Emerging pollutants characterization, mitigation and toxicity assessment of sewage wastewater treatment plant- India: A case study

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Abstract

India faces major challenges related to fresh water supply and the reuse of treated wastewater is an important strategy to combat water scarcity. Wastewater in Gorakhpur, India, is treated by a decentralised wastewater treatment system (DEWATS) and the treated wastewater is reused in the rural area. This research provides important scientific data that ascertain the safety of wastewater reuse in this region. The physicochemical characteristics, including pigment, ionic strength, BOD, COD, TDS, TSS, salinity, total N, ammonium N, phenolics, heavy metals, and sulphate, of the inlet and outlet sewage water samples (SWWs) from a wastewater treatment facility was conducted. These parameters were found to be significantly over the national limit. The inlet and outlet samples were further characterised by using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR) and gas chromatography-mass spectrometry (GC–MS). SEM showed microstructure and the presence of various metals, polymers, and other co-pollutants in the samples and FT-IR confirmed the presence of aldehyde, hard liquor, and nitrogen molecules in the SWW's discharge. Many endocrine disruptors and potentially mutagenic chemical substances (e.g., Dodecane, Hexadecane, Octadecane etc.) were identified in the outlet SWW by the GC-–MS analysis. Toxicity of the SWW was assessed via phytotoxicity assessment using Phaseolus mungo L. and histological and biochemical analyses of *Heteropneustes fossilis* in a 24-h exposure study. Results confirmed the wastewater was harmful and inhibited germination of P. mungo L. by >80% compared to the control, destroyed gill laminae and significantly increased oxidative stress (above 5% increase in catalase production) in *H. fossilis*. This work clearly demonstrated that the quality of the treated wastewater in Gorakhpur was poor and immediate action is needed before it can be discharged or reused.

Keywords: wastewater, metals, organometallic compounds, endocrine disruptors, phytotoxicity, cytotoxicity

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1. Introduction

In India, a larger proportion of urban and rural inhabitants rely only on groundwater. The chemical composition of groundwater is heavily influenced by bedrock composition, dissolution, contributions from surrounding aquifers, surface soil percolation rate, and anthropogenic activities (Haghnazar et al., 2022; Paikaray and Chander, 2022). Sewage wastewater contamination of groundwater is a serious environmental and health challenge (Ayat et al., 2021). Rapid industrial development and expansion, population growth, urbanization, insufficient sewage systems, urban and agricultural runoffs, and human and animal wastes all contribute to sewage wastewater contamination (Minhas et al., 2022; Manikandan et al., 2022; Krishna et al., 2022). The top three sewage wastewater producers are the United States, India, and China. In India, the Central Pollution Control Board (CPCB) estimated that sewage wastewater generation ranges from 200 to 600 g/day per capita in cities based on factors such as socioeconomic status (CPCB, 2000, 2006). The sewage discharge composition changes according to geographic location and style of living (Tripathi et al., 2021d, 2021e). Before sewage is discharged into the receiving water, it must be properly treated to remove suspended particles, organic wastes, and nutrients. Agricultural activities, such as fertilizer use and pesticide spraying in the fields, also change the chemical composition of sewage wastewater (Liu et al., 2021). As a result, it is critical to monitor sewage effluent regularly to establish an appropriate water management system. More than 90 % of the sewage wastewater is used for agricultural irrigation, the remaining supply is recycled and reused for drinking water (Ali et al., 2019; Sishodia et al., 2016). However, it is difficult in developing countries to obtain reliable wastewater reuse and recycling information due to a lack of records. The production of sewage effluent is frequently linked to economic, demographic, and social variables. The improper management and maintenance of sewage wastewater (SWW) in developing nations is a recognized cause of environmental harm (Ferronato and Torretta, 2019). Poor sewage wastewater management degrades the ecosystem, resulting in pollution of the water, land, and air. Treated sewage wastewater is an important water source in subtropical and water scare regions. Sewage cleansing and conservation are critical in many mid-latitude areas (Mayes et al., 2021; Daily et al., 2021), arid and semi-arid countries such as India (Tripathi et al., 2021a, 2021b). Sewage recycling is practised in North India: Uttarakhand (Ravindra et al., 2019), Delhi (Dimri et al., 2021), Uttar Pradesh (Singh et al., 2019); Middle India: Maharashtra (Singh et al., 2019) and South India: Andhra Pradesh (Ravindra et al., 2019). Gorakhpur in the state of Uttar Pradesh uses decentralised wastewater treatment (DEWATS) to treat their wastewater and reuse them in rural areas (Verma et al., 2022). A number of environmental regulatory bodies throughout the globe have concluded that SWW is detrimental to human and environmental health, including the World Health Organization, UNEP and EPA (US EPA), therefore, the safety of the treated wastewater in Gorakhpur needs to be determined and established.

Many studies examined point data on physicochemical properties to determine if treated sewage effluent could be reused. One of the cost-effective and efficient methods is to conduct regular environmental monitoring of plants in the affected areas (Dailianis et al., 2021; Blaise et al., 2018). The Phaseolus mungo L. seed germination test is widely considered one of the simplest, most precise and affordable phytotoxicity assays for industrial effluent (Barathi et al., 2020; Ibrahim et al., 2022). It is important to measure the susceptibility of animals to toxicants, as well as assess the degree of damage to target organs that may cause physiological, biochemical, and behavioural abnormalities (Tripathi et al., 2022a, 2022b). Information on the toxicity and impacts on specific local species is needed to augment risk assessment studies. *Heteropneustes fossilis* is a freshwater fish with a high yield potential, often found in ponds, ditches, swamps, marshes, and in the rice fields of Southeast Asia (Khanom et al., 2022; Samim et al., 2022). This species has become increasingly popular due to its delicious taste, appealing market price, and medicinal and nutritional value. It has been proven to be a good candidate for aquaculture due to its very hardy nature. The presence of the accessory respiratory organs also enables this species to survive additional few hours even if outside the water (Billah et al., 2022; Saha et al., 2022). Although *H. fossilis* breeds in confined waters during the monsoon months, they can also breed in ponds, derelict ponds, and ditches when sufficient rainwater accumulates, which makes this fish one of the most susceptible species to exposure to aquatic pollutants. In the present study, as a model fish, fry of freshwater stinging catfish, *H. fossilis* was selected to evaluate the LC50 mediated toxicity. Histopathological observations of major organs and changes in haematological parameters were investigated to understand the probable threats elicited by this wastewater during the early life stages of this fish in the wild. The aim of this research is to establish scientific data concerning the physicochemical properties in the SWWs at a 30 MLD sewage treatment facility in Gorakhpur, India, and to investigate the toxicity of the contaminants using a germination study of *Phaseolus mungo* L. and histological and biochemical analyses of *Heteropneustes fossilis*. Studies on the quality of SWW treated by DEWATS reported mainly on their physicochemical properties and performance (e.g., Singh et al., 2019; Verma et al., 2022). Few studies on SWW measured both phytotoxicity and piscine toxicity, the information gathered will be useful for environmental pollution management and monitoring. This detailed study will provide much-needed information on the aquatic toxicity of SWW to improve their management and safe reuse in the region.

