RESEARCH ARTICLE

4-amino Antipyrine Spectrophotometric and Chromatographic Gas Determination of Phenols in the Water of Southern Iraq along with the Density Functional Theory Study

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ABSTRACT

Different methods based on spectrophotometric and chromatography were summarized for trace level determination of phenolics. Total phenols in the Tigris and Shatt –Al-Arab Rivers were determined using UV-vis Spectrophotometry at 500 nm after distillation, complexation with 4-amino antipyrine(4-AAP) in alkaline solution in the presence of potassium ferricyanide, then extraction by chloroform. Water samples from six stations were collected during the winter and summer of 2017–19. The seasonal and spatial variation concentrations ranged values 0.00982–0.132 mg/L. In winter, values of phenol were highest, but lower values appeared in the summer. The result has shown that the phenol concentrations in all stations were more than 0.001 mg/L in the World Health Organization (WHO) case and 0.005 mg/L in the Iraqi Standard for Drinking Water Quality (ISDWQ).

Gas chromatography (GC) – flame ionization detection (FID) method is commonly used for quantification of phenolic compounds such as (Phenol;2,3 -DimethylPhenol; 2,3,5,6 -TetramethylPhenol;2,4,5-trichloroPhenol;2,6-DibromoPhenol; 3,4- DichloroPhenol; P-nitro phenol and Pentachloro Phenol) in river water. Water samples were collected from three different stations of the Euphrates River (Al-chibayish channel) during summer (2017). Various sample stations containing phenol's concentration and its derivative in river water were obtained in the range of 0.013–24.183 ng/L. The highest concentrations found in the period examined were those of p-nitrophenol (24.183 ng/L), pentachlorophenol (1.285 ng/L), and 3,4-Dichloro Phenol (0.228 ng/L).

Conclusion: The other phenolic compounds studied did not record any concentration in this sample. Substituent effects on the physical and chemical properties of phenol and its derivatives were studied using density functional theory (DFT)[B3LYP/6-31G (d, p)].

Keywords: Phenolic compounds, River water, Spectrophotometry.

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INTRODUCTION

Phenols and their substitutes are one of a large type of organic compounds that nowadays chemists and experts are considered very important environmental pollutants for their contaminations. They can enter the environment through different resources, such as anthropogenic activity and natural processes.[1,2] They have many analytical methods, including GC and spectrophotometry, to determine phenolic compounds in differences in an aqueous environment. GC is one of the methods mainly used for the determination of phenol and its derivative, while the most common spectrophotometry method is used for the determination of the total phenol concentration in a river, ground, sewage, seawater, and wastewater based on the potassium ferricyanide as oxidative coupling reaction of phenols with 4-amino antipyrine(4-AAP) in alkaline solution. This method can determine phenol and substituted phenols in water rivers.[3-5] Tigris, Euphrates, and Shatt-Al-Arab rivers are three main sources of drinking, agricultural and industrial water for part of southern Iraq serving a population of approximately six million people settled in Maysan, Thi-Qar (Al-chibayish city), and Basrah provinces. These rivers are usually affected by agricultural, sewage effluent, discharges of domestic wastewater, industrial effluent discharge, oil plants, wastes from hospitals, electrical power stations, and paper mills which are discharged to sewers without any treatment process. They have been computational chemistry DFT is one of the most used methods to predict the reactivates of a wide variety of phenol and their derivatives.[6,7] The present work aimed to evaluate the pollution level concentration of phenols in the Tigris, Euphrates, and Shatt-Al-Arab rivers.

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EXPERIMENTAL

Sampling Description

Water samples were collected seasonally from 30 cm depths at selected six stations alongside Tigris River within Maysan province and Shatt Al-Arab river within Basra governorate from 2017–19, as shown in Figure 1. One liter of water sampling was collected in amber glass bottles to determine phenol compounds and acidified with 4 mL of concentrated H_2SO_4, then kept in a cooler container in a refrigerator for 24 h. water samples were collected at three stations from the river Euphrates (Al-chibayish city) within the Thi-Qar governorate from 2017, as they are shown in Figure 2.

