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Assessment of Oued Seybouse Pollution by Anionic Surfactants and their Ecotoxicological Effects on the Freshwater Crustaceans Case of *Daphnia magna*

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ABSTRACT

This study was conducted to assess both biological and chemical pollution of Oued Seybouse (Guelma). To achieve that, indicator bacteria of faecal pollution were isolated and counted and physico-chemical parameters of such watershed including anionic surfactants were measured. Also, acute toxicity of sodium dodecyl sulphate (as an ionic surfactant) was tested on *Daphnia magna.* According to our results, it was observed that: The values of physico-chemical parameters of pollution were not within the permissible limits as the concentrations of total phosphorus and nitrate exceeded 7 mg/l and 52 mg/l, respectively. The concentration of an anionic surfactant did not exceed the accepted norm but it showed toxic effect on aquatic organisms such as *Daphnia magna* especially after prolonged exposure to 0.750 mg/l. Regarding the bacterial load, it largely exceeded the accepted standards for the three types of bacteria which are indicators of fecal contamination. In conclusion, this pollution is a consequence of the solid and liquid wild discharges that have a direct and significant impact on the quality of water throughout Oued Seybouse.

INTRODUCTION

Surface water has become the most important natural source of water for domestic, agricultural and industrial purposes in many countries of the world. Surface water is usually rain water that collects in Lake, River, or Stream [1]. The exponential development of industrial, agricultural and urban activities leads the growing spill of many substances in the environment. The aquatic compartment is generally the final receptacle of many of these compounds that threaten the functioning and sustainability of the ecosystem. This widespread pollution due to the presence of various types of contaminants has led to a gradual deterioration of water quality and threatening of biodiversity and balance in the aquatic ecosystems [2]. The particular vulnerability of freshwater biodiversity also reflects the fact that fresh water is a resource that can be extracted, diverted, or contaminated in ways that can affect its value as a habitat for organisms [3]. Surfactants are produced and used in several million tons per year, 56% are used for household detergents, 27% for technical and agricultural industries, 9% for industrial detergency, 8% for personal hygiene. Surfactants are mainly of four types: anionic, cationic, zwitterionic and nonionic. Anionic surfactants are used in detergent formulations, and the predominant classes of anionic surfactants are branched alkylbenzene sulfonate (ABS) and linear alkylbenzene sulfonate (LAS) [4]. It is likely that of the massive amounts of surfactants used domestically, most end up in wastewater flows. Surfactants are harmful to human beings, fishes and vegetation. Due to the toxic nature, their presence creates many health hazards like dermatitis. They create unpleasant taste and smell when present at a certain concentration. They are responsible for causing foams in rivers and effluent treatment plants (ETPs). Surfactants cause short-term as well as long-term changes in the ecosystem. They reduce the surface tension of water and allow aqueous solutions to spread and penetrate more easily. This property in water adversely affects aquatic life, e.g., by altering the properties of a fish gill and changing the way the fish takes other substances. Some surfactants are biodegradable under aerobic conditions, but many are not biodegradable under anaerobic conditions and found in sewage sludge and river sediments [5]. The species *Daphnia magna* is sensitive to a wide range of pollutants and is representative of freshwater organisms [6-8]. It has become a model organism for ecotoxicological bioassays. The aims of this study was to evaluate the spatial and temporal variation of the bacteriological and physico-chemical parameters

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of Oued Seybouse, and determine the acute toxicity of anionic (LAS) on *Daphnia magna*. The monitoring was carried out on three sampling points covering the region of Guelma and lasted an annual cycle.

MATERIALS AND METHODS

Location and Morphology of Study Area

Located in Northeastern of Algeria, the Seybouse watershed covers a total area of about 6471 km^2, it is divided into six main sub-watersheds. It is the second largest basin, after that of the Medjerda in the Eastern part of North Africa [9]. Oued Seybouse is formed shortly before its entry into the commune of Guelma (20 Km) (36° 26.587"N, 007° 18.740"E), by the junction of Oued Cherf and Oued Bouhamdan. It then crosses a fairly narrow defile and penetrates into the vast Guelma watershed as traverses its entire length in a markedly West-East direction **(Figure 1)**. The Seybouse in this region is located in the North-East, a few kilometers from the chef town of the region. It drifts slightly in South-East after encountering the Guelma depression, and its tertiary watershed consisting of sedimentary terrain of the Cretaceous, Oligocene, Pliocene and Quaternary Mio [10]. The flow of this section shows a remarkable increase, the groundwater joined the surface water through resurgences which can be the resources of the tributaries like Chaabet Zimba, Oued Boussera and Oued Hlia which join the Seybouse on its right border in this section. The vegetation is mainly includes: *Juncus* sp., *Typhia* sp., *Phragmites communis*, *Tamarix* sp., *Neriumo leander*, *Lemna minor*, *Melissa officinalis*, *Senecio* sp., *Sonchus oleraceus*. The most common macroinvertebrates in this region are: molluscs (Pulmonata), arthropods (Hemiptera) (Personal inventory).

