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Quantity and quality of surfactants in sediment of Tigris River, Baghdad, Iraq

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ABSTRACT

Detergents (surfactants) are one of the environmental risks that cause adverse effects on aquatic ecosystems. Due to less attention on their effects in Iraqi aquatic ecosystems, concentrations of surfactants in sediment were determined to assess their potential risks in Tigris River. Four sites were selected to collect sediment samples from February 2017 to February 2018. Anionic (AS) and nonionic surfactants (NS) were extracted from sediment by ultrasonic device and their concentrations were measured by using colorimetric determination Photo lab S12 and high-performance liquid chromatography. In addition, multiple physicochemical parameters were also measured. Results showed that both AS and NS were detectable in all sites for all months with slight variations, suggesting high washing activities that release directly to the river, causing surfactants to adsorb and accumulate in the sediment for a long time before degradation by microbial communities. High NS concentrations were recorded in the dry season for May 2017 at Al-Dora Bridge, suggesting that the NS, especially their degradation products (nonylphenol), were widespread pollutants compared with AS in the dry season. We recommend that these compounds must be monitored and further investigated in order to determine their effects on fish and other aquatic organisms.

Keywords: Cluster analysis; Detergents; Lotic system; Physico-chemical parameters; Water pollution

1. Introduction

Most of the worldwide detergents used are composed of 50%–60% anionic surfactant (AS) and 40% nonionic surfactant (NS) [1]. Surfactants (detergents) are an abbreviation of surface-active agents. They are organic compounds with one or more hydrocarbon chains and either hydrophilic or hydrophobic nature [2]. Surfactants are widely used for the purpose of washing and other applications such as their uses as emulsifiers, pesticide formulations, fibers, wetting agents, cosmetics and treatment of textiles [3].

Surfactants are classified into anionic, cationic and nonionic according to the charge of the hydrophilic part. A specific structure of surfactant molecules is applied in different domestic and industrial purposes. Thus, they are introduced into the soil, water and sediment elements of the environment in different ways. Surfactants are undergoing numerous physico-chemical processes such as sorption, degradation and transition freely [4–6]. They have high sorption in sediments, which reflects their intense concentration [7,8]. In addition, they are released into the river from the wastewater treatment plants and their solid wastes. These compounds differ in their actions and interaction with other components in the environment [9]. An environmental hazard of surfactants is represented by bioaccumulation, which adversely affects the biological components of the ecosystem, e.g. toxicity and endocrine balance disorder. They also increase melting of organic pollutants in water that can lead to migration and accumulation in various environmental compartments [8].

Contamination of sediment represents a serious environmental problem worldwide. Poor environmental management in the past resulted in widespread sources and incidental events, making the sediment as a sink for various pollutants [10]. Surfactants are widely distributed in the environment which was considered safe at low concentrations and as organic pollutants; their toxicities have

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to drown extensive attention [11]. However, this research was conducted to investigate the quantity and quality of the surfactant concentration in the sediment river and their distribution, in addition, this study provides baseline information on the effect of household and industrial effluent containing surfactants on physico-chemical parameters of a particular water body and comparing such data with known standards.

2. Materials and methods

2.1. Study area

This study was carried out along Tigris River within Baghdad city in specific locations for the period from February 2017 to February 2018. Water and sediment samples were collected on a monthly basis for 5 months of the wet season and 8 months of the dry season. Sampling time was between 8.30 am to 2.30 pm hours. To cover the Tigris river within Baghdad city from North to South, four sites were selected for sample collection; one site upstream: Al-Muthanna Bridge (site 1), two sites at midstream: Al-Sarrafia Bridge (site 2) and Al-Shuhada Bridge in the North of Baghdad city (site 3) and one site at downstream: Al-Dora Bridge in the South of Baghdad city (site 4); (Fig. 1). Global positioning system (Table 1) determined the position of the study sites.