Chemicals and Standard Solutions

All chemicals were of analytical reagent grade from Merck, BDH, and GC grade; double distilled water was used throughout the experiments.

Preparation of standard phenol stock solutions (1000 mg/L) by dissolving one gram of phenol in 1L of freshly deionized water and Preparation of standard solutions of various concentrations by diluting the stock solution with deionized water.

The 4-amino antipyrine stock solution was prepared by dissolving 2.0 g of 4-amino antipyrine in 100 mL of deionized water.

Preparation of the ammonia buffer solution by dissolving 6.75 g of ammonium chloride in 57 mL of ammonia and diluting to 100 mL with deionized water.

Preparation of potassium ferricyanide stock solution by dissolving 8.0g of $K_3Fe(CN)_6$ in 100 mL distilled water.

The standard materials of phenol [phenol(Ph);2,3-DimethylPhenol (2,3-DMP);2,3,5,6-TetramethylPhenol(2,3,5,6-TMP);2,4,5-Trichlorophenol(2,4,5-TCP);2,6-DibromoPhenol(2,6-DBP);3,4-DichloroPhenol(3,4-DCP);p-

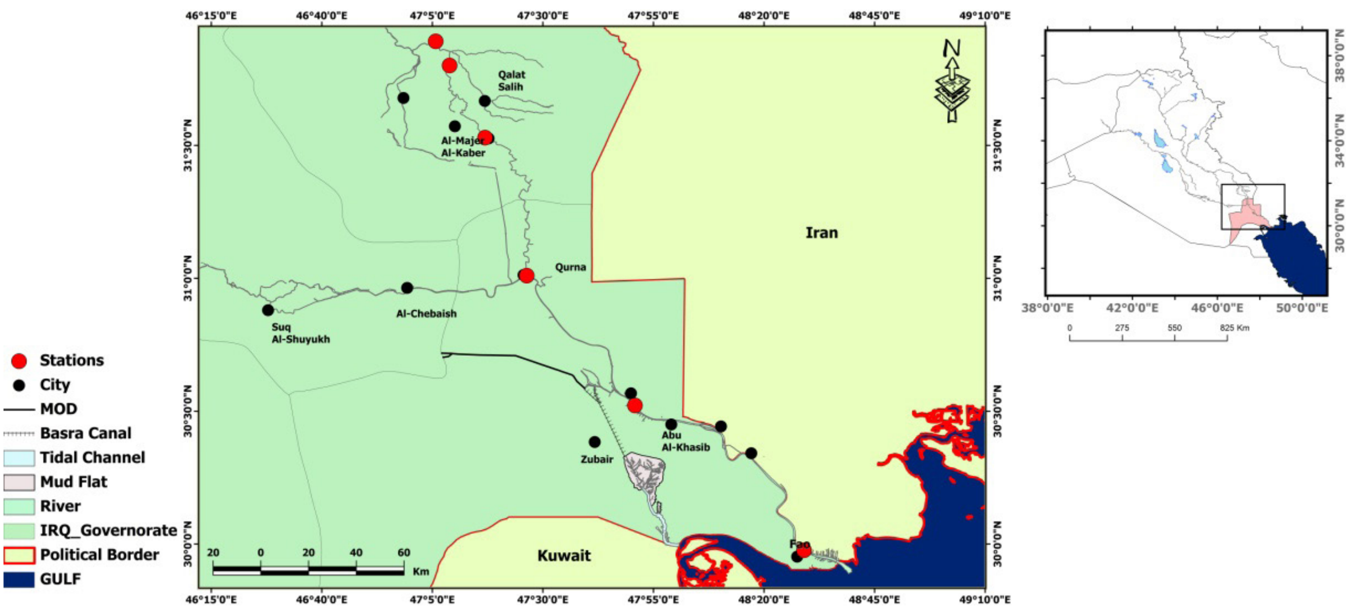


Figure 1: Map of Southern Iraq showing the sampling stations.

Figure 2: Map of Euphrates river showing the locations of the sampling stations.

nitrophenol(P-NP)and pentachlorophenol were obtained from Supelco (Bellefonte, PA, USA).