2. Materials and methods

2.1 Sampling site

The sewage effluent sampling site was situated around the 30MLD STP in Gorakhpur (Latitude- 26.713291 and Longitude- 83.408798) (Fig. 1), and the control was taken from upstream where the river water did not mix with the DEWATS effluent (Saleh et al., 2021). The 30MLD STP received wastewater around 30 million litres per day (MLD). The wastewater was taken from the inlet and outlet in pre-sterilized 20-20 L plastic jerrycans (Tarsons Products Pvt. Ltd.). To avoid any thermal deterioration, the samples were collected in duplicate and stored at 4 °C. It was transported to the laboratory on the same day and the physicochemical analyses were carried out within 24 h. The SWWs were evaluated using major water quality metrics in this study and compared with the guideline values published by the National Environmental Management Authority (NEMA).

2.2 Reagents and chemicals

Researchers from Merck, SRL (Sisco Research Laboratories in India), and Sigma-Aldrich collaborated to offer the compounds for GC–MS analysis, as well as other reagents and precursor chemicals (St. Louis, MO, USA). All the reagents and chemicals used in this study were of Analytical Grade (Sigma-Aldrich, St. Louis, MO, USA).

2.3 Physicochemical analysis

The physicochemical analysis will be made according to the standard methods for the examination of wastewater (American Public Health Association Vision/Mission, 2011). The pH of the collected effluent sample was measured by Orion ion meter (Model - 960), COD by open reflux method, BOD by 5-day method, total solid (TS) by drying method, total nitrogen (TN) by (TOC – Vcsn analyser Shimadzu, Japan). Phosphate and sulphate were measured by vanadomolybdo – phosphoric acid colorimetric and $BaCl_2$ precipitation methods respectively. Different ions e.g. sodium, nitrate, chloride and potassium ion will be analysed by ion meter (Orion ion meter - 960) by their selective electrode. Heavy metals in the effluent were analysed by means of acid digestion (1HNO_3:3HCL), following the standard method for the examination of water and wastewater (American Public Health Association Vision/Mission, 2011) after this, the metal analyses were carried out on inductively coupled plasma (ICP) spectrophotometer (IRIS Intrepid II XDL: Thermo Electron, Waltham, Mass., USA).

2.4 SEM-EDXS analysis

Using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDXS), the surface morphology of dissolved contaminants in SWW samples was investigated. The specimens were cooled in desiccators after oven-dried for 4 to 5 h at 600 °C in aluminium foil in a muffle furnace (Merck KGaA, Darmstadt, Germany). The samples were then surface coated with palladium and gold and attached to the electron micropore stubs. Controlled field emission SEM (JOEL JSM-6330f, JOEL Ltd. Dearborn Rd, USA) with a WVEX-EDS sensor network was used to analyse the material's morphology in the SEM/EDXS study (Asberry et al., 2014; Tripathi et al., 2021c, 2021d).

2.5 Ultraviolet–visible and Fourier transform infrared spectroscopic analyses

A double-beam spectrophotometer (Evolution-201, Thermo Scientific, USA) was used to analyse the UV absorption patterns of different pollutants in the SWW at room temperature, within a wavelength range of 200–700 nm. The samples were also examined using Fourier transform infrared spectroscopy (FTIR) (Nexus-890 from Thermo Electron Co., Japan) (Tripathi et al., 2021a, 2021b, 2021c; Chandra et al., 2018).

2.6 Detection of organic pollutants

The continuous phase extraction (liquid–liquid extraction) approach was utilized to pre-treat the SWW samples (Tripathi et al., 2021a) and the organic contaminants present were analysed using GC–MS analysis obtaining low detection limits (between 0.01 and 6.4 ng g^{-1}).

2.6.1 Derivatisation of the extracted sample

GC vials were filled with 300 L of the SWWs extract and nitrogen gas was used to evaporate it to dryness. It was also employed to derivatize the 300-µl samples with the addition of pyridine and trimethylsilyl BSTFA and TMCS. It was heated to 70 °C for 30 min with intermittent stirring of the residue before being injected for GC–MS measurement as previously described and indicated (Tripathi et al., 2022a, 2022b; Chandra and Kumar, 2017).

2.6.2 GC-MS analysis

Derivation of trimethylsilyl (TMS) of the SWW organic components was carried out according to Chandra and Kumar (2017). Briefly, Trimethylsilyl BSTFA (TMCS) was used to treat 300 mL of samples in this technique where they were heated to 70 °C for 30 min stirring from time to time. A functionalized sample aliquot (2.0 mL) was automatically fed into a GC–MS (Thermo Scientific Trace GC-Ultra Gas Chromatograph, USA) (Chandra and Kumar, 2017). The organic compounds in this study were compared to the mass spectra and retention periods in the NIST library (https://www.nist.gov/).

2.7 Phytotoxicity assay through seed germination experiment in SWW

The *Phaseolus mungo* L. seed germination test was used to assess the phytotoxicity of the SWW samples. Seed germination was assessed using various effluent concentrations as previously described (David Noel and Rajan, 2015). Ten sterilized seeds were evenly spaced in ten separate Petri dishes. For the dishes, SWW concentrations of 25%, 50%, and 100% (v/v) were added to the dishes; tap water served as the control. The dishes were incubated at 28 °C until sprouting (Tripathi et al., 2021a). Three duplicates of each concentration were carried out to ensure statistical significance. The percent toxicity and stress resistance index was calculated using the formulae below (Quaratino et al., 2007).

$$Percentage\ Phytotoxicity\ (\%) = \frac{radical\ length\ of\ the\ control - radical\ length\ of\ test\ sample}{radical\ length\ of\ the\ control} \times 100 \quad (1)$$

$$Index\ of\ tolerance\ (\%) = \frac{mean\ length\ of\ the\ longest\ root\ in\ the\ treatment}{mean\ length\ of\ the\ longest\ root\ in\ the\ control} \times 100 \quad (2)$$

2.8 Toxicity of SWWs on *Heteropneustes fossilis*

The toxicity test was carried out in compliance with the ethical committee's local and national guidelines for animal research.

2.8.1 Fish collection, maintenance and experimental setup

During the respawning season (July), 35–45 g of young and strong juvenile *Heteropneustes fossilis* were purchased from a local fish market (Mishra and Chaube, 2017). The fish were acclimatized for a week in the laboratory at a regular photoperiod and temperature (12:12 h, light to dark, and 25 °C). During the acclimation stage, the fish were fed goat liver dietary carbohydrate regularly. The toxicity of the SWW was assessed by determining the LC50 in a 24-h exposure experiment. The fish were separated into four groups of 10 fish each (n = 2) in duplicate. The SWWs were given to three groups (1.0%, 5.0%, or 10%), while the fourth group was kept in plain freshwater as a control. The LC50 was calculated using mortality data collected after 24 h. The gills and liver of the fish were taken after they were killed in the lab. Gills were preserved in Bouin's fixative for anatomical inspection, and livers were maintained at -20 °C until further study. Catalase activity was used to assess the stress level generated by wastewater exposure.

