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Evaluation of Mutagenicity of Refinery Waste Effluents

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ABSTARCT

The mutagenicity evaluation of refinery wastewater from Mathura (Uttar Pradesh), was carried out by Ames Salmonella/microsome test. Effluents were concentrated using XAD resins and extracted using dichloromethane (DCM), chloroform and hexane solvents. Different natural mixes were recognized by GC-MS investigation in the tested samples. TA98 strain was found to be most responsive with and without metabolic activation. XAD concentrated wastewater samples showed more mutagenicity as compared to liquid-liquid extracts (chloroform, hexane and dichloromethane extracts).

Keywords: Ames test, Extraction, Mutagenicity, Refinery

I. INTRODUCTION

Petroleum is one of the most important sources of energy on this planet. In any case, the oil processing stages, identified with various phases of generation have been leading several environmental impacts, basically the arrival of toxins into water systems. Effluents from petroleum comprises of mixes from unique petroleum stock and also metallic (Zn, Cr, Cu, Ni, Pb, Va) and non-metallic constituents. Phenols are additionally a noteworthy portion of refinery effluents. Besides, among the hydrocarbons present in raw petroleum, the polycyclic aromatic hydrocarbons (PAHs) are the absolute most perilous natural contaminants due to their toxic, cancer causing, and mutagenic impacts.

Hazardous substances competent of altering the DNA of living organisms might occur below the detection limit, however act as genotoxins in these low concentrations. Both Phenols and PAHs are known to initiate genotoxcity even at low concentrations. This has raised worry for the genotoxic and carcinogenic impacts related with the release of industrial wastes and effluents.

Usually physical and chemical measurements are performed for hazard and risk appraisal of contaminated water/soil samples but chemical investigation alone may not be adequate for biological evaluation. Toxicity evaluation utilizing biological assays is a notable technique for complex toxins. Prior information of key contaminants is not required and intuitively toxic quality of the compounds is reflected by the results.

By utilizing both pro- and eukaryotic test systems will fortify and correlate the results to ensure if the chemicals truly have any dangerous effects on the genes. Ames test, *Salmonella*/microsome test is broadly utilized as a part of examining the mutagenic impacts of chemicals. It is not only one of the most dependable short-term bacterial test systems but also cheap and exceptionally fast [1].

In the present study the genotoxicity of refinery wastewaters from Mathura, was performed by the bacterial reverse mutation assay in *Salmonella typhimurium* TA97a, TA98, TA100, TA102, and TA104 strains with and without metabolic activation.

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II. MATERIALS AND METHODS

2.1.Sample Collection

Mathura refinery was authorized in the year 1982 as India's sixth oil refinery, settled between Delhi and Agra; the refinery at Mathura U.P. (India) is located at 27˚30'N 77˚41'E 27.5'N 77.68'E. The effluents samples were collected from the encompassing zone of refinery, in sterile glass bottles according to the method depicted by APHA [2].

2.2.XAD-extraction

For the extraction of organic compounds, 1 liter of refinery wastewater was collected. Before concentration the water samples were filtered through two membrane filters with pore sizes of 8 µm and 0.45 µm. The adsorption of organic constituents on the XAD-resins was performed as suggested by Wilcox and Williamson [3]. The adsorbed material was then eluted with 20 ml of acetone (HPLC-grade). This elute was dissipated to dryness and reconstituted in DMSO (HPLC-grade) such that 5 ml of concentrate was proportional to 1 liter of original sample. Samples were filter sterilized through 0.45 µm filter and stored at - 20°C until further testing.

2.3.Liquid-liquid extraction

Extraction of effluents was performed with three different organic solvents, namely dichloromethane (DCM), n-hexane, and chloroform (HPLC-grades) as given in APHA [2]. Concentrates were filtered through 0.45 µm membrane filter before they were utilized for genotoxicity testing and GC-MS examination.

2.4.Bacteria used in the study

The *Salmonella typhimurium* strains were received from National Institute of Hygienic Sciences, Division of Genetics and Mutagenesis, Tokyo, Japan. The bacterial strains were maintained in frozen stocks and grown as suggested by Maron and Ames [1].The characteristics of the bacterial strains are given in Table. 1.

