure of APHA (1998), standardized field analysis kit and portable instruments. Dissolved oxygen was measured by modified Winkler's method. Effluent and crop field soil samples were brought in glass containers and stored in cold room for use in laboratory experimental work. Measurement of heavy metals of the collected soil, water, effluent and animal samples were carried out by following the protocols of Wanntorp and Dyfverman (1955) and Yoshida *et al*. (1976). Mercury, cadmium and lead in digested samples were estimated with the help of Atomic Absorption Spectrophotometer. Live fish respiration rate studies were conducted on the site following the Winkler's method of dissolved oxygen measurement (APHA, 1998). The

Table 1: (Field Study) Comparison and analyses of physico-chemical variables of the sample collected from the effluent canal before joining the river Nagavalli at JayKaypur, Odisha at different time periods. (Data presented are the mean ± standard deviation; NA = Not available)

Parameters of study (mg l^{-1})	Year wise study by the authors.				
	2015	2017	2018	2019	2021
Colour	Deep Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Odour	Pungent, Strong H_2S	Pungent, Strong H_2S	Pungent, Rotten egg	Pungent. Filthy	Pungent. Filthy
pH	7.8±0.5	7.5±0.6	7.4±0.4	7.9±0.4	7.8±0.05
Temp. °C	32.5±0.7	32.2±0.4	30.8±0.8	31.2±1.2	32.2±0.6
Dissol. O_2	1.6±0.5	1.8±0.3	2.8±0.6	1.5±0.4	1.8±0.05
Copper	0.11±0.04	0.09±0.05	NA	NA	0.03±0.01
Cadmium	0.39±0.11	0.31±0.05	0.29±0.04	0.32±0.09	0.28± 0.07
Mercury	0.38±0.12	0.49±0.06	0.39±0.08	0.46±0.14	0.38±0.11
Lead	0.12±0.06	0.09±0.03	0.11±0.04	NA	0.06±0.03

Table 2: ANOVA ratio test for the Table 1

Single Factor (Column)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.218486	4	0.054621	0.00040023	1	2.689628
Within Groups	4094.252	30	136.4751			
Total	4094.47	34				
Single Factor (Row)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4090.997	6	681.8328	5495.612	1.2E-41	2.445259
Within Groups	3.47392	28	0.124069			
Total	4094.47	34				

whole body respiration rate of the crop field fish was estimated following the procedure as suggested by Panigrahi and Misra (1980).

Results

Table 1 represents the physico-chemical properties of the discharged effluent waste sampled from the effluent canal at different time periods. The table showed the comparison of effluent analyses data at different time periods indicating no significant change in physico-chemical status. All the parameters studied showed significantly higher values much more than the values indicated by PCBs. The ANOVA test indicated the existence of strong similarity between columns of different periods of study and non similarity of different parameters of study in between rows (Table 2). Significant similarity between years indicated that the industry probably never tried to improve the quality of the effluent by using different treatment protocols. The correlation coefficient analysis indicated the non-existence of significant correlation between parameters of study (Table 3). Periodic and constant monitoring of the discharged effluent revealed minor variation in select parameters studied during 2015 to 2021. The odour of discharged effluent is pungent with rotten egg smell and the colour ranged from deep yellowish brown to yellowish brown during 2015 to 2021.

Table 3: Correlation coefficient analysis of parameters of Paper mill effluent studied at different years (2015 to 2021)

	pH	Temp.	DO	Copper	Cadmium	Mercury	Lead
pH	1						
Temp.	0.325	1					
DO	-0.821	-0.6208	1				
Copper	0.013	0.858	-0.422	1			
Cadmium	0.368	0.461	-0.478	0.683	1		
Mercury	-0.112	-0.0659	-0.317	0.066	-0.112	1	
Lead	-0.606	0.292	0.478	0.567	0.306	-0.422	1

Table 4: The analyses of physico-chemical parameters of the effluent collected from effluent canal at the joining point (S1), and 1 km from the meeting point on the opposite side of the river near crop fields, where the effluent mixed river water is used for irrigation (S2) of the crop field and crop field soil contaminated (E) and crop field soil uncontaminated soil (C)

