



A review on effect of sugar mill effluents on histopathology in freshwater fish

Maya Devi*, Chandrakala, Alka Mishra

Assistance Professor, Department of Zoology, Govt. V. Y. T. PG. Autonomous College, Durg (C.G.), Raipur Chhattisgarh, India

Abstract

Sugar mill effluents, an important cause of industrial pollution, are a severe threat to aquatic life, especially freshwater fish. The effluents carry high amounts of organic and inorganic pollutants, including heavy metals, pesticides, and other toxic compounds, which can cause severe histopathological changes in fish. High levels of contaminants are found in water quality parameters, especially in chemical oxygen demand (COD) and biological oxygen demand (BOD). This review focuses on changes in vital organs such as gills, liver, kidney, Heart, stomach, intestine, and Ovary. These studies indicate that continuous exposures lead to damage of gill lamellae, degeneration of the liver, and renal impairment, loss of striation in cardiac muscle, disintegration of the epithelial duct, hydropic degeneration at the tips of the villi and thickening of ovarian wall, after all affecting fish health, growth, and survival. The extent of these effects changes depends on effluent concentration, exposure time, and fish species. Such information on the histopathological changes can serve as indicators of fish health, ecosystem stability, and environmental disturbances.

Keywords: Sugar mill effluents, histopathological change, physiochemical

Introduction

The environmental pollution issue arising from industrial growth mainly involves the disposal of industrial waste, which can be solid, liquid, or gaseous. Each of these waste types poses a risk of polluting water (Kisku *et al.* 2000; Barman *et al.* 2000) [41]. Chemical pollution in many Indian rivers is the result of rapid industrialization as these rivers serve as temporary reservoirs for sewage waste water and are affected by industrial effluents (Singh D.P *et al.*, 2018) [36] (Upadhyay, S.K. 2017) [37] (Babita *et al.* 2019) [38]. The majority of Indian sugar industries use the double sulphitation process to produce white sugar. The production process involves several steps, starting with sugarcane crushing to extract juice, clarification to separate impurities, evaporation to thicken the juice by evaporating water, crystallization to create sugar crystals, and centrifugation to remove the sugar crystals from the molasses. These processes ensure the manufacture of high-purity refined white sugar. (Limbani, & Shah, 2018) [43].

Sugar industries in India: Sugar is a vital component of the daily diet for many people and plays an important role in maintaining a balanced diet. These industries are the backbone of Indian industrial development (Latha *et al.* 2018) [1]. The sugar industry is a vital agro-based sector in India, significantly influencing the rural economy and contributing to the national economy. It operates seasonally for 120 to 200 days annually (Saranraj and Stella 2014, Gunkel *et al.* 2007, Memon *et al.* 2006, Latha *et al.* 2018) [1, 2, 3, 4]. India has been known as the original home of sugar and sugar cane. Sugar industries rank second among the agro-based industries in India (Sahu *et al.* 2017, Saranraj and Stella 2014, Gunkel *et al.* 2007) [2, 3, 39]. India's sugar industry is characterized by the growth of sugar factories and the expansion of sugar-related industries (Sahu *et al.* 2017) [39]. The sugar industry plays a crucial role in

economic development and job creation in many developing countries across Asia, Africa, and South America. It processes over 70% of the world's sugarcane to produce raw sugar (Patil and Kulkarni 2022) [5]. The primary products of the sugarcane industry are crystalline sugar and bioethanol, which is produced through the fermentation and distillation of sugarcane juice and molasses. Significant by-products include bagasse, the solid residue left after juice extraction, and stillage, the liquid waste generated during the distillation process (Siddiqui and Waseem 2012) [6]. According to Kohle, *et al.* (2008) [7] there are two categories of sugar manufacturing processes: carbonation and sulphitation. Most sugar factories in India use the double sulphitation process to produce plantation white sugar. Sugar industries have initiated economic growth by establishing various facilities in their regions, such as dairies, distilleries, paper mills, and poultry farms (Deshmukh 2014) [8].

