Research article

Factors affecting the performance of a pharmaceutical wastewater treatment plant: Characterization of effluent and environmental risk

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A B S T R A C T

Pharmaceutical industries produce a huge volume of emerging pollutants (EPs) that pose a threat to the aqueous environment. Biological processes have shown their inefficacy in treating many pharmaceutical products. The study assessed physicochemical parameters, EPs, heavy metals in pharmaceutical industrial wastewater, and the removal efficiency (RE) of an aerobic biological treatment plant. The study also assessed the contamination levels and risk using several indices, such as the Canadian Council of Ministers of the Environment Water Quality Index (*CCME-WQI*), heavy metal pollution index (*HPI*), heavy metal evaluation index (*HEI*), and risk quotients index (*RQs*). The study found that the treated water quality was poor, having antibiotics, nonsteroidal anti-inflammatory drugs, and others, along with several transformation products (TPs) and heavy metals, which were unsafe for consumption with high environmental risk. The analysis results showed that the RE for TSS, BOD_5, COD, TDS, and EC were found to be 91.80%, 86.81%, 72.29%, 72.20%, and 65.60%, respectively, where the values of BOD_5, COD, NO_3^-, and PO_4^{3-} in the effluent were still higher than the permissible limits of the ECR (2023). However, the RE for heavy metals was in the order of Cu (84.62%) > Fe (65.04%) > Mn (63.3%) > Zn (60.58%) > Cd (53.85%) > Ni (54.12%) > Pb (42.42%) > Cr (38%), where Cr and Cd concentrations were still higher than the permissible limit of DoE (2019). The Pearson correlation and PCA suggested that EC, TDS, TSS, DO, BOD_5, and COD were the most correlating and contributing variables. This study argued that metal-ligand behaviors mainly affect the removal efficiency of the treatment plant by lowering the removal rate of heavy metals and pharmaceutical products.

1. Introduction

Pharmaceutical emerging pollutants (EPs), especially antibiotics, steroids, and hormones in aquatic environments, have become a global environmental issue as they are found to be toxic, persistent, and non-biodegradable, which pose significant risks to aquatic life and human health [1]. The traditional wastewater treatment processes have proved to be less efficient at entirely removing EPs, such as selected pharmaceuticals, personal care products (PCPs), perfluorinated compounds (PFCs), and pesticides, due to their variety, extreme low concentrations, and unique characteristics [2]. Due to the limited effectiveness of traditional wastewater treatment plants (WWTPs) and the direct discharging of untreated wastewater to make more profit, pharmaceutical EPs, especially antibiotics, are

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directly discharging into water bodies continuously. The adverse effects caused by the increasing amount of antibiotics released into the environment cannot be ignored, as antibiotic-resistant microbes may evolve [3]. Alimba et al. (2019) suggested that the pollutants in pharmaceutical effluents are emerging carcinogens and mutagens that are capable of increasing genome instability, altering blood cell indices, and causing pathological lesions in fish tissues [4]. Pharmaceutical effluent also contains toxic heavy metals and metalloids, including Cr, Ni, Co, Cu, Cd, Pd, and As [4,5]. These toxic heavy metals are found to be extremely toxic to aquatic organisms even at very low concentrations, which can cause significant histopathological alterations in the tissues of aquatic organisms, such as fish [6,7]. Though the conventional biological WWTP is the most cost-effective method, it is found that only polar contaminants are removed from the final discharged effluent in the biological process [8]. Many researchers have shown excellent COD removal efficiency by biological WWTPs. Khan and Mostafa (2011) showed that about 75% removal of COD from pharmaceutical wastewater can be achieved by employing hydraulic residence times of 15 days in an aerobic biological reactor [9]. Zhou et al. (2006) showed that the COD removal efficiency of biological WWTP was as high as 97.8%, however, the removal efficiencies of two antibiotics, namely ampicillin and aureomycin were, less than 10% [10]. It is more important to remove toxic nonbiodegradable chemicals, such as antibiotics, by WWTPs than just to achieve high COD removal efficiency.

The pharmaceutical industrial effluent containing antibiotics is pre-treated by heating at a pH above 12 with sodium hydroxide (NaOH) to break down β-lactam rings and convert simpler compounds before subjected to biological treatment (Fig. 1). Therefore, a necessity exists to investigate antibiotic degradation associated with organic matter removal in wastewater treatment processes. This study aimed to assess the physicochemical parameters, heavy metals, and EPs in pharmaceutical industrial wastewater and the removal efficiency of the aerobic biological WWTP. This study investigated the impacts of the discharged effluent. The correlation among the variables was assessed using Pearson correlation and PCA analyses.

2. Materials and methods

2.1. Sampling site

The study selected a pharmaceutical industry in Bogura city (24.83° N and 89.37° E), the northern part of Bangladesh. Several industries discharge untreated effluent into the Korotoa River, which passes through the industrialized city of Bogura, and poses a huge threat to the environment [6,11]. The pharmaceutical industry was purposefully chosen, and necessary permission was obtained from the authority. The industry has an aerobic biological plant followed by multi-layer and activated carbon filters to treat wastewater with a capacity of 5000 L per day.

2.2. Sample collection and extraction analysis

Two influent samples (one containing antibiotics and the other non-antibiotic tablets) and a combined effluent sample were collected into separate high-density PVC bottles and were properly labeled, filtered, and preserved by adding sodium azide (1 g/L) and kept at 4 °C until the extraction process was started. The samples were extracted by the liquid-liquid extraction (LLE) process using dichloromethane [12,13].

