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Study of correlations between microbiological and physicochemical parameters of drinking water quality in El kolea city (Agadir, Morocco): Using multivariate statistical methods

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Abstract

El kolea city, located in south-east of Agadir, supply a drinking water to its habitants for many years ago from wells after treatment by chlorination. Near to these wells exist a considerable number of septic tanks and cesspools used as sanitation system. For this reason. physicochemical and microbiological pollution of groundwater generated by the infiltration of sewage from cesspools were studied. The aim of this study is to determine the correlation among the measured variables using multivariate analysis. Data including microbiological and physicochemical parameters gathered in ten groups were collected from three monitored sites, for a period of two months. Principal Component Analysis (PCA) and Cluster Analysis (CA) were applied to the collected data on groundwater quality to get better information's about the quality of groundwater consumed by El kolea population and to found correlations between studied parameters. The results of One-way ANOVA test show a lack of difference between months for all studied parameters except for T and NH_4^+. Three studied sites don't show any difference between them for pH, NO_3^- and NH_4^+. Conversely to the other studied parameters, a significant difference is demonstrated. Results of PCA and CA shows physiochemical component (F1) and microbial component (F2) which explain 90.91% of the total dataset.

1. Introduction

Multivariate statistical methods are used to reveal complex relationships in large variety of environmental situation [1]. It could be the appropriate tool for data reduction and interpretations of multi-constituent physicochemical measurements [2]. Multivariate statistical techniques have been applied to characterize and evaluate freshwater quality; and to verify spatiotemporal variations caused by natural and anthropogenic factors linked to seasonality [3]. Many studies in literatures have used multivariate statistical techniques to assess and characterize groundwater quality. Lu et al., were successfully applied CA method to spatially categorize the collected water samples in Southwestern Taiwan. to elucidate key parameters associated with the occurrence of elevated Arsenic pollution in groundwater [4]. Factors analysis was used to explain the groups given by CA method. Another work concerns 81 groundwater samples were collected from three cities in Punjab (Pakistan), and 16 physicochemical parameters were subjected of multivariate statistical techniques such as factor analysis, cluster analysis and discriminant analysis [5]. In this work, five factors (salinization, alkalinity, temperature, domestic waste and chloride) explained 74 % of the total variance in water quality data set using Factor Analysis. Hierarchical cluster analysis revealed three clusters (relatively less polluted, moderately polluted and highly polluted sites) from nine sampling stations of each city and ten significant parameters were identified by using Discriminant Analysis (calcium, ammonia, sulphate,

sodium, electrical conductivity, chloride, temperature, total hardness and turbidity) [5]. Affum et al., were used Principal Component Analysis (PCA) on groundwater quality in the Western Region of Ghana to explain 93.3 % of the total variance with five factors. The first principal component (PC_1) described salinity and anthropogenic contaminants (Cl^-, Na^+, Mg^{2+}, conductivity, TDS and SO_4^{2-}) and the second principal component (PC_2) described contribution of water hardness (Calcium ion, K^+, HCO_3^-, alkalinity and pH) and anthropogenic contaminants described by others components PC_3, PC_4 and PC_5 [6].

PCA is a multivariate technique used in several studies to reduce the dimensionality of data and to determine linear relationships between variables [3,7]. This linear combination obtained from original variables transformed by PCA is called principal components (PC) [8]. Indeed, decomposition of correlation matrix is used to normalized each variable to a unite variance and contributes to build PCA [9]. The objective of Cluster Analysis method is to study the similarities and differences between studied objects and used to classify objects into clusters and give a relationship between entire data set [10]. Dendrogram is a graph of clusters which reduces the dimensionality of original data and gives us a global vision of the groups of objects and their proximity [8].