Sources of sugar mill effluent: Wastewater from the mill house often contains oil and grease, which result from spills on the floor caused by machinery and equipment. During floor washing, these contaminants are washed away along with the water. During the process, a large amount of water is used and as an outcome, industry give rice to wastewater on a regular basis. Water is primarily needed in sugar mills for various purposes, including cooling barometric condensers, feeding boilers, preparing lime, and diluting substances in evaporators (Memon *et al.* 2006) [4]. The effluents generated are primarily organic, containing small amounts of inorganic material. The main discharges include wastewater, bagasse, press mud, and molasses (Latha *et al.* 2018) [1].

The effluent consists mainly from floor washing and condensate water, the leakage in valves and glands of the pipeline introduce sugarcane juice, syrup and molasses into

the effluent (Prakash and Capoor 2018) ^[9]. The fast-growing industrialization now in progress spells an unpredictable threat to humanity, domestic animals, fishes and wildlife through its wastes. The enforcement of strict environmental laws and regulations on the industrial waste discharge (Maleki A. *et al.*, 2005, Ebrahimpour M. *et al.*, 2010) ^[10, 11]. Effluents from sugar cane factories released onto land and surface water bodies impact water quality, soil health, and also contaminate groundwater through the percolation of water-soluble pollutants (Deshmukh 2014) ^[8]. Discharging untreated effluent into water bodies depletes dissolved oxygen, and harms aquatic life. If untreated effluent is discharged on land, then decaying organic solids, oil and grease clog the pores of the soil (Prakash and Capoor 2018) ^[9].

The rise of sugar industrialization, along with high urbanization and population growth, has significantly unprecedented environmental degradation (Deshmukh 2014) ^[8]. A typical sugar mill effluent can contain carbonaceous sugar matter, fiber and lignin, cellulose, lime, lactic acid, oils and phosphate of Calcium (David and Ray, 1966) ^[12]. Type of compound used in clearing bleaching processes in sugar mill such as lime and Sulphur dioxide also adds to the higher calcium and sulphate content of the effluent (Devarajan *et al.* 2015) ^[13]. Fish are great bio indicator organisms for the determination of impacts of harmful substances such as pesticides, waste and heavy metals in aquatic ecosystems; therefore, they can be utilized in bio toxicity assays to find out aquatic hazards (Xia C. *et al.*, 2018) ^[14].

Fish behavior changes due to effluent are the changes in fish activity, movement, feeding, and social interactions that result from the impact of pollutants or waste released into water bodies. Industrial, agricultural, and sewage effluents can release toxins, heavy metals, chemicals, and excess nutrients into aquatic environments. The fish were observed to be erratic in swimming, experiencing jerky movements, rapid opercular activity, and attempted leaping out of the water, they also showed fish covering their body surfaces with a thick layer of mucus (Agrawal & Pandey, 2014; Sarwade, 2015) ^[44, 45]. Increasing the effluent concentration beyond 15% at both sites led to unusual behavior in fish over 96 hours. The fish displayed erratic movements, tried jumping out of the water, and showed rapid gill movements to access atmospheric air (Prakash and Verma, 2020) ^[46]. Other researchers have reported similar effects in fish exposed to heavy metals Srivastava and Prakash, 2018 & 2019; Prakash and Verma, 2019b) ^[9, 47, 48]. Ghosh and Konar (1992) ^[49] found in their study that the growth and fecundity of fish were greatly reduced by this effluent.

Physicochemical test of sugar mill effluents

The analysis of sugar mill effluents showed that various water quality parameters, such as temperature (30°C), turbidity (84.7 NTU), pH (8.1), electrical conductivity (5530 μ S/cm), BOD (6856 mg/L), COD (7432 mg/L), total dissolved solids (2516 mg/L), chloride (1894 mg/L), total alkalinity (254 mg/L), total hardness (342 mg/L), sulfate (540 mg/L), phosphate (224 mg/L), total acidity (45 mg/L),

calcium (364 mg/L), and magnesium (151 mg/L), were all found to exceed the permissible limits set by the Bureau of Indian Standards. This indicates that wastewater from the sugar industry is highly polluted, making it unsuitable for irrigation or public use (Shivappa, *et al.*, 2007) ^[50].