2.3. Analysis with LC-MS

The extracted samples were analyzed using a Liquid chromatography coupled with mass spectrometry (LC–MS 2020, Shimadzu, Japan) in the laboratory of the Bangladesh Council of Scientific and Industrial Research, Rajshahi (BCSIR), Bangladesh, according to
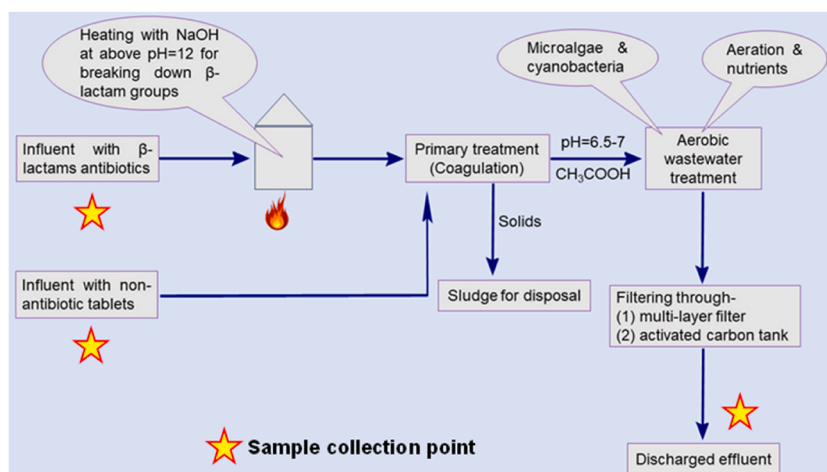

Fig. 1. Sample collection point and operational diagram of the WWTP in Bogura city of Bangladesh.

the standard method of analysis [14,15].

2.4. Physicochemical parameters determination

The temperature, pH, EC, and DO were measured in situ using a digital multimeter (YSI Pro 1030 and Lutron PDO-519). The other physicochemical parameters, such as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total hardness (TH), total suspended solids (TSS), total dissolved solids (TDS), chlorides, sulfates, nitrates, phosphates, sodium, potassium, calcium, and magnesium were analyzed using the standard methods of APHA (2005) [16].

2.5. Heavy metals analysis

The concentrations of metals and metalloids were determined by using a flame atomic absorption spectrometer (AAS) (SHIMADZU, AA-6800) in the Central Science Laboratory of the University of Rajshahi, Bangladesh, according to the standard methods of analysis [17,18].

2.6. FTIR analysis

Different functional groups of organic compounds present in the influent and effluent were identified by an FT-IR (PerkinElmer Spectrum 100 FTIR) machine at the Central Science Laboratory of Rajshahi University according to the standard method [12].

2.7. Indexing methods

Several water quality indices for the discharged effluent were calculated to assess the environmental impacts and the contamination levels, which are stated below.

2.7.1. CCME-WQI
The treated water quality was assessed by the Canadian Council of Ministers of the Environment Water Quality Index (*CCME-WQI*) method [19]. The *CCME-WQI* consists of three main factions, F1 (scope), F2 (frequency), and F3 (amplitude). These were calculated using Eqs. (1), (2) and (6). Finally, *CCME-WQI* values were calculated using Eq. (7).
F1 is the percentage of the variables that exceeded their objective or standard limit relative to the total number of variables.

$$F1(scope) = \frac{No.\ of\ failed\ variables}{Total\ no.\ of\ variables} \times 100 \tag{1}$$

F2 is the percentage of the total number of failed tests relative to the total number of tests, which is the sum of each variable tested multiplied by the number of times that variable tested.

$$F2\ (frequency) = \frac{No.\ of\ failed\ tests}{Total\ no.\ of\ tests} \times 100 \tag{2}$$

F3 (amplitude) is calculated with the help of the normalized sum of excursion (nse), which is calculated either by Eq. (3) or (4) depending on the conditions. When the test value must not exceed the objective or standard limit, Eq. (3) is used.

$$Excursion = \frac{Failed\ test\ value}{Objective} - 1 \tag{3}$$

In cases where the test value must not fall below the objective, Eq. (4) is to be used.

$$Excursion = \frac{Objective}{Failed\ test\ value} - 1 \tag{4}$$

The normalized sum of excursion (nse) is calculated by Eq. (5).

$$nse = \frac{\sum_{i=1}^{n} Excursion}{Total\ no.\ of\ tests} \tag{5}$$

Finally, F3 is calculated using Eq. (6).

$$F3\ (amplitude) = \frac{nse}{0.01(nse) + 0.01} \tag{6}$$

The *CCME-WQI* water quality index value is then calculated using Eq. (7).

$$CCMEWQI = 100 - \left(\frac{\sqrt{F1^2 + F2^2 + F3^2}}{1.732} \right) \tag{7}$$

The *CCME-WQI* criteria are as follows: *CCME* = 95–100 = Excellent; *CCME* = 80–94 = Good; *CCME* = 65–79 = Fair; *CCME* = 45–64 = Marginal; *CCME* = 0–44 = Poor [20].

2.7.2. Heavy metal pollution index (HPI) analysis

The overall water quality of the treated water and its suitability for human consumption were assessed using the heavy metal pollution index (*HPI*) according to Eqs. (8)–(10) [6,21]. This study used Bangladesh standard permissible value for water quality parameters [39]. The *HPI* values were calculated using the following equations:

$$HPI = \frac{\sum_{i=1}^{i=n} W_i \times Q_i}{\sum_{i=1}^{i=n} W_i} \tag{8}$$

Where Q_i is the subindex of the ith parameter, W_i is the unit weight of the ith parameter, and n is the number of parameters considered. The unit weight of the ith parameter (W_i) and the subindex (Q_i) of the ith parameter are given by the equations:

$$W_i = \frac{K}{S_i} \tag{9}$$

$$Q_i = \frac{(M_i - I_i)}{(S_i - I_i)} \times 100 \tag{10}$$

Where Mi is the monitored value of the metal of the ith parameter in ppb, Si is the standard permissible in ppb and Ii is the ideal value of the ith parameter in ppb. The *HPI* value of water less than 100 is considered safe for drinking, while water is considered unsafe for drinking, when the *HPI* value of water is greater than 100 [6].