Sampling Stations

The stations studied were selected on the basis of the volume of wastewater discharged into the Oued Seybouse. We have located these rejection points as follows:

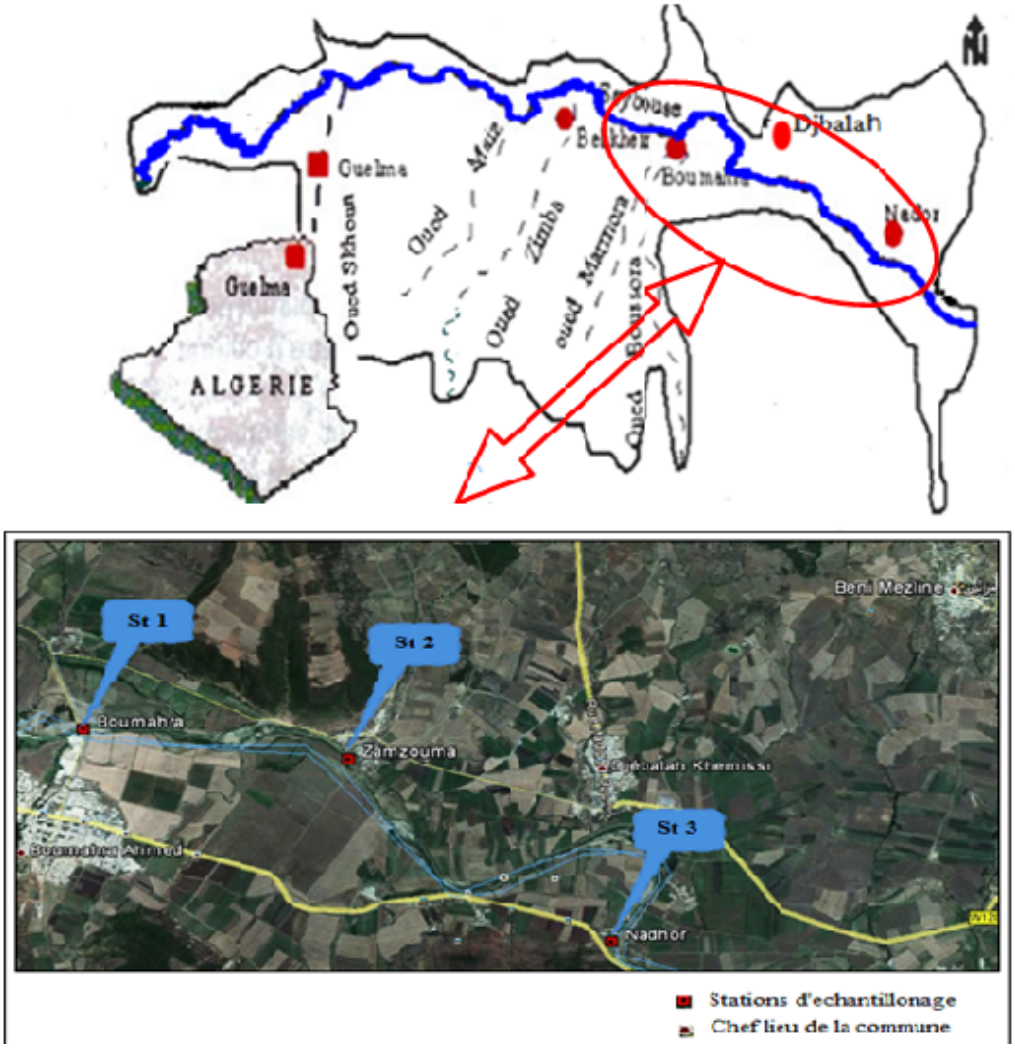

Figure 1. Geographical location of the Oued Seybouse (Guelma watershed) and location of sampling stations.

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• Station 01 (Boumahra) (36° 28' 5.54"N 7° 30'59.47" E); Oued Seybouse in the municipality of Boumahra Ahmed with a daily volume of wastewater discharged estimated as 1591 m^3/day, and three discharge points. The population reached 19900 inhabitants in 2008.