2.8.2 Histological and catalase study

The snipped gill sections were dehydrated through a succession of calibrated ethanol after 24 h in the Bouin's fixative and then embedded in filtered paraffin (54 to 62 °C). The rotator microtome (Weswox, India) was used to slice paraffin-embedded gills into 5 m-thick sections. After passing through a series of calibrated water and ethanol, the slices were double-stained with hematoxylin and eosin, washed in xylene, and mounted in DPX to photomicrograph. The activity of the antioxidant enzyme catalase (CAT) in liver tissues was measured and analysed (Keramati et al., 2010). In a nutshell, 0.1 mL of supernatant was added to 2.9 mL of freshly manufactured 30 Mm H_2O_2 in phosphate buffer (pH 7.0), and the optical density was monitored for 1.0 min at 240 nm with a UV–Vis's spectrophotometer (EVOLUTION 201, Thermo Scientific).

2.9 Statistical analysis

Each experiment was done in triplicate to ensure accuracy. All data are provided as a mean standard deviation (SD) value, with the SD having been computed using Microsoft Excel (version 2007, Microsoft®, USA). Student's *t*-test in SPSS was used to compare the means of many physicochemical properties for the control and pollutants that had been degraded (version 22.0; SPSS Inc., Chicago, IL, USA). The Newman–Keuls tests were used to compare the results from different groups, and a one-way ANOVA was used to establish the statistically significant results (P 0.001) and (P 0.05). Tukey's analysis was also performed using GraphPad software (GraphPad Software, San Diego, CA, USA) after one-way ANOVA to see whether the results varied or differed (Kim, 2014; Chandra et al., 2018).

3. Results and discussion

3.1 Physicochemical characterisation of SWW

The SWWs were brown in color and had an affected odour. As indicated in Table 2, the levels of BOD, COD, and other pollution parameters were extremely high in the inlet sample of SWWs and decreased in the outlet sample. However, the values of the outlet samples remain significantly higher than the regulatory bodies' (USEPA, 2002) industrial discharge permitted limits and their detected method and instruments shown in (Table 1). As previously documented in other wastewater research, the presence of a significant amount of dissolved organic matter, as well as calcium, magnesium, and nitrogen compounds (Table 2), may have contributed to the high BOD and COD of effluent (Choi et al., 2017; Tripathi et al., 2021a; Chandra et al., 2008a, 2008b; Sankaran et al., 2014). Different soluble salts and heavy metals combine to form organometallic compounds with complicated structures (Sankaran et al., 2014; Chandra et al., 2018). Furthermore, the proliferation of BOD, COD, TSS, TDS, volatile solids, total N, phenolics, sulfates, and phosphate might be attributed to huge amounts of unidentified chemicals and hazardous chemicals created during the processing of wastewater (Aniyikaiye et al., 2019). The elevated concentrations of heavy metals in the SWW could be attributed to the deterioration of metallic connections, digester vessels, and, to a slightly lesser extent, raw materials conveyed by sewage wastewater (Bortoletto et al., 2018). The results indicated the wastewater treatment plant was not sufficiently effective in improving the quality of the wastewater. The high levels of metals remaining in the wastewater were a great concern, especially in terms of wastewater reuse, such as the irrigation of crops.

Table 1
Methods/instruments for physical and chemical parameter analysis.

Sr. no.	Parameters	Analysis methods/instruments
1.	pH	pH meter
2.	Electrical conductivity	Conductivity method, electrical conductivity meter
3.	Turbidity	Turbidity meter/nephelometer
4.	Color	Sensory test
5.	BOD	5-day method/ BOD Sensor
6.	COD	colorimeter or spectrophotometer/open reflux and closed reflux.
7.	Total Nitrogen	Nitrogen analyzers/The Kjeldahl method
8.	Carbonates	Titration (standard solution of strong acid)
9.	Bicarbonates	Titration (standard solution of strong acid)
10.	Alkalinity	Titration (standard solution of strong acid)
11.	Calcium	Calcium meters
12.	Chloride	Titration (silver nitrate solution)
13.	Fluoride	Fluoride electrode method (water + zirconyl xylenol orange complex reagent)
14.	Hardness	Titration (standard solution of strong acid)
15.	Magnesium	EDTA (ethylenediaminetetraacetic acid)
16.	Sodium	Flame photometer
17.	Nitrate	Visible spectrophotometer
18.	Potassium	Flame photometer
19.	Sulphate	Titration (barium chloride)
20.	TDS	TDS meter/ gravimetric method

3.2 Morphological examination of pollutants

The analyses indicated that both the inlet and outlet SWW samples have a sophisticated and distinctive form, as illustrated in Fig. 2a and b. The SEM analysis showed enlarged rods that were cylindrical in shape in the outlet samples, whereas globular-like structures were found in the inlet samples, commonly discovered in SWW (Speiser et al., 2001; Manohara and Belagali, 2017). The residue of thermal decomposition resulted in the stretched and porous look of SWW samples. The morphology of a rod and brick shape might be due to polymers or cellulose observed in SWW samples. Likewise, observations on the crystalline texture of organic molecules with their whole structure have been published previously (Remond et al., 2002; Herman et al., 2016). Earlier findings by Graminha et al. (2008) and Abdel-Shafy and Mansour (2018) revealed the existence of metallic complexes of several organic contaminants. The complicated structure of several heavy metals, polymers, and other co-pollutants was generally seen in SWW samples. SWW samples of different pore shapes and sizes were found to be anisotropic. SWW samples were much finer and more uniformly scattered. The elemental composition of the effluent was confirmed by EDX-elemental analysis. The wastewater's high salt content showed that it may be a serious pollutant in both terrestrial and aquatic environments.

The presence of residual metals in the outlet samples as indicated by the physicochemical and SEM analyses suggests the SWW requires further treatment before it can be discharged safely into the aquatic environment. For example, bioremediation involving autochthonous bacteria and phytoremediation using native plants can provide sustainable and low-cost treatment as they had been shown to be effective in reducing the metal levels (Tripathi et al., 2021b, 2022a; Sharma et al., 2021).