The major issues facing the population of the world is the availability of safe drinking water for domestic use. Several factors hinder the achievement of this objective, such as pollution from high urbanization and lack of adequate sanitation. El kolea city, situated in the south east part of Agadir with 18127 households [11] suffered for many years from lack of adequate sanitation. Twelves water supplier associations assures the water supply services from wells equipped with pump motors and storage tanks. The quality of this water is controlled periodically by Water and Electricity National Office. Water branch (WENO-WB). After chlorination treatment in storage tanks or directly in wells, water is transported by distribution network system to reach houses. Daily infiltration of sewage from cesspools without treatment throughout a calcareous soil pollute a groundwater quality. This inadequate situation will cause several waterborne diseases such as cholera. gastroenteritis. typhoid. dysentery and diarrhea [12]. Diarrhea disease related to lack of safe drinking water and shortage of adequate sanitation in many areas of the world i.e. 696 million of African children under five years of age were sick with diarrhea and cause 46 per cent of child deaths yearly [13].

The safety of domestic drinking water is assured when fecal pollution indicators are not detected in drinking water samples [14]. For this reason. total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) were measured in drinking water samples. In addition. seven physicochemical parameters are measured also (temperature (T), pH, nitrite nitrogen (NO_2^-), nitrate nitrogen (NO_3^-), ammoniacal nitrogen (NH_4^+), electrical conductivity (EC) and turbidity (Tur).

The aim of this work was to study the correlation among the measured variables and to find more detailed information's about water quality parameters in this city using PCA and Cluster Analysis.

2. Materials and methods

2.1. Study area and samples collection

To study physicochemical and microbiological impact of sewage from cesspools on drinking water quality. three studied sites are chosen in the El kolea city. Taddart's Well (TW) is located south-west of El Kolea, far from the intense agglomerations and located near the agriculture activities. Chorafa Lkhmaïss's Well (CLW) exist near the center of El Kolea in the most populous area and Widadiat Lkhir's Well (WLW) is moderately populated and located northwest of El Kolea in the city of Laazibe. During May 2016 and June 2016, two sampling campaigns were performed at these three wells. For microbiological and physicochemical analysis, water samples were collected in sterile bottles of 500 mL manually, aseptically and in triplicate. Samples were stored in an ice box at a 4°C in obscurity and transported to the laboratory for analysis within 24 hours after sampling [15]. Geographical position of three studied sites are shown in Figure.1. More details on experimental part are described in our previous paper [16].

According to the Moroccan standard recommendations and analysis methods references of Rodier [15,17]. measurements of physicochemical and microbiological parameters for drinking water are shown in (Table.1).

2.2. Statistical analysis

The results were analyzed by One-way ANOVA and the homogenous groups are determined by Tukey HSD Test ($\alpha = 0.05$). The Principal Components Analysis and Cluster analysis was used to study correlations among the variables. This statistical process is carried out using StatSoft. Inc. (2014). STATISTICA (data analysis software system). version 12.