• Station 02 (Zamzouma) (36° 27' 50.8 7"N 7° 32'40.95" E); Oued Seybouse upstream of the municipality of Djebalah Khemissi with a daily volume of wastewater discharged estimated as 404 m^3/day, and two points of discharge. The population was estimated as 5044 inhabitants in 2008.

• Station 03 (Nadhor) (36° 25' 14.1 6"N 7° 36'53.28" E); Oued Seybouse upstream of the municipality of Beni Mezline with a daily volume of wastewater discharged estimated as 447 m^3/day , and Two points of discharge. The population reached 5582 inhabitants in 2008, knowing that this station is far from the discharges of the sewage.

Bacteriological Analysis

Our work relied on counting indicators of fecal contamination; total coliform (TC), fecal coliform (FC) and fecal streptococci (FS), using the technique of indirect estimation of bacterial cells in liquid medium (NPP method: most probable number NFT 90-400) [11]. For collecting bacterial samples, the previously sterilized sampling bottles were immersed in water. Then they were opened at a depth of 25 cm, rinsed 2 to 3 times with the water before being filled and closed in the water [12]. They were transported directly to the laboratory at a temperature ≤4° c to avoid bacterial proliferation [11].

Physico-Chemical Parameters

The following parameters were measured:

- Total phosphorus.
- Nitrates [11].
- Anionic surfactants according to ISO 7875 with modification [13].
- Suspended matters by the filtration method [11].
- Temperature, pH, conductivity, salinity and dissolved oxygen were measured *in situ* (electrochemical method), using a multiparameter "Inolab 750*wtw*".

Toxicity of Anionic Surfactants on *Daphnia magna*

The selection of a biological indicator is often based on their ease of rearing, their sensitivity and predictable manner of response to the contaminants [14,15]. *Daphnia* individuals used in this test were belonging to the 3rd generation; of 7 days old and their size was between 0.5 and 0.8 mm. This species was kindly identified by professor; Samraoui. B (University of Guelma). The test involved assessing the acute toxicity of an anionic surfactant by measuring the death rate (%) (Immobilisation test) of *Daphnia* after 24 and 48 hours of treatment [15,16]. For this purpose, five individuals of *Daphnia* were introduced into each control tube (containing 10 ml of culture medium) or in each treated tube (containing 10 ml of culture medium and the anionic surfactant sodium dodecyl sulfate (CH 3 - (CH 2) 11 -O-SO 3 Na)), at different concentrations (Max=0.750 mg/l and Min=0.150 mg/l). (I tested only the highest and lowest concentrations I found in this study).

RESULTS AND DISCUSSION

Bacteriological Analysis

The present results revealed the omnipresence of total coliforms in the three studied stations with a variation from a station to another **(Figure 2)**. Obviously the load of the total coliforms was superior to the proposed standards of water contamination estimated at 10^6 germ/ml for all stations. In addition, significant temporal variations were recorded showing fluctuations during the study period; with a maximum of 5×10^7 germ /ml during the warm period while the cold period was characterized by a minimum of 0.1×10^6 germ ml. Our results agreed with the work of they found that the load of heterotrophic aerobic bacteria and enterobacteria was related to water temperature [17,18].