Bhatt and Verma, (2018) ^[51] studied the physico-chemical properties of sugar mill effluent showed brown color, unpleasant odor, and high temperature (29°C). The pH was slightly acidic (6.3). Total hardness (200 mg/L) was within permissible limits. Turbidity (76.1 NTU), COD (400 mg/L), BOD (275 mg/L), TDS (565 mg/L), nitrate (132 mg/L), sulfate (70mg/L), iron (5mg/L), calcium ion (65mg/L), and magnesium ion (29mg/L). According to the limits suggested by Bureau of Indian Standards, almost all the parameters have been found to be very high and well above the permissible limits.

Bhoramdeo Sugar Mill wastewater revealed high levels of BOD (457.1 mg/L) and COD (1600 mg/L), pointing to a serious issue with organic pollution. After treatment at the effluent treatment plant (ETP), these numbers decreased to 273.9 mg/L and 960 mg/L, respectively. The TDS decreased from 849.7 mg/L to 458.3 mg/L due to the treatment processes, however, the TSS decreased from 117 mg/L to 225.3 mg/L. The pH level increased from 4.66 to 6.14 and turbidity decreased from 287.3 NTU to 29.99 NTU, improving the water clarity significantly. Also, the concentration of ions such as chloride, calcium, magnesium, nitrate, sodium, potassium and bicarbonate was also reduced (Lahare and Yadav, 2021) ^[52].

The analysis of the sugar effluents reveals, according to (Siddiqui *et al.*, 2012) ^[6] Colour and Temperature: Untreated effluent is dark brown, while treated effluent is lighter, impacting photosynthesis in aquatic plants. High untreated effluent temperature (40°C), exceeding the permissible discharge limit (35°C), negatively affecting germination processes. pH and Dissolved Oxygen (DO): Cleaning processes (e.g., using PO₄ acids and SO₂) lower pH (5-7) in both untreated and treated effluent, adversely impacting crop growth. DO levels are significantly low in untreated (1.30 ppm) and treated effluent (2.30 ppm), failing to meet BIS standards (4–6 ppm). BOD and COD: Untreated effluent has a high BOD (~100 ppm), reduced to 88 ppm after treatment. Untreated effluent (35ppm) and treated effluent (255ppm) COD, indicate toxic conditions and the presence of biological matter in groundwater samples. TDS and TS: Seasonal variations influence TDS, with lower levels during the rainy season. TS level found in treated and untreated effluent from 2700ppm to 2010ppm and High TDS in treated and untreated effluent (from 2980ppm to 1920ppm) can increase soil salinity and risk groundwater contamination with saline water. Chlorides and Sulphates: Chloride levels decrease after treatment (from 210 ppm to 175 ppm), within BIS limits. Sulphate levels also decrease after treatment from 760ppm to 420ppm.

Doke *et al.* (2011) ^[15] evaluated the impact of treated wastewater effluents from a sugar industry on seed germination for *Vigna angularis* (Mung), *Vigna cylindrical* (Chavali), and *Sorghum cernum* (Jowar) across various effluent concentrations (0%, 20%, 40%, 60%, 80%, and 100%). Physico-chemical analysis of the effluent showed a

low pH (4.35), high total dissolved solids (720 mg/L), and elevated chemical oxygen demand (1330 mg/L), indicating significant inorganic and organic content with an acidic nature. The findings revealed a decline in germination percentages and germination values as the concentration of the effluent increased for all seed types tested.

Environmental Impact of Sugar Mill Effluent

Sugar factory effluents significantly impact the environment by altering the physico-chemical properties and biodiversity of aquatic ecosystems. They pose serious health risks to rural and semi-urban populations relying on stream and river water for agriculture and domestic use. Additionally, these effluents can cause fish mortality and damage crops like paddy when wastewaters enter agricultural land (Baruah *et al.* 1993) ^[42].