2.2. Water sampling and analysis

Duplicate water samples (1 L) were collected from the surface layer (depth 20–30 cm) in stopper fitted clean polyethylene bottles pre-washed with distilled water. Before filling with water samples, the polyethylene bottles were rinsed several times with the river water. Samples were analyzed for physical and chemical properties immediately after collection. Air temperature (AT), electrical conductivity (EC), turbidity (Tur.), water flow (WF), water temperature (WT) and pH were measured in the field. Samples were analyzed for other properties immediately after collection. Salinity‰, total dissolved solids (TDS), total suspended solids (TSS), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), organic matter percent (OM%) and total organic carbon percent (TOC%) were measured in the laboratories of Environmental Research Center, University of Technology, Iraq. All tests have been done in accordance with the standard methods [12].

2.3. Sediment sampling, extraction and analysis

Sediment samples were collected using an Ekman Grab (n = 3) for each site from the river sediment to 5 cm depth. Excess water was eliminated and enough volume of 10%

Table 1
Geographical positions (GPS) of the study sites

Site number	Site name	Coordinates	
		Longitude (E)	Latitude (N)
1	Al-Muthanna Bridge	44°34′55.50″	33°42′83.22″
2	Al-Sarrafia Bridge	44°37′36.01″	33°35′37.53″
3	Al-Shuhada Bridge	44°38′79.03″	33°33′79.59″
4	Al-Dora Bridge	44°45′02.84″	33°28′96.82″

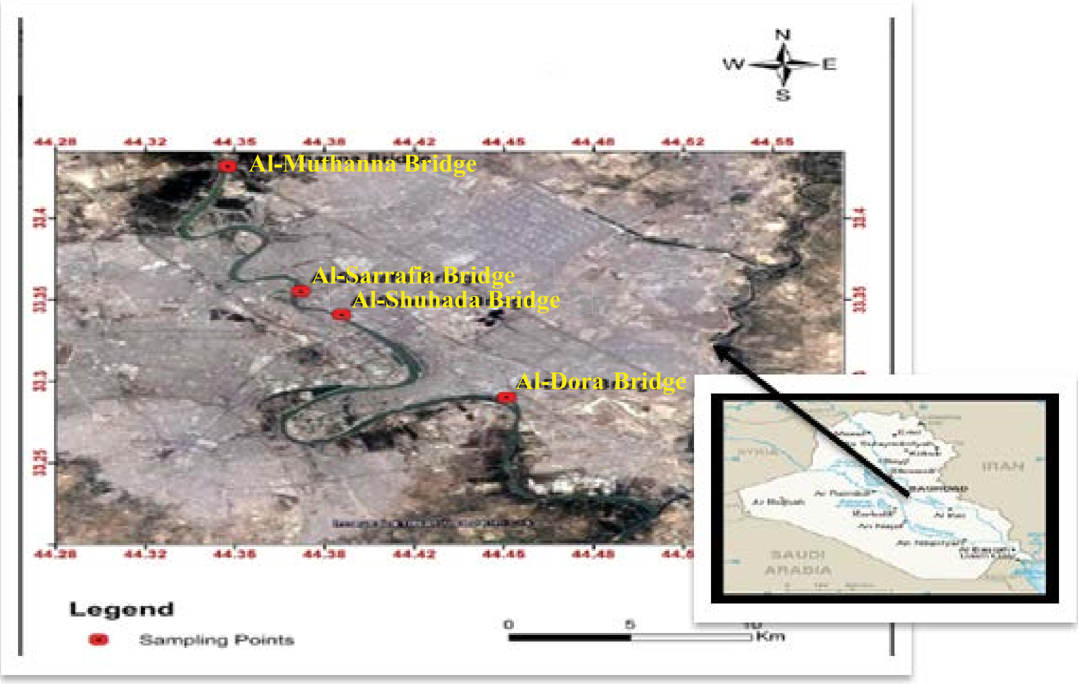


Fig. 1. Study sites in Tigris River, Baghdad city.

formalin was added to submerge the sediment for preservation when transferred to the laboratory by using glass jars. Aluminum foil washed with methanol was placed over the mouth of the jar and then placed in the lid to prevent contamination of the sample. In the laboratory, sediment samples were reserved at about 4°C until analysis of surfactants [13].