Spatio-temporal Variation of Bacteriological Parameters

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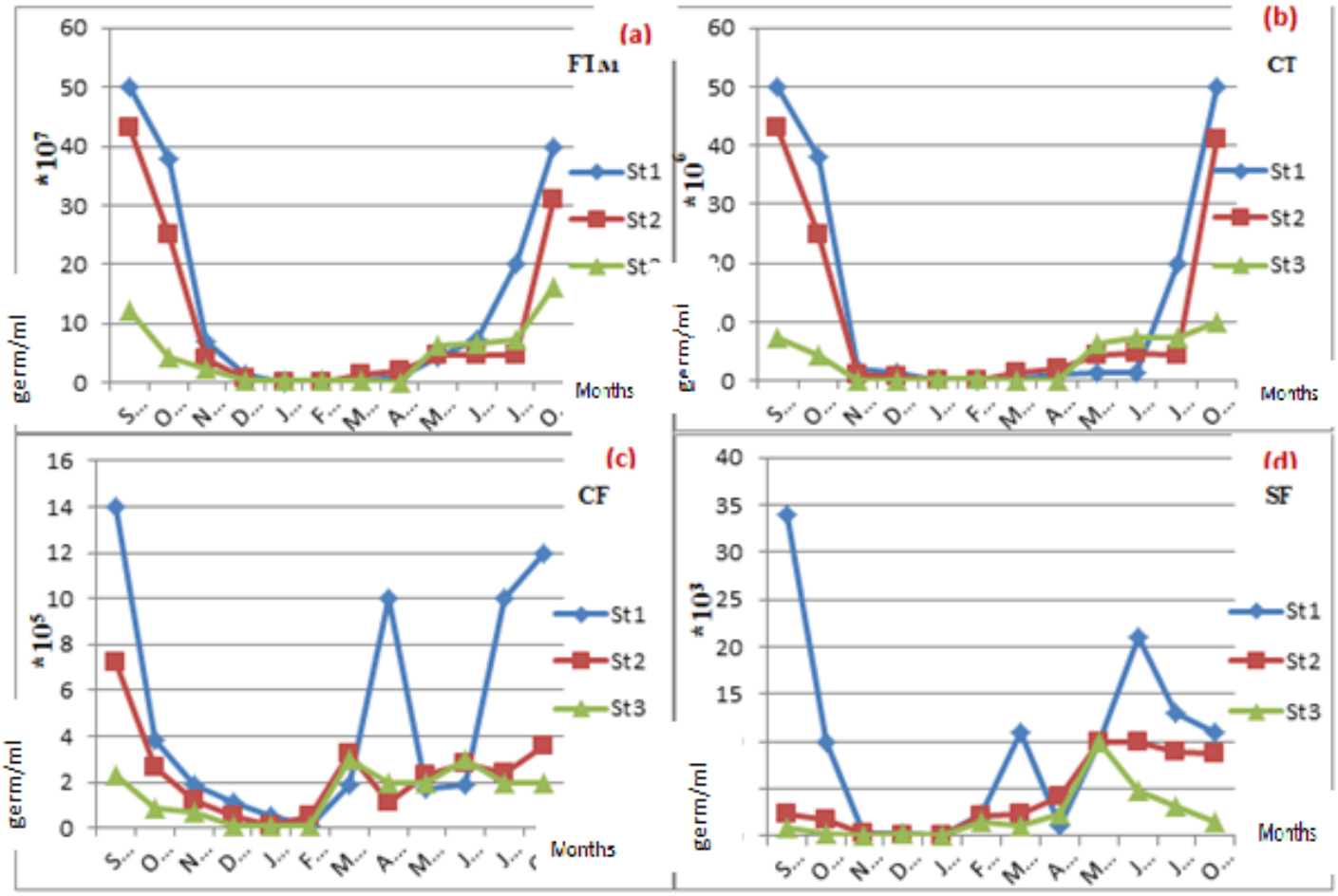

Figure 2. (a) Total mesophilic flora; (b) total coliforms; (c) fecal coliforms; (d) fecal streptococci.

Regarding fecal coliform as indicators of fecal contamination, the omnipresence was observed in three stations Their number was less than that of TC. The load of fecal coliforms showed that the Oued Seybouse underwent considerable fecal contamination. However a significant spatial variation in the load of FC was found Stations 1 and 2 (St1 and St2) which received very large daily volumes of sewage of domestic origin were marked by high concentrations of FC; 1.4×10^6 and 0.72×10^6 germ/ml, respectively. On the other hand, the remote station 3 wastewater discharges and downstream of a rejection of the tiles manufacturing unit which released gypsum, cement-lime and other dyes influencing bacteria was marked by low concentrations of CF 0.01×10^6 germ /ml. Seasonal variations of enteric bacteria can be partly explained by the influence of changes in hydro-meteorological conditions [19]. For fecal streptococci (FS) (bacteria indicative of fecal contamination); the lowest numbers in the three stations were recorded in January, as the number of germs decreased considerably with to a minimum of 0.1×10^3 germ/ml in St3. In general, the spatial pattern of fecal streptococci (FS), predominated or exceeded that of total coliform (TC), and fecal coliform (FC). These results confirmed the fecal contamination of Oued Seybouse water. The comparison of the three stations on the basis of the content of total coliform and bacteria indicative of fecal contamination showed that the Oued Seybouse contained significant bacterial load, with concentrations above the acceptable standards of bacteriological water quality.

Physico-chemical Parameters

Temperature (T)

Water temperature plays an important role in modifying chemical and physical properties as well as biological reactions **(Figure 3a).** In general, the temperatures measured in the present work reflected the seasonal periods in relation to the weather conditions, namely the highest temperature in hot period (from 21.5 to 26.2°C), and the lowest temperature in the cold period (from 10 to 14°C). These temperature values correspond to the Algerian standard for swage, which is 30°C [20].

pH

It's a factor dependent on natural and environmental conditions such as vegetation cover, rock nature, soil substrate and

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human activity [21,22]. Its value ranges from 6 to 8.5 in natural waters [23]. The pH results measured at all sampling points during the study period ranged from 6.16 to 8.6 (slightly neutral to moderately alkaline) **(Figure 3b)**.