Physico-chemical parameters	Different stations of study			
	Station-S1 (Meeting point)	Station-S2 (Irrigation water)	Uncontaminated crop field soil	Contaminated crop field soil (E)
Colour	Pale-yellowish brown	Light yellow	Deep brown silt clay muddy	Yellowish deep brown
Soil structure	=	=	clay sandy, muddy	Wet silt, clay sandy, muddy
Odour-	Pungent, strong H_2S smell	Filthy smell	Normal	Filthy smell
Temperature	26.8±2.1°C	22.8±0.8°C	21.2±0.6°C	22.4±0.3°C
pH	7.8±0.6	8.2±0.4	7.9±0.4	8.4±0.4
Soil moisture	=	=	71.3%;	79.4%;
Cond. mho/cm	1694.7±17.5	674.2±18.8	=	=
DO- mg/l	2.1±0.8	4.6±1.1	=	=
BOD - mg/l	108.3±11.3	26.1±2.8	=	=
COD- mg/l	276.4± 12.5	81.4±8.5	=	=
TSS- mg/l	819.3±19.6	118.2±12.4	=	=
TDS- mg/l	1387.9±22. 7	185.2±8.8	=	=
Chlorides	311.5±14.2 mg/l	71.4 ±14.3 mg/l	44.5±8.2 mg/gdw	152.5±4.6 mg/gdw
Soil Alkalinity	=	=	144.6±12.6 mg/gdw	156.6±11.2 mg/gdw
Hardness-	514.2±21.6 mg/l	174.5±5.6 mg/l	=	=
Fluoride	1.89±0.8 mg/l	Not Detectable	=	=
Total –N-NO_3	2.26±0.5 mg/l	2.88 ±0.92 mg/l	6.32±2.1 mg/g dw	9.58±1.4 mg/g dw
Total PO_4	1.26± 0.3 mg/l	2.18±0.38 mg/l	=	=
Total SO_4-	132.1±9.8 mg/l	31.5± 6.1 mg/l	11.2±3.2 mg/g dw	26.8±2.1 mg/g dw
Sodium-	61.8 ±5.2 mg/l	24.1± 2.7 mg/l	21.1±8.5 mg/g dw	29.6±9.5 mg/g dw
Potassium-	49.5±3.8 mg/l	15.2 ± 1.8 mg/l	35.2±3.6 mg/g dw	42.6±7.1 mg/g dw
Mercury-	0.46±0.07 mg/l	0.46±0.07 mg /l	ND	2.41±0.3 mg/g dw
Cadmium -	0.32±0.05 mg/l	0.07±0.01 mg /l	ND	2.85±0.09 mg/gdw
Lead-	0.18±0.03 mg/l	0.06±0.02 mg /l	ND	0.98±0.11 mg/g dw

DW=Dry weight; ND= Not detectable

The pH ranged from 7.4±0.4 to 7.9±0.4 and the temperature ranged between 30.8 ± 0.8°C and 32.5 ± 0.7°C. The dissolved oxygen present in the discharged effluent ranged from 1.5 ± 0.5 to 2.8 ± 0.6 mg l^{-1} far less than the dissolved oxygen of normal water of a river. Significant amount of copper, cadmium, lead and mercury was recorded in the year 2015. In 2015, the effluent showed 0.11 ± 0.04 mg of copper l^{-1}, 0.39 ± 0.11 mg of cadmium l^{-1}, 0.38 ± 0.12 mg mercury l^{-1} and 0.12 ±

0.06 mg of lead l^{-1} in the effluent sample collected. In 2017, the effluent showed 0.09 ± 0.05 mg of copper l^{-1}, 0.31 ± 0.05 mg of cadmium l^{-1}, 0.49 ± 0.06 mg mercury l^{-1} and 0.09±0.03 mg of lead l^{-1} in the effluent sample. In 2018, the effluent showed 0.29 ± 0.04 mg of cadmium l^{-1}, 0.39 ± 0.08 mg mercury l^{-1}, 0.11 ± 0.04 mg of lead l^{-1} and presence of copper was not detected in the effluent sample collected. In 2019, the effluent sample showed 0.32 ± 0.09 mg of cadmium l^{-1}, 0.46 ± 0.14 mg mercury l^{-1}, and presence of lead and copper was not detected (Table 1). In 2021, the effluent sample showed 0.28 ± 0.07 mg of cadmium l^{-1}, 0.38 ± 0.11 mg mercury l^{-1}, 0.03±0.01 mg of copper l^{-1} and 0.06±0.03 mg of lead l^{-1} was detected. Non detection of heavy metals is a good sign but the very availability of heavy metals in a significant quantity in the effluent warrants attention.