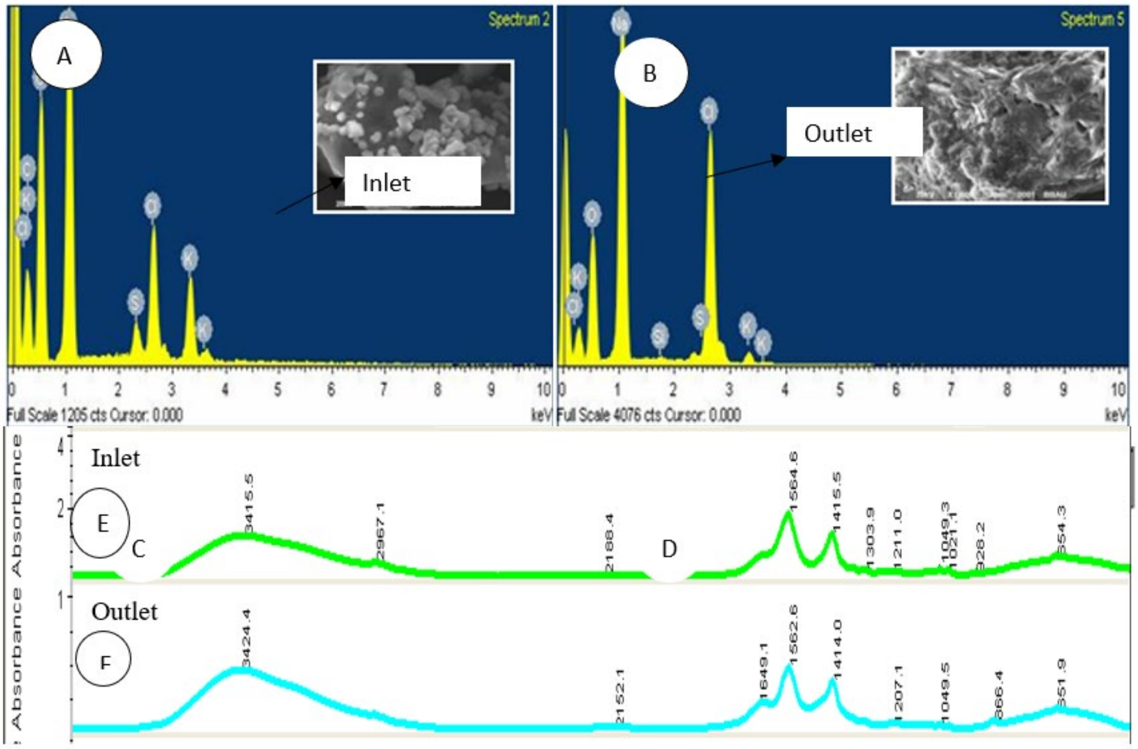


Fig. 2 (A- B) UV-Vis spectral analysis of inlet and outlet sample **(C- D)** Surface topology and elemental analysis of SWWs by SEM EDAX**, (E- F)** Fourier transforms infrared (FTIR) spectra of inlet and outlet sample of SWWs.

3.3 Ultraviolet-visibility and functional groups of compounds

The scanning of UV–Vis’s absorption spectra absorption peaks in the region of 200–250 nm is shown in Fig. 2c, d. This revealed a polymerized organic molecule as a complex color material with UV absorption. Although the absorption band had been at 220 nm, 225 nm, and 235 nm, the spectral measurement'’ absorbance maximum was at λmax = 230 nm. The defining attribute of a chemical once the polymerization procedure is completed during its production has always been maximum (Billaud et al., 2004). A combination of additional contaminants was revealed by the modest peaks in the absorption spectrum. The results of previous investigations (Echavarria-Pinto et al., 2013; Chandra et al., 2018) have likewise corroborated this finding. This study demonstrated how organic molecules were degraded with increasing dilution. Fig. 2c, d shows how little the absorbance intensities at 230 nm in SWW's primary product changed. There was also a dominant band at 230 nm, which was previously described to demonstrate the number of early complex chemicals in the polymerized product (Gu et al., 2010). This ultraviolet area is where a variety of chemical compounds have been produced in the early stages of the polymerization process at the SWW with optical absorption (Echavarria-Pinto et al., 2013). FT-IR spectroscopy was used to analyse the molecular orbitals and covalent bonds in the SWW samples. Organic, robust and weak bonds, functional groups, and polymers were found in both the inlet and outflow samples. As can be seen in Fig. 2e, the spectra of SWW samples are displayed in the infrared, together with their respective absorption peaks, for illustration. Functional groups with strong and weak bonds in organic compounds and polymers were analysed in the inlet and exit SWW using the FT-IR range (3500 to 500 cm-−1). There was broadband at 3415.5 cm-−1 in the inlet sample and 3424.4 cm-−1 in the output sample, with values of 2152.1, 1649.1, 1562.6, 1414.0, 1207.1, 1049.5, 866.4, and 851.9 cm-−1, respectively. The O—H stretch indicated there was an alcohol hydroxyl group and a considerable amount of alcohol. Many organic compounds were identified in the inlet and outflow SSW samples, which had a bandwidth range of 3315.5 to 2967.9 cm-−1 (Table 2). There were functional groups, such as C—N or C—C triple bonds, in the 2188.4 and 1564.6 cm-−1 broadband area. The broadband area with bond N==C==S stretching strength of medium at 1564.6 and 1415.5 cm-−1 demonstrated the presence of functional groups, particularly isothiocyanate. Absorption at 2152.1 and 14,164.0 cm-−1 distinguished the functional group of alkenes and aliphatic C—H stretching in CH3, especially C==C==C stretching and C—H, O—H. Because of its great energy and precious resources recovery potential and the cheap cost, sewage wastewater is an excellent long-term resource. The FT-IR data showed a shift in functional groups as a consequence of the exchange of metabolites in SWW during biodegradation. The functional groups C=C, C=O, and S=O, stretching, where amide, ketone, and quinones were identified, correlated with the maximum. Infrared spectra had been used to investigate various bonds and functional groups in complex SWW samples (Haberhauer et al., 2000). The structural and functional features of natural organic substance groups agreed with a study by Singh et al. (2019). The metabolization of organic components in the wastewater treatment process was indicated by the various functional groups found in the inlet and output samples. Chemical characterization and ecotoxicological analyses were carried out to identify the influence of the metabolites on aquatic biota due to their potentially dangerous nature.

Table 2
Physico-chemical characteristics of inlet and outlet SWW samples.

S. no.	Parameter	Inlet SWWs	Outlet SWWs	Permissible limit (USEPA, 2002)	
1.	pH	7.53 ± 0.01	7.01 ± 0.01	8.00 ± 0.01[a]	7.54 ± 0.01
2.	BOD (mg L^{-1})	8000.11 ± 00.11	7687.15 ± 11.18	40.00	47.00 ± 0.00
3.	COD (mg L^{-1})	12,432.54 ± 0.65	10,150.32 ± 0.05	121.00[b]	79.00 ± 0.01
4.	TS (mg L^{-1})	6200 ± 2.00	5401 ± 9.00	–	152 ± 0.01
5.	TDS (mg L^{-1})	22,321.21 ± 1.50	17,765.86 ± 0.55	50–70	70 ± 0.00
6.	VS (mg L^{-1})	1002.32 ± 1.01	776.15 ± 1.02		20 ± 0.01
7.	Chloride (mg L^{-1})	2021 ± 1.30	1600 ± 0.44	750.00[b]	11.82 ± 0.01
8.	Total Nitrogen	999.3267 ± 4.65	754.31 ± 1.43		9.90 ± 0.00
9.	Sulphate (mg L^{-1})	7654.00 ± 1.11	4332.00 ± 4.54	750.00	
	Trace Elements				
i.	Mn (mg L^{-1})	6.76 ± 0.07	5.00 ± 0.08	0.20[b]	0.15
ii.	Cr (mg L^{-1})	2.90 ± 0.027	1.09 ± 0.065	0.05[b]	0.01
iii.	Zn (mg L^{-1})	10.65 ± 0.10	8.11 ± 0.01	2.00	1.28
iv.	Cu (mg L^{-1})	1.90 ± 0.01	1.05 ± 0.09	0.50[a]	0.19
v.	Fe (mg L^{-1})	123.65 ± 0.36	120.98 ± 0.00	2.00[a]	1.45
vi.	Pb (mg L^{-1})	1.48 ± 0.00	1.11 ± 0.00	0.05	0.02
vii.	Cd (mg L^{-1})	BDL	BDL	BDL	BDL
viii.	Ni (mg L^{-1})	3.01 ± 0.00	2.19 ± 0.01	0.10	0.04
ix.	Na (mg L^{-1})	66.23 ± 0.07	55.59 ± 0.07	0.04[a]	0.01
x.	K (mg L^{-1})	45.21±	35.45±	0.09[b]	0.02

All values are mean ($n = 3$) ± SD in mg l^{-1} except color intensity (Co-Pt unit), pH, and temperature (°C),
(BOD): - at $p < 0.001$, [b]Less significant at $p < 0.05$.