Table 1. Characteristics of Ames *Salmonella* strains

Strain designation	Relevant Genetic Markers
Ames Tester Strains	
TA97a	*uvrB, hisD661, bio, rfa*, R-factor plasmid-pkM101, frame shift mutation at G-C site
TA98	*uvrB, hisD3052, bio, rfa*, R-factor plasmid-pkM101 frame shift mutation at G-C site
TA100	*uvrB, hisG46, bio, rfa*, R-factor plasmid-pkM101, base pair substitution mutation at G-C site
TA102	*rfa*, R-factor plasmid-pkM101, multicopy plasmid paQ1 containing hisG428 auxotrophic marker and Tet^r, transition mutation at A-T site
TA104	*uvrB, hisG428, rfa*, R-factor plasmid-pkM101, transition mutation at A-T site

2.5.Salmonella Mutagenicity Test

The preincubation test was executed as depicted by Maron and Ames (1983) with a few modifications [4]. Five doses of each water extracts i.e. 1, 5, 10, 20 and 40 µl/plate (0.2, 1, 2, 4 and 8 ml-identical wastewater/plate respectively) were plated in triplicate with 0.1 ml of the bacterial culture. After incubation of the test samples and bacterial culture for 30 min at 37°C, 2.0 ml top agar containing traces of histidine and biotin was added and were poured on minimal glucose agar plates. Plates were incubated at 37°C for 48-72 h. Negative and positive

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controls were included in each assay. The negative control plates contained bacteria and DMSO. Methyl methane sulphonate and sodium azide used as positive controls. All the water extracts were also tested in the presence of S9 microsomal fraction, to which 20 µl of S9 liver homogenate mix per plate was included. For classifying the results as positive the criteria used was similar to those of Vargas et al. [5]: number of revertants double the spontaneous yields accompanied by a reproducible dose-response curve.

III. STATISTICAL ANALYSIS

Mutagenic Index

The number of his+ revertants in the test sample was compared to the negative control by its mutagenic index value.

$$\text{Mutagenic index} = \frac{Number\ \ of\ his^{+}revertants\ \ induced\ \ in\ the\ sample}{Number\ \ of\ his^{+}revertants\ \ induced\ \ in\ the\ negative\ \ control}$$

Mutagenic potential (m)

Mutagenic potential was calculated by the initial linear portion of the dose response curve with tester strains. Slope (m) was obtained by least square regression of the initial linear portion of the curve of initial dose-response.

Induction factor (Mi)

The induction factor for various tester strains for different water extracts was evaluated as follows:

Mi = *ln n-c/c*

Where n is the number of revertants in the sample and c is the number of revertants in solvent control.

Results

GC-MS analysis of wastewater extracts revealed presence of various organic compounds (Table 2). The mutagenicity of XAD-concentrated and liquid-liquid extracted wastewater samples was assessed utilizing *Salmonella typhimurium* strains. The reversion of *Salmonella typhimurium* strains with XAD-concentrated water samples is summarized in Table 3. It was found that XAD extracts showed maximum response with TA98 strain with and without S9 fraction. An increase in the reversion of tester strains was observed up to dose level of 20 µl/plate and declined at a dose of 40 µl/plate. Among all the strains tested, TA98 showed maximum mutagenic index of 13.0 (without S9) and 12.1 (with S9) with basic fraction of DCM, while acidic fraction of DCM exhibited mutagenic index of 11.1 (without S9 fraction) and 11.4 (with S9 fraction) in the TA98 strain. Similarly, TA98 showed maximum response in terms of induction factor (Mi) and slope (m) of the initial linear dose-response curve as determined by linear regression analysis. It was observed that TA98 showed maximum response with and without metabolic activation. The order of responsiveness in view of the mutagenic index and induction factor for samples was TA98 > TA97a > TA100 > TA102 > TA104.

Table 2. Compounds identified in wastewaters using GC-MS

Sample	NIST library ID
Site 1 (hexane extract)	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester Bis (2-ethylhexyl) phthalate Propane, 2-chloro- 1-chloro-2-nitro- 1,9-Nonanediol Pyrido[2,3-d] pyrimidine-2, 4 (1H, 3H)

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Site 1 (acidic DCM fraction)	Phenol, 2,4- bis (1,1-dimethylethyl)- Di-n-octyl phthalate 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester Nonane, 2,2,4,4,6,8,8-heptamethyl- Cyclopentane, undecyl-
Site 1 (basic DCM fraction)	Phenol, 2,4- bis (1,1-dimethylethyl)- Di-n-octyl phthalate 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester 6-Octen-1-ol, 3,7-dimethyl-