2.7.3. Heavy metal evaluation index (HEI)

The *HEI* analysis was done to assess the level of pollution of the discharged effluent with respect to heavy metals, calculated based on Eq. (11) [22].

$$HEI = \sum_{i=1}^{n} \frac{H_C}{H_{mac}} \tag{11}$$

Where H_c and H_{mac} are the monitored values and maximum admissible concentration (MAC) of the ith parameter in ppb, respectively. The applied parameters and constants for the calculation of *HEI* were done according to WHO guidelines [40]. The proposed *HEI* criteria are as follows: pollution level is considered low, when $HEI < 10$, medium, when $HEI = 10–20$, and high, when $HEI > 20$ [22].

2.7.4. Hazard quotients (HQs)

The Hazard Quotients (*HQs*), also called Risk Quotients (*RQs*), is calculated using Eq. (12).

$$RQ = \frac{MEC}{PNEC} \tag{12}$$

Where the unit for the measured environmental concentration (*MEC*) and the predicted no effect concentration (*PNEC*) is ppb. When PNEC is not available, which is a constant for each chemical, estimated PNECs can be derived from the values of effect concentration, EC50 or lethal concentration, LC50 reported in the literature, divided by an appropriate uncertainty factor (assessment factor, AF), which varies from 10 to 1000 [23,24]. The *RQ* for the mixture based on MEC/PNEC ratios given by Eq. (13) is well-accepted and extensively used model [25].

$$RQ_{MEC/PNEC} = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} \tag{13}$$

The proposed *RQ* criteria are as follows: $RQ < 0.1$ meaning minimum risk; $0.1 \leq RQ < 1.0$ meaning intermediate risk and $RQ \geq 1.0$ meaning high environmental risk [26].

2.8. Statistical analysis

Pearson's correlation matrix and principal component analysis (PCA) were used to assess the correlations among the variables. For PCA analysis, factors with an eigenvalue greater than 1 were extracted. The first principal component (PC1) is equal to the weighted (factor loading) linear combination of the initial variables that account for the greatest variability [27,28]. Pearson correlations and PCA were calculated using IBM SPSS Statistics software version 2022.

3. Results and discussion

The descriptive results of the physicochemical parameters, heavy metal concentrations, and EPs are presented in Tables 1–5. To assess the pollution level, the water quality parameters of the discharged effluent were compared with the standards of the Bangladesh Environmental Conservation Rules 2023 (ECR, 2023) and the Department of Environment, Bangladesh Water Quality Standard 2019 (DoE, 2019) [41,42]. Since the two influents of the WWTP are combined before they are subjected to the biological treatment (Fig. 1), the values of each parameter for the two influents were added to evaluate the performance of the WWTP except color, temperature, pH, and DO.

3.1. Physicochemical parameters

This study exposed that all the physicochemical parameters for discharged effluent were found below the permissible limit set by the ECR (2023) with the exception of BOD_5, COD, nitrate, and phosphate (Tables 1, 2 and 5).

3.1.1. Physical parameters (color, temp., EC, TSS, TDS)

The discharged effluent was colorless and had a temperature of 31 °C, which was just above the guidelines provided by the DoE (2019). However, the ECR (2023) did not set any temperature range, instead, it suggested that the temperature of the discharged effluent should be a maximum of 5 °C higher than the temperature of the reservoir. The value of EC of the treated effluent was 501 µS/cm, which was within the permissible standard (1200 µS/cm) of the ECR (2023). The TSS value of the effluent was found to be 47 mg/L, which was within the permissible limit of the ECR (2023), whereas this value exceeded the standard permissible limit of the DoE (2019) (Table 1). However, the TDS value for the effluent was found within the standard limits of both the ECR (2023) and DoE (2019) (Table 1).

3.1.2. Chemical parameters (pH, TH, DO, BOD_5, COD)

The pH of the discharged effluent was found to be acidic in nature (6.35), which was within the allowable range of both the ECR (2023) and DoE (2019) (Table 1).

The TH value of the discharged effluent was 355 mg/L, suggesting that the water was very hard. However, this value did not exceed the permissible limit of 500 for the TH according to the guidelines set by the DoE (2019) (Table 1). The DO value was found within the standard range of 4–6 set by the ECR (2023), while the value was lower than the standard value of DO set by the DoE (2019) (Table 1). Both the BOD_5 and COD values for the effluent were found to be high above their standard permissible limits, suggesting that very high concentrations of organic and metal-ligand complex compounds were present in the discharged effluent, which was confirmed by the FTIR and LC-MS analyses (Tables 1 and 4 and Fig. 2).

3.1.3. Chemical parameters (anionic)

The nitrate concentration in the effluent was found to exceed the standard permissible limits of both the ECR (2023) and DoE (2019) (Table 2). The values of chloride and sulfate were 160.20 and 113.61 mg/L, respectively, for the effluent, which were within the standard limits set by both the ECR (2023) and DoE (2019). However, the phosphate concentration was found to be nearly three times higher than the allowable limits set by both standards (Table 2).

3.1.4. Chemical parameters (cationic)

The effluent was found to have very little Na and K, which were far below their standard limits (Table 2). The concentration of Ca was also found to be within the maximum allowable limit of the DoE (2019). However, the concentration of Mg (97.63 mg/L) was found to be very high above the permissible limit of the DoE (2019) (Table 2).

3.2. Emerging pollutants in influent and effluent

The LC-MS analysis showed that both the influent and effluent had a very large number of EPs, though the number of EPs in the

Table 1
Physicochemical parameters of the influent and effluent.

Type	Color	Temp. (°C)	pH	EC (µS/cm)	Concentrations (mg/L)					
					TSS	TDS	TH	DO	BOD_5	COD
Influent-1	Greenish yellow	32	8.90	605	340	610	66	3.20	93.40	287.28
Influent-2	Brownish yellow	33	6.62	853	233	1235	740	1.60	410.55	515.69
Total	–	–	–	1458	573	1845	806	2.40	503.95	802.97
Effluent	Colorless	31	6.35	501	47	513	355	4.80	66.75	222.5
ECR (2023)	150 Hazen	–	6–9	1200	100	2100	–	4–6	30	200
DoE (2019)	15 Hazen	20–30	–	–	10	1000	500	6	0.2	4
RE (%)	–	–	–	65.60	91.80	72.20	55.90	–	86.81	72.29

Influent-1 = antibiotics; Influent-2 = non-antibiotic tablets; RE = removal efficiency.