3.4 Organic pollutants characterisation

Fig. 3 shows the GC–MS/MS spectrographs of organic chemicals recovered with ethyl acetate from the SWWs samples. Tables 2 and 3 present the chemical compounds identified based entirely on the mass-to-charge proportion (m/z) at different retention times (RTs) in the inlet and outlet samples, respectively. The majority of the synthesized derivatives seem to have been lingering secondary metabolites due to the formation of complexes with each other in the existence of divalent cations with feedstock, which include numerous saturated fats such as hexadecenoic acid, octadecanoic acid, propanoic acid, dodecanoic acid, and nonacosane, that were derived from primary commodities and generated wastewater (Chandra and Kumar, 2017; Chandra et al., 2018). Some of these substances were also classified as endocrine-disrupting chemicals (EDCs) in the current study, according to the USEPA screening programme list (U.S. Environmental Protection Agency US EPA, 2012; Tripathi et al., 2022c). The EDC characteristics of numerous pollutants released by this industry remain unknown. Other substances found in the wastewater were either bacterial metabolic products or chemical additives, which can behave as mycotoxins, carcinogens, or EDCs, in addition to metals and other metalloids in released wastewater. The presence of these harmful chemicals must be removed or brought down to an acceptable level before the treated wastewater can be reused safely and prevent the chemicals from entering the human food chain.

Table 3
Identified organic pollutants by GC–MS in ethyl acetate extracts from inlet SWW sample site. (RT = Retention time, SI = Similarity Index, RSI = Reverse similarity index).

S. no.	RT	Inlet compound name	Probability	Function Vs toxicity	SI	RSI
1.	8.19	Sulfurous acid, di(2-ethylhexyl) ester	4.63	Ingestion causes burning of the mouth and throat	625	865
2.	9.25	Dodecane, 5,8-diethyl-	21.55	gastrointestinal symptoms such as nausea	490	603
3.	11.62	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	16.93	Impaired insulin signaling	466	534
4.	13.02	Heptadecane, 9-hexyl-	30.08	Burning, itching	509	559
5.	14.09	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	13.01	difficulty swallowing, vomiting	463	516
6.	15.01	5-Keto-2,2-dimethylheptanoic acid,	16.40	Irritation to eye, can cause severe burn	619	657
7.	16.39	Dodecane, 5,8-diethyl-	17.64	Skin and eye irritation, urge to vomit, Abdominal cramps	461	593
8.	17.88	Hexadecane	5.77	Skin, Eye and Respiratory Irritations	777	820
9.	18.08	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5á)-	6.72	elevated levels have a negative cardiac impact	427	479
10.	20.70	Benzene, (1-butylheptyl)-	28.07	Cause nausea, indigestion, gas, diarrhea,	497	711
11.	20.90	Benzoic acid, 3,5-dimethyl- ester	6.38	Flushing, sweating, nausea, vomiting	410	503
12.	21.32	Benzene, (3-octylundecyl)-	31.16	Skin irritations, itching, redness	475	531
13.	21.95	Nonadecane, 2-methyl-	5.95	Harmful for respiratory tract irritation,	762	834
14.	25.60	Tetradecane, 2-methyl-	5.65	Vomiting, abdominal cramps and diarrhea	750	805
15.	25.86	Phorbol 12,13-dihexanoate	4.87	Burning, itching, stinging, redness,	417	446
16.	27.73	Palmitic Acid, TMS derivative	85.67	Skin, Eye and Respiratory Irritations	614	737
17.	28.90	Hexacosane, 1-iodo-	6.65	Nausea, bloating, and diarrhea.	726	783
18.	29.00	2,5-Furandione, dihydro-3-octadecyl	10.95	Constipation, upset stomach etc.	490	511
19.	32.40	Octadecane, 5,14-dibutyl	12.01	Shortness of breath, headache,	419	470
20.	35.53	Cholestan-3-one, cyclic 1,2-ethanediylaetal	11.39	May cause coughing, wheezing	401	470
21.	37.67	2,4-Imidazolidinedione,5-[3,4-bis[(trimethylsilyl)	6.12	Some skin problems	352	391
22.	41.94	Octadecane, 1,1′-[1,3-propanediylbis(oxy)] bis-	10.36	Burning of the mouth and throat,	363	408
23.	42.49	Acetamide, N-[5-(diethylamino)-2-[(2,4-dinitrophenyl) azo]-4 methoxyphenyl]-	12.84	Gastrointestinal symptoms such as nausea,	360	402
24.	46.31	Orotic Acid, 3TBDMS derivative	8.30	Impaired insulin signaling	342	462