Table 3. Reversion of *Salmonella* tester strains in the presence of XAD-concentrated wastewater samples

		Doses (µl/plate)							
Strain	**S9**	**Control**	**1**	**5**	**10**	**20**	**40**	**Mi**	**m**
TA97a	-	96 ± 7	194 ± 10 (2.0)	258 ± 9 (2.6)	315 ± 10 (3.2)	361 ± 8 (3.7)	307 ± 9 (3.2)	1.01	4.08
	+	98 ± 5	202 ± 11 (2.1)	272 ± 8 (2.8)	331 ± 7 (3.4)	375 ± 10 (3.8)	315 ± 13 (3.2)	1.04	4.12
TA98	-	37 ± 4	168 ± 7 (4.5)	203 ± 8 (5.4)	271 ± 12 (7.3)	400 ± 14 (10.8)	346 ± 12 (9.3)	2.26	6.6
	+	40 ± 6	183 ± 13 (4.6)	218 ± 10 (5.5)	294 ± 15 (7.4)	428 ± 14 (10.7)	365 ± 16 (9.1)	2.27	6.9
TA100	-	131 ± 9	236 ± 11 (1.8)	295 ± 11 (2.2)	368 ± 13 (2.8)	448 ± 12 (3.4)	360 ± 10 (2.7)	0.88	4.7
	+	136 ± 11	248 ± 15 (1.8)	310 ± 13 (3.2)	382 ± 11 (2.8)	467 ± 10 (3.4)	384 ± 8 (2.8)	0.89	5.07
TA102	-	238 ± 10	346 ± 13 (1.4)	378 ± 10 (1.5)	443 ± 16 (1.8)	491 ± 13 (2.0)	430 ± 13 (1.8)	0.06	3.63
	+	248 ± 14	360 ± 14 (1.5)	390 ± 20 (1.6)	465 ± 12 (1.9)	508 ± 19 (2.0)	455 ± 15 (1.8)	0.05	3.9
TA104	-	337 ± 15	408 ± 12 (1.2)	448 ± 14 (1.3)	514 ± 16 (1.5)	561 ± 16 (1.6)	526 ± 14 (1.5)	-0.41	4.0
	+	344 ± 12	423 ± 18 (1.2)	460 ± 22 (1.3)	531 ± 18 (1.6)	581 ± 11 (1.7)	541 ± 17 (1.6)	-0.37	4.1

Values in parentheses are mutagenic index; Mi induction factor; m mutagenic potential

Table 4. Reversion of *Salmonella* tester strains in the presence of acidic fraction of dichloromethane extracted wastewater sample

		Doses (µl/plate)							
Strain	**S9**	**Control**	**1**	**5**	**10**	**20**	**40**	**Mi**	**m**
TA97a	-	93 ± 6	190 ± 11 (2.0)	238 ± 11 (2.5)	302 ± 11 (3.2)	345 ± 10 (3.7)	310 ± 9 (3.3)	0.99	4.3
	+	98 ± 5	200 ± 8 (2.0)	250 ± 13 (2.6)	321 ± 8 (3.3)	362 ± 7 (3.7)	325 ± 6 (3.3)	0.99	4.5
TA98	-	35 ± 3	130 ± 6 (3.7)	197 ± 7 (5.6)	258 ± 10 (7.3)	390 ± 9 (11.1)	338 ± 10 (9.6)	2.31	6.8
	+	36 ± 5	146 ± 5	208 ± 9	271 ± 16	411 ± 11	354 ± 14	2.34	7.1

			(4.1)	(5.7)	(7.5)	(11.4)	(9.8)		
TA100	-	129 ± 10	215 ± 10 (1.7)	265 ± 10 (2.0)	336 ± 8 (2.6)	400 ± 12 (3.1)	347 ± 11 (2.6)	0.74	4.6
	+	132 ± 9	226 ± 15 (1.7)	281 ± 22 (2.3)	355 ± 9 (2.7)	418 ± 17 (3.2)	366 ± 17 (2.8)	0.77	4.9
TA102	-	241 ± 11	317 ± 9 (1.3)	354 ± 12 (1.4)	414 ± 11 (1.7)	473 ± 11 (1.9)	418 ± 13 (1.7)	-0.03	4.4
	+	259 ± 12	330 ± 10 (1.3)	373 ± 17 (1.4)	428 ± 20 (1.7)	490 ± 22 (1.9)	431 ± 23 (1.7)	-0.11	3.6
TA104	-	334 ± 13	394 ± 13 (1.1)	430 ± 15 (1.2)	492 ± 14 (1.4)	536 ± 13 (1.6)	500 ± 15 (1.4)	-0.50	3.6
	+	338 ± 16	400 ± 19 (1.2)	452 ± 25 (1.3)	513 ± 17 (1.5)	554 ± 18 (1.6)	518 ± 24 (1.5)	-0.64	3.9