Electrical conductivity

We observed two types of spatio-temporal variations of electrical conductivity; one dominated by values lower than 1500 µS/cm and the other marked by a large increase recording values greater than 2000 µS/cm. This strong mineralization in the hot season was due to urban wastewater discharge, known by their high load of dissolved salts and erosion phenomenon. In effect the low flow velocity phenomenon and prolonged contact time between the water and marine clays, favors the dissolution of salts and gives highly mineralized waters [24]. On the other hand, during the rainy season, the decline in electrical conductivity levels was generally linked to flooding in the Oued Seybouse (dilution phenomenon) **(Figure 3c)**.

Salinity

The salinity values measured in all stations were from 0.3 to 0.8 mg/l **(Figure 3d)**. The high salinity levels were recorded during dry periods, probably due to the high evaporation of water. Also, the clayey nature of the soil and the probable contamination induced by urban water could increase the salinity. On the other hand, a drop in salinity values was observed during the winter months. This decrease was due to the dilution effect, which proved that urban waste water was the main origin of the salinity of Oued Seybouse water in Guelma region.

Dissolved Oxygen (DO)

Generally, the concentration of dissolved oxygen in natural surface waters is less than 10 mg/l [25]. Dissolved oxygen values obtained in this study ranged from 2.8 mg/l and 12.7 mg/l **(Figure 3e)**. Station 3 located in downstream and characterized by a low input of urban wastewater recorded values between 3.1 and 12.7 mg/l. On the other hand, DO concentration in station 1 and 2, was slightly low, varying from 2.8 to 9.36 mg/l. This decrease in dissolved oxygen was probably related to municipal wastewater rich in organic matter which was degraded by aquatic microorganisms consuming a substantial portion of dissolved oxygen. In addition, dissolved oxygen concentration was subjected to diurnal and seasonal variations which were partly due to temperature fluctuations, photosynthetic activity and water flow [25].

Suspended Matter (SM)

The analysis of the temporal variation showed that SM concentrations were increased in most stations and ranged between 40 and 360 mg/l **(Figure 3f)**. The highest concentrations were recorded during the winter period and fluctuated between 150 and 360 mg/l. the moderate rainfalls on soil weakened by climatic conditions create strong mechanical erosion.

Nitrate (NO_3)

Analysis of nitrate concentrations in water of Oued Seybouse showed that the lowest concentration 2.51 mg/l was recorded at station 3, whereas the highest concentration exceeding the limit value of 50 mg/l established by the Algerian standard was recorded at station 1 as 52.73 mg/l [20]. However, a slight spatial variation between the different sampling stations was observed **(Figure 3g)**. Seasonal variations in nitrate concentrations at the three studied stations were previously recorded by studies of Berzas, Neal, House [26-28].

Nitrites (NO_2)

Nitrite concentrations observed during our study period and at the three stations ranged from 0.09 to 1.3 mg/l. The highest value was noticed at station 3 with 1.3 mg/l **(Figure 3h)**. High concentrations of nitrites often indicate the presence of toxic materials. Nitrites are especially harmful to young fish [29]. Nevertheless, these concentrations remain below the critical value of 3 mg NO^{2-}/l.