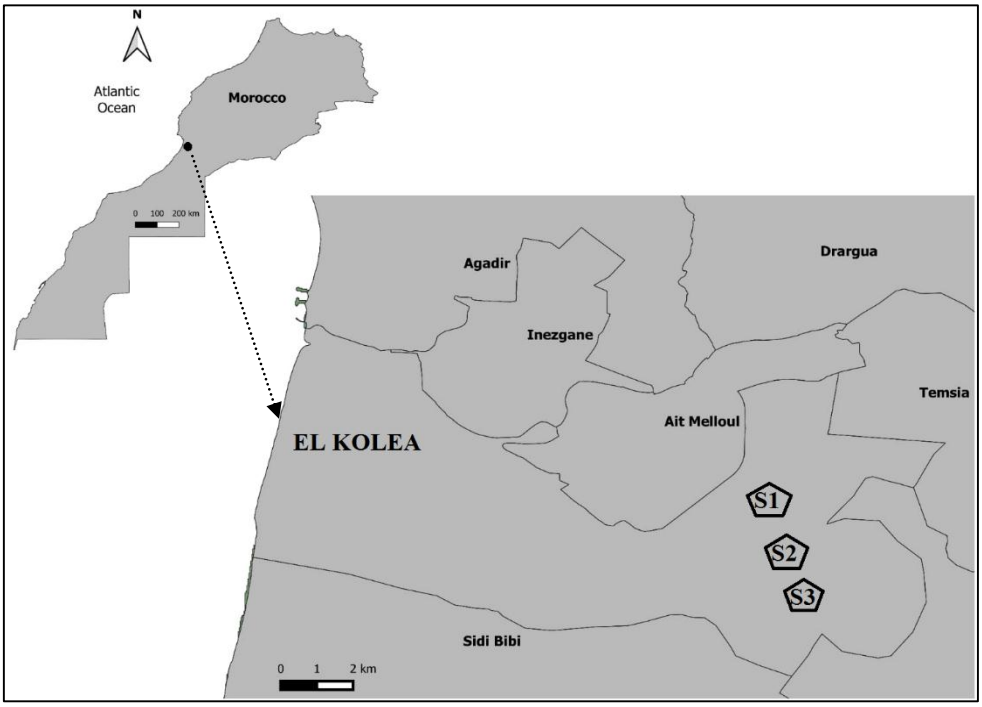

Figure.1: Map of EL Kolea city (S_1: WLW, S_2: CLW, S_3: TW)

2.1. Physicochemical and microbiological analysis

Table.1: Physicochemical and bacteriological analysis methods

Measured parameters	Analysis methods references
Temperature (°C)	NM 03.7.008. 1989
pH	NM 03.7.009. 2001
Electrical conductivity (µS/cm)	NM 03.7.011. 2001
Nitrite nitrogen (mg/l)	Rodier et al. 2009 [15]
Nitrate nitrogen (mg/l)	
Ammoniacal nitrogen (mg/l)	
Turbidity (NTU)	
Total coliform (CFU /100 ml)	
Fecal coliform (CFU /100 ml)	
Fecal streptococci (CFU /100 ml)	

3. Results and discussion

3.1 Descriptive statistics

Univariate descriptive statistics were used to present the complete data set which corresponds to ten studied parameters for eighteen samplings, mean, maximum, minimum, median, and standard deviation were used to describe it. Means of all of our parameters were compared to Moroccan standards (Table.2). Box and Whisker plot in figure.2 represent mean, mean ± standard error and mean ± standard deviation of all studied parameters. In three sites, mean of both temperature and pH are within Moroccan guidelines (T = 26.58 °C, pH = 7.45). In addition, mean of electrical conductivity, NO_2^-, NO_3^-, NH_4^+

and turbidity (814.44 µS / cm; 0.018 mg / L; 13.07 mg / L; 0.0045 mg/ L and 0.5478 NTU respectively) are all lower than Moroccan guidelines. Whilst, the mean of CT, CF and SF (747.05 CFU / 100mL, 878.38 CFU / 100mL and 382.50 CFU / 100mL) exceed Moroccan standards for drinking water. As consequence, physicochemical parameters in the collected samples was above acceptable limits, but it must be highlighted that bacteriological samples don't respect acceptable limits (0 UFC/100 mL). The logics explanations for this polluted situation are cited in our previous studies [16].

Table.2: Descriptive statistics for the total data set.

Variables	Units	Samples	Mean	Min	Max	Median	Standard deviation	Moroccan Drinking Water Guidelines [17]
T	°C	18	26.58	23	30	26.45	1.65	25 - 30
pH	-	18	7.45	7.3	7.58	7.445	0.0707	6.5 < pH < 8.5
EC	µS / cm	18	814.44	744	864	843	51.13	2700
NO_2^-	mg / L	18	0.018	0.011	0.03	0.017	0.007	0.5
NO_3^-	mg / L	18	13.07	7	35.9	11	8.29	50
NH_4^+	mg / L	18	0.0045	0	0.017	0.002	0.0057	0.5
Tur	NTU	18	0.54	0.29	1.03	0.375	0.3076	5
CT	CFU / 100mL	18	747.05	8	1140	1100	526.61	0
CF	CFU / 100mL	18	878.38	10	1430	1242	633.08	0
SF	CFU / 100mL	18	382.50	5.00	1069	96.5	477.10	0