Stock standard solutions (1000 µg/mL) of each phenol were prepared in methanol and kept dark, and standard preparation solutions of each compound by diluting the stock solution with methanol.

Determination of total Phenol in Tigris and Shatt Al-Arab Rivers

Using the calibration curves of total phenols prepared in this study, the concentration of phenols in the rivers was determined according to the literature.[8-9]

Instrument and Operating Conditions

The water samples were detected using a Shimadzu gas chromatograph fitted with a flame ionization detector (GC-FID-2014). A capillary column of 50 m x 0.22 mm i. d. 0.2 µm film thickness was used, the gas chromatography (GC-FID) oven temperature was held at 80°C for 1.5 minutes, then increased to 230°C at a rate of 6°C/min and then to 270°C at 10°C/min, and finally held isothermal for 6 minutes: the injector temperature (290°C) and the detector temperatures (300°C). Nitrogen gas was used as a carrier and became flow rates at 11 mL/ min. One microliter was manually injected using a 20% split injection.

Extraction Procedure

Using stabilizing agent of 10% $CuSO_4$ solution were added to water samples at three stations, then take 100 mL from each water sample was transferred into a conical flask was extracted with dichloromethane (50 mL) in 30 minutes. by shaking at pH = 2, using H_3PO_4.[10] After that, the solution (aqueous layer and dichloromethane layer) was transferred to the separatory funnel and then stand for 10 minutes., then the aqueous layer was drained, leaving the dichloromethane layer (extract) in the separatory funnel. The extract was dried with anhydrous Na_2SO_4, then pre-concentrated of extract using an evaporative concentrator. Finally, take (1-2 mL)volume From each concentrated sample was injected into the GC-FID instrument, and different peaks of phenol and its derivatives were obtained in the GC. The phenol was identified and quantified by comparing its retention time and peak area with that of the known concentration of a standard solution, which was also injected into the GC system under the same conditions.

Computational Chemistry Methods

They have been DFT method was used to calculate the physical and chemical properties of the substituted phenol like LUMO, LUMO, total energy, heat of formation (HF), dipole moment, electronic chemical potential (µ), hardness, Softness (S), Polarizability (η), global electrophilicity index (ω), ΔG, ΔH, ΔS, and pKa parameters.[11-13]

RESULTS AND DISCUSSION

In general, the reaction of phenol with 4-amino antipyrine(4-AAP) in the alkaline medium in the presence of hexacyanoferrate (III) as oxidant to obtain the *p*-quinoid compound as a result of the reaction.[14] The reaction is shown in Scheme 1.

Oxidative coupling reactions for quinone imine dyes were proposed by Faust *et al.*,[15] and the mechanism pathway is illustrated in Figure 3.

The mean concentration of phenol in winter and summer are presented in Table 1. Phenolic compounds were in the sampling stations' (0.00982–0.132 mg/L) levels. The seasonal and mean values calculated from six months in each station were listed. It can be seen that, in Tigris and Shatt AL-Arab rivers, we show at station 1, 12 km upstream of the Tigris river, the phenol mean value was (0.0175 mg /L). The concentration of phenol was (0.0928 mg /L) at station 2. The higher concentration of phenolic compounds in these stations may be attributed to more discharge of effluent from domestic sewage drainage systems onto the Tigris river at this station. Therefore not completely mixed and high-density organic materials exist in sewage water. These are industrial, agricultural, and hospital waste, washing wastes on both sides of the river without treatment near this station, and bacteria used for organic compounds analysis. This is probably related to organic matter loads discharged into the river from the area near the sewage effluent and agricultural land. Comparable results were obtained by other authors.[16-19]

At station 3, 43.3 km, the concentration of total phenols was (0.0206 mg/L) due to dilution occurring and microbial degradation during flowing in the stream.