Table 2
Physicochemical parameters of the influent and effluent.

Type	Concentrations in mg/L							
	Na	K	Ca	Mg	NO_3^-	Cl^-	PO_4^{3-}	SO_4^{2-}
Influent-1	0.93	0.82	7.2	13.82	81	144.14	11.05	118.91
Influent-2	0.46	0.33	16.8	201.02	257	318.23	49.3	156.76
Total	1.40	1.15	24	214.84	338	462.37	60.35	275.67
Effluent	0.39	0.51	6.4	97.63	26	160.20	17.85	113.61
ECR (2023)	–	–	–	–	10	600	5	–
DoE (2019)	200	12	75	35	10	600	6	400
RE (%)	72.2	55.6	73.3	54.6	92.3	65.4	70.4	58.8

Table 3
Key findings from the LC-MS and FTIR analyses of the effluent and influent.

Category	Influent-1	Influent-2	Effluent
No. of compounds found	70	35	28
No. of TPs	38	18	17
Common TPs	Two TPs common to effluent at $m/z = 58$ and 223; RT = 3.45 and 2.37 respectively; both in positive ESI mode	Two TPs common to effluent at $m/z = 219$ and 223; RT = 4.98 in negative ESI and 2.6 in positive ESI mode respectively	Four TPs common to influents
Average m/z	237.6	298.9	238.6
β-lactam ring	**Present** FTIR: 1768 cm^{-1} LC-MS: Amoxicillin, Flucoxacillin etc.	**Absent**	**Present** FTIR: 1785 cm^{-1} LC-MS: Amoxicillin, Flucoxacillin etc.
Metal-oxygen bond (M − O), metal-nitrogen bond (M − N)	**Present** FTIR: 622 cm^{-1} for M-N; 470 cm^{-1} for M-O LC-MS: TPs	**Present** FTIR: 594 cm^{-1} for M-N; 484 cm^{-1} for M-O LC-MS: TPs	**Present** FTIR: 594 cm^{-1} for M-N; 468 cm^{-1} for M-O LC-MS: TPs

Table 4
LC-MS analysis data of effluent.

Possible Compounds	Empirical Formula	MW (g/mol)	Mode	RT (min)	$(M \pm zA)^{z\pm}$ (m/z)	Intensity
Amoxicillin	$C_{16}H_{19}N_3O_5S$	365	–	6.667	364	644
Benzyl benzoate	$C_{14}H_{12}O_2$	212	+	11.85	214	69673
Cetirizine	$C_{21}H_{25}ClN_2O_3$	389	–	0.567	388	53451
Drotavarine HCl	$C_{24}H_{32}ClNO_4$	434	+	8.133	435	64737
Fluconazole	$C_{13}H_{12}F_2N_6O$	306	+	2.433	307	528
Flucoxacillin	$C_{19}H_{17}ClFN_3O_5S$	454	+	2.583	472	68534
Ibuprofen	$C_{13}H_{18}O_2$	206	+	9.183	245	57202
Lactic acid	$C_3H_6O_3$	90	+	5.35	108	11238
Losartan potassium	$C_{22}H_{22}ClKN_6O$	461	–	1.167	460	22522
Metformin	$C_4H_{11}N_5$	129	+	9.983	132	17485
Propylhydroxybenzoate sodium	$C_{10}H_{12}NaO_3$	202	+	2.283	203	125095
TP	–	–	–	13.567	274	3432
TP	–	–	+	12.2	117	15601
TP	–	–	+	14.067	140	85417
TP	–	–	–	3.05	402	83431
TP	–	–	+	3.45	58	142925
TP	–	–	+	7.617	191	105618
TP	–	–	–	3.1	393	44952
TP	–	–	–	3.517	171	54219
TP	–	–	–	4.983	219	36382
TP	–	–	–	7.883	80	36749
TP	–	–	+	2.367	223	14506
TP	–	–	+	5.15	210	14654
TP	–	–	–	5.25	109	4180
TP	–	–	–	7.117	85	4653
TP	–	–	–	12.533	210	5356
TP	–	–	–	2.567	405	19335
TP	–	–	+	11	67	113336

z = 1,2,3, etc.; A = H and M = molar mass.

Table 5
Heavy metal concentrations in influent and effluent.

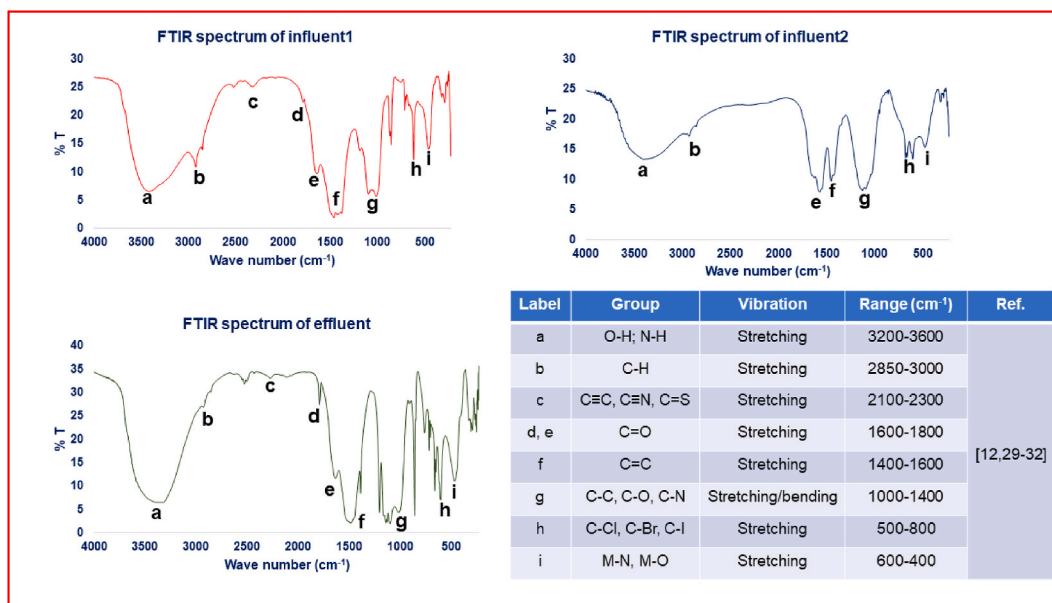| Type	Concentrations in mg/L							
	Cr	Mn	Fe	Ni	Cu	Zn	Pb	Cd
Influent-1	0.036	0.061	0.754	0.039	0.006	0.046	0.014	0.009
Influent-2	0.064	0.059	0.824	0.046	0.007	0.058	0.019	0.004
Total	0.100	0.120	1.578	0.085	0.013	0.104	0.033	0.013
Effluent	0.062	0.044	0.552	0.039	0.002	0.041	0.019	0.006
ECR (2023)	0.50	2	3	1	3	5	0.1	0.02
DoE (2019)	0.05	0.1	1	0.1	1	5	0.05	0.005
RE (%)	38	63.3	65.04	54.12	84.62	60.58	42.42	53.85

