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Physicochemical study of opium industry effluent and toxicity prediction using *in silico* method

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Industrial effluent discharge is a major concern in developing countries like India. Opioids and alkaloids serve as raw materials for production of important pharmaceutical drugs like codeine, morphine, papaverine, thebaine, etc. Proper effluent discharge and waste management from opioids processing and similar industries is still a challenge. The present study focuses on collection, characterization and toxicity prediction of pollutants found in opioids processing industries in India. The wastewater was collected from Government Opium Alkaloid Works, Ghazipur, Uttar Pradesh, India. The collected effluent was subjected to physicochemical characterization, identification and quantification of different pollutants. Physicochemical tests revealed Chemical oxygen demand of 10015 mg/L, Biological oxygen demand 3870 mg/L, and Total dissolved solid (TDS) 1275 mg/L, with an pH of 9.47 having dark brown appearance accompanied by a pungent odour. Gas chromatography mass spectrometry followed by Fourier transform infrared spectroscopy was performed to identify the chemical composition of the effluent which revealed the presence of butanol, toluene, methyl isocyanide, 2-methylpentane, and benzeamine, N, N-dimethyl in major concentrations. Toluene is one of the hazardous pollutants found in up to 5.17% in the effluent. Prolonged exposure to toluene leads to disorders of muscles, cardiovascular effects, permanent neuropsychiatric effects, renal tubular damage, and sudden death. The toxicity of toluene was predicted by Toxicity estimation software tool. The software provided the LD_{50} value for oral rat as 1.77 $-\log_{10}$ mol/kg and for human 0.28 $-\log_{10}$ mol/kg. The authors propose to develop a methodology to bioremediate toluene from wastewater to prevent harmful effects on aquatic and higher living organisms.

Keywords: Alkaloids,Opium effluent, Pollutants, Toluene, Waste management

Since ancient times, opium poppy (*Papaver somniferum*L.) has been recognized for its medicinal properties. As a major subsidiary crop worldwide, this plant continues to influence political, social, and economic spheres. The commercial supply of strong analgesics and anesthetics such as morphine, codeine, and semisynthetic derivatives like oxycodone, noscapine, thebaine, papaverine, buprenorphine and naltrexone is limited to permitted growth of poppy[1]. On the other hand, unrestrained extraction has caused contaminants to be released into the environment over time. Pollution has a detrimental impact on living organisms and is a major global environmental problem. Unrestrained urbanization over the past few decades has made this problem worse, largely because industrial waste and sewage is being improperly dumped into the water bodies. Hazardous organic pollutants are being released as a result of various anthropogenic activities, which adversely affects the quality of surface and groundwater[2]. Since all organizations need water for their operations, which in turn produces a significant amount of wastewater. Regrettably, a large amount of this effluent is frequently released into watercourses without being properly treated or with insufficient treatment, which pollutes these water bodies[3].

In particular, the complex structural components found in wastewater have contributed to persistence of the problem with robust effluent from production and processing plants of alkaloid[4]. Alkaloid processing plants are prominent in generating large amounts of effluent, on an average these plants release 24500-30500 L/day, since it is the sole viable source of medicinal alkaloids, including codeine, a cough suppressant, and morphine a painkiller, opium poppy has therapeutic value, furthermore, its effluent contains numerous harmful chemicals such as toluene, butanol, and other alkaloid impurities which on long term exposure causes serious damage to aquatic as

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well as terrestrial organisms. When opium is ingested, its alkaloid components or their derivatives leads to analgesic, hypnotic, antitussive, or antidiarrheal conditions, furthermore[5] chronic exposures to these pollutants can lead to disorders of muscles, cardiovascular effects, irritation to eyes, skin, damage to Central Nervous System (CNS), respiratory tract, and many others as listed in (Table 1)[6].