Contaminated site analysis:

Table 4 indicated the physico-chemical status of the samples tested indicating presence of heavy metals. No heavy metal was detected in the control crop field soil where the upstream river water was used for irrigation. Significant amount of mercury, cadmium and lead was available in the contaminated crop fields (Table 4). The river water at Station-S1 showed 0.46±0.07 mg of mercury/l, 0.32±0.05 mg of cadmium/l and 0.18±0.03 mg of lead/l and station S2 showed 0.08±0.03 mg of mercury/l, 0.07±0.01mg of cadmium/l and 0.06±0.02 mg of lead/l. All the above values were significant from environment point of view as the obtained values were much more than the prescribed limit. The contaminated crop field receiving the polluted water from the river contained significant amount of mercury to the tune of 2.41±0.3 mg/g dry weight, cadmium to the tune of 2.85±0.09 mg/g dry weight and lead to the tune of 0.98±0.11 mg/g dry weight (Table 4). Higher enrichment of heavy metals was noted in the contaminated crop fields when compared to station S1 and S2.

Heavy metal accumulation in crop field fish: In the crop fields of Jaykaypur, Rayagada, we found 9 varieties of fishes, earthworm and fresh water snail. These animals were collected and the whole animal was digested for heavy metal analysis. The size of the fishes was very small. It was not possible to dissect out brain, liver and gill to estimate heavy metals as the available quantities were significantly less. The whole body analysis indicated significant accumulation of mercury, cadmium and lead and heavy metals as a whole. Lead was not detected in swamp barb, Tankgoby and Slender Rasbora might be due to very small in size and insignificant accumulation. In case of Swamp barb fish, 0.74±0.14 µg of mercury/g dry wt and 0.81±0.21 µg of cadmium/g dry wt accumulation was recorded. In case of Tankgoby fish, 0.88±0.18 µg of mercury/g dry wt and 0.74±0.14 µg of cadmium/g dry wt accumulation was recorded. In case of Spotted snakehead fish, 0.85±0.32 µg of mercury/g dry wt, 1.16±0.35 µg of cadmium/g dry wt and 0.64±0.16 µg of lead/g dry wt accumulation was recorded (Table 5). All the obtained values were statistically significant and the values were much more than the stipulated limit prescribed by Food and Safety Organization. In absence of information pertaining to exact age of the collected fishes, it was not possible to compare the dry weight data and somatic indices data. It was also not possible to carry all the live fishes to the laboratory for tissue slice respiration studies.

In case of Fossil cat fish, 0.45±0.08 µg of mercury/g dry wt, 0.38±0.06 µg of cadmium/g dry wt and 0.21±0.05 µg of lead/g dry wt deposition was recorded. In case of Dwarf snake head fish, 0.76±0.06 µg of mercury/g dry wt, 0.82±0.11 µg of cadmium/g dry wt and 0.28±0.07 µg of lead/g dry wt deposition was recorded. In case of slender Rasbora fish, 0.38±0.05 µg of mercury/g dry wt and 0.35±0.04 µg of cadmium/g dry wt bioaccumulation was recorded and residual lead was not detected. Other than fish, in case of earthworm, 2.18±0.22 µg of mercury/g dry wt, 2.65±0.14 µg of cadmium/g dry wt and 0.91±0.32 µg of lead/g dry wt bioaccumulation was recorded. In case of fresh water snail, 1.16 ± 0.17 µg of mercury/g dry wt, 1.46 ± 0.12 µg of

Table 5: Changes in mercury, cadmium and lead residue content of animals collected from the contaminated crop fields located on the opposite side of the contaminated Nagavalli river bank. Animal samples collected during cultivation time (after 180days of sowing of crop seedlings) (ND=Not detectable)

S. No.	Name of animal sample with family	Status - Whole animal	Residual heavy metal content in whole animal tissue= μg/g dry weight.		
			Hg	Cd	Pb
1	E: Spotted snakehead; *Channa punctatus*, Bloch	Exposed	0.85±0.32	1.16±0.35	0.64±0.16
2	E; Tank goby; *Glossogobius giuris*, Hamilton	Exposed	0.88±0.18	0.74±0.14	ND
3	E: Fossil catfish; *Heteropneustes fossilis*,Bloch	Exposed	0.45±0.08	0.38±0.06	0.21±0.05
4	E: Swamp barb; *Puntius chola,* Hamilton	Exposed	0.74±0.14	0.81±0.21	ND
5	E-Razor Belly Minnow; *Salmostoma bacaila,* Hamilton	Exposed	0.32±0.07	0.35 ±0.09	0.28±0.16
6	E-Molacarplet; *Amblytharyngodon mola,* Hamilton	Exposed	0.28±0.11	0.17 ±0.06	0.14 ±0.05
7	E: Slender Rasbora; *Rasbora daniconius,* Hamilton	Exposed	0.38±0.05	0.35±0.04	ND
8	E: Dwarf snakehead; *Channa gachua,* Hamilton	Exposed	0.76±0.06	0.82±0.11	0.28±0.0.07
9	E: Stripped dwarf catfish; *Mystus vittatus,* Bloch.	Exposed	0.51±0.12	0.49±0.18	0.19±0.11
10	E:-Fresh water Snail; *Pila globosa*	Exposed	.16±0.17	1.46±0.12	0.81±0.23
11	E:-Earthworm; *Pheretima posthuma*	Exposed	2.18±0.22	2.65±0.0.14	0.91±0.32