For AS, the excess water was removed before drying in the oven at 80°C for 16 h. From dry sediments (10 g, large stones and grit not included) surfactant compounds were extracted by Ultrasonic water bath (ISOLAB, Germany) with methanol at 50°C (240 V, 3 A, 50 Hz). Three 10 min extractions (50 ml and 2 × 40 ml) were performed with the sediment separated from the extract by centrifugation. Then the combined extract was concentrated to 2 ml [13].

For NS, sediment samples were homogenized by sieving through a 2 mm stainless steel sieve before extraction. The surfactant compound was extracted from the sediment by using ultrasonic water bath using a mixture of methanol-dichloromethane (7:3, v/v). Then elutes were evaporated with a gentle stream of nitrogen gas and reconstituted with 1 ml methanol [14].

After extraction, both AS and NS were measured using photometer Photo lab S 12 (PHD) and high-performance liquid chromatography system model (HPLC) (Syknm-S1122, Germany). The standard solutions used in this study were 4-dodecylbenzenesulfonic acid for AS and 4-nonylphenyl-polyethylene glycol for NS.

2.4. Statistical analysis

Results were subjected to descriptive statistical analysis and to one-way analysis of variance. Probabilities less than 0.05 ($P < 0.05$) were considered statistically significant, also cluster analysis (CA) is used to sort cases, data or objects into groups or cluster using the Statistica Release 7 software. While the principle component analysis done to extract the important information as a set of unrelated variables and the results presented with graphs plotting the projections of the units onto the components, along with the loading of the variables. The importance of each component is expressed by the variance (i.e., Eigenvalue) of its projection or by the proportion of the variance explained [15,16].

3. Results and discussion

The range of some water physico-chemical parameters from Tigris River as compared to the Iraqi River Maintaining System Law [17] and Canadian Council of Ministers of the Environment (CCME) [18,19] guidelines are reported in Table 2.

The range of AT 12.55°C–43.73°C, while the WT showed a noticeable seasonal trend with a minimum value of 10.36°C recorded in the wet season and a maximum value of 30.11°C in the dry season. AT was within the weather rates of Baghdad city [20] during the study period and. WT was also within the

Table 2
Physico-chemical characteristics of Tigris River for wet and dry seasons

Parameters	Range		Mean	Standard deviation	Standard values	
	Minimum	Maximum			Law 25/1967	CCME
Physical parameters						
Air temperature (AT) (°C)	12.55 (w)	43.73 (d)	26.27	±9.64	–	–
Elictrical conductivity (EC) (µs/cm)	580.50 (d)	1,108.75 (w)	876.27	±148.05	0.5–1.0	–
Salinity (S) ‰	0.20 (d)	0.48 (w)	0.35	±0.08	–	–
Total dissolved solids (TDS) (mg/L)	362.75 (d)	711.75 (w)	563.87	±105.47	1,000	500
Total suspended solids TSS (mg/L)	3.00 (d)	84.50 (d)	18.58	±22.05	60	–
Turbidity (Tur) (NTU)	10.61 (w)	193.75 (d)	67.83	±65.36	5	5
Water flow (WF) (m/s)	0.31 (w)	0.71 (d)	0.47	±0.13	–	–
Water temperature (WT) (°C)	10.36 (w)	30.11 (d)	21.59	±6.83	>35	15
Chemical parameters (standard unit)						
pH	7.43 (d)	8.25 (w)	7.75	±0.22	6–9.5	6.5–9
Nutrients (mg/L)						
Nitrite (NO_2)	0.01 (d)	0.45 (d)	0.11	±0.12	0.06	0.06
Nitrate (NO_3)	0.64 (d)	8.97 (d)	4.18	±2.82	15	13
Phosphate (PO_4)	0.07 (w)	1.52 (w)	0.66	±0.43	0.4	0.1
Organic						
Biological oxygen demand (BOD) (mg/L)	0.53 (w)	3.67 (d)	1.5	±0.79	>5	–
Chmical oxygen demand (COD) (mg/L)	3.75 (w)	88.25 (d)	36.73	±34.9	>100	–
Dissolved oxygen (DO) (mg/L)	4.63 (d)	11 (w)	6.18	±2.06	<5	5.5–9
Organic matter (OM) (%)	0.43 (d)	5.55 (d)	1.7	±1.44	–	–
Total organic carbon (TOC) %	0.27 (d)	2.24 (w)	0.88	±0.56	–	–