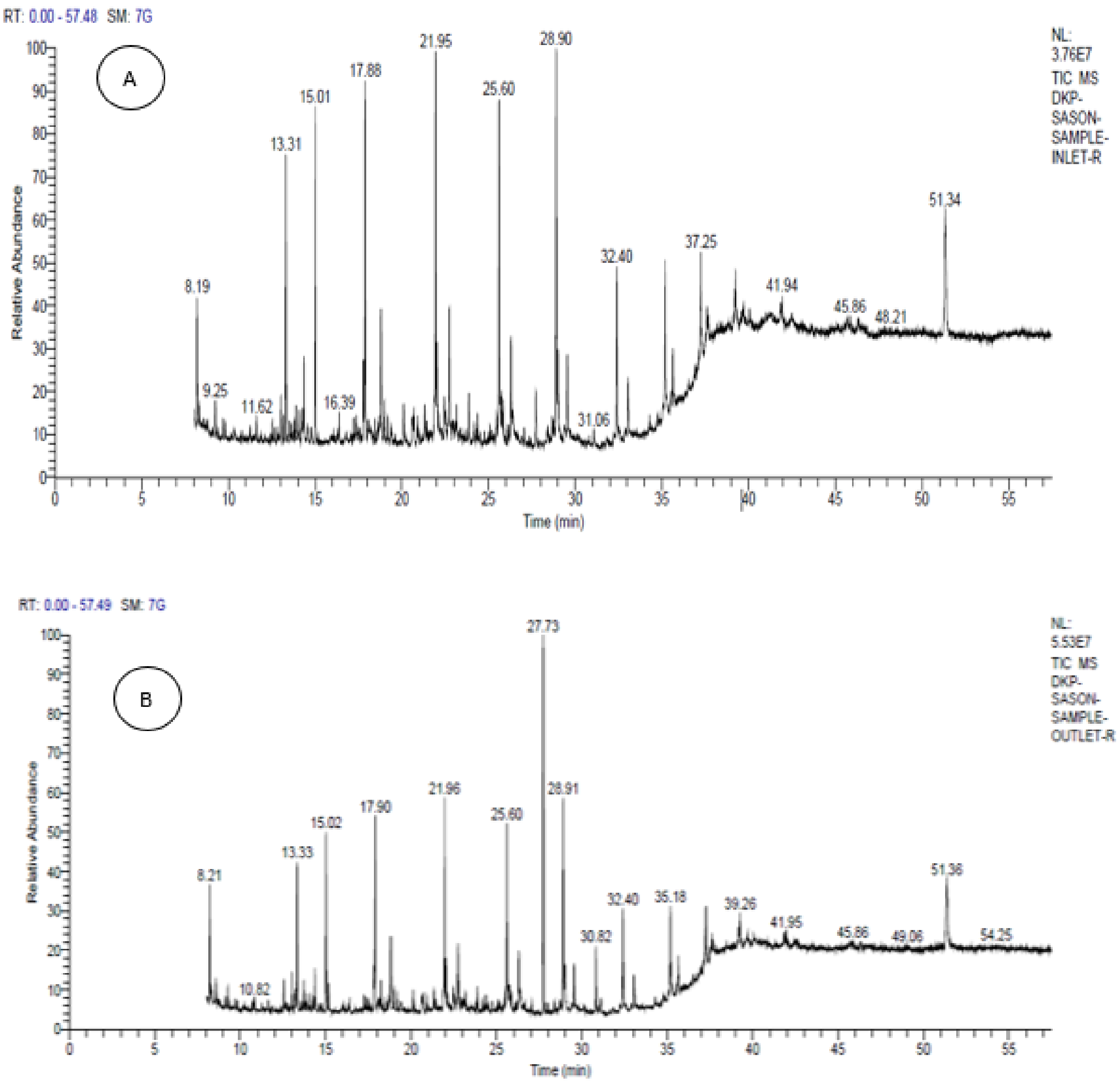


Fig. 3. Gas chromatography–mass spectrometry analysis of (a) inlet and (b) outlet SWW sample.

3.5 Phytotoxicity assessment

In comparison with the control, *Phaseolus mungo* L. seedling growth in the presence of SWW decreased by 82 and 88% after sprouting in inlet and outlet SWWs, respectively (Fig. 4). Due to the obvious greater pollution load, SWWs showed increased germination toxicity. Seed germination was inhibited in the germination experiments, which was attributable to the α-amylase enzyme inhibiting chemicals (Obuotor and Odeyemi, 2017). Similar findings have been observed in previous research (Tripathi et al., 2021a; Sourkova et al., 2020). The reaction of the seed to numerous contaminants during germination activity is a critical step in the growth of plants (Balestri et al., 2019; Zayneb et al., 2015). In this investigation, the SWW samples had a significant influence on seed germination characteristics. When Phaseolus mungo L. roots were exposed to varied concentrations of SWW samples, their root length and germination % were affected due to the high salt concentration (Table 1). Higher values indicate a higher germination efficiency and rate of germination; therefore, the germination index (GI) can be employed as a phytotoxicity indicator's includes toxins, both organic and inorganic, that can prevent seeds from germinating. According to previous studies, SWW with increased total salt concentrations generated oxidative pressure, which resulted in germination inhibition (Ramana et al., 2002). Compared to the control, all of the examined seeds treated with varying doses of SWW had significantly lower root length or damage (Fig. 4). Heavy metals, phenolics, organic contaminants, and high salt concentrations are all present in SWW. Even at relatively low concentrations (25%) of SWW, seed germination and plumule development were reduced. Seed germination, plumule, and radical development were further suppressed at greater doses, and blackening of the embryo point was observed. According to one-way ANOVA, the toxicity results at varied doses exhibited substantial variance. The amylase activity decreased as the concentration of SWW increased. Amylase is responsible for the conversion of starch into maltose, which is then converted into glucose by alpha-glucosidase to provide energy to the germinating seed. In treated plots with 25% (v/v) SWW, the optimal α-amylase activity was found to be 0.6 U. Furthermore, an increase in SWW by 50% reduced α-amylase expression by >10%. The suppression of α-amylase activity might be related to the penetration of organic pollutants as well as the cumulative toxic impact of metallic compounds on the seed as a result of the effluent concentration's endosmosis effect. Additionally, shoots and root length inhibition revealed a negative impact on plant growth hormones (i.e., indole acetic acid (IAA), gibberellic acid, and cytokinins), which seemed to be important for shoots and root lengthening. This resulted in the suppression of plant growth hormones at high effluent concentrations, as well as a negative influence on germination percentage, as reported in separate research (Chandra et al., 2008a, 2008b). Because the harmful effects of heavy metals on seed germination are well recognized, high concentrations of heavy metals owing to exceeding allowed values in SWW might potentially be attributed to the toxicity of the tested seed (Mahimairaja and Bolan, 2004).

