PHYSICO-CHEMICAL AND BACTERIOLOGICAL CHARACTERIZATION OF OUED SEYBOUSENORTH-EASTERN ALGERIA

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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 fecal 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 can be toxic on aquatic organisms. 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.

Key words: Water pollution, anionic surfactant and Oued Seybouse.

Résumé

Cette étude a été menée pour évaluer la pollution biologique et chimique de l'Oued Seybouse (Guelma). Pour cela. nous avons dénombré et isolé lestrois types de bactéries indicatrices d'une contamination fécale. ainsi que le suivi de la variation spatio-temporelle des paramètres physico-chimiques de ce bassin hydrographique. y compris les tensioactifs anioniques. D'après les résultats obtenus. il en ressort que: Les valeurs des paramètres physico-chimiques de la pollution sont largement élevés par rapport aux normes admissibles puisque les concentrations de phosphore total et de nitrate ont dépassé les limites autorisées 7 mg/L et 52 mg/L respectivement. Les concentrations de tensioactif anionique n'ont pas dépassé la norme admissible mais ils peuvent être toxiques pour les organismes aquatiques. En ce qui concerne la charge bactérienne. elle a dépassé largement les normes acceptées pour les trois types de bactéries indicatrices de contamination fécale.

En conclusion, cette pollution est une conséquence des rejets sauvages solides et liquides qui ont un impact direct et significatif sur la qualité de l'eau de l'Oued Seybouse.

Mots clés: Pollution de l'eau, tensioactifs anioniques et 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

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contaminants has led to a gradual deterioration of the water quality and threatening of biodiversity and equilibrium in the aquatic ecosystems [2].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 [3].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 alkylbenzenesulfonate (ABS) and linear alkylbenzenesulfonate (LAS)[4]. The aims of this study were to evaluate the spatial and temporal variation of the bacteriological and physic-chemical parameters of waters of Oued Seybouse. In order to identify the main factors of degradation of this ecosystem. 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 [5].

Oued Seybouse is formed shortly before its entry into the commune of Guelma (20 km) (N 36.435999. E7.450166), 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 (Fig. 1). The OuedSeybouse 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 Mio-Quaternary [6].

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 ChaabetZimba. OuedBoussera and OuedHlia which join the OuedSeybouse on its right border in this section.The vegetation mainly includes: *Juncus*sp.. *Typhia*sp, *Phragmitescommunis*. *Tamarix*sp, *Neriumoleander*, *Lemna minor*, *Melissa officinalis*, *Senecio*spand. *Sonchusoleraceus*. The most common macroinvertebrates in this region are: molluscs (Pulmonata), arthropods (Hemiptera) (Personal data).

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: (Fig. 1).

Bacteriological analysis

For the realization of our work we have performed a monthly sampling during a year of study where we have sampled 36 simples. Our main objectif 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) [7],[8].