Industrial effluents, especially from sugar mills, are usually discharged without treatment, causing environmental problems because of their unpleasant odor, color, and pollution. Farmers using these effluents for irrigation observe adverse effects on plant growth, crop production, and soil quality. Contaminants like chloride, sulphate, phosphate, magnesium, and nitrate further contribute to the harmful impact, affecting the appearance, taste, and smell of water. These polluted waters are dangerous for plants, animals, and humans, attracting the interest of researchers studying their impact on seed germination and crop yields. (Ozoh, & Oladimeji (1984) ^[40].

Soil pollution is caused by the disposal of industrial waste. The chemical industries, which include tanneries, paper, textiles, atomic power plants, and electric power plants, release pollutants with organic, inorganic, and non-biodegradable materials. These toxic chemicals alter soil parameters, leading to a decline in soil fertility (Kisku, *et al.* 2000) ^[41].

Histopathological changes

In Gill

The gills of *Rasbora Daniconius* treated with sub-lethal concentrations of sugar factory effluent showed curling, degeneration and breaking down of epithelial cells of the secondary gill lamellae, hypertrophy, destruction of blood cells, ruptured blood capillaries and nuclei were some prominent alterations in the gill region (Kakade, 2017) ^[18]. In the gills, liver and muscles of fish, Cr accumulation was found of 0.33-12.5 mg Kg⁻¹ d.w. respectively. In general, it has been noted that fish gills accumulate more Cr than other organs and in earlier studies was reported by Yousafzai *et al.*, 2010, Nwajei *et al.*, 2012, Osman and Kloas, 2010, Coetzee *et al.*, 2002) ^[19, 20, 21, 22, 25].

Cadmium chloride as a toxic agent induces histopathological alterations in the gills that are normally structured in the control fish, whereas in the experimental fish treated with LC50, hypertrophy of the lamellar epithelium, destruction of gill lamellae, and blood congestion were observed (Bais & Lokhande, 2012) ^[23].

In Liver

According to the Bais & Lokhande (2012) ^[23] the control fish has normal hepatic tissue with round nuclei in the

hepatocytes and granular cytoplasm. In contrast, experimental fish exposed to LC50 for 96 hours show degenerative changes, hepatocellular dissociation, necrosis, and hypertrophy in the liver.

The liver of fish exposed lethal concentration for 96 hrs. at LC50 (9.5%). The central vein shrinkage in fish treated with 1.9% of paper mill effluent sublethal concentration for 30 days was observed. The destructive hemorrhages into sinusoids were identified. Swelling of hepatocytes was critical that the polygonal shape of hepatocytes was lost and nuclei were swollen, hypertrophy, vacuoles form in hepatic cell. Degeneration of hepatocytes nuclei were observed in some areas. Sublethal concentrations (0.95%) of paper mill effluent exposed fish suffered visible pathological alterations after 30 days of exposure. This is characterized by the massive swelling of hepatic cells along with nuclear hypertrophy. The tissue frequently demonstrated rupture of the sinusoid and hemorrhages. At some sites, degeneration of the hepatocyte's nuclei was also detected (Pathan 2020) ^[24].

Fe showed the highest concentration among metals accumulated in the organs and tissues of *Channa punctatus*. Its levels varied between 182.33 and 1760.83 mg/kg dry weight (d.w.), with the liver having the greatest accumulation and the muscle the lowest. Similar observation were also observed for *Clarias gariepinus* (Osman, and Kloas, 2010) ^[22, 25], *Labeo rohita* (Javed and Usmani, 2011) ^[26], *Mastacembelus armatus* (Javed M. and Usmani N. 2013c) ^[17] and *Tincatinca* (Tekin-Ozan, *et al.* 2005) ^[27]. The concentration of Zn in organ / tissues of fish *C. punctatus* ranged from 0.44 – 93.33 mg Kg⁻¹d.w. The maximum levels were recorded in gills and minimum in liver. The maximum amount of Zn in *C. punctatus* was also found in the gills by other workers. (Javed and Usmani, 2012b, Shukla *et al.*, 2007) ^[28, 29].