– = not applicable or available, w = wet season, d = dry season.
Law 25/1967 = Iraqi River Maintaining System Law.
CCME = Canadian Council of Management of the Environment.

permissible limit and showed a positive correlation with AT during wet and dry seasons (Table 2). This result is consistent with the previously reported role of AT for the heat budget of Tigris River [21].

EC in the aquatic ecosystem is a good indicator assessment of TDS in water [12]. In the present study, an EC range between 580.50 and 1,108.75 µs/cm in dry and wet seasons, respectively, indicated levels higher than the permissible standards. This suggests higher concentrations of main ions in the wet season than those of dry season due to mechanisms that can possibly include increasing surface runoff, return irrigation WF, the salinity of soil and increasing human activities [22].

Salinity (S‰) concentration ranged between 0.2‰ and 0.48‰ in the dry and wet season, respectively. While the TDS ranged from 362.75 mg/L in the dry season to 711.75 mg/L in the wet season. The highest values for both S‰ and TDS are within the permissible value. Higher values of these parameters in the environment might result from increasing surface runoff, the geologic formation that the river passes through, increasing in evaporation rate and increasing in human activities, which can collectively cause increased ions concentrations [23].

The lowest value of TSS was 3.00 mg/L in the dry season and the highest value was 84.50 mg/L in the dry season, while turbidity ranged between 10.61 and 193.75 NTU in wet and dry seasons, respectively. The presence of a wide variety of material, such as silt, decaying plant and animal matter, industrial wastes and sewage can cause an increase of TSS level. High concentrations of suspended solids can cause many problems for stream health and aquatic life [24,25]. Sediments in rivers reflect suspended solids which depend on discharge [12]. Turbidity showed values clearly above the permissible limits, with turbid water being visible by eye, possibly due to the presence of organic, inorganic matter, microbes, silt, algae, etc [26].

The highest value of WF was recorded in a dry season of 0.71 m/s (Table 2). WF determines the degree and type of deposition and thus the nature of sediment [27]. WF is an important force that moves the pollutants into regions far from their origin. Melting snow in summer causes an increase in flow rate, which explains much lower levels of pollutants measured in sediment [28].

The value of pH was between 7.43 in the dry season and 8.25 in the wet season, which suggest that the river water is often slight alkaline and within the limiting value for the living of aquatic life [29,30].

Nutrients include nitrite (NO_2), nitrate (NO_3) and phosphate (PO_4). Concentration ranges recorded in dry and wet seasons were 0.01–0.45 mg/L for NO_2, 0.64–8.97 mg/L for NO_3 and 0.07–1.52 mg/L for PO_4, respectively. NO_2 and NO_3 are naturally-occurring ions present in water as a part of the nitrogen cycle. For all of the three nutrients, concentrations in wet season were remarkably higher than those in dry season except PO_4 in the wet season. NO_2 showed higher concentration than the standards in the dry season, while NO_3 was highly decreased as compared to permissible limits for both wet and dry seasons (Table 2). NO_3 is known to be more stable than NO_2 [31]. Reasons for the decreased concentration of nitrate in Tigris River were possibly due to the reported uptake process by microbial activity, especially during the summer season where most of the biological processes take place during the hot season [32]. For the concentration of PO_4, it was higher than the permitted level of aquatic life for both seasons and the highest value was recorded in the wet season. PO_4 is an important nutrient in the water body and only in the soluble form, inorganic phosphorus, can be directly utilized by aquatic biota [33]. Phosphorus occurs through natural or anthropogenic activities. Natural sources include atmospheric deposition, natural dissolution of rocks and minerals, weathering of soluble inorganic materials, decomposition of biomass, runoff and sedimentation. An anthropogenic source includes fertilizers, detergents, animal wastes, wastewater and septic system effluent, and industrial discharge [34].