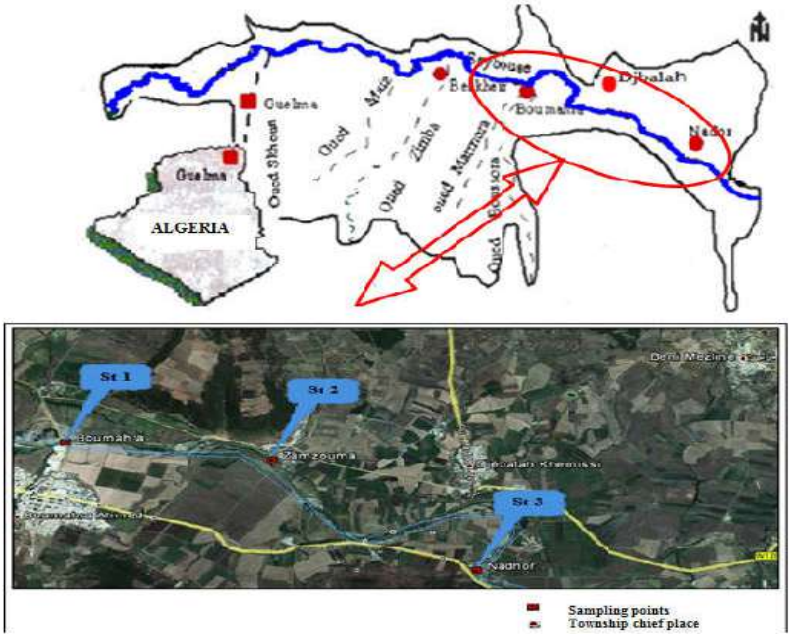

Fig. 1-Geographical location of the Oued Seybouse (Guelma watershed) and sampling stations position

Tableau 1: Sampling points and their coordinates.Sampling stations.

	Site name	Geographic coordinates	wastewater discharged m^3/d	Inhabitants in 2008
Station1 (St1)	Boumahra	36° 28' 5.54"N 7° 30' 59.47" E	1591	19.900
Station 2 (St2)	Zamzouma	36° 27' 50.8 7"N 7° 32' 40.95" E	404	5.044
Station 3 (St3)	Nadhor	36° 25' 14.1 6"N 7° 36' 53.28" E	447	5.582

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 [9]. They were transported directly to the laboratory at a temperature ≤4°Cto avoid bacterial proliferation.

Physico-chemicalparameters

Concerning, physico-chemical analyzes we have adopted the same sampling strategy carried for bacteriological analyzes, with a total of 36 samples and 3 replicates for each sample during the analyzes. The following parameters were measured:

- Total phosphorus according to [8].
- Nitrates according to [8].
- Nitrites according to [8].
- Anionic surfactants according to ISO 7875 with modification [10].
- Suspended matters by the filtration method [8].

-Temperature, pH, conductivity, salinity

and dissolved oxygen were measured in situ (electrochemical method), using a multiparameter "Inolab*750wtw*"

Statistical analysis

The results of physico-chemical and microbial soil parameters were evaluated by the LSD test (least significant difference) ($p<0,05$) using tow-way Anova . A Pearson correlation test and principal components analysis (PCA) were also applied to highlight the relationship between the physical-chemical and microbial soil variables (XlStat-Pro. V.7.5.2)

Results and discussion

Bacteriological analysis

The present results (Fig. 2), revealed the omnipresence of total coliforms in the three studied stations, with a variation from a station to another (Fig. 2 (b)). Obviously, the load of total coliforms was higher than 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 0.1×10^6germ ml. Our results agreed with the work of Fernandez-Alvarez [11] and Chahlaoui [12] they found that the load of heterotrophic aerobic bacteria and enterobacteria was related to water temperature.