Fig. 2. FTIR spectra of effluent and influent water residue.

effluent was reduced to 28 from 105 in the influent (Table 3). The data presented in Table 3 showed that nearly half of the EPs are transformation products (TPs) in influent and effluent, of which only 4 TPs were common. The analyzed data suggested that the discharged effluent contained β-lactam antibiotics (Table 4) as well as other toxic compounds, along with a number of TPs, which could be the metal complexes of pharmaceutical ligands. EPs were characterized by LC-MS and FTIR analyses, as illustrated below.

3.2.1. FTIR analysis

The FTIR spectra of influent and effluent samples were found to have identical absorption bands, suggesting that the compounds with similar functional groups were present in the influent and effluent. The broad absorption bands in the range of 3150–3600 cm^{-1} denoted by 'a' in Fig. 2, corresponded to the asymmetric and symmetric stretching vibrations of O–H and N–H bonds present in alcohols, carboxylic acids, amines, amides, and imides [29,30].

Both influent and effluent contained this characteristic peak, suggesting that the discharged effluent carried these compounds and was mixed with the aquatic system. The low-intensity peaks for the C–H stretches were in the range of 2900–2950 cm^{-1} (b in Fig. 2). The weak absorption bands at 2269 and 2296 cm^{-1} were due to the stretching vibrations of C≡C, C≡N, and C=S bonds, suggesting that the different types of chemical compounds were present in the influent and effluent (c in Fig. 2). The absorption peaks at 1768 and 1785 cm^{-1} were for the stretching vibrations of C=O bond in β-lactam rings, suggesting that β-lactam antibiotics were present in the influent-1 and effluent (d in Fig. 2). The absorption peaks observed in the range of 1630–1660 cm^{-1} (e in Fig. 2) were due to the stretching vibrations, which indicated the presence of C=O bonds other than β-lactams, which was clear evidence for the existence of different types of organic compounds, such as aldehydes, ketones, carboxylic acids, acid halides, amides, esters, etc. [31]. The appearance of these peaks in relatively lower fields (Fig. 2), suggested that the metal-ligand coordination bonds were present [30,32]. The heterocyclic stretching vibration of the C=N bond was also observed in the range of 1630–1660 cm^{-1}, suggesting that organic compounds with a C=N bond might also be present [33]. The medium intensity absorption bands in the range of 1410–1485 cm^{-1} were due to the stretching vibrations of the C=C bond (f in Fig. 2). The strong absorption bands in the fingerprint region within the range of 1050–1100 cm^{-1} (g in Fig. 2) were for the stretching or bending vibrations of the C–C, C–O, and C–N bonds [29,31]. Several

absorption bands in the range of 1000–1400 cm^{-1} suggested that C–F bond was also present [31]. The medium intensity absorption bands observed between 600 and 650 cm^{-1} (h in Fig. 2), due to the stretching vibrations of C–Cl, C–Br, and C–I vibrations, indicated that the halogenated compounds were present in the influent and effluent [12,29].

The low-intensity bands in the region of 400–480 cm^{-1} were attributed to metal-nitrogen (M − N) and metal-oxygen (M − O) vibrations [30]. The LC-MS analysis suggested that both influent and effluent samples contained a very large number of transformation products (Table 3). From the above discussion, it can be said that the majority of the transformation products (TPs) could be metal-ligand complex compounds, since the pharmaceutical products are strong ligands that can combine with metal ions in varying proportions, such as 1:1, 1:2, 1:3, 1:4, and mixed ligand complexes too [34]. These complexes are highly stable and toxic to aquatic biota, and due to their inertness, they can manage to bypass removal mechanisms [34,35].

3.2.2. LC-MS analysis

Due to the presence of a large number of unknown TPs and the complex nature of influent and effluent, quantitative assessment was not done. The LC-MS analysis result presented in Table 3 shows that the total number of compounds in the influent was 105 (70 in influent-1 and 35 in influent-2) with an average *m/z* of 268.3, suggesting high molecular weight compounds were present in the influent. However, the number of compounds was reduced to 28 in the effluent, with an average *m/z* of 238.6, suggesting there were still a significant number of high molecular weight compounds in the discharged effluent. Mainly, three types of compounds, such as chemicals used as reagents in manufacturing processes, manufactured products, and TPs produced before or during treatment processes, can exist in the influent and effluent samples (Table 4).

For assigning a particular chemical for each *m/z* value, retention time (RT) and masses of (M − H)$^-$, (M + H)$^+$, (M+2H)$^{2+}$, etc., were matched (Table 4).

The assigned possible compounds, including organochlorine compounds, are polar, which have either replaceable protons to be deprotonated (-ESI mode) or lone paired electrons (bases) to be protonated (+ESI mode) (Table 4). Some compounds have the ability to be protonated or deprotonated depending on the conditions, so they can appear in both ESI modes. Moreover, some compounds have the ability to accept/release more than one proton. Therefore, multiple signals were obtained for the same molecule. To avoid overcounting or errors in the measurements, each possibility was carefully analyzed and reset. Each sample was scanned five times in both ESI modes.