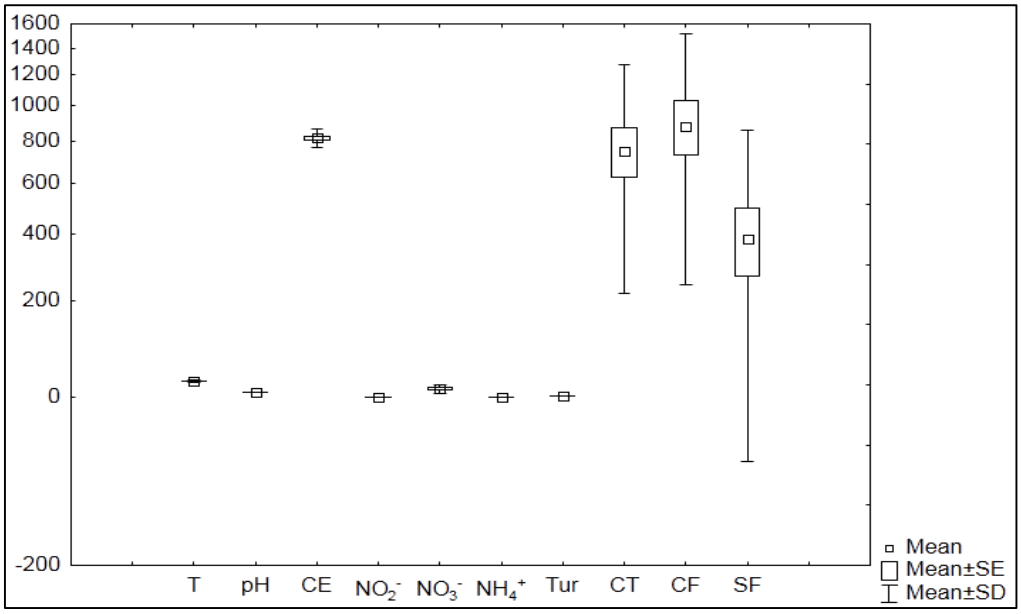

Figure.2 : Box & Whisker plot of studied parameters in groundwater samples.

3.2 One-way ANOVA test

One-way ANOVA results are shown in Table 3. With regard to the study of difference between three studied sites and between two months, significant differences ($p < 0.05$) were observed in (T) between months and between sites. This difference may be due to specificity of each month and each site concerning climat conditions. No significant differences are observed in pH both in month and sites ($p > 0.05$). All pH values ranged from 7.40 to 7.45 for all sites and this fluctuation may indicat contamination by sewage and agriculture activities [18,19,20]. Electrical conductivity concentration shows significant differences between sites ($p < 0.05$), while, no significant difference is observed between months. The origine of electrical conductivity may be from the presence of chloride, phosphate and nitrate nitrogen which comes from failing sewage system [19]. For NO_2^-, significant differences are observed between sites, while, no significant difference is observed between months ($p > 0.05$). In addition, no significant difference is observed between months and between sites for NO_3^- ($p > 0.05$),

nitrate contamination may correlated with agriculture activities and livestock production [21,22]. NH_4^+ showed no significant difference between months but significant differences are observed between sites. Many processes (metabolic. agriculture and industrial) produce ammonia in the environment areas as ammonium ion NH_4^+ which is very soluble in water and in the pH range of natural waters [21]. With regard to the study of turbidity, no significant difference is observed between months, but significant differences is observed between sites. This difference can be attribuated to microorganisms adsorbed onto small particles i.e. clays which raised the turbidity of water [23,24]. It is also related to transport and bioavailability of polluants materials in natural waters [25]. The concentration of microbiological indicators (TC, FC, FS) were different between sites ($p < 0.05$), while no significant difference is observed between months. Raw sewage discharges and effluents rich with high loads of microorganisms could explain this polluted situation [26].