Organics investigated in the present study include BOD, COD, DO, OM% and TOC%. Ranges recorded in wet and dry seasons were 0.53–3.67 mg/L for BOD, 3.75–88.25 mg/L for COD, while the DO concentrations were ranged 4.63–11.00 mg/L in the dry and wet season, respectively.

BOD is a measure of the amount of oxygen consumed by the bacteria that are a decomposing OM to both waste and surface water [33]. The present study showed that BOD value is within the permissible limits [17]. COD is a measure of the number of chemicals, usually organics that consume DO [35]. All values in wet and dry seasons were within the permissible limits and they are in agreement with those found by previous investigations [36]. DO supports the biological life in water and its fluctuations depend on temperature and algal population [37]. Increasing the flow rate of a water body will increase the amount of DO in the water because the flow rate increases the diffusion or movement of oxygen into the water from the atmosphere. The amount of OM in the water affects DO levels by lowering it [38]. In the present study, the DO level was within the permissible limit (Table 2).

The present study also demonstrated that OM% ranged between 0.43% and 5.55% in the dry season, while TOC% ranged from 0.27% to 2.24% in dry and wet seasons, respectively. Estimation of TOC% in sediment and soil samples is an important parameter for assessment the quality of the environment. OM is present in soil, land, aquatic, and sediment components of the environment. The presence of these compounds in the sediment leads to their binding with metal ions causing the formation of soluble or insoluble complexes. These complexes, in turn, interact with minerals, present in the sediment to form particles that can be absorbed to other pollutants [39]. Due to the proportionality between TOC content and OM which has an affinity for trace AS and NS contaminants, TOC should be used as an indicator of river pollution [40]. In this study, the highest values were recorded for OM% in dry season and TOC% in the wet season (Table 2). Urano et al. [41] showed that the adsorption abilities of the sediment seemed to be independent on the surface area of sediment but are more related with the organic carbon contents. They also found that the values for the adsorption of AS and NS on the microbiota are equal to the values for the adsorption on sediment. OM in the sediment provides matrices for sorbing hydrophobic compounds and releasing them slowly and incompletely [14].

The soil texture results were variable among the study sites as follows (Table 3): in S1 it was clay loam (40.4% silt, 30.6 sand% and 29% clay), S2 it was clay loam (37.7% silt, 34.8% sand and 27.5% clay), S3 it was clay loam (38.6% silt, 33% clay and 28.4%) and silty clay loam (51.6% silt, 37% clay and 44.4% sand) at site 4 [42,43].

Descriptive analysis for AS and NS concentrations in sediment from the Tigris River sites during the study period by using Photo lab S12 (PHD) and HPLC are demonstrated in Table 4. These analysis is presented knowing that no standard has been found, whether Iraqi or international, about the permissible limits of the presence of the surfactants in river sediments [44].

Using PHD, concentrations of AS ranged from 41.83 to 72.05 μg/g in the wet season, while the minimum concentrations of NS were not detectable in the wet or dry season, while the maximum concentrations was recorded 56.19 μg/g in the dry season. HPLC results showed that AS concentrations ranged from 10.80 to 135.74 μg/g in the dry season. The minimum concentration of NS was not detectable in the wet season and the highest concentration was 467.31 μg/g in the dry season.

The different charges of surfactants are the main important factor to absorption these compound on sediment, so the cationic surfactants (CS) can undergo sorption in sediment much greater in contrast to AS such as sodium dodecylbenzene sulfonate (SDBS) [45]. It has been shown that the coefficient of equilibrium distribution for CS is twice greater than that for SDBS. Also, low organic carbon content and neutral pH provide CS with greater sorption capability to the sediment, due to the electrostatic interactions between the positively charged ammonium groups ($[(CH_3)_3NR]^+$), forming the heads of CS, and the overall negatively charged surface of the sediment. SDBS shows lower sorption than the CS due to the negative charge of SDBS as compared to CS that have positively charged [45]. Thus the adsorption of surfactants types in sediment can be put in the order as Cationic > Nonionic > Anionic. However, using PHD in this study recorded not detectable concentrations of NS in 9 of the 13 months of the study period (3 in wet season and 6 in dry season), in contrast to higher concentrations of AS. Lif and Hellsten [46] explained that the NS have an amide group which represent a small part of the total volume of NS, but their production and application are increasing because of good chemical stability with rapid biodegradation and fairly simple manufacturing processes that are mainly based on renewable raw materials.