Cultivation and processing of poppy plant is permitted only under strict government regulations worldwide; however, illegal processing plants also exists which in turn releases a large amount of contaminants into the environment. Availability of literature, regarding treatment strategies of effluent from the opium processing industries is very less. Integrated Aerobic Membrane Bioreactor Nano-filtration (MBR-NF) has been used for the treatment of opium wastewater where it uses an aerobic membrane bioreactor to separate treated wastewater from diffused and unsettled biomass, and have been able to reduce Chemical oxygen demand (COD) from 32000 mg/L to 2500 mg/L and by using Nano-filtration (NF) removal of total Nitrogen[11]. [12]Kantoğlu& Ergun[12] revealed how the presence of methanol reduces the rate at which wastewater's alkaloids decompose due to radiation. They observed that irradiation leads to complete degradation of alkaloids, to oxygenated and methoxy group containing organic compounds as degraded products. Wang *et al.*[13] focused on using chemical ways to break down morphine in opium poppy processing waste by composting. After 30 days, the morphine concentrations of all treatments had dropped below the detection limit.Noorjahan[14] worked on physicochemical characterization and biodegradation of pollutants present in effluent. The authors reported isolation and identification of non-native bacillus sp. showing reduction in impurities by 95% and also stated the scope of re-usage of treated wastewater for agricultural purposes.Wijekoon and Facchini[1] investigated importance of Opium poppy for several benzylisoquinoline alkaloids. Kunukcu*et al.*[15] evaluated degradation of organic contaminants found in opium alkaloid wastewaters by anaerobic as well as aerobic biological degradation process and achieved 39% removal of using anaerobic process where as aerobic process led to 75% removal of organic load.Aydin*et al.*[16] chosen modern techniques for treating resilient effluent by Up-flow Anaerobic Sludge Blanket (UASB) Reactor and Sequential Batch Reactor (SBR) along with chemical treatment and observed that process of fenton's oxidation is more suitable for treatment of impurities present in opium wastewater. Biological treatment methods have been recommended because these are less expensive, more efficient moreover environmentally friendly than physical, chemical and other approaches for degradation of pollutants which are more expensive and less efficient.[17,18].

Table 1 — The major pollutants and their toxic effects with degradation methods

Compound	Toxicity	Bacteria/Method for degradation
2-Metyl pentane[7]	Mildly irritating to eyes, respiratory tract, and skin Toxic to aquatic life Cause effect on CNS React with Lysine damage protein structure	Methanogens Corny bacterium *Nocardia* sp.
Methyl Isocyanide[8]	Chest pain Lacrimation Eyelid edema Unconsciousness	Pseudomonas Pseudomonas alcaligens
Toluene[9]	Highly irritating to eyes, skin Toxic to aquatic life Cause membrane damage to leaf	Pseudomonas Bacillus Actinomyces
Butanol[10]	Cause effect on CNS [ildly irritating to eyes, respiratory tract, and skin	*Enterobacter* sp.

Present study is only the first part of a long-term study focussing on physicochemical characterization of opium effluent discharged by Government Opium & Alkaloid Works (GOAW), Ghazipur, Uttar Pradesh, India. The study also focuses on identification and quantification of major pollutants found in discharge of the effluent. The analysis revealed presence of various compounds, including 1-butanol, toluene, pentane, 2-methyl, benzeamine, N, N-dimethyl, methyl isocyanide, phenol. Presence of toluene in the effluent is a concern as long term exposure to toluene leads to acidosis, renal failure[19], acute asthma attack[20]. Toluene is a volatile organic compound (VOCs) which vaporizes into the atmosphere and becomes one of the component leading formation of photochemical smog. Photochemical smog is a brownish-grey haze caused by the action of solar UV- radiation in atmosphere polluted with hydrocarbons and oxides of nitrogen[21,22].

Due to toulenes' high toxicity and persistence, toluene is classified as a priority pollutant by the U.S. Environmental Protection Agency (EPA). The paper also presents the effects of toluene using Toxicity

Estimation Software Tool (TEST) providing values of lethal dose [LD_{50}], lethal concentration [LC_{50}] for rats and humans[23].

Material and Methodologies

General Description of Sampling Site

Government Opium & Alkaloid Works (GOAW) is one of the oldest opium factories in Asia, spreading over an area of 45 acres, situated on high banks of river Ganga in city of Ghazipur, Uttar Pradesh, India. Currently, there are only two opium processing factories in operation in India, one at Ghazipur and another at Neemuch (Madhya Pradesh), both the factories are under the direct administrative control of the Ministry of Finance, Government of India.

Sample collection

Proper way of collecting samples is crucial in obtaining efficient data; sample of opium effluent of dark brown colour with heavy smell was collected in the morning around 10:30 with an average temperature of 32℃ having geographical locations of longitudes 25.584042℃ and latitude 83.577019℃. GOAW factory runs 365 days, discharging effluent at the rate of 36500-44500 L/year containing morphine sulphate in majority, codeine phosphate, narcotine, thebaine, papaverine, etc. Plant has no recovery unit to recover these or useful components before being discharged into the nearby riverine system.