Spatiotemporal Variation Of Physico-chemical Parameters

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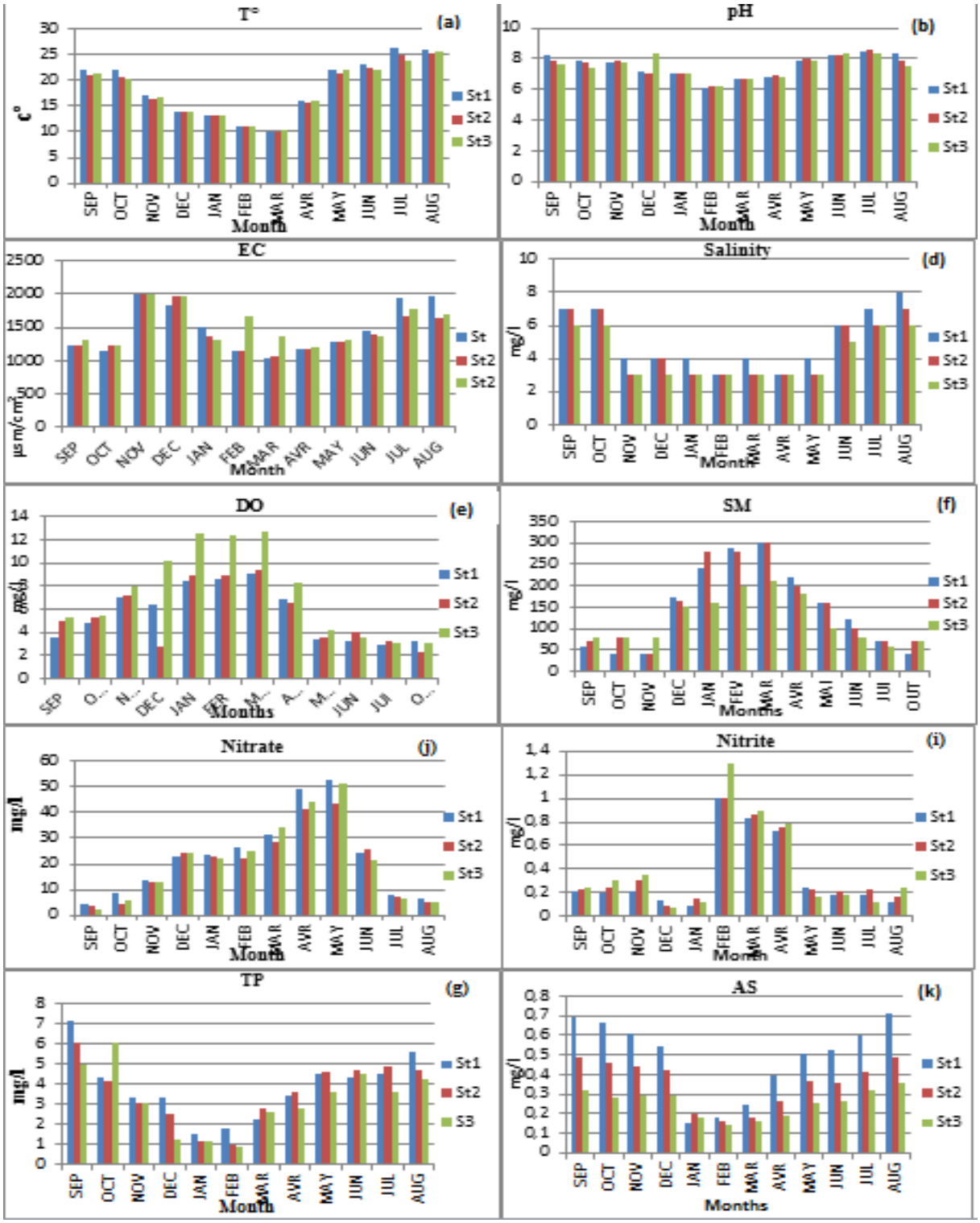

Figure 3. (a) Temperature; (b) pH; (c) electrical conductivity; (d) salinity; (e) dissolved Oxygen; (f) suspended mater; (g) nitrates; (h) nitrites; (i) total phosphorus; (j) anionic surfactants.

Total Phosphorus (TP)

The increase in phosphorus fluxes in surface water results from: The intensification of population pressure and agricultural activities. Phosphorus values above 0.5 mg/l indicate polluted water according to ANRH. The total phosphorus concentrations observed during the study period were high and varied between 0.89 and 7.18 mg/l **(Figure 3i)**. Unlike nitrates, phosphate levels during the dry period were higher than those observed in winter, marked by the intensity of the climatic factor (precipitation), that can affect the total phosphorus concentration in Seybouse water (dilution phenomenon), suggesting that urban wastewater was the main source of phosphorus pollution [26,30-32].

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Anionic Surfactants (AS)

The highest concentrations in the four sampling seasons were encountered at station 1. These concentrations decreased from 0.713 mg/l in August to 0.146 mg/l in February. This decreasing gradient was in relation to the increase in rainfall amounts, This decreasing gradient was related to the increase in rainfall, which led to the increase the water level of Oued Seybouse especially at the stations located near the sources of sewage in the Boumahra Ahmed and Zamzouma regions **(Figure 3j)**. The lowest concentrations 0.385 and 0.146 mg/l were observed during the three sampling seasons at station 3 which is apart from the agglomerations. The sewage of St1 and St2 which reached this station after a few kilometers ran very slowly, due to a very low slope, and the majority of the degradable products had time to disappear. On the other hand, during the flood period, there were no marked differences in concentration between the three stations (except in March), which can be explained by the decrease in the activity of microorganisms degrading the organic matter due to temperature, as well as the increase of the Oued Seybouse flow. These low concentrations were generally at levels where the flora and fauna disturbances may occur, with a concentration ranging from 50 to 100 µg/l [33]. In light of this distribution, it appears that enrichment is not conditioned solely by inputs but also by climatic conditions. Overall, the levels of anionic surfactants observed during the study period were lower than the acceptable standard (10 mg/l) [20].