cadmium/g dry wt and 0.81±0.23 μg of lead/g dry wt bioaccumulation was recorded (Table 5). Fishes, snail and earthworm were also collected from the uncontaminated crop fields of a different locality (far away from the industry and contaminated Nagavalli river) indicated absence of toxic heavy metals like Cd, Hg and Pb. The data indicated that all the collected animals available in the contaminated crop fields accumulated heavy metals, while inhabiting in the crop fields. The very presence of heavy metals in the fish body and the contaminated fish being consumed by farmers and tribal people is significant and needs attention. The bioaccumulation factor (BAF) values for Hg, Cd and Pb in case of Swamp barb was calculated to be 0.307, 0.284 and 0, respectively when we have considered soil as the source of heavy metal. The BAF values for Hg, Cd and Pb in case of Tank goby was calculated to be 0.365, 0.259 and 0, respectively. The BAF values for Hg, Cd and Pb in case of Spotted snakehead was calculated to be 0.352, 0.407 and 0.653, respectively. The BAF values for Hg, Cd and Pb in case of Fossil catfish was calculated to be 0.186, 0.133 and 0.214, respectively. The BAF values for Hg, Cd and Pb in case of Dwarf snakehead was calculated to be 0.315, 0.287 and 0.285, respectively. The BAF values for Hg, Cd and Pb in case of Slender Rasbora was calculated to be 0.157, 0.122 and 0, respectively. The BAF values for Hg, Cd and Pb in case of Stripped dwarf catfish was calculated to be 0.211, 0.171 and 0.193, respectively. The BAF values for Hg, Cd and Pb in case of earthworm was calculated to be 0.904, 0.929 and 0.928, respectively. The BAF values for Hg, Cd and Pb in case of snail was calculated to be 0.066, 0.512 and 0.826, respectively. It was observed that earthworm showed higher BAF

Fig.L.1.: Changes in dissolved oxygen consumption by fishes collected from the contaminated and control crop fields.

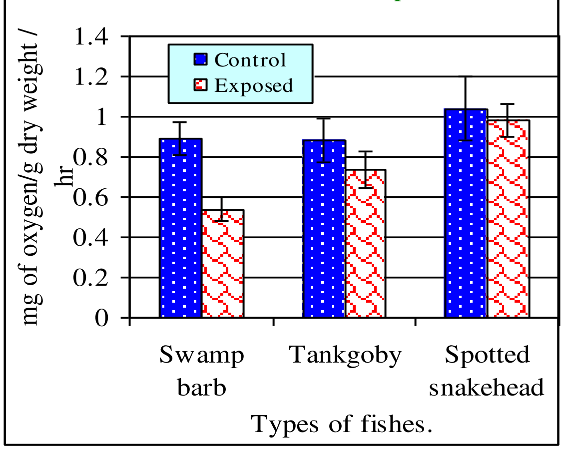


Fig.L.2: Changes in whole body oxygen uptake in control and contaminated fishes collected from the crop fields.

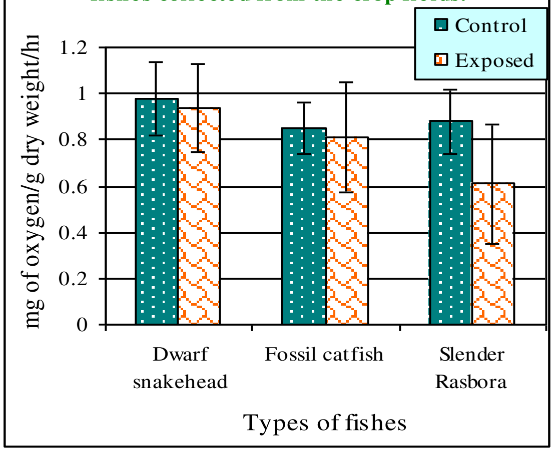


Fig.L.3: Changes in whole body oxygen uptake by different fishes collected from contaminated and control crop fields.