The number of TPs in the influent samples was 56 (38 in influent-1 and 18 in influent-2), while the number of TPs in the effluent was 17 (Table 3). The most interesting thing was that only 4 TPs were common in both influents and effluent, suggesting TPs formed before the treatment processes underwent some chemical changes (Table 3). Some of the compounds in the effluent characterized by the FTIR and LC-MS analyses are presented in Table 4. Among the identified drugs, some cephalosporins or β-lactam antibiotics, such as amoxicillin and flucoxacillin were present in the discharged effluent (Table 4). Other notable drugs in the effluent were nonsteroidal anti-inflammatory drugs (ibuprofen) and antihistamine drugs (cetirizine), along with some other types of drugs (Table 4). These pharmaceuticals are highly toxic to microorganisms and small fish along with other aquatic biota [36]. The data in Table 4 shows that half of the identified chemicals in the discharged effluent were TPs, which could be the metal complexes of pharmaceutical ligands (Fig. 2). Many reports suggest that these TPs are more toxic than their parent compounds [37,38,49].

3.3. Heavy metals in influent and effluent

The data presented in Table 5 show that the concentrations of all the heavy metals were within their respective permissible limits set by both the ECR (2023) and DoE (2019) except for Cr and Cd. The value of Cr in the effluent was 0.0624 mg/L, which is within the standard limit of the ECR (2023) for discharged effluent, whereas the value is higher than the standard limit set by the DoE (2019) for surface water quality (Table 5).

Similarly, the Cd concentration was found within the permissible limit of the ECR (2023), whereas the value was somewhat higher than the maximum allowable limit set by the DoE (2019) (Table 5).

3.4. Performance of the treatment plant

The pharmaceutical industry's WWTP has mainly two sections; the aerobic biological treatment section for removing organic compounds and a multi-layer filter followed by activated carbon filters for removing heavy metals (Fig. 1).

3.4.1. Biological process

It is difficult to track individual organic compounds, determine their concentrations, and evaluate their removal percentages due to the very complex nature of influent and effluent and the presence of a large number of TPs. Therefore, the study assessed the performance of the biological treatment quantitatively by COD and BOD$_5$ reduction capacity and qualitatively by the analyses of FTIR and LC-MS. The COD removal efficiency of the WWTP was found to be 72.29%, while the removal efficiency for BOD$_5$ was 86.81% (Table 1). The low COD removal efficiency as compared to BOD$_5$, suggested that the treated water contained a significant amount of non-biodegradable organic and metal-ligand complex compounds, which were discharged into the aquatic system. During the sample digestion period in the COD determination process, these non-biodegradable compounds were oxidized on heating with an acidic potassium dichromate solution, which increased the value of COD. The analyzed effluent contained 28 compounds, of which 11 were manufactured and/or used as reagent compounds, the remaining 17 compounds were TPs (Table 4). The total number of compounds found in the influents was 105 (Table 3). Therefore, the removal efficiency in terms of the number of compounds reduced 73.3%.

The term average m/z, which is introduced for the first time in this study, can be used to assess the performance of the biological treatment process, since successful biodegradation of any large molecule produces smaller molecules.

The average m/z values for the influent and effluent were 268.3 (average of influent-1 and influent-2) and 238.6, respectively, suggesting that there were still a significant number of high molecular weight compounds in the discharged effluent. However, only four TPs were common in both influent and effluent samples, suggesting that TPs formed before the treatment processes underwent some chemical changes during the treatment processes (Table 3). The removal efficiency of TSS was found to be 91.8%, indicating that almost all suspended solids were removed during the activated sludge process in the biological treatment section (Table 1). The removal percentage of EC was 65.6%, while the removal of TDS was found to be 72.2%. Since the nitrate ion (NO_3^-) is easily biodegradable [43], the maximum removal rate for nitrate was found to be 92.3%. The removal efficiency for other anionic parameters was as follows: Cl^- (65.4%), PO_4^{3-} (70.4%), and SO_4^{2-} (58.8%) (Table 2).

