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Impact of 30 days exposure of whole Paper mill effluent (WRPBILE) on nucleic acid profile in the liver and gonad of freshwater teleost *Mystusvittatus* during annual reproductive cycle

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Abstract

The present study has been undertaken to investigate the biochemical alterations in teleost fish *Mystusvittatus* after chronic exposure to sublethal concentrations of paper mill effluent for 30 days. a quantitative estimation of DNA and RNA material was made in liver and gonadal tissues throughout the reproductive cycle of the fish. The biochemical variables studied in the control fish showed the maximum values during the spawning phase as compared to other phases of the reproductive cycle of the fish. The changes produced in the nucleic acid content on account of chronic exposure of the fishes for 30 days to 0.4 (40%) and 0.8(80%) of 96 h LC_{50} of WRBBILE stress were found to be close dependent, being relatively much higher in case of 0.8 WRPBILE when compared to 0.4 WRPBILE. This phenomenon was observed during the three phases of the annual reproductive cycle of the fish. The DNA as well as RNA contents in liver, testis and ovary tissues showed a reduction in case of both the sublethal concentration of effluent in all the three phases of the reproductive cycle. The changes produced by WRPBILE stress were found to statistically very significant in all the phases except in the case of RNA content of testis during the post spawning phase of the fishes exposed to 0.4 WRPBILE. The present study concludes stress induced depletion might be due to degradation of cells, nuclear material and metabolic dysfunction in response to WRPBILE toxicity in the fish.

Keywords:*WRPBILE, stress-induced, spawning phase, annual reproductive cycle, chronic, exposure.*

Introduction

Pulp and paper mill effluents are major sources of pollution to aquatic habitats. In addition to producing bad taste and odor in receiving water, these discharges also cause such ecological hazards as oxygen depletion. Pulp and paper mill waste waters are known to be very toxic for fish population. Numerous studies have revealed significant effect of pulp and paper mill effluents on fish health and fish populations. Some of these effects observed were skin disruption, liver dysfunction, kidney damage, abnormal blood chemistry, effects on growth and reproduction, delayed sexual maturation (Whittle and flood 1977; Andersson et al. 1988; Harding *et al*.1988; Khan *et al*. 1992; Forlin*et al*.1995; Jeney*et al*. 1996; Mishra *et al*. 2011). The major components isolated from paper mill effluents include resin acids, ditespene alcohol, lignin degradation products, chlorophenols, furans, dioxins, and inorganic materials like sulfides, chlorides, phosphates, nitrates, calcium, sodium, organochlorine compounds and certain heavy metals, have also been extracted. Various workers have carried out the extensive research in order to understand the biochemical and physiological changes in a number of fishes under the effect of toxic paper mill effluent (Mcleay and Brown, 1974; Oikari*et al*.1984; Klocpper– sams*et al*.1994; Forlin*et al*.1995; Jeney*et al*.1996; Mishra *et al*. 2010). Liver being the chief metabolic centre and main detoxifying organ, particularly harmful for the body .Among other fish tissues, gonadal tissue is particularly important. Healthy gonads being vital tissue for reproductive success, any biochemical anomaly in their tissue would adversely affect the reproductive performance of the fishes and thus, threaten the natural survival of fish populations. The effects of WRPBILE on nucleic acid contents and other biochemical constituents in liver and gonads can vary between mills which adopt different pulping ,Bleaching and effluent treatment

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technologies. However, due to the unavailability of such published data on such effects, the correlation between the toxic components in WRPBLIE and the biochemical effects in liver and gonadal tissues of the stressed fish is much difficult.

The main objective of the present investigation was to examine the biochemical alterations in the liver and gonadal tissues of *Mystusvittatus* caused under the stress of paper mill effluent by exposing the fishes in whole mill effluent for 30 days .The biochemical affects of chronic exposure to sublethal concentrations of WRPBILE on the nucleic acid contents (DNA and RNA), of these tissues were observed through the annual reproductive cycle of the fish.