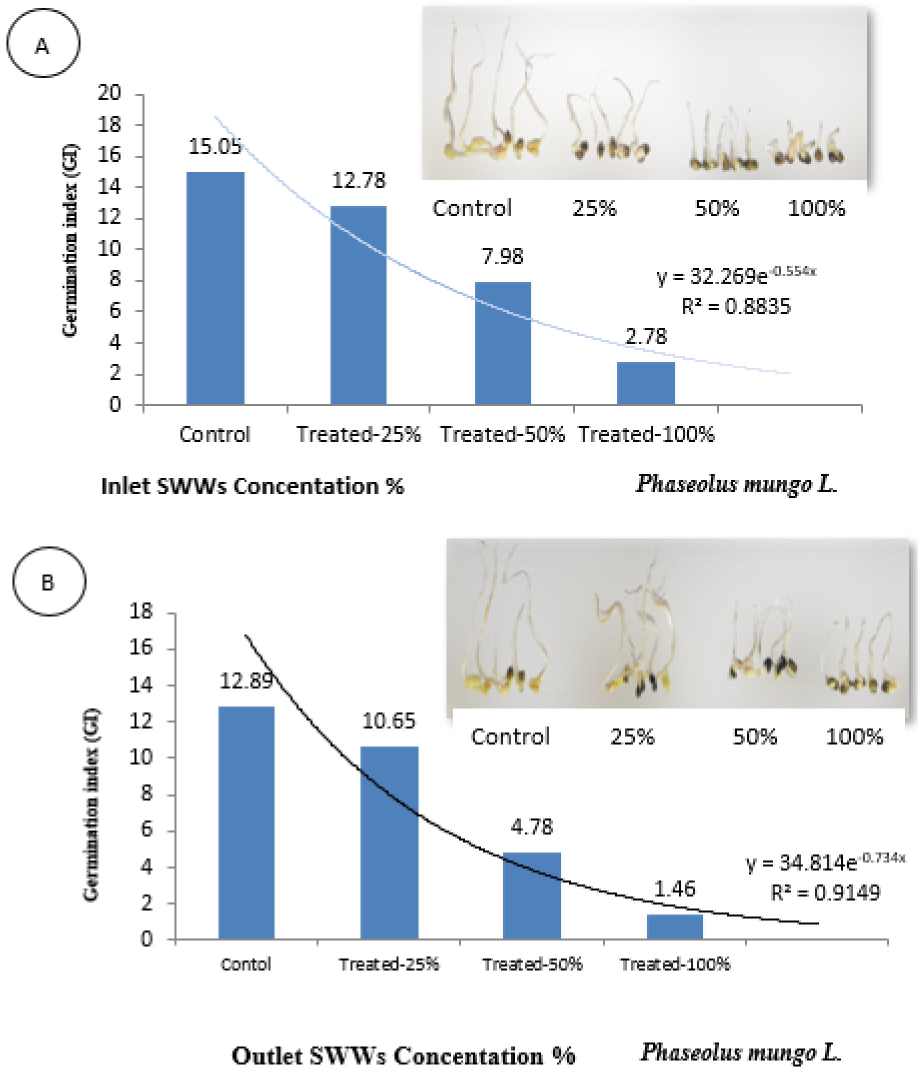


Fig. 4. Phytotoxicity evaluation of inlet (a) outlet (b) 30MLD SWW sample on *Phaseolus mungo* L. seed germination test at different concentrations.

3.6 Fish toxicity

The SWW was found to be extremely toxic to *H. fossilis*, despite being a well-known tenacious common freshwater fish (Fig. 5). During the 24-h incubation period, the estimated LC50 based on the mortality percentage was 1.432 % inlet SWWs and 0.932% outlet SWWs. The percentage of mortality increased significantly as the concentration of SWWs increased. A previous study also reported on the fish toxicity of the effluent inlet and outlet wastewater samples (Tripathi et al., 2021a). The effluent toxicity was well depicted in the photomicrograph of gills after 24 h. (Fig. 5). Vacuolation and degradation of main and secondary lamellae and epithelial cells were among the abnormalities. A complete loss of integrated gill tissue structure was found at a concentration of 5% inlet SWWs. Furthermore, after 24 h, higher concentrations of SWWs resulted in a higher than 5% rise in CAT activity in the fish's liver, indicating toxicity (Fig. 5b). This study suggested that oxidative defence mechanisms were activated in response to the production of free radicals induced by effluent exposure and the salinity shown in Table 4 (Billah et al., 2022; Saha et al., 2022). These new findings were validated by previous SWW toxicity data in terms of lethal concentrations, nutritional biochemistry, and haematological markers. After a 24-h exposure to SWWs, the LC50 in *H. fossilis* was 3.663% with 95% confidence limits of 9.231% and 0.143%. This corroborated earlier toxicity results for fish toxicity in the presence of other contaminants (Khanom et al., 2022; Samim et al., 2022). The treated wastewater (outlet) caused similar histological damage to a resilient fish species as the inlet raises concern about the safety of the treated SWW in the aquatic environment and the suitability of its reuse. (See Table 5.)

Table 4

Identified organic pollutants by GC–MS in ethyl acetate extracts from Outlet SWW sample. (RT = Retention time, SI = Similarity Index, RSI = Reverse similarity index).

S. no.	RT	Outlet Compound name	Probability	Function Vs toxicity	SI	RSI
1.	**8.21**	L-(+)-Lactic acid, 2TMS derivative	21.62	Bloating or cramping	587	665
2.	**8.57**	Hexanoic acid, TMS derivative	36.88	Difficulty swallowing, vomiting and stomach pain	564	853
3.	**9.26**	Dodecane, 5,8-diethyl-	16.20	Diarrhea and skin problem	465	571
4.	**10.81**	Pentacosane, 13-phenyl-	11.33	Stinging, redness,	376	426
5.	**13.22**	Glycerol, 3TMS derivative	12.24	Irritation may occur	524	665
6.	**15.02**	8,10-Dioxaheptadecane	7.55	Pain at the injection site.	539	595
7.	**17.89**	Heptadecane, 2,6,10,15-tetramethyl-	5.23	Constipation and erectile dysfunction	723	782
8.	**21.33**	Heptadecane, 9-hexyl-	15.19	Mainly effect gastrointestinal tract	421	492
9.	**21.96**	Hexadecane, 2,6,11,15-tetramethyl-	4.85	Shortness of breath,	701	761
10.	**25.60**	Heptadecane	9.20	Skin, eye and respiratory irritations	721	781
11.	**25.86**	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	24.21	Elevated levels have a negative cardiac impact	487	551
12.	**26.30**	Heptadecane, 9-hexyl-	10.96	Can cause severe burn, when inhaled, person's nose and throat	522	621
13.	**27.06**	Octadecane, 5,14-dibutyl-	12.73	Shortness of breath, cough, and sore throat	443	560
14.	**27.73**	Palmitic Acid, TMS derivative	96.57	Vomiting and stomach pain	845	854
15.	**28.42**	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	18.78	Cardiac impact, difficulty breathing	423	529
16.	**29.01**	Octadecane, 2-bromo-	11.74	Elevated levels have a negative cardiac impact	514	543
17.	**29.54**	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	7.38	Cardiac impact, tired feeling, fast or irregular heartbeats	576	620
18.	**31.11**	Methyl glycocholate, 3TMS derivative	3.39	Stinging, redness, or irritation	308	387
19.	**32.40**	Eicosane, 7-hexyl-	7.52	Vomiting, irritation	652	702
20.	**35.18**	Heptadecane, 9-octyl-	6.15	Constipation and erectile dysfunction	592	660
21.	**39.26**	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	23.67	Shortness of breath, headache, tired feeling, fast or irregular heartbeats	419	564
22.	**41.95**	Stearic acid, 3-(octadecyloxy)propyl ester	10.55	Urge to vomit, abdominal cramps	396	440
23.	**45.87**	Astaxanthin	13.91	Abdominal pain & cramps	374	389