In Kidney

Exposure to sugar mill effluent (25.75 ml/25L) induced progressively severe kidney damage over time. Control samples showed normal kidney structure, but after 24 hours of treatment (25.75 ml/25L effluent), slight damage appeared, including pyknosis (shrinking of the cell nucleus) and slight vacuolization of the renal cells. By 48 hours, damage escalated with increased vacuolization, hemorrhages, and lipid globules, indicating significant cellular stress. By 72 hours, the kidney tissues exhibited sustained injury, with pronounced pyknosis, persistent lipid globules, and necrosis, suggesting ongoing tissue degeneration. The most severe histological changes occurred after 96 hours, marked by extensive cellular degeneration, extensive hemorrhage, significant vacuolization, pronounced edema, and necrosis. The damage was widespread, with profound hemorrhage, extensive necrosis, large vacuolization, and pronounced edema defining the worst conditions (Prakash & Capoor 2018) ^[9].

Exposure to paper mill effluents significantly affected fish kidneys. At sublethal concentrations (1.9%) for 30 days, severe necrosis caused disintegration of hemopoietic tissue, glomerulus blockage with stained blood, dilated renal

tubules with pyknotic nuclei, and broken blood capillaries. At 0.95% concentration for 30 days, less severe effects were observed, including hypertrophy of hemopoietic tissue with necrosis and dilated renal tubules with pyknotic nuclei, showing tubular atrophy. The severity increased with higher concentrations, demonstrating progressive kidney damage (Pathan 2020) [24].

The Fish's kidney changes over time were measured after the mercuric chloride (16.7ppb) exposure on 7,28,63, and 90 days. Renal lesions revealed tubular nephrosis, karyolytic necrosis of tubular epithelial cells, mild to moderate multifocal acute lesions, and dilation or shrinkage of Bowman's capsule and glomerulus. Active mercury elimination and the rapid phases may explain the presence of epithelial cell necrosis in the posterior kidney (Banerjee and Bhattacharya, 1994) [30].

Exposed fish presented skin and gill erosion, changes in kidney Malpighian corpuscle structure, spleen hemosiderosis was more intense, and increased neoplastic lesions were observed. (Pacheco, & Santos, 2002) [31].

In Heart: The study observed pathological alterations in the structure of the fish heart at different times of treatment durations with effluent from the sugar mill. The control group showed normal heart structures such as endocardium, myocardium, and epicardium with well-oriented cardiac muscles and nuclei. In the treated groups (24 hours, 48 hours, 72 hours, 96 hours, and 1 week), the fish heart exhibited significant histological changes, with the prominent effects observed after 1 week of exposure to sugar mill effluent. Changes include splitting of muscle fibers, dislocation of nuclei, muscular atrophy, pigment accumulation, loss of striation in cardiac muscle, destruction of longitudinal tissues, and necrosis (Prakash and Capoor, 2018) [9]. Areechon and Plumb (1990) [32] reported a similar finding after malathion exposure in fish, Almeida *et al.*, (2001) [33] reported it in freshwater fish affected by cadmium and Zhang *et al.*, (2005) [34] reported it in goldfish exposed to heavy metals.

In Ovary: The ovary of *Rasbora daniconius* exhibited considerable degenerative changes such as Complete absorption of oocytes, ruptured zona radiata with remnants in the lumen, and degeneration of oocyte cytoplasm showing what appeared to be liquification and clumping. Thickening of ovarian wall and cytoplasmic vacuolation with lack of nucleus in mature oocytes on treatment with paper mill effluent (Pathan *et al.*, 2012) [35].

In Stomach: The control fish stomach has a normal epithelial duct, submucosa, and blood capillaries. The experimental group treated with LC50 for 96 hours showed signs of hyperchromasia in the epithelial cells, breakdown of the epithelial duct, and gastric mucosal desquamation (Bais & Lokhande, 2012) [23].