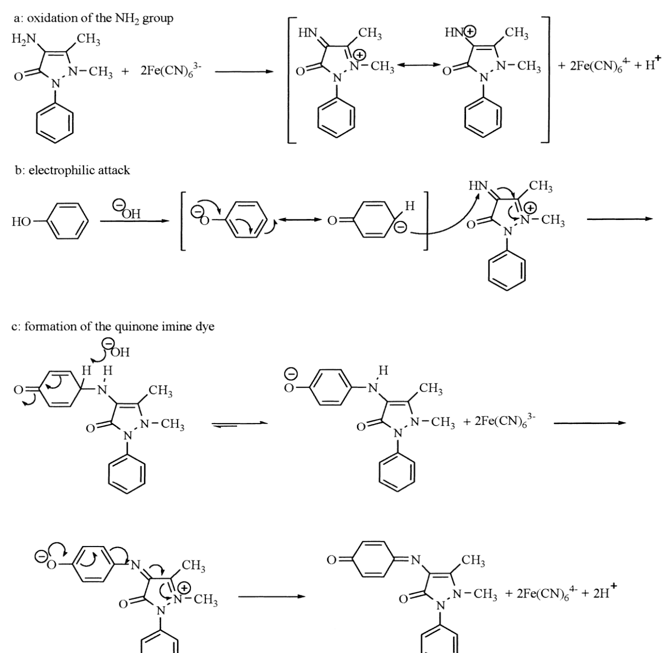
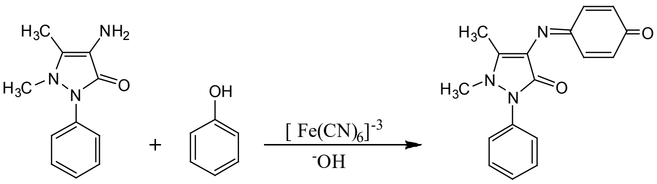


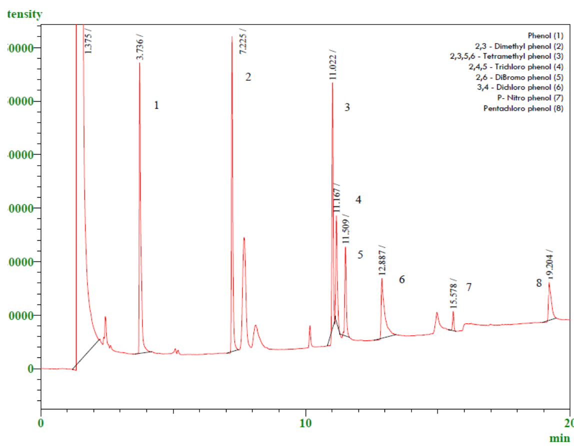
Figure 3: The mechanism proposed for the reaction of 4-amino antipyrine (4-AAP) with phenol.



Scheme 1: reaction of phenol with 4-AAP in alkaline medium

Table 1: Seasonal and mean values of total phenols (mg /L) in six stations.

Sample stations	*Seasonal mean*						*Mean*
	Summer	*Winter*	*Summer*	*Winter*	*Summer*	*Winter*	
1	0.0182	0.0254	0.0179	0.0201	0.00982	0.0141	0.0175
2	0.0932	0.132	0.0841	0.103	0.0572	0.0875	0.0928
3	0.0205	0.0293	0.0189	0.0249	0.0111	0.0192	0.0206
4	0.0253	0.0475	0.0201	0.0389	0.0175	0.0273	0.0294
5	0.0837	0.102	0.0689	0.0991	0.0431	0.0737	0.0784
6	0.0531	0.0737	0.0378	0.0653	0.0207	0.0389	0.0482
Range	0.0182-0.0932	0.0254-0.132	0.0179-0.0841	0.0201-0.103	0.00982-0.0572	0.0141-0.0875	
Mean	0.049	0.0683	0.0412	0.0585	0.0265	0.0434	
Year	2017		2018		2019		



Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Name
1	1.375	482230533	90314739	99.818			
2	3.736	224402	54115	0.046			
3	7.225	205225	58492	0.042			
4	11.022	145215	45749	0.030			
5	11.167	76471	19759	0.016			
6	11.509	63471	16579	0.013			
7	12.887	100191	11148	0.021			
8	15.578	11873	3555	0.002			
9	19.204	50547	7029	0.010			
Total		483107928	90531165				

Figure 4: Standard Calibration Curve of Phenol.