Table.3: One-way ANOVA results and Tukey HSD Test between sites and between months

		T	pH	CE	NO_2^-	NO_3^-	NH_4^+	Tur	TC	FC	FS
Sites	**P.W.L**	25.16^a	7.40^a	844.33^b	0.023^b	11.00^a	0.0016^a	0.30^a	23.66^b	12.83^a	14.66^a
	P.C.L	27.00^{ab}	7.49^a	744.83^a	0.012^a	10.98^a	0.0085^a	0.37^b	1105.83^a	1381.66^c	1036.16^c
	P.REF	27.60^b	7.45^a	854.16^c	0.019^{ab}	17.23^a	0.0033^a	0.96^c	1111.66^a	1240.66^b	96.66^b
	F	5.3226	2.3412	597.57	6.904	1.1544	2.9003	748.06	7338.3	4509.4	7138.4
	P	0.017	0.13	0.00	0.007	0.34	0.08	0.00	0.00	0.00	0.00
Months	**May**	25.62^a	7.45^a	812.66^a	0.020^a	9.81^a	0.00093^a	0.53^a	734.66^a	862.88^a	386.55^a
	June	27.55^b	7.44^a	816.22^a	0.016^a	16.33^a	0.0081^b	0.56^a	759.44^a	893.88^a	378.44^a
	F	9.08	0.1274	0.0205	1.718	3.13	11.38	0.056	0.0093	0.01	0.0012
	P	0.008	0.725	0.8879	0.208	0.095	0.0038	0.814	0.924	0.92	0.972

P: p-value. F: Factorial. ($p < 0.05$). Values having the same letter in the same column are not significantly different

3.3 Multivariate analysis results

3.3.1 *Principal Component Analysis*

The analysis of the physicochemical and bacteriological data carried out in the analyzed waters of the El Kolea city is carried out by PCA. The used PCA matrix contains six elements (three sites and two sampling campaigns) and ten studied variables. A total of observations without missing values were thus selected for further analysis with the two multivariate methods. The number of variables is equal to the number of components in PCA, but in our study, two maximum components are chosen and the other components were omitted. All of the two components F_1 and F_2 contribute to the total inertia with a percentage of 90.91 % of total data set. The first component (F_1) cumulates 47.63 % of the total variance and the second component (F_2) accounted for about 43.28 % of the total variance of the data set (Table 4). The PCA analysis reduce the dimensionality of the total data from 10 to 2 axis and generate 80 % of reduction and resulted in 9.09 % loss of information contained in the dimensions. This method presupposes primarily that the main components of the samples with larger dispersions are the most interesting directions and that the variability associated with these directions corresponds to the researched information [27,28].

The variables that primarily contributed positively to the first component (F_1) were EC, NO_2^- contrary to NO_3^-, pH, SF which correlate negatively with this axis. The second component (F_2) had negative loadings for T, Tur, TC, FC and positive loading to the NH_4^+. Thus, the first principal component can be interpreted as physicochemical contamination because it was mainly related to the most significant variables being NO_2^- and EC. But, the second component indicate microbial component since it ‘is linked to TC and FC (Table.5). The strength of the associations between the parameters is determined by the study of the linear correlations bi-varied between these parameters [29]. The correlation matrix of the ten quantitative variables measured during the study period (May 2016 and June 2016) is shown in (Table.5). Each variable that present a value equal or greater than 0.60 were considered as significant [30].

It should be noted that a high positive association is observed between water temperature and successively total coliform. fecal coliform and fecal streptococci ($r = 0.88$; $r = 0.88$ and $r = 0.61$ respectively) on the one hand and high negative association is noted between temperature and

ammonium nitrogen on the other hand (r = - 0.64). The pH is negatively associated with electrical conductivity (r = -0.63) and positively associated with nitrate nitrogen and fecal streptococci (r = 0.68. r = 0.60). A high negative association exists between electrical conductivity and both fecal streptococci and NO_3^- (r = -1, r = - 0.63) and positive association between EC and NO_2^- (r = 0.82). Nitrite nitrogen is negatively correlated with both nitrate nitrogen and fecal streptococci (r = -0.80, r = -0.80). and associated positively with Turbidity (r = 0.66). Nitrate nitrogen are negatively correlated with Turbidity (r = -0.95) and positively associated with ammoniacal nitrogen (r = 0.77) (Table.5).