STATISTICAL STUDY

Pearson's analysis established the following matrix **(Table 1)**. A positive and highly significant correlation between nitrates and DO (r = 1.00*) was recorded. The nitrate ion (NO_{3-}) is the principal form of inorganic nitrogen found in natural water; it is the final stage of oxidation of nitrogen. The nitrite ion (NO_{2-}) was readily oxidized to nitrate ion in the presence of oxygen. The significant positive correlation between total coliforms and temperature (r=0.67*) was consistent with the results of concerning the water of the Boufekrane and Ouislane Oueds in Morocco [34]. This correlation was due to the fact that indigenous bacteria were the dominant components of the total population in polluted rivers.

Table 1. Matrix of correlations..

Variables	FTM	CT	CF	SF	T°	pH	CE	Salinité	Pt	Nitrate	Nitrite	TAA	OD	MES
FTM	1.000													
CT	0.310	1.000												
CF	0.313	1.000	1.000											
SF	0.313	1.000	1.000	1.000										
T°	0.400	0.668	0.669	0.669	1.000									
pH	0.365	0.972	0.972	0.972	0.825	1.000								
CE	0.379	0.946	0.946	0.946	0.873	0.996	1.000							
Salinité	0.394	0.895	0.895	0.895	0.930	0.975	0.991	1.000						
Pt	0.496	0.789	0.790	0.790	0.819	0.860	0.874	0.882	1.000					
Nitrate	-0.529	-0.679	-0.680	-0.680	-0.402	-0.644	-0.620	-0.577	-0.438	1.000				
Nitrite	-0.643	-0.360	-0.362	-0.362	-0.630	-0.475	-0.512	-0.557	-0.441	0.395	1.000			
TAA	-0.506	-0.622	-0.623	-0.623	-0.338	-0.581	-0.555	-0.511	-0.350	0.995	0.381	1.000		
OD	-0.531	-0.667	-0.668	-0.668	-0.394	-0.633	-0.609	-0.567	-0.421	1.000	0.405	0.997	1.000	
MES	-0.533	-0.651	-0.653	-0.653	-0.384	-0.618	-0.595	-0.553	-0.399	0.998	0.418	0.998	0.999	1.000

Also, a significant positive correlation between anionic surfactants (AS) and nitrates (r=0.99*) was observed, this correlation could be explained by the use of nitric acid as a main component in the production of this detergent for their descaling properties. On the other hand, a significant negative correlation between the total coliforms and the AS surfactants (r=-0.62) was observed, which explained the influence of the AS on the bacterial load of the water. These findings were in accordance with the study of Stavskaya which showed that the anionic surfactants had an antimicrobial effect depending on their chemical structure [35].

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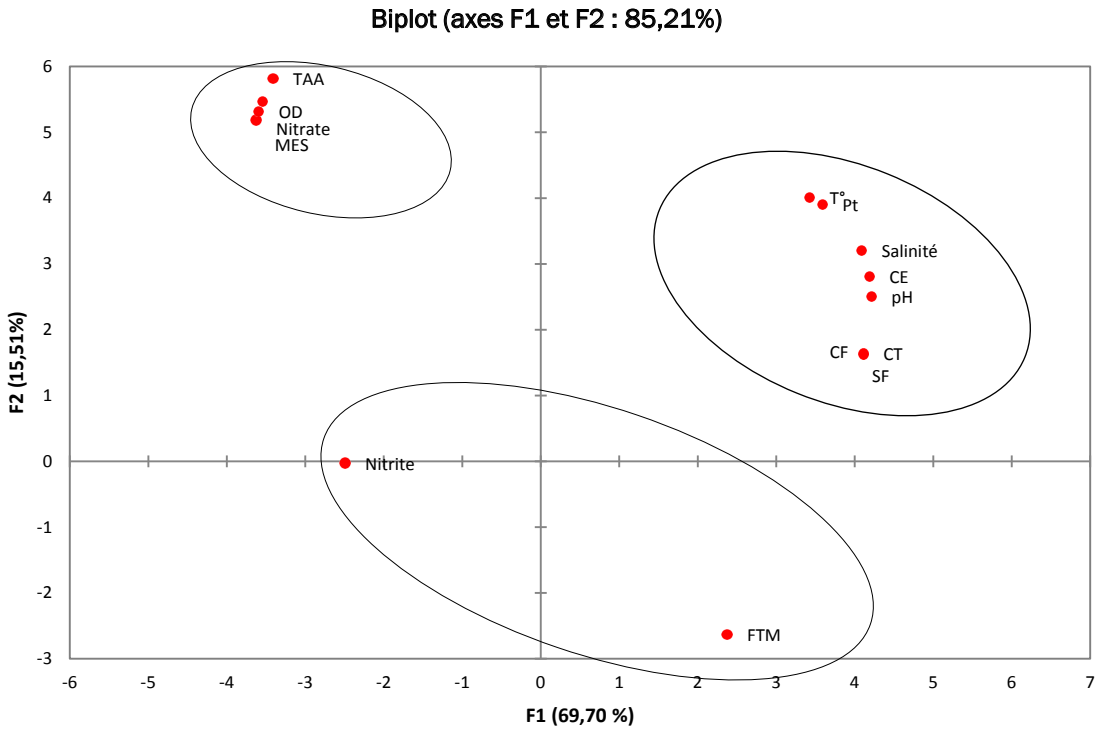