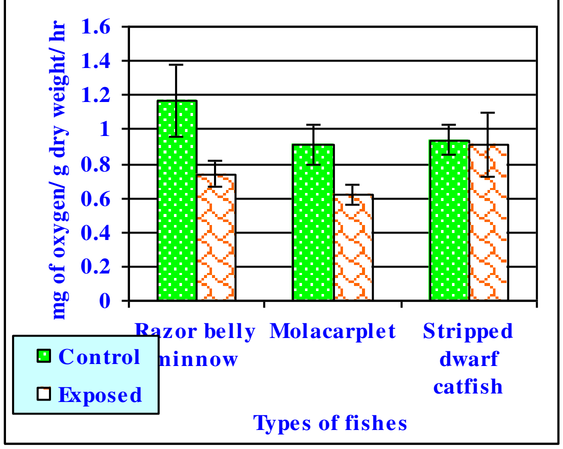
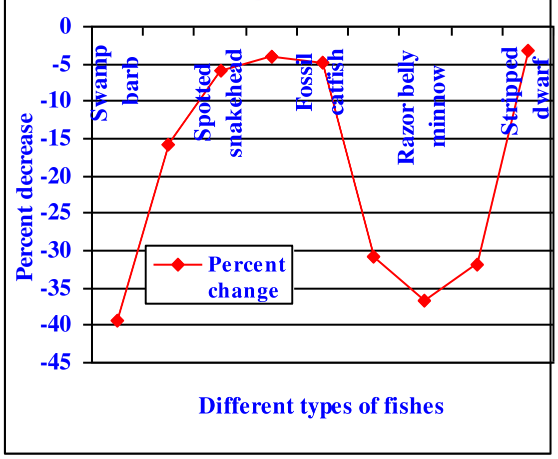


Fig. L.4: Percent change in whole body oxygen uptake in different exposed fresh water fish collected fromthe contaminated crop fields.



values indicating highest accumulation of all the three heavy metals followed by the BAF value in stripped dwarf catfish. BAF value for mercury accumulation was lowest in case of snail. Slender Rasbora showed the lowest BAF for all the three heavy metals tested. The BAF values for Hg, Cd and Pb in case of fishes calculated showed very high values when we have considered crop field water as the source of the heavy metals. The BAF values for Hg, Cd and Pb in case of other animals like earthworm and snail calculated showed very high values when we have considered crop field effluent mixed water as the source of the heavy metals.

Respiration rate studies on fish collected from the crop fields:

The available fishes were trapped carefully by wooden trappers and collected in hand without causing any injury to the fish. Fishes were allowed to rest without causing any type of physical and chemical stress. After the fishes settled, fishes were transferred to normal water in 2L conical flasks and hermetically sealed for one hour by covering the conical flasks by a glass lid. The initial dissolved oxygen of normal water used for the experiment was measured. The collected fishes were allowed to live for 1 h and then the exposed water was processed for dissolved oxygen analysis. The difference in dissolved oxygen between the initial reading and final reading was the amount of oxygen consumed by the fish in 1 h/g dry weight of fish. The whole body respiration rate experiments of collected fish were conducted at the site of collection. The whole body respiration rate of fresh water fishes inhabiting crop fields collected from the contaminated crop fields showed significant variation compared to control fish. The whole body oxygen uptake of Swamp barb decreased significantly from 0.89 ±0.08 mg of O_2 consumed/g dry weight/h (uncontaminated fish) to 0.54±0.06 mg of O_2 consumed/g dwt (dry weight)/h (contaminated fish) showing 39.3% decrease on whole body respiration rate. The whole body oxygen uptake of Tankgobi decreased significantly from 0.88±0.11 mg of O_2 consumed/g dwt/h (control fish) to 0.74±0.99 mg of O_2 consumed/g dwt/ h (contaminated fish) showing 15.9% decrease of whole body respiration rate. The whole body oxygen uptake of Spotted snakehead decreased significantly from 1.04±0.16 mg of O_2 consumed/g dry weight/h (Control fish) to 0.98±0.08 mg of O_2 consumed/g dwt/h (contaminated fish) showing 5.8% decrease on whole body respiration rate (Figs. L1, L4). The whole body oxygen uptake of Dwarf snakehead decreased significantly from 0.98±0.16 mg of O_2 consumed/g dwt/h (Control fish) to 0.94±0.19 mg of O_2 consumed/g dwt/h (contaminated fish) showing 4.1% decrease on whole body respiration rate (Figs. L-2, L4).