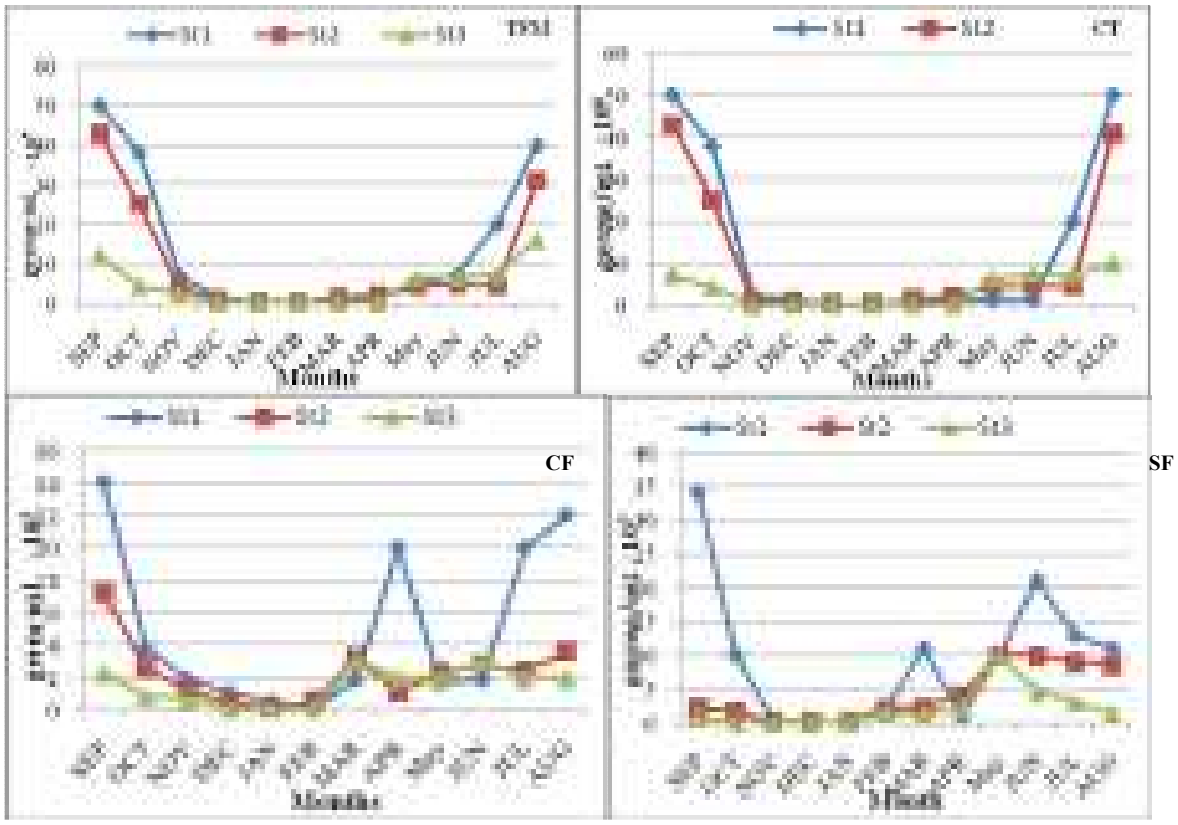

Fig. 2-Spatio-temporal variation of bacteriological parameters; (TMF): total mesophilic flora (TC): total coliforms. (FC): fecal coliforms (FS): fecal streptococci.

Regarding, fecal coliform as indicators of fecal contamination, their presence was observed in three stations (Fig. 2 (c)). The load of fecal coliforms showed that the Oued Seybouse underwent considerable fecal contamination.

However, a significant spatiotemporal variation in the load of FC was found (Fig. 2 (c)). Stations St1 and St2 which received very large daily volumes of sewage of domestic origin were marked by high concentrations of FC; 1.4 $\times 10^6$ and 0.72 $\times$ 10^6 germ/mL respectively. On the other hand, the remote station St3 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 FC 0.01 $\times$ 10^6 germ/mL.

Seasonal variations of enteric bacteria can be partly explained by the influence of changes in hydro-meteorological conditions [13]. 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 $\times$ 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

pH: It's a factor dependent on natural and environmental conditions such as vegetation cover, rock nature, soil substrate and human activity[14.15].Its value ranges from 6 to 8.5 in natural waters[16.17]. The pH results measured at all sampling points during the study period ranged from 6.16 to 8.6 (slightly neutral to moderately alkaline) (Fig. 3).

Temperature (T°): Water temperature plays an important role in modifying chemical and physical properties as well as biological reactions. In general, the temperatures 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 (from10 to 14 °C) (Fig. 3). These temperature values correspond to the Algerian standard for swage which is 30 °C [17].

Electrical conductivity (EC): 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 (Fig. 3). 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 fact 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 [18]. 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).

Salinity: The salinity values measured in all stations were from 0.3 to 0.8 mg/L (Fig. 3). 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 [19] Dissolved oxygen values obtained in this study ranged from 2.8 mg/Land 12.7 mg/L (Fig. 3). Station St 03 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 St 01 and St 02 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 [19].