Material and Methods

Adult and healthy freshwater teleost *Mystusvittatus* (body lenth10.6 ± 0.28 cm and weight 55.3± 2.6 gm) of both the sexes were collected from a local uncontaminated freshwater resources in Basti city area, immediately brought to the research centre in open containers being field with sufficient quantity of water show that the stress produced by handling and transportation may be minimized .The fishes were acclimatized in 50 liter glass aquaria to laboratory condition for 3 to 4 week at natural temperature in the acclimation tanks field with dechlorinated tap water (pH 7.1-7.2; dissolved Oxygen 7.6 ppm ; free carbon dioxide 12.4 ppm; Total alkalinity 112.0 ppm). The fishes were fed upon dried earthworms and a mixture of equal parts of dried shrimps and roasted flour. The whole test effluent test sample was collected from Rayana Paper Board Industries Limited, Maghar, Santkabir Nagar Uttar Pradesh (India), for the study of toxicological responses the test samples were collected from 3 spots during the morning shift of the normal course of mill operation. the samples were mixed thoroughly and brought to the research centre in sealed polyethylene containers ,the chemical characteristics of effluent sample analyzed according to the procedures recommended by American Public Health Association (2005), with in 24h of collection ,every month (Table I). The acute toxicity of effluent to the perch was studied in terms of 96h – LC50 by using the static bioassay procedures as outlined by USEPA (1989) for each acute toxicity bioassay, a minimum of 8 concentration of test sample was used and 20 test fishes were used for each concentration. Controls were also run separately during experimentation, using normal dechlorinated tap water. No food was supplied to either the control or the experimental test fishes during the toxicity experiments. The toxicity test experiments were conducted every month of the annual reproductive cycle and 96h LC_{50} value were determined using regression analysis. For evaluating the effects on tissue biochemistry, the test fishes were exposed for a period of 30 days in two sublethal concentrations i.e. 0.4(40%) and 0.8(80%) of the 96 h LC_{50} determined during the mortality studies .The healthy test fishes, selected from the acclimation tank, were divided in three groups of 60 fishes each. The first group was exposed to 0.4 of 96 h LC_{50} and the second group to 0.8 of 96h LC_{50} WRPBILE sublethal concentration .The 3rd group was placed in unpolluted dechlorintedtapwater, and served as control. The experiments was repeated 5 times .after the completion of 30 days exposure period ,the fishes from all the 3 groups were taken out for the sampling of liver and gonadal tissue.Ten fishes were used for each determination the levels of deoxyribose nucleic acid (DNA)and ribose nucleic acid (RNA) in the liver ,testis and ovary tissues were estimated according to the standard method of Schneider (1957),using calf thymus DNA (standard) and Diphenylamine reagent for DNA ,and Yeast RNA hydrolysis (standard) and orcinol reagent for RNA estimation. Homogenates were prepared in 5% TCA (1 mg/ml W/V) at 90˚C , centrifuged at 5000 g for 20 min ,the supernatant being used for estimating the nucleic acid levels the nucleic acid concentration was measured in the 3 phases of the annual reproductive cycle of the fish and 10 were recorded in each spawning phase.

A Photocolourimeter (Systonics) was employed for biochemical colorimetric estimation. The standard deviation (± SD), and standard errs (±SE), were calculated and tested for significant according to the statistical methods outlined by Snedecor (1961). To test the significance of differences between the mean experimental and the corresponding mean control values, the Student's t-test was applied as described by Campbell (1974).

Results and Discussion

The DNA and RNA contents of liver ,Ovary testis of control *M. Vittatus* were found to rise during the spawning phase as compared to the pre-spawning and Post spawning phases of the annual

reproductive cycle of the fish .Chronic exposure to sublethal concentration (i.e.0.4 and 0.8 of 96h-LC_{50}) of WRPBILE produced a mark reduction in the DNA and RNA content of Liver Testis and ovary of *M .Vittatus* during all phases of annual reproductive cycle .The changes produced by WRPBILE stress in DNA and RNA amounts were found to be statistically very significant ($p<0.05$ or 0.0010 in all the phases of the fishes exposed to 0.4 WRPBILE.(Table I and II).Nucleic acid, especially RNA is initially associated with protein synthesis, usually in positive correlation, the tissues of fish (Love, 1980 b). Hence any increased in nucleic acid concentration would imply increased protein synthesis. It has been mentioned that a general build up of protenious material, leading to higher protein and amino acidlevels, in *M. vittatus* during spawning phase explains that protein is mostly synthesized in liver from where it is transported to o other organs(Mishra, *et al*. 2010). It is also known that a protein reserve is specially needed in the gonadal growth and gamete formation. Hence, and increased protein synthesis, as reflected by higher nucleic acid content would be mostly most likely to occurs in maturing gonad tissues. Thus the presently observed higher nucleic acid concentrations in the spawning *M. vittatus* would appear to reflecting an accelerated DNA-RNA synthesis which the spawning condition induces in these fishes. At the same time, DNA being the genetic material, its increase may be expected during gametogenesis.