The whole body oxygen uptake of Fossil catfish decreased significantly from 0.85 ±0.11 mg of O_2 consumed/ = dry weight/h (Uncontaminated fish) to 0.81±0.24 mg of O_2 consumed/g dwt/h (contaminated fish) showing 4.7% decrease on whole body respiration rate (Figs. L1, L4). The whole body oxygen uptake of Slender Rasbora decreased significantly from 0.88 ±0.14 mg of O_2 consumed g dwt/h (Control fish) to 0.61±0.26 mg of O_2 consumed/g dwt/h (contaminated fish) showing 30.7% decrease on whole body respiration rate (Figs. L2, L4). The whole body oxygen uptake of Razor belly minnow decreased significantly from 1.17±0.21 mg of O_2 consumed/g dwt/h (Control fish) to 0.74±0.08 mg of O_2 consumed/g dwt/h (contaminated fish) showing 36.8% decrease on whole body respiration rate (Figs. L3, L4). The whole body oxygen uptake of Molacarplet decreased significantly from 0.91 ±0.12 mg of O_2 consumed/g dwt/h (Control fish) to 0.62±0.06 mg of O_2 consumed/g dwt/h (contaminated fish) showing 31.9% decrease on whole body respiration rate. The whole body oxygen uptake of Stripped dwarf catfish decreased significantly from 0.94 ±0.09 mg of O_2 consumed/g dwt/h (Control fish) to 0.91±0.19 mg of O_2 consumed/g dwt/h (contaminated fish) showing 3.2% decrease on whole body respiration rate (Figs. L3, L4). From the data it is very clear that Swamp barb is very sensitive and the impact of contaminated water was highest. Out of 9 fishes caught from the contaminated field, Stripped dwarf catfish and Dwarf snakehead fish were tolerant and could resist the contamination.