The concentrations of total phenol (0.0784 mg/L) in Shatt Al-Arab river at station 5 are greater than those taken from 4 and 6 river water stations. Because of discharge effluent from Shatt AL-Arab branches, including (sewage water, industrial waste, pesticides, herbicides, and burning of fuels). At the same time, the lowest values of total phenols are in stations 4 and 6. Maybe due to these stations being away from the discharge of effluent from Shatt AL-Arab branches and dilution occurring during flowing in the Shatt AL-Arab.

The highest concentrations of phenol in the Tigris and Shatt AL-Arab rivers were appeared during winter, while the lowest concentration appeared during the summer season from 2017 to 2019, respectively. Due to the climatic condition of Iraq, rapid photolysis, volatilization, and biological effect.[20,21] It was observed that the phenol value for 2017 which higher compared to phenol value for 2018 and 2019. This is due to the decreased flow rate, which is leading to increasing in concentration. The total phenol concentrations in the Tigris and Shatt AL-Arab rivers at six stations were highest than the

Table 2: Concentration of Phenol and its derivatives in the Euphrates Rivers (Al-chibayish chanal) obtained from three water samples.

No.	*Component*	*ng/L, station 1*	*ng/L, station 2*	*ng/L, station 3*
1	Phenol	0.059	0.024	0.030
2	2,3 Dimethyl Phenol	N.D	0.020	0.059
3	2,3,5,6 Tetra methyl phenol	ND	0.013	0.064
4	2,4,5 trichloro phenol	ND	0.065	0.070
5	2,6 Dibromo phenol	0.089	0.067	0.043
6	3,4 Dichloro phenol	0.228	ND	0.135
7	P-nitro phenol	24.183	9.646	16.371
8	Pentachloro phenol	1.285	0.375	0.427
	Sum	26.148	10.361	17.199

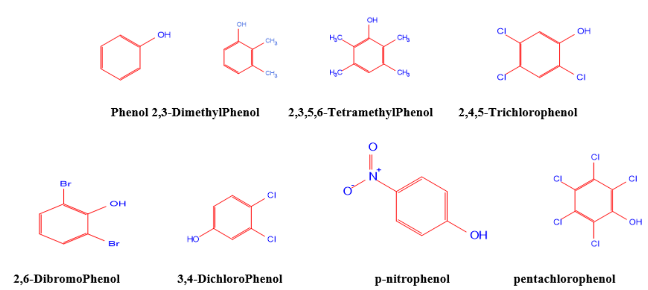


Figure 5: Structure of phenol and its derivatives in the Euphrates Rivers (Al-chibayish chanal)

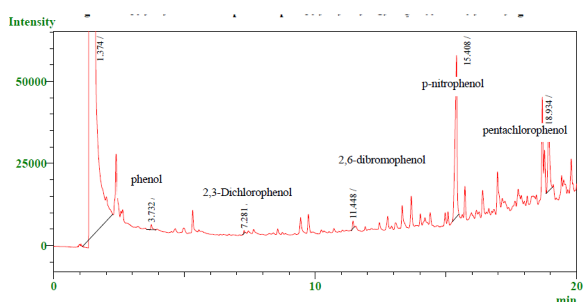
recommended standards by the World Health Organization (WHO) and (ISDWQ) [WHO].[22-24]

The standard calibration curve of phenol and their derivate is shown in Figure 4. Determining phenol and its derivatives at three different stations in the Euphrates River (Al-chibayish chanal) matched the standard calibration curve. The concentrations and structure compounds, physical, and chemical properties of phenol; 2,3 -Dimethyl phenol; 2,3,5,6 -Tetra methyl Phenol; 2,4,5 -trichloro Phenol; 2,6-Dibromo Phenol; 3,4- Dichloro Phenol; P-nitro phenol and pentachlorophenol are summarized in Figure 5 and Tables 2, and 3) respectively. For all the samples (Figures 6-8); The concentrations for phenol in the range of 0.024 to 0.059 ng/L;2,3 -Dimethyl Phenol in the range of 0.020 to 0.059 ng/L; 2,3,5,6 -Tetra methyl Phenol in the range of 0.013 to 0.064 ng/L; 2,4,5 -trichloro Phenol in the range of 0.065

Table 3: Physical and chemical properties of phenols and their derivatives were calculated using DFT methods.