Table.4: Eigenvalues of correlation matrix and related statistics

Component	Eigenvalue (Eig)	% Total variance	Cumulative Eig	Cumulative %
F_1	4.763349	47.63349	4.76335	47.6335
F_2	4.328440	43.28440	9.09179	90.9179

Table.5: Factors models and correlation matrix

Variables	F_1	F_2	T	pH	EC	NO_2^-	NO_3^-	NH_4^+	Tur	TC	FC	FS
T	-0.16	**-0.41**	1.00	0.20	-0.57	-0.28	-0.09	**-0.64**	0.37	**0.88**	**0.88**	**0.61**
pH	**-0.35**	0.05		1.00	**-0.63**	-0.56	**0.68**	0.43	-0.51	0.08	0.13	**0.60**
EC	**0.43**	0.14			1.00	**0.82**	**-0.63**	-0.17	0.42	-0.51	-0.58	**-1.00**
NO_2^-	**0.41**	-0.03				1.00	**-0.80**	-0.41	**0.66**	-0.14	-0.22	**-0.80**
NO_3^-	**-0.38**	0.24					1.00	**0.77**	**-0.95**	-0.32	-0.24	0.59
NH_4^+	-0.19	**0.41**						1.00	**-0.89**	**-0.70**	**-0.64**	0.12
Tur	0.30	**-0.35**							1.00	0.57	0.50	-0.37
TC	-0.10	**-0.46**								1.00	**1.00**	0.56
FC	-0.14	**-0.45**									1.00	**0.62**
FS	**-0.42**	-0.16										1.00

The values in bold are different from 0 to a level of significance α = 0.05

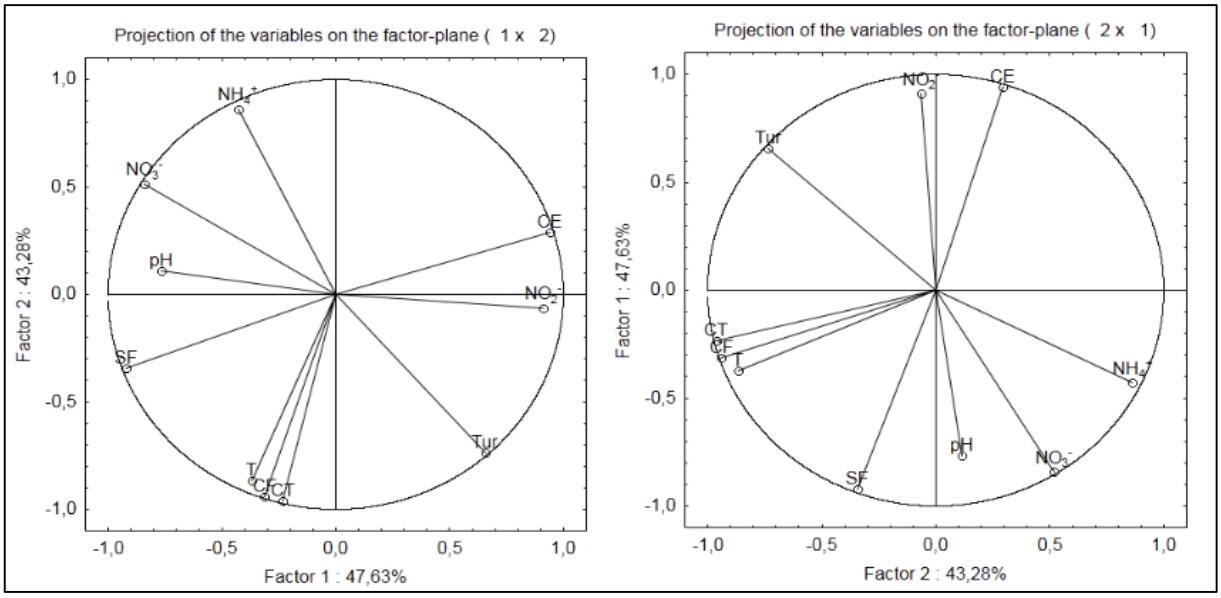

Figure.4: Biplots of the two axes of PCA performed on microbiological and physicochemical variables