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(Fig. 3). 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 (NO3**)**: Analysis of nitrate concentrations in water of Oued Seybouse showed that the lowest concentration 2.51 mg/L was recorded at station St 03. whereas the highest concentration exceeding the limit value of 50 mg/L established by the Algerian standard[16]was recorded at station St 01 as 52.73 mg/L(Fig. 3). However a slight spatial variation between the different sampling stations was observed. Seasonal variations in nitrate concentrations at the three studied stations were previously recorded by studies of Berzas [20].Neal [21] and House [22].

Nitrites (NO2**)**: 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 St3 with 1.3 mg/L (Fig. 3. High concentrations of nitrites often indicate the presence of toxic materials. Nitrites are especially harmful to young fish [23]. Nevertheless, these concentrations remain below the critical value of 3 mg NO2 / L [23].

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 [24], (National agency of water resources).

The total phosphorus concentrations observed during the study period were high and varied between 0.89 and 7.18 mg/L (Fig. 3).

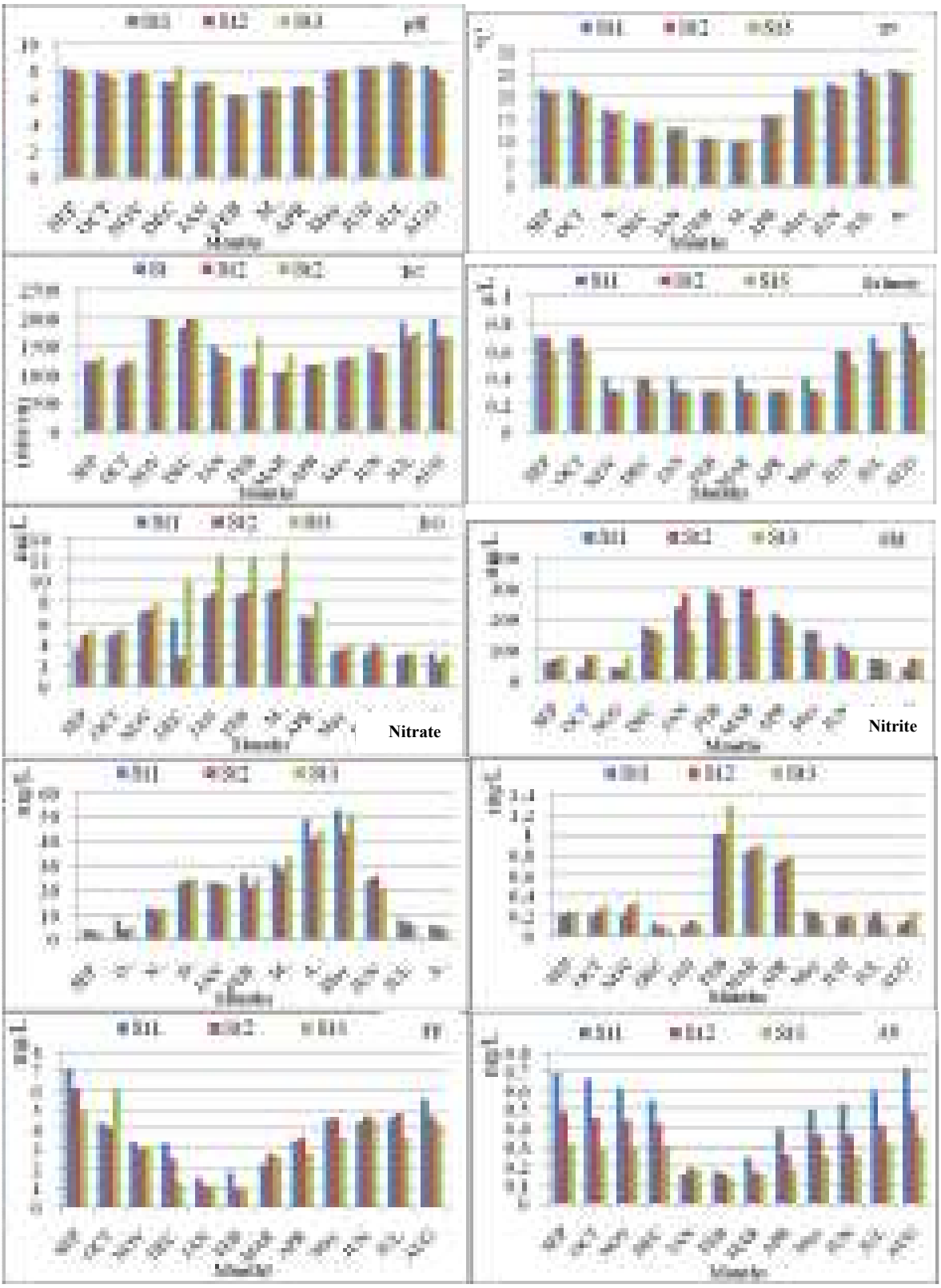

Fig.3- Spatiotemporal variation of waterphysic-chemical parameters; **a**): pH. **b**): Temperature. **c**): electrical conductivity. **d**): Salinity. **e**): Dissolved Oxygen. **f**): Suspended Mater.**g**): Nitrates. **h**): Nitrites. **i**): Total phosphorus and **j**): Anionic Surfactants.

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 [21,25, 26 and 27].

Anionic surfactants (AS): The highest concentrations in the four sampling seasons were encountered at station 1. These concentrations decreased from 0.713 mg/Lin 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 (Fig. 3). 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 according to Thoumelin [28]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) [17].

Statistical results

Processing data by ANOVA two-way analysis for water physic-chemical and bacteriological parameters between months and study sites, revealed a significant difference ($p<0.05$) of T°, pH, EC, DO, and Salinity between factors F1 and F2. Significant differences were recorded between factors F1 and F2 with respect to NO3. NO2, TP and AS. A significant difference was also observed between Factors F1 and F2 concerning bacterial parameters (bacterial biomass) (Fig.4).

