Bulletin of Environment, Pharmacology and Life Sciences

Online ISSN 2277-1808
Bull. Env. Pharmacol. Life Sci. Volume 2 [3] February 2013: 17-23

Website: www.bepls.com

REVIEW ARTICLE

A Review on Toxicity of Paper Mill Effluent on Fish

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ABSTRACT
Pulp and paper mill, controversially, is one of the largest and major contributors of pollution in aquatic environment, affecting aquatic organisms in general and fishes in particular. Several field and laboratory studies were conducted worldwide on effects of both treated and untreated paper mill effluents on fish. The studies showed wide ranges of toxicity at organismal as well as at population levels. Several workers discussed endpoints of paper mill toxicity in fish ranging from acute and behavioral toxicity, developmental and growth studies, immune toxicity, haematotoxicity, genotoxicity, histotoxicity as well as effects on enzymes, reproductive and endocrine disruption. The present study is an attempt to review toxic effects of paper mill effluents in fish.
Keywords: *effluent, toxic, lethal, chronic, fish*

Received 22/02/2013 Accepted 16/03/2013

INTRODUCTION

Globally, pulp and paper industry is considered as one of the most polluting industry [1,2] contributing 100 million kg of toxic pollutants that are being released every year in the environment [3]. In India, the paper industry has been one of the major sources of aquatic pollution [4]. The pulp and paper Industry is one of the oldest industries in this country and there has been tremendous expansion of these industries during the last 25 years. Effluents from pulp and paper mills are highly toxic and are a major source of aquatic pollution. Many chemicals have been identified in effluents which are produced at different stages of papermaking. Their toxic nature is derived from the presence of several naturally occurring and xenobiotic compounds which are formed and released during various stages of papermaking. The pulp and paper mills rank high in terms of water use during paper production on the other hand they contributing to pollution loads in rivers through effluent discharge [5]. Waste and wastewaters are generated from both of pulp and bleaching processes. The major problems of the wastewaters are high organic content, dark brown coloration, adsorbable organic halide and toxic pollutants. Toxic dyes, bleeching agents, salts, acids and alkalis are present in effluents discharged from pulp and paper industries. Heavy metals such as cadmium, copper, zinc, chromium are present in pulp and paper mill effluent that ultimately released into aquatic environment [6]. Dichloroguicol, trichloroguicol, tetrachloroguicol and chlorinated phenols are major contaminants found in the effluent released from pulp and paper mill which are toxic to fish fauna [7, 8]. Various acute and chronic effects of pulp and paper mill effluent in fish fauna have been noticed by several workers. Walden (1976) reviewed the pulp and paper industry as it affects aquatic biology [9]. The present study, thus, is an attempt in reviewing toxic effects of paper mill effluents in fish.

ACUTE TOXICITY AND BEHAVIOURAL RESPONSE

The static bioassay procedures to study the toxicity of pulp and paper mill effluent to a variety of fishes have been reported [10]. The toxicity, in terms of LC_{50} values, was found to vary a great deal for species tested. In a study on *Anabus testudineus, Channa punctatus and Clarias batrachus*, the result showed that *Anabus testudineus* was most susceptible to paper mill effluent, while *Channa punctatus* and *Clarias batrachus* were comparatively resistant [11]. Bleached kraft mill effluent concentrations of 8 to 12% up to 20% caused increased mortality after 72 hours of exposure in striped bass (*Morone saxatilis*) [12]. The LC_{50} values of the prepared concentration of paper mill effluent for 24, 48, 72 and 96-h were found at 11, 10.5, 10.1 and 9.5% respectively in *Rasbora*

daniconius [4]. In a study, toxicity of paper mill effluent to fish *Puntius sophor* was reported. Tests were conducted in two groups, first group were aerated while the second group second were not aerated. 96 h LC_{50} for *Puntius sophor* was estimated to be 1.5% whereas in the second test it was recorded at 16.5% [13]. 96-h LC_{50} value of paper and pulp mill effluent to fingerlings of *Oreochromis mossambicus* was reported to be 6% [14].

The toxicity and behavioral changes in *Rasbora daniconius* exposed to lethal concentration of paper mill effluent were also studied [4]. At different concentrations, erratic swimming, jerky movement, rapid opercular movement, leaping out of water and thick mucous covering over the whole body surface was documented. Similar results were observed when *Labeo rohita* and *Channa punctatus* were exposed to paper mill effluents [15]. Mishra *et al.,* (2011) evaluated the acute toxicity of paper mill effluent on the behavioral responses in *Mystus vittatus* [16].