Table I: Chemical characteristics of Rayana Paper Board Industries Limited, effluent), Maghar, Santkabirnagar (U.P.), India, samples to which *M. vittatus* were exposed for 30 days. Data based on the Samples taken during the morningshift of the normal course of mill operation (i.e. 8am).

Parameters	**Variable constituents through the reproductive phases (Mean values)**		
(mg/l)	**Pre-spawning phase (Feb-May)**	**spawning phase (June-Sept)**	**Post spawning phase (Oct-Jan)**
Color	Dark Brown	Dark Brown	Dark Brown
Sodium	350	320	351
Chloride	422	450	425.7
Sulphate	2.34	5.8	3.5
Nitrate	7.6	7.3	7.2
Nitrogen	1.8	6.5	3.8
Phosphate	0.78	0.64	0.69
pH	7.3	7.4	7.4
Alkalinity	162	175	170
Suspended solid	5021	4642	4257
Total solid	6136	5867	1015
Tem	23.5	28	26.5
BOD	552	538	497
COD	2370	2548	2326
Fe	9.6	13.5	10.4
Mg	1.9	1.65	1.28
K	8.6	6.5	4.4
Cu	0.12	0.15	-
Total Cr	-	0.078	0.069
Mn	0.34	0.072	0.52
CO	-	0.001	0.00
Zn	0.06	0.08	0.106
Cd	0.027	0.017	0.018

Table II: DNA content µg/l of the liver , Ovary and Testis of *M.Vittatus* exposed to sublethal concentrations of WRPBILE 0.4 and 0.8% of LC_{50} for 30 days during the different phases of annual reproductive cycle .Values expressed as mean ± S.E. of Ten observations ;test sample(Table I used)

Tissues	Pre-Spawning Phase 96h-LC_{50}:43.8 (% v/v)			Spawning Phase 96h-LC_{50}:38.7 (% v/v)			Post-Spawning Phase 96h-LC_{50}:50.8 (% v/v)		
	Exposure conditions			Exposure conditions			Exposure conditions		
	Control	0.4% of 96h LC_{50}	0.8% of 96h LC_{50}	Control	0.4% of 96h LC_{50}	0.8%of 96h LC_{50}	Control	0.4% of 96h LC_{50}	0.8% of 96h LC_{50}
Liver									
Mean	28.15	25.61*	17.61**	32.68	27.27**	18.99**	23.97	20.94**	17.21**
±SE	0.18	0.44	0.45	0.43	0.61	0.35	0.52	0.38	0.66
P.C.	-	-9.02	-37.43	-	-16.63	-41.92	-	-12.6	-28.2
Ovary									
Mean	26.83	23.65*	19.19**	30.31	25.14*	20.62**	21.74	18.28**	15.42**
±SE	0.81	0.54	0.31	0.79	1.18	0.67	0.434	0.29	0.64
P.C.	-	-11.9	-28.5	-	-17.09	-32.04	-	-5.8	-28.9
Testis									
Mean	24.6	18.20**	18.08**	26.57	18.08**	15.18**	20.25	17.15**	14.55**
±SE	0.41	0.72	0.43	0.66	0.85 -	1.21	0.46	0.42	0.62
P.C.	-	-26.02	-26.50	-	3.95	-42.9	-	-15.3	-28.15

P. C. : Percent change from corresponding control
***P< 0.05> ,**P<0,001> Student's 't'-test (Campbell,1974)**

Table III: R.N.A. content µg/l of the liver, Ovary and Testis of *M.Vittatus* exposed to sublethal concentrations of WRPBILE 0.4 and 0.8% of LC_{50} for 30 days during the different phases of annual reproductive cycle .Values expressed as mean ±S.E. of Ten observations; test sample (Table I used)