Values in parentheses are mutagenic index; Mi induction factor; m mutagenic potential

Table 5. Reversion of *Salmonella* tester strains in the presence of basic fraction of dichloromethane extracted wastewater sample

			Doses (µl/plate)						
Strain	**S9**	**Control**	**1**	**5**	**10**	**20**	**40**	**Mi**	**m**
TA97a	-	89 ± 6	181 ± 10 (2.0)	228 ± 9 (2.5)	284 ± 11 (3.1)	312 ± 8 (3.5)	276 ± 8 (3.1)	0.91	3.5
	+	92 ± 6	195 ± 12 (2.1)	246 ± 10 (2.7)	300 ± 8 (3.3)	330 ± 10 (3.6)	295 ± 6 (3.2)	0.95	3.7
TA98	-	29 ± 4	119 ± 7 (4.1)	167 ± 8 (5.7)	241 ± 6 (8.3)	377 ± 12 (13.0)	319 ± 10 (11.0)	2.48	6.7
	+	33 ± 7	130 ± 14 (3.9)	180 ± 5 (5.5)	262 ± 15 (8.0)	398 ± 17 (12.1)	336 ± 5 (10.2)	2.40	6.9
TA100	-	124 ± 7	208 ± 8 (1.6)	260 ± 11 (2.0)	316 ± 8 (2.5)	390 ± 11 (3.1)	338 ± 13 (2.7)	0.76	4.5
	+	131 ± 10	224 ± 16 (1.7)	274 ± 10 (2.1)	331 ± 12 (2.5)	411 ± 10 (3.1)	350 ± 18 (2.7)	0.76	4.6
TA102	-	239 ± 10	289 ± 13 (1.2)	331 ± 10 (1.3)	400 ± 9 (1.6)	468 ± 13 (1.9)	411 ± 12 (1.7)	-0.04	4.0
	+	247 ± 10	300 ± 13 (1.2)	352 ± 9 (1.4)	417 ± 13 (1.7)	485 ± 15 (2.0)	430 ± 20 (1.7)	-0.04	4.2
TA104	-	328 ± 12	371 ± 14 (1.1)	427 ± 13 (1.3)	478 ± 17 (1.4)	523 ± 14 (1.5)	485 ± 14 (1.4)	-0.52	3.5
	+	334 ± 9	392 ± 19 (1.2)	443 ± 22 (1.3)	496 ± 16 (1.5)	538 ± 12 (1.6)	504 ± 13 (1.5)	-0.49	3.6

Values in parentheses are mutagenic index; Mi induction factor; m mutagenic potential

The response of various strains based on slope (m) of the initial linear dose response was obtained by the least square regression analysis. It was discovered that TA98 shows maximum value of the slope followed by TA100 in all the extracts of the refinery wastewater. The order of responsiveness of various strains in terms of slope for XAD extract, acidic and basic fraction of DCM extracts of effluent with and without S9 fraction was as under: TA98 > TA100 > TA97a > TA104 > TA102 (Table 3-5), while for hexane and chloroform extracts without S9 were as follows: TA98 > TA100 > TA97a > TA102 > TA104 (Table 6 and 7). It was observed that reversion of the strains increases significantly with increasing doses in comparison to the negative control suggesting a dose-dependent mutagenicity. The results obtained revealed that XAD has higher mutagenic index, induction factor, and slope (m) of the response with increasing doses in comparison to liquid-liquid extracts. The results indicate

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that toxicity of different samples can be arranged as follows: XAD-concentrated > dichloromethane extracted water samples > hexane extracted water samples > chloroform extracted water samples.