Pearson's correlation tests of water physicochemical and bacteriological parameters of different sampling points are represented in the following matrix (Tab.II).

A positive and highly significant correlation between nitrates and DO ($r = 0.87$, $p<0.05$) was recorded. The nitrate ion (NO3) is the principal form of inorganic nitrogen found in natural water; it is the final stage of oxidation of nitrogen. The nitrite ion (NO2) was readily oxidized to nitrate ion in the presence of oxygen.The significant positive correlation between total coliforms and temperature ($r = 0.67$ $p<0.05$) was consistent with the results of Aboulkacem [29], concerning the water of the Boufekrane and OuislaneOueds in Morocco. This correlation was due to the fact that indigenous bacteria were the dominant components of the total population in polluted rivers.

Table 2: Matrix of correlations between water physicochemical and bacteriological parameters of 03 sampling points.

Variables	TMF	TC	FC	FS	T°	pH	EC	Salinity	TP	NO3	NO2	AS	DO	SM
TMF	1													
TC	0.3102	1												
FC	0.3132	1.0000	1											
FS	0.3132	1.0000	1.0000	1										
T°	0.3998	0.6679	0.6685	0.6685	1									
pH	0.3646	0.9717	0.9719	0.9719	0.8247	1								
EC	0.3793	0.9458	0.9460	0.9460	0.8735	0.9957	1							
Salinity	0.3945	0.8950	0.8953	0.8953	0.9298	0.9750	0.9914	1						
TP	0.4964	0.7892	0.7900	0.7900	0.8192	0.8599	0.8742	0.8816	1					
NO3	-0.5289	-0.6790	-0.6800	-0.6800	-0.4020	-0.6443	-0.6204	-0.5773	-0.4377	1				
NO2	-0.6432	-0.3604	-0.3622	-0.3622	-0.6302	-0.4747	-0.5117	-0,5567	-0.4409	0.3945	1			
AS	-0.5061	-0.6224	-0.6234	-0.6234	-0.3380	-0.5810	-0.5554	-0.5109	-0.3504	0.9953	0.3809	1		
DO	-0.5307	-0.6673	-0.6683	-0. 6683	-0.3943	-0. 6330	-0.6094	-0.5670	-0.4211	0. 9997	0.4050	0.9971	1	
SM	-0.5325	-0.6515	-0.6526	-0,6526	-0.3840	-0.6177	-0.5946	-0,5530	-0.3993	0.9982	0.4181	0.9983	0.9994	1

TMF : total mesophilic flora ; TC : total coliform ; FC fecal coliform ;FS : fecal streptococcus ; T° : temperature ; EC : electrical conductivity ; TP : total phosphorus ; NO3 : nitrate ; NO2 : nitrite ; AS : anionic surfactant ; DO : dissolved oxygen; SM: suspended mater.