3.4.2. Adsorption process

This study found that the removal efficiency of heavy metals in the WWTP was in the order of Cr (38%) < Pb (42.42%) < Cd (53.85%) < Ni (54.12%) < Mg (54.6%) < Zn (60.58%) < Mn (63.3%) < Fe (65.04%) < Ca (73.3%) < Cu (84.62%) (Tables 2 and 5). For the removal of heavy metals in the WWTP, two successive filters, such as multi-layer and activated carbon filters are used. These filters are capable of removing heavy metals in varying percentages depending on the effect of pH, temperature, organic pollutants, coexisting ions (anions/cations), and initial concentrations of the heavy metal ions [44]. The pH of the discharged effluent was 6.35, which was acidic in nature (Table 1). Lower pH values reflect a high concentration of H^+ in water, which competes with heavy metal ions for ion exchange and electrostatic interaction on the adsorbent sites, decreasing the adsorption efficiency of heavy metals [44]. The high concentrations of cations, such as, K^+, Na^+, Ca^{2+}, and Mg^{2+} in the influent can also compete with heavy metal cations, decreasing heavy metal adsorption on the adsorbent surface. The concentrations of these cations in influent, especially Ca^{2+} and Mg^{2+} concentrations were found to be very high (Table 2), which might decrease the heavy metal removal efficiency. Moreover, anions would interact with heavy metal cations to form coordination ions or precipitation, and compete with heavy metal anions for adsorption sites [44]. The high concentrations of NO_3^-, Cl^-, PO_4^{3-}, and SO_4^{2-} in the influent (Table 2), suggested that the removal of heavy metals might be affected by these anions. The presence of a strong ligand, especially chelating agents like EDTA, citrate, antibiotics, etc., inhibits adsorption on adsorbents because of strong complex formation with the metal ions or by competing with the surface for available adsorption sites. Complex formation occurs at a higher pH for the elimination of protons (H^+) formed during the reaction process [34,35]. In the WWTP, the influent was heated at a pH above 12 with sodium hydroxide (NaOH), which provided favourable conditions to form metal complexes with pharmaceutical products. Metal complexes are thermodynamically very stable, inert, less biodegradable, and toxic to microorganisms [48,54]. Valentina Uivarosi (2013) showed that pharmaceutical chelating agents have a greater affinity for forming more stable chelates with hard Lewis's acids like trivalent cations (Al^{3+}, Fe^{3+}, etc.) [34]. The study also showed that the formation constant values for stable chelates of different pharmaceutical products, including antibiotics were in the order of $Al^{3+} > Fe^{3+} > Cu^{2+} > Fe^{2+} > Zn^{2+} > Mg^{2+} > Ca^{2+}$, suggesting that the stability of the chelates would follow the same order [34]. However, the water solubility of the metal complexes is equally important since the insoluble compounds would be separated during the sludge process. In the presence of high organic contents, Cr is expected to be in the Cr^{3+} form, which can form very strong chelates with pharmaceutical chelating agents. The removal efficiency of Cr was found to be the lowest among the heavy metals, suggesting the presence of water soluble chelates of the Cr^{3+} ion. This fact was also supported by the FTIR and LC-MS analyses. The presence of absorption bands below 600 cm^{-1} in the FTIR spectra of influent and effluent was clear evidence of M − N or M − O bonds (i in Fig. 2). Further, the presence of a large number of TPs in the effluent suggested that these TPs could be stable chelates that easily bypassed the adsorption processes [34,35]. Despite the borderline Lewis's acid characteristic, Pb^{2+} was found to have a low removal percentage (42.42%), suggesting that it formed mostly water-soluble stable complexes that could manage to escape being absorbed. The removal percentages for other borderline Lewis's acids were found between 54% and 73%, except for Cu, which is quite an acceptable range described in the literature [44]. Cu^{2+} is a borderline Lewis's acid that can be reduced to Cu^+ in the presence of reducing agents like iodide (I^-), which was highly expected to be in pharmaceutical wastewater (Fig. 2). Cu^+ forms labile or less stable complexes, which could be easily absorbed on the adsorbent surface, thereby increasing its removal percentage [45]. The average removal percentage of alkali metals (Na, K) was 63.8%, which was in accordance with the EC removal percentage (Tables 1 and 2). From the above discussion, it can be said that metal-ligand behaviors dominate the removal efficiency of heavy metals. In this way of forming stable water soluble chelates, both organic compounds and heavy metals can manage to bypass the removal processes, thereby lowering the removal efficiency of COD, BOD$_5$, and some of the heavy metals.

To reach a concrete conclusion about metal-ligand interaction, which lowers the removal efficiency of WWTP, data on both laboratory and different wastewater analyses are required. If the transformation mechanism of all pollutants is known accurately, their successful removal process can be achieved by further research on this issue.

3.5. Environmental impact assessment

The *CCME-WQI* analysis suggested that the treated effluent water quality was poor, indicating various pollutants were present in the discharged effluent, which is harmful to the aquatic biota.

The treated effluent contained β-Lactam antibiotics, nonsteroidal anti-inflammatory drugs, and TPs, along with toxic heavy metals (Tables 4 and 5). The most widely used antibiotics in clinical medicine are β-lactam antibiotics. Resistance of microbes to β-Lactams is increasing worldwide, which may become a severe threat because they have very low toxicity and are used to treat a broad range of infections [46,47]. Antibiotics in high concentrations cause harmful effects on the environment in three ways. Firstly, the antibiotics

present in wastewater kill microorganisms by damaging their metabolic activities or causing toxicity. Those microorganisms actually help impair the waste in the treatment process. Secondly, antibiotics contaminate microbial ecosystems. Thirdly, antibiotics present in such a wide range in the environment lead to the development of antibiotic-resistant microorganisms [3]. Pollutants in pharmaceutical effluents are emerging carcinogens and mutagens that are capable of increasing genome instability, altering blood cell indices, and causing pathological lesions in fish tissues [4,55]. This is a serious threat to the normal functioning of aquatic ecosystems and the survival of aquatic biota. Pharmaceutical effluent also contains varying concentrations of toxic heavy metals and metalloids. These metals and metalloids are found to be extremely toxic to aquatic organisms, even at very low concentrations, which can cause significant histopathological alterations in the tissues of aquatic organisms such as fish [6,7].

In many countries, even in Bangladesh, the river surface water is used for domestic and drinking purposes [51]. The treated effluent of the WWTP is directly discharged into the river system. Therefore, the suitability of the discharged effluent for drinking was assessed using the HPI index method, where the standards for domestic and drinking water supply were used.

The *HPI* analysis results showed that the discharged effluent was unsafe for human consumption for domestic and drinking purposes (Table 6). However, the *HEI* analysis for the heavy metal pollution revealed that the treated water was in the low pollution category considering the heavy metal concentrations. The *RQs* analysis for mixtures of heavy metals showed that they had a high environmental risk (Table 6). Moreover, the presence of some antibiotics, nonsteroidal anti-inflammatory drugs, and a number of TPs (Table 4) suggested that the discharged effluent had significant environmental impacts.

The WWTP managed to develop the CCME-WQI value by 52.10%, the HPI value by 53.16%, the HEI value by 53.25%, and $RQ_{MEC/PNEC}$ value considering only eight heavy metals and metalloids by 40.56%. Despite these developments, the discharged effluent fell under poor water quality, which was unsafe for consumption and had a high environmental risk (Table 6).

3.6. Statistical analysis

To investigate the correlations between variables and the most significant variables contributing to the pollution of the discharged effluent, Pearson correlation matrix and principal component analysis (PCA) were used.