EFFECTS ON DEVELOPMENT AND GROWTH

The paper mill effluent has the potential to cause developmental effects in fish. The paper mill effluent exposure induced abnormalities including conditions wherein, the yolk sac get protruded up to the head region, deformities in eyes, spinal curvature, abnormal head and overall stunted growth were observed during the a study in *Cyprinus carpio* larvae [17]. In a study on, *Cyprinus carpio* hatching was preponed by 3-h, 2-h and 1-h in 4%, 2% and 1% exposed paper mill effluent respectively. Dose related mortality was observed was also obserdved in the same study. Amongst successful hatchlings, teratism was observed. Paper mill effluent altered axial curvature, caused head deformities, tail malformations, edema in pericardial region, microphthalmia, fused eyes/ single eye, deformity in the fin region, gills disorganised in the head region and delay in yolk absorption in embryo. Enlarged yolk sac leading death of larvae is another frequently observed developmental anomaly in fish. Hatching success can also significantly declined in mill effluent treated embryos of *Cyprinus carpio* [18].

The paper mill effluent could cause 50% growth reduction for experimental downstream brown trout fish, compared with the group upstream of the mill [19]. On investigating the histochemical components of the liver of *Rasbora daniconius* on exposure to paper mill effluent, metabolic crisis and impairment in fish liver were observed [4]. The depleted levels of protein, lipid and glycogen reflect stressful conditions and points towards exhaustion of cell- energy to meet high demand of fish in stressful condition [20]. In a study in striped seabream, *Lithognathus mormyrus,* protein content of gonads and flesh showed directly proportional reduction with effluent concentration compared to control. Seabream protein is characterized by high values of aspartic and glutamic amino acids. Flesh of seabream has high values of alanine and lycine, while ovaries and testis have higher values of leucine, isoleucine and arginine. Total amino acid content decreased by increasing effluent concentrations. The study concluded that the rate of breakdown of protein was higher than the anabolic processes during effluent exposure [21].

IMPACT ON IMMUNE SYSTEM

On investigating the effects of bleached paper mill effluent on selected physiological and hematological endpoints in fingerling largemouth bass (*Micropterus salmoides*)., the results indicated that in-stream exposure to elemental-chlorine-free pulp and paper mill effluents produce a generalized stress response, leading to potential immune suppression in fish [22]. Serum immunoglobin levels were also reported to be decreased in papermill effluent exposed fish, *Rutilus rutilus* [23]. The effect of effluent exposure on the organ weight and organ cellularity and also investigated the influence of temperature variation on the humoral immune response using the plaque-forming cell (PFC) assay in freshwater fish *Channa punctatus* (Bloch) were also investigated [24]. Results demonstrated a suppressive effect of chemical constituents of paper and pulp mill effluent on the immune functions. They also recognized a modulatory effect of the temperature on immune functions.

HAEMATOTOXICITY

The effect of paper mill effluent on haematology was investigated in several studies. In *Tor putitora* red blood corpuscle count and haemoglobin decreased significantly whereas increasing trend in white blood corpuscle count at all concentrations has been observed. Pathological alterations of erythrocytes in blood smears included deformation sticking, lysis and abnormal presence of senile

erythrocytes were observed [25]. These changes might be attributed to structural damage of RBC membranes resulting in haemolysis and destruction in haemoglobin production [26]. *Lithognathus mormyrus*, exposed to sublethal concentrations (20 ml/L) of paper mill effluent for 4-week, showed erythrocytes count decreased significantly followed by reduction in both haemoglobin content and packed cell volume ensuing in macrocytic hypochromic anemia. The corpuscular indices, as mean corpuscular volume and mean corpuscular heamoglobin increased with effluent concentrations compared to control. Also the results indicated that the mean corpuscular heamoglobin concentration decreased significantly. These changes in corpuscular indices, MCV and MCH, compensate the decrease in RBC, Hb and PCV values resulting in macrocytic hypochromic anemia. The total white blood cells count increased significantly with increasing effluent concentration. Blood smears and kidney prints revealed that increasing waste concentration caused increase in small lymphocytes and neutrophiles, that emphasize the compensatory and defensive reaction of fish to effluent [20]. In another study, juvenile catfish, *Clarias gariepinus* exposed to paper mill effluent under field condition revealed that fish exposed to effluent discharge site had lower haemoglobin count, red blood cell count, packed cell volume and higher concentrations of mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and white blood cell indicating that test fish suffered haemolytic anaemia and leucocytosis [27].