Figure 4. Factorial diagram 1 × 2 of the ACP applied to the data on physico-chemical and bacteriological quality of Oued Seybouse (Guelma).

Multivariate statistical analysis through the PCA (Principal Component Analysis) in its 1x2 factorial design that brings together 8.21% of the information **(Figure 4)**. We can differentiate three groups of parameters more or less distinct. A group influenced by temperature, containing Pt, pH, EC, FC, TC, FS and salinity; the increase in water temperature favored the growth of bacteria, knowing that the majority of the bacterial flora is mesophilic. High temperatures can affect salinity, pH and electrical conductivity of water; this increase causes stirring of the ions and cations, which leads to an increase in the electrical conductivity of the water. So we can say that this group (Pt, pH, EC, FC, TC, FS), expresses the effect of climate factor (temperature) on the physic-chemical and bacteriological parameters of water. A group opposite to the first along the y-axis, was influenced by dissolved oxygen, it was represented by AS, Nitrate and SM. We can say that this group expresses the redox phenomenon in water. The decomposition of a biodegradable organic matter discharged into water bodies by microorganisms resulting in consumption of dissolved oxygen in water. The nitrate ion is the final stage of the oxidation of nitrogen in the presence of oxygen. Last group opposite the two first groups along the abscise axis composed of nitrite and FTM.

Acute Toxicity Test of Anionic Surfactants on *Daphnia magna*

Table 2. Results of acute toxicity test.

Treatment	Initial Nb of Daphnia	After 24 h Mortality rate (%)	After 48 h Mortality rate (%)
[C]T=0 mg/l	25	0.0%	0.0%
[C]Min=0.150 mg/l	25	1.4%	1.4%
[C]Max=0.750 mg/l	25	3.12%	7.28%

The results of the acute toxicity test showed that the concentrations that were recorded during the study period in all sampling stations were toxic to *Daphnia magna*, but they were far from the lethal concentration (LC50). The studies of reported that the values of LC50 or EC50 varied between 0.6 and 30 mg/l [36,37]. However, most of LC50 or EC50 were between 1 and 10 mg / l. A 20% mortality after 48 hours of treatment with the concentration (0.750 mg/l) was observed, it increased from 8% after 24 hours to 28% after 48 hours **(Table 2)**. This indicated that a prolonged contact time could have more harmful effects on *Daphnia magna*, these results were in agreement with the studies of they reported chronic toxicity values between 0.1 and 10 mg/l [38-40].

CONCLUSION

The assessment of physico-chemical parameters and microbiological indicators of pollution at the three sampling stations reflected an urban pollution of Oued Seybouse. This pollution was characterized by high levels of phosphorus and nitrates 7.18 7.18 mg/l and 52.73 52.73 mg/l, respectively and widely exceeded acceptable standards. The concentrations of anionic surfactants were slightly higher but did not exceed the Algerian standard. These values recorded in various stations showed that their

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effects may appear at a concentration of 0.75 mg /l on the freshwater crustacean *Daphnia magna.* The bacteriological quality of the three sampling stations is of fecal types (presence of fecal coliforms, fecal streptococci ...), and their concentrations are above the permissible standards. In perspective, this study offers us a vast field of investigation. Concerning the ecotoxicity of surfactants, it is necessary to carry out further complementary research to further deepen this approach. The use of other animal and plant species as well as the chronic toxicity test will help to understand the effect of these elements on the aquatic ecosystem. Finally, the wastewater treatment stations play a very important role in the purification of water; it is very interesting to test the effectiveness of this station towards surfactants treatment.

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