Tissue	Pre-Spawning Phase 96h-LC_{50}:43.8 (% v/v)			Spawning Phase 96h-LC_{50}:38.7 (% v/v)			Post-Spawning Phase 96h-LC_{50}:50.8 (% v/v)		
	Exposure conditions			Exposure conditions			Exposure conditions		
	Control	0.4% of 96h LC_{50}	0.8% of 96h LC_{50}	Control	0.4% of 96h LC_{50}	0.8%of 96h LC_{50}	Control	0.4% of 96h LC_{50}	0.8% of 96h LC_{50}
Liver									
Mean	32.95	30.48**	25.25**	35.99	30.41**	24.88**	28.15	25.5**	20.38**
±SE	0.28	0.46	0.13 -	0.33	0.44	0.35	0.31	0.31	0.44
P.C.	-	-7.8	23.4	-	-15.5	-30.9	-	-9.41	-27.6
Ovary									
Mean	38.08	35.83*	30.73**	41.6	38.63*	29.2**	31.55	30.54**	26.97**
±SE	0.213	0.19	0.15	0.15	0.14	0.13	0.93	0.35	0.1
P.C.	-	-5.81	-19.26	-	-7.14	-29.81	-	-3.2	-14.52
Testis									
Mean	29.82	25.84**	21.43**	33.16	25.62**	21.91**	24.24	19.83**	17.06**
±SE	0.36	0.44	0.39-	0.63	0.71	1.88	0.45	0.35	0.44
P.C.	-	-13.34	28.14	-	-22.74	-33.93	-	- 18.06	-29.51

P. C. : Percent change from corresponding control
***P< 0.05> ,**P<0,001> Student's 't'-test (Campbell,1974)**

The nucleic acid synthesis appeared to be greater in the ovary than in the tissues of these fish. The RNA:DNA ratio observed in control *M. vittatus* was found to be 1.25±0.03 in case of testis and 1.45±0.05 in case of ovary ,the difference between the two being statistically significant ($p<0.005$). so ,while the increase observed in the content of both the nucleic acid would be positioning at a general increase in cell number and size in the growing and dividing gonad tissues of spawning ,the comparatively greater rise in RNA concentration of the ovary could be a pointer to greater growth and accumulation on the material in the developing ova when compared to sperms .After chronic exposure for 39 days to both the sublethal concentration of WRPBILE, a dose- dependent reduction in DNA and RNA material in Liver, ovary and testis of *M. vittatus* was seen during all the phase of the annual reproductive cycle. The reduction in 0.8 sublethalconcentration was always generally higher as compared to 0.4 WRPBILE sublethal concentrations. There are many records of various environmental and other factors producing significant reduction in the nucleic acid amount of liver and gonads of fishes (Rath and Mishra ,1980; Shukla and Pandey ,1986; Kumar and Ansari ,1986; Pandey and Narain ,1990).A close parallelism was seen between protein level on one hand and DNA – RNA level on the other. Hence any reduction in the amount of nucleic acid would imply a reduced synthesis of protein (Buckley,1980; Barron and Adelman, 1984; Mishra , *et al* . 2010) .Reduced nucleic acid content could , infact, be a major cause for the under production of protein levels of these stressed fishes .regarding the causative factors involved in the lowering of nucleic acid level in *M. vittatus* ,both under production and loss could be contributing . Histopathological damage to tissues would naturally result in a loss of nucleic acid result in the destroyed cells. Possibility of stress that induced tissue damage in stressed *M. vittatus* could not be avoided. Under production of RNA would be inevitable in the event of DNA reduction because, as in the case of mammals, RNA synthesis in developing and growing fish tissues also depends upon the amount of available DNA template (Zeitoun, *et al*.1977). DNA molecule in particular seems to be susceptible to stress including environmental stress .Nucleic acid loss on account of damage to RNA and / or DNA materials, caused by stress –induced chromosomal abnormalities, has been reported in the tissues subjected to environmental poisons (Fowler, 1977; Adam's, *et al.* 1992; Marlasca, *et al*. 1998; Sumath, *et al*. 2001; Cavas and Gozukara, 2003). It is also suggested (Fowler, 1977), that stressed could inhibit DNA repair process as well, and thus lead to its dimunition.

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