GENOTOXICITY

Pulp and paper mill effluent compounds pollute the aquatic environment and are responsible for increased biochemical alterations and genotoxicity in aquatic organisms such as fish. Resin acids can cause teratogenicity in fish larvae. Resin acids accumulate in sediments and become bioavailable to fish. Short term exposure to low concentrations of bleached kraft pulp mill effluent induced genotoxic effects in sea bass, including erythrocytic micronuclei and nuclear abnormalities [28]. A study was made to see the genotoxic effect of an effluent from a paper mill located at Kurukshetra, India, was tested in *Channa punctatus*, a fresh water fish. The fish were exposed to the effluent for 24, 48, 72 and 96 hours. The remarkable chromosomal aberrations observed in the treated specimens included centromeric gaps, chromatid break, attenuation, acentric fragments, pycnosis, polyploidy and chromosomal gaps. The results of the study clearly points genotoxic potential of the paper mill effluent on the fish chromosomes [29]. Analysis the genotoxic effects of a "complex mixture" of anthropogenic chemical toxins and reveals a significant level of genetic damage in Androscoggin River smallmouth bass that increases in a downstream gradient in parallel with toxic equivalent concentrations. Individual concentrations of recognized, chemical toxins in Androscoggin River fish tissues are measured on an annual basis as part of a State monitoring program [30]. In a study, adult European eels (*Anguilla anguilla*) were exposed to bleached kraft pulp and paper mill effluent collected at the river Vouga, and its sediment (water-soluble fraction). The genotoxicity as blood/liver DNA strand breaks and erythrocytic nuclear abnormalities induced on European eel were determined. It was found that liver DNA integrity was significantly decreased at 24 h exposure to E25 and E50, and 72 h to E50 [31]. A study of wild Chinook salmon exposed to paper mill effluent detected increased DNA adducts in liver microsomes [32]. Gravato and Santos (2002) determined the effects of pulp mill effluent on population genetic structure of redbreast sunfish (*Lepomis auritus*) [33].The level of genetic diversity was higher in the populations, genetic distances among populations have been altered by contaminant exposure, the genetic diversity (within and among populations) populations may have been affected by altered gene flow and mutational processes as a result of pulp mill effluent discharge [34].

IMPACT ON ENZYMES

In a study in roach, *Rutilus rutilus*, alkaline phosphatise, serum GOT and GPT increased in fish exposed to mill effluent [35]. Mature white sucker (*Catostomus commersoni*) captured from a reference site and exposed to bleached kraft mill effluent in cages had 3- to 12-fold Mixed function oxidase (MFO) induction while immature white sucker and juvenile rainbow trout exposed to bleached kraft mill effluent had over 60-fold MFO induction above reference fish [36]. The MFO induction was reversible as white sucker captured from the effluent and kept in clean water for 4 d had marked decreases in liver MFO activity [37]. While, exposures of juvenile white sucker exposed to bleached kraft mill effluent showed Ethoxyresorufin-*O*-deethlyase (EROD) induction peaked in 8 d

and remained elevated until 14 d, whether or not the fish were removed to depurate in clean water. In a survey using 31 samples of secondary-treated PMEs from 8 different mills, rainbow trout MFO activity was most consistently induced after exposure to kraft mill effluent (both bleached and unbleached) [38]. Rainbow trout exposed for 4 d to 10% final effluent showed significant MFO induction, < 2 to 15.5 fold above control fish MFO [39]. Wood pulp leachates and wood condensates in black liquors also induced MFO in laboratory exposures of rainbow trout [39, 40].