✓ Cluster Analysis

The similarity degrees study between the different studied variables is made by the use of the Ascending Hierarchical Classification (AHC) on the basis of the physicochemical and bacteriological quality of the prospected well waters. This study could to distinguish two different clusters shown in dendrogram (**Figure 5)**. The first cluster (A) represent the microbiological component (F_2) previously described in PCA study. Cluster (A) is characterized by the presence of fecal indicators FC. TC and FS associated with turbidity. The rise of turbidity was previously linked to bacteria which adsorbed onto clay particles. The second cluster (B) includes turbidity, pH, T, NO_3^-, NO_2^-, NH_4^+ and represent physicochemical component (F_1) presented in PCA study.

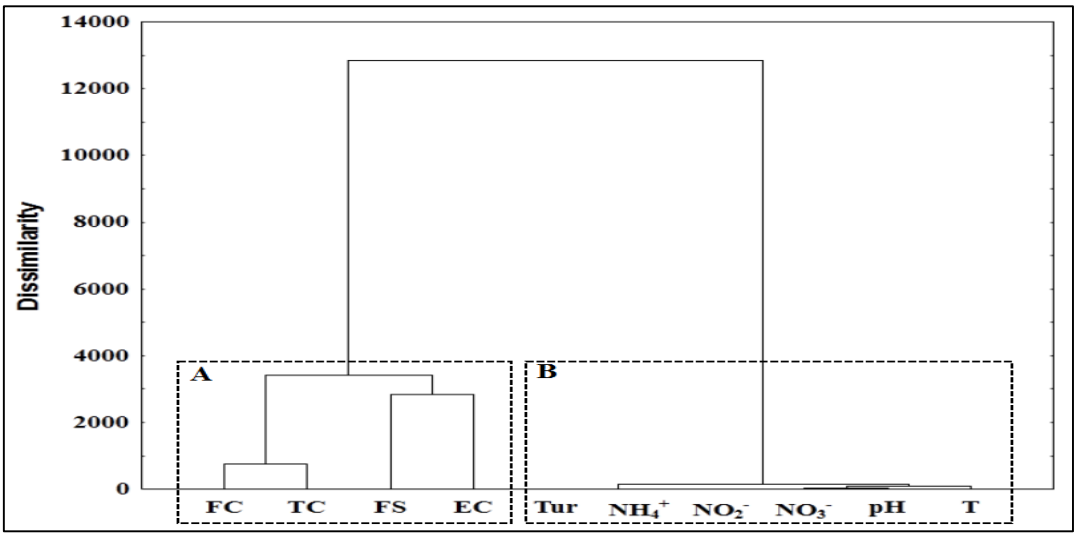

Figure 5: Dendrogram obtained by application of the Ward's method

Conclusion

This paper revealed that three monitored sites of drinking water in El kolea (Taddart , Chorafa Lkhmaïss and Widadiat Lkhir's wells) are influenced by multiple natural and anthropgenic factors such as fecal bacteria and nitrogen pollution. These wells are polluted with fecal bacteria coming undoubtedly from sewage of cesspools used as sanitation system. While all of physicochemical parameters are within Moroccan standards. Used ANOVA show existence of difference between sites and between months for some studied parameters, due to specificity and characteristics of each site and each month. Principals Component Analysis and Cluster Analysis were the multivariate analysis techniques which revealed some specific features of the data structure. Two principal components were accounted for 90.91 % of the total variance. The first component was identified as the physicochemical component with 47.63 % of contribution and the second component with 43.28 % of contribution was linked to microbial load. Physicochemical component formed mostly by NO_2^- and EC and the microbial component is related to TC and FC. PCA results were confirmed with cluster analysis obtained by application of Ward's method. Two clusters of variables detected corresponding to the two components previously identified in PCA studies.

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