Compounds	*E_1 (a.u)*	*Di (Debye)*	*HOMO (au).*	*LUMO (au)*	*Energy band gap ΔE*	*Hardness (η)*	*Chemical potential (μ)*	*Softness (S)*
phenol	-307.550	1.360	-0.01296	-0.22629	0.21333	0.1066	0.1196	9.380
p-Nitrophenol	-511.978	5.432	-0.07803	-0.25130	0.17327	0.0866	0.1646	11.54
2,6- dibromo phenol	-1123.069	1.461	-0.01557	-0.21925	0.20368	0.10181	0.11741	9.819
3,4-DiChloro-phenol	-1226.663	1.151	-0.02928	-0.23228	0.203	0.1015	0.1307	9.852
2,4,5-TriChloro-phenol	-1686.251	1.471	-0.03637	-0 .23498	0.19861	0.0993	0.1358	10.070
Penta chlorophenol	-2605.415	2.049	-0.04798	-0.24387	0.19589	0.0979	0.1459	10.214
2,3-DiMethylphenol	-386.113	1.680	0.00127	-0.20385	0.20512	0.1026	0.1013	9.75
2,3,5,6-TetraMethyl phenol	-425.434	1.444	0.00661	-0.20073	0.207343	0.1036	0.0970	9.652

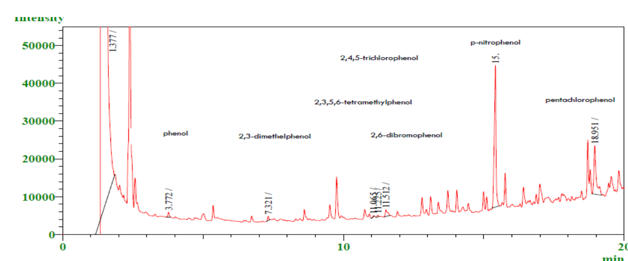
Compounds	*electrophilicity index (ω)*	*Polarizability*	*PKA*	*ΔG, kJ/ mol*	*Henry's Law*	*Heat from kJ/mol,*	*ΔH(Kcal/ mol)+ΔS(Cal/ mol.K)*
phenol	0.0670	62.41	9.98	-32.94	4.64	-96.48	69 + 74
p-Nitrophenol	0.1564	76.85	7.14		1.61		72. + 85.
2,6- dibromo phenol	0.0668	68.09	6.89	-23.56	5.44	-66.76	45 + 78
3,4-DiChloro-phenol	0.0837	78.84	7.89	-76.06	4.9	-150.9	59 + 88.
2,4,5-TriChloro-phenol	0.0926	89.17	7.02	-97.62	5.03	-178.11	53 + 92
Penta chlorophenol	0.1086	109.69	4.9	-140.74	5.29	-232.53	42 + 96
2,3-DiMethylpheno	0.050	82.13	10.6	-35.36	4.55	-160.7	106 + 87
2,3,5,6-TetraMethyl phenol	0.0454	94.21	10.89	-37.78	4.47	-224.92	124 + 94



Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Name
1	1.374	444228640	84338926	99.914			
2	3.732	5798	1571	0.001			
3	7.281	2306	879	0.001			
4	11.448	6050	2220	0.001			
5	15.408	260442	49089	0.059			
6	18.934	100180	19299	0.023			
7	36.369	6292	1544	0.001			
Total		444609708	84413528				

Figure 6: Gas Chromatogram of phenols from Euphrates River (Al-chibayish chanal), Sample 1.