Characterization of Opium effluents

Physicochemical study

Evaluation of physicochemical factors which are included in discharge limitations and have the potential to harm the receiving environment, included parameters like pH, temperature, Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total discharge solid (TDS), total nitrogen content, total phosphorous and Total suspended solids (TSS). Organic components were characterised using Fourier transform infrared (FTIR) spectroscopy,Gas chromatography mass spectrometry (GCMS). For determination of pH, and TDS, Sentwin pH meter, Labtronics TDS meter LT25, were used, while UTS BOD incubator FC 344, COD digester Hanna HI 839800, Centrifuge-Remi R4C, were used according to standard procedures in the laboratory for determination of COD, and BOD[24].

GCMS analysis

Fifty milliliters (mL) of opium effluent was treated three times with an equivalent volume of hexane in order to examine presence of organic elements in effluent[25]. Using hexane and trimethyl silyl, the effluent was collected and derivatized. In GCMS [Agilent Technologies 7697A Headspace sampler (HS GCMS)], a silylated sample (1 µL) was injected. At a temperature of 250°C in the column with a flow rate of 1.1 mL min^{-1}, the injection step was run in direct injection mode. Retention time (RT) in minutes and mass spectra from full scan mode (m/z 45–800) was used to identify the components [26].

FTIR analysis

To determine the different organic functional groups and asses the possible chemical composition, FTIR was performed. Fifty µL of effluent was used for FTIR using Diamond ATR. The investigation were carried out on FTIR with spectrum scan of 500–4000 cm^{-1} (Shimadzu IR Affinity 1S)[26].

Toxicity prediction by "*in silco*" method

TEST, an open-source program developed by the US Environmental Protection Agency (EPA), was used to perform "Insilico." experiments. Versions 5.1.2 and 4.2.1 of TEST comprise a broad range of models intended to assess acute toxicity thresholds using of structural analogue analysis or multivariate regression[23]. TEST uses this large collection of descriptors to help with toxicity assessments. The software features endpoint models, including the 50% fatal concentration for fathead minnows after 96 h, the 50% lethal concentration (LC_{50}) for daphnia magna after 48 h, the 50% lethal dose (LD_{50}) for oral rats, and tetrahymena pyriformis after 50 h. For its predictive algorithms, the TEST software uses training data from the ECOTOX database. To evaluate acute toxicity, specifically the oral rat LD_{50} endpoint, TEST utilizes four Quantitative structure-activity relationship (QSAR) approaches, Hierarchical clustering, Nearest neighbour, Consensus for predicting the LC_{50} and LD_{50}[27]. The rat-to-human extrapolation is considered by converting toxicity results in accordance with standard recommendations for dosage conversion based on body surface area. The software computes LD_{50} values for oral administration in rats. The human dose is calculated by dividing the rat dose by a ratio of 6.2 [28].

Result and Discussion

Physicochemical analysis

The plant produces 36500-44500 L/year of effluent, having dark brown appearance and a high organic content, with COD 10,015 mg/L and BOD 3,870 mg/L

whereas TDS 1,275 mg/L, pH of 9.47, values as given in (Table 2). These results were supported by data published in literature with COD 30000 mg/L, BOD 16625 mg/L, pH 5, TSS 555 mg/L, VSS 382 mg/L [29], also studies led by Erdem *et al*.[30] has obtained BOD of 10151 mg/L, and COD of 32995 mg/L.

GCMS analysis

The chromatogram in (Fig. 1 —A) depicts the GCMS analyses untreated effluent. Retention time was used to identify the presence of various organic pollutants. The analysis revealed presence of compounds, including 1-butanol (RT = 10.01),