Table 6. Reversion of *Salmonella* tester strains in the presence of hexane extracted wastewater sample

			Doses (µl/plate)						
Strain	**S9**	**Control**	**1**	**5**	**10**	**20**	**40**	**Mi**	**m**
TA97a	-	82 ± 6	138 ± 6 (1.6)	162 ± 8 (1.9)	221 ± 9 (2.6)	275 ± 9 (3.3)	241 ± 9 (2.9)	0.85	3.7
	+	90 ± 3	152 ± 7 (1.7)	175 ± 18 (1.9)	240 ± 6 (2.7)	294 ± 8 (3.7)	259 ± 11 (2.9)	0.82	3.8
TA98	-	35 ± 5	147 ± 8 (4.2)	200 ± 7 (5.7)	267 ± 10 (7.6)	312 ± 14 (8.9)	278 ± 11 (7.9)	2.1	4.8
	+	36 ± 7	164 ± 19 (4.6)	221 ± 10 (1.6)	288 ± 17 (8)	330 ± 10 (9.2)	296 ± 10 (8.2)	2.1	4.9
TA100	-	130 ± 9	178 ± 11 (1.3)	243 ± 11 (1.8)	330 ± 8 (2.5)	371 ± 12 (2.8)	339 ± 10 (2.6)	0.61	4.7
	+	137 ± 13	193 ± 14 (1.4)	256 ± 9 (1.9)	342 ± 12 (2.5)	390 ± 11(2.9)	357 ± 15 (2.6)	0.62	4.9
TA102	-	241 ± 10	283 ± 13 (1.1)	325 ± 9 (1.3)	386 ± 11 (1.6)	439 ± 13 (1.8)	394 ± 13 (1.6)	-0.19	3.6
	+	248 ± 12	300 ± 18 (1.2)	341 ± 12 (1.4)	400 ± 7 (1.6)	452 ± 15 (1.8)	412 ± 13 (1.7)	-0.18	3.7
TA104	-	325 ± 11	359 ± 19 (1.1)	418 ± 12 (1.2)	472 ± 16 (1.4)	516 ± 16 (1.5)	478 ± 16 (1.4)	-0.53	2.9
	+	331 ± 8	380 ± 20 (1.2)	432 ± 19 (1.3)	488 ± 18 (1.5)	536 ± 25 (1.6)	500 ± 21 (1.5)	-0.48	2.7

Values in parentheses are mutagenic index; Mi induction factor; m mutagenic potential

Table 7. Reversion of *Salmonella* tester strains in the presence of chloroform extracted wastewater samples

			Doses (µl/plate)						
Strain	**S9**	**Control**	**1**	**5**	**10**	**20**	**40**	**Mi**	**m**
TA97a	-	94 ± 6	147 ± 10 (1.5)	200 ± 6 (2.1)	255 ± 7 (2.7)	300 ± 6 (3.1)	250 ± 8 (2.6)	0.78	3.4
	+	98 ± 10	160 ± 7 (1.6)	217 ± 9 (2.1)	270 ± 9 (2.8)	315 ± 15 (3.2)	270 ± 15 (2.8)	0.79	3.6
TA98	-	32 ± 5	92 ± 7 (2.8)	150 ± 8 (4.6)	218 ± 10 (6.8)	336 ± 9 (10.5)	279 ± 10 (8.7)	2.25	5.4
	+	36 ± 4	110 ± 9 (3.1)	172 ± 10 (4.8)	237 ± 11 (6.6)	350 ± 8 (9.7)	295 ± 8 (8.2)	2.17	5.9
TA100	-	128 ± 8	189 ± 11 (1.4)	234 ± 11 (1.8)	271 ± 8 (2.1)	365 ± 10 (2.8)	300 ± 11 (2.3)	0.61	3.9
	+	130 ± 9	200 ± 12 (1.5)	250 ± 9 (1.9)	293 ± 8 (2.3)	381 ± 15 (2.9)	321 ± 10 (2.5)	0.66	4.2
TA102	-	244 ± 9	275 ± 13 (1.1)	316 ± 12 (1.2)	377 ± 11 (1.5)	446 ± 13 (1.8)	400 ± 15 (1.6)	-0.18	3.8
	+	251 ± 7	294 ± 16 (1.6)	330 ± 13 (1.3)	398 ± 17 (1.6)	463 ± 12 (1.8)	428 ± 20 (1.7)	-0.17	4.3
TA104	-	325 ± 13	363 ± 14 (1.1)	400 ± 14 (1.2)	452 ± 14 (1.3)	500 ± 16 (1.5)	460 ± 13 (1.4)	-0.61	3.1
	+	331 ± 8	384 ± 20 (1.2)	424 ± 21 (1.3)	474 ± 16 (1.4)	518 ± 19 (1.6)	471 ± 21 (1.4)	-0.57	2.9

Values in parentheses are mutagenic index; Mi induction factor; m mutagenic potential

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The net revertants per liter for the most responsive strains TA97a, TA98 and TA100 are presented in Table 8. The XAD concentrated sample showed maximum number of net revertants per liter for TA98 with (1070000) and without S9 fraction (1000000).