Table 3
Soil texture of sediment samples

	Soil percentage			Soil class
Site	Clay%	Silt%	Sand%	Soil texture
S1	29	40.4	30.6	Clay loam
S2	27.5	37.7	34.8	Clay loam
S3	33	38.6	28.4	Clay loam
S4	37	51.6	11.4	Silty clay loam

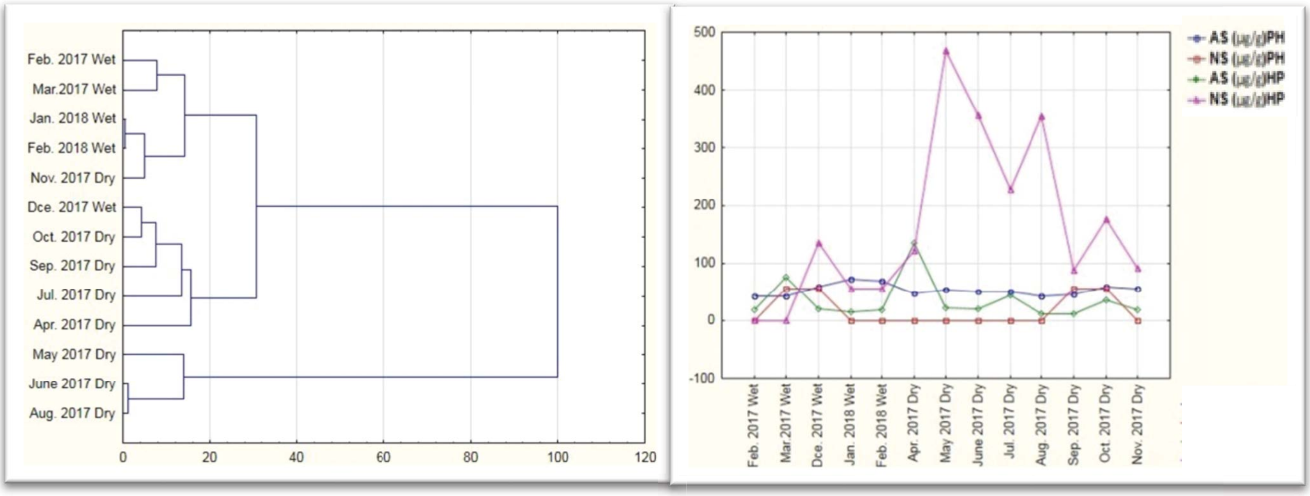
A *T*-test analysis was used to compare the two measurement methods (PHD and HPLC). The results showed no significant differences at $P < 0.05$ ($t = 0.088$) between the results obtained by the two devices for AS measurement, while significant differences were obtained at $p < 0.05$ ($t = 0.004$) for NS measurement.

Two clusters diagram shows (Fig. 2a) during the wet and dry season. Whereas two highest values 72.05 and 69.71 μg/g for AS by PHD, (ND) for NS by PHD, (14.83 and 18.20 μg/g) for AS (by HPLC) and (56.17 and 55.03 μg/g) for NS (by HPLC) were obtained during wet season. In dry season was recorded ND for NS (by HPLC) especially in June, August 2017 and May 2017, which suggesting a marked seasonal variation. The seasonal dependence is clearly shown in Fig. 2b. Concentrations of NS and AS (by HPLC) recorded the highest values in the dry season in contrast to the wet season with lower temperatures. This can be attributed either to lower inputs of pollutants (surfactants) to the river or to more efficient biodegradation of compounds studied in river water during the dry season. Marcomini et al. [47] showed significant seasonal differences in concentrations of linear alkylbenzene sulfonate and nonylphenol ethoxylates in Lake Venice, mainly due to increased biodegradation at temperatures greater than 20°C (late spring and summer). In 1994, Ahel et al. [48] and co-workers observed the similar seasonal variation of nonylphenol (NP), lipophilic nonylphenol monoethoxylate and nonylphenol diethoxylate (NP_1EO, and NP_2EO) in the Glatt River (Switzerland).