Discussion

Tripathy *et al*. (2022) reported that effluent of JK Paper mill is highly toxic and affected the whole flora and fauna of the area. The effluent of Paper mill altered the physico-chemical status of the river basin. The contaminated river was used for irrigation of crop fields located near both the sides of river. The crop fields receiving contaminated river water were seriously affected (Tripathy *et al*., 2022; Tripathy and Panigrahi, 2023a, b). The same authors also reported that this contaminated river water contained toxic heavy metals, which were absorbed and retained by the crop, non-crop and algal flora of crop fields. The pulp and paper mill effluent pose acute and chronic effects to fish species due to the complex nature of effluent containing heavy metals and endocrine disrupting chemicals. Patel *et al.* (2013) worked on JK pulp and paper mill treated effluent and mentioned that the effluent was used for irrigation for some crop plants and reported that the effluent improved the soil fertility and organic carbon, increase in soil salinity and sodicity but forgot to mention about heavy metal accumulation in crop plants and rice field inhabiting animals like fishes, earthworm and snail. The same authors suggested for irrigation of semi tolerant crops but warned that continuous use of effluent should be monitored regularly to avoid a disaster. Dey *et al*. (2018) reported that the paper mill effluent was extremely toxic even in very low concentrations to fishes. Abnormalities like hepatic and histological damage in *Cyprinus carpio* fingerlings were observed by the same authors. Long term exposure to Cd could lead to lung cancer; kidney diseases, bone damage in humans if rice from the contaminated soil is consumed (Zhang *et al*., 2021). MacDonald *et al.,* (2020) reported that the effluent affects fish by means of reduction in sex hormone levels, gonad size and delayed maturity. Sharma *et al.* (2020) reported the presence of endocrine disrupting chemicals with genotoxic compounds which were not biodegradable in biological treatment of pulp and paper mill effluent and induced chromosomal aberration and phytotoxicity in natural fauna. The above authors suggested tertiary treatment mostly proper biological treatment of the effluent prior to discharge into the environment. Kaur *et al.* (2021) reported that the toxic chemicals of the pulp and paper mill effluent affect adversely on the zooplanktons and fishes. Aquatic biota, fish serve as important indicator for water quality and human health assessments as they can bioaccumulate contaminants to higher magnitudes (Stahl *et al*., 2021). Heavy metals like Hg, Pb, Cd, Cr, As, disrupt cellular events like growth, differentiation, damage-repairing and apoptosis (Balali-Mood *et al*., 2021). These cause DNA methylation, defects in DNA repair resulting into genomic instability and carcinogenicity. Hg accumulates primarily at protein fraction in fishes. Consumption of contaminated fishes with Hg, Cd and Pb makes way to human exposure to mercury, cadmium and lead poisoning (Nong *et al*., 2021). The study of Fernandez-Trujillo *et al.* (2021) indicated that heavy metal and metalloid pollution brought potential risk for the fishes inhabiting the freshwaters. Hg, Cd and Pb are toxic metals prone to be accumulated in fish muscles and tissues. The accumulation of heavy metals depends upon temperature variation in the environment and acute and chronic doses vary accordingly. Increase in temperature and low pH can result in intense accumulation of Hg, whereas suppresses the penetration of Cd and Pb due to increase in salt concentration. The experiment indicated high bioaccumulation ability of heavy metals in the fishes tested which reduced their dietary quality (Ravanbakhsh *et al*., 2020). The same authors also indicated that metals are accumulated through bioconcentration process and the toxicity can enter into human food chain after prolonged consumption. The aquatic heavy metal pollution affects the organisms morphologically as well as metabolically which depends upon the amount of pollutants released to the ecosystem, bioavailability, absorption and retention and transfer through food chain. Large discharge leads to high mortality and low discharge results in accumulation of metals in the inhabitants of the contaminated site (Malik *et al*., 2020). Mercury can be converted into highly toxic methyl-mercury by microbial action consumed by fish. The dual toxicity can cause serious health damages to fish like hepatic tissue damage, ulceration, fin rot, gill disease, etc. The decline of native crop field fish species of Chandilli, Rayagada area can be a potential reason of heavy metal presence. The behavior and impact observed in the fishes collected from the contaminated crop fields was almost similar to the experimental fish conducted in the laboratory *in vivo* (Tripathy and Panigrahi, 2023b). Apykhtina *et al.* (2018) indicated that acute exposure to excessive amount of cadmium can cause damage to lungs, kidney, liver and reproductive organs. The chronic exposure of cadmium has nephro-toxic, immuno-toxic and osteo-toxic effects. Fish can be suggested as bio-indicators of heavy metal pollution. The continuous industrialization and by different agricultural practices, different heavy metals were released to the coastal waters of Ondo state, Nigeria (Olusola and Festus, 2015), where high levels of heavy metals were found in gills and eyes of the fish tested and lowest in the muscles as the heavy metals target the metabolically active tissues. The muscle showed relatively lower metabolic activity than gills and the presence of heavy metals in water, sediment and tissues of fish species can be a serious concern for the human use. In the present study the authors also studied the impact of effluent mixed river water which was used for irrigation to contain toxic metals. These toxic metals slowly get enriched in the crop fields. This study indicated the impact of effluent river water used in irrigation on the fresh water fishes inhabiting in the crop fields and observed that these small fresh water fishes were slowly disappearing from crop fields and no more they are available in the contaminated crop fields. We observed significant accumulation of heavy metals like mercury and cadmium and lead in fish body sampled from the crop field contaminated sites. The physiological activity of the fishes was greatly reduced due to the impact of paper mill effluent. Tripathy *et al.* (2022) reported the impact of Paper mill effluent mixed river water on the crop plants and non crop plants inhabiting crop fields. The heavy metal like Hg, Pb and Cd contamination in the muscles of five fish species were studied (Mahjoub *et al*., 2021). The same authors also reported that fishes at higher trophic level accumulate higher trace metals. The concentrations were generally higher in summer than winter seasons. Hence it was suggested to reduce the consumption of carnivorous fishes as they accumulated higher mercury and cadmium metals compared to others. In the present investigation, lead accumulation was noted in mud eating fishes like *Channa puctatus, Heteropneustes fossilis* and *Channa gaucha* compared to other fishes. It seems free lead was not available for absorption by other fishes and the above mentioned fishes could absorb lead from the bottom sediments where lead got precipitated. Variation in heavy metal accumulation was observed in different fishes. The absorption and accumulation of heavy metals is probably dependent on the metabolic activity of the fishes, duration of exposure and age of the fishes. In the present investigation, it was observed that the heavy metals in combination (Cd + Pb + Hg) with the paper mill waste have serious impact on living organisms inhabiting crop fields. Boudjema *et al*.(2022) indicated that heavy metal interactions can affect bioaccumulation and consequently toxicity. The same authors also agreed to the earlier reports that competition between chemically similar ions (Azizi *et al*., 2018) for the binding sites can significantly affect bioaccu-mulation of heavy metals and our findings and interpretations are in conformity with this. In addition, we are of the opinion that due to interaction of other chemicals present in the effluent waste and chemically similar heavy metal ions might have competed for absorption and might have interacting with binding sites leading to less absorption and retention of heavy metals in fish body.