REPRODUCTIVE DYSFUNCTION

A great deal of research has been conducted over years, and revealed that fish exposed to pulp and paper mill effluents affect fish reproduction. Bioactive substances in paper mill effluent can cause reproductive dysfunction [10]. They worked on the sources and characterization of bioactive substances from pulp and paper mill effluents and their environmental impacts on fishes. They documented that these bioactive substances originate from wood and are derived from lignin and terpenoids, and are liberated during pulp digestion. It also revealed that these bioactive substances had the potential to affect the fish reproduction. Sepúlveda *et al.,* (2009) studied the reproductive efficiency of largemouth bass, *Micropterus salmoides,* treated with pulp and paper mill effluents [41]. Their study revealed that a significant reduction in reproductive efficiency of parent and fry growth and survival. This decline may have been caused by an increased frequency of deformities, in conjunction with alterations of growth. These changes could have resulted from alterations in egg quality because of failure of parental reproductive organs. Largemouth bass exposed for periods of 7 days to 2 months to 10-80% bleached paper mill effluent had decreased circulating steroids [42]. Development in a Baltic fish community was studied when exposed to bleached pulp mill effluent**.** An effect of enrichment, indicated by high catches of cyprinids, was visible in the vicinity of the mill outlet. Sexual maturation was delayed, gonad development was retarded, average growth rate was faster, and the condition factor was higher in perch caught in the vicinity of the effluent outlet, than in perch (*Perca fluviatilis*) captured elsewhere. It is concluded that substances inhibiting reproduction and causing growth disturbance were still present in the effluents in effective concentrations [43]. *In vitro* and competitive assays was done on goldfish (*Carassius auratus*) brain tissues, the extracts were screened for their ability to interact with enzymes and receptors involved in gamma-aminobutyric acid (GABA), dopamine, glutamate, and acetylcholine-dependent neurotransmission, and documented a novel and plausible mechanism by which pulp and paper mills effluents impair fish reproduction by interacting with neurotransmitter systems [44]. Effects of treated bleached kraft mill effluent on eggs and pro-larvae of striped bass (*Morone saxatilis*) established that concentrations ranging from 12% to 20% caused adverse effects [12]. Most research has shown that exposure to paper mill effluent decreased the levels of circulating steroids in fish. Exposure of mature male goldfish to bleached sulphite mill effluent for 21 d caused a drop in testosterone production by goldfish testes to one-seventh of control fish [45]. Bleached kraft mill effluent from Alberta, Canada, after 8-days of exposure decreased male goldfish circulating testosterone [46]. Mummichog exposed for one month to to 1% treated bleached kraft mill effluent reduced plasma testosterone in male and female fish [47]. Two native fish species (*Trichomycterus areolatus* and *Percilia gillissi*) of Central Chile were investigated for effects at both the molecular and individual level due to the discharge of effluent from a tertiary treated elemental chlorine-free pulp mill into a fluvial system over three seasons and established possible links in the reproductive alterations observed at the sub individual and individual levels that could explain the changes observed at the population level [48]. Male goldfish exposed to 100% bleached kraft mill effluent had a 60% and 10% drop in testis production of testosterone, and a 74% and 41% drop in blood testosterone, for 9- and 16- exposures, respectively [45]. White sucker, exposed to bleached kraft mill effluent showed depressions of sex steroid hormones [49].

HISTOPATHOLOGY

A prolonged toxicity study was done in young European eel (*Anguilla anguilla*) to evaluate the effects of bleached kraft pulp mill effluent. Exposed fish showed skin and gill disruption as well as kidney malpighian corpuscle alterations, revealed intense spleen hemosiderosis and neoplastic lesions [50]. The effect of paper mill effluent on the morphology of fish *Heteropneustes fossilis* (Bloch) showed excessive mucous secretion and epidermal lesions during the period of exposure to effluent [51]. Ovary of *Rasbora daniconius* showed marked degenerative changes like complete absorption of

oocytes, broken zona radiata, degeneration of oocytes, cytoplasmic liquification and clumping, thickening of ovarian wall and disappearance of nucleus in mature oocytes on exposure to paper mill effluent [52]. *Rasbora daniconius* revealed swollen hapatocytes, nuclear hypertrophy, rupture sinusoids, hemorrhages, vacuolation in hepatic cells and broken central vein in liver, kidney showed hypertrophy of hematopoietic tissues, cell necrosis [53]. In a study on histological analysis of the liver samples of brown trout, clear structural differences between the upstream and downstream experimental groups were observed. The percentage of normal liver cells was 68% in the upstream group, whereas the corresponding percentage in the downstream fish from the effluent discharge was 35%, which shows clear histopathological changes due to papermill effluent [19].

CONCLUSION

Pulp and paper mills seem to pose great risk to fish species in terms both acute and chronic effects. Plenty of works have been carried on the effects of pulp and paper mill effluents in Canadian region and some from European zone, but not enough data are available from Asian region. However, all the available works is of opinion that pulp and paper mill pose serious threat to aquatic biota. The problem is aggravated due to the complex nature of paper mill effluent as it may contain mixtures of several potentially threatening substances including heavy metals and endocrine disrupting chemicals. Therefore, it is absolutely necessary to regulate the discharge of effluent in water bodies besides taking adequate steps to treat the effluent.

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ISSN2277 1808

HOW TO CITE THIS ARTICLE: Sangeeta Dey, Manabendra Dutta Choudhury and Suchismita Das. A Review on Toxicity of Paper Mill Effluent on Fish. Bull. Env. Pharmacol. Life Sci. 2 [3] February 2013: 17-23