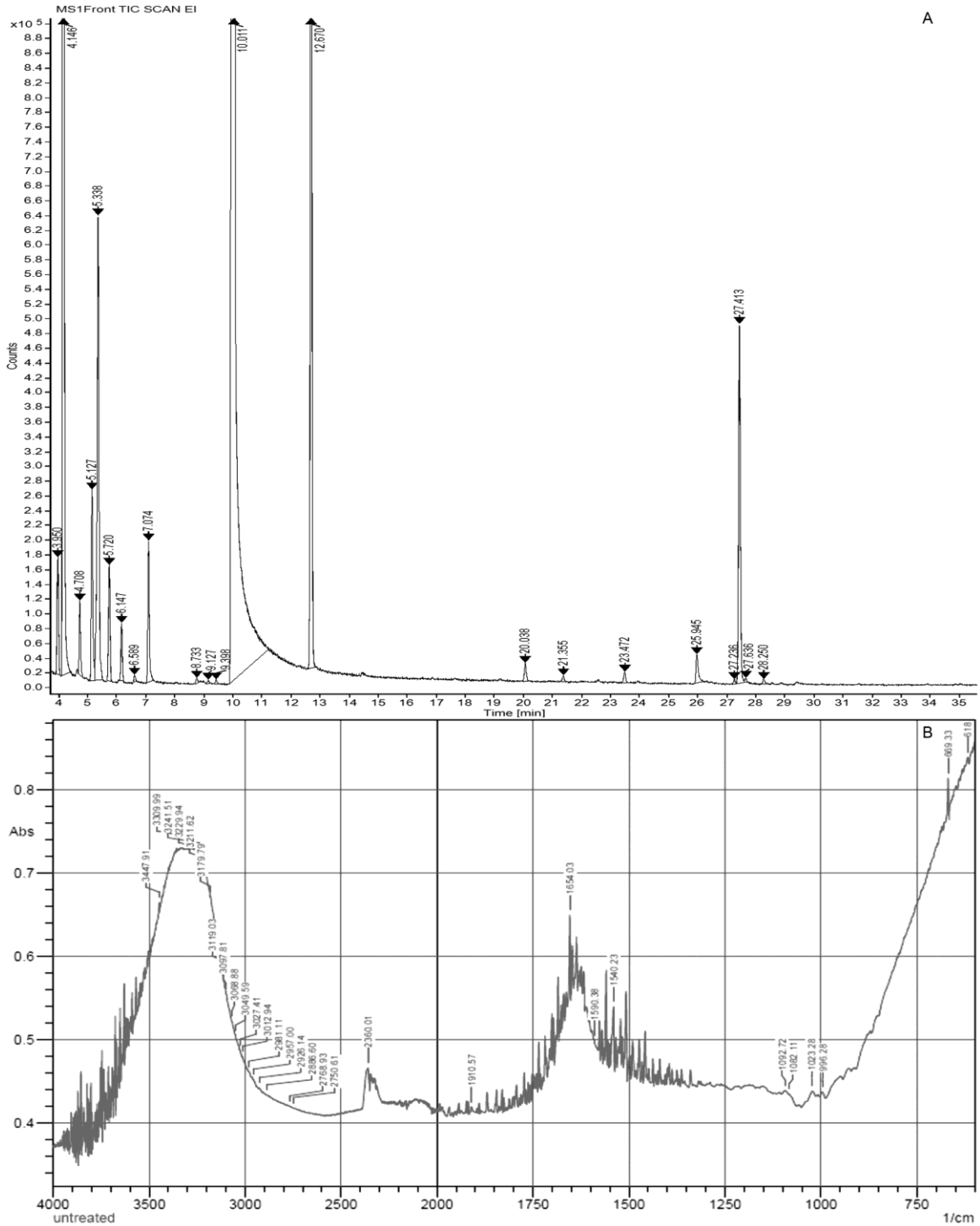

Fig. 1 — (A) GC-MS chromatogram; and (B) FTIR analysis of untreated opium effluent

Table 2 — Physicochemical characterisation of untreated Opium effluents

Parameter	Experimental value
Physical	
Colour	Dark Brown
Odour	Strong
Taste	NA
Chemical	
pH	9.47
TDS	1275 mg/L
TS	116505 mg/L
BOD	3870 mg/L
COD	10015 mg/L
Total Nitrogen as N	38.5 mg/L
Total Phosphorus as P	5.0 mg/L
TVS	67105 mg/L

[TDS, Total dissolved solids; TS, Total solids; BOD, Biological oxygen demand; COD, Chemical oxygen demand; TVS, Total volatile solids]

toluene (RT = 12.67), pentane,2-methyl (RT = 5.34), benzeamine, N,N-dimethyl (RT = 27.41), methyl isocyanide (RT = 5.13), phenol (RT = 25.95). The findings suggest that the predominant toxic compounds identified in the effluent were 1, 2 methyl pentane, methyl isocyanide, toluene and butanol. The authors were first to report the GCMS characterization of the Opioid Processing Plant however, there are studies reporting usage of chromatographic and other techniques for identification of chemical constituents of the discharged effluent [9].