Also, a significant positive correlation between anionic surfactants (AS) and nitrates (r = 0.99 p<0.05) 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 p<0.05) was observed which explainmd the influence of the AS on the bacterial load of the water. These findings were in accordance with the study of Stavskaya [30] which showed that the anionic surfactants had an antimicrobial effect depending on their chemical structure.

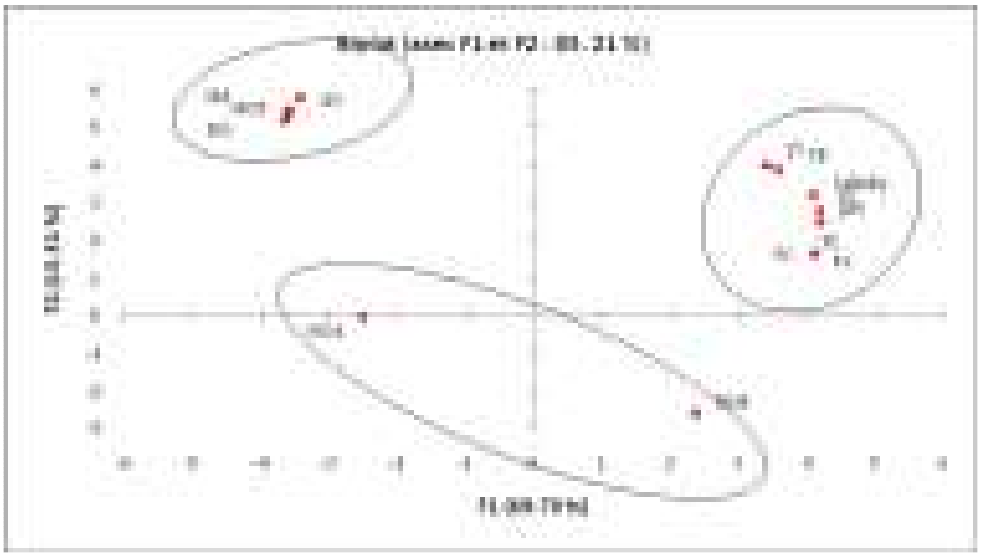

Fig. 5. - Factorial diagram 1 × 2 of the PCA applied to the data on physic-chemical and bacteriological quality of Oued Seybouse (Guelma).

Multivariate statistical analysis using Principal Component Analysis (PCA) in its 1x2 factorial design collected 85.21% of the information. We can differentiate three groups of parameters more or less distinct (Fig.5).

A group influenced by the pH containing TP, EC, FC, TC, FS and salinity; pH obtained results are close to neutrality or slightly alkaline. These valuesfavored the growth of bacteria knowing that the majority of the bacterial flora is mesophilic prefer a pH varied between 6 to 8. The variation of salinity, T° and electrical conductivity can affect pH of water; this increasecauses stirring of the ions and cations which leads to an increase in the electrical conductivity of the water, which causes changes in pH values. So we can say that this group (TP, pH, EC, FC, TC and FS) expresses the effect of physic factor (pH), on the physic-chemical and bacteriological parameters of water.

A group opposite to the first along component was influenced by anionic surfactant; it was correlated with DO, NO3 and SM. We can say that this group expresses the biodegradation 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 first component composed of nitrite and TFM.

Conclusion

The assessment of physic-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/Land 52.73 52.73 mg/L. respectively and widely exceeded acceptable standards.

The bacteriological contamination 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 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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