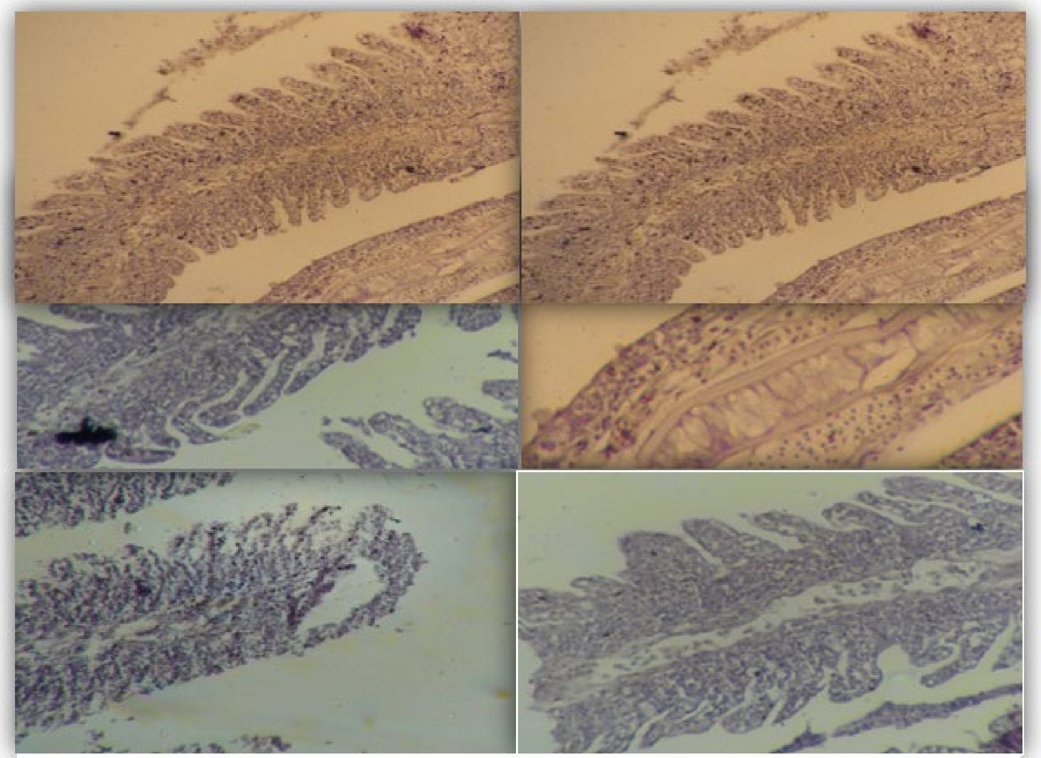
Table 5

The table shows the water quality parameters among the treatments during the experimental period. LC_{50} values with its 95% confidence limit (upper and lower) for 24 h during reproductive phase in freshwater catfish, *Heteropneustes fossilis* exposed to different concentration of inlet and Outlet (SWW; 1%, 5%, 10%). Each group had ten fish in duplicates for 24 h. Estimation performed through probit analysis of SPSS.

Parameters	Experimental unit			
	Control	Inlet SWW	Outlet SWW	
DO (mgL^{-1})	3.4 ± 0.51	6.5 ± 0.48	5.8 ± 0.50	
pH	7.1 ± 0.15	8.0 ± 0.10	8.0 ± 0.10	
Ammonia (mgL^{-1})	0.097 ± 0.062	0.073 ± 0.071	0.048 ± 0.069	
Temperature (°C)	21.76 ± 0.51	28.66 ± 0.46	23.8 ± 0.51	
Phase	**Test duration (hr)**	**LC_{50} (%)**	**95% confidence limits**	
			Upper	**Lower**
Pre-spawning phase	24	3.663	9.231	0.143

4. Limitations of this technology in India

There are a lot of issues with the technology used for treating sewage wastewater, such as the need for more room and energy and the disposal of sludge. In addition, there are significant practical challenges associated with these technologies, including inefficient removal of complex organic compounds, the inability to process wastewater in excess of the set design capacity, and a dearth of appropriately trained personnel. These operational and technical limitations of existing wastewater treatment systems have prompted the development of new classes of advanced wastewater treatment technologies. Membrane technologies and advanced oxidation processes are essential components of tomorrow's wastewater treatment systems (iii) Sludge production should be reduced, and if any is produced, it should be put to good use rather than dumped in a landfill (iv) Cost-effective adsorption materials (v) less chemical or bio flocculant applications (vi) a new category of nanomaterials for wastewater treatment. Despite the vast amount of published research on the aforementioned topics, there are still knowledge gaps in the published literature that prevent adequate solutions to the problems associated with new wastewater treatment methods from being implemented. Effective wastewater treatment and more reuse and recycling of treated water may arise from the employment of innovative wastewater technologies in tandem with more traditional methods.



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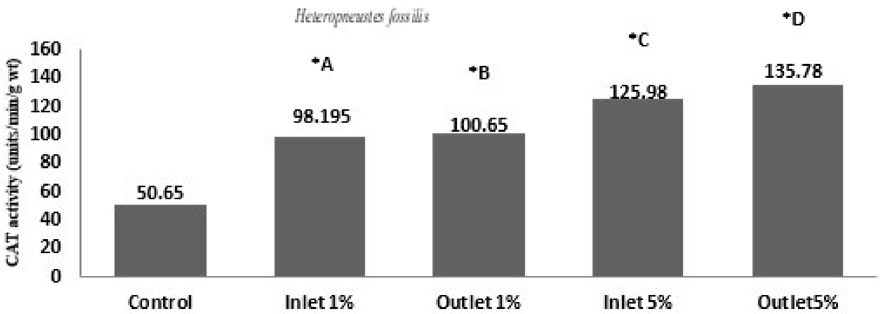

B

Fig. 5A. Effect of different concentrations of inlet and outlet 30 MLD SWWs (1%, 5%,) on gill histopathology of the freshwater catfish *Heteropneustes fossilis* after 24 h. (a & b) Control, (c & e) 1% inlet SWWs; 5% inlet SWWs (d & f) 1% outlet SWWs; 5% outlet SWWs. PL, primary lamellae; SL, secondary lamellae; EC, epithelial cell; PLD, primary lamellae degeneration; SLD, secondary lamellae degeneration; V, vacuolation; DE, damaged epithelium; LPL, loss of primary lamellae; LSL, loss of secondary lamellae; LE, loss of epithelium. [Image captured at 20 ×; hematoxylin and eosin stained]. **B** Catalase activity (units/min/g wt) in the liver of *Heteropneustes fossilis* at different concentrations of inlet and outlet 30 MLD SWWs (Control, 1%, 5 %,). Each group had ten fish in duplicates for 24 h. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA (*$P < 0.001$) and Newman– Keuls test ($P < 0.05$; A, B, C). Groups superscripted with different letters are significantly different in intergroup comparison

5. Conclusion

India is the seventh-largest nation in terms of land and the second-largest country in terms of population (nearly 1.2 billion). The wastewater generated by the population is either discharged into the environment or reused for irrigation due to water scarcity. The effectiveness of the wastewater treatment facility is therefore paramount in protecting the environment and human health. According to the findings in this physicochemical and toxicological study, various organic monomers, metals, and other co-pollutants were detected in both the inlet and outlet SWW samples, and several carcinogenic and EDCs compounds were detected in the outlet (treated) samples, their discharged into the environment could impact the biota directly or indirectly. The ability of chemicals to remain in the environment was confirmed by FT-IR and GC-–MS. The toxicity tests using *P. mungo* L. and *H. fossilis* showed the inherent toxicity of the treated wastewater. Our results provided scientific evidence that the sewage treatment plant (STP) in Gorakhpur was not sufficiently effective in removing the toxic compounds present in the wastewater. Investment in a suitable, long-term treatment technique, such as bioremediation using autochthonous bacteria and phytoremediation using native plants, is urgently needed to reduce pollutant loading in SWW, lower the potential harm to human and environmental health, and to allow for the safe reuse of the treated wastewater.

Ethical approval

Any of the authors' investigations with human participants or animals are not included in this article. The toxicity text, on the other hand, was done using freshwater fish (*Heteropneustes fossilis*) in accordance with national ethics committee norms and permission was given by the BBAU ethics committee.

Authors statements

There are no conflicts in any authors. The corresponding author has all the authority regarding the manuscript on behalf of all authors.

Declaration of competing interest

None.

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