In Intestine: According to Bais & Lokhande, (2012) [23], in the control fish, the intestine had normal structures, such as columnar epithelium, circular muscle fibers, and a normal submucosa. However, the experimental group exposed to

LC50 for 96 hours showed degenerative changes like hydropic degeneration in the tips of the villi, loss of mucosal fold structural integrity, and mucosal epithelial changes, such as cloudy swelling and necrosis.

Conclusion

Wastewater from sugar mills contributes significantly to water pollution, creating a major risk to the health of freshwater ecosystems and fishes. This review shows that these wastes are rich in organic matter, heavy metals and harmful compounds, which can significantly alter water quality indicators such as BOD, COD, pH and dissolved oxygen levels. When fishes are continuously exposed to untreated or poorly treated wastewater, it can cause severe damage to their vital organs like gills, liver, kidneys, heart, stomach, intestines and ovaries. These harmful changes can prevent the growth, reproduction and survival of fishes, finally causing to a loss of biodiversity and ecosystem balance. To prevent prolonged ecological damage, it is necessary to effectively treat sugar mill wastewater, strictly adhere to environmental regulations and adopt sustainable industrial practices. Future research should aim to create cost-effective and environmentally friendly treatment technologies as well as assess the recovery potential of affected aquatic species.

Reference

1. Latha A, Vardhini M, Monisha R, Shobika B. Environmental Impacts of Sugarcane Industry – A Case Study on Kurungulam Mills in Thanjavur, India. *IJERT*,2018:6(02).
2. Saranraj P, Stella D. Impact of Sugar Mill Effluent to Environment and Bioremediation: A Review. *World Applied Sciences Journal*,2014:30(3):299-316,
3. Gunkel G, Kosmol J, Sobral M, Rohn H, Montenegro S, Aureliano J. *et al.* Sugar cane industry as a source of water pollution-Case study on the situation in Ipojuca River, Pernambuco, Brazil. *Water, Air, and Soil Pollution*,2007:180(1-4):261-269.
4. Memon AR, Soomro SA, Ansari AK. Sugar Industry Effluent–Characteristics and Chemical Analysis. *J. App. Em. Sc*,2006:1(2):152-157.
5. Patil MAK, Kulkarni MS. A Review on Sugar Factory Wastewater: Parameters and it's Treatment Technologies. *IRJET*,2022:09(06).
6. Siddiqui WA, Waseem M. A Comparative Study of Sugar Mill Treated and Untreated Effluent- A Case Study. *Oriental Journal of Chemistry*,2012:28(4):1899-1904.
7. Kohle AS, Sarode AG, Ingale SR. Study of effluent from Sugarcane industry. *Sodh, Samiksha aur Mulyankan*, 2008, 0974-2832.
8. Deshmukh KK. Environmental Impact of Sugar mill Effluent on the Quality of Groundwater from Sangamner, Ahmednagar, Maharashtra, India. *Research Journal of Recent Sciences*,2014:3:385-392.
9. Prakash S, Capoor A. Sugar Mill Effluent Induced Histological Changes in Kidney of *Channa punctatus*. *Journal of Advanced Laboratory Research in Biology*,2018:9(1):32-35.