Peak Table - Channel 1

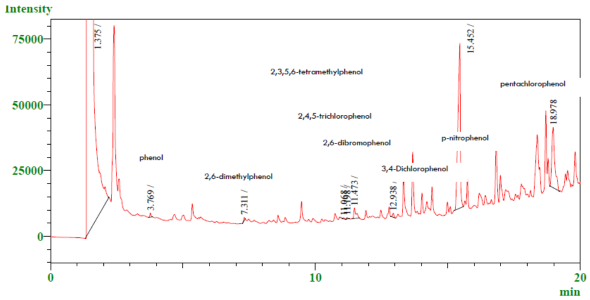
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Name
1	1.377	480981640	97301686	99.947			
2	3.772	4427	1313	0.001			
3	7.321	3500	1145	0.001			
4	11.065	909	313	0.000			
5	11.225	1402	446	0.000			
6	11.512	9535	1744	0.002			
7	15.412	161225	37368	0.034			
8	18.951	75426	12769	0.016			
Total		481238064	97356784				

Figure 7: Gas Chromatogram of phenols from Euphrates River (Al-chibayish chanal), Sample 2

to 0.070 ng/L; 2,6- Dibromo Phenol in the range of 0.043 to 0.089 ng/L; 3,4 Dichloro Phenol in the range of 0.135 to 0.228 ng/L; P-nitro phenol in the range of 9.646 to 24.183 ng/L and Pentachloro Phenol in the range of 0.375 to 1.285 ng/L; A distinctly higher content of phenols is observed at the stations 1 and 3, while at station 2 the total concentration of phenols is at the lowest level.

Figures 6 show the concentration of phenolic compounds found in river water station 1. situated along the Euphrates River (Al-chibayish chanal) with heavy traffic. There the highest concentration found in the period examined were those of p-nitrophenol (24.183ng/L), pentachlorophenol (1.285 ng/L), and 3,4-DichloroPhenol (0.228ng/L);2,3 -Dimethyl Phenol; 2,3,5,6 -Tetra methyl Phenol;2,4,5-trichlorophenol were not detected in this sample. A sampling station 2, Figure 7, the concentration of p-nitrophenol was as high as 9.646 ng/l. This particular compound was detected in all samples analyzed. Other phenolic compounds were detected at much lower levels. While 3,4-DichloroPhenol was not detected in this sample.

A sampling station 3 (Figure 8), the most common phenolic compound was phenol with a minimum concentration of 0.030 and p-nitrophenol with a maximum concentration of 16.371 ng/L. The contamination of Euphrates rivers (Al-chibayish chanal) by phenolic compounds may most probably be due to the discharge of domestic sewage water, while the contamination in the river was decreased, phenolic compounds can be bound to natural matrixes, particularly



Peak Table - Channel 1

Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Name
1	1.375	510764630	96439057	99.881			
2	3.769	4308	1505	0.001			
3	7.311	3184	1243	0.001			
4	11.068	1491	441	0.000			
5	11.198	1747	579	0.000			
6	11.473	23922	4039	0.005			
7	12.938	5854	1530	0.001			
8	15.452	390916	62427	0.076			
9	18.978	177091	22948	0.035			
Total		511373143	96533769				

Figure 8: Gas Chromatogram of phenols from Euphrates River (Al-chibayish chanal), Sample 3.

sediments or diluted, volatility, biological.[25-27] The phenol and its derivatives concentrations in the Euphrates river (Al-chibayish chanal) at three different stations were lower than the recommended standards by the World Health Organization (WHO) and Canadian DWQ.[28-29]

The predicted values for the phenol and substituted phenol are summarized in (Table 2)

They have been DFT was used to predict the physical and chemical properties of phenol and its derivatives. It has proved to be successful as a working method. It can see chemical reactivity such as Total energy, HOMO, LUMO, heat of formation, dipole moment, electronic chemical potential (μ), hardness, Softness(S), Polarizability (η), global electrophilicity index (ω), ΔG, ΔH, ΔS and pKa parameters, maybe the Theoretical parameters' values for phenol and substituted phenol were important in this study so; we can understand the relationship between structures and properties without needed for The experimental values.

CONCLUSIONS

The contamination of Tigris, Shatt –Al- Arab and Euphrates Rivers (Al-chibayish chanal) by phenolic compounds were high, due to direct discharge of industrial and sewage water without treatment to rivers, so must removal or treatment of toxin phenolic compounds in wastewater before discharging it into the rivers, as well as these compounds have dangerous effect on healthy human being.

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