In the present study, we attempted for the first time to study the impact of heavy metal containing effluent on crop field inhabiting fish and we found significant amount of mercury, cadmium and lead accumulation in different fish bodies collected from the contaminated crop fields. It was observed that these three heavy metals accumulation in fish body caused fish death after 4 months of exposure in the crop fields towards the last phase of the crop. In the present study the fish body gets heavy metals from two sources and the heavy metals concentration is significantly high compared to first trophic level organisms. The piscivorous fishes belonging to third trophic level of the food chain accumulated significantly much higher level of heavy metals due to their feeding habit of eating fishes with higher accumulation of heavy metals and from the environment. The heavy metal concentration was much higher than the second trophic level organism leading to biomagnification of heavy metals. Once the heavy metal concentration in the fish body reaches the thresh hold limit, the impact can be clearly visible and symptoms of poisoning can be marked visibly. Lower accumulation of heavy metals in the fish body will no doubt show signs of toxicity but death of the contaminated fishes can only occur when the concentration of heavy metals are high and the impact is intense. The accumulation mechanism of heavy metals occurs as a result of combination of uptake, metabolism and excretion (Boudjema *et al*., 2022). Metals can become toxic when their uptake is not balanced by excretion processes and detoxifying systems (Castaldo *et al*., 2020). We agree with the findings of Boudjema *et al*. (2022) and Castaldo *et al.* (2020). Many reports from our laboratory indicated that most of the reports published earlier were based on individual impact analysis of heavy metals on different organisms. The present investigation is a combined effect of effluent waste study on fishes under field and laboratory conditions. Both the observed data showed strong similarity, except that the laboratory experimental fish showed high inhibition than the crop field tested fish. The difference was probably due to the environmental impact and interaction which might have reduced the toxicity. The accumulation of heavy metals in fishes depend strongly on the exposure period, age of the fish, metabolic efficiency and the type of food they eat. Monitoring, analyzing and characterizing all types of toxicants including heavy metals in aquatic environments indicated the water resource condition, status, usability of water for drinking and agricultural purposes and protection of plants, animals and most important is environment and human health. All the paper industries should be very careful and should monitor the effluent regularly and must remove all the heavy metals present in the effluent waste by proper biological treatment by using the protocols of bioremediation suggested by many authors.

Conclusion

The Paper mill effluent is deadly toxic and discharged into water bodies without proper biological treatment as it contained significant amount of heavy metals like Hg, Cd and Pb. The contaminated river water is used for irrigation of the surrounding crop fields. The crop inhabiting organisms like crop plants, non-crop plants, algal mass, fresh water fishes, earthworms and snail accumulated significant amount of heavy metals. The impact was severe as observed from the data. The effluent significantly altered the whole body fish respiration and accumulated significant amount of heavy metals in their tissues. The effluent needs to be treated physically, chemically and by potent biological organisms which have shown efficiency to survive in heavy metal contaminated environments. Stripped dwarf catfish (*Mystus vittatus,* Bloch) accumulated 1.19 µg of heavy metals/g dry weight showed 3.2% insignificant depletion in respiration rate; Dwarf snakehead (*Channa gachua,* Hamilton) accumulated 1.86 µg of heavy metal/g dry weight showed 4.1% insignificant depletion in respiration rate; Fossil catfish (*Heteropneustes fossilis*, Bloch) accumulated 1.04 µg of heavy metal /g dry weight showed 4.7% insignificant depletion in respiration rate and Spotted snakehead (*Channa punctatus*, Bloch) accumulated 2.65 µg of heavy metal/g dry weight showed 5.8% insignificant depletion in respiration rate. These four types of fishes available in the crop fields are more resistant compared to other fishes. In addition these fishes adopt bimodal gaseous exchange where gills are rudimentary and depend less on gill breathing for oxygen consumption. Significant amount of toxicant is generally absorbed by gills where gaseous exchange takes place in a gill breathing fish. These fishes are also mud dwellers and feed on soft mud (humus+soil). The precipitated heavy metals on the crop field soil enter the fish body through the food. These fishes can be used for reclamation of Paper mill effluent along with micro and macrophytes for a cleaner and safer environment.

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