10. Maleki A, Mahvi AH, Vaezi F, Nabizadeh R. Ultrasonic degradation of phenol and determination of the oxidation by-products toxicity. *Iran. J. Environ. Health Sci. Eng.*2005(2):201–206.
11. Ebrahimpour M, Alipour H, Rakhshah S. Influence of water hardness on acute toxicity of copper and zinc on fish. *Toxicol. Ind. Health*,2010;26(6):361– 365.
12. David A, Ray P. Studies on the pollution of river Daha (N. Bihar) by sugar and distillery wastes. *Environ. Health*,1966;8(1): 6-35.
13. Devarajan M, Priya R, Sasikala M, Babu M, Ganapathi N, Vedapuri A. *et al.* Toxicity and behavioural changes in freshwater fish *Tilapia mosaambica* exposed to sugar mill effluent. *Int. J. Adv. Res. Biol. Sci*,2015;2(10):207-211.
14. Xia C, Fu L, Liu H, Chen L, Liu Y. Aquatic toxic analysis by monitoring fish behavior using computer vision: A recent progress. *J. Toxicol*,2018(1):251924 ref.48.
15. Doke KM, Khan EM, Rapolu J, Shaikh A. Physico-chemical analysis of sugar industry effluent and its effect on seed germination of *Vigna angularis*, *Vigna cylindrical* and *Sorghum cernum*. *Annals of Environmental Science*,2011;5:7-11.
16. *International Journal of Pharmaceutical and Biological Archives*,2(5):1469-1472.
17. Javed M, Usmani N. Investigation on accumulation of toxicants and health status of freshwater fish *Channa punctatus*, exposed to sugar mill effluent. *Int J Zoo Res*,2013;3(1):43-8.
18. Kakade VB. Study on toxic impact of sugar Factory Effluent on the Gill of the Fresh Water fish *Rasbora Daniconius*. *IJEAB*,2017;2(4). <http://dx.doi.org/10.22161/ijeab/2.4.56>
19. Yousafzai AM, Chivers DP, Khan AR, Ahmad I, Siraj ML. Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. *Pakistan J Zoo*,2010;42:537–544.
20. Nwajei GE, Obi-Iyeke GE, Okwagi P. Distribution of selected trace metal in fish parts from the River Nigeria. *Res J Rec Sci*,2012;1(1):81-84.
21. Coetzee L, Preez HH, Vuren JV. Metal concentrations in *Clarias gariepinus* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. *Water SA*,2002;28:433–448.
22. Osman AG, Kloas W. Water quality and heavy metal monitoring in water, sediments, and tissues of the African Catfish *Clarias gariepinus* (Burchell, 1822) from the River Nile, Egypt. *Journal of Environmental Protection*,2010;1(04):389.
23. Bais UE, Lokhande MV. Effect of cadmium chloride on histopathological changes in the freshwater fish *Ophiocephalus striatus* (Channa). *International Journal of Zoological Research*,2012;8(1): 23.
24. Pathan TS. Histopathological Changes in Liver and Kidney of Freshwater Fish, *Rasbora Daniconius* Exposed to Sub lethal Concentration Paper mill Effluent. *J. Exp. Zool. India*,2020;23(1):41-46.
25. Osman AG, Kloas W. Water quality and heavy metal monitoring in water, sediments, and tissues of the African Catfish *Clarias gariepinus* (Burchell, 1822) from the River Nile, Egypt. *Journal of Environmental Protection*,2010;1(04):389.
26. Javed M, Usmani N. Accumulation of heavy metals in fishes: a human health concern. *Int J Environ Sci*,2011;2(2):659–670.
27. Tekin-Ozan S, Kir I. Comparative study on the accumulation of heavy metals in different organs of tench (*Tinca tinca* L. 1758) and plerocercoids of its endoparasite *Ligula intestinalis*. *Parasitology research*,2005;97:156-159.
28. Javed M, Usmani N. Toxic effects of heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) to the haematology of *Mastacembelus armatus* thriving in Harduaganj Reservoir, Aligarh, India. *Global J Medical Res*, 2012;12(8):59–64.
29. Shukla V, Dhankhar M, Prakash J, Sastry KV. Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. *J Environ Bio*,2007;28(2):395-397.
30. Banerjee S, Bhattacharya S. Histopathology of kidney of *Channa punctatus* exposed to chronic nonlethal level of elsan, mercury and ammonia. *Ecotoxicol. Environ. Saf*,1994;29(3):265- 275.
31. Pacheco M, Santos MA. Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). *Ecotoxicology and Environmental Safety*,2002;53(3): 331-347.
32. Arechon N, Plumb JA. Sublethal effects of malathion on channel catfish, *Ictalurus punctatus*. *Bull. Environ. Contam. Toxicol*,1990;44(3):435-442.
33. Almeida JA, Novelli EL, Dal Pai Silva M, Júnior RA. Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environ. Pollut*,2001;114(2):169-175.
34. Zhang YM, Huang DJ, Wang YQ, Liu JH, Yu R, Long J. *et al.* Heavy metal accumulation and tissue damage in goldfish, *Carassius auratus*. *Bull. Environ. Conta. Toxicol*,2005;75(6):1191-1199.
35. Pathan TS, Thate PB, Shinde SE, Sonawane DL. Histopathological effects of paper mill effluent in the ovary of a fresh water fish, *Rasbora daniconius*, *Journal of Fisheries and Aquaculture*,2012;3(1):29-32.
36. Singh DP, Upadhyay SK, Sharma V, Kumar N. Effect of endosulfan on ATPase activity in liver, Kidney and muscles of *Channa punctatus* and their recovery response. *Bulletin of Pure & Applied Sci- Zoology*, 2018;37(1):21-26.
37. Upadhyay SK, Environmental impact on helminth parasites of fresh water garfish from river Yamuna at Allahabad, Uttar Pradesh, India. *Proc. Zool. Soc. India*,2017;16(2):63-75.
38. Babita Panwar P, Upadhyay SK. A review on ecosegregation and parasitocenosis of *Helminthes* perspective to health and sustainability. *Bulletin of Pure*