Table 4
Surfactant concentrations in sediment of the Tigris River for wet and dry seasons

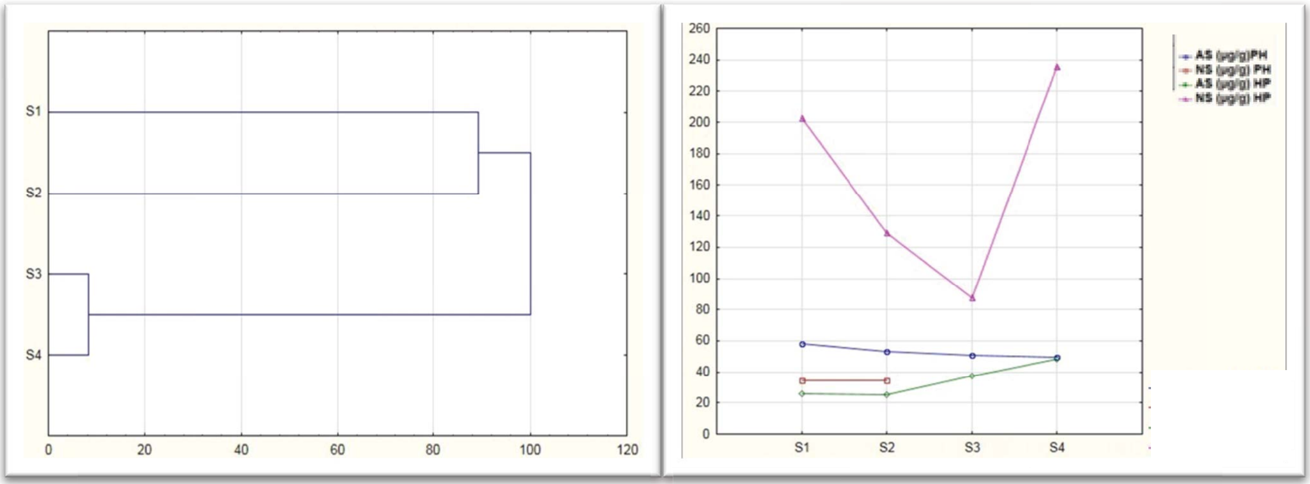
Parameters	Range		Mean	Standard Deviation	Standard values	
	Minimum	Maximum			Law 25/1967	CCME for rivers
Photo lab (μg/g)						
Anionic surfactant (AS)	41.83 (w)	72.05 (w)	52.85	9.88	–	–
Nonionic surfactant (NS)	ND (w&d)	56.19 (d)	17.12	26.73	–	–
HPLC (μg/g)						
Anionic surfactant (AS)	10.80 (d)	135.74 (d)	34.15	35.32	–	–
Nonionic surfactant (NS)	ND (w)	467.31 (d)	163.80	147.38	–	–

– = not applicable or available, w = wet season, d = dry season.
Law 25/1967 = Iraqi River Maintaining System Law.
CCME = Canadian Council of Management of the Environment.



a **b**

Fig. 2. (a) Cluster diagram of temporal of the sampling period for wet and dry seasons and (b) temporal variation of surfactants in sediment for wet and dry seasons.



a **b**

Fig. 3. (a) Cluster diagram of spatial clustering of sampling sites for wet and dry seasons and (b) spatial variation of surfactants in sediment for dry and wet seasons.