FTIR analysis

In this study, an in-depth analysis of untreated opium effluent was conducted using FTIR Spectroscopy. FTIR was done to identify various organic functional groups and identification of probable pollutants in effluent. Fifty µL of effluent was used for FTIR using Diamond ATR. The analysis was carried out on FTIR spectrum scan of 500–4000 cm^{-1}. A comprehensive examination involving 30 scans per run revealed a spectrum with various absorbance peaks, ranging from 618.21 cm^{-1} to 3958.1 cm^{-1}. The observed peaks were indicative of the presence of toxic compounds with peak value, as sulfonate (1339.62 cm^{-1}), sulphonamides (1350.23 cm^{-1}-1363.73 cm^{-1}), isothiocyanate (2025.89 cm^{-1}-2089.96 cm^{-1}), azide (2134.33 cm^{-1}-2143.01 cm^{-1}), carbodiimide (2134.33 cm^{-1}-2143.01 cm^{-1}) and thiocyanate (2167.12 cm^{-1}) collectively expressed in the range of 1339.62- 2167.12 cm^{-1}, these results are supported by the literature[31]. Presence of alkaloid compounds, toxic elements and complex species were identified by slope analysis and FTIR spectra[32]. The authors observed bifurcated grease waste peaks at 2926 cm^{-1} and 2855 cm^{-1} which reduce remarkably[31]. This identification of specific chemical constituents provides valuable insights into the nature of the sample. Additionally, the study unveiled structural possibility due to these compounds denoted as N-H bending, C=C stretching, C-H stretching, N-H stretching (strong, broad), O-H bending,S=O stretching, N=C=S stretching, N=N=N stretching, N=C=N stretching and S-C≡N stretching, with strong appearance validate the presence of these toxic compounds. The findings presented herein (1B), contribute to a deeper understanding of the chemical composition within the untreated opium effluent sample.

Table 3 — Toxicity values of Toluene on different end points

End points	Methods	Predicted value -log10 (mol/kg)	Exp. value -log10 (mol/kg)
Oral rat	Hierarchical clustering	2.13	
LD_{50}	Nearest Neighbour	1.40	2.16
	Consensus	1.77	
Human	Hierarchical clustering	0.34	
LD_{50}	Nearest Neighbour	0.26	NA
	Consensus	0.28	
Fathead	Hierarchical clustering	3.44	
Minnow	Nearest Neighbour	3.86	3.43
LC_{50} (96 h)	Consensus	3.47	
Daphnia	Hierarchical clustering	3.00	
Magna	Nearest Neighbour	3.98	3.00
LC_{50}(48 h)	Consensus	3.64	

Toxicity prediction results

The software, TEST was further employed in order to estimate the toxicity of pollutants ("*In silico*") using the QSAR-based Consensus method. The LD_{50} value of toluene was calculated using various clustering algorithms (Hierarchical clustering, Nearest Neighbour, Consensus) for LD_{50} for Oral rat, Humans, Fathead Minnow LC_{50} (96hr), Daphnia Magnaas shown (Table 3). Rat-to-human extrapolation is calculated by translating toxicity data in accordance with prescribed dosage conversion criteria that take body surface area into account the rat LD_{50} in this study is 1.77 is close to several published work[33–35], LD_{50} for human is 0.34, 0.26, 0.28 [all the values are in -log10mol/kg] from Hierarchical clustering, Nearest Neighbour, Consensus respectively in these methods consensus provides the most reliable result. The human LD_{50} 0.28 (Consensus TEST) values are similar to data available in literature[36].

Conclusion

Contamination of the aquatic sources disturbs marine life and it harms the terrestrial organisms also.

Semi processed or untreated discharge of effluent of opium processing or associated industries is a concern. To assess different types of pollutants of Opium processing industry, authors collected the effluent discharged by the Government Opium Alkaloid Works (GOAW), Ghazipur, India. Physicochemical characterization of this effluent yielded high values of COD, BOD and TDS of 10015, 3870 and 1275 mg/L, respectively, signifying high pollution levels therefore appropriate and effective treatment method is needed for effluent treatment before disposal. The contaminants identified through GCMS were majorly toluene, methyl isocyanide, 2-methyl pentane, etc. in effluent. Functional groups like N=C=S stretch (2089.96), N=C=N stretch (2134.33), S=O stretch (1707.08) and functional groups like sulfonate, sulphonamides , isothiocyanate , azide , carbodiimide ,thiocyanate, alkene, secondary alcohols, aliphatic ether, conjugated aldehydes, alkene and halo compounds were identified using FTIR. In addition to this toxicity prediction was done using "Insilico." by using TEST software. Using TEST human LD_{50} values were predicted to be 0.34, 0.26, and 0.28 ($-\log_{10}$ mol/kg), respectively. The Consensus approach, notably, yielded the most trustworthy outcome. With all these experimental techniques used in laboratory and computational approach narrate the toxic behavior of opium effluents.From the current study toluene and methylisocyanide were found to be major pollutants.

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Conflict of Interest

Authors declare no competing interests.

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