- and Applied Sci. Zool,2019:38A:40-51.
39. Sahu O, Rao DG, Gopal R, Tiwari A, Pal D. "Treatment of wastewater from sugarcane process industry by electrochemical and chemical process: Aluminum (metal and salt)"Science Direct,2017:7:50–62.
 40. Ozoh PT, Oladimeji AA. Effects of Nigeria dyestuff effluent on germination latency, growth, and gross growth of *Zea mays*. Bulletin of environmental contamination and toxicology,1984:33:215-219.
 41. Kisku GC, Barman SC, Bhargava SK. Contamination of soil and plants with potentially toxic elements irrigated with mixed industrial effluent and its impact on the environment. *Water, air, and soil pollution*,2000:120:121-137.
 42. Baruah AK, Sharma RN, Borah GC. Impact of sugar mill and distillery effluents on water quality of river Gelabil Assam. *Indian Journal of Environmental Health*,1993:35(4):288-293.
 43. Limbani P, Shah M. Impact of Sugar Industry Effluent on Nearby Water Body: A Review. *IJTSRD*,2018:2(2): 1456-1459.
 44. Agrawal V, Pandey N. Toxicity and behavioural changes in freshwater fish *Labeo rohita* exposed to amlai paper mill effluent. *Gurukul Shodh Srijan*, 2014:1(1).
 45. Sarwade JP. Toxicity and behavioural changes in freshwater fish, *Puntius stigma* exposed to paper mill effluent. *Int. J. Innovation in Biological and Chemical Sciences*,2015:6:36-44.
 46. Prakash S, Verma AK. Impact of Arsenic on Protein Metabolism of a fresh water cat fish, *Mystus vittatus*. *Uttar Pradesh Journal of Zoology*,2020:41(5):16-19.
 47. Srivastava NK, Prakash S. Morphological, behavioural and haematological alterations in catfish, *Clarias batrachus* (Linn.) after acute zinc toxicity. *International Journal on Biological Sciences*,2018:9(1):72-78.
 48. Prakash S, Verma AK. Acute toxicity and Behavioural responses in Arsenic exposed *Mystus vittatus* (Bloch). *International Journal on Agricultural Sciences*, 2019:10(1):01-0310.
 49. Ghosh TK, Konar SK. Effect of Sugar mill complex effluent on fish Growth and Reproduction. *Environment and Ecology*,1992:10(4):794-803.
 50. Shivappa D, Puttaiah ET, Kiran BR. Physico-chemical characteristics of sugar mill effluent. *J. Industrial Pollution Control*,2007:23(2):217-221.
 51. Bhatt C, Verma R. Physico-Chemical Analysis of Sugar Mill Effluent of Kabirdham (CG), 2018.
 52. Lahare G, Yadav M. Waste Water Management in Bhoramdeo Sugar Mill Factory. *International Journal for Research in Applied Science and Engineering. Technology*,2021:9(4).