3.6.1. Pearson correlation

A very strong negative linear relationship was found between DO and temperature (-1.0), DO and TDS (-0.98), and DO and EC (-0.97) at significant levels of 0.01 and 0.05 (2-tailed), respectively. A very strong positive correlation was also found between EC and TDS (0.99), TDS and BOD (0.99), TDS and COD (0.99), BOD and COD (0.99) at significant levels of 0.05. Among the cationic parameters, a strong positive linear relationship was found between Ca^{2+} and Mg^{2+} (0.86), while among the anionic parameters, a strong positive correlation was found between PO_4^{3-} and SO_4^{2-} (0.96) at significant levels of 0.05. Among the heavy metals, a very strong positive linear relationship was also found between Mn and Fe (0.93) and Pb and Cr (0.99) at significant levels of 0.05. Each pair of the positively correlated variables will change linearly, which means that if the concentration of one variable is increased, the concentration of the other will also increase. Similarly, each pair of negatively correlated variables will change their concentrations in opposite directions [6].

3.6.2. Principal component analysis (PCA)

Principal components (PCs) were extracted using eigenvalues greater than 1 [6]. Two principal components (PCs) were extracted using a correlation matrix, which accounts for almost 100% of the total sample variance (Table 7). The first principal component (PC1) is strongly positively correlated (>0.75) with Temperature, EC, TDS, BOD, COD, Ca^{2+}, NO_3^-, Cl^-, PO_4^{3-}, SO_4^{2-}, Fe, and Ni, which has the greatest variability (66.21%) (Table 7). The first principal component is also strongly negatively correlated with DO. However, the second principal component (PC2) is strongly positively correlated with pH, TSS, K^+, Cu, and Cd. A strong negative correlation was also found in the PC2 with Cr and Pb (Table 7).

Positively correlated variables, either in PC1 or PC2, will vary together, that is, if one variable increases, the remaining ones tend to increase as well. Negatively correlated variables will show the opposite trends. In the future, for any development of WWTP, these trends must be considered for an effective solution.

4. Conclusion

The study results showed that the removal percentages for the major water quality parameters of the WWTP were in the order of TSS (91.80%) > BOD_5 (86.81%) > COD (72.29%) > TDS (72.20%) > EC (65.60%). The removal percentages of the cationic parameters were in the order of Ca^{2+} (73.30%) > Na^+ (72.20%) > K^+ (55.60%) > Mg^{2+} (54.60%), while the removal percentages for the anionic parameters were in the order of NO_3^- (92.30%) > PO_4^{3-} (70.40%) > Cl^- (65.40%) > SO_4^{2-} (58.80%). The removal rates for the heavy metals were in the order of Cu (84.62%) > Fe (65.04%) > Mn (63.3%) > Zn (60.58%) > Cd (53.85%) > Ni (54.12%) > Pb (42.42%) > Cr (38%). The four vital parameters, such as BOD_5, COD, NO_3^-, and PO_4^{3-} failed to achieve the standard permissible limits of the ECR (2023). The analysis of the treated effluent with different water quality indicators suggested that the discharged effluent was poor in quality, and fell within the high environmental risk category in terms of heavy metals only without considering the threat associated with EPs. The Pearson correlation and PCA analysis showed that EC, TDS, TSS, DO, BOD_5, COD, and some other parameters were the most correlating and contributing variables. This study argued that metal-ligand behaviors dominate the removal efficiency of heavy metals as well as pharmaceutical products. Therefore, it is necessary to install more modern and efficient effluent treatment plants, such as oxidation, for the removal of pharmaceutical products. It is highly recommended to carry out urgent research on the Korotoa

Table 6
Water quality of the influent and discharged effluent assessed by different indices.

Index method	Value (before treatment)	Water quality	Value (after treatment)	Water quality	Remarks
CCME-WQI	24.17	Poor category	36.76	Poor category	Considering all parameters, 52.10% development
HPI	400.38	Unsafe for drinking	187.53	Unsafe for drinking	Considering heavy metal pollution, 53.16% development
HEI	8.92	Low pollution	4.17	Low pollution	Considering heavy metal pollution, 53.25% development
$RQ_{MEC/PNEC}$[a]	34.05	High environmental risk	20.24	High environmental risk	Considering heavy metal pollution only, 40.56% development

[a] PNEC (ppb): Cr = 3.4; Mn = 209.88; Fe = 78; Ni = 44.3; Cu = 68.66; Zn = 87; Pb = 61.41; Cd = 57.33 [50,52,53].

Table 7
Rotated component matrix after Varimax Rotation.

PC1				PC2		
Temp	0.98	NO_3^-	0.99	Temp	0.21	NO_3^-	−0.09
pH	−0.11	Cl^-	0.92	pH	0.99	Cl^-	−0.39
EC	1	PO_4^{3-}	0.89	EC	−0.02	PO_4^{3-}	−0.46
TSS	0.45	SO_4^{2-}	0.98	TSS	0.89	SO_4^{2-}	−0.20
TDS	0.98	Cr	0.27	TDS	−0.19	Cr	−0.96
TH	0.73	Mn	0.67	TH	−0.69	Mn	0.75
DO	−0.98	Fe	0.88	DO	−0.21	Fe	0.46
BOD	0.97	Ni	0.98	BOD	−0.24	Ni	−0.18
COD	0.99	Cu	0.33	COD	−0.10	Cu	0.94
Na^+	−0.09	Zn	1	Na^+	0.99	Zn	0.02
K^+	−0.55	Pb	0.21	K^+	0.84	Pb	−0.98
Ca^{2+}	0.97	Cd	−0.58	Ca^{2+}	−0.24	Cd	0.82
Mg^{2+}	0.71	–	–	Mg^{2+}	−0.70	–	–
Eigenvalues	16.55				8.45		
% of Variance	66.21				33.79		
Cumulative %	66.21				100		

River and the river bank area for groundwater since antibiotics, other pharmaceuticals, and heavy metals may slowly accumulate in the aquatic systems.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [M.G. Mostafa], upon reasonable request.

CRediT authorship contribution statement

Md Anowar Hossen: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **G.S. Sattar:** Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **M.G. Mostafa:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Md Anowar Hossen reports financial support, administrative support, and equipment, drugs, or supplies were provided by University of Rajshahi. M.G. Mostafa reports a relationship with University of Rajshahi that includes: board membership and employment. NA If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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