Table 8. Net revertants/liter for the most responsive TA97a, TA98 and TA100 strains

Strain	S9 fraction	Net Revertants/l				
		XAD-concentrated water sample	Hexane extracted water sample	Dichloromethane Acidic extract	Dichloromethane Basic extract	Chloroform extracted water sample
TA97a	-	902500	602500	826250	780000	750000
	+	937500	735000	905000	825000	787500
TA98	-	1000000	780000	975000	942500	840000
	+	1070000	825000	1027500	995000	875000
TA100	-	1120000	927500	1000000	975000	912500
	+	1167500	975000	1045000	1027500	952500

IV. DISCUSSION

Genotoxicity testing of surface waters or industrial effluents using a set of bioassays demonstrates that these samples contain various unidentified and unregulated toxicants that may cause harm and carcinogenicity of high magnitude [6, 7]. With the advent of industry, refinery has been seen as a major source of environmental contaminants since after the beginning of the twentieth century [8]. In surroundings of refinery, unrefined oils (primarily PAHs) and phenols are the significant portions. Some high molecular weight PAHs, for example, benzo(a)pyrene, dibenzo(a, h)anthracene, benzo(b)fluoranthene, and benzo(k)fluroranthene are famous for their mutagenicity and clastogenicity.

Ames plate incorporation assay with refinery wastewater showed a significant mutagenicity with all the analyzer strains. However TA98 and TA100 were observed to be most responsive strains with test tests in connection to acceptance factor both with and without metabolic initiation. (Table 3-7). Based on the slope of the initial dose response, which is usually taken as valid indicator of the mutagenic response the trend of sensitivity of tester strains was different, TA98 and TA100 or TA97a occupied the first place in order of sensitivity in the presence and absence of S9 mix respectively. Sensitivity of TA97a, TA98 and TA100 suggest presence of frame shift and base pair substitution mutagen respectively in the test samples. Employing a set of different strains instead of a single strain for mutagenicity testing of complex samples is useful since single strain would not clearly determine the different classes of mutagens present in the contaminated samples. A significant increase in the revertant frequency in presence of S9 fraction suggests that the test samples were having substances that are convertible to more risky mutagens after metabolic initiation.

Significant mutagenicity was found with XAD concentrates when compared with liquid-liquid extracts (Table 8). XAD resins can generally adsorb a broad class of mutagenic mixes, including polycyclic aromatic hydrocarbons, aryl amines, nitro mixes, quinolines, anthraquinones, and so on. Adsorption brought after by elution with solvents is helpful in extracting all the polar and non polar toxic chemicals and mutagens/genotoxicants [9]. Therefore, most mutagens are expected to be concentrated by XAD resins. Aleem and Malik [10] tested genotoxicity of the Yamuna River water at Okhla (Delhi), India, their Ames assay results revealed that the XAD-concentrated water samples had maximum mutagenicity with TA98 strain both in the

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absence and presence of S9 mix. Liquid-liquid extracted samples were also found mutagenic however to a lesser degree compared with XAD concentrates.

Earlier studies [11-13] have demonstrated that both PAHs and phenols could cause genotoxicity in animals and human beings, even at low concentrations [14, 15]. Masood and Malik [16] reported mutagenicity of tannery wastewaters of Kanpur City, India and observed significant mutagenic response on TA98, TA97a and TA100 strains. Gupta and Ahmad [17] assessed the genotoxicity of refinery wastewater and they demonstrated genotoxic and cytotoxic potential of the tested samples. The genotoxic impact of effluents from an oil refinery was assessed in *Oreochromis niloticus* (Pisces: Perciformes) employing the micronucleus test [18]. The results showed that refinery effluents had genotoxic potential. According to Hamoutene et al. [19], PAHs present in the refinery effluents are responsible for DNA damages.

V. CONCLUSIONS

Present study confirmed the presence of various organic pollutants in the tested refinery effluents. Mutagenicity assessment using Ames assay indicates presence of frame shift and base pair substitution mutagens in the contaminated effluents. XAD resins were found to be efficient for concentration of various polar and non-polar mutagens.

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