The cluster diagram (Fig. 3a) shows two clusters during the study period. The first cluster is consisted of two sub-clusters; first, the pair of S3–S4: In these sites, the closely high concentrations of AS and NS by HPLC (37.5 and 235.79 μg/g, respectively) were recorded. However, PHD measurement showed no detectable concentrations for NS. Second, the pair of S1–S2: at S1, the closely highest values for AS were recorded by PHD (57.88 and 53.17 μg/g). In addition, S2 was recorded the closely highest values for NS by PHD (34.32 and 34.17 μg/g).

A clear variation was observed for NS (HPLC) in which two highest values of S1 and S4 were recorded. The lowest values were observed at S2 and S3 for NS (HPLC). Nevertheless, there was no clear variation observed to other surfactants during the study period (Fig. 3b), which indicates that S1 and S4 recorded the highest pollution levels for NS (by HPLC) as compared to the lowest in S2 and S3, suggesting these sites as hot spots with point sources of municipal and industrial discharged.

The correlation matrix results recorded the following strong correlations ($P < 0.05$) (Table 5): AT showed strongly negative correlation with NS (PHD) ($r = –0.997$), and a strong positive correlation with AS (HPLC) ($r = 0.999$). The correlation matrix also showed high level of correlation between NS measured by PHD and TSS ($r = 0.998$), COD ($r = 0.998$) and NO_2 ($r = 0.999$).

Table 5
Correlation between physico-chemical and surfactants in Tigris river sediment during the study period

Parameters	AT (°C)	TSS (mg/L)	COD (mg/L)	NO_2 (mg/L)
AS (µg/g) PHD	–0.778	–0.170	–0.294	–0.206
	$P = 0.432$	$P = 0.892$	$P = 0.810$	$P = 0.868$
NS (µg/g) PHD	–0.997	–0.697	–0.783	–0.723
	$P = 0.050$	$P = 0.509$	$P = 0.428$	$P = 0.485$
AS (µg/g) HPLC	0.999	0.720	0.803	0.745
	$P = 0.029$	$P = 0.488$	$P = 0.407$	$P = 0.465$
NS (µg/g) HPLC	0.787	0.998	0.9975	0.999
	$P = 0.423$	$P = 0.036$	$P = 0.045$	$P = 0.012$

– Correlation marked is significant at $p < 0.05$.

Table 6
Correlation between soil texture and surfactants in sediment

Parameters	Clay %	Silt %	Sand %
AS (µg/g) PHD	0.269	0.191	–0.232
	$P = 0.73$	$P = 0.81$	$P = 0.77$
AS (µg/g) HPLC	0.940	0.807	–0.897
	$P = 0.06$	$P = 0.19$	$P = 0.10$
NS (µg/g) HPLC	0.389	0.824	–0.678
	$P = 0.61$	$P = 0.18$	$P = 0.32$

– Analysis done according to temporal variation for all 4 sites.

Cano and Dorn [49] and Brownawell et al. [50] reported that the sorption of alcohol ethoxylate surfactant is better correlated with a clay content of the sediment than to the organic carbon content. The correlation matrix between soil texture and presence of AS and NS in the sediment of Tigris River was illustrated in Table 6. The results, however, showed no significant correlation obtained.

4. Conclusions

Based on the findings of the present study, several conclusions can drown. First, AS and NS were detected in the sediment of Tigris River for both seasons and sites by two measurements methods, which suggest that the sediment acts as a sink for AS and NS in the river. Second, NS and, most probably, their degradation products, nonylphenol, are widespread pollutants in the aquatic environment, especially in dry season. The highest concentration in the dry season was due to the high temperature that probably led to higher activity of microbial organisms for degradation of compounds than in wet season. Surfactants can be used as indictors for the presence of organic pollutants in sediment, due to enhanced sorption of organic pollutants in sediment. Surfactants can be also used to remove the organic compounded in pollinated soils. Soil class has no effect on the efficiency of the adsorption and/or precipitation of AS and NS on river sediment. HPLC or PHD can be used with similar efficiency to measure AS in river sediment, while it is more efficient and sensitive to use